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UNFURLING THE DIVERSITY
OF THE NEOTROPICAL
FERN GENUS *DANAEA*
(MARATTIACEAE)

Venni Keskiniva



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*Show me something I can kick,
and hit with rocks,
and set fire to,
and leave out in the rain,
and think about,
and if it's still standing after all that then maybe,
just maybe,
I'll start to believe in it,
but not till then.*

– Frances Hardinge, Fly By Night

UNIVERSITY OF TURKU

Faculty of Science

Department of Biology

Biology

VENNI KESKINIVA: Unfurling the diversity of the Neotropical fern genus *Danaea* (Marattiaceae)

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ABSTRACT

Tropical rainforests are at the same time some of the most species-rich and the least known terrestrial ecosystems. They are also under immense human pressure. Lack of information about species taxonomy, their distributions, and their relationships to environmental variables hinders conservation efforts. In Amazonia, the world's largest rainforest, ferns have been used as indicators of the soil, which determines the forest types of the region. That is why this dissertation focuses on *Danaea* (Marattiaceae), a fern genus with a distribution across tropical America, whose species seem to show soil specialization. However, the unclear state of taxonomy of the genus has led to a situation where individuals have been identified mainly at the level of three subgenera, which has inhibited the use of these ferns as indicators.

I investigated what species *Danaea* contains and where they occur by examining variation in morphology and molecular phylogenetics together with biogeography. I also studied the significance of hybridization as an evolutionary force within the genus. My research was based on phylogenetic analyses and herbarium samples from across the distribution of the genus. I accepted 79 species and 8 hybrids in *Danaea*, of which 24 species and 6 hybrids were described as new to science. *Danaea* is the most species rich around the Andean mountains and Central America.

The difficult taxonomy of *Danaea* can be explained by hybridization as well as convergent evolution and/or conservation of morphology. A taxonomic revision utilizing molecular data helped separate the morphologically challenging genus into evolutionarily, biogeographically, and morphologically consistent species, which can be used in future research employing a new identification key.

Hybridization appears to act both as a homogenizing and diversifying force within *Danaea*. One of the hybrids I found is a result of one of the possibly deepest known hybridization events in the biological world.

My dissertation showed how molecular phylogenetic methods can accelerate the description of diversity. Many of the new species were already endangered at the time of description. Furthermore, following my revision the distribution areas of *Danaea* species are generally narrower than previously thought.

KEYWORDS: biodiversity, new species, taxonomy, phylogenetics, hybridization, Neotropics

TURUN YLIOPISTO

Matemaattis-luonnontieteellinen Tiedekunta

Biologian Laitos

Biologia

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TIIVISTELMÄ

Trooppiset sademetsät ovat samaan aikaan sekä lajirikkaimpia että huonoiten tunnettuja maaekosysteemeitä. Tämän lisäksi niihin kohdistuu suuria käyttöpaineita. Puutteellinen tieto lajien taksonomiasta, niiden levinneisyydestä ja niiden suhteesta ympäristömuuttujiin hankaloittaa niiden suojelua. Amazoniassa, maailman suurimmassa sademetsässä, saniaisia on käytetty ilmentämään maaperän ominaisuuksia, jotka määräävät alueen metsätyyppejä. Siksi tämä väitöskirja keskittyykin trooppisessa Amerikassa esiintyvään saniaissukuun *Danaea* (Marrattiaceae), jonka lajeilla vaikuttaa esiintyvän erikoistumista maaperän mukaan. Suvun taksonomian epäselvä tila on kuitenkin johtanut tilanteeseen, jossa yksilöt on määritetty lähinnä kolmen alasukun tasolle, mikä on haitannut näiden saniaisten käyttöä ilmentäjinä.

Selvitin mitä lajeja *Danaea*-suku sisältää ja missä nämä esiintyvät tarkastelemalla morfologista ja molekyylifylogeneettistä vaihtelua yhdessä eliömaantieteen kanssa. Tutkin myös risteytymisen merkitystä evolutiivisena voimana suvussa. Tutkimukseni perustuivat fylogeneettisiin analyyseihin sekä herbaarionäytteisiin koko suvun levinneisyysalueelta. Päädyin hyväksymään 79 lajia ja 8 risteymää suvussa, joista 24 lajia ja 6 risteymää kuvasin tieteelle uutena. Suurin *Danaea*-lajirikkaus on Andien vuoriston ympäristössä sekä Keski-Amerikassa.

Taksonomian vaikeutta suvussa selittävät risteytyminen sekä konvergenttinen evoluutio ja/tai morfologian konservoituminen. Molekyylifylogeneetiikkaan nojaava revisio auttoi erottamaan morfologisesti hankalan suvun evolutiivisesti, eliömaantieteellisesti ja morfologisesti johdonmukaisiksi lajeiksi, joita voidaan käyttää tulevaisuudessa tutkimuksissa hyödyntäen nyt julkaisemaani määrittäyskaavaa.

Risteytyminen vaikuttaa toimivan sekä homogenisoivana että diversiteettiä lisäävänä voimana *Danaea*-suvussa, ja yksi löytämistäni risteymistä on mahdollisesti yksi syvimmistä tunnetuista hybridisaatioista eliömaailmassa.

Väitöskirjani osoitti, kuinka molekyylifylogeneettiset menetelmät voivat vauhdittaa luonnon monimuotoisuuden kuvaamista. Monet uusista lajeista paljastuivatkin jo kuvaamishetkellä uhanalaisiksi. Revisioni myötä *Danaea*-lajien levinneisyysalueet ovat ylipäättään kapeampia kuin aiemmin on luultu.

ASIASANAT: fylogeneetiikka, hybridisaatio, monimuotoisuus, Neotropiikki, uudet lajit, taksonomia.

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List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

- I Keskiniva, J. S., & Tuomisto, H. Six new species of *Danaea* (Marattiaceae) and the synonymisation of *Danaea quebradensis*. *Kew Bulletin*, 2022; 77(1): 189-210.
- II Keskiniva, V., & Tuomisto, H. *Danaea* (Marattiaceae) keeps diversifying, part 1: eighteen new species. *Willdenowia*, 2024; 53: 173-228
- III Keskiniva, V., Tuomisto, H., Lehtonen, S. *Danaea* (Marattiaceae) keeps diversifying, part 2: phylogeny and identification key for 81 taxa. *Willdenowia*, 2024; 53: 229-255.
- IV Keskiniva, V. Lehtonen, S., Testo, W., Consortium, GoFlag, Tuomisto, H. Rampant hybridization in an old tropical fern genus (*Danaea*, Marattiaceae). *Manuscript*.

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1 Introduction

Here, in the 21st century, one can still go to one of the most thoroughly researched tropical rainforests in the world, enter the back yard of a biological station, and pick up a species of fern unknown to science. This is a story of those ferns.

1.1 Tropics as a research environment

In many ways, research in the tropics today is concerned with questions that were at the forefront of research in temperate regions a hundred years ago. What species are there? Where do they occur? The answers are necessary for all other research of tropical ecosystems and evolution. These are also largely the questions that this thesis is concerned with.

Tropical rainforests are simultaneously some of the most species rich and the poorest known land ecosystems (Barlow et al., 2018; Feeley, 2015). These forests are also threatened by human development and climate change (IPBES, 2019). They are disappearing at an alarming rate, which causes a significant threat to global biodiversity. However, current conservation areas are not always chosen to best protect biodiversity (Schulman et al., 2007). Efficient planning of conservation areas is hindered by missing information on species distributions and how they relate to environmental variation.

Many species in tropical forests are rare (Coddington et al., 2009; Lim et al., 2012, Hubbell, 2013; Enquist et al., 2019). Combined with a remarkable species richness, a limited amount of field trips, and a scarcity of taxonomists specializing in tropical taxa, this leads to a situation where many species are still undescribed (Collen et al., 2008; Hortal et al., 2015). Species that are thought to be common and widespread often turn out to be species complexes (Damasco et al., 2021).

Taxonomy, or the systematic classification of organisms, lags behind in the tropics in part due to undersampling when compared with temperate regions (Collen et al., 2008). Taxonomy in tropical groups is often based on few specimens from disjunct areas and thus contains a lot of filling in the gaps. Defining species limits without a clear picture of variation between and within populations can be difficult.

The largest remaining area of tropical rainforest is Amazonia in the Neotropics. Soils largely drive the floristic patterns of this climatically rather uniform area

(Tuomisto & Poulsen, 1996; Phillips et al., 2003; Tuomisto et al., 2014; 2016; 2019; Baldeck et al., 2016; Bañares-de-Dios et al., 2022) and ferns of the understory have been used as indicators of edaphic (soil) and floristic variation in the Amazon rainforest (Ruokolainen et al., 2007; Tuomisto et al., 2016; 2019). Ferns have light spores that can disperse along air currents, making them more likely to colonize all suitable growing sites than is the case with angiosperms, which are often dependent on animals to transfer their seeds (Tryon, 1986). As a result, fern distribution appears to correlate more closely with environmental variables than the distribution of angiosperms does, making them good indicators of soil properties (Jones et al., 2014; Tuomisto et al., 2016).

The use of ferns as indicators of forest types in Amazonia is one of the reasons, why the focus of this thesis is on the Neotropical fern genus *Danaea*. *Danaea* species appear to have different edaphic niches in Amazonia, which is why they could be good indicators of soil nutrient levels (Tuomisto & Poulsen, 1996). Identifying *Danaea* species has been difficult, however, necessitating taxonomic research on the group whether one is primarily focused on exploring species ecology and their indicator properties, or simply on cataloguing the diversity of tropical forests.

1.2 Integrative taxonomy and cryptic species

Species is a fundamental concept of taxonomy and all other biological sciences (de Queiroz, 2005). Species can be defined as independent evolutionary lineages, or lineages of metapopulations through time (Wiley, 1978; de Queiroz, 2005; 2007). These ‘evolutionarily significant units’ (Moritz 1994) are also the natural targets for conservation biology (Carstens et al., 2013).

Scientific names of species are integral in connecting all biological information of a species together (Wheeler, 2004). They are discussed in the realm of taxonomy and published in species descriptions. Species description as a term may evoke the image of taxonomy as a descriptive field, but each species description is a hypothesis of separately evolving entities based on discontinuities of unique traits (Nixon & Wheeler, 1990). These are always theoretical, as it is not possible, or indeed sensible, to look at all individuals in all populations (Wheeler, 2004). Species hypotheses are tested against other species and specimens in a taxonomic revision.

The delimitation of a species in practice is no easy task. For practical and historical reasons most species descriptions are based on trying to find morphologically separable groups (Padial et al., 2010). Recently molecular phylogenetic trees, which show the relationships of organisms through time, have made it possible to link taxonomy with evolutionary history. Species as independent evolutionary lineages are expected to diverge genetically and thus form monophyletic groups (groups containing a common ancestor and all its descendants)

in a molecular phylogeny (de Queiroz, 2007). As both morphology and genomes are expected to diverge during the evolutionary divergence of lineages, they can be used in conjunction to build stronger species hypotheses (de Queiroz, 2005; Padial et al., 2010). In fact, integrative taxonomy combines as many lines of evidence as possible to draw limits between species, including phylogeography, comparative morphology, population genetics, ecology, development, and behaviour (Dayrat, 2005).

In short, our confidence in a species hypothesis is generally higher when supported by broad biological evidence than when supported by only one kind of data. However, in the case of cryptic species, incongruences in molecular and morphological data can lead to species hypotheses that are stronger than those supported by congruence (Fišer et al., 2018). Two or more species can be considered cryptic if they have been classified as a single species because they are at least superficially morphologically indistinguishable (Bickford et al., 2007). The existence of cryptic species can be explained with recent divergence, niche conservatism, and morphological convergence (Fišer et al., 2018).

Identifying new species requires a thorough understanding of the existing diversity in the relevant group, but collectors and herbarium managers may lack this depth of knowledge. Taxonomic revisions are rarely conducted and outside them experts do not have the time to systematically go through collections of different herbaria. Consequently, new plant species that have been collected often sit for decades in herbaria without anyone ‘discovering’ them (Fontaine et al., 2012). Indeed, it has been estimated that more than half of plant species unknown to science have already been collected (Bebber et al., 2010). Integrative taxonomy approaches could potentially speed the discovery and description of new species.

1.3 Hybridization and evolution

Hybridization, or the interbreeding of individuals from genetically distinct populations, is an important evolutionary force (Ellstrand & Schierenbeck, 2000; Seehausen, 2004; Abbott et al., 2013; Todesco et al., 2016). For instance, hybridization can lead to speciation (Abbott et al., 2013), which is most commonly associated with genome duplication, as this directly leads to reproductive isolation (Soltis & Soltis, 2009). Hybrids can combine the traits of their parental species in different ways, forming intermediate traits or entirely new evolutionary innovations (Rieseberg et al., 1999). Whereas evolution usually works on small scale variation in a population, hybridization makes it possible combine functional blocks of traits into new combinations, enabling adaptive radiations into new ecological niches (Anderson & Stebbins, 1954; Seehausen, 2004). On the other hand, hybridization has been seen as a homogenizing force that assures the continuation of gene flow

and can stop or even reverse the speciation process (Abbott et al., 2013; Rothfels et al., 2015). Excessive gene flow can result in genetic swamping and even lead to the extinction of rare taxa (Todesco et al., 2016).

Hybridization in plants is common but does not occur universally in all lineages, with most hybridization events happening in relatively few genera (Ellstrand et al., 1996; Whitney et al., 2010). Evidence drawn principally from temperate floras suggests that hybridization is especially prevalent in ferns (Whitney et al., 2010), perhaps due to their genetic stability (Schneider et al., 2015; Clark et al., 2016) and abiotic method of dispersal (Ellstrand et al., 1996; Mitchell et al., 2019). The deepest known hybrids, or hybrids between lineages diverged the longest time ago, are also known from ferns (Liu et al., 2020). Weaker reproductive barriers in ferns have been suggested to have contributed to the smaller number of fern species in comparison to flowering plants (Rothfels et al., 2015).

Tropical hybrids have mostly received scant systematic attention (Schley et al., 2022). It has been difficult to say whether the scarcity of tropical fern hybrids is due to differences in hybridization tendency between tropical and temperate regions, or due to fewer studies on hybridization in the tropics (Liu et al., 2020).

1.4 The genus *Danaea*

1.4.1 Evolutionary history

The Neotropical fern genus *Danaea* consists of 10 cm to 3 m tall terrestrial plants with simple or pinnate (rarely bipinnate) leaves (Fig. 1). *Danaea* are part of the ancient group of Marattioid ferns (marattioids). Marattioid tree ferns dominated swamp forests especially in the western Euramerica near the end of the Carboniferous period, circa 300 Mya (DiMichele & Phillips, 2002), creating a part of the coal deposits that are today used as fossil fuels. The golden age of marattioid diversity was during the Palaeozoic (DiMichele & Phillips, 2002; Lehtonen et al., 2017; Rothwell et al., 2018). Only one family, Marattiaceae, has survived to modern times in the wet tropical regions (Murdock 2008a).

Marattioids are eusporangiate, which means that their sporangia arise from several epidermal cells, and most phylogenomic analyses resolve them as the sister lineage to leptosporangiate ferns, whose sporangia arise from a single cell (see Lehtonen et al., 2020 and references therein). Extant marattioids, Marattiaceae, can be diagnosed with the naked eye by their sporangia being fully or partially fused into synangia, their complex polycyclic stem vasculature, the stipules that flank leaves basally, and the prominent, fleshy swellings (pulvini) at nodes or bases of segments of leaves (Murdock 2008a).

The generic classification of Marattiaceae has differed between authors, but most recently Murdock (2008a) revised the family with the aid of molecular data to contain six genera; the paleotropical *Marattia* Sw; *Danaea* Sm. and *Eupodium* J.Sm. in the Neotropics; *Angiopteris* Hoffm. and *Ptisana* Murdock in the paleotropics; and *Christensenia* Maxon in the Indo-Malayan region (Murdock, 2008a). Out of these, *Danaea* has been rather consistently resolved as a sister to the other genera (Murdock, 2008b, Lehtonen et al., 2020, Zhao et al., 2023).

Timing the divergence of *Danaea* from the rest of the extant Marattiaceae is difficult; there is a wealth of marattioid fossils from the Carboniferous, but next to nothing has been found since the beginning of the Cretaceous ca. 150 Mya (Rothwell et al., 2018; Lehtonen et al., 2020). Estimates for the divergence of *Danaea* have varied in timed phylogenies between as early as late Triassic to as late as the late Cretaceous, 75–220 Mya (Smith et al., 2010; Testo & Sundue, 2016; Lehtonen et al., 2017; Lehtonen et al., 2020; May et al., 2021; Nitta et al., 2022). The ancient age of *Danaea* in combination with an anomalously slow rate of molecular evolution (Soltis et al., 2002) makes it an interesting group for looking at hybridization, as it could potentially contain events of deep hybridization. Widespread reticulate evolution could also explain why *Danaea* species are so difficult to identify morphologically.

A previous study focusing on the evolutionary relationships in *Danaea* found that *Danaea* is monophyletic and can be split into three well supported clades (Christenhusz et al. 2008), which Christenhusz (2010) associated with the three subgenera that had been described by Presl (1845); *D.* subg. *Arthrodanaea*, *D.* subg. *Danaea* and *D.* subg. *Holodanaea* (Fig. 1). Out of these, *D.* subg. *Arthrodanaea* is a sister to the other two subgenera (Christenhusz et al., 2008, Christenhusz, 2010). The phylogeny also revealed possible patterns of allopatric and sympatric speciation in *Danaea* (Christenhusz et al. 2008). However, resolution was lacking in some clades, and not all of the species in the genus were sampled. More accurate information on the taxonomy, geographical distributions, and ecology together with a wider sampling of taxa and more variable genes could further clarify the evolutionary history of *Danaea*.

1.4.2 Morphology

Danaea differ from other ferns in the family Marattiaceae by generally having pulvini both at nodes and at petiole bases (vs only at petiole bases), by having usually



Figure 1. Subgenera of *Danaea*. **A** *D. cuspidata* from *D.* subg. *Holodanaea*; **B** *D. lepieurii* from *D.* subg. *Arthrodanaea*; **C** *D. kessleri* from *D.* subg. *Danaea*. © Hanna Tuomisto

once-pinnate leaves (vs bipinnate or more complex) with opposite pinnae (vs alternate), and by the leaves being dimorphic, with the fertile leaves having the lower surfaces covered in sori that are sunken into the lamina (Christenhusz et al., 2008; Murdock, 2008a). An erect rhizome (Fig. 2 A) seems to be the ancestral form in the genus, perhaps reflecting the tree-like past of marattioids (Christenhusz et al., 2008; Murdock 2008a). The erect rhizome has evolved into creeping or ascending several times in the history of the genus (Fig. 2; Christenhusz et al., 2008).

The subgenera identified in the phylogeny in Christenhusz et al. (2008) had also previously been identified based on divergent morphology (Christenhusz & Tuomisto, 2005). Species of *Danaea* subg. *Arthrodanaea* are under 1 m tall, lack proliferous buds, have an erect rhizome, few pairs of pinnae (0–8), entire pinna margins, and usually nodose petioles. Species of *D.* subg. *Danaea* grow to the largest sizes, being over 1 m tall as adults (up to 3 m in some species). They usually have many pairs of pinnae (2–24), no nodes on the petiole, lack proliferous buds, and have creeping dorsiventral rhizomes, but exceptions to each of these traits exist. Species of *D.* subg. *Holodanaea* are more variable than those of the other two subgenera, having for example species with translucent or strongly bicolorous leaves or crispate pinna margins. They are under 1 m tall and usually have many pairs of pinnae (0–29), serrate pinna apices, nodose petioles, and proliferous buds at the leaf apex. Rhizomes in *D.* subg. *Holodanaea* vary from erect to ascending or creeping (Fig. 2).

1.4.3 Ecology and biogeography

Danaea have rather long life spans, with sporophytes of one of the smallest species in the genus, *D. wendlandii* from *D.* subg. *Holodanaea*, estimated to live at least 23 years, reaching sexual maturity only at about 13 years (Sharpe, 1993). Leaves in *D. wendlandii* emerge sequentially at a rate of about 1.6 leaves per year (Sharpe, 1993) and senesce over time (Sharpe, 2023). A long-term study of taxa from *D.* subg. *Arthrodanaea* and *D.* subg. *Danaea* showed mean leaf ages of ca. 3–5 years (Sharpe, 2023). I have seen a rhizome of *D. sellowiana* with at least 40 leaf bases (Mynssen 1074, TUR), corresponding to an estimated maximum age of at least 25 years for *D.* subg. *Danaea* as well. The long generation times of Marattiaceae could in part explain their slow rate of molecular evolution (Soltis et al., 2002), as has been suggested in tree ferns (Korall et al., 2010).

The distribution of *Danaea* spans most of the tropical Americas, from southern Mexico through Central America, the Greater and Lesser Antilles and tropical South America, south to Bolivia, Paraguay and the Atlantic coast of Brazil (Christenhusz & Tuomisto, 2005). *Danaea* likely arrived to its current area of distribution from the ancestral Eurasian range of Marattiaceae through North-America while these



Figure 2. The different rhizome habits of *Danaea*. **A** An erect rhizome with leaf and root bases arranged radially (*D. leprieurii* from *D. subg. Arthrodanaea*); **B** A creeping rhizome with leaf and root bases arranged dorsiventrally and leaf bases in two rows (*D. ampla* from *D. subg. Danaea*); **C** A creeping rhizome with leaf and root bases arranged dorsiventrally and leaf bases in more than two rows (*D. grandifolia* from *D. subg. Danaea*); **D** An ascending rhizome with leaf and root bases arranged radially (*D. riparia* subg. *Holodanaea*). **A, B, D:** © Hanna Tuomisto **C:** © Michael Kessler

continents were still connected (Lehtonen et al., 2020). Two fossils from the Paleogene of North America have been described as *Danaea*; *D. coloradensis* Knowlton (Knowlton, 1923) and *D. borealis* Pabst (Pabst, 1968), but their identities have been questioned (Collinson, 2001). *Danaea coloradensis* looks similar to the fertile pinnae of modern *D.* subg. *Holodanaea*, but was considered to be an “object of unknown affinities” by MacGinitie (1969), and *D. borealis* is represented by sterile material that cannot be determined to family level (Collinson, 2001).

Danaea thrive in the humid shade of the undergrowth of tropical rainforests, often at the edges of creeks or streams (Christenhusz & Tuomisto, 2005). They have been collected from sea level up to 2600 meters, but appear to be especially species rich at 100–1000 meters (Christenhusz, 2006). Different *Danaea* species appear to grow on soils with different nutrient status in Amazonia (Tuomisto & Poulsen, 1996; Christenhusz et al., 2008) and could be used as indicators of forest types (Salovaara et al., 2004; Ruokolainen et al., 2007; Tuomisto et al., 2016; 2019). However, because identifying *Danaea* species is challenging, it is difficult to accurately document ecology and, as a result, indicator characteristics.

Danaea species grow in tropical environments which are under pressure from humans (IPBES, 2019), and some species are therefore likely to be under threat of extinction. Targeted conservative measures cannot be directed to a species before it is first described.

1.4.4 Taxonomy and phylogenetics

Morphological differences between species in *Danaea* are often continuous rather than qualitative and a combination of several traits is usually needed to definitively diagnose a species (Christenhusz, 2010). This has led to wildly incongruent species delimitations in *Danaea* (Tuomisto & Moran, 2001; Rolleri 2004; Christenhusz 2010; Rojas-Alvarado, 2013). Some groups are so difficult, that it has not been possible to divide them into clearly separatable species, resulting in a wide range of variation being accepted within a single species. Many species complexes contain potentially undescribed species. For instance, Christenhusz et al. (2008) found that *D. nodosa*, which had previously been considered to be a morphologically variable and geographically widespread species, was actually a polyphyletic complex of geographically more restricted lineages. The taxonomic confusion within *Danaea* has led to a situation, where most specimens are identified under only a handful of names.

An integrative approach to taxonomy utilizing both comparative morphology and molecular methods has previously helped in delimiting species in the morphologically difficult fern genera *Metaxya* C.Presl and *Saplichlaena* J.Sm. (Cárdenas et al., 2016; 2019), and could be expected to work for *Danaea* as well.

The previous phylogenetic study on *Danaea* (Christenhusz et al., 2008) found conflicting evidence to the broad species delimitations of Rolleri (2004), who lumped all but 18 species in the genus (Tuomisto & Moran (2001) recognized 18 species for Ecuador alone). The phylogeny in Christenhusz et al. (2008) was based on three plastid loci from only 31 species, and a wider taxonomic and genomic sampling could help in resolving species complexes in *Danaea*.

1.5 Aims of the thesis

This dissertation aims to revise the tropical fern genus *Danaea* by integrating traditional taxonomy with phylogenetics to understand patterns of diversity, distribution, and hybridization within the genus.

In Chapters **I**, **II**, and **III** I wanted to answer those most basic of questions on which the rest of biological research is built; “What species are there?” and “Where do they occur?”. My aim was to delimit species in *Danaea* in such a way that they would represent evolutionary lineages in order to be useful in further ecological and evolutionary studies, and to make the revised taxonomy available to facilitate further research using *Danaea*.

In Chapter **IV** my aims were threefold. Firstly, I quantified the extent of hybridization in *Danaea* to compare rates of hybridization between temperate and tropical ferns. Secondly, hybridization can work as both a diversifying and a homogenizing force, so I looked at the effect of hybridization on *Danaea* diversity. Lastly, as an early diverging genus with a slow rate of molecular evolution, events of deep hybridization could happen in *Danaea*, so I aimed to estimate the time of divergence of parental lineages.

2 Materials and Methods

2.1 Herbarium and field data

To clarify the taxonomy of *Danaea*, I, together with prof. Hanna Tuomisto, studied the original type specimens of almost all described *Danaea* taxa, as well as more than 3000 herbarium specimens across the distribution of the genus (**I, II, III**, Fig. 3). This was made possible by extensive loans from herbaria around the world, as well as the increasing number of collections available as photographs online. The sampling was spatially heterogenous, with specimen densities mirroring the favourite destinations of researchers throughout history (Fig. 3). For example, more specimens were collected in both the Central America and the Lesser Antilles than in the whole of Brazil. It can be difficult to separate whether a species is actually rare, or if the scarcity of specimens is due to the species' area of distribution being poorly collected.

There were some limitations to our observations of the herbarium data. Firstly, several species were represented by only a handful of collections, which mostly made it impossible to look at within and between population variation in morphology. Secondly, herbarium material seldom includes juvenile plants, and because of this we mostly focused on characteristics of adult plants. We also limited our exploration of the material to traits observable with the naked eye. These traits, such as leaf shape, have been observed to work in species delimitation in *Danaea* before. As previous studies on *Danaea* have not found microscopic traits useful in species delimitation within this group, we opted not to use them (Christenhusz, 2007).

Some traits in *Danaea*, such as lamina color and rhizome habit, are best observed in the field. Thankfully, many of the species had been collected by my co-authors or other colleagues who also provided field observations and images of fresh material.

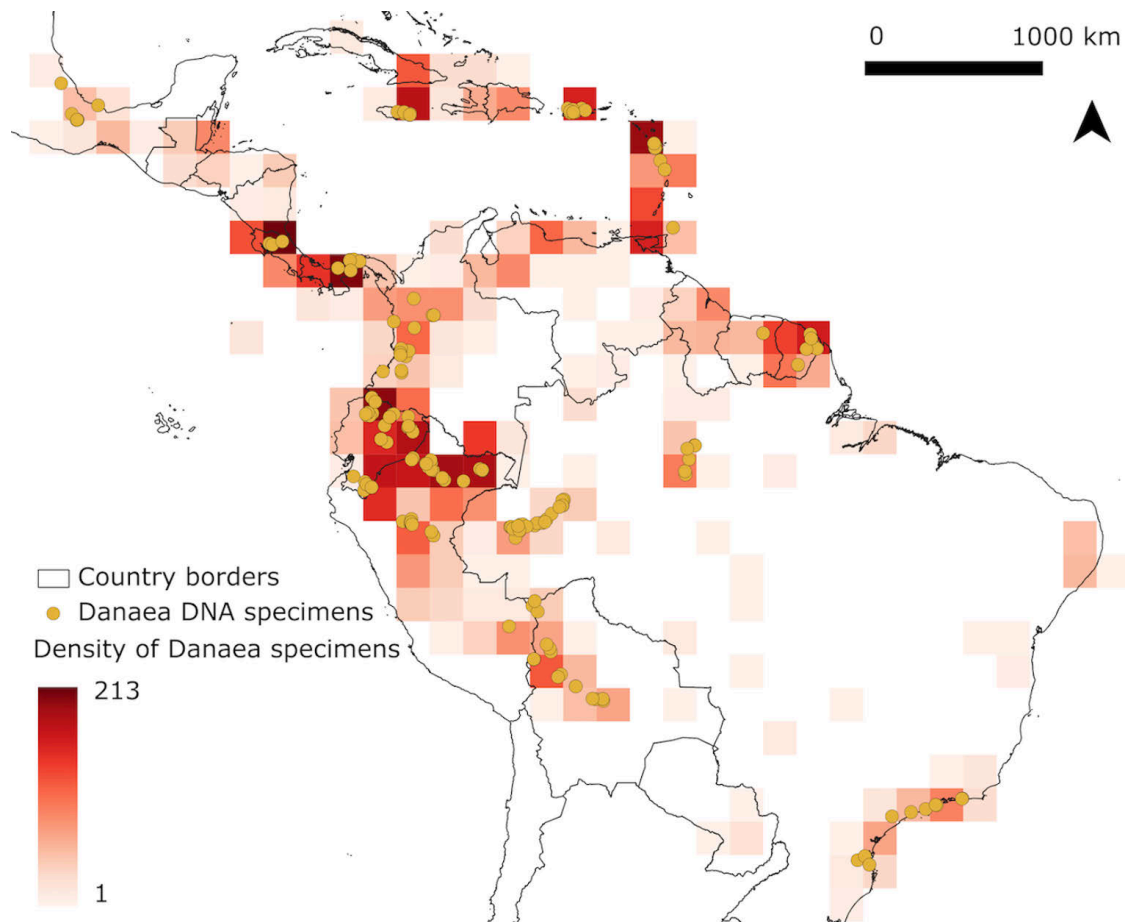


Figure 3. Distribution in tropical America of *Danaea* specimens used in this dissertation (III). Density of all available material is shown as a count of unique collection numbers per $2^\circ \times 2^\circ$ grid cell, and DNA specimens used in phylogeny are shown as dots.

2.2 Genomic data

Plants have three types of genomes with distinct evolutionary trajectories; the nuclear, the plastid and the mitochondrial genome. Plastid DNA has traditionally been used in plant systematics, as plastid genomes are smaller than nuclear genomes and less variable in size than mitochondrial genomes (Smith & Keeling, 2015), making them easier to sequence, assemble, and analyse. The majority of land plants have plastids with highly-conserved structures, which facilitate comparative studies across a wide range of taxa (Wicke et al., 2011). The plastid genome is inherited uniparentally from the maternal progenitor in ferns (Gastony & Yatskievych, 1992; Vogel et al., 1998), making it easier to track individual lineages from the plastid genome in comparison to the biparentally inherited nuclear genome, which is under constant recombination.

For the systematic study of *Danaea* in Chapter III, we produced a plastid dataset of four loci for 68 species of *Danaea* (Fig. 3), including samples from each of the

five other genera in the family Marattiaceae. For most species we had more than one collection throughout the distribution of the species to cover intraspecific variation.

Because the nuclear genome contains haplotypes (groups of DNA variants inherited together) from both parents, it facilitates the study of hybridization and reticulate evolution (Bloesch et al., 2022). The larger size and variability of the nuclear genome in relation to the plastid genome could also make it more suited for studying relationships of closely related taxa. However, researchers focusing on ferns have faced challenges in accessing and utilizing nuclear data, mainly because of the lack of an annotated genome sequence and the complexities associated with the large, highly repetitive genomes characteristic of many fern species (Wolf et al., 2015).

To look at the extent of hybridization and introgression in *Danaea*, in Chapter IV we used a nuclear dataset of 20 taxa obtained with target enrichment methods. These methods use short RNA (or DNA) probes corresponding to the targeted loci to bind DNA (or RNA) from sequencing libraries. The bound DNA is then sequenced, while much of the unbound DNA is discarded (Gnirke et al., 2009). Target enrichment methods can obtain data from hundreds of phylogenetically informative nuclear loci at a relatively low cost (Breinholt et al., 2021).

The targets used to produce the *Danaea* dataset were originally designed to enable comparison across all flagellate land plant lineages, and thus contained relatively conserved exons found within single- or low-copy nuclear loci (Breinholt et al., 2021). The enrichment protocol typically also yields sequence data from the more variable flanking regions that can help in resolving relationships among closely related taxa (Breinholt et al., 2021). I matched reads of the target enrichment dataset of *Danaea* to references for each target locus that also contained some of the more variable flanking regions to acquire a final dataset of 356 nuclear loci using Hybpiper (Johnson et al., 2016).

To further look at the extent of hybridization in *Danaea* in Chapter IV, I identified potential hybrids based on unexpected placement of samples in the plastid phylogeny of Chapter III and sequenced one variable nuclear locus for 19 additional samples. To compare plastid and nuclear phylogenies in Chapter IV, we produced plastid sequences for the samples not present in the phylogeny in Chapter III.

2.3 Molecular analyses

2.3.1 Reconstructing phylogenies

Molecular phylogenetic methods can be used to infer and visualize potential species boundaries (DeSalle & Goldstein, 2019), as well as evolutionary history. I built

phylogenies based on plastid data to help in delimiting species and identifying hybrids in *Danaea* in Chapters **III** and **IV**.

There are different philosophies and statistical methodologies for tree building in phylogenetics, and the choice of method depends on the study question as well as the computational resources available. The most commonly used character-based methods are maximum parsimony, maximum likelihood, and Bayesian inference. Comparison of empirical datasets (Rindal & Brower, 2011) indicates that the topology of best trees from the same data is the same for all well supported branches irrespective of which of these three statistical methods is chosen. Specific traits of the data can end up being more relevant for choice of method. In my case the plastid phylogeny in Chapter **III** contained a number of genetically very similar specimens due to broad within species sampling. This led to a situation where Bayesian Inference runs failed to converge. As I also wanted to estimate the best model of evolution from the data, I used a maximum likelihood instead of a maximum parsimony approach.

A multiple sequence alignment is usually made prior to phylogenetic analysis, and the choice of an alignment method can have a larger effect on the final tree than the choice of a tree-building method (Wong et al., 2008). I tested the effect of different alignment methods, as two of the plastid loci in Chapter **III** were intergenic spacers, which have variation in sequence lengths that makes them less straightforward to align than exonic regions. The phylogenies based on the different alignment methods were congruent in all well supported nodes, indicating the robustness of the data to different alignment methods.

When sequences have variable lengths, a multiple sequence alignment will contain gaps. These are often treated as missing data in phylogenetic analyses (Machado et al., 2021). However, gaps often represent actual events of insertion or deletion in the genome, drastic events that should be more conserved signals of evolutionary history than single nucleotide mutations (Dessimoz & Gil, 2010; Nagy et al., 2012). I coded gaps as binary data for the intergenic spacers in Chapter **III**, as variation in sequence lengths was phylogenetically informative in *Danaea* and even used in the DNA diagnoses in Chapter **II**.

For phylogenetic analysis, sequence data is usually partitioned to model different molecular evolution in different parts of the genome. Sequence data is often partitioned by genes, non-coding regions, and codon positions in genes. This likely poorly represent actual modes of evolution at different loci, so I also partitioned the data based on rates of evolution estimated from the alignments in Chapter **III** (Rota et al., 2018). I estimated the best models of molecular evolution with ModelFinder (Kalyaanamoorthy et al., 2017) and used them in building a phylogenetic tree with the maximum likelihood program RAxML-NG (Kozlov et al., 2019).

For Chapter **IV** I did a more simple analysis of the plastid data with the inbuilt model selection of the maximum likelihood program IQTREE2 (Johnson et al., 2016) and without coding gaps as binary data. The result of this analysis was essentially the same tree as in Chapter **III**, indicating the robustness of the data to missing gap data and different evolutionary models.

We used the plastid phylogeny of Chapter **III** as a guideline for the evolutionary history in the genus, and delimited species in part by extrapolating from the specimens in the phylogeny to specimens for which DNA had not been analysed. The new species described in Chapters **I** and **II** are based on our revision that utilized molecular phylogenetics, even if in Chapter **I** we only described the species that could be readily separated based on morphology alone.

2.3.2 Delimiting species with the aid of DNA

From a growing concern over threats to biodiversity stems a desire to describe as many species as possible as quickly and accurately as possible (Wiens, 2007). This has led to proposals to describe species based on DNA (Tautz et al., 2003) and even to use DNA as type material, an issue under heated debate (Thiele et al., 2023). Automatic DNA-based methods have also been developed for species delimitation, but different methods can give widely incongruent species numbers (Petzold & Hassanin, 2020). Multispecies coalescent methods, for instance, have been shown to inflate species numbers by recognizing population-level structure as species boundaries (Sukumaran & Knowles, 2017).

Few descriptions of plant species include DNA characters explicitly in the diagnosis (Renner, 2016). Recently Hassemer et al. (2020) advocated for excluding all traits except morphological ones from the species diagnosis, asserting that other traits do not offer “the most succinct and accessible means” for identifying a taxon. In the case of cryptic taxa, however, morphological traits fail to result in a definitive diagnosis (Bickford et al., 2007).

In Chapter **II** we used morphological differences to delimit species, but in addition I identified the diagnostic genetic differences for many of the new species with DNAdiagnoser (Vences et al., 2021), and included a list of these in the diagnosis portions of the species descriptions. My reasons for doing this were twofold. Firstly, not all of the species that were genetically unique could be unambiguously diagnosed based on morphology due to continuous and overlapping morphology. Secondly, our species delimitations were often strongly based on phylogenetic deduction in addition to comparative morphology, and I wanted the reasoning to be explicit.

2.3.3 Identifying hybrids

As nuclear DNA is biparentally inherited, hybrids have nuclear haplotypes from both parental lineages (Bloesch et al., 2022). In Chapter IV, I used a pipeline designed to separate these divergent haplotypes to identify hybrids and their parental lineages.

Different parts of the genome have different evolutionary histories, and gene trees (trees obtained from different loci) are often in conflict with the true species tree (Degnan & Rosenberg, 2009). The cause of this discordance can be hybridization, but also incomplete lineage sorting (Edwards, 2009), which can be modelled by the multi-species coalescent model (Pamilo & Nei, 1988). Because of computational limitations, most coalescent-based analyses are performed using summary methods, which infer the species tree from a set of gene trees (Roch & Warnow, 2015). I estimated the maximum likelihood phylogeny for each of the recovered loci in the nuclear dataset of *Danaea* and built a summary coalescent tree based on these with ASTRAL III (Zhang et al., 2018). I removed poorly supported branches from the gene trees before the coalescent analysis, as these can create an unnecessary source of error (Simmons & Gatesy, 2021).

To infer if samples contained very divergent haplotypes indicating a hybrid origin, I mapped single nucleotide polymorphisms (SNPs) using HybPhaser 2.0 (Nauheimer et al., 2021). The putative hybrids with high numbers of SNPs were mapped to references of major clades in the coalescent phylogeny, and the ones that mapped in high proportions to several references were chosen for haplotype phasing. After phasing the haplotypes by matching reads to the parental references with Hybpiper (Johnson et al., 2016), I had reads that represented haplotypes that hybrids inherited from their parents (Nauheimer et al., 2021). I then built a coalescent phylogeny that showed the divergent placements of the hybrid haplotypes.

To confirm the hybrid identity of the specimens with discordant placement in the chloroplast phylogeny of Chapter III, I extracted variants for each accession of the additional single nuclear locus using vsearch (Rognes et al., 2016). I also included the phased (for hybrids) and non-phased (for non-hybrids) sequences of the same locus from the larger nuclear analysis to create a taxonomically wider nuclear dataset. I performed a phylogenetic analysis similarly to the analysis of the plastid data in Chapter IV.

2.4 Building an identification key

Taxonomy is charged with the scientific tasks of delineating and classifying species, as well as the tasks that are in service to the community; naming species and providing tools to identify them (Dayrat, 2005). One aim of our work was to have the revised taxonomy of *Danaea* accessible to the wider scientific community. For this purpose we built an online identification key to the genus.

With traditional dichotomic keys, the user often runs into a brick wall if the specimen being identified lacks some necessary identifying features, for instance the fertile parts. To facilitate ease of identification we built a multi access key, which can be started with any trait (**III**). I took several morphological measurements across the variation of each species for the identification key, concentrating on characteristics of adult plants that could be seen with the naked eye in herbarium specimens.

2.5 Assessing conservation statuses

Due to the advancing loss of biodiversity as well the scarcity of resources for conservation, conservation actions have to be prioritised (Bachman et al., 2019). Knowing the extinction risk of a species helps in this prioritisation, as it indicates how immediate conservation actions need to be (Bachman et al., 2019). The International Union for Conservation of Nature's (IUCN) Red List of Threatened Species (hereafter Red List) is an important global resource for conservation (Rodrigues et al., 2006) that collects scattered data on the extinction risks of species into assessments that make the data easier to reach for decision makers (Collar, 1996). However, only a small subset of earth's biodiversity has been assessed, with tropical species and vascular plants being especially poorly represented (Bachman et al., 2019).

Red List assessments are based on quantitative criteria that are designed to categorize species according to their likelihood of extinction (Bachman et al., 2019). I assessed conservation statuses for each new species of *Danaea* (**I**, **II**) according to Red List criteria (IUCN, 2012). I based my assessments on the number of specimens collected, the number of locations where the species was found, the estimated quality of these habitats now and in the future, as well as the extent of occurrence (measured as the area of the minimum convex polygon drawn around the occurrence points of a species), and the area of occupancy (measured as the total area of 2 km grid cells occupied by the species) of the species.

3 Results

We set out to revise the taxonomy of *Danaea* to find out what species there are in the genus and where they occur. We described 24 new species in *Danaea* (I, II) and clarified synonymizations in the genus (I, II), in the end accepting 79 species as distinct. In most cases splitting species complexes into distinct species led to narrower geographical ranges (I, II, III). *Danaea moritziana*, for example, went from covering the entire latitudinal range of *Danaea* to only being found in the Venezuelan Andes (III). In a few cases new interpretations of species limits led to a wider geographical ranges, such as the range expansion of *D. trinitatensis* from a single island to occurring throughout Central America and the Caribbean (III).

Many of the new species were widespread and abundant, but many were also rare, endemic to a small area, and under threat from deforestation (I, II). I found that 7 of the new species probably belong in a threatened Red List category (CR: *Danaea ubatubensis* and *D. velona*; EN: *D. nasua* and *D. peruviana*; VU: *D. antioquiiana*, *D. pumila*, and *D. tenuicaulis*). I also placed four of the new species into the Near Threatened (NT) category (*D. alba*, *D. dilatata*, *D. erosa*, and *D. polypinna*). This left 13 of the 24 newly described taxa sufficiently abundant and widespread to be placed in the LC category.

We revised the species limits in *Danaea* to be concordant with a well resolved plastid phylogeny we published (III). We first noticed several of the new species in the plastid phylogeny (III), but managed to find morphological differences for most of these (II). Few species had clear cut qualitative differences, but a combination of traits made identification possible. Only one species was truly morphologically indistinguishable when looked at with the naked eye (*Danaea cuspidopsis*). We decided to split it from *D. cuspidata* because it was genetically distant and its inclusion would have made *D. cuspidata* polyphyletic (II).

New species were found especially in *Danaea* subg. *Holodanaea* (15 species), with only three species described from *D.* subg. *Arthrodanaea* and five species described from *D.* subg. *Danaea* (III). New species were described from almost all of the major clades of *D.* subg. *Holodanaea*, with the exception of the *D. excurrens* and the *D. acuminata* clades (Fig. 5 in III). However, both of these clades contained specimens that we were not able to identify to a species. The clade with the most

new species was the geographically and morphologically heterogeneous *D. cuspidata* clade, which had a total of seven new species, all of which had a distribution in Central America or the adjacent Pacific coast of Colombia and Ecuador.

We published nuclear phylogenies of *Danaea* where hybrid haplotypes were phased and found a relatively high rate of hybridization in *Danaea*, with at least 8 well supported hybrids out of 79 species (IV). We found two hybridization derived species likely capable of reproduction. We also found that one subgenus, *D. subg. Arthrodanaea*, had overall high signals of hybridization. We estimated how old *Danaea* hybrids could be based on the phylogeny, and found two hybrids between deeply divergent subgenera (IV).

We published an online identification key to the genus that included all of the 79 species as well as two of the hybrids (III). We found that the best diagnostic features in separating *Danaea* species are generally characteristics of the rhizome, size, number of nodes on the petiole, and the size, shape and margin serrations of the pinnae (I; II; III).

4 Discussion

4.1 Taxonomy of *Danaea*

The taxonomy of the genus *Danaea* has been unstable, with only 26 species recognized in the 90's, but 53 new species described after the turn of the millennium, 24 of which are described in this dissertation (I, II, III, Fig.4). We consider over half of the names published before 2000 to be synonyms (Fig. 4).

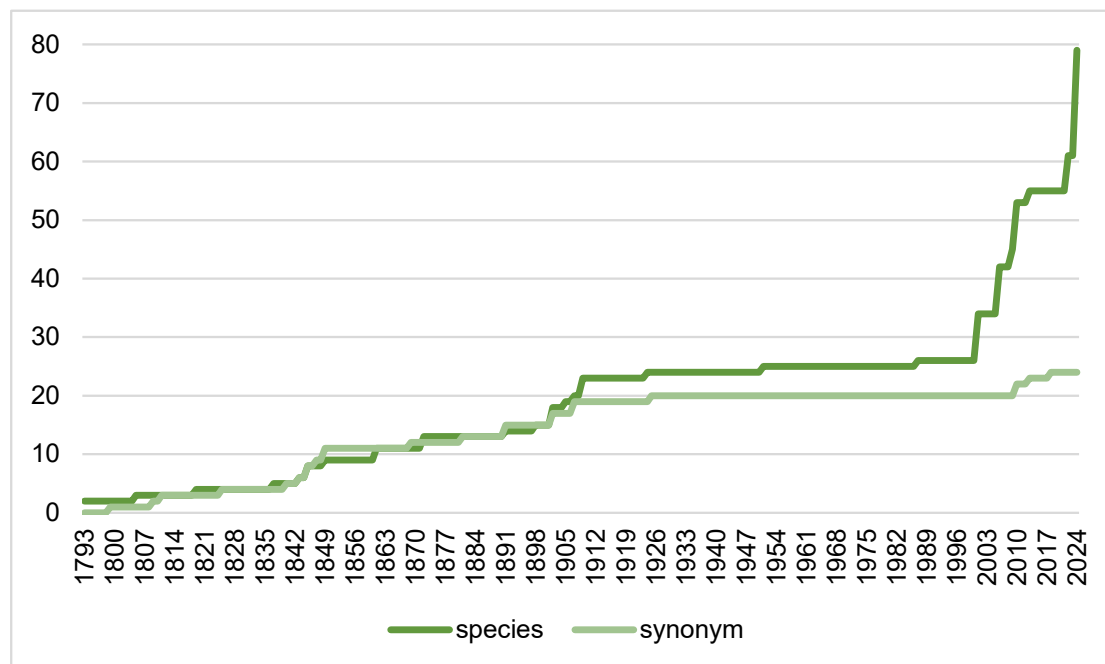


Figure 4. The total number of published species names available in the genus *Danaea* through time, separated into names that are currently considered valid species and names currently considered to be synonyms.

We may seem to have done a lot of splitting, but we believe these species represent true evolutionary lineages. Historically the problem of difficult morphology has been solved by lumping all but the clearly distinct cases. Indeed, we could have lumped all morphologically difficult complexes, e.g. the *D. moritziana*-complex, the *D. nodosa*-complex and the *D. geniculata*-complex, and many of the

new species would have become superfluous. However, the morphologically defined complexes were distributed across all the major clades within their respective subgenera, and to keep the species monophyletic, one would have to lump even more species than has been done before (III). This would make the species morphologically so variable as to render them unidentifiable.

This dissertation is a step towards systematically sound species in *Danaea*, where previous species complexes have been split into (usually) monophyletic and morphologically separable species. My approach is inevitably subjective, and another taxonomist could have ended up with wider or narrower species circumscriptions and consequently more or fewer species. As a guideline for my delimitation work was the usability of the species in field work. I accepted cryptic species only when combining them would have resulted in polyphyletic species.

In the previous thesis work focused on the genus (Christenhusz 2007), *Danaea* species were said to have a lot of morphological plasticity. With my narrower species delimitations the within species morphology is more stable. Nevertheless, differences between *Danaea* species are rarely qualitative and often continuous, and a combination of several traits is usually needed to definitely diagnose a species (III). This means that *Danaea* can still be difficult to identify to a species. The identification key published in Chapter III should make species identification easier.

Christenhusz (2010) suggested that the reason morphology is so difficult in *Danaea* is because the speciation is still in process. A possible example of such a group is the rather poorly resolved and sympatric *Danaea acuminata* clade in subg. *Holodanaea* (III). However, this does not seem to be the case for most of the morphologically difficult previous species complexes, as we have several cases of morphologically similar but genetically distant species. In these cases, either the morphology is conserved, hybridization is rampant, or the same morphology has arisen several times in convergent evolution.

The spatial distribution of *Danaea* collections is biased (Fig. 5). Especially high collection numbers can be found at the sites of popular biological stations and research facilities in rainforests, such as La Selva Biological Station in Costa Rica and Barro Colorado Island Research Station in Panama. Collection projects by active herbaria can lead to high collection numbers. For example, many of the numerous *Danaea* specimens from Ecuador were collected during a long-term collaboration between AAU (Denmark) and QCA (Ecuador). High collection numbers can also be found in the French overseas departments of Guadeloupe and French Guiana. Collection density in Amazonia is generally very low, but where intensive fern inventories have been made, such as in some areas of northern Peru (Tuomisto et al., 2003; Salovaara et al., 2004; Higgins et al., 2011), most *Danaea* species have likely been found. In Colombia, particularly in the Chocó area, many locations have been sparsely sampled yet have yielded a high number of species.

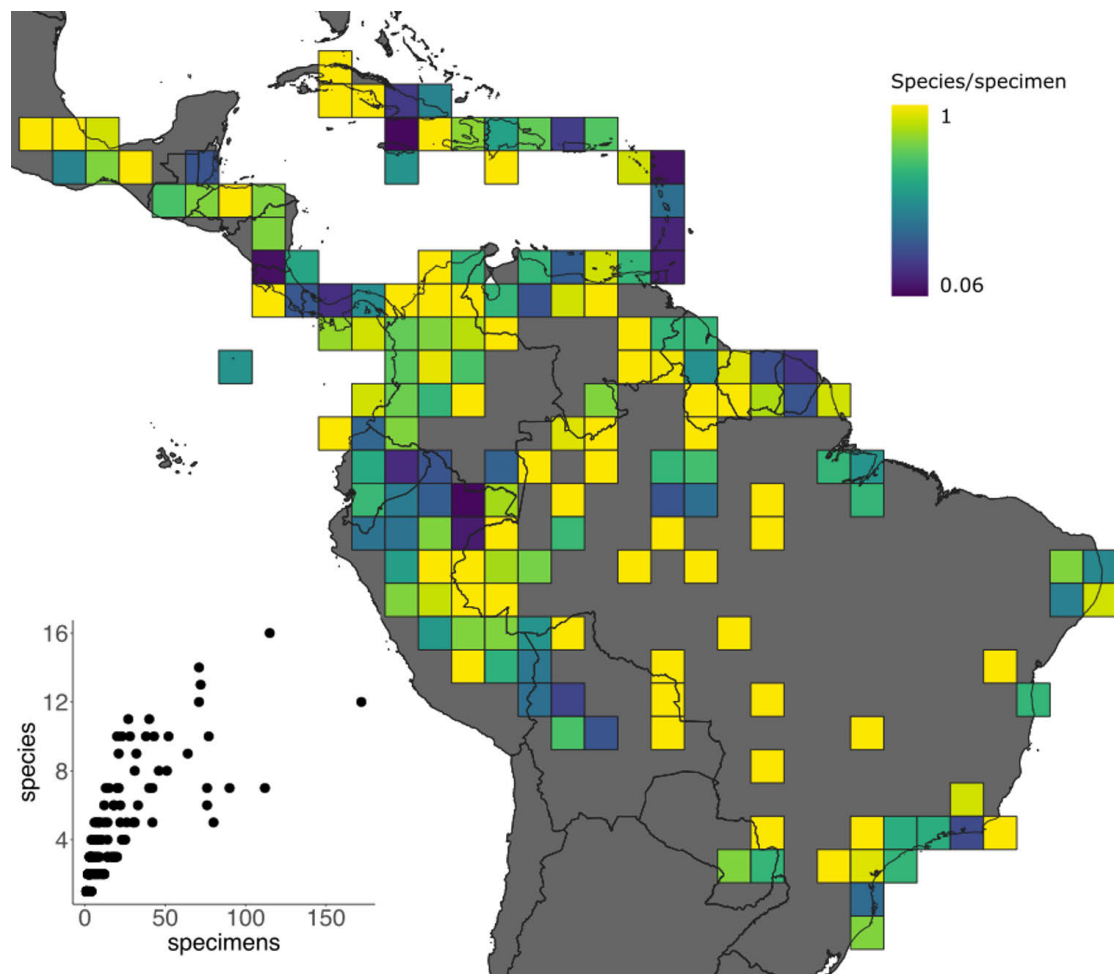


Figure 5. Number of identified *Danaea* species divided by number of unique collection numbers per $2^{\circ} \times 2^{\circ}$ grid cell.

Some of the new species were collected only during one expedition in a poorly collected location, and some *Danaea* species are represented by a single specimen. As many species are rare, they are usually represented by singletons when first collected (Lim et al., 2012). Taxonomists are usually reluctant to describe a species based on a single specimen, which increases the lag between the collection and the description of a species (Fontaine et al., 2012). This problem could be alleviated by doing more field work to get more specimens. Rare species like the newly described *D. velona* and *D. ubatubensis* may be more difficult to find but constitute over a third of the global biodiversity (Enquist et al., 2019).

In addition to the importance of field work, this thesis again highlights the key role of taxonomic work in recognizing biodiversity. Some of the new species were growing in well documented places, but were thought to belong to other, already described species. Many of the species described here could be considered cryptic sensu Bickford et al. (2007); they would not have been discovered without the

molecular data. For example the new species *Danaea ampla* and *D. panamensis* can be found in Barro Colorado Island, one of the most intensively researched rainforest in the world, but were identified as *D. nodosa* along with most material in their subgenus. Morphological differences between these species were recognized as consistent only after evolutionary relationships had been clarified with genomic data (II, III). The rate at which cryptic species are found in DNA based systematics suggests that molecular data should be routinely used in taxonomic research (Bickford et al., 2007). This thesis also shows how the use of molecular data in taxonomy allows for an accelerated pace of species discovery and description (Fig 4), as well as more confidence in the results.

Characteristics of the rhizome are taxonomically informative in *Danaea*. Given the long life spans of *Danaea* (see Introduction), I would not generally recommend collecting their rhizomes even if these contain relevant information for species identification (I, II, III). Instead, I would recommend either photographing the rhizome or making a note on the relevant characteristics (Fig. 2); Is the rhizome erect, ascending or creeping?; Are the leaf and root bases arranged radially or dorsiventrally on the rhizome?; If the rhizome is clearly creeping and dorsiventral, are the leaves in two or more rows?

The new circumscription increased species diversity in *Danaea* greatly, and at the same time the species became restricted to much smaller areas (Table 1). This is important knowledge for biodiversity conservation. Large unknowns still remain, as even after describing 24 new species, we were still left with some 20 taxa with unofficial species numbers only. Because of this I estimate *Danaea* to consist of at least 100 species; that is twice the number estimated in the previous thesis work focused on the genus less than 20 years ago (Christenhusz, 2007).

4.2 Hybridization in *Danaea*

Hybridization has mostly been looked at in the context of temperate taxa, and little has been known of the importance of hybridization in tropical lineages (Schley et al., 2022). Nevertheless, a trend of less hybridization in tropical ferns in comparison to temperate ferns was reported by Liu et al. (2020). In contrast to this trend, I found high rate of hybridization in a tropical fern genus (IV) and suggested that that the apparent paucity of tropical hybrids is another case of observation bias. The estimated hybridization rate of up to 18 % in *Danaea* would be high even for ferns, which typically have high rates of hybridization (Whitney et al., 2010).

The rate of hybridization has important evolutionary consequences for lineages, as it can create novel diversity. Hybridization has increased species diversity in *Danaea*; two species of *Danaea* (*D. trifoliata* and *D. wendlandii*), or 11 % of the tested 19 species, turned out to likely be hybridization derived species (IV).

Hybridization can also enable gene flow between distant relatives, and it can be a possible force confusing morphology in *Danaea*. The morphologically most difficult subgenus, *D. subg. Arthrodanaea*, was also the one with the highest signals for hybridization across the genus (IV).



Figure 6. The hybrid *Danaea* \times *ushana* (centre) and its parent species *D. nigrescens* (left) and *D. simplicifolia* (right) in French Guiana (IV). © Samuli Lehtonen

Danaea is one of the oldest extant plant genera, and I found evidence of some of the deepest hybrids known to science. One such example is *Danaea* \times *ushana* (Fig. 6), which was thought to belong in *D. subg. Danaea* based on morphology, but grouped in *D. subg. Arthrodanaea* in the plastid phylogeny (III). Nuclear data revealed it to be a hybrid between the two subgenera (IV) that diverged approximately 27–83 Mya (Testo & Sundue, 2016; Nitta et al., 2022). Another example of deep intersubgeneric hybridization first noticed in the plastid phylogeny was found between *D. wendlandii* and *D. subg. Danaea*, making it a hybrid of a hybrid derived species (IV). My results corroborate previous studies showing that ferns can have low reproductive barriers, allowing them to hybridize between lineages diverged as long ago as the Cretaceous (Rothfels et al., 2015; Lehtonen, 2018; Liu et al., 2020).

There were several specimens that we hypothesized to be hybrids, but did not have genetic or cytological data to test. For instance, one of the new species described, *Danaea erosa*, is a possible hybrid between *D. wendlandii* and *D. crispa*

(II). It has been found growing alongside both of these species, and is intermediate in morphology between them. I also treat *D. draco* as a synonym of *D. polymorpha* in this dissertation (III) but some specimens in Trinidad previously identified as *D. draco* and now identified as *D. polymorpha* might instead be hybrids between *D. polymorpha* and *D. trinitatensis*, both of which occur in these same locations. These specimens combine the size, colour and general shape of *D. polymorpha* with the thin texture and sinuate tip typical to *D. trinitatensis* from Trinidad.

Hybridization can greatly complicate the interpretation of phylogenetic trees. Evolutionary history is commonly modelled as a strictly bifurcating tree, but would often more accurately be described by a reticulate web. A simple exploration of nuclear data with a phylogenetic network analysis can already reveal potential hybrids (Huson & Bryant, 2006). For example, all of the three hybrids or hybridization derived species for which we had multi locus nuclear data were placed in intermediate locations in a phylogenetic network (Fig. 7). The strength of nuclear data is that it can reveal events of reticulate evolution, but this strength is not fully harnessed if the data is not phased. Phasing clearly showed the divergent haplotypes inherited by hybrids in *Danaea* (IV).

In the end, all of the accessions that we identified as possible cases of contamination due to their unexpected placement in the plastid phylogeny turned out to be likely hybrids. In addition, some taxa that we did not even suspect to be hybrids were revealed as such upon examination of the nuclear genome. Hybridization in vascular plants is common, widespread, and leads to frequent gene flow (Ellstrand et al., 1996; Whitney et al., 2010), and I suggest that it should always be considered as a possible driver of observed incongruent patterns in phylogenies.

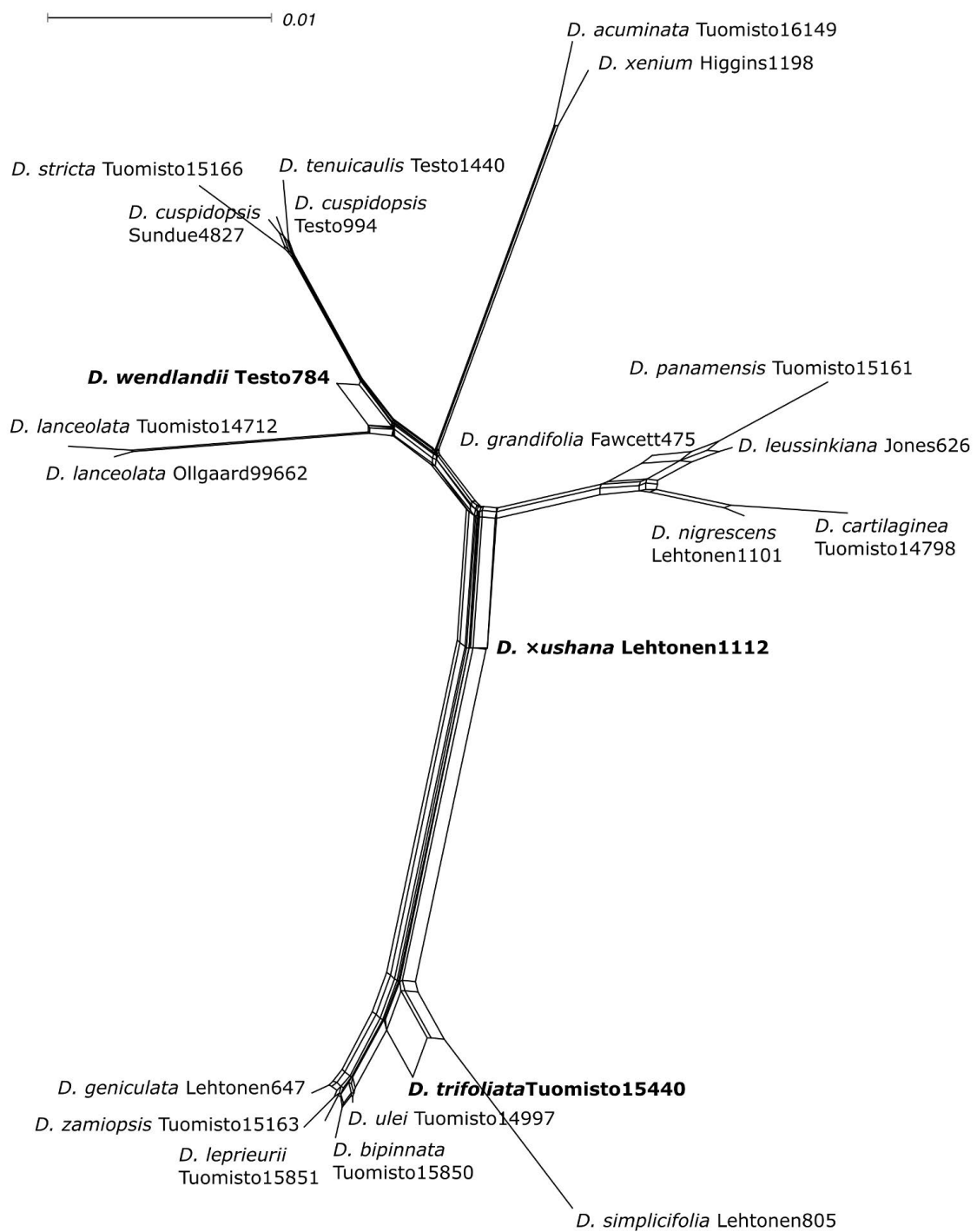


Figure 7. A phylogenetic network of the multi locus nuclear dataset in **IV**, made with SplitsTree 4 (Huson & Bryant, 2006). Presumed hybrid accessions are in bold.

4.3 Patterns of diversity in *Danaea*

The highest diversity of *Danaea* species can be found around the Andean mountains and in the southern Central America (Fig. 8). Many of the new species were also described from these areas (I, II). Areas of especially high endemism can be found in Costa Rica, Panama, the Lesser Antilles, French Guiana, Chocó, the western Amazonia, and between the Cordilleras in Colombia. However, some of these were also among the best collected localities (Fig. 5).

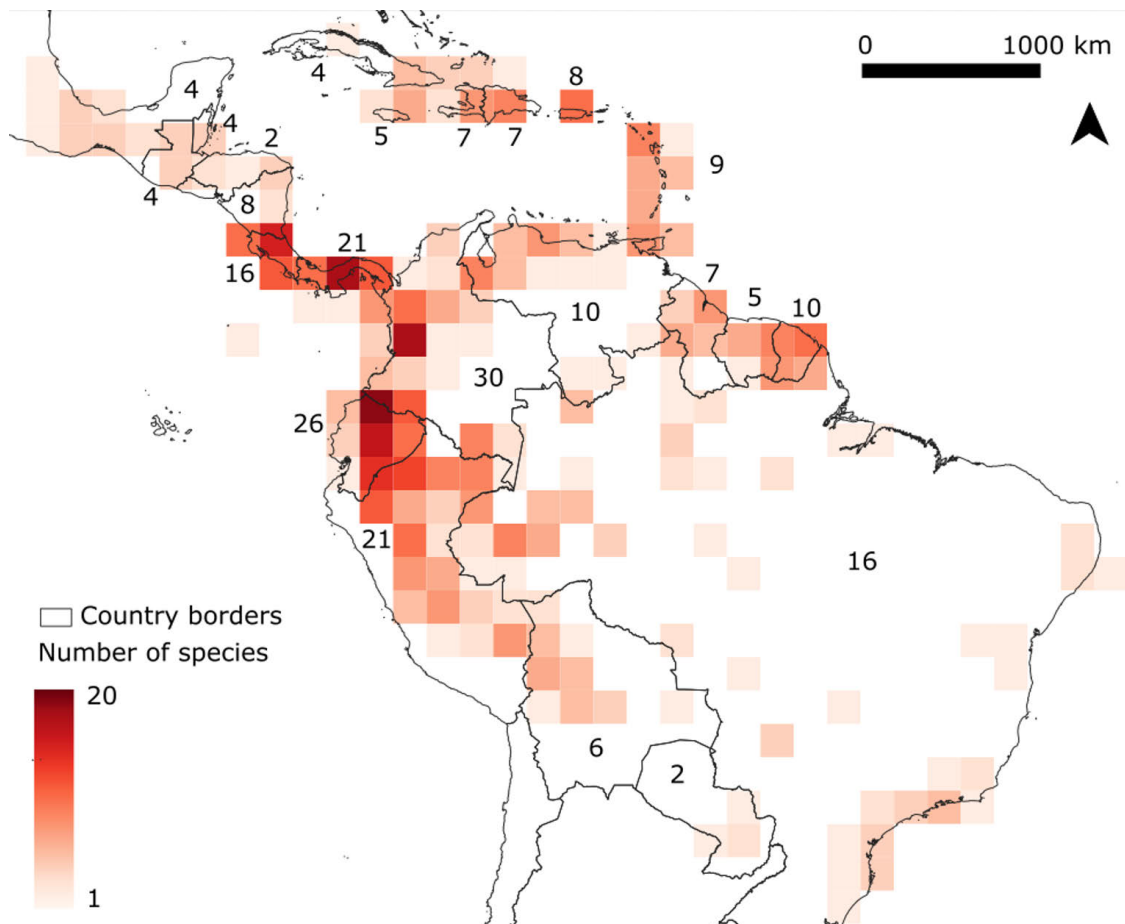


Figure 8. Diversity of *Danaea* per 2° × 2° grid cell (III); number of species that were coded as likely occurring in the identification key have been added by country (except for the Lesser Antilles, which are represented by one number).

How much have the patterns of diversity changed after our new species delimitations? The best point of comparison is Christenhusz (2007), the previous thesis focusing on *Danaea* upon which this work largely builds. Table 5 (on page 39) of that thesis shows the distributions of each *Danaea* species by country. I made a similar table for easy reference on species distributions by country (Table 1), as species distributions have changed quite a bit since then due to changes in species

delimitation, splitting off of new species, and accumulation of new specimens. Comparison of these two tables shows that species numbers have grown the most in Panama, having an increase of 6 to 21 species of *Danaea*, or 15 more species now than 17 years ago. Species numbers have also grown by 11 species in Colombia (19 to 30) and Costa Rica (5 to 16), with a more moderate growth of 13 to 16 species in Brazil, 21 to 26 in Ecuador, and 15 to 21 in Peru. The Caribbean islands and the eastern part of the Guianas were noted as species rich areas in Christenhusz (2007), but species numbers in these areas have changed little, and they now seem species poor in comparison. In other words, the species rich areas have been getting richer and the species poor areas are staying poor. The areas with the highest species richness have also likely been the ones with the most unsorted complexes.

Half of *Danaea* species can be found growing above 1000 m and a fourth above 1500 m (III). A pattern of high species richness in the mountains is mirrored in other ferns (Suissa et al., 2021). In *Danaea* this pattern is driven largely by *D.* subg. *Holodanaea*, in which 16 out of 43 species grow above 1500 m and 27 above 1000 m. In contrast, in *D.* subg. *Danaea* only three species out of 20 have been found above 1500 m and 9 above 1000 m. No species have been found growing above 1500 m in *D.* subg. *Arthrodanaea*, and only five out of 16 have been found above 1000 m.

Suggested mechanisms driving mountain biodiversity include the density of diverse niches (Rahbek et al., 2019) and rapid mountain uplift generating new habitats into which species can disperse and diversify (Antonelli et al., 2018). *Danaea* subg. *Holodanaea* contains many species that are endemic to small areas and appear to have turnover with elevation (I, II, III). Species within this subgenus generally have the highest genetic differences, suggesting a faster rate of evolution. This could also be explained by the plants in this subgenus often having the smallest statures and thus likely shorter lifespans.

The pattern of high species diversity along the Andes and in Central America is contrasted by *Danaea* subg. *Arthrodanaea*, which has centres of diversity in the Guiana shield (eight out of the ten species occurring there are from this subgenus) and the lowlands of Western Amazonia (III). Only one species in *D.* subg. *Arthrodanaea* (*D. arbuscula*) reaches higher elevations (up to 1500 m). I hypothesize that a lack of adaptations to high elevations could in part explain the lower species diversity in *Danaea* subg. *Arthrodanaea* in comparison to the other two genera. I also hypothesize that hybridization has played a role in homogenizing this subgenus and pushing down the species numbers. The species in this subgenus are generally more widespread than species in the other two subgenera, and often have sympatric distributions in Amazonia, giving ample opportunity for gene flow. This subgenus is also the most difficult morphologically, genetically the least diverged, and has the highest signals for hybridization (III, IV).

Previous studies have found that fern species diversity increases with soil fertility (Tuomisto & Poulsen, 1996, Tuomisto et al., 2014). This seems to be true for *Danaea* as well, where for instance the Western Amazonian lowlands have more species than the poorer soil areas of central Amazonia and the Guiana shield (III; Zuquim et al., 2023).

In addition to possibly driving species diversity in the genus, the Andean mountains are a significant dispersal barrier for *Danaea*. Only two species occur on both sides of the mountain range; *Danaea erecta* and *D. andina*, and both of these occur high in the mountains, even above 2000 m (II, III). Again, the subgenus *Arthrodanaea* has not managed to jump over or disperse around the Andes, and is missing from the diversity hotspot of Chocó, which contains an exceptionally high species diversity and rate of endemism in *Danaea*.

Chocó is a region on the north-western coast of South and Central America (III; Pérez-Escobar et al., 2019) which receives one of the highest rainfalls in the world. This, alongside the highly dissected elevational niches of the Andes, apparently makes it ideal for diversification of the moisture and shade loving *Danaea* ferns. Of the new species occurring in the Chocó region, *D. nasua*, *D. tenuicaulis*, and *D. velona* were first recognized as distinct due to their elongated drip tips, an adaptation to the wet conditions that has developed several times (I, II). Another such adaptation seems to be the translucent leaves of the new species *D. gracilis* and the previously described *D. tenera*, which are also endemic to the area (I).

Patterns of tropical biodiversity is still poorly understood because of shortfalls in knowledge on taxonomy and species distributions (Hortal et al., 2015; IPBES, 2019). To overcome these shortfalls more work on the taxonomy of tropical organisms and more field trips to the tropics are needed (Collen et al., 2008; Feeley, 2015).

Table 1. Species occurrence of *Danaea* in Neotropical countries, with the species described in this dissertation in bold (part 1).

	Belize	Bolivia	Brazil	Colombia	Costa Rica	Cuba	Dominican Republic	Ecuador	French Guiana	Guatemala	Guyana	Haiti	Honduras	Jamaica	Lesser Antilles	Mexico	Nicaragua	Panama	Paraguay	Peru	Puerto Rico	Suriname	Venezuela
<i>Danaea acuminata</i>			X					X												X			
<i>Danaea alansmithii</i>				X																			
<i>Danaea alata</i>															X								X
<i>Danaea alba</i>				X														X					
<i>Danaea ampla</i>	?				?								?			X	?	X					
<i>Danaea andina</i>				X				X												X			
<i>Danaea antillensis</i>															X								
<i>Danaea antioquiiana</i>				X																			
<i>Danaea arbuscula</i>	X	X	X	X	X		X	X	X	X	X	X		X	X		X	X		X	X		X
<i>Danaea betancurii</i>				X				X												X			X
<i>Danaea bicolor</i>								X															
<i>Danaea bipinnata</i>			X	X				X												X			
<i>Danaea carillensis</i>					X																		
<i>Danaea cartilaginea</i>		X	X	X				X												X			X
<i>Danaea chococcala</i>				X														X					
<i>Danaea crispa</i>					X													X					
<i>Danaea cuspidata</i>									X							X	?						
<i>Danaea cuspidopsis</i>				X	X			X									?	X					
<i>Danaea danaeipinna</i>			X						X	X												X	
<i>Danaea dilatata</i>									X	X													
<i>Danaea elongata</i>		X						X													?		
<i>Danaea epilithica</i>					x																		
<i>Danaea epiphytica</i>				?				X															
<i>Danaea erecta</i>				X	X			X													X		
<i>Danaea erosa</i>					X																		
<i>Danaea excurrentis</i>		X	X																	?			
<i>Danaea falcata</i>								X													X		
<i>Danaea geniculata</i>				X					X														
<i>Danaea gracilis</i>				X				X										X					
<i>Danaea grandifolia</i>				X			X					X									X		X
<i>Danaea humilis</i>				X				X										X		X			X
<i>Danaea imbricata</i>								X															
<i>Danaea inaequilatera</i>				X				X										X					
<i>Danaea jamaicensis</i>						X	X					X		X							X		
<i>Danaea jenmani</i>						X	X					X		X							X		
<i>Danaea kalevala</i>																X							
<i>Danaea kessleri</i>								X															
<i>Danaea lanceolata</i>				X				X													X		
<i>Danaea latipinna</i>				X				X															
<i>Danaea lepreurii</i>			X	X				X	X											X			

Table 1. Species occurrence of *Danaea* in Neotropical countries, with the species described in this dissertation in bold (part 2).

	Belize	Bolivia	Brazil	Colombia	Costa Rica	Cuba	Dominican Republic	Ecuador	French Guiana	Guatemala	Guyana	Haiti	Honduras	Jamaica	Lesser Antilles	Mexico	Nicaragua	Panama	Paraguay	Peru	Puerto Rico	Suriname	Venezuela
<i>Danaea teussinkiana</i>					X												X						
<i>Danaea lingua-cervina</i>				X																X			
<i>Danaea longicaudata</i>							X																
<i>Danaea lucens</i>				X																			
<i>Danaea mazaana</i>															X								
<i>Danaea megaphylla</i>				X													X						
<i>Danaea moritziana</i>				X																			X
<i>Danaea nasua</i>				X			X																
<i>Danaea nigrescens</i>		X	X	X			X	X	X						X					X		X	X
<i>Danaea nodosa</i>	X				X	X	X		X		X	?	X		X	?	?				X		
<i>Danaea oblanceolata</i>																				X			
<i>Danaea opaca</i>			X																				
<i>Danaea panamensis</i>																X	X						
<i>Danaea pumila</i>				X													X						
<i>Danaea peruviana</i>																				X			
<i>Danaea × plicata</i>					X																		
<i>Danaea polymorpha</i>															X								
<i>Danaea polypinna</i>					X												X						
<i>Danaea pterorachis</i>					X												?						
<i>Danaea riparia</i>																				X			
<i>Danaea robbinsmoranii</i>					X																		
<i>Danaea sellowiana</i>			X																X				
<i>Danaea simplicifolia</i>			X						X	X					X							X	
<i>Danaea stricta</i>				X													X						
<i>Danaea tenera</i>				X			X																
<i>Danaea tenuicaulis</i>				X																			
<i>Danaea trichomanoides</i>							X														X		
<i>Danaea trinitatensis</i>	X			X	X	X			X	X	X	X	X	X	X	X	X				X	X	X
<i>Danaea trifoliata</i>			X						X	X											X	X	X
<i>Danaea tuomisloana</i>					X																		
<i>Danaea ubatubensis</i>			X																				
<i>Danaea ulai</i>			X	X													X		X				
<i>Danaea urbanii</i>							X					X									X		
<i>Danaea × ushana</i>								X															
<i>Danaea vanderwerffii</i>																		X					
<i>Danaea velona</i>				X																			
<i>Danaea vivax</i>																				X			
<i>Danaea wendlandii</i>					X												X						
<i>Danaea xenium</i>				X			X													X			
<i>Danaea ypori</i>								X															
<i>Danaea zariopsis</i>																	X						

5 Conclusions

This dissertation again highlights the importance of poorly known tropical ecosystems for global biodiversity. I found two dozen new fern species in just one genus, *Danaea* (I, II). Some of these species were large and abundant, at best covering ground for kilometres on end. However, many of the new species were rare, geographically restricted, and under threat from deforestation. I assessed the likelihoods of extinction for the new species, but many other species in the genus are likely also threatened. These assessments followed the Red List criteria, but I did not formally publish them in the Red List. This would be a necessary step to make the assessments accessible for decision makers.

Taxonomy in *Danaea* has been inconsistent, and for good reason; taxonomy based on morphology alone can be misleading in this genus (III). Convergent evolution, morphological conservatism and/or hybridization seems to be widespread, and it can be difficult to tell which morphological characteristics delimit a species (III, IV). Species borders can be visualized on a molecular phylogeny and subsequently the diagnostic morphological differences can be pinpointed (II, III). Cryptic species and hybrids can also be detected through molecular phylogenetics (II, III, IV).

With a more than tripling of the number of species, a full taxonomic revision of *Danaea* is in order. This is in the works for Flora Neotropica. Prior to that, we included a list of synonyms and species that we accept as distinct in the Results section of Chapter III. All species have been measured for morphological variation for the identification key, so species identification in the genus can hereafter easily follow the taxonomy presented in this dissertation (III).

With a solid taxonomy based on evolutionarily coherent species, it is now possible to look at evolution, ecology and historical biogeography in *Danaea*. A timed phylogeny focusing on the genus would be the next step to delve deeper into evolutionary history of *Danaea*. Next to nothing is known of the ecological niches of *Danaea* species, so niche modelling would also be a logical next step. Much ecological data on *Danaea* species has been collected especially in Amazonia, and analysis of the niches of Amazonian species of *Danaea* would facilitate their use as

indicator species there. Niches could also be modelled onto a phylogeny to look at niche evolution and to understand the drivers of diversification in the genus.

I started this introduction with a mental image of finding a new fern species in the back yard of a biological station. This more or less happened with *Danaea*, several times (II). But where would be the best place in the world to find new species of *Danaea*? My answer would be Colombia, where many of the biogeographical areas relevant to *Danaea* meet, where the combination of mountains, rich soils, and high precipitation likely drive diversification of ferns in this genus, and where many areas remain poorly collected. Indeed, several unnamed species collected during recent expeditions await description from this area.

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