RESEARCH ARTICLE

Rampant hybridization in an old tropical fern genus (*Danaea*, Marattiaceae)

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Abstract The number of hybrids has been reported to be lower in tropical than temperate ferns, but patterns of hybridization are poorly known in the tropics. We examine hybridization in the Neotropical fern genus *Danaea* (Marattiaceae) using a phylogenomic approach with single or low-copy nuclear and chloroplast loci and haplotype phasing. We find a high hybridization rate of 10%–18% for *Danaea*, similar to the rates observed for temperate ferns. We find further evidence for the previously proposed low reproductive barriers in ferns, enabling hybridization and even the production of hybridization-derived species between lineages separated possibly as far back as the Cretaceous. We found that *Danaea trifoliata* and *D. wendlandii* are fertile, hybridization-derived species between lineages diverged potentially 14–72 million years ago (mya) and 23–62 mya, respectively. We also confirm that the previously recognized species *D.* ×*ushana* is a likely sterile hybrid between *D. simplicifolia* (*D.* subg. Arthrodanaea) and *D. nigrescens* (*D.* subg. *Danaea*), which diverged potentially 27–83 mya. We find that hybridization may function as a homogenizing force in *Danaea*, as the subgenus with the highest signals for hybridization (*D.* subg. *Arthrodanaea*) also has the fewest species that are the least morphologically and genetically diverged. We describe a new hybrid between *D. wendlandii* (*D.* subg. *Holodanaea*) and *D.* subg. *Danaea* as *D.* ×*deltoidea*.

Keywords diversity; haplotype phasing; introgression; Neotropics; phylogenomics; reticulate evolution

Supporting Information may be found online in the Supporting Information section at the end of the article.

■ INTRODUCTION

Hybridization can have important evolutionary consequences, as it has been linked to adaptive radiation, speciation (Seehausen, 2004; Abbott & al., 2013), invasion, and even extinction (Ellstrand & Schierenbeck, 2000; Todesco & al., 2016). Evidence drawn principally from temperate plant taxa suggests that ferns and lycophytes are particularly prone to hybridization (Barrington & al., 1989; Wood & al., 2009; Whitney & al., 2010; Sigel, 2016). In a dataset of 37,000 species of temperate plants (Whitney & al., 2010), the studied fern genera had an average frequency of 0.17 hybrids per non-hybrid species in contrast to 0.06 in the studied angiosperm genera (both sterile hybrids and hybridization-derived species were counted). The rate of hybridization is related to the strength of reproductive barriers, and in the case of ferns it can be connected both to their genomic stasis (Schneider & al., 2015; Clark & al., 2016) and to their dispersal by wind, which is less targeted than biotic vectors (Ellstrand & al., 1996; Mitchell & al., 2019). Low reproductive barriers in ferns have also been suggested to explain the low diversity of ferns in relation to flowering plants via a low birth rate of new species, as populations take longer to achieve complete genetic separation (Rothfels & al., 2015).

The time since divergence of two lineages may be positively correlated with the strength of the reproductive barriers between them (Whitney & al., 2010; Rothfels & al., 2015; Mitchell & al., 2019). The deepest recorded hybridization events known are all intergeneric hybrids of ferns, with estimated divergence times of parental taxon lineages of up to 100 million years ago (mya) in ×*Osmuntonia* Hong M.Liu & al. and ×*Osmunasium* Hong M.Liu & al. (Liu & al., 2020). Other examples of deep intergeneric hybrids in ferns are ×*Dryostichum* W.H.Wagner (Wagner & al., 1992)

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between lineages diverged 65–99 mya, ×*Cystocarpium* Fraser-Jenk. (Fraser-Jenkins, 2008) between lineages diverged 58–77 mya, and ×*Lindsaeosoria* W.H.Wagner (Wagner, 1993) between lineages diverged 45–80 mya (Schuettpelz & Pryer, 2009; Lehtonen & al., 2012, 2017; Rothfels & al., 2015; Le Péchon & al., 2016; Testo & Sundue, 2016; Regalado & al., 2017; Lehtonen, 2018).

Liu & al. (2020) found that some temperate fern lineages have more hybrids than expected, and several tropical lineages lack them. This pattern could arise from differences in modes of speciation in temperate and tropical zones (Haufler & al., 2000) or from unequal efforts to record hybrids between the two (Schley & al., 2022). Tropical species are generally less well studied and represented by fewer herbarium specimens than temperate ones, and a case study of the flora of Michigan found a positive correlation between observed hybridization rates and the number of available herbarium collections (Beddows & Rose, 2018).

Here we examine hybrid diversity in the strictly Neotropical fern genus *Danaea* Sm. (Marattiaceae) to study the role of hybridization in tropical ferns. Estimates for the divergence of *Danaea* from the rest of the extant Marattiaceae have varied in timed phylogenies between as early as late Triassic to as late as the late Cretaceous, 75–220 mya (Smith & al., 2010; Testo & Sundue, 2016; Lehtonen & al., 2017, 2020; May & al., 2021; Nitta & al., 2022).

Hybridization has been examined in Marattiaceae with transcriptome data, and Zhao & al. (2023) suggested that the challenges systematists face in the family could primarily stem from incomplete lineage sorting and hybridization. Hybrids between potentially deeply divergent lineages have been previously found in the paleotropical marattialean genus *Angiopteris* (Hsieh & al., 2008; Wang & al., 2021; Zhao & al., 2023).

Within *Danaea*, two cases of hybridization have been widely cited in the literature. Hybridization has been postulated between *D. jamaicensis* Underw. and *D. jenmanii* Underw., as triploid specimens of *D. jenmanii* have been found in a likely hybrid population in Jamaica, whereas other cytologically studied specimens of *D. jenmanii* were tetraploid ($4n = 160$; Walker, 1966, 1985). The ploidy of *D. jamaicensis* remains unknown, but the spores of *D. jenmanii* have also been reported to be abnormal (Rolleri, 2004), and morphologically intermediate forms between *D. jamaicensis* and *D. jenmanii* can often be found.

The second case is *Danaea* ×*plicata* Christ, which was recently proposed to be a hybrid (Moran & Grayum, 2018). Leaf division is one of the most conspicuous characteristics of ferns, and the discovery of hybrids seems to be more likely in cases where the leaf division is clearly different between the parent species and intermediate in their hybrid (Sorojsrisom & al., 2023). Indeed, *D.* ×*plicata*, is proposed to be a hybrid between the simple-leaved *D. carillensis* Christ and the pinnate *D. crispa* Endrés & Rchb.f.

A few potential new examples of hybridization were suggested on the basis of discordance between morphological characteristics of some specimens and their placement in a chloroplast phylogeny (Keskiniva & al., 2024). Some of these were postulated to be hybrids between the morphologically and genetically clearly distinct subgenera of *Danaea*; *D.* subg. *Arthrodanaea* C.Presl, *D.* subg. *Danaea*, and *D.* subg. *Holodanaea* C.Presl. Marattiaceae have been hypothesized to have an anomalously slow rate of molecular evolution (Soltis & al., 2002), which could facilitate deep hybridization between lineages.

Instead of showing traits intermediate of the parent species, hybrids may also be morphologically very similar to one of the parents. In these cases, hybrids can be revealed when phylogenies are based on molecular data, as then they might be grouped with the parent that is morphologically divergent. However, most phylogenies to date have been inferred from sequences of the chloroplast genome, which is maternally inherited in ferns (Stein & Barrington, 1990; Gastony & Yatskievych, 1992). As a result, any hybrids that are morphologically similar to the maternal parent are likely to be overlooked, even when included in a phylogenetic study. Since nuclear data includes haplotypes inherited from both parents, they can show all the alleles inherited by hybrids and hence reveal both parental lineages (Nauheimer & al., 2021).

Here we examine the importance of hybridization as an evolutionary force in *Danaea* using both nuclear and chloroplast sequence data. We aimed to both reveal possible cryptic hybrids and test the status of putative hybrids that had been proposed based on plastid data.

■ MATERIALS AND METHODS

Sampling and sequencing. — Here we test for hybridization in *Danaea* by analysing both nuclear and chloroplast sequence data from the same samples. We use two different nuclear datasets, a multi-locus and a single-locus dataset, and sequences from four different chloroplast regions (Appendix 1).

For the multi-locus dataset, we chose one sample for each identified species for which we had silica material. These covered all major clades and were evenly split between the three subgenera. DNA was extracted at the University of Florida using a modified CTAB approach (Breinholt & al., 2021). The DNA samples were delivered to RAPiD Genomics (Gainesville, Florida, U.S.A.) for library prep, target enrichment using the GoFlag 408 probe set, and sequencing, all following protocols described in Breinholt & al. (2021). This generated raw sequence reads from ca. 400 single or low-copy loci of 22 *Danaea* specimens representing 20 taxa, hereafter referred to as the multi-locus data.

Potential hybrids and their putative parents were identified from the chloroplast phylogeny published in Keskiniva & al. (2024) based on placement on the phylogeny that was discordant with morphology. For this set of potential hybrids and their presumed parental species we generated the single-locus data by sequencing a highly variable region flanking the exonic target region of locus 369 of the GoFlag probe set (Breinholt & al., 2021). This region was selected because it had conserved regions for primers to attach to and a variable

part in the middle that was short enough (~330 bp) to be covered from both ends. Primers (forward GGTCATCTTTG TCAGCCCCA; reverse TTCTGAACCCACAGGCAGAC) were designed with Primer3 (Untergasser & al., 2012). PCR and Targeted Illumina MiSeq 2 × 300 bp sequencing was performed by Macrogen (Seoul, South Korea/Amsterdam, the Netherlands; www.macrogen.com). The single-locus data was generated for 19 specimens, including 5 potential hybrids and their putative parent species and additional samples from *Danaea* subg. *Danaea* to increase the taxonomic coverage for that subgenus. The single-locus dataset was augmented by sequences of the same locus obtained from the multi-locus data (see below) for a resulting dataset of 39 specimens.

The chloroplast dataset was generated for the same sampling as the nuclear datasets. We used chloroplast sequences of the plastid genes *rbcL* and *atpB*, and the non-coding *rpl32-trnL* and *trnL-F* regions published in Christenhusz & al. (2008) and Keskiniva & al. (2024), augmenting the dataset with new sequences generated with the protocol used in Keskiniva & al. (2024). For the samples we failed to get silica material for, we assembled plastid fragments from the trimmed multi-locus data (see below) in Geneious Prime v.2021.1.1.

Trimming and sequence assembly. — For all nuclear data, sequence reads were first trimmed by removing Illumina adapters and low-quality bases and reads using Trimmomatic v.0.39 (Bolger & al., 2014; illuminaclip 2:30:10, leading 20, trailing 20, sliding window 4:20, trimmed reads of less than 30 bases excluded).

HybPiper v.2.0 (Johnson & al., 2016) was used to assemble sequences for each locus in the multi-locus dataset. Including non-coding regions can help in solving relationships at shallow phylogenetic scales, where low sequence divergence is expected (McKain & al., 2018). To this end, we used sequences from the GoFlag pipeline (Breinholt & al., 2021) containing target (i.e., conserved exons) and flanking (i.e., mostly non-coding) regions as references for the sequence assembly using HybPiper. Some of the reference sequences overlapped and were cut downstream of the exon to avoid overlap. We chose the longest *Danaea* sequence from the GoFlag pipeline as reference for each locus. Because our reference sequences already contained the flanking regions, we did not use the intronerate function of HybPiper. Sequences were assembled for up to 387 loci with an average of 386 loci per sample.

For the single-locus data, we used VSEARCH v.2.23 (Rognes & al., 2016) and cutadapt v.3.4 (Martin, 2011) to combine reverse and forward reads, retain sequences 260–360 bp long, dereplicate the data, remove chimeras, create variants, and filter out variants with less than 5% abundance in the dereplicated and cleaned data.

Data analysis. — Hybrids typically have divergent parental alleles, and therefore a high proportion of single nucleotide polymorphisms (SNPs) (Nauheimer & al., 2021). We analysed the composition of SNPs in the multi-locus data to infer allele divergence (AD, percentage of SNPs across all loci) and locus heterozygosity (LH, percentage of loci with SNPs), high values of which suggest a hybrid origin (Nauheimer & al., 2021). To this end, HybPhaser v.2.0 (Nauheimer & al., 2021) was used to map SNPs with a coverage of at least 10×, an allele frequency of at least 0.15, and alternative allele occurrence in at least 4 reads. Loci with a number of SNPs above 1.5× the interquartile range above the third quartile for all loci were considered to be putative paralogs and were removed, and 351 loci were retained. Consensus sequence lists were generated for each locus and aligned with MAFFT v.7.5 (Katoh & Standley, 2013). Columns with more than 50% gaps were removed using TrimAl v.1.4.1 (Capella-Gutierrez & al., 2009), resulting in a total alignment length of 247,881 bp (suppl. Appendix S1).

Phylogenetic analysis. — To account for incomplete lineage sorting among loci, we used a two-step summary coalescent method for the multi-locus data, where we first inferred gene trees and then used these to estimate a species tree (Zhang & al., 2018). Fully resolved gene trees estimated from loci with low variation can introduce error to coalescent tree estimations (Simmons & Gatesy, 2021), and a Shimodaira-Hasegawa-like approximate likelihood-ratio test (SH-like aLRT) (Guindon & al., 2010) was used as a criterion to collapse unsupported nodes into polytomies in the gene trees before estimating the species tree.

IQ-TREE2 v.2.3.4 (Minh & al., 2020b) was used to perform a maximum likelihood analysis for each aligned locus of the multi-locus dataset separately with a substitution model selected using ModelFinder (Kalyaanamoorthy & al., 2017) and SH-like aLRT calculated for each node. Nodes with 0% SH-like aLRT support were collapsed with the nw-ed function in Newick utilities (Junier & Zdobnov, 2010; Simmons & Gatesy, 2021). The collapsed gene trees were combined, and ASTRAL III (Zhang & al., 2018) was used to estimate a coalescence-based species tree. IQ-TREE2 v.2.3.4 (Minh & al., 2020b) was subsequently used to estimate gene concordance factors (percentage of informative gene trees containing the same branch), and site concordance factors (percentage of informative alignment sites that support the branch, Minh & al., 2020a).

The chloroplast data was aligned with MAFFT v.7.5 (Katoh & Standley, 2013), concatenated, and two datasets were made; one corresponding with the samples in the multi-locus (suppl. Appendix S2) and one in the single-locus (suppl. Appendix S3) dataset. Maximum likelihood phylogenetic analyses were conducted for both datasets with IQ-TREE2 (Minh & al., 2020b) using ModelFinder (Kalyaanamoorthy & al., 2017) to select the substitution model and estimating support using 1000 ultrafast bootstrap replicates (Hoang & al., 2018).

Clade association and phasing. — To assess if the haplotypes inherited from each parent were different, as would be expected of hybrids, reads were first mapped to references in different parts of the phylogeny. Hybrids contain reads that associate with both of their parent clades, and thus accessions that map to multiple clade references in high proportions and have high LH (>90%) and AD (>2%) represent putative

hybrids (Nauheimer & al., 2021). We used phasing as implemented in HybPhaser (Nauheimer & al., 2021) to map reads to the potential parental clades flagged in the earlier step to separate the divergent reads. The final phased reads theoretically represent the haplotypes that a hybrid inherited from its parents.

To this end, we chose eight references that represented major clades in the ASTRAL phylogeny and that had low LH and AD, making them unlikely to be hybrids themselves (Nauheimer & al., 2021). HybPhaser with BBSplit (BBMap v.38.47; https://sourceforge.net/projects/bbmap/) was used to map reads to all eight references and record the proportion of reads that mapped to each reference. Accessions that mapped to multiple clade references in high proportions and had an LH >90% and an AD >2% were chosen for phasing. HybPhaser was then used to map reads with BBSplit to the references with high read associations, and the reads that mapped unambiguously to one reference saved in separate files according to their association (reads matching similarly well to multiple references were saved in all read files).

HybPiper and HybPhaser were subsequently used with the same settings as above to assemble and generate consensus sequence lists of the phased accessions. These were combined with the non-phased data, removing the non-phased accessions of the samples that were phased. The resulting sequence lists were aligned and trimmed (suppl. Appendix S4), and phylogenetic analyses were performed exactly as for the non-phased data to generate an ASTRAL tree.

For analysis of the single-locus, phased sequences of L369 from analyses of the multi-locus data were trimmed to the same length as the single-locus data and combined with the variants from VSEARCH. For the resulting dataset of 64 sequences of 39 specimens (suppl. Appendix S5), tree analysis was performed exactly as for the chloroplast data.

■ RESULTS

Locus heterozygosity and allele divergence. — In the multi-locus data, 10 of the 22 accessions had both high LH (>90% of loci had SNPs) and an AD (percentage of SNPs across all loci) above 1% (Fig. 1). Of these 10 samples, 3 had a high AD (>2%) and reads that mapped unambiguously and in high proportions to two reference clades, indicating that these were hybrids between lineages in the respective clades. These were *Danaea ushana* Christenh., *D. trifoliata* Rchb. ex Kunze and *D. wendlandii* Rchb.f. (Fig. 1).

After the apparent hybrids *Danaea ushana*, *D. trifoliata* and *D. wendlandii* were removed, the three subgenera had differing signals for hybridization or introgression, with AD differing significantly between them (Fig. 1, two-way ANOVA $p < 0.05$). *Danaea* subg. *Holodanaea* had the lowest signal for hybridization (mean AD = 0.5%), *D.* subg. *Arthrodanaea* the highest (mean AD = 1.3%), and *D.* subg. *Danaea* intermediate (mean AD = 1.0%). Differences in LH were not significant, but the LH values were less informative than the AD values for our dataset containing flanking regions, as most loci were variable.

Splitting of divergent haplotypes. — In the multi-locus data, the phased accessions of *Danaea ushana*, *D. trifoliata* and *D. wendlandii* were resolved into multiple parts of the phylogeny with strong support, showing divergent haplotypes from their hybrid origins (Fig. 2A). *Danaea ushana* grouped with both *D. simplicifolia* Rudge (*D.* subg. *Arthrodanaea*) and *D. nigrescens* Jenman (*D.* subg. *Danaea*), *D. trifoliata* grouped both with *D. simplicifolia* and the rest of *D.* subg. *Arthrodanaea*, and *D. wendlandii* grouped both with *D. lanceolata* Tuomisto & Keskiniva and in a clade containing *D. tenuicaulis* Tuomisto & Keskiniva, *D. stricta* Tuomisto & Keskiniva, and *D. cuspidopsis* Keskiniva & Tuomisto.

Based on an earlier chloroplast phylogeny (Keskiniva & al., 2024), a single nuclear locus was sequenced for some potential hybrids and their putative parents. In the phased single-locus nuclear phylogeny (Fig. 3A), the potential *Danaea wendlandii* × *D.* subg. *Danaea* hybrid (*Tuomisto 13811*) had three variants; the same two variants as *D. wendlandii*, and one that was present in the non-Amazonian species of *D.* subg. *Danaea*, including *D. nodosa* (L.) Sm. and *D. pterorachis* Christ. The potential *D. alba* × *D. panamensis* hybrid *Jones 742* had three variants of the locus; two grouping with *D. alba* Keskiniva & Tuomisto with good support, one in a polytomy containing *D. panamensis* Keskiniva & Tuomisto. The potential *D. bipinnata* × *D. leprieurii* hybrid (*Tuomisto 14481*) had two variants; one grouping with *D. leprieurii* Kunze and one

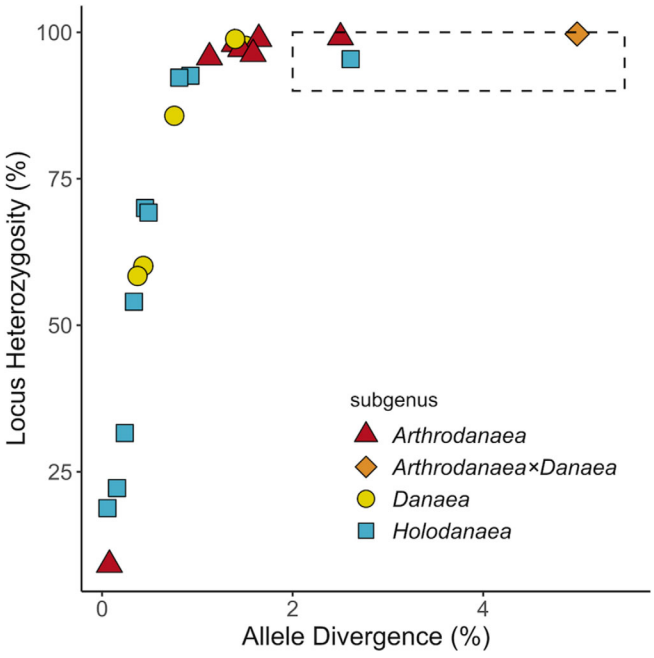

Fig. 1. Scatterplot showing the relationship between locus heterozygosity and allele divergence in the nuclear data of 351 loci. Dotted rectangle showing LH (>90%) and AD (>2%).

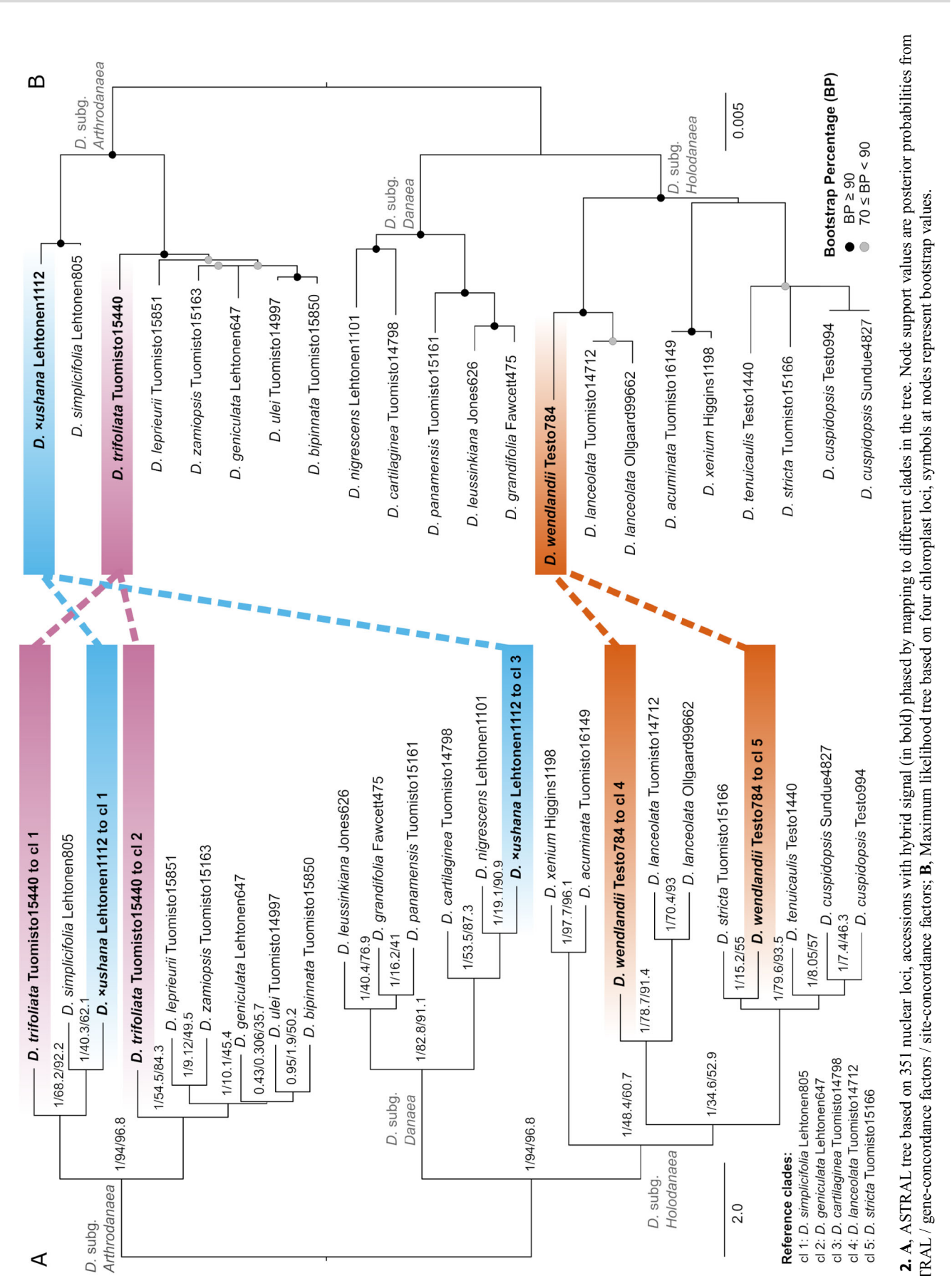

Fig. 2. A, ASTRAL tree based on 351 nuclear loci, accessions with hybrid signal (in bold) phased by mapping to different clades in the tree. Node support values are posterior probabilities from ASTRAL / gene-concordance factors / site-concordance factors; **B**, Maximum likelihood tree based on four chloroplast loci, symbols at nodes represent bootstrap values.

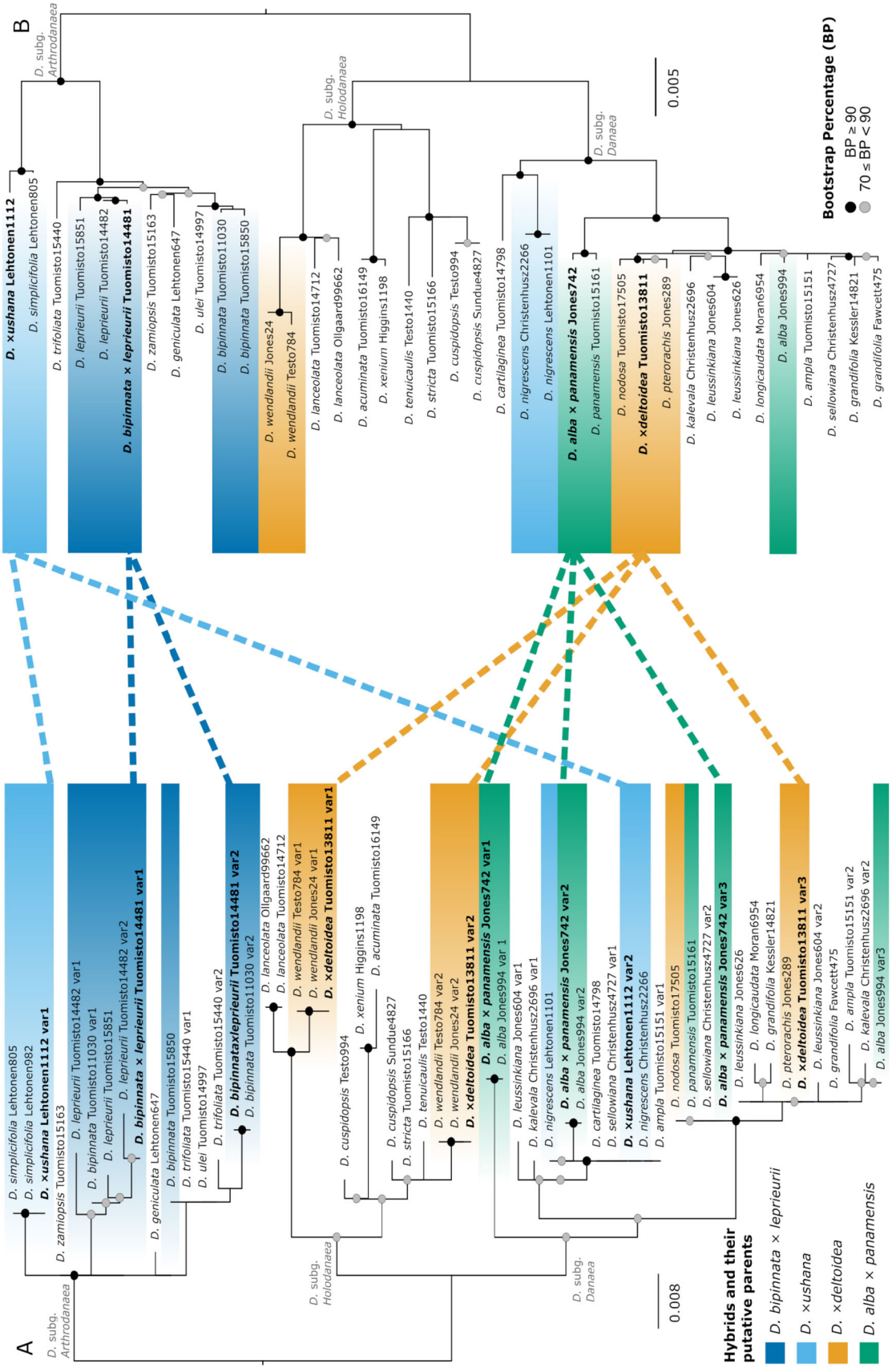

Fig. 3. Maximum likelihood (ML) trees with putative hybrids in bold and their putative parents with the same background colour. Symbols at nodes represent bootstrap values. **A**, ML tree based on a single variable nuclear locus with diverging alleles phased; **B**, ML tree based on four chloroplast loci.

with *D. bipinnata* Tuomisto. *Danaea bipinnata* had two variants shared with *D. leprieurii*, but in addition it had one variant that it shared with *Tuomisto 14481*, which *D. leprieurii* did not have.

Aside from the hybrids, the phylogeny based on the multi-locus data was congruent with the chloroplast phylogeny in all well-supported branches (Fig. 2A,B). We had both single-locus and multi-locus data for two specimens (*Danaea panamensis Tuomisto 15161* and *D. ushana Lehtonen 1112*), and both datasets had identical alleles for the single locus.

In the single-locus data, there were divergent variants of the locus within several of the samples, with some possessing up to three variants and others only one (Fig. 3). For instance, the Amazonian species of *Danaea* subg. *Danaea* (*D. nigrescens* and *D. cartilaginea* Christenh. & Tuomisto) had only one variant, whereas many of the non-Amazonian species had an additional variant.

■ DISCUSSION

Hybrids. — The splitting of divergent haplotypes showed evidence of hybridization events between *Danaea* subg. *Arthrodanaea* and subg. *Danaea* and *D.* subg. *Danaea* and subg. *Holodanaea*, as well as potential hybridization events within all three subgenera. These are discussed below.

***Danaea nigrescens* × *D. simplicifolia* (Fig. 4). —** We suggest that *Danaea ushana* is a hybrid between *D. nigrescens* and *D. simplicifolia* and should be treated as a hybrid rather than a species. The two specimens of *D.* ×*ushana* (*Lehtonen 1112*, *Boudrie 3764*) in the chloroplast phylogeny of Keskiniva & al. (2024) grouped with *D. simplicifolia*, indicating this is the maternal progenitor. However, morphologically *D.* ×*ushana* conforms with species of *D.* subg. *Danaea*. In the nuclear phylogeny where haplotypes were phased, *D.* ×*ushana* grouped with both *D. simplicifolia* and *D. nigrescens* (Fig. 2A). All three taxa occur in the same geographical area, with *D.* ×*ushana* having been found only in a small area in French Guiana (Fig. 5). Despite being very similar in morphology to *D. nigrescens*, it presents somewhat intermediate morphological traits by having a creeping rhizome with leaf bases in three or more rows (vs creeping with leaf bases in two rows in *D. nigrescens*, erect and radial in *D. simplicifolia*), few pairs of fertile pinnae (3–4 vs 6–14 in *D. nigrescens*, usually none in *D. simplicifolia*), and sterile pinnae with entire apices (almost always serrate in *D. nigrescens*, entire in *D. simplicifolia*).

***Danaea wendlandii* × *D.* subg. *Danaea* (Fig. 6). —** Two specimens from Costa Rica (*Tuomisto 13811*, *Jones 606*) originally identified as *Danaea* aff. *wendlandii* from *D.* subg *Holodanaea* grouped into *D.* subg. *Danaea* in the chloroplast phylogeny of Keskiniva & al. (2024). These specimens are larger than typical specimens of *D. wendlandii* but much smaller than any species in *D.* subg. *Danaea*, with small and narrow fertile pinnae typical of *D.* subg. *Holodanaea* (Fig. 6). In the phased single-locus nuclear phylogeny,

Fig. 4. The hybrid *Danaea* ×*ushana* and its parent species in French Guiana. **A**, *D. nigrescens*; **B**, *D.* ×*ushana*; **C**, *D. simplicifolia*. © Samuli Lehtonen 2013.

Tuomisto 13811 grouped with both *D. wendlandii* and *D. pterorachis* (Fig. 3A). It had three divergent alleles, two of which were also present in *D. wendlandii*, suggesting this to be the paternal progenitor. The third allele was present in the non-Amazonian species of *D.* subg. *Danaea*, including *D. nodosa* and *D. pterorachis*, which co-occur with the putative hybrid and group with it in the chloroplast phylogeny. Therefore, it seems safe to assume one of these (perhaps more likely *D. pterorachis*) to be the maternal progenitor. As the hybrid can be readily recognized, there are several collections known from an area of active research (La Selva biological station in Costa Rica), and one of its parents is rather confidently known, we describe and name it below.

***Danaea bipinnata* × *D. leprieurii*. —** *Danaea bipinnata* is generally larger than *D. leprieurii* (Tuomisto & Moran, 2001). Two specimens from Amazonia (*Tuomisto 14481* from Peru, *Tuomisto 15855* from Brazil) that had intermediate characters between the two species but were unusually large even for *D. bipinnata* were grouped with *D. leprieurii* in the chloroplast phylogeny of Keskiniva & al. (2024).

In the phased single-locus phylogeny, *Tuomisto 14481* had two variants; one grouping with *D. leprieurii* and one with *D. bipinnata* (Fig. 3A). As all these taxa co-occur and *Tuomisto 14481* also presents intermediate traits between the two prospective parental species, we suggest that it is a *D. bipinnata* × *D. leprieurii* hybrid, with *D. leprieurii* as the maternal progenitor.

***Danaea alba* × *D. panamensis*. —** *Danaea alba* and *D. panamensis* were recently described from *D.* subg. *Danaea* based on morphology and chloroplast DNA, which showed them as clearly distinct (Keskiniva & Tuomisto, 2024). They co-occur in Panama, however, and one juvenile specimen (*Jones 742*) that was morphologically identical to juveniles of *D. alba* (with light coloured, elliptical pinnae) grouped with *D. panamensis* (with dark, oblong juvenile pinnae) in the chloroplast phylogeny.

In the phased single-locus nuclear phylogeny (Fig. 3), *Jones 742* had three variants of the locus; two grouping with *Danaea alba* with good support, one in a polytomy containing *D. panamensis*. As all these taxa co-occur, and in the chloroplast phylogeny *Jones 742* groups with *D. panamensis* but shares alleles with both prospective parents and is morphologically indistinguishable from *D. alba*, we suggest that it is a *D. alba* × *D. panamensis* hybrid, with *D. panamensis* as the maternal progenitor. This could also be an example of introgression; based on data only from one locus, we can only speculate that there must have been mixing at some point.

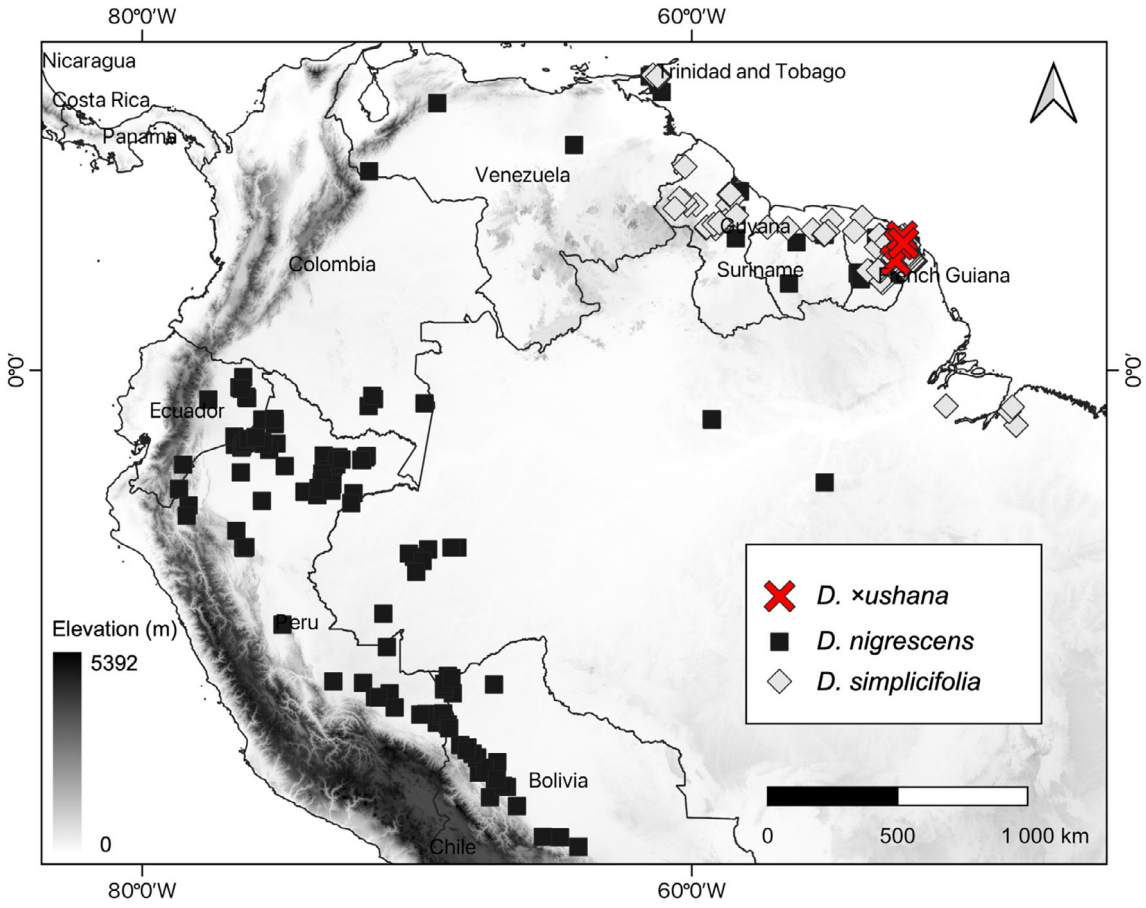

Fig. 5. Distribution of the hybrid *Danaea* ×*ushana* and its parent species.

***Danaea trifoliata* (Fig. 7). —** *Danaea trifoliata* is rather widespread in Amazonia. It has an unusually plastic morphology; fertile plants can have sterile leaves that are simple or pinnate with up to four lateral pinna pairs, and fertile leaves that are simple or have up to two lateral pinna pairs (Fig. 7). In the chloroplast phylogeny, *D. trifoliata* was in a well-supported clade containing *D. antillensis* Christenh. and *D. trinitatensis* Christenh. & Tuomisto (Keskiniva & al., 2024).

In the nuclear data, a specimen of *Danaea trifoliata* from Brazil (*Tuomisto 15440*) had high AD and LH. In the phased nuclear phylogeny (Fig. 2), it grouped both with *D. simplicifolia* and the rest of *D.* subg. *Arthrodanaea*, but it was not identical to either. It seems that at least this specimen of *D. trifoliata* is a hybrid of uncertain parentage. As *D. trifoliata* was monophyletic in the chloroplast phylogeny (Keskiniva & al., 2024) and is abundant across a wide distribution, we suggest that it is a fertile species derived from a previous hybridization event.

***Danaea wendlandii*. —** In the chloroplast phylogeny of Keskiniva & al. (2024), *Danaea wendlandii* grouped with good support into a clade containing the morphologically similar *D. gracilis* Tuomisto & Keskiniva and *D. lanceolata*. In the nuclear data a specimen of *D. wendlandii* (*Testo 784*) from Costa Rica showed high AD and LH, and in the phased nuclear phylogeny (Fig. 2), it grouped both with *D. lanceolata* and in a clade containing *D. tenuicaulis*, *D. stricta*, and *D. cuspidopsis*, but was not identical to any of these. Another regular-looking *D. wendlandii* specimen (*Jones 24*) in the single-locus phylogeny (Fig. 3) had the same two alleles as *Testo 784*, as did the *D. wendlandii* × *D.* subg. *Danaea* hybrid (*Tuomisto 13811*).

Danaea wendlandii evidently spreads to new locations through spores, establishing readily on the vertical soil surfaces of uprooted trees (Sharpe, 1993). In addition, it is the parent of another hybrid (described below) and has been observed to produce normal spores (Rolleri, 2004). *Danaea wendlandii* is monophyletic in the chloroplast phylogeny

Fig. 6. The hybrid *Danaea* ×*deltoidea* and its putative parent species. *Danaea* ×*deltoidea* is a cross between *D. wendlandii* and either *D. pterorachis* or *D. nodosa*. **A**, *D.* ×*deltoidea*, Costa Rica; **B**, *D. wendlandii*, Costa Rica; **C**, *D. pterorachis*, Costa Rica; **D**, *D. nodosa*, Mexico (*Tuomisto 17508*). © Hanna Tuomisto, 2001, 2017.

and abundant in Costa Rica. The divergent haplotypes found in *D. wendlandii* here point to no obvious parent species. The ability to procreate as well as divergent haplotypes that seem to have been evolving separately from other species suggest that *D. wendlandii* is a fertile species derived from a previous hybridization event between unknown parent species. This makes the *D. wendlandii* × *D.* subg. *Danaea* hybrid described below a hybrid of a hybridization derived species.

General discussion. — We set out to assess the potential importance of hybridization as an evolutionary force in tropical ferns, as hybridization has been linked to diversification and speciation (Seehausen, 2004; Abbott & al., 2013). We sampled 20 taxa (one-fifth of the estimated total number of species) in the tropical fern genus *Danaea*. We found that three of the previously recognized species (15% of the sampled diversity) likely have a hybrid origin, one of them involving hybridization between subgenera. In addition, we identified three other probable hybrids, which had already been suspected because of their unexpected position in a chloroplast phylogeny.

Hybridization can also work as a homogenizing force (Rothfels & al., 2015), and we found evidence of this in *Danaea*. Species in *Danaea* subg. *Arthrodanaea* had especially high values of LH and AD and had accessions that mapped to multiple clade references, which indicates hybridization or introgression (Nauheimer & al., 2021). This subgenus is the one that has been the most challenging taxonomically, as species delimitation is made difficult by morphologically variable species with fuzzy and overlapping diagnostic characters. Our results show that these problems have a genetic basis: almost all of the species were genetically similar and had poorly resolved relationships in both the nuclear and chloroplast phylogenies. This is also reflected in patterns of diversity and biogeography: *D.* subg. *Arthrodanaea* has fewer species but a larger proportion of them with wide, sympatric distributions than the other subgenera in *Danaea*.

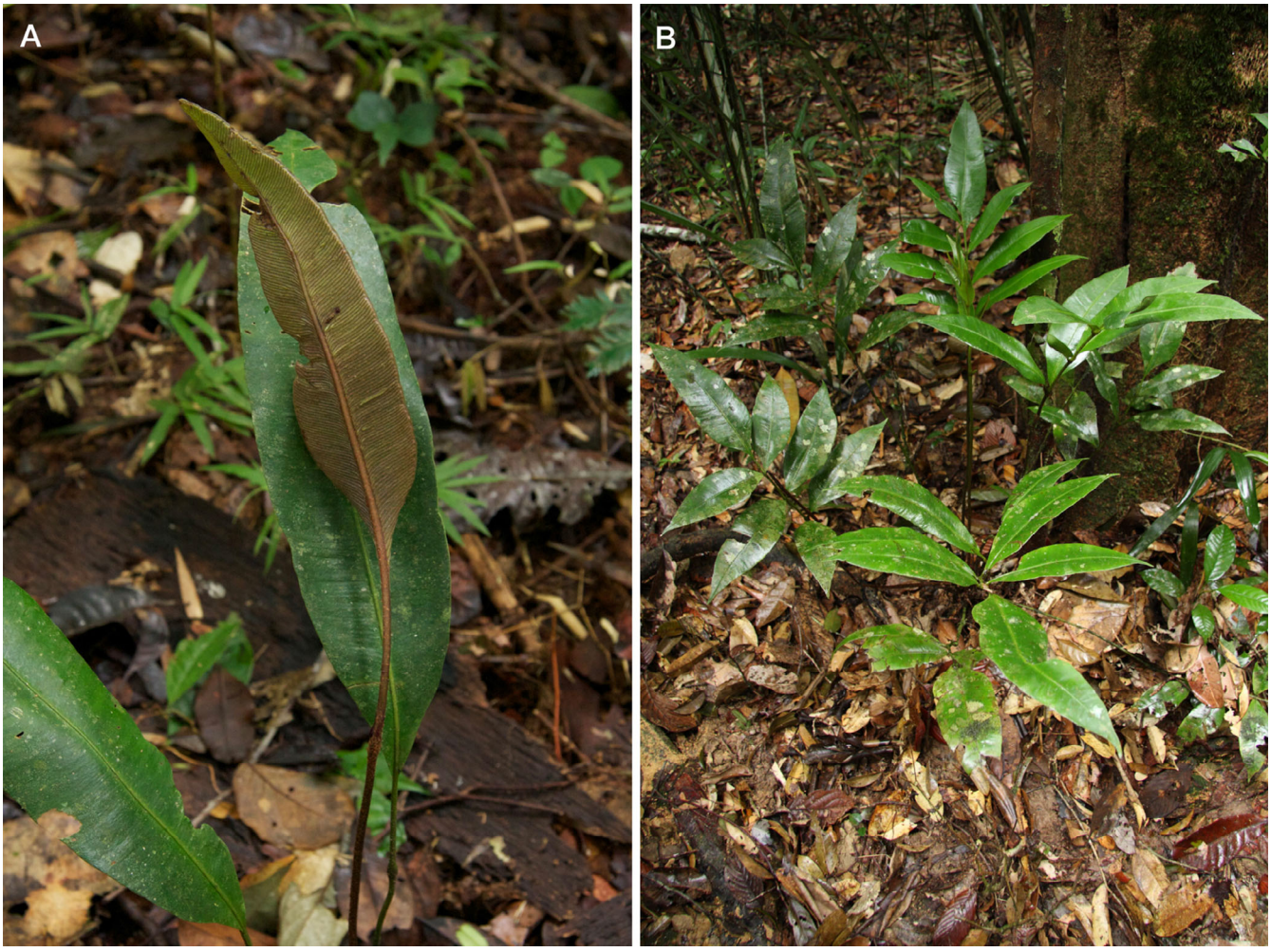

Fig. 7. Examples of the variable morphology of *Danaea trifoliata* in Brazil, Amazonas (both specimens group into *D. trifoliata* in Keskiniva & al., 2024). **A**, Fertile plant with simple leaves (*Tuomisto 16549*); **B**, Pinnate plant (*Tuomisto 15717*). — A, © Gabriel Moulatlet 2012; B, © Hanna Tuomisto 2008.

Another possible case of introgression is found in Costa Rica, where we hypothesize the existence of a hybrid swarm between *Danaea nodosa* and *D. wendlandii*, with *D.* ×*deltoidea* (nothosp. nov., described below) and *D. pterorachis* representing different stages of backcrossing. We hypothesize this because, while *D. pterorachis* is similar to *D. nodosa* in gross morphology and these two taxa were indistinguishable in the chloroplast phylogeny of Keskiniva & al. (2024), *D. pterorachis* presents some traits typical of *D. wendlandii* that are not seen in *D. nodosa* (nodes on the petiole, leaves in several rows on the rhizome). However, we found no evidence of hybridization in *D. pterorachis* based on the single nuclear locus. If *D. pterorachis* does turn out to be a hybrid between *D. wendlandii* and *D. nodosa*, the name *D. pterorachis* should also be used for *D.* ×*deltoidea* (Art. H.4.1., Turland & al., 2018), even though the two are morphologically very different.

Temperate fern lineages have been suggested to hybridize more than tropical lineages (Liu & al., 2020). The tropical fern genus *Danaea* contains 79 described species, of which two appear to be self-sustaining species of hybrid origin (*D. trifoliata*, *D. wendlandii*), and two were described as species before their hybrid status was noticed (*D.* ×*plicata*, *D.* ×*ushana*). In addition, four hybrids have been discovered with molecular and/or morphological and cytological support (*D. alba* × *D. panamensis*, *D. bipinnata* × *D. leprieurii*, *D. wendlandii* × *D.* subg. *Danaea*, *D. jenmanii* × *D. jamaicensis*). Overall, these discoveries give a rate of 10% hybrids per nonhybrid species in *Danaea*, which is lower than the 17% average in temperate ferns, but higher than the 6% average in temperate angiosperms (Whitney & al., 2010). It is likely that the hybridization rate in *Danaea* is actually higher, because a taxonomic revision of *Danaea* (Keskiniva & al., 2024) uncovered several additional specimens with intermediate morphology and/or incongruent placement in the chloroplast phylogeny that could be the result of the following hybridization events: *D. acuminata* Tuomisto & R.C.Moran × *D. vivax* Christenh. & Tuomisto, *D. erecta* Tuomisto & R.C. Moran × *D. kessleri* Keskiniva & Tuomisto, *D. imbricata* Tuomisto & R.C.Moran × *D. trichomanoides* T.Moore, *D. polymorpha* Lepr. ex Baker × *D. trinitatensis* Christenh. & Tuomisto, *D. wendlandii* Rchb.f. × *D. crispa* Endrés & Rchb.f., and the intersubgeneric *D. cuspidata* Liebm. × *D. nodosa* (L.) Sm. The confirmation of these will require further study, as they were not included in our current nuclear dataset.

It is noteworthy that all the specimens we flagged as potential hybrids in the chloroplast phylogeny were confirmed as hybrids by nuclear DNA. If all the remaining suspected but untested hybrids were confirmed, the hybridization rate in *Danaea* would increase to 18%, which would be similar to (and even slightly higher than) the average rate in temperate ferns (Whitney & al., 2010). This topic needs further study to see if the pattern of high hybridization rates is unique to *Danaea* or if hybridization in tropical fern groups has been generally underestimated.

Given that hybridization is considered an exception and is only detected if specifically tested for, even the 18% hybridization rate is likely an underestimate. *Danaea* are often rare and endemic to poorly collected areas, and hybrids can be morphologically cryptic (such as the *D. alba* × *D. panamensis* hybrid described above, which is morphologically identical to *D. alba*). When hybrid morphology is unique it may be considered a separate species, but if its morphology is closer to the paternal than the maternal parent, misplacement in a chloroplast phylogeny can hint at hybrid status (as was the case with *D.* ×*ushana*). However, if morphology matches the maternal parent, morphological and chloroplast data give no indication of hybridization. Here we focused on specimens that looked misplaced in a chloroplast phylogeny, so we probably underestimate overall hybridization rate.

The ability of nuclear data to show the complexity of evolutionary histories is a strength that can be lost if genomic data is not phased. Phasing clearly showed the divergent haplotypes of hybrids in *Danaea*. In addition, it showed a tentative biogeographical pattern between Amazonian and non-Amazonian species of *D.* subg. *Danaea*, where the Amazonian species had only one, perhaps the ancestral, variant of a nuclear locus, and many of the non-Amazonian species had an additional variant, perhaps due to a later duplication event. The dataset was small, however, and this story needs to be looked at with more data.

Ferns have especially high rates of hybridization (Barrington & al., 1989; Wood & al., 2009; Whitney & al., 2010; Sigel, 2016) and the deepest hybrids among vascular plants (Rothfels & al., 2015; Lehtonen, 2018; Liu & al., 2020). This indicates weak reproductive barriers, which in turn have been associated with genomic stasis (Schneider & al., 2015; Clark & al., 2016). The family Marattiaceae has been suggested to have an anomalously slow rate of molecular evolution in comparison to other plants (Soltis & al., 2002), and we found some of the potentially deepest known hybridization events in any group of organisms in *Danaea*. The timing of evolutionary events in Marattiaceae is problematic due to it being an isolated branch without intermediate fossils (Lehtonen & al., 2020). For a point of reference, however, we found two timed phylogenies with sufficient resolution to speculate on the timing of the divergence between the parental lineages of the deepest *Danaea* hybrids listed here. In these two plastid phylogenies, the split of the parental lineages occurred 27–83 mya for *D.* ×*ushana*, 14–72 mya for *D. trifoliata*, 23–62 mya for *D. wendlandii* × *D.* subg. *Danaea*, and 17–50 mya for *D. wendlandii* (Testo & Sundue, 2016; Nitta & al., 2022). To compare, the divergence time of the other known deep fern hybrids in the same phylogenies were 73–77 mya (×*Cystocarpium*), 70–88 mya (×*Lindsaeosoria*), 89–105 mya (×*Dryostichum*), 59–98 mya (×*Osmuntonia*) and 48–94 mya (×*Osmunasium*).

If *Danaea trifoliata* and *D. wendlandii* are both fertile, hybridization-derived species as our research suggests, they could be some of the deepest hybridization derived species of any organism, with the parental lineages of *D. trifoliata* potentially having diverged as far ago as the Cretaceous.

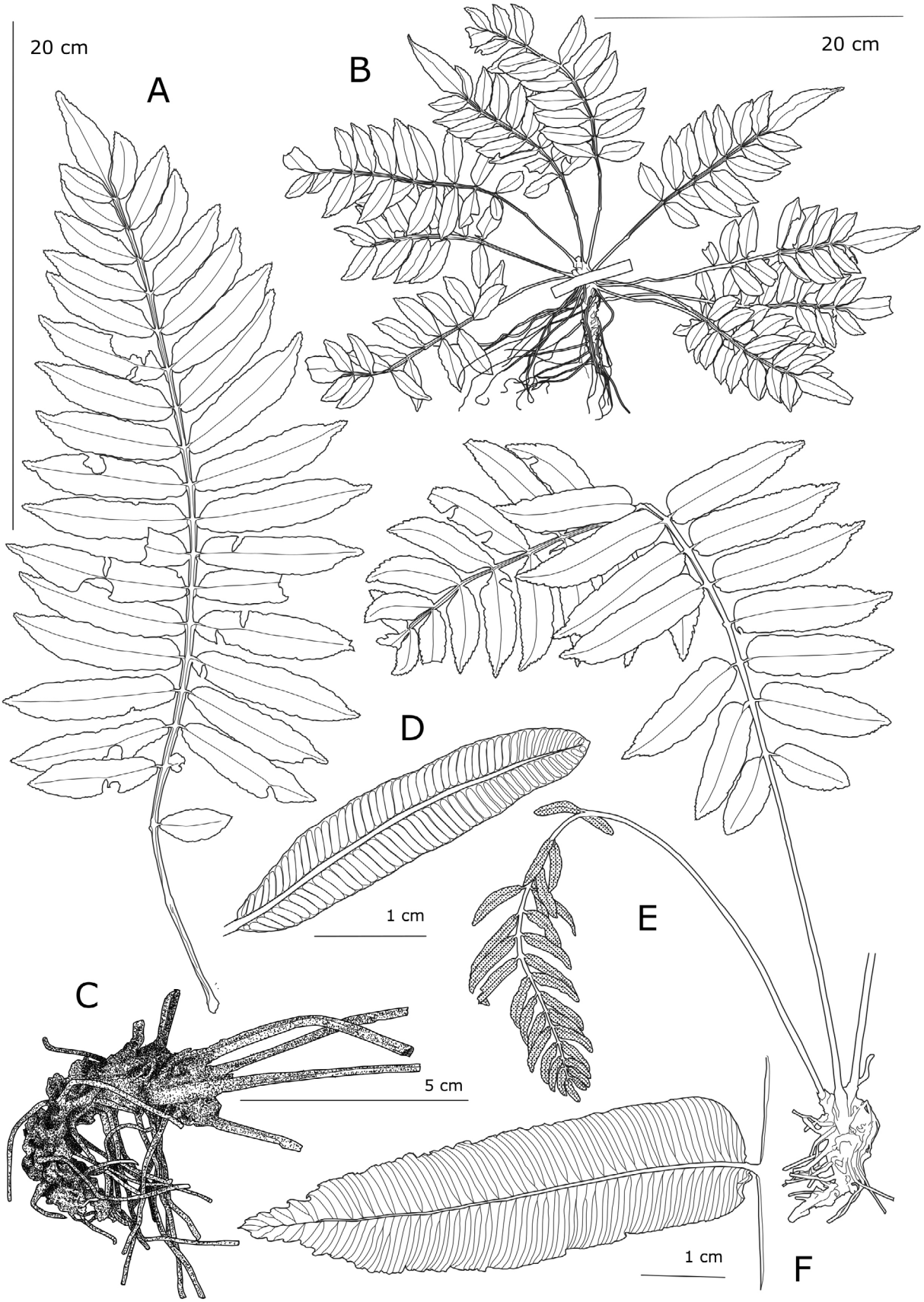

Fig. 8. *Danaea ×deltoidea*: **A**, Sterile leaf; **B**, Juvenile; **C**, Decumbent rhizome; **D**, Fertile pinna; **E**, Sterile and fertile leaf; **F**, Sterile pinna. **A** & **D**, *W. Kupper 478* (M); **B**, *W. Kupper 360* (M); **C**, *H. Tuomisto 13811* (TUR); **E** & **F**, *M. Jones 32* (TUR). — Drawn by Venni Keskiniva.

■ TAXONOMIC TREATMENT

Danaea* ×*deltoidea Keskiniva & Tuomisto, **nothosp. nov.** – Holotype: COSTA RICA. Heredia, Sarapiquí. La Selva biological station, 10°26′N 84°01′W, 50–150 m a.s.l., 4 Jul 2001, *M. Jones 32* (CR barcode 0241381!; isotypes: TUR!, USJ n.v.).

The new nothospecies is illustrated in Figs. 6 and 8.

Diagnosis. – Similar to *Danaea wendlandii* Rchb.f., but differs in larger leaves (26–41 cm vs 13–25 cm long); lateral pinnae that are longer (5.5–7.7 cm vs 1.9–4.1 cm) and wider (1.3–1.7 cm vs 0.6–1.1 cm); terminal pinna more often present and then longer (2.6–5.0 cm vs 1.7–2.0 cm) and wider (1.1–1.4 cm vs 0.5–0.6 cm); more pinna pairs (13–18 vs 10–15); cuspidate or acuminate pinna apices (vs obtuse to acute).

Description. – *Rhizomes* creeping to decumbent, with leaves spirally and roots on one side, 1.0–2.0 cm in diam., to 8 cm long. *Sterile leaves* 26–41 cm long; *petioles* 4.5–22.5 cm long, with 0–3 nodes, winged in distal part; *laminae* 21–35 cm × 11–14 cm, 13–18 pinna pairs, imparipinnate or paripinnate, lanceolate, proximal pinnae sometimes slightly more distant and somewhat reduced, medial pinnae 1.0–1.5 cm apart, almost concolorous, (dark) green adaxially, (slightly) lighter green abaxially, texture thin, rachises winged, wings to 0.7–1.0 mm wide; *terminal pinnae* 2.6–5.0 cm × 1.1–1.4 cm, lanceolate, bases acute, apices 0.4–1.0 cm long, acuminate, apical margins crenate, crenulate or serrulate; *largest lateral pinnae* 5.5–7.7 cm × 1.3–1.7 cm, 3.5–4.5 times as long as wide without apex, parallel-sided, perpendicular to rachis, bases asymmetrical, obtuse (or obtuse proximally, acute distally), apices 0.0–0.8 cm long, acuminate, cuspidate (or acute), apical margins crenate, crenulate, or serrate; *veins* 13–20 per cm, mostly forked at the costa, sometimes above. *Fertile leaves* 27–37 cm long; *petioles* 15–20 cm long, 0–1 nodes; *laminae* 12–16 cm × 4–7 cm, 13–18 pinna pairs, imparipinnate or paripinnate, parallel-sided; *terminal pinnae* 2.6–5.0 cm × 1.1–1.4 cm, linear-lanceolate, bases acute, apices acute to obtuse; *largest lateral pinnae* 2.6–5.0 cm × 0.2–0.8 cm, linear-oblong, bases symmetrical, obtuse, apices obtuse to acute. *Juveniles* with elliptic to lanceolate laminae, terminal pinnae lanceolate, often longest, lateral pinnae oblong, apices cuspidate to acute.

Etymology. – Deltoidea is Latin for triangular, referring to the shape of the lamina in relation to the more parallel-sided *Danaea wendlandii*.

Distribution and habitat. – Has been found in two locations in Costa Rica (Provs. Limón and Heredia), from 50 to 600 m a.s.l. (Fig. 9).

Additional specimens examined. – COSTA RICA. **Heredia:** Sarapiquí, La Selva biological station, 10°26′N 84°01′W, 50–150 m a.s.l., 21 Aug 2001, *Jones, M. 218* (CR, TUR); Sarapiquí, La Selva biological station, 10°26′N 84° 01′W, 50–150 m a.s.l., 14 Sep 2001, *Jones, M. 266* (TUR); Sarapiquí, La Selva biological station, 10°26′N 84°01′W, 50–150 m a.s.l., 8 Jul 2002, *Jones, M. 513* (TUR); Sarapiquí, La Selva biological station, 10°26′N 84°01′W, 50–150 m a.s.l., 2 May 2007, *Jones, M. 606* (TUR); Sarapiquí, 10°26′N

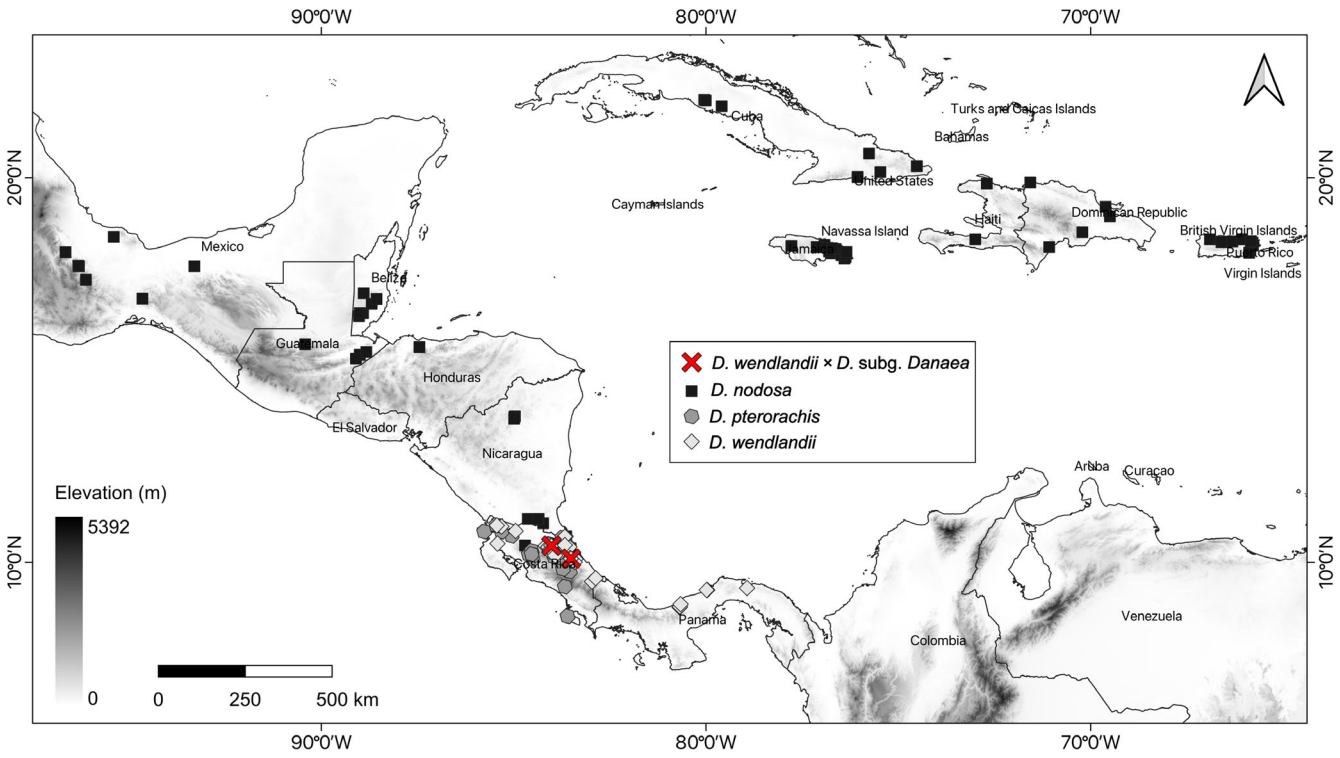

Fig. 9. Distribution of the hybrid *Danaea* ×*deltoidea* and its putative parent species.

84°01′W, 50–150 m a.s.l., 1 Dec 2001, *Tuomisto, H. 13811* (CR, TUR); Carillo, 600 m a.s.l., 9 Jan 1932, *Kupper, W. 360* (M); **Limón:** Siquirres, 400 m a.s.l., 9 Feb 1932, *Kupper, W. 478* (M); Siquirres, 400 m a.s.l., 18 Apr 1932, *Kupper, W. 1095* (M).

Notes. – *Danaea* ×*deltoidea* is a hybrid between *D. wendlandii* and either *D. nodosa* or *D. pterorachis* (the latter was treated in some previous publications as *D. media* Liebm., but this is now considered a synonym of *D. nodosa*; Keskiniva & Tuomisto, 2024). In appearance, *D.* ×*deltoidea* resembles *D. wendlandii* of *D.* subg. *Holodanaea* but in the chloroplast phylogeny it forms a clade with *D. nodosa* and *D. pterorachis* of *D.* subg. *Danaea*. A phased phylogeny based on a variable nuclear locus grouped this hybrid with *D. wendlandii* as well as *D. pterorachis* (Fig. 3). *Danaea* ×*deltoidea* has been found in two locations in Costa Rica, both near the proposed parent species (Fig. 9).

Danaea ×*deltoidea* is a much smaller plant than *D. nodosa* or *D. pterorachis* (sterile leaves <45 cm vs >90 cm long, largest lateral pinna <8 cm vs >14 cm long) or indeed any of the species in *D.* subg. *Danaea* (the smallest being *D. longicaudata* Tuomisto, which has leaves >50 cm long). In addition, *D.* ×*deltoidea* has short and narrow fertile pinnae typical of *D.* subg. *Holodanaea*, a usually nodose petiole (nodes absent from the petiole of *D. nodosa*), and a radial rhizome (dorsiventral in *D. nodosa* and *D. pterorachis*).

■ CONCLUSIONS

We found a high rate of hybridization of 10%–18% in a strictly tropical fern group. The upper estimate is similar to the ones recorded for temperate ferns. Further research on tropical fern groups is needed to see if *Danaea* is an exception to the larger pattern, or if the paucity of tropical fern hybrids reported in Liu & al. (2020) may be more due to a lack of systematic attention towards tropical ferns than actual differences in reproductive or evolutionary processes between temperate and tropical ferns.

In one subgenus of *Danaea*, we found evidence of introgression functioning as a homogenizing force that reduces species numbers and makes them more difficult to separate. On the other hand, we also found evidence of hybridization as a diversity-promoting force, as two well-established species turned out to have a hybrid origin.

The two hybridization derived, fertile species were between lineages that could have diverged already 50 mya. One of the hybrids and one of the hybridization derived species we discovered were between parental lineages that potentially diverged already in the Cretaceous. This adds to a growing body of evidence that ferns appear to have low reproductive barriers, allowing hybridization and even the birth of hybridization derived species between lineages that diverged a very long time ago (Rothfels & al., 2015; Lehtonen, 2018; Liu & al., 2020).

■ AUTHOR CONTRIBUTIONS

VK, HT and SL conceived the original idea for the study. VK, HT, and GFC obtained funding; HT, SL and WT provided field observations and specimens; VK produced and analysed the data from herbarium specimens; VK, SL, and GFC generated the data from DNA samples; VK, SL, WT, and GFC analysed and interpreted the data; and VK wrote the manuscript. All authors participated in revising the manuscript and have approved the final version.

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■ LITERATURE CITED

Abbott, R., Albach, D., Ansell, S., Arntzen, J.W., Baird, S.J.E., Bierne, N., Boughman, J., Brelsford, A., Buerkle, C.A., Buggs, R., Butlin, R.K., Dieckmann, U., Eroukhmanoff, F., Grill, A., Cahan, S.H., Hermansen, J.S., Hewitt, G., Hudson, A.G., Jiggins, C., Jones, J., Keller, B., Marczewski, T., Mallet, J., Martinez-Rodriguez, P., Möst, M., Mullen, S., Nichols, R., Nolte, A.W., Parisod, C., Pfennig, K., Rice, A.M., Ritchie, M.G., Seifert, B., Smadja, C.M., Stelkens, R., Szymura, J.M., Väinölä, R., Wolf, J.B.W. & Zinner, D. 2013. Hybridization and speciation. *J. Evol. Biol.* 26: 229–246. https://doi.org/10.1111/j.1420-9101.2012.02599.x

Barrington, D.S., Haufler, C.H. & Werth, C.R. 1989. Hybridization, reticulation, and species concepts in the ferns. *Amer. Fern J.* 79: 55–64. https://doi.org/10.2307/1547160

Beddows, I. & Rose, L.E. 2018. Factors determining hybridization rate in plants: A case study in Michigan. *Perspect. Pl. Ecol. Evol. Syst.* 34: 51–60. https://doi.org/10.1016/j.ppees.2018.07.003

Bolger, A.M., Lohse, M. & Usadel, B. 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30: 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Breinholt, J.W., Carey, S.B., Tiley, G.P., Davis, E.C., Endara, L., McDaniel, S.F., Neves, L.G., Sessa, E.B., von Konrat, M., Chantanaorrapint, S., Fawcett, S., Ickert-Bond, S.M., Labiak, P.H., Larraín, J., Lehnert, M., Lewis, L.R., Nagalingum, N.S., Patel, N., Rensing, S.A., Testo, W., Vasco, A., Villarreal, J.C., Williams, E.W. & Burleigh, J.G. 2021. A target enrichment probe set for resolving the flagellate land plant tree of life. *Applic. Pl. Sci.* 9: e11406. https://doi.org/10.1002/aps3.11406

Capella-Gutiérrez, S., Silla-Martínez, J.M. & Gabaldón, T. 2009. trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25: 1972–1973. https://doi.org/10.1093/bioinformatics/btp348

Christenhusz, M.J.M., Tuomisto, H., Metzgar, J.S. & Pryer, K.M. 2008. Evolutionary relationships within the neotropical,

eusporangiate fern genus *Danaea* (Marattiaceae). *Molec. Phylogen. Evol.* 46: 34–48. https://doi.org/10.1016/j.ympev.2007.09.015

Clark, J., Hidalgo, O., Pellicer, J., Liu, H., Marquardt, J., Robert, Y., Christenhusz, M., Zhang, S., Gibby, M., Leitch, I.J. & Schneider, H. 2016. Genome evolution of ferns: Evidence for relative stasis of genome size across the fern phylogeny. *New Phytol.* 210: 1072–1082. https://doi.org/10.1111/nph.13833

Ellstrand, N.C. & Schierenbeck, K.A. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? *Proc. Natl. Acad. Sci. U.S.A.* 97: 7043–7050. https://doi.org/10.1073/pnas.97.13.7043

Ellstrand, N.C., Whitkus, R. & Rieseberg, L.H. 1996. Distribution of spontaneous plant hybrids. *Proc. Natl. Acad. Sci. U.S.A.* 93: 5090–5093. https://doi.org/10.1073/pnas.93.10.5090

Fraser-Jenkins, C.R. 2008. *Taxonomic revision of three hundred Indian subcontinental pteridophytes: With a revised census list; A new picture of fern-taxonomy and nomenclature in the Indian subcontinent*. Dehra Dun: Bishen Singh Mahendra Pal Singh.

Gastony, G.J. & Yatskievych, G. 1992. Maternal inheritance of the chloroplast and mitochondrial genomes in cheilanthoid ferns. *Amer. J. Bot.* 79: 716–722. https://doi.org/10.1002/j.1537-2197.1992.tb14613.x

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst. Biol.* 59: 307–321. https://doi.org/10.1093/sysbio/syq010

Haufler, C.H., Hooper, E.A. & Therrien, J.P. 2000. Modes and mechanisms of speciation in pteridophytes: Implications of contrasting patterns in ferns representing temperate and tropical habitats. *Pl. Spec. Biol.* 15: 223–236. https://doi.org/10.1111/j.1442-1984.2000.00042.x

Hoang, D.T., Chernomor, O., Haeseler, A. von, Minh, B.Q. & Vinh, L.S. 2018. UFBoot2: Improving the ultrafast bootstrap approximation. *Molec. Biol. Evol.* 35: 518–522. https://doi.org/10.1093/molbev/msx281

Hsieh, T.H., Chang, Y.H., Chiou, W.L. & Huang, Y.M. 2008. Chromosome numbers of five species of the Marattiaceae in Taiwan. *Taiwan J. Forest Sci.* 23: 335–339.

Johnson, M.G., Gardner, E.M., Liu, Y., Medina, R., Goffinet, B., Shaw, A.J., Zerega, N.J.C. & Wickett, N.J. 2016. HybPiper: Extracting coding sequence and introns for phylogenetics from high-throughput sequencing reads using target enrichment. *Applic. Pl. Sci.* 4: 1600016. https://doi.org/10.3732/apps.1600016

Junier, T. & Zdobnov, E.M. 2010. The Newick utilities: High-throughput phylogenetic tree processing in the UNIX shell. *Bioinformatics* 26: 1669–1670. https://doi.org/10.1093/bioinformatics/btq243

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., Haeseler, A. von & Jermiin, L.S. 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Meth.* 14: 587–589. https://doi.org/10.1038/nmeth.4285

Katoh, K. & Standley, D.M. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molec. Biol. Evol.* 30: 772–780. https://doi.org/10.1093/molbev/mst010

Keskiniva, V. & Tuomisto, H. 2024. *Danaea* (Marattiaceae) keeps diversifying, part 1: Eighteen new species. *Willdenowia* 53: 173–228. https://doi.org/10.3372/wi.53.53303

Keskiniva, V., Tuomisto, H. & Lehtonen, S. 2024. *Danaea* (Marattiaceae) keeps diversifying, part 2: Phylogeny and identification key for 81 taxa. *Willdenowia* 53: 229–255. https://doi.org/10.3372/wi.53.53304

Le Péchon, T., Zhang, L., He, H., Zhou, X.-M., Bytebier, B., Gao, X.-F. & Zhang, L.-B. 2016. A well-sampled phylogenetic analysis of the polystichoid ferns (Dryopteridaceae) suggests a complex biogeographical history involving both boreotropical migrations and recent transoceanic dispersals. *Molec. Phylogen. Evol.* 98: 324–336. https://doi.org/10.1016/j.ympev.2016.02.018

Lehtonen, S. 2018. ×*Lindsaeosoria flynnii* (Lindsaeaceae): Another confirmed example of deep hybridization among the ferns. *Amer. Fern J.* 108: 7–18. https://doi.org/10.1640/0002-8444-108.1.7

Lehtonen, S., Wahlberg, N. & Christenhusz, M.J. 2012. Diversification of lindsaeoid ferns and phylogenetic uncertainty of early polypod relationships. *Bot. J. Linn. Soc.* 170: 489–503. https://doi.org/10.1111/j.1095-8339.2012.01312.x

Lehtonen, S., Silvestro, D., Karger, D.N., Scotese, C., Tuomisto, H., Kessler, M., Peña, C., Wahlberg, N. & Antonelli, A. 2017. Environmentally driven extinction and opportunistic origination explain fern diversification patterns. *Sci. Rep.* 7: 4831. https://doi.org/10.1038/s41598-017-05263-7

Lehtonen, S., Poczai, P., Sablok, G., Hyvönen, J., Karger, D.N. & Flores, J. 2020. Exploring the phylogeny of marattialean ferns. *Cladistics* 36: 569–593. https://doi.org/10.1111/cla.12419

Liu, H.-M., Schuettpelz, E. & Schneider, H. 2020. Evaluating the status of fern and lycophyte nothotaxa in the context of the Pteridophyte Phylogeny Group classification (PPG I). *J. Syst. Evol.* 58: 988–1002. https://doi.org/10.1111/jse.12641

Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal* 17(1): 10–12. https://doi.org/10.14806/ej.17.1.200

May, M.R., Contreras, D.L., Sundue, M.A., Nagalingum, N.S., Looy, C.V. & Rothfels, C.J. 2021. Inferring the total-evidence timescale of marattialean fern evolution in the face of model sensitivity. *Syst. Biol.* 70: 1232–1255. https://doi.org/10.1093/sysbio/syab020

McKain, M.R., Johnson, M.G., Uribe-Convers, S., Eaton, D. & Yang, Y. 2018. Practical considerations for plant phylogenomics. *Applic. Pl. Sci.* 6: e1038. https://doi.org/10.1002/aps3.1038

Minh, B.Q., Hahn, M.W. & Lanfear, R. 2020a. New methods to calculate concordance factors for phylogenomic datasets. *Molec. Biol. Evol.* 37: 2727–2733. https://doi.org/10.1093/molbev/msaa106

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., Haeseler, A. von & Lanfear, R. 2020b. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molec. Biol. Evol.* 37: 1530–1534. https://doi.org/10.1093/molbev/msaa015

Mitchell, N., Campbell, L.G., Ahern, J.R., Paine, K.C., Giroldo, A.B. & Whitney, K.D. 2019. Correlates of hybridization in plants. *Evol. Lett.* 3: 570–585. https://doi.org/10.1002/evl3.146

Moran, R.C. & Grayum, M.H. 2018. The hybrid nature of *Danaea plicata* (Marattiaceae): A Costa Rican endemic. *Brittonia* 70: 31–39. https://doi.org/10.1007/s12228-017-9504-2

Nauheimer, L., Weigner, N., Joyce, E., Crayn, D., Clarke, C. & Nargar, K. 2021. HybPhaser: A workflow for the detection and phasing of hybrids in target capture data sets. *Applic. Pl. Sci.* 9: e1144. https://doi.org/10.1002/aps3.11441

Nitta, J.H., Schuettpelz, E., Ramírez-Barahona, S. & Iwasaki, W. 2022. An open and continuously updated fern tree of life. *Frontiers Pl. Sci. (Lausanne)* 13: 909768. https://doi.org/10.3389/fpls.2022.909768

Regalado, L., Schmidt, A.R., Appelhans, M.S., Ilsemann, B., Schneider, H., Krings, M. & Heinrichs, J. 2017. A fossil species of the enigmatic early polypod fern genus *Cystodium* (Cystodiaceae) in Cretaceous amber from Myanmar. *Sci. Rep.* 7: 14615. https://doi.org/10.1038/s41598-017-14985-7

Rognes, T., Flouri, T., Nichols, B., Quince, C. & Mahé, F. 2016. VSEARCH: A versatile open source tool for metagenomics. *PeerJ* 4: e2584. https://doi.org/10.7717/peerj.2584

Rolleri, C.H. 2004. Revisión del género *Danaea* (Marattiaceae—Pteridophyta). *Darwiniana* 42: 217–301.

Rothfels, C.J., Johnson, A.K., Hovenkamp, P.H., Swofford, D.L., Roskam, H.C., Fraser-Jenkins, C.R., Windham, M.D. & Pryer, K.M. 2015. Natural hybridization between genera that diverged from each other approximately 60 million years ago. *Amer. Naturalist* 185: 433–442. https://doi.org/10.1086/679662

Schley, R.J., Twyford, A.D. & Pennington, R.T. 2022. Hybridization: A 'double-edged sword' for Neotropical plant diversity. *Bot. J. Linn. Soc.* 199: 331–356. https://doi.org/10.1093/botlinnean/boab070

Schneider, H., Liu, H., Clark, J., Hidalgo, O., Pellicer, J., Zhang, S., Kelly, L.J., Fay, M.F. & Leitch, I.J. 2015. Are the genomes of royal ferns really frozen in time? Evidence for coinciding genome stability and limited evolvability in the royal ferns. *New Phytol.* 207(1): 10–13. https://doi.org/10.1111/nph.13330

Schuettpelz, E. & Pryer, K.M. 2009. Evidence for a Cenozoic radiation of ferns in an angiosperm-dominated canopy. *Proc. Natl. Acad. Sci. U.S.A.* 106: 11200–11205. https://doi.org/10.1073/pnas.0811136106

Seehausen, O. 2004. Hybridization and adaptive radiation. *Trends Ecol. Evol.* 19: 198–207. https://doi.org/10.1016/j.tree.2004.01.003

Sharpe, J.M. 1993. Plant growth and demography of the Neotropical herbaceous fern *Danaea wendlandii* (Marattiaceae) in a Costa Rican rain forest. *Biotropica* 25: 85–94. https://doi.org/10.2307/2388981

Sigel, E.M. 2016. Genetic and genomic aspects of hybridization in ferns. *J. Syst. Evol.* 54: 638–655. https://doi.org/10.1111/jse.12226

Simmons, M.P. & Gatesy, J. 2021. Collapsing dubiously resolved gene-tree branches in phylogenomic coalescent analyses. *Molec. Phylogen. Evol.* 158: 107092. https://doi.org/10.1016/j.ympev.2021.107092

Smith, S.A., Beaulieu, J.M. & Donoghue, M.J. 2010. An uncorrelated relaxed-clock analysis suggests an earlier origin for flowering plants. *Proc. Natl. Acad. Sci. U.S.A.* 107: 5897–5902. https://doi.org/10.1073/pnas.1001225107

Soltis, P.S., Soltis, D.E., Savolainen, V., Crane, P.R. & Barraclough, T.G. 2002. Rate heterogeneity among lineages of tracheophytes: Integration of molecular and fossil data and evidence for molecular living fossils. *Proc. Natl. Acad. Sci. U.S.A.* 99: 4430–4435. https://doi.org/10.1073/pnas.032087199

Sorojsrisom, E.S., Alvarado-Rodríguez, O. & Testo, W. 2023. *Goniopteris ×tico* (Thelypteridaceae): A new hybrid fern from Costa Rica. *Syst. Bot.* 48: 471–481. https://doi.org/10.1600/036364423X17000842213632

Stein, D.B. & Barrington, D.S. 1990. Recurring hybrid formation in a population of *Polystichum ×potteri*: Evidence from chloroplast DNA comparisons. *Ann. Missouri Bot. Gard.* 77: 334–339. https://doi.org/10.2307/2399548

Testo, W. & Sundue, M. 2016. A 4000-species dataset provides new insight into the evolution of ferns. *Molec. Phylogen. Evol.* 105: 200–211. https://doi.org/10.1016/j.ympev.2016.09.003

Todesco, M., Pascual, M.A., Owens, G.L., Ostevik, K.L., Moyers, B.T., Hübner, S., Heredia, S.M., Hahn, M.A., Caseys, C., Bock, D.G. & Rieseberg, L.H. 2016. Hybridization and extinction. *Evol. Applic.* 9: 892–908. https://doi.org/10.1111/eva.12367

Tuomisto, H. & Moran, R.C. 2001. Marattiaceae. Pp. 21–68 in: Harling, G. & Andersson, L. (eds.), *Flora of Ecuador*, vol. 66 (6–10). Gothenburg: Department of Systematic Botany, University of Göteborg.

Turland, N.J., Wiersema, J.H., Barrie, F.R., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T.W., McNeill, J., Monro, A.M., Prado, J., Price, M.J. & Smith, G.F. (eds.) 2018. *International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017*. Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books. https://doi.org/10.12705/Code.2018

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M. & Rozen, S.G. 2012. Primer3—New capabilities and interfaces. *Nucl. Acids Res.* 40: e115. https://doi.org/10.1093/nar/gks596

Wagner, W.H. 1993. New species of Hawaiian pteridophytes. *Contr. Univ. Michigan Herb.* 19: 63–82.

Wagner, W.H., Wagner, F.S., Reznicek, A.A. & Werth, C.R. 1992. ×*Dryostichum singulare* (Dryopteridaceae), a new fern nothogenus from Ontario. *Canad. J. Bot.* 70: 245–253. https://doi.org/10.1139/b92-034

Walker, T.G. 1966. IX.—A cytotaxonomic survey of the Pteridophytes of Jamaica. *Earth Environm. Sci. Trans. Roy. Soc. Edinburgh* 66: 169–237. https://doi.org/10.1017/S0080456800023516

Walker, T.G. 1985. Cytotaxonomic studies of the ferns of Trinidad. 2. The cytological and taxonomical implications. *Bull. Brit. Mus. (Nat. Hist.), Bot.* 13: 149–249.

Wang, T., Zhang, G.L., Yan, Y.H., Sun, D.K., Shu, J.P., Duan, J.Q., Zhao, G.H., Zhang, G.S., Xiang, J.Y. & Wang, K.L. 2021. *Angiopteris sugongii* (Marattiaceae), a new diploid species with transitional morphology between *Angiopteris* (sen. str.) and *Archangiopteris*. *Phytotaxa* 516: 275–282. https://doi.org/10.11646/phytotaxa.516.3.6

Whitney, K.D., Ahern, J.R., Campbell, L.G., Albert, L.P. & King, M.S. 2010. Patterns of hybridization in plants. *Perspect. Pl. Ecol. Evol. Syst.* 123: 175–182. https://doi.org/10.1016/j.ppees.2010.02.002

Wood, T.E., Takebayashi, N., Barker, M.S., Mayrose, I., Greenspoon, P.B. & Rieseberg, L.H. 2009. The frequency of polyploid speciation in vascular plants. *Proc. Natl. Acad. Sci. U.S.A.* 106: 13875–13879. https://doi.org/10.1073/pnas.0811575106

Zhang, C., Rabiee, M., Sayyari, E. & Mirarab, S. 2018. ASTRAL-III: Polynomial time species tree reconstruction from partially resolved gene trees. *B. M. C. Bioinf.* 19: 153. https://doi.org/10.1186/s12859-018-2129-y

Zhao, J., Zhou, X., Fang, S., Zhu, Z., Li, Y., Yu, H. & He, Z. 2023. Transcriptome-based study on the phylogeny and hybridization of marattialean ferns (Marattiaceae). *Plants (Switzerland)* 12: 2237. https://doi.org/10.3390/plants12122237

Appendix 1. Voucher information and accession numbers.

The list of accessions used in this study is presented as follows: ***Taxon*** and authorship, origin, *collector and collection number* (herbarium), SRA accession number for multi-locus data, GenBank accession number(s) for single-locus data (followed by SL if sequences are from Targeted Illumina sequencing of the single locus, and ML if sequences were extracted from the multi-locus data), *atpB*, *rbcL*, *rpl32-trnL*, and *trnL-F*. A dash (–) indicates information that was unavailable. Sequences generated for this study are marked with asterisk (*). The other sequences were published in Christenhusz & al. (2008), Breinholt & al. (2021), and Keskiniva & al. (2024).

Danaea acuminata Tuomisto & R.C.Moran, Brazil, Amazonas, *Tuomisto, H. 16149* (SP, TUR), *SRR29519718, *PQ631653 (ML), OR541134, OR541674, OR550920, OR550677; ***D. alba*** Keskiniva & Tuomisto, Panama, Panama, *Jones, M. 994* (TUR, US), –, *PQ631654, *PQ631655, *PQ631656 (SL), OR541139, OR541678, OR550926, OR550682; ***D. alba* × *D. panamensis***, Panama, Panama, *Jones, M. 742* (TUR), –, *PQ631657, *PQ631658, *PQ631659 (SL), OR541302, OR541851, OR551147, OR550869; ***D. ampla*** Keskiniva & Tuomisto, Panama, Panama, *Tuomisto, H. 15151* (PMA, TUR, UC), –, *PQ631660, *PQ631661 (SL), OR541141, OR541680, OR550928, OR550684; ***D. bipinnata*** Tuomisto, Brazil, Amazonas, *Tuomisto, H. 15850* (SP, TUR), *SRR29519715, *PQ631664 (ML), OR541168, OR541705, OR550963, OR550715; ***D. bipinnata*** Tuomisto, Peru, Loreto, *Tuomisto, H. 11030* (AMAZ, TUR, USM), –, *PQ631662, *PQ631663 (SL), OR541167, OR541704, OR550961, OR550714; ***D. bipinnata* × *D. leprieurii***, Peru, Loreto, *Tuomisto, H. 14481* (AMAZ, TUR, USM), –, *PQ631665 (SL), OR541311, OR541860, OR551158, OR550880; ***D. cartilaginea*** Christenh. & Tuomisto, Peru, Loreto, *Tuomisto, H. 14798* (AAU, AMAZ, NY, TUR, UC, USM), *SRR29519724, *PQ631667 (ML), OR541174, OR541712, OR550971, OR550722; ***D. cuspidopsis*** Keskiniva & Tuomisto, Colombia, Antioquia, *Sundue, M. 4827* (VT), *SRR29519707, *PQ631668 (ML), *PQ522164, *PQ522171, –, *PQ564635; ***D. cuspidopsis*** Keskiniva & Tuomisto, Panama, Panama, *Testo, W. 994* (VT), *SRR29519716, *PQ631669 (ML), *PQ522165, *PQ522172, –, –; ***D. ×deltoidea*** Keskiniva & Tuomisto, Costa Rica, Heredia, *Tuomisto, H. 13811* (CUZ, TUR, USM), –, *PQ631702, *PQ631703, *PQ631704 (SL), –, OR541859, OR551156, OR550878; ***D. geniculata*** Raddi, Brazil, Sao Paulo, *Lehtonen, S. 647* (SP, TUR), *SRR29519711, *PQ631670 (ML), *PQ522166, *PQ522173, *PQ540715, *PQ564636; ***D. grandifolia*** Underw., Puerto Rico, Adjuntas, *Fawcett, S. 475* (VT), SAMN14888190, *PQ631671 (ML), *PQ522167, *PQ522174, *PQ540716, *PQ564637; ***D. grandifolia*** Underw., Colombia, Valle del Cauca, *Kessler, M. 14821* (TUR), –, *PQ631672 (SL), OR541215, OR541758, OR551029, OR550771; ***D. kalevala*** Christenh., Martinique, *Christenhusz, M.J.M. 2696* (NY, TUR), –, *PQ631673, *PQ631674 (SL), EU221708, EU221770, OR551041, EU221838; ***D. lanceolata*** Tuomisto & Keskiniva, Ecuador, Sucumbios, *Øllgaard, B. 99662* (AAU, NY, QCA, TUR, UC), *SRR29519725, *PQ631675 (ML), *PQ522168, *PQ522175, –, *PQ564638; ***D. lanceolata*** Tuomisto & Keskiniva, Peru, Loreto, *Tuomisto, H. 14712* (AMAZ, TUR, UC, USM), *SRR29519713, *PQ631676 (ML), OR541229, OR541775, OR551050, OR550787; ***D. leprieurii*** Kunze, Brazil, Amazonas, *Tuomisto, H. 15851* (INPA, SP, TUR), *SRR29519709, *PQ631679 (ML), OR541237, OR541783, OR551061, OR550797; ***D. leprieurii*** Kunze, Peru, Loreto, *Tuomisto, H. 14482* (AMAZ, TUR, USM), –, *PQ631677, *PQ631678 (SL), OR541233, OR541779, OR551057, OR550793; ***D. leussinkiana*** Christenh., Costa Rica, *Jones, M. 626* (TUR), *SRR29519706, *PQ631682 (ML), OR541244, OR541790, OR551069, OR550804; ***D. leussinkiana*** Christenh., Costa Rica, Heredia, *Jones, M. 604* (TUR), –, * PQ631680, *PQ631681 (SL), OR541243, OR541789, OR551068, OR550803; ***D. longicaudata*** Tuomisto, Ecuador, Pichincha, *Moran, R.C. 6954* (NY), –, *PQ631683 (SL), EU221711, EU221773, OR551071, EU221842; ***D. nigrescens*** Jenman, French Guiana, *Lehtonen, S. 1101* (CAY, TUR), *SRR29519714, *PQ631685 (ML), OR541251, OR541798, OR551082, OR550813; ***D. nigrescens*** Jenman, French Guiana, *Christenhusz, M.J.M. 2266* (CAY, TUR), –, *PQ631684 (SL), –, –, *PQ540717, *PQ564639; ***D. nodosa*** (L.) Sm., Mexico, Oaxaca, *Tuomisto, H. 17505* (TUR, XAL), –, *PQ631686 (SL), OR541273, OR541819, OR551105, OR550835; ***D. panamensis*** Keskiniva & Tuomisto, Panama, Panama, *Tuomisto, H. 15161* (AAU, PMA, TUR, UC, Z), *SRR29519720, *PQ631687 (SL), OR541291, OR541837, OR551123, OR550853; ***D. pterorachis*** Christ, Costa Rica, Heredia, *Jones, M. 289* (CR, TUR), –, *PQ631688 (SL), EU221713, EU221775, OR551128, EU221844; ***D. sellowiana*** C.Presl, Brazil, Santa Catarina, *Christenhusz, M.J.M. 4727* (TUR), –, *PQ631689, *PQ631690 (SL), OR541294, OR541843, OR551134, OR550861; ***D. simplicifolia*** Rudge, French Guiana, *Lehtonen, S. 805* (CAY, TUR), *SRR29519710, *PQ631691 (ML), *PQ522169, *PQ522176, *PQ540718, *PQ564640; ***D. simplicifolia*** Rudge, French Guiana, *Lehtonen, S. 982* (CAY, TUR), –, *PQ631692 (SL), –, –, –, –; ***D. stricta*** Tuomisto & Keskiniva, Panama, Panama, *Tuomisto, H. 15166* (PMA, TUR), *SRR29519717, *PQ631693 (ML), OR541313, OR541862, OR551161, OR550883; ***D. tenuicaulis*** Tuomisto & Keskiniva, Colombia, Narino, *Testo, W. 1440* (VT), *SRR29519723, *PQ631694 (ML), *PQ522170, *PQ522177, –, *PQ564641; ***D. trifoliata*** Rchb. in Kunze, Brazil, Amazonas, *Tuomisto, H. 15440* (SP, TUR), *SRR29519708, *PQ631695, *PQ631696 (ML), OR541319, OR541868, OR551168, OR550889; ***D. ulei*** Christ, Peru, Loreto, *Tuomisto, H. 14997* (AMAZ, TUR, USM), *SRR29519722, *PQ631697 (ML), OR541332, OR541881, OR551181, OR550902; ***D. ×ushana*** Christenh., French Guiana, *Lehtonen, S. 1112* (CAY, TUR), *SRR29519705, *PQ631706, *PQ631707 (SL), OR541335, OR541884, OR551184, OR550905; ***D. wendlandii*** Rchb.f., Costa Rica, Heredia, *Testo, W. 784* (VT), *SRR29519712, *PQ631700, *PQ631701 (ML), –, *PQ522178, *PQ540719, *PQ564642; ***D. wendlandii*** Rchb.f., Costa Rica, Heredia, *Jones, M. 24* (TUR), –, *PQ631698, *PQ631699 (SL), EU221733, EU221800, OR551191, EU221870; ***D. xenium*** Christenh. & Tuomisto, Peru, Loreto, *Higgins, M. 1198* (AMAZ, TUR), *SRR29519721, *PQ631705 (ML), OR541340, OR541890, OR551192, OR550912; ***D. zamiopsis*** Christenh. & Tuomisto, Panama, Panama, *Tuomisto, H. 15163* (AAU, BM, MO, NY, PMA, TUR, UC, Z), *SRR29519719, *PQ631708 (ML), OR541343, OR541893, OR551195, OR550915.