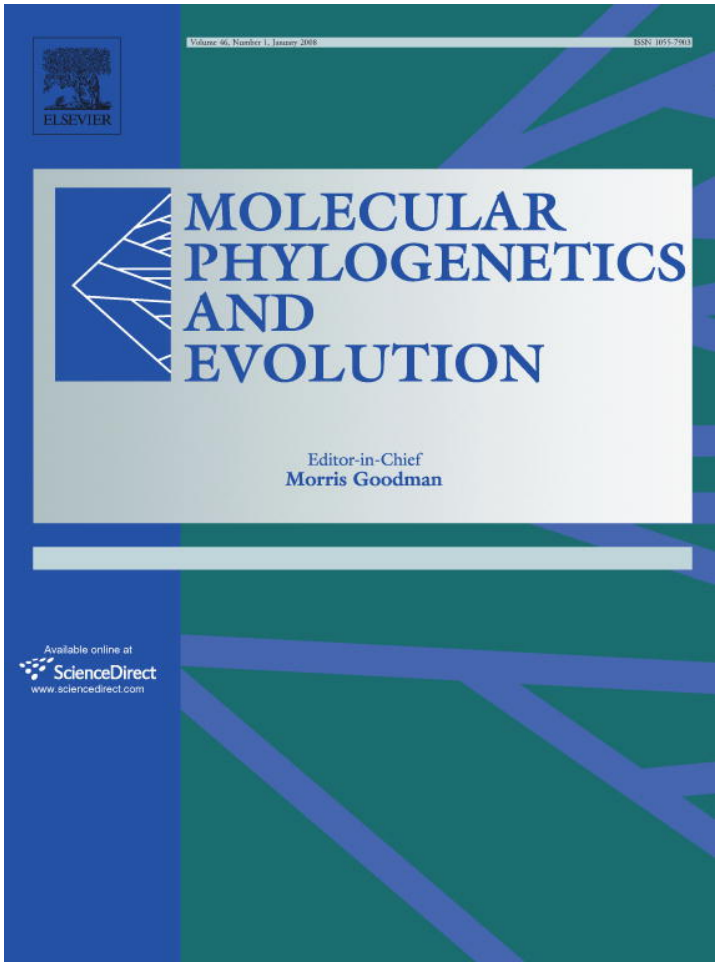

Available online at www.sciencedirect.com
ScienceDirect
Molecular Phylogenetics and Evolution 46 (2008) 34–48
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Evolutionary relationships within the Neotropical, eusporangiate fern genus *Danaea* (Marattiaceae)

M.J.M. Christenhusz [a], H. Tuomisto [a,*], J.S. Metzgar [b], K.M. Pryer [b]

[a] *Department of Biology, University of Turku, FI-20014 Turku, Finland*
[b] *Department of Biology, Duke University, Durham, NC 27708, USA*

Received 1 December 2006; revised 17 August 2007; accepted 17 September 2007
Available online 29 September 2007

Abstract

Genera within the eusporangiate fern family Marattiaceae have long been neglected in taxonomic and systematic studies. Here we present the first phylogenetic hypothesis of relationships within the exclusively Neotropical genus *Danaea* based on a sampling of 60 specimens representing 31 species from various Neotropical sites. We used DNA sequence data from three plastid regions (*atpB*, *rbcL*, and *trnL-F*), morphological characters from both herbarium specimens and live plants observed in the field, and geographical and ecological information to examine evolutionary patterns. Eleven representatives of five other marattioid genera (*Angiopteris*, *Archangiopteris*, *Christensenia*, *Macroglossum*, and *Marattia*) were used to root the topology. We identified three well-supported clades within *Danaea* that are consistent with morphological characters: the "*leprieurii*" clade (containing species traditionally associated with the name *D. elliptica*), the "*nodosa*" clade (containing all species traditionally associated with the name *D. nodosa*), and the "*alata*" clade (containing all other species). All three clades are geographically and ecologically widely distributed, but subclades within them show various distribution patterns. Our phylogenetic hypothesis provides a robust framework within which broad questions related to the morphology, taxonomy, biogeography, evolution, and ecology of these ferns can be addressed.

Keywords: *AtpB*; *Danaea*; Edaphic specialization; Ferns; Marattiaceae; Molecular phylogeny; Neotropics; *rbcL*; Speciation; Systematics; *trnL-F*

1. Introduction

Recent evidence (Pryer et al., 2001a, 2004; Renzaglia et al., 2000; Schuettpelz et al., 2006) indicates there are five major extant lineages of ferns, now recognized in four classes (sensu Smith et al., 2006): whisk ferns and ophioglossoid ferns (Psilotopsida), horsetails (Equisetopsida), marattioid ferns (Marattiopsida), and leptosporangiate ferns (Polypodiopsida). The closest living relatives to leptosporangiate ferns are the marattioid ferns, together with horsetails (Pryer et al., 2001a, 2004; Wikström and Pryer, 2005). These two eusporangiate fern lineages have excellent fossil records extending into the Carboniferous (Hill and Camus, 1986; Liu et al., 2000), and they live on today, although represented by much fewer species than the leptosporangiate ferns.

The Marattiopsida consists of two families: the Asterothecaceae, which are extinct, and the Marattiaceae, which have both fossil and extant members (Sporne, 1962). Extant Marattiaceae comprise approximately 200 species in six genera, with a center of diversity in the Asian tropics. Three genera (*Archangiopteris*, *Christensenia*, *and Macroglossum*) are restricted to that area, one (*Angiopteris*) extends to Australia, Japan, Madagascar, and Polynesia (and is naturalized from cultivation in Hawai'i, Jamaica, and Costa Rica), and one (*Marattia*) is pantropical. *Danaea*, the focus of this study, is the only marattioid genus restricted to the Neotropics. *Danaea* is represented throughout the humid areas of tropical America, from southern Mexico to Southern Brazil, on the Antilles and Cocos Island (Fig. 1).

* Corresponding author. Fax: +358 2 333 5730.
E-mail address: hanna.tuomisto@utu.fi (H. Tuomisto).

1055-7903/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2007.09.015

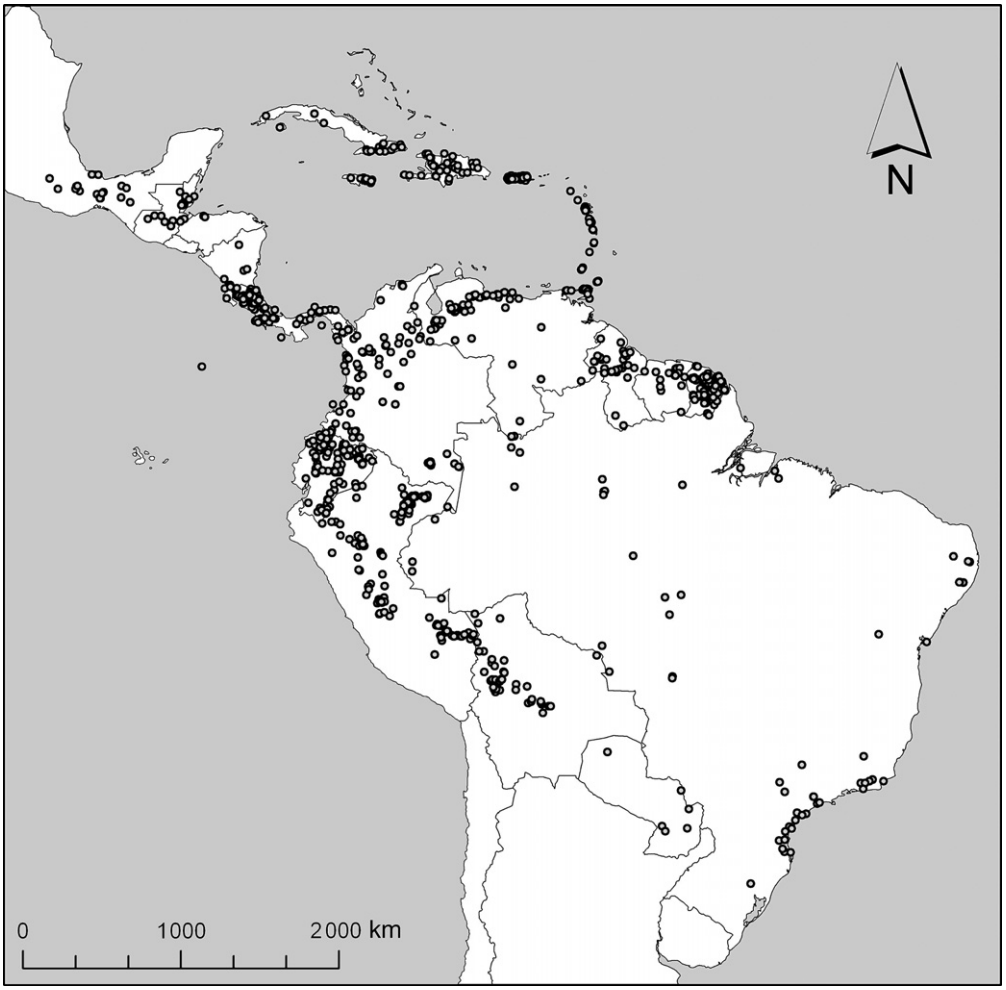

Fig. 1. Distribution of *Danaea* based on herbarium specimens (see Section 2 for source herbaria). Collection localities were plotted on the Digital Basemap of the Americas (Bletter et al., 2003).

All marattioid genera possess a combination of distinctive morphological characters that together serve to distinguish Marattiaceae from all other ferns. These include a complex polycyclic stelar structure (Brebner, 1901), rhizomes with fleshy or papery stipule-like outgrowths on each side of the petioles, presence of swollen nodes on rachises and often on petioles, and eusporangia. In addition, most genera have free venation (*Christensenia* has reticulate venation, Fig. 2b), and three of the genera (*Christensenia*, *Danaea*, and *Marattia*) have their sporangia grouped into synangia (Fig. 2b–e, j, and l).

The degree of leaf dissection is a straightforward visual character that helps to distinguish among marattioid genera (Sporne, 1962). *Danaea* is unique in that most species have once-pinnate leaves with opposite pinnae (Fig. 3); some species have simple leaves (Fig. 3e and k), and a few are bipinnate, often irregularly so (Fig. 3g and h). *Archangiopteris*, *Macroglossum*, and *Marattia rolandi-principis* Rosenstock also have once-pinnate leaves, but their pinnae are always alternately arranged. The leaves in *Angiopteris* are typically bipinnate, and those of most *Marattia* are bipinnate or more complex (in some species up to four times pinnate). *Christensenia* is unusual in having palmately compound leaves, reticulate venation, and radially symmetrical synangia, and has therefore at times been placed in its own family, the Christenseniaceae (Ching, 1940).

The first attempt to investigate evolutionary trends in Marattiales was published by Stidd (1974), who compared stelar structures among various fossil and extant genera. Hill and Camus (1986) examined generic relationships using a cladistic analysis of morphological characters, and proposed a new classification for Marattiales. In that study, they hypothesized that *Christensenia* was sister to a clade comprising *Danaea*, *Marattia*, and *Angiopteris*. Later phylogenetic studies based on DNA sequence data, which included representatives from Marattiaceae, consistently showed *Danaea* as sister to a clade uniting *Marattia* and *Angiopteris* (Hasebe et al., 1995; Pryer et al., 1995, 2001b, 2004).

Danaea has received scant systematic attention. Presl (1845) divided *Danaea* into three sections, but subsequent authors rarely accepted these. In their monograph on Marattiaceae, Vriese and Harting (1853) excluded *Danaea*

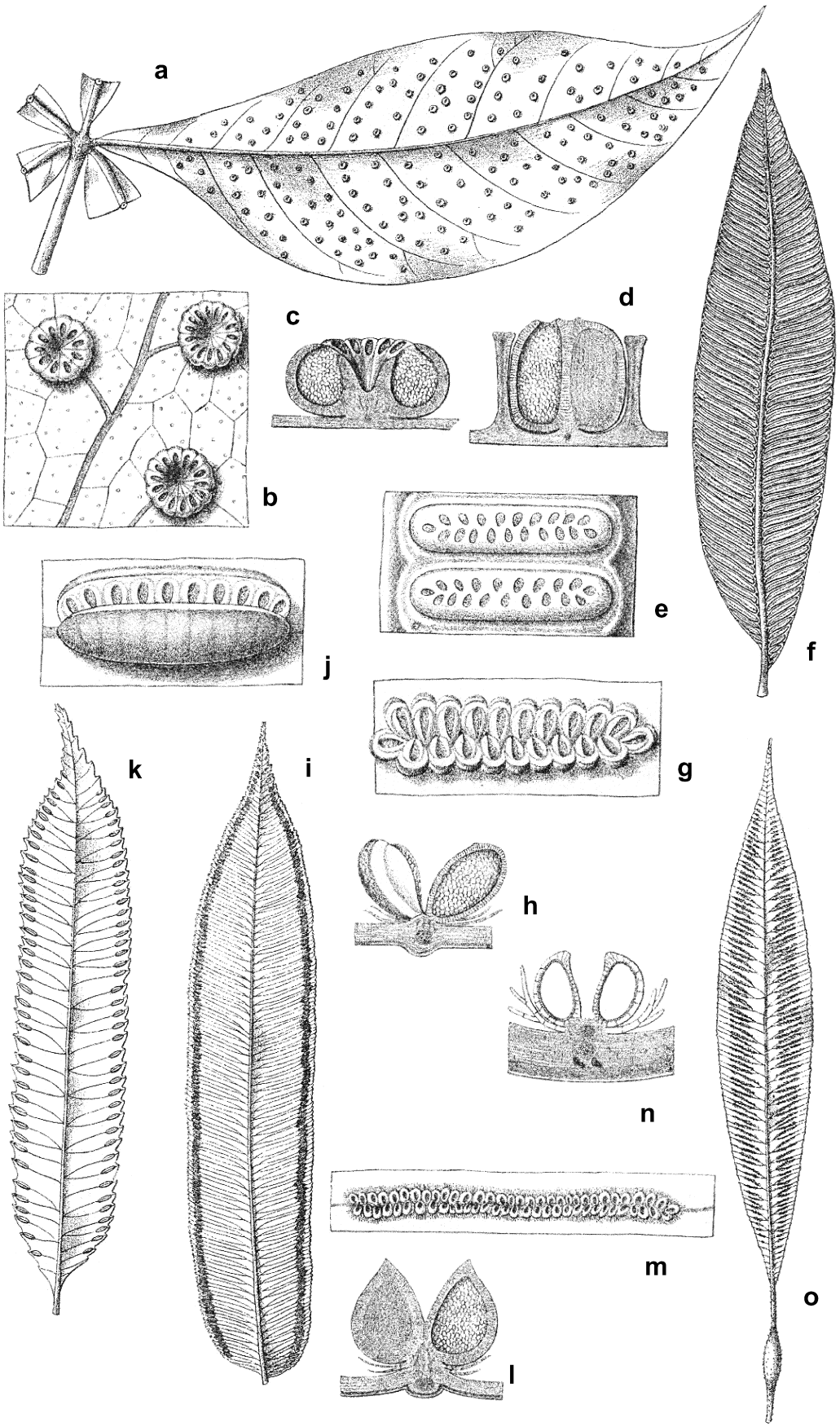

Fig. 2. Examples of fertile lateral pinnae (a, f, and o) or pinnules (i and k), sori (g, h, m, and n), and synangia (b–e, j, and l) in extant genera of Marattiaceae. (a–c) *Christensenia*; (d–f) *Danaea*; (g–i) *Angiopteris*; (j–l) *Marattia*; (m–o) *Archangiopteris* (reproduced from Bittner, 1902).

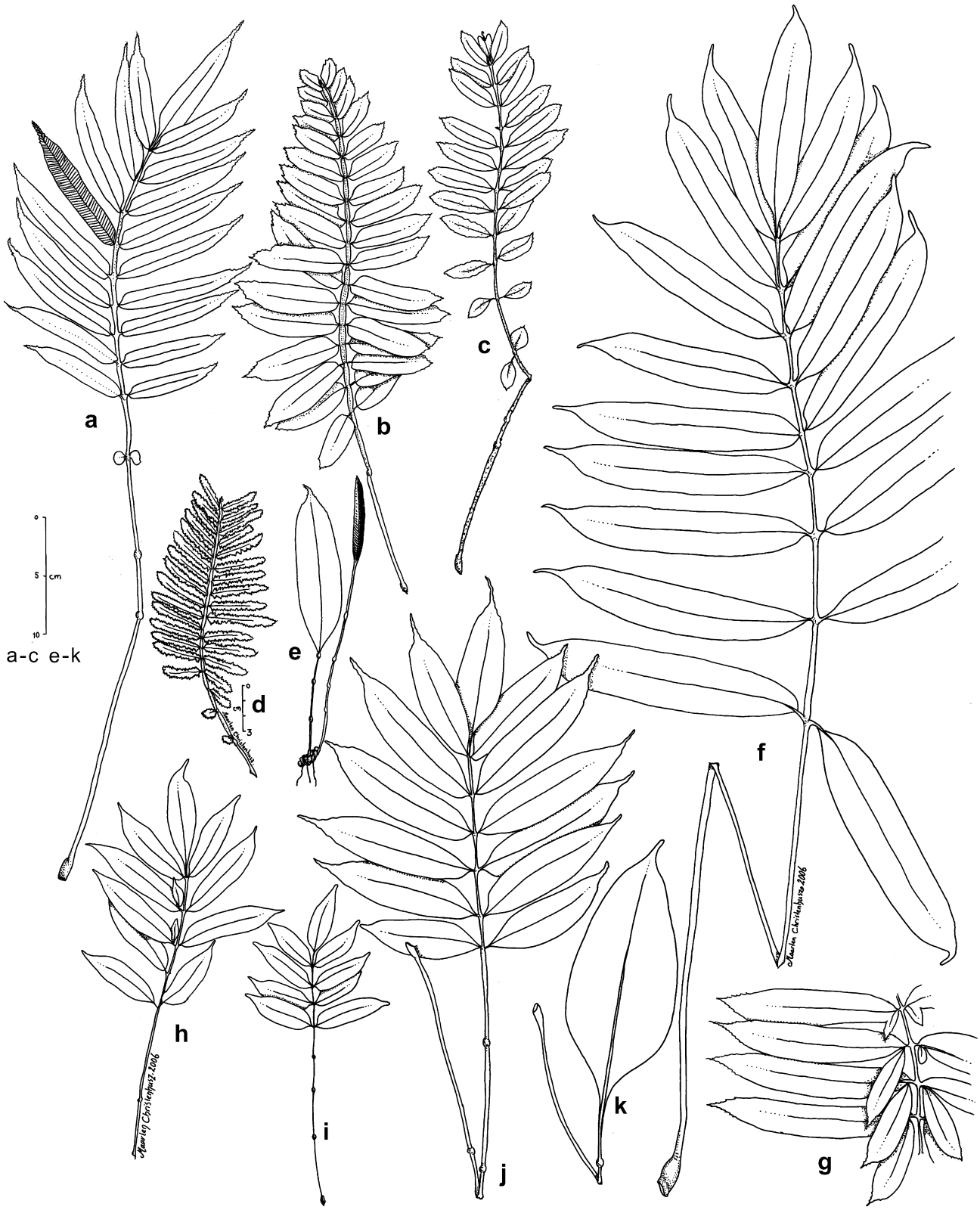

Fig. 3. Examples of leaf shape in selected *Danaea* species. (a–e) "*alata*" clade; (f–g) "*nodosa*" clade; (h–k) "*leprieurii*" clade. (a) *D. alata*, Martinique, *Christenhusz 2711* (TUR); (b) *D. oblanceolata*, Peru, *Tuomisto 5715* (TUR); (c) *D. jenmanii*, Jamaica, *Christenhusz 2990* (TUR); (d) *D. crispa*, Panama, *Folsom 1889* (PMA); (e) *D. carillensis*, Costa Rica, *Brade 30* (US); (f) *D. grandifolia*, Puerto Rico, *Christenhusz 3505* (TUR); (g) *D. nodosa*, Jamaica, lateral pinnae of bipinnate leaf, *Christenhusz 3194* (TUR); (h) *D. bipinnata*, Ecuador, *Tuomisto 11650* (TUR); (i) *D. leprieurii*, French Guiana, *Christenhusz 2427* (TUR); (j) *D. antillensis*, Guadeloupe, *Christenhusz 2730* (TUR); (k) *D. simplicifolia*, French Guiana, *Christenhusz 2275* (TUR).

because they, as did many taxonomists at the time, placed the genus in its own family, the Danaeaceae (Agardh, 1822). Underwood (1902) review of *Danaea* excluded most of the South American species, and a later version of his work (Underwood, 1909) explicitly concentrated on the North American species. Since then, many floristic treatments have commented on the need for a critical review of the genus (e.g., Morton, 1951; Kramer, 1978; Camus, 1995).

Altogether, 64 *Danaea* species have been described to date, but because their relationships have been little studied, the extent of taxonomic synonymy has not been completely resolved yet. Recent floristic studies have resulted in considerable taxonomic flux in the genus. Tuomisto et al. (2001) recognized 18 species in Ecuador, eight of which were newly described, and Christenhusz (2006) and Christenhusz and Tuomisto (2006) described another eight new species from Peru, the Lesser Antilles, and French Guiana. However, in a recent revision, Rolleri (2004) opted for a much broader species concept and recognized only 17 species in the entire genus. After extensive herbarium work and study of original type specimens of almost all published species, we currently estimate that the genus consists of approximately 50 species. However, this number is subject to change because some species complexes are still unresolved.

Danaea is generally confined to moist, shaded habitats in lowland and montane tropical rain forests, cloud forests, and elfin woodlands. Recent ecological studies in Amazonia have revealed that some *Danaea* species have a relatively narrow ecological range, such that different species are found on different soil types (Ruokolainen and Tuomisto, 1998; Tuomisto and Poulsen, 1996). In combination with other ferns, *Danaea* species have been used as indicators of different forest types (Salovaara et al., 2004; Tuomisto et al., 2003), but several taxonomic and nomenclatural problems in *Danaea* need to be resolved before this can become common practice.

Species circumscription based only on morphological characters can be quite tricky in *Danaea*, because many of the characters are quantitative rather than qualitative, and can vary even within species. Furthermore, important characters that facilitate species identification in the field (e.g., rhizome habit; posture, color, and texture of leaves) are not well preserved on dried specimens, which complicates herbarium studies. Herbarium specimens of the larger species (adult *Danaea* leaves range from 10 to 300 cm long) often consist only of leaf fragments. Few specimens include a preserved rhizome or an adequate description of it.

In this paper, we draw on recent field studies by two of us (HT and MC) that have provided new ecological and morphological information, as well as freshly collected silica-dried leaf material for DNA studies. We use DNA sequence data from three plastid markers to examine species relationships within *Danaea*. Our results are used to make evolutionary inferences in light of what we know about the morphology, ecology, and biogeography of these ferns.

2. Materials and methods

2.1. Taxon sampling

Herbarium specimens of *Danaea* were examined from A, AAU, AMAZ, B, BBS, BM, BR, C, CAY, COAH, CUZ, DUKE, E, F, FBG, FI, G, GB, GH, GOET, GUAD, H, IJ, K, KSP, L, LZ, M, MAPR, MICH, MO, NY, P, PI, PR, PRC, QCA, QCNE, S, SJ, SP, TUB, TUR, U, UC, UCWI, UPR, UPRRP, UPS, US, USM, W, WU, YU, and Z (herbarium acronyms follow Holmgren and Holmgren, 1998-present). Field observations on morphology and ecology of the species were made in Colombia, Costa Rica, Ecuador, French Guiana, Guadeloupe, Jamaica, Peru, Puerto Rico, and Suriname.

We included 31 different species of *Danaea* in our DNA analyses, of which 26 had been studied by us in the field. When possible, multiple DNA accessions were sampled across the geographic range of a species (Table 1). Our species concept is based on morphological discontinuities between species, and the application of species names is based on comparison of our material with the original type specimens. Three taxa that we identified as morphologically distinct, but are as yet unnamed, are here referred to as *Danaea* sp. A, sp. B, and sp. C. Our outgroup sampling includes nine species, with at least one representative from each of the other five genera of Marattiaceae. Table 1 lists all species sampled for DNA and includes voucher information, GenBank numbers, and Fern DNA database numbers for each accession.

2.2. DNA isolation, amplification, and sequencing

Genomic DNA was extracted from silica-dried leaf material using a DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA). For each taxon, three plastid regions (*atpB*, *rbcL*, and *trnL-F*) were amplified separately using the polymerase chain reaction (PCR), following established protocols (Pryer et al., 2001b). Amplicons were cleaned using either QIAquick columns (Qiagen) or Montage columns (Millipore, Billerica, MA, USA), according to the manufacturer's protocol. To ensure the internal integrity of sequences, sequencing reactions were carried out for both strands of the purified PCR products (to obtain both forward and backward sequencing) using Big Dye Terminator Cycle Sequencing reagents (Applied Biosystems, Foster City, California, USA). Amplification and sequencing primer information is provided in Table 2. Sequences were processed using ABI 3700 and ABI 3730XL automated sequencers (Applied Biosystems), and all sequencing reads were evaluated for possible contamination using the NCBI nucleotide–nucleotide BLAST (blastn) tool (Altschul et al., 1997). Except for two *Danaea* sequences (*Sharpe s.n.*, UC, as '*D. elliptica*'), published in earlier studies (Pryer et al., 2001a; Des Marais et al., 2003; Pryer et al., 2004), all 200 sequences newly reported here were generated specifically for this study (Table 1).

Table 1
Specimens included in this study; species names, voucher information, geographical origin, Genbank accession numbers, and Fern DNA database numbers[a] are listed

Species	Voucher (Herbarium)	Source	GenBank Accession Nos. [a]			Fern DNA Database No.[b]
			atpB	*rbcL*	*trnL-F*	
Angiopteris angustifolia C. Presl	Hortus Botanicus Leiden, acc. nr. 8088 (TUR)	Cultivated	EU221678	EU221738	EU221806	2574
A. evecta (G. Forst.) Hoffm.	*Christenhusz 2992* (IJ, TUR)	Jamaica	EF463485	EU221739	EU221807	2569
A. evecta	Hortus Botanicus Leiden, acc. nr. 960127 (TUR)	Malaysia	EU221679	EU221740	EU221808	2575
A. sp.	Botanical Gardens Utrecht (TUR)	Philippines	EU221680	EU221741	EU221809	2576
Archangiopteris itoi Shieh	*Walker* 356 (UC)	Taiwan	EU221681	EU221742	EU221810	2832
Christensenia aesculifolia (Blume) Maxon	*Walker* 354 (UC)	Malaysia	EU221682	EU221743	EU221811	2833
Danaea acuminata Tuomisto & R.C. Moran	*H. Tuomisto 10507* (AAU, F, K, MO, NY, QCA, QCNE, TUR, U, UC, US); Type	Ecuador	EU221683	EU221744	EU221812	2627
D. alata Sm.	*M. Christenhusz 2711* (TUR)	Martinique	EU221684	EU221745	EU221813	2568
D. alata	*M. Kessler 12908* (GOET, TUR)	Tobago	EU221685	EU221746	EU221814	2563
D. antillensis Christenh.	*M. Christenhusz 2730* (TUR); Paratype	Guadeloupe	EU221686	EU221747	EU221815	2656
D. antillensis	*M. Christenhusz 2747* (BM, P, TUR, UC); Type	Guadeloupe	EU221687	EU221748	EU221816	2628
D. arbuscula Christenh. & Tuomisto	*M. Christenhusz 2074* (AAU, AMAZ, B, BM, K, L, NY, TUR, P, UC, USM); Type	Peru	EU221688	EU221749	EU221817	2640
D. arbuscula	*M. Christenhusz 2760* (TUR)	Guadeloupe	—	—	EU221818	2813
D. bipinnata Tuomisto	*H. Tuomisto 10634* (AAU, NY, QCA, QCNE, TUR); Type	Ecuador	EU221689	EU221750	EU221819	2638
D. bipinnata	*H. Tuomisto 11650* (TUR); Paratype	Ecuador	—	EU221751	EU221820	2600
D. carillensis H. Christ	*T. Lemieux 2344* (CR)	Costa Rica	EU221690	EU221752	EU221821	2616
D. cartilaginea Christenh. & Tuomisto	*H. Tuomisto 11684* (QCA, QCNE, TUR); Paratype	Ecuador	EU221691	EU221753	EU221822	2630
D. crispa Endrés in Rchb. f.	*R. C. Moran 6349* (CR, INB, NY, USJ)	Costa Rica	EU221692	EU221754	EU221823	2613
D. crispa	*A. R. Smith 2594* = P. Hammond s.n. (UC)	Costa Rica	EU221693	EU221755	EU221824	822
D. cuspidata Liebm.	*B. Boyle 5971* = *Chapotin 13* (CR, INB, USJ, NY)	Costa Rica	EU221694	EU221756	EU221825	2607
D. erecta Tuomisto & R.C. Moran	*M. Lehnert 1203* (GOET, TUR)	Ecuador	EU221695	EU221757	EU221826	2771
D. falcata Tuomisto & R.C. Moran	*H. Tuomisto 10832* (AAU, K, NY, QCA, QCNE, TUR, UC); Type	Ecuador	EU221696	EU221758	EU221827	2599
D. geniculata Raddi	*M. Christenhusz 1938* (AMAZ, TUR, USM)	Peru	EU221697	EU221759	EU221828	2629
D. geniculata	*M. Jones 100* (CR, TUR)	Costa Rica	EU221698	EU221760	EU221829	2580
D. geniculata	*M. Jones 101* (CR, TUR)	Costa Rica	EU221699	EU221761	—	2581
D. geniculata	*M. Jones 137* (CR, TUR)	Costa Rica	EU221700	EU221762	EU221830	2582
D. geniculata	*H. Tuomisto 13255* (TUR)	Peru	EU221701	EU221763	EU221831	2590
D. geniculata	*H. Tuomisto 13590* (TUR)	Peru	EU221702	EU221764	EU221832	2589
D. grandifolia Underw.	*M. Christenhusz 3505* (MAPR, TUR)	Puerto Rico	EU221703	EU221765	EU221833	2762
D. grandifolia	*M. Christenhusz 3439* (MAPR, TUR, UPRRP)	Puerto Rico	EU221704	EU221766	EU221834	2641
D. jenmanii Underw.	*M. Christenhusz 2990* (IJ, TUR)	Jamaica	EU221705	EU221767	EU221835	2643
D. jenmanii	*M. Christenhusz 3373* (IJ, TUR)	Jamaica	EU221706	EU221768	EU221836	2763
D. jenmanii	*M. Christenhusz 3514* (MAPR, TUR)	Puerto Rico	EU221707	EU221769	EU221837	2644
D. kalevala Christenh.	*M. Christenhusz 2696* (BM, P, NY, TUR, UC); Type	Martinique	EU221708	EU221770	EU221838	2567
D. leprieurii Kunze	*M. Christenhusz 2427* (CAY, TUR)	French Guiana	EU221709	EU221771	EU221839	2844
D. leprieurii	*H. Tuomisto 11397* (TUR)	Peru	EU221710	EU221772	EU221840	2766
D. leprieurii	*M. Christenhusz 2150* (AMAZ, TUR, USM)	Peru	—	—	EU221841	2593
D. longicaudata Tuomisto	*R. C. Moran 6954* (UC)	Ecuador	EU221711	EU221773	EU221842	2772
D. mazeana Underw.	*M. Christenhusz 3371* (IJ, TUR)	Jamaica	EU221712	EU221774	EU221843	2773
D. media Liebm.	*M. Jones 289* (CR, TUR)	Costa Rica	EU221713	EU221775	EU221844	2584

(*continued on next page*)

Table 1 (*continued*)

Species	Voucher (Herbarium)	Source	GenBank Accession Nos. [a]			Fern DNA Database No.[b]
			atpB	*rbcL*	*trnL-F*	
D. media	*M. Jones 169* (CR, TUR)	Costa Rica	—	EU221776	EU221845	2583
D. nodosa (L.) Sm.	*M. Christenhusz 3194* (IJ, TUR, UCWI)	Jamaica	EU221714	EU221777	EU221846	2780
D. nodosa	*M. Christenhusz 3309* (IJ, TUR)	Jamaica	—	EU221778	EU221847	2784
D. nodosa	*M. Kessler 13000* (GOET, TUR)	Bolivia	EU221715	EU221779	EU221848	2564
D. nodosa	*H. Tuomisto 11304* (TUR)	Peru	EU221716	EU221780	EU221849	2786
D. nodosa	*M. Christenhusz 2596* (BBS, TUR)	Suriname	EU221717	EU221781	EU221850	2789
D. nodosa	*I. Jimenez 1979* (GOET, TUR)	Bolivia	EU221718	EU221782	EU221851	2631
D. nodosa	*M. Christenhusz 2266* (CAY, TUR)	French Guiana	EU221719	EU221783	EU221852	2565
D. nodosa	*H. Tuomisto 13084* (TUR)	Peru	EU221720	EU221784	EU221853	2587
D. nodosa	*M. Christenhusz 1904* (TUR, USM)	Peru	EU221721	EU221785	EU221854	2573
D. nodosa	*M. Christenhusz 1949* (TUR, USM)	Peru	EU221722	EU221786	EU221855	2572
D. nodosa	*H. Tuomisto 11934* (TUR)	Ecuador	—	EU221787	EU221856	2793
D. oblanceolata Stolze	*H. Tuomisto 11915* (TUR)	Ecuador	EU221723	EU221788	EU221857	2601
D. polymorpha Baker	*M. Christenhusz 2746* (TUR)	Guadeloupe	EU221724	EU221789	EU221858	2595
D. simplicifolia Rudge	*M. Christenhusz 2275* (CAY, TUR)	French Guiana	EU221725	EU221790	EU221859	2802
D. simplicifolia	*M. Christenhusz 2415* (CAY, TUR)	French Guiana	EU221726	EU221791	EU221860	2566
D. simplicifolia	*M. Christenhusz 2428* (CAY, TUR)	French Guiana	EU221727	EU221792	EU221861	2594
D. sp. A	*M. Jones 542* (CR, TUR)	Costa Rica	—	EU221793	EU221862	2636
D. sp. B	*M. Christenhusz 2107* (AMAZ, TUR, USM)	Peru	EU221728	EU221794	EU221863	2571
D. sp. B	*J. Sharpe s.n.* (UC)	Puerto Rico	AF313540	AF313578	EU221864	451
D. sp. C	*M. Christenhusz 2339* (CAY, TUR)	French Guiana	EU221729	EU221795	EU221865	2775
D. trichomanoides Spruce ex T. Moore	*M. Lehnert 1542* (GOET, TUR)	Ecuador	EU221730	EU221796	EU221866	3075
D. trifoliata Rchb. in Kunze	*M. Christenhusz 2606* (BBS, TUR)	Suriname	EU221731	EU221797	EU221867	2809
D. urbanii Maxon	*M. Christenhusz 3506* (TUR)	Puerto Rico	EU221732	EU221798	EU221868	2705
D. vivax Christenh. & Tuomisto	*M. Christenhusz 2002* (AAU, AMAZ, B, BM, GOET, L, P, NY, S, TUR, U, UC, US, USM); Type	Peru	—	EU221799	EU221869	2810
D. wendlandii Rchb. f.	*M. Jones 24* (CR, TUR)	Costa Rica	EU221733	EU221800	EU221870	2578
Macroglossum smithii (Racib.) Campbell	*R. Whitehead 338* (UC)	Malaysia	EU221734	EU221801	EU221871	2834
Marattia alata Sw.	*M. Christenhusz 3266* (IJ, TUR)	Jamaica	EF463486	EU221802	EU221872	2570
M. laxa Kunze	*A. R. Smith 2566* (UC)	Mexico	EU221735	EU221803	EU221873	459
M. laxa	*M. Christenhusz 1313* (TUR)	Mexico	EU221736	EU221804	EU221874	2577
M. weinmanniifolia Liebm.	*A. R. Smith 2567* (UC)	Mexico	EU221737	EU221805	EU221875	461

[a] — = Data not available for this voucher.

[b] Permanent record numbers in http://www.pryerlab.net/DNA_database.shtml.

Table 2
Primers used to amplify and sequence *atpB*, *rbcL*, and *trnL-F*, and appropriate references

Primer name	Primer sequence (5′ → 3′)	Amp (A)/Seq (S)	Reference
atpB			
ATPB1419F	CRACATTTGCACATYTRGATGCTAC	S	Wolf (1997)
ATPB672F	TTGATACGGGAGCYCCTCTWAGTGT	A/S	Wolf (1997)
ATPB910R	TTCCTGYARAGANCCCATTTCTGT	S	Pryer et al. (2004)
ATPE384R	GAATTCCAAACTATTCGATTAGG	A/S	Pryer et al. (2004)
ESATPB274F	ACGGGAGCTCCTCTWAGTGTTCC	A/S	Schuettpelz[a]
ESATPE45R	ATTCCAAACWATTCGATTWGGAG	A/S	Nagalingum et al. (2007)
ESRBCL26R	GCTTTAGTCTCCGTTTGTGGTGACAT	A	Korall et al. (2007)
rbcL			
1379R	TCACAAGCAGCAGCTAGTTCAGGACTC	A/S	Pryer et al. (2001b)
AF	ATGTCACCACAAACAGAGACTAAAGC	A/S	Hasebe et al. (1994)
ESRBCL1361R	TCAGGACTCCACTTACTAGCTTCACG	A	Korall et al. (2006)
ESRBCL1F	ATGTCACCACAAACGGAGACTAAAGC	A	Korall et al. (2006)
ESRBCL645F	AGAYCGTTTCYTATTYGTAGCAGAAGC	S	Korall et al. (2006)
ESRBCL663R	TACRAATARGAAACGRTCTCTCCAACG	S	Korall et al. (2006)
JYDS5	CTCTCTATCAATAACAGCATGCAT	S	Pryer et al. (2001b)
trnL-F			
TRNFF	ATTTGAACTGGTGACACGAG	A/S	Taberlet et al. (1991)
TRNLC	CGAAATCGGTAGACGCTACG	A/S	Taberlet et al. (1991)
TRNLD	GGGGATAGAGGGACTTGAAC	S	Taberlet et al. (1991)
TRNLE	GGTTCAAGTCCCTCTATCCC	S	Taberlet et al. (1991)

[a] Primer designed by Eric Schuettpelz (Duke University), published here with permission.

2.3. Sequence alignment

Sequence fragments obtained as chromatograms were edited and assembled into contiguous alignments using Sequencher (Gene Codes, Ann Arbor, Michigan, USA). For each of the three amplified regions, the resulting consensus sequences for each taxon were aligned manually using MacClade version 4.05 (Maddison and Maddison, 2005). The alignments for *atpB* and *rbcL* were straightforward because no insertions or deletions were present. Although insertions and deletions were present in the *trnL-F* alignment, no ambiguously aligned regions were identified and gaps were coded as missing data. Alignments are deposited in TreeBASE (http://www.treebase.org/treebase/), ID number SN3659-16603.

2.4. Data set combinability assessment and phylogenetic analyses

Each plastid region was analyzed independently with PAUP* version 4.0b10 (Swofford, 2002) using an equally weighted maximum parsimony bootstrap approach (Felsenstein, 1985) to assess clade support. For *rbcL* and *trnL-F*, the bootstrap analysis consisted of 1000 replicates each with 10 random-addition-sequence replicates and tree bisection and reconnection (TBR) branch swapping. For *atpB*, the bootstrap analysis consisted of 200 replicates, each with five random-addition-sequence replicates and TBR branch swapping, to limit the search time. To assess the compatibility of the results from the three plastid regions, a procedure was invoked in which topological conflict among trees resulting from the bootstrap analyses of the individual data sets was examined (Mason-Gamer and Kellogg, 1996). Using a significance threshold of 70%, the bootstrap consensus trees were compared visually for conflict. No topological conflict among well-supported nodes was detected among data sets using this method. Therefore, the single-partition data sets of *trnL-F*, *rbcL*, and *atpB* were combined into a single alignment and analyzed simultaneously.

The combined data set was analyzed using a Bayesian/Markov Chain Monte Carlo (B/MCMC) approach, using MrBayes version 3.1 (Huelsenbeck and Ronquist, 2001), with each plastid region having its own model of sequence evolution ($GTR + I + G$ for *atpB* and *trnL-F*; $HKY + I + G$ for *rbcL*) as determined using a hierarchical likelihood ratio test in Modeltest version 3.1 (Posada and Crandall, 1998). Four independent B/MCMC analyses were conducted, each with data partitioned by locus, flat priors, and four chains. The chains were run for 10 million generations apiece and were sampled every 1000 generations. Following completion, the sampled trees from each analysis were plotted against their likelihood to identify the point where the likelihoods converged on a maximum value. All trees prior to this convergence (2500 trees representing 2,500,000 generations for each of the four analyses) were discarded as the "burn-in"-phase. Because all four analyses converged on the same maximum, the post-"burn-in" trees (30,000 total trees) from each analysis were pooled, and a majority-rule consensus was calculated to obtain a topology with average branch lengths (Fig. 4), as well as posterior probabilities for all resolved nodes.

The combined data set was also analyzed using maximum parsimony (MP) and maximum likelihood (ML). The MP heuristic analysis was run for 1000 random-addition-sequence replicates with TBR branch swapping, followed by 500 bootstrap replicates, each with five

random-addition-sequence replicates. The ML heuristic analysis was run for 500 random-addition-sequence replicates with TBR branch swapping. To shorten the ML bootstrap analysis, 100 separate ML bootstrap analyses were performed, each with one bootstrap replicate and one random-addition-sequence search replicate. Trees were pooled from all 100 ML bootstrap analyses to calculate the bootstrap frequencies. The ML analyses utilized the K81uf + I + G sequence evolution model and parameters, as determined by Modeltest.

3. Results

3.1. Phylogenetic analyses

The MP analysis of the combined three-locus dataset (*atpB*, *rbcL*, and *trnL-F*) resulted in 749,076 equally parsimonious trees (753 steps, CI = 0.746, RI = 0.952), with deep-level nodes that were well-resolved in the strict consensus tree (not shown). The ML analysis produced 22 most likely trees ($-\ln L = 9383.90589$), which also yielded

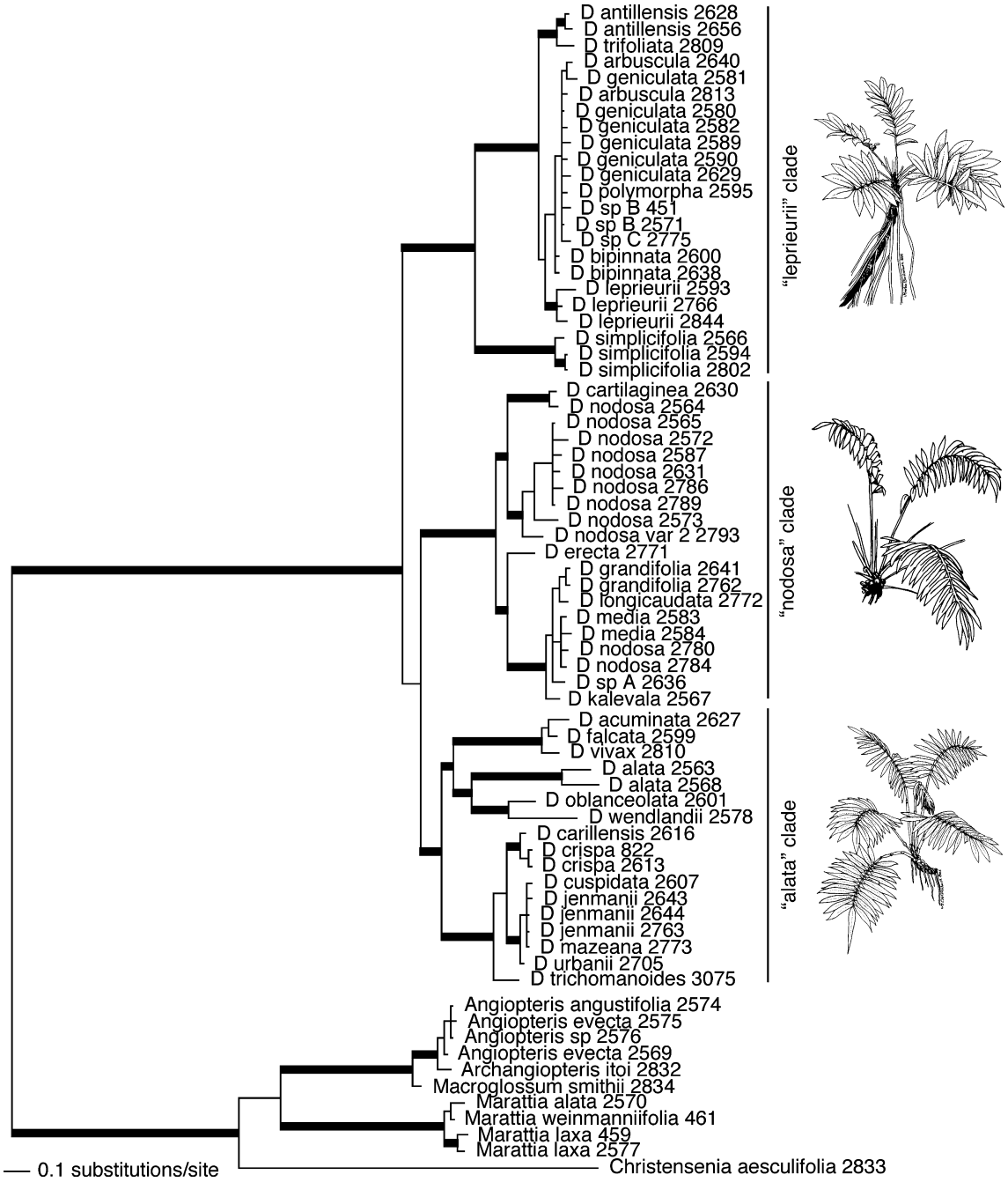

Fig. 4. Phylogenetic relationships of *Danaea* species. The topology is a majority-rule consensus tree with average branch lengths from a Bayesian analysis of three plastid regions (*atpB*, *rbcL*, and *trnL-trnF*). Branches that are heavily thickened indicate strong support (PP $\geqslant$ 95, MLBS $\geqslant$ 70, and MPBS $\geqslant$ 70). The tree was rooted with *Archangiopteris*, *Angiopteris*, *Christensenia Macroglossum*, and *Marattia*. The three main clades discussed in the text are indicated.

a strict consensus tree (not shown) with well-supported deep-level relationships. The B/MCMC analysis resulted in a majority-rule consensus tree with robust supra-specific relationships observed with all three measures of support (Bayesian PP $\geqslant$ 95, ML^{BS} and $MP^{BS} \geqslant 70$; Fig. 4). No conflicts between significantly supported nodes in the MP, ML or B/MCMC trees were found, therefore we focus here on the phylogenetic relationships from the Bayesian analysis.

The monophyly of *Danaea* is well-supported ($MP^{BS} = 100$, $ML^{BS} = 100$, and PP = 100). Within *Danaea*, three clades are consistently supported (Fig. 4): the "*leprieurii*" clade ($MP^{BS} = 100$, $ML^{BS} = 100$, and PP = 100), the "*nodosa*" clade ($MP^{BS} = 100$, $ML^{BS} = 100$, and PP = 100), and the "*alata*" clade ($MP^{BS} = 78$, $ML^{BS} = 89$, and PP = 100). Although the "*nodosa*" and "*alata*" clades are sister in the B/MCMC analysis, the support for this relationship is low ($MP^{BS} = 90$, $ML^{BS} = 80$, and PP = 85).

3.2. The "leprieurii" clade

Species in the "*leprieurii*" clade are allied to what has been traditionally called with the name *D. elliptica* Sm, and were considered conspecific by many authors. However, the type of *D. elliptica* is in fact a juvenile of *D. nodosa*, so the name *D. elliptica* is a synonym of *D. nodosa* and cannot be used (Christenhusz and Tuomisto, 2006).

All representatives of the "*leprierii*" clade are intermediate in size (leaf length 0.3–1 m). They are further characterized by erect, radially symmetric rhizomes, entire pinna margins, usually nodose petioles, absence of proliferous buds in the leaf apex, and few (usually <6, invariably <10) pairs of lateral pinnae (Fig. 5). The species in this clade are quite similar morphologically and differ mainly in pinna shape and size, rhizome height and diameter, and the number of pinnae and of nodes on the petiole.

Danaea simplicifolia (Fig. 3k) is well-supported ($MP^{BS} = 100$, $ML^{BS} = 100$, PP = 100) as sister to the rest of the "*leprieurii*" clade (Fig. 4). It is morphologically distinct from the other species in this clade in having simple leaves. Two other subclades within "*leprieurii*" receive robust support: the species pair *D. antillensis* (Fig. 3J) + *D. trifoliata*, and the species *D. leprieurii* (Fig. 3I). The resolution obtained among the other taxa in this clade is rather poor.

3.3. The "nodosa" clade

All members of the "*nodosa*" clade are large plants (leaf length 1–3 m, Fig. 3f), usually with many pairs of lateral pinnae, and generally lacking petiole nodes. Otherwise their morphology is quite variable (Fig. 5). Several authors have considered all taxa in the "*nodosa*" clade to be conspecific with the type species of the genus, *D. nodosa*, but our results suggest otherwise.

Our results split the "*nodosa*" clade into two well-supported subclades: one ($MP^{BS} = 82$, $ML^{BS} = 83$, PP = 100) includes accessions from Amazonia and the Guianas, and the other ($MP^{BS} = 86$, $ML^{BS} = 91$, PP = 100) accessions from Central America, the Pacific coast of South America, the Andes, and the Caribbean (Fig. 5). The Amazonian and Guianan material (identified here as *D. nodosa* and *D. cartilaginea*) is uniform in having creeping, dorsiventral rhizomes less than five centimeters thick, along which leaves arranged in two rows. However, *D. nodosa* and *D. cartilaginea* differ clearly in that the latter has fewer pinna pairs and larger pinnae with a remarkably thick texture and a cartilaginous margin. The extra-Amazonian material has been identified as seven different species. Five of these differ from typical *D. nodosa* in obvious rhizome characteristics (erect in *D. erecta* and *D. longicaudata*, massive and multi-rowed in *D. grandifolia*, *D. kalevala*, and *D. media*), and one species is less clearly distinct by characteristics of the pinnae (*D.* sp. A). Specimens identified as *D. nodosa* are found in both subclades of the "*nodosa*" clade, indicating that what is currently considered as one species on morphological grounds is actually polyphyletic.

3.4. The "alata" clade

Members of the "*alata*" clade are morphologically more variable than those of the other two main clades, and most of its species have been recognized by virtually all who have studied *Danaea*. Plants of the "*alata*" clade are usually small to intermediate in size (leaf length 0.1–1 m), with leaf arrangement radial or nearly so, although the rhizome may be creeping, ascending, or erect. Leaves have serrulate or erose pinna margins, nodose petioles, and often proliferous buds at the apex (Fig. 5).

The "*alata*" clade has two well-supported subclades, the *D. acuminata*/*D. wendlandii* clade ($MP^{BS} = 74$, $ML^{BS} = 76$, PP = 98) and the *D. carillensis*/*D. trichomanoides* clade ($MP^{BS} = 100$, $ML^{BS} = 100$, PP = 100; Fig. 4). The former is further divided into two well-supported subclades. One consists of three Amazonian species (*D. acuminata*, *D. falcata*, and *D. vivax*; $MP^{BS} = 100$, $ML^{BS} = 100$, PP = 100; Figs. 4 and 5) that have creeping rhizomes and falcate, sharply serrate pinnae, but that differ in leaf size and in the size, width, and number of pinnae. The other subclade comprises three species spanning different geographical ranges, including *D. alata* (Fig. 3a), *D. oblanceolata* (Fig. 3b), and *D. wendlandii*; $MP^{BS} = 84$, $ML^{BS} = 87$, PP = 100; (Fig. 5). These species are morphologically rather similar and differ mainly in the number and size of pinnae, and the presence or absence of proliferous buds. In the *D. carillensis*/*D. trichomanoides* clade, *D. carillensis* (Fig. 3e) and *D. crispa* (Fig. 3d) are sister taxa. Both are small plants from Central America, but they differ in morphology: *D. carillensis* has simple, denticulate, leathery leaves and *D. crispa* has pinnate, erose, membranaceous leaves. The core of this subclade consists of several species whose delimitations are not yet resolved, and whose phylogenetic relationships remain unclear.

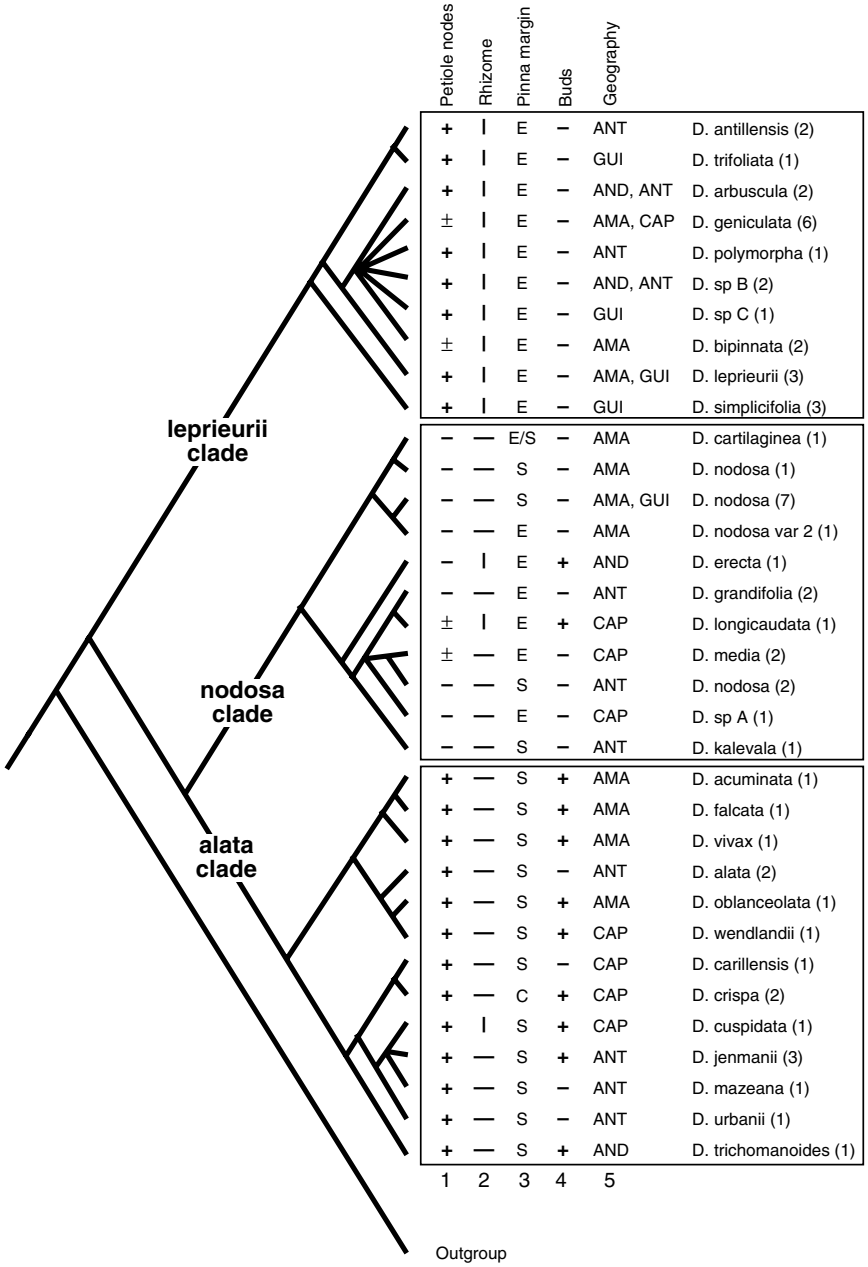

Fig. 5. The distribution of selected morphological and geographical characters within *Danaea*. The tree topology is the same as in Fig. 4, except that most multiple occurrences of the same species have been trimmed for clarity. The three main clades discussed in the text are indicated, and the number of specimens sequenced for each species is shown in parentheses. The characters are as follows, from left to right. 1. Nodes on the petiole: +, present; –, absent; ±, present or absent. 2. Rhizome habit: —, creeping with all roots on the ventral side; |, erect with both leaves and roots arranged radially. 3. Margins of pinna apex: S, serrulate or denticulate; C, crispate-erose; E, entire or sinuate. 4. Proliferous buds in leaf apex: +, present; –, absent. 5. Geographical origin of the analyzed specimens: AMA, Amazonian (lowlands); AND, Andean (submontane or montane); ANT, Antillean; CAP, Central American or along the Pacific coast of South America; GUI, Guianan.

4. Discussion

4.1. Phylogenetic relationships within Danaea

The monophyly of *Danaea* has never been disputed, and it is further corroborated by our results. Within *Danaea* we find three well-supported clades, the "*leprieurii*", "*nodosa*", and "*alata*" clades, which correspond with the morphologically defined groups of Christenhusz and Tuomisto (2005). If these clades are recognized at the rank of section, the names of Presl (1845) will have to be applied to them. Presl (1845) recognized three sections in *Danaea*: section *Arthrodanaea*, section *Holodanaea*, and section *Eudanaea*. Section *Arthrodanaea* included only *D. leprieurii*, and the name can thus be applied to our "*leprieurii*" clade. Section *Holodanaea* was lectotypified by Rolleri et al. (2003) with *D. alata*, and therefore that name can be applied to our "*alata*" clade (with the exclusion of *D. sellowiana* C. Presl,

which is morphologically similar to *D. nodosa*). The third section was named *Eudanaea*, and the species that Presl placed in it are found across all three clades of our phylogeny. Because this section contains the type species of the genus, *D. nodosa*, it automatically gets the section name *Danaea*, and this name is applicable to our "*nodosa*" clade. We have refrained from using Presl's section names in the present paper because our phylogenetic results do not agree with the original circumscriptions, and the sections would need to be redefined to enable coherent use of the section names in the future.

Up to this point, we have used a purely morphological species concept for *Danaea*. However, the availability of DNA sequence data now makes it possible to also consider genetic relatedness and monophyly as criteria for species circumscription. Our results lend genetical support to the recognition of several recently described *Danaea* species, but are in conflict with the broad species concept of Rolleri (2004). For example, four of the species in our "*alata*" clade were synonymized by Rolleri under *D. moritziana*: *D. acuminata*, *D. falcata*, *D. cuspidata*, and *D. urbanii*. This is not consistent with our phylogenetic results, because if a species were to be both monophyletic and circumscribed so broadly that it includes both *D. acuminata* and *D. cuspidata*, then all the other species in the "*alata*" clade should belong to this species as well (Fig. 4).

Danaea nodosa is an especially interesting case, because here morphological data are clearly inadequate for the identification of genetically monophyletic lineages. Our phylogeny includes eight species in the "*nodosa*" clade, each of which we find morphologically distinguishable. Rolleri (2004) merged six of these species under *D. nodosa* itself, but recognized *Danaea cartilaginea* as distinct (at the time it was called *D. ulei* H. Christ, following Tuomisto et al., 2001; *D. cartilaginea* was described by Christenhusz and Tuomisto, 2006, after it was proven to be morphologically distinct from the type of *D. ulei*). *Danaea media* was synonymized with *D. elliptica* by Rolleri, who used the name *D. elliptica* in the traditional sense (i.e. to include all species of the "*leprieurii*" clade except *D. simplicifolia*), but in our phylogeny the specimens of *D. media* form a polytomy with the Jamaican specimens of *D. nodosa*.

The specimens that we presently identify as *Danaea nodosa* display a wide range of morphological variation, but we have been unable to find clear morphological discontinuities that could be used as criteria to assign the specimens to more than one species. Because the Jamaican *D. nodosa* is deeply embedded in the extra-Amazonian subclade, our phylogenetic analyses suggest that *D. nodosa* is polyphyletic in its current circumscription. Moreover, *D. cartilaginea* is in our phylogeny deeply embedded within the Amazonian-Guianan subclade of the "*nodosa*" clade, so *D. nodosa* will also become paraphyletic if no other species are segregated from it.

The lectotype of *D. nodosa* is from Haiti (Underwood, 1909), and morphologically the Haitian specimens closely resemble those from Jamaica. If species are to be monophyletic, then the Jamaican material should probably be treated as true *D. nodosa*, and the Amazonian and Guianan material would need a new name, or possibly more than one new name. The oldest available name with a continental type amongst the proposed synonyms of *D. nodosa* is *D. sellowiana*. However, the applicability of this name is not yet certain, because the type specimen collected in Rio de Janeiro is incomplete and therefore not morphologically conclusive. We have seen little other material from Atlantic Brazil, and we do not have DNA sequence data from this region. Further studies on this complex are clearly necessary.

Within the "*leprieurii*" clade, it has been suggested that *D. trifoliata* is a subspecies of *D. simplicifolia*, because trifoliate individuals of *D. simplicifolia* resemble *D. trifoliata* (Moore, 1861). However, *D. trifoliata* always has more than one petiole node, and its leaves are green abaxially rather than whitish as in *D. simplicifolia*. Our molecular analysis resolved the two species to different well-supported subclades of the "*leprieurii*" clade. *Danaea antillensis* (Fig. 3j) and *D. polymorpha* have long been considered conspecific (as *D. elliptica* sensu Proctor, 1977), but *D. antillensis* was recently segregated and described as a new species on morphological grounds (Christenhusz, 2006). Our results are consistent with recognizing these as two different species, because they were resolved to different subclades of the "*leprieurii*" clade (Fig. 4). The phylogenetic analyses also provide support for the recognition of *D. erecta* (Tuomisto et al., 2001), within the "*nodosa*" clade.

Twelve described species could not be included in this study because no extractable material was available to us. Based on morphological characters it seems obvious that most belong to the "*alata*" clade (*D. excurrens*, *D. humilis*, *D. imbricata*, *D. moritziana*, *D. plicata*, *D. riparia*, and *D. tenera*), two to the "*leprieurii*" clade (*D. lingua-cervina* and *D. ulei*), and two to the "*nodosa*" clade (*D. latipinna* and *D. ushana*). The placement of *Danaea bicolor* is ambiguous, because it combines morphological characters of different clades. Molecular data will be necessary to resolve its position in the phylogeny.

4.2. Morphological character evolution in Danaea

Stidd (1974) suggested that the ancestral state of leaf dissection in the Marattiales is multiple times pinnate, because highly divided leaves are found in most fossil Marattiales. Under this scenario, the simpler leaves would have evolved through reduction. We find this scenario likely given the fossil evidence and the results of our phylogenetic analyses. The two species of *Danaea* with mature leaves that are simple rather than pinnate, *D. simplicifolia* (Fig. 3k) and *D. carillensis* (Fig. 3e), belong to different major clades in the genus ("*leprieurii*" and "*alata*", respectively). This can be explained most parsimoniously by assuming that each species evolved simple leaves independently through reduction from a pinnate ancestor.

This interpretation is supported by the observation that both simple-leaved species can (but rarely do) produce one

pair of lateral pinnae. In addition, several other *Danaea* species, from across all three clades, have been observed to occasionally produce more highly dissected leaves than is the norm for the species. Bipinnate leaves (usually incompletely so) are common in *D. bipinnata* (Fig. 3h) from Amazonia, and they have also been observed in *D. nodosa* (Fig. 3g) from Jamaica, *D. urbanii* from Puerto Rico, and *D. geniculata* from Colombia. The repeated occurrence of once-pinnate leaves in normally simple-leaved species, and of bipinnate leaves in normally once-pinnate species, can be explained more parsimoniously as an occasional reversal to an ancestral state than the repeated evolution of a new trait.

All juvenile Marattiaceae have leaves that are less dissected than leaves of conspecific adult plants. The *Danaea* species that we observed in the field have simple leaves in their juvenile stages, and as the plant grows it first produces leaves with one pair of lateral pinnae; gradually, the number of lateral pinnae increases to the number typical for the species (Tuomisto and Groot, 1995; Tuomisto et al., 2001). The size at which the first pinnate leaves are produced is species-specific, and varies from about 1 cm in several species of the "*alata*" clade to about 40 cm in *D. cartilaginea* of the "*nodosa*" clade. This general ontogenetic pattern is similar to what we have also observed in *Angiopteris evecta* and *Marattia alata* in Jamaica. Given this and the fossil evidence, we suggest that the once-pinnate and simple leaves of *Danaea* probably evolved through neoteny, i.e. by the plants attaining reproductive maturity when still morphologically juvenile.

The rhizome habit in *Danaea* varies between a fully erect rhizome where both leaves and roots are arranged spirally, and a creeping, dorsiventral rhizome, where all leaves are on the dorsal side and all roots on the ventral side. Various intermediate forms also exist; for example, creeping rhizomes where the leaves are arranged spirally but all roots are on the ventral side. Creeping, dorsiventral rhizomes, which are prevalent in the "*nodosa*" clade, are also found in the outgroup genera *Christensenia* and *Archangiopteris*. Erect, radial rhizomes are found in some of the outgroup genera, in all species of the "*leprieurii*" clade, and in some species of the "*nodosa*" and "*alata*" clades (Fig. 5); therefore, radial erect rhizomes are likely to be the ancestral state. This interpretation is supported by fossil evidence, because most fossil taxa known (such as *Psaronius*) were erect, tree-like ferns (Sporne, 1962).

For characteristics without a fossil record, it is more difficult to polarize the character states. We suggest that petiole nodes were not present in ancestral Marattiaceae, because in extant genera they occur only in *Danaea* and *Archangiopteris*. Petiolar nodes are absent in most species of the "*nodosa*" clade, whereas species of the other two clades mostly have nodes. Petiole nodes probably evolved from pinna-bearing nodes through the reduction of the pinnae. In some species, such as *D. alata* (Fig. 3a) and *D. crispa* (Fig. 3d), the proximal pinnae are much reduced in size in relation to other pinnae. On the other hand, the single petiolar node in *D. simplicifolia* (Fig. 3k) may occasionally produce a pair of lateral pinnae, in which case the petiole becomes nodeless.

Although species with a membranaceous leaf texture are restricted to the "*alata*" clade (*D. crispa*, *D. trichomanoides*, and *D. wendlandii*), they are found in three different subclades, so it is likely that this character has evolved repeatedly.

4.3. Biogeographical and ecological considerations

The three major clades of *Danaea* are widely distributed in the Neotropics, and each spans almost the entire geographical range of the genus. This is not immediately apparent from Fig. 5, because the figure shows the geographical origin of the specimens used in the phylogenetic study rather than the global geographical ranges of the species. This is because we have too little information from some areas, such as southern Brazil and adjacent areas, to establish which species occur there. The subclades within the three major clades, however, differ widely in their geographical ranges.

In the "*leprieurii*" clade, both *D. antillensis* and *D. polymorpha* occur in Guadeloupe, but because they were resolved to different subclades, they probably did not evolve in situ from a common ancestor, but colonized the island independently. *Danaea antillensis* is well-supported as sister to the Guianan *D. trifoliata* (Figs. 4 and 5), but the sister of *D. polymorpha* is not resolved.

The main division within the "*nodosa*" clade is geographical, and it separates the Amazonian-Guianan species from the Antillean, Andean and Central American-Pacific species, which suggests allopatric differentiation in this clade (Fig. 5). The Amazonian *D. nodosa* and *D. cartilaginea* are sympatric, but in the extra-Amazonian clade, sister species appear to be mainly allopatric.

In the "*alata*" clade, the two main clades are widely distributed (Fig. 5), but the smaller subclades show various geographical patterns. *Danaea carillensis* and *D. crispa* are restricted to high elevations in Costa Rica and Panama, and *D. acuminata*, *D. falcata*, and *D. vivax* are all found in a limited area in western Amazonia. These are potential examples of sympatric speciation. On the other hand, *D. alata*, *D. oblanceolata*, and *D. wendlandii*, which form a well-supported subclade (Fig. 4), are widely separated geographically.

In some cases, closely related species appear to differ in their ecological distribution. In the Amazonian lowlands, *D. cartilaginea* and *D. nodosa* differ in soil preferences; the former grows on poor loamy soils and the latter on richer clayey soils (personal observations; Tuomisto and Poulsen, 1996). In the same way, *D. leprieurii* is often found together with *D. cartilaginea* on the loamy soils, whereas *D. bipinnata* grows on intermediate substrates and may be found together with any of the other three species. Similar differences in edaphic distribution between closely related species have been found in other Amazonian plants (see Gentry, 1981 for *Passiflora*; Schulman et al., 2004 for

Clidemia of the Melastomataceae; Fine et al., 2005 for Protieae of the Burseraceae; Tuomisto, 2006 for *Polybotrya* ferns).

Elevation is often important for species distributions, and we have observed in the field that two or three species of the same clade can replace each other along an elevational gradient. On Guadeloupe, for example, *D. alata* is found in lowland forests and *D. mazeana* in montane cloud forests (both in the "*alata*" clade). Four species of the "*leprieurii*" clade occur in Guadeloupe, three of which (*D. antillensis*, *D. geniculata*, and *D. polymorpha*) are found in lowland and mid-altitude forests and one (*D. arbuscula*) in cloud forests.

4.4. Conclusions

Our results indicate that there are three strongly supported clades within *Danaea*, and each of these clades is morphologically recognizable and distinguishable from the others. Several subclades within these main lineages also received strong support. Both allopatric and sympatric patterns were found in different subclades of *Danaea*, and in some cases, ecological differences between closely related species were observed. Our results also indicate that *Danaea nodosa*, which has traditionally been considered a morphologically variable and geographically widespread species, is actually a polyphyletic assemblage of geographically more restricted and genetically distinct lineages that are not easily distinguished on morphological grounds. More accurate information on ecological and geographical distributions together with a more complete sampling of taxa and the sequencing of more variable genes can be expected to further clarify the taxonomy and evolutionary history of this interesting fern genus.

Acknowledgments

This study was supported by the Academy of Finland (grants to Hanna Tuomisto) and the US National Science Foundation (Grant No. DEB-0347840 to Kathleen M. Pryer). We are grateful to Michel Boudrie, Mirkka Jones, Michael Kessler, Marcus Lehnert, Robbin C. Moran, Andrew Murdock, and Alan R. Smith for providing silica-dried samples and voucher specimens. We thank Eric Schuettpelz for designing a new primer that was published here and used to amplify and sequence *atpB*, Milja Tammi for producing the map in Fig. 1, and Alan R. Smith and Andrew Murdock for useful comments on the manuscript. We also thank Lisa Bukovnik, Frank Kauff, Jolanta Miadlikowska, David Negrotto, Michal Skakuj, and Soili Stenroos for their help, comments, and support.

References

Agardh, C.A., 1822. Danaeaceae Aphorismi Botanici 8. Lund, Sweden.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, M., Lipman, D.J., 1997. Gapped blast and psi-blast: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Bittner, G., 1902. Marattiaceae. In: Engler, A., Prantl, K. (Eds.), Die natürlichen Pflanzenfamilien. I (IV), pp. 432–434.

Bletter, N., Janovec, J., Brosi, B., Daly, D.C., Thomas, W., Peters, C., 2003. A Digital Basemap of the Americas, for Mapping and Analyzing Plant Distributions (CD-ROM). New York Botanical Garden, NY, USA.

Brebner, G., 1901. On the Anatomy of *Danaea* and other Marattiaceae. Ann. Bot. 16, 517–552, plates XXII–XXIII.

Camus, J.M., 1995. Marattiaceae. In: Moran, R.C., Riba, R. (Eds.), Flora Mesoamericana. Pteridofitas, Psilotaceae a Salviniaceae, vol. 1. Univ. Nacional Autónoma de México, Ciudad, Universitaria, pp. 48–51.

Ching, R.C., 1940. Christenseniaceae. A new fern family in tropical Asia. Bull. Fan Mem. Inst. Biol. 10, 227–229.

Christenhusz, M.J.M., 2006. Three new species of Danaea (Marattiaceae) from French Guiana and the Lesser Antilles. Ann. Bot. Fennici 43, 212–219.

Christenhusz, M.J.M., Tuomisto, H., 2005. Some notes on the taxonomy, biogeography and ecology of Danaea (Marattiaceae). Fern Gaz. 17, 217–222.

Christenhusz, M.J.M., Tuomisto, H., 2006. Five new species of *Danaea* (Marattiaceae) from Peru and a new status for *D. elliptica*. Kew Bull. 61, 17–30.

Des Marais, D.L., Smith, A.R., Britton, D.M., Pryer, K.M., 2003. Phylogenetic relationships and evolution of extant horsetails, Equisetum, based on chloroplast DNA sequence data (rbcL and trnL-F). Int. J. Plant Sci. 164, 737–751.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Fine, P.V.A., Daly, D.C., Villa Muñoz, G., Mesones, I., Cameron, K.M., 2005. The contribution of edaphic heterogeneity to the evolution and diversity of Burseraceae trees in the western Amazon. Evolution 59, 1464–1478.

Gentry, A.H., 1981. Distributional patterns and an additional species of the Passiflora vitifolia complex: Amazonian species diversity due to edaphically differentiated communities. Plant Syst. Evol. 137, 95–105.

Hasebe, M., Omori, T., Nagazawa, M., Sano, T., Kato, M., Iwatsuki, K., 1994. *rbcL* gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. Proc. Natl. Acad. Sci. USA 91, 5730–5734.

Hasebe, M., Wolf, P.G., Pryer, K.M., Ueda, K., Ito, M., Sano, R., Gastony, G.J., Yokoyama, J., Manhart, J.R., Murakami, N., Crane, E.H., Haufler, C.H., Hauk, W.D., 1995. Fern phylogeny based on *rbcl* nucleotide sequences. Am. Fern J. 85, 134–181.

Hill, C.R., Camus, J.M., 1986. Evolutionary cladistics of marattialean ferns. Bull. Br. Mus. (Nat. Hist.) Bot. 14, 219–300.

Holmgren, P.K., Holmgren, N.H., 1998-present (continuously updated). Index Herbariorum. New York Botanical Garden. Available from: <http://sweetgum.nybg.org/ih/>.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Korall, P., Pryer, K.M., Metzgar, J., Schneider, H., Conant, D.S., 2006. Tree ferns: monophyletic groups and their relationships as revealed by four protein-coding plastid loci. Mol. Phylogenet. Evol. 39, 830–845.

Korall, P., Conant, D.S., Metzgar, J.S., Schneider, H., Pryer, K.M., 2007. A molecular phylogeny of scaly tree ferns (Cyatheaceae). Am. J. Bot. 94, 873–886.

Kramer, K.U., 1978. The pteridophytes of Suriname, an enumeration with keys of the ferns and fern-allies. Uitgaven Natuurw. Studiekring Suriname Ned. Antillen 93, 1–198.

Liu, Z.H., Hilton, J., Li, C.S., 2000. Review on the origin, evolution and phylogeny of Marattiales. Chin. Bull. Bot. 17, 39–52.

Maddison, D.R., Maddison, W.P., 2005. MacClade Version 4.07: Analysis of Phylogeny and Character Evolution. Sinauer, Sunderland, Massachusetts, USA.

Mason-Gamer, R.J., Kellogg, E.A., 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). Syst. Biol. 45, 524–545.

Moore, T.M., 1861. Index Filicum 2. Pamplin, London, UK, pp. 287.

Morton, C.V., 1951. A new fern of the genus *Danaea* from Colombia. J. Wash. Acad. Sci. 41, 276.

Nagalingum, N.S., Schneider, H., Pryer, K.M., 2007. Molecular phylogenetic relationships and morphological evolution in the heterosporous fern genus *Marsilea*. Syst. Bot. 32, 16–25.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Presl, C.B., 1845. Genera Filicacearum, Supplementum Tentaminis Pteridographiae. Filiorum Amadei Haase, Prague, Czech Republic, pp. 34–39.

Proctor, G.R., 1977. Flora of the Lesser Antilles, vol. 2. Pteridophyta. Arnold Arboretum, Harvard University, USA.

Pryer, K.M., Schneider, H., Smith, A.R., Cranfill, R., Wolf, P.G., Hunt, J.S., Sipes, S.D., 2001a. Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. Nature 409, 618–622.

Pryer, K.M., Schuettpelz, E., Wolf, P.G., Schneider, H., Smith, A.R., Cranfill, R., 2004. Phylogeny and evolution of ferns (monilophytes) with a focus on the early leptosporangiate divergences. Am. J. Bot. 91, 1582–1598.

Pryer, K.M., Smith, A.R., Hunt, J.S., Dubuisson, J.-Y., 2001b. *rbcL* data reveal two monophyletic groups of filmy ferns (Filicopsida: Hymenophyllaceae). Am. J. Bot. 88, 1118–1130.

Pryer, K.M., Smith, A.R., Skog, J.E., 1995. Phylogenetic relationships of extant ferns based on evidence from morphology and *rbcL* sequences. Am. Fern J. 85, 205–282.

Renzaglia, K.S., Duff, R.J., Nickrent, D.L., Garbary, D.J., 2000. Vegetative and reproductive innovations of early land plants: implications for a unified phylogeny. Philos. Trans. R. Soc. London Ser. B 355, 769–793.

Rolleri, C.H., 2004. Revisión del género Danaea (Marattiaceae—Pteridophyta). Darwiniana 42, 271–301.

Rolleri, C.H., Lavalle, M.C., Mengascini, A., Rodríguez, M., 2003. Sistemática de los heléchos maratiáceos (Marattiales-Marattiaceae), Rev. Museo La Plata, Bot. 16 (117), 1–21.

Ruokolainen, K., Tuomisto, H., 1998. Vegetación natural de la zona de Iquitos. In: Kalliola, R., Flores Paitán, S. (Eds.), Geoecología y desarrollo amazónico: Estudio integrado en la zona de Iquitos, vol. 114. Perú. Annal. Univ. Turkuensis Ser. A II, pp. 253–365.

Salovaara, K.J., Cárdenas, G.G., Tuomisto, H., 2004. Forest classification in an Amazonian rainforest landscape using pteridophytes as indicator species. Ecography 27, 689–700.

Schuettpelz, E., Korall, P., Pryer, K.M., 2006. Plastid atpA data provide improved support for deep relationships among ferns. Taxon 55, 897–906.

Schulman, L., Koivunen, H., Ruokolainen, K., 2004. Spatio-ecological niche segregation of two sympatric species of Clidemia (Melastomataceae) in western Amazonian non-flooded rainforests. Folia Geobot. 39, 143–160.

Smith, A.R., Pryer, K.M., Schuettpelz, E., Korall, P., Schneider, H., Wolf, P.G., 2006. A classification for extant ferns. Taxon 55, 705–731.

Sporne, K.R., 1962. The Morphology of Pteridophytes. Hutchinson, London, UK, pp. 127–135.

Stidd, B.M., 1974. Evolutionary trends in Marattiales. Ann. Mo. Bot. Gard. 61, 388–407.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods). Sinauer, Sunderland, Massachusetts, USA.

Taberlet, P., Gielly, L., Pautou, G., Bouvet, J., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. 17, 1105–1109.

Tuomisto, H., 2006. Edaphic niche differentiation among *Polybotrya* ferns in Western Amazonia: implications for coexistence and speciation. Ecography 29, 273–284.

Tuomisto, H., Groot, A.T., 1995. Identification of the juveniles of some ferns from western Amazonia. Am. Fern J. 85, 1–28.

Tuomisto, H., Moran, R.C., 2001. Marattiaceae. In: Harling, G., Andersen, L. (Eds.), Flora of Ecuador, vol. 66, pp. 23–68.

Tuomisto, H., Poulsen, A.D., 1996. Influence of edaphic specialization on pteridophyte distribution in neotropical rain forests. J. Biogeogr. 23, 283–293.

Tuomisto, H., Ruokolainen, K., Aguilar, M., Sarmiento, A., 2003. Floristic patterns along a 43-km long transect in an Amazonian rain forest. J. Ecol. 91, 743–756.

Underwood, L.M., 1902. American Ferns V: a review of the genus *Danaea*. Bull. Torrey Bot. Club 29, 669–679.

Underwood, L.M., 1909. Marattiaceae. In: North American Flora. New York Botanical Garden, NY, USA, 16 (1), pp. 17–21.

de Vriese, W.H., Harting, P., 1853. Monographie des Marattiacées Noothoven van Goor. Leiden and Arnz, Düsseldorf, The Netherlands and Germany.

Wikström, N., Pryer, K., 2005. Incongruence between primary sequence data and the distribution of a mitochondrial atp1 group II intron among ferns and horsetails. Mol. Phylogenet. Evol. 36, 484–493.

Wolf, P.G., 1997. Evaluation of atpB nucleotide sequences for phylogenetic studies of ferns and other pteridophytes. Am. J. Bot. 84, 1429–1440.