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The Relationships of Papuasian Cyatheaceae to New World Tree Ferns

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ABSTRACT.—Phylogenetic hypotheses about the Cyatheaceae have been based largely on either New World or Old World species. In this study we examine molecular data for species from both hemispheres. Chloroplast DNA restriction site variation has been compared among 23 species of Cyatheaceae from Papuasia and the Pacific and 61 species from the New World. Parsimony analysis of the variation documented supports the existence of three major clades, and reveals a correlation between phylogenetic relationship and biogeography. Species from the Neotropics appear derived with respect to those from the Paleotropics in the *Alsophila* clade; in this clade species from Papua New Guinea and Lord Howe Island are basal to those from the American tropics. A similar relationship is tentatively suggested for the *Cyathea* clade, where the sole Old World representative, (*C. howeana*) from Lord Howe Island, is basal to the remainder of the clade. This finding extends the worldwide distribution of the *Cyathea* clade across the Pacific. No clear correlation between phylogeny and biogeography of the *Sphaeropteris* clade was observed based on current data. These results suggest that additional sampling from other regions in the Old World tropics may further elucidate the evolutionary history of this group of plants.

The Cyatheaceae (the scaly tree ferns) are a family of approximately 500 species occurring throughout the wet tropics, particularly in cool montane rain forests. Regions that are especially rich in species include the Greater Antilles, Central America, the northern part of the Andes (including Venezuela, Colombia, Ecuador, and Peru), Madagascar, Borneo, Sumatra, the Philippines, and New Guinea (Tryon, 1970; Tryon and Gastony, 1975). Most species have restricted ranges; few occur in more than one of these regions. Whereas the geographic ranges of species are, for the most part, fairly well known, the ranges of genera are not because there is a lack of agreement on generic boundaries. This lack of consensus on generic circumscription is illustrated by studies of Tryon and Tryon (1982), Holttum and Edwards (1983), and Lellinger (1987) in which six, one, and four genera were recognized, respectively. Recent studies of chloroplast DNA restriction site variation in New World Cyatheaceae (Conant et al., 1994; Stein et al., in press) have demonstrated the existence of three well-defined evolutionary lineages (the *Alsophila* clade, the *Cyathea* clade, and the *Sphaeropteris* clade). The *Alsophila* clade is represented in all centers of tree fern diversity; however, the clade is most diverse in Malesia. The *Sphaeropteris* clade is also most diverse in Malesia, but is absent from Africa

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and Madagascar and represented in the neotropics by only eight species, the *S. horrida* group (Tryon, 1971). The distribution of the *Cyathea* clade is controversial. This clade includes the genera *Trichipteris, Cyathea,* and *Cnemidaria,* all of which Tryon and Tryon (1982) believed were restricted to the American tropics. Holttum (1982), however, argued that the range of this evolutionary lineage extended beyond the American tropics. He proposed that *Cy. decurrens* (Hook.) Copel. (*Alsophila decurrens* Hook., of Tryon, 1970) and its allies in the islands of the Western Pacific were more closely related to members of the *Cyathea* clade from Central and South America than to members of the *Alsophila* clade as indicated by Tryon (1970). This would suggest that the *Cyathea* clade is not restricted to the Neotropics, but extends westward through the islands of the Pacific to Australia and New Ireland.

Hypotheses about the phylogeny of the Cyatheaceae have been influenced by an Old World (Holttum) or a New World (Tryon) perspective. To bring a more global point of view to our ongoing investigation of tree fern phylogeny, we have obtained new cpDNA restriction site mutation data from the tree ferns of Papua New Guinea and Lord Howe Island. Combining these data with those previously obtained (largely from New World taxa) permits a broader examination of clade membership and the investigation of biogeographic patterns in the group. Specifically, our analysis addresses the following questions: 1) Is the *Cyathea* clade restricted to the New World? 2) Is there a close alliance of the *S. horrida* complex with Papuan species? 3) Are the three major clades identified earlier maintained as separate lineages? 4) Are *Sphaeropteris* and *Cyathea* the derived sister lineages as suggested by earlier analyses?

Materials and Methods

Total DNA was extracted from fresh, liquid-nitrogen frozen or silica gel-dried leaf tissue (Stein, 1993). The study set included 84 species of Cyatheaceae from the Caribbean, Central and South America, Papua New Guinea, Lord Howe Island, Australia and New Zealand (Table 1). Two outgroup species, *Lophosoria quadripinnata* (Lophosoriaceae) and *Dicksonia gigantea* (Dicksoniaceae), both from the American Tropics, were also included. DNAs were digested with 20 restriction enzymes: *Apa*I, *Ava*I, *Bam*HI, *Bcl*I, *Bgl*I, *Bst*EII, *Eco*RV, *Hind*III, *Mlu*I, *Nru*I, *Pst*I, *Pvu*II, *Sac*I, *Sac*II, *Sal*I, *Sca*I, *Sma*I, *Sph*I, *Stu*I, and *Xho*I. Electrophoresis, Southern blotting, nick translation, and hybridizations were carried out as in Stein (1993).

Detailed restriction site maps were constructed for *A. bryophila, A. salvinii, Cy. furfuracea, S. cooperi, L. quadripinnata,* and *D. gigantea* (Conant et al., 1994; Stein et al., in press) using cloned *Adiantum capillus-veneris* cpDNA fragments (Hasebe and Iwatsuki, 1990). Restriction site variation was assessed using eight *Adiantum* probe groups that combined two or three adjacent cpDNA fragments (Table 2) in the hybridization experiments.

Data were analyzed with the computer program PAUP (Phylogenetic Analysis Using Parsimony, Version 3.1.1; Swofford, 1992) on a MacIntosh Quadra 950. Because the number of species was so large, only heuristic searches could be

TABLE 1. Species of Cyatheaceae included in this study. *Lophosoria quadripinnata* (Lophosoriaceae) and *Dicksonia gigantea* (Dicksoniaceae) are outgroup species. Voucher specimens of each species are deposited at the Lyndon State College Herbarium (LSC), and a duplicate set at the Harvard University Herbaria (GH). Species placed in the genus *Nephelea* by Tryon (1970) are designated with an asterisk. Plants were collected from Costa Rica (CR), the Dominican Republic (DR), Honduras (HO), Lord Howe Island (LHI), Papua New Guinea (PNG), and Puerto Rico (PR). LSCG and QBG are the Lyndon State College greenhouse and the Quail Botanical Garden, Encinitas, California, respectively. Voucher specimen numbers cited are those of Conant. Two vouchers are cited for *Sphaeropteris brunei*, because the collection number on the DNA sample was lost. All species were collected in duplicate so the DNA had to come from one of the two specimens cited.

Species	Abbreviation	Source	Voucher
1. *Alsophila abbottii* (Maxon) R. Tryon	ALabb	DR	4305
2. *A. amintae* Conant	ALami	PR	4318
3. *A. brevipinna* (Benth.) R. Tryon	ALbre	LHI	4667
4. *A. brooksii* (Maxon) R. Tryon	ALbro	PR	4310
5. *A. bryophila* R. Tryon	ALbry	PR	4322
6. *A. coactilis* (Holtt.) R. Tryon	Alcoa	PNG	4589
7. *A. cuspidata* (Kunze) Conant*	ALcus	CR	4427
8. *A. erinacea* (H. Karst.) Conant*	ALeri	CR	4386
9. *A. ferdinandii* R. Tryon	ALfer	LHI	4666
10. *A. firma* (Baker) Conant*	ALfir	HO	4364
11. *A. foersteri* (Rosenst.) R. Tryon	ALfoe	PNG	4646
12. *A. fulgens* (C. Chr.) Conant*	ALful	DR	4287
13. *A. geluensis* (Rosenst.) R. Tryon	ALgel	PNG	4661
14. *A. gleichenioides* (C. Chr.) R. Tryon	ALgle	PNG	4584
15. *A. hooglandii* (Holtt.) R. Tryon	ALhoo	PNG	4650
16. *A. imrayana* (Hook.) Conant	ALimr	VE	4466
17. *A. jimeneziana* Conant*	ALjim	DR	4287
18. *A. minor* (D.C. Eaton) R. Tryon	ALmin	DR	4297
19. *A. muelleri* (Baker) R. Tryon	ALmue	PNG	4582
20. *A. nigrolineata* (Holtt.) R. Tryon	ALnil	PNG	4636
21. *A. nigropaleata* (Holtt.) R. Tryon	ALnip	PNG	4647
22. *A. polystichoides* (Christ) Conant*	ALpol	CR	4391
23. *A. portoricensis* (Kuhn) Conant*	ALpor	PR	4321
24. *A. pseudomuelleri* (Holtt.) R. Tryon	ALpse	PNG	4586
25. *A. salvinii* Hook.	ALsal	HO	4365
26. *A. urbanii* (Brause) R. Tryon	ALurb	DR	4301
27. *A. woodwardioides* (Kaulf.) Conant*	ALwoo	DR	4312
28. *Cnemidaria amabilis* (Morton) R. Tryon	CNama	VE	4495
29. *Cn. choricarpa* (Maxon) R. Tryon	CHcho	CR	4414
30. *Cn. consimilis* Stolze	CNcon	VE	4497
31. *Cn. grandiflora* (Willd.) Proctor	CNgra	VE	4487
32. *Cn. horrida* (L.) C. Presl	CNhor	PR	4343
33. *Cn. karsteniana* (Klotzsch) R. Tryon	CNkar	VE	4471
34. *Cn. mutica* (Christ) R. Tryon	CNmut	CR	4385
35. *Cn. spectabilis* (Kunze) R. Tryon	CNspe	VE	4482
36. *Cyathea andina* (H. Karst.) Domin	CYand	PR	4334
37. *Cy. arborea* (L.) Smith	CYarb	PR	4345
38. *Cy. caracasana* (Klotzsch) Domin	CYcar	PR	4326
39. *Cy. delgadii* Sternb.	CYdel	VE	4455
40. *Cy. divergens* Kunze	CYdiv	CR	4384
41. *Cy. fulva* (Mart. & Gal.) Fée	CYful	CR	4397
42. *Cy. furfuracea* Baker	CYfur	PR	4325

TABLE 1. Continued.

Species	Abbreviation	Source	Voucher
43. *Cy. holdridgeana* Nisman & L.D. Gómez	CYhol	CR	4406
44. *Cy. howeana* Domin	CYhow	LHI	4665
45. *Cy. multiflora* Sm.	CYmul	VE	4425
46. *Cy. parvula* (Jenman) Domin	CYpar	PR	4332
47. *Cy. speciosa* Willd.	CYspe	VE	4475
48. *Cy. suprastrigosa* (Christ) Maxon	CYsup	CR	4402
49. *Cy. tenera* (Hook.) T. Moore	CYten	PR	4333
50. *Dicksonia gigantea* H. Karst.	DIgig	CR	4400
51. *Lophosoria quadripinnata* (J.F. Gmel.) C. Chr.	LOqua	PR	4339
52. *Sphaeropteris aeneifolia* (Alderw.) R. Tryon	SPaen	PNG	4578
53. *S. angiensis* (Gepp) R. Tryon	SPang	PNG	4629
54. *S. atrox* (C. Chr.) R. Tryon	SPatr	PNG	4606
55. *S. brunei* (Christ) R. Tryon	SPbru	CR	4388/4392
56. *S. concinna* (Baker) R. Tryon	SPcon	PNG	4623
57. *S. cooperi* (F. Muell.) R. Tryon	SPcoo	LSCG	4304
58. *S. elongata* (Hook.) R. Tryon	SPelo	CR	4410
59. *S. glauca* (Blume) R. Tryon	SPgla	PNG	4622
60. *S. horrida* (Liebm.) R. Tryon	SPhor	HO	4363
61. *S. insignis* (D.C. Eaton) R. Tryon	SPins	DR	4396
62. *S. magna* (Copel.) R. Tryon	SPmag	PNG	4630
63. *S. medullaris* (G. Forst.) Bernh.	SPmed	QBG	4303
64. *S. parianensis* Windisch	SPpar	VE	4496
65. *S. procera* (Brause) R. Tryon	SPpro	PNG	4645
66. *S. robusta* (Watts) R. Tryon	SProb	LHI	4663
67. *S. senilis* (Klotzsch) R. Tryon	SPsen	VE	4479
68. *S. tomentosissima* (Copel.) R. Tryon	SPtom	PNG	4581
69. *Trichipteris armata* (Sw.) R. Tryon	TRarm	PR	4328
70. *T. borinquena* (Maxon) R. Tryon	TRbor	PR	4341
71. *T. cordata* (Klotzsch) R. Tryon	TRcor	VE	4473
72. *T. decomposita* (H. Karst). R. Tryon	TRdec	VE	4440
73. *T. gibbosa* (Klotzsch) Barrington	TRgib	VE	4469
74. *T. kalbreyeri* (Baker) R. Tryon	TRkal	VE	4448
75. *T. microdonta* (Desv.) R. Tryon	TRmic	HO	4357
76. *T. microphylla* (Klotzsch) R. Tryon	TRmip	VE	4468
77. *T. nigripes* (C. Chr.) Barrington	TRnig	CR	4417
78. *T. pauciflora* (Kuhn) R. Tryon	TRpau	VE	4445
79. *T. procera* (Willd.) R. Tryon	TRpro	PR	4338
80. *T. scabriuscula* (Maxon) R. Tryon	TRsca	HO	4346
81. *T. schiediana* (C. Presl) R. Tryon	TRsch	HO	4368
82. *T. steyermarkii* R. Tryon	TRste	VE	4500
83. *T. stipularis* (Christ) R. Tryon	TRsti	CR	4395
84. *T. trichiata* (Maxon) R. Tryon	TRtri	CR	4422
85. *T. tryonorum* (Riba) R. Tryon	TRtry	VE	4465
86. *T. ursina* (Maxon) R. Tryon	TRurs	CR	4423

executed. Searches were carried out with the SIMPLE, CLOSEST and AS IS addition sequences with ten trees held at each step during stepwise addition. When simple addition sequences were employed, *A. abbottii* served as the reference taxon. Starting trees were also constructed in MacClade (Maddison and

TABLE 2. Chloroplast DNA restriction site mutations scored in 84 species of Cyatheaceae and the outgroup taxa *Lophosoria quadripinnata* (Lophosoriaceae) and *Dicksonia gigantea* (Dicksoniaceae) based on 20 enzymes. No phylogenetically significant mutations were found for the enzymes *BstE* II, *Mlu*I, and *Pvu*II. Only synapomorphies are listed. Mutations are numbered according to the alphabetical listing by enzyme (No.) and by the actual column in the data matrix (Col. Matrix). Mutations are listed with ancestral state first and derived state last. Species with mutated DNAs are listed as assigned by PAUP in the tree in Fig. 1. Mutations were polarized using *Lophosoria quadripinnata* and *Dicksonia gigantea*. Probe groups correspond to *Pst*I fragments of cpDNA from *Adiantum capillis-veneris* (Hasebe and Iwatsuki, 1990).

No.	Col. Matrix	Enzyme	Probe Group	Mutation	Species with Mutated DNAs
1.	58	*Apa*I	A9 + 5	7.0 + 10.0 = 17.0	28–35, 47
2.	59	*Apa*I	A10 + 4 + 17	13.3 = 5.5 + 7.8	28–43, 45–49, 58, 64, 67, 69–86
3.	63	*Apa*I	A19 + 6 + 12 + 3 + 20 + 16	9.6 = 4.2 + 5.4	28–31, 33–35, 39–41, 43, 45, 47–48, 58, 64, 67, 71–78, 80–86
4.	56	*Ava*I	A11 + 1	6.9 = 0.9 + 6.0	28–49, 52–86
5.	57	*Ava*I	A3 + 20 + 16 + 11 + 1	2.9 + 6.4 = 9.3	28–49, 58, 64, 67, 69–86
6.	41	*BamH*I	A11 + 1	6.5 = 1.7 + 4.8	12, 17, 27
7.	42	*BamH*I	A11 + 1	6.5 = 2.3 + 4.2	28–36, 38–49, 64, 67, 70–79, 81–82, 86
8.	44	*BamH*I	A9 + 5	3.9 + 6.9 = 10.8	28–29, 32, 34–35, 47
9.	43	*BamH*I	A9 + 5	9.7 = 2.8 + 6.9	9, 13, 15, 21, 28–49, 52–80, 82–86
10.	79	*BamH*I	A19 + 6 + 12	16.3 = 6.3 + 10.0	73, 82
11.	80	*BamH*I	A19 + 6 + 12	16.0 = 3.2 + 12.8	1–2, 4–5, 7–8, 10, 12, 16–18, 22–23, 26–27
12.	45	*BamH*I	A19 + 6 + 12	3.7 = 0.7 + 3.0	45, 77, 86
13.	81	*BamH*I	A10 + 4 + 17	1.35 + 1.35 = 2.7	58, 80, 83–85
14.	51	*Bcl*I	A2 + 15	2.5 = 0.9 + 1.6	52–57, 59–63, 65–66, 68
15.	55	*Bcl*I	A11 + 1	5.0 + 9.3 = 14.3	52–57, 59–63, 65–66, 68–69, 80, 83–85
16.	52	*Bcl*I	A11 + 1	9.3 = 1.0 + 8.3	1–27
17.	53	*Bcl*I	A9 + 5	1.8 + 4.6 = 6.4	28–35, 47
18.	54	*Bcl*I	A8 + 13 + 2 + 15	11.4 = 3.6 + 7.8	1–24, 26–27, 44
19.	26	*Bgl*I	A2 + 15	25.0 = 10.0 + 15.0	69, 80, 83–85
20.	25	*Bgl*I	A2 + 15	26.0 = 8.0 + 18.0	1–2, 4–5, 7–8, 10, 12, 16–18, 22–23, 26–27
21.	85	*Bgl*I	A2 + 15	45.4 = 19.4 + 26.0	1–2, 4–24, 26–27
22.	86	*Bgl*I	A8 + 13	19.6 = 4.8 + 14.8	13, 15, 21
23.	27	*Bgl*I	A11 + 1	28.5 = 13.5 + 15.0	28–49, 52–86
24.	46	*EcoR*V	A19 + 6 + 12	7.1 = 1.7 + 5.4	1–24, 26–27
25.	6	*EcoR*V	A19 + 6 + 12	9.3 = 2.2 + 7.1	1–35, 37–49, 52–86
26.	47	*EcoR*V	A8 + 13	11.7 = 5.2 + 6.5	44, 52–57, 59–63, 65–66, 68
27.	48	*EcoR*V	A4 + 10 + 17	17.7 = 7.2 + 10.5	58, 69, 80, 83–85
28.	49	*Hind*III	A19 + 6 + 12	5.5 = 1.9 + 3.6	1–2, 4–5, 12, 17–18, 23, 26–27
29.	39	*Hind*III	A19 + 6 + 12	1.7 + 1.8 = 3.5	52–57, 59–62, 65–66, 68, 79
30.	91	*Hind*III	A8 + 7 + 14	13.4 = 4.2 + 9.2	28–49, 52–86
31.	92	*Hind*III	A8 + 7 + 14	1.2 + 9.2 = 10.4	28–43, 45–49, 53, 58, 63–64, 67, 69–78, 80–86
32.	93	*Hind*III	A8 + 7 + 14 + 9 + 5	1.2 + 4.2 = 5.4	46, 56, 60–61, 79

TABLE 2. Continued.

No.	Col. Matrix	Enzyme	Probe Group	Mutation	Species with Mutated DNAs
33.	50	*Hind*III	A9 + 5	0.8 + 1.0 = 1.8	1–24, 26–27
34.	72	*Nru*I	A8 + 13	27.0 = 5.5 + 21.5	1–2, 4–5, 12, 17–18, 23, 26–27, 51
35.	30	*Nru*I	A8 + 13	26.5 = 10.0 + 16.5	45, 64, 67, 70–73, 75–77, 79, 81–82, 86
36.	31	*Nru*I	A8 + 13 + 2 + 15	6.5 + 27.0 = 33.5	52–57, 59–63, 65–66, 68
37.	32	*Nru*I	A3 + 20 + 16	9.5 = 2.0 + 7.5	3, 9, 13–15, 19, 21, 24–25, 28–49, 52–59, 62–86
38.	33	*Nru*I	A11 + 1	18.2 = 4.2 + 14.0	52–57, 59–63, 65–66, 68
39.	34	*Nru*I	A9 + 5	0.9 + 11.6 = 12.5	38–43, 48–49, 74, 78
40.	35	*Nru*I	A9 + 5	15.3 = 1.8 + 13.5	46, 69, 83–85
41.	89	*Nru*I	A9 + 5	1.0 + 3.4 = 4.4	44, 52–54, 57, 59, 62, 66, 68
42.	74	*Nru*I	A18 + 7 + 14	15.3 = 5.2 + 10.1	1–35, 37–44, 47–49, 63, 74, 78
43.	1	*Pst*I	A18 + 7 + 14	1.8 + 6.3 = 8.1	1–24, 26–27, 51
44.	64	*Pst*I	A18 + 7 + 14	19.6 = 6.6 + 13.0	28, 30–33, 35, 40–41, 47
45.	65	*Pst*I	A18 + 7 + 14	19.6 = 8.1 + 11.5	1–2, 4–5, 12, 17–18, 23, 26–27
46.	66	*Pst*I	A18 + 7 + 14	0.6 + 21.4 = 22.0	52–57, 59–63, 65–66, 68
47.	3	*Pst*I	A3 + 20 + 16	7.9 + 8.1 = 16.0	1–2, 4–5, 7–8, 10–12, 16–18, 22–23, 26–27, 37, 55
48.	67	*Pst*I	A3 + 20 + 16	6.8 + 7.4 = 14.2	1–27
49.	2	*Pst*I	A9 + 5	8.0 = 0.6 + 7.4	28, 30–49, 58, 60, 64, 67, 69–82, 84–86
50.	19	*Sac*I	A3 + 20 + 16	22.0 = 9.0 + 13.0	1–2, 4–8, 10–12, 14, 16–20, 22–24, 26–27
51.	20	*Sac*I	A11 + 1	13.7 = 6.7 + 7.0	28–43, 45–49, 58, 64, 67, 69–86
52.	22	*Sac*I	A9 + 5	8.8 = 1.3 + 7.5	52–57, 59–63, 65–66, 68
53.	21	*Sac*I	A9 + 5	5.7 + 8.7 = 14.4	1–49, 52–86
54.	23	*Sac*I	A2 + 15	3.4 + 24.0 = 27.4	1–24, 26–27, 64, 67, 71–72
55.	24	*Sac*I	A8 + 13	26.0 = 11.0 + 15.0	29, 34
56.	87	*Sac*II	A9 + 5 + 10 + 4 + 17	13.0 = 6.4 + 6.6	9, 13, 15, 21
57.	28	*Sac*II	A10 + 4 + 17	12.0 + 17.0 = 29.0	69, 80, 83–85
58.	29	*Sac*II	A11 + 1	14.1 = 6.5 + 7.6	1–24, 26–27
59.	73	*Sac*II	A11 + 1 + 18 + 7 + 14	3.2 + 14.1 = 17.3	37, 69, 80, 83–85
60.	10	*Sal*I	A10 + 4 + 17	33.0 = 13.0 + 20.0	29, 32, 34–37, 42–43, 45, 47, 58, 64, 67, 69–73, 75–86
61.	84	*Sal*I	A19 + 6 + 12	4.6 + 10.5 = 15.1	31, 52–57, 59–63, 65–66, 68
62.	11	*Sal*I	A11 + 1	19.8 = 5.8 + 14.0	1–15, 17–24, 26–49, 58, 64, 67, 69–86
63.	9	*Sal*I	A9 + 5	4.8 = 2.4 + 2.4	28–49, 58, 64, 67, 69–86
64.	36	*Sca*I	A8 + 13	10.4 = 3.0 + 7.4	1–2, 4–5, 7–8, 10, 12–13, 15–18, 21–23, 26–27
65.	90	*Sca*I	A8 + 13	18.6 = 8.0 + 10.6	60–61
66.	77	*Sca*I	A8 + 13 + 2 + 15	18.6 = 3.2 + 15.4	1–2, 4–5, 12, 17–18, 23, 26–27
67.	78	*Sca*I	A8 + 13 + 2 + 15	18.6 = 5.5 + 13.1	28–43, 45–49, 58, 64, 67, 69–86
68.	75	*Sca*I	A8 + 13 + 2 + 15	1.0 + 10.4 = 11.4	38–41, 43, 48–49, 74, 78
69.	76	*Sca*I	A8 + 13 + 2 + 15	13.1 = 1.1 + 12.0	28–37, 42–43, 45, 47–48, 58, 64, 67, 69, 71–73, 75–86

TABLE 2. Continued.

No.	Col. Matrix	Enzyme	Probe Group	Mutation	Species with Mutated DNAs
70.	37	*Sca*I	A19 + 6 + 12	14.2 = 3.6 + 10.6	28–49, 58, 64, 67, 69–86
71.	38	*Sca*I	A11 + 1	25.0 = 8.0 + 17.0	1–27, 52–57, 59–63, 65–66, 68
72.	40	*Sca*I	A9 + 5	3.1 = 1.3 + 1.8	1–2, 4–5, 12, 16–18, 23, 26–27
73.	61	*Sma*I	A11 + 1	22.5 = 3.5 + 19.0	28–31, 33–35, 37, 44–46, 64, 67, 70–73, 75–77, 79, 81–82, 86
74.	62	*Sma*I	A11 + 1	21.8 = 3.8 + 18.0	45, 58, 77, 86
75.	60	*Sma*I	A11 + 1	22.5 = 1.5 + 21.0	58, 69, 80, 83–85
76.	18	*Sph*I	A11 + 1	11.5 = 3.0 + 8.5	28–49, 58, 64, 67, 69–86
77.	12	*Stu*I	A8 + 13	23.0 = 9.0 + 14.0	9, 45, 77, 86
78.	13	*Stu*I	A10 + 4 + 17	8.5 = 2.1 + 6.4	28–49, 58, 64, 67, 69–86
79.	14	*Stu*I	A11 + 1	19.0 = 6.3 + 12.7	38–41, 49, 74
80.	69	*Stu*I	A11 + 1	18.6 = 5.6 + 13.0	1–2, 4–5, 12, 17–18, 23, 26–27, 45, 64, 67, 71–73, 75–77, 79, 81–82, 86
81.	70	*Stu*I	A11 + 1	19.0 = 2.4 + 16.6	28–35, 46–47
82.	15	*Stu*I	A9 + 5	2.1 + 5.0 = 7.1	45–46, 77, 86
83.	16	*Stu*I	A3 + 20 + 16	1.8 + 4.4 = 6.2	28–49, 58, 64, 67, 69–86
84.	71	*Stu*I	A3 + 20 + 16	0.6 + 7.8 = 8.4	37, 58, 69, 80, 83–85
85.	17	*Stu*I	A19 + 6 + 12	17.6 = 2.6 + 15.0	1–2, 4–5, 7–8, 10, 12, 16–18, 22–23, 26–27
86.	4	*Xho*I	A8 + 13	11.3 = 3.8 + 7.5	28–49, 52–86
87.	5	*Xho*I	A3 + 20 + 16	8.8 = 1.0 + 7.8	29, 32, 34, 36, 43, 45, 47, 57, 64, 67, 70–73, 75–77, 79, 81–82, 86
88.	7	*Xho*I	A19 + 6 + 12	16.9 = 8.3 + 8.6	25, 79
89.	8	*Xho*I	A19 + 6 + 12	16.8 = 6.8 + 10.0	58, 69, 80, 83–85
90.	68	*Xho*I	A9 + 5	0.7 + 6.0 = 6.7	29, 69, 80, 83–85
91.	82	*Xho*I	A9 + 5	10.3 = 3.5 + 6.8	9, 13, 15, 21
92.	83	*Xho*I	A9 + 5	2.1 + 10.3 = 12.4	1–2, 4–5, 7–8, 10, 12, 16–18, 22–23, 26–49, 58, 64, 67, 69–86

Maddison, 1992) and then imported into PAUP for branch swapping. The Tree Bisection Reconnection (TBR) algorithm was used for branch swapping on each of the starting trees. The MULPARS, COLLAPSE ZERO-LENGTH BRANCHES, and STEEPEST DESCENT options were in effect with MAXTREES set at 20,000. Majority-rule consensus trees were computed for each set of 20,000 trees and the trees compared for common monophyletic groups. All character reconstructions were obtained using the ACCTRAN character-state optimization option. All searches were carried out using Wagner parsimony with the constraint that the ingroup is a monophyletic group (Stein et al., in press).

Random addition sequences were also used to obtain a broader distribution of starting trees upon which to carry out branch swapping with TBR (Maddison et al., 1992). Up to five equally parsimonious trees were saved at the end of each replication. After 100 replications, majority-rule consensus trees were computed from all trees stored in memory. Twenty-five searches (100 replications

each) were carried out. The frequencies of various monophyletic groups in each search were tabulated, and mean frequencies of these groups computed.

The degree of support for monophyletic groups was also evaluated via bootstrapping (Felsenstein, 1985; Sandersen, 1989). Again, because of the number of taxa, only heuristic searches could be carried out. We used the AS IS addition sequence, and held one tree at each step during stepwise addition. The TBR algorithm was used to carry out branch swapping. The STEEPEST DESCENT and MULPARS options were not in effect. At the end of 100 replications, a majority rule consensus tree was constructed.

Tryon and Tryon (1982), Nishida (1989), and Conant et al. (1994) had indicated that *Lophosoria* and *Dicksonia* are the closest relatives of the Cyatheaceae, and noted that it is uncertain which of these taxa is closer to the scaly tree ferns. Therefore, we have used both taxa as outgroups in polarizing the mutations in our data set and compared searches using both species to those in which either was used as the outgroup.

The lengths of trees with alternative arrangements of the major clades were determined using the constraints option in PAUP and with MacClade.

Results

In this study we have compared cpDNA restriction site variation in 84 species of Cyatheaceae. Sixty-one of these taxa are from the American tropics, representing about 33% of the species diversity in the New World (Tryon and Tryon, 1982). Twenty-three species were from Malesia and the Islands of the Western Pacific, accounting for about 10% of the diversity from that region (Holttum, 1963; 1964). From this comparison, we found 92 variable restriction sites that were phylogenetically informative (Table 2).

The shortest trees found were 160 steps long. Because the shortest tree obtained is dependent on the starting tree, we used a variety of different heuristic search procedures in analyzing the data. This included searches where 20,000 trees were generated from a single starting tree and searches that involved many starting trees derived from random addition sequences. Random addition sequences enabled us to obtain a wide variety of starting trees (2500) in order to broadly sample tree space. Overall, more than 32,767 trees (the maximum number that can be stored in memory in PAUP) of 160 steps were found. This is not unexpected, because there were only 92 characters available to resolve relationships among 84 taxa. When a monophyletic group was present in 95% or more of all minimal trees observed, we considered it a feature of the set of most parsimonious trees that merited discussion. These monophyletic groups are supported by one or more synapomorphies.

We have also carried out bootstrap analyses of our data set in order to obtain another measure of the degree of support for monophyletic groups. In the discussions below we have referred to the mean frequency among consensus trees, the number of synapomorphies supporting a group, and the bootstrapping values for various monophyletic lineages. All of these parameters are considered together to gain an understanding of the degree of support for a group.

With the addition of the Old World taxa, the data continue to support the existence of three major evolutionary lineages in the family: The *Alsophila, Cyathea,* and *Sphaeropteris* clades (Fig. 1). These lineages are present in 100% of the consensus trees and are supported by 7, 12, and 7 synapomorphies, with bootstrapping values of 80, 62, and 85%, respectively. The results reported here are based on searches in which both *Lophosoria* and *Dicksonia* were used as outgroups (Fig. 1), but were very similar to those using either taxon to polarize the mutations.

Within the *Alsophila* clade, the most derived group consists of ten species from the Caribbean. This group is present in 100% of the consensus trees, is supported by 5 synapomorphies, and has a bootstrapping value of 100%. These species, together with other American *Alsophila* (with the exception of A. *salvinii*), form a larger monophyletic group that is present in 100% of the consensus trees, is supported by 5 synapomorphies, and has a bootstrapping value of 99%. American *Alsophila* is derived with respect to other *Alsophila* species analyzed from Papua New Guinea and Lord Howe Island. Together, these American and Old World species form a group which is present in 100% of the consensus trees, supported by 7 synapomorphies, and occurs in 80% of bootstrap replicates. However, *A. salvinii,* from southern Mexico and Central America, lies outside this clade. *Alsophila salvinii* is linked to the base of the *Alsophila* clade by two mutations. We are currently making plans to collect putative relatives of A. *salvinii* in Borneo to clarify the relationship of this species to other members of the family.

Tryon and Tryon (1982) argued that the taxa within the *Cyathea* clade were restricted to the American tropics and that *Cy. decurrens* and its allies in the western Pacific were members of the genus *Alsophila.* Holttum (1982), however, suggested that the latter species were related to *Cy. multiflora* and its allies in Central and South America. In our analysis, *Cy. howeana,* a member of the *Cy. decurrens* group endemic to Lord Howe Island, clusters with the *Cyathea* clade in 100% of the consensus trees, shares 12 synapomorphies with other members of this clade, but is present in only 62% of the bootstrap replicates. If *Cy. howeana* is indeed a member of the *Cyathea* clade as hypothesized by Holttum (1982), rather than a member of the *Alsophila* clade as suggested by Tryon and Tryon (1982), this would extend the geographic distribution of the *Cyathea* clade through the islands of the Pacific. However, moving *Cy. howeana* to the *Alsophila* clade increases tree length by only one step. Thus the data only weakly suggest that *Cy. howeana* is a member of the *Cyathea* clade. Additional data might better resolve this problem, but it is interesting to note that in the shortest trees *Cy. howeana* is basal to all New World members of the *Cyathea* clade. Thus it might be that, as in the *Alsophila* clade, the New World species are more derived than those of the Old World.

In the *Sphaeropteris* clade, there are 14 species belonging to *Sphaeropteris* subgenus *Sphaeropteris* section *Sphaeropteris.* Within this section, the cpDNA data provide evidence for two monophyletic groups. One, including two American species, *S. horrida* and *S. insignis,* plus *S. concinna* from Papua New Guinea, is present in 94% of the consensus trees, is supported by 1 syn-

apomorphy, and has a bootstrapping value of 62%. Tryon (1971), in a study of the American tree ferns allied to *S. horrida,* stated that, "The relation of the *S. horrida* group to species such as *S. medullaris* of New Zealand and *S. concinna* of New Guinea is so close that it must be accepted as a true (evolutionary) neotropic-paleotropic relationship." The cpDNA data support a close relationship of *S. horrida* and *S. insignis* to *S. concinna,* confirming, at least in part, Tryon's prediction. *Sphaeropteris brunei,* a member of the *S. horrida* group from Central America, appears to lie outside this group, as it lacks one mutation present in *S. horrida, S. insignis,* and *S. concinna.* However, *S. medullaris,* the most basal member of the *Sphaeropteris* clade, does not appear to be a close relative of the *S. horrida* group, as suggested by Tryon. The other monophyletic group, including 6 species from Papua New Guinea, *S. cooperi* from Queensland, and *S. robusta* from Lord Howe Island, is present in 96% of the consensus trees, is supported by 1 synapomorphy, and has a bootstrapping value of 52%. Although members of this group share only one synapomorphy, the cohesive geographical distribution suggests that it may be a natural evolutionary lineage restricted to Papuasia.

It is important to determine the relationships of the major clades to one another in order to resolve long standing arguments about the direction of morphological character evolution in the family. Conant et al. (1994), in a study of 23 species of Cyatheaceae, reported that trees in which the *Cyathea* and *Sphaeropteris* clades were positioned as derived sister groups were four to five steps shorter than alternative arrangements of the clades. Stein et al. (in press), in an analysis of 63 tree fern species, also found that trees with the *Cyathea* and *Sphaeropteris* clades as sister groups were more parsimonious than other arrangements, although in their study, the difference was only one or two steps. Adding still more species to the data matrix has undermined the support for one arrangement of the major clades over the others. In this study of 84 species of Cyatheaceae, no single arrangement of the major clades is most parsimonious. We are currently sequencing conserved genes to search for characters that will help to resolve the relationships of the three major clades.

In summary, we find that in two of the three major clades of the Cyatheaceae the basal-most members are from the Old World or are New World species that belong to a primarily Old World group (such as A. *salvinii*). It is premature to reach conclusions about the center of origin of the family because we have not yet included species from several floristically important regions of the paleotropics. The evidence available from the fossil record, particularly from the study of spores (Mohr and Lazarus, in press), suggests that the derived genus *Cnemidaria,* now restricted to the Neotropics, once had a much broader distribution in Antarctica, Australia, and Asia. Thus, current geographical distributions of tree fern taxa are not necessarily indicative of past histories. However, an improved understanding of the geographic distribution and biogeographic history of the Cyatheaceae depends on an appreciation of their phylogeny. Significant insights into the evolution of this group will only come from data sets representing both hemispheres, such as the results reported here.

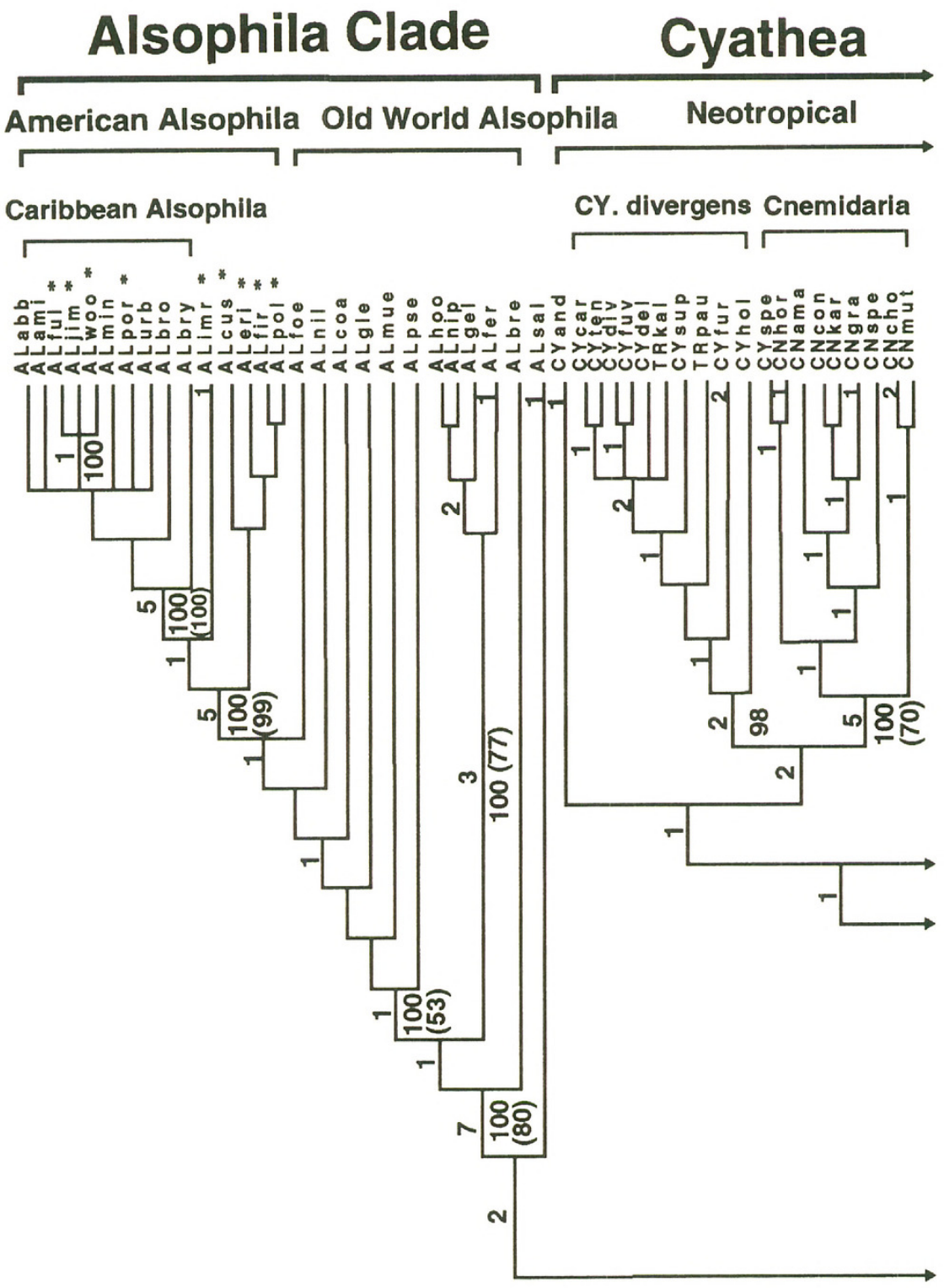
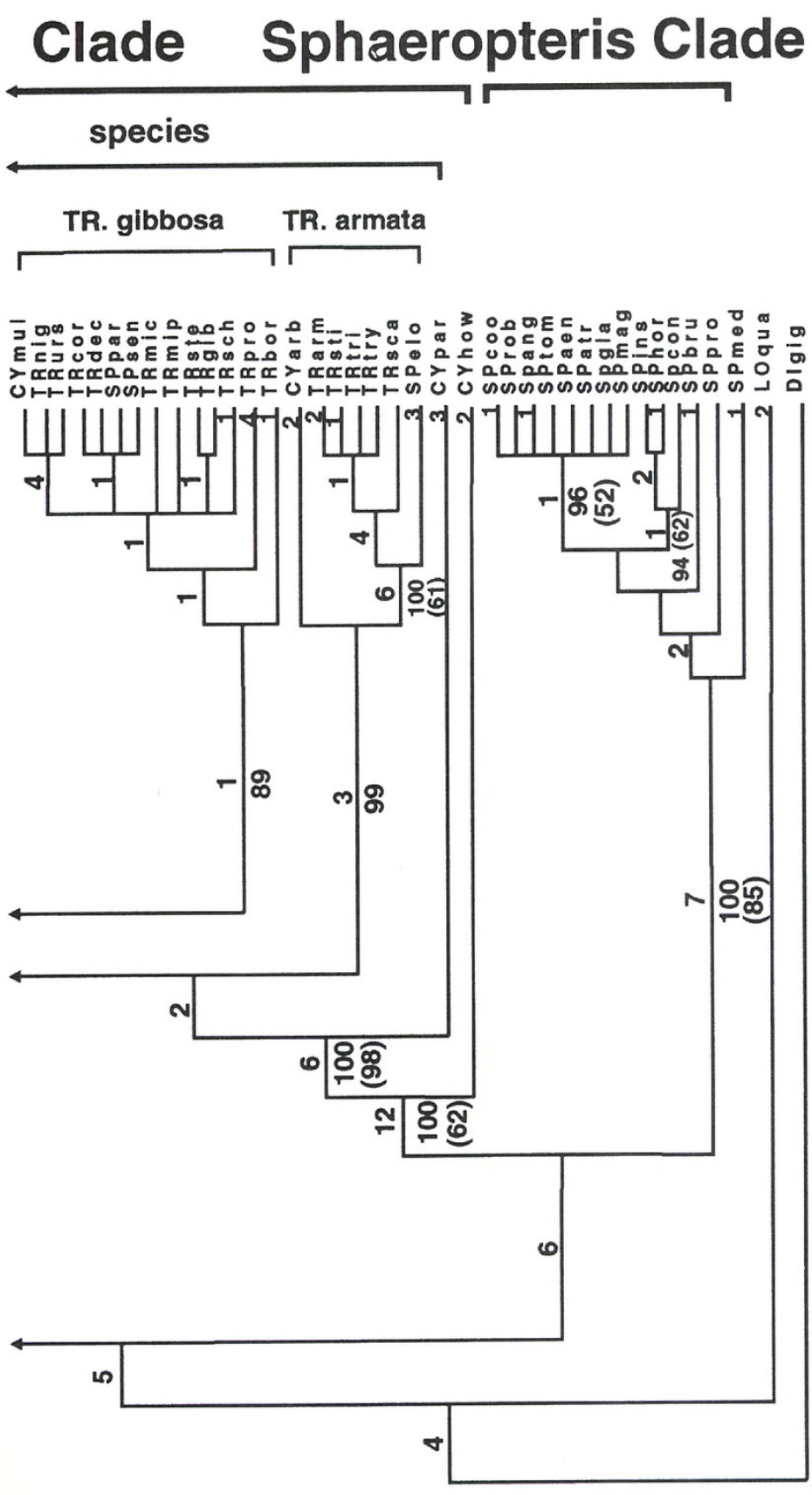


FIG. 1. A representative tree derived from an analysis of chloroplast DNA restriction site mutations in the Cyatheaceae. The length is 160 steps (excluding autapomorphies). The consistency index is 0.575, and the retention index is 0.939. The major clades and monophyletic groups discussed in the text are indicated with brackets at right. Species with asterisks in the *Alsophila* clade were included in the genus *Nephelea* by Tryon and Tryon (1982). The numbers above the nodes indicate the number of restriction site mutations. Numbers below the nodes are the mean frequency of monophyletic groups in 25 heuristic searches (each of 100 replications) using random addition sequences and bootstrapping values (in parentheses). See Table 1 for list of species names



that correspond to abbreviations. The tree was constrained so that all Cyatheaceae were together and outgroup species were excluded from the ingroup.

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