Aliso: A Journal of Systematic and Floristic Botany

Volume 17 | Issue 2 Article 4

1998

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Aliso, 17(2), pp. 131–144

CHEILANTHOID FERNS (PTERIDACEAE: CHEILANTHOIDEAE) IN THE SOUTHWESTERN UNITED STATES AND ADJACENT MEXICO—A MOLECULAR PHYLOGENETIC REASSESSMENT OF GENERIC LINES

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ABSTRACT

Cheilanthoids are the most commonly encountered fern species of the arid southwest and other xeric habitats throughout the world. *Cheilanthes, Notholaena, Pellaea,* and *Bommeria* are the best known southwestern genera, but some authors recognize segregate genera such as *Argyrochosma, Aspidotis, Astrolepis,* and *Pentagramma*. Others reject distinctions among some of these genera as artificial, leaving cheilanthoid generic concepts in a state of flux. This unsettled taxonomy is often attributed to morphological homoplasy associated with adaptation to xeric habitats, suggesting the need for new analyses that do not depend on potentially misleading morphology. Nucleotide sequences of the maternally inherited, chloroplast-encoded *rbcL* gene from 57 species that bear on the relationships of the cheilanthoids of the southwest were cladistically analyzed under the optimality criterion of maximum parsimony. The results provide new insights into phylogenetic relationships and generic circumscriptions of these ferns. Mexican *Llavea cordifolia* is rejected from the cheilanthoids, traditional *Cheilanthes, Notholaena,* and *Pellaea* are polyphyletic, and the segregations of *Argyrochosma, Aspidotis, Astrolepis,* and *Pentagramma* are supported. To assess confidence in these conclusions, results of the *rbcL*-based analysis are compared with those based on ITS sequences of biparentally inherited nuclear ribosomal DNA (nrDNA) for a subset of cheilanthoid taxa. These two data sets yield remarkably congruent topologies at shallower phylogenetic levels, suggesting that previous taxonomic problems in this group may indeed be attributable to difficulties in interpreting the taxonomic significance of morphological characters. Disagreement at deeper levels of the topologies suggests the need to incorporate data from less rapidly evolving nrDNA regions.

Key words: *Bommeria, Cheilanthes, ferns, ITS, nuclear ribosomal DNA, Notholaena, Pellaea, Pityrogramma,* Pteridaceae, *rbcL.*

INTRODUCTION

Cheilanthoid ferns are among the most fascinating elements of the flora of the southwestern United States and adjacent Mexico. In part this is because they defy the stereotypic view of ferns as plants of cool, mesic woodlands by thriving in the xeric and semixeric habitats that are at least seasonally characteristic of this region. The apogamous lifestyle (Gastony and Windham 1989) of many of these taxa minimizes the impact of hot, dry conditions on the relatively delicate gametophyte generation because apogamy both shortens the time from spore to sporophyte (Whittier 1970) and eliminates gametophytic dependence on free water for fertilization by motile sperm. Among the features thought to adapt many cheilanthoid sporophytes to such habitats are an indument of wax or insulating scales or trichomes on the leaves, reflexion of the leaf margin to form a false indusium over the sori, and the ability to die back to the rhizome and survive the driest periods in dormant condition. Convergence in adaptive morphology associated with xeric habitats is often credited with responsibility for legendary problems in assessing natural evolutionary lineages in cheilanthoids and for our consequent inability to develop an acceptable generic taxonomy (Tryon and Tryon 1973, 1982 p. 248; Lellinger 1989 p. 111).

Cheilanthoid ferns constitute subfamily Cheilanthoideae of the homosporous fern family Pteridaceae, a subfamily virtually worldwide in distribution but particularly richly developed in southwestern North America (Tryon and Tryon 1973; Tryon et al. 1990). Prior to newly available molecularly based insights into phylogenetic relationships in this group (Gastony and Rollo 1995), the most authoritative, current, worldwide taxonomy recognized 12 genera of cheilanthoid ferns (Table 1, adapted from Tryon et al. 1990).

Subfamily Cheilanthoideae is difficult to circumscribe morphologically (see key and description in Tryon et al. 1990, pp. 231–232, 240–241). In general, the sori are borne on the distal region of the veins, but in some cases they extend inward from the leaf margin. In most genera, the sori are protected by having parts or all of the margin of the leaflet reflexed over the sori to form a false indusium, and variant states of this character have been used to define the three most frequently encountered cheilanthoid genera in the southwest, *Cheilanthes, Pellaea,* and *Notholaena.* In

Table 1. Synopsis of the generic classification of cheilanthoid ferns (Pteridaceae subfamily Cheilanthoideae) according to the most recent worldwide treatment by Tryon et al. (1990), with the number of species included in the present study given in parentheses. Several species from beyond the southwestern U.S. and adjacent Mexico were included in a previous smaller data set by Gastony and Rollo (1995) and are incorporated into the present analysis to provide a broader phylogenetic context for the southwestern species.

CHEILANTHES Sw.: essentially worldwide in distribution, about 150 or more species. Segregate genera not accepted by Tryon et al. (1990) but discussed in this paper are *Aleuritopteris* Fée, *Cheiloplecton* Fée, *Mildella* Trevis., *Sinopteris* C. Chr. & Ching, and *Aspidotis* Copel. (20, including representatives of the preceding generic segregates).

ADIANTOPSIS Fée: tropical America, about seven species. Old World species sometimes referred to *Adiantopsis* were consigned to *Cheilanthes* by Tryon and Tryon (1982). Alan R. Smith (pers. comm.) notes a potential relationship between *A. madagascariensis* (Baker) Diels and American *A. chlorophylla* (Sw.) Fée but views the resemblance of *A. linearis* Bonap. to American *Adiantopsis* as superficial. The relationships of these two Madagascar species have not yet been tested with molecular data. None occur in the southwest, (0).

NOTHOLAENA R. Br.: America, about 40 species. Two sections are recognized: sect. *Notholaena* with 22 species and sect. *Argyrochosma* with 17 species, the latter elevated to generic rank by Windham (1987) but not accepted by Tryon et al. (1990), (9, including 4 of sect. *Argyrochosma*).

PELLAEA Link: North and South America, Africa and eastward to India and New Zealand; also northeastern Spain and the Azores, about 35 species. Four sections are recognized: sect. *Pellaea* with about 16 species in America and one in Africa, sect. *Ormopteris*, with six species of South America, sect. *Holcochlaena* with about 10 species from Africa to India and Ceylon, and sect. *Platyloma* with three species from India and Ceylon east to Tasmania, New Zealand, and New Caledonia, (12, including representatives of all sections except *Ormopteris*).

DORYOPTERIS J. Sm.: tropical America to the Old World, eastward to New Guinea, 25 species. Two sections are recognized, sect. *Lytoneuron* with 13 species in America and sect. *Doryopteris* with seven in America and five from Madagascar east to New Guinea, (1 of sect. *Doryopteris*).

PARACETERACH (F. v. Mueller) Copel.: Old World in the Canary Islands and Madeira, southern Europe, Ethiopia, northern India to China, and Australia, seven species, (0).

HEMIONITIS L.: tropical America, seven species, (3).

BOMMERIA E. Fourn.: America, from the southwestern U. S. to Costa Rica, four species (2).

TRACHYPTERIS H. Christ: South America and the Galapagos Islands, and Madagascar, three species (1).

CONIOGRAMME Fée: Old World from tropical Africa east to the Himalayas, China, Java, and Japan, and in the Pacific to the Hawaiian Islands and Samoa, 20 or more species (1).

CRYPTOGRAMMA R. Br.: primarily boreal and austral, in the U. S. and Canada northwest to Alaska, in Europe from Spain to the Balkans, Scandinavia, and the Urals, and eastward to the Himalayas, China, and northeast Asia, also southern Chile and adjacent Argentina, two species (0).

LLAVEA Lag.: Mexico and Guatemala, one species (1).

Cheilanthes the reflexed margin is said to be discontinuous, in *Pellaea* continuous, and in *Notholaena* the margin is said to be not reflexed over the sori or only slightly so. Unfortunately these characters of leaf margin morphology are so variable as to render these circumscriptions meaningless. Gastony and Rollo (1995) reviewed the frustration of pteridologists from the time of Copeland (1947) who have tried to decipher natural evolutionary lineages that can be circumscribed as genera, subgenera, etc. That history of frustration is synopsized by a statement in the most recent taxonomic summary of cheilanthoid ferns on a worldwide basis: ". . . evolutionary lines within the subfamily and the relations of these to other groups in the Pteridaceae are scarcely known" (Tryon et al. 1990, p. 241). Molecular insights into cheilanthoid phylogeny by Gastony and Rollo (1995) suggest that we do not have good generic characters in cheilanthoids because we do not have good genera in cheilanthoids.

Mindful of the attribution of taxonomic problems in cheilanthoids to homoplastic adaptive morphology (Tryon and Tryon 1973, 1982; Lellinger 1989), Gastony and Rollo (1995) used newly available molecular data, presumably unaffected by adaptation to arid habitats, to gain new insight into evolutionary lines within cheilanthoids. They included 26 ingroup taxa in their study of cheilanthoid phylogeny based on nucleotide sequences of the *rbcL* gene. The present study more than doubles their database to include 53 ingroup taxa. Many of these are directly relevant to the flora of the southwest because they occur in this region. The relevance of others is indirect by providing a more complete context in which to assess relationships among these southwestern species. In addition, this study presents a first attempt to assess confidence in the results of the *rbcL* data set by comparing a subset of the *rbcL* results with those of a comparable molecular data set derived from nuclear DNA. Gastony and Rollo (1996) provided a preliminary report of these comparative findings based on analysis of a variant and incompletely aligned *rbcL*/ITS data set for some of the species in the present study.

MATERIALS AND METHODS

This study provisionally follows the taxonomy of the most recent worldwide treatment of cheilanthoid ferns by Tryon et al. (1990), summarized in Table 1. Sporophytes collected directly from nature or grown from spores taken from specimens provided by colleagues were the sources of DNAs used to generate the nucleotide sequences in this study (Table 2), except that the sequence of *Cheilanthes* (*Doryopteris*) *concolor* was supplied by Mitsuyasu Hasebe via GenBank accession U05621. DNA extractions, polymerase chain reaction (PCR) amplifications, sequencing reactions, *rbcL* primers, and *rbcL* outgroup selection were as reported by Gastony and Rollo (1995). Primers used in PCR amplifications and sequencing of the internal transcribed spacer (ITS) regions of nuclear ribosomal

DNA are presented in Table 3; in addition M13 primers were used in sequencing single-stranded cloned templates as noted in Gastony and Rollo (1995).

All 52 taxa in Table 2 (plus *Cheilanthes concolor* noted above) were used as the ingroup in the *rbcL* analysis, including *Pityrogramma calomelanos* and *P. trifoliata* which were treated as part of the ingroup in order to test the affinities of *P. triangularis,* as discussed below. Maximum parsimony analysis was carried out with PAUP*4.0d54, a test version of the forthcoming new release of PAUP (see Swofford 1993), on a Power Macintosh 8500/120 (using TBR branch swapping, MULPARS, and steepest descent). A subset of these species (identified with asterisks in Table 2) was used to generate ITS sequence data. Ingroup and outgroup designation for the ITS analysis is based on the results of the *rbcL* analysis in Fig. 1, with the clade of species 1–26 (*Pellaea andromedifolia* to *Cheilanthes lanosa*) serving as the ingroup and the well defined sister clade of species 27–46 (*Notholaena sulphurea* to *Pityrogramma triangularis*) serving as outgroup. From these clades ITS sequences were obtained from ingroup species 1, 2, 4–14, 16–19, 21, and 23, and the outgroup was represented by species 27, 28, 35, and 38. By using *rbcL* sequences from this same subset of Fig. 1 species and conducting independent maximum parsimony analyses with PAUP*4.0d54 (with TBR branch swapping, MULPARS, and steepest descent), cladistic analyses of these two molecular data sets were directly comparable. Because of numerous insertions and deletions (indels), the ITS sequences required alignment. This was carried out with Clustal W within the software program SeqPup (Gilbert 1995; <ftp://iubio.bio.indiana.edu/molbio/seqpup/>), followed by visual inspection and a few realignments by hand. Two regions (8 base pairs and 9 base pairs, respectively) were excluded from the analyzed ITS sequences because their alignment appeared particularly arbitrary. Indels were not used as characters in the ITS data set. Analyses of the full *rbcL* data set and of the ITS and *rbcL* subsets required heuristic searches, and 1000 random addition sequence replicates were used in each analysis to avoid being confined to potential suboptimal islands of most parsimonious trees. Support for clades resolved by the foregoing basic analyses was determined by the bootstrap option of PAUP, using a random addition sequence with TBR, MULPARS, and steepest descent suboptions in effect. Bootstrapping the full *rbcL* data set used 100 random addition replicates; 500 random addition replicates were used in bootstrapping the ITS subset and its comparable *rbcL* subset.

RESULTS AND DISCUSSION

Full rbcL *Data Set*

During the first random addition sequence replicate, PAUP found a single most parsimonious tree (Fig. 1) of 1462 steps with a consistency index (CI) of 0.423, a retention index (RI) of 0.614, and a rescaled consistency index of 0.260. No new minimal trees were found during any of the next 999 random addition sequence replicates, suggesting that local optima insularity (Maddison 1991) is not a problem in this data set and that all of the most parsimonious trees have been found (Swofford 1993, p. 35). Of the 1321 *rbcL* nucleotide positions used as characters, 862 were constant, 143 were variable but parsimony-uninformative, and 316 were parsimony-informative.

Taxa rejected from the cheilanthoid ingroup.—As noted by Gastony and Rollo (1995) *Coniogramme* was included in subfamily Cheilanthoideae by Tryon et al. (1990) but was placed well outside the cheilanthoids and sister to all other Pteridaceae plus Vittariaceae in the global analysis of fern phylogeny by Hasebe et al. (1995). This indicates that *Coniogramme* should be excluded from the cheilanthoids and instead entered as a distant member of the outgroup. In order not to prejudice the results of PAUP's analysis of this much larger data set of cheilanthoids, however, our searches were conducted with *Coniogramme* alternatively specified as a member of the outgroup or as a member of the cheilanthoid ingroup. Results were identical under both alternatives. Just as shown by Hasebe et al.'s (1995) global *rbcL* analysis involving many fewer cheilanthoid taxa, *Coniogramme* (Fig. 1, species 53) is rejected from the cheilanthoids and placed within the outgroup in the present analysis. Similarly, as in the previous analysis with a smaller data set of cheilanthoid taxa (Gastony and Rollo 1995), PAUP was unable to place *Llavea* (species 52) in the ingroup. Instead *Llavea* is sister to *Coniogramme* in the outgroup in a clade strongly supported by 26 synapomorphies and a 100% bootstrap value. This result for *Llavea* was obtained whether *Coniogramme* was defined as part of the outgroup or was treated as a member of the cheilanthoid ingroup.

Yatskievych et al. (1990) recently reassessed the taxonomy of the genus *Pityrogramma,* generally placed outside the cheilanthoids in Pteridaceae subfamily Taenitidoideae. They concluded that the *P. triangularis* complex of the southwestern United States and adjacent Mexico merits recognition as an independent genus *Pentagramma,* and they formalized a suggestion by Tryon and Tryon (1982 p. 218) that this southwestern complex be transferred to subfamily Cheilanthoideae. To test this new taxonomy, three species of *Pityrogramma* (including southwestern *P. triangularis*) were included in the present analysis as elements of the cheilanthoid ingroup. PAUP's analysis could not maintain *P. calomelanos* and *P. trifoliata* (species 54, 55) as members of a monophyletic ingroup. Instead it placed them in the outgroup sister to

Table 2. Sources of living material of ingroup species and three putative outgroup species of *Pityrogramma* used to generate *rbcL* sequences for this study. Outgroup species *Adiantum pedatum, Ceratopteris thalictroides, Platyzoma microphyllum, Coniogramme japonica,* and *Vittaria lineata* and ingroup species *Cheilanthes concolor* were obtained as in Gastony and Rollo (1995). Taxonomy follows that of Tryon et al. (1990). Voucher herbarium specimens are deposited at the Indiana University herbarium (IND) except for *Trachypteris pinnata* in Brazil (SJRP). Asterisks following the species names identify the subset of species constituting the ingroup (*) and outgroup (**) for the ITS and directly comparable *rbcL* analyses as explained more fully in the text.

Species	Provenance	Collector(s) & number	Collection date
Bommeria ehrenbergiana (Klotzsch) Underw.	Hidalgo, Mexico	*Yatskievych & Gastony 89-203*	17 Jul 1989
B. hispida (Mett. ex Kuhn) Underw.	Jeff Davis Co., Texas	*Gastony 87-3A*	14 Mar 1987
Cheilanthes aemula Maxon*	Tamaulipas, Mexico	*Yatskievych & Gastony 89-222*	18 Jul 1989
C. alabamensis (Buckley) Kunze	Nuevo León, Mexico	*Yatskievych & Gastony 89-226A*	19 Jul 1989
C. albofusca Baker**	Yunnan, China	*Li & Xiang S-4L*[a]	18 Jan 1990
C. allosuroides Mett.*	Jalisco, Mexico	*Yatskievych & Gastony 89-237*	21 Jul 1989
C. aurea Baker	Oaxaca, Mexico	*Yatskievych & Gastony 89-256*	26 Jul 1989
C. beitelii Mickel*	Oaxaca, Mexico	*Yatskievych & Gastony 89-265*	26 Jul 1989
C. bonariensis (Willd.) Proctor*	Michoacán, Mexico	*Yatskievych & Gastony 89-246*	23 Jul 1989
C. californica (Hook.) Mett.	Monterey Co., California	*Kirkpatrick s.n.*	7 May 1989
C. chipinquensis Knobloch & Lellinger*	Nuevo León, Mexico	*Knobloch 1996B MSC*[a]	16 May 1964
C. cochisensis (Goodd.) Mickel*[b]	Coahuila, Mexico	*Yatskievych & Gastony 89-227*	19 Jul 1989
C. decora (Brack.) R. M. Tryon & A. F. Tryon	Kauai, Hawaii	*Flynn s.n.*	29 Sep 1989
C. duclouxii (H. Christ) Ching in C. Chr.	Yunnan, China	*Li & Xiang S-18L*[a]	15 Feb 1990
C. horridula Maxon*	Nuevo León, Mexico	*Gastony 90-10-1*	26 Oct 1990
C. intramarginalis (Kaulf. ex Link) Hook. var. *serratifolia* (Hook. & Baker) Hall & Lellinger	Hidalgo, Mexico	*Yatskievych & Gastony 89-207*	17 Jul 1989
C. lanosa (Michx.) D. C. Eaton	Martin Co., Indiana	*Hegeman s.n.*	17 Jun 1989
C. lendigera (Cav.) Sw.	Cochise Co., Arizona	*Yatskievych 89-432*	28 Dec 1989
C. leucopoda Link	Tamaulipas, Mexico	*Gastony 90-10-4A*	27 Oct 1990
C. myriophylla Desv.	San Luis Potosí, Mexico	*Brown 83-31-4*	25 Jan 1983
C. rigida (Sw.) Mett.	Puebla, Mexico	*Yatskievych & Gastony 89-284*	28 Jul 1989
C. rufa D. Don	Yunnan, China	*Li S-20L*[a]	15 Feb 1990
Doryopteris pedata (L.) Fée var. *palmata* (Willd.) Hicken	Hidalgo, Mexico	*Riba 1746*	15 Jul 1989
Hemionitis elegans Davenp.	Oaxaca, Mexico	*Yatskievych & Gastony 89-258*	26 Jul 1989
H. levyi E. Fourn.	Oaxaca, Mexico	*Yatskievych & Gastony 89-253*	25 Jul 1989
H. palmata L.**	Provenance unknown	*Greenhouse specimen*	Unknown
Llavea cordifolia Lag.	Nuevo León, Mexico	*Yatskievych & Gastony 89-224A*	19 Jul 1989
Notholaena aschenborniana Klotzsch	Tamaulipas, Mexico	*Yatskievych & Gastony 89-221*	18 Jul 1989
N. candida (M. Martens & Galeotti) Hook. var. *copelandii* (C. C. Hall) R. M. Tryon**	Tamaulipas, Mexico	*Gastony 90-10-2*	26 Oct 1990
N. dealbata (Pursh) Kunze*	McDonald Co., Missouri	*Yatskievych & Smith 90-233*[c]	11 Jul 1990
N. delicatula Maxon & Weath.*	Nuevo León, Mexico	*Yatskievych & Gastony 89-229*	20 Jul 1989
N. fendleri Kunze	Sandoval Co., New Mexico	*Sullivan, Drummond, & Fitzpatrick s.n.*	3 Jun 1989
N. pilifera R. M. Tryon*	Morelos, Mexico	*Yatskievych & Gastony 89-287*	29 Jul 1989
N. rosei Maxon	Oaxaca, Mexico	*Yatskievych, Windham, & Ranker 83-453*	28 Dec 1983
N. sulphurea (Cav.) J. Sm.**	Puebla, Mexico	*Yatskievych & Gastony 89-248*	24 Jul 1989
N. trichomanoides (L.) Desv.	Provenance unknown	Amer. Fern Soc. spore exch. #660[a]	Unknown
Pellaea andromedifolia (Kaulf.) Fée*	Orange Co., California	*Gastony 86-8*	15 Mar 1986
P. boivinii Hook.	Prov. Fianarantsoa, Madagascar	*Liede & Conrad 2626*	30 Jan 1990
P. breweri D. C. Eaton*	Moffat Co., Colorado	*Naumannn 257*	30 Jun 1988
P. calidirupium Brownsey & Lovis*	Christchurch, New Zealand	*Lovis s.n.*	Unknown
P. calomelanos (Sw.) Link	Provenance unknown	Amer. Fern Soc. spore exch. #661[a]	Unknown
P. cordifolia (Sessé & Moc.) A. R. Sm.*	Jeff Davis Co., Texas	*Gastony 87-3*	14 Mar 1987
P. glabella Mett. ex Kuhn var. *missouriensis* Gastony*	Carter Co., Missouri	*Gastony 83-41*	14 Mar 1983
P. notabilis Maxon*	Nuevo León, Mexico	*Gastony & Yatskievych 86-22*	16 Sep 1986
P. ovata (Desv.) Weath.	Llano Co., Texas	*Gastony & Yatskievych 86-45*	4 Oct 1986
P. pringlei Davenp.*	Morelos, Mexico	*Gastony 87-11-16*	24 Nov 1987

Table 2. Continued.

Species	Provenance	Collector(s) & number	Collection date
P. rotundifolia (Forst.) Hook.*	Indiana University greenhouse specimen	*Gastony s.n.*	Unknown
P. rufa A. F. Tryon*	Boonstevlei, South Africa	*Bean B5 & s.n.5*	Nov 1985
Pityrogramma calomelanos (L.) Link	Oaxaca, Mexico	*Yatskievych & Gastony 89-251*	25 Jul 1989
P. triangularis (Kaulf.) Maxon var. *maxonii* Weath.	Cochise Co., Arizona	*Yatskievych 89-431*	28 Dec 1989
P. trifoliata (L.) R. M. Tryon	Oaxaca, Mexico	*Yatskievych & Gastony 89-252*	25 Jul 1989
Trachypteris pinnata (Hook. f.) C. Chr.	Prov. Santa Cruz, Bolivia	*Windisch 6088* SJRP	18 May 1991

[a] DNA was extracted from a sporophyte grown from the spores from this source.
[b] In *Astrolepis* this is *A. cochisensis* (Goodd.) Benham & Windham subsp. *chihuahuensis* Benham (Benham 1992).
[c] Also vouchered at MO.

Ceratopteris of Pteridaceae subfamily Ceratopteridoideae (Fig. 1), thereby supporting their traditional placement outside of subfamily Cheilanthoideae. Clarification of their relationship to other members of traditional subfamily Taenitidoideae must await a broader study of that group. These two *Pityrogramma* species are very strongly united by 50 synapomorphies and a 100% bootstrap value, but their close association specifically with *Ceratopteris* should be regarded as only provisional, given the very long autapomorphic branch length of *C. thalictroides* and the weak bootstrap support for this outgroup clade. *Pityrogramma triangularis* (species 46), on the other hand, did nest robustly within the cheilanthoid ingroup, just above the basalmost ingroup clade of *Bommeria* species (47–49) in Fig. 1. This very strongly supports removal of the *P. triangularis* complex from *Pityrogramma* to *Pentagramma* and the placement of *Pentagramma* in subfamily Cheilanthoideae.

Pellaea.—*Pellaea* is represented in this analysis by 12 species assigned to three sections by Tryon and Tryon (1982). Species 1–5 and 8–10 in Fig. 1 are traditionally placed in *Pellaea* section *Pellaea.* With the exception of South African *P. rufa* (species 2) and Chilean *P. myrtillifolia* (not included in this analysis), section *Pellaea* is primarily distributed in the southwestern United States and Mexico, although the ranges of some species of this section extend beyond that region (Tryon 1957, 1968; Tryon and Britton 1958). *Pellaea rotundifolia* and *P. calidirupium* (species 6, 7) are New Zealand species of section *Platyloma.* In separate clades in the lower half of Fig. 1 are African *P. boivinii* (37) and *P. calomelanos* (45), both of section *Holcochlaena. Pellaea* in the traditional sense of Tryon et al. (1990) is clearly polyphyletic. As noted by Tryon and Tryon (1982), each section of *Pellaea* was at one time recognized as a separate genus. This *rbcL* study suggests that if taxonomy is to reflect phylogeny, at least some of these sections will again require generic recognition.

Even largely southwestern *Pellaea* section *Pellaea* is polyphyletic. Species 1–5 of this section at the top of Fig. 1 are sister to a clade containing *Pellaea* section *Platyloma* (species 6, 7) from New Zealand. The remaining three species of *Pellaea* section *Pellaea* (8–10) are sister to *Cheilanthes* species 11 and 12 recently segregated as the genus *Astrolepis,* whose distribution ranges from California to Oklahoma and southward along the Cordillera to Argentina, with outlying stations in Georgia and the West Indies (Benham and

Table 3. Attributes of PCR amplification and sequencing primers used in the ITS portion of this study. P indicates PCR primer; S indicates sequencing primer.

Primer		Direction	5′ sequence 3′	Position from 5′ end of (subunit)
P/S	ITS5p[a]	F	GGA AGG AGA AGT CGT AAC AAG G	1767–1788 (18S)
P	C28C	R	GCT ATC CTG AGG GAA ACT TCG G	974–995 (26S)
S	ITS3p[b]	F	GCA TCG ATG AAG AAC GTA GC	35–54 (5.8S)
S	ITS2p[c]	R	GCT ACG TTC TTC ATC GAT GC	35–54 (5.8S)
S	RJ5.8S	R	CTC GAT GGA ACA CGG GAT TCT GC	79–101 (5.8S)
S	ITS4[d]	R	TCC TCC GCT TAT TGA TAT GC	41–60 (26S)

[a] Corrected from the original fungal primer of White et al. (1990) to be plant specific at the sixth (T → G) and eighth (A → G) positions from the 5′ end.
[b] Corrected from the original fungal primer of White et al. (1990) to be plant specific at the seventeenth (C → T) position from the 5′ end.
[c] Corrected from the original fungal primer of White et al. (1990) to be plant specific at the fourth (G → A) position from the 5′ end.
[d] Unchanged from White et al. (1990)

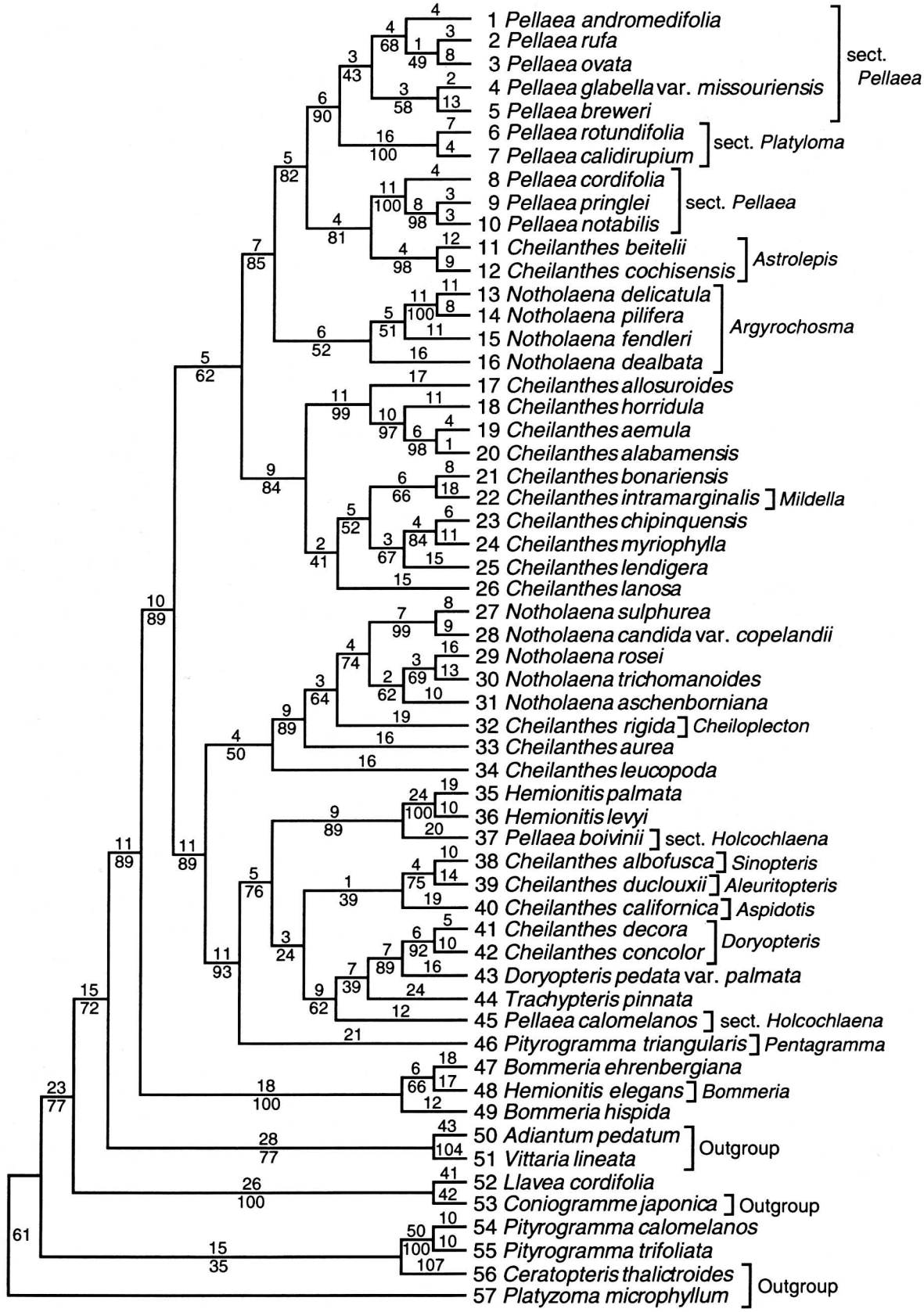

Fig. 1. The single most parsimonious tree based on *rbcL* nucleotide sequences of the taxa in Table 2. The number of synapomorphies supporting each clade and autapomorphic branch lengths for terminal taxa are indicated above the lines. Numbers below lines represent bootstrap percentages based on 100 replicates. Species are numbered 1–57 to facilitate discussion in the text. Taxonomy and nomenclature follow the treatment of Tryon et al. (1990), which presents the most recent comprehensive treatment of cheilanthoids on a worldwide basis. Information at brackets indicates sectional taxonomy within *Pellaea,* alternative generic taxonomies discussed in the text, and species designated as the outgroup. Note that maximum parsimony was unable to place *Llavea cordifolia* (52), *Pityrogramma calomelanos* (54), or *P. trifoliata* (55) as members of a monophyletic ingroup, relegating them instead to positions within the outgroup.

Windham 1992). These authors thought the base chromosome number of $x = 29$ common to *Astrolepis* and *Pellaea* arose independently in the two groups because it was not previously apparent that *Pellaea* and *Astrolepis* are phylogenetically closely related. The *rbcL* phylogeny in Fig. 1 indicates that in this case $x = 29$ is a synapomorphy resulting from common ancestry.

Argyrochosma *and* Notholaena.—The entire upper clade discussed above (species 1–12 of Fig. 1) is sister to a group of predominantly southwestern and Mexican species until recently considered species of *Notholaena* section *Argyrochosma* (Tryon and Tryon 1982). Windham (1987) segregated these *Notholaena* species as the genus *Argyrochosma,* partly because of their unique chromosome base number of $x = 27$, and he hypothesized that *Argyrochosma* is the sister group of southwestern *Pellaea.* This sister relationship is supported by *rbcL.* Furthermore, *rbcL* deeply separates these *Argyrochosma* species from the rest of *Notholaena* (species 27–31) sensu Tryon and Tryon (1982), robustly supporting Windham's generic taxonomy.

Tryon's (1956) revision of *Notholaena* was based on unpublished work by Charles A. Weatherby that took a broad view of the genus. Tryon and Tryon (1982) removed the first 21 species of Tryon's (1956) revision to *Cheilanthes.* Among those 21 excised species are *C. beitelii* and *C. cochisensis* (Fig. 1, species 11, 12) that this analysis shows to have been correctly removed from *Notholaena* because they nest with the *Pellaea* species toward the top of the tree, deeply removed from the *Notholaena* clade represented by species 27–31. As discussed above, these two species should also be removed from *Cheilanthes* to *Astrolepis* as done by Benham and Windham (1992) and Benham (1992). This analysis also shows that another of the 21 excised species (*Notholaena aurea* (Poir.) Desv.) was correctly removed to *Cheilanthes* as *C. bonariensis* (Fig. 1, species 21), as discussed below. Species 27–31 of Fig. 1, considered elements of *Notholaena* section *Notholaena* by Tryon and Tryon (1982), form a fairly well supported clade to which three species of *Cheilanthes* discussed below are successively sister.

Cheilanthes *sensu lato.*—*Cheilanthes* is a complex genus of 150 or more species and is worldwide in distribution according to its last comprehensive treatment by Tryon et al. (1990). *Cheilanthes* in this sense is represented by species 11, 12, 17–26, 32–34, and 38–42 in Fig. 1. Species 11 and 12 are those segregated as *Astrolepis* discussed above, a clearly appropriate generic segregation by Benham and Windham (1992). Species 17–26 are American, largely southwestern, species of *Cheilanthes* and are discussed below. *Cheilanthes* species 32–34 and 38–42 are deeply separated from the foregoing in a clade robustly supported by 11 synapomorphies and a 89% bootstrap value. Of these, species 32–34 are American and are with varying degrees of confidence associated with *Notholaena* (species 27–31). *Cheilanthes rigida* (32) of Mexico is one of several species whose affinities within *Cheilanthes* were unclear to Tryon and Tryon (1982). It is sometimes recognized as the genus *Cheiloplecton,* one of the segregate genera not accepted by Tryon et al. (1990). *Cheilanthes aurea* (33) of Mexico, has a yellow wax on the underside of its leaves, not unlike some species of *Notholaena* with which it shares this clade. *Cheilanthes leucopoda* (34) of the Edwards Plateau of Texas and of Mexico is another of the several species whose affinities within the genus were unclear to Tryon and Tryon (1982). A morphological doctoral study by Reeves (1979) placed it into *Cheilanthes* subgenus *Cheilanthes* along with *C. lanosa* (26) in the deeply separated clade above it. The long autapomorphic branch lengths of species 32–34 suggest that the closest affinities of these species are not yet determined and that incorporation of additional species may clarify their affinities. *Cheilanthes* species 38, 39 are Asian and are segregated by some authors as separate genera *Sinopteris* and *Aleuritopteris,* respectively, but their generic segregation from *Cheilanthes* was not accepted by Tryon et al. (1990). Species 40 is *Cheilanthes californica,* maintained in *Cheilanthes* by Tryon et al. (1990), but often recognized as one of the three California species of *Aspidotis* (Smith 1975). Its association with the two Asian species is very weakly supported in this analysis and will probably be modified by incorporation of additional species. Hawaiian *Cheilanthes decora* (41) and pantropical *C. concolor* (42) were transferred to *Cheilanthes* from their previous placement in *Doryopteris* (Tryon 1942) by Tryon and Tryon (1982). Their strong association with *Doryopteris pedata* var. *palmata* (43) indicates that their true affinity is with *Doryopteris* rather than with *Cheilanthes* and that their 1982 transfer should be reversed. Clearly *Cheilanthes* in the broad sense of Tryon et al. (1990) is polyphyletic and warrants fragmentation into several genera.

This *Cheilanthes/Doryopteris* alliance (species 41–43) is sister to *Trachypteris pinnata* (44). *Trachypteris* is locally Andean with disjunct distributions in Brazil and the Galapagos and with a putatively congeneric species (*T. drakeana* C. Chr.) in Madagascar (Tryon and Tryon 1982). The pedate form of the mature lamina of Andean *T. induta* and the typically pedate but heteroblastic and unusually variable lamina architecture of *Doryopteris* were noted by Tryon and Tryon (1982). The *rbcL* cladistic relationship of species 41–44 (Fig. 1) suggests that this leaf architecture may be synapomorphic, although it must be noted that bootstrap support for this clade is quite weak. Tryon and Tryon (1982) also hypothesized a relationship between *Doryopteris* and *Pellaea*, particularly citing features of

South American *Pellaea pinnata* (of *Pellaea* section *Ormopteris,* not included in this analysis). The cladistic relationship between *Pellaea calomelanos* (45) and the *Doryopteris/Trachypteris* alliance (41–44) at first suggests some degree of molecular support for their observations. However, as discussed above, *Pellaea* as circumscribed by Tryon and Tryon (1982) is polyphyletic. Of the *Pellaea* species examined here, only *P. calomelanos* (species 45 of Fig. 1) relates to the *Doryopteris/Trachypteris* alliance, and it is deeply separated from its fellow member of *Pellaea* section *Holcochlaena* (*P. boivinii,* species 37 of Fig. 1) and from *Pellaea* sections *Pellaea* and *Platyloma* (species 1–10 at the top of Fig. 1).

Southwestern Cheilanthes.—The largely southwestern species of *Cheilanthes* included in this analysis (species 17–26) form their own clade that is fairly strongly supported by nine synapomorphies and a 84% bootstrap value. Species 18–20 have been recognized as part of the "*alabamensis* group" (Windham and Rabe 1993) in part because of their synapomorphic base chromosome number of $x = 29$, whereas $x = 30$ is typical of *Cheilanthes. Cheilanthes allosuroides* (17; widespread on dry, rocky slopes in Mexico) is very strongly grouped with the three species of the *alabamensis* group (18–20) in this analysis. We are unaware of a well-documented chromosome count for *C. allosuroides* but anticipate that it may be based on 29 rather than 30, given its *rbcL* relationship with the *alabamensis* group seen here. Its potential membership in the *alabamensis* group is supported by the observation of Mickel and Beitel (1988) that it shares morphological features with *C. notholaenoides* and *C. cucullans,* which are species of the *alabamensis* group according to Reeves (1979). *Cheilanthes bonariensis* (21), ranging from Arizona to Texas and southward to South America and the West Indies, was known as *Notholaena aurea* until it was transferred to *Cheilanthes* by Tryon and Tryon (1982). The wisdom of this transfer is endorsed by *rbcL*'s placement of species 21 deeply within *Cheilanthes.* Mexican *Cheilanthes intramarginalis* (22) is interpreted by some as a member of the segregate genus *Mildella* (Hall and Lellinger 1967), but *rbcL* (Fig. 1) indicates that that taxonomy would render this group of southwestern *Cheilanthes* paraphyletic. Species 23–25 are southwestern and Mexican species treated by Reeves (1979) as members of *Cheilanthes* subgenus *Physapteris,* a monophyletic group in this analysis. *Cheilanthes lanosa,* of Texas and the eastern U.S., was said by Reeves (1979) to represent *Cheilanthes* subgenus *Cheilanthes,* the subgenus to which he also attributed *C. leucopoda* (34) of the *Notholaena* clade. According to *rbcL* (Fig. 1) these two species cannot represent a monophyletic subgenus.

Bommeria.—*Bommeria* species (47–49) constitute the basalmost clade in the cheilanthoids. Gastony and Rollo (1995) noted that *rbcL* strongly supports the transfer of *Hemionitis elegans* to *Bommeria* originally proposed by Ranker (1990) and Ranker and Haufler (1990). The present analysis adds *Hemionitis palmata* and *Bommeria hispida* (the only southwestern species of *Bommeria* north of Mexico) to the species of these genera analyzed by Gastony and Rollo (1995). *Hemionitis elegans* remains robustly nested within *Bommeria* (species 47–49), forming a clade supported by 18 synapomorphies and 100% bootstrap support whereas *H. palmata* and *H. levyi* (species 35, 36) form their own very strong grouping in a deeply separated clade.

Assessing Confidence in the rbcL *Phylogeny*

Does analysis of *rbcL* nucleotide sequences provide the long-sought key to understanding phylogenetic lineages and generic circumscriptions in cheilanthoid ferns, or does *rbcL* simply replace our unsatisfactory grasp of cheilanthoid phylogeny based on morphology with a different but equally impaired view based on molecular data? This question needs to be addressed because of a potential problem with phylogenetic reconstruction based on chloroplast DNA (cpDNA). Although the lineages of sexually reproducing species are based on biparental inheritance, cpDNA phylogenies, including those based on the cpDNA *rbcL* gene, are maternal, biparental, or paternal depending on how the chloroplast genome is inherited. Gastony and Yatskievych (1992) reviewed the paths of cpDNA inheritance in higher plants and demonstrated that the chloroplast genome is maternally inherited in cheilanthoid ferns. Work with angiosperms has shown that hybridization, introgression, and lineage sorting can yield erroneous reconstructions of species lineages based on the maternally inherited chloroplast genome (Rieseberg and Soltis 1991; Doyle 1992). The frequency of allopolyploid hybrid speciation in extant ferns is well known (Wagner 1954, 1973; Wagner and Wagner 1980), and it is reasonable to infer that such speciation has occurred in the past as well. This has led some to interpret the high chromosome numbers of ferns relative to those of angiosperms as a legacy of paleopolyploidy (reviewed in Gastony 1991). Reticulate evolution resulting from paleopolyploidy would not be tracked by the matrilineal chloroplast genome. Consequently cpDNA gene trees in ferns may be highly discordant with species trees based on nucleus-encoded biparentally inherited characters such as morphology.

One might therefore ask why we find unexpected *rbcL* phylogenetic relationships in cheilanthoids, such as the separation of southwestern *Pellaea* section *Pel-*

laea into two clades with one clade sister to *Pellaea* section *Platyloma* from New Zealand and the other clade sister to *Astrolepis* (Fig. 1). These may occur 1) because species relationships based on biparentally inherited, nucleus-encoded morphology are correct but matrilineal *rbcL* phylogenies do not track species relationships, or 2) because traditional morphological characters are homoplastic (perhaps resulting from adaptation to xeric habitats) or have been misinterpreted, yielding misleading information about species relationships, whereas *rbcL* is more insightful.

We can test these alternative explanations of unexpected findings without relying on potentially homoplastic morphological data by determining whether results based on matrilineal *rbcL* agree with those based on biparentally inherited, nucleus-encoded *molecular* data. The best nucleus-encoded molecular data source at present is nuclear ribosomal DNA (nrDNA). Because of their utility in angiosperms at the suprageneric level (Baldwin 1992) we analyzed the internal transcribed spacer (ITS) regions (ITS1 and ITS2) of nrDNA as a source of nuclear molecular data directly comparable to the data from *rbcL.* The structure of the 18S–26S nrDNA repeat unit including the ITS regions is illustrated by Baldwin (1992). In cheilanthoid ferns ITS1 is about 338 bp long and ITS2 is about 351 bp long. Concerted evolution renders the many copies of these genes homogeneous within individuals (Hillis et al. 1991), rendering them phylogenetically useful (Sanderson and Doyle 1992).

The cheilanthoid ingroup *rbcL* phylogeny in Fig. 1 provides a well defined hypothesis of *rbcL* relationships that can be tested with ITS data. The major clade from *Pellaea andromedifolia* to *Cheilanthes lanosa* (species 1–26) contains several of the unexpected taxon positionings noted earlier, and 19 of its species were used as a test ingroup from which ITS sequences were determined (Table 2). The well-defined sister clade from *Notholaena sulphurea* to *Pityrogramma* (*Pentagramma*) *triangularis* (27–46) provided the four species used as an outgroup (Table 2). Parallel analyses of this subset of taxa, based respectively on *rbcL* and ITS, yielded results that are directly comparable and permit us to determine whether molecular data from maternally and biparentally inherited genomes generate concordant or discordant conclusions about cheilanthoid phylogeny.

Subset analyses.—The *rbcL* analysis of this subset of species yielded a single most-parsimonious tree (Fig. 2A) in the first replicate. During the next 999 random addition sequence replicates, no new minimal trees were found, suggesting that local optima insularity is not a problem in this *rbcL* subset. This tree is 412 steps long (CI = 0.631 and RI = 0.690), and its topology is precisely the same as in the *rbcL* tree in Fig. 1 except that fewer species are included in Fig. 2A. Of the 1321 *rbcL* characters used, 1093 were constant, 85 variable characters were parsimony-uninformative, and 143 were parsimony-informative.

The ITS analysis of this same subset of species also yielded a single most-parsimonious tree (Fig. 2B) in the first replicate. No new minimal trees were found during the next 999 random addition sequence replicates, suggesting that local optima insularity is also not a problem in this data set. This tree is 961 steps long (CI = 0.633 and RI = 0.553). Of the 771 ITS characters used, 355 were constant, 171 variable characters were parsimony-uninformative, and 245 were parsimony-informative.

Direct rbcL *vs. ITS comparison.*—Comparison of Figs. 2A and 2B shows that both the *rbcL* and ITS data sets find the same terminal clades and in some cases the same clades at one or two levels deeper. Both data sets group the top four species of *Pellaea* section *Pellaea* (*P. andromedifolia* to *P. breweri*) in exactly the same way. Both data sets group *Pellaea rotundifolia* and *P. calidirupium* of section *Platyloma.* Both identically group the remaining three species of *Pellaea* section *Pellaea* (*P. cordifolia* to *P. notabilis*), the two species of *Cheilanthes/Astrolepis,* the three species of *Notholaena/Argyrochosma,* and the five species of southwestern *Cheilanthes allosuroides* to *C. chipinquensis*; and both group southwestern *Cheilanthes* into identical subclades of three species and two species, respectively. In the outgroup, both data sets group the two *Notholaena* species into a single clade that is sister to the remaining two outgroup species. Thus there is remarkable concordance between results with the chloroplast-based and the nucleus-based data sets.

The chloroplast and nuclear data sets significantly do not agree, however, with regard to the deeper relationships of the clades they found in common: how the two elements of *Pellaea* section *Pellaea,* and *Pellaea* section *Platyloma,* and *Cheilanthes/Astrolepis,* and *Notholaena/Argyrochosma,* and southwestern *Cheilanthes* relate to one another. Since both data sets find the same terminal clades, the terminal clades in common can be collapsed to single summary lines in Fig. 3 in order to facilitate comparisons at the deeper levels of the *rbcL* and ITS trees. For example, in Fig. 3 the top line of each tree summarizes the identical clade of four species (*Pellaea andromedifolia, P. rufa, P. glabella* var. *missouriensis,* and *P. breweri*) found by both data sets. By comparing the relative positions of these collapsed summary clades, one easily sees how the deeper level placements of these identical clades differ in the *rbcL* tree versus the ITS tree. Bootstrap support is indicated below the lines in Fig. 3. The inferred phylogenetic relationships among the summary clades differ sharply in the two trees. For

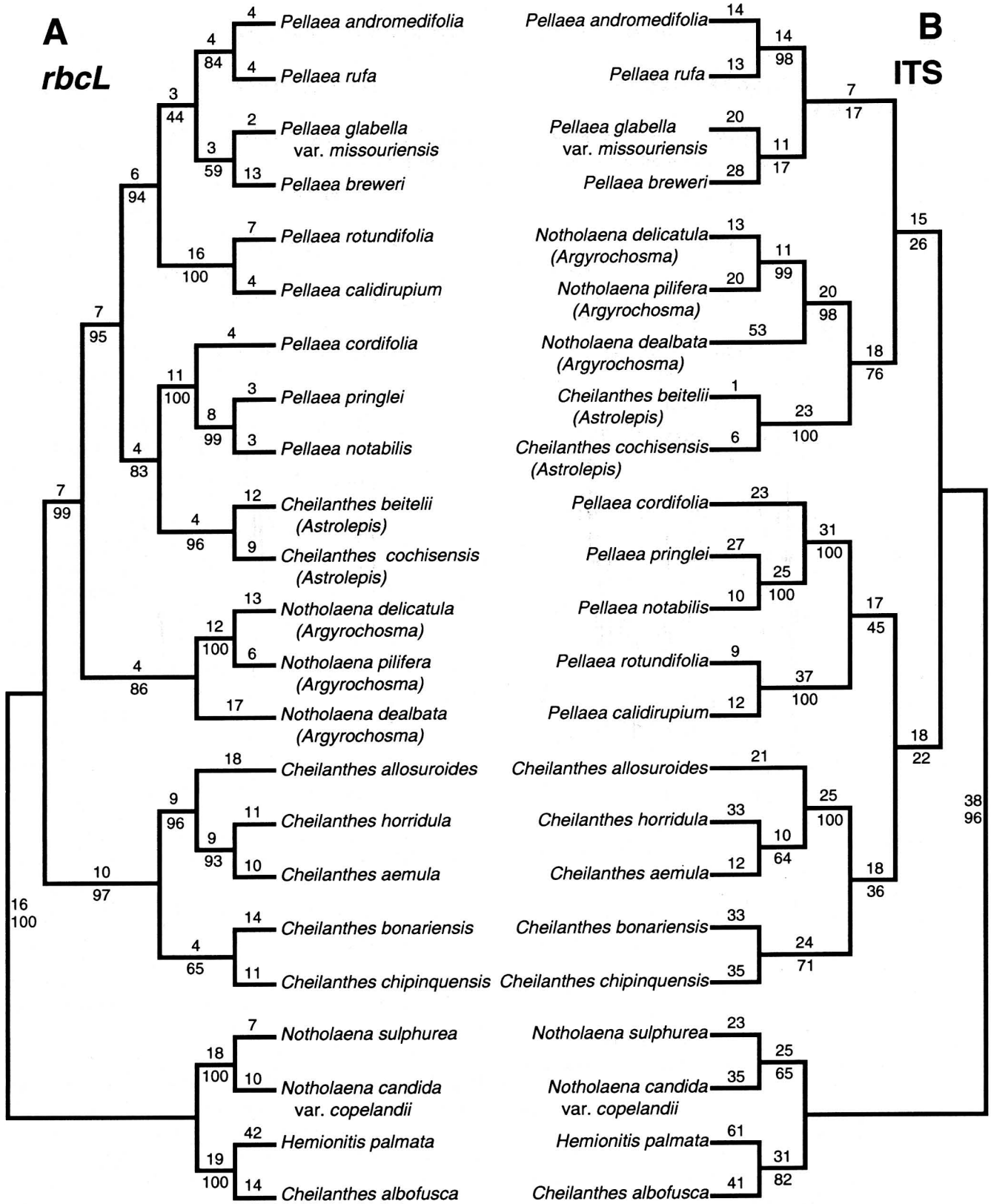

Fig. 2A, B. Single most parsimonious trees found for the subset of cheilanthoid taxa asterisked in Table 2. Branch lengths are indicated above the lines and bootstrap values are noted below the lines.—A. Tree based on analysis of *rbcL* sequences.—B. Tree based on analysis of ITS sequences.

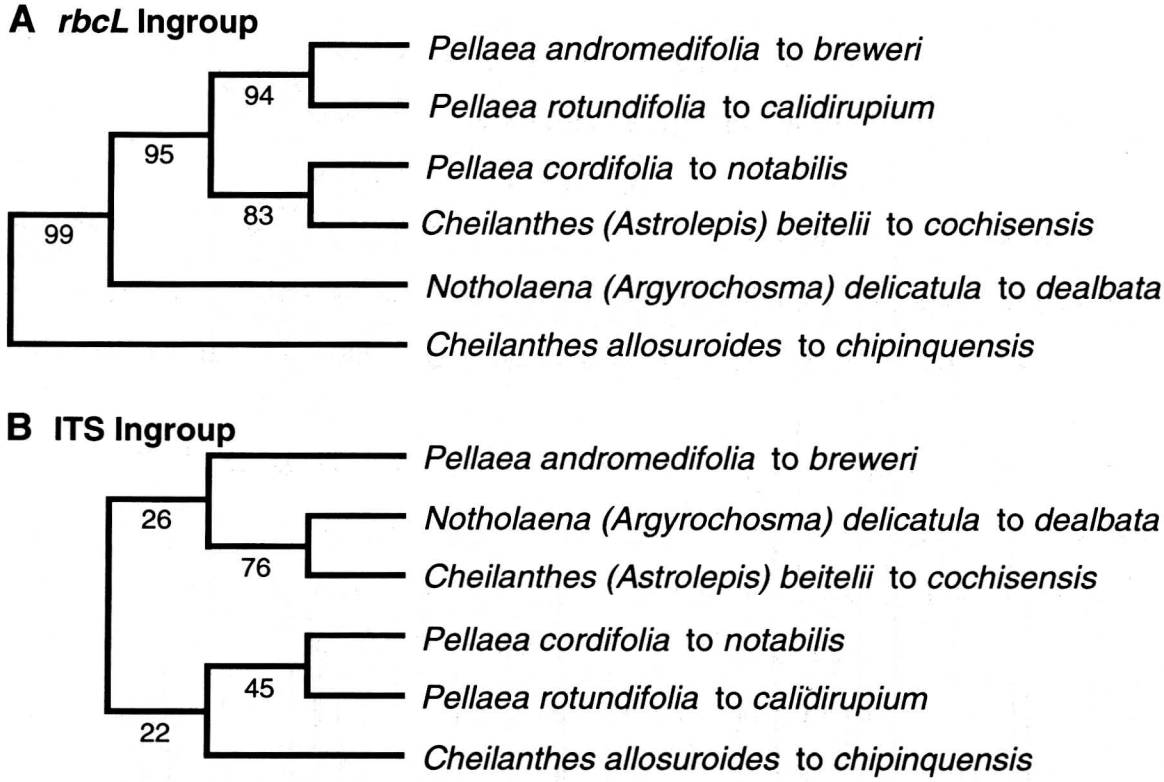

Fig. 3A, B. Ingroup portions of the trees in Figs. 2A and 2B condensed to facilitate comparisons of deeper level relationships among identical terminal and subterminal clades identified by the respective chloroplast and nuclear data sets. Clades that are identical in Figs. 2A and 2B are here collapsed to a single line as explained in the text. Bootstrap support for clades is indicated below the lines.—A. Clade arrangement inferred from *rbcL* data.—B. Clade arrangement inferred from ITS data.

example, in the *rbcL* tree of Fig. 3A the clade of *Pellaea* section *Platyloma* (*P. rotundifolia* to *P. calidirupium*) is sister to *Pellaea* section *Pellaea*'s clade of *Pellaea andromedifolia* to *breweri* with a 94% bootstrap confidence value. In the ITS tree of Fig. 3B, however, *Pellaea* section *Platyloma* is sister to *Pellaea* section *Pellaea*'s clade of *Pellaea cordifolia* to *notabilis* with a mere 45% bootstrap value. In the *rbcL* tree the *Notholaena/Argyrochosma* clade is sister to the *Pellaea*–to–*Cheilanthes/Astrolepis* clade with a 99% bootstrap value, whereas in the ITS tree, *Notholaena/Argyrochosma* is sister to only *Cheilanthes/Astrolepis* with a lesser 76% bootstrap value. Based on *rbcL,* the *Cheilanthes allosuroides* to *C. chipinquensis* clade is sister to everything else in the ingroup with a 100% bootstrap value (Fig. 2A) whereas based on ITS this *Cheilanthes* clade is sister to two *Pellaea* clades with a mere 22% bootstrap value (Fig. 2B, 3). Both data sets indicate that southwestern *Pellaea* section *Pellaea* is polyphyletic.

The *rbcL* gene is well known to be relatively slowly evolving and well suited to studies at generic and familial levels of divergence in ferns (Hasebe et al. 1995; Gastony and Ungerer 1997) and at even deeper levels in angiosperms (Chase et al. 1993). Sequence divergence values for *rbcL* for the subset of taxa used in Fig. 2A ranged from 0.5% to 5.5% for the ingroup taxa and from 0.5% to 7.1% for the entire subset of ingroup plus outgroup taxa (Table 4).

ITS, on the other hand, evolves relatively rapidly and in ferns is seen here to be particularly useful within genera or subdivisions of genera, although it is also useful at deeper levels in angiosperms (Baldwin 1992). Sequence divergence values for the two ITS regions for the subset of taxa used in Fig. 2B ranged from 1.1% to 20.1% for the ingroup taxa and from 1.1% to 23.0% for the entire subset of ingroup plus outgroup taxa (Table 4). Combined ITS1 and ITS2 sequence divergences for Compositae subtribe Madiinae (Baldwin 1992) ranged from 0.5% to 15.0% for the ingroup taxa and from 0.5% to 20.5% for the entire set of ingroup plus outgroup taxa.

Conclusions.—As a result of this first analysis of ITS regions of ferns, we now know that many ITS regions appear to be only arbitrarily and unreliably aligned at suprageneric levels in these ferns. Thus the inability of ITS to resolve the same deeper clades as *rbcL,* and the very weak bootstrap support for the ITS data at these deeper levels is now not surprising. ITS simply is not an appropriate molecule for use at these deeper phylogenetic levels in ferns. The concordance between biparentally inherited ITS and maternally inherited *rbcL* at less deep levels of the phylogeny, how-

Table 4. Sequence divergence values for the subset of taxa in Fig 2A, B. Numbers above diagonal are percentage differences for *rbcL*. Numbers below diagonal are percentage differences for the aligned dataset of ITS1 plus ITS2. Taxon names are abbreviated from the full names in Table 2 as follows: the abbreviated generic name is followed by the first four letters of the specific epithet (e.g., "*C. allo*" = "*Cheilanthes allosuroides*").

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1 *C. beit*	—	1.6	4.6	4.9	3.8	3.9	4.5	2.9	3.6	3.3	2.7	2.5	2.3	2.4	2.2	2.7	2.7	3.1	3.6	4.9	4.8	6.8	4.8
2 *C. coch*	1.1	—	4.5	4.8	3.6	3.5	4.3	3.2	3.6	3.1	2.4	2.4	2.0	2.3	2.1	2.3	2.3	2.7	3.2	4.6	4.8	6.8	4.8
3 *C. aemu*	12.7	13.1	—	2.7	2.7	2.8	1.6	4.6	4.8	4.3	4.1	4.5	3.9	4.2	4.5	4.8	4.8	4.5	4.9	4.6	4.8	6.5	5.2
4 *C. allo*	13.6	14.1	5.0	—	3.2	3.1	2.8	4.3	4.9	4.4	4.8	4.5	4.5	4.7	4.7	5.1	5.1	5.2	5.5	5.1	5.1	6.5	5.2
5 *C. bona*	10.8	11.8	11.2	11.5	—	1.9	2.9	3.7	4.4	3.7	3.4	3.9	3.4	3.3	3.5	3.5	3.6	3.6	4.2	4.6	4.4	5.8	4.4
6 *C. chip*	14.3	15.1	12.8	13.1	10.9	—	3.0	3.9	4.2	3.9	3.8	3.8	3.6	3.7	3.8	3.8	3.8	4.3	4.8	4.5	4.5	6.2	4.2
7 *C. horr*	14.6	14.8	7.2	9.8	13.0	15.7	—	4.5	4.6	4.1	4.3	4.5	4.2	4.2	4.3	4.6	4.6	4.7	5.1	5.3	5.4	6.7	5.5
8 *N. deal*	14.7	15.3	16.1	16.0	16.8	19.7	18.4	—	3.2	2.7	3.3	3.0	3.1	2.9	2.8	3.3	3.3	3.8	4.3	4.8	4.9	6.6	5.2
9 *N. deli*	7.9	9.0	12.3	11.5	10.1	13.8	13.3	12.4	—	1.4	3.6	3.7	3.5	3.4	3.4	3.9	3.9	4.0	4.5	5.4	5.1	7.1	5.4
10 *N. pili*	10.1	10.9	13.3	12.8	11.9	15.6	14.9	12.9	5.1	—	3.2	3.4	3.2	3.0	3.0	3.3	3.4	3.7	4.2	4.9	4.6	6.7	5.1
11 *P. andr*	9.2	9.3	10.8	11.0	10.6	13.7	13.0	14.3	8.9	10.6	—	1.8	1.0	0.6	2.6	2.8	3.0	2.0	2.4	4.8	4.8	6.3	4.8
12 *P. brew*	11.1	11.3	10.8	12.5	9.9	13.9	13.5	16.3	11.2	12.4	9.2	—	1.1	1.8	2.6	2.9	2.9	2.7	2.9	4.5	4.5	6.6	5.0
13 *P. glab*	9.1	10.0	10.7	10.2	11.3	13.9	13.8	14.6	8.8	10.4	7.1	7.8	—	1.0	2.2	2.7	2.7	2.0	2.2	4.3	4.4	6.1	4.5
14 *P. rufa*	8.1	8.1	10.9	10.6	10.1	13.3	12.5	13.6	8.2	9.8	4.3	9.4	7.5	—	2.3	2.3	2.5	2.0	2.4	4.8	4.8	6.3	4.8
15 *P. cord*	11.9	12.7	12.3	11.5	13.6	14.9	15.1	17.8	12.8	13.4	11.9	12.2	10.9	11.1	—	1.1	1.1	3.0	3.4	5.2	4.8	6.5	5.2
16 *P. nota*	12.8	13.2	12.7	11.2	12.6	15.9	16.0	18.4	12.9	13.8	10.9	12.6	11.3	11.4	8.3	—	0.5	2.9	3.5	5.5	5.1	6.8	5.5
17 *P. prin*	15.7	16.2	15.3	14.8	15.6	17.0	17.9	20.1	15.9	16.5	14.4	13.9	13.1	13.0	11.3	6.0	—	3.0	3.5	5.5	5.3	7.0	5.5
18 *P. cali*	13.3	13.8	14.2	14.1	13.1	13.9	14.8	16.6	11.7	13.9	12.0	13.3	10.3	11.7	12.4	12.2	14.3	—	0.8	5.1	5.1	6.8	5.2
19 *P. rotu*	13.3	14.2	13.3	13.9	12.2	13.5	14.6	17.7	12.0	13.8	13.2	12.8	11.0	13.5	13.2	14.1	16.4	3.5	—	5.5	5.7	7.1	5.6
20 *N. cand*	15.1	15.1	16.0	15.4	13.2	17.1	18.6	20.4	14.2	15.8	15.5	15.3	15.1	13.9	15.0	15.3	18.3	15.9	16.4	—	1.3	5.8	4.2
21 *N. sulp*	13.0	13.0	14.7	14.5	13.2	17.3	17.4	18.4	12.6	14.6	13.2	14.7	13.7	12.3	14.5	14.6	16.5	15.5	17.3	10.0	—	6.0	4.1
22 *H. palm*	16.7	16.7	18.9	18.0	19.3	19.9	17.7	23.0	17.4	18.6	19.1	20.4	19.0	18.4	17.3	20.5	21.3	19.2	19.7	19.5	16.7	—	4.3
23 *C. albo*	16.3	16.3	17.7	17.4	15.9	18.6	19.0	19.9	13.7	15.8	14.9	17.5	14.7	14.6	15.9	16.4	18.7	16.7	17.6	15.1	13.2	17.1	—

ever, generates confidence that *rbcL* is tracking real species phylogenies, not simply matrilineal gene phylogenies.

Maternally inherited *rbcL* data and biparentally inherited nonmorphological nuclear data do agree at divergence levels where both *rbcL* and the ITS regions of nuclear ribosomal DNA evolve at appropriate rates. Remarkable congruence is found for terminal and slightly deeper clades based on both data sets. Moreover, *rbcL*'s unexpected finding that southwestern *Pellaea* section *Pellaea* is polyphyletic—that it is broken into two deeply separated clades—is also found by nuclear ribosomal DNA. Support for clades at deeper levels of the phylogeny, where the two data sets do not agree, is much stronger for *rbcL* than for ITS.

One may therefore conclude that 1) at appropriate levels of divergence, matrilineal chloroplast-encoded *rbcL* is concordant with biparental nucleus-encoded molecular data in cheilanthoids, 2) unexpected *rbcL* findings are attributable to shortcomings of traditional morphology-based studies, and 3) *rbcL* does reliably track species phylogeny, enabling us to begin to unweave the tangled web of generic lineages and phylogenetic relationships in cheilanthoid ferns. To resolve the deeper levels of cheilanthoid phylogeny with nuclear molecular data, future attention will focus on the 18S region of nrDNA already applied by Wolf (1995) to ferns of the Dennstaedtiaceae and on other potentially informative regions.

ACKNOWLEDGMENTS

We appreciate the assistance of George Yatskievych in obtaining many of the ingroup plants in this study. We thank P. Anne Bean, Jianwei Li, Tim Flynn, Adrian Hegeman, Ruth Kirkpatrick, Irving Knobloch, J. D. Lovis, Tamara Naumann, Ramón Riba, Kelly Sullivan, Sigrid Leide, and Paulo Windisch for supplying the plant material indicated in Table 2, David Swofford for permission to use test versions of PAUP 4.0, Bruce G. Baldwin and Alan R. Smith for helpful comments in review, and Aaron Liston for verifying that our ITS sequences are not fungal. This study was supported by National Science Foundation grant DEB–9307068 to GJG.

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