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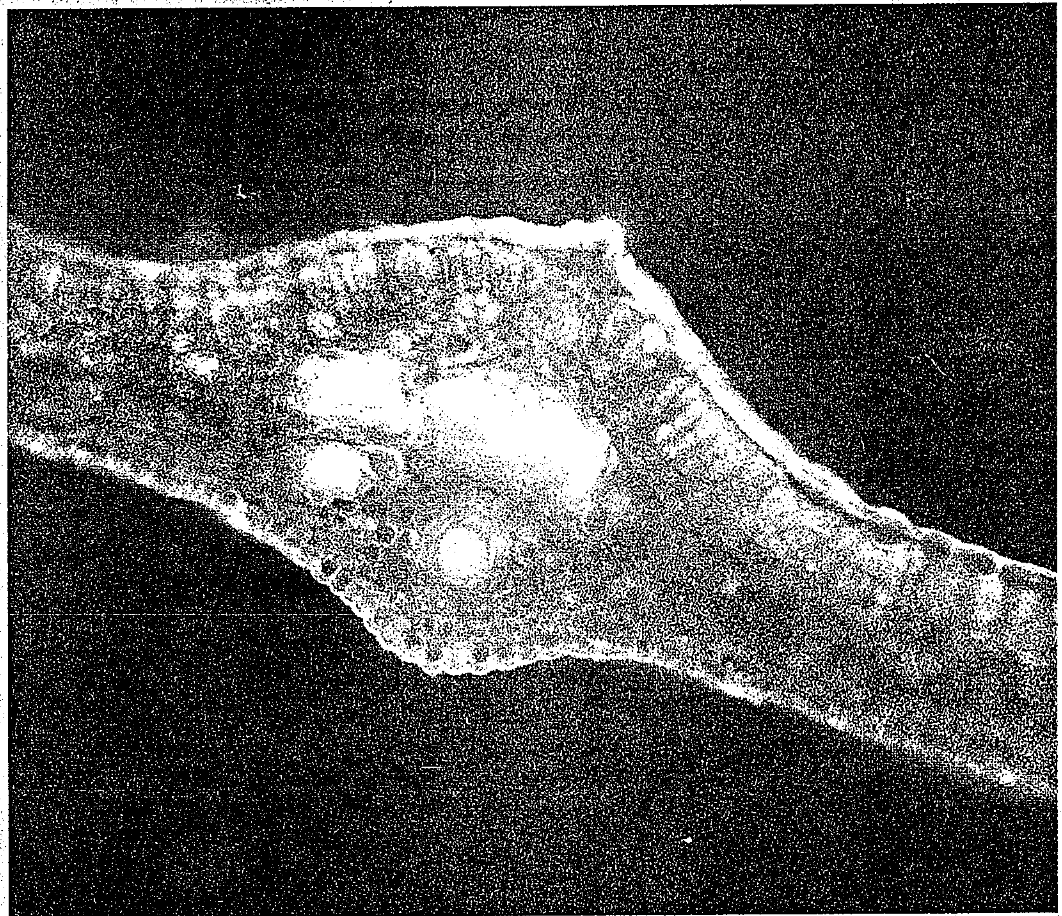

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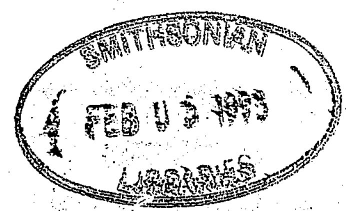

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Phylogeny of some Species of *Cyrtandra* (Gesneriaceae) Inferred from the *atpB/rbcL* cpDNA Intergene Region

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Abstract: The PCR-amplified *atpB/rbcL* spacer region of ten *Cyrtandra* species and representatives of the genera *Didymocarpus*, *Didissandra*, and *Monophyllaea* was sequenced and used for phylogenetic analyses. In *Cyrtandra* 83 variable positions were found over a length of 890bp. The data suggest that the Samoan species included in our study originated by at least two independent colonization events, while for the Hawaiian species the situation is not clear yet. With respect to generic relationships, *Monophyllaea* shows considerable divergence (9.9%) from the remaining taxa and thus is used as outgroup. *Didymocarpus* is most closely related to *Cyrtandra*, their genetic divergence (2.2%) being only slightly higher than the average distance within *Cyrtandra* (1.9%). The promoter regions for the *atpB* and *rbcL* genes, which have been described from Rubiaceae, were identified and found to be located at homologous positions. Of the two *atpB* promoters found in Rubiaceae only the distal one appears functional, the proximal promoter has been eroded by mutations and is disrupted in *Didissandra* by a 14bp insertion.

Key words: Gesneriaceae, *Cyrtandra*, Malesia, Hawaii, Samoa, cpDNA, *atpB/rbcL* intergene region, *atpB* promoter, phylogeny.

Introduction

The genus *Cyrtandra* comprises more than 600 species (cf. Smith, 1991) ranging from the Nicobar Islands in the west to Hawaii, the Marquesas, and the Society Islands in the east, and from the islands south of Japan southwest to Sumatra and Java, and southeast to Queensland.

Information on most species of the genus is scarce. Morphological polymorphism is found in several of the better known taxa and hybridization between species has been reported (see, e.g. Wagner et al., 1990; Smith et al., 1996c, for taxa from the Hawaiian Islands). The only overall survey of *Cyrtandra* dates back to Clarke (1883); more recent surveys and subdivisions have been attempted on a regional basis only (e.g. Gillett, 1967, 1973; Smith, 1991; Wagner et al., 1990).

To obtain a better understanding of speciation and radiation patterns in the genus, multidisciplinary studies were started at Vienna University: ecology (Luegmayr and Kiehn, 1991; Kiehn, 1993), pollination, germination and growth patterns (Kiehn, in prep.), (micro)morphology (Luegmayr and Kiehn, 1991; Luegmayr, 1993a, 1993b; Mühlbauer and Kiehn, 1997; Schlag-Edler and Kiehn, in press), embryology (Svoma and Kiehn, 1993, 1995), and karyology (Luegmayr and Kiehn, 1991; Kiehn, 1995; Kiehn and Weber, in press). *Cyrtandra* species from different regions of the distribution area are cultivated at the Vienna University Botanical Garden (HBV) and are now available for molecular analyses.

Chloroplast DNA (cpDNA) has been widely employed for phylogenetic inference at lower taxonomic levels in angiosperms, although the vast majority of investigations dealt with restriction site variation (Palmer et al., 1988; Crawford et al., 1992a; Soltis et al., 1992; Zunk et al., 1996). In cpDNA the nucleotide substitution rate is much lower than in nuclear DNA (Wolfe et al., 1987). Thus, in order to maximize the yield of observed nucleotide differences at a lower taxonomic level, sequencing of non-coding cpDNA has been used by various groups (Manen et al., 1994; Böhle et al., 1994; Natali et al., 1996). Up to now, the only molecular data available for *Cyrtandra* originate from investigations of RAPD-variation among the Hawaiian species (Smith et al., 1996b, 1996c).

The present paper gives a phylogenetic analysis for 10 species of *Cyrtandra* based on DNA sequence comparison of the spacer region between the *ATP-synthetase-ß-subunit (atpB)* gene and the *ribulose-1,5-biphosphate carboxylase* subunit (*rbcL*) gene of the chloroplast genome. The structure of this cpDNA region is commented on by Manen and Natali (1996). Our investigations aim to assess the degree of interspecific variation and to contribute to the phylogeny of the genus *Cyrtandra*. Special interest is focused on possible implications for the geographic distribution and Pacific islands colonization. Using the *atpB/rbcL* region as a marker, it should, e.g. be possible to trace the origins of the Hawaiian taxa and their relationships to species from outside Hawaii. Wagner et al. (1990) suggested independent colonization events giving rise to the different sections of Hawaiian *Cyrtandra*. This suggestion was discussed by Smith et al. (1996c). They stated, on the basis of their molecular data, that multiple colonization events are potentially important in terms of the hybridization pattern observed in Hawaiian *Cyrtandra*. Cladistic analyses of cpDNA data resulting in different clades for the Hawaiian species would provide further support for this hypothesis, if those clades were separated by taxa from other islands.

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According to an alternative hypothesis, assuming a single founder event followed by explosive diversification, the Hawaiian species should form a single clade. The basis taxa of this clade should then also be found on the Hawaiian Islands (see discussion below). Representatives of three other genera of Gesneriaceae-Cyrtandroideae are included in the study as outgroups. Their relationships are also discussed.

Materials and Methods

Plant material

Samples of 500 mg fresh leaves of plants grown in the greenhouse of the Vienna University Botanical Garden (HBV) were collected and stored at −80 °C until extraction. A list of the species studied is given in Table **1**. Voucher specimens are deposited at the herbaria listed in Table **1**.

DNA extraction, amplification, and sequencing

Total DNA was extracted according to Doyle and Doyle (1987). DNA was extracted from single individuals and treated with RNAse. The entire region covering the noncoding spacer between *atpB* and *rbcL* was amplified using polymerase chain reaction (PCR). Five different primers were employed (binding region and direction of amplification are given in brackets):

C1, 5′-GAAGTAGTAGGATTGATTCTC (*atpB* gene 5′-> 3′);
C2, 5′-TACAGTTGTCCATGTACCAG (spacer, 5′-> 3′);
C3, 5′-GACATGAGAGGTAACAAC (spacer, 5′-> 3′);
C4, 5′-CCCTACAACTCATGAATTAAG (*rbcL* gene, 3′-> 5′);
C5, 5′-TCATTATTTCTATCTTATT (spacer, 3′-> 5′).

Primers C1, C2, C3, and C4 correspond to oligo 2, 5, 8, and 7 respectively used in the study of Manen et al. (1994), primer C5 was designed in the course of the present investigation. The highest yield of PCR product was obtained using the following conditions: The 100 μl PCR reaction contained 72.5 μl of sterile water, 10 μl of 10 x *Taq* polymerase reaction buffer, 2 mM (4 μl of 50 mM stock) magnesium chloride, 0.2 mM (2 μl of 10 mM stock) of each dNTP (total 8 μl), 0.25 μM (2 μl of 50 mM stock) of each primer (total 4 μl, C1 and C2), 2.5 units (0.5 μl of 5 units/μl) of *Taq* DNA polymerase and 2–8 ng (1 μl of 2–8 ng/μl) of purified total DNA. Reaction mixtures were sealed with one or two drops of mineral oil to prevent evaporation during thermal cycling.

Small amounts of PCR products (5 μl) were visualized with ethidium bromide after agarose gel electrophoresis using a mini-gel system. A single band corresponding to a 1kb fragment was detected. No substantial length variation was observed among species. PCR-generated DNA was purified by electrophoresis on agarose gel. After staining the gel with ethidium bromide, the agarose block containing DNA was excised from the gel. Concentrated DNA was recovered from the agarose using Elu-Quick glass pulver kit. The DNA was checked for purity on an agarose gel.

Sequences were generated by standard dideoxy chain termination reaction, using the snap cooling method to obtain single stranded DNA from the template. Annealing and ^{35}S-labelling reactions were carried out with T7 sequencing kit (Pharmacia). Single stranded DNA was sequenced using primers C1, C2, C3, C4, and C5. DNA was separated on a 6% acrylamide gel. Gels were transferred to 3 MM Whatman paper, vacuum dried at 80 °C for 2 h and exposed to X-ray film (Kodak) for 24 to 72 h. Both strands of the *atpB/rbcL* spacer region were analysed in all species studied.

Data analysis

The sequences have been deposited in the GenBank data base under the following accesion numbers: U91304 (*Cyrtandra calyptibracteata*), U91309 (*C. compressa*), U91310 (*C. falcifolia*), U91306 (*C. grandiflora*), U91308 (*C.* cf. *milnei*), U91311 (*C. paludosa*), U91305 (*C. pendula*), U91314 (*C. pogonantha*), U91307 (*C. sandwicensis*), U91303 (*C. splendens*), U91313 (*Didissandra frutescens*), U91312 (*Didymocarpus antirrhinoides*), and U91315 (*Monophyllaea horsfieldii*). Sequences were aligned with the program Clustal V (Higgins et al., 1992) and the alignment was improved by hand. Maximum parsimony trees were constructed from the data set using PAUP 3.1.1 (Swofford, 1993). Search mode: heuristic search, tree-bisection-reconnection branch swapping performed. In the tree shown, gaps were treated as missing data. Including gaps as an additional character state did not change the topology of the tree (not shown). Bootstrap values were obtained with branch-and-bound search (100 replicates).

Table 1 Species used in the study and their origin. Abbreviations of herbaria follow Holmgren et al. (1981).

Species	Origin	Collector, voucher No., and herbarium of deposition
Cyrtandra calyptibracteata Bakh. f.	Indonesia, Java: Arjuno Mt.	Van Balgooy 6193 (L, WU)
C. compressa C. B. Clarke	Western Samoa, Savaii: Mt. Matavanu	Kiehn & al. MK-940823–2/1 (WU)
C. falcifolia A. Gray	Western Samoa, Savaii: Mt. Matavanu	Kiehn & al. MK-940823–3/1 (WU)
C. grandiflora Gaud.	U.S.A., Hawaii, Oahu: Manoa Cliff Trail	Kiehn & Kiehn MK-940910–2/2 (BISH, WU)
C. cf. *milnei* A. Gray	Fiji, Viti Levu: Mt. Korombamba	Vodonaivalu & al. MK-940807–1/1 (SUVA, WU)
C. paludosa Gaud. var. *paludosa*	U.S.A., Hawaii, Big Island: Stainback Hw.	Smith 2913 (SRP)
C. pendula Blume	Malaysia: Negeri Sembilan, Jeram Toi	Weber & Antonysamy 860730–1/2 (WU)
C. pogonantha A. Gray	Western Samoa, Upolu: Mt. Fogalepolu	Kiehn & al. MK-940819–3/1 (WU)
C. sandwicensis (H. Lév.) St. John & Storey	U.S.A., Hawaii, Oahu: Manoa Cliff Trail	Kiehn & al. MK-900722–2/1 (BISH, PTBG, WU)
C. splendens C. B. Clarke	Indonesia, Borneo: Sarawak	cult. in HBV sub No. GS-89–04 (WU)
Didissandra frutescens (Jack) C. B. Clarke	Malaysia: Perak, Maxwell's Hill	Weber 950905–1/1 (WU)
Didymocarpus antirrhinoides A. Weber	Malaysia: Johore, Gunung Ledang	Weber 840717–1/1 (WU)
Monophyllaea horsfieldii R. Br.	Malaysia: Selangor, Batu Caves	cult. in HBV; seeds received from BG Edinburgh (WU)

Results

Variation within Cyrtandra

The sequences of the *atpB/rbcL* spacer regions of ten *Cyrtandra* species were aligned to the corresponding sequences from *Didymocarpus antirrhinoides, Didissandra frutescens* (both tribe Didymocarpeae), and *Monophyllaea horsfieldii* (tribe Epithemateae). The complete sequence alignment (957bp) comprising the data from 13 taxa (not shown) served as a basis for the phylogenetic study. To illustrate the variation within *Cyrtandra*, a consensus sequence was deduced from the 10 *Cyrtandra* sequences, which is thought to approach the ancestral sequence for the genus. The infrageneric alignment of the ten *Cyrtandra* sequences spans a length of 890bp. The 83 variable positions (9.3%) found within the genus are compiled in Fig. **1**. Positional information relates to the intergeneric alignment in Fig. **2**, where the complete *Cyrtandra* consensus sequence is presented together with the *atpB/rbcL* spacer regions from *Didymocarpus, Didissandra*, and *Monophyllaea*. One unique 1bp insertion in *C. sandwicencis* has no matching site in the intergeneric alignment. It is designated by the nucleotide number of the preceding position in Fig. **1** and marked by the letter "i". The length of the sequences was rather uniform ranging from 878bp (*C. compressa* and *C.* cf. *milnei*) to 885bp (*C. pogonantha*). In order to obtain an optimal alignment of the *Cyrtandra* sequences, gaps (with a maximum length of 3bp) had to be introduced at 27 positions (3.0%). All 3bp gaps result from deletions as revealed by comparison with the outgroup sequences in Fig. **2**. Two 3bp gaps are shared by several taxa: *C. compressa, C.* cf. *milnei*, and *C. falcifolia* have a common gap at position 749–751, the gap at position 758–760 is found in *C. compressa, C.* cf. *milnei*, and *C. sandwicensis*. The remaining gaps are due to 1–2bp indels. Three of them (at positions 179, 411, and 455) occur in more than one taxon and thus may be used as informative characters. An insertion of a single A at position 179 is shared by *C. compressa, C.* cf. *milnei, C. falcifolia*, and *C. paludosa*. The insertion of T at position 455 is located in a section with variable numbers of Ts (4–5) and therefore does not appear to be useful for phylogenetic interpretation. The same applies for a gap of variable length (0 to 2bp) at positions 570–571 at the end of an oligo-A stretch. Base substitutions occur at 60 sites (6.7%), 16 of them are phylogenetically informative. Compared to the *Cyrtandra* consensus sequence, *C. pogonantha, C. calyptibracteata*, and *C. splendens* deviate at 6–8 positions (Table **2**). The highest number of changes with respect to the consensus sequence is recorded in *C.* cf. *milnei* (24). On average the divergence among *Cyrtandra* species measures 1.9% (Table **3**).

Table 2 Differences with respect to the *Cyrtandra* consensus sequence. del = deletions, ins = insertions, sub = substitutions, total = number of altered positions.

	del	ins	sub	total
C. splendens	2	–	5	7
C. pogonantha	–	–	6	6
C. calyptibracteata	2	1	6	9
C. pendula	2	2	15	19
C. sandwicensis	8	2	7	17
C. grandiflora	3	2	5	10
C. paludosa	2	2	10	14
C. compressa	8	1	8	17
C. cf. *milnei*	8	1	15	24
C. falcifolia	4	1	15	20

Variation among the investigated genera

The alignment of the sequences obtained for *Didymocarpus antirrhinoides, Didissandra frutescens*, and *Monophyllaea horsfieldii* compared with the *Cyrtandra* consensus sequence has a length of 956bp (Fig. **2**). The sequences are well conserved among the four genera and alignment is readily achieved. Although there is no considerable variation in total length (880–899bp), the sequences differ by several large insertions and deletions. The largest deletion (18bp at position 740–757) and the largest insertion (14bp at position 277–290) occur in *Didissandra*. The 14bp insertion is located within one of the putative promoter regions of the *atpB* gene (see below). *Cyrtandra* and *Didymocarpus* share an 8bp deletion at position 338–345.

The pairwise distances between the 13 investigated taxa are presented in Table **3**. At the generic level *Didymocarpus* stands next to *Cyrtandra* (2.2%), followed by *Didissandra* (2.9%). The divergence between *Cyrtandra* and *Didymocarpus* is only slightly higher than the infrageneric divergence among the *Cyrtandra* species. *Monophyllaea* (9.9%) is clearly separated

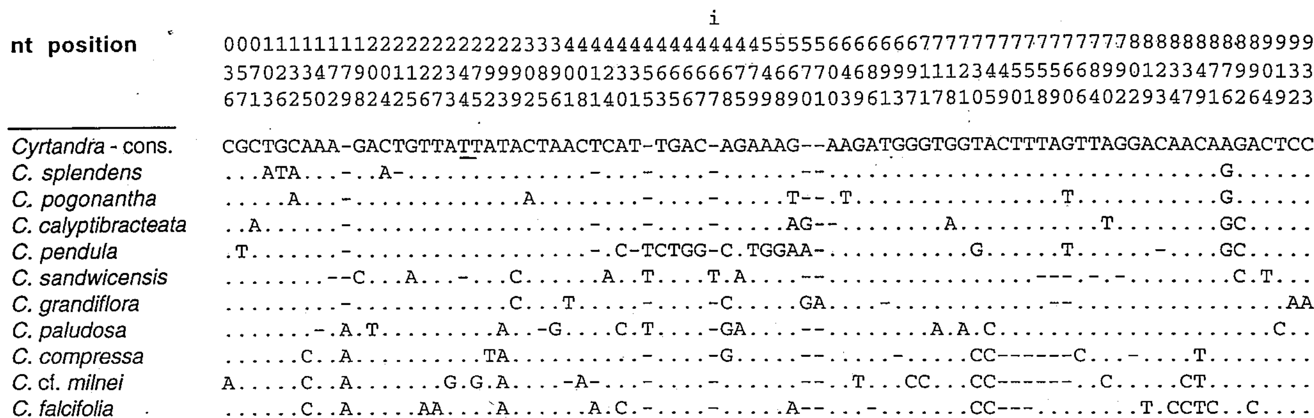

Fig. 1 Variable sites in *Cyrtandra*. Positional information relate to the sequence alignment shown in Fig. **3**. i = unique 1bp insertion in *C. sandwicensis*, nt = nucleotide. Gaps are indicated by dashes (–), identical nt positions by dots (.).

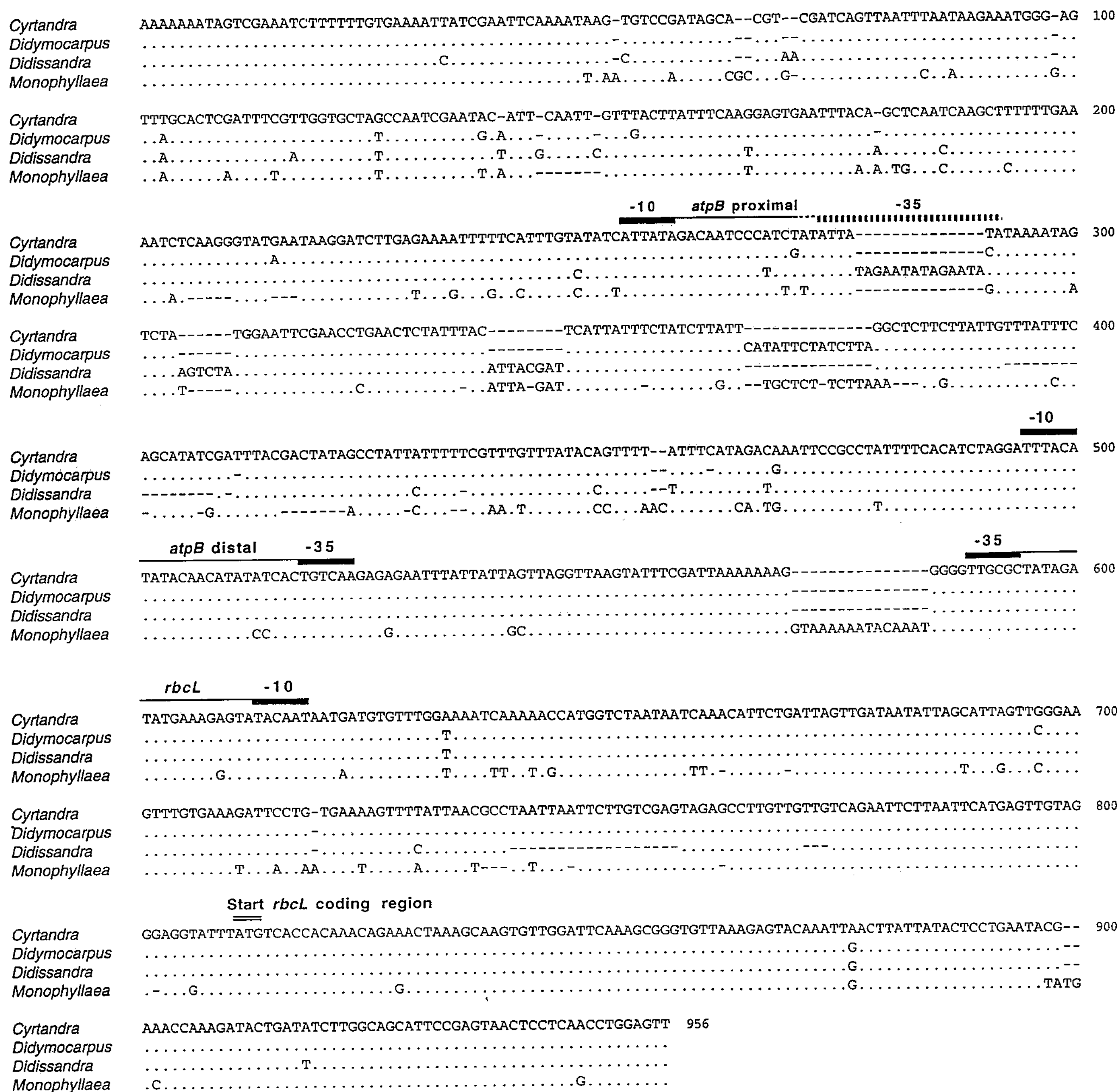

Fig. 2 Alignment of the sequences obtained for *Didymocarpus antirrhinoides*, *Didissandra frutescens*, and *Monophyllaea horsfieldii* compared with the *Cyrtandra* consensus sequence (length: 956bp). The promoter regions for the *atpB* and *rbcL* genes described for Rubiaceae are indicated. The start of the coding region of the *rbcL* gene at position 811 is marked. Gaps are indicated by dashes (–), identical nt positions by dots (.).

from the other taxa and, therefore, appears as a suitable outgroup for the rooting of the cladogram.

Phylogeny

For the phylogenetic analysis a maximum parsimony dendrogram was constructed using *Monophyllaea* as an outgroup. Four shortest trees with a length of 173 steps were obtained (consistency index = 0.896, retention index = 0.660). The topology of the dendrogram presented in Fig. 3 is consistent with the strict consensus tree deduced from the 4 shortest trees, which varied only with respect to branching order and length of the terminal branches of the taxa *C. pogonantha*, *C. calyptibracteata*, and *C. pendula*. Based on six informative substitutions (165, 186, 247, 430, 449, and 467), the tree reveals *Didymocarpus* as sister group of *Cyrtandra*. The close relationship between these two genera is further corroborated by a common 8bp deletion (338–345), three 1bp deletions (69, 179, and 311), and one 1bp insertion (435). The alternative branching, with *Cyrtandra* and *Didissandra* as the closest relatives, is supported by only two informative substitutions (468, 696) and one common 14bp gap (365–378) with respect to *Didymocarpus*. Since the sections corresponding to the gap differ considerably between *Didymocarpus* and *Monophyllaea* (3 unmatched positions and 2 mismatches), two independent insertions seem more likely, assuming that the 14bp gap in

Table 3 Pairwise distances between 13 taxa. The length of the aligned sequence from the *atpB/rbcL* intergene region and the 5'-section of the *rbcL* gene measures 957bp. Absolute distances are given below the diagonal, mean distances (in%, adjusted for missing data) above the diagonal.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 *Cyrtandra falcifolia*	–	1.9	1.6	2.3	2.4	2.6	2.8	2.2	2.5	2.4	3.1	3.4	10.5
2 *C.* cf. *milnei*	17	–	1.4	2.3	2.1	2.4	3.1	2.2	2.3	2.3	3.0	3.3	10.4
3 *C. compressa*	14	12	–	1.4	1.4	1.7	2.3	1.6	1.6	1.6	2.1	2.8	9.7
4 *C. paludosa*	20	20	12	–	1.6	1.7	2.5	1.8	1.9	1.8	2.3	2.7	10.0
5 *C. grandiflora*	21	18	12	14	–	1.1	2.0	1.2	1.2	1.3	1.8	2.6	9.8
6 *C. sandwicensis*	23	21	15	15	10	–	2.2	1.3	1.5	1.5	1.9	3.0	10.1
7 *C. pendula*	25	27	20	22	18	19	–	1.8	1.9	2.2	2.7	3.2	10.0
8 *C. calyptibracteata*	19	19	14	16	11	11	16	–	1.1	1.1	1.8	2.6	9.6
9 *C. pogonantha*	22	20	14	17	11	13	17	10	–	1.0	1.9	2.7	9.8
10 *C. splendens*	21	20	14	16	11	13	19	10	9	–	1.4	2.2	9.0
11 *Didymocarpus antirrhinoides*	27	26	18	20	16	17	24	16	17	12	–	2.6	9.1
12 *Didissandra frutescens*	29	28	23	23	22	25	27	22	23	18	22	–	8.9
13 *Monophyllaea horsfieldii*	89	88	82	85	83	85	85	81	83	76	78	73	–

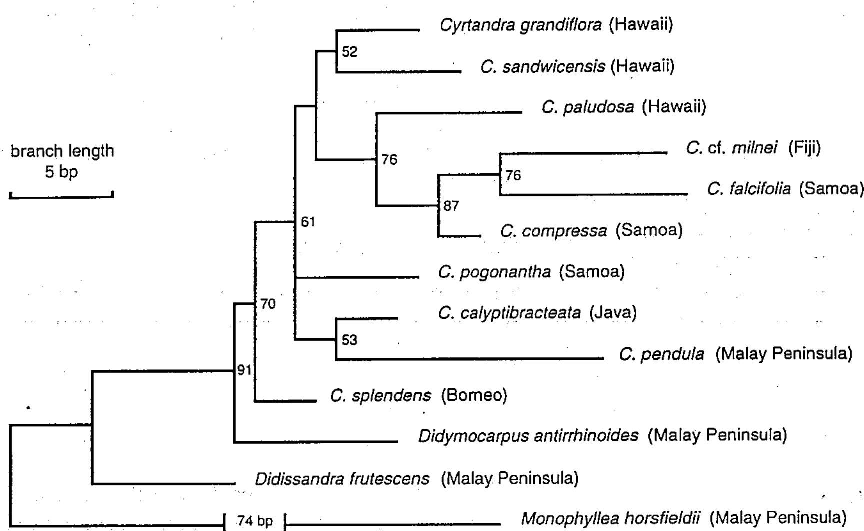

Fig. 3 Maximum parsimony dendrogram (PAUP 3.1.1) based on the alignment of 13 sequences (CI = 0.896). Bootstrap values > 50 are given at the nodes (100 replicates). Branch lengths are drawn to scale. The terminal branch of the outgroup *Monophyllaea horsfieldii* measures 74bp.

Cyrtandra and *Didissandra* represents the ancestral situation. Within *Cyrtandra*, the dendrogram places *C. splendens* at the most basal position as the sister group to all other *Cyrtandra* species. An unresolved polytomy comprises *C. pogonantha* (Samoa), a cluster with *C. calyptibracteata* (Java) and *C. pendula* (Malaysia), and a cluster of Pacific taxa. In the latter cluster, the Hawaiian species *C. grandiflora* and *C. sandwicensis* form one clade. They come next to a cluster consisting of *C. paludosa* (Hawaii), *C. compressa* (Samoa), *C.* cf. *milnei* (Fiji), and *C. falcifolia* (Samoa). This is supported by two derived substitutions (293 and 745) and one 1bp insertion (179). Within the cluster, the triad comprising *C. compressa*, *C.* cf. *milnei* and *C. falcifolia* shares three derived substitutions (135, 730, and 849) and one 3bp deletion (749–751). *C.* cf. *milnei* and *C. falcifolia* appear as sister taxa.

Promoters and rcbL*-coding region*

Promoter regions for both the *atpB* and the *rbcL* gene have been described by Manen and Natali (1996) within the *atpB/rbcL* spacer region of Rubiaceae. The two detected promoters for the *atpB* gene are both present in some of the investigated rubiaceous taxa, but the distal promoter is lacking in the tribe Rubieae due to a large 152bp deletion. In the Gesneriaceae studied here (Fig. 2), the distal promoter (495–523) is strongly conserved, not a single substitution being found in the sequences of 13 taxa. On the other hand, the proximal promoter (252–292) is truncated and most likely non-functional. Only the –10 region and 12bp towards the –35 region are conserved, whereas the –35 region itself shows no similarity to the proximal *atpB* promoter sequence in Rubiaceae as described by Manen and Natali (1996). In *Didissandra*, the presumptive –35 region is further destroyed by a 14bp insertion. The promoter of the *rbcL* gene, which is located at positions 589–618, is perfectly conserved in all taxa analysed in our investigation.

The sequence amplified for the study of the *atpB/rbcL* spacer extends at the 3'-side into the adjacent coding region and thus contains the first 48 codons of the *rbcL* gene (811–956). There are only two substitutions (1.4%) in the sequences of the *Cyrtandra* species, *Didymocarpus*, and *Didissandra*, as compared to the spacer region, where 4.9% variable sites among 714bp were found (excluding gaps > 3bp). Both substitutions are conservative: A synonymous substitution at position 886 in *Cyrtandra* and a non-synonymous at position 918 in *Didissandra* causing the replacement of isoleucine by phenylalanine (both hydrophobic). More variation occurs in

Monophyllaea. With respect to the reading frame of *Cyrtandra* synonymous substitutions are found at positions 828, 897, and 947. A non-synonymous substitution at position 902 would lead to the replacement of glutamate by the chemically equivalent aspartate. However, the insertion of 2bp (TG) at position 899–900 causes a frame shift in codon 30. Although no stop-codon is found in the part of the sequence determined so far, it is unlikely that the copy of the *rbcL* gene of *Monophyllaea* amplified should be able to produce a functional protein. In *C. pendula* the original reading frame is destroyed by a 1bp deletion at position 823. Here again the frame shift does not lead to a translational stop signal within the sequenced region.

Discussion

Variation within Cyrtandra

The average divergence within the genus *Cyrtandra* (1.9%) is nearly as high as the average pairwise distance between the *Cyrtandra* species and *Didymocarpus* (2.2%). The observed degree of variation within the genus is expected to provide useful data for phylogenetic considerations. The 3bp gaps shared by several taxa are remarkable: The gap at position 749–751, found in *C. compressa*, *C.* cf. *milnei*, and *C. falcifolia*, seemingly is a synapomorphy indicating monophyly of this group. At position 758–760 another 3bp gap is shared by *C. compressa*, *C.* cf. *milnei*, and *C. sandwicensis*, whereas a 2bp gap (759–760) is found in *C. grandiflora*. This second gap is difficult to interpret and partly contradicts the relationship derived from the other gap at position 749–751 and the common substitutions. The most likely hypothesis is that independent deletions have occurred in different lineages. Alternatively, a 3bp deletion may be assumed in the common ancestor of six species followed by independent insertions.

Variation among the investigated genera

Due to the limited number of species included in this study interpretations of relationships between genera still are preliminary. Greater sampling, especially among the tribes Didymocarpeae and Epithemateae (= Klugieae, see Burtt, 1997) is needed to prove the trends discussed here. Remarkably, the average pairwise distance among *Cyrtandra* species is only slightly smaller than the average genus distance to the investigated *Didymocarpus* species. This could indicate a close relationship between the tribes Didymocarpeae and Cyrtandreae. A possible explanation is comparatively recent separation of the genera of those tribes followed by parallel intensive speciation. The latter has been postulated on the basis of morphological characters for the genus *Cyrtandra* on the Hawaiian Islands (Wagner et al., 1990). On the other hand, there is considerable distance of the three genera of Didymocarpeae and Cyrtandreae to *Monophyllaea* and thus to the tribe Epithemateae. These results are in accordance with molecular analyses of *ndhF*-sequences (Smith et al., 1996a, 1997) revealing monophyly of only the tribe Epithemateae within the Cyrtandroideae. Morphological studies (Weber, 1976a–c, 1977a–b, 1978a–b, 1982, 1988; Smith, 1997) also indicate a separate position of the Epithemateae. *Monophyllaea* thus qualifies as a good outgroup for the other three genera. In further studies on the *atpB/rbcL* spacer region the other genera of the Epithemateae will be included.

Phylogeny

There are several implications of the dendograms (Fig. 3) regarding phylogeny and colonization pattern of *Cyrtandra*. The branching pattern clearly indicates several colonization events for the Samoan *Cyrtandra* species, as *C. pogonantha* and the clade comprising *C. compressa* and *C. falcifolia* are separated by the Hawaiian taxa. Another interesting result is that the Hawaiian *C. grandiflora* and *C. sandwicensis* appear in the same clade, although supported only by a low bootstrap value. *C. paludosa*, the third investigated species from Hawaii, clearly is part of a second clade including taxa from Fiji and Samoa. This could be seen in accordance with the hypothesis of Wagner et al. (1990) that the Hawaiian *Cyrtandra* species originate from several colonization events. However, another interpretation is possible as well, postulating a migration from Hawaii (*C. paludosa*) to Samoa (*C. compressa*, *C. falcifolia*) and Fiji (*C.* cf. *milnei*). At the basis of the dendrogram, there are mainly taxa from Malesia (*C. splendens*: Borneo; *C. calyptibracteata*: Java; *C. pendula*: Peninsular Malaysia), and only one taxon from East Polynesia (*C. pogonantha*: Samoa). At present, this makes a Malesian origin of the genus more probable. However, species from the central region of the distribution area (mainly the Philippines and New Guinea) have to be included in the studies before a final statement can be made.

Functionally important sections

The investigated part of the *rbcL* coding region shows very little variation and seems to be strictly conserved by selective pressure. Although frame shift mutations were found in the sequence of *C. pendula* and *Monophyllaea*, there is no indication for a higher substitution rate in the rest of the coding sections analysed so far, as would be expected in a silenced pseudogene. Therefore it can be concluded that the observed frameshift is either the result of a PCR artefact or occurred rather recently, giving rise to heteroplasmy in the individuals used in this study.

The lack of the distal *atpB* promoter in some members of the Rubiaceae was interpreted by Manen and Natali (1996) as a possible reason for the loss of woodiness in those taxa. In all investigated Gesneriaceae only the distal *atpB* promoter is functional whereas the proximal *atpB* promoter is truncated. Thus, a second predominantly herbaceous group is characterized by only a single intact *atpB* promoter. Further investigations on plant groups with a shift from a herbaceous to a woody habit and *vice versa* could reveal the merit of the theory of Manen and Natali (1996).

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