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Molecular phylogenetics of *Alternanthera* (Gomphrenoideae, Amaranthaceae): resolving a complex taxonomic history caused by different interpretations of morphological characters in a lineage with C_4 and C_3–C_4 intermediate species

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Alternanthera (Amaranthaceae) is a diverse genus (80–200 species) largely restricted to the American Tropics. With *Pedersenia* and *Tidestromia*, it makes up the 'Alternantheroid clade' in Gomphrenoideae. Parsimony and Bayesian analyses of nucleotide sequences of nuclear (ITS) and plastid (*rpl16*, *trnL-F*) and morphological characters identify that the capitate stigma of *Alternanthera* is a synapomorpy within the Alternantheroids. Within *Alternanthera*, two major clades were resolved, both of which were marked by otherwise homoplasious characters of the gynoecium: Clade A [99% jackknife (JK); 1.0 posterior probability (PP)] with nine species and Clade B (60% JK; 0.98 PP) with 22 species. Four subclades (B1–B4), strongly supported statistically, were identified in Clade B. Previous subgeneric classifications of *Alternanthera* appear artificial in light of our new molecular phylogenetic analyses. Most major lineages are congruently resolved by nuclear and plastid data but some incongruence between the nrITS and plastid phylogenetic trees suggests hybridization may have played a role in the rampant speciation in *Alternanthera*. Whereas C_4 photosynthesis appears to have evolved in a single clade, the position of *A. littoralis* var. *maritima* (C_3) in this clade may be explained by hybrid speciation rather than a reversal from C_4 to C_3. All C_3–C_4 intermediates belong to a different clade that also contains C_3 species, but species limits, including the widely studied *A. tenella*, are unclear. The clade including *A. tenella* and *A. halimifolia* contains most of the species endemic to the Galápagos whereas *A. nesiotes*, also endemic to the islands, is nested among widespread American taxa. This suggests that the Galápagos radiation of *Alternanthera* may have arisen from at least two independent colonization events followed by a subsequent radiation in the former lineage. *Botanical Journal of the Linnean Society*, 2012, **169**, 493–517.

ADDITIONAL KEYWORDS: Caryophyllales – classification systems – Galápagos – gene trees – Neotropics – photosynthetic pathways – reconstructing character evolution – reticulate evolution.

INTRODUCTION

Alternanthera Forssk. is the second largest genus in subfamily Gomphrenoideae of Amaranthaceae (Eliasson, 1990; Townsend, 1993). The highest diversity is found in South America (Mears, 1977), but many

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species also occur in the Caribbean, Central America and Mexico. About 20 new taxa were rather recently described from Argentina, Paraguay and Brazil (Pedersen, 1997, 2000). Estimates of species numbers in different treatments and by different authors vary from 80 (Mears, 1977) to over 100 (Townsend, 1993; Borsch, 2001) or 200 (Robertson, 1981; Eliasson, 1987, 1990; Siqueira, 2004). These considerably deviating numbers are largely the result of differing points of view on species limits in alpha-taxonomic treatments and indicate the need for more thorough studies including molecular approaches in *Alternanthera*. Thirteen indigenous *Alternanthera* spp. occur on the Galápagos Islands (nine endemic) making it one of the most species-rich genera on the archipelago (Eliasson, 1988, 2004). Several species are native to the Old World, occurring in Africa, Asia and Australia (Robertson, 1981), whereas a few others were introduced from the New World and have now become invasive weeds (e.g. *A. caracasana* Kunth, *A. paronychioides* A.St-Hil., *A pungens* Kunth and *A. sessilis* (L.) DC.; Robertson, 1981; Eliasson, 1987). Economically, *A. bettzichiana* (Regel) Voss is commonly used as an ornamental for its colourful foliage (Robertson, 1981; Eliasson, 1987); *A. tenella* Colla is reportedly used in Brazil as an anti-inflammatory remedy (Guerra *et al*., 2003); and *A. repens* (L.) Link (= *A. pungens*) is used in Mexico to treat gastrointestinal infections because of its tested antiprotozoal activity (Tapia-Pérez *et al*., 2003).

Alternanthera spp. are annual or perennial herbs, shrubs, small trees or rarely vines (Robertson, 1981; Borsch, 2001). They are characterized by subglobose to short-cylindrical inflorescences with dense solitary flowers, the stamens basally united in a cup and alternating with long, laciniate, small triangular or rarely obsolete pseudostaminodia and capitate stigmas (Eliasson, 1987; Townsend, 1993).

Alternanthera has long been of interest to physiologists because of the occurrence of C_3–C_4 intermediate and C_4 species (Devi, Rajagopalan & Raghavendra, 1995; Chinthapalli *et al*., 2000; Gowik *et al*., 2006; Sage *et al*., 2007). Whereas Sage *et al*. (2007) provided $\delta^{13}C$ carbon isotope values for a large number of *Alternanthera* spp., they included only three out of at least 17 C_4 species in their reconstruction of the evolution of photosynthetic pathways in Amaranthaceae. It was evident that C_4 photosynthesis is derived within *Alternanthera*, but so far there are no hypotheses on the origin of *A. tenella*, which as a C_3–C_4 intermediate is comparatively studied with the C_4 *A. pungens* (e.g. Gowik *et al*., 2006).

Apart from considerable morphological variation among populations of many species and high phenotypic plasticity that has led to the description of many infraspecific taxa such as forms, varieties and subspecies in *Alternanthera* (e.g. Moquin-Tandon, 1849; Pedersen, 1967, 1990), generic concepts have shifted considerably in treatments published during the past two centuries. This was due to the fact that different authors gave different emphasis to individual morphological characters and treated them as diagnostic without insight into possible homoplasy. As a result, there is an enormous number of names that may exceed our conservative estimates of species diversity by three or four times, and complicates the study of *Alternanthera*. Mears (1977) attempted to clarify typification issues and proposed various lectotypes of nine widespread species of *Alternanthera*, but his planned revision of the genus was never carried out, and the most comprehensive taxonomic treatment of *Alternanthera* is that of Moquin-Tandon (1849).

The generic concept employed by Moquin-Tandon (1849) was rather broad, including *Pedersenia* Holub (= *Trommsdorffia* Mart.), whereas Martius (1826) and Endlicher (1836–1840) recognized individual parts of *Alternanthera* as distinct genera. *Alternanthera* as currently widely accepted goes back to Schinz (1893), later adopted by Townsend (1993) who circumscribed the genus like Moquin-Tandon (1849) but excluded *Pedersenia* (for an overview of classification systems see Table 1).

The phylogenetic position of *Alternanthera* in Gomphrenoideae has been demonstrated in recent phylogenetic studies of Amaranthaceae (Müller & Borsch, 2005; Sánchez-del Pino, Borsch & Motley, 2009). Thus, far the best sampling included only 13 *Alternanthera* spp. (Sánchez-del Pino *et al*., 2009) using *trnL-F* and *rpl16* sequence data. They inferred a plastid tree for Gomphrenoideae and provided strong evidence [93% jackknife (JK); 1.0 posterior probablity (PP)] for an 'Alternantheroid clade' that includes *Alternanthera* (99% JK; 1.0 PP) as sister to a clade comprising *Pedersenia* (= *Trommsdorffia*) and *Tidestromia* Standl. (= *Cladothrix* Nutt. ex Moq.; Sánchez-del Pino *et al*., 2009). A *matK*/*trnK* analysis focusing on *Pedersenia* also indicated the monophyly of *Alternanthera* but did not resolve an 'Alternantheroid clade', indicating a position of *Pedersenia* sister to a lineage formed by *Pfaffia* Mart. and relatives plus *Gomphrena* L. and relatives, all together in a polytomy with *Alternanthera* and *Tidestromia* (Borsch, Ortuño & Nee, 2011).

The goal of the present study is to provide a first insight into phylogenetic relationships in the genus *Alternanthera* and to provide a comprehensive overview on the complex history of classification as a basis for future taxonomic treatments reflecting natural entities. To test for reticulate patterns in evolution of species diversity, trees from the nuclear ribosomal internal transcribed spacer (nrITS) region were compared with trees inferred from data sets of the highly

Table 1. Traditional classifications proposed for the genus *Alternanthera* and related genera

Martius (1826)	Endlicher (1836–1840)	Moquin-Tandon (1849)	Bentham & Hooker (1880)	Schinz (1893)	Schinz (1934)	Townsend (1993)
Alternanthera	*Alternanthera*	*Alternanthera*	*Alternanthera*	*Alternanthera*	*Alternanthera*	*Alternanthera*
					Subgenus *Eualternanthera*	
		Section *Trommsdorffia*		*Iresine**		*Pedersenia*† *Iresine*§
		Section *Dassiera*			Section *Dassiera*	
		Section *Allaganthera*	Section *Allaganthera*		Section *Allaganthera*	
			Section *Lithophila*	*Lithophila*		*Lithophila*
		Section *Cladothrix*	*Cladothrix*	*Cladothrix*		*Tidestromia*‡
	Teleianthera¶	*Telanthera*	*Telanthera*		Subgenus *Telanthera*	
Bucholzia	Section *Bucholzia*	Section *Bucholzia*	Section *Bucholzia*		Section *Bucholzia*	
Brandesia	Section *Brandesia*	Section *Brandesia*	Section *Brandesia*		Section *Brandesia*	
Mogiphanes	Section *Mogiphanes*	Section *Mogiphanes*	*Mogiphanes*		Section *Mogiphanes*	

*Including members of *Trommsdorffia*.
†Including *Trommsdorffia*.
‡Including *Cladothrix*.
§Narrower concept.
¶Name probably misspelled by the author for *Telanthera*.

performing non-coding plastid regions *trnL-F* and *rpl16*. Molecular phylogenetic hypotheses were then used to reconstruct the evolution of selected morphological characters considered diagnostic in previous classification systems and to obtain first insight into the evolution of photosynthetic pathways and biogeography in *Alternanthera*.

MATERIAL AND METHODS

Taxon sampling

The ingroup contains 33 samples representing a total of 31 out of the *c*. 80–200 described *Alternanthera* spp. (Eliasson, 1987; Townsend, 1993; Appendix 1). The sampling approach was designed to include all previously described sections (Table 1) and was also guided by the comprehensive taxonomic treatment of Moquin-Tandon (1849) to cover morphological diversity. To account for possible reticulate speciation and incomplete lineage sorting in a group with unreliable species classification, the same individuals were used for each data set, with the exceptions of *Tidestromia carnosa* (Steyerm.) I.M.Johnst. and *T. lanuginosa* (Nutt.) Standl., both outgroup taxa for which two individuals each were used (Appendix 1). Vouchers are deposited at B, GB, MEXU and NY (Appendix 1).

Outgroup taxa included *Pedersenia cardenasii* (Standl.) Holub, *Pedersenia* cf. *hassleriana* (Mart.) Holub, *Tidestromia carnosa*, *T. lanuginosa* and *T. valdesiana* Sanch. Pino & Flores Olv., and were selected based on recent molecular analyses of *trnL-F* and *rpl16* data for Gomphrenoideae (Sánchez-del Pino *et al*., 2009). Parsimony reconstructions that required a single taxon as the outgroup (and placing of the root) used *T. valdesiana* for this purpose.

Morphology

The data matrix consisted of 11 characters. All characters were treated as unordered (non-additive) and equally weighted. Morphological characters and states are given in Appendices 2 and 3 and were optimized using Winclada.

C_4 and C_3–C_4 photosynthesis

To obtain insight into the phylogenetic distribution of currently known types of photosynthetic pathways in *Alternanthera*, data were taken from published works. The major source for isotope and, with some limitations, anatomical data was Sage *et al*. (2007) who examined a large number of *Alternanthera* spp. Two taxa [*A. tenella* and *A. ficoidea* (L.) P.Beauv., but see discussion below] were considered as C_3–C_4 intermediate species based on Devi *et al*. (1995). The three different kinds of photosynthetic pathways were coded as unordered states and optimized using Winclada on the respective gene trees (the plastid DNA tree is presented here in Fig. 1) to estimate the number and position of C_4 and intermediate lineages in *Alternanthera*.

DNA extraction, amplification and sequencing

Total genomic DNA was isolated from leaf tissue, dried in silica gel or taken from herbarium specimens. DNA extraction followed the Qiagen Plant DNeasy (Qiagen Inc.) manufacturer's protocol and Fast PrepTM method (Qbiogen Inc.) or used a modified CTAB protocol for silica-dried samples (Borsch *et al*., 2003). DNA extraction from herbarium material included 30 μL of β-mercaptoethanol and 30 μL of highly purified proteinase K solution (Roche) added to the recommended 400 mL of AP1 lysis buffer with constant mixing and incubation at 42 °C for 12–24 h based on the methods of Motley, Wurdack & Delprete (2005).

The polymerase chain reaction (PCR) was performed with 25-μL reactions containing 1 × Taq buffer with 1.5 mM $MgCl_2$, 1 mM dNTP mix (2.5 mM each), 0.4 μM of each primer, 1 U *Taq* DNA polymerase (Qiagen) and 1 μL of DNA template. To improve amplification, bovine serum albumin (0.25 μg μL^{-1}), dimthyl sulphoxide (10%) or betaine (1 M) were added. Ex TaqTM DNA polymerase (hot-start version; Takara Mirus Bio) was used to amplify difficult samples. Amplification and cycle sequencing reactions were run on a Gene Amp PCR system 9600 (Applied Biosystems). Double-stranded DNA templates were amplified for two plastid regions (*rpl16*, *trnL-F*) and nuclear ITS. All PCR and cycle sequencing reactions were run on a Gene Amp PCR system 9600 (Applied Biosystems). Amplification and sequencing of the *trnL-F* region was carried out using primers c and f of Taberlet *et al*. (1991), sometimes complemented with the internal universal forward sequencing primer trnL460F (Worberg *et al*., 2007) to produce reads of the *trnL-F* spacer. The *rpl16* intron was amplified using primers designed by Asmussen (1999) and another primer based on the reverse complement of *rp116*-584R (5′-TTCATTGGGTGGGAGGCGGAA-3′) was designed at NYBG. Two primers, forward (5′-CCTTATCATTAGAGGAAGGAG-3′) and reverse (5′-ATGCTTAAAYTCAGCGGGT-3′; modified from White *et al*., 1990; Baldwin *et al*., 1995), were used to amplify the ITS region. The PCR conditions for amplifications of the *trnL-F* region were: one cycle at 97 °C for 2 min; 30 cycles each at 94 °C for 1 min, 48 °C for 2 min and 72 °C for 2 min; and one cycle at 72 °C for 16 min, hold at 4 °C. PCR conditions for amplifications of the *rpl16* intron were: one cycle at 94 °C for 3 min; 30 cycles each at 93 °C for 1 min, 55 °C for

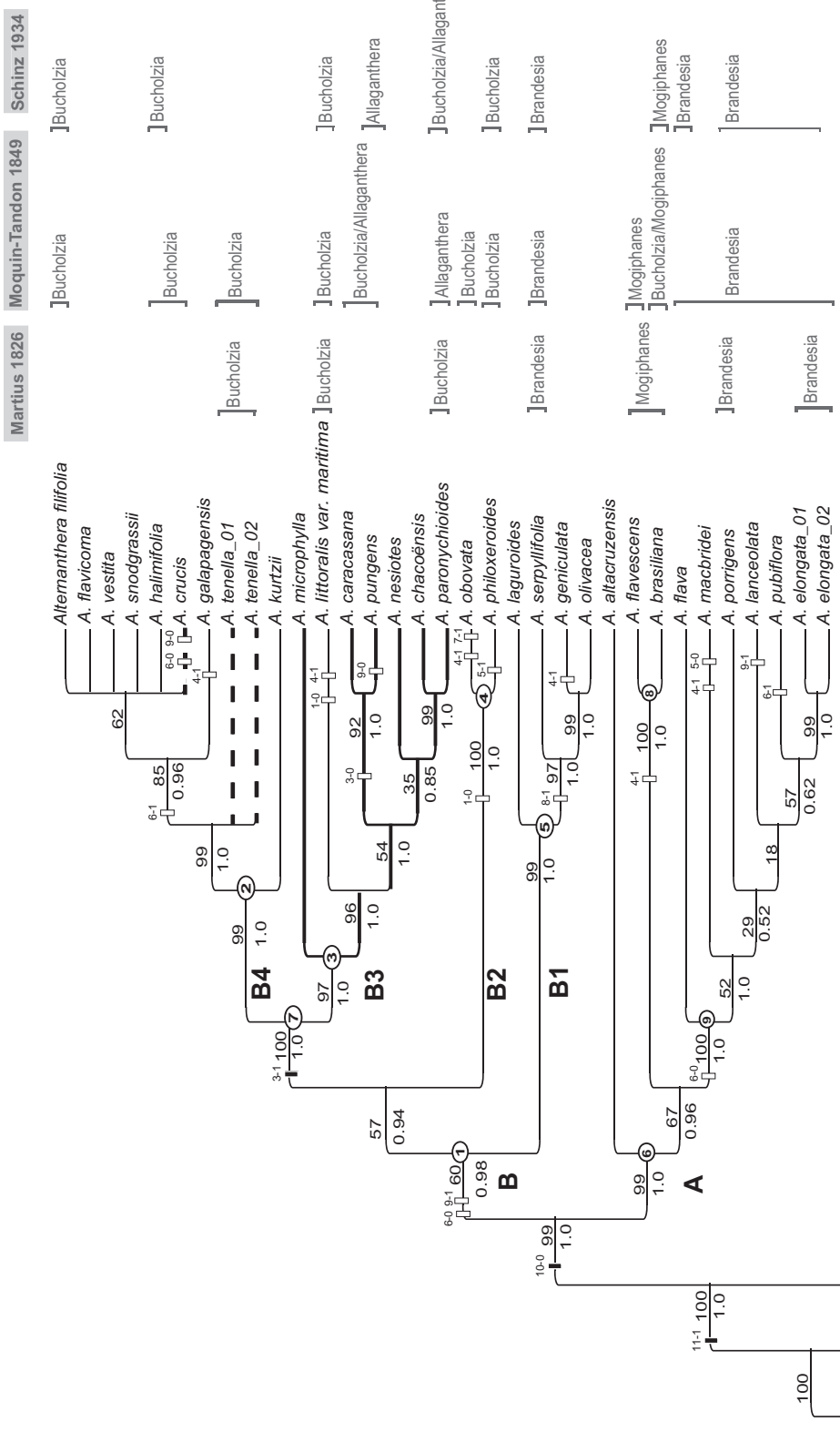

Figure 1. The single MPT from the combined analysis using *trnL-F* and *rpl16* data indicating sections proposed in *Alternanthera* by Martius (1826), Moquin-Tandon (1849) and Schinz (1934). Solid black bars represent homologies and white bars represent homologous characters. The C_4 branches are indicated in bold and C_3–C_4 as dashed lines. Numbers are jackknife values (above and posterior probabilities (below). The two major clades (A and B) and the four subclades of B (B1–B4) are also denoted on the tree.

1 min and 72 °C for 1.5 min; and one cycle at 72 °C for 5 min, hold at 4 °C. The temperature profiles for ITS amplifications were: one cycle at 97 °C for 50 s, 30 cycles each at 97 °C for 50 s, 53 °C for 50 s and 72 °C for 1 min 50 s, and a final extension at 72 °C for 7 min.

Amplified products were purified with the QIAquick PCR Purification Kit (Qiagen Inc.). Purified products were cycle sequenced with dye terminator ABI Prism Ready reaction mix v.3.1 (Applied Biosystems). Sequences were electrophoresed on an ABI 377XL DNA automated sequencer.

Sequence alignment and indel coding

Sequences were edited in Sequencher version 4.1 for PC (Gene Codes). Edited sequences were automatically pre-aligned with Clustal X v. 2.0.12 (Larkin *et al*., 2007) using default settings. The alignments were then adjusted by eye using the BioEdit Sequence Alignment Editor v 7.0.9 (Hall, 1999) and PhyDe version 0.9971 (Müller *et al*., 2010). The alignment method followed the criteria for homology assessment suggested by Borsch *et al*. (2003). These criteria account for microstructural changes involving from one to many nucleotides in a single mutational step. Hotspots (Borsch *et al*., 2003) or regions with uncertain primary homology were excluded in phylogenetic analyses (the number of regions and total number of positions excluded in the analyses are indicated below and in Table 3). Substitutions within repeats were coded with ambiguity codes. Gaps were coded as binary characters using the 'simple gap coding' method (Simmons & Ochoterena, 2000). The program SeqState version 1.4.1 (Müller, 2005) was employed to score indels automatically.

Phylogenetic analysis

Four data sets were prepared for the phylogenetic analyses and the number of aligned positions and parsimonious informative characters are given in Table 2. Specimen details for the data sets of *trnL-F*, *rpl16* (each 38 terminals) and ITS (37 terminals) are listed in Appendix 1. Data sets were analysed independently for each of the three genomic partitions and simultaneous analyses were conducted for the combined data sets of plastid regions.

Parsimony analysis

Constant invariable characters were deactivated. Heuristic parsimony analyses were conducted using Nona (Goloboff, 1993) spawned by Winclada (Nixon, 1999). TBR branch swapping on Wagner trees were conducted from 10 000 random taxon addition sequences with 10 trees held in memory for each of the replicate initiations expanding the memory to 100 000 for further TBR (h 100 000; mult* 10 000; ho/10).

JK branching support was calculated by Nona using Winclada with 10 000 replications with 100 search replications and 10 trees held in memory with the next parameters (mult*100; ho/10; max*). In this paper JK values are described as high (85–100%), moderate (75–84%) or low (≤ 74%).

Bayesian analysis

Bayesian analysis was conducted with the program MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). Modeltest 3.7 (Posada & Crandall, 1998) was used to select the best-fit model of nucleotide substitution for the present data based on the Akaike Information Criterion (AIC; Akaike, 1974) and analyses were performed for five structurally and functionally different genomic regions. Data were partitioned into the *rpl16* intron (group II), *rpl16* exon, *trnL* intron (group I), *trnL-F* spacer and ITS with an individual model assigned to each partition. The TVM+G model of substitution was selected for the *rpl16* intron and *trnL-F* spacer data sets, TIM+G for the *rpl16* exon, K81uf+I+G for the *trnL* intron and the SYM+I+G model for the ITS partition. For the simultaneous analysis of plastid markers, MrBayes was run for 5000 000 generations. PP distributions of trees and branch lengths were obtained using the Markov Chain Monte Carlo (MCMC) method (Metropolis *et al*., 1953; Hastings, 1970) and transferred on the resulting phylogenetic trees. Four chains were run with a temperature setting of 0.2. Chains were sampled every 100 generations after burn in, which was set at 5000 generations when PP reached stable log likelihood values of the model and tree. Starting trees for two independent runs were randomly selected. A majority rule consensus tree was then obtained using all saved trees.

Congruence of data sets

The Incongruence Length Difference (ILD) test (Farris *et al*., 1995) assesses character conflict between data sets and calculates the significance of that conflict. The null hypothesis is that conflict between data sets is no greater than conflict among random partitions of the combined data set. The use of various data types provides phylogenetic information that can either converge towards the same phylogenetic tree or show discrepancies leading to conflicting conclusions (Darlu & Lecointre, 2002). Darlu & Lecointre (2002), Lee (2001) and van der Niet & Linder (2008) suggested that the ILD test is

Table 2. Sequence variation among the *trnL-F, rpl16* and ITS regions

Molecular marker	Nc	Length range	Mean length	SD	%div. (range)	ts/tv ratio	%var.	% inf. (char. inf.)	%GC	In.	inf. in.
trnL-F (total)	1202	626–1059	880.053	77.723	3.014 (0.000–5.288)	0.715 (0.000–5.000)	14.143	8.07	31.064		
trnL-intron	754	391–655	623.421	49.873	3.08 (0.000–5.424)	0.546 (0.000–3.000)	16.446	8.886	27.731		
trnL-spacer	398	162–371	206.711	60.576	3.323 (0.000–8.889)	1.979 (0.000–8.000)	11.307	7.286	37.7		
trnL-F (excluding hotspots)	1084	582–964	809.079	72.924	2.969 (0.000–5.007)	0.729 (0.000–4.000)	13.653	8.026	32.929	66	31
trnL-intron	663	353–598	564.316	45.475	2.996 (0.000–5.351)	0.552 (0.000–2.000)	15.686	8.597 (57)	29.411	46	22
trnL-spacer	370	149–348	194.842	58.409	3.421 (0.000–8.876)	1.951 (0.000–8.000)	11.622	7.838 (30)	39.972	18	9
rpl16 (total)	1398	943–1170	1,120.90	38.471	3.82 (0.000–7.192)	0.727 (0.000–3.500)	20.529	12.303	29.99		
rpl16-intron	1258	803–1030	981.579	37.345	3.984 (0.000–7.200)	0.66 (0.000–3.000)	21.065	12.719	28.51		
rpl16-exon	140	115–140	138.316	3.867	2.748 (0.000–7.914)	1.567 (0.000–5.000)	15.714	8.571 (12)	39.96	1	0
rpl16 (excluding hotspots)	1238	894–1085	1,045.08	34.726	3.492 (0.000–7.003)	0.788 (0.000–5.000)	16.64	9.935	31.47	95	44
rpl16-intron	1097	754–945	905.763	33.505	3.613 (0.000–6.980)	0.708 (0.000–5.000)	16.773	10.119 (111)	30.09	94	44
ITS (total)	645	584–611	596.432	4.979	15.361 (0.000–27.087)	1.102 (0.000–4.500)	48.682	38.605	55.694		
ITS1	251	213–231	225.568	2.656	21.245 (0.000–36.697)	1.159 (0.000–4.000)	64.542	52.59	54.679		
5.8S	163	162–163	162.973	0.162	1.826 (0.000–4.908)	2.224 (0.000–6.000)	9.816	5.521 (9)	54.909	1	0
ITS2	232	196–219	207.892	3.54	19.767 (0.000–37.073)	1.02 (0.000–11.000)	58.621	46.121	57.397		
ITS (excluding hotspots)	624	566–595	579.703	4.948	14.911 (0.000–26.748)	1.143 (0.000–4.200)	47.596	37.5	55.735	64	26
ITS1	239	202–223	216.189	2.958	21.401 (0.000–37.143)	1.198 (0.000–5.000)	64.435	52.72 (128)	54.163	35	19
ITS2	222	189–211	200.541	3.43	18.74 (0.000–36.181)	1.076 (0.000–10.000)	57.207	44.595 (99)	58.1	28	7

Nc, number of characters; SD, standard deviation of mean length; %div. (range), percentage of pairwise sequence distance (uncorrected p distance, overall means, lowest and highest values in parentheses); ts/tv ratio, ratio of transitions to transversions; %var., percentage of variable positions; %inf. (char. inf.), percentage of parsimony-informative positions (number of potentially parsimony-informative characters in parentheses); %GC, GC content; In., number of indels; inf. in., number of informative indels.

still suitable as an explanatory method to detect significant incongruence and it produces more accurate results than other tests.

In this study, congruence among data sets was evaluated observing topological congruence (Figs 1, 3). In addition, quantitative congruence among data sets was tested with the ILD test. The incongruence test value was calculated using Nona in Winclada. One thousand replications were executed with 10 searches per replication holding 10 trees for each search and holding 100 trees with the next parameters (100 replications, 10 mult per rep; holding 10 trees per mult; hold 100 trees for 'hold*'). Uninformative characters were removed from the analyses before running the ILD test (Lee, 2001).

RESULTS

Sequence variability of the *trnL-F* region

Statistical values of the sequences included in the molecular matrices are summarized in Table 2. The total length of the *trnL-F* sequence comprised 1202 bp positions. The *trnL-F* spacer was more variable in range (162–371 bp), in variable sites (45 characters, 11.3%), in transitions/transversions (ts/tv) ratio and in GC percentage than the *trnL* intron (Table 2). However, the *trnL* intron has 40 more potentially phylogenetically informative characters, 57 nucleotides (46% of the proportion of the variable sites) and 22 indels (70% of the total number of indels). These values may be affected by a large indel (ranging from 169 to 178 bp) in the *trnL-F* spacer, which was previously proposed as a potential synapomorphic character for *Alternanthera* (Sánchez-del Pino *et al*., 2009). Six hotspot regions in the *trnL* intron and two in the *trnL-F* spacer were identified (Table 3). Excluding the 5′*trnL* exon and hotspots, the aligned matrix length of the *trnL-F* sequence included a total of 1084 bp positions.

Sequence variability of the *rpl*16 region

The total length of *rpl16* comprised 1398 characters and the *rpl16* intron included 155 potentially parsimony-informative sites (Table 2). Of these, 111 are point mutations (41.8% of the proportion of the variable sites) and 44 are indels (100%), whereas the *rpl16* exon contained only 12 bp that were potentially parsimony-informative characters (54.5% of the proportion of the variable sites). Six hotspots were found in the *rpl16* intron (Table 3). The aligned length of the *rpl16* comprised a total of 1238 bp positions (excluding hotspots).

Sequence variability of the ITS region

The total length of the ITS sequence (ITS1+5.8S+ ITS2) is 645 bp (Table 2). From two species (*A. flavicoma*, *A. pubiflora*) no clean sequences could be obtained from herbarium material, so they could not be included in the analysis. Compared with ITS2, the ITS1 spacer had a higher ts/tv ratio, a higher percentage of variable sites and possessed 41 more potentially parsimony-informative characters. The ITS1 ranged from 213 to 231 bp, of which 163 bp were variable (65%), and 128 nucleotides (78.5% of the proportion of the variable sites) and 19 indels were identified as potentially parsimony-informative characters (Table 2). The ITS2 region ranged from 196 to 219 bp, of which 137 were variable (59%) and 99 nucleotides (72.2% of the proportion of the variable sites) and seven indels were found to be potentially phylogenetically informative. Low divergence was found in the 5.8S gene with only nine potentially parsimony-informative characters (represent-

Table 3. Positions of hotspots and exons in *trnL-F, rpl16* and ITS regions

trnL-F region	*rpl16* region	ITS
trnL 5′ exon 1–12	*rpl16* intron	ITS 1
trnL intron		
H1. 73–79 poly A	H1. 166–191 poly T and G	H1. 54–65
H2. 122–128 poly A	H2. 231–272 poly A	
H3. 157–174 poly A	H3. 307–323 poly A	
H4. 343–346 poly A	H4. 751–770 poly T	
H5. 399–416 poly A	H5. 880–893 poly A	
H6. 509–533 poly A and T	H6. 1086–1127 poly A and T	
trnL 3′ exon 755–804		ITS2
		H2. 436–445
H7. 840–854 poly T		
H8. 1079–1091 poly T		

Table 4. Taxa with polymorphic nucleotide sites and length polymorphisms in ITS

Taxon	ITS1	5.8S	ITS2	NPST
A. altacruzensis	1	0	0	1
A. brasiliana	3	0	1	4
A. caracasana	0	0	1	1
A. elongata	0	0	1	1
A. filifolia	1	0	0	1
A. flavescens	1	0	2	3
A. galapagensis	0	1	0	1
A. kurtzii	5	0	6	11
A. laguroides	1	0	0	1
A. littoralis var. *maritima*	0	1	0	1
*A. tenella*_01	2	0	0	2
*A. tenella*_02	1	0	2	3

NPST, total number of polymorphic sites.

ing 55.2% of the variable sites). One hotspot region was observed in each of the ITS1 and ITS2 spacers, respectively. The total aligned length of ITS included 624 positions (excluding hotspots; Table 3). About one-third of the ITS sequences showed polymorphic sites that hint to introgression or hybridization (divergent paralogues; Table 4). This was by far strongest in the sequence of *A. kurtzii* (AC617) with 11 polymorphic sites.

Trees obtained from plastid *trnL-F* and *rpl16* sequences

Parsimony analysis of the combined plastid *trnL-F* and *rpl16* data yielded a single most-parsimonious tree (MPT) with a length of 476 steps (CI = 0.70, RI = 0.89; Fig. 1). Both maximum-parsimony (MP) and Bayesian analyses of the plastid DNA data set resolved a strongly supported monophyletic *Alternanthera* (99% JK, 1.0 PP) and revealed two major clades in *Alternanthera*.

Clade A (Node 6 in Figs 1, 2). Apart from *A. macbridei* Standl., this highly supported (99% JK, 1.0 PP; Fig. 1) clade includes species with long, simple or compound pedunculate inflorescences. All nine species have globose stigmas with distinctive carpel demarcations [except for *A. lanceolata* (Benth.) Schinz; character 9(1); Appendix 2]. The species forming Clade A are predominantly distributed in South America with several extending to Central America [*A. brasiliana* (L.) Kuntze, *A. pubiflora* Kuntze] and Florida (*A. flavescens* Kunth; Figs 1, 2).

Clade B (Node 1 in Figs 1, 2). This clade includes species that have entire stigmas without distinctive carpel demarcations [character 9(1); Appendix 2], except for *A. crucis* Bold. and *A. pungens*. This clade is weakly supported in both plastid (Fig. 1; 60% JK, 0.98 PP) and includes four well-supported subclades (nodes 2–5). Most of the species included in this clade [subclades B2–B4 (57% JK, 0.94 PP); Fig. 1] have sessile, axillary inflorescences either solitary or grouped into two- to five-flowered spikes. However, species with pedunculate inflorescences or both sessile and pedunculate inflorescences also occur in this clade [subclade B1 and *A. philoxeroides* (Mart.) Griseb.].

Within Clade B, there are four subclades (Clades B1–B4).

Clade B1 (Node 5; 99% JK, 1.0 PP) consists of four species [*A. geniculata* Urb., *A. laguroides* (Standl.) Standl., *A. olivacea* Urb. and *A. serpyllifolia* Urb.; Figs 1, 2] which have an erect habit, flowers usually arranged along a rachis to form slender spicate inflorescences, and are predominately distributed in Central America and the Caribbean islands. However, *A. laguroides* differs in having a more globose spicate inflorescence and a distribution restricted to Central America.

Clade B2 (Node 4; 100% JK, 1.0 PP) comprises two species (*A. obovata* Millsp. and *A. philoxeroides*; Figs 1, 2), which are procumbent herbs and possess sessile, globose or cylindrical inflorescences. These species also share a preference for aquatic environments. The latter species is widespread, being native in the New World and invasive in the Palaeotropics (Mears, 1977).

Clade B3 (Node 3; 97% JK, 1.0 PP) includes seven species [*A. caracasana*, *A. chacoënsis* Morong ex Morong & Britton, *A. littoralis* Beauv. ex Moq. var. *maritima* (Mart.) Pedersen, *A. microphylla* R.E.Fr., *A. nesiotes* I.M.Johnst., *A. paronychioides* and *A. pungens*; Figs 1, 2] that are procumbent plants and mostly have sessile, globose or cylindrical inflorescences similar to species in Clade B2. Two species, *A. caracasana* and *A. pungens*, are widespread throughout the Neotropics and invasive in the Old World. One variety of *A. littoralis* P.Beauv. occurs along the east coasts of tropical America and three varieties in the west coast of tropical Africa (Pedersen, 1990). *Alternanthera microphylla* is an endemic to the Prepuna of the Andes.

Clade B4 (Node 2; 99% JK, 1.0 PP) consists of nine species [*A. crucis*, *A. filifolia* (Hook.f.) J.T.Howell, *A. flavicoma* (Andersson) J.T.Howell, *A. galapagensis* (Stewart) J.T.Howell, *A. halimifolia* Standl. ex Pittier, *A. kurtzii* Schinz ex Pedersen, *A. snodgrassii* (B. L. Rob.) J.T.Howell, *A. tenella* and *A. vestita* (Andersson) J.T.Howell; Figs 1, 2). Except for *A. tenella* and *A. crucis*, which have a herbaceous habit, the species in Clade B4 are shrubby. Several of these taxa are distributed in the Galápagos Islands and some are indigenous to Central America and South America.

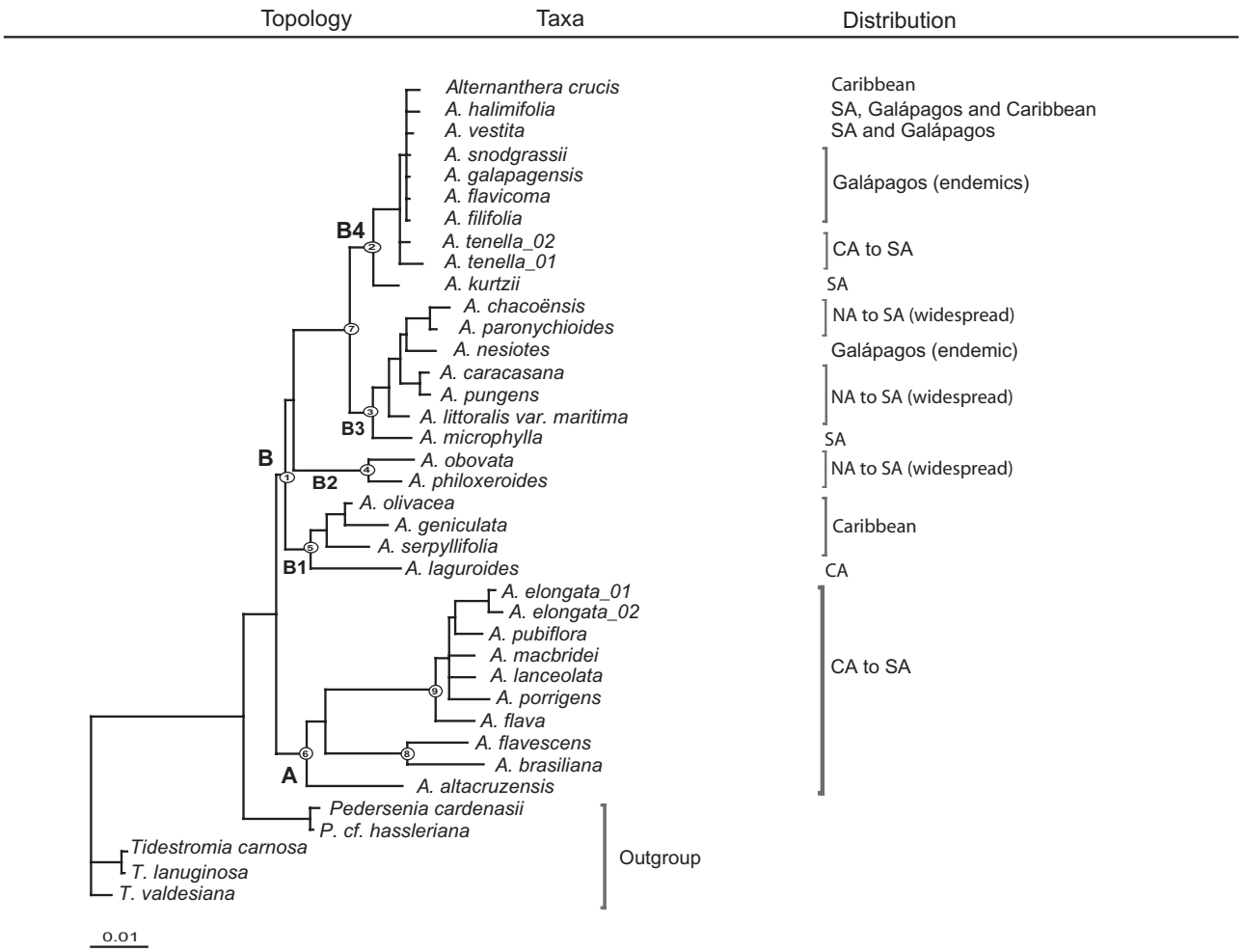

Figure 2. Fifty per cent majority-rule tree from the Bayesian analysis of combined *trnL-F* and *rpl16* data with distributions of *Alternanthera* taxa. NA, North America; SA, South America; CA, Central America. The two major clades (A and B) and the four subclades of B (B1–B4) are also denoted on the tree.

Morphological character mapping

The single MPT resulting from the plastid DNA data shows character state transformations of 11 morphological characters listed in Appendix 2. The morphological characters resolved as either homologous or homoplasious for this analysis are depicted in Figure 1.

Distribution of photosynthetic pathways

The plesiomorphic condition is represented by C_3 photosynthesis. C_4 photosynthesis occurs in one lineage represented by widespread species (Clade B3; Fig. 1), except for *Alternanthera littoralis* var. *maritima* (= *A. maritima*), which is C_3. Two intermediate C_3–C_4 species, *A. crucis* and *A. tenella*, are found in Clade B4.

Trees obtained from nuclear ITS sequences

Parsimony analysis of the nuclear ITS data resulted in three MPTs of 788 steps in length (CI = 0.55, RI = 0.80). The strict consensus tree of the ITS data (L = 789, CI = 0.55, RI = 0.80; Fig. 3) shows a topology largely consistent with that from the analysis of plastid data albeit with lower support for major clades. However, some individual species show different placement as compared with the plastid DNA tree. The ITS tree congruently reveals the two major clades, Clades A (Node 6) and B (Node 1), and strongly supports the monophyly of *Alternanthera* (99% JK). In general, the same four subclades (B1–B4; Nodes 2–5) are resolved (with the exception of the placement of *A. littoralis* var. *maritima*).

A comparison between MP and Bayesian analyses (Fig. 3) of the ITS dataset indicates some topological

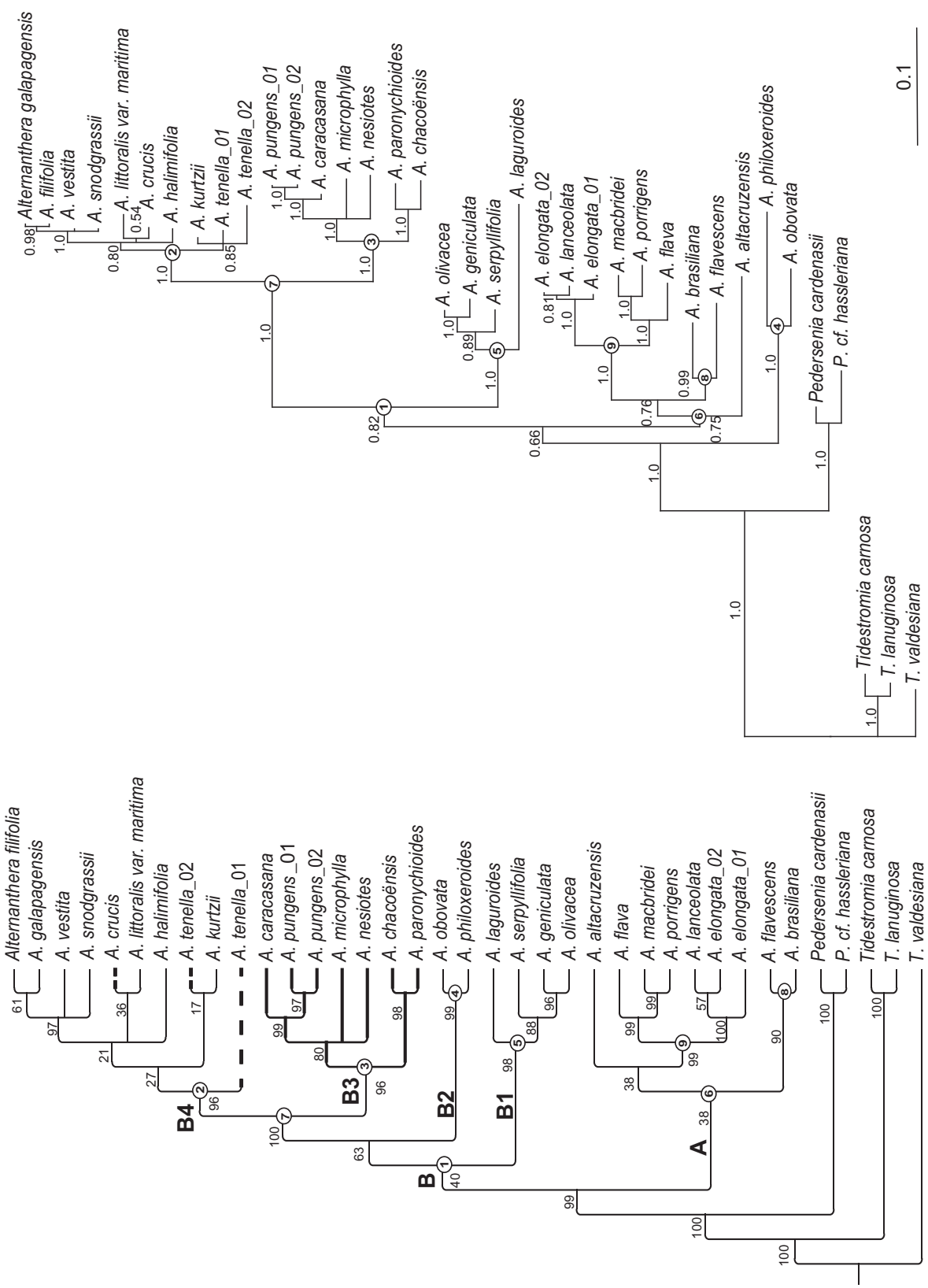

Figure 3. I, the strict consensus trees of 3 MPTs (L = 789 steps, CI = 0.55, RI = 0.80) resulting from the parsimony analysis of ITS data. Numbers below each branch are jackknife values. The two major clades (A and B) and the four subclades of B (B1–B4) are also denoted on the tree. The C_4 branches are indicated in bold and C_3–C_4 as dashed lines. II, fifty per cent majority-rule tree from the Bayesian analysis produced from the ITS data with posterior probabilities above the branches.

inconsistency in weakly supported parts of the tree. Thus, a clear nuclear-based hypothesis on the relationships of the four subclades (B1–B4) and regarding species relationships within Clade A is not possible using ITS alone.

Comparisons between plastid DNA and ITS data sets

All analyses indicated the monophyly of the genus *Alternanthera*. However, there was an area of inconsistency between the two genomic data sets. The inconsistency mentioned above was the position of *A. littoralis* var. *maritima*. The plastid DNA data placed *A. littoralis* var. *maritima* with six other species in Clade B3 (Node 3, 97% JK; Fig. 1), which includes *A. caracasana*, *A. chacoënsis*, *A. microphylla*, *A. nesiotes*, *A. paronychioides* and *A. pungens*, but the ITS data resolved *A. littoralis* var. *maritima* sister to *A. crucis*, in Clade B4 (Node 2, 96% JK; Fig. 3).

Incongruence test

Comparison of plastid DNA and ITS (Figs 1, 3) trees suggested some conflicts among the data partitions from the nuclear and plastid genome compartments. The ILD test was conducted to evaluate if there was significant conflict in signal between plastid DNA and ITS, and between plastid DNA and morphology data. Incongruency was significant ($P = 0.0099$), so a combined analysis of the three molecular data sets and morphology (Appendix 2) was not performed in this study.

DISCUSSION

Phylogenetic relationships in *ALTERNANTHERA*

The use of nrITS, morphology and plastid DNA (*trnL-F*, *rpl16*) confirmed previous hypotheses that the genus *Alternanthera* (100% JK) is monophyletic (Müller & Borsch, 2005; Sánchez-del Pino *et al*., 2009). Contrary to the widely accepted concept of *Alternanthera* developed by Schinz (1893), previous classification systems recognizing several independent genera such as *Brandesia*, *Bucholzia*, *Mogiphanes* or *Telanthera* (Table 1) are thus not supported by phylogenetic data. This study indicates that *Alternanthera* comprises two major clades (Clades A and B), which are also supported by gynoecium characters (Figs 1–3).

Clade A (Node 6; Figs 1–3): Most of the species included in this clade were historically classified in (genus or section) *Brandesia*, and a few in *Bucholzia* and *Mogiphanes* by several authors (Martius, 1826; Endlicher, 1836–1840; Moquin-Tandon, 1849; Schinz, 1934). Among these species, some authors (Martius, 1826; Moquin-Tandon, 1849) placed *A. flavescens* Kunth in (genus or section) *Mogiphanes* and *A. brasiliana* was assigned in sections *Bucholzia*/*Mogiphanes* (Moquin-Tandon, 1849). These two species form a strongly supported clade (100% JK, 1.0 PP; Fig. 1) and both have pedicellate flowers, a homoplasious character [character 4(1); Appendix 2]. Pedicellate flowers were a key character in the diagnosis of the genus *Mogiphanes* as proposed by Martius (1826), and later adopted as a section by Endlicher (1836–1840) and Schinz (1934). However, the character occurs several times in the evolution of *Alternanthera* such that it is not a synapomorphy and therefore *Mogiphanes* is not a well-defined taxon.

The remaining species of Clade A are included in a well-supported subclade (Node 9; 100% JK, 1.0 PP) of species placed in section *Brandesia* following Moquin-Tandon's (1849) and Schinz's (1934) systems. This subclade includes *A. elongata* (Willd.) Schinz, *A. flava* (L.) Mears, *A. lanceolata*, *A. macbridei* and *A. porrigens* Kuntze supported by the homoplasious character of long style [character 6(0); Appendix 2] with a reversal condition for *A. pubiflora*, which is supported by the presence of short styles [character 6(1); Appendix 2].

Alternanthera altacruzensis Suess., formally placed in section *Bucholzia* by Suessenguth (1950), is sister to the species belonging to sections *Mogiphanes* and *Brandesia*. It shares morphological characters with both clades and differs mainly in its inflorescence type. It has three long pedunculate heads originating from a single axis, whereas all other species in the clade have more or less branched thyrsoid synflorescences.

Clade B1 (Node 5; Figs 1–3): This clade (99% JK, 1.0 PP; Fig. 1) includes four species. Two, *A. serpyllifolia* and *A. geniculata*, were formerly assigned to *Brandesia* by several authors (Martius, 1826; Endlicher, 1836–1840; Moquin-Tandon, 1849; Moore, 1895; Schinz, 1934; Fig. 1). *Alternanthera serpyllifolia*, *A. geniculata*, *A. olivacea* and *A. costaricensis* (the last-named species was not sampled here) were placed in a separate genus '*Jamesbondia*' by A. J. Mears (unpubl. data). However, other than annotating many herbarium specimens with this invalid name, his proposal was never published. Nevertheless, molecular data resolved a strongly supported '*Jamesbondia*' clade (97% JK, 1.0 PP; Fig. 1), which was sister to *A. laguroides*. The '*Jamesbondia*' taxa are suffrutescent, procumbent or prostrate perennials (Standley, 1917). Personal observations of herbarium material suggested that this group of species share the presence of long-cylindrical inflorescences, which are different from globose inflorescences (that characterize all the other *Alternanthera* spp.) in

which flowers emerge from a common point [homoplasious character 8(1); Appendix 2]. Among these '*Jamesbondia*' spp., *A. geniculata* has stipitate flowers [homoplasious character 4(1); Appendix 2], whereas *A. olivacea* has sessile flowers. Both have long styles, small stigmas and ligulate, laciniate pseudostaminodia. Their closely related sister species, *A. serpyllifolia*, differs by having bracts, bracteoles, and tepals with thick midnerves, pistils with short styles and flowers lacking pseudostaminodia. *Alternanthera laguroides* is sister to these three species and shares with them the presence of a long styled pistil and globose, long papillate glandulose stigmas.

Clade B2 (Node 4; Figs 1–3): A. philoxeroides and *A. obovata*, both formerly placed in section *Bucholzia* (Moquin-Tandon, 1849; Schinz, 1934; Fig. 1), form a strongly supported clade (100% JK, 1.0 PP; Fig. 1). They share the presence of glabrous tepals [homoplasious character 1(0); Appendix 2] and are characterized by a procumbent perennial habit, obovate leaves and mostly sessile, globose or cylindrical–globose inflorescences. *Alternanthera obovata* further has two overall homoplasious floral features, i.e. pedicellate flowers [character 4(1); Appendix 2] and crenate pseudostaminodia [character 7(1); Appendix 2]. *Alternanthera philoxeroides* is an aquatic or subaquatic (Mears, 1977) perennial herb with ascending or decumbent stems (Standley, 1937; Duke, 1961), whereas *A. obovata* grows in aquatic environments (I. Sánchez-del Pino, pers. observ.), and can be either prostrate or decumbent herbs (Standley, 1917).

Clade B3 (Node 3; Figs 1–3): The species in this clade have been placed in sections *Bucholzia* and in *Allaganthera* (Martius, 1826; Endlicher, 1836–1840; Moquin-Tandon, 1849; Schinz, 1934; Fig. 1). The clade includes *A. microphylla* as sister to the rest of the species *A. caracasana*, *A. chacoënsis*, *A. littoralis* var. *maritima*, *A. nesiotes*, *A. paronychioides* and *A. pungens* based in the combined plastid DNA tree. *Alternanthera littoralis* var. *maritima* is a prostrate perennial with succulent, ovoid leaves and regularly apically fimbriate pseudostaminodia (Mears, 1977) and occurs along the Atlantic coast of Africa and Tropical America (Pedersen, 1990). *Alternanthera littoralis* var. *maritima* is characterized by two homoplasious features: absence of trichomes on tepals [character 1(0); Appendix 2] and presence of pedicellate flowers [character 4(1); Appendix 2]. In the plastid DNA tree it diverges second after *A. microphylla* in clade B3 (Fig. 1). Alternatively, in the nrITS tree (Fig. 3) *A. littoralis* var. *maritima* is nested in subclade B4 sister to *A. crucis*. *Alternanthera littoralis* var. *maritima* may be of hybrid origin as will be discussed in more detail below.

Alternanthera nesiotes is a subshrub (Jørgensen, 1999) species from the Galápagos islands and is closely related to *A. chacoënsis* and *A. paronychioides*, which are prostrate perennial herbs (Figs 1–3). *Alternanthera caracasana* and *A. pungens* are sister species, which share many morphological characteristics. The close relationship between these species was noted by others (e.g. Standley, 1917; Eliasson, 1987). Eliasson (1987) distinguished *A. caracasana* from *A. pungens* by its shorter tepals with almost non-pungent tips and proportionally narrower leaves. After further evaluation of morphological characters, it was determined that the characters distinguishing the species are the apex of the tepals and bracteoles and tepal and leaf size (Sánchez-del Pino, Flores Olvera & Valdés, 1999). *Alternanthera pungens* has a longer bracteole midrib and the tepals have long pungent tips, whereas *A. caracasana* has bracteoles and tepals with acute to apiculate apices. *Alternanthera caracasana* and *A. pungens* (92% JK) grouped together in this study based on one homoplasious character: midrib of bracteoles not prominent [character 3(0), Appendix 2]. Other sister species (99% JK, 1.0 PP) in the subclade, *A. chacoënsis* and *A. paronychioides*, have been variously treated by authors in the past. Pedersen (1967) described six varieties of *A. paronychioides*, including *A. paronychioides* var. *chacoënsis*. Mears (1977) treated *A. chacoënsis* as a synonym of *A. paronychioides*, but Pedersen (1990) retained *A. chacoënsis* as a species. To clarify species limits further, a geographically representative sampling of populations from both entities will be needed.

Clade B4 (Node 2; Figs 1–3): Most of the species included in this highly supported subclade (99% JK, 1.0 PP; Fig. 1) were referred to *Bucholzia* by several authors (Martius, 1826; Endlicher, 1836–1840; Moquin-Tandon, 1849; Schinz, 1934; Fig. 1). The single MPT from the plastid DNA data sets shows that *A. crucis*, *A. filifolia*, *A. flavicoma*, *A. halimifolia*, *A. snodgrassii* and *A. vestita* were grouped together in a weakly supported clade (62% JK). Although *Alternanthera filifolia*, *A. flavicoma* and *A. vestita* share many morphological characters, such as inflorescence types and several floral characters, they can be identified based on leaf shape. The latter has elliptic-lanceolate, oblanceolate or narrowly obovate leaves and the former two species have narrowly linear lanceolate leaves (Eliasson, 1971). *Alternanthera filifolia* is a highly variable species with numerous infraspecific taxa (Howell, 1933; Eliasson, 1971). Howell (1933) stated that *A. filifolia* might be a pubescent variant of *A. flavicoma* and noted it was not easy to distinguish the two species. Along the

same lines, Mears's annotations on herbarium specimens show that he considered *A. flavicoma* to be a subspecies or form of *A. filifolia*, but a nomenclatural change was never published. Later, Eliasson (1990) mentioned that some species closely related to *A. filifolia*, such as *A. flavicoma*, represent branches of the same evolutionary tree and could perhaps be accommodated as subspecies of *A. filifolia*. The sister species to this unresolved clade is *A. snodgrassii*, which shares their shrubby habit. Howell (1933) and Eliasson (1971) suggested that *A. snodgrassii* is closely related to *A. vestita*. These authors mentioned that both species differ mainly in trichome type: *A. snodgrassii* has simple and *A. vestita* has stellate trichomes. However, the type of trichomes is the same for both species based on our observations.

Alternanthera halimifolia is a perennial with stems either spreading and rooting at the nodes, or ascending and forming bushes up to 1 m in height (Eliasson, 1971), and is sister to the shrubby species endemic (*A. filifolia*, *A. flavicoma* and *A. snodgrassii*) and indigenous (*A. vestita*) to the Galápagos. Leaf size was thought to be an important taxonomic character that characterized *A. halimifolia*. Eliasson (1971) mentioned that differences in leaf size seem to be in direct response to the environment. Howell (1933), based on this morphological variation, considered the Galápagos population to be a subspecies of the populations from Pacific coast of South America. In the present study, our results based on polytomy suggest that it is possible to recognize only one species. However, the identity of *A. halimifolia* is not entirely clear and treatments vary from treating it as an endemic from the Lomas of Peru (Borsch, 1993) to a widespread species in many parts of the Neotropics.

Alternanthera crucis, which is part of the clade of the five bushy species from the Galápagos, shares the character leaf-type with *A. halimifolia*. In fact, Duke (1961) considered *A. crucis* as a synonym of *A. halimifolia*. However, in this study two homoplasious characters distinct from *A. halimifolia* support *A. crucis*: long styles [character 6(0); Appendix 2; Fig. 1] and stigmatic surface with distinctive carpel demarcations [character 9(0); Appendix 2; Fig. 1].

Alternanthera galapagensis is the closely related sister species to *A. crucis* and the five bushy species from the Galápagos. *Alternanthera galapagensis* is supported by the homoplasious character pedicellate flower insertion [character 4(1); Fig. 1]. This species is a low-growing shrub endemic to the Galápagos with semi-succulent, glaucous leaves (Eliasson, 1990). *Alternanthera tenella* is sister taxon to the species from the Galápagos and *A. crucis*. *Alternanthera tenella* and *A. crucis* differ from all the Galápagos species by being herbaceous rather than woody. This suggests that *Alternanthera* is another classic case of derived secondary woodiness associated with insular species (Carlquist, 1962, 1974, 2010a, b).

Incongruence of plastid and nuclear data and possible evidence for reticulate evolution

Comparisons between tree topologies show a conflict with regard to the position of four species in the plastid and nuclear trees (Figs 1, 3). The variable position of *A. littoralis* var. *maritima* in trees derived from biparentally and maternally inherited markers suggests a possible hybrid origin.

Hybridization has often been favoured as a mechanism to explain tree incongruence in plants, and incongruence between plastid and nuclear data is often attributed to introgression and hybrid speciation (Baldwin *et al*., 1995; Morrell & Rieseberg, 1998; Widmer & Baltisberger, 1999; Hamzeh & Dayanandan, 2004; Kim & Donoghue, 2008). Similar incongruent results have been demonstrated in the closely related genus *Tidestromia*. Chromosome evidence related to number and the form of meiotic division indicated hybridization in some members of *Tidestromia* (Sánchez-del Pino & Motley, 2010). It is interesting to note that Turner (1994) stated that although two-thirds of the genera of the Amaranthaceae remain to be counted, polyploidy appears to be common in the family. He also suggested that dysploidy ($7 \leftarrow 8 \rightarrow 9, 10, 11$) and amphiploidy ($17 = 8 + 9$) play a role in Amaranthaceae and in the entire order Caryophyllales.

Other species, such as *A. altacruzensis*, *A. galapagensis* and *A. tenella*, have inconsistent placements in the plastid and nuclear trees (Figs 1, 3). However, these minor incongruences are confined to a single clade in which branch support is low, and differences are basically related to sister relationships among species in the same clades. These results may not be related to introgression, but possibly to inadequate characters, stochastic errors, horizontal gene transfer, lineage sorting or heterogeneous rates of molecular evolution (Baldwin *et al*., 1995; Kim & Donoghue, 2008). Therefore, our results are not conclusive with regard to reticulate patterns. ITS data will have to be compared with other nuclear markers to get better understanding of species relationships.

Evolution of diagnostic morphological characters of *Alternanthera* and its major lineages

Diagnostic characters at the generic and sectional ranks include several vegetative characteristics and many floral structures (Martius, 1826; Endlicher, 1836–1840). The characteristic features of *Alternanthera* have changed along with its taxonomic history

such that after a re-evaluation, Eliasson (1987) suggested that the genus is a natural group characterized by a combination of floral and pollen characteristics.

Here we corroborate Eliasson's proposal that the only morphological character that supports the monophyly of *Alternanthera* is the synapomorphy related to stigma form. Our data indicate that probably the only useful characters to recognize major clades in *Alternanthera* are related to pistil characters and inflorescence type. Several species observed in this study that form the major Clade A were described by Moquin-Tandon (1849) as having bilobed, inconspicuously subbilobed or capitate stigmas. Detailed observations of the sampling used in this study suggested that this characteristic is related to a stigma with distinctive carpel demarcations (excluding *A. lanceolata*; character 9, Appendix 2), whereas the species in Clade B have stigmas lacking distinctive carpel demarcations (excluding *A. crucis* and *A. pungens*). Although this attribute resolved as homoplasious, features of the gynoecium should be studied carefully with scanning electron microscopy to examine their potential importance in the taxonomy and classification of *Alternanthera* (see also Appendix 2; Fig. 2).

ALTERNANTHERA AND MONOPHYLETIC INFRAGENERIC ENTITIES

The circumscription of *Alternanthera* has varied considerably (Table 1) over time among different authors. Taxonomic problems in *Alternanthera* began with the designation of the type. Forsskål (1775) proposed the genus *Alternanthera* in the *Flora Aegyptiaco Arabica* without mentioning the species type on the page on which the genus was described. However, a single species name is mentioned for the genus among the list of *Triandra* on page LIX as *Alternanthera achyranthes*. Mears (1977) mentioned that Lamarck validly published the identity of the type species of *Alternanthera* in 1753. Then, the type species of *Alternanthera* was designated as *A. sessilis* (L.) DC., which has the basionym *Gomphrena sessilis* L. (Melville, 1958; Mears, 1977). The situation was complicated by an incorrect designation of the type species of *Achyranthes* L. by Standley (1915). Mears (1977) later explained that for many years it was thought that *Alternanthera* Forsskål was based on *Achyranthes repens* L. Then, Standley (1915) considered *Achyranthes repens* to be the type species of *Achyranthes* and he placed most of the species of *Alternanthera* in *Achyranthes* while transferring the species of *Achyranthes* to *Centrostachys* Wallich (Bullock, 1957; Melville, 1958; Mears, 1977; Robertson, 2003). Standley's (1915) circumscription of *Alternanthera* including *Achyranthes* was so artificial that most taxonomists never adopted it. The widely accepted classification system of Amaranthaceae by Schinz (1893, 1934), later refined by Townsend (1993), placed *Achyranthes* in subfamily Amaranthoideae whereas *Alternanthera* was located in subfamily Gomphrenoideae. The distant positions of both genera also appear in recent molecular phylogenetic analyses of Amaranthaceae (Müller & Borsch, 2005; Sánchez-del Pino *et al*., 2009).

Many early classifications recognized segregate genera. The first classification for *Alternanthera* and related genera (*Brandesia*, *Bucholzia* and *Mogiphanes*) was proposed by Martius (1826). Later, several authors (Endlicher, 1836–1840; Moquin-Tandon, 1849; Bentham & Hooker, 1880) recognized *Alternanthera* and *Telanthera* R.Br. as different genera, whereas others treated these groups as subgenera (Schinz, 1934) and others still recognized them as sections of *Alternanthera*. Endlicher (1836–1840) recognized three sections (*Bucholzia* Mart., *Brandesia* Mart. and *Mogiphanes* Mart.) within *Telanthera*. Moquin-Tandon (1849) accepted Endlicher's (1836–1840) subgeneric classification of *Telanthera* and proposed four sections in *Alternanthera* (*Trommsdorffia* Mart., *Dassiera* Moq., *Allaganthera* Mart. and *Cladothrix* Nutt.). Bentham & Hooker (1880) followed Moquin-Tandon's circumscription but differed in that they recognized only two of the three sections in *Telanthera* (*Bucholzia* and *Brandesia*) and two in *Alternanthera* (*Allaganthera* as already proposed by Moquin-Tandon and a new section *Lithophila*).

The classifications proposed for *Alternanthera* by Martius (1826) and Endlicher (1836–1840) primarily used pseudostaminodium shape and flower pedicels (present or absent) along with other several flower morphology to define generic or infrageneric units. Moquin-Tandon (1849) recognized infrageneric taxa based on sexual expression, stem habit, inflorescence type, stamen number and fusion, stigma shape, tepal features, style size and pseudostaminodium shape. Schinz (1934) used the characters stamen filaments and pseudostaminodia shape as his diagnostic units.

Martius (1826) first described the diagnostic characters of three genera (*Mogiphanes*, *Brandesia* and *Bucholzia*) but it was Moquin-Tandon (1849) who published an extensive list of *Alternanthera* spp. following the diagnosis of Endlicher's sections. It is important to emphasize that species described after Moquin-Tandon's (1849) classification are included in this study and have no sectional designation in Figure 1. However, information about sectional designation for most of the species included in this sampling was obtained from the original descriptions.

The analyses with plastid DNA (*trnL-F*, *rpl16*) and ITS used in this study confirm the findings of Sánchez-del Pino *et al*. (2009) on the monophyly of *Alternanthera* (100% JK, 1.0 PP). Nevertheless, it indicated that the subgeneric classifications for *Alternanthera* proposed in the past by Martius (1826), Endlicher (1836–1840), Moquin-Tandon (1849) and Schinz (1934; Fig. 1) do not reflect monophyletic groups. Some sections previously recognized for *Alternanthera* were elevated to the generic level [e.g. *Lithophila* Swartz (= Section *Lithophila*) and *Cladothrix* (= Section *Cladothrix*)], whereas some former *Alternanthera* spp. (= Section *Trommsdorffia*) were transferred to *Iresine* P.Browne (Endlicher, 1836–1840; Moquin-Tandon, 1849; Bentham & Hooker, 1880; Schinz, 1893, 1934; Townsend, 1993). Townsend (1993) adopted this concept. Despite the considerable variation of *Alternanthera* spp. in life form, Eliasson (1987) argued that the genus in the latter circumscription seems to be a natural taxon characterized by capitate stigmas and a dodecahedral pollen form. We found that in addition to some floral characteristics (stigmatic characteristics resolved relevant clades in this study), the inflorescences types and life forms are important features in the taxonomy of the genus.

Our results suggest that two major clades can be recognized within *Alternanthera* based on stigma surface, bracteole form and also inflorescence type. Within Clade B, Subclades B3 and B4 (Node 7, Fig. 1) are supported by the synapomorphy bracteoles with midnerve prominently keeled [character 3(1); Appendix 2]. Bracteoles in some taxa become strongly curved and boat-shaped. This character was used by Martius (1826) to distinguish *Bucholzia* (including species with concave bracteoles) from *Mogiphanes* and *Brandesia* (including species with carinate bracteoles). However, in general molecular data do lend some support to Martius's classification. Much further study and sampling will be needed before a new infrageneric classification can be proposed.

This study did find that the species assigned by A. J. Mears (unpubl. data) to the invalid genus '*Jamesbondia*' form a monophyletic group and share some common morphological characters and a similar distribution. However, species of '*Jamesbondia*' are nested within *Alternanthera* based on the trees obtained from using plastid (*trnL-F*, *rpl16*), nuclear (ITS) and morphology data and perhaps will be a useful subgeneric lineage.

Evolution of C_4 and C_3–C_4 photosynthesis in *Alternanthera*

The clade including Amaranthaceae *sensu stricto* and Chenopodiaceae [together treated as Amaranthaceae in APG III 2009; however, in light of ongoing multigene studies (T. Borsch *et al*. pers. observ.) of the group the authors prefer to retain the family name Chenopodiaceae in addition to Amaranthaceae] are the major lineage with C_4 species in eudicots (Kadereit *et al*., 2003; Sage, Christin & Edwards, 2011). Phylogenetic analyses in the Amaranthaceae and allies (e.g. Kadereit *et al*., 2003; Müller & Borsch, 2005; Akhani, Edwards & Roalson, 2007; Sage *et al*., 2007) and character state mapping indicated no fewer than 16 independent C_4 lineages. *Alternanthera* is one of those but was only represented by a few species in phylogenetic reconstructions (Sage *et al*., 2007). Much earlier, several authors had examined C_4 and C_4–C_3 intermediate species in *Alternanthera* with the aim of understanding the molecular basis and evolution of photosynthetic pathways (Rajendrudu, Prasad & Rama Das, 1986; Devi *et al*., 1995; Chinthapalli *et al*., 2000; Gowik *et al*., 2006). The most extensive survey using $\delta^{13}C$ values was that of Sage *et al*. (2007) who analysed 87 *Alternanthera* spp. and found that 17 (19.5%) had C_4 metabolism. The carbon isotope ratios of the three previously identified C_3–C_4 intermediate taxa were in the same range as the C_3 species.

Our results depict all C_4 species in Clade B3 (Figs 1, 3) with several of the species widely distributed in tropical and subtropical America, whereas *A. microphylla* is an endemic of dry chaparral vegetation at high elevations in the central Andes (Borsch, Ortuño & Nee, in press). *Alternanthera littoralis* var. *maritima* as a member of this clade shows the C_3 pathway. *Alternanthera littoralis* (= *A. maritima*) grows in moist dunes, with three varieties considered to be vicariant along the coasts of the Caribbean, South America and western tropical Africa, respectively (Pedersen, 1990), and has succulent leaves without Kranz anatomy (T. Borsch, pers. observ.). If C_4 photosynthesis is coded as an individual unordered character state as in our study, a reversal from a C_4 ancestor of Clade B3 to C_3 photosynthesis in *A. littoralis* requires the same number of steps as an independent acquisition of C_4 photosynthesis in *A. microphylla*, if optimized on the plastid DNA tree. Losses of C_4 appear to be rare based on the optimization of C_4 photosynthesis as a character state on phylogenetic trees, but it is so far unclear if there is a mechanism that would explain a reversal from C_4 to C_3 (Christin, Freckleton & Osborne, 2010). However, further taxon sampling is also needed to confirm the position of *A. microphylla*, which might in fact be resolved in the same clade but further away once all species are included, or ancient close C_3 relatives might today be extinct. When the nuclear ITS tree is used to reconstruct character evolution, there is clearly only one C_4 origin in *Alternanthera*, in subclade B3 (see Fig. 3). Because incongruence of nuclear

and plastid trees with respect to the position of *A. littoralis* points to reticulate evolution, it may even be possible that the species arose through hybridization of a C_3 and a C_4 ancestor, with the C_3 ancestor as the maternal parent.

According to the current literature (summarized in Sage *et al*., 2007), three species are considered C_3–C_4 intermediates: *A. crucis*, *A. ficoidea* and *A. tenella*. *Alternanthera tenella* was considered to be widespread at relatively low elevations through many parts of the Neotropics (Borsch, 2001), and in light of our trees does not appear to be monophyletic. Unfortunately, our samples of *A. tenella* were not included in any physiological study, so their precise photosynthetic pathway is not known. However, it is also unclear to which genotype the material cultivated in India belongs, which was the source for currently available physiological and biochemical data. A pure correlation of such primary research data to our individuals here identified as *A. tenella* is in such a situation rather speculative. *Alternanthera crucis* (a species considered to be endemic to the Caribbean) is a close relative to our samples of *A. tenella* but so is the plant included here from Peru to represent *A. halimifolia* (the species is considered to be C_3; Sage *et al*., 2007). The case of *A. ficoidea* ['*A. ficoides* (L.) R.Br.' in Chinthapalli *et al*., 2000], however, cannot be discussed further because the plants used in physiological studies may have been misidentified. The name *A. ficoidea* has indeed been the source of profound confusion (often with *A. tenella*) but could clearly be shown to be a synonym of *A. paronychioides* (Mears, 1977; Eliasson, 1987; Borsch, 2001), a distantly related C_4 species. A lack of resolution and support in our trees based on the current limited taxon and character sampling allows no firm conclusions about evolutionary patterns of the C_3–C_4 intermediates and the morphologically closely allied C_3 taxa. Nevertheless, based on the results of this study it appears that the evolution of photosynthesis in *Alternanthera* does not exhibit a stepwise development from C_3–C_4 intermediates to C_4. However, *A. kurtzii*, which is the sister group, is most likely of hybrid origin (parental taxa are unknown); thus, the more derived position of the putative C_3–C_4 intermediate taxa in the plastid tree may not depict the true origin of this photosynthetic pathway if a C_3 species is the paternal parent and C_3 is dominant. The survey of Sage *et al*. (2011) indicates that the C_3–C_4 intermediate type does not often occur in immediate relatives, suggesting that it often evolves independently from C_4 in other lineages of angiosperms including *Alternanthera*.

Implications for biogeography

Although distribution patterns in South and Central America, Mexico and the Caribbean are complex and biogeographical results will probably be strongly influenced by denser taxon sampling, the phylogenetic trees for *Alternanthera* suggest some specific biogeographical scenarios. One regards the colonization of the Galápagos Islands and the subsequent radiation of species. Many colonizers in the Galápagos appear to be from weedy ancestral species that could succeed in the islands by inhabiting varied and disturbed environments. Several weedy *Alternanthera* spp. have been reported in the Galápagos Islands (*A. caracasana*, *A. lanceolata* and *A. sessilis*; Eliasson, 1990) and nine are considered endemic to the Galápagos (Jørgensen, 1999; Eliasson, 2004). In this study, the phylogenetic trees resolved the strongly supported Clade B4 (96–99% JK, 1.0 PP; Figs 1, 3), including six species that are either endemic or indigenous to the Galápagos Islands; the Galápagos endemics are *A. filifolia*, *A. flavicoma*, *A. galapagensis* and *A. snodgrassii* in Clade B4 and *A. nesiotes* in Clade B3. *Alternanthera halimifolia*, *A. tenella* and *A. vestita* are species that also occur in South America (in Subclade B4).

The most parsimonious hypothesis based on optimizing distributions of the species for the origin of Galápagos *Alternanthera* spp. based on our data suggests two independent introductions to the Galápagos Islands and two back migrations to the mainland. Because of the poor branch support in Clade B4, the geographical optimization in the clade is limited. In fact, Carlquist (1974) indicated that Galápagos *Alternanthera* spp. most probably represented two or possibly more introductions whereas Eliasson (2004) proposed, based on morphological features, that the endemic species could be traced back to two or possibly three successful colonization events.

In this study, the phylogenetic trees using plastid DNA and ITS suggest that it is possible to hypothesize a single introduction from an *A. kurtzii*- or *A. tenella*-like ancestor in Clade B. *Alternanthera tenella* occurs from southern Mexico through Central America and the Caribbean Islands to Bolivia and southern Brazil (Burger, 1983) and *A. kurtzii* occurs from Bolivia to Brazil (Pedersen, 1967). *Alternanthera flavicoma*, *A. filifolia*, *A. galapagensis* and *A. snodgrassii*, which are endemics to the Galápagos, and two more widespread species (*A. halimifolia* and *A. vestita*) that occur in the Galápagos (Eliasson, 1971; Fournet, 2002; DeFilipps & Maina, 2003) probably share a common ancestor with this lineage. Therefore, if *A. halimifolia* and *A. vestita* are derived from these Galápagos endemics it would mean that there have been two back migrations to the mainland. The alternative is that *A. halimifolia* and *A. vestita* each represent separate introductions or that one or the other may have given rise to the remaining taxa in the radiation (Fig. 2).

The second introduction is an *A. nesiotes* ancestor, a species endemic to the Galápagos Islands (Jørgensen, 1999). Because *A. nesiotes* is resolved in a subclade consisting of species that are distributed throughout the Americas (Clade B3; Fig. 2), it must be a separate distinct introduction to the archipelago.

This study suggests that it might be possible for Galápagos *Alternanthera* spp. to have affinities with taxa occurring in Chile, Peru and Mexico. Eliasson (1985, 1990, 2004) already hypothesized that two or three Galápagos *Alternanthera* spp. are morphologically more similar to plants from Chile and southern Peru than to species from the geographically closer Ecuador. Galápagos *Alternanthera* spp. must be the result of long-distance dispersal. It is known that the organisms best adapted for long-distance dispersal are weedy plants (Carlquist, 1965). In addition, the estimated age for Amaranthaceae ranges from 83 Ma (Magallón, Crane & Herendeen, 1999) to 104–111 Ma (Wikström, Savolainen & Chase, 2001). The family is therefore much older than the relatively young Galápagos Islands (3–4 Ma; McMullen, 1987), which are volcanic in origin and have never been in contact with the continental mainland. Amaranthaceae is the sixth largest family of vascular plants in the Galápagos Islands and is represented by 29 species in seven genera (Stewart, 1911; Eliasson, 1990). This suggests that the rate of speciation in the group was fast or that there were multiple introductions of the family to the archipelago. The closest relatives of the Galápagos flora appear to have affinities with South America, and to a lesser extent with Mexico and Central America, and only occasionally with the Caribbean Islands (Carlquist, 1965). Porter (1984) confirmed this and pinpointed many colonizers from South America, in particular the Andean region. For flowering plants, he indicated that birds and wind are the main dispersers. Eliasson (2004) suggested that species of subfamily Gomphrenoideae, which is strongly established on the South American mainland, were probably transported to the Galápagos, most likely by birds.

Regarding species of Central America and the Caribbean, Clade B1 (Fig. 2) only includes species distributed in that area. The islands of the West Indies extend 200 km south of North America (Florida), east of Central America and South America to Venezuela (Fritsch & McDowell, 2003). Although all of these areas are close to the Caribbean islands they were not connected to the continents when Caribbean floras and faunas were being established (Carlquist, 1974). Long-distance dispersal is the predominant biogeographical explanation for groups (e.g. Rubiaceae) in the Caribbean islands (Fritsch & McDowell, 2003). *Alternanthera laguroides* from Central America is sister to the Caribbean species. *Alternanthera serpyllifolia*, *A. olivacea* and *A. geniculata* occur in the Caribbean islands (Standley, 1917), and only *A. olivacea* has been collected outside the islands in Brazil, Venezuela, Costa Rica, Nicaragua and Panama (Burger, 1983). It seems likely that there was one long-distance dispersal introduction to the Caribbean islands with affinities to Central America followed by radiation and dispersal throughout the Caribbean. Because *A. olivacea* is nested within the Caribbean species, its existence in Central and South America probably reflects migration to the continent.

CONCLUSIONS AND FUTURE WORK

The present study provides a comprehensive picture of the overall relationships in *Alternanthera* (Amaranthaceae, Gomphrenoideae). Our data establish its monophyly and identify several major lineages in the genus, but future work should focus on increased taxon sampling. We have aimed at representing all previously recognized taxonomic entities above the species level and covering morphologically deviating species (e.g. those provisionally placed in '*Jamesbondia*') and are therefore content to provide a solid first picture on the relationships and evolution of this large Neotropical genus. However, available recent studies of *Alternanthera* were limited, either regionally (in the context of treatments for national floras) and in the analysis of only gross morphological characters.

A truly integrative molecular approach with a dense sampling of populations across the possible range of species will therefore be essential to delimit species in *Alternanthera* and to assess the diversity in this genus robustly. The situation may be complicated by introgression and hybridization, underlining the need for generating a spectrum of molecular data on well-documented individual plants that will at the same time be analysed morphologically and anatomically. Such work is underway to set the base for a modern monograph of *Alternanthera* for *Flora Neotropica* (I. Sánchez-del Pino, L. Senna, T. Borsch, pers. comm.).

For a better understanding of the evolution of photosynthetic pathways, it will be important to clearly document the plant materials studied biochemically and physiologically. With the lack of a proper taxonomic treatment, especially for subclade B4 (*A. tenella* and relatives), the evolutionary position of plant individuals should be determined by generating sequence data from markers otherwise used in phylogenetic analyses, ideally by studying wild material from documented origins and by keeping voucher specimens that can later be analysed for features of the phenotype. In this way it may be possible to link the origin of C_3–C_4 intermediate types with certain speciation processes in *Alternanthera*.

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APPENDIX 1

Taxon sampling and voucher information. Species name, vouchers information from the NYBG (DNA bank accessions starting with ISP) and B (DNA bank accessions starting with AC) databases, DNA bank accession, and GenBank accession numbers for taxa used in this study. NA (sample not able to be amplified[1] or used for the analyses[2])

Taxon	Voucher specimen	DNA bank accession	*trnL-F*	*rpl16*	ITS (ITS1/ITS2)
Alternanthera altacruzensis Suess.	Bolivia, *Nee & Vargas 43479* (NY)	ISP127	EF688732	EF688659	JQ403572
Alternanthera brasiliana (L.) Kuntze	Bolivia, *Gonzales 147* (NY)	ISP116	JQ315137	JQ403544	JQ403565
Alternanthera caracasana Kunth	Mexico, *Sánchez-del Pino* et al. *20 (MEXU)*	ISP64	EF688733	EF688662	JQ403581
Alternanthera chacoënsis Morong ex Morong & Britton	Bolivia, *Nee & Coimbra 40161* (NY)	ISP182	JQ315138	JQ403550	JQ403578
Alternanthera crucis Bold.	Puerto Rico, *Taylor 9531& Lodge* (NY)	ISP181	EF688735	EF688663	JQ403577
(01) *Alternanthera elongata* (Willd.) Schinz	Bolivia, *Beck 11078* (LPB, NY)	ISP171	EF688736	EF688664	JQ403576
(02) *Alternanthera elongata* (Willd.) Schinz	Bolivia, *Borsch & Ortuño 3617* (B, LPB)	AC618	JQ315139	JQ403534	JQ403555
Alternanthera filifolia (Hook.f.) J.T.Howell	Ecuador (Galápagos), *Eliasson & Eliasson 1668* (GB)	ISP106	JQ315140	JQ403539	JQ403560
Alternanthera flava (L.) Mears	Mexico, *Nee & Taylor 28763* (NY)	ISP117	EF688737	EF688665	JQ403566
Alternanthera flavescens Kunth	Mexico, *Martínez s.n.* (NY)	ISP118	EF688738	EF688666	JQ403567
Alternanthera flavicoma (Andersson) J.T.Howell	Ecuador (Galápagos), *Eliasson & Eliasson 1888* (GB)	ISP111	JQ315141	JQ403540	NA[1]
Alternanthera galapagensis (Stewart) J.T.Howell	Ecuador (Galápagos), *Eliasson & Eliasson 726* (GB)	ISP112	EF688739	EF688667	JQ403561
Alternanthera geniculata Urb.	Dominican Republic, *Alain & Liogier 26490* (NY)	ISP102	JQ315142	JQ403537	JQ403558
Alternanthera halimifolia Standl. ex Pittier	Peru, *FLSP2171* (NY)	ISP199	EF688740	EF688668	JQ403579
Alternanthera kurtzii Schinz ex Pedersen	Bolivia, *Borsch & Ortuño 3629* (B, LPB)	AC617	JQ315143	JQ403533	JQ403554
Alternanthera laguroides (Standl.) Standl.	Costa Rica, *Taylor 17394* (NY)	ISP152	EF688741	EF688669	EU567664
Alternanthera lanceolata (Benth.) Schinz	Costa Rica, *Barringer* et al. *2270 (NY)*	ISP119	JQ315144	JQ403545	JQ403568
Alternanthera littoralis Beauv. ex Moq. var. *maritima* (Mart.) Pedersen	Bahamas Islands, *Correll & Popenoe 45459* (NY)	ISP129	JQ315145	JQ403548	JQ403574
Alternanthera macbridei Standl.	Peru, *Cowan* et al*., 4276 (NY)*	ISP90	JQ315146	JQ403552	JQ403582
Alternanthera microphylla R.E.Fr.	Bolivia, *Borsch & Ortuño 3670* (B, LPB)	AC619	JQ315147	JQ403535	JQ403556
Alternanthera nesiotes I.M.Johnst.	Ecuador (Galápagos), *Eliasson & Eliasson 2057* (GB)	ISP113	JQ315148	JQ403541	JQ403562
Alternanthera obovata Millsp.	Mexico, *Ventura 1314* (NY)	ISP164	JQ315149	JQ403549	JQ403575

APPENDIX 1 *Continued*

Taxon	Voucher specimen	DNA bank accession	*trnL-F*	*rpl16*	ITS (ITS1/ITS2)
Alternanthera olivacea Urb.	Brazil, *Van Proosdij 1105* (NY)	ISP128	EF688744	EF688672	JQ403573
Alternanthera paronychioides A.St.-Hil.	USA, *Thomas 1141179* (NY)	ISP2	JQ315150	JQ403551	JQ403580
Alternanthera philoxeroides (Mart.) Griseb.	USA, *Thomas and Amason 142585* (NY)	ISP121	EF688745	EF688673	JQ403569
Alternanthera porrigens Kuntze	Peru, *Weigend* et al. *544 (NY)*	ISP122	JQ315151	JQ403546	JQ403570
Alternanthera pubiflora Kuntze	Panama, *Burch* et al. *1176 (NY)*	ISP123	JQ315152	JQ403547	NA[1]
Alternanthera pungens Kunth	Brazil, *Agra* et al. *2084 (NY)*	ISP125	EF688746	EF688674	JQ403571
Alternanthera pungens	USA, Borsch, Pratt & Müller 3449 (B, ISC)	AC061	NA[2]	NA[2]	JQ403553
Alternanthera serpyllifolia Urb.	Dominican Republic, *Alain & Liogier 11185* (NY)	ISP104	JQ315153	JQ403538	JQ403559
Alternanthera snodgrassii (B.L.Rob.) J.T.Howell	Ecuador (Galápagos), *Eliasson & Eliasson 1810* (GB)	ISP114	JQ315154	JQ403542	JQ403563
(01) *Alternanthera tenella* Colla	Brazil, *Nee 42581* (NY)	ISP119	EF688747	EF688675	EU567665
(02) *Alternanthera tenella* Colla	Cuba, *Borsch 3951* (B, HAJB)	AC620	JQ315155	JQ403536	JQ403557
Alternanthera vestita (Andersson) J.T.Howell	Ecuador (Galápagos), *Eliasson & Eliasson 1912* (GB)	ISP115	JQ315156	JQ403543	JQ403564
Pedersenia cardenasii (Standl.) Holub	Bolivia, *Borsch & Ortuño 3504* (B, LPB)	ISP187	EF688782	EF688712	EU567666
Pedersenia cf. *hassleriana* (Mart.) Holub	Bolivia, *Borsch & Ibisch 3532* (B, LPB)	ISP188	EF688783	EF688713	EU567667
Tidestromia carnosa (Steyerm.) I.M.Johnst.	Mexico, Flores *et al*. HF 02-22 (MEXU)	ISP37	EF688789	EF688720	EU567668
	Mexico, *Sánchez-del Pino* et al. *70 (MEXU)*	ISP14			
Tidestromia lanuginosa (Nutt.) Standl.	Mexico, *Flores* et al. *HF 02-19 (MEXU)*	ISP30-31	EF688791	EF688722	EU567670
	Mexico, *Flores* et al. *HF 02-18 (MEXU)*	ISP36			
Tidestromia valdesiana Sánch. Pino & Flores Olv.	Mexico, *Flores* et al. *HF 02-33 (MEXU)*	ISP35	EF688796	EF688726	EU567675

APPENDIX 2

Characters and character states used in the study.

1. Tepal trichomes on tepals. 0 = absent, 1 = present. Trichomes on tepals are present in most of the taxa and absent in *Pedersenia hassleriana*, *Alternanthera littoralis* var. *maritima*, *A. obovata*, *A. philoxeroides* and a few representatives of *A. paronychioides*.

2. Kind of tepal trichomes form on tepals. 0 = simple, 1 = dendritic, 2 = barbed. Type of trichomes was characterized by Sánchez-del Pino & Flores Olvera (2006). This study follows the same descriptions. The simple form was the most common for the sampling. Dendritic trichomes are present in *Tidestromia carnosa*, *T. lanuginosa*, *Alternanthera altacruzensis*, *A. kurtzii*, *A. crucis*, *A. brasiliana*, *A. flavescens*, *A. flavicoma*, *A.*

galapagensis and *A. halimifolia*. Barbed trichomes are present in *A. caracasana*, *A. pungens* and *T. valdesiana*. The character is coded as not applicable in *Pedersenia hassleriana*, *A. littoralis* var. *maritima*, *A. obovata* and *A. philoxeroides*.

3. Bracteoles midnerve shape. 0 = not keeled, 1 = prominently keeled. Midrib of bracteoles in some taxa is sharply keeled and became strongly curved, having a boat-shaped form. Prominent midribs of bracteoles are present in *Alternanthera filifolia*, *A. flavicoma*, *A. galapagensis*, *A. halimifolia*, *A. littoralis* var. *maritima*, *A. nesiotes*, *A. paronychioides*, *A. chacoënsis*, *A. kurtzii*, *A. crucis*, *A. snodgrassii*, *A. tenella* and *A. vestita*. The remaining species have a distinct midrib but never prominent so that bracteoles are flattened or convex. This character is not applicable in *A. brasiliana*.

4. Flower insertion. 0 = sessile, 1 = pedicellate. Flowers in *Alternanthera* can be sessile to short-pedicellate within the bracteoles. Previous authors (Martius, 1826; Endlicher, 1836–1840; Moquin-Tandon, 1849; Bentham & Hooker, 1880; Schinz, 1934) recognized the presence of flowers stipitated with short and sulcate pedicels as diagnostic of section *Mogiphanes*. However, this character is shared for species of *Alternanthera* placed in other sections, suggesting that the character is not diagnostic of sections within *Alternanthera*. Therefore, pedicellate flowers are present in *A. brasiliana*, *A. flavescens*, *A. galapagensis*, *A. geniculata*, *A. macbridei*, *A. littoralis* var. *maritima* and *A. obovata*. The remaining species have sessile flowers.

5. Inflorescence insertion. 0 = sessile, 1 = pedunculate. Inflorescence architecture helps to characterize two main groups within *Alternanthera*. There is a group with spikes sessile and mostly axillary. This group corresponds to *A. caracasana*, *A. chacoënsis*, *A. filifolia*, *A. flavicoma*, *A. galapagensis*, *A. geniculata*, *A. halimifolia*, *A. macbridei*, *A. littoralis* var. *maritima*, *A. kurtzii*, *A. crucis*, *A. nesiotes*, *A. obovata*, *A. paronychioides*, *A. pungens*, *A. serpyllifolia*, *A. snodgrassii*, *A. tenella*, *A. vestita* and *Tidestromia*. Species that have inflorescences either pedunculate or sessile are common in *A. laguroides*, *A. olivacea*, *A. porrigens* and *A. pubiflora*. The remaining species have spikes pedunculate, arranged in synflorescences of thyrsoid paracladia following correct terminology according to Acosta *et al*. (2009).

Inflorescences either sessile or pedunculate has been a character used along the infrageneric classification of *Alternanthera* proposed in the past by several authors (Martius, 1826; Endlicher, 1836–1840; Moquin-Tandon, 1849; Bentham & Hooker, 1880; Schinz, 1934) and the character is still useful to recognize groups within the genus.

6. Style length. 0 = long, 1 = short. Size of styles has been an important character at section level based on Schinz (1934). Long styles are present in *A. kurtzii*, *A. crucis*, *A. elongata*, *A. flava*, *A. geniculata*, *A.laguroides*, *A. macbridei*, *A. lanceolata*, *A. obovata*, *A. olivacea*, *A. philoxeroides*, *A. porrigens*, *A. serpyllifolia*, *A. tenella* and *Tidestromia valdesiana*. Short styles occur in the remaining species.

7. Pseudostaminodial margin. 0 = laciniate, 1 = crenate. The diagnostic character for *Alternanthera* is the presence of laciniate pseudostaminodia. However, few species within *Alternanthera* have crenate pseudostaminodia and shorter than the common form in the genus as occurs in *A. altacruzensis*, *A. caracasana*, *A. chacoënsis*, *A. obovata*, *A. paronychioides*, *A. pungens*, *Pedersenia*, *Tidestromia lanuginosa* and *T. carnosa*. This character is inapplicable in *A. serpyllifolia* and *T. valdesiana* because the structure is lacking in these taxa.

8. Flower arrangement along rachis. 0 = dense, 1 = loose. Inflorescence units of *Alternanthera* are flowers crowded along the rachis in globose spikes (dense) whereas other taxa have few flowers arranged along the rachis forming slender spikes (loose). The latter is common in *A. geniculata*, *A. olivacea* and *A. serpyllifolia* as well as the outgroup taxa *Pedersenia hassleriana*. This is inapplicable in *Tidestromia* because it is a dichasium.

9. Stigmatic surface. 0 = with distinctive carpel demarcations, 1 = without distinctive carpel demarcations. Some taxonomic descriptions at species (Moquin-Tandon, 1849) and section level (Schinz, 1934) suggested that some groups within *Alternanthera* have capitate stigma whereas others have bilobed or obscure bilobed stigmas. Observations suggested that some stigmas have small stigmatic surface and dense hairy glandulous stigmatic area that make stigmas look hairy. Other taxa have a larger stigmatic surface that does not look hairy and carpel lines are visible so that stigmas seem bilobed or more divided. Taxa that have distinctive carpel demarcations in the stigma surface are *A. altacruzensis*, *A. brasiliana*, *A. crucis*, *A. elongata*, *A. flava*, *A. flavescens*, *A. macbridei*, *A. porrigens*, *A. pubiflora*, *A. pungens* and the outgroup taxa *Pedersenia* and *Tidestromia*.

10. Stigma form. 0 = capitate; 1 = bilobed. Stigma bilobed is the common form in most of the outgroup taxa such as *Tidestromia* and *Pedersenia*. Species of *Alternanthera* have a capitate stigma which never has two evident deeply lobes as in the outgroup taxa.

11. Pollen exine surface. 0 = psilate, 1 = ornamented. Psilate pollen is common in *Tidestromia* whereas the remaining taxa have ornamented pollen.

APPENDIX 3

Morphological data matrix of *Alternanthera* and outgroups (OG). Characters and coding are detailed in Appendix 2. '?' represents missing data.

	Character states										
Taxon	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**
Alternanthera altacruzensis	1	1	0	0	1	1	1	0	0	0	1
Alternanthera brasiliana	1	1	–	1	1	1	0	0	0	0	1
Alternanthera caracasana	1	2	0	0	0	1	1	0	1	0	1
Alternanthera chacoënsis	1	0	1	0	0	1	1	0	1	0	1
Alternanthera crucis	1	1	1	0	0	0	0	0	0	0	1
(01) *Alternanthera elongata*	1	0	0	0	1	0	0	0	0	0	1
(02) *Alternanthera elongata*	1	0	0	0	1	0	0	0	0	0	1
Alternanthera filifolia	1	0	1	0	0	1	0	0	1	0	1
Alternanthera flava	1	0	0	0	1	0	0	0	0	0	1
Alternanthera flavescens	1	1	0	1	1	1	0	0	0	0	1
Alternanthera flavicoma	1	1	1	0	0	1	0	0	1	0	1
Alternanthera galapagensis	1	1	1	1	0	1	0	0	1	0	1
Alternanthera geniculata	1	0	0	1	0	0	0	1	1	0	1
Alternanthera halimifolia	1	1	1	0	0	1	0	0	1	0	1
Alternanthera kurtzii	1	1	1	0	0	0	0	0	1	0	1
Alternanthera laguroides	1	0	0	0	0,1	0	0	0	1	0	1
Alternanthera lanceolata	1	0	0	0	1	0	0	0	1	0	1
Alternanthera littoralis var. maritima	0	–	1	1	0	1	0	0	1	0	1
Alternanthera macbridei	1	0	0	1	0	0	0	0	0	0	1
Alternanthera microphylla	?	?	?	?	?	?	?	?	?	?	1
Alternanthera nesiotes	1	0	1	0	0	1	0	0	1	0	1
Alternanthera obovata	0	–	0	1	0	0	1	0	1	0	1
Alternanthera olivacea	1	0	0	0	0,1	0	0	1	1	0	1
Alternanthera paronychioides	0,1	0	1	0	0	1	1	0	1	0	1
Alternanthera philoxeroides	0	–	0	0	1	0	0	0	1	0	1
Alternanthera porrigens	1	0	0	0	0,1	0	0	0	0	0	1
Alternanthera pubiflora	1	0	0	0	0,1	1	0	0	0	0	1
Alternanthera pungens	1	2	0	0	0	1	1	0	0	0	1
Alternanthera serpyllifolia	1	0	0	0	0	0	–	1	1	0	1
Alternanthera snodgrassii	1	0	1	0	0	1	0	0	1	0	1
(01) *Alternanthera tenella*	1	0	1	0	0	0	0	0	1	0	1
(02) *Alternanthera tenella*	1	0	1	0	0	0	0	0	1	0	1
Alternanthera vestita	1	0	1	0	0	1	0	0	1	0	1
Pedersenia cardenasii (OG)	1	0	0	0	1	1	1	0	0	1	1
Pedersenia cf. *hassleriana* (OG)	0	–	0	0	1	1	1	1	0	1	1
Tidestromia carnosa (OG)	1	1	0	0	0	1	1	–	0	1	0
Tidestromia lanuginosa (OG)	1	1	0	0	0	1	1	–	0	1	0
Tidestromia valdesiana (OG)	1	2	0	0	0	0	–	–	0	1	0