

Phylogeny, diversity, and distribution in *Exostema* (*Rubiaceae*): implications of morphological and molecular analyses

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Abstract: The neotropical genus *Exostema* comprises 25 species of trees and shrubs, ranging in distribution from Bolivia to Mexico and throughout the West Indies, with most species endemic to the Greater Antilles. Infrageneric relationships and species-level patterns of evolution were investigated in phylogenetic analyses using morphological, molecular, and combined data sets. All data sets resolved three main species groups which correspond to the three sections recognized by MCDOWELL (1996). However, the analyses of ITS sequence data placed the two South American species basal to the three main clades. Otherwise, the morphological and molecular data are highly compatible, and produce a more robust yet consistent phylogeny in the combined data analysis. Morphological evolution in *Exostema* involves many specializations for xeric habitats, reflecting repeated ecological shifts from moist forest to exposed, seasonally dry environments during the diversification of the genus. Both moth and bee pollination syndromes are found in *Exostema*, and shifts in pollination ecology appear pivotal to the differentiation of the three sections. Biogeographically, *Exostema* likely originated in South America and migrated via Central America to the Greater Antilles, where the morphological diversification and speciation are most extensive.

The genus *Exostema* (PERSOON) BONPLAND (*Rubiaceae*) comprises 25 species of trees and shrubs ranging in distribution from Peru to Mexico, throughout the islands of the Caribbean, to the Bahamas and Florida Keys (MCDOWELL 1995). Originally described as a subgenus of *Cinchona* (PERSOON 1805: 196–197) and recognized as a distinct genus by BONPLAND (1807), *Exostema* is distinguished by its long-exserted stamens with basifixed, linear anthers, and filaments attached basally in the corolla tube. The narrowly cylindrical corolla tube and strap-like, recurved corolla lobes are putative synapomorphies for the genus, supported by the chromosome number $n=13$, found in *Exostema* but otherwise uncommon in the *Rubiaceae* (KIEHN 1995). Although *Exostema*, like *Cinchona*, has winged seeds in biloculate capsules, and has similar medicinal and anti-malarial properties (NOSTER & KRAUS 1990; MATA & al. 1987, 1988, 1990), recent studies have demonstrated

Table 1. Species of *Exostema* and their distributions, arranged by section (McDOWELL 1996) with key sectional characters noted. Voucher specimens for DNA samples listed with collector names (C CROAT; M McDOWELL; R ROVA & al.; S STONE & STONE; W WOYTKOWSKI) and herbarium (D Duke; S Stockholm; U Uppsala) abbreviated. EMBL accession numbers given for ITS 1 and ITS 2 sequences

Taxon	Distribution	Voucher	Accession no.
Sect. <i>Exostema</i> (inflorescence axillary, flowers solitary or few, 2–10 cm long)			
<i>E. acuminatum</i> URB.	N Hispaniola	M4410 D	Z95500, Z95519
<i>E. caribaeum</i> (JACQ.) ROEM. & SCHULT.	C. Amer., Mexico, Antilles, Florida	M4908 D	Z95497, Z95517
<i>E. glaberrimum</i> BORHIDI & M. FERNÁNDEZ ZEQ.	E Cuba		not sequenced
<i>E. lancifolium</i> BORHIDI & ACUÑA	E Cuba	M4958 D	Z95503, Z95522
<i>E. nitens</i> URB.	C Hispaniola	M4997 D	Z95501, Z95520
<i>E. purpureum</i> GRISEB.	E Cuba	M4844 D	Z95502, Z95521
<i>E. salicifolium</i> GRISEB.	W Cuba	M4973 D	Z95499, Z95518
<i>E. spinosum</i> (VAVASS.) KRUG & URB.	E Cuba, N Hispaniola	M4987 D	Z95498, Z95516
Sect. <i>Brachyantha</i> (inflorescence terminal, flowers many, 1–3 cm long)			
<i>E. corymbosum</i> (RUIZ & PAV.) SPRENG.	Andean Peru	W5721 U	Z95513, Z95532
<i>E. ixoroides</i> (HOOK. f.) T. McDOWELL, ined.	Cuba	M4917 D	Z95511, Z95530
<i>E. mexicanum</i> A. GRAY	C. America, Mexico	S4019 D	Z95512, Z95531
<i>E. myrtifolium</i> GRISEB.	E Cuba	M4968 D	Z95509, Z95528
<i>E. parviflorum</i> L. RICH.	Hispaniola	M4984 D	Z95510, Z95529
<i>E. selleanum</i> URB. & EKMAN.	S Hispaniola, SE Cuba		not sequenced
S. <i>Pitonia</i> DC. (inflorescence terminal, flowers usually many, 4–21 cm long)			
<i>E. angustifolium</i> (SW.) ROEM. & SCHULT.	S Hispaniola		not sequenced
<i>E. brachycarpum</i> (SW.) ROEM. & SCHULT.	Jamaica	M4673 D	Z95508, Z95527
<i>E. ellipticum</i> GRISEB.	Cuba, Hispaniola, Puerto Rico	M5000 D	Z95507, Z95526
<i>E. lineatum</i> (VAHL) ROEM. & SCHULT.	S Hispaniola	M4353 D	Z95506, Z95525
<i>E. longiflorum</i> (LAMB.) ROEM. & SCHULT.	Cuba, N Hispaniola	M4990 D	Z95504, Z95523
<i>E. maynense</i> POEPP.	W Amazonia, Ecuador-Bolivia	C19370 S	Z95514, Z95533
<i>E. polyphyllum</i> URB. & EKMAN	SW Hispaniola		not sequenced
<i>E. rotundatum</i> GRISEB.	E Cuba		not sequenced
<i>E. sanctae-luciae</i> (KENTISH) BRITTON	Lesser Antilles		not sequenced
<i>E. stenophyllum</i> BRITTON	E Cuba	M4875 D	Z95505, Z95524
<i>E. triflorum</i> (W. WRIGHT) G. DON. (Outgroup)	Jamaica		not sequenced
<i>Coutarea hexandra</i> (JACQ.) K. SCHUM.	S. America, C. America, Mexico	R2072 S	Z95515, Z95534

that these genera are not closely related, and *Exostema* is now excluded from the tribe *Cinchoneae* (ANDERSSON & PERSSON 1991, BREMER & JANSEN 1991, ANDERSSON 1995). *Exostema* appears closely related to *Coutarea* and various genera associated with *Portlandia*, which share the characteristic stamen structure and spinulose pollen of *Exostema* (ANDERSSON & PERSSON 1991, B. BREMER 1992, ROBBRECHT 1993). *Exostema* is a morphologically and ecologically diverse genus, including large rainforest trees, thorny microphyllous shrubs, and many endemics of serpentine and rheophytic habitats.

Taxonomic background. Generic and sectional level taxonomy of *Exostema* has recently been reviewed and a revised infrageneric classification presented (Table 1; MCDOWELL 1996). Three sections were recognized, readily distinguished by inflorescence position, flower number and flower length, and supported by a preliminary phylogenetic analysis of morphological and molecular characters. The one other modern infrageneric classification of *Exostema* was proposed by BORHIDI (BORHIDI & FERNÁNDEZ 1989), and recognized five sections. BORHIDI's subdivisions differ from those in Table 1 only in the placement of three morphologically divergent species. BORHIDI placed the small-leafed shrub *E. polyphyllum* in the monotypic sect. *Polyphyllae* BORHIDI, and segregated the two long-flowered rheophytes *E. longiflorum* and *E. stenophyllum* into sect. *Longiflorae* BORHIDI, whereas MCDOWELL included these species in sect. *Pitonia* DC. The phylogenetic analyses presented in this article provide a test of these infrageneric classifications. These analyses expand upon the preliminary studies (MCDOWELL 1996) with molecular data for ten additional species and extended sampling of morphological characters.

Ecological diversity. Ecological diversity is great among the 25 species of *Exostema*. Habitats range from Amazonian rain forest slopes at 120 m elevation, to Andean shrublands at 2800 m, to coastal thorn-scrub thickets and wet rocky streambanks in the Antilles. *Exostema* species are most often found on limestone substrates, but several distinctive serpentine-endemic species occur in eastern Cuba. Variation in habit and leaf morphology is also extreme. The Amazonian species *E. maynense* grows to over 30 m tall and over 1 m in diameter, with thinly chartaceous leaves to 20 cm long; *E. spinosum* (Fig. 1a), endemic to dry thickets in Hispaniola and eastern Cuba, is a thorny shrub whose stiff, coriaceous leaves seldom exceed 7 mm in length. The four rheophytic species, including *E. stenophyllum* and *E. longiflorum* (Fig. 1e), found only on rocks of fast flowing streams and endemic to Cuba and Hispaniola, are all small shrubs with narrowly lanceolate leaves, a common habit and leaf-shape for rheophytes (STEENIS 1981). The rare (and likely extinct) rheophyte *E. polyphyllum* of southwestern Haiti has leaves about 1 cm long and only 1 to 3 mm in width. In serpentine-endemic species of *Exostema*, such as the shrub *E. myrtifolium* (Fig. 1c) the leaves are thicker, shorter and rounder, a common characteristic of plants restricted to these ultramafic substrates (BORHIDI 1991).

Floral diversity. The key morphological feature distinguishing *Exostema* species from closely-related taxa is the corolla: a narrow tube with narrow, recurved lobes, bearing conspicuously exerted stamens (Fig. 1f, *E. caribaeum*). Nevertheless, there is considerable variation in floral traits among *Exostema* species. Inflorescences may be terminal, compound cymes of 20 to 100 flowers



Fig. 1. Diversity among some *Exostema* species. *a* *Exostema spinosum* with capsules dehiscent; *b* *E. parviflorum*, older flowers turning pale yellow; *c* *E. myrtifolium*, with flowers and fruit, domatia visible on leaf undersides; *d* *E. ellipticum*, with flowers aging to pink-purple; *e* *E. longiflorum*, with flowers and green fruit; *f* *E. caribaeum*, with newly opened flower white, older flower aging to tan

(Fig. 1b, *E. parviflorum*), or axillary with only one or few flowers (e.g. *E. caribaeum*). The entire corolla may be less than 1 cm long, as in *E. myrtifolium* or may exceed 20 cm in length, as in the rheophytes *E. longiflorum* and *E. stenophyllum*. Various patterns of color change occur in the genus. The white flowers turn to pink and then dark purple-red in most of the long-flowered species (shown in *E. ellipticum*, Fig. 1d), while the short flowers of *E. parviflorum* (Fig. 1b) and other species turn from white to yellow or tan with age. Flowers are often very fragrant, with a sweet *Lonicera*-like smell. The longer flowers generally are open and fragrant at night, and the short flowers typically open and fragrant during the day. These and other floral features suggest that distinct lepidopteran and hymenopteran pollination syndromes have developed in various lineages within *Exostema*.

Distribution. The distribution of *Exostema* species presents intriguing biogeographical questions. In western South America there occur just two species: *E. maynense*, from western Amazonia at the base of the Andes, is found from Ecuador to Bolivia, at elevations to 500 m; *E. corymbosum*, is known only from the Andes of Peru, at elevations of 1000 to 2800 m. Central America and Mexico share one endemic species, *E. mexicanum*, found from Panama to Tamaulipas (central Mexico), from sea level to 1000 m. Also occurring through this region is the widespread *E. caribaeum*, which is the only species found throughout the islands of the Caribbean and nearby Florida and the Bahamas. The remaining twenty-one species of *Exostema* are endemic to the Antilles, and except for *E. sanctae-luciae*, a Lesser Antilles species, they are all restricted to the four islands of the Greater Antilles: Cuba, Hispaniola, Jamaica, and Puerto Rico. By far the greatest diversity of *Exostema* species occurs in Cuba and Hispaniola, which have eighteen endemics among a total of twenty *Exostema* species. The Cuban and Hispaniolan *Exostema* species include the most morphologically distinctive species in the genus, the extremely modified xerophytes, rheophytes and serpentine-endemics.

Objectives. The primary objective of this investigation is to examine, with both morphological and molecular characters, the phylogenetic relationships among the species of *Exostema*. The results of these phylogenetic analyses will be used to test the monophyly of current infrageneric groupings, and to address several broader biological questions. How have the distinctive ecological specializations arisen in the genus? What are the patterns of morphological evolution for vegetative and reproductive characters? Where did the genus originate, and how has it reached its present distribution? Finally, what may have caused the pronounced radiation and diversification of *Exostema* species in the islands of the Greater Antilles?

Materials and methods

To investigate evolutionary relationships within *Exostema*, and to provide a phylogenetic framework for the interpretation of ecological, morphological, and biogeographical features of the genus, two independent data sets were developed, based on morphological and molecular characters. The morphological data matrix was based on extensive examination of herbarium material, field observations, and selected microscopic features, taken from both vegetative and reproductive plant structures. The character analysis for this

data provides the basic descriptive information for interpreting patterns of morphological change within the genus. To provide an independent test of phylogenetic relationship among *Exostema* species, base sequences from the internally transcribed spacer (ITS) region of the nuclear ribosomal DNA were examined for 18 species of *Exostema* and for the outgroup, *Coutarea hexandra*. The ITS region, which has been used in many phylogenetic studies in recent years (reviewed in BALDWIN & al. 1995), is convenient due to its high copy numbers, its relatively high rate of evolution, and its juxtaposition to highly conserved regions coding for rDNA. The two data sets were analyzed separately and in a combined matrix in cladistic analyses to examine relationships in *Exostema*, and to compare their alternative resolutions.

Morphological data. The data matrix for the morphological analysis consists of 25 taxa and 37 morphological and anatomical characters. Species recognized in *Exostema* (Table 1) are based on a taxonomic revision of the genus (McDOWELL, unpubl.). Twenty-four of the twenty-five species of *Exostema* are included in the present analysis. One species, *Exostema glaberrimum* BORHIDI & M. FERNÁNDEZ ZEQ., known only from the type collection and marginally distinct from *E. lancifolium*, was excluded from the final data matrix, due to an excessive number of missing character states and its general similarity to the latter species. *Coutarea hexandra* (JACQ.) K. SCHUM. was selected as the outgroup. It is considered closely related to *Exostema* for the following reasons. The general morphology, as well as pollen and wood features of *Coutarea* and *Exostema* are similar (AIELLO 1979, KOEK-NOORMAN & HOGEWEG 1975). Both genera have historically been placed together, in the tribe *Cinchoneae* and recently removed to an alliance of genera associated with *Portlandia* (ANDERSSON & PERSSON 1991, ROBBRECHT 1993). Cladistic studies of the *Cinchoneae* s. l. using morphological and anatomical characters (ANDERSSON & PERSSON 1991) and of selected genera across the *Rubiaceae* with both morphological and molecular data (BREMER & STRUWE 1992) have placed *Exostema* and *Coutarea* s. l. as closely related taxa.

The morphological character analysis was based on examination of over 3000 herbarium specimens including all major collections for *Exostema* species, field observations of fifteen species and microscopic study of pollen and leaf anatomy for most species. The characters used in this analysis and their character states are given in Table 2, and briefly described in the following paragraphs. The morphological data matrix is shown in Appendix 1. Characters were selected to avoid the use of non-independent features, and no two characters are coded identically across all species.

Character states were delimited to allow unambiguous coding as much as possible. Traits which showed extreme variation within taxa, (e.g. leaf size, which varies in length by a factor of 3 to 4 in many species), were avoided. Quantitative characters (e.g. flower length or seed number) were coded for two or more states according to clear discontinuities between the species' ranges compared across all taxa. Measurements used to determine size classes for state coding of quantitative characters are the normal size ranges for a given feature, which encompass at least 80% of the variation observed in each species, and are based on examinations made during taxonomic revision of *Exostema*. The size classes used reflect discontinuities between these species' ranges. Although the character state boundaries used are continuous for certain size features (e.g. flower length: 1–4 cm, 4–13 cm, 13–21 cm) most species' ranges fall well within a single character state size class, and only rarely do individual specimens exceed the size boundaries given their species' character state. In the few situations in which two or more character states do occur in one species the character was coded as polymorphic for the states found. Polymorphic coding was seldom necessary, (except for the unusually variable outgroup species *Coutarea hexandra*), and totally in less than 2% of the character coding for ingroup species.

Table 2. Characters used in morphological analysis. (asterisk indicates characters ordered in alternative analyses)

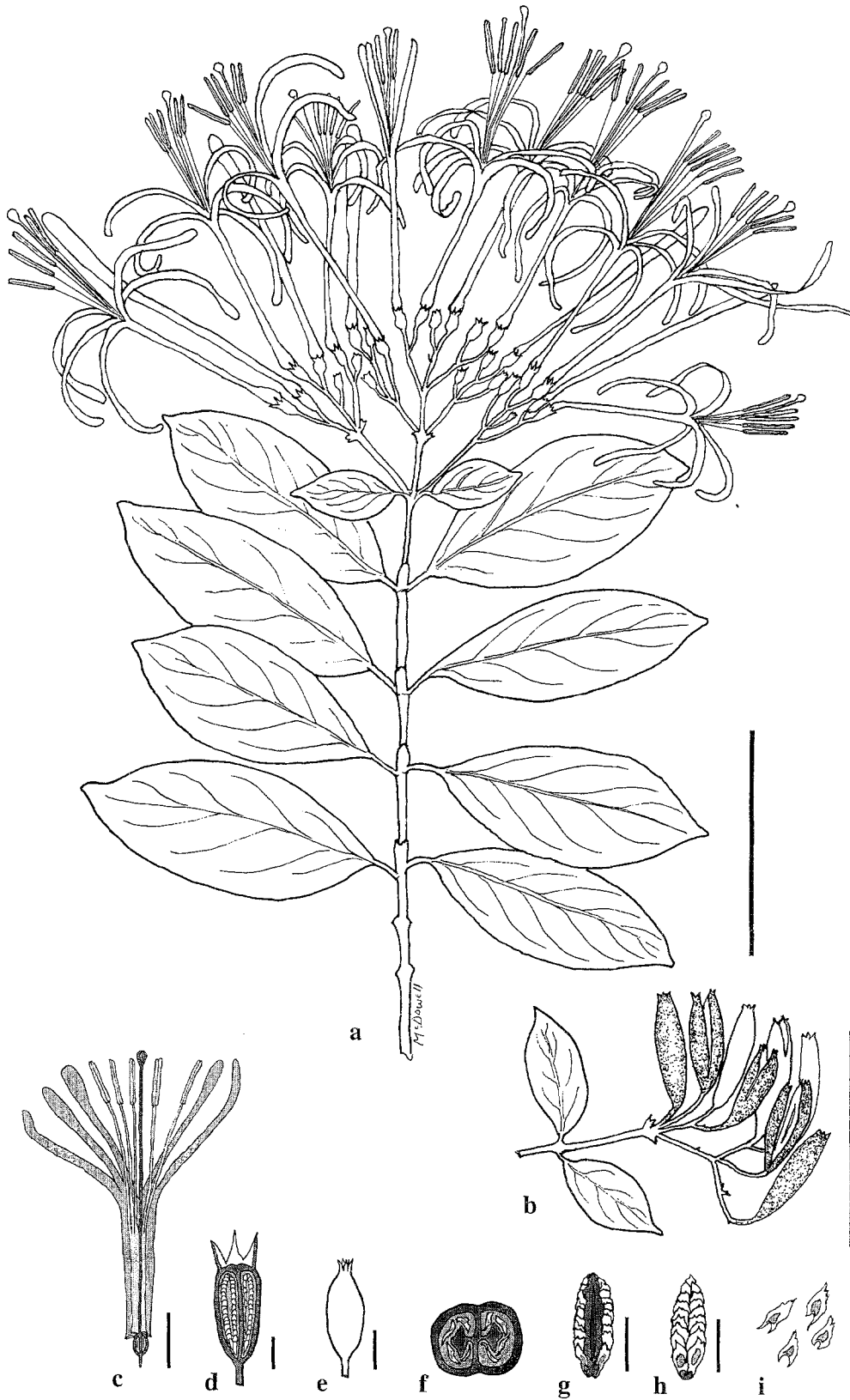
Character number	Character states			
	0	1	2	3
Vegetative features:				
1. Habit*	trees > 5 m high	trees, shrubs, 2–5 m	small shrubs < 2 m	
2. Stem pubescence	present	absent		
3. Lenticels	absent	white sulcate	black punctate	
4. Stipule fusion	free at base	lower 1/3–1/2 sheathing		
5. Stipule edge	no hyaline margin	with hyaline margin		
6. Leaf shape	narrowly lanceolate	elliptic	rotundate	
7. Leaf thickness*	thinly chartaceous	thickly chartaceous	coriaceous	
8. Leaf pubescence	absent	present		
9. Vein number	3–6	7–10		
10. Domatia	absent	present		
11. Hypodermis	absent	present		
12. Stomata size*	15–23 μ	24–28 μ	29–32 μ	
Reproductive features				
13. Inflorescence	terminal	axillary		
14. Flower number*	many: 10–100	few: 2–9	1	
15. Peduncle thickness	slender: 0.3–1.0 mm	stout: 1–2.5 mm		
16. Merosity*	6	5		4
17. Flower length*	short: 1–4 cm	long: 4–13 cm	very long: 13–21 cm	
18. Hypanthium lg.	3–6 mm	1–3 mm		
19. Calyx tube	present	absent		
20. Calyx lobe shape	triangular or obtuse	lanceolate to digitate		
21. Corolla aging	pink to maroon	pale yellow to tan		
22. Corolla exterior	glabrous	pubescent		
23. Corolla interior	glabrous	finely pubescent		
24. Corolla division	tube exceeding lobes	lobes, tube similar	lobes exceeding tube	
25. Filament in bud	straight	strongly curved		
26. Filament pubesc.	absent	present towards base		
27. Anther length*	long: 15–25 mm	medium: 5–14 mm	short: 1–4 mm	
28. Pollen size*	20–22 μ	24–26 μ	27–31 μ	
29. Style extension	exceeding stamens	shorter than stamens		
30. Stigma position	at tip of style	linear from style tip		
31. Capsule length	6–35 mm	3–5 mm		
32. Capsule shape	strongly flattened	slightly flattened	broadened	
33. Capsule lenticels	absent	conspicuous		
34. Seed wing	entire	dissected	absent	
35. Seed number *	2–10	10–20	20–100	100–400
36. Seed alignment	acro-basipetal	basipetal	acropetal	horizontal
37. Seed length	3–7 mm	1–3 mm		

Fifteen multi-state (three or four state) characters were used. Analyses were done with all characters unordered, and with a group of nine multi-state characters ordered as indicated by asterisks in Table 2. Only characters coding for simple number (e.g. flower merosity, seed number) or size traits (e.g. leaf thickness, anther length) were ordered. Notes on particular characters (with character number in brackets) are listed after Table 2.

Molecular data. The molecular analysis includes 18 of the 24 *Exostema* species represented in the morphological data set, as well as the outgroup *Coutarea hexandra*. The species selected represent the entire range of morphological, geographical, and ecological diversity in the genus, except for the rare (or extinct) species *E. polyphyllum*, for which leaf material was unavailable. Total DNA was extracted from fresh, air dried or silica gel dried leaf material, or (for three species) from herbarium specimens, using a modified CTAB extraction (DOYLE & DOYLE 1987). Earlier extractions used c. 1 g dry leaf material with repeated chloroform extractions, and micro-pore purification of total DNA. Later extractions followed a mini-prep procedure using 0.02 to 0.06 g leaf material with a single chloroform extraction and purification of DNA by QIAex and QIAquick kits (QIAGEN). The internally transcribed spacer (ITS) regions 1 and 2 and intervening 5.8S rDNA were amplified by polymerase chain reaction (PCR) following the protocol of BALDWIN (1992), with temperatures adjusted to 94°, 54°, and 70°. Primers “ITS 2, 3, 4, and 5” (BALDWIN 1992, WHITE & al. 1990) were used. Double-stranded DNA was amplified first, using equal amounts of primer pairs 2 & 5 for ITS 1 and 3 & 4 for ITS 2, or in later samples primers 5 & 4 for the entire ITS region. Single stranded DNA was amplified from double stranded PCR product using a 20:1 ratio of excess to limiting primer. Both directions were amplified and sequenced. Single stranded PCR product was cleaned with QIAquick kit according to manufacturers instructions. Sequencing reactions were done with S-35 and Sequenase T-7 (Perkin-Elmer) and T7 polymerase (Pharmacia-Upjohn) according to manufacturer’s protocols, using 45° temperature for the extension step. Acrylamide wedge gels (0.2–0.6 mm thick) were used following standard protocols. Sequences were read manually and rechecked twice using McVector light box and gel reading apparatus. Base positions which appeared to have more than one band, (due to presumed polymorphisms or sequencing artifact) were scored polymorphic for bands present using standard DNA coding. Sequences were aligned manually. The molecular character matrix used is shown in Appendix 2. EMBL accession numbers for the ITS sequences are given in Table 1. Aligned sequences are available from the first author.

Data analyses. The morphological, molecular, and combined data matrices were analyzed using PAUP 3.1.1 (SWOFFORD 1993) with several Macintosh computers (Centris, Quadra, Power-PC). Heuristic searches using the TBR option were performed with random taxon sequence addition and one hundred replicates. Taxa with multiple states for a character were treated as polymorphic using the PAUP option “multi-states = polymorphic”, which assigns each of the states to the multi-state taxa. Gaps in the ITS sequences were coded as missing. This is an effective approach for recovering well-supported topological patterns while avoiding the possible redundancy of various alternative schemes for coding insertion/deletions in ITS sequence data (YUAN & al.

Fig. 2. *Exostema ellipticum*. *a* habit; *b* infructescence with mature and dehiscent capsules; *c* dissected flower, showing stamen attachment at base of corolla tube and filaments convivent towards base; *d* longitudinal section through hypanthium showing septum, two locules and vertically imbricate seeds; *e* outline of capsule; *f* cross sectional diagram of capsule, showing seeds surrounding peltate placenta; *g* adaxial view of mature seeds on placenta; *h* abaxial view of seeds on placenta; *i* winged seeds. Drawn from MCDOWELL 4437, 5000 Bars: *a*, *b* 5 cm; *c*–*e*, *g*–*i* 1 cm



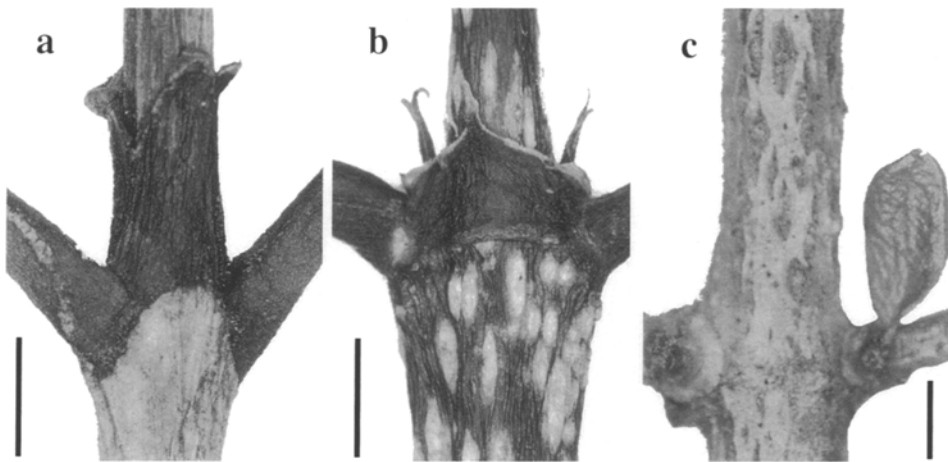


Fig. 3. Stem features of *Exostema* spp. used in morphological analysis: *a* lenticels absent, stipules sheathing (*E. ellipticum*); *b* white sulcate lenticels, stipules not sheathing (*E. mexicanum*); *c* black punctate lenticels, stipules not shown (*E. spinosum*). *a* MCDOWELL 4437; *b* STONE & STONE 4019; *c* MCDOWELL 4439. Bars: 1 cm

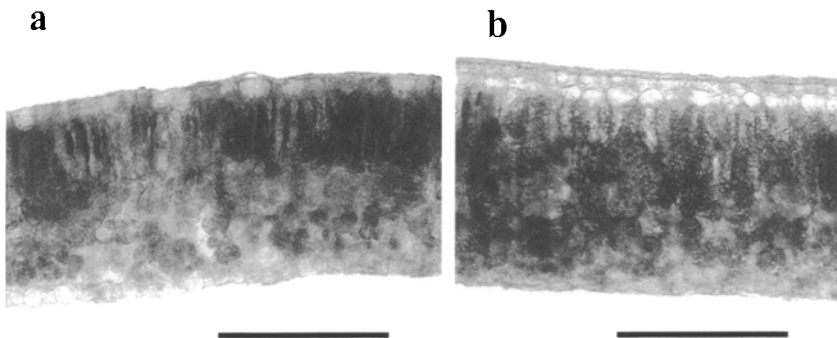


Fig. 4. Leaf transections of *Exostema* spp. used for morphological analysis: *a* single epidermal layer, no hypodermis (*E. caribaeum*); *b* epidermal and hypodermal layers present (*E. ixoroides*). *a* MCDOWELL 4908; *b* MCDOWELL 4917. Bars: 100 μ m

1996, BAUM & al. 1994, WOJCIECHOWSKI & al. 1993). Separate analyses of the ITS data with gap and missing data positions excluded resulted in similar but slightly less resolved topologies and consensus trees as those for the regular ITS analyses.

In the molecular analysis all characters were unordered. The morphological and combined data matrices were analyzed with all characters unordered and with nine multi-state morphological characters ordered, as described above (see “morphological data” methods and Table 2). All analyses were first performed with characters unweighted. Reweighted analyses (FARRIS 1969), using the rescaled consistency index (RC) (FARRIS 1989) and base weight of 1000, were performed using PAUP after the unweighted analyses, to provide an objective means for choosing among equal length trees (CARPENTER 1988). The most parsimonious trees for the unweighted analyses were filtered using the strict consensus tree of the reweighted analysis as a constraint, to identify minimum length trees consistent with both weighted and unweighted analyses. Similarly, the results of analyses

including ordered characters were used to identify identical trees among the analyses with all character unordered. BREMER branch support (BREMER 1988) values were determined for the morphological, molecular, and combined data phylogenies using the AutoDecay 2.9.3 program (ERIKSSON 1995). Bootstrap values (FELSENSTEIN 1985) for 2000 replicates, consistency indices (for informative characters only), and pair-wise distance between taxa were determined using PAUP. Relative base composition (C-G A-T ratios) were computed with MacClade (MADDISON & MADDISON 1992). The MacClade program was also used to show distributions of selected character states on phylogenetic trees.

Results

Morphological analysis. Analyses of the morphological data matrix with all characters unordered produced 60 shortest trees of 145 steps each (calculated for “informative characters” only), with the following length and fit measures: CI=0.517, HI=0.628, RI=0.755, RC=0.391. The strict consensus tree from this analysis (Fig. 5) resolves three principal clades, which correspond to the three sections of *Exostema* (MCDOWELL 1996). Within each of these clades the most basal taxa are the species from South America (*E. maynense*, *E. corymbosum*) and middle America (*E. mexicanum*, *E. caribaeum*). The results are similar when the

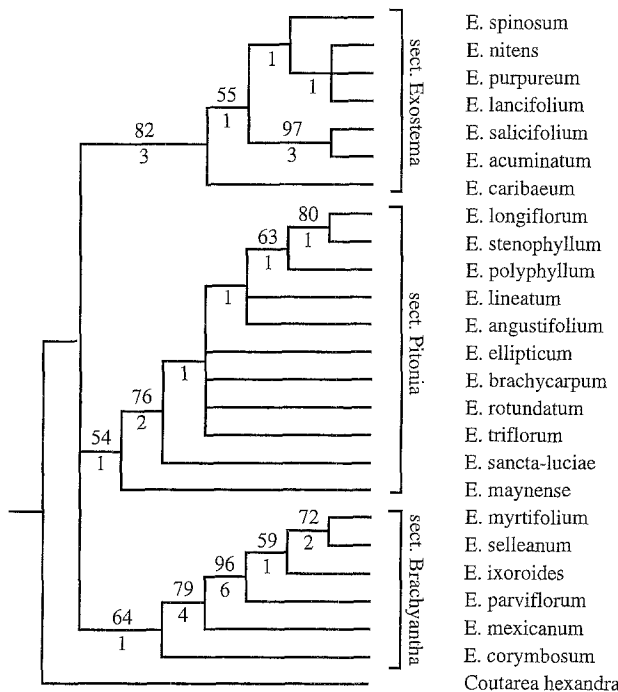


Fig. 5

Fig. 5. Strict consensus tree for morphological data of *Exostema* with all characters unordered. Bootstrap values above 50% (for 2000 replicates) shown above branches, branch support values shown beneath branches

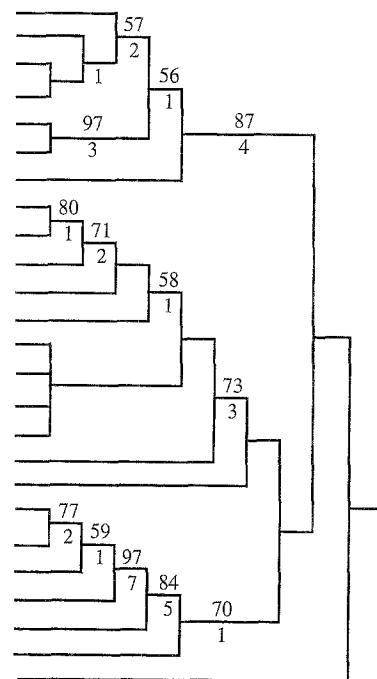


Fig. 6

Fig. 6. One of 60 shortest trees for morphological data of *Exostema* with nine characters ordered. Bootstrap values above 50% (for 2000 replicates) shown above branches, branch support values shown beneath branches

morphological data was analyzed with nine multi-state characters ordered: 60 most parsimonious trees of 147 steps each, with CI=0.510, HI=0.633, RI=0.758, RC=0.387. One most parsimonious tree represented in both ordered and unordered analyses is given in Fig. 6. Branch support values and bootstrap percentages (calculated with nine ordered characters) are shown above and below appropriate branches.

Analyses with characters reweighted produced 15 and 12 most parsimonious trees for the “all characters unordered” and “nine characters ordered” data sets respectively. The strict consensus trees with unordered and ordered characters are identical, and are nearly equivalent to Fig. 6 with slightly less resolution within sections. All reweighted and most unweighted analyses of the morphological data show the seven species of sect. *Exostema* (with axillary inflorescences of one or few flowers) forming a sister group to the terminal-flowered species, as in Fig. 6. The terminal-flowered species form two groups, comprising the six short-flowered species of sect. *Brachyantha* DC. and the eleven long-flowered species of sec. *Pitonia*. Relationships within sectt. *Exostema* and *Brachyantha* are rather well resolved, as indicated by the branch support and bootstrap values in Fig. 6. In the third group (“sect. *Pitonia*”) the five shrub species form one clade nested among the mostly unresolved tree species. In all most parsimonious trees the four species found in continental South and Central America, (*E. maynense*, *E. corymbosum*, *E. mexicanum* and *E. caribaeum*) appear as the most basal clades of their respective sections.

Molecular analyses. The ITS 1 and 2 regions in the 18 species of *Exostema* investigated range in length from 224 to 226 and 225 bases respectively. Pairwise distances between *Exostema* species range from 0.175 to 17.5 % of bases (excluding gaps) for ITS 1, and from 0 to 10.4 % for ITS 2. This amount of ITS variation is similar to the levels of divergence within genera reported by other researchers (e.g. YUAN & KÜPFER 1995, OXELMAN & LIDÉN 1995; reviewed in BALDWIN & al. 1995). The ratio of bases C-G to A-T for ITS 1 & 2 (excluding ambiguous sites and gaps) averaged 60:40 and 62:38 respectively. The aligned sequences for all species and the outgroup gave a total of 85 informative characters, 45 in ITS 1 and 40 in ITS 2 and the adjacent 5.8S region (Appendix 2).

Analyses of the molecular data matrix produced nine shortest trees of 159 steps each, with the following length and fit measures (calculated for “informative characters” only): CI=0.730, HI=0.415, RI=0.811, RC=0.591. The reweighted analysis of the ITS sequence data produced three most parsimonious trees. The strict consensus trees for the reweighted and unweighted analyses were identical. The strict consensus tree for these analyses is given in Fig. 7, with branch support values shown above and bootstrap percentages shown below appropriate branches (calculated for unweighted analysis).

The ITS consensus tree differs from the morphology based trees in the placement of the two South American species. The ITS phylogeny places the Amazonian species *E. maynense* as the basal clade in genus, and the Andean species *E. corymbosum* next, as the sister clade to the remaining, northern-hemisphere species. These northern species form three principal groups, which, excepting the exclusion of the South American species, correspond to the three sections of *Exostema* recognized by MCDOWELL (1996). Among these three clades

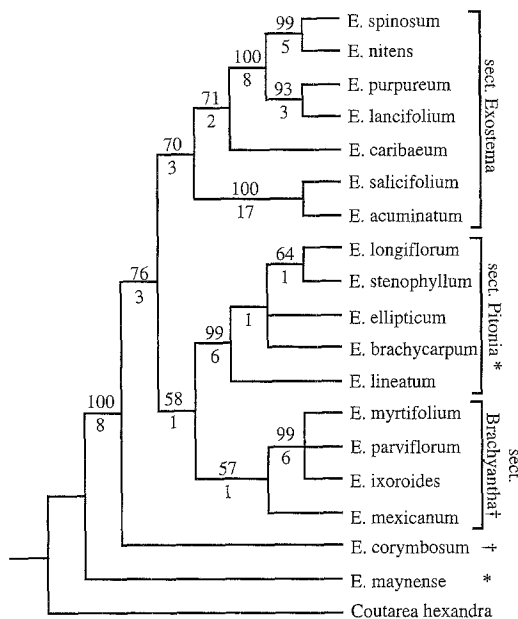


Fig. 7. Molecular analysis of *Exostema*, strict consensus tree. Bootstrap values above 50% (for 2000 replicates) shown above branches, branch support values shown beneath branches

the axillary-flowered species (*sect. Exostema*) form a sister group to the remaining terminal-flowered species. The terminal-flowered species are divided into two well-supported clades: those with short flowers (*sect. Brachyantha*, less *E. corymbosum*); and those with long flowers (*sect. Pitonia*, less *E. maynense*). Several species pairs are strongly supported in the ITS phylogeny, as the consensus tree and branch support values indicate. Among axillary-flowered species, *E. salicifolium* and *E. acuminatum*, *E. spinosum* and *E. nitens*, and *E. purpureum* and *E. lancifolium* form species pairs. The long-flowered rheophytes *E. longiflorum* and *E. stenophyllum* are paired, and three species with short, terminal flowers, *E. ixoroides*, *E. parviflorum*, and *E. myrtifolium* also form a well supported group in the ITS phylogeny.

Combined data analyses. The unweighted analysis of the combined morphological (with all characters unordered) and ITS data yielded 297 most parsimonious trees of 315 steps each, with the following length and fit measures: CI=0.606, HI=0.533, RI=0.758, RC=0.460. When analyzed with nine morphological characters ordered, the combined data set produced 49 shortest trees of 316 steps each, with very similar length and fit measures: CI=0.608, HI=0.532, RI=0.758, RC=0.461. The strict consensus for these 49 trees is given in Fig. 8, with branch support values and bootstrap percentages indicated. The “all characters unordered” analysis produced a similar but slightly less resolved strict consensus tree. The reweighted analyses, with and without use of ordered characters, produced six and twelve shortest trees respectively, and their strict consensus trees are nearly identical, differing only in arrangement of the tree taxa of *sect. Pitonia*. Figure 9 shows a most parsimonious tree represented in all analyses (with and without ordered characters, with and without reweighting) of the combined data. It is very similar to the consensus trees for these analyses. This tree is well resolved (except for the tree species of *sect. Pitonia*), and maintains

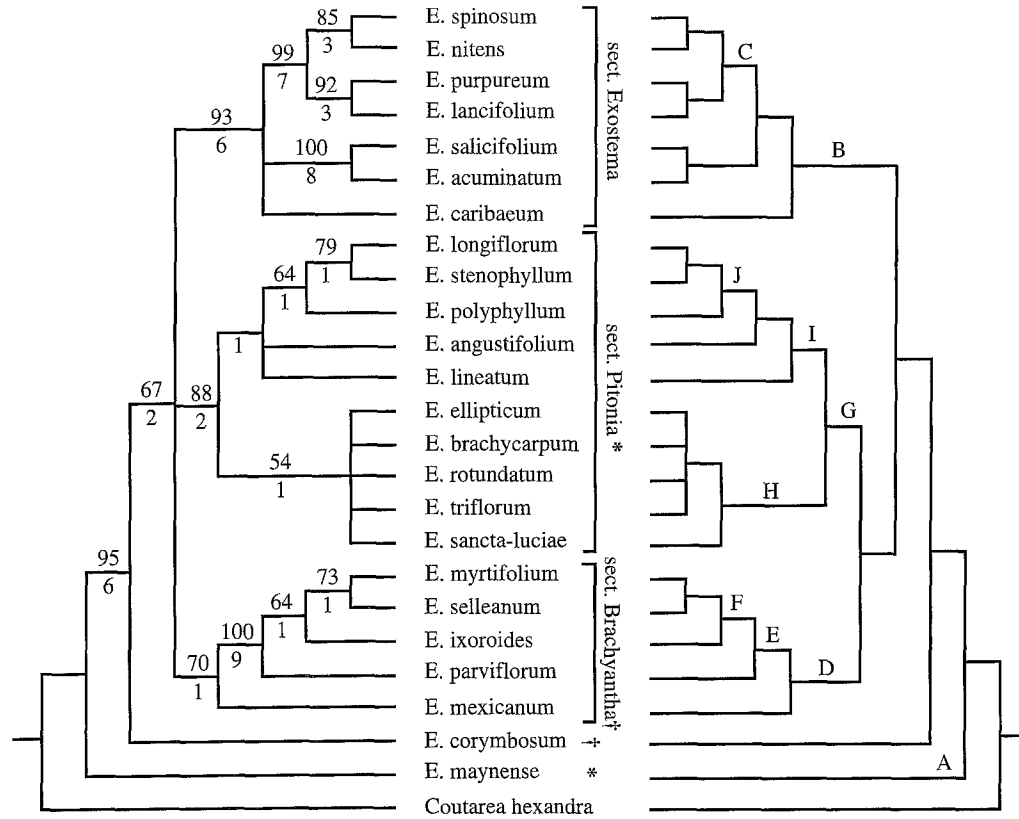


Fig. 8

Fig. 9

Fig. 8. Strict consensus tree for combined morphology and ITS data of *Exostema*. Bootstrap values above 50% (for 2000 replicates) shown above branches, branch support values shown beneath branches

Fig. 9. One of 49 shortest trees for the combined morphology and ITS data of *Exostema*. Letters refer to clades discussed in text

nearly all the groups represented in the ITS phylogeny. As in the ITS tree, the species *Exostema maynense* and *E. corymbosum* appear as basal clades in the genus, the remaining species are divided into axillary and terminal-flowered clades, and the terminal-flowered species form well supported long- and short-flowered groups. The combined data tree is also congruent with the morphological phylogenies, except for the exclusion of *E. corymbosum* from the sect. *Brachyantha* clade and *E. maynense* from the sect. *Pitonia* clade. The length of the combined data tree (316 steps) is only ten steps longer than the combined lengths of the morphological and ITS trees, an indication that these data sets are highly concordant.

Discussion

The analyses based upon morphological data resolve three clades within the genus *Exostema*, as proposed in the subgeneric classification of McDOWELL (1996). The

phylogenies produced from the molecular and combined data analyses differ from the morphology based trees in their placement of the two South American species, *E. maynense* and *E. corymbosum*. The morphology based trees place these species basal within the sectt. *Pitonia* and *Brachyantha* respectively; the molecular and combined data analyses place these species as the basal clades of the entire genus. All analyses – morphological, molecular and combined data – divide the remaining, northern-hemisphere species into three groups, easily distinguished by inflorescence and flower traits, and corresponding to the sectt. *Exostema*, *Pitonia*, and *Brachyantha* (with or without the two South American species).

The South American species are undoubtedly among the most ancestral clades in the genus, as indicated by both morphological and molecular analyses. The Antillean species, on the other hand, include the most morphologically derived and ecologically specialized species of the genus. Morphological diversity and ecological variation within each of the three major clades in *Exostema* reflect a transition to increasingly dry environments, and the colonization of riverine and serpentine habitats. These evolutionary processes underlie the radiations of numerous endemic species in the Greater Antilles. Shifts in pollination ecology are also evident. The derivation of bee pollination from moth pollination appears to have taken place early in the diversification of the genus.

In the following discussion these patterns of character evolution and their underlying ecological processes are evaluated in the context of *Exostema* phylogeny, using the combined data tree in Fig. 9. This topology, with three principal groups subtended by the Andean and Amazonian species, is well supported in the combined data analysis, due to the strong support of the ITS data for the basal position of the South American species. The total length of the combined data phylogeny is only 3% longer than the sum of the morphological and molecular trees, an indication of the high level of congruence between these analyses. Alternatives to the combined data phylogeny are also noted when interpreting certain characters or relationships. In this discussion letters in brackets refer to clades on Fig. 9. Morphological and ecological features are interpreted using characters from the cladistic analysis. Numbers in brackets refer to morphological characters included in the data matrix (Appendix 1) and listed in Table 2. This discussion proceeds from the basal clades to the more distal groups, then addresses the major evolutionary trends and biogeographic patterns in *Exostema*.

The basal position of *Exostema maynense*. The position of *E. maynense* [A] as the most basal lineage in *Exostema* is strongly supported in the combined data phylogeny. The ITS data provide the most support for this relationship, however in the morphological analyses *E. maynense* is unambiguously basal among species of sect. *Pitonia*, and actually placed in a basal polytomy in the strict consensus tree for the “characters ordered” morphological analysis. *Exostema maynense* displays several plesiomorphic morphological features which distinguish it from its congeners, particularly in its flower and fruit. It is the only *Exostema* species with six-merous flowers [16], a trait found in the outgroup *Coutarea hexandra*. The occurrence of six-merous flowers in *E. maynense* has long been overlooked, since the species was originally described and illustrated as five-merous (POEPPIG 1845: 31–32). Nevertheless, photographs of the type specimen (POEPPIG s.n., “types of

the Berlin Herbarium no. 251" GH) and examination of available collections shows that this species, with its conspicuously exerted, 3 cm long anthers is consistently six-merous. A reduction in merosity is a common trend in floral evolution (RONSE DECRAENE & SMETS 1994), and in *Exostema* independent reductions from five to four-merous flowers have occurred in sections *Exostema* and *Brachyantha*. Other plesiomorphies of the flower of *E. maynense* are the relatively long calyx tube [19], the slight pubescence of the filaments [26] and the long anthers [27]. Several unusual fruit and seed characters provide further evidence of ancestral morphological traits in *E. maynense*. The fruits of this species (known from only a single herbarium collection and the protologue) are large [31], round, distinctly flattened narrowing the septum [32], and conspicuously lenticellate [33], with many seeds in each locule [35]. The imbricate, winged seeds, which are vertically aligned, are attached to a central placenta, with some seeds extending upwards and some downwards in the strongly flattened locule. This fruit and seed structure closely resembles that found in the outgroup *Coutarea hexandra* (illustrated in detail in AIELLO 1979). The acro-basipetal seed arrangement [36] also occurs in *E. corymbosum*, and a similar alignment is seen in a few other species (*E. salicifolium*, *E. acuminatum*). Plesiomorphic vegetative features found in *E. maynense* (and some other *Exostema* species) include its lenticellate stems [3] and large, elliptic [6] thinly chartaceous [7] and many veined [9] leaves with moderately large stomata [12]. The large tree habit [1] of *E. maynense* may also be plesiomorphic but cannot be inferred from the present analysis, since the outgroup *Coutarea* occurs as both a large (to 30 m high) and a small tree.

The monophyly of sect. *Exostema*. The combined data analysis, and both morphological and ITS phylogenies, unequivocally support the monophyly of sect. *Exostema* [B], which includes all of the axillary-flowered *Exostema* species. All but one of these species are endemic to the islands of Cuba and Hispaniola. The widespread *E. caribaeum* ranges from Costa Rica to Mexico and throughout the West Indies. Synapomorphies for sect. *Exostema*, in addition to its inflorescence position [13], include the reduction from many to solitary or few flowers [14], pubescence within the corolla tube [23], large pollen [28] and leaves with fewer lateral veins [9]. Within sect. *Exostema* several species groups are well supported by both morphological and molecular data sets. The sister species relationship of *E. acuminatum* and *E. salicifolium*, endemics to Hispaniola and western Cuba respectively, is clear in the combined data tree. It is strongly supported by ITS data (particularly apparent in the ITS 1 region), which is consistent with their morphological similarities. Indeed, the original description of *E. salicifolium* (GRISEBACH 1866), based on just three collections, included both entities, but as more material became available the distinctive Hispaniolan species *E. acuminatum* was also recognized (URBAN 1903). Although the two species are easily distinguished by differences in leaf size and shape, and the coloration of flowers and stems, they share synapomorphies including narrow leaves [6], deeply lobed corollas [24] and broadened fruits [32] with seeds extending both upwards and downwards from the placenta [36]. Another well supported species group [C] includes *E. purpureum* and *E. lancifolium*, endemic to northeastern Cuba, *E. nitens*, a local endemic of central Hispaniola, and the xerophyte *E. spinosum*, found in

coastal thorn scrub thickets of Hispaniola and southeastern Cuba. Coriaceous leaves [7] and small flowers [17] are the morphological synapomorphies for this clade, which is additionally supported by ITS data. The species *E. purpureum* and *E. lancifolium* are parapatric endemics of the serpentine zone of Oriente, Cuba. *Exostema spinosum* and *E. nitens* are another strongly supported species pair in the combined data tree, due chiefly to the ITS data. This result is unexpected, for the species *E. nitens* (endemic to central Hispaniola) is morphologically very similar to *E. purpureum* (endemic to eastern Cuba). *Exostema spinosum* occupies the driest habitats of any *Exostema* species, and is morphologically an anomalous, derived member of the genus. It was formerly placed by in the genus *Catesbaea*, owing to its thorny stems, very small leaves, and tetra-merous flowers, and was the latest species to be transferred into *Exostema* (URBAN 1897). Although there are no morphological synapomorphies for these two (allopatric) species, both are native to rocky habitats of northern Hispaniola.

The monophyly of sect. *Brachyantha*, possibly excluding *Exostema corymbosum*. Section *Brachyantha* comprises six species with short flowers and terminal inflorescences. In the combined data analysis the five northern hemisphere species form a well supported clade [D], but the South American *E. corymbosum* is excluded from this group, and appears as the second most basal clade in the genus. This separation of *E. corymbosum* is based mainly on ITS characters: in the morphological phylogeny *E. corymbosum* is well supported as the basal lineage of sect. *Brachyantha*. The close overall similarity in morphology between *E. corymbosum* and northern representatives of sect. *Brachyantha* has been emphasized in the original descriptions of the Antillean (RICHARD 1807: 132–135) and Mexican species (GRAY 1861) of this group. All these species have short flowers [17] in dense terminal inflorescences, and share traits associated with reduced flower size: a short hypanthium [18], filaments strongly curved in bud [25], and stamens exceeding the style [29]. In addition, these species all have fragrant, day-opening flowers with linear stigmas [30] and corolla coloration changing to pale yellow with age [21]. Together, the floral characteristics of sect. *Brachyantha* suggest a syndrome of bee pollination (supported by the first author's field observations for most species), in contrast to the lepidopteran pollination syndrome found among the long-flowered *Exostema* species. This bee pollination syndrome was first derived in *E. corymbosum*, and may represent a pollinator shift associated with the transition from lowland rain forest habitat to the dryer montane shrub-savannah environment.

All five northern species of sect. *Brachyantha* [D] grow in steep, rocky, seasonally dry habitats, and character evolution within the group reflects a series of adaptations to these dryer conditions. The morphological synapomorphies for this northern hemisphere group involve reductions in flower and fruit size: shortened anthers [27], smaller pollen [28], and fewer seeds [35] with basipetal alignment [36]. Relationships among these species are well resolved in the combined data tree, and also show a trend towards increasingly reduced size of vegetative and reproductive structures. The West Indian, Cuban, and Hispaniolan species form a strongly supported sister clade [E] to the mainland species *Exostema mexicanum*, present in both molecular and morphological phylogenies. Synapomorphies for the Antillean species include reduced leaf vein number [9], and smaller size of the

calyx tube [19], corolla lobes [24], capsule [31], and seeds [37]. Further changes in leaf and flower traits distinguish the most xeric species of sect. *Brachyantha* [F]: a reduction to tetra-merous flowers [16], and in *E. myrtifolium* and *E. selleanum* a change in leaf shape [6] to rotundate, and leaf thickness [7] to coriaceous. These species are endemics to serpentine and dry limestone regions of Cuba and Haiti, and their shrub habit, small coriaceous leaves, and small, few-seeded fruits are specializations for their very xeric environments.

The monophyly of sect. *Pitonia*, probably excluding *Exostema maynense*.

Section *Pitonia* comprises eleven species with long flowers in terminal inflorescences, ten endemic to the Antilles, and *E. maynense*, from western Amazonia. As discussed above, *E. maynense* appears as the basal clade in *Exostema*, and in the combined data phylogeny is separated from the remaining species of sect. *Pitonia*. The Antillean species form a monophyletic group with strong support in all analyses [G], distinguished by their glabrous [2], non-lenticellate stems [3] with long sheathing stipules [4], and their rather long capsules [31], with very many seeds [35] aligned acropetally [36]. Five of these species, trees of moist, forested slopes, are morphologically and ecologically very similar, and (apart from their geographic distributions) are distinguished mostly by small differences in leaf shape and texture. As expected, relationships among these marginally distinct species are not well resolved [H], and in the combined data phylogeny strict consensus tree they form a five-taxa polytomy.

The other five species of sect. *Pitonia* are all shrubs, and all except *Exostema lineatum* are rheophytes, growing only on rocks in fast flowing streams. These five species form a clade [I] in the combined data analysis, sharing as synapomorphies their shrub habit [1] and smaller seed size [37]. *Exostema lineatum*, which in flower and fruit much resembles the tree species of this section, is basal among these shrub species, and the four rheophytes appear monophyletic due to the synapomorphy of lanceolate leaf shape [6]. Lanceolate leaves are a typical feature of rheophytes (STEENIS 1981), and, like the small shrub habit, may be adapted to sustaining the force of frequent flooding. The most basal of the four rheophytic species, the Haitian endemic *E. angustifolium*, is morphologically intermediate between *E. lineatum* and the remaining rheophytes, with longer leaves, many-flowered inflorescences [14] and relatively long corolla lobes [24], like *E. lineatum*, but similar in stem pubescence [2] and leaf shape [6] to its sister rheophytes.

The three species *E. polyphyllum*, *E. longiflorum*, and *E. stenophyllum* (clade J) are perhaps the most morphologically distinctive species in the genus, which led BORHIDI & FERNÁNDEZ (1989) to separate them into their own sections. *Exostema polyphyllum* was named in 1922 on the basis of two collections (EKMAN 9212a & 10248) from the Massif de la Hotte, a remote and biologically distinct mountain peninsula of southwestern Haiti (URBAN 1921). It has not been collected since, despite botanical expeditions to the region, and with the severe human disturbance to the local ecology this interesting species is now likely to be extinct. Its tiny leaves, under 1 cm long and less than 3 mm wide, are the smallest in the genus, and its terminal inflorescence bears a solitary flower. Seeds of *E. polyphyllum* show a transition from winged to unwinged [34]: the wing is rudimentary or absent, and the seed arrangement approaches the horizontal alignment seen in the species with unwinged seeds. The loss of seed wings is a common trend among rheophytes in

general (GENTRY 1979) and probably aids in dispersal by water instead of by air. The species *E. longiflorum* and *E. stenophyllum* are distinguished by their very long corollas [17], which may exceed 21 cm in length, with long anthers [27]. Their seeds are unwinged [34] and very numerous [35], and obviously disperse effectively by water, as evidenced by the abundance of linear populations along streams. Their long, narrow corolla tubes, nocturnal anthesis, and reddening color change suggests pollination by sphingid moths, which could nectar feed in a trap-line manner from the linear, stream-side populations. While *E. longiflorum* is widely distributed across Cuba and northern Hispaniola, *E. stenophyllum* is endemic to the serpentine region of northeastern Cuba. Distinguished by its smaller, coriaceous leaves, glabrous stems, and contracted stipule shape, *E. stenophyllum* provides a classic example of a restricted serpentine-endemic derived from a widespread sister species.

Major trends in the morphological diversification of *Exostema*. The great morphological diversification in *Exostema* may be attributed to two main processes: specializations for xeric environments, and specializations involving pollination biology. These processes have affected each of the three principal species groups and both continental and island species, and have occurred in both calcareous, serpentine, and rheophytic habitats. The effects of these processes are visible in all aspects of character evolution in *Exostema*. To illustrate these patterns, the character state distributions for selected characters are indicated on the combined data analysis strict consensus tree in Figs. 10 and 11.

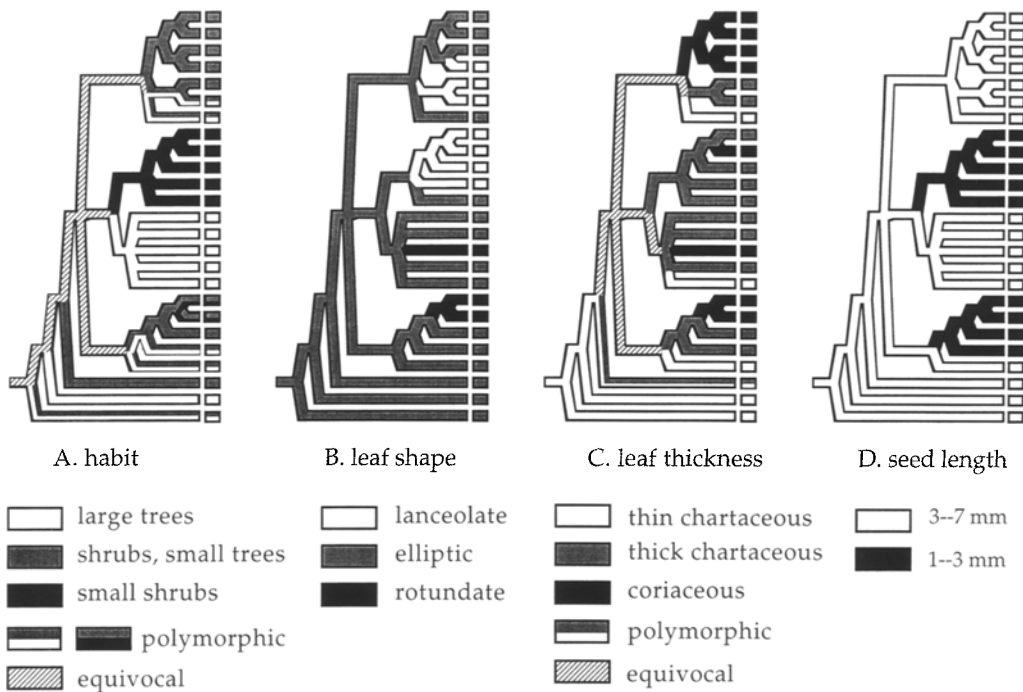


Fig. 10. Vegetative character state distributions in *Exostema* shown on combined data strict consensus tree. Branches leading to taxa with more than one character state are shown as polymorphic for states present

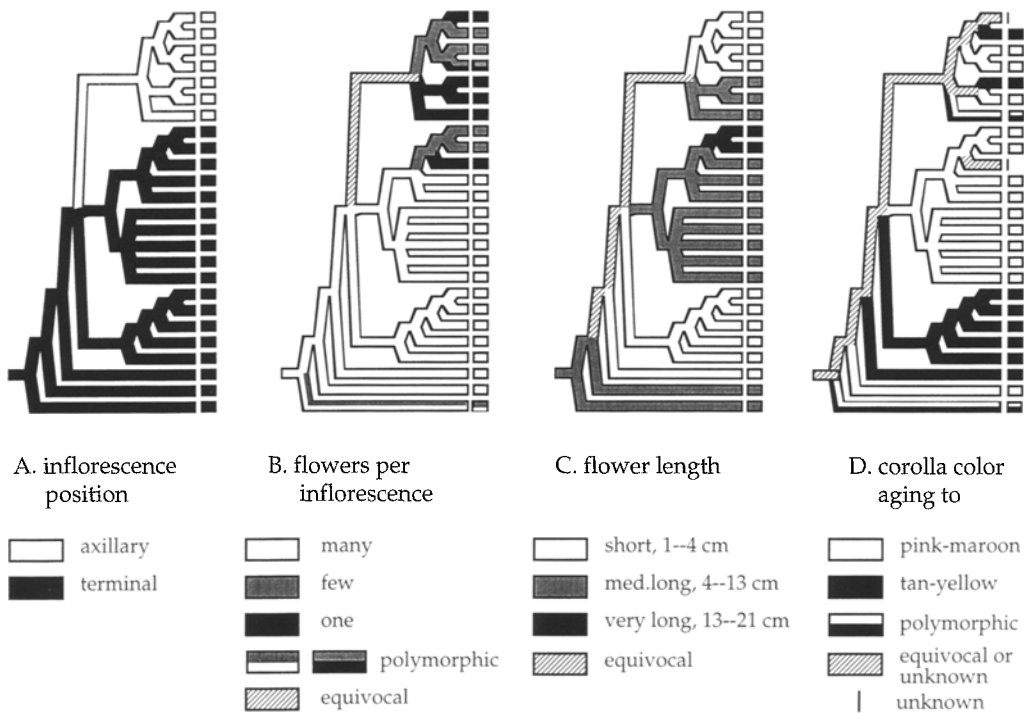


Fig. 11. Inflorescence character state distributions in *Exostema* shown on combined data strict consensus tree. Branches leading to taxa with more than one character state are shown as polymorphic for states present

Specializations for dry environments. The many “xeromorphic” modifications involve reductions in the size and number of vegetative and reproductive structures, and are most evident among the Cuban and Hispaniolan species. The shrub habit (Fig. 10A) and smaller, often rotundate leaves (Fig. 10B) are characteristic of drought- and serpentine-adapted plants, and have arisen repeatedly and in separate lineages. In many of these *Exostema* species the leaves are thickened or coriaceous (Fig. 10C), with heavier cuticle and an extra cell layer (hypodermis) above, and with smaller stomata beneath. Reductions in flower size and merosity, and in fruit size, seed size (Fig. 10D) and seed number occur among the various groups of Antillean species. Tetra-merous flowers are derived in the most xeric, small leaved members of sectt. *Exostema* (*E. spinosum*) and *Brachyantha* (e.g. *E. ixoroides*), and these are among the shortest flowers in the genus. The capsules of *E. ixoroides* plants which grow in the driest parts of eastern Cuba may be just 3 mm long and contain only two seeds per locule. The diversification of rheophytic species in sect. *Pitonia* may also be considered a consequence of the increasingly drier climates in Cuba and Hispaniola. Whereas the other species of sect. *Pitonia* inhabit moist forested slopes, often overlooking streams, the rheophytes often grow in drier, open regions, where the rocky streambeds are the only moist habitats. *Exostema polyphyllum*, with its tiny leaves and single terminal flower, shows this “xeric rheophyte” tendency; the small, coriaceous leaves of rheophytic *E. stenophyllum* appear to be a similar response to serpentine-induced water stress.

The trend towards more xeric environments and associated morphologies is also evident among the continental species of *Exostema*. The most basal species, *E. maynense* is a large rain forest tree, with large leaves, flowers and fruits. The next clade, Andean species *E. corymbosum*, is a montane shrub with much smaller flowers and fruits, and in the Meso-American *E. mexicanum* flower and fruit size is further reduced.

Specializations associated with pollination biology. Two major pollination syndromes occur among species of *Exostema*. The long-flowered species of sect. *Pitonia* and *Exostema* present a typical lepidopteran pollination syndrome. These flowers (e.g. *E. ellipticum*, Fig. 1d) open at night with a strong, sweet fragrance, their white corolla lobes directing moths towards the copious nectar which is produced at the base of the long, narrow corolla tube. The exerted, linear anthers are held so that they contact the feeding moth, and the capitate stigmatic surface is positioned to receive pollen from the visiting insect. At dawn the corolla changes to a deep pink-maroon color, making it invisible to the next evening's visitors, and during the day no fragrance is emitted. The short-flowered species of sect. *Brachyantha* display a bee pollination syndrome. Their flowers (e.g. *E. parviflorum*, Fig. 1b) are open and fragrant during the day, with a much shorter corolla tube, and turn pale yellow with age, typically after one day. The linear stigmatic surface is well aligned to receive pollen from the visiting bees, which move along each anther from base to tip, gathering pollen into their leg-pouches. Among the lepidopteran syndrome species the rheophytes *E. longiflorum* (Fig. 1e) and *E. stenophyllum* display a more specialized sphingid moth syndrome, with a reduced number of flowers and extremely long (9–16 cm) corolla tubes. Among the long-flowered species of the axillary-flowered sect. *Exostema* is derived a group of short-flowered species. Some of these, such as *E. nitens*, have 2–7 flowers per axil, with corollas about 3 cm long, open during the day, and fading to a pale yellow, presenting the typical bee syndrome. Another species of this group, *E. spinosum*, which has even smaller white flowers, with no noticeable fragrance or color change, may have some alternative pollinator more suited to its arid environment.

Viewing flower and pollination traits phylogenetically, the derivation of axillary inflorescences occurred only once in the genus (Fig. 11A), and distinguishes sect. *Exostema*. Within sect. *Exostema* alternative states occur for many pollination traits, such as flower number (Fig. 11B) and size (Fig. 11C), merosity, stigma position, and corolla color change (Fig. 11D). The terminal-flowered sect. *Brachyantha* and *Pitonia* are each uniform for their style shape and stigma position and their flower color change, which reflect their alternative bee and moth pollination syndromes. Other floral traits, including flower number, size, merosity, etc. have been variable within these sections.

The biological mechanisms for alternative color changes typically involve distinct biochemical systems: anthocyanins for changes to red or purple, and carotenoids for changes to yellow or orange (WEISS 1995). Nevertheless, both color changes occur in at least one *Exostema* species (*E. caribaeum*), in the outgroup *Coutarea hexandra*, and among the sister species *E. acuminatum*/*E. salicifolium*, as can be seen on herbarium specimens as well as in direct field observations. It seems that some species and perhaps lineages are polymorphic, and maintain

alternative color change systems. In this context it is curious that two *Exostema* species named for (and described with) purple flowers in fact almost always have white flowers which age to pale yellow, not to purple. *Exostema purpureum* GRISEBACH, described with corollas purple (GRISEBACH 1866: 125–126), and *E. bicolor* POEPPIG (= *E. corymbosum*), described with corollas green turning purple, normally have white flowers with no noticeable changes to purple seen in field or herbarium studies (T. M. pers. obs.).

While the occurrence of various pollination syndromes in *Exostema* is evident, it is not clear precisely when, or how many times the moth and bee syndromes may have evolved. The funnellform flower of the outgroup *Coutarea hexandra* suggests bat pollination, although this is likely a derived condition. In the combined data phylogeny of *Exostema* the two basal, South American species display both moth (*E. maynense*) and bee (*E. corymbosum*) syndromes. Among the remainder of the genus, sect. *Pitonia* has flowers characteristic of moth-pollination, sect. *Brachyantha* has typical bee-pollinated flowers, and sect. *Exostema* includes both types of pollination syndromes. It is possible (and consistent with the combined data phylogeny) that the long moth-pollinated flowers are plesiomorphic in the *Exostema*, and that shorter flowers arose, perhaps consequent to the Andean orogeny, with the speciation of the montane shrub *E. corymbosum*. Subsequent derivations of short flowers and bee pollination would most likely also have occurred in sect. *Exostema*. Alternatively, a polymorphic condition for flower color may be ancestral, with certain lineages becoming fixed for either purple or yellow corolla colors. In any case, it appears that the derivation of pollination-related traits began early in the evolutionary history of *Exostema*, and it will be necessary to resolve the deepest branches of *Exostema* phylogeny in order to determine the pollinator transitions involved.

Biogeography of *Exostema*. The combined data phylogeny strongly suggests a South American origin for the genus *Exostema*, with the Amazonian and Andean species forming the two most basal clades (Fig. 12). The distribution of the outgroup *Coutarea hexandra*, from Argentina and French Guiana to Central America and Mexico, suggests a continental, possibly South American origin for *Exostema*, but by itself is not persuasive evidence. The “Ancestral Areas index” (K. BREMER 1992) was designed to elucidate the region of origin for natural groups on the basis of their phylogenetic relationship and modern distributions. Using this method with the combined data phylogeny for *Exostema*, the Ancestral Areas index for South America is 1, as compared to 0.5 for the Antilles and 0.2 for Central America-Mexico, which supports a South American origin for the genus. The group of short-flowered species (sect. *Brachyantha*) presents a straightforward pattern of South American origin (*E. corymbosum*), Central American migration (*E. mexicanum*), and subsequent Antillean radiation (four species of Cuba and Hispaniola), possibly reaching the Greater Antilles via the closely adjacent regions of Yucatan and western Cuba. The biogeographical connection between the South American ancestral clades and Antillean species of sect. *Exostema* are less clear. In the combined data phylogeny *E. caribaeum*, with a widespread Caribbean distribution, is basal among the axillary-flowered species, suggesting a possible Central American migration of this lineage en route to the Greater Antilles. The Antillean species of sect. *Pitonia* seem morphologically close to *E. maynense*, but

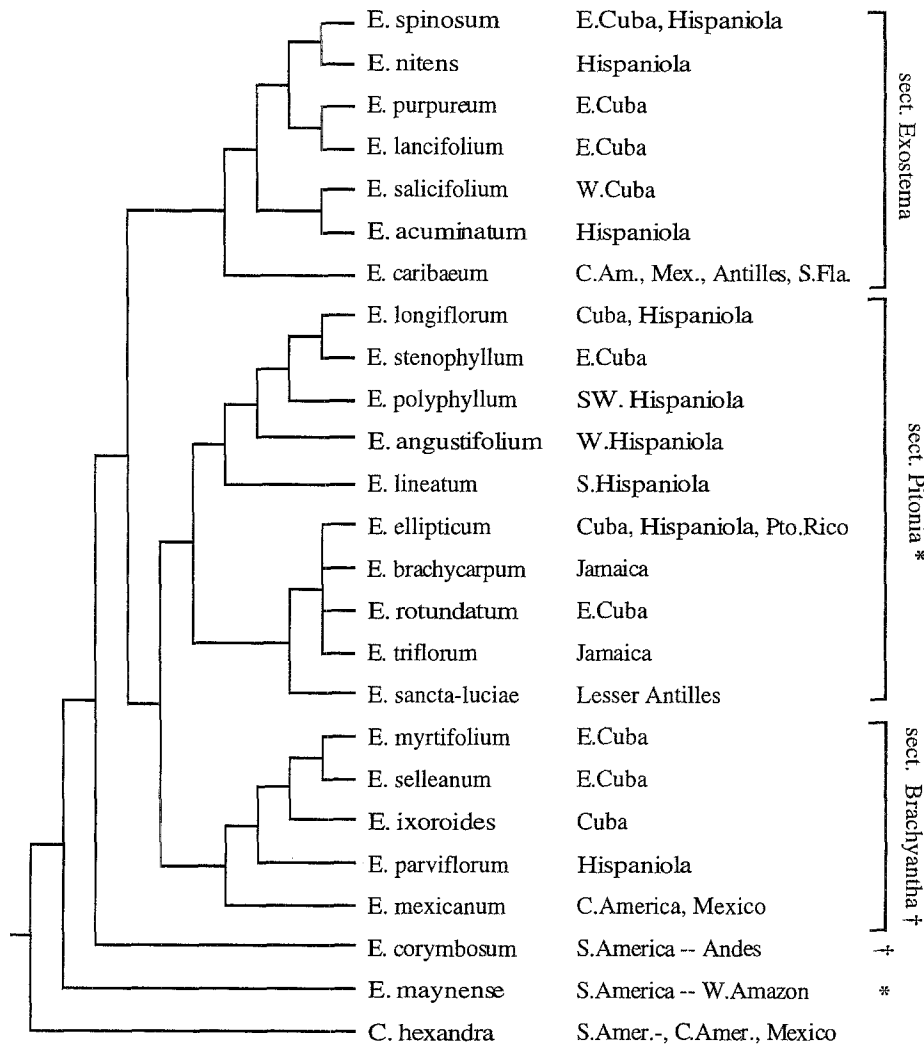


Fig. 12. Distribution of *Exostema* species shown beside one of 49 shortest trees for the combined data analysis

the absence of this group from Central America leaves open the question of how (and when) the group arrived in the Antilles. Whether one concludes that *Exostema* reached Central America and the Antilles by one or by more than one independent dispersal depends on the resolution of the basal nodes within the genus. In the combined data analysis the position of *E. corymbosum* is critical for this determination of biogeographical history. Because the placement of *E. corymbosum* in the combined data phylogeny differs from its position in the morphological phylogeny and relies on relatively few ITS characters, a final assessment of these ancient avenues of *Exostema* biogeography must await additional data.

Within the Antilles, the biogeography of *Exostema* reflects this region's complex geological history. The islands of Cuba and Hispaniola are each composed of at least six separate land masses, which have been variously joined to and divided from the mainland and each other since the mid-Cretaceous era

(ITURRALDE-VINENT 1994, MANN & LAWRENCE 1991, PINDELL & BARRETT 1990). The endemic rich mountainous and serpentine regions of eastern Cuba and south-west Hispaniola resulted from the collisions of these island fragments and their underlying geological plates. The long isolation of Jamaica, and the geologically recent fusion of the Massif de la Hotte to southwestern Hispaniola are consistent with the presence of endemic *Exostema* species (e.g. *E. brachycarpum*, *E. polyphyllum*) in those areas. The formerly contiguous terrains of eastern Cuba and northern Hispaniola share certain species (e.g. *E. spinosum* and *E. longiflorum*) absent from other parts of those islands. This demonstrates the historical connection between these islands, and the biogeographical separation of regions within a single island, such as “south island” and “north island” Hispaniola. The several endemic species on the serpentine zone of northeastern Cuba is another aspect of the adaptive radiation of *Exostema* into the drought and mineral stressed environments of the Greater Antilles.

In the broad view, the migration, diversification, and species radiation in *Exostema* is very consistent with the processes described by GENTRY (1982) underlying the great proliferation of Neotropical species. According to this scenario, the Andean orogeny and consequent shifts to shrub habit and bee pollination produced the great explosion of neotropical diversity. An ancestral lowland Neotropical flora dominated by relatively few, widespread rain forest species, typically large trees, with lepidopteran pollinators, was gradually modified during the Andean orogeny (tens of millions of years), into the much more speciose and morphologically diverse upland (Andean) flora, characterized by shrubs and lianas, with bee pollinated flowers, and drier, more restricted habitats. Within the genus *Exostema*, the ancestral lowland element is characterized by the Amazonian species *E. maynense*, a large rain forest tree, with a long tubular, “lepidopteran syndrome” flower. The highland element is represented first by the Andean species *E. corymbosum*, a shrub or small tree with short, “bee syndrome” flowers. The great species radiations associated with the transition to the dryer, geographically and ecologically fragmented montane habitats, discussed by GENTRY (1982) with reference to the Andean flora, took place in *Exostema* in the region of the Greater Antilles. There, particularly in the areas of present-day Cuba and Hispaniola, the processes of mountain building and island vicariance and accretion, combined with a progressively drier climate during the Quaternary and recent periods (HAMMEN 1974, 1981; TAYLOR 1993) produced new environments and niches. The ecological diversity found among the Antillean species of *Exostema*, much like the overall diversity of neotropical angiosperms, may be seen as a consequence of these long term geological and climatological processes.

Taxonomic consequences. The subdivision of *Exostema* into three sections by MCDOWELL (1996) was based primarily upon morphological characters, with support from molecular data for a small sample of *Exostema* species. The present study supports this sectional classification except for the placement of the two South American species. The ITS DNA data indicates that these two species (*E. maynense* and *E. corymbosum*) are basal clades within *Exostema*, which have originated prior to the divergence of the three main species groups or sections. Given this conflict between morphological and ITS based phylogenies, the exact relationships of the two South American species remain unresolved, but their

relatively basal position is supported by both types of data. To clearly resolve whether these South American species are indeed the basal lineages for the entire genus, or are simply basal within their respective sections, will require more information. Additional characters, such as sequence data from an alternative gene (a chloroplast gene such as *matK* or *trnL* might be appropriate) or morphological data from additional, unexamined structures (e.g. seed exotesta structure) could test these alternative resolutions. The inclusion of additional outgroup taxa could also help resolve these relationships within *Exostema*.

The conflict among phylogenies derived from morphological and molecular data may reflect problems in either data set. The coding of morphological character states and the non-independence of functional traits can bias the morphological based phylogenies. Potential problems in the molecular data may include insufficient sampling for variation within taxa, non-independence of certain sites or mutations, and the saturation of certain sites with multiple mutations. Conflicts between phylogenies based upon ITS data and other types of molecular and morphological characters suggest that ITS data should be interpreted cautiously (OXELMAN 1996).

Both morphological and molecular analyses support the placement of the morphologically anomalous species *Exostema longiflorum*, *E. stenophyllum*, and *E. polyphyllum* within sect. *Pitonia*. The separation of those species into sect. *Longiflorae* and *Polyphyllae* (BORHIDI & FERNÁNDEZ 1989) obscures this relationship and makes the remainder of sect. *Pitonia* a paraphyletic group. Thus, the inclusion of these species within sect. *Pitonia* is appropriate.

The three section classification, with the uncertain placement of *Exostema maynense* and *E. corymbosum*, reflects the major phylogenetic groupings in *Exostema*. Among these three clades the axillary-flowered sect. *Exostema* may be the sister to the terminal-flowered sects. *Pitonia* and *Brachyantha*, however this hierarchy is not maintained in some of the morphological analyses. Given the present information and the uncertainty regarding the most basal lineages, it seems appropriate to maintain the three section classification on a provisional basis.

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Notes on character coding:

[1] Some species occur only as large trees or small shrubs, others (e.g. *E. mexicanum*, *E. parviflorum* and *Coutarea hexandra*) may often grow and reproduce both as shrubs and as tall (to > 20 m) trees. Similarly, two species (*E. myrtifolium*, *E. selleanum*) typically

occur as both large (2 m or taller) and very small (less than 1 m) shrubs. Such species are coded as polymorphic with two states.

[2] Pubescence in *Exostema* species is often a variable trait and a particular structure may be pubescent or glabrous depending on soils, climate, age etc. Some species, however, are always glabrous, never showing pubescence on a given structure. Accordingly, pubescence characters are coded as glabrous only when the species is always glabrous on the relevant structure. Species are coded as pubescent if the relevant structure is pubescent sometimes or always. Hairs in *Exostema* are one-celled, non-articulate and tubular. Various structures, e.g. stems [2], leaves [8], and corolla exterior [22] and interior [23] show distinct patterns of pubescence in different species, and no two pubescence characters are scored the same for all species.

[3] Raised, white to gray sulcate lenticels are conspicuous on young stems of many species. In others, young stems have small black punctae or dots visible in longitudinal fissures; some smooth stems have neither of these modifications (Fig. 3).

[4] The interpetiolar stipule, which may be strongly sheathing or nearly free at base, was scored as sheathing if the stipule usually encircled the stem for at least 3 mm, or 1/3 to 1/2 half of its length (Fig. 3).

[5] Stipules of some species bear a conspicuous hyaline margin or fringe. Species were scored "0" if such structures were not evident (hyaline margin present in Fig. 3 b).

[6] Leaf shape states are delimited by the blade length:width ratio. $L/W > 3$, narrowly lanceolate; $L/W 2-3$, narrowly to broadly elliptic; $L/W 0.7-1.3$, rotundate.

[8] The degree and disposition of leaf blade pubescence may vary within pubescent species: it is often most dense on the underside of the blade, particularly along the leaf veins, but may also cover both surfaces of the entire blade. In glabrous species the leaf blade is never pubescent.

[9] The number of secondary veins to each side of the midrib of the leaf blade is relatively consistent within species. *Exostema spinosum*, which has lateral veins nearly obsolete, but when visible often numbering 3, was scored "1". This trait provides an alternative to using measurements of leaf blade length, a highly plastic character, which within many species vary by a factor of 4.

[10] Several sorts of domatia, including pit, crypt and tuft domatia, are found at vein axils of the leaf underside. Though their occurrence, position and density vary considerably within species, but their absence from various species is a consistent trait.

[11] The leaf blade, viewed in cross section by light microscope, may have a single layer of upper epidermal cells between the cuticle and palisade parenchyma cells (0); or may have an additional hypodermal layer between epidermis and palisade parenchyma (1) (Fig. 4).

[12] Measurements of stomata diameter were made along the axis between the paired stomata. Mean and range for 10-20 measurements from a mature leaf were used to score this character.

[13] Terminal inflorescences occur at the ends of branches or at the ends of lateral shoots in the uppermost axils of branches; axillary inflorescences are always born in leaf axils, with no interruption in stem growth.

[14] The inflorescence may bear many, few or solitary flowers. Many-flowered inflorescences usually have at least 15-20 flowers, while the few-flowered peduncles seldom bear more than seven flowers. *Exostema lancifolium* often bears solitary flowers but occasionally produces two flowers per axil, so was coded polymorphic.

[16] Species coded for 6- or 5-merous flowers are consistently so, but those coded as 4-merous may sometimes have 5-merous flowers as well.

[17] Flower length measured from hypanthium base to corolla tip.

[20] Species with the calyx lobes united into a brief tube 1–2 mm long were scored for tube present, while those with calyx lobes essentially free to hypanthium, or united for less than 0.5 mm were scored for tube absent.

[21] The shape and size of the calyx lobes were coded broadly triangular or obtuse when < 2 mm long and much shorter than the hypanthium; those coded lanceolate to digitate had lobes > 2 mm long, about equal to or longer than the hypanthium.

[21] The corolla tube and lobes of all species are white at anthesis, but most change color after one day or less. The corolla changes to pink or dark purple in many night-flowering species, while the day-flowering species often age to pale yellow. *Exostema polyphyllum*, (color change unknown) were scored “?”.

[22, 23] The outer surface of the corolla tube and lobes may be glabrous or more or less pubescent, and the inside of the corolla tube is sometimes finely pubescent towards the base. These characters vary independently.

[24] The tubular corolla may be more or less deeply divided by the narrow lobes, and was coded according to the ratio of lobe to tube lengths as follows: lobes < (0.7) tube: corolla lobes much shorter than the tube; lobes = (0.7–1.3) tube: lobes and tube similar in length; lobes > (1.5) tube: lobes much longer than the tube.

[25] In the mature, pre-anthesis corolla bud the filaments may be straight or may be conspicuously bent into a tight, semi-circular curve just beneath the attachment of the linear, basifixed stamen. The long S-curve condition in the outgroup *Coutarea hexandra* is distinctly different from either of these states, and was coded “?”.

[28] Equatorial diameter of dry pollen was measured from SEM, using the mean and ranges for ten normal grains.

[30] The stigmatic surface may be localized at the end of a more or less clavate to capitate style, or may extend in two lines from the tip of an essentially linear style, for a distance similar to the length of anthers.

[32] The locules may be strongly compressed narrowing the septum, so that the capsule is wide and strongly flattened in cross section, or may be slightly flattened with the septum slightly narrower than the width of the capsule, often creating a sulcus between locules and the capsule bilobed in cross section. In the broadened capsule the septum is widened, and the capsule is slightly ellipsoid in cross section.

[34] The margin of the membranaceous seed wing may be entire, often with a heavy rim, or much dissected and with no rim. *Exostema polyphyllum*, in which the dissected seed wing is sometimes obsolete and some seeds are unwinged, was regarded as transitional, and coded polymorphic.

[36] The alignment of seeds in the mature capsule and their attachment to the placenta is highly variable in *Exostema*. In the acro-basipetal alignment seeds are attached at their wing margin, and are directed upwards, downwards and outwards from the central placenta. In the basipetal seeds are attached to the placenta from above and extend downwards, while in the acropetal state the seeds are attached from beneath and extend upwards. The horizontally imbricate seeds extend outwards from the placenta.

Appendix 1. Morphological data matrix (in this list “7”=“0,1”, “8”=“1,2”, “9”=“2,3”, multiple states treated as polymorphic)

	10	20	30
<i>E. acuminatum</i>	7120001000	0202011011	0012011200 0200100
<i>E. angustifolium</i>	2001001111	??10011010	0101001100 0101221
<i>E. brachycarpum</i>	0101011011	1010111001	0000001100 0101220
<i>E. caribaeum</i>	7010110101	0202011001	7011011200 0800120
<i>E. corymbosum</i>	1010117111	0110110110	1101111111 0010100
<i>E. ellipticum</i>	0101011011	1010111001	0001001100 0101220
<i>E. ixoroides</i>	1010111101	1010020101	1100102011 1107011
<i>E. lancifolium</i>	1020102000	??08010011	00?1001201 0100020
<i>E. lineatum</i>	2101011111	1010011010	0001001100 0101221
<i>E. longiflorum</i>	2001001101	1011112010	0000000100 0102331
<i>E. maynense</i>	0111110011	0110101011	0001010100 0010200
<i>E. mexicanum</i>	7010110111	0010110111	1101102011 0110010
<i>E. myrtifolium</i>	8010122101	1010020101	1100102011 1107011
<i>E. nitens</i>	1120112000	0201010011	1011011201 0100120
<i>E. parviflorum</i>	7110011101	1010010101	1000102011 1107011
<i>E. polyphyllum</i>	2001101000	??12011111	?0?0001100 0108291
<i>E. purpureum</i>	1020112000	0101010011	0011011201 0100120
<i>E. rotundatum</i>	0101022001	1010111001	0001001100 0101220
<i>E. salicifolium</i>	1020001000	0202011011	1012011200 0200100
<i>E. sanctaluciae</i>	0101010011	1010111011	0001001100 0101220
<i>E. selleanum</i>	8010122101	1010020101	10?0102011 1100711
<i>E. spinosum</i>	1020112000	0102020001	?011011200 0100120
<i>E. stenophyllum</i>	2101102001	1011112011	0000000100 0102331
<i>E. triflorum</i>	0101011011	?010011101	0001001100 0101220
<i>Coutarea</i>	7010110171	0117101010	7110?10201 0010800

Appendix 2. Molecular data matrix (phylogenetically informative characters only; ? missing bases and gaps)

ITS 1	10	20	30	40
<i>E. acuminatum</i>	GCCACCCATT	CGG?CGGTCC	ATGCCCTGGA	CCTGTGTCCC CGTTC
<i>E. brachyanthum</i>	GYTGTACGTC	TAATCACTTT	AYGCTTTAGA	?TTACGTCTG CGTT?
<i>E. caribaeum</i>	GTCGTCCGTT	TAACCACTCT	GTGTTTTAGA	CCCATGTTTT CGTTA
<i>E. corymbosum</i>	GCCGTCCGTC	TAACCACTTC	ACGCTCAAGA	?TTATGTCTG CAWTA
<i>E. ellipticum</i>	?TTGTACGTC	TAATCACTTT	ATGCTTTAGA	?TTACGTCTG CGTT?
<i>E. ixoroides</i>	TTCGTCTGCC	CGACCACTTT	ATGTTTTGGA	?TTATGCCTG CGWTA
<i>E. lancifolium</i>	?TCGCCCGTT	TTACTACACT	GTACTTTAAA	GTTATTTTTG TATTA
<i>E. lineatum</i>	GTTGTACGTC	TAATCACTTC	ATGCTTTAGA	?TTACGTCTG CGTT?
<i>E. longiflorum</i>	GTTGTACGTC	TAATCACTTT	ATGTTTTAGA	?TTACGTCTG CGTT?
<i>E. maynense</i>	?CCGTTGCGC	TAATCACTTC	GCGCTTAATA	?TTGTGTCTG CATCA
<i>E. mexicanum</i>	TTCGTCCGTC	CAACCACTTC	ATGTTCTAGA	?TCATGTCTG CGTTA
<i>E. myrtoides</i>	GTCGTCTGCC	CGACCACTTT	ATGTTTTGGA	?TTATGCCTG YGTTA
<i>E. nitens</i>	GTCGCCCGTT	TAATCACACT	GTGCTTTAAG	?TTCTTTTTG TATTA
<i>E. parviflorum</i>	?TCGTCTGCC	CGACCACTTT	ATGTTTTGGR	?TTATGCCTG CGTTA
<i>E. purpureum</i>	???????TT	TTACTACACT	GCACTTTAAA	GTAT?TTTG TATTA
<i>E. salicifolium</i>	?CMACCCATT	CGGCCGGTCC	ATGCCCTGGA	CCTGTGTCCC CGTCC
<i>E. spinosum</i>	GTCGCC?GTT	TAATYGCCT	GTGCTTTAAA	?TTCTTCTTG TAT??
<i>E. stenophyllum</i>	GTTGTACGTC	TAATCACTTT	ATGTTTTAGA	?TTACGTCTG CGTT?
<i>C. hexandra</i>	ACCGTTCGCC	TAATCACTTC	GCGCTCAATA	?TTGTGTCTG CATTA
ITS 2	10	20	30	40
<i>E. acuminatum</i>	ACA????GCT	CGCTGAGCTG	TAGCCGGCGC	CTCCGAGGGM
<i>E. brachyanthum</i>	ACACCCCGAT	TTTTGAATAG	TATCCGTTGC	CTC???????
<i>E. caribaeum</i>	AC??CSGGCT	TGCTGAGTTG	TAGCCGACGC	CGTTGGGATC
<i>E. corymbosum</i>	A??CYCCGCC	TGTTGGATTG	TATTCTGCGC	CGCTAAGAAC
<i>E. ellipticum</i>	ACA?CCCGAT	TTTTGAATAR	TATC??????	???????????
<i>E. ixoroides</i>	A??TCCACT	TGTTGAATAG	TATCTGTTGC	CTCTAAGAAC
<i>E. lancifolium</i>	TAACCCCGCT	TGCTAAGTTG	TAGTCGACGC	CATTAAGAAA
<i>E. lineatum</i>	ACACCCCGAT	TTTTGAATAG	TATCCGTTGC	CTCTAAAAAC
<i>E. longiflorum</i>	ACACCCCGAT	TTTTGAAAGT	TATCCGTTGC	CTCTGA????
<i>E. maynense</i>	ACACCCCGCT	CGTTGAAHAG	AGTT?GA?SS	CGCTGA????
<i>E. mexicanum</i>	ACACTCCACC	TGTCGGATAG	TGTCCGCCGC	CTCTAGGAAC
<i>E. myrtoides</i>	A??C?NCACT	TGTTGAATAG	TATCTGTTGC	CTCTAAGAAC
<i>E. nitens</i>	TATCCCGCT	TGCTGAGTTG	TATTCAACGC	TATTAAGAAC
<i>E. parviflorum</i>	ACAC?CCACT	TGTTGAATAG	TATCTGTTGC	CTCTAAGAAC
<i>E. purpureum</i>	TGT?CCCGCT	TGCTAAGTTG	TAGTCGACGC	C?????????
<i>E. salicifolium</i>	ACACCCCGCT	CGCCGA?TG	TAGCCGGCGC	CTCCGAGGGC
<i>E. spinosum</i>	??TCCSGCT	TGCTGAGTTG	TATTCAACGC	TATTAAGAAC
<i>E. stenophyllum</i>	ACACCCCGAT	TTTTGAATAA	TATCCGTTGC	CTCTGAAAAC
<i>C. hexandra</i>	ACATCCCGCT	CGTTGAATAG	AGTTTGACSS	CGCTAAGAAC

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