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PHYLOGENETIC RELATIONSHIPS WITHIN *SENNA* (LEGUMINOSAE, CASSIINAE) BASED ON THREE CHLOROPLAST DNA REGIONS: PATTERNS IN THE EVOLUTION OF FLORAL SYMMETRY AND EXTRAFLORAL NECTARIES[1]

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Senna (Leguminosae) is a large, widespread genus that includes species with enantiostylous, asymmetric flowers and species with extrafloral nectaries. Clarification of phylogenetic relationships within *Senna* based on parsimony analyses of three chloroplast regions (*rpS16*, *rpL16*, and *matK*) provides new insights on the evolution of floral symmetry and extrafloral nectaries. Our results support the monophyly of only one (*Psilorhegma*) of the six currently recognized sections, while *Chamaefistula*, *Peiranisia*, and *Senna* are paraphyletic, and monotypic *Astroites* and *Paradictyon* are nested within two of the seven major clades identified by our molecular phylogeny. Two clades (I, VII) include only species with monosymmetric flowers, while the remaining clades (II–VI) contain species with asymmetric, enantiostylous flowers, in which either the gynoecium alone or, in addition, corolla and androecium variously contribute to the asymmetry. Our results further suggest that flowers were ancestrally monosymmetric with seven fertile stamens and three adaxial staminodes, switched to asymmetry later, and reverted to monosymmetry in clade VII. Fertility of all 10 stamens is a derived state, characterizing the *Psilorhegma* subclade. Extrafloral nectaries evolved once and constitute a synapomorphy for clades IV–VII ("EFN clade"). These nectaries may represent a key innovation in plant defense strategies that enabled *Senna* to undergo large-scale diversification.

Key words: Cassiinae; extrafloral nectaries; floral asymmetry; key innovation; *matK* gene; *rpL16* intron; *rpS16* intron; *Senna*.

Formerly included in *Cassia* L., *Senna* (Leguminosae, Caesalpinioideae, Cassiinae) is a large, widespread, and diverse genus characterized by a distinctive floral morphology and the presence of extrafloral nectaries (referred to as EFNs from here on) in numerous species. *Senna* displays a high diversity of habits, including herbs, shrubs, treelets, tall trees, and lianas, and has successfully colonized a wide range of habitats in different climates and latitudes. Of the approximately 350 species currently ascribed to the genus, 80% occur on the American continent, while most of the remaining members are found in tropical Africa, Madagascar, and Australia, with only a few species in southeastern Asia and some on the Pacific Islands (Irwin and Barneby, 1982; Randell and Barlow, 1998). No *Senna* species are native to Europe, although several of them have long been used in the European medical tradition (e.g., Colladon, 1816).

Shifting taxonomic boundaries mark the history of traditional systematic treatments of *Senna*. These shifts are best explained by the difficult taxonomic interpretation of morphological variation in *Senna*. For example, the high degree of specialization typical of the buzz-pollinated *Senna* flowers complicates the identification of traits that can be unambiguously used for taxonomic purposes. The yellow, nectarless flowers offer pollen as a reward to their pollinators, usually large female bees of different genera, for example, *Xylocopa* (Dulberger, 1981; Irwin and Barneby, 1982; Gottsberger and Silberbauer-Gottsberger, 1988). The heteranterous flowers of *Senna* generally have 10 stamens; the three adaxial stamens are typically staminodial, while the remaining seven, or fewer, are fertile. The fertile stamens are poricidal and differentiated into two sets: one set of four middle stamens (between the adaxial staminodes and abaxial stamens; see also Fig. 3A), which bees buzz to extract food pollen, and a second set of two or three (often longer) abaxial stamens, whose pollen is deposited on the bee's body during buzzing and transported to the stigma of other flowers (Buchmann, 1974; Gottsberger and Silberbauer-Gottsberger, 1988; Carvalho and Oliveira, 2003). Furthermore, many species of *Senna* have asymmetric flowers, with the gynoecium deflected either to the left or to the right within the same inflorescence (Dulberger, 1981; Irwin and Barneby, 1982; Gottsberger and Silberbauer-Gottsberger, 1988). This type of floral asymmetry is known as enantiostyly.

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In many enantiostylous *Senna* species, corolla and androecium additionally contribute to the floral asymmetry.

Typically found on leaves and rarely also on pedicels, EFNs are another characteristic feature of many *Senna* species. They occur in ca. 76% of the American species (Irwin and Barneby, 1982), numerous Australian species, more rarely in African species, but apparently in no Southeast Asian species. These organs attract ants, which feed on the nectar and protect the plant against herbivores, thus forming an opportunistic ant–plant interaction or mutualism (e.g., Heil and McKey, 2003). Although recent monographic treatments explored the taxonomic utility of EFNs in *Senna* (Irwin and Barneby, 1982; Randell, 1988, 1989), little is known about their specific distribution, anatomy, and evolutionary significance in the genus.

Species of *Senna* were formerly included among the approximately 600 species of *Cassia* s.l. (Irwin and Turner, 1960). Subsequent taxonomic treatments subdivided this large genus into the smaller *Cassia* s. str., *Chamaecrista* Moench, and *Senna*, and ascribed these three genera to subtribe Cassiinae (Irwin and Barneby, 1981, 1982). In the most recent monograph, *Senna* comprised approximately 260 species (Irwin and Barneby, 1982), which later increased to 350, mainly as a result of new nomenclatural combinations in non-American taxa (Randell and Barlow, 1998). The separation of *Senna* from *Cassia* was confirmed by further taxonomic (e.g., Randell, 1988, 1989, 1990; Singh, 2001), structural (Endress, 1994; Tucker, 1996b), and phenetic studies (Boonkerd et al., 2005). Published molecular phylogenies, comprising only 11 species of *Senna*, supported its monophyly (Bruneau et al., 2001; see also Herendeen et al., 2003, based on both molecular and morphological data). In these phylogenies, Cassiinae are included in a large clade formed by other caesalpinioids (Caesalpinieae and Cassieae, subtribe Ceratoniinae) and the Mimosoideae (see also Wojciechowski et al., 2004). Although the recent survey on legumes by Lewis et al. (2005) considered Cassiinae monophyletic (as Cassieae s. str.), relationships among the three genera *Cassia*, *Chamaecrista*, and *Senna* remain unclear.

The most recent classification of *Senna* (Irwin and Barneby, 1982) relied greatly on Bentham's (1871) taxonomic revision of *Cassia*, specifically of *Cassia* subgenus *Senna*. Although Irwin and Barneby (1982) focused only on the American species, which represent the majority of the genus, their classification was soon adopted in subsequent treatments of *Senna* in other continents (discussed next). Irwin and Barneby (1982) divided *Senna* into six sections: *Astroites* (1 species, abbreviated to "sp." from here on), *Chamaefistula* (c. 140 spp.), *Paradictyon* (1 sp.), *Peiranisia* (c. 55 spp.), *Psilorhegma* (c. 30 spp.), and *Senna* (c. 20 spp.). They also recognized 35 series, which increased to 38 with more recent revisions of the Australian (Randell, 1988, 1989, 1990) and Indian species of *Senna* (Singh, 2001; see Appendix 1). This recent classification of *Senna* emphasized the importance of floral morphology, while former classifications (with *Senna* included in *Cassia*) focused on fruit structure (e.g., Bentham, 1871). At a lower taxonomic level, the occurrence, location, and form of the EFNs were used in both older and more recent classifications of *Senna* (or as *Cassia*) to characterize several series or groups of series (Bentham, 1871; Irwin and Barneby, 1982).

In their classification of *Senna*, Irwin and Barneby (1982) proposed several hypotheses of evolutionary trends in floral morphology, focusing on androecium, corolla, and floral architecture. The fertility of all 10 stamens was used to distinguish section *Psilorhegma* from all other sections, which have only seven or fewer fertile stamens and three adaxial staminodes. The kind of androecium typical of *Psilorhegma* was considered to represent the ancestral state, justifying the "basal" position of *Psilorhegma* within *Senna*. Conversely, the highly asymmetric condition of the androecium and corolla characteristic of flowers in *Peiranisia* was used to interpret *Peiranisia* as the most derived section, while the flowers of sections *Chamaefistula*, *Astroites*, *Senna*, and *Paradictyon* were considered to represent intermediate evolutionary stages (Irwin and Barneby, 1982; see also Fig. 3 A–C). The monotypic sections *Astroites* and *Paradictyon* consist of *Senna villosa* and *S. paradictyon*, respectively. Although *S. villosa* shares some floral features with sect. *Chamaefistula*, and *S. paradictyon* with sections *Senna* and *Peiranisia*, the two species were assigned each to its own section because some of their morphological features are found nowhere else in the genus (Irwin and Barneby, 1982). These unique features include the lomentaceous pod and stellate hairs of *S. villosa* and the xylopodium and parallel sepal venation of *S. paradictyon*.

The diversity of floral symmetries and structures raises interesting questions on the evolution of floral morphology in *Senna*. Irwin and Barneby (1982) suggested that floral asymmetry evolved in relation to bee pollination in more than one evolutionary line within *Senna*, but did not test this hypothesis, nor did other researchers. The relationship between enantiostyly and pollination biology has been investigated only in a few species of *Senna* (e.g., Fontanelle, 1979; Dulberger, 1981; Gottsberger and Silberbauer-Gottsberger, 1988; Carvalho and Oliveira, 2003). Other morphological studies, which focused on a few species, examined the specialization of the stigma (Owens and Lewis, 1989; Dulberger et al., 1994), the structure of the poricidal stamens (e.g., Lassaigne, 1979; Endress, 1994; Tucker, 1996a), and floral development (Tucker, 1996b).

The presence of EFNs represents another fascinating evolutionary aspect of *Senna* morphology. Most taxonomic treatments of *Senna* considered EFNs as an "archaic feature," present in the "basal" sections *Psilorhegma* and *Chamaefistula*, but lost in more "advanced" lines, i.e., sections *Senna*, *Paradictyon* and part of *Peiranisia* (Irwin and Barneby, 1982; Randell, 1989). Although little is known about EFNs in *Senna*, experimental studies in other angiosperms, including many legume taxa, demonstrated that the mutualistic relationship between EFNs and the ants that feed on them protects the plant from herbivores, thus enhancing plant fitness (e.g., Koptur, 1979; Barton, 1986; Rutter and Rausher, 2004). Nevertheless, the implications of these recent findings for the evolutionary history of *Senna* have not been addressed. An explicit phylogenetic framework within *Senna* would allow us to investigate the proposed ancestral nature of the EFNs and the single vs. multiple origins of these structures within the genus, compare the sizes of sister clades formed by species with or without EFNs, and discuss the potential evolutionary role of EFNs in light of their value for plant defense.

The major aim of the present study is to generate a molecular phylogeny of the genus *Senna* to be compared with current classification systems (Irwin and Barneby, 1982; Randell, 1988, 1989, 1990; Singh, 2001) and elucidate patterns of morphological evolution. More specifically, we address the following questions: (1) Are the sections *Chamaefistula*,

Peiranisia, *Psilorhegma*, and *Senna*, as defined by Irwin and Barneby (1982), monophyletic? (2) Are the monotypic *Astroites* and *Paradictyon* embedded in other sections or do they represent isolated lineages? (3) Which morphological traits are congruent with the clades defined by the molecular phylogeny? (4) Does our molecular tree support the hypotheses of floral evolution proposed by Irwin and Barneby (1982)? (5) Did EFNs evolve once or multiple times? (6) Are the EFNs associated with species-richer clades?

MATERIALS AND METHODS

Taxonomic sampling—Of the 98 taxa (101 samples) considered in this study, 87 were ascribed to *Senna*, and the remainder to other caesalpinioids (discussed later). The 81 *Senna* species (approximately one fourth of the genus) represent all six sections and 24 of 38 recognized series (Irwin and Barneby, 1982; Randell, 1988, 1989, 1990; Singh, 2001; Appendix 1). Eleven of the 14 series unrepresented in our study are monotypic, while each of the remaining three series includes up to three species. Most samples were field-collected in Argentina, Australia, Bolivia, Brazil, Mexico, Panama, Paraguay, South Africa, and the United States, while a few were received from European and Australian botanic gardens. The broad infraspecific morphological variation of *Senna acuruensis*, *S. multijuga*, and *S. hirsuta* recognized at the varietal rank (Irwin and Barneby, 1982), prompted us to sample several accessions of these species. The 11 outgroups, selected on the basis of published phylogenies, comprise species of *Cassia*, *Caesalpinia*, *Chamaecrista*, *Delonix*, and *Gleditsia* (Bruneau et al., 2001; Kajita et al., 2001; collection and voucher information provided in Appendix 2).

DNA extraction, amplification, and sequencing—Total genomic DNA was extracted from silica gel dried leaves and petals and from seeds. Tissue samples were homogenized using a Retsch MM 2000 shaker (Retsch, Haan, Germany). DNA was extracted using the Dneasy Plant Mini Kit (Qiagen, Basel, Switzerland), the protocol by Eichenberger et al. (2000), and the Extract-N-Amp Seed PCR Kit (Sigma, St. Louis, Missouri, USA). Three chloroplast regions (*rpL16* intron, *rpS16* intron, and the *matK* gene) were sequenced for all taxa. Target regions were amplified by the polymerase chain reaction (PCR; Mullis and Faloona, 1987) on a Biometra TGradient thermocycler or a Biometra T1 thermocycler (Biometra, Göttingen, Germany). We used primer F71 (which anneals at position 71 of the flanking 3′ exon; Jordan et al., 1996) and the internal primer R1516 (Baum et al., 1998) to amplify a partial sequence of the *rpL16* intron; primers rpsF and rpsR2 (Oxelman et al., 1997) to amplify the complete *rpS16* intron, and primers matK3R and matK3F (Sang et al., 1998) to amplify the central portion of the *matK* gene. Annealing positions of the *matK* primers are described in Sang et al. (1998). Primer sequences are listed in Table 1. The thermal cycling program for the amplification (TCPA) of the *rpL*16 and *rpS16* introns consisted of the following steps: premelting at 95°C/4 min followed by 35 cycles with melting at 95°C/30 s, annealing at 51.3°C/1 min, extension at 72°C/1 min 50 s (*rpL*16) and at 1 min 30 s (*rpS16*). The TCPA for the *matK* gene was: premelting at 95°C/4 min followed by 5 cycles with melting at 95°/30 s, annealing at 42°C/1 min, extension at 72°C/1 min 42 s, and then 30 cycles with annealing at 50°C. All reactions ended with a final extension at 72°C/10 min followed by a holding step at 4°C. PCR products were electrophoresed on 1% agarose gels containing ethidium bromide and examined under UV light. Successfully amplified PCR products were usually purified with the QIAquick PCR purification Kit (Qiagen), although shrimp alkaline phosphatase and exonuclease I (USB Corp., Cleveland, Ohio, USA) or the precipitation protocol by Dunn and Blattner (1987) were also employed.

Cycle-sequencing reactions were performed using the same primers (see Table 1) and the Big Dye Terminator (Perkin-Elmer, Applied Biosystems, Applera Europe BV, Rotkreuz, Switzerland), or the DYEnamic Et-terminator Kit (Amersham, Buckinghamshire, UK) for Brazilian samples. Three additional internal primers, rpL16Fa rpL16Fb, and rpL16 Ra (Table 1), were designed from the conserved sites of the aligned *rpL16* sequences from our taxa. Cycle sequencing products obtained using the Big Dye Terminator were cleaned with Microspin G-50 (Amersham Pharmacia Biotech Europe, Dübendorf, Switzerland) using multiscreen plates to remove excess Big Dye Terminator before loading on the automated sequencer ABI PRISM 3100 Genetic Analyzer (Perkin-Elmer). The cycle-sequencing products obtained with the DYEnamic Et-terminator Kit were directly loaded on the automated sequencer. The software Sequencher (versions 3.1.1 and 4.2; Gene Codes, 1998) was used to edit and assemble complementary strands. Base positions were individually checked for agreement between the complementary strands. Sequences were visually aligned in MacClade 4.0 (Maddison and Maddison, 2000). Sequence accession numbers are provided in Appendix 2.

Phylogenetic reconstruction—Cladistic analyses were performed using the maximum parsimony (MP) optimization criterion of the software package PAUP*: phylogenetic analyses using parsimony (*and other methods), version 4.0b10 (Swofford, 2002). Only informative characters were included, and all characters and character states were weighted equally (Fitch, 1971). Insertion/deletions (indels) were directly included in the overall analysis of the aligned matrices. Missing parts of a few sequences were replaced by question marks (see details in the Results, Size and structure of the molecular data sets).

The individual data matrices for each of the three DNA regions and a combined data matrix were analyzed by employing a heuristic search strategy with 1000 random taxon-addition replicates, holding 10 trees at each step, tree-bisection-reconnection (TBR) branch swapping with STEEPEST DESCENT and MULTREES options in effect, and saving a maximum of 30 trees for each replicate. Branch support was estimated by bootstrap resampling (Felsenstein, 1985) with 1000 replicates, holding 10 trees at each step, simple addition sequence, and TBR branch swapping with STEEPEST DESCENT and MULTREES options in effect, saving a maximum of 30 trees in each replicate. Branch lengths of the trees derived from the combined data set were calculated by including all characters.

Our outgroups comprise species of *Cassia*, *Caesalpinia*, *Chamaecrista*, *Delonix*, and *Gleditsia* (see Appendix 2). To investigate whether *Senna* is monophyletic, we initially rooted the tree with *Gleditsia* alone, because recent molecular phylogenies identified *Gleditsia* as sister to a large clade that included all our sampled genera (Bruneau et al., 2001; Wojciechowski et al., 2004). Because this analysis supported the monophyly of *Senna*, we proceeded to keep only *Senna* species in the ingroup for the final analysis. The 11 outgroups were allowed to be paraphyletic with respect to the ingroup.

RESULTS

Size and structure of the molecular data sets—The length of the aligned combined data set included 2909 nucleotide positions (ntps), 501 (17.2%) of which were potentially parsimony informative (see Table 2). The partial sequences of the *matK* gene (620 aligned ntps), provided the highest percentage of informative positions (19.3%), while the *rpL16* (1209 aligned ntps) and *rpS16* (1080 aligned ntps) intron sequences were slightly less informative, with 18.6% and 14.4% informative positions, respectively. Absolute sequence length of the *rpL16* intron ranged from 797 ntps (*S.* indet. ser. *Subverrucosae*) to 962 ntps (*Gleditsia triacanthos*), sequences of the *rpS16* intron ranged from 839 ntps (*S. paradictyon*) to

TABLE 1. Primer sequences for the cpDNA regions used in this study.

Primer	5′ to 3′
For *rpL16* intron:	
F71	GCTATGCTTAGTGTGTGACTCGTTG
R1516	CCCTTCATTCTTCCTCTATGTTG
rpL16Fa	ATCTCTACTACAGAACCG
rpL16Fb	TTTKGGGTTATAGTTGATG
rpL16Ra	CTATARAACTAATAACCAAC
For *rpS16* intron:	
rpsF	GTGGTAGAAAGCAACGTGCGACTT
rps2R	TCGGGATCGAACATCAATTGCAAC
For *matK* gene:	
matK3F	AAGATGCCTCTTCTTTGCAT
matK3R	GATCCGCTGTGATAATGAGA

Note: Primers rpL16Fa, rpL16Fb, and rpL16Ra were used exclusively for cycle sequencing.

TABLE 2. Description of cpDNA data sets and resulting trees (excluding uninformative characters).

cpDNA region/matrix	Aligned length	Informative ntps (% of aligned ntps)	No. of steps	CI	RI	No. of trees
rpL16 intron	1209	225 (18.6)	511	0.568	0.849	13530
rpS16 intron	1080	165 (14.4)	335	0.570	0.856	18150
matK gene (part)	620	120 (19.4)	262	0.599	0.840	2917
Combined	2909	501 (17.2)	1136	0.562	0.840	29582

Note: ntps = nucleotide positions, CI = consistency index, RI = retention index.

908 ntps (*S. obtusifolia*), whereas partial sequences of the *matK* gene included 620 ntps.

Despite repeated efforts to optimize PCR conditions, we were unable to generate *matK* sequences for *S. aversiflora*, *S. odorata*, and one of the two accessions of *S. acuruensis* var. *acuruensis* (accession LQ 9201; see Appendix 2), and *rpL16* intron sequences for *S. alata*, *S. venusta*, and *Caesalpinia gilliesii*. Three sequences in the *rpL16* intron aligned data set are incomplete: *S. artemisioides* (110 missing ntps at the 3′ end), *S. cardiosperma* (141 missing ntps at the 3′ end), and *S. oligoclada* (161 missing ntps at the 3′ end). Also two sequences in the *rpS16* intron aligned data set are incomplete, *Caesalpinia gilliesii* (107 missing ntps at the 5′ end and 32 ntps at the 3′ end) and *Gleditsia sinensis* (181 missing ntps at the 3′ end).

Phylogenetic reconstruction—Descriptive values for the trees resulting from the analyses of the separate and combined data sets are listed in Table 2. The strict consensus trees generated from each separate data set showed no major topological conflicts (see Fig. 1). Because the few observed topological discrepancies involved clades with weak branch support (bootstrap support [BS] $<$ 50%), we combined all matrices in a combined data set that was used for MP analysis (Brower, 1996; Nixon and Carpenter, 1996). Minor topological discrepancies involved members of clades III (which formed a clade only in the *rpL16* strict consensus tree), IV, and VII. The tree derived from the combined data set showed increased resolution and branch support (discussed next; see also Figs. 1, 2).

Analyses of the combined data matrix using *Gleditsia* alone as outgroup supported the monophyly of *Senna*. Therefore, the final analyses reported here included only species of *Senna* in the ingroup (see Fig. 2). MP analyses of the combined data set resulted in 29582 trees of 1136 steps, consistency index [CI] of 0.562 and retention index [RI] of 0.840, excluding uninformative characters. When all characters were included, the trees had a length of 1578 steps, CI = 0.674, and RI = 0.840. One of the MP phylograms (including all characters) is shown in Fig. 3. Combination of the data sets clearly increased resolution and branch support in the resulting tree. For example, in the *Senna* clade the number of branches with BS above 80% approximately doubled in the combined tree as compared with the trees from the separate data sets. In addition, the number of branches with 100% BS increased to 16 in the combined tree, while only three branches had 100% BS in the trees from the separate data sets. The strict consensus (Fig. 2) of the MP trees derived from the combined matrix supported the sister relationship between *Cassia fistula* and the *Senna* clade (90% BS). Analysis of the combined matrix also increased the support for the monophyly of *Senna* (100% BS), in comparison with the lower support retrieved from the separate data sets (58–87% BS). Within *Senna*, four species of section *Chamaefistula* (clade I) form the sister clade to the rest of the genus, which comprises clades II to VII.

None of the three chloroplast regions was sufficiently variable to resolve relationships within *Senna* (see Figs. 1 and 2). This result is consistent with the findings of numerous studies, which have demonstrated that best phylogenetic resolution is often achieved through a combination of several cpDNA regions in the same data set (Mast et al., 2001; Schönenberger and Conti, 2001; Simões et al., 2004; Shaw et al., 2005).

DISCUSSION

Comparison between the molecular phylogeny and Irwin and Barneby's classification—Parsimony analysis of the combined data matrix supports the monophyly of *Senna* as defined by Irwin and Barneby (1981, 1982; see Fig. 2), thus confirming the results of previous molecular phylogenetic studies, which included only a few *Senna* species (Bruneau et al., 2001; see also Herendeen et al., 2003). Our limited taxon sampling outside of *Senna* does not allow us to make any conclusive remarks concerning the monophyly and relationships of Cassiinae, although our results favor the sister relationship between *Cassia* and *Senna* (Bruneau et al., 2001), rather than between *Chamaecrista* and *Senna* (e.g., Herendeen et al., 2003).

Sectional delimitations within *Senna*, as defined by Irwin and Barneby (1982), are largely incongruent with our tree (Fig. 2). Only section *Psilorhegma* is supported as monophyletic (82% BS), whereas sections *Chamaefistula*, *Peiranisia*, and *Senna* are paraphyletic. The largest section, *Chamaefistula*, is splintered into several lineages over the entire phylogenetic tree. Section *Peiranisia* is divided into two clades, and *Senna chloroclada*, ascribed to *Peiranisia*, groups instead with other species of *Chamaefistula*. The monotypic *Astroites* (*S. villosa*) is embedded within a clade comprising species of *Chamaefistula*, while the monotypic *Paradictyon* (*S. paradictyon*) is embedded within sect. *Senna*. Thus, our phylogenetic results do not support the conclusion that these monotypic sections represent isolated lineages within *Senna* (Irwin and Barneby, 1982).

Although most of Irwin and Barneby's (1982) sectional delimitations are in conflict with our molecular results, series circumscriptions are often congruent with the molecular tree topology, as in the case of series *Aphyllae*, *Bacillares*, *Basiglandulosae*, *Deserticolae*, *Galeottianae*, *Isandrae*, and *Laxiflorae* (72–100% BS; Fig. 2).

Phylogenetic relationships within Senna—The discussion of the seven major clades supported by the combined molecular

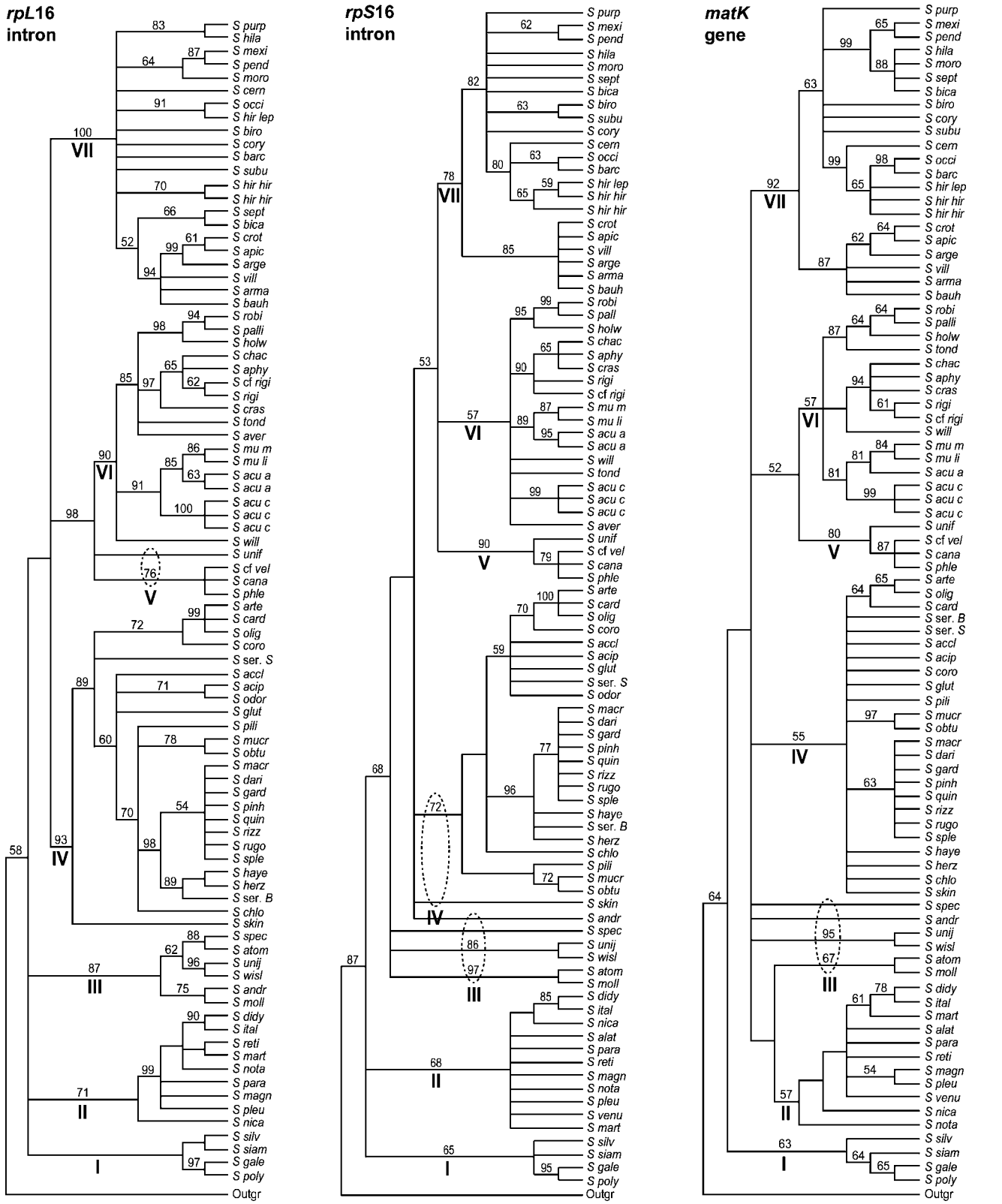

Fig. 1. Strict consensus trees resulting from parsimony analyses of each individual matrix (see Table 2). The outgroup species (Outgr) are listed in Fig. 2. Bold Roman numerals indicate major clades; dotted ellipses show the taxa that form a clade in the combined tree (Fig. 2).

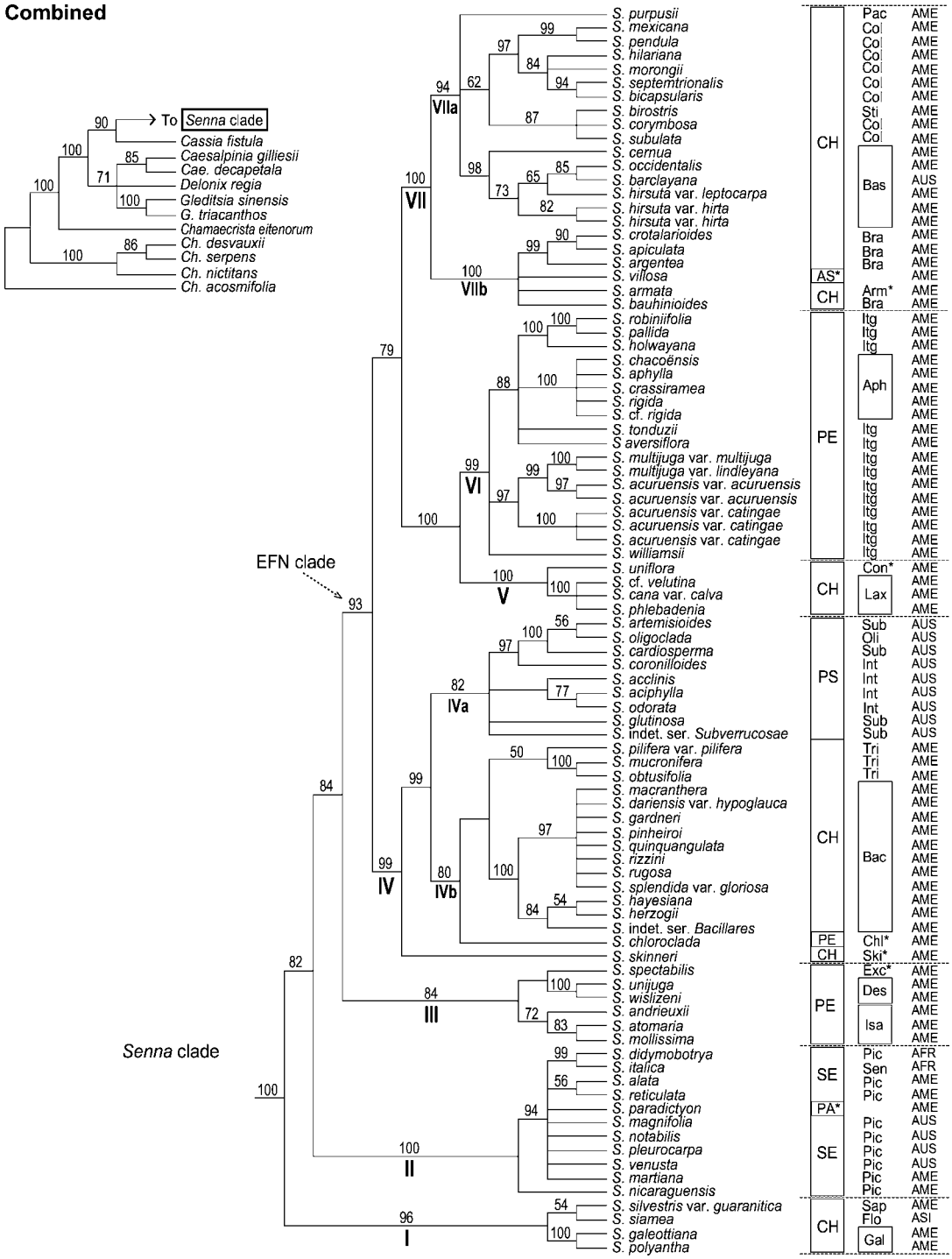

Fig. 2. Strict consensus tree resulting from parsimony analyses of the combined matrix (see Table 2). Bootstrap support [BS] values >50% are reported above the branches. Bold Roman numerals below the branches indicate major clades and subclades. *Senna* species, sections, series, and geographic distribution are listed to the right of the tree (see Appendix 1 for full section and series names; AFR, Africa; AME, America; ASI, Asia; AUS, Australia). Monophyletic series are framed; monotypic sections and series are marked with an asterisk. Dashed horizontal lines show delimitations of the major clades. Outgroups are listed in the partial tree on the left.

phylogeny (Fig. 2) will focus on the morphological synapomorphies that are congruent with these clades, with special emphasis on floral architecture and symmetry, and EFNs.

Clade I—Clade I, including only species of section *Chamaefistula,* is sister to the rest of *Senna* (Fig. 2). This clade contains species ascribed to the American series *Galeottianae* and *Sapindifoliae*, and to the Asian series

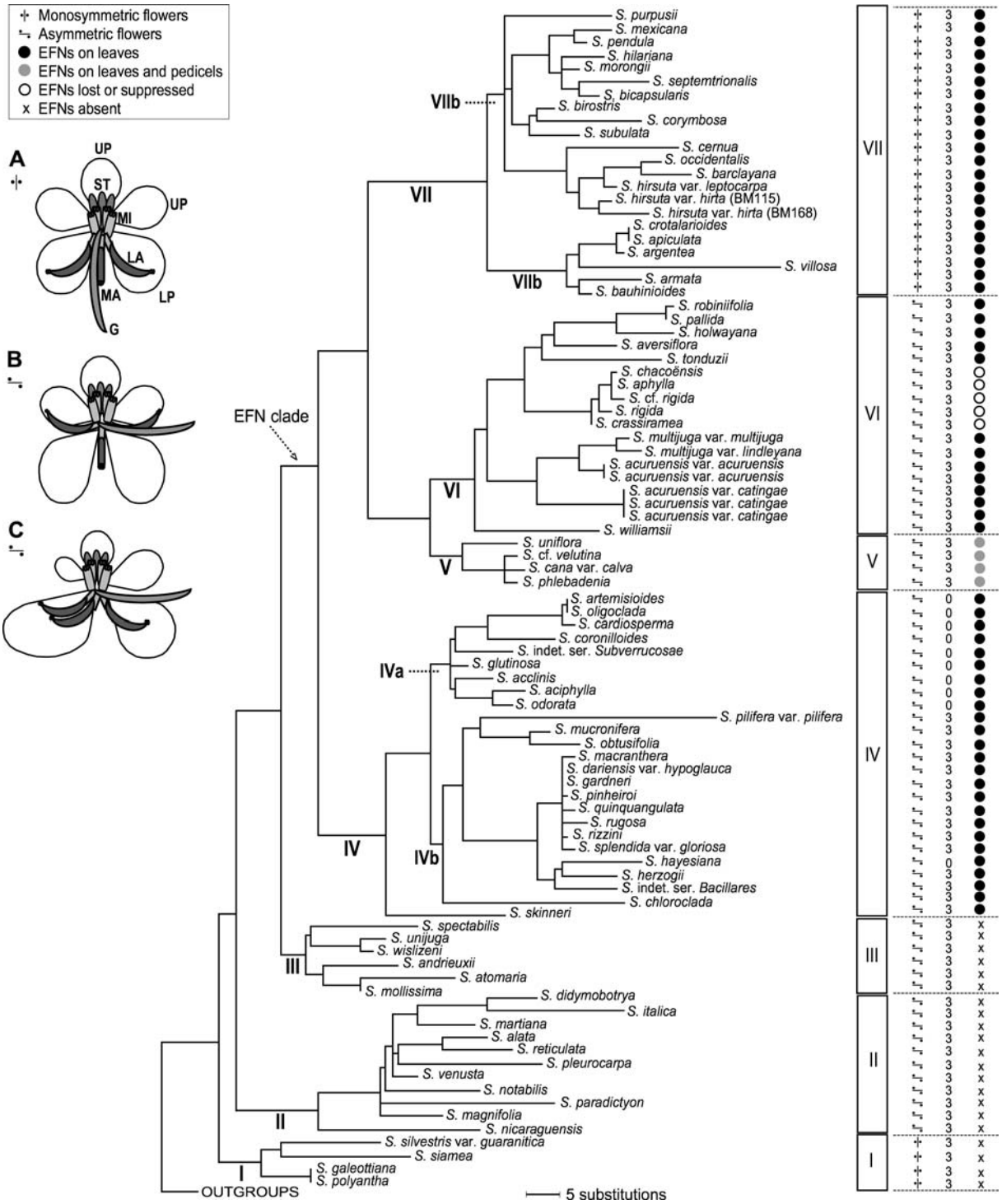

Fig. 3. One of the most parsimonious trees resulting from the analyses of the combined matrix (1578 steps; CI = 0.674; RI = 0.840; all characters included). Bold Roman numerals below the branches indicate major clades and subclades. A branch-length scale is reported below the tree. Outgroups are listed in Fig. 2. The symbols to the right of the boxes representing the major clades indicate, respectively: floral symmetry, number of adaxial staminodes, extrafloral nectaries (EFNs). Dashed horizontal lines show delimitations of the major clades. A–C. schematic diagrams of floral symmetry patterns in *Senna* species: (A) Monosymmetric flower (*S. silvestris*, sect. *Chamaefistula*, clade I); AL, abaxial lateral stamen; AM, abaxial median stamen; G, gynoecium; LP, lower petal; MI, set of four middle stamens; ST, set of three adaxial staminodes; UP, upper petal; sepals not shown. (B) Asymmetric flower in which only the gynoecium is involved in the floral asymmetry (*S. nicaraguensis*, sect. *Senna*, clade II). (C) Asymmetric flower in which also petals and stamens are involved in the floral asymmetry (*S. pallida,* sect. *Peiranisia*, clade VI).

Floridae. These three series comprise trees and treelets lacking EFNs (Fig. 3). Their monosymmetric flowers have seven fertile stamens and three adaxial staminodes. This androecial organization is also characteristic of most remaining clades, except for IV and VII (see Fig. 3A). In some flowers, the gynoecium may be slightly deflected to one side. Because carpel deflection is inconsistent in these species, their flowers are not considered truly enantiostylous.

Previous taxonomic treatments of *Senna* identified similarities in the habit, corolla, and androecium of the species included in clade I (Bentham, 1871; Irwin and Barneby, 1982). However, these species were ascribed to different series in section *Chamaefistula* mainly based on allopatric ranges of distribution and morphological differences among their hypanthia and fruits (Irwin and Barneby, 1982).

Clade II—Clade II is formed by Australian, American, and African species of section *Senna*, except for the South American *S. paradictyon,* the only species ascribed to section *Paradictyon* (Fig. 2). Therefore, our results suggest that sect. *Senna* would be monophyletic, if *S. paradictyon* were included in it. The clade comprises shrubs and treelets characterized by asymmetric flowers and lacking EFNs (Fig. 3). Floral asymmetry appears to be a synapomorphy for the super clade comprising clades II–VII, with a possible reversal to monosymmetric flowers in clade VII. In clade II, floral asymmetry affects only the gynoecium, while the androecium and corolla are monosymmetric. These flowers represent the simplest variant of enantiostyly, involving the lateral deflection of the gynoecium only (see Fig. 3B). The petals partially enclose the reproductive organs during the initial stages of anthesis and unfold during the later stages, as in *S. didymobotrya* (Dulberger, 1981; B. Marazzi, personal observation) or never unfold, as in *S. alata*. The orientation of the two lateral abaxial stamens is characteristic for this clade and has been used to distinguish sect. *Senna* from sect. *Chamaefistula* (Irwin and Barneby, 1982; see also Fig. 3A, B). Of the three abaxial stamens, one is median and two are lateral; these latter ones are curved laterally, so that their anthers face each other, resembling the arms of tongs.

The presence of a xylopodium (discussed later), bracteoles on the pedicels (otherwise absent in *Senna*), and parallel venation of the sepals (otherwise palmately veined in *Senna*) are autapomorphies of *S. paradictyon* and prompted its inclusion in a separate, monotypic section (Irwin and Barneby, 1982). In contrast, Bentham (1871) included *S. paradictyon* (as *Cassia paradictyon*) in his section *Chamaesenna* ser. *Pictae*, which later became ser. *Pictae* of section *Senna* in Irwin and Barneby's (1982) revision, although *S. paradictyon* was excluded from it. The xylopodium, a cylindrical, lignified region of the stem that is located just above the soil line, gives rise to new sprouts. This interesting structure of *S. paradictyon* may represent an adaptation to fire, which plays an important ecological role in the renewal of cerrado vegetation (Ratter et al., 1997), where *S. paradictyon* typically occurs. The cerrado is a vegetation type of central Brazil, extending marginally into Paraguay and Bolivia, characterized by savanna woodlands (e.g., Ratter et al., 1997; Pennington et al., 2000).

Clade III—Clade III is formed by a part of the polyphyletic section *Peiranisia* and combines the southern North American and northern Central American series *Deserticolae* and *Isandrae* and the monotypic South American series *Excelsae*. All species of this clade are treelets and shrubs lacking EFNs and characterized by enantiostylous flowers, in which the floral asymmetry also involves the corolla and, in some species, the androecium (Fig. 3). Enantiostyly affects petals and stamens in different ways, for example: (1) the upper petals are more or less reduced, (2) one or both lower petals are strongly modified in shape and size, (3) the abaxial stamens are on the opposite side of the deflected carpel, or (4) the androecium is nearly monosymmetric. Therefore, many variants of enantiostyly are represented in the clade. The observation that floral asymmetry assumes different variants in this and other clades (discussed later) and that it was derived independently multiple times in *Senna* suggests that such variants probably originated through different evolutionary and developmental pathways, therefore they are not strictly homologous.

The highly asymmetric corolla, in which one or both lower petals are strongly modified in shape and size, was used to distinguish section *Peiranisia* from sections *Chamaefistula* and *Senna* (Irwin and Barneby, 1982; see also Figs. 3A-C). Our results divide *Peiranisia* into two well-supported clades (III and VI; BS = 84% and 99%, respectively), except for *S. chloroclada* of the monotypic series *Chlorocladae*, which is included in clade IV. Irwin and Barneby's (1982) classification used androecial traits and the absence of EFNs to distinguish the series of *Peiranisia* in clade III from the other series of the section, i.e., series *Chlorocladae* in clade IV, and *Interglandulosae* in clade VI (Fig. 2). In addition, other characteristics of the androecium, such as minor differences in the size and form of the stamens, were used to distinguish the series in clade III from each other (Irwin and Barneby, 1982).

Clade IV—In clade IV, one species of section *Chamaefistula*, *S. skinneri,* is sister to a clade formed by subclades IVa and IVb. Subclade IVa comprises only members of section *Psilorhegma*, which is thus supported as monophyletic by our analyses. In subclade IVb, one species of section *Peiranisia*, *S. chloroclada*, is sister to a clade comprising species of *Chamaefistula*, series *Bacillares* and *Trigonelloideae* (subclade IVb; Fig. 2). All species in subclade IVa (sect. *Psilorhegma*) occur exclusively in Australia, while the other members of clade IV are American. Clade IV includes herbs, shrubs, and treelets with asymmetric flowers and bearing EFNs on the leaves (Fig. 3). Floral asymmetry involves corolla and androecium, in addition to the gynoecium. One or both lower petals are often strongly concave, and, in a few species, they surround the reproductive organs and can be additionally modified in shape and size (e.g., *S. chloroclada*). In some species, the flowers are slightly rotated on their axis and attain an oblique position (e.g., *S. aciphylla*, *S. obtusifolia*), further deflecting the gynoecium, thus emphasizing enantiostyly. Hairs were observed on the anthers of both middle and abaxial stamens in species of clade IV, but not of other clades. While hairs are normally rare on the anthers of caesalpinioids (Endress and Stumpf, 1991; Tucker, 1996a), in *Senna* they may represent a synapomorphy for clade IV.

The fertility of all 10 stamens in the androecium, traditionally used to circumscribe section *Psilorhegma* (Bentham, 1871, as *Cassia*; Irwin and Barneby, 1982), represents a unique synapomorphy for subclade IVa (see Figs. 2 and 3). In section *Psilorhegma*, the stamens are more or less all similar in shape, but in some species (e.g., *S. artemisioides*) the lateral

abaxial stamen opposite to the deflected carpel is bigger. In the remaining species of clade IV (with seven or rarely fewer fertile stamens), the abaxial stamens present two kinds of arrangement: either they are almost equal in size to the middle stamens, and, in this case, the androecium is nearly monosymmetric, or they are much longer, and, in this case, the median abaxial stamen is deflected to the opposite side of the gynoecium, making the androecium asymmetric.

The 11 sampled species of series *Bacillares* (sect. *Chamaefistula*) are all included in subclade IVb (Fig. 2). Series *Bacillares,* comprising approximately 50 species of shrubs and treelets, is characterized by leaves consistently having two pairs of leaflets (Irwin and Barneby, 1982). Members occur in tropical and subtropical areas of the American continent and have colonized a wide range of habitats, from the dry cerrado to the humid tropical forest. Some species, e.g., *S. macranthera*, display a high level of morphological variation, which prompted the description of many infra-specific varieties (Irwin and Barneby, 1982). Interestingly, Irwin and Barneby (1982) did not consider the flowers of *Bacillares* as enantiostylous, but rather as having a "centrically (or subcentrically) oriented pistil," and interpreted the asymmetric corolla and the median pistil as a "variation of simple zygomorphy." Flowers of one species, *S. hayesiana*, lack the adaxial staminodes and the abaxial stamens, displaying only the four middle stamens (Irwin and Barneby, 1982; Marazzi, personal observation; Fig. 3). Our molecular phylogeny corroborates the monophyly of *Bacillares* and its subdivision into two well-supported subclades (BS values of 97% and 84%; Fig. 2): one subclade consists of a large polytomy with short terminal branches, suggesting a possibly recent radiation of this group; the other subclade comprises only a few species at the tips of longer terminal branches (*S. hayesiana*, *S. herzogii*, and *S.* indet. ser. *Bacillares*; Fig. 3). We were unable to identify any morphological or biogeographic patterns congruent with these subclades.

In our chloroplast DNA phylogeny, *S. chloroclada*, traditionally ascribed to section *Peiranisia*, appears to be more closely related to species of *Chamaefistula* in clade IV (80% BS) than to species of *Peiranisia* (Fig. 2). This subaphyllous species was viewed as representing a transitional stage between the aphyllous and the leafy species (Burkart, 1943). Irwin and Barneby (1982) placed *S. chloroclada* in *Peiranisia,* ser. *Chlorocladae* for its habit, inflorescence, asymmetric corolla, and pod similar to that of *Peiranisia,* ser. *Aphyllae*. However, they also mentioned that the anthers of *S. chloroclada* resemble those of *S. mucronifera*, assigned to sect. *Chamaefistula*. Our results suggest that the morphological similarities between *S. chloroclada* and section *Peiranisia*, ser. *Aphyllae* have independent evolutionary origins. The observation that *S. chloroclada* is associated with a rather long branch (29 nucleotide substitutions in one of the MP trees; see Fig. 3), the unusual combination of traits similar to those found in members of disparate clades (clades III, IV, and VI), and its distribution restricted to the Gran Chaco (including Argentina, Bolivia and Paraguay) justify its isolated taxonomic position.

Senna skinneri, ascribed to the monotypic series *Skinnerae* (sect. *Chamaefistula*), is sister to the remainder of clade IV. This species shares some morphological traits with species of both subclades IVa and IVb. Its flowers resemble those of many species of subclade IVb, but the leaves are more similar to those of subclade IVa in having more than three pairs of leaflets. Irwin and Barneby (1982) suggested that *S. skinneri* had an unspecified "close resemblance" and "genetic affinity" to series *Coriaceae* and *Laxiflorae* or was related to ser. *Spinescentes* based on similarities in pod morphology (the three series belonging to section *Chamaefistula*). Series *Coriaceae* and *Spinescentes* are not represented in our molecular phylogenetic study (see Appendix 1), and series *Laxiflorae* is included in clade V (Fig. 2). Therefore, the inclusion of representatives from series *Coriaceae* and *Spinescentes* will be necessary to clarify the phylogenetic position of *S. skinneri* in the genus.

Clade V—The strongly supported clade V (100% BS) contains the monotypic series *Confertae* (*S. uniflora*) and members of series *Laxiflorae*, both of section *Chamaefistula* (Fig. 2). The two series are strikingly different in habit and floral structure. *Senna uniflora* is a widespread, weedy, self-pollinated herb with small enantiostylous flowers and a monosymmetric corolla. Conversely, *Laxiflorae* comprise shrubs or treelets restricted to Brazil (except for one species extending into Bolivia and Paraguay), with rather showy enantiostylous flowers and a slightly asymmetric corolla characterized by one lower petal slightly modified in form and more concave than the other lower petal (Irwin and Barneby, 1982). The unusual angular shape and marginal teeth of the leaflets typical of *S. phlebadenia* (series *Laxiflorae*) form a combination of traits unique in *Senna* and indeed in Cassiinae (Irwin and Barneby, 1985). A synapomorphic feature for clade V is the presence of EFNs at the base of the pedicels (Fig. 3), in addition to the occurrence of similar nectaries on the leaves. Because pedicellar nectaries are restricted to *S. uniflora* and series *Laxiflorae*, Irwin and Barneby (1982) suggested that *S. uniflora* was a "specialized offshoot" derived from series *Laxiflorae* or from the common ancestor of *S. uniflora* and series *Laxiflorae*. The molecular tree supports this latter conclusion.

Series *Laxiflorae* seems to be still poorly known, and its species number has increased in the past decades: *S. phlebadenia* (Irwin and Barneby, 1985) was added to the four traditionally recognized species (*S. australis*, *S. cana*, *S. lechriosperma*, and *S. velutina*; Irwin and Barneby, 1982), and recently, a new species was discovered (A. Araujo and V. C. Souza, University of São Paulo, Brazil, unpublished data). In addition, species delimitation appears to be problematic in this series because flowers of *Laxiflorae* are morphologically similar, and fruit characteristics are needed for species identification (Irwin and Barneby, 1982). The inclusion of more taxa ascribed to *Laxiflorae* would allow us to further test the sister relationship between *S. uniflora* and *Laxiflorae* and to further explore species boundaries in *Laxiflorae*.

Clade VI—Clade VI comprises series *Aphyllae* and *Interglandulosae* of section *Peiranisia*, with *Aphyllae* forming a strongly supported (100% BS) monophyletic group nested within *Interglandulosae*. This exclusively American clade comprises species of shrubs and treelets bearing EFNs on the leaves and strongly asymmetric flowers (Figs. 2 and 3). Floral asymmetry affects the corolla and the androecium, in addition to the gynoecium (see Fig. 3C). Both lower petals are strongly modified in shape and size, and in most species one or both are incurved in front of the reproductive structures, hiding all or part of them. These petals were suggested to function as an

"androecial shield" (Irwin and Barneby, 1982), but this proposed function has not yet been convincingly demonstrated. The upper petals may be more or less reduced. The abaxial stamens are often all deflected to the opposite side of the gynoecium.

The shrub, treelet, or tree species of section *Peiranisia* series *Interglandulosae* occur mainly in more or less wet habitats (usually with a pronounced dry season), ranging from northeastern Brazil and northern South America to Central America and Mexico (Irwin and Barneby, 1982). In many species, the leaves have up to more than 14 leaflet pairs and, in others, the upper petals are more or less reduced, while the anthers of the abaxial stamens have peculiar long beaks. In contrast to the distribution of *Interglandulosae*, members of *Aphyllae* occur in dry areas of northern and northwestern Argentina, southeastern Bolivia, and adjacent northwestern Paraguay (Irwin and Barneby, 1982). These species are highly xerophytic shrubs with usually aphyllous and photosynthetic stems, a combination of features unique among *Senna* species. Leaves are only present in young shoots (Bravo, 1978; Irwin and Barneby, 1982; Marazzi, personal observation). The scales present on the stems have been interpreted as reduced leaves (Bentham, 1871; Burkart, 1943; Bravo, 1978) and stipules (Irwin and Barneby, 1982). The upper petals are not reduced, and the anthers of the abaxial stamens appear beakless.

The samples of *S. acuruensis* (restricted to northeastern Brazil) and *S. multijuga* (ranging from northeastern Brazil to northern South America and Mexico) form a strongly supported clade (97% BS; Fig. 2). *Senna acuruensis* is paraphyletic in the cpDNA phylogeny. The broad range of morphological variation characterizing *S. acuruensis* and *S. multijuga* prompted the recognition of several infraspecific varieties (Irwin and Barneby, 1982). These two species are distinguished mainly by the habit (large trees in *S. multijuga* vs. spindly shrubs in *S. acuruensis*) and by the presence of viscid hairs on the axis of the inflorescence in *S. acuruensis*. Variation in the number of leaflet pairs has been used to recognize three varieties in *S. acuruensis*: plants with more than 14 pairs of leaflets (multifoliolate) have been assigned to var. *acuruensis*, while plants with less than 14 pairs of leaflets (few-foliolate) have been assigned to var. *catingae* and var. *interjecta*. In *S. multijuga*, all varieties have more than 14 pairs of leaflets. Irwin and Barneby (1982) proposed that *S. acuruensis* is the closest relative of *S. multijuga* and that this species pair is closely related to the other species of the same series (*S. aristeguietae* and *S. trachypus*, both few-foliolate) and to *S. mutisiana*, *S. williamsii*, and one form of *S. pallida* (all three taxa are multifoliolate). Our phylogenetic analyses support the monophyly of a multifoliolate group and a few-foliolate group, suggesting that the two sampled varieties of *S. acuruensis* may in fact represent different species (Fig. 2). However, because our sampling included only two out of three and two out of five described varieties in *S. acuruensis* and *S. multijuga*, respectively, it is premature to draw any final conclusions on species circumscriptions in this complex.

Clade VII—Clade VII comprises the monotypic section *Astroites* (*S. villosa*) and species of section *Chamaefistula*. Most species are American and one, *S. barclayana*, is Australian (Randell, 1988; Fig. 2). Members of clade VII are mainly herbs and shrubs, sometimes weeds, with monosymmetric flowers and bearing one or more EFNs on the leaves (Fig. 3). The clade is divided into two strongly supported subclades (VIIa, 94% BS, and VIIb, 100% BS; Fig. 2).

Subclade VIIa includes *S. purpusii* (ser. *Pachycarpae*), a group of species mainly of ser. *Coluteoideae* (with *S. birostris* of ser. *Stipulaceae* nested within) and the monophyletic ser. *Basiglandulosae* (all of section *Chamaefistula*). Their flowers have sometimes six, instead of seven, fertile stamens, because the median abaxial stamen is highly reduced and sterile or absent (Irwin and Barneby, 1982). In some species, the gynoecium may be slightly deflected to the right or to the left, but such flowers are not considered truly enantiostylous because they occur in inflorescences formed also by exactly monosymmetric flowers (see discussion of clade I). *Senna hirsuta* is paraphyletic in our cpDNA phylogeny (Fig. 2). This species comprises many varieties (Irwin and Barneby, 1982), which previous taxonomic studies considered to represent different species (as belonging to *Cassia*; e.g., Bentham, 1871). In our phylogenetic tree, series *Stipulaceae*, represented only by *S. birostris*, is embedded into series *Coluteoideae* (Fig. 2). Irwin and Barneby (1982) created *Stipulaceae* to accommodate those species of section *Chamaefistula* displaying a mixture of seed and fruit characteristics typical of series *Coluteoideae* and *Pachycarpae* (Irwin and Barneby, 1982). These species were previously included in *Cassia* ser. *Pachycarpae*, which was therefore "diagnostically inseparable" from *Coluteoideae* (Bentham, 1871). Expanded taxon sampling in series *Pachycarpae* and *Stipulaceae* is necessary to clarify the relationships among series *Coluteoideae, Pachycarpae*, and *Stipulaceae*.

Subclade VIIb comprises the monotypic section *Astroites* (*S. villosa*), the monotypic series *Armatae* (*S. armata*) of section *Chamaefistula*, and species of sect. *Chamaefistula* ser. *Brachycarpae*. Irwin and Barneby (1982) noticed similarities between the flowers of *S. armata* and *S. villosa* and those of ser. *Brachycarpae*, but placed *S. armata* in its own series, *Armatae*, because of its xeromorphic habit, and *S. villosa* in its own section, *Astroites,* because of its lomentaceous pod and uncommon stellate hairs. In contrast, Bentham (1871) grouped *S. villosa* (as *Cassia villosa*) together with *S. uniflora* (as *C. sericea*) of clade V.

The most evident morphological difference between subclades VIIa and VIIb concerns the androecium. In the first subclade, the six to seven fertile stamens are differentiated into a middle and an abaxial set, with the four middle stamens notably shorter than the abaxial ones. In contrast, in the second subclade, all fertile stamens have more or less the same length and shape.

Evolutionary aspects of floral morphology—The current classification of *Senna* reflects Irwin and Barneby's (1982) interpretation of evolutionary trends in the floral morphology of the genus, focusing in particular on androecium, corolla, and floral architecture. In their view, fertility of all 10 stamens represented the ancestral condition of the androecium in *Senna*, and they assigned all species with this trait to sect. *Psilorhegma*. All remaining sections were characterized by the supposedly derived state with seven or fewer fertile stamens. Within these sections, the highly asymmetric flowers of *Peiranisia* were considered the most derived state of floral architecture, in contrast with the ancestral, monosymmetric flowers of *Chamaefistula* (Irwin and Barneby, 1982; see also Randell, 1989, for a detailed list of "primitive" vs. "advanced" character states). However, Irwin and Barneby (1982) did

acknowledge the difficulty of identifying a clear evolutionary progression in the floral morphology of extant species of *Senna* because the presence of strongly specialized features, either in the corolla or in the androecium, produced a mosaic of ancestral and derived states in any given species that hampered the recognition of unequivocal synapomorphies. The cpDNA phylogeny presented here suggests that the morphological features used by Irwin and Barneby (1982) for their classification evolved independently multiple times, thus limiting their taxonomic utility.

For example, the position of section *Psilorhegma*, embedded in clade IV, does not support Irwin and Barneby's (1982) conclusion that the fertility of all ten stamens represents the ancestral condition of the androecium in *Senna*, but rather a synapomorphy of a well supported subgroup of *Senna* (see discussion of clade IV; Fig. 2). The observation that the androecium consists of seven or fewer fertile stamens and three staminodes in all sections of *Senna*, except for *Psilorhegma*, suggests that this androecial organization probably characterized the ancestral *Senna* flower.

Different patterns of floral symmetry characterize different clades (or subclades) of the molecular phylogeny presented in this study. The species with monosymmetric flowers group into two clades (I and VII), whereas the species with asymmetric flowers form the remaining clades II to VI (Fig. 3). Because flowers in *Cassia* s.str., the putative sister of *Senna* (this study and Bruneau et al., 2001), are monosymmetric (Irwin and Barneby, 1982), the flowers of *Senna* were probably ancestrally monosymmetric, with a later switch to asymmetry. Therefore, the monosymmetry of flowers in clade VII probably represents a reversal to the ancestral condition. The polyphyly of section *Peiranisia,* split over three different clades, suggests that strong floral asymmetry, affecting corolla, androecium, and gynoecium, evolved independently more than once (see Fig. 2).

Floral asymmetry in *Senna* affects gynoecium, androecium, and corolla, but the three whorls do not always simultaneously contribute to the asymmetry. The gynoecium alone, both gynoecium and corolla, or gynoecium, corolla and also androecium can be involved, as in clades II, III, and III–VI, respectively. Clade III is characterized by several different variants of floral asymmetry. Because all asymmetric flowers observed in this study are enantiostylous, we doubt the existence in ser. *Bacillares* of non-enantiostylous flowers with the floral asymmetry affecting only the corolla, as reported by Irwin and Barneby (1982). The existence of different variants of asymmetric flowers and the independent switches to these variants inferred from the molecular phylogeny suggest that our traditional interpretation of floral asymmetry in *Senna* may be inadequate, for it subsumes different morphological, evolutionary, and, probably, developmental pathways. In upcoming structural and developmental studies, we will investigate in depth the diversity of asymmetric patterns in *Senna* flowers and with future optimization analyses will evaluate possible scenarios for the evolution of diverse types of floral symmetry.

Evolutionary role of extrafloral nectaries—Many species of *Senna* bear EFNs on the leaves, and a few species also bear them on the pedicels. The great variation in the shape, number, and location of the EFNs has been used for series circumscription in several *Senna* classifications (e.g., as *Cassia* in Bentham, 1871; Irwin and Barneby, 1982). Recent monographic treatments viewed the presence of EFNs as an "archaic feature," for EFNs are present in the supposedly "basal" section *Psilorhegma,* but absent in the more "advanced" sections *Senna*, *Paradictyon*, and part of *Peiranisia* (Irwin and Barneby, 1982; Randell, 1989, 1990). However, our phylogenetic results reject this interpretation as all sampled species with EFNs form a strongly supported clade, the "EFN clade" (93% BS; Figs. 2 and 3), embedded within *Senna*. Therefore, the presence of EFNs constitutes a clear synapomorphy for the four clades IV–VII (Fig. 3). EFNs appear to have evolved once in *Senna*, being later lost or suppressed in the highly xerophytic series *Aphyllae*, where they have never been observed. The presumed absence of EFNs in *Aphyllae* (Irwin and Barneby, 1981) may be related to the absence of normally developed leaves in the adult plants (see also the discussion of clade VI). However, EFNs may be present in young shoots of *Aphyllae* where leaves are produced, but the developmental morphology of young shoots in this series has not yet been investigated.

The distribution of EFNs is associated with strong clade-size disparity (Fig. 3). The difference in clade size is unlikely to be an artifact of taxon sampling because we sampled all sections and series according to species number (see Appendix 1, Appendix 2). The EFN clade, including 61 species, is sister to the EFN-less clade III, which includes only six species (Fig. 3). This disparity corresponds to a 10-fold difference in clade size. Because sister clades are the products of the same cladogenetic event and, by definition, of equal age (Hennig, 1966), the observed disparity in species numbers could reflect different rates of speciation and/or extinction within each clade. Molecular dating analyses to evaluate the age of the respective crown groups will be necessary to clarify the link between diversification rates and clade size disparity. Interestingly, species with EFNs and species without EFNs occur together in some continents, i.e., America (most species) and Australia (especially subclade IVa; Fig. 2). However, species included in the EFN clade have colonized a wider range of habitats and climates, including rain forests, savannas, cerrados, and deserts, and display a higher diversity of habits, including trees, treelets, shrubs, lianas, and herbs (often ruderals or weeds), than species lacking EFNs. The ant–plant protective mutualism and its likely positive effect on plant fitness might explain, at least in part, the higher species richness of the EFN clade in *Senna*, and the greater diversity of habitat and habits observed for the clade.

Plants with EFNs offer nectar to ants, which, in return, protect the plant from herbivores and seed predators (Tilman, 1978; Smiley, 1986). The documented effects of such protection on plant fitness include decreased damage due to herbivory (Stephenson, 1982; Koptur et al., 1998, Rutter and Rausher, 2004), increased plant growth and survival (Buckley, 1983; Barton, 1986; Kelly, 1986), and higher seed set (Koptur, 1979; Barton, 1986; Oliveira, 1997). Ants were observed to feed on the EFNs of *Senna* species (e.g., Schupp and Feener, 1991; Marazzi, personal observation). Exclosure experiments are in progress to test whether ants visiting EFNs contribute to decreased herbivory and higher seed production in *S. mexicana* (Koptur, 2004). Many genera in Leguminosae have EFNs, but few researchers have investigated the interaction with ants in any detail (see McKey, 1989, for a review). For example, studies on *Acacia* provided the first convincing evidence for the protective function of the mutualism (Janzen, 1967). Other studies have shown the mutualistic relationship with ants in *Inga* (Koptur, 1984, 1994; Wickers, 1993) and in *Chamae-*

crista (Barton, 1986; Kelly, 1986; Rutter and Rausher, 2004), confirming the positive effects of the mutualism on plant fitness.

The mutualism in *Senna* has not yet been experimentally investigated. However, because the morphology of the EFNs in *Senna* and *Chamaecrista* is similar (Pascal et al., 2000), we expect the EFNs in *Senna* to play a protective role similar to that documented in *Chamaecrista* and other legume taxa. Therefore, it is probable that the evolution of EFNs conferred higher fitness to the EFN clade, increasing survival, reproduction, dispersal, and potential for adaptation. Consequently, the evolution of EFNs might have played a key role in producing a higher diversification rate, thus explaining, at least in part, the observed size difference between the EFN clade and the EFN-less clades, in particular clade III (Figs. 2 and 3). To summarize, the available phylogenetic and ecological evidence suggests that EFNs in *Senna* may represent a key innovation in plant defense strategies that promoted large-scale diversification and colonization of a wide range of habitats and climates in different continents, especially in America and Australia.

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APPENDIX 1. Classification of *Senna* used in the present study based on Irwin and Barneby (1982), Randell (1988, 1989, 1990), and Singh (2001). The order of the sections is after Irwin and Barneby (1982), while series are listed alphabetically. Asterisks indicate monotypic sections or series. Rectangular brackets include abbreviations of sections and series listed in Fig. 2 and in Appendix 2; series not represented in this study are labeled with [n.r.].

Section *Psilorhegma* (Vogel) H.S. Irwin & Barneby [PS]
- Series *Davidsonae** V. Singh [n.r.]
- Series *Interglandulosae* (Benth.) Randell [Int]
- Series *Oligocladae* Randell [Oli]
- Series *Subverrucosae* (Benth.) Randell [Sub]
- Series *Sulfureae** V. Singh [n.r.]

Section *Chamaefistula* (Collad.) H.S. Irwin & Barneby [CH]
- Series *Armatae** H.S. Irwin & Barneby [Arm]
- Series *Bacillares* (Benth.) H.S. Irwin & Barneby [Bac]
- Series *Basiglandulosae* (Collad.) H.S. Irwin & Barneby [Bas]
- Series *Brachycarpae* (Benth.) H.S. Irwin & Barneby [Bra]
- Series *Coluteoideae* (Collad.) H.S. Irwin & Barneby [Col]
- Series *Confertae** (Benth.) H.S. Irwin & Barneby [Con]
- Series *Coriaceae* (Benth.) H.S. Irwin & Barneby [n.r.]
- Series *Floridae* (Benth.) H.S. Irwin & Barneby [Flo]
- Series *Galeottianae* H.S. Irwin & Barneby [Gal]
- Series *Harleyanae** H.S. Irwin & Barneby [n.r.]
- Series *Laxiflorae* (Benth.) H.S. Irwin & Barneby [Lax]
- Series *Nanae** H.S. Irwin & Barneby [n.r.]
- Series *Pachycarpae* (Benth.) H.S. Irwin & Barneby [Pac]
- Series *Sapidifoliae* H.S. Irwin & Barneby [Sap]
- Series *Skinneranae** H.S. Irwin & Barneby [Ski]

Series *Spinescentes* H.S. Irwin & Barneby [n.r.]
Series *Stipulaceae* H.S. Irwin & Barneby [Sti]
Series *Temperatae* H.S. Irwin & Barneby [n.r.]
Series *Tharpia** (Britton & Rose) H.S. Irwin & Barneby [n.r.]
Series *Trigonelloideae* (Collad.) H.S. Irwin & Barneby [Tri]
Series *Trolliflorae** H.S. Irwin & Barneby [n.r.]
Section *Astroites H.S. Irwin & Barneby [AS]**
Section *Senna* Mill. [SE]
Series *Aculeatae** H.S. Irwin & Barneby [n.r.]
Series *Pictae* (Benth.) H.S. Irwin & Barneby [Pic]
Series *Senna* Mill. [Sen]
Series *Spinigerae** H.S. Irwin & Barneby [n.r.]
Section *Paradictyon H.S. Irwin & Barneby [PA]**
Section *Peiranisia* (Raf.) H.S. Irwin & Barneby [PE]
Series *Aphyllae* (Benth.) H.S. Irwin & Barneby [Aph]
Series *Auriculatae** (Benth.) V. Singh [n.r.]
Series *Chlorocladae** H.S. Irwin & Barneby [Chl]
Series *Deserticolae* H.S. Irwin & Barneby [Des]
Series *Egregiae** H.S. Irwin & Barneby [n.r.]
Series *Excelsae** (Benth.) H.S. Irwin & Barneby [Exc]
Series *Interglandulosae* (Benth.) H.S. Irwin & Barneby [Itg]
Series *Isandrae* H.S. Irwin & Barneby [Isa]

APPENDIX 2. Taxa used in this study, GenBank accession numbers for the three chloroplast regions studied, source, and voucher information. A dash indicates the region was not sampled. See Appendix 1 for full section and series names of *Senna* species. Voucher specimens are deposited in the following herbaria (in alphabetic order): ANBG (= CBG) = Australian National Botanic Gardens, BGB = Botanical Garden of the University of Basel, BGM = Botanischer Garten der Universität München, CTES = Instituto de Botánica del Nordeste, Corrientes, HUEFS = Universidad Estadual de Feira de Santana, KPBG = Kings Park and Botanic Garden, Perth, LPB = Herbario Nacional de Bolivia, La Paz, MEXU = Universidad Nacional Autónoma de México, MT = Université de Montréal, NMC = New Mexico State University, Las Cruces, PBIB = Parco Botanico Isole di Brissago, PERTH = Department of Conservation and Land Management, Perth, PMA = Universidad de Panamá, PY = Museo Nacional de Historia Natural de Paraguay, RBGA = Royal Botanic Garden Mount Annan, SI = Instituto de Botánica Darwinion, San Isidro, STRI = Smithsonian Tropical Research Institute, Balboa, SYD = University of Sydney, Z = University of Zürich and Botanical Garden.

Taxon; Section, Series [given only for *Senna* species]; *rpL16*, *rpS16*, *matK*; Source; Voucher specimen.

Caesalpinia decapetala (Roth) Alston; AM086721; AM086910; AM086828; Cult. PBIB 2003/77; *Marazzi BM137*, garden, Z. ***C. gilliesii*** (Hook.) Benth.; —, AM086914, AM086829, Wild; *Marazzi et al. BM131*, Argentina, Tucuman, CTES, Z.

Cassia fistula L.; AM086721, AM086915, AM086830; Cult. at the roadside; *Marazzi & Flores BM177*, Mexico, Oaxaca, MEXU, Z.

Chamaecrista acosmifolia (Benth.) H.S. Irwin & Barneby; AM086567, AM086584, AM086602; Wild; *Conceiçao & Marazzi AC1129*, Brazil, Bahia, HUEFS, Z. ***C. desvauxii*** (Collad.) Killip; AM086715, AM086911, AM086831; Wild; *Marazzi et al. BM013*, Paraguay, San Pedro, PY, CTES, Z. ***C. eitenorum* var. *regana*** (H.S. Irwin & Barneby) H.S. Irwin & Barneby; AM086566, AM086585, AM086603; Wild; *Conceiçao & Marazzi AC1133*, Brazil, Bahia, HUEFS, Z. ***C. nictitans*** Moench; AM086721, AM086912, AM086832; Wild; *Marazzi et al. BM034*, Paraguay, Alto Paraná, PY, CTES, Z. ***C. serpens*** Greene; AM086717, AM086913, AM086833; Wild; *Marazzi & Flores BM179*, Mexico, Oaxaca, MEXU, Z.

Delonix regia (Bojer) Raf.; AM086721, AM086916, AM086834; Cult. at the roadside; *Marazzi & Flores BM183*, Mexico, Oaxaca, MEXU, Z.

Gleditsia sinensis Lam.; AM086719, AM086917, AM086835; Cult. in Alte Botanische Garten Z s.n.; *Marazzi BM188*, garden, Z. ***G. triacanthos*** L.; AM086720, AM086918, AM086836; Cult. in Alte Botanische Garten Z s.n.; *Marazzi BM189*, garden, Z.

Senna acclinis (F. Muell.) Randell; PS, Int; AM086721, AM086922, AM086837; Seed Bank RBGA 20020785; *Johnstone 1137*, Australia, New South Wales, SYD. ***S. aciphylla*** (Benth.) Randell; PS, Int; AM086722, AM086923, AM086838; Seed Bank RBGA 884115; *D'Aubert 432*, Australia, New South Wales, SYD. ***S. acuruensis* var. *acuruensis*** (Benth.) H.S. Irwin & Barneby; PE, Itg; AM086568, AM086586, AM086604; Wild; *Queiroz & Marazzi LQ 9198*, Brazil, Bahia, HUEFS, Z. ***S. acuruensis* var. *acuruensis*** (Benth.) H.S. Irwin & Barneby; PE, Itg; AM086569, AM086587, —; Wild; *Queiroz & Marazzi LQ 9201*, Brazil, Bahia, HUEFS, Z. ***S. acuruensis* var. *catingae*** (Harms) H.S. Irwin & Barneby; PE, Itg; AM086571, AM086589, AM086606; Wild; *Queiroz & Marazzi LQ 9173*, Brazil, Bahia, HUEFS, Z. ***S. acuruensis* var. *catingae*** (Harms) H.S. Irwin & Barneby; PE, Itg; AM086570, AM086588, AM086605; Wild; *Queiroz & Marazzi LQ 9177*, Brazil, Bahia, HUEFS, Z. ***S. acuruensis* var. *catingae*** (Harms) H.S. Irwin & Barneby; PE, Itg; AM086572, AM086590, AM086607; Wild; *Queiroz & Marazzi LQ 9205*, Brazil, Bahia, HUEFS, Z. ***S. alata*** (L.) Roxb.; SE, Pic; —, AM086924, AM086839; Wild; *Marazzi & al. BM026*, Paraguay, Caaguazú, PY, CTES, Z. ***S. andrieuxii*** (Benth.) H.S. Irwin & Barneby; PE, Des; AM086723, AM086925, AM086840; Wild; *Marazzi & Flores BM162*, Mexico, Puebla, MEXU, Z. ***S. aphylla*** (Cav.) H.S. Irwin & Barneby; PE, Aph; AM086724, AM086926, AM086841; Wild; *Marazzi et al. BM084*, Argentina, Santiago del Estero, CTES, Z. ***S. apiculata*** (M. Martens & Galeotti) H.S. Irwin & Barneby; CH, Bra; AM086725, AM086927, AM086842; Wild; Marazzi & Flores BM170, Mexico, Puebla, MEXU, Z. ***S. argentea*** (Kunth) H.S. Irwin & Barneby; CH, Bra; AM086726, AM086928, AM086843; Wild; *Marazzi & Flores BM175,* Mexico, Oaxaca, MEXU, Z. ***S. armata*** (S. Watson) H.S. Irwin & Barneby; CH, Arm; AM086727, AM086929, AM086844; Wild; *Schönenberger JS751*, USA, California, Z[a]. ***S. artemisioides*** (DC.) Randell; PS, Sub; AM086728, AM086919, AM086845; Cult. s.n. Z; *Marazzi BM002*, garden, Z. ***S. atomaria*** (L.) H.S. Irwin & Barneby; PE, Des; AM086729, AM086930, AM086846; Wild; *Marazzi & Flores BM173*, Mexico, Oaxaca, MEXU, Z. ***S. aversiflora*** (Herbert) H.S. Irwin & Barneby; PE, Itg; AM086573, AM086591, —; Wild; *Queiroz & Marazzi LQ 9204*, Brazil, Bahia, HUEFS, Z. ***S. barclayana*** (Sweet) Randell; CH, Bas; AM086730, AM086931, AM086847; Cult. PBIB 2003/76; *Marazzi BM136*, garden, Z. ***S. bauhinioides*** (A. Gray) H.S. Irwin & Barneby; CH, Bra; AM086731, AM086932, AM086848; Wild; *Spellenberg & Brouillet 12700*, USA, New Mexico, MT, NMC. ***S. bicapsularis*** (L.) Roxb.; CH, Col; AM086732, AM086933, AM086849; Wild; *Marazzi & Alvárez BM159*, Republic of Panama, Coclé, PMA, STRI, Z. ***S. birostris* var. *hookeriana*** (Hook.) H.S. Irwin & Barneby; CH, Sti; AM086733, AM086934, AM086850; Wild; *Marazzi et al. BM090*, Argentina, Tucumán, CTES, Z. ***S. cana* var. *calva*** H.S. Irwin & Barneby; CH, Lax; AM086574, AM086592, AM086608; Wild; *Conceiçao & Marazzi 1132*, Brazil, Bahia, HUEFS, Z. ***S. cardiosperma*** (F. Muell.) Randell; PS, Sub; AM086734, AM086935, AM086851; Seed Bank KPBG 952264; *Sweedman S2938*, Australia, Western Australia, KPBG, PERTH. ***S. cernua*** (Balb.) H.S. Irwin & Barneby; CH, Bas; AM086735, AM086936, AM086852; Wild; *Marazzi et al. BM007*, Paraguay, Caaguazú, PY, CTES, Z. ***S. chacoënsis*** (L. Bravo) H.S. Irwin & Barneby; PE, Aph; AM086736, AM086937, AM086853; Wild; *Marazzi et al. BM083*, Argentina, Santiago del Estero, CTES, Z. ***S. chloroclada*** (Harms) H.S. Irwin & Barneby; PE, Chl; AM086737, AM086938, AM086854; Wild; *Marazzi et al. BM128*, Argentina,

Salta, CTES, Z. ***S. coronilloides*** (Benth.) Randell; PS, Int; AM086738, AM086939, AM086855; Seed Bank RBGA 842721; *Rodd 4219*, Australia, Queensland, SYD. ***S. corymbosa*** (Lam.) H.S. Irwin & Barneby; CH, Col; AM086739, AM086940, AM086856; Cult. in private garden; *Marazzi et al. BM103*, Argentina, Tucumán, CTES, Z. ***S. crassiramea*** (Benth.) H.S. Irwin & Barneby; PE, Aph; AM086740, AM086941, AM086857; Wild; *Marazzi et al. BM120*, Argentina, Jujuy, CTES, Z. ***S. crotalarioides*** (Kunth) H.S. Irwin & Barneby; CH, Bra; AM086741, AM086942, AM086858; Wild; *Marazzi & Flores BM163*, Mexico, Puebla, MEXU, Z. ***S. dariensis* var. *hypoglauca*** H.S. Irwin & Barneby; CH, Bac; AM086742, AM086943, AM086859; Wild; *Marazzi & Alvárez BM153*, Republic of Panama, Coclé, PMA, STRI, Z. ***S. didymobotrya*** (Fresen.) H.S. Irwin & Barneby; SE, Pic; AM086743, AM086920, AM086860; Cult. Z 19700009; *Marazzi BM002*, garden, Z. ***S. galeottiana*** (M. Martens) H.S. Irwin & Barneby; CH, Gal; AM086744, AM086944, AM086861; Wild; *Marazzi & Flores BM165*, Mexico, Puebla, MEXU, Z. ***S. gardneri*** (Benth.) H.S. Irwin & Barneby; CH, Bac; AM086575, AM086593, AM086609; Wild; *Queiroz LQ 7866*; Brazil, Bahia, HUEFS. ***S. glutinosa***[b] (DC.) Randell; PS, Sub; AM086745, AM086945, AM086862; Seed Bank KPBG 952287; *Demarz 4803*, Australia, Western Australia, unk. ***S. hayesiana*** (Britton & Rose) H.S. Irwin & Barneby; CH, Bac; AM086746, AM086946, AM086863; Wild; *Marazzi & Alvárez BM150*, Republic of Panama, Panamá, PMA, STRI, Z. ***S. herzogii*** (Harms) H.S. Irwin & Barneby; CH, Bac; AM086747, AM086947, AM086864; Wild; *Morrone & Belgrano 5084*, Bolivia, Santa Cruz, LPB, SI, CTES. ***S. hilariana*** (Benth.) H.S. Irwin & Barneby; CH, Col; AM086748, AM086948, AM086865; Wild; *Marazzi et al. BM027*, Paraguay, Alto Paraná, PY, CTES, Z. ***S. hirsuta* var. *hirta*** (Benth.) H.S. Irwin & Barneby; CH, Bas; AM086749, AM086949, AM086866; Wild; *Marazzi & Flores BM168*, Mexico, Puebla, MEXU, Z. ***S. hirsuta* var. *hirta*** (Benth.) H.S. Irwin & Barneby; CH, Bas; AM086750, AM086950, AM086867; Wild; *Marazzi et al. BM115*, Argentina, Salta, CTES, Z. ***S. hirsuta* var. *leptocarpa*** (Benth.) H.S. Irwin & Barneby; CH, Bas; AM086751, AM086951, AM086868; Wild; *Marazzi et al. BM065*, Paraguay, San Pedro, PY, CTES, Z. ***S. holwayana* var. *holwayana*** (Rose) H.S. Irwin & Barneby; PE, Itg; AM086752, AM086952, AM086869; Wild; *Marazzi & Flores BM161*, Mexico, Puebla, MEXU, Z. ***S.* indet. ser. *Bacillares***; CH, Bac; AM086753, AM086953, AM086870; Wild; *Marazzi & Alvárez BM160*, Republic of Panama, Panamá, PMA, STRI, Z. ***S.* indet. ser. *Subverrucosae***[c]; PS, Sub; AM086754, AM086954, AM086871; Seed Bank KPBG 952265; *Demarz 5523*, Australia, Western Australia, unk. ***S. italica*** Mill.; SE, Sen; AM086755, AM086955, AM086872; Wild; *Zietsmann 4345*, Republic of South Africa, Free State, NMB, Z. ***S. macranthera* var. *nervosa*** (Vogel) H.S. Irwin & Barneby; CH, Bac; AM086756, AM086956, AM086873; Cult. at the roadside; *Marazzi et al. BM082*, Paraguay, Caaguazú, PY, CTES, Z. ***S. magnifolia*** (F. Muell.) Randell; SE, Pic; AM086757, AM086957, AM086874; Seed Bank RBGA 861394; *Rodd 4526*, Australia, Western Australia, unk. ***S. martiana*** (Benth.) H.S. Irwin & Barneby; SE, Pic; AM086576, AM086594, AM086610; Wild; *Queiroz LQ 7916*, Brazil, Bahia HUEFS. ***S. mexicana*** (Jacq.) H.S. Irwin & Barneby; CH, Bas; AM086758, AM086958, AM086875; Cult. BGM 96/3360; *Marazzi BM006*, garden, Z. ***S. mollissima*** (Willd.) H.S. Irwin & Barneby; PE, Des; AM086759, AM086959, AM086876; Wild; *Marazzi & Flores BM181*, Mexico, Oaxaca, MEXU, Z. ***S. morongii*** (Britton) H.S. Irwin & Barneby; CH, Col; AM086760, AM086960, AM086877; Wild; *Marazzi et al. BM130*, Argentina, Salta, CTES, Z. ***S. mucronifera*** (Benth.) H.S. Irwin & Barneby; CH, Tri; AM086761, AM086961, AM086878; Wild; *Marazzi et al. BM019*, Paraguay, Caaguazú, PY, CTES, Z. ***S. multijuga* var. *lindleyana*** (Gardner) H. S. Irwin & Barneby; PE, Itg; AM086577, AM086595, AM086611; Wild; *Queiroz & Marazzi LP 9226*, Brazil, Bahia, HUEFS, Z. ***S. multijuga* var. *multijuga*** (Rich.) H.S. Irwin & Barneby; PE, Itg; AM086762, AM086962, AM086879; Cult. in private garden; *Marazzi & Alvárez BM151*, Republic of Panama, Panamá, PMA, STRI, Z. ***S. nicaraguensis*** (Benth.) H.S. Irwin & Barneby; SE, Pic; AM086763, AM086963, AM086880; Wild; *Marazzi & Flores BM185*, Mexico, Chiapas, MEXU, Z. ***S. notabilis*** (F. Muell.) Randell; SE, Pic; AM086764, AM086964, AM086881; Seed Bank RBGA 872886; *Johnstone 37*, Australia, Northern Territory, SYD. ***S. obtusifolia*** (L.) H.S. Irwin & Barneby; CH, Tri; AM086765, AM086965, AM086882; Wild; *Marazzi et al. BM024*, Paraguay, Caaguazú, PY, CTES, Z. ***S. occidentalis*** (L.) Link; CH, Bas; AM086766, AM086966, AM086883; Wild; *Marazzi et al. BM060*, Paraguay, Caaguazú, PY, CTES, Z. ***S. odorata*** (Morris) Randell; PS, Int; AM086767, AM086967, —; Cult. ANBG 68349; s.n., garden, CBG. ***S. oligoclada*** (F. Muell.) Randell; PS, Oli; AM086768, AM086968, AM086884; Seed Bank RBGA 880070; collector unk., Australia, unk. ***S. pallida*** (Vahl) H.S. Irwin & Barneby; PE, Itg; AM086769, AM086969, AM086885; Wild; *Marazzi & Flores BM178*, Mexico, Oaxaca, MEXU, Z. ***S. paradictyon*** (Vogel) H.S. Irwin & Barneby; PA; AM086770, AM086970, AM086886; Wild; *Marazzi et al. BM028*, Paraguay, Alto Paraná, PY, CTES, Z. ***S. pendula*** (Willd.) H.S. Irwin & Barneby; CH, Col; AM086771, AM086971, AM086887; Wild; *Marazzi et al. BM117*, Argentina, Salta, CTES, Z. ***S. phlebadenia*** H.S. Irwin & Barneby; CH, Lax; AM086578, AM086596, AM086612; Wild; *Stapf 209*, Brazil, Bahia, HUEFS. ***S. pilifera*** (Vogel) H.S. Irwin & Barneby; CH, Tri; AM086772, AM086972, AM086888; Wild; *Marazzi et al. BM011*, Paraguay, Caaguazú, PY, CTES, Z. ***S. pinheiroi*** H.S. Irwin & Barneby; CH, Bac; AM086579, AM086597, AM086613; Wild; *Queiroz 9210*, Brazil, Bahia, HUEFS. ***S. pleurocarpa*** (F. Muell.) Randell; SE, Pic; AM086773, AM086973, AM086889; Seed Bank KPBG 930575; *Demarz 12081*, Australia, Western Australia, unk. ***S. polyantha*** (Collad.) H.S. Irwin & Barneby; CH, Gal; AM086774, AM086974, AM086890; Wild; *Marazzi & Flores BM172*, Mexico, Oaxaca, MEXU, Z. ***S. purpusii*** (Brandegee) H.S. Irwin & Barneby; CH, Pac; AM086775, AM086921, AM086891; Cult. BGB 3585/96-P; *Marazzi BM004*, garden, Z. ***S. quinquangulata*** (Rich.) H.S. Irwin & Barneby; CH, Bac; AM086580, AM086598, AM086614; Wild; *Queiroz & Marazzi LQ 9220*, Brazil, Bahia, HUEFS, Z. ***S. reticulata*** (Willd.) H.S. Irwin & Barneby; SE, Pic; AM086776, AM086975, AM086892; Wild; *Marazzi & Alvárez BM154*, Republic of Panama, Coclé, PMA, STRI, Z. ***S. rigida*** (Hieron.) H.S. Irwin & Barneby; PE, Aph; AM086777, AM086976, AM086893; Wild; *Marazzi et al. BM108*, Argentina, Salta, CTES, Z. ***S. cf. rigida***; PE, Aph; AM086778, AM086977, AM086894; Wild; *Marazzi et al. BM104*, Argentina, Salta, CTES, Z. **S. rizzinii** H.S. Irwin & Barneby; CH, Bac; AM086581, AM086599, AM086615; Wild; Conceição & Marazzi 1126, Brazil, Bahia; HUEFS, Z. ***S. robiniifolia*** (Benth.) H.S. Irwin & Barneby; PE, Itg; AM086779, AM086978, AM086895; Cult. BGM 98/3500w; *Marazzi BM005*, garden, Z. ***S. rugosa*** (G. Don) H.S. Irwin & Barneby; CH, Bac; AM086582, AM086600, AM086616; Wild; *Giulietti 2337*, Brazil, Bahia, HUEFS. ***S. septemtrionalis*** (Viviani) H.S. Irwin & Barneby; CH, Col; AM086780, AM086979, AM086896; Cult. BGM s.n.; *Marazzi BM140*, garden, Z. ***S. siamea*** (Lam.) H.S. Irwin & Barneby; CH, Flo; AM086781, AM086980, AM086897; Cult., Causeway, Panamá City; *Marazzi & Alvárez BM157*, Republic of Panama, Panamá, PMA, STRI, Z. ***S. silvestris* var. *guaranitica*** (Chodat & Hassl.) H.S. Irwin & Barneby; CH, Sap; AM086782, AM086981, AM086898; Wild; *Marazzi et al. BM068*, Paraguay, San Pedro, PY, CTES, Z. ***S. skinneri*** (Benth.) H.S. Irwin & Barneby; CH, Ski; AM086783, AM086982, AM086899; Wild; *Marazzi & Flores BM176*, Mexico, Oaxaca, MEXU, Z. ***S. spectabilis*** (DC.) H. S. Irwin & Barneby; PE, Exc; AM086784, AM086983, AM086900; Wild; *Marazzi et al. BM029*, Paraguay, Alto Paraná, PY, CTES, Z. ***S. splendida* var. *gloriosa*** H.S. Irwin & Barneby; CH, Bac; AM086583, AM086601, AM086617; Wild; *Araújo 1566*, Brazil, Ceará, HUEFS. ***S. subulata*** (Griseb.) H.S. Irwin & Barneby; CH, Col; AM086785, AM086984, AM086901; Wild; *Potzner & Belgrano 427*, Argentina, Jujuy, SI. ***S. tonduzii*** (Standl.) H.S. Irwin & Barneby; PE, Itg; AM086786, AM086985, AM086902; Wild; *Marazzi & Flores BM187*, Mexico; Chiapas, MEXU, Z. ***S. uniflora*** (Mill.)

H.S. Irwin & Barneby; CH, Con; AM086787, AM086986, AM086903; Wild; *Marazzi & Flores BM186*, Mexico, Chiapas, MEXU, Z. ***S. unijuga*** (Rose) H.S. Irwin & Barneby; PE, Des; AM086788, AM086987, AM086904; Wild; *Marazzi & Flores BM167*, Mexico, Puebla, MEXU, Z. ***S.* cf. *velutina*** (Vogel) H.S. Irwin & Barneby; CH, Lax; AM086789, AM086988, AM086905; Wild; *Morrone & Belgrano 4988*, Bolivia, Santa Cruz, LPB, SI, CTES. ***S. venusta*** (F. Muell.) Randell; SE, Pic; —, AM086989, AM086906; Seed Bank RBGA 881196; *McCarthy 224*, Australia Western Australia, unk. ***S. villosa*** (Mill.) H.S. Irwin & Barneby; AS; AM086790, AM086990, AM086907; Wild; *Marazzi & Flores BM174*, Mexico, Oaxaca, MEXU, Z. ***S. williamsii*** (Britton & Rose) H.S. Irwin & Barneby; PE, Itg; AM086791, AM086991, AM086908; Wild; *Marazzi & Alvárez BM158*, Republic of Panama, Panamá, PMA, STRI, Z. ***S. wislizeni*** (A. Gray) H.S. Irwin & Barneby; PE, Des; AM086792, AM086992, AM086909; Wild; *Marazzi & Flores BM169*, Mexico, Puebla, MEXU, Z.

[a] Voucher: fixed material in 70% ethanol.

[b] *Senna glutinosa*: plant material received with the name *Senna* form *glutinosa*.

[c] *Senna* indet. ser. *Subverrucosae*: plant material received with the invalid species name of *S. nemophila* most likely corresponding to *Senna* form '*oligophylla*' (R. Johnstone, Royal Botanic Garden Mount Annan , N.S.W., Australia, personal communication).