DIVERSITY IN ANTHERS AND STIGMAS IN THE BUZZ-POLLINATED GENUS *SENNA* (LEGUMINOSAE, CASSIINAE)

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The large genus *Senna* (Cassiinae, Leguminosae) is an outstanding example of floral structural specialization associated with buzz pollination. This specialization is expressed especially in the androecium (with a high diversity of anther elaborations) and gynoecium (with diversity in stigma shape). The floral structure of 69 species from all major clades of *Senna* was studied, focusing on heteranthery, anther dehiscence, pore position, extension of the lateral furrow of the thecae, cell wall thickening in the anther tip, and stigma diversity (especially position, form, size, and structure of the orifice and presence of a chamber). Filament union is reported for the first time in the genus; it involves the seven adaxial androecial organs, a pattern unique in legumes. Our investigations identified novel morphological characteristics that are congruent with the clades supported by the molecular phylogeny. Anthers of abaxial stamens with the least differentiated dehiscence pattern, i.e., two separate pores and separate thecae, are found in most major clades (I, III–V, VII). Anthers with apically confluent thecae, forming a shared chamber, and/or with a single pore by confluence of two pores, represent specialized patterns (clades II, IV, VI, VII). Diverse anther tips may reflect different strategies of pollen dispersal; anther pore position may influence pollen flow directions. Anther tip elongation in the abaxial stamens and constriction between the thecae and the anther tip may influence the speed and/or amount of the released pollen.

Keywords: poricidal anther dehiscence, buzz pollination, functional morphology, heteranthery, pollen dispersal, stamen union.

Introduction

Senna (Cassiinae, Leguminosae) is a large, diverse, and widespread genus of ca. 350 species with showy yellow nectarless flowers that are buzz pollinated by pollen-collecting bees. They display an array of floral traits typically related to the specialized pollination biology, including heteranthery (i.e., different kinds of stamens in a flower), poricidal anthers (i.e., dehiscence restricted to apical pores), point-tipped stigmas, enantiostyly (i.e., deflection of the single carpel to the left or right), and asymmetric corolla and androecium (Buchmann 1974; Delgado Salinas and Sousa Sánchez 1977; Dulberger 1981; Irwin and Barneby 1981; Gottsberger and Silberbauer-Gottsberger 1988; Owens and Lewis 1989; Dulberger et al. 1994). In addition to species with enantiostylous, asymmetric flowers, there are also species with monosymmetric (zygomorphic) flowers.

Although the specialized floral traits of *Senna* have intrigued researchers for a long time (Müller 1883; Burck 1886), their role in pollination biology has been investigated only in a few species (Buchmann 1974; Delgado Salinas and Sousa Sánchez 1977; Fontanelle 1979; Dulberger 1981; Gottsberger and Silberbauer-Gottsberger 1988; Carvalho and Oliveira 2003; Laporta 2003). This is also true for their

floral morphology, in which specializations of the stigma (Owens and Lewis 1989; Dulberger et al. 1994), structure of the poricidal stamens (Venkatesh 1957; Lasseigne 1979; Endress 1994; Tucker 1996a), and floral development (Tucker 1996b) were studied. Most of these studies compare *Senna* with *Cassia* L. and *Chamaecrista* Moench (the three genera belonging to subtribe Cassiinae, formerly known as *Cassia* s.l.; Irwin and Barneby 1981, 1982), which are all usually characterized by yellow and nectarless, buzz-pollinated flowers (Delgado Salinas and Sousa Sánchez 1977; Gottsberger and Silberbauer-Gottsberger 1988). Such flowers are otherwise unusual in legumes. The evolution of floral diversity in *Senna* is highly intriguing and still enigmatic. Molecular phylogenetic work by Marazzi et al. (2006) does not support previous interpretations of morphological evolution and current classification systems of *Senna* (Irwin and Barneby 1982; Randell 1988, 1989, 1990; Singh 2001; see Marazzi et al. 2006; table 1). This work is part of a larger project aimed at understanding floral evolution within *Senna* and focuses on stamen and gynoecium diversity in the genus, whereas floral symmetry will be treated elsewhere.

The androecium and, in particular, the poricidal anther tips have been recognized as the most diverse and interesting floral parts of *Senna* (Venkatesh 1957; Fontanelle 1979; Lasseigne 1979; Irwin and Barneby 1981; Tucker 1996a). The typically five-merous flowers of *Senna* have two androecial whorls with up to four different kinds of stamens. The three adaxial stamens (1) are usually staminodial and produce pollen only in the species of *Senna* section *Psilorhegma*

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Table 1

Taxa Used in This Study, Source, and Voucher Information

Taxon	Clade	Section	Source	Voucher no.	Locality and herbaria
Senna aciphylla (Benth.) Randell	IVa	PS	Cult.	CGB 9900061	Garden CBG, CBG, Z
S. acuruensis var. *acuruensis* (Benth.) H.S. Irwin & Barneby	VI	PE	Wild	Queiroz & Marazzi LQ 9198	Brazil, Bahia, HUEFS, Z
S. acuruensis var. *catingae* (Harms) H.S. Irwin & Barneby	VI	PE	Wild	Queiroz & Marazzi LQ 9177	Brazil, Bahia, HUEFS, Z
S. alata (L.) Roxb.	II	SE	Cult.	Vanni & Marazzi 4516	Argentina, Corrientes, CTES, G
S. alata (L.) Roxb.	II	SE	Wild	Marazzi et al. BM026	Paraguay, Caaguazú, PY, CTES, Z
S. andrieuxii (Benth.) H.S. Irwin & Barneby	III	PE	Wild	Marazzi & Flores BM162	Mexico, Puebla, MEXU, Z
S. aphylla (Cav.) H.S. Irwin & Barneby	VI	PE	Wild	Marazzi et al. BM084	Argentina, Santiago del Estero, CTES, Z
S. apiculata (M. Martens & Galeotti) H.S. Irwin & Barneby	VIIb	CH	Wild	Marazzi & Flores BM170	Mexico, Puebla, MEXU, Z
S. argentea (Kunth) H.S. Irwin & Barneby	VIIb	CH	Wild	Marazzi & Flores BM175	Mexico, Oaxaca, MEXU, Z
S. armata (S. Watson) H.S. Irwin & Barneby	VIIb	CH	Wild	Schönenberger JS751	USA, California, Z
S. artemisioides (DC.) Randell	IVa	PS	Cult.	Marazzi BM002	Garden BGZ s.n., Zurich, Z
S. atomaria (L.) H.S. Irwin & Barneby	III	PE	Wild	Marazzi & Flores BM173	Mexico, Oaxaca, MEXU, Z
S. aversiflora (Herbert) H.S. Irwin & Barneby	VI	PE	Wild	Queiroz & Marazzi LQ 9204	Brazil, Bahia, HUEFS, Z
S. barclayana (Sweet) Randell	VIIa	CH	Cult.	Marazzi BM136	Garden PBIB 2003/76, Z
S. birostris (Hook.) H.S. Irwin & Barneby[a]	VIIa	CH	Wild	Marazzi et al. BM090	Argentina, Tucumán, CTES, Z
S. cana var. *calva* H.S. Irwin & Barneby	V	CH	Wild	Conceiçao & Marazzi 1132	Brazil, Bahia, HUEFS, Z
S. cernua (Balb.) H.S. Irwin & Barneby	VIIa	CH	Wild	Marazzi et al. BM007	Paraguay, Caaguazú, PY, CTES, Z
S. chacoënsis (L. Bravo) H.S. Irwin & Barneby	VI	PE	Wild	Marazzi et al. BM083	Argentina, Santiago del Estero, CTES, Z
S. chloroclada (Harms) H.S. Irwin & Barneby	IVb	PE	Wild	Marazzi et al. BM128	Argentina, Salta, CTES, Z
S. chloroclada (Harms) H.S. Irwin & Barneby	IVb	PE	Wild	Schinini & Marazzi 35285	Argentina, Formosa, CTES, Z
S. chrysocarpa (Desv.) H.S. Irwin & Barneby	unc	CH	Cult.	Prévost 4915	French Guyana, Cayenne, CAY, Z
S. corymbosa (Lam.) H.S. Irwin & Barneby	VIIa	CH	Cult.	Marazzi et al. BM103	Argentina, Tucumán, CTES, Z
S. crassiramea (Benth.) H.S. Irwin & Barneby	VI	PE	Wild	Marazzi et al. BM120	Argentina, Jujuy, CTES, Z
S. crotalarioides (Kunth) H.S. Irwin & Barneby	VIIa	CH	Wild	Marazzi & Flores BM163	Mexico, Puebla, MEXU, Z
S. dariensis var. *hypoglauca* H.S. Irwin & Barneby	IVb	CH	Wild	Marazzi & Alvárez BM153	Republic of Panama, Coclé, PMA, STRI, Z
S. didymobotrya (Fresen.) H.S. Irwin & Barneby	II	SE	Cult.	Marazzi BM002	Garden BGZ 19700009, Z
S. galeottiana (M. Martens) H.S. Irwin & Barneby	VIIa	CH	Wild	Marazzi & Flores BM165	Mexico, Puebla, MEXU, Z
S. hayesiana (Britton & Rose) H.S. Irwin & Barneby	IVb	CH	Wild	Marazzi & Alvárez BM150	Republic of Panama, Panamá, PMA, STRI, Z
S. hebecarpa (Fernald) H.S. Irwin & Barneby	unc	CH	Cult.	s.n.	Garden BGM, Z
S. hilariana (Benth.) H.S. Irwin & Barneby	VIIa	CH	Wild	Marazzi et al. BM027	Paraguay, Alto Paraná, PY, CTES, Z
S. hirsuta var. *hirta* (Benth.) H.S. Irwin & Barneby	VIIa	CH	Wild	Marazzi et al. BM115	Argentina, Salta, CTES, Z
S. hirsuta var. *leptocarpa* (Benth.) H.S. Irwin & Barneby	VIIa	CH	Wild	Marazzi et al. BM065	Paraguay, San Pedro, PY, CTES, Z
S. holwayana var. *holwayana* (Rose) H.S. Irwin & Barneby	VI	PE	Wild	Marazzi & Flores BM161 and 166	Mexico, Puebla, MEXU, Z
S. indet. ser. Bacillares	IVb	CH	Wild	Marazzi & Alvárez BM160	Republic of Panama, Panamá, PMA, STRI, Z
S. italica Mill.	II	SE	Wild	Zietsmann 4345	Republic of South Africa, Free State, NMB, Z
S. macranthera cf. var. *nervosa* (Vogel) H.S. Irwin & Barneby	IVb	CH	Cult.	Vanni & Marazzi 4543	Argentina, Misiones, CTES, G
S. macranthera var. *nervosa* (Vogel) H.S. Irwin & Barneby	IVb	CH	Cult.	Marazzi et al. BM082	Paraguay, Caaguazú, PY, CTES, Z
S. mollissima (Willd.) H.S. Irwin & Barneby	III	PE	Wild	Marazzi & Flores BM181	Mexico, Oaxaca, MEXU, Z
S. morongii (Britton) H.S. Irwin & Barneby	VIIa	CH	Wild	Marazzi et al. BM130	Argentina, Salta, CTES, Z
S. morongii (Britton) H.S. Irwin & Barneby	VIIa	CH	Wild	Schinini & Marazzi 35279	Argentina, Chaco, CTES, G

Species	Clade	Section	Status	Voucher	Locality
S. mucronifera (Benth.) H.S. Irwin & Barneby	IVb	CH	Wild	Marazzi et al. BM019	Paraguay, Caaguazú, PY, CTES, Z
S. multijuga var. *lindleyana* (Gardner) H.S. Irwin & Barneby	VI	PE	Wild	Queiroz & Marazzi LP 9226	Brazil, Bahia, HUEFS, Z
S. multijuga var. *multijuga* (Rich.) H.S. Irwin & Barneby	VI	PE	Cult.	Marazzi & Alvárez BM151	Republic of Panama, Panamá, PMA, STRI, Z
S. nicaraguensis (Benth.) H.S. Irwin & Barneby	II	SE	Wild	Marazzi & Flores BM185	Mexico, Chiapas, MEXU, Z
S. obtusifolia (L.) H.S. Irwin & Barneby	IVb	CH	Wild	Marazzi et al. BM024	Paraguay, Caaguazú, PY, CTES, Z
S. obtusifolia (L.) H.S. Irwin & Barneby	IVb	CH	Wild	Vanni & Marazzi 4514	Argentina, Corrientes, CTES, G
S. occidentalis (L.) Link	VIIa	CH	Wild	Marazzi et al. BM060	Paraguay, Caaguazú, PY, CTES, Z
S. odorata (Morris) Randell	Iva	PS	Cult.	ANBG 68349	Australia, CBG
S. pallida (Vahl) H.S. Irwin & Barneby	VI	PE	Wild	Marazzi & Flores BM178	Mexico, Oaxaca, MEXU, Z
S. paradictyon (Vogel) H.S. Irwin & Barneby	II	PA	Wild	Marazzi et al. BM028	Paraguay, Alto Paraná, PY, CTES, Z
S. pendula (Willd.) H.S. Irwin & Barneby	VIIa	CH	Wild	Marazzi et al. BM117	Argentina, Salta, CTES, Z
S. pilifera var. *pilifera* (Vogel) H.S. Irwin & Barneby	IVb	CH	Wild	Marazzi et al. BM011	Paraguay, Caaguazú, PY, CTES, Z
S. pilifera var. *pilifera* (Vogel) H.S. Irwin & Barneby	IVb	CH	Wild	Vanni & Marazzi 4513	Argentina, Corrientes, CTES, G
S. pinheiroi H.S. Irwin & Barneby	IVb	CH	Wild	Queiroz 9210	Brazil, Bahia, HUEFS.
S. polyantha (Collad.) H.S. Irwin & Barneby	I	CH	Wild	Marazzi & Flores BM172	Mexico, Oaxaca, MEXU, Z
S. purpusii (Brandegee) H.S. Irwin & Barneby	VII	CH	Cult.	Marazzi BM004	Garden BGB 3585/96-P, Z
S. quinquangulata (Rich.) H.S. Irwin & Barneby	IVb	CH	Wild	Queiroz & Marazzi LQ 9220	Brazil, Bahia, HUEFS, Z
S. rgida (Hieron.) H.S. Irwin & Barneby	VI	PE	Wild	Marazzi et al. BM108	Argentina, Salta, CTES, Z
S. rizzinii H.S. Irwin & Barneby	IVb	CH	Wild	Conceição & Marazzi 1126	Brazil, Bahia, HUEFS, Z
S. robinifolia (Benth.) H.S. Irwin & Barneby	VI	PE	Cult.	Marazzi BM005	Garden BGM 98/3500w, Z
S. rugosa (G. Don) H.S. Irwin & Barneby	IVb	CH	Wild	Queiroz & Marazzi LQ 9181	Brazil, Bahia, HUEFS
S. septemtrionalis (Viviani) H.S. Irwin & Barneby	VIIa	CH	Cult.	Marazzi BM140	Garden BGM s.n., Z
S. siamea (Lam.) H.S. Irwin & Barneby	I	CH	Cult.	Marazzi & Alvárez BM157	Panamá City, Panamá, PMA, STRI, Z
S. silvestris var. *guaranitica* (Chodat & Hassl.) H.S. Irwin & Barneby	I	CH	Wild	Marazzi et al. BM068	Paraguay, San Pedro, PY, CTES, Z
S. skinneri (Benth.) H.S. Irwin & Barneby	IV	CH	Wild	Marazzi & Flores BM176	Mexico, Oaxaca, MEXU, Z
S. spectabilis (DC.) H.S. Irwin & Barneby	III	PE	Wild	Marazzi et al. BM029	Paraguay, Alto Paraná, PY, CTES, Z
S. spectabilis var. *spectabilis* (DC.) H.S. Irwin & Barneby	III	PE	Wild	Vanni & Marazzi 4530	Argentina, Misiones, CTES, G
S. surattensis (Burm.f.) H.S. Irwin & Barneby	unc	PS	Cult.	Endress 03-91	Garden PTGB, USA, Hawaii, Z
S. tonduzii (Standl.) H.S. Irwin & Barneby	VI	PE	Wild	Marazzi & Flores BM187	Mexico, Chiapas, MEXU, Z
S. uniflora (Mill.) H.S. Irwin & Barneby	V	CH	Wild	Marazzi & Flores BM186	Mexico, Chiapas, MEXU, Z
S. unijuga (Rose) H.S. Irwin & Barneby	III	PE	Wild	Marazzi & Flores BM167	Mexico, Puebla, MEXU, Z
S. villosa (Mill.) H.S. Irwin & Barneby	VIIb	VS	Wild	Marazzi & Flores BM174	Mexico, Oaxaca, MEXU, Z
S. williamsii (Britton & Rose) H.S. Irwin & Barneby	VI	PE	Wild	Marazzi & Alvárez BM158	Republic of Panama, Panamá, PMA, STRI, Z
S. wislizeni (A. Gray) H.S. Irwin & Barneby	III	PE	Wild	Marazzi & Flores BM169	Mexico, Puebla, MEXU, Z

Note. Clades or subclades of *Senna* by Marazzi et al. (2006) and section by Irwin and Barneby (1982) (AS, *Astroites*; CH, *Chamaefistula*; PA, *Paradictyon*; PE, *Peiranisia*; PS, *Psilorhegma*; SE, *Senna*; unc, clade uncertain). Acronyms of herbaria or botanical gardens (in alphabetic order): CBG (= ANBG) = Australian National Botanic Gardens; BGB = Botanical Garden of the University of Basel; BGM = Botanischer Garten der Universität München; BGZ = Botanischer Garten der Universität Zürich; CAY = Institut de Recherche pour le Développement (IRD), Cayenne; CTES = Instituto de Botánica del Nordeste, Corrientes; G = Conservatoire et Jardin Botaniques de la Ville de Genève; HUEFS = Universidad Estadual de Feira de Santana; MEXU = Universidad Nacional Autónoma de México; PBIB = Parco Botanico Isole di Brissago; PMA = Universidad de Panamá; PTGB = National Tropical Botanical Garden, Kauai; PY = Museo Nacional de Historia Natural de Paraguay; SI = Instituto de Botánica Darwinion, San Isidro; STRI = Smithsonian Tropical Research Institute, Balboa; Z = University of Zurich and Botanical Garden. Cult. = cultivated.

[a] Compare var. *jallaguensis* based on Laporta et al. (2006).

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(Irwin and Barneby 1981). The remaining fertile stamens form (2) a set of four middle "feeding" stamens (with pollen collected for larval provision) and (3) a set of three usually longer abaxial "pollinating" stamens (with pollen for pollination), in which (4) the median stamen may be smaller than the lateral ones, highly reduced, and sterile or even absent in several species. Anthers are slightly dorsifixed, with apical dehiscence through two separate pores or two pores that are confluent into one pore. A more or less deep lateral furrow (nonfunctional stomium) usually runs from the lower end of each dehiscence pore down along the entire length of each theca. In many species, the anthers are variably beaked; i.e., they have a more or less elongated sterile anther tip (Venkatesh 1957; Lasseigne 1979). Some Senna species have unusual, long tubular beaks that are not found in other buzzed caesalpinioids and are indeed rare in buzz-pollinated angiosperms.

As characteristic in legumes, flowers of Senna have a single carpel, usually more or less curved upward and with a short stipe. Although the gynoecium is less diverse than the androecium, there is some variability in the deflection of the carpel (not discussed here) and, in particular, in the unusual stigma structure with enclosed stigmatic surface. Dulberger (1981) first described the extremely small orifice at the end of the style. Two types of stigma have been recognized in Senna (and other Cassiinae): (i) a chambered type, with the receptive surface enclosed in a cavity, the entrance of which is more or less closed; and (ii) a craterlike type, with the receptive surface not enclosed (Owens and Lewis 1989; Dulberger et al. 1994; Endress 1994). Hairs may variously fringe the entrance of the cavity, and a drop of secretion may occur at the outlet. In contrast to other angiosperms with an orifice at the end of a stylar canal, in Senna the cells surrounding the orifice are not part of the tissue that lines the cavity and produces the secretory material (Owens 1985; Dulberger et al. 1994). Moreover, the cuticle of these cells apparently does not allow pollen germination, which occurs in the secretion droplet within the cavity (Owens 1985; Dulberger et al. 1994).

Several features of the androecium but almost no features of the gynoecium were used for taxonomic purposes in Senna (e.g., Bentham 1871, as Cassia; Irwin and Barneby 1982). For example, the fertility of all stamens distinguishes sect. Psilorhegma from all other sections in Senna. In the previous interpretations of evolutionary trends in the floral morphology of the genus (Irwin and Barneby 1982), fertility of all 10 stamens and anthers with two pores represented the ancestral condition of the androecium. In contrast, Marazzi et al. (2006) suggested that flowers of Senna ancestrally had seven (or fewer) fertile stamens and three adaxial staminodes, with the condition of the androecium previously considered as derived. Thus, fertility of all stamens represents a synapomorphy of Psilorhegma that supports its monophyly (Marazzi et al. 2006). It remains unclear whether two anther pores represent the ancestral state in Senna and a single pore by confluence of two represents the derived state. The orientation of the abaxial stamens used to distinguish sect. Senna from sect. Chamaefistula by Irwin and Barneby (1982) is characteristic for clade II of Senna in the study by Marazzi et al. (2006), which includes the species of sect. Senna and Senna paradictyon of sect. Paradictyon. In these flowers, the two lateral long abaxial anthers are curved laterally so that their anthers face each

other, resembling the arms of tongs. Section Chamaefistula is polyphyletic (Marazzi et al. 2006). On the basis of stigma morphology, Owens and Lewis (1989) divided their examined Senna species into five groups, which were, however, not congruent with any of Irwin and Barneby's (1982) sections or series. Heteranthery and poricidal dehiscence have not yet been explored in the genus in a systematic context.

Floral diversity in Senna is associated with the specialized pollination mode, which has been described in more or less detail in a number of studies. Except for the occurrence of apomixis in the S. artemisioides species complex (as Cassia nemophila group; Randell 1970) and self-compatibility in S. silvestris (Carvalho and Oliveira 2003), Senna species are generally self-incompatible and require a pollinator to set seeds (e.g., Fontanelle 1979; Dulberger 1981; Laporta 2003). The pollinators, bees belonging mostly to Xylocopa but also to Bombus, Centris, Epicharis, and Melipona, extract the pollen by vibrating the flowers (i.e., buzz pollination; Buchmann 1974; Delgado Salinas and Sousa Sánchez 1977; Fontanelle 1979; Dulberger 1981; Gottsberger and Silberbauer-Gottsberger 1988; Carvalho and Oliveira 2003; Laporta 2003; van Kleunen and Johnson 2005). The thecae act as a resonating chamber, where pollen grains bounce when vibrated, gaining kinetic energy until they are forced through the apical pore (Buchmann and Hurley 1978).

It is not clear whether the feeding anthers of Senna provide only feeding pollen for the bee and the pollinating anthers only pollinating pollen for plant reproduction. For example, in S. silvestris, the feeding pollen, significantly smaller than the pollinating pollen, is not viable for plant reproduction (Carvalho and Oliveira 2003). In S. corymbosa, pollen tubes of pollinating pollen grow faster than those of feeding pollen (Laporta 2003), and in S. didymobotrya, all anthers are fertile and provide viable feeding pollen, suggesting no separation of anther function (Dulberger 1981).

Electrostatic forces seem to play an important role in buzz pollination (e.g., Corbet et al. 1982, 1988). The minute point-tipped stigma of Senna species touches or approaches the sites of the bee's body from which pollen was not removed by grooming. The ratio of the area of pollen presentation on the bee's body to the area of pollen reception on the stigma approximates 10,000 in two studied Senna species (Dulberger 1981), suggesting an imprecise mechanism of pollen delivery aided by electrostatic forces that promote pollen capture and its adhesion to the minute stigmatic tip (Dulberger et al. 1994).

The major aim of this study is to provide new structural data on the androecium and gynoecium of Senna, focusing on anthers and stigmas, to understand floral diversity and evolution within the genus. We will address the following specific questions. (1) What patterns of heteranthery can be recognized in the androecium of Senna species? (2) What patterns can be identified in the diversity of stamens and anther dehiscence? (3) Do other types of stigma exist in addition to chambered versus craterlike stigmas? (4) Do the investigated features provide any synapomorphies congruent with the new infrageneric relationships supported by the molecular phylogeny of Senna by Marazzi et al. (2006)? (5) Do our results support the interpretations on the evolution of the androecium by Irwin and Barneby (1982)? (6) What are the implications of our results on anther and stigma diversity to

improve our understanding of pollination and floral evolution in Senna?

Material and Methods

Our taxonomic sampling included 69 Senna species (one or more individuals per species), almost half of which were selected for detailed structural studies with scanning electron microscopy (SEM) and serial microtome sectioning, while the other species were investigated with stereomicroscopy. Sixty-six Senna species represent the clades of the molecular phylogeny of the genus by Marazzi et al. (2006; based on 83 species) and the diversity of morphological patterns observed. The remaining three species, Senna chrysocarpa, S. hebecarpa, and S. surattensis, were not considered by Marazzi et al. (2006).

To evaluate the consistency of the observed morphological patterns within a species, we studied additional accessions that were available for a number of species (S. alata, Vanni & Marazzi 4516; S. chloroclada, Schinini & Marazzi 35285; S. holwayana, Marazzi & Flores BM166; S. macranthera cf. var. nervosa, Vanni & Marazzi 4543; S. morongii, Schinini & Marazzi 35279; S. obtusifolia, Vanni & Marazzi 4514; S. pilifera var. pilifera, Vanni & Marazzi 4513; S. spectabilis var. spectabilis, Vanni & Marazzi 4530; these accessions were not used in Marazzi et al. 2006). Data for the androecium of S. reticulata and S. martiana are from Lasseigne (1979) and from color photographs made in the field by the first author, respectively. A list of the specimens studied and voucher information is given in table 1.

Flowers at anthesis and buds at different stages were fixed and stored in 70% ethanol. They were investigated with light microscopy (LM), including serial microtome sectioning, and

SEM (Hitachi S4100 FESEM; 20 kV). Standard specimen preparation procedures were used for osmium tetroxide–impregnated samples for SEM studies. Specimens for serial microtome sectioning were embedded in Kulzer's Technovit 2-hydroethyl methacrylate (Igersheim 1993) and sectioned with a Microm HM 335 rotary microtome (Microm International GmbH, Walldorf) and conventional microtome knife (grade D); transverse section series were cut at 7 μm, stained with ruthenium red and toluidine blue (Weber and Igersheim 1994), and mounted in Histomount on glass slides. Fixed floral material and slides are deposited at the Institute of Systematic Botany of the University of Zurich (Z), Switzerland.

Results

Anther Dehiscence Pattern of Abaxial Stamens

The two lateral abaxial stamens belong to the inner androecial whorl, and the median abaxial stamen belongs to the outer one. In this study, we primarily focus on the abaxial stamens because of the higher diversity in these organs compared with the middle stamens. In particular, we study the lateral abaxial stamens, which are fertile in almost all species (except Senna hayesiana; see below). The median abaxial stamen is similar to the lateral stamens in most Senna species (clades III–VI, VIIb) or, in other species, it is smaller (species of clades I, VIIa), smaller and slightly different in shape (species of clade II), highly reduced and sterile (species of clade VIIa), or absent (S. hayesiana, clade IV, other species of clade VIIa).

Clade I. Anthers of the lateral abaxial stamens have two apical dehiscence slits or (sometimes in S. siamea) one V-shaped slit by confluence of two and point inward (fig. 1A, 1B). The
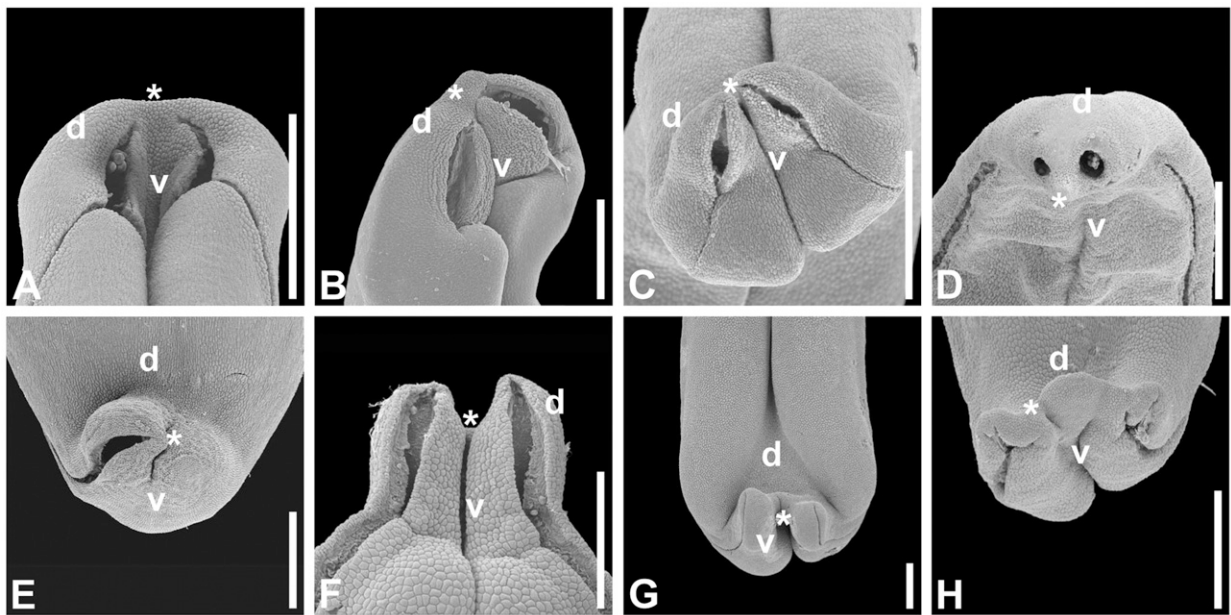

Fig. 1 Anther tips of lateral abaxial stamens in Senna species of clades I–III. A, Senna polyantha (I); B, S. siamea (I); C, S. italica (II); D, S. paradictyon (II); E, S. nicaraguensis (II); F, S. unijuga (III); G, S. mollissima (III); H, S. wislizeni (III). v = ventral anther side; d = dorsal anther side. End of anther tip marked with asterisk. Scale bars = 500 μm.

lateral furrow (nonfunctional stomium) is continuous, i.e., runs from the lower end of each dehiscence slit down along the entire length of the theca (*S. polyantha*; fig. 1*A*; fig. 2*A*, sections *v–vii*), is not continuous, i.e., is present only along the sides of the thecae between the pollen sacs, without being connected to the slits (*S. silvestris* var. *guaranitica*), or is absent (*S. siamea*; fig. 1*B*). The thecae are not confluent, and pollen of each theca is released by the dehiscence slit of that theca (fig. 2*A*; see also *S. silvestris* in Fontanelle 1979) or through the V-shaped slit by confluence of two (Venkatesh 1957). One hypodermal cell layer adjacent to the stomial region is specialized to serve the mechanical role in dehiscence: the cells are relatively narrow, radially elongated, and strongly thick walled (fig. 2*A*, sections *ii–vi*; see also Venkatesh 1957). Other hypodermal cells in the anther are also strongly thick walled (fig. 2*A*), so a continuous hypodermal cell layer of thickened cell walls is present throughout the anther. The vascular bundle extends almost up to the upper end of the slits (fig. 2*A*, sections *ii–vii*).

Clade II.   Anthers of the lateral abaxial stamens have two dehiscence pores (*S. italica*, fig. 1*C*; *S. paradictyon*, fig. 1*D*) or only one pore, while the other expected pore is always absent on the side away from the median plane (*S. nicaraguensis*, fig. 1*E*). The anther tip is bent in such a way that the pores point inward (*S. paradictyon*, fig. 1*D*), more or less upward (*S. alata*, *S. didymobotrya*, *S. italica*, fig. 1*C*), or outward (*S. nicaraguensis*, fig. 1*E*). The lateral furrow is continuous (fig. 1*C*, 1*E*; fig. 2*C*) or not continuous (*S. paradictyon*, figs. 1*D*, 2*B*; cf. sections *vi*, *vii*). The thecae are apically confluent, forming a shared chamber by decay of the tissue, and pollen traverses this chamber and is released through two separate pores (fig. 2*B*) or the single pore in *S. nicaraguensis*. Although in *S. nicaraguensis* one of the two pores is not formed, its canal is still present (fig. 2*C*, sections *ii–iv*). Thick-walled specialized hypodermal cells, as described in clade I, surround most of the sterile anther tip in *S. nicaraguensis* and, together with thick-walled hypodermal and subhypodermal cells, form an evenly thick layer throughout the anther wall (fig. 2*C*,
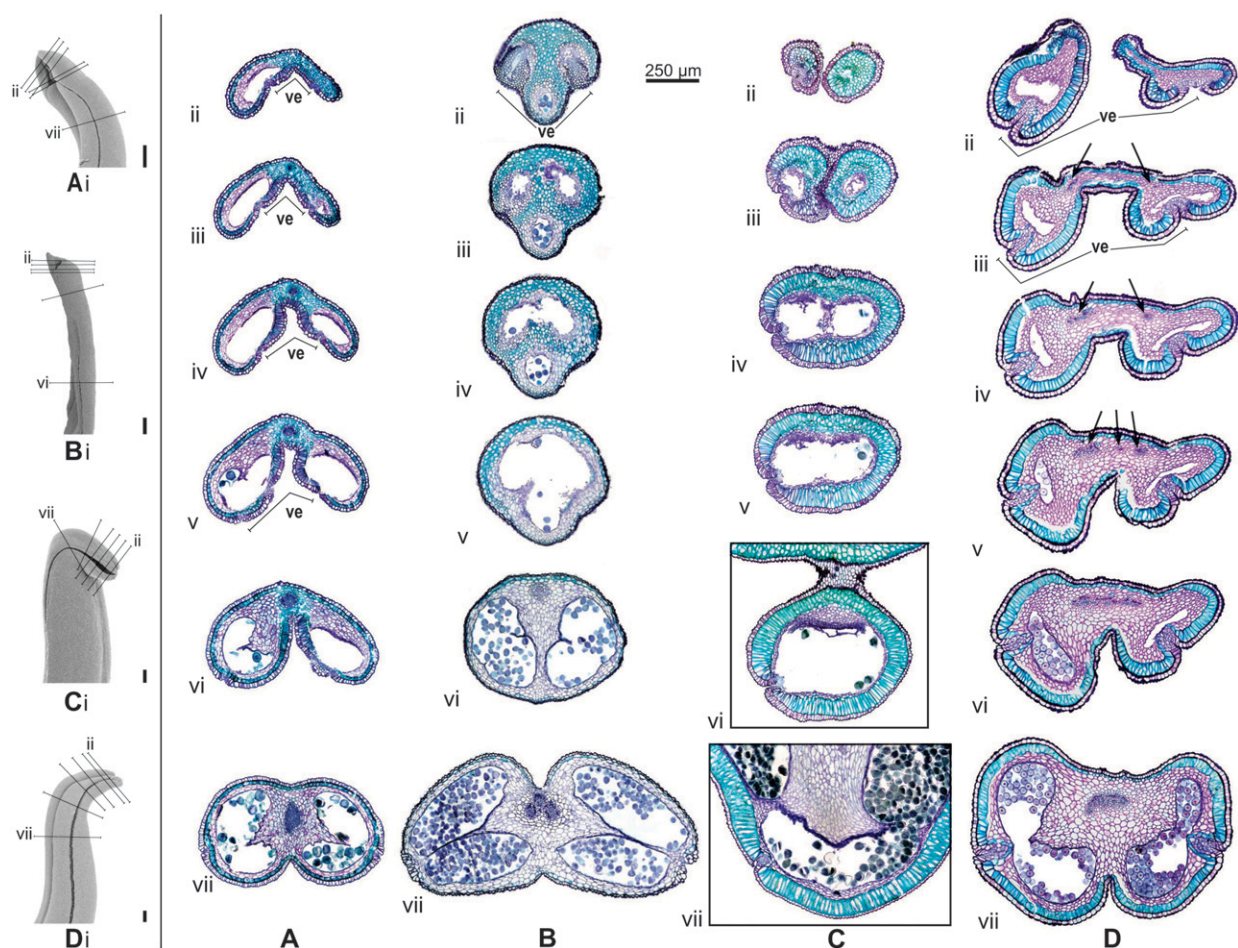

Fig. 2   Transverse section series of anther tips of abaxial stamens in *Senna* species of clades I–III. *A*, *Senna polyantha* (I); *B*, *S. paradictyon* (II); *C*, *S. nicaraguensis* (II); *D*, *S. wislizeni* (III). Ventral side (*ve*) of anther on left side in SEM micrographs and on lower side or as indicated in transverse sections. Arrows indicate branching vascular bundles in *S. wislizeni*. Lines on SEM micrographs indicate the approximate level of the transverse sections. Scale bars = 250 *μ*m.

sections *iv–vii*). *Senna paradictyon* lacks the specialized elongated hypodermal cells, and the evenly thick layer throughout the anther wall is less conspicuous than in *S. nicaraguensis* (fig. 2B). The vascular bundle does not extend into the sterile anther tip (fig. 2B, 2C).

Clade III. Anthers of the lateral abaxial stamens have two dehiscence slits in all studied species (see fig. 1F, 1G). The slits point inward (*S. unijuga*, fig. 1F) or more or less outward (*S. andrieuxii*, *S. atomaria*, *S. mollissima*, fig. 1G; *S. spectabilis*, *S. wislizeni*, fig. 1H). The lateral furrow is continuous (fig. 1F–1H). The thecae are not confluent, and pollen of each theca is released by the dehiscence slit of that theca (fig. 2D). One layer of hypodermal cells adjacent to the stomium is specialized as described in clade I, whereas the walls of other hypodermal cells are strongly thickened throughout the anther wall (*S. mollissima*, *S. wislizeni*, fig. 2D, sections *iii–vii*). The vascular bundle extends into the anther tip, and, in *S. wislizeni*, it branches close to the upper end of the dehiscence slits (fig. 2D, sections *iii–v*).

Clade IV. This clade is composed of *S. skinneri* and a clade comprising subclades IVa and IVb (Marazzi et al. 2006). Species of subclade IVa have all the stamens fertile, whereas *S. skinneri* and species of subclade IVb have seven (or fewer) fertile stamens. Flowers of *S. hayesiana* (subclade IVb) display only the four middle stamens at anthesis, while they lack the adaxial staminodes and the abaxial stamens. Staminodes and highly reduced abaxial stamens are present but fall off before anthesis. Anthers of the lateral abaxial stamens have two apical slits (*S. skinneri*, fig. 3A; species of subclade IVa, fig. 3B; species of subclade IVb: *S. chloroclada* [accession 35285], *S. macranthera* var. *nervosa*, fig. 3C; *S. pinheiroi*, *S. rizzinii*), or the two slits are confluent into a more

or less U-shaped pore (other species of subclade IVb: *S. chloroclada* [accession BM128], fig. 3D; *S. dariensis* var. *hypoglauca*, *S. mucronifera*, fig. 3E; *S. obtusifolia*, *S. pilifera*, *S. quinquangulata*, fig. 3F; *S. rugosa*). The pores or slits point inward (*S. skinneri*, fig. 3A; species of subclade IVa, fig. 3B), or the anther tip is bent (subclade IVb), and the pores point thus more or less upward (*S. mucronifera*, fig. 3E; *S. quinquangulata*, fig. 3F) or outward (*S. macranthera* var. *nervosa*, fig. 3C). The lateral furrow is continuous (species of subclade IVa, fig. 3B, and *S. macranthera* var. *nervosa* of subclade IVb), not continuous (*S. skinneri*, other species of subclade IVb: *S. chloroclada*, fig. 3D; *S. mucronifera*, fig. 3E; *S. obtusifolia*, *S. pilifera*), or absent (*S. quinquangulata*, subclade IVb, fig. 3F). Either the thecae are not confluent, and pollen of each theca is released by the dehiscence slit of that theca (*S. skinneri*, fig. 4A), or they are apically confluent, forming a shared chamber (species of subclades IVa and IVb), and pollen traverses this chamber and is released either through the two separate slits (fig. 4B) or through two separate short canals opening in the U-shaped confluent pore (figs. 3E, 4C). The shared chamber may be absent in *S. quinquangulata* because of lacking anther tip elongation (fig. 3F). A few species have unusually long tubular anther tips, almost up to 2.5 mm long (*S. mucronifera*, fig. 4Ci; *S. pilifera*). One layer of hypodermal cells adjacent to the stomium is specialized as described in clade I, and the walls of other hypodermal cells and several layers of subhypodermal cells are also strongly thickened (*S. skinneri*, fig. 4A, sections *ii–iv*; *S. mucronifera* of subclade IVb, fig. 4C), or only one layer of hypodermal cells is thick walled throughout the anther wall (*S. aciphylla* of subclade IVa, fig. 4B). A pollen sac placentoid is present at least in *S. skinneri* (fig. 4A, section *vii*) and apparently in
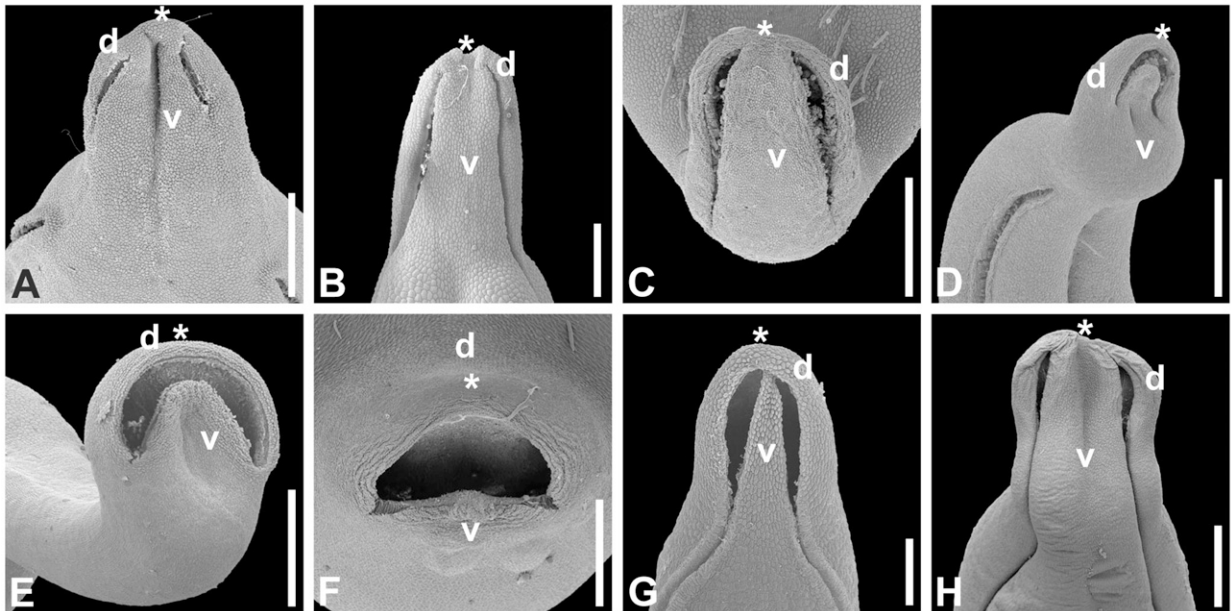

Fig. 3 Anther tips of abaxial stamens in *Senna* species of clades IV and V. *A*, *Senna skinneri* (IV); *B*, *S. aciphylla* (IVa); *C*, *S. macranthera* var. *nervosa* (IVb); *D*, *S. chloroclada* (IVb); *E*, *S. mucronifera* (IVb); *F*, *S. quinquangulata* (IVb); *G*, *S. uniflora* (V); *H*, *S. cana* var. *calva* (V). *v* = ventral anther side; *d* = dorsal anther side. End of anther tip marked with asterisk. Scale bars = 500 μm.
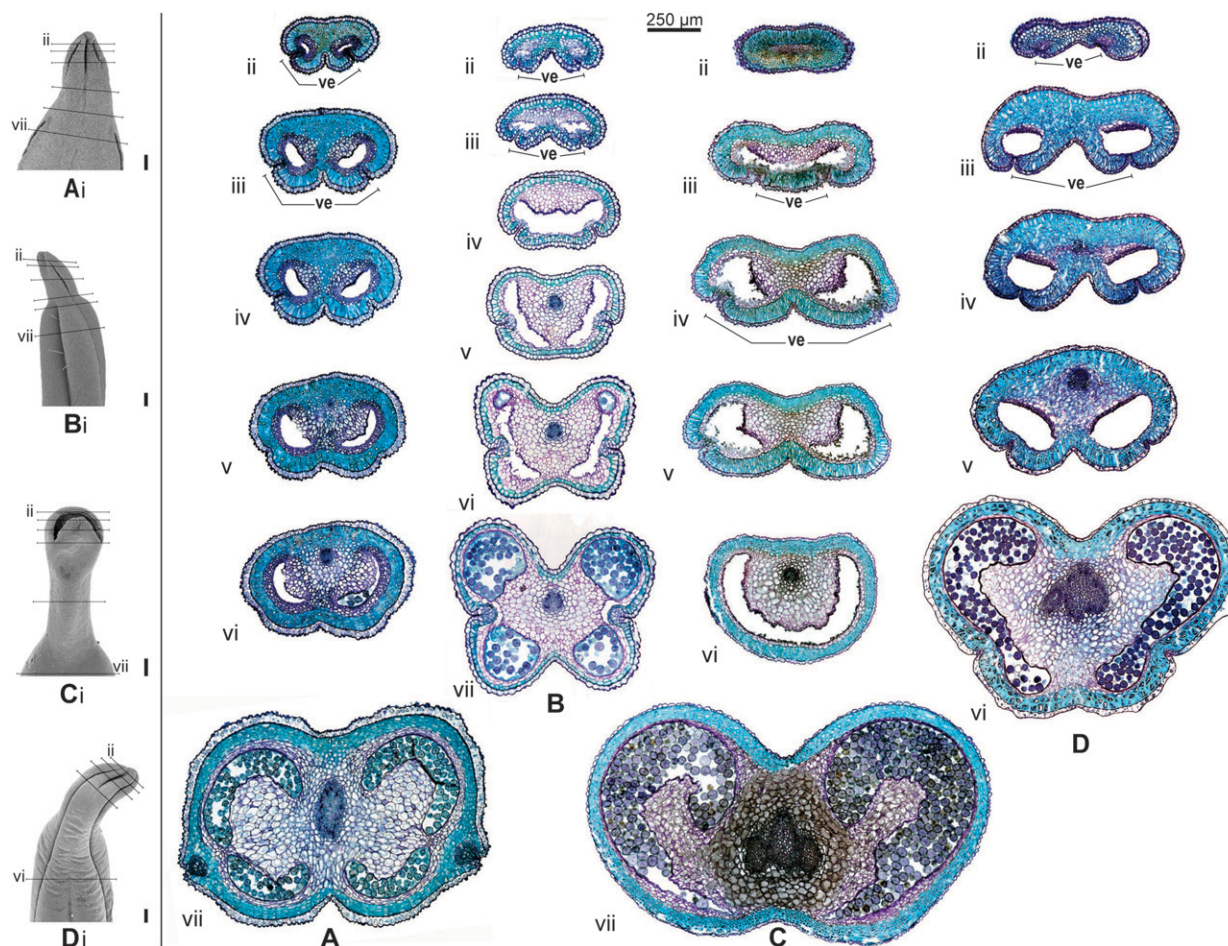
Fig. 4 Transverse section series of anther tips of abaxial stamens in *Senna* species of clades IV and V. *A*, *Senna skinneri* (IV); *B*, *S. aciphylla* (IVa); *C*, *S. mucronifera* (IVb); *D*, *S. cana* var. *calva* (V). Ventral side (*ve*) of anther on left side in SEM micrographs and on lower side or as indicated in transverse sections. Lines on SEM micrographs indicate the approximate level of the transverse sections. Scale bars = 250.

S. mucronifera (fig. 4C, section *vii*). The vascular bundle extends almost up to the sterile anther tip (fig. 4A, sections *iv*, *v*; fig. 4B, sections *iii*, *iv*; fig. 4C, sections *iv*, *v*).

Clade V. Anthers of the abaxial stamens have two apical slits that point inward (fig. 3G, 3H). The lateral furrow is continuous (fig. 3G, 3H). The thecae are not confluent in *S. cana* var. *calva*, and the pollen of each theca is released by the dehiscence slit of that theca (fig. 4D). The pattern of wall thickenings of the hypodermal and subhypodermal cells in *S. cana* var. *calva* (fig. 4D, sections *iii–v*) is as described for *S. skinneri* in clade IV (fig. 4A, sections *ii–iv*). A pollen sac placentoid is present in *S. cana* var. *calva* (fig. 4D, section *vi*). The vascular bundle extends up to the level of the dehiscence slits (fig. 4D, sections *iii*, *iv*).

Clade VI. Anthers of the lateral abaxial stamens have two apical slits or pores (*S. acuruensis* var. *acuruensis*, fig. 5A; *S. aphylla*, *S. chacoënsis*, fig. 5B; *S. crassiramea*, *S. multijuga* var. *lindleyana* and var. *multijuga*, fig. 5C; *S. rigida*, fig. 5D; *S. williamsii*, fig. 5E), or the two slits are confluent into a single U-shaped pore (*S. acuruensis* var. *catingae*, fig. 5F; *S. aversiflora*, fig. 5G; *S. holwayana*, *S. pallida*, *S. robiniifolia*, *S. tonduzii*, fig. 5H). The lateral furrow is continuous (*S.*

acuruensis var. *acuruensis*, fig. 5A; *S. williamsii*, fig. 5E) or is not continuous in the other 12 taxa studied of clade VI (fig. 5B–5D, 5F–5H). The thecae are apically confluent, forming a shared chamber. Pollen traverses this chamber and is released either through more or less short canals ending into the separate slits (fig. 6A) or through the single U-shaped pore (fig. 6B). Most species of clade VI have unusually long tubular anther tips, almost up to 2–4 mm long (*S. acuruensis*, *S. aversiflora*, *S. holwayana*, *S. multijuga*, *S. pallida*, *S. tonduzii*, *S. williamsii*; see fig. 5A, 5C, 5E–5H). In *S. tonduzii* (fig. 6B, sections *ii–v*), the pattern of cell wall thickenings of the hypodermal and other subhypodermal cells is as described for *S. skinneri* (clade IV, fig. 4A, sections *ii–iv*) and for *S. cana* var. *calva* (clade V, fig. 4D, sections *iii–v*). In contrast, anther tips of *S. chacoënsis*, lacking the specialized hypodermal cells adjacent to the stomium described for clade I, have hypodermal and a few layers of subhypodermal cells that appear uniformly and strongly thick walled (fig. 6A, sections *ii–vi*). In the anther below the elongated tip, there is only one layer of such thick-walled cells (fig. 6A, sections *vii*, *viii*). A pollen sac placentoid is present at least in *S. tonduzii* (fig. 6B, section *vii*). The vascular bundle extends either almost to the
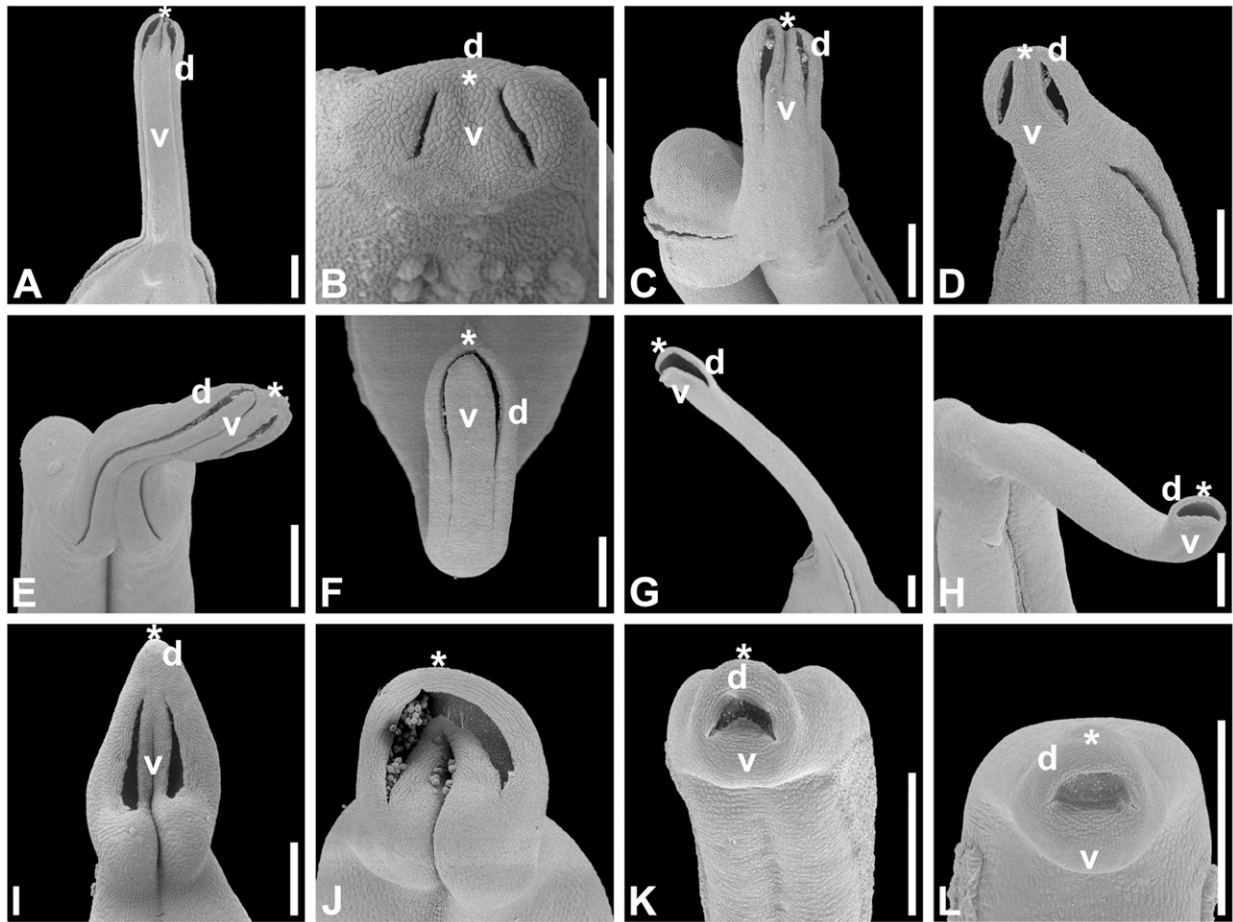
Fig. 5 Anther tips of lateral abaxial stamens in *Senna* species of clades VI and VII. *A, Senna acuruensis* var. *acuruensis* (VI); *B, S. chacoënsis* (VI); *C, S. multijuga* var. *multijuga* (VI); *D, S. rigida* (VI); *E, S. williamsii* (VI); *F, S. acuruensis* var. *catingae* (VI); *G, S. aversiflora* (VI); *H, S. tonduzii* (VI); *I, S. cernua* (VIIa); *J, S. pendula* (VIIa); *K, S. apiculata* (VIIb); *L, S. villosa* (VIIb). v = ventral anther side; d = dorsal anther side. End of anther tip marked with asterisk. Scale bars = 500 μm.

shared chamber (fig. 6*A*, sections *vi, vii*) or within part of the sterile anther tip (fig. 6*B*, sections *iii, iv*).

Clade VII. Clade VII is divided into subclades VIIa and VIIb, which differ in androecial structure. In subclade VIIa, 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, whereas in subclade VIIb, all fertile stamens have more or less the same length and shape (Marazzi et al. 2006). Anthers of the lateral abaxial stamens have two apical pores (species of subclade VIIa: *S. barclayana, S. cernua,* fig. 5*I; S. hirsuta, S. occidentalis* [see also Venkatesh 1957], *S. purpusii*) or a U- or V-shaped pore by confluence of two pores (species of subclade VIIa: *S. birostris* var. *hookeriana, S. corymbosa, S. hilariana, S. morongii, S. pendula,* fig. 5*J; S. septemptrionalis*; species of subclade VIIb, fig. 5*K,* 5*L*). The lateral furrow is present only along the sides and is not continuous with the pores (see fig. 5*I*–5*L*). Either the thecae are not confluent (species of subclade VIIa), and pollen of each theca is released by the dehiscence pore of that theca (figs. 5*I,* 6*C*) or by the U-shaped single pore by confluence (fig. 5*J*), or the thecae are apically confluent,

forming a shared chamber (species of subclade VIIb, fig. 5*K,* 5*L*; fig. 6*D*). Thick-walled specialized hypodermal cells, as described in clade I, surround the dehiscent pores and, together with strongly thick-walled hypodermal cells, form an evenly thick layer throughout the anther, which may be more (fig. 6*C*) or less (fig. 6*D*) conspicuous. A pollen sac placentoid is present at least in *S. cernua* (fig. 6*C,* section *vi*). The vascular bundle extends almost to the sterile anther tip (fig. 6*C,* sections *iv, v*) or to the shared chamber (fig. 6*D,* sections *v, vi*).

Species of uncertain affinity. The androecium of *S. chrysocarpa* has seven stamens, in which the abaxial stamens are slightly larger than the middle ones, and three (adaxial) staminodes. Anther tips of the abaxial stamens in *S. chrysocarpa* are similar to those of species in subclade IVb and are characterized by two dehiscence pores pointing more or less outward, with the lateral furrow apparently not connected to the pores. The androecium of *S. hebecarpa* also has seven fertile stamens, but the abaxial ones are conspicuously longer than the middle ones. Anther tips of abaxial stamens in *S. hebecarpa* are similar to those of species of subclade VIIb (especially *S. purpusii*), with two dehiscence pores pointing
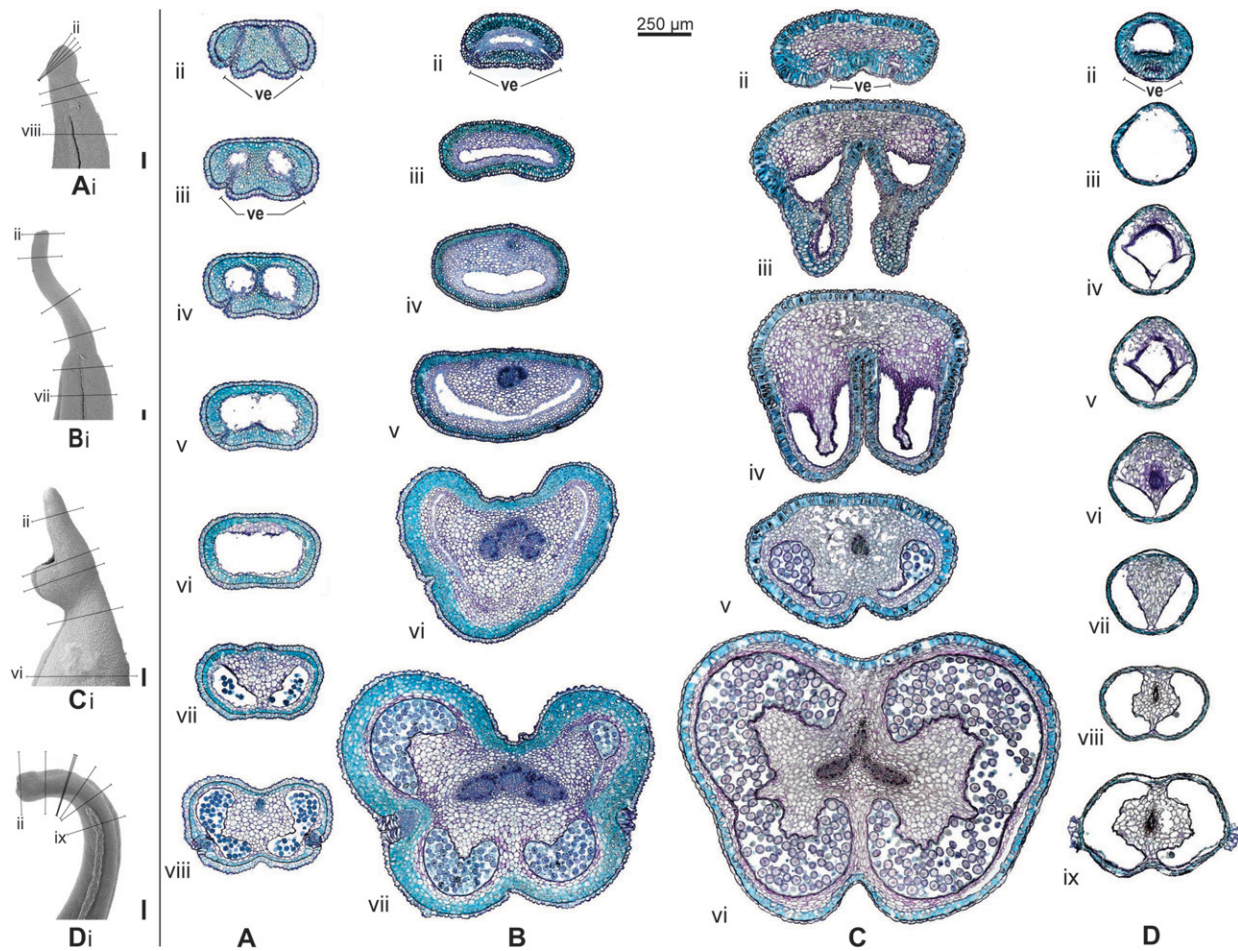
Fig. 6 Transverse section series of anther tips of abaxial stamens in *Senna* species of clades VI and VII. *A, Senna chacoënsis* (VI); *B, S. tonduzii* (VI); *C, S. cernua* (VIIa); *D, S. villosa* (VIIb). Ventral side (*ve*) of anther on left side in SEM micrographs and on lower side or as indicated in transverse sections. Lines on SEM micrographs indicate the approximate level of the transverse sections. Scale bars = 250 μm.

inward and the thecae apically not confluent. In contrast, *S. surattensis* has all stamens fertile, as typical of species of subclade IVa (*Senna* sect. *Psilorhegma*). The anther tips of abaxial stamens in *S. surattensis* are in fact similar to those of other studied species of subclade IVa (e.g., *S. aciphylla*, figs. 3*B*, 4*B*).

Anther Dehiscence Pattern of Middle Stamens

The two posterior middle stamens belong to the inner androecial whorl, and the two anterior middle stamens belong to the outer whorl. These four stamens are similar in shape and size, and the anther tip is usually not elongated, in contrast to the abaxial stamens (see "Anther Dehiscence Pattern of Abaxial Stamens"), and the thecae are not confluent in most species.

Clade I. Anthers have two apical slits (*S. polyantha*, fig. 7*A*; *S. silvestris* var. *guaranitica*) or a single pore by confluence (*S. siamea*, fig. 7*B*). The anther tip is bent in such a way that the pores point more or less outward. The lateral furrow is continuous (*S. polyantha*, fig. 7*A*), not continuous (*S. silvestris* var. *guaranitica*), or absent (*S. siamea*, fig. 7*B*).

Clade II. Anthers have two apical pores (see fig. 7*C*–7*E*). The pores point inward (*S. italica*, fig. 7*E*), or the anther tip is bent in such a way that the pores point either more or less upward (*S. nicaraguensis*, fig. 7*C*) or outward (*S. paradictyon*, fig. 7*D*). The lateral furrow is continuous. In *S. paradictyon*, the anther tip is elongated, and the thecae may thus be confluent and form a shared chamber, as in the abaxial stamens (see above).

Clade III. Anthers have two apical pores (see fig. 7*F*, 7*G*). The pores point outward. The lateral furrow is continuous.

Clade IV. Anthers have two apical pores (species of subclade IVa, species of subclade IVb: *S. dariensis, S. macranthera* var. *nervosa*, fig. 7*H*; *S. mucronifera*, fig. 7*I*) or a single pore by confluence of two (*S. skinneri*, fig. 7*J*). The lateral furrow is continuous (species of subclade IVa) or not continuous (*S. skinneri*, fig. 7*J*; species of subclade IVb, see fig. 7*H*, 7*I*). In species of subclade IVa, anthers of the middle stamens are similar to those of the abaxial stamens.

Clade V. Anthers have two apical pores (see fig. 7*K*). The pores point more or less upward or outward. The lateral furrow is continuous.
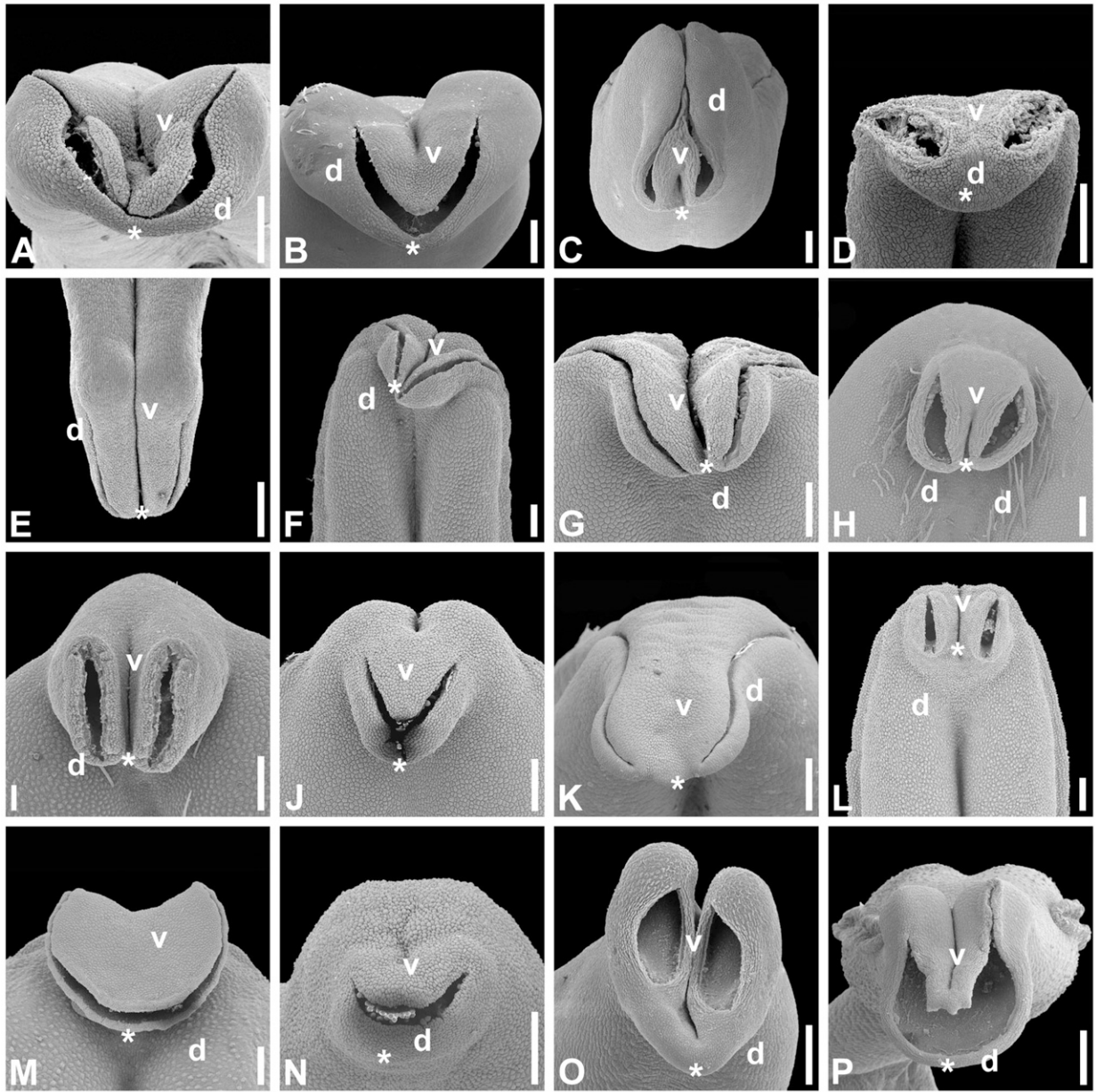
Fig. 7 Anther tips of middle stamens in *Senna* species of clades I–VII. *A, Senna polyantha* (I) (middle left stamen of inner whorl [iml]); *B, S. siamea* (I) (iml); *C, S. nicaraguensis* (II) (middle right stamen of outer whorl [omr]); *D, S. paradictyon* (II) (iml); *E, S. italica* (II) (middle right stamen of inner whorl [imr]); *F, S. unijuga* (III) (middle left stamen of outer whorl [oml]); *G, S. wislizeni* (III) (omr); *H, S. macranthera* var. *nervosa* (IVb) (iml); *I, S. mucronifera* (IVb) (iml); *J, S. skinneri* (IV) (imr); *K, S. cana* var. *calva* (V) (oml); *L, S. multijuga* var. *multijuga* (VI) (imr); *M, S. aversiflora* (VI) (omr); *N, S. tonduzii* (VI) (omr); *O, S. hirsuta* var. *leptocarpa* (VIIa) (iml); *P, S. pendula* (VIIa) (iml). *v* = ventral anther side; *d* = dorsal anther side. End of anther tip marked with asterisk. Scale bars = 250 μm.

Clade VI. Anthers have two apical pores (*S. acuruensis* var. *acuruensis, S. aphylla, S. chacoënsis, S. crassiramea, S. holwayana, S. multijuga* var. *lindleyana* and *var. multijuga*, fig. 7L; *S. rigida, S. robiniifolia,* and *S. williamsii*) or a single, more or less U-shaped pore by confluence of two (*S. acuruensis* var. *catingae, S. aversiflora,* fig. 7M; *S. pallida, S. tonduzii,* fig. 7N). The pores point outward. The lateral furrow is continuous (*S. crassiramea, S. holwayana, S. pallida*) or not continuous (see, e.g., *S. multijuga* var. *multijuga,* fig. 7L; *S. tonduzii,* fig. 7N).

Clade VII. Anthers have two apical pores (species of subclade VIIa: *S. barclayana, S. cernua, S. corymbosa, S. hirsuta,* fig. 7O; *S. occidentalis, S. purpusii*) or a U- or V-shaped single pore by confluence of two (species of subclade VIIa: *S. birostris* var. *hookeriana, S. hilariana, S. morongii, S. pendula,* fig. 7P; *S. septemtrionalis*; species of subclade VIIb). The lateral furrow runs only along the side and is not connected to the pore (see fig. 7O, 7P), except in *S. corymbosa.* The thecae are confluent in subclade VIIb, forming a shared chamber as in the abaxial stamens (see above).

Species of uncertain affinity. Anther tips of *S. chrysocarpa* are similar to anther tips of species of subclade IVb (especially to *S. macranthera* var. *nervosa*), with two dehiscence pores pointing outward and the lateral furrow not connected to the pores but only along the sides of the thecae. Anther tips in *S. hebecarpa* are similar to those of species of subclade VIIb (especially to *S. purpusii*), with two dehiscence pores pointing outward. Anther tips in *S. surattensis* are similar to those of other species studied of subclade IVa, with two dehiscence pores.

Staminodes

In most *Senna* species except for sect. *Psilorhegma*, the three adaxial stamens are sterile (i.e., do not produce pollen), and in some species of subclade VIIa, the median abaxial stamen is also sterile. This median staminode is of different form than the other staminodes. The two (adaxial) lateral staminodes belong to the outer androecial whorl, whereas the median one belongs to the inner whorl. These staminodes have rudimentary, flattened anthers (fig. 8). In the two lateral staminodes, anthers may be asymmetric, since their two thecae are of different lengths (e.g., fig. 8A, 8B, 8D–8G, 8J, 8N), but the median staminode is usually symmetric (e.g., fig. 8C, 8H, 8I, 8K–8M, 8O, 8R). The staminodes may be arrowlike (e.g., fig. 8C, 8H, 8O), spatulate to petaloid (e.g., fig. 8F, 8G, 8I, 8S), more or less strongly twisted (fig. 8E, 8Q), or highly reduced (e.g., fig. 8P, 8T). The lateral furrow is more or less conspicuous.

Development of Filament Union and Lateral Furrow

In *S. tonduzii*, the stamens are united (fig. 9); this feature is here reported for the first time in the genus *Senna*. The filaments of the (adaxial) staminodes are united, and the left and right lateral staminodes are congenitally united with the filaments of the left and the right middle stamens, respectively. These latter ones are also united pairwise with each other. Filament union extends almost up to the attachment of the anthers. The organs appear still free in young buds (fig. 9A). Union originates by zonal growth of the common base of the organs, appearing first between lateral staminodes and middle stamens of the inner androecial whorl (fig. 9B) and then also between the other organs (fig. 9C). In contrast, the abaxial stamens remain free (fig. 9D).

In many species, the lateral furrow in mature stamens is not connected to the dehiscence pores (see above). We studied the development of abaxial stamens in one such species, *S. mucronifera* of clade IVb, to explore the extension of the lateral furrow before anther tip elongation. When the anther tip starts to elongate, the lateral furrow—in the form of a weak depression—still extends along the entire length of the thecae (fig. 9E). This weak lateral depression disappears along the sterile anther tip during the elongation of the anther tip, whereas it remains along the sides of the thecae, becoming deeper until the mature lateral furrow is formed (fig. 9F).

Stigma

Either the stigmatic surface in *Senna* is enclosed in a chamber (i.e., chambered stigma; species of clades II–VII; fig. 10C–10G, 10I–10L; fig. 11B–11I, 11L) or it is exposed (i.e.,

craterlike stigma; species of clades I, IVa, V, VIIa; fig. 10A, 10B, 10H; fig. 11A, 11J). Chambered stigmas point usually inward (fig. 10C–10G, 10I–10L; fig. 11C–11I, 11K; *S. armata* of subclade VIIb) or rarely upward (*S. cana* var. *calva*, fig. 11B). Craterlike stigmas also point either inward (fig. 10A, 10B, 10H) or upward (fig. 11A, 11L). In general, the stigmatic orifice may be more or less punctiform (i.e., almost closed; fig. 10E, 10F, 10J), circular (fig. 10C, 10H, 10K, 10L; fig. 11A, 11E, 11F, 11H, 11I, 11L), elliptical (fig. 10A, 10B, 10G; fig. 11B–11D, 11G, 11J, 11K), or slitlike (fig. 10I). The diameter of the orifice of chambered stigmas varies from almost 0 (*S. mollissima*, fig. 10F) to ca. 1.4 mm (*S. quinquangulata*; measured on fig. 10K). One layer of hairs (species of clades IV–VII; fig. 10G, 10H, 10J–10L; fig. 11A–11H, 11K) or several layers (species of clades I, VIIa; fig. 10A, 10B; fig. 11J) may fringe the stigmatic orifice, or hairs are absent (species of clades II, III, IVa; figs. 10C–10F, 10I; fig. 11I, 11L). Secretion is especially visible and copious in the SEM samples of species with a craterlike stigma and several layers of fringing hairs (fig. 10A, 10B; fig. 11J) but is more or less obvious also in other craterlike stigmas (figs. 10H, 11A) and in chambered stigmas (fig. 10E, 10F; fig. 11B, 11C, 11E, 11F, 11H, 11K, 11L). Secretion may be produced also from the stigma of *S. macranthera* var. *nervosa*, since a large amount of pollen grain seems to stick on its stigmatic orifice (fig. 10L), and even from stigmas of species whose SEM samples apparently lack the secretion (fig. 10C, 10G, 10I–10K; fig. 11D, 11G, 11I), since it might have been washed out during the preparation of the samples.

Discussion

Diversity of the Stamens

Androecia of buzz-pollinated flowers are characterized by different types and degrees of structural specialization associated with the unusual pollination mode (see Buchmann 1983). The androecium of *Senna* is probably one of the best examples to illustrate this specialization. Although only a few species of Cassiinae were studied previously, *Senna* has been regarded as the genus with the greatest androecial diversity among Cassiinae (Venkatesh 1957; Lasseigne 1979). Our results support and extend this view. We found that diversity in the androecium of *Senna* species pertains to (1) patterns of heteranthery (including anther fertility and size difference among sets of fertile stamens), (2) dehiscence patterns (i.e., two pores or slits, or one pore by confluence of two; presence or absence of a shared chamber and separate canals; and extension of the vascular bundle), (3) pointing direction of the pores (i.e., inward, upward, or outward), (4) extension of the lateral furrow (i.e., from the ends of the dehiscent pores along the entire thecae or only along the sides of the thecae), (5) cell wall thickening patterns in the anther tip, and (6) stamen union. We also observed diversity in the form of anther base, but since it is not directly related to anther dehiscence, we do not discuss it further here.

Heteranthery in *Senna*, with up to four kinds of stamens, has fascinated researchers for a long time (e.g., Burk 1906). We investigated the variation observed in fertility versus sterility (i.e., no pollen production), presence or absence of
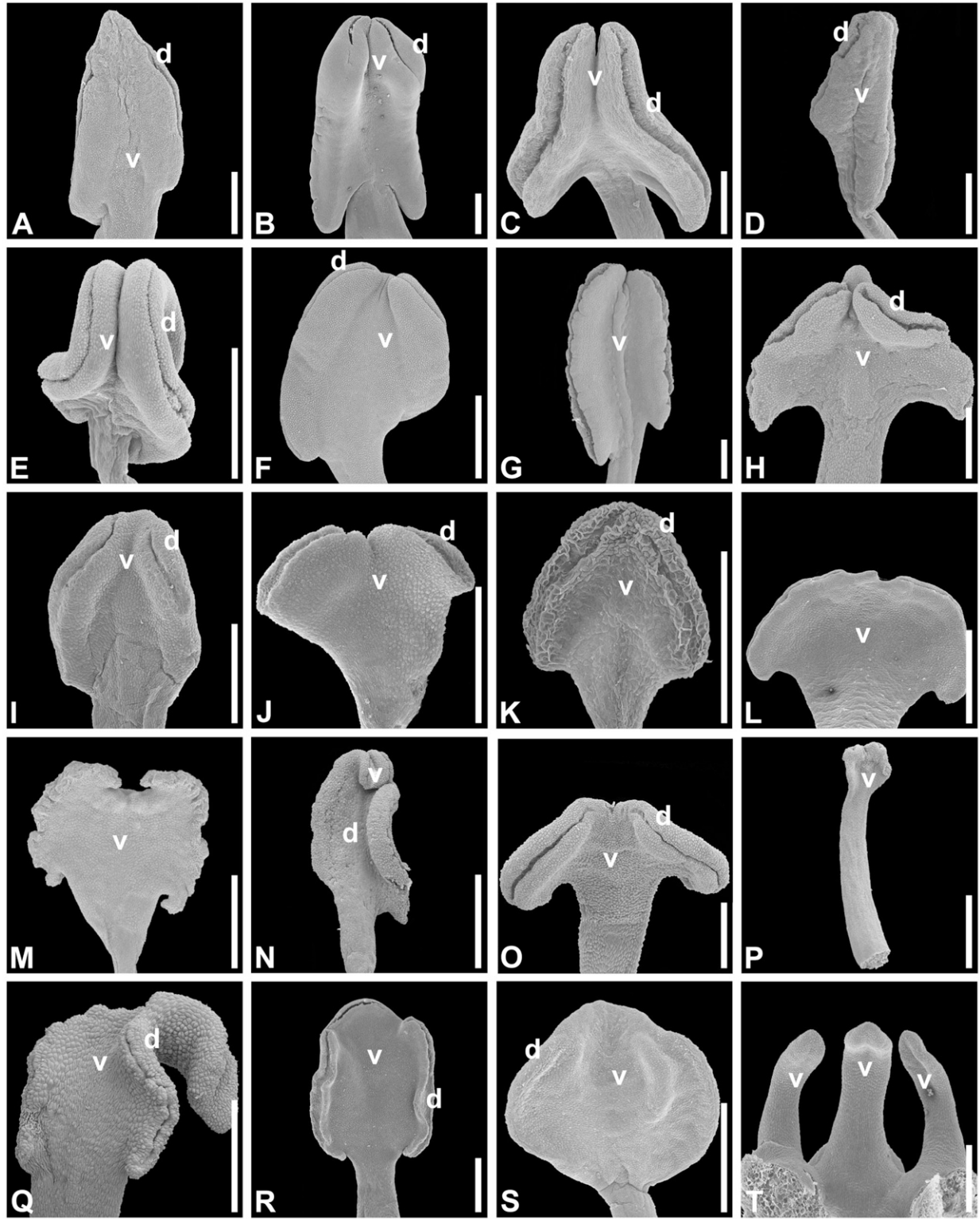
Fig. 8 Staminodes of *Senna*. *A*, *Senna polyantha* (I); *B*, *S. silvestris* var. *guaranitica* (I); *C*, *S. nicaraguensis* (II); *D*, *S. paradictyon* (II); *E*, *S. italica* (II); *F*, *S. atomaria* (III); *G*, *S. wislizeni* (III); *H*, *S. unijuga* (III); *I*, *S. skinneri* (IV); *J*, *S. artemisioides* (IVa); *K*, *S. mucronifera* (IVb); *L*, *S. hayesiana* (IVb); *M*, *S. macranthera* var. *nervosa* (IVb); *N*, *S. cana* var. *calva* (V); *O*, *S. tonduzii* (VI); *P*, *S. holwayana* (VI); *Q*, *S. pallida* (VI); *R*, *S. chacoënsis* (VI); *S*, *S. hirsuta* var. *leptocarpa* (VIIa); *T*, *S. villosa* (VIIb). *v* = ventral anther side; *d* = dorsal anther side. End of anther tip marked with asterisk. Scale bars = 500 µm for *A–J*, *L–T*; scale bar = 250 µm for *K*.
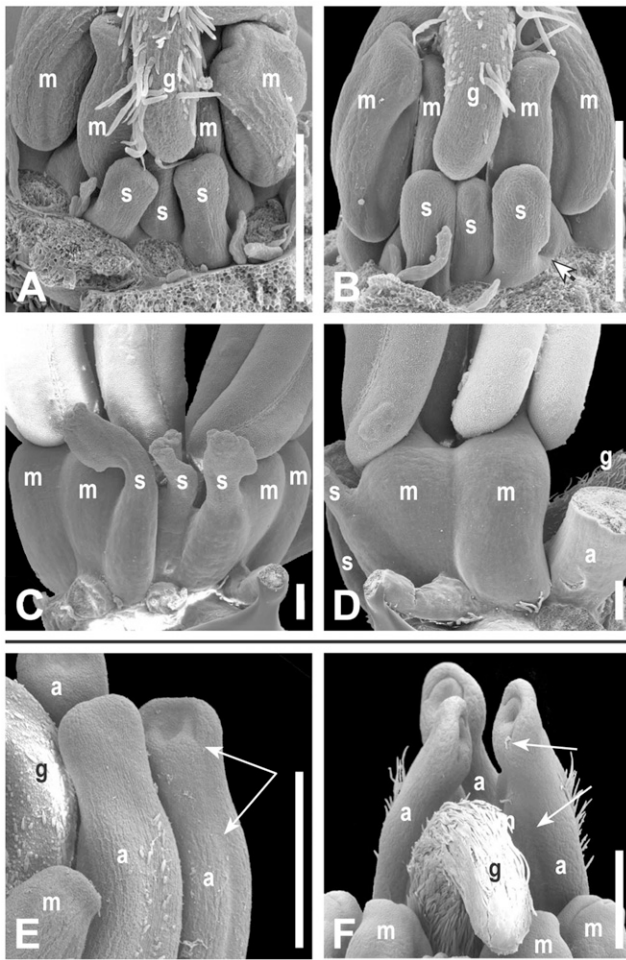
Fig. 9 Filament union and development of discontinuous anther lateral furrow in *Senna*. *A–D*, Filament union in *Senna tonduzii*. *A*, Adaxial view of free filaments in young bud. *B*, Adaxial view of later stage of development, with beginning filament union indicated by arrowhead. *C*, Adaxial view of united filaments at anthesis. *D*, Lateral view of united versus free filaments at anthesis. *E*, *F*, Development of discontinuous lateral anther furrow in *S. mucronifera*. *E*, Adaxial view of young abaxial stamens; continuous lateral furrow indicated by united arrows. *F*, Lateral view of abaxial stamens at older stage; lateral furrow not connected to the pores indicated by separate arrows. Petals removed in all images. *a* = abaxial stamen; *g* = gynoecium; *m* = middle stamen; *s* = staminode. Scale bars = 500 μm.

organs, and size difference between middle and abaxial anthers. We found seven patterns (fig. 12, *i–vii*): (*i*) all stamens fertile (subclade IVa; in some species, one lateral abaxial stamen may be larger than the other ones); (*ii*) seven fertile stamens and three adaxial staminodes (species of clades I–VII), in which all abaxial stamens are longer than the middle stamens and their anthers are of similar size (clades IIIa, VI, VIIa); (*iii*) the median abaxial stamen is smaller than the lateral ones but still longer than the middle ones (clades I, II); (*iv*) the abaxial and the middle stamens are of similar size (clades IIIb, IVb, VIIb); (*v*) only six fertile stamens, in which the abaxial stamens are longer than the middle ones, and three adaxial and the median abaxial organs staminodial

(clade VIIa); (*vi*) similar to pattern *v*, but the median abaxial organ is absent (clades VIIa); (*vii*) only four middle stamens are fertile, and six organs are staminodial, falling off at anthesis (*Senna hayesiana*, subclade IVb). It should be noted that in pattern *ii* of clade VI, although the anthers of the abaxial stamens are of similar size, their filaments may be of different lengths, and the stamens can thus have different sizes.

Anthers display diverse dehiscence patterns. They may have two dehiscent slits or pores or a single pore by confluence of two, which is more or less V or U shaped. The anther tip (i.e., upper portion of anthers, above the pollen sacs) may be elongated, especially in abaxial stamens, and the thecae may be apically confluent, forming a shared chamber. The pollen thus traverses the chamber and is released by passing through two separate canals and then the pores or directly either by the two separate pores or the single pore by confluence of two. The shared chamber and the canals are formed by decay of the tissue in the sterile anther tip, called resorption tissue by Venkatesh (1957). The vascular bundle more or less extends into the sterile anther tip. According to the number of pores or slits, presence or absence of a shared chamber and of separate short canals, and the extension of the vascular bundle, we found six patterns in *Senna* (fig. 12, patterns *A–F*): (*A*) two separate pores, no shared chamber, vascular bundle almost or up to the anther tip; (*B*) two separate pores, shared chamber and separate canals present, vascular bundle almost to the chamber; (*C*) two separate pores, shared chamber present but canals absent, vascular bundle almost to the chamber; (*D*) a single pore by confluence of two, no shared chamber, vascular bundle almost to the single pore; (*E*) a single pore by confluence, shared chamber and separate canals present, vascular bundle almost to the single pore; and (*F*) a pore by confluence of two, shared chamber present but canals absent, vascular bundle almost to or into part of the chamber.

The morphologically least differentiated pattern *A* (two separate pores and separate thecae) is present at least partially in almost all clades except clades II and VI (fig. 12). Pattern *D*, with one pore by confluence and separate thecae, is restricted to a few species in clades I, IV, and VII. Clades with even more specialized patterns, characterized by a shared chamber (gray boxes in fig. 12), are in some cases sister to clades characterized by the least differentiated pattern *A*. Patterns *B* and *E*, with canals present, may be considered as morphologically intermediate between the least differentiated patterns *A* and *D* and the most specialized patterns *C* and *F*.

Among the sets of fertile stamens, the abaxial stamens display all six patterns, whereas the middle ones, likely because of no anther tip elongation, apparently display mostly patterns *A* and *D*, which lack the shared chamber. Exceptions are species of subclade VIIb, in which all anthers display pattern *F*, and possibly certain species of clade IVb, where microtome sections by Venkatesh (1957) show that middle stamens of *S. obtusifolia* have a shared chamber but no anther tip elongation. The unusual tubular anther tips, up to several millimeters long in some species of clades IVb and, especially, VI, all have a shared chamber (patterns *B*, *C*, *E*, *F*), and most have a single pore by confluence (patterns *E*, *F*). Therefore, apical confluence of the thecae into a shared chamber seems to be a precondition for the tubular anther
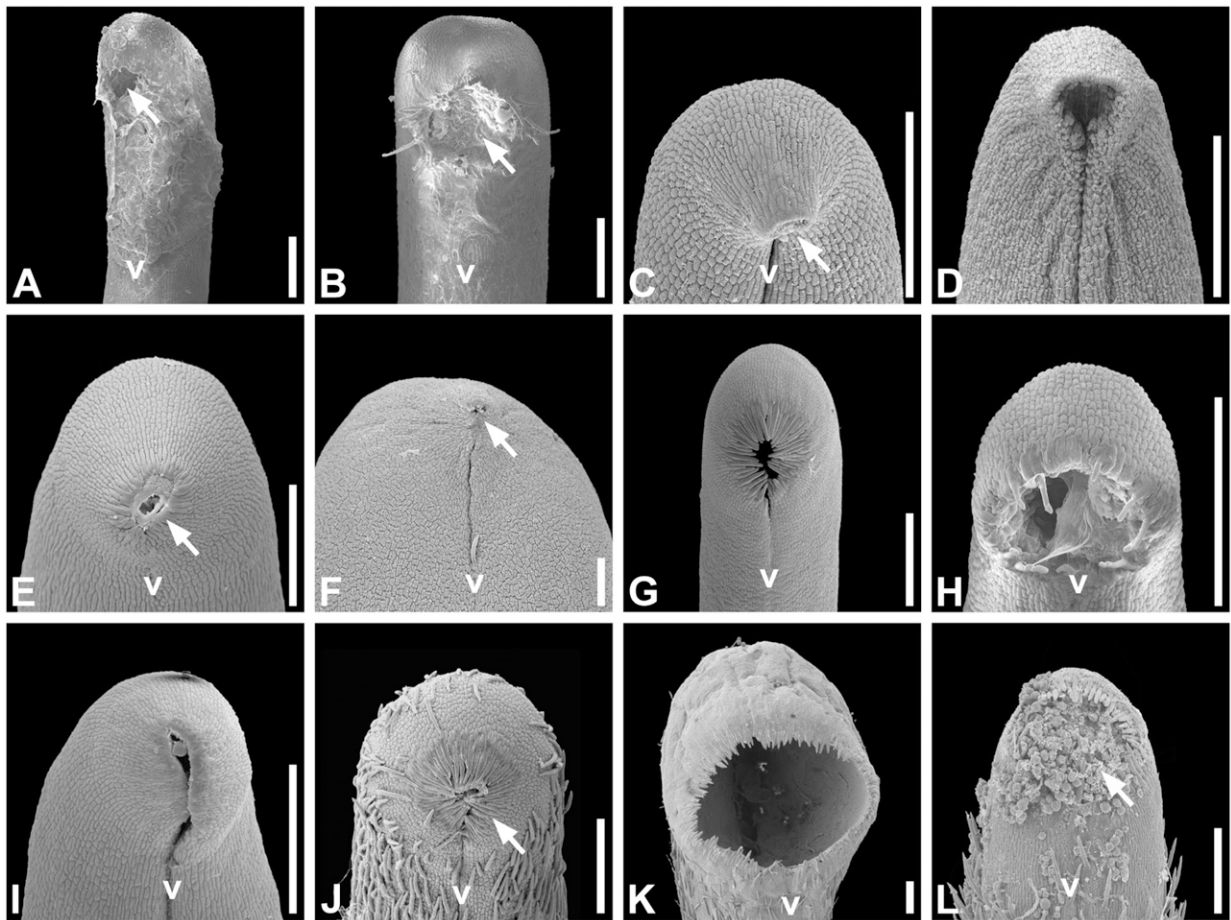
Fig. 10 Stigmas of *Senna* species of clades I–IV. *A, Senna polyantha* (I); *B, S. siamea* (I); *C, S. paradictyon* (II); *D, S. nicaraguensis* (II); *E, S. wislizeni* (III); *F, S. mollissima* (III); *G, S. skinneri* (IV); *H, S. artemisioides* (IVa); *I, S. aciphylla* (IVa); *J, S. mucronifera* (IVa); *K, S. quinquangulata* (IVb); *L, S. macranthera* var. *nervosa* (IVb). *v* = ventral anther side; *d* = dorsal anther side. Stigmatic orifice or surface indicated by arrow. Scale bars = 250 µm.

tip elongation. In addition to anther dehiscence, middle and abaxial stamens may also differ in size. We found the following patterns: the abaxial stamens are much longer and more arched than the middle stamens and have (i) a different pattern of anther dehiscence, usually corresponding to one of the specialized patterns B, C, E, or F (clades II, IVb, VI, and VIIa; fig. 12), or, more rarely, (ii) the same pattern of dehiscence (clades I, IIIa), or (iii) middle and abaxial stamens are of approximately the same size and share the same dehiscence pattern, which often corresponds to the less differentiated patterns A and D (subclades IIIb, IVa, IVb; fig. 12).

We found three more or less distinct pointing directions of the pores (fig. 12, *a–c*): (*a*) inward (pores on the ventral anther side), (*b*) upward (pores on the anther top), and (*c*) outward (pores on the dorsal anther side). According to these patterns, pores pointing inward are directed toward the flower center, whereas pores pointing upward or outward are directed away from the flower center. Therefore, the pointing direction of the pores directly affects the direction of pollen release during buzzing.

Three different types of lateral furrows can be identified in the anthers (fig. 12, *1–3*): (*1*) a continuous lateral furrow,

i.e., the lateral furrow running from the lower end of each dehiscence slit or pore along the entire length of the theca (species of clades I–VI); (*2*) a noncontinuous lateral furrow, i.e., present only along the sides of the thecae between the pollen sacs (species of clades I, II, IV, VI, VII); or (*3*) no lateral furrow, i.e., with the anther sides smooth (a few species of clades I, IV). Evidence from our developmental studies on *S. mucronifera* (fig. 9E, 9F), indicates that a noncontinuous lateral furrow (pattern *2*) appears to have the same early developmental pathway as the continuous lateral furrow (pattern *1*). At the beginning of the anther tip elongation in young bud, the lateral furrow appears as a weak depression running from the end of the pore along the entire length of the thecae, resembling an early stage of pattern *1* (fig. 9E). This weak depression successively disappears in the part of the elongating sterile anther tip, while it develops further along the sides of the thecae (fig. 9F).

As in many other buzz-pollinated angiosperms (Endress 1994, 1996), the endothecium is lacking, while hypodermal and subhypodermal cells are strongly thick walled. Venkatesh (1957) first described anther cell wall thickenings and the role of such cells during dehiscence. He recognized two kinds
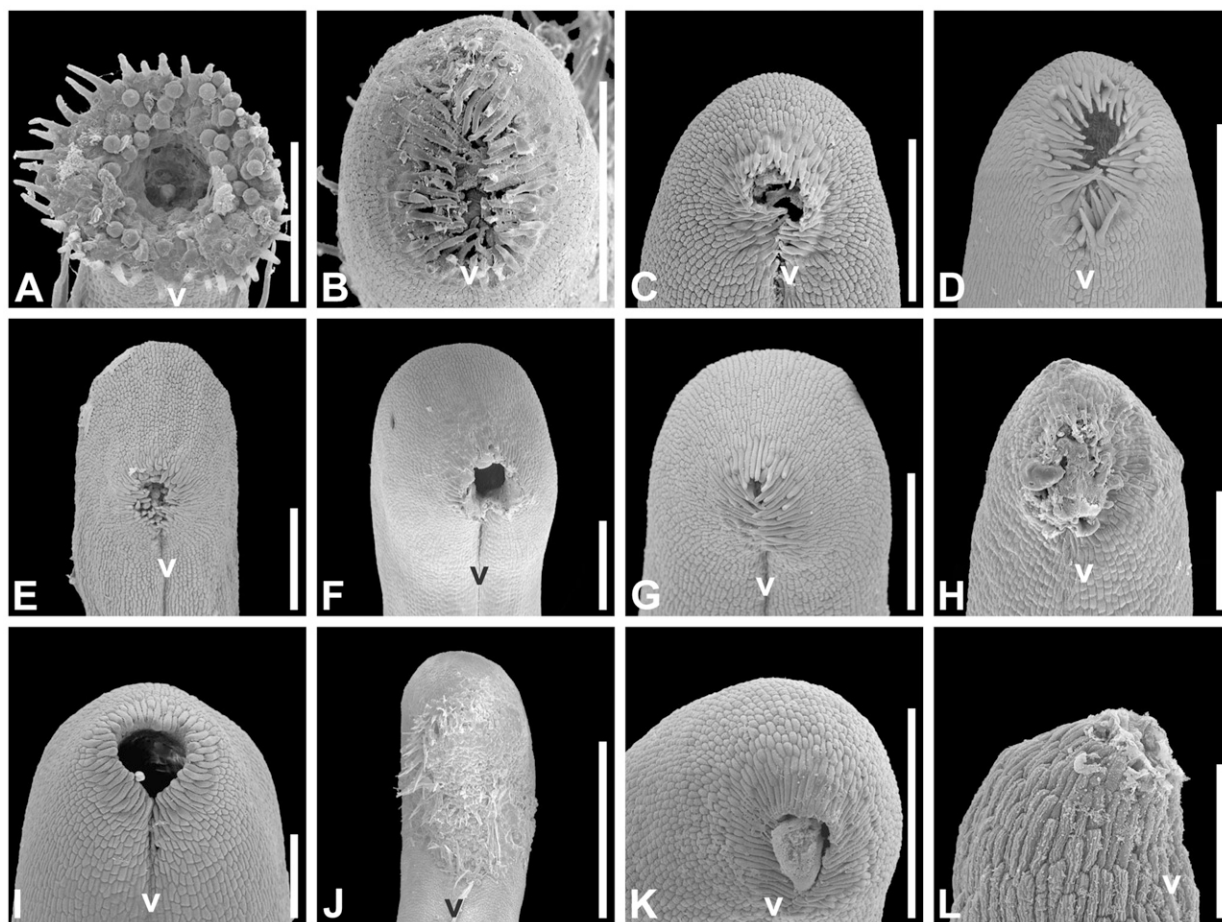
Fig. 11 Stigmas of *Senna* species of clades V–VII. *A, Senna uniflora* (V); *B, S. cana* var. *calva* (V); *C, S. williamsii* (VI); *D, S. pallida* (VI); *E, S. multijuga* var. *multijuga* (VI); *F, S. acuruensis* var. *acuruensis* (VI); *G, S. acuruensis* var. *catingae* (VI); *H, S. rigida* (VI); *I, S. aversiflora* (VI); *J, S. hirsuta* var. *leptocarpa* (VIIa); *K, S. pendula* (VIIa); *L, S. villosa* (VIIb). v = ventral anther side; d = dorsal anther side. Scale bars = 250 μm.

of thick-walled cells: a specialized kind of subhypodermal radially elongated cells, usually adjacent to the region of the stomium, and a nonspecialized kind of hypodermal and also subhypodermal cells in other anther parts. Although this study does not focus on thickening patterns, we observed a trend between the size of the anthers of abaxial stamens and the thickening pattern of anther walls. In large and stiff anthers, there are several layers of strongly thick-walled hypodermal and subhypodermal cells continuous all around the anther wall (e.g., *S. cana* var. *calva*, fig. 4D; *S. mucronifera*, fig. 4C; *S. nicaraguensis*, fig. 2C; *S. skinneri*, fig. 4A; *S. tonduzii*, fig. 6B). In contrast, in less stiff anthers (e.g., *S. paradictyon*, fig. 2B) or in small anthers (e.g., *S. aciphylla*, fig. 4B; *S. apiculata*, fig. 6D; *S. chacoënsis*, fig. 6A; *S. polyantha*, fig. 2A), there is only one layer of thick-walled hypodermal cells continuous all around the anther.

Reported here for the first time in *Senna*, filament union is a unique feature of *S. tonduzii* (fig. 9A–9D) and is rare in caesalpinioids. The filaments of the three staminodes and of the four stamens are united almost up to the attachment of the anthers, whereas the abaxial stamens are free (fig. 12, *vii*). United filaments occur also in flowers of species of the

distantly related caesalpinioid genera *Amherstia*, *Barnebydendron*, *Brownea*, *Eperua*, *Eurypetalum*, *Stemonocoleus*, *Baikiaea*, *Tessmannia*, *Sindoropsis*, *Sindora*, and *Tamarindus* (Endress 1994; Tucker 2000; Fougère-Danezan 2005; Detarieae s.l. clade; e.g., Wojciechowski et al. 2004). However, in these and especially in several papilionoids, filament union involves abaxial organs, while the adaxial median organ is free. Therefore, the pattern of filament union observed in *S. tonduzii* is unique in the family. Congenital union of the filaments is unusual in buzz-pollinated flowers, whereas postgenital union of the anthers is more common, for example, to form a tube as in *Lycopersicon* (see Endress 1994).

Diversity of the Stigma

Stigmas of buzz-pollinated flowers are often point-tipped (e.g., Endress 1994). The diversity observed in point-tipped stigmas of *Senna* species prompted Owens and Lewis (1989) to divide them into five groups. These groups were not congruent with the sectional boundaries of the current classification of *Senna* by Irwin and Barneby (1982) and were not considered in subsequent studies (Dulberger et al. 1994).
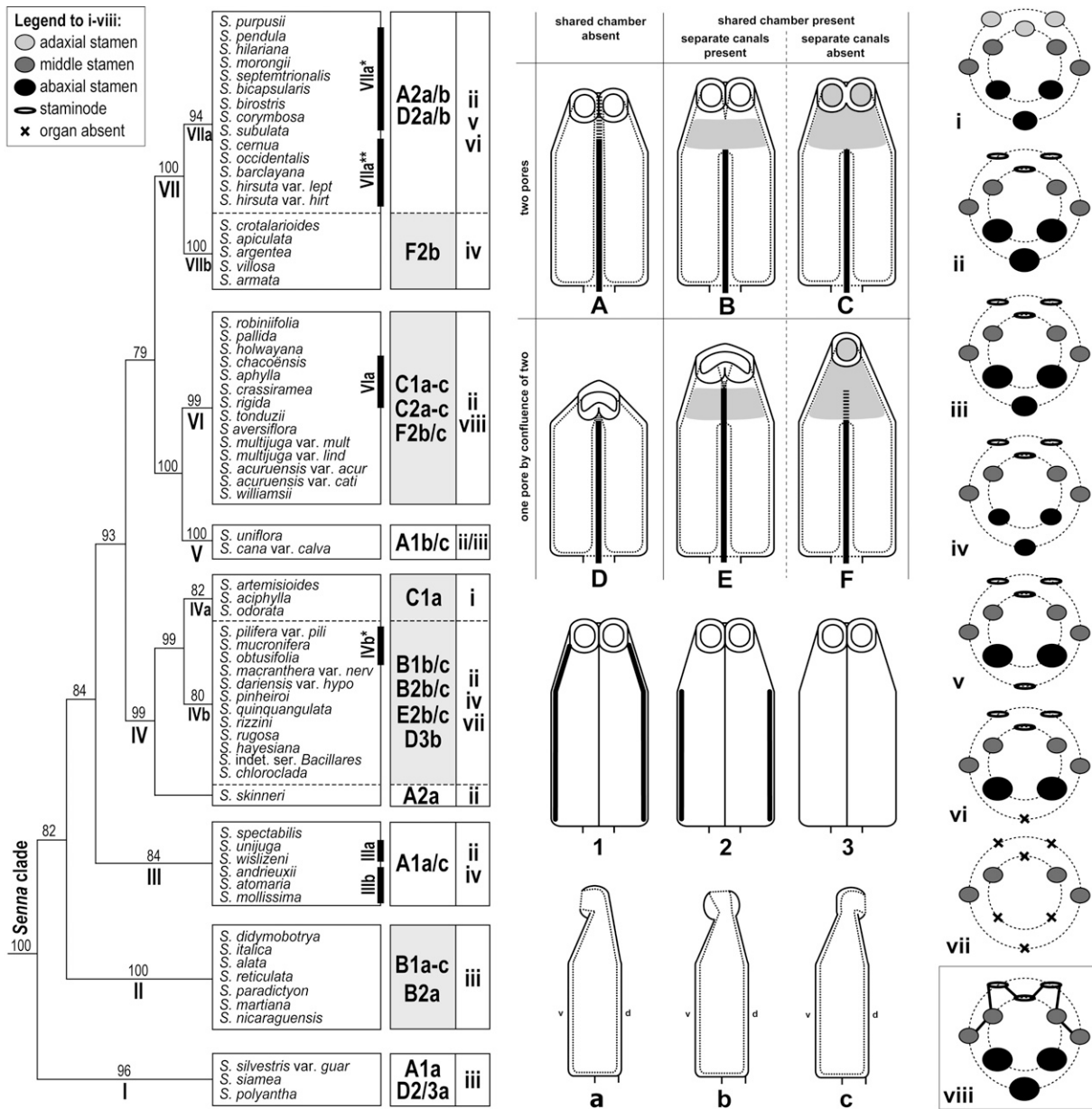
Fig. 12 Diagrams of different androecium patterns in *Senna* and their distribution on the molecular phylogenetic tree (simplified from Marazzi et al. 2006, their fig. 2). In the phylogenetic tree, bootstrap values are above and clade numbers below the branches. Major clades represented by boxes, subclades separated by dashed lines, and other major subclades supported in this study indicated by a vertical bold line. Relationships within clades or subclades not shown. Only species studied in this article are listed (species in the same order as in Marazzi et al. 2006, their fig. 2). In the boxes on the right of the tree, patterns shown in the diagrams are listed for clades and subclades. Gray boxes, patterns of anther dehiscence with shared chamber; open boxes, patterns lacking shared chamber. *A–F*, Patterns of anther dehiscence (area of shared chamber in gray; vascular bundle in black). *1–3*, Extension of the lateral furrow (bold line). *a–c*, Orientation of the anther tip and pointing direction of the dehiscence pore(s) or slits. *i–vii*, Patterns of heteranthery; *viii*, stamen union in *Senna tonduzii*.

Adding our results, diversity in the stigmas of *Senna* species includes presence or absence of a chamber (i.e., chambered vs. craterlike stigma); orifice position, form, size, and structure; and presence or absence of a secretion (figs. 10, 11). We did not find other kinds of stigmas in addition to the chambered and craterlike ones, but we observed a higher diversity in the position or pointing directions of the stigmas than pre-

viously recognized (Owens and Lewis 1989): inward in most chambered and several craterlike stigmas and upward in several craterlike stigmas but only in one chambered stigma.

Implication for Systematics of Senna

Molecular phylogenetic analyses of *Senna* (Marazzi et al. 2006) did not support the current classification system of

Senna based on Irwin and Barneby (1982), because most sectional boundaries (except for the monophyletic sect. *Psilorhegma*) were not congruent with the seven major clades and a number of subclades of the molecular phylogeny. Our study on anther tips, in particular of abaxial (pollinating) stamens, provides support for several of the clades and subclades of *Senna* (Marazzi et al. 2006). Anther tip morphology is constant in clades III and V (combination of patterns A1) and subclades VIIb and IVa (patterns F2b and C1a, respectively), whereas the remaining clades I, II, IV, VI, and VII have diverse anther dehiscence patterns (see fig. 12). The results of the morphological analyses presented here support most of the current series (Irwin and Barneby 1982) that are monophyletic in Marazzi et al. (2006) (see black longitudinal bars in fig. 12, from top). Subclade VIIa*, equivalent to ser. *Coluteoideae* but including also *S. birostris* of ser. *Stipulaceae*, is characterized by pattern D2; subclade VIIa**, equivalent to ser. *Basiglandulosae*, is characterized by A2; subclade VIa, equivalent to ser. *Aphyllae*, is characterized by C2b, lacking the long tubular anther tips of the other species of clade VI; subclade IVb*, equivalent to ser. *Trigonelloideae*, is characterized by E2; subclade IIIa, equivalent to ser. *Deserticolae*, is characterized by abaxial stamens longer than the middle ones; in contrast, subclade IIIb, equivalent to ser. *Isandrae*, is characterized by abaxial stamens as long as the middle ones.

On the basis of the combination of anther traits described in this study, we identified *S. chrysocarpa*, *S. hebecarpa*, and *S. surattensis*, the three additional species not considered in the molecular phylogeny of *Senna* (Marazzi et al. 2006), as probable members of subclade IVb, clade VII, and subclade IVa, respectively. Although molecular phylogenetic analyses are necessary to clarify the position of these species within these clades, anther traits proved to be taxonomically useful, as previously suggested (Venkatesh 1957; Lasseigne 1979; Irwin and Barneby 1982).

In most cases where multiple accessions were investigated, it was possible to conclude that patterns of anther dehiscence appeared to be consistent within species. However, in some species, especially those characterized by broad morphological variation and, hence, subdivided in multiple varieties by previous taxonomists, patterns of anther dehiscence were variable. For example, our structural results are consistent with the paraphyly of the *S. acuruensis* complex, as suggested by Marazzi et al. (2006). Abaxial stamens of *S. acuruensis* var. *acuruensis* are more similar to those of the sister species *S. multijuga*, with two dehiscence pores, than to those of *S. acuruensis* var. *catingae*, with a single U-shaped pore by confluence of two (fig. 5A, 5C, 5F). *Senna birostris* includes seven varieties (Irwin and Barneby 1982; Laporta et al. 2006). According to recent taxonomic work (Laporta et al. 2006), *S. birostris* var. *hookeriana* is distinguished from the newly described var. *jallaguensis* on the basis of the number of anther pores: two in var. *hookeriana* and a single pore in var. *jallaguensis*. Another species with different anther dehiscence patterns is *S. chloroclada* (subclade IVb): abaxial anthers of our Paraguayan accession have two apical slits, whereas those of the Argentinean accession have a single U-shaped pore by confluence (fig. 3D). However, unlike *S. acuruensis* and *S. birostris*, in *S. chloroclada*, these different patterns of anther dehiscence are not ascribed to different varieties.

Evolutionary Aspects of the Androecium

Previous interpretations of evolutionary trends in the floral morphology of *Senna* considered the fertility of all stamens (pattern *i*, fig. 12), typical of *Senna* section *Psilorhegma*, to be the ancestral condition within the genus (Irwin and Barneby 1982; Randell 1988). Molecular phylogenetic studies, however, did not support this hypothesis (Marazzi et al. 2006). In fact, the position of section *Psilorhegma*, embedded in clade IV, suggested that fertility of all stamens is a synapomorphy of subclade IVa and likely represents a derived condition in *Senna*. Conversely, an androecium formed by seven or fewer fertile stamens and three staminodes, a condition found in all remaining clades (patterns *ii–vii*, fig. 12), probably characterized the ancestral *Senna* flower. Character optimization studies are necessary to evaluate which of the patterns *ii–vii* may represent the ancestral condition of the androecium.

Anthers with a single pore were previously considered to be derived from anthers with two pores in *Senna* (Irwin and Barneby 1982; Randell 1988). Anthers with two pores (fig. 12, patterns *A–C*) occur in all species of clades II and III and in many species of the other major clades (I, IV–VII), whereas anthers with a single pore occur in some species of unrelated clades I, IVb, VI, VIIa*, and VIIb. However, anthers with two pores or a single pore do not necessarily represent the same pattern of anther dehiscence: two pores occur in patterns *A–C*, while a single pore occurs in patterns *D–F* (fig. 12). These patterns are distinguished from one another by other features, such as presence or absence of a shared chamber and short canals, in addition to the number of anther pores. This means that, for example, anthers with two pores of pattern *A* may not be homologous to anthers with two pores of pattern *C*. Therefore, structural differences among anther dehiscence patterns should be considered when reconstructing the evolution of the number of anther pores within a phylogenetic framework.

Implications for Pollination Biology and Floral Evolution

Several structural specializations of *Senna* flowers—including different kinds of stamens (middle feeding stamens vs. abaxial pollinating stamens), diverse pointing directions of the dehiscence pores, and different kinds of point-tipped stigmas—probably evolved in relation to buzz pollination. Our observation in *Senna* that anther tips of abaxial stamens are more diverse and more specialized than those of the middle stamens may be related to a separation of function between feeding anthers (with pollen used as food for bee larvae) and pollinating anthers (with pollen used for plant reproduction). The separation of function may in turn be related to the intrafloral behavior of the pollen-collecting bees. In particular, the positioning of the bees on the androecium may have influenced the evolution of anther morphology and of diverse strategies of pollen release.

Although little is known about the details of pollination biology in *Senna*, studies on a few species have shown that in order to vibrate the flowers, bees usually clasp with their legs the middle stamens only, which then eject the pollen toward the ventral side or the legs of the bees, while the abaxial anthers eject the pollen in other directions, such as toward the

flanks or the dorsal side of the bees (e.g., Delgado Salinas and Sousa Sánchez 1977; Gottsberger and Silberbauer-Gottsberger 1988; Westerkamp 2004). This situation is found in flowers that have middle and abaxial stamens of different sizes, where the bees clasp only the shorter anthers of the middle stamens and not the anthers of the much longer and often arched abaxial stamens. These abaxial stamens show the greatest anther tip diversity in *Senna*. In flowers with stamens of the same size, anther morphology is similar and usually of the least differentiated patterns *A* and *D* (fig. 12), and pollen release is always on the ventral side or on the legs of the bees. All anthers appear thus to function as feeding anthers. In these flowers, the bees may clasp not only the anthers of the middle stamens but also those of the abaxial stamens. Therefore, the clasping of anthers by the bees seems to have favored the evolution of rather unspecialized anther dehiscence patterns, releasing pollen to the ventral side and/or legs of the bees, where anther tip elongation is thus probably useless. In contrast, the abaxial stamens, which are not clasped by the bees, have undergone a remarkable divergent evolution of anther tip specializations. Of particular interest are the different pointing directions of their pores (i.e., inward, upward, outward; fig. 12, patterns *a–c*) and types of anther tip elongation, with important implications for pollen discharge and dispersal.

The orientation of the pores directly affects the direction of pollen release. Pores pointing inward likely release pollen toward the flower center and thus toward the body of the bees. In this case, the morphology of the anthers would allow control of the direction (downward or forward) of the ejected pollen flow. In contrast, pores pointing upward or outward likely release the pollen in the opposite direction. In this case, we expect the pollen to be ejected toward the petals, which should be positioned to reduce loss of pollen from the flower. The pollen flow is thus ricocheted by the petals toward the body of the bees (e.g., Delgado Salinas and Sousa Sánchez 1977; Westerkamp 2004).

Tubularly elongated sterile anther tips are rare in angiosperms but occur in numerous taxa with buzz-pollinated flowers. A tubular anther tip structure ensures a precise direction of pollen ejection, since only those pollen grains moving exactly in the direction of the tube will be released by the anthers (Buchmann and Hurley 1978). To our knowledge, in addition to *Senna*, tubular anther tips are present in four families, including Elaeocarpaceae/Tremandraceae (e.g., in *Platytheca galioides* and *Tetratheca thymifolia*: Matthews and Endress 2002), Ericaceae (e.g., in *Demosthenesia cordifolia*: Luteyn 1978; *Diplycosia* spp.: Sleumer 1967; *Vaccinium* spp.: Sleumer 1967; Danet 2005), Melastomataceae (e.g., *Tococoa* spp.: Michelangeli 2006), and Solanaceae (e.g., *Solanum mortonii*: Hunziker 2001; *Solanum quitoense*: B. Marazzi, personal observation; and *Solanum rostratum*: Todd 1882). The most specialized anther tips in *Senna* have apically confluent thecae, which form a shared chamber and may be variably elongated (up to several millimeters long) or constricted at their base (close to the fertile thecae). However, diverse kinds of elongation and constriction may also reflect different strategies of pollen discharge, which influence (1) the speed of the pollen flow ejected and/or (2) the amount of pollen released by the vibrations of the bees.

In the first case, anther tip elongation may funnel the pollen flow, causing it to be accelerated and ejected at a higher speed. This may be seen by blowing into the wider side of a horn: the airflow is compressed toward the narrower side of the horn and will thus be released with an increased speed. The decreasing lumen toward the narrower end causes compression of the pollen flow. An increase in the speed of the pollen flow would then allow the pollen to have enough speed to ricochet against the petals, which vibrate and contribute to rebound the pollen, finally adhering to the body of the bees. Flowers with long and tubular anthers with upward- and/or outward-pointing pores have strongly concave lower petals, which are often also highly modified in both shape and size. The corolla of these flowers is thus highly asymmetric. Work in progress will test whether there is a correlation between the strong petal modification and specialized patterns of anther dehiscence.

In the second case, the constriction between the thecae and the sterile anther tip in *Senna* may influence the amount of pollen released. The narrower the constriction, the smaller the amount of released pollen, a condition that favors the discharge of pollen in multiple, smaller packages and makes pollen available for successive pollinator visits. This mechanism, known as packaging, influences the amount of pollen presented on the anthers (Harder and Thomson 1989). Pollen packaging has been described, for example, in flowers of *Penstemon* s.l. (Scrophulariaceae; Castellanos et al. 2006). Anthers of bee-pollinated penstemons have narrower dehiscence openings and present their pollen more gradually than anthers of hummingbird-adapted relatives. Gradual pollen discharge should compensate the inefficiency of pollen delivery by frequent and wasteful pollinators, for example, bees, as compared to more efficient pollinators, for example, hummingbirds, which do not groom pollen from their body (Castellanos et al. 2003, 2006). However, experimental studies conducted on buzz-pollinated species (but not on *Senna* species) showed that pollen removal increased with flower age (e.g., Harder and Barclay 1994). In this mechanism, known as dispensing, pollen is gradually presented by restricting the amount of pollen removed from a package during a single visit (Harder and Thomson 1989).

Stiffness of anthers and filaments and filament union in *S. tonduzii* are other features that appear to have evolved in relation to buzz pollination, because they allow the bee to firmly clasp the anthers and improve the transmission of the vibrations from its body to all stamens, thus causing pollen release. We observed that anther wall thickening appears to be correlated with flower size. Abaxial anthers of small flowers tend to have only one layer of thick-walled cells, whereas those of larger flowers tend to have several layers (see above). The relation between anther wall thickenings and flower size may be interpreted as a consequence of the need of anthers to be sufficiently stiff to act as a resonating chamber for pollen release by buzzing and may be associated with particular features of the size of visiting bees and/or the intensity of vibrations. As Buchmann and Hurley (1978) showed that the intensity of vibrations increases with the size of the insect, we would expect bees of different sizes to be unequally efficient in pollen removal from the same flower. Measurements of pollen removal from *S. reticulata* showed that large bees removed

more pollen in one visit (ca. 15%–20% of total initial available pollen) than did smaller bees (Snow and Roubik 1987).

The role of the staminodes in buzz pollination is enigmatic. Gottsberger and Silberbauer-Gottsberger (1988) observed that bees sometimes bite the staminodes to avoid dropping out of the flowers while buzzing. However, they may also play a role in pollinator attraction. In fact, many *Senna* species display staminodes with flat, enlarged, and often yellow or brownish sterile anthers, e.g., in species of clades I, II, III, V, and VI and subclade VIIa. In contrast, the staminodes of other species apparently lack such a function, for they are highly reduced and rather cryptic (subclade IVb and some of clade VI).

Most of the diversity we observed in the stigma involves its shape (i.e., chambered or craterlike), the hairs fringing the stigmatic orifice, and presence or absence of secretion. The minute, point-tipped stigmas touch or approach such sites of the body of the bees where pollen is not groomed. Owens and Lewis (1989) suggested that the hairs fringing the stigmatic orifice may remove pollen from the bee's body, acting as a fine comb, while Dulberger et al. (1994) suggested that electrostatic forces may aid pollen capture and its adhesion to those stigmatic tips lacking hairs. However, while brushing and electrostatic forces may be instrumental for pollen transport to the stigma, they may not serve any special function to promote pollen movement into the stigmatic chamber, for which the vibrations of the bees appear to be necessary (Dulberger 1981; Dulberger et al. 1994; Laporta 2003; van Kleunen and Johnson 2005). Instead, the fringing hairs are probably a device for protecting the stigmatic secretion (Dulberger et al. 1994). Most stigmas are associated with a long and often strongly arched gynoecium, and the stylar tip may even be strongly inflected (Owens and Lewis 1989; Dulberger et al. 1994). We observed that most of these long gynoecia have a chambered or inward-pointing craterlike stigma. In contrast, upward-pointing craterlike stigmas appear to be associated with short gynoecia of species that are common weeds. These weeds have small and probably autogamous flowers (e.g., *S. uniflora*: Irwin and Barneby 1982; clade V, fig. 11*A*; and the group of *S. apiculata*, *S. argentea*, and *S. crotalarioides*, subclade VIIb). Dulberger et al. (1994) suggested that the diversity in inflection of the style tip and stigma position and orientation evolved in relation to the specific size and positioning of the bees visiting the flowers. Whether there is an additional correlation between the stigma type and breeding system has not yet been explored.

Acknowledgments

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