

## Phylogeny of prickly poppies, *Argemone* (Papaveraceae), and the evolution of morphological and alkaloid characters based on ITS nrDNA sequence variation

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Received June 15, 1998

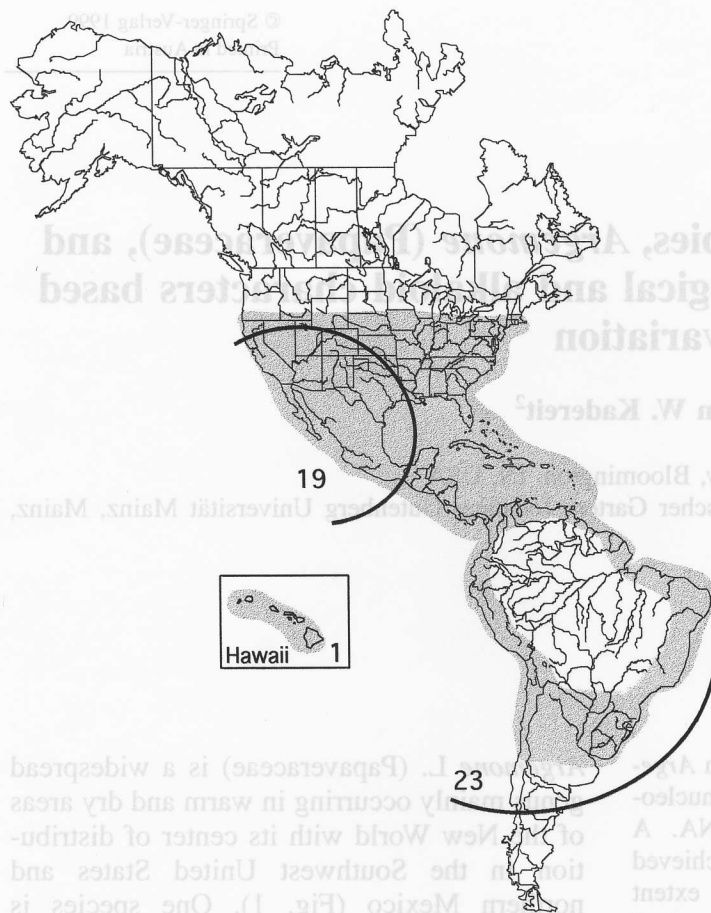
Accepted November 3, 1998

**Abstract.** Evolutionary relationships within *Argemone* (Papaveraceae) were inferred from nucleotide variation in nuclear ribosomal DNA. A complete representation of the genus was achieved by using herbarium material to a large extent (74%). Four distinct clades can be recognized based on the molecular results. The support for the different clades varies greatly due to a very uneven distribution of characters. Although some clades are largely unresolved, some unexpected relationships, for example the sister group relationship of the relict species *A. subintegrifolia* to the rest of *Argemone*, were found. The evolution of morphological and alkaloid characters against the background of the obtained phylogeny is discussed. Whereas the morphological characters provide some support for the phylogeny found, the alkaloid characters turned out not to contain much phylogenetic information. Past difficulties in recognizing phylogenetic relationships within *Argemone* result from largely continuous morphological variation and from parallelisms correlated with and probably caused by polyploidization.

**Key words:** Papaveraceae, Papaveroideae, *Argemone*. Polyploidy, alkaloids, nrDNA, ITS, molecular phylogeny.

*Argemone* L. (Papaveraceae) is a widespread genus mainly occurring in warm and dry areas of the New World with its center of distribution in the Southwest United States and northern Mexico (Fig. 1). One species is endemic to Hawaii and some weedy species are naturalized elsewhere. The genus comprises annual and perennial herbs or rarely shrubs with mostly incised leaves and is well defined by a combination of morphological characters. *Argemone* species have three (rarely four) sepals with conspicuously elongated sepal horns, a double 3-merous corolla, numerous (30–200) stamens, a paracarpous gynoeceum that is formed by 3–7 carpels, and capsular fruits that open by apical valves. Additionally, most parts of the plants are distinctively prickly (Fig. 2).

After a critical revision (Schwarzbach in prep., Table 1), the previously described 32 species (Ownbey 1958, 1961; Powell 1972; Johnston 1976; Sorarú 1976; McDonald 1991) were reduced to 24. Although the monophyly of *Argemone* has never been questioned, inter- and intrageneric relationships have been

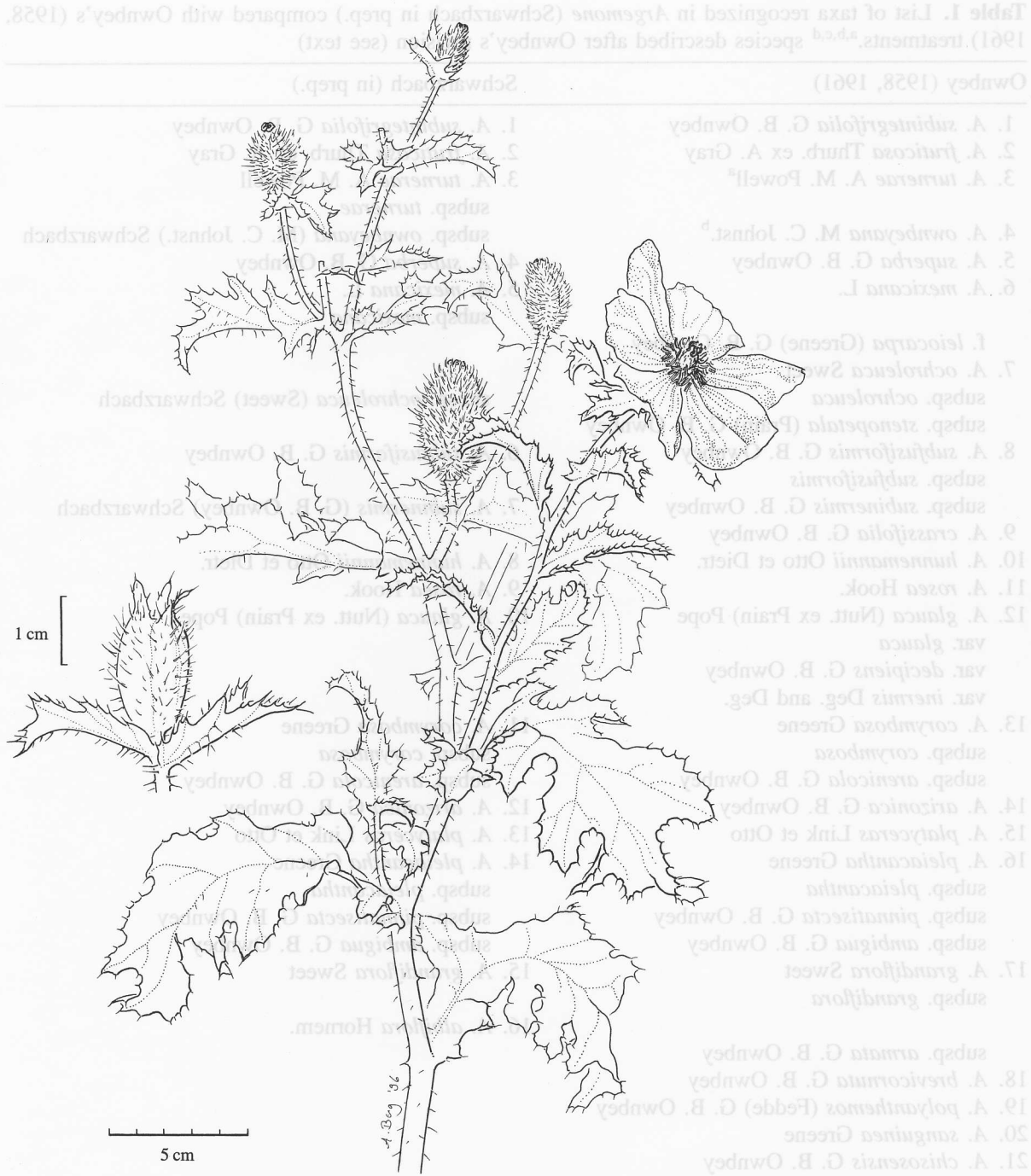


**Fig. 1.** Distribution of *Argemone* in the New World (shaded area). Numbers refer to number of species occurring within circled area

controversial. Ownbey (1958) proposed a close relationship of the genus to *Papaver* L. and *Meconopsis* Vig., which are distributed mainly in the Old World. Other studies based on morphological characters (Fedde 1909, 1936; Prain 1895; Kadereit et al. 1994) placed the New World genus *Romneya* Harv. close to *Argemone*. A previous RFLP study (Schwarzbach and Kadereit 1995), however, suggested that *Argemone* is sister group of *Arctomecon* Torr. and Frem., a genus comprising three species endemic to the Mojave desert in the southwestern United States. Within subfam. Papaveroideae, *Argemone* belongs to a clade which contains only New World genera. Besides *Argemone*, these are *Romneya*, *Arcto-*

*mecon*, *Canbya* Parry ex A. Gray, *Platystemon* Benth., *Hesperomecon* Greene and *Meconella* Nutt. in Torr. and A. Gray. The latter three were formerly included in subfam. Platystemonoideae (Ernst 1967, Kadereit 1993, Loconte et al. 1995) but have been shown to have evolved from within the Papaveroideae (Schwarzbach and Kadereit 1995). The entire group is sister to a clade of mainly Old World Papaveroideae, which comprises *Meconopsis*, *Papaver*, *Roemeria* Medik. and the monotypic New World *Stylomecon* G. Taylor.

The present study examines intragenetic relationships on the basis of nuclear ribosomal DNA (nrDNA) sequence data, which could not be resolved in an earlier chloroplast DNA



**Fig. 2.** *Argemone platyceras*. Insert shows a flower bud with conspicuous sepal horns

RFLP analysis (Schwarzbach and Kadereit 1995). The internal transcribed spacer (ITS) regions of the nrDNA have been shown in several studies to provide enough information

to resolve relationships at the species level (e.g. Baldwin 1993, Wojciechowski et al. 1993, Baldwin et al. 1995, Ray 1995, Wen and Zimmer 1996). Specifically, the relatively

**Table 1.** List of taxa recognized in *Argemone* (Schwarzbach in prep.) compared with Ownbey's (1958, 1961) treatments.<sup>a,b,c,d</sup> species described after Ownbey's revision (see text)

Ownbey (1958, 1961)	Schwarzbach (in prep.)
1. <i>A. subintegrifolia</i> G. B. Ownbey	1. <i>A. subintegrifolia</i> G. B. Ownbey
2. <i>A. fruticosa</i> Thurb. ex A. Gray	2. <i>A. fruticosa</i> Thurb. ex A. Gray
3. <i>A. turnerae</i> A. M. Powell <sup>a</sup>	3. <i>A. turnerae</i> A. M. Powell subsp. <i>turnerae</i>
4. <i>A. ownbeyana</i> M. C. Johnst. <sup>b</sup>	subsp. <i>ownbeyana</i> (M. C. Johnst.) Schwarzbach
5. <i>A. superba</i> G. B. Ownbey	4. <i>A. superba</i> G. B. Ownbey
6. <i>A. mexicana</i> L.	5. <i>A. mexicana</i> L. subsp. <i>mexicana</i>
f. <i>leiocarpa</i> (Greene) G. B. Ownbey	
7. <i>A. ochroleuca</i> Sweet subsp. <i>ochroleuca</i> subsp. <i>stenopetala</i> (Prain) G. B. Ownbey	subsp. <i>ochroleuca</i> (Sweet) Schwarzbach
8. <i>A. subfusiformis</i> G. B. Ownbey subsp. <i>subfusiformis</i> subsp. <i>subinermis</i> G. B. Ownbey	6. <i>A. subfusiformis</i> G. B. Ownbey
9. <i>A. crassifolia</i> G. B. Ownbey	7. <i>A. subinermis</i> (G. B. Ownbey) Schwarzbach
10. <i>A. hunnemannii</i> Otto et Dietr.	8. <i>A. hunnemannii</i> Otto et Dietr.
11. <i>A. rosea</i> Hook.	9. <i>A. rosea</i> Hook.
12. <i>A. glauca</i> (Nutt. ex Prain) Pope var. <i>glauca</i> var. <i>decipiens</i> G. B. Ownbey var. <i>inermis</i> Deg. and Deg.	10. <i>A. glauca</i> (Nutt. ex Prain) Pope
13. <i>A. corymbosa</i> Greene subsp. <i>corymbosa</i> subsp. <i>arenicola</i> G. B. Ownbey	11. <i>A. corymbosa</i> Greene subsp. <i>corymbosa</i> subsp. <i>arenicola</i> G. B. Ownbey
14. <i>A. arizonica</i> G. B. Ownbey	12. <i>A. arizonica</i> G. B. Ownbey
15. <i>A. platyceras</i> Link et Otto	13. <i>A. platyceras</i> Link et Otto
16. <i>A. pleiacantha</i> Greene subsp. <i>pleiacantha</i> subsp. <i>pinnatisecta</i> G. B. Ownbey subsp. <i>ambigua</i> G. B. Ownbey	14. <i>A. pleiacantha</i> Greene subsp. <i>pleiacantha</i> subsp. <i>pinnatisecta</i> G. B. Ownbey subsp. <i>ambigua</i> G. B. Ownbey
17. <i>A. grandiflora</i> Sweet subsp. <i>grandiflora</i>  subsp. <i>armata</i> G. B. Ownbey	15. <i>A. grandiflora</i> Sweet
18. <i>A. brevicornuta</i> G. B. Ownbey	16. <i>A. albiflora</i> Hornem.
19. <i>A. polyanthemos</i> (Fedde) G. B. Ownbey	
20. <i>A. sanguinea</i> Greene	
21. <i>A. chisosensis</i> G. B. Ownbey	
22. <i>A. albiflora</i> Hornem. subsp. <i>albiflora</i> subsp. <i>texana</i> G. B. Ownbey	
23. <i>A. burkartii</i> Sorarú <sup>c</sup>	
24. <i>A. arida</i> Rose	17. <i>A. arida</i> Rose
25. <i>A. squarrosa</i> Greene subsp. <i>squarrosa</i> subsp. <i>glabrata</i> G. B. Ownbey	18. <i>A. squarrosa</i> Greene
	19. <i>A. glabrata</i> (G. B. Ownbey) Schwarzbach

**Table 1** (continued)

26. <i>A. echinata</i> G. B. Ownbey	20. <i>A. echinata</i> G. B. Ownbey
27. <i>A. subalpina</i> J. A. McDonald <sup>d</sup>	21. <i>A. aurantiaca</i> G. B. Ownbey
28. <i>A. aurantiaca</i> G. B. Ownbey	22. <i>A. munita</i> Dur. et Hilg. subsp. <i>gracilentata</i> (Greene) Schwarzbach
29. <i>A. gracilentata</i> Greene	
30. <i>A. munita</i> Dur. et Hilg. subsp. <i>munita</i> subsp. <i>argentea</i> G. B. Ownbey subsp. <i>robusta</i> G. B. Ownbey subsp. <i>rotundata</i> (Rydb.) G. B. Ownbey	subsp. <i>munita</i> subsp. <i>argentea</i> G. B. Ownbey subsp. <i>robusta</i> G. B. Ownbey
31. <i>A. hispida</i> A. Gray	23. <i>A. rotundata</i> Rydb.
32. <i>A. aenea</i> G. B. Ownbey	24. <i>A. hispida</i> A. Gray hybrid between <i>A. albiflora</i> × <i>A. mexicana</i> ?

small size of ITS1 allowed us to amplify this marker from herbarium material DNA and to achieve almost complete representation of the genus. About 3/4 of the taxa were available only as herbarium material.

On the basis of our molecular phylogeny we will trace the evolution of morphological characters and demonstrate that the few characters with discontinuous variation available in the genus provide some support for the relationships found. We also address the question of whether the intrageneric groups proposed on the basis of alkaloid contents (Foote 1932; Slavík and Slavíková 1963; Stermitz and Seiber 1966; Stermitz 1967, 1968; Stermitz et al. 1969, 1971, 1973a, b, 1974; Stermitz and Coomes 1969; Bandoni et al. 1972, 1975; Benn and Mitchell 1972; Coomes et al. 1973; Stermitz and Williams 1973) are in any way consistent with our findings.

In comparison to earlier hypotheses about species relationships in *Argemone* our study revealed some patent cases of morphological parallelism. These and their possible causes are discussed.

## Materials and methods

**Taxa and outgroup selection.** Species delimitation in *Argemone* has been problematic. Based on morphological characters and geographical distri-

bution, all 24 species and all except one subspecies were sampled and altogether 43 individuals of *Argemone* were sequenced (Table 2). Geographically widespread and morphologically variable taxa (*Argemone albiflora*, *A. mexicana*, *A. rotundata*) were represented by several individuals from different populations. At least one representative of all other clades previously found (Kadereit et al. 1994, Schwarzbach and Kadereit 1995, Hoot et al. 1997) within the subfamily Papaveroideae was included in the study. *Papaver rhoeas* L. and *Meconopsis delavayi* (Franch.) Franch ex Prain were chosen as representatives of a well-supported 'Old World' clade (Jork and Kadereit 1995) and *Arctomecon humilis* Coville, *Romneya coulteri* Harv. and *Canbya aurea* S. Wats. as 'New World' representatives. *Bocconia frutescens* L. and *Macleaya cordata* (Willd.) R. Br. as members of a different subfamily (*Chelidonioideae*, Blattner and Kadereit in press), were used as outgroup. Altogether ITS sequences for 50 individuals were analyzed. Of the 43 *Argemone* individuals one was grown in the greenhouse and ten were collected in the field. The remaining 32 samples (74%) were available only as herbarium material (Table 2). Morphological characters were observed either from live plants during field trips or from herbarium specimens.

**Experimental strategy for the molecular study.** Total DNA from the fresh material was extracted using the CTAB method of Doyle and Doyle (1987) modified by the addition of sodium metabisulfite 2% w/v to the CTAB buffer. The herbarium material DNA was extracted using the

**Table 2.** Sources of sequenced taxa in alphabetical order, voucher information or literature reference, material characterization h herbarium material, f fresh or frozen material, s silica gel dried material, and GenBank accession numbers. Information about collection permits for endangered plants and for plants from protected areas can be obtained from the first author upon request. Synonymous plant names are given in brackets

Taxon	Voucher	Plant material	GenBank acc.#
<i>Arctomecon humilis</i>	Schwarzbach 93/66-28.6.1993 USA, Utah, Washington Co., 5 mi, W of St. George (MJG)	f	AF057646
<i>Argemone albiflora</i> 1 (= <i>A. grandiflora</i> subsp. <i>armata</i> )	Ownbey and Ownbey 1411-16.7.1950 Mexico, Tamaulipas, 22.3 mi NW of C. Victoria (MIN)	h	AF057673
<i>A. albiflora</i> 2 (= <i>A. sanguinea</i> )	Abrigo s.n.-25.4.1963 U.S.A., Texas, La Salle Co., 5 mi S of Cotulla (TEX)	h	AF057686
<i>A. albiflora</i> 3 (= <i>A. albiflora</i> subsp. <i>albiflora</i> )	D'Arcy 1506-16.4.1967 U.S.A., Florida, Alachua Co., roadside (MO)	h	AF057662
<i>A. albiflora</i> 4 (= <i>A. polyanthemos</i> )	Schwarzbach 93/25-1.6.1993 USA, New Mexico, Dona Ana Co., E of Las Cruces, 2 mi W of Organ (MJG)	f	AF057685
<i>A. albiflora</i> 5 (= <i>A. chisosensis</i> )	Schwarzbach 93/40-7.6.1993 USA, Texas, Brewster Co., Big Bend National Park Panther junction (MJG)	f	AF057670
<i>A. albiflora</i> 6 (= <i>A. albiflora</i> subsp. <i>texana</i> )	Ownbey and Ownbey 1650-27.3.1952 USA, Texas, Fayette Co., Schulenburg city limits (MIN)	h	AF057663
<i>A. albiflora</i> 7 (= <i>A. brevicornuta</i> )	Ownbey and Ownbey 1848-18.9.1953 Mexico, Chihuahua, 19.6 mi E of Hidalgo del Parral on road to Jiménez (MIN)	h	AF057668
<i>A. arida</i>	Ownbey and Ownbey 2040-25.10.1953 Mexico, San Luis Potosi, 0.1 mi SW of San Luis Potosi (MIN)	h	AF057664
<i>A. arizonica</i> 1	Schwarzbach 93/52-17.6.1993 USA, Arizona, Coconino Co., Grand Canyon National Park, South Rim, S Bright Angel trail (MJG)	f	AF057665
<i>A. arizonica</i> 2	Schwarzbach 93/59-22.6.1993 USA, Arizona, Coconino Co., Grand Canyon National Park, North Rim, N Bright Angel trail to Roaring Springs (MJG)	f	AF057666
<i>A. aurantiaca</i>	Ownbey and Ownbey 1397-12.7.1950 USA, Texas, Hays Co., 6.3 mi NE of San Marcos (MO)	h	AF057667
<i>A. corymbosa</i> subsp. <i>arenicola</i>	Schwarzbach 9354-18.6.1993 USA, Arizona, Coconino Co., Hwy 89 at Cedar Ridge (MJG)	f	AF057660
<i>A. corymbosa</i> subsp. <i>corymbosa</i>	Pavlik 227-31.5.1980 USA, California, Inyo Co., Panamint dunes, Panamint Valley, N end (DAV)	h	AF057661

Table 2 (continued)

<i>A. echinata</i> 1	Stanford, Retherford, Northcraft 280-10.7.1941 Mexico, Coahuila, at Fraile, 59 km S of Saltillo (UC)	h	AF057671
<i>A. echinata</i> 2 (= <i>A. subalpina</i> )	Nesom, Norris, Villareal, Carranza 7140-25.8.1989 Mexico, Nuevo Leon, on N side of Peña Nevada complex (MO)	h	AF057689
<i>A. fruticosa</i>	Chiang, Wendt, Johnston 9469-23.9.1972 Mexico, Coahuila, Canyon Escondido (CAS)	h	AF057650
<i>A. glabrata</i> (= <i>A. squarrosa</i> subsp. <i>glabrata</i> )	Davis 289-11.6.1974 USA, New Mexico, Lincoln Co., 2 mi W of Roswell (MO)	h	AF057687
<i>A. glauca</i> (= <i>A. glauca</i> var. <i>decipiens</i> )	Gustafson 2740-8.7.1982 USA, Hawaii, about 0.2 mi SE of Mauna Kea State Park (RSA)	h	AF057651
<i>A. grandiflora</i> (= <i>A. grandiflora</i> subsp. <i>grandiflora</i> )	Benn s.n.-6.1967 Mexico, Hidalgo, Zimapan (MIN)	h	AF057674
<i>A. hispida</i>	Soine s.n.-8.1960 USA, Colorado, Boulder Co., near Boulder (MIN)	h	AF057675
<i>A. hunnemannii</i>	Worth and Morrison 15813-13.10.1938 Chile, Antofagasta, ca. 10 km E of Taltal (GH)	h	AF057659
<i>A. mexicana</i> subsp. <i>mexicana</i> 1	Cooley 2539-28.2.1954 USA, Florida, Lee Co., Sanibel Island (GH)	h	AF057669
<i>A. mexicana</i> subsp. <i>mexicana</i> 2	Schwarzbach 92/1-23.7.92 Botanical Garden Mainz, Germany (MJG)	f	AF057652
<i>A. mexicana</i> subsp. <i>mexicana</i> 3	Cooley 8792-19.4.1962 West Indies, St. Kitts (GH)	h	AF057653
<i>A. mexicana</i> subsp. <i>ochroleuca</i>	Ownbey and Ownbey 1443a-22.7.1950 (MIN)	h	AF057654
<i>A. munita</i> subsp. <i>argentea</i>	Schwarzbach 93/2-26.3.1993 USA, California, San Bernardino Co., 10.2 mi S of Needles, Lobecks Pass (MJG)	f	AF057676
<i>A. munita</i> subsp. <i>gracilentata</i> (= <i>A. gracilentata</i> )	Ownbey and Ownbey 2079a-7.5.1954 Mexico, sonora, 3.7. mi S of Villa de Seris, Hermosillo (MIN)	h	AF057672
<i>A. munita</i> subsp. <i>robusta</i>	Ownbey and Ownbey 2177-2.7.1954 USA, California, Orange Co. (MO)	h	AF057677
<i>A. platyceras</i>	Tlapa and Ubierna 1759-14.3.1988 Mexico, Puebla, Cholula (TEX)	h	AF057681
<i>A. pleiacantha</i> subsp. <i>ambigua</i>	Ownbey and Ownbey 1830-11.9.1953 USA, Arizona, Yavapai Co., 0.8 mi NE of Prescott (MIN)	h	AF057683
<i>A. pleiacantha</i> subsp. <i>pleiacantha</i>	Hutchins 10745-13.6.1983 USA, New Mexico, Catron Co., 7 mi N of Alma (UNM)	h	AF057684

Table 2 (continued)

Taxon	Voucher	Plant material	GenBank acc.#
<i>A. pleiacantha</i> subsp. <i>pinnatisecta</i>	Schwarzbach 93/27-2.6.1993 USA, New Mexico, Otero Co., near La Luz, Fresnal Canyon (MJG)	f	AF057682
<i>A. rosea</i>	Werdermann 105-7.11.1923 Chile, Coquimbo, Rivadavia (SI)	h	AF057658
<i>A. rotundata</i> 1 (= <i>A. munita</i> subsp. <i>rotundata</i> )	Schwarzbach 93/10-23.5.1993 USA, California, Kern Co., 11 m E of South Lake (MJG)	f	AF057678
<i>A. rotundata</i> 2 (= <i>A. munita</i> subsp. <i>rotundata</i> )	Schwarzbach 93/61-23.6.1993 USA, Arizona, Coconino Co., 10 mi S of Fredonia (MJG)	f	AF057679
<i>A. rotundata</i> 3 (= <i>A. munita</i> subsp. <i>rotundata</i> )	Schwarzbach 93/81-29.6.1993 USA, Nevada, Esmeralda Co., Hwy. 266, ca. 10 mi W of Jct. 95 (MJG)	f	AF057680
<i>A. squarrosa</i> (= <i>A. squarrosa</i> subsp. <i>squarrosa</i> )	Ogzewalla s.n.-1956 USA, Colorado, Prowers Co., 11 mi E of Lamar (MIN)	h	AF057688
<i>A. subfusiformis</i> (= <i>A. subfusiformis</i> subsp. <i>subfusiformis</i> )	Walle and Sparre 476-12.11.1946 Argentina, Tucuman, Yerba Buena (S)	h	AF057656
<i>A. subinermis</i>	Troels, Myndel, Pedersen 70-11.12.1947 Argentina, Chacra near Estancia Santa Theresa (C)	h	AF057657
<i>A. subintegrifolia</i>	Moran 13057-27.4.1966 Mexico, Sonora, SW corner of San Esteban Island (CAS)	h	AF057647
<i>A. superba</i>	Ownbey 4170-22.6.1968 Mexico, San Luis Potosi, 14 mi E of Rio Verde (MIN)	h	AF057655
<i>A. turnerae</i> subsp. <i>ownbeyana</i> (= <i>A. ownbeyana</i> )	Chiang, Wendt, Johnston 9728-20.10.1972 Mexico, Chihuahua, 28.5 km W of Ojinaga (LL)	h	AF057649
<i>A. turnerae</i> subsp. <i>turnerae</i> (= <i>A. turnerae</i> )	Chiang, Wendt, Johnston 9872-24.10.1972 Mexico, Chihuahua, 14 km NW of the Presa del Granero reservoir lake (LL)	h	AF057648
<i>Bocconia frutescens</i>	Blattner and Kadereit (in press)	s	AJ001951
<i>Canbya aurea</i>	Schwarzbach 93/10-7/1993 USA, Nevada, Humboldt Co. (MJG)	s	AF098921
<i>Macleaya cordata</i>	Blattner and Kadereit (in press)	f	AJ001953
<i>Meconopsis delavayi</i>	Blattner and Kadereit (in press)	s	AJ001967
<i>Papaver rhoeas</i>	Jork 95/4-31.5.1995 Germany, Campus University Mainz (MJG)	f	AF098920
<i>Romneya coulteri</i>	Schwarzbach 93/7 Germany, Botanical Garden Mainz (MJG)	f	AF098922



miniprep method described by Rogers (1994, 'method A'). Usually 5–50 mg of dried plant material was used for DNA isolation. For some material extra cleaning steps were required. An additional purification step with QIAGEN G/20 genomic-tip columns (QIAGEN GmbH, Hilden, Germany; protocol available from the first author upon request) could improve the PCR amplification success in many cases. DNA amplifications were performed in 50 µl reactions containing 1.5 U *Taq* polymerase (Appligene), reaction buffer supplied by Appligene, 1.5 mM MgCl<sub>2</sub>, 25 pmol of each primer (ITS 5 and 2; White et al. 1990) and 0.2 mM of each dNTP. The PCR profile was 30 sec at 95 °C, 30 sec at 55 °C and 1 min at 72 °C for 32 cycles with a final cycle of 30 sec at 95 °C, 1 min at 55 °C and 8 min at 72 °C. Separate PCR runs (3–6) were pooled and the product was separated from residual primers, dNTPs and *Taq* Polymerase by running the entire product on a low melting gel. The sample was recovered by using the QIAquick Gel Extraction kit (QIAGEN GmbH, Hilden, Germany). Part of the purified sample (60–120 ng) was used for the cycle sequencing reaction following the protocol of the DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Inc.). Unincorporated dye terminators were removed through a double phenol-chloroform extraction and the DNA was dried under vacuum. The samples were run on a 6% acrylamide gel using an ABI 373 DNA Sequencing System (Applied Biosystems, Inc.). Both strands were sequenced.

**Sequence analysis.** The DNA sequences obtained were assembled and consensus sequences were constructed using SeqEd 1.0.3. (Applied Biosystems). All sequences were deposited at GenBank (see Table 2 for accession numbers). Most mutations were nucleotide substitutions, thus allowing manual alignment (alignment available from the first author upon request). Gaps were coded as missing and informative insertions and deletions were added as absent/present information to the data matrix. The aligned sequences were exported to PAUP\* (test version d64, kindly provided by D. L. Swofford) and analyzed using the heuristic search algorithm, with the MULPARS option on, TBR branch swapping, and 100 RANDOM additions. Strict and 50% majority rule consensus trees were calculated from all most

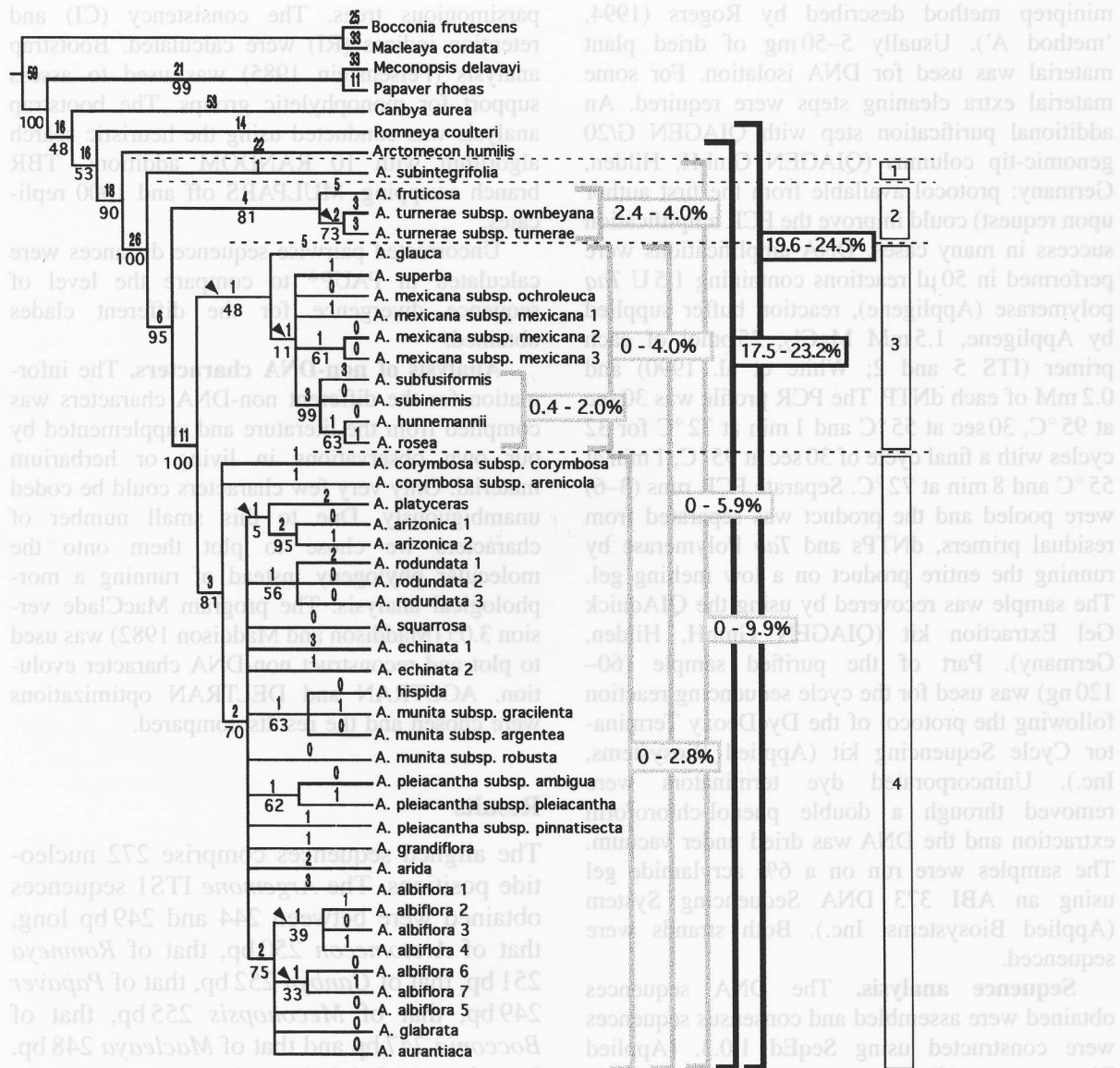
parsimonious trees. The consistency (CI) and retention indices (RI) were calculated. Bootstrap analysis (Felsenstein 1985) was used to assess support for monophyletic groups. The bootstrap analysis was conducted using the heuristic search algorithm with 10 RANDOM additions, TBR branch swapping, MULPARS off and 1000 replicates.

Uncorrected pairwise sequence distances were calculated in PAUP\* to compare the level of sequence divergence for the different clades obtained.

**Analysis of non-DNA characters.** The information for the different non-DNA characters was compiled from the literature and supplemented by our own observations in living or herbarium material. Only very few characters could be coded unambiguously. Due to this small number of characters we chose to plot them onto the molecular phylogeny instead of running a morphological analysis. The program MacClade version 3.05 (Maddison and Maddison 1982) was used to plot and reconstruct non-DNA character evolution. ACCTRAN and DELTRAN optimizations were chosen and the results compared.

## Results

The aligned sequences comprise 272 nucleotide positions. The *Argemone* ITS1 sequences obtained were between 244 and 249 bp long, that of *Arctomecon* 250 bp, that of *Romneya* 251 bp, that of *Canbya* 252 bp, that of *Papaver* 249 bp, that of *Meconopsis* 255 bp, that of *Bocconia* 247 bp and that of *Macleaya* 248 bp. Insertions and deletions (indels) are between one and four bp long. Within *Argemone* all indels are one bp long and only one deletion (synapomorphy of the South American species *A. subinermis*, *A. subfusiformis*, *A. rosea* and *A. hunnemannii*) and two insertions (synapomorphy of *A. fruticosa* and *A. turnerae* and a synapomorphy of all *Argemone* species except *A. subintegrifolia*, *A. fruticosa* and *A. turnerae*) are phylogenetically informative. Altogether 145 (124 without indels) parsimony informative characters were found. Excluding the several outgroups reduced the number of



**Fig. 3.** One of 840 equally most parsimonious trees identical to the majority rule tree. Arrows indicate branches collapsing in the strict consensus tree. The trees are 364 steps long, CI is 0.632 (excluding autapomorphies), RI is 0.74. *Bocconia* and *Macleaya* as members of a different subfamily (Chelidonioideae) were used as outgroup. The numbers below the branches indicate bootstrap values, number of transformations are shown above branches. Numbers on gray bars indicate the minimum-maximum for sequence divergence (uncorrected distances) for the marked group. Numbers on black bars indicate the minimum-maximum distance between *Arctomecon/Argemone* and *Romneya/Argemone*. Boxes on the right refer to the four major clades discussed in the text

informative characters within *Argemone* to 36 (33 without indels). Parsimony analysis resulted in 840 equally most parsimonious trees, each 364 steps long with a consistency

index of 0.632 (excluding uninformative characters) and a retention index of 0.74. Figure 3 shows the 50% majority rule consensus tree containing all compatible

groupings, which is identical to one of the 840 most parsimonious trees. Branches that collapse in the strict consensus tree are indicated with arrows.

For unknown reasons ITS2 could be amplified only for a small subset of species. The phylogenetic analysis of ITS1 and ITS2 of these species resulted in a phylogeny (not shown) congruent with that obtained from ITS1 only.

The calculation of pairwise distances (only nucleotide changes were considered) results in a relatively large distance between *Argemone* and *Arctomecon*, the closest extant relative of the prickly poppies (17.5–23.2%). Within *Argemone* we found pairwise distances between 0–9.9% (Fig. 3). The variation within *Argemone* is within a range found by other researchers. For example, Wojciechowski et al. (1993) found sequence differences of 0–10.2% among 26 species of *Astragalus* L., Schultheis and Baldwin (1994) obtained 0–7.8% for 11 species of *Fouquieria* Kunth., and Donoghue and Baldwin (1993) detected 0–13.6% in *Viburnum* L.. Phylogenetically informative characters are very unevenly distributed in our study; for example, only 0–2.8% of sequence divergence can be found in the largest clade containing 13 of the 24 species of *Argemone*. The variation of sequence distances in the different clades is shown in Fig. 3.

The major results can be summarized as follows (Fig. 3):

1. The genus *Argemone* is a monophyletic genus with a bootstrap support of 100%.

2. The genus *Arctomecon* is the closest extant genus to *Argemone*, the bootstrap support for the monophyly of the entire clade is 90%. The relative position of the other outgroups to each other is not entirely clear.

3. *A. subintegrifolia* is sister to the rest of the genus. Monophyly of the rest of the genus is supported by a bootstrap value of 95% (clade 1).

4. *A. fruticosa*/*A. turnerae* form a well-supported clade separate from *A. subintegrifolia* and sister to all other *Argemone* species (clade 2).

5. *A. glauca*/*A. superba*/*A. mexicana*/*A. subfusiformis*/*A. subinermis*/*A. hunnemanni*/*A. rosea* form a monophyletic group. Their monophyly, however, is not well supported with a bootstrap value of 48% (clade 3).

6. Within the largest and geographically most widespread clade two subspecies of *A. corymbosa* are separate from the remaining species. Whereas the molecular data do not show monophyly of this species, unique morphological characters such as fruit and leaf shape clearly justify their treatment as one species. This clade has a bootstrap support of 81% (clade 4).

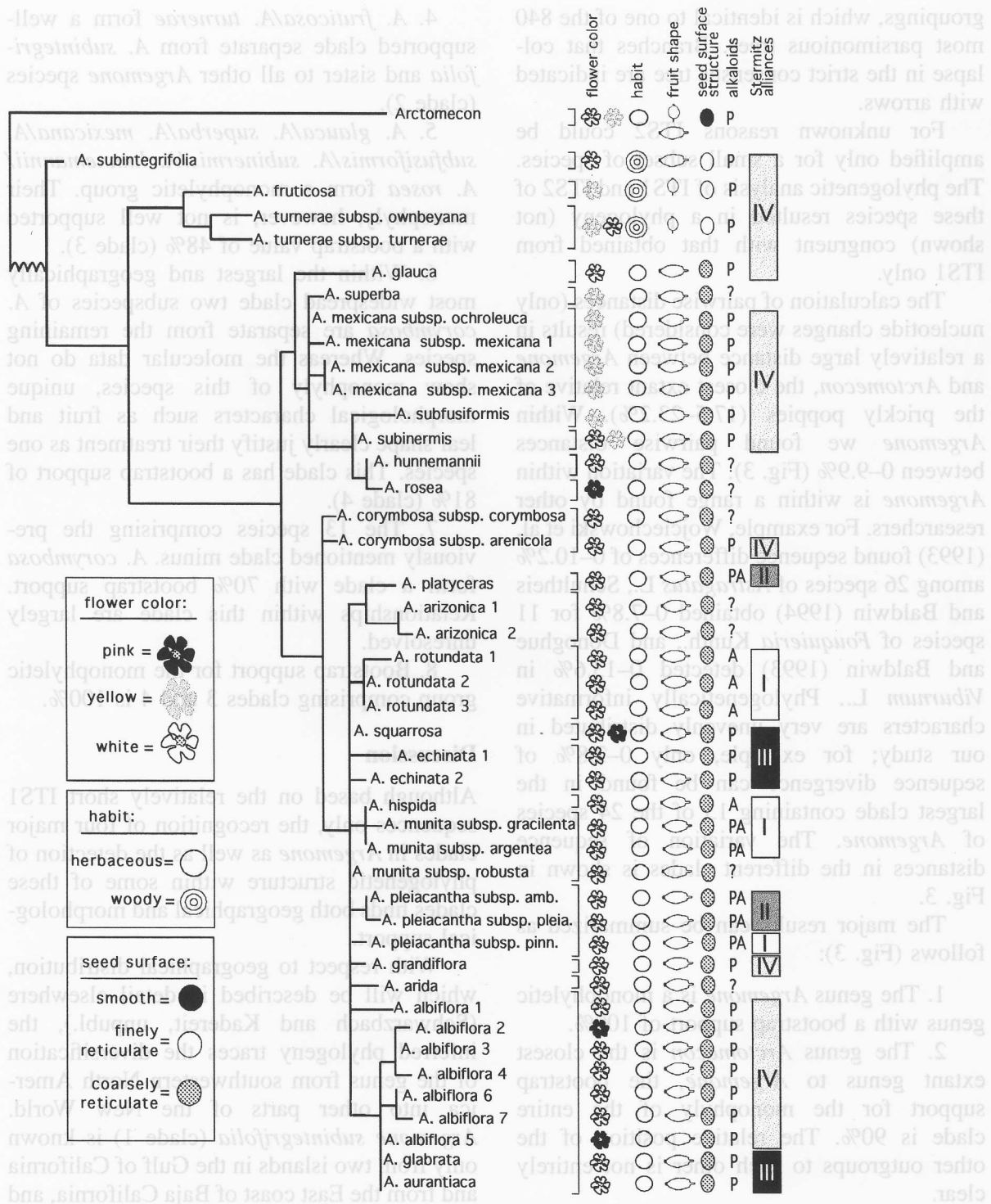
7. The 13 species comprising the previously mentioned clade minus *A. corymbosa* form a clade with 70% bootstrap support. Relationships within this clade are largely unresolved.

8. Bootstrap support for the monophyletic group comprising clades 3 and 4 is 100%.

## Discussion

Although based on the relatively short ITS1 sequences only, the recognition of four major clades in *Argemone* as well as the detection of phylogenetic structure within some of these clades finds both geographical and morphological support.

With respect to geographical distribution, which will be described in detail elsewhere (Schwarzbach and Kadereit, unpubl.), the inferred phylogeny traces the diversification of the genus from southwestern North America into other parts of the New World. *Argemone subintegrifolia* (clade 1) is known only from two islands in the Gulf of California and from the East coast of Baja California, and *A. fruticosa* and *A. turnerae* (clade 2) are rare endemics in NE Mexico. Clade 3 contains one species from Hawaii (*A. glauca*) as well as Caribbean, Mexican and Central American



**Fig. 4.** Morphological and chemical characters plotted onto the ITS phylogeny. To illustrate the unequal branch lengths the phylogeny is drawn as a phylogram. Only *Arctomecon* as closest relative is shown as outgroup. P protoberberine and protopine alkaloids, A pavines. Bars on the right show alliances as previously described based on a combination of alkaloid and morphological characters

(*A. superba* through *A. mexicana*) and South American (*A. subfusiformis* through *A. rosea*) species, and clade 4 is widely distributed in the USA and Mexico. Within clade 4 the basal *A. corymbosa* is found in dune habitats in the Mojave Desert.

In the following discussion of the evolution of discontinuous morphological and chemical characters it will become obvious that some morphological support for our phylogeny can be found.

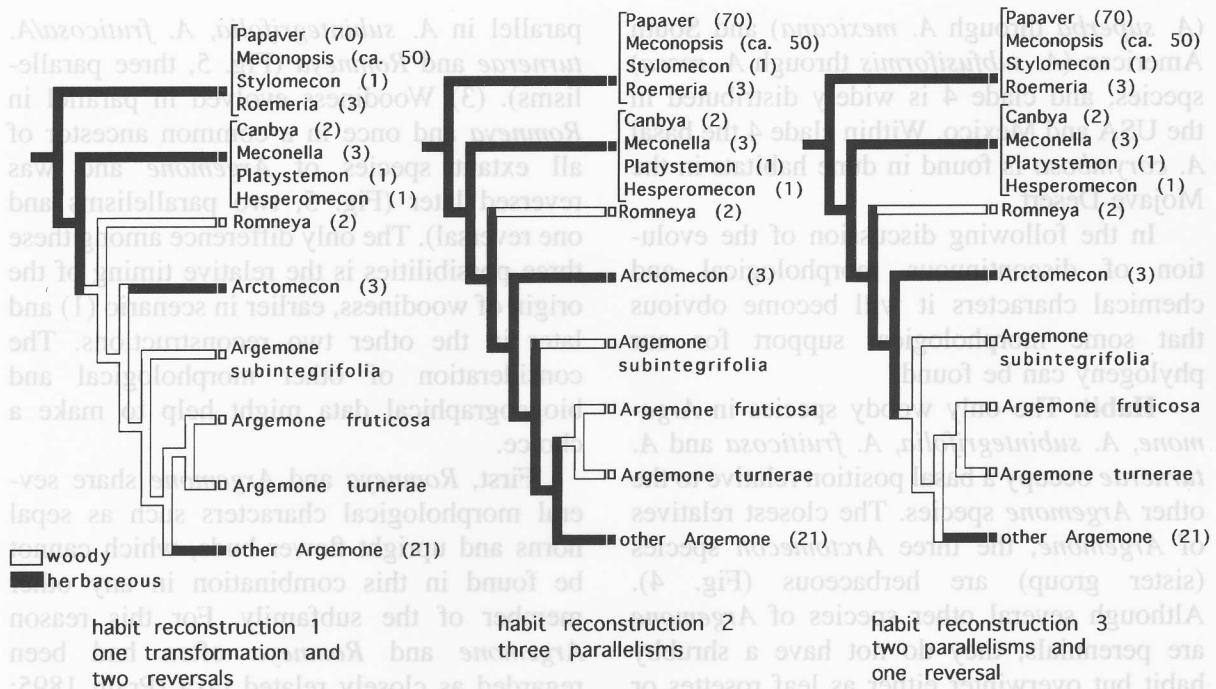
**Habit.** The only woody species in *Argemone*, *A. subintegrifolia*, *A. fruticosa* and *A. turnerae* occupy a basal position relative to the other *Argemone* species. The closest relatives of *Argemone*, the three *Arctomecon* species (sister group) are herbaceous (Fig. 4). Although several other species of *Argemone* are perennials, they do not have a shrubby habit but overwinter either as leaf rosettes or roots. Woodiness is not restricted to certain habitats or climatic zones but woody and herbaceous taxa grow in sympatry. Our results show that the woody taxa do not form a monophyletic group (Fig. 4). Assessment of the character state of the outgroup as plesiomorphic is cautioned against, because *Arctomecon* is a very specialized taxon and therefore is very likely to show an accumulation of derived features (see also Donoghue and Cantino 1984, Meacham 1984, Donoghue and Maddison 1986, Baum and Estabrook 1996). For this reason we included all lineages of the subfamily Papaveroideae as found in earlier analyses of the Papaveraceae (Jork and Kadereit 1995, Schwarzbach and Kadereit 1995, Hoot et al. 1997) as outgroups for the determination of character state polarity (Fig. 5).

This resulted in three different but equally parsimonious optimizations of the evolution of woodiness (Fig. 5). (1) Woodiness is plesiomorphic in the entire clade containing *Romneya*, *Arctomecon* and *Argemone*. Reversals occurred in *Arctomecon* and the herbaceous clade of *Argemone* (Fig. 5, one transformation and two reversals). (2) Woodiness evolved in

parallel in *A. subintegrifolia*, *A. fruticosa*/*A. turnerae* and *Romneya* (Fig. 5, three parallelisms). (3) Woodiness evolved in parallel in *Romneya* and once in a common ancestor of all extant species of *Argemone* and was reversed later (Fig. 5, two parallelisms and one reversal). The only difference among these three possibilities is the relative timing of the origin of woodiness, earlier in scenario (1) and later in the other two reconstructions. The consideration of other morphological and biogeographical data might help to make a choice.

First, *Romneya* and *Argemone* share several morphological characters such as sepal horns and upright flower buds, which cannot be found in this combination in any other member of the subfamily. For this reason *Argemone* and *Romneya* often had been regarded as closely related taxa (Prain 1895; Fedde 1909, 1936). According to their relationship, however, these character states should be interpreted as plesiomorphic. *Arctomecon* is very different from the above two genera and shows a large number of morphological autapomorphies. Its uniquely cuneate leaves densely covered by long hairs and arranged in rosettes have been interpreted as adaptations to extremely dry habitats (Schwarzbach and Kadereit 1995). This may suggest that the herbaceous habit of *Arctomecon* also is a derived adaptive character, and that the shrubby habit shared by the basal two clades of *Argemone* and *Romneya* is the plesiomorphic state that was present in a common ancestor of the clade as suggested by reconstruction 1 (Fig. 5).

Second, biogeographical data also may support the 'two reversals' hypothesis. As briefly described above, *Argemone subintegrifolia* is only known from two islands in the Gulf of California and two populations along the East coast of Baja California, and *A. fruticosa* as well as *A. turnerae* are rare endemics from NE Mexico. *Romneya* comprises two rare species endemic to the coastal regions of southern California and northern



**Fig. 5.** Three equally parsimonious reconstructions for the evolution of woody habit in *Argemone* shown for the subfamily Papaveroideae. Reconstruction 1: change from herbaceous to woody habit in a common ancestor of *Romneya*, *Arctomecon* and *Argemone*; then two reversals to herbaceous habit in *Arctomecon* and the largest *Argemone* clade. Reconstruction 2: woody habit evolved three times in parallel in *Romneya*, *Argemone subintegrifolia* and in the common ancestor of *A. fruticosa* and *A. turnerae*. Reconstruction 3: woody habit evolved two times in parallel in *Romneya* and the common ancestor of all *Argemone* species, followed by a reversal to herbaceous habit in the largest *Argemone* clade comprising 21 species. Numbers in parentheses indicate number of species for the different genera

Baja California, which are known to contain several relict species (Raven and Axelrod 1978, Fiedler 1995). Based on this geographical distribution, *Romneya* and the woody *Argemone* are best interpreted as rare relicts (Schwarzbach and Kadereit, unpubl.). Additionally, all herbaceous taxa occur in relatively young habitats. *Arctomecon* grows mainly on gypsum soils in the Mojave desert (Turner and Powell 1979, Sheldon Thompson and Smith 1997) and the herbaceous species of *Argemone* are widely distributed in the relatively young desert areas of North and South America (Axelrod 1979, 1983; Thorne 1986) or as weedy and mainly annual species throughout

the warmer regions of the New World. Although relictual distribution does not necessarily imply that the relictual species show throughout plesiomorphic character states, in case of the New World poppies our character interpretation nicely agrees with tree topology, geological and geographical data.

Based on these distributional patterns, ecology and morphological characters, we favor the interpretation of woodiness as an 'old' character retained in *Romneya* and the two shrubby lineages of *Argemone*. The herbaceous habit in *Arctomecon* and the majority of *Argemone* can best be understood as a loss of woodiness perhaps in adaptation to

very dry conditions (*Argemone* and *Arctomecon*) or to a colonizing ecology (*Argemone*). Consequently, the herbaceous habit can be regarded as a synapomorphy of clades 3 and 4 of *Argemone* (Fig. 4).

Apart from habit all other vegetative characters either vary continuously in the genus or are autapomorphic and therefore are not discussed further. An example for autapomorphic vegetative characters is leaf shape, which allows unambiguous identification of some species (e.g. *A. subintegrifolia*, *A. grandiflora*).

**Flower.** The flowers of *Argemone* are almost completely uniform. As described above, they have three (rarely four) sepals with conspicuously elongated sepal horns (Fig. 2), the corolla is 3-merous (3 + 3), the number of stamens is large (mostly > 50), and the paracarpous gynoecium is formed by 3–7 carpels. The petals vary in size and color and the flowers are 4–15 cm in diameter when fully expanded. Unless fixed in the genus, these characters vary widely within and among species and cannot be used for species delimitation. A general trend towards increased petal size and stamen number, however, can be observed in some of the polyploid species (*A. grandiflora*, *A. squarrosa*, *A. glabrata*).

Flower color is more distinct and has been used as an important character for the delimitation of species (Ownbey 1958). It has not been clear whether this is a useful character for the identification of larger clades. Most species have exclusively white flowers, and yellow or pink flower color is less frequent. A few species have exclusively yellow (*A. mexicana*, *A. fruticosa*, *A. superba*, *A. subfusiformis*) or pink flowers (*A. rosea*). More frequently, pink or yellow colored individuals co-occur with pure white individuals. Because *Romneya* and most species of *Arctomecon* (one yellow-flowered species is nested within white-flowered ones; unpublished results) as well as *A. subintegrifolia*, *A. glauca* and *A. corymbosa* have white flowers,

this can be interpreted most parsimoniously as the plesiomorphic character state (Fig. 4). Yellow flower color evolved at least twice in the *A. fruticosa* and *A. mexicana* clades or might, with some reservation, be interpreted as a homoplastic synapomorphy of both clade 2 and clade 3 excluding *A. glauca*. The sometimes white-flowered individuals of *A. subinermis* and the exclusively white flowers of *A. hunnemannii* are interpreted as character reversals. Both of these species are South American in distribution and evolutionarily derived within clade 3. The pink-flowered *A. rosea* is a rare endemic known only from one Chilean locality. Additionally, pink-flowered populations of the widespread *A. albiflora* are known from southern Texas and northeastern Mexico. However, many of these populations are not exclusively pink-flowered but contain some white-flowered individuals.

When comparing pink and white flowers, the color difference does not seem to be an adaptation to different pollinators. Comparative studies of these two flower morphs revealed a very similar pollinator spectrum. The flowers are not specialized and a large variety of different pollinators can be found (Nichols 1982, Schneider and Nichols 1984, Cresson 1986, Schneider et al. 1987).

Although all non-fixed flower characters mostly show continuous variation, differences in the shape of the sepal horns are sometimes autapomorphic and useful for identifying some species (e.g. *A. platyceras*, *A. pleiocantha*).

**Fruit.** The paracarpous gynoecium in *Argemone* is formed by 3–7 carpels, and the fruit is a capsule opening with apical valves. Fruit characters, especially shape and prickliness, have played an important role in the delimitation of species (Prain 1895; Ownbey 1958, 1961). Because fruit characters often may be adaptive, they can be misleading when similarity is used as evidence for close relationship. Recently, several examples have been described where the use of fruit characters led to the description of para- or

polyphyletic groups. Examples for this are *Heterogaura* Rother./*Clarkia* Pursh. (Onagraceae; Sytsma and Gottlieb 1986), *Roemeria*/*Papaver* (Papaveraceae; Kadereit and Sytsma 1992, Kadereit et al. 1997) and *Anemone* L./*Pulsatilla* Miller (Ranunculaceae; Hoot et al. 1994, Hoot 1995).

Density and shape of prickles are highly variable characters and cannot be subdivided into discrete states. There is a trend towards branched fruit prickles in the polyploid species (*A. aurantiaca*, *A. squarrosa*, *A. glabrata*, *A. echinata*), but exceptions, such as the tetraploid *A. grandiflora*, are known. Occasionally, prickle characters as autapomorphies are useful for the delimitation of species.

However, fruit shape can be considered a character with discrete states (Fig. 4). *Argemone fruticosa* and *A. turnerae* have more or less globose fruits, and all other species have ovoid to ellipsoid capsules. In *Arctomecon* both fruit types can be observed, but the globose shape occurs only in the morphologically most derived species (*A. humilis*). *Romneya* has elliptic fruits although these are relatively broader than those in *Argemone*. Because the most basal species of *Arctomecon*, as well as *Argemone subintegrifolia*, have an elliptic fruit shape, this can most parsimoniously be interpreted as the plesiomorphic character state retained in most species of *Argemone*. Therefore the globose capsules can be considered a synapomorphy of *Argemone fruticosa* and *A. turnerae* (Fig. 4).

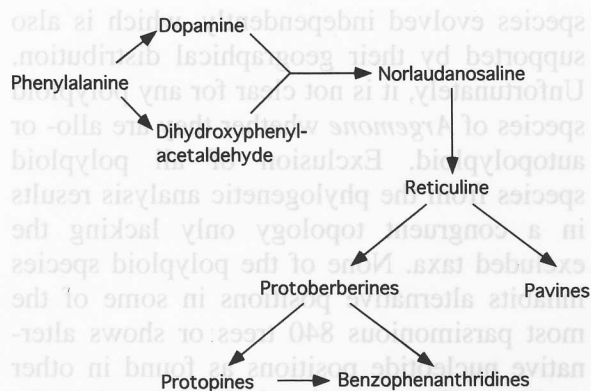
**Seed.** Gunn and Seldin (1976) studied seed characters for all North American members of the Papaveraceae. We completed this analysis by examining the South American and Hawaiian species of *Argemone*. Whereas size and shape of the seeds are highly variable characters impossible to translate into a discrete character matrix, a finely vs. coarsely reticulate surface pattern can be distinguished unambiguously (Gunn and Seldin 1976). Finely reticulate surfaces are shared by *A. subintegrifolia*, *A. fruticosa* and *A. turnerae*. *Romneya* seeds have a minutely

reticulate surface, and *Arctomecon* seeds show no reticulation (Gunn and Seldin 1976). *Arctomecon* is also distinguished from *Argemone* and *Romneya* by the presence of an aril. The finely reticulate surface therefore is best interpreted as plesiomorphic state, and the coarse reticulation can be regarded as a synapomorphy of our clades 3 and 4. The evolution of a coarsely reticulate seed surface in derived taxa has also been observed in Papaveraceae subfam. Eschscholioideae, in which the shrubby relict genus *Dendromecon* has a minutely reticulate seed surface, while the herbaceous and clearly derived *Eschscholzia* has a more conspicuous reticulation pattern (Clark and Jernstedt 1978).

Of the four discrete morphological characters discussed, woody habit and finely reticulate seeds are shared by clades 1 and 2 of *Argemone* (Figs. 3 and 4) and have been interpreted as plesiomorphic character states. Alternatively if they are regarded as evidence for a sister group relationship between these two clades, a cladogram two steps longer than the shortest cladograms results. Although this is not much, we prefer to interpret these two clades of *Argemone* as done above because this is supported by all molecular and morphological evidence available.

**Alkaloids.** The alkaloid contents of *Argemone* and *Arctomecon* have been studied in detail by several authors (Foote 1932; Slavík and Slavíková 1963; Stermitz and Seiber 1966; Stermitz 1967, 1968; Stermitz et al. 1969, 1971, 1973a, b, 1974; Stermitz and Coomes 1969; Bandoni et al. 1972, 1975; Benn and Mitchell 1972; Coomes et al. 1973; Stermitz and Williams 1973; Raynie et al. 1990, 1991). Because *Argemone* often has been regarded as a close relative of *Papaver*, it seemed to be an interesting target in the search for new alkaloids for medicinal use. Researchers have been interested in pavine alkaloids, which have been shown to be effective in alleviating both pain and heart arrhythmia (Stermitz et al. 1969). Papaveraceae also are known to be rich in protopines, as are four





**Fig. 6.** Biosynthetic relationships of the isocholinoalkaloids derived from reticuline (redrawn from Preininger 1986)

other angiosperm families, namely Berberidaceae, Fumariaceae, Ranunculaceae and Rutaceae (Cordell 1981). As first proposed by Slavík and Slavíková (1963), *Argemone* can be subdivided into two groups based on the presence of certain alkaloids. The first includes species containing pavine alkaloids, and the second is characterized by the presence of protoberberines or derivatives, mainly protopines and benzophenanthridines. These two alkaloid groups are products of different biosynthetic pathways (Fig. 6). Stermitz et al. (1969) elaborated this subdivision and described four alliances in the genus. This subdivision was based on type and composition of alkaloids as well as on morphology. Unfortunately, these authors did not specify which morphological characters they used. Their alliance (I) contains mainly pavines, alliance (II) is intermediate, and alliances (III)–(IV) contain mainly protoberberines and protopines. Alliances (III) and (IV) were separated based on morphological characters. The alliance (III) species (*A. squarrosa*, *A. glabrata*, *A. aurantiaca*, *A. echinata*) are polyploid species and are morphologically very distinct by having fruits with branched prickles and by being strongly branched. In these characters they are very different from the alliance (IV) taxa. Stermitz

et al. (1969) suggested that the pavine type alliance (I) represents the oldest in the genus, and they also proposed a phylogenetic sequence of (I) → (II) → (IV). The position of alliance (III) could not be determined by these authors.

In *Argemone*, as far as known, pavines are found only in species of our clade 4. The alliances described by Stermitz et al. (1969) do not coincide with the clades recognized by us (Fig. 4), and the presence of pavines does not appear to be an ancestral character as proposed by these authors. The relict species, *A. subintegriifolia*, *A. fruticosa* and *A. turnerae* are in their alliance (IV), considered to be most derived. Stermitz et al. (1969) regarded the presence of pavines as plesiomorphic because only *Eschscholzia* shares this character with *Argemone*. *Eschscholzia* has been regarded as ancestral in the Papaveraceae because it shares characters with the Fumariaceae (e.g. Fedde 1936). Although subfam. Eschscholzioideae is basal in the Papaveraceae Hoot et al. (1997), *Eschscholzia* is nested well within the subfamily and does not occur near the base. The co-occurrence of pavines in *Eschscholzia* and parts of *Argemone* is best interpreted as the result of parallel evolution. Interestingly, the species of *Argemone* with pavines have almost exactly the same geographical distribution as *Eschscholzia*. Therefore the presumably parallel evolution of pavines might have an environmental cause.

Protopines and protoberberines, which were regarded by Stermitz et al. (1969) as a synapomorphy of their supposedly derived alliance (IV), are the most widespread alkaloids in the Papaveraceae and are present in almost all genera (Preininger 1986, Raynie et al. 1990). According to their distribution in the family and within *Argemone* they are best interpreted as comprising a plesiomorphic character state.

The different classes of alkaloids can be further subdivided on the basis of their substitution patterns. By plotting these more exact characters onto the phylogeny (not

shown) we could not find any alkaloid type uniquely supporting one of the different clades. Accordingly, alkaloids seem to be extremely homoplasious in *Argemone*.

The apparent incongruence of alkaloid composition and our phylogenetic hypothesis might result from experimental artifacts such as taxonomic misidentifications (in our experience c. 40% of herbarium specimens are wrongly identified!), the use of different plant parts or the same parts in different developmental phases (Bandoni et al. 1975, Robinson 1979, Raynie et al. 1991), possibly from a strongly adaptive component in alkaloid variation as was proposed above for pavinines, or merely a random sorting of character states. Without further examinations it cannot be discriminated between these hypotheses.

**Morphological parallelism and polyploidy.** As evident from a comparison of our phylogeny with the distribution of morphological characters, conspicuous parallelism either in individual characters or in overall appearance can be found particularly among the polyploid species. Examples for this already described above are the presence of branched prickles in *A. aurantiaca*, *A. echinata*, *A. glabrata* and *A. squarrosa*, or the trend towards larger flowers and a larger number of stamens in *A. glabrata*, *A. grandiflora* and *A. squarrosa*. In fact, the similarities between *Argemone squarrosa* and *A. glabrata* led Ownbey (1958) to treat them as conspecific subspecies. The two taxa share a similar fruit shape and fruit armature, and they also have the same vegetative branching pattern. Their flowers are large and they have a larger number of stamens than other species of *Argemone*. They differ in the crisply hispid leaf surface which characterizes *A. squarrosa* but is absent in *A. glabrata*. Our molecular data show that *A. glabrata* is part of the *A. albiflora* clade. Although the relationships of *A. squarrosa* are unresolved, it is not sister species of *A. glabrata* as would be required for their treatment as one species. The molecular results rather suggest that these two polyploid

species evolved independently, which is also supported by their geographical distribution. Unfortunately, it is not clear for any polyploid species of *Argemone* whether they are allo- or autopolyploid. Exclusion of all polyploid species from the phylogenetic analysis results in a congruent topology only lacking the excluded taxa. None of the polyploid species inhabits alternative positions in some of the most parsimonious 840 trees or shows alternative nucleotide positions as found in other analyses (Sang et al. 1995) that might be indicative of their possible parental species. We are however not sure if we obtained as a result of concerted evolution an ITS1 gene tree that is not identical to the species tree (Wendel et al. 1995, Seelanan et al. 1997). Due to the lack of informative characters we were not able to produce a resolved tree based on sequences of a chloroplast spacer region (*trnL-F*, unpublished results). More chloroplast sequence data will hopefully shed light on these unanswered questions in the near future.

Whereas parallelism in characters such as flower size and stamen number may well be the direction and immediate result of polyploidization, other characters such as prickle morphology, fruit shape and branching pattern may require additional explanations. Homoplasy in these characters may represent an example for the concept of underlying synapomorphies (Tuomikoski 1967, Brooks 1996). Brundin (1972) and Saether (1977) suggested that parallelisms are not only produced by non-inherited factors such as selection but also by inherited factors called canalized evolutionary potentials (Brooks 1996). Brooks (1996) described this as follows: "...this means that among groups within a larger clade, and within this clade only, certain homoplasious changes occur repeatedly. The appearance of any of these traits conforms to a phylogenetic pattern of homoplasy, but the evolutionary capacity to produce it actually evolved only one time, in the common ancestor of the larger clade containing all the

species exhibiting the trait." In *Argemone* this might mean that the appearance of identical traits in unrelated species might in some way or other be triggered by polyploidization.

A similar pattern of parallel evolution in polyploids as in *Argemone* has been found in *Montanoa* (Asteraceae; Funk 1982, Brooks and McLennan 1991, Brooks 1996). Whereas five species of *Montanoa* are trees, the others are shrubs (21 species) or vines (4). Funk (1982) found that the shrubby habit is the plesiomorphic character state and the tree form evolved four times in parallel. All five tree species share similar morphological characters and are polyploid.

In order to detect the above type of homoplasy it is necessary to look for character independence. This can be done by plotting one set of characters onto phylogenies constructed on the basis of another set of characters as we did for the group under study instead of including nonindependent morphological characters in the tree search. One might argue that by using a total evidence approach (with which we generally agree) we also would be able to identify parallel evolution simply by identifying the homoplastic nature of characters, for example the parallel increase of flower and fruit size. However, this can only be unambiguously detected if the alternative data set alone (in our case the ITS sequences) produces a resolved phylogeny. Since especially clade 4 which contains all but one polyploid species is completely unresolved due to a lack of informative molecular characters, adding even a small number of nonindependent morphological characters would change this topology. This would mean that all polyploid species with large flowers and large fruits form a clade. But these characters are always correlated with the polyploid nature and therefore possibly nonindependent. By looking at the very few morphological characters that are not correlated with polyploidy and especially by looking at geographical patterns it does not make any sense to us to regard all polyploid

species as a monophyletic group. In our understanding a total evidence approach is only adequate if one can avoid introducing a large number of noise in form of highly homoplasious nonindependent characters to the combined character set. As our results show it is very difficult to detect tree incongruencies if one data set results in an unresolved topology based on the lack of informative characters, because every other topology is congruent with this unresolved tree.

In conclusion it seems necessary to us to include other sources of evidence such as biogeography, geological information and especially in our study ploidy level in the interpretation of tree topologies to detect and understand homoplasies. Although these sources of evidence alone because of a very small number of codable characters cannot resolve a phylogeny they might be helpful in detecting underlying patterns causing homoplasies or being helpful in discriminating between alternative evolutionary scenarios.

We gratefully acknowledge the many helpful comments on the manuscript by the two reviewers Richard Olmstead and Bengt Oxelman. André Chanderbali and Jane Whitehill (University of Missouri and Missouri Botanical Garden, St. Louis) made many helpful comments on an earlier version of the manuscript. We thank Aaron Liston, Corvallis, Oregon State University, for kindly providing help and lab space for part of the molecular work. We also thank the various people who helped us to obtain collection permits and to collect the plant material, and the directors of B, BISH, BM, C, CAS, DAV, DS, F, GH, JEPS, K, LIL, LL, MICH, MIN, MO, ND, P, PH, RSA, S, SD, SI, TEX, UC, UNM, US, WS for the loan of herbarium material. Anke Berg kindly drew Fig. 2. This work was supported by a grant from the German Research Foundation (DFG) to JWK. A grant for a visit to Aaron Liston's lab was given to AES by the German Academic Exchange Service (DAAD) as well as a graduate student stipend by the Landesgraduiertenförderung.

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