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PHYLOGENY OF AMARANTHACEAE AND CHENOPODIACEAE AND THE EVOLUTION OF C_4 PHOTOSYNTHESIS

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A phylogenetic analysis of Chenopodiaceae and Amaranthaceae was carried out using sequence variation of the chloroplast gene *rbc*L. Our sampling included 108 species of these two families along with 29 species of Caryophyllales serving as outgroups. Phylogeny inferences with maximum parsimony and maximum likelihood indicate that the two families form a well-supported monophyletic clade that is sister to Achatocarpaceae. Despite extensive sampling, we found that the relationship between Chenopodiaceae and Amaranthaceae remains unclear as a result of short and weakly supported basal branches. The clearly monophyletic Polycnemoideae (traditionally considered a subfamily of Chenopodiaceae) appear as sister to Amaranthaceae *sensu stricto*. Within Amaranthaceae, most major lineages inferred except Gomphrenoideae and Celosieae do not correspond to recognized subfamilies and tribes. *Bosea* and *Charpentiera* branch first in the Amaranthaceae. Within Chenopodiaceae, the genera of Betoideae occur in basal and largely unresolved positions. The remaining Chenopodiaceae are divided into three major clades of unclear relationship: Chenopodioideae (Atripliceae s.str., Chenopodieae I-III); Corispermoideae (Corispermeae); and Salicornioideae (Haplopeplideae, Salicornieae), Suaedoideae (Suaedeae, Bienertieae), and Salsoloideae (Camphorosmeae, Sclerolaeneae, Salsoleae I-II). The *rbc*L tree is discussed also with regard to historical classifications and morphological support for the major clades. The molecular results are used to elucidate the evolution of C_4 photosynthesis in the two families. C_4 photosynthesis has evolved independently at least three times in Amaranthaceae and at least 10 times in Chenopodiaceae. A survey of C_4 leaf anatomy revealed 17 different leaf types that in most cases mark an independent origin of C_4 photosynthesis. The application of a molecular clock indicates an age of C_4 photosynthesis of 11.5–7.9 Ma in *Atriplex* (Chenopodioideae) and 21.6–14.5 Ma in subfamily Salsoloideae.

Keywords: Amaranthaceae, Chenopodiaceae, phylogeny, systematics, C_4 photosynthesis, C_4 leaf anatomy.

Introduction

Amaranthaceae and Chenopodiaceae constitute the most diverse lineage (ca. 180 genera and 2500 species) of the Caryophyllales and have long been regarded as two closely related families (Brown 1810; Bentham and Hooker 1880; Baillon 1887; Volkens 1893; Ulbrich 1934; Aellen 1965–1968; Behnke 1976; Thorne 1976; Carolin 1983; Kühn et al. 1993). Numerous studies on the morphology, anatomy, and phytochemistry of the two families revealed a number of shared, mostly derived features. These include minute sessile flowers arranged in cymose inflorescences; a five-merous, imbricate, uniseriate perianth; a single whorl of epitepalous stamens; a single basal ovule; pantoporate pollen; chenopodiad embryogeny; sieve elements with P-type plastids but without a central protein crystalloid; occurrence of the betacyanins amaranthin and celosianin; and presence of 6,7-methylenedioxyflavonol and isoflavones (Hegnauer 1964, 1989; Wohlpart and Mabry 1968; Behnke 1976; Natesh and Rau 1984; Sandersson et al. 1988; Rodman 1990, 1994; Behnke and Mabry 1994; see also Judd and Ferguson 1999). The two families have mostly been treated as separate entities although most authors admitted difficulties in identifying distinguishing characters. However, Baillon (1887) treated Chenopodiaceae and Amaranthaceae as one family (table 1), as was later suggested also by Malligson (1922) on the basis of serological studies. Recently, it has again been proposed to merge both families into one family Amaranthaceae (APG 1998; Judd et al. 1999).

The Position of Amaranthaceae/Chenopodiaceae within Caryophyllales

Traditionally, Caryophyllaceae subf. Paronychioideae were assumed to be the closest relatives of Amaranthaceae/Chenopodiaceae (Bentham and Hooker 1880). However, recent phylogenetic analyses based on morphological characters (Rodman 1994), *rbc*L (Manhart and Rettig 1994; Savolainen et al. 2000*b*), and *mat*K sequence data (Cuénoud et al. 2002; Hilu et al. 2003) have identified the small neotropical family Achatocarpaceae as sister to the Amaranthaceae/Chenopodiaceae.

The Amaranthaceae/Chenopodiaceae-Achatocarpaceae clade (for convenience, we refer to this as the ACA clade) clearly belongs to the core Caryophyllales (Cuénoud et al. 2002) and, as such, is part of the Centrospermae as traditionally circumscribed (Cronquist and Thorne 1994). Early analyses of *rbc*L sequence data (Albert et al. 1992;

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Table 1

Historical Classifications of Chenopodiaceae and Amaranthaceae

Moquin-Tandon 1840	Moquin-Tandon 1849	Baillon 1887	Bentham and Hooker 1880	Volkens 1893/Schinz 1893	Ulbrich 1934/Schinz 1934	Kühn et al. 1993/ Townsend 1993	This article
Chenopodeae	Salsolaceae	Chénopodiacées	Chenopodiaceae	Chenopodiaceae	Chenopodiaceae	Chenopodiaceae	Chenopodiaceae
Cyclolobeae	Cyclolobeae		Cyclolobeae	Cyclolobeae	Cyclolobeae		
					Chenopodioideae	Chenopodioideae	Chenopodioideae
Anserinae	Chenopodieae	Chenopodiées	Euchenopodieae	Chenopodieae	Chenopodieae	Chenopodieae	Chenopodieae I-III[a]
Spinaciae	Spinacieae		Atripliceae	Atripliceae	Atripliceae	Atripliceae	Atripliceae[a]
Camphorosmeae	Camphorosmeae		Camphorosmeae	Camphorosmeae	Camphorosmeae	Camphorosmeae	→ Salsoloideae (see below)
			Chenoleae			Sclerolaeneae	→ Salsoloideae (see below)
					Corispermoideae		Corispermoideae
Corispermeae	Corispermeae		Corispermeae	Corispermeae	Corispermeae	Corispermeae	Corispermeae
					Betoideae		Betoideae
					Beteae	Beteae	Beteae I–IV[a]
				Beteae	Hablitzieae		
					Polynemoideae	Polynemoideae	→ Amaranthaceae
	(sub Amaranthaceae Polycnemeae, subtr.)	Polycnémées	Polycnemeae	Polycnemeae	Polycnemeae	Polycnemeae	
					Salicornioideae	Salicornioideae	Salicornioideae
Salicornieae	Salicornieae	Salicorniées	Salicornieae	Salicornieae	Halopeplideae	Halopeplideae	Halopeplideae
					Salicornieae	Salicornieae	Salicornieae
Spirolobeae	Spirolobeae		Spirolobeae	Spirolobeae	Spirolobeae		
					Suaedoideae	Salsoloideae	Suaedoideae
Suaedinae	Suaedeae	Salsolées	Suaedeae	Suaedeae	Suaedeae	Suaedeae	Suaedeae
					Bienertieae		Bienertieae
					Salsoloideae		Salsoloideae
Salsoleae	Salsoleae		Salsoleae	Salsoleae	Salsoleae	Salsoleae	Salsoleae I–II[a]
					Nucularieae		Camphorosmeae (incl. Sclerolaeneae)[a]
					Sarcobatoideae		
	(dubia sedis: *Sarcobatus*)	Sarcobatées	Sarcobatideae	Sarcobatideae	Sarcobateae	Sarcobateae	→ excl. as Sarcobataceae
			Eubaselleae	→ excluded			
			Boussingaultieae	→ excluded			
	Amaranthaceae		Amaranthaceae	Amaranthaceae	Amaranthaceae	Amaranthaceae	Amaranthaceae
				Amaranthoideae	Amaranthoideae	Amaranthoideae	amaranthoids I+II[a]
	Celosieae	Célosiées	Celosieae	Celosieae	Celosieae	Celosieae	Celosieae
	Achyrantheae	Amarantées	Amarantheae	Amarantheae	Amarantheae	Amarantheae	Amarantheae I–IV[a]
				Gomphrenoideae	Gomphrenoideae	Gomphrenoideae	Gomphrenoideae
	Gomphreneae	Gomphrénées	Gomphreneae	Gomphreneae	Gomphreneae	Gomphreneae	Gomphreneae (incl. Pseudoplantageae)[a]
				Guillemineae			
					Brayulineae		
						Pseudoplantageae	
		Microtéées	→ excluded				
		Leucastérées	→ excluded				

[a] New tribal classification necessary.

Giannasi et al. 1992; Rettig et al. 1992; Chase et al. 1993; Manhart and Rettig 1994) had already indicated that Polygonaceae, Plumbaginaceae, Frankeniaceae, Tamaricaceae, Ancistrocladaceae, Dioncophyllaceae, Droseraceae, Nepenthaceae, and a few other families form a monophyletic group together with Centrospermae. This was largely confirmed by 18S nuclear ribosomal DNA (Soltis et al. 1997), *atp*B (Savolainen et al. 2000*a*), and *mat*K sequences (Cuénoud et al. 2002; Hilu et al. 2003). The whole clade was reclassified as an expanded Caryophyllales by APG (1998), and the Caryophyllales s.str. (=Centrospermae) were called core Caryophyllales by Cuénoud et al. (2002).

Combined analyses (Savolainen et al. 2000*a*; Soltis et al. 2000; Cuénoud et al. 2002) provided solid evidence for a basal position of Asteropeiaceae within core Caryophyllales and suggest a split of the remaining families in two sister clades, one comprising the ACA clade and Caryophyllaceae, the other including Nyctaginaceae, Phytolaccaceae, Aizoaceae, Cactaceae, Portulacaceae, and Molluginaceae, along with several isolated genera (=higher core Caryophyllales). However, the relationship of the ACA clade to the Caryophyllaceae is not yet sufficiently clear. Depending on the data set, either both are sister to each other (Savolainen et al. 2000*b*; Soltis et al. 2000; Cuénoud et al. 2002) or Caryophyllaceae appear basal to a lineage comprising the ACA clade and the higher core Caryophyllales (Savolainen et al. 2000*a*; Cuénoud et al. 2002; Hilu et al. 2003).

Phylogenetic Relationships between Amaranthaceae and Chenopodiaceae

Molecular systematic studies of the Caryophyllales in which Amaranthaceae and Chenopodiaceae were represented by more than two taxa either identified them as sister families (Giannasi et al. 1992; Rettig et al. 1992; Downie and Palmer 1994; Cuénoud et al. 2002) or found Amaranthaceae nested within Chenopodiaceae (Manhart and Rettig 1994; Downie et al. 1997; Cuénoud et al. 2002). Combined *rbc*L and partial *mat*K data yielded particularly high statistical support for two monophyletic lineages (100% bootstrap for Amaranthaceae, 99% bootstrap for Chenopodiaceae; Cuénoud et al. 2002), but sampling in that study was limited (Amaranthaceae: *Celosia*, *Amaranthus*, *Froelichia*; Chenopodiaceae: *Spinacia*, *Atriplex*). Phylogenetic analyses using phenotypic characters (Scott 1977*a*; Carolin 1983; Rodman 1990) provided arguments for a paraphyletic Chenopodiaceae. However, in a more recent cladistic analysis, Rodman (1994) again came to the conclusion that the two families are sister to each other. Taken together, the reunion of the two families as Amaranthaceae proposed by APG (1998) and Judd et al. (1999) clearly requires further substantiation.

Classification of Amaranthaceae

Amaranthaceae comprise ca. 70 genera and 800 species, mainly distributed throughout tropical and subtropical latitudes. Only a few genera occur in temperate regions, the most prominent of which is *Amaranthus*. Centres of diversity are Central and South America, tropical and South Africa, and Australia. The family contains annuals, herbaceous perennials, shrubs, woody lianas (e.g., *Hebanthe*, *Sericostachys*), and even small trees. Inflorescences are either complex cymose structures or the cymes are reduced to a single flower subtended by one bract and two bracteoles.

Important contributions to the systematics of Amaranthaceae were made by Martius (1826), Moquin-Tandon (1849), Schinz (1893, 1934), Suessenguth (1934), and Cavaco (1962; table 1). The currently accepted classification by Townsend (1993) is based on Schinz (1893, 1934), who recognized two subfamilies, namely Gomphrenoideae, with 2-locular anthers, and Amaranthoideae, with 4-locular anthers, and four tribes (table 1). The Pseudoplantageae (of Gomphrenoideae) with amaranthoid floral morphology but 2-locular anthers were considered intermediate between the two subfamilies (Eliasson 1988; Townsend 1993). Cavaco (1962) presented a new system largely based on inflorescence characters and embryology, with two additional subfamilies (Brayulineoideae, Celosioideae). However, this classification was not accepted by later authors (Eliasson 1988; Townsend 1993). A recent survey of the pollen morphology of Amaranthaceae points to the polyphyly of most of Schinz's tribes (Borsch 1998).

Classification of Chenopodiaceae

Chenopodiaceae comprise ca. 110 genera with ca. 1700 species. They are predominantly found in arid to semiarid, saline, disturbed, and agricultural habitats of temperate and subtropical regions (maps in Zhu 1996). Only few genera are also present in the Tropics, e.g., *Chenopodium*, *Halosarcia*, and *Suaeda*. Most species of the family are annuals or subshrubs. Herbaceous perennials, shrubs, small trees, and lianas are restricted to only few genera.

The taxonomic history of Chenopodiaceae is characterized by numerous rearrangements at the subfamily level (table 1). The first subdivision (Meyer 1829) was based on seed structure, which can be exalbuminous with a spiral embryo (Spirolobeae) or albuminous with a peripheral embryo (Cyclolobeae). These two subgroups were adopted by many authors but were given different names and ranks (see table 1). Based on ideas of Volkens (1893), Ulbrich (1934) raised the number of subfamilies to eight, namely Polycnemoideae, Betoideae, Chenopodioideae, Corispermoideae, Salicornioideae, Sarcobatoideae, Suaedoideae, and Salsoloideae. This classification was generally accepted. However, in more recent accounts, some of Ulbrich's subfamilies were abandoned (Williams and Ford-Lloyd 1974; Kühn et al. 1993; Judd and Ferguson 1999). Scott (1977*a*, 1977*b*) attempted to reinstate Salsolaceae Moq. and Salicorniaceae J. Agardh as separate families, but this was generally rejected by subsequent authors. Agreement was also reached on the reclassification of the Australian genus *Dysphania* R. Br. within Chenopodioideae (Eckardt 1967, 1968) after it had been included in Illecebraceae (Bentham and Hooker 1880) or separated as the monotypic Dysphaniaceae (Pax 1927).

The position of *Polycnemum* and a few related genera has long been controversial. All or some of these have been included in Chenopodiaceae (Dumortier 1827; Bentham and Hooker 1880; Volkens 1893; Ulbrich 1934; Aellen 1965–1968; Kühn et al. 1993), Caryophyllaceae (Moquin-Tandon 1837), or Amaranthaceae (Endlicher 1837; Boissier 1879; Black 1924; Soriano 1944). In many accounts, Polycnemeae

were considered as morphological intermediates between Chenopodiaceae and Amaranthaceae (Bentham and Hooker 1880; Volkens 1893) and sometimes even as a group bridging the gap to the Paronychieae of Caryophyllaceae (Ulbrich 1934; Aellen 1965–1968; Kühn et al. 1993).

Two genera that were traditionally classified within Chenopodiaceae have been excluded and established as separate families because of deviant phenotypic characters. These are the monotypic *Halophytum* (formerly Salicornioideae; Soriano 1946) and *Sarcobatus*, comprising two species (Behnke 1994, 1997). Subsequent molecular phylogenetic studies confirmed their status as distinct lineages not closely related to the Amaranthaceae-Chenopodiaceae alliance (Downie et al. 1997; Cuénoud et al. 2002).

Distribution and Evolution of C_4 Taxa in Amaranthaceae and Chenopodiaceae

One prominent feature shared by Amaranthaceae and Chenopodiaceae is the frequent occurrence of C_4 photosynthesis as proven by carbon isotope determinations ($\delta^{13}C$ values) and leaf anatomical studies (Akhani et al. 1997; Sage et al. 1999 and references therein; Jacobs 2001; R. F. Sage, unpublished survey of C_4 taxa in Amaranthaceae). According to recent counts, C_4 photosynthesis occurs in 45 genera and ca. 550 species of Chenopodiaceae and in 10 genera and ca. 250 species of Amaranthaceae (Sage and Monson 1999; Sage 2001; R. F. Sage, unpublished data). Whereas both families together contain ca. 50% of all C_4 species known among eudicots, other families of the core Caryophyllales contain only modest numbers of C_4 species: Portulacaceae 70 spp./2 gen., Caryophyllaceae 50 spp./1 gen., Aizoaceae 30 spp./5 gen., Nyctaginaceae 25 spp./3 gen., and Molluginaceae 3 spp./1 gen. (Sage 2001). At present, ca. 6000 C_4 species are known for monocots (401 genera of Poaceae, Cyperaceae, and Hydrocharitaceae) and 1600 C_4 species for eudicots (86 genera from 15 families [Sage 2001]). It has been estimated that C_4 photosynthesis evolved at least 31 times in 18 different angiosperm families (Kellogg 1999; Sage 2001).

Most Chenopodiaceae and Amaranthaceae prefer habitats in which C_4 plants are favored and often dominant, i.e., warm temperate and tropical grasslands, savannas, sand dunes, salt marshes, semideserts, and deserts. Large C_4 genera of the Amaranthaceae/Chenopodiaceae lineage that have diversified in such habitats are, e.g., *Gomphrena*, *Amaranthus*, *Atriplex*, *Salsola*, and *Suaeda*. In Chenopodiaceae and Amaranthaceae, C_4 photosynthesis also occurs in numerous subshrubs, shrubs, and rarely even in small trees, whereas the majority of C_4 species in other families are herbaceous. While the leaf anatomy of C_4 species in Amaranthaceae is incompletely known (but see Carolin et al. 1978; Ruthsatz and Hofmann 1984), the leaf anatomy of C_4 species in Chenopodiaceae has been studied intensively (Carolin et al. 1975, 1982; Shomer-Ilan et al. 1975; Voznesenskaya 1976; Butnik 1984, 1995; Gamaley 1984, 1985; Voznesenskaya and Gamaley 1986; Butnik et al. 1991, 2001; P'yankov et al. 1997; Fisher et al. 1997; Freitag and Stichler 2000, 2002; Voznesenskaya et al. 2001*a*, 2001*b*, 2002) even before the physiological background of C_4 photosynthesis was known (Volkens 1887; Monteil 1906; Khatib 1959). Together, these studies document an astonishing diversity in C_4 leaf anatomy that surpasses the diversity of C_4 types found in grasses and suggests a multiple origin of C_4 photosynthesis even at lower systematic levels. Thus, several large genera in both families contain C_3 as well as C_4 species, e.g., *Atriplex*, *Kochia*, *Bassia*, *Suaeda*, and *Salsola* in Chenopodiaceae and *Aerva*, *Alternanthera*, and *Gomphrena* in Amaranthaceae. However, the monophyly of several of these genera is doubtful.

Studies of C_4 leaf architecture and the occurrence of C_4 taxa in several of the traditional subfamilies suggested multiple origins of C_4 photosynthesis in Chenopodiaceae. The classical papers of Carolin et al. (1975, 1982) list four C_4 leaf types that have evolved independently from C_3 leaves and a fifth type that probably is derived from a simpler C_4 type. On the basis of anatomical studies and biochemical data, Freitag and Stichler (2002) hypothesized four separate derivations of C_4 leaf types only within the small subfamily Suaedoideae. A recent molecular phylogenetic analysis of nuclear ITS and noncoding chloroplast DNA sequences provided independent evidence for this hypothesis (Schütze et al. 2003). Multiple origins of C_4 photosynthesis were also proven by molecular analyses in other families, e.g., 10 times in Poaceae (GPWG 2001; Giussani et al. 2001), four times in Cyperaceae (Soros and Bruhl 2000), three times in Asteraceae (Karis and Ryding 1994; Kim and Jansen 1995; Kopriva et al. 1996; see also Kellogg 1999), and at least twice in Zygophyllaceae (Sheahan and Chase 1996).

Like in Asteraceae, Brassicaceae, Cyperaceae, Molluginaceae, Hydrocharitaceae, and Poaceae (Sage and Monson 1999 and references therein), anatomical and physiological C_3-C_4 intermediates have also been documented for Amaranthaceae (*Alternanthera*: Rajendrudu et al. 1986; Devi and Raghavendra 1993) and Chenopodiaceae (*Salsola*: Voznesenskaya et al. 2001*a*).

Aims of This Study

The aims of this phylogenetic analysis are (1) to clarify the relationships of Amaranthaceae and Chenopodiaceae, (2) to test the monophyly of all currently recognized subfamilies and tribes and to propose relevant classificatory adjustments in cases of clear evidence by molecular and morphological characters, and (3) to trace the evolution of C_4 photosynthesis as a biologically highly relevant complex of characters that may have played a crucial role in the diversification of the Amaranthaceae/Chenopodiaceae lineage. To achieve this, we performed an extensive sampling including 108 species of both families representing 78 genera. The taxa sampled were carefully selected to cover the morphological diversity of both families and thus all presumed major lineages. All three genera of the Polycnemoideae were included because the members of this subfamily share morphological similarities with both families, show a number of unique characters, and had not been studied at the DNA level before.

The plastid *rbc*L gene was chosen for comparative sequencing for several reasons. First, in addition to revealing deep-level relationships among angiosperms (Chase et al. 1993; Olmstead and Palmer 1994), *rbc*L has been successfully applied to family- and genus-level phylogenetic questions in a wide range of taxa (Price and Palmer 1993; Olmstead and

Sweere 1994; Hoot et al. 1995; Endress et al. 1996; Bremer et al. 1999; Prince and Parks 2001). Second, *rbc*L fragments are comparatively easy to amplify and sequence even from difficult templates (Savolainen et al. 2000*b*). Third, *rbc*L sequences are already available from several taxa of Caryophyllales, providing a rich source for outgroups. Accompanying leaf anatomical studies were carried out to document the diversity of C_4 leaf types in the different lineages of Chenopodiaceae. Finally, the rate of *rbc*L sequence evolution was determined for Chenopodiaceae and calibrated by several fossils in an attempt to estimate the age of C_4 photosynthesis in this family.

Material and Methods

Sequence Analysis

Leaf samples were acquired as herbarium, silica-dried, or fresh material, or they were preserved in saturated NaCl-CTAB solution, supplemented with 200 mM sodium ascorbate (Rogstad 1992; S. Jacobs, personal communication). The latter method yielded extraordinary good quality and quantity of DNA especially for the succulent taxa. Extraction of total genomic DNA was performed by using NucleoSpin plant DNA extraction kits (Macherey-Nagel, Düren, Germany) following the manufacturer's specifications or by a modified CTAB method (Borsch et al. 2003).

*Rbc*L sequences were obtained for 137 species. Of these, 110 are new, and 27 were taken from GenBank (see table 2 for accession numbers and information on vouchers). For each taxon, two or three overlapping fragments were PCR amplified and sequenced using standard *rbc*L primers (1F 5′-ATGTCACCACAAACAGAAACTAAAGC-3′, 875F 5′-GCAGTTATTGATAGACAGA-3, 955F 5′-CGTCTATCTGGTGGAGATC-3′, 579R 5′-AAATCAAGTCCACCGCG-3′, 1460R 5′-CTTTTAGTAAAAGATTGGGCCGAG-3′). Two internal primers were designed for this study (507F 5′-TATTGGGATGCACTATTAAAC-3′, 1024R 5′-ATCAACAAARCCTAAAGTAATATC-3′).

PCR amplifications were performed using the following reaction mix: 2 mM $MgCl_2$, 200 μM dNTP, 1 pmol primer, 0.025 U/μL *Taq* polymerase, 4% DMSO, and ca. 1 ng/μL DNA in a buffer provided by the manufacturer of the polymerase. Grant Autogene II or Biometra T3 thermocyclers were programmed as follows: pretreatment of 60 s at 94°C, followed by 35 cycles of 18 s at 94°C, 30 s at 55°C, 60 s at 72°C, and a posttreatment of 78 s at 55°C and 8 min at 72°C. For difficult templates such as DNA isolated from 10-yr-old herbarium material of *Pseudoplantago*, an additional 7-min denaturation step at 95°C (hot start) was included, and a highly sensitive *Taq* polymerase (Amplitaq Gold and gold buffer, Applied Biosystems) was used. Amplification products were checked on 0.8% agarose gels. PCR products were usually purified directly with the PCR product purification kit of Macherey-Nagel. In those samples where the test gel showed a smear, total PCR products were gel purified using Macherey-Nagel or QiaGen gel extraction kits. A few sequences remained incomplete because of amplification problems (see table 2 for information on missing data).

Purified, double-stranded PCR products were sequenced directly, using the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). Fivefold diluted PCR primers were used as sequencing primers. For cycle sequencing, thermocyclers were programmed as follows: preheating for 60 s at 96°C, 27 cycles of 6 s at 96°C, 12 s at 55°C, 4 min at 60°C, and posttreatment of 18 s at 51.4°C, 4 min at 60°C. Extension products were purified by ethanol/sodium acetate or isopropanol precipitation and electrophoresed on ABI 310, 373, or 377 automated sequencers. Forward and reverse sequences were compared and edited, and consensus sequences initially aligned using Sequencher 4.1. The alignment was straightforward since no indels occurred. The sequences were trimmed at both ends to avoid missing data. Each sequence starts with nucleotide position 64 of the translated region and ends with position 1406 (Zurawski et al. 1981)

Four representatives of the noncore Caryophyllales (Cuénoud et al. 2002), namely *Limonium spectabile* (Plumbaginaceae), *Frankenia pulverulenta* (Frankeniaceae), *Drosophyllum lusitanicum* (Droseraceae), and *Simmondsia chinensis* (Simmondsiaceae), were defined as outgroups. The ingroup contained 30 Amaranthaceae, 78 Chenopodiaceae, two Achatocarpaceae, 10 Caryophyllaceae, 11 higher core Caryophyllales belonging to other families, and one representative each of Physenaceae and Asteropeiaceae (table 2).

Maximum parsimony (MP) analyses were performed with PAUP* (Swofford 2002) in 100 replicated heuristic searches using random stepwise addition of taxa and tree-bisection-reconnection (TBR) branch swapping. Node support was assessed by 1000 bootstrap replicates with TBR swapping, random addition of taxa, retaining a maximum number of 600 trees in each replicate. For convenience in presenting and discussing our results, bootstrap support of 50%–74% is considered low, 75%–84% moderate, and >85% high (Chase et al. 2000).

Maximum likelihood (ML) analyses were performed as follows. The appropriate model of DNA substitution for the inference of phylogenetic relationships under ML was estimated using Modeltest 3.06 software (Posada and Crandall 1998). The GTR (general time-reversible) model was chosen with gamma distribution set to 0.727. The rate matrix was set to AC 1.2417, AG 2.9825, AT 0.4342, CG 1.0826, CT 3.9377, and GT 1.0. Heuristic search settings were set to stepwise random addition of taxa and TBR swapping. The search was aborted after 74,500 rearrangements.

Calibration and Application of a Molecular Clock

Estimation of divergence time was restricted to the Chenopodiaceae excluding Betoideae. Because nonsynonymous substitutions are likely to be nonclocklike in *rbc*L (Xiang et al. 2000), they were excluded from the subsequent analyses. To this end, the nucleotide sequence was translated into the amino acid sequence, and nonsynonymous substitutions were identified using MacClade (3.08a; Maddison and Maddison 1999). A global likelihood ratio test (Felsenstein 1988) was conducted with the reduced nucleotide matrix. This was achieved by calculating log-likelihood scores for trees with and without a molecular clock enforced. Nonsignificance at the 0.01 level between tree topologies, indicating that a molecular clock cannot be rejected, was assessed with Modeltest 3.06 (Posada and Crandall 1998). Because this first global likeli-

Table 2

Taxa Sampled Including Vouchers and GenBank Accession Numbers for the Sequences Generated in This Study

Family, subfamily, tribe, and species[a]	DNA source (garden, field origin, voucher)	GenBank accession no.
Amaranthaceae, Amaranthoideae, Amarantheae:		
Achyranthes aspera L.	S. Jacobs 8660; NW of Charters Towers, Queensland, Australia (NSW)	AY270048
Aerva javanica (Burm. f.) Schultes	E. Fischer s.n.; Bot. Gard. Bonn, from Madagascar (BONN, BG 12712)	AY270050
Amaranthus greggii S. Wats.	D. Pratt, K. Müller, & Th. Borsch 207; Texas, U.S.A. (ISC, BONN)	AY270055
Amaranthus tricolor L.	Rettig et al. 1992	X53980
Bosea yervamora L.	K. Müller 751; Tenerife, Canary Islands (BONN)	AY270069
Calicorema capitata (Moq.) Hook.f.	C. Neinhuis s.n.; Namibia (BONN)	AY270070
Chamissoa altissima (Jacq.) Kunth	E. Zardini & L. Guerrero 42592; Paraguay (BONN, MO)	AY270073
Charpentiera obovata Gaudich.	B. A. Prigge 15251; Hawaii, U.S.A. (LA)	AY270074
Charpentiera ovata Gaudich.	B. A. Prigge 15252; Hawaii, U.S.A. (LA)	AY270075
Nototrichium humile Hillebr.	B. A. Prigge 15249; Hawaii, U.S.A. (LA)	AY270111
Pandiaka angustifolia (Vahl) Hepper	J. Müller 324; Burkina Faso (FR)	AY270115
Ptilotus manglesii (Lindl.) F. Muell	Th. Borsch 3543; Australia (BONN, Bot. Gard. Bonn 12999)	AY270121
Pupalia lappacea A. Juss.	Th. Borsch 3544; (BONN, Bot. Gard. Bonn 16784)	AY270122
Sericostachys scandens Gilg et Lopr.	E. Fischer s.n.; Rwanda (BONN)	AY270134
Amaranthaceae, Amaranthoideae, Celosieae:		
Celosia argentea L.	Bot. Gard. Mainz (no voucher)	AY270072
Deeringia amaranthoides (Lam.) Merrill	E. Moore 746; Guam, Philipp. Sea (BONN, Bot Gard. Bonn 18100)	AY270085
Hermbstaedtia glauca (Wendl.) Reichenb. ex Steudel	C. Neinhuis s.n.; Namibia (BONN)	AY270099
Pleuropetalum sprucei (Hook. f.) Standley	Th. Borsch 3547; (BONN, Bot. Gard. Bonn 16484)	AY270117
Amaranthaceae, Gomphrenoideae, Gomphreneae:		
Alternanthera caracasana Kunth	Th. Borsch, D. Pratt, & K. Müller 3433; Texas, U.S.A. (ISC, BONN)	AY270053
Alternanthera pungens Kunth	Th. Borsch, & D. Pratt, & K. Müller 3449; Texas, U.S.A. (ISC, BONN)	AY27054
Blutaparon vermiculare (L.) Mears	Th. Borsch, D. Pratt, & K. Müller 3444; Texas, U.S.A. (ISC, BONN)	AY270067
Froelichia floridiana (Nutt.) Moq.	J. S. Clement and T. J. Mabry, unpublished data	AF132089 (22 bp missing)
Gomphrena elegans Mart.	Th. Borsch 3545; from Bot. Gard. Meise 07-4052 (BONN)	AY270088
Gomphrena haageana Klotzsch	Bot. Gard. Mainz (no voucher)	AY270089
Gomphrena serrata L.	Th. Borsch & B. Summers 3221; Florida, U.S.A. (BONN, VPI)	AY270090
Guilleminea densa (Willd.) Moq.	Th. Borsch, D. Pratt, & K. Müller 3437; Texas, U.S.A. (ISC, BONN)	AY270091
Hebanthe occidentalis (R. E. Fr.) Borsch & Pedersen	E. Zardini 45377; Paraguay (BONN, MO)	AY270097
Iresine palmeri S. Wats.	Th. Borsch, D. Pratt, & K Müller 3445; Texas, U.S.A. (ISC, BONN)	AY270101
Tidestromia lanuginosa (Nutt.) Standl.	Th. Borsch, D. Pratt, & K. Müller 3439; Texas, U.S.A. (ISC, BONN)	AY270141
Amaranthaceae, Gomphrenoideae, Pseudoplantageae:		
Pseudoplantago friesii Suess.	T. M. Pedersen 15792; Argentina (CTES, C)	AY270120
Chenopodiaceae, Chenopodioideae, Atripliceae:		
Atriplex coriacea Forssk.	H. Freitag 19.596; Eastern desert, Wadi Hof, Egypt (KAS)	AY270045
Atriplex halimus L.	J. Hensen s.n., 31.03.01; Salinas Santa Palo, SE Spain (KAS)	AY270059
Atriplex patula L.	Hudson et al. 1990	X15925
Atriplex rosea L.	Hudson et al. 1990	
Atriplex spongiosa F. Muell.	Hort. Bot. Berg. Stockholm	AY270060
Atriplex undulata (Moq.) D. Dietr.	M. E. Múlgura 2005; La Pampa, Argentina (SI, KAS)	AY270061
Axyris prostrata L.	G. & S. Miehe 96-140-04; Gobi Altai, Mongolei (Hb. Miehe, KAS)	AY270062
Halimione pedunculata (L.) Aellen	G. Kadereit 2000/202; Kattegat, Denmark (MJG)	AY270093
Krascheninnikovia ceratoides(L.) Gueldenst.	B. Dickoré 12752; Nanga Parbat area, Pakistan (Hb. Dickoré, KAS)	AY270105
Microgynoecium tibeticum Hook. f.	B. Dickoré 4284; Qinghai, C. Tibet, China (Hb. Dickoré, KAS)	AY270107
Scleroblitum atriplicinum (F. Muell.) Ulbr.	S. Jacobs 8724; Lake Pinaroo, North Far Western Plains, New South Wales, Australia (NSW)	AY270044 (52bp missing)
Spinacia oleracea L.	Zurawski et al. 1981	
Chenopodiaceae, Chenopodioideae, Beteae:		
Acroglochin chenopodioides Schrad.	Bot. Gard. Kassel (KAS); seeds from Jard. Bot. Lyon	AY270049
Aphanisma blitoides Nutt. ex Moq.	S. Junak SR-987; Santa Rosa Island, Santa Barbara County, California, U.S.A. (SBBG)	AY270057
Beta *vulgaris* L. subsp. *maritima* Thell.	G. Kadereit 99/255; Baltic Sea, Germany (MJG)	AY270065
Beta nana Boiss. & Heldr.	K. Tan s.n.; Mt. Vardhousia, Sterea, Greece (C, KAS)	AY270064
Hablitzia tamnoides M. Bieb.	Th. Borsch 3546; Bot. Gard. Bonn 3609–90 (BONN)	AY270092
Oreobliton thesioides Durieu & Moq.	J. Poelt s.n., 22.04.1982; S Tunisia (M)	AY270113
Chenopodiaceae, Chenopodioideae, Camphorosmeae:		
Bassia dasyphylla (Fisch. & C.A. Mey.) Kuntze	G. & S. Miehe 96-203-02; Gobi Altai, Mongolia (Hb. Miehe, KAS)	AY270150
Bassia sedoides (Pall.) Asch.	H. Freitag 28.035; Uralsk, NW Kazakhstan (KAS)	AY270063
Camphorosma monspeliaca L.	H. Freitag 28.133; Prov. Guŕyev (Atyrau), Kazakhstan (KAS)	AY270071

Table 2

(Continued)

Family, subfamily, tribe, and species[a]	DNA source (garden, field origin, voucher)	GenBank accession no.
Chenoleoides tomentosa (Lowe) Botsch.	H. Freitag 27.256; Lanzarote, Canary Islands (KAS)	AY270076
Dissocarpus paradoxus (R. Br.) Ulbr.	S. Jacobs 8712; South Far Western Plains, New South Wales, Australia (NSW)	AY270151
Kochia americana Wats.	S.-W. Breckle 2756; Utah, U.S.A. (Hb. Breckle)	AY270103
Kochia prostrata (L.) Schrad.	H. Freitag 28.254; Volgograd, SE Russia (KAS)	AY270104
Panderia pilosa Fisch. & C.A. Mey.	H. Freitag & G. Kothe 18.894; Kalat, Baluchistan, Pakistan (KAS)	AY270114
Chenopodiaceae, Chenopodioideae, Chenopodieae:		
Chenopodium acuminatum Willd.	G. & S. Miehe 96-060-5; Gobi Altai, Mongolia (Hb. Miehe, KAS)	AY270077
Chenopodium auricomum Lindley	S. Jacobs 8655; North Western Plains, New South Wales, Australia (NSW)	AY270078
Chenopodium bonus-henricus L.	Bot. Gard. Mainz	AY270079
Chenopodium botrys L.	H. Freitag & N. Adigüzel 28.769; Konya Prov., Turkey (KAS, GAZI)	AY270080
Chenopodium cristatum (F. Muell.) F. Muell.	S. Jacobs 8653; North Western Plains, New South Wales, Australia (NSW)	AY270046
Chenopodium desertorum (J. Black) J. Black ssp. *anidiophyllum* (Aellen) Paul. G. Wilson	S. Jacobs 8650; North Western Plains, New South Wales, Australia (NSW)	AY270042
Chenopodium foliosum Asch.	G. Stöber 42; Yasin, Pakistan (GOET)	AY270081
Chenopodium frutescens C. A. Mey.	A. Korolyuk s.n., 23.6.2000; Tuva, Russia (NS)	AY270082
Chenopodium sanctae-clarae Johow	Roy. Bot. Gard. Kew, from Juan Fernandez Islands (K)	AY270043
Dysphania glomulifera (Nees) Paul G. Wilson	S. Jacobs 8738; North Western Plains, New South Wales, Australia (NSW)	AY270086
Holmbergia tweedii Speg.	Zardini et al. 21619; Rio Verde, Paraguay (K)	AY270100 (128 bp missing)
Monolepis nuttaliana Greene	Bot. Gard. Kassel; seeds from Univ. Hohenheim (KAS)	AY270108
Rhagodia drummondi Moq.	N. Schmalz 194 (52); Hayden, Western Australia (MJG)	AY270124
Teloxys aristata (L.) Moq.	B. Neuffer & H. Hurka 11.727; Ulaanbaatar, Mongolia (Hb. Hurka, KAS)	AY270140
Chenopodiaceae, Chenopodioideae, Corispermeae:		
Agriophyllum squarrosum (L.) Moq.	H. Freitag 28.196a; Prov. Astrakhan, SE Russia (KAS)	AY270051
Anthochlamys multinervis Rech.f.	H. Freitag 13.979; Kavir National Parc, Mobarakiyeh, Iran (KAS)	AY270056
Corispermum filifolium C. A. Mey.	H. Freitag & N. Adigüzel 28.702, Samsun, Prov. Çarambe (KAS, GAZI)	AY270084
Chenopodiaceae, Chenopodioideae, Sclerolaeneae:		
Maireana brevifolia (R. Br.) Paul G. Wilson	D. Brandes 20.2.1999; Fuerteventura, Canary Islands (Hb. Brandes, KAS)	AY270106
Sclerolaena obliquicuspsis (R. Anders.) Ulbr.	N. Schmalz 85 (15); Norseman, Western Australia (MJG)	AY270132
Chenopodiaceae, Polycnemoideae, Polycnemeae:		
Hemichroa diandra R. Br.	Blaylock 383; 140 km NNW Adeleide, South Australia (AD, M)	AY270098
Nitrophila occidentalis S. Wats.	D. Pratt 204; Utah, U.S.A. (ISC, BONN)	AY270109
Polycnemum majus A. Br. f.	Bot. Gard. Mainz	AY270118
Polycnemum perenne Litv.	M. Nabiev & U. Pratov 124; S-Kirgistan, Mayli-sai (TASH)	AY270119 (34 bp missing)
Chenopodiaceae, Salicornioideae, Halopeplideae:		
Allenrolfea occicentalis Kuntze	M. Piep & S. Long 120; Utah, U.S.A. (UTC)	AY270052
Halopeplis amplexicaulis Ung.-Sternb.ex Ces., Passer. & Gibelli	G. Kadereit et al. 2002/14; Laguna de Guallar, Spain (MJG)	AY270095
Kalidium caspium Ung.-Sternb.	H. Freitag 30.022; Syr-Darya distr., S Tashkent Uzbekistan (KAS)	AY270102
Chenopodiaceae, Salicornioideae, Salicornieae:		
Arthrocnemum macrostachyum (Moric.) K. Koch	H. Freitag & N. Adigüzel 28.846; SE Adana, Seyhan Prov., Turkey (KAS, GAZI)	AY270058
Halocnemum strobilaceum (Pall.) M. Bieb.	H. Freitag & N. Adigüzel 28.783; near Konya, Turkey (KAS, GAZI)	AY270094 (26 bp missing)
Halosarcia indica (Willd.) Paul G. Wilson	M. A. Khan & B. Grul s.n., 10.03.2000; Gadani, SW Pakistan (KUH, KAS)	AY270096
Salicornia dolichostachya Moss	K. Scheelke s.n., Aug. 2001; Spiekeroog, North Sea, Germany (no voucher)	AY270125
Sarcocornia utahensis (Tidestr.) A. J. Scott	D. Pratt 196; Utah, U.S.A. (ISC)	AY270126
Sarcocornia blackiana (Ulbr.) A. J. Scott	N. Schmalz 190 (S 49); Hayden, Western Australia (MJG)	AY270149 (324 bp missing)
Pachycornia triandra (F. Muell.) J. Black	S. Jacobs 8702; South Far Western Plains, New South Wales, Australia (NSW)	AY270047
Sclerostegia moniliformis Paul G. Wilson	N. Schmalz 184 (S 43); Lake King, Western Australia, (MJG)	AY270133
Tecticornia australasica (Moq.) Paul G. Wilson	S. Jacobs 8685; N. of Townsville, Queensland, Australia (NSW)	AY270139
Chenopodiaceae, Salsoloideae, Salsoleae:		
Climacoptera crassa (M.Bieb.) Botsch.	H. Freitag 30.115; Gulistan distr., SSW of Tashkent, Uzbekistan (KAS)	AY270083 (74 bp missing)
Girgensohnia oppositiflora (Pall.) Fenzl	H. Freitag & S. Rilke 26.282; Alma-Ata distr., Samsy, Kazakhstan (KAS)	AY270087
Noaea mucronata (Forssk.) Asch. & Schweinf.	H. Freitag & N. Adigüzel 28.716; Çorum, 16 km WSW of Sungurlu, Turkey (KAS)	AY270110
Ofaiston monandrum (Pall.) Moq.	H. Freitag 28.078; Lake Shalkar, NW Kazakhstan (KAS)	AY270112
Petrosimonia nigdensis Aellen	H. Freitag & N. Adigüzel 28.730; Eski3ehir, SW Polatli, Turkey (KAS, GAZI)	AY270116
Raphidophyton regelii (Bunge) Iljin	V.I. Baranov 364, Karatau, Kazakhstan (TASH)	AY270123
Salsola canescens (Moq.) Spach	H. Freitag 28.800; Aksaray Prov., S edge of Tuz Gölü, Turkey (KAS, GAZI)	AY270127 (20 bp missing)

Table 2

(Continued)

Family, subfamily, tribe, and species[a]	DNA source (garden, field origin, voucher)	GenBank accession no.
Salsola genistoides Juss.ex Poir.	J. Hensen, s.n., 1.04.2001; Campo de Tabernas, SE Spain (KAS)	AY270128
Salsola kali L.	G. Kadereit 1999/211; Baltic Sea, Germany (MJG)	AY270129
Salsola laricifolia Litv. ex Drobov	K. Helmecke s.n., 9.7.1973; Omnogobi Aimaq, Mongolia (HAL)	AY270130 (13 bp missing)
Salsola vermiculata L.	H. Freitag 27.234; Campo de Nijar, SE Spain (KAS)	AY270131
Sympegma regelii Bunge	H. Kürschner & M. Sonnentag 00-548; Prov. Gansu, ca. 90 km NW Zhang Ye, China (BSB, KAS)	AY270138
Chenopodiaceae, Salsoloideae, Suaedeae:		
Bienertia cycloptera Bunge	H. Akhani s.n., 16.11.2000; Kavir protected area near Mobarakiyeh, Iran (Hb. Akhani, KAS)	AY270066
Borszczowia aralocaspica Bunge	Bot. Gard. Kassel, from E Kazakhstan (Ogar 25.9.2000) (KAS)	AY270068 (369 bp missing)
Suaeda altissima (L.) Pall.	H. Freitag & N. Adigüzel 28.601; near Erzincan, Turkey (GAZI, KAS)	AY270135
Suaeda crassifolia Pall.	H. Freitag, 30.130; near Gulistan, SW of Tashkent, Uzbekistan (KAS)	AY270136
Suaeda maritima ("*macrocarpa*") (L.) Dumort.	Bot. Gard. Kassel, from North Sea coast, Wucherer 1996 (KAS)	AY270137
Achatocarpaceae:		
Achatocarpus praecox Griseb.	Bot. Gard. Berlin	AY270142
Phaulothamnus spinescens A. Gray	Manhart and Rettig 1994	M97887
Aizoaceae:		
Sesuvium verrucosum Rafin.	Clement and Mabry 1996	AF132098 (22 bp missing)
Tetragonia tetragonioides (Pall.) Kuntze	Clement and Mabry 1996	AF132094 (22 bp missing)
Asteropeiaceae:		
Asteropeia micraster H.Hallier	D. E. Soltis, P. S. Soltis, and M. W. Chase, unpublished data	AF206737 (48 bp missing)
Basellaceae:		
Anredera cordifolia (Ten.) Steenis	Bot. Gard. Mainz	AY270147
Cactaceae:		
Pereskia aculeata Mill.	D. E. Soltis, P. S. Soltis, and M. W. Chase, unpublished data	AF206805
Caryophyllaceae, Alsinoideae:		
Arenaria drummondii Shinners	Rettig et al. 1992	M83541
Cerastium glomeratum Thuill.	Rettig et al. 1992	M83542
Scleranthus annuus L.	Th. Borsch 3389; Rheinland-Pfalz, Germany (BONN)	AY270145
Stellaria media Cyrill.	Rettig et al. 1992	M62570
Caryophyllaceae, Caryophylloideae:		
Dianthus caryophyllus L.	Giannasi et al. 1992	M77699
Silene gallica L.	Rettig et al. 1992	M83544
Caryophyllaceae, Paronychioideae:		
Herniaria glabra L.	Clement and Mabry 1996	AF132091 (22 bp missing)
Illecebrum verticillatum L.	Th. Borsch & K. Müller 3541; Nordrhein-Westfalen Germany (BONN)	AY270143
Polycarpon tetraphyllum L.	Bot. Gard. Mainz	AY270144
Spergula rubra (L.) J. et C. Presl.	Bot. Gard. Mainz	AY270146
Didiereaceae:		
Alluaudia procera Drake	Rettig et al. 1992	M62563
Molluginaceae:		
Mollugo verticillata L.	Rettig et al. 1992	M62566
Nyctaginaceae:		
Bougainvillea glabra Choisy	Manhart and Rettig 1994	M88340
Physenaceae:		
Physena spec.	Morton 1997	Y13116 (27 bp missing)
Phytolaccaceae:		
Phytolacca americana L.	Rettig et al. 1992	M62567
Stegnospermataceae:		
Stegnosperma halimifolia Benth.	Rettig et al. 1992	M62571
Portulacaceae:		
Portulaca grandiflora Hook.	Rettig et al. 1992	M62568
Sarcobataceae:		
Sarcobatus vermiculatus Torr.	Ickert-Bond. 1121, Arizona, U.S.A.	AY270148 (370 bp missing)
Droseraceae:		
Drosophyllum lusitanicum Link.	Albert et al. 1992	L01907
Frankeniaceae:		
Frankenia pulverulenta L.	Fay et al. 1997	Z97638 (40 bp missing)
Plumbaginaceae:		
Limonium spectabile (Svent.) Kunkel & Sunding	Fay et al. 1997	Z97646 (64 bp missing)
Simmondsiaceae:		
Simmondsia chinensis (Link) C. K. Schneid.	Hoot et al. 1999	AF093732

Note. Sources of sequences that were already in GenBank are mentioned with a reference instead of the voucher specimen including the accession number. Herbarium acronyms are according to Index Herbariorum.

[a] Classification after Townsend (1993) (Amaranthaceae), Kühn et al. (1993) (Chenopodiaceae), and Bittrich (1993*b*) (Caryophyllaceae).

hood ratio test did not show rate constancy, relative rate tests (Wu and Li 1985) were conducted using the program K2WuLi (Jermiin 1996) to evaluate rate constancy of *rbc*L sequence evolution in Chenopodiaceae. Rate constancy was tested for the Salicornioideae/Suaedoideae/Salsoloideae clade (with *Corispermum* defined as outgroup) and for the Chenopodioideae clade (with *Acroglochin* defined as outgroup). Pairwise comparisons were used to identify taxa with highly deviating substitution rates. These were removed from the matrix used in a second likelihood ratio test, starting with the taxon with the highest or lowest *z* score. Rate constancy among lineages was then again tested with global likelihood ratio tests (Felsenstein 1988) with recalculated ML models separately for Chenopodioideae, Salsoloideae, and Suaedoideae/Salicornioideae.

To obtain an overview of the fossil record of the two families, we screened the literature for descriptions of macro- and pollen fossils of Chenopodiaceae and Amaranthaceae. For Amaranthaceae, we started from the review of Muller (1981), and for Chenopodiaceae, we used the card files of D. H. Mai (unpublished data). Three fossils proved to be sufficiently documented and reliably identified and were used as calibration points for this analysis (table 3; fig. 1). Fossil 1 contains fossils that are 0.7-mm-long *Chenopodium*-like seeds from south Germany. They were dated to the Lower Miocene (23.3–16 Ma) and resemble seeds of members of subg. *Chenopodium*. However, these seeds cannot be assigned to any of the terminal clades but most likely represent the crown group of Chenopodieae I (position of the calibrated node, fig. 1). Fossil 2 is the oldest record of pollen belonging to the Chenopodiaceae/Amaranthaceae alliance and was found in Canada. The pantoporate pollen with more than 40 pores per grain was dated to the Upper Cretaceous (Maestrichtian, 86–65 Ma). A more precise placement within the alliance is not possible. However, it is more likely that it belongs to Chenopodiaceae because it was found in the transitional environment between continental and marine facies where younger records of Chenopodiaceae are also concentrated. Brackish or saline habitats in temperate zones are typical of extant Chenopodiaceae while Amaranthaceae are virtually absent from such habitats. Fossil 3 is a pollen record of Chenopodiaceae/Amaranthaceae from the United States and was dated to the Paleocene (65–56.5 Ma; position of the calibrated node of fossil 2 and 3, fig. 1).

Anatomical and Morphological Analyses

In parallel studies, the traditional morphological characters were cross-checked. Special emphasis was given to leaf anatomy. After screening material from all relevant groups by hand sections, selected species were studied in detail by microtome sections prepared according to standard methods (for details, see Freitag and Stichler 2000). The material was taken from wet-conserved material collected during field studies or from living plants cultivated in the greenhouse. For the screening procedures, herbarium material was also used.

Our naming of leaf types differs from the traditional terminology introduced by Carolin et al. (1975) in two respects. First, wherever necessary, leaf types are defined more precisely by citing the name of the representative genus, section, or species. For example, we have chosen the names "*Salsola kali* type" and "*Salsola soda* type" instead of "salsoloid type," as used in the terminology of Carolin et al. (1975), because both types differ from each other (see fig. 3*E*) and from all C_3 species of genus *Salsola*. Second, we also take into account the presence or absence of hypodermis and of sclerenchyma, the peripheral or subperipheral position of small vascular bundles, and the shape of the leaf blade. Our system, which was already used in the parallel article on the phylogeny of Suaedoideae (Schütze et al. 2003), also differs from the descriptive terminology of Butnik (1995). To our experience, this refined terminology of C_4 leaf types allows a better comparison between related C_3 and C_4 taxa. Types that look like minor variants of the traditional types described by Carolin et al. (1975) may have a strong taxonomic significance and a functional meaning. For instance, a hypodermis, if present, usually functions in water storage or deposition of crystals, and the shift from a succulent C_4 leaf to a scleromorphous needle or spine has far-reaching consequences for the survival of taxa in arid environments.

Results

In this study, new *rbc*L sequences were obtained for 103 Amaranthaceae/Chenopodiaceae and for seven species from other Caryophyllales. The *rbc*L sequences of *Amaranthus tricolor* and *Froelichia floridana* (Amaranthaceae), *Atriplex rosea*, *Atriplex patula*, and *Spinacia oleracea* (Chenopodiaceae), and 22 sequences from other Caryophyllales were taken from GenBank (table 2).

Phylogenetic Analysis

The data matrix comprised 1343 characters, 844 of which were constant and 499 (37.2%) were variable. Of the variable characters, 356 (71.3%) were potentially parsimony informative. Mean base frequencies were distributed as follows: A: 0.27145, C: 0.19671, G: 0.24135, T: 0.29049. A total of 18,910 shortest trees of 2080 steps were found on two islands with a consistency index (CI) of 0.34 and a retention index (RI) of 0.713. To illustrate relative branch lengths, one of the shortest trees comprising the full set of taxa is shown (fig. 1). The strict consensus tree is provided for the ACA clade only (fig. 2). Of the 499 variable sites, ca. 66% were mutations of the third codon position. The number of transformations in the variable third positions ranged from one to 20, but one to four transformations were most common (62%). A heuristic search excluding mutations at the third codon position resulted in a largely unresolved tree (not shown).

The ML analysis (not illustrated) resulted in a tree topology that differs from the MP tree (fig. 1) at only two positions; *Acroglochin chenopodioides* is sister to a clade comprising Corispermoideae, Salsoloideae, Suaedoideae, and Salicornioideae, and Salsoleae I is sister to a clade comprising Salsoleae II and Camphorosmeae where Camphorosmeae is nested within Salsoleae II. The topologies of both trees are described and discussed in detail in the "Discussion."

Molecular Clock

Seventy-three changes of amino acids were identified in our Chenopodiaceae *rbc*L sequences. The corresponding 219 nucleotide sites were excluded from the estimation of divergence

Table 3

Macro- and Pollen Fossils of Chenopodiaceae/Amaranthaceae Used for Calibrating the Molecular Clock

Fossil	Age and origin of the fossil	Calibrated node (fig. 1)	Reference
1. *Parvangula randeckensis*; seeds	Lower Miocene (23.3–16 Ma); Germany: Randecker Mar, Tübingen	Crown of Chenopodieae I	Gregor 1982
2. *Polyporina cribaria*; pollen	Upper Cretaceous (86–65 Ma, Maestrichtian), Canada	Root of Chenopodioideae	Srivastava 1969
3. *Chenopodipollis multiplex*; pollen	Paleocene (65–56.5 Ma), U.S.A.	Root of Chenopodioideae	Nichols and Traverse 1971

time. There was no rate constancy among lineages for the Chenopodiaceae excluding Betoideae. However, rate constancy could be achieved for Chenopodioideae and Salsoloideae, separately. Four taxa of Chenopodioideae and two taxa of Salsoloideae had to be removed because of strongly deviating z scores in the relative rate tests (table 4). For Salicornioideae/Suaedoideae, significant results in the likelihood tests were obtained only after the exclusion of many more taxa (including most of the C_4 species). Therefore, the age of C_4 photosynthesis in this clade could not be estimated.

For the Chenopodioideae, two fossils (1 and 2/3) were used to calibrate the molecular clock (fig. 1; table 3). The use of fossil 1 resulted in a substitution rate of $2.8–4.1 \times 10^{-9}$ synonymous substitutions per site per year and calibrated the root of Chenopodioideae to 65.1–44.7 Ma. Fossil 3 calibrating the root of Chenopodioideae between 65 and 56.5 Ma resulted in a similar substitution rate ($2.8–3.3 \times 10^{-9}$ synonymous substitutions per site per year). This congruency is the first argument for not using the lower age of fossil 2 (86 Ma) for calibration. A second argument is the occurrence of further pollen records of Chenopodiaceae at 65 Ma. The synonymous substitution rate obtained for Chenopodiaceae was subsequently used to estimate the age of C_4 lineages in Salsoloideae where we observed rate constancy among lineages but have no reliable fossils for calibration. The analysis settings and the results for Chenopodioideae and Salsoloideae are shown in table 4.

Anatomical Results

Fifteen anatomically different C_4 leaf types that might be significant in a functional and/or evolutionary respect were distinguished in Chenopodiaceae (A–E in fig. 3) and two compiled for Amaranthaceae (F, G in fig. 3). Most of these types are known from previous studies by different authors, and a few new ones discovered in the course of our project were already described in detail elsewhere (Freitag and Stichler 2000, 2002). All earlier descriptions were compared with the present results. The variation of C_4 leaf anatomy in Chenopodiaceae and Amaranthaceae is summarized (table 8).

Discussion: Systematics of Amaranthaceae and Chenopodiaceae

Monophyly of Amaranthaceae and Chenopodiaceae and Their Position in the Caryophyllales

The monophyly of Amaranthaceae and Chenopodiaceae is well supported (94% bootstrap) and congruent with trees inferred from other data sets (Giannasi et al. 1992; Rettig et al. 1992; Downie and Palmer 1994; Rodman 1994; Downie et al. 1997; Cuénoud et al. 2002). There are a number of morphological and anatomical synapomorphies uniting Chenopodiaceae and Amaranthaceae as summarized in the introduction. This result is in agreement with the traditional view, provided that *Sarcobatus* and *Halophytum* are excluded.

Our analyses further confirm the sister group relationship of Amaranthaceae/Chenopodiaceae to Achatocarpaceae (ACA clade) with moderate (80% bootstrap) support. Achatocarpaceae is a small, poorly known family of shrubs and small trees comprising *Achatocarpus* Triana (five spp.) and *Phaulothamnus* A. Gray (one sp.) occurring from Texas, California, and northwest Mexico to Paraguay and Argentina. Achatocarpaceae have been linked with Phytolaccaceae mainly because of the presence of racemose inflorescences and berries in both families (Heimerl 1934; Bittrich 1993*a*). The close relationship of Achatocarpaceae to Chenopodiaceae and Amaranthaceae was first discovered by Manhart and Rettig (1994) and Rodman (1994) on the basis of *rbc*L sequences and morphological data, respectively. Rodman (1994) described a single, unique synapomorphy for these three families, which is aperturate pollen without furrows. However, pollen of Achatocarpaceae, with its poorly defined pores and a scabrate tectum (Nowicke 1994), is so different from all other Caryophyllales that this statement requires further investigation. Other phenotypic synapomorphies for the ACA clade are currently unknown, although there are some trends such as the preponderance of uniovulate ovaries. Nevertheless, this character state seems to be homoplastic in core Caryophyllales as well because Celosieae of Amaranthaceae are mostly multiovulate and Paronychioideae of Caryophyllaceae are mostly uniovulate. Bentham and Hooker (1880) even treated *Achatocarpus* (*Phaulothamnus* was described five years later) as a member of tribe Amarantheae within Amaranthaceae based on its uniovulate ovaries and bilocular anthers but also admitted differences in *Achatocarpus* such as the higher number (10–20) of stamens.

According to our *rbc*L tree, the Caryophyllaceae are sister to the ACA clade (73% bootstrap). The increased sampling over Savolainen et al. (2000*b*) in *rbc*L of both the ACA clade and the Caryophyllaceae led to an increased support of the sister group relationship of the two (jackknife < 50% in Savolainen et al. 2000*b*). The results underscore that increased sampling can be beneficial (Graybeal 1998). Knowing the sister group of the ACA clade is very important to assess character

evolution in Amaranthaceae and Chenopodiaceae because Achatocarpaceae are probably unique in many characters such as pollen morphology and thus might not show plesiomorphic states. The 10 representatives of Caryophyllaceae included cover all three subfamilies recognized by Bittrich (1993*b*), i.e., Alsinoideae (four gen. out of 28: *Arenaria*, *Cerastium*, *Stellaria*, *Scleranthus*), Caryophylloideae (two gen. out of 24: *Dianthus*, *Silene*), and Paronychioideae (four gen. out of 34: *Spergularia*, *Polycarpon*, *Herniaria*, *Illecebrum*). While the monophyly of the Caryophyllaceae is well supported (97% bootstrap), none of its three subfamilies seems to be monophyletic (fig. 1). Paronychioideae were traditionally regarded as closely related to Chenopodiaceae/Amaranthaceae, especially to Polycnemoideae (Ulbrich 1934; Aellen 1965–1968; Kühn et al. 1993). Bentham and Hooker (1880) classified the genera of Paronychioideae as Illecebraceae distinct from Caryophyllaceae, the latter of which were considered to be distinguished by petaliferous flowers, multiovulate ovaries, and capsules. The authors also suggested affinities of Illecebraceae to Amaranthaceae and Chenopodiaceae. According to our data, Paronychioideae clearly belong to Caryophyllaceae and—except *Spergularia*—form a basal grade.

Relationships between Amaranthaceae and Chenopodiaceae

The relationship between Chenopodiaceae and Amaranthaceae is only poorly resolved in the *rbc*L tree. Branches at the base of the Amaranthacaceae/Chenopodiaceae lineage are short (fig. 1) and largely collapse in the strict consensus tree (fig. 2). This lack of resolution is not caused by an overall lack of variability in *rbc*L as is evident from the well-resolved terminal clades. However, possible reasons include a fast radiation of major lineages during the early diversification of the group that did not allow for the accumulation of numerous mutations in *rbc*L as well as patterns of homoplasy in *rbc*L that obscure historic signal for deeper nodes. Further studies are needed for clarification. Taxon sampling can probably be only slightly improved to break down long branches (Graybeal 1998) since especially the basal lineages of Betoideae and Polycnemoideae are already well represented. Moreover, it will be difficult to predict whether *Bosea* (not supported with *rbc*L) is really the first branching Amaranthaceae until all other genera, many of which are hardly available, from the former Amaranthoideae are sampled. Interestingly, a similar weak resolution of the basal branches is evident in an *ndh*F analysis of the two families (Pratt 2003).

Several major lineages are resolved in the strict consensus tree (fig. 2). These are the Chenopodioideae; a clade comprising Salicornioideae, Suaedoideae, and Salsoloideae; the Corispermoideae; and a clade uniting Amaranthaceae and Polycnemoideae. The three former clades collectively include the vast majority of Chenopodiaceae as traditionally recognized, albeit without statistical support. This large clade appears in a basal polytomy with an Amaranthaceae-Polycnemoideae clade and the genera currently classified as Betoideae, most of which form isolated lineages.

In the ML analysis, the Betoideae (except *Acroglochin*) represent the most basal branch of the Chenopodiaceae (not shown, same topology as MP tree in fig. 1), and morphological data provide further support for an affiliation of Betoideae with Chenopodiaceae (table 5). Like all Chenopodiaceae, Betoideae have sepaloid tepals (herbaceous, at least along the dorsal vein) in contrast to petaloid tepals (white or pigmented, scarious or papyraceous) in Amaranthaceae and Polycnemoideae. The filaments of Chenopodiaceae and Betoideae are inserted on a hypogynous disc, a rim, or tepal bases but are not united into a filament tube like in Polycnemoideae and Amaranthaceae. The Betoideae are likely to be relics of an old stock. This may explain the unresolved tree topology and the relatively long terminal branches leading to all five genera (fig. 1). The considerable genetic distance between genera is also reflected by their morphological and physiological distinctness and by their disjunct distribution. *Beta* is restricted to the Mediterranean region, *Oreobliton* to the mountains of northwest Africa, *Hablitzia* to Transcaucasia, *Acroglochin* to the Himalayan region, and *Aphanisma* to California.

Although bootstrap values were below 50% for the Amaranthaceae-Polycnemoideae clade (fig. 2), there are several morphological characters in support of this clade (see table 5). The crucial role of the Polycnemoideae for clarifying relationships between Amaranthaceae and Chenopodiaceae is evident from their changing family assignment in traditional classification systems (see also "Introduction"). Polycnemeae were recognized as a distinct tribe by Dumortier (1827) and were later raised to subfamilial level (Ulbrich 1934) within Chenopodiaceae. Moquin-Tandon (1849) treated *Polycnemum*, *Nitrophila*, and *Hemichroa* as subtribe Polycnemeae of tribe Achyrantheae (corresponding to Amarantheae *sensu* Schinz) within the Amaranthaceae. Polycnemoideae differ from other Chenopodiaceae in their conspicuous chartaceous tepals, a short but distinct filament tube (fig. 173*d* in Ulbrich 1934) as present in most Amaranthaceae, and the position of the stomata, which are arranged in parallel to the midveins of leaves (Khatib 1959; Aellen 1965). Furthermore, *Polycnemum* has 2-locular anthers unlike any chenopod but present in Gomphrenoideae. Polycnemoideae are also distinguishable from all other members of both families by normal secondary growth (Ulbrich 1934). Taken together, our data are in favor of a transfer of Polycnemoideae from Chenopodiaceae to Amaranthaceae. This is supported by first results of a *ndh*F analysis of both families where the monophyly of Amaranthaceae and Polycnemoideae receives low bootstrap support (Pratt 2003).

Amaranthaceae and Chenopodiaceae have recently been united as Amaranthaceae s.l. based on the assumption that the Chenopodiaceae are paraphyletic in relation to Amaranthaceae (APG 1998; Judd et al. 1999). Molecular data, including our dense sampling of *rbc*L, however, so far are inconsistent as to the exact relationships of both families (Cuénoud et al. 2002; this study). Even provided that Polycnemoideae are included in Amaranthaceae, our *rbc*L tree does not give unequivocal support to the recognition of the remainder of Chenopodiaceae as a monophyletic lineage that would justify its classification as a separate family. Further investigations of the basal groups, in particular Betoideae, with additional genes are envisaged to address the outstanding questions. Until these are resolved, we follow the traditionally recognized families.

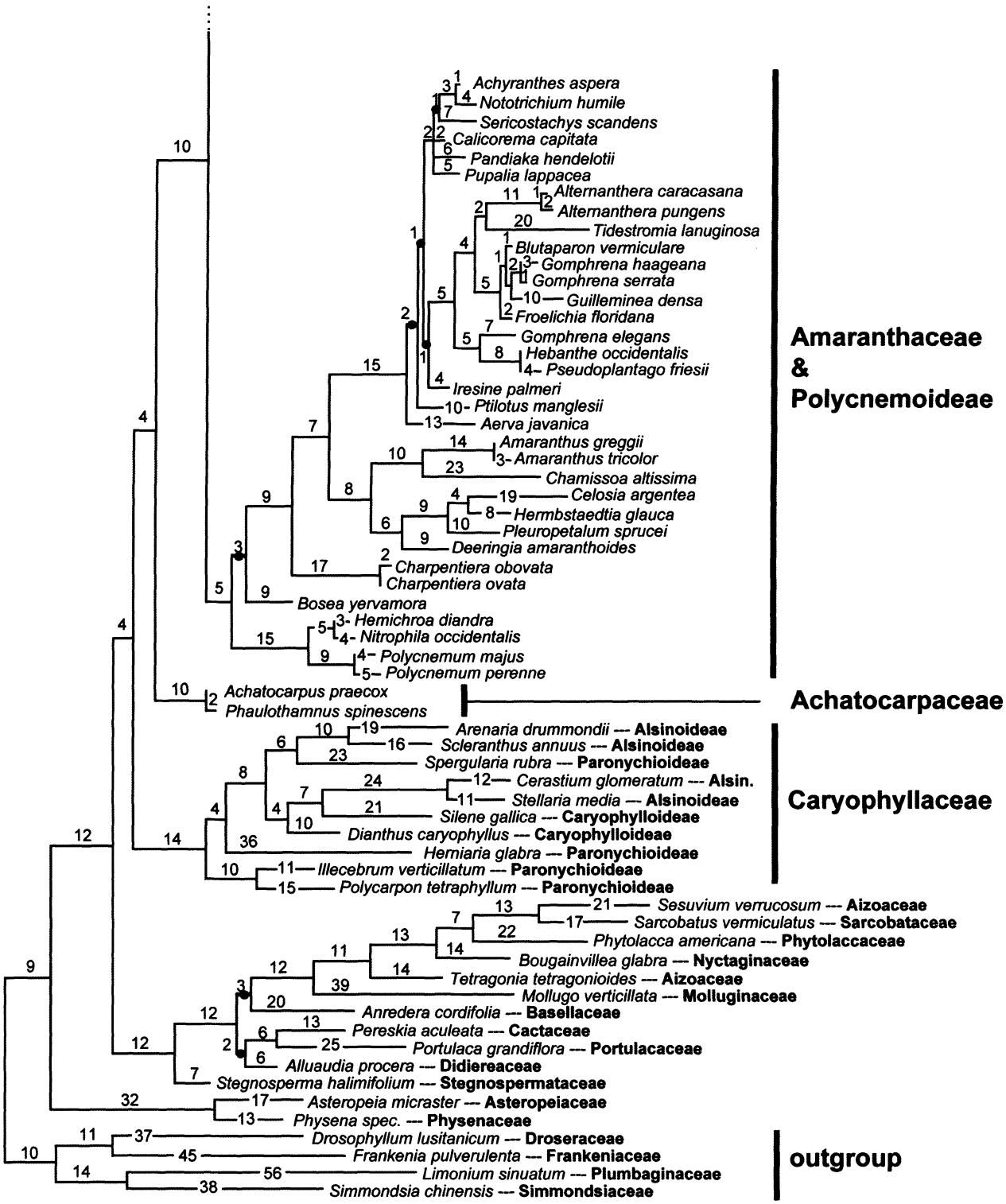

Fig. 1 One of 18,910 equally parsimonious trees obtained from the MP analysis of 137 *rbc*L sequences. Numbers refer to character changes along branches. Branches marked with a dot collapse in the strict consensus. The position and age of two calibrated nodes for the molecular clock analysis are indicated.

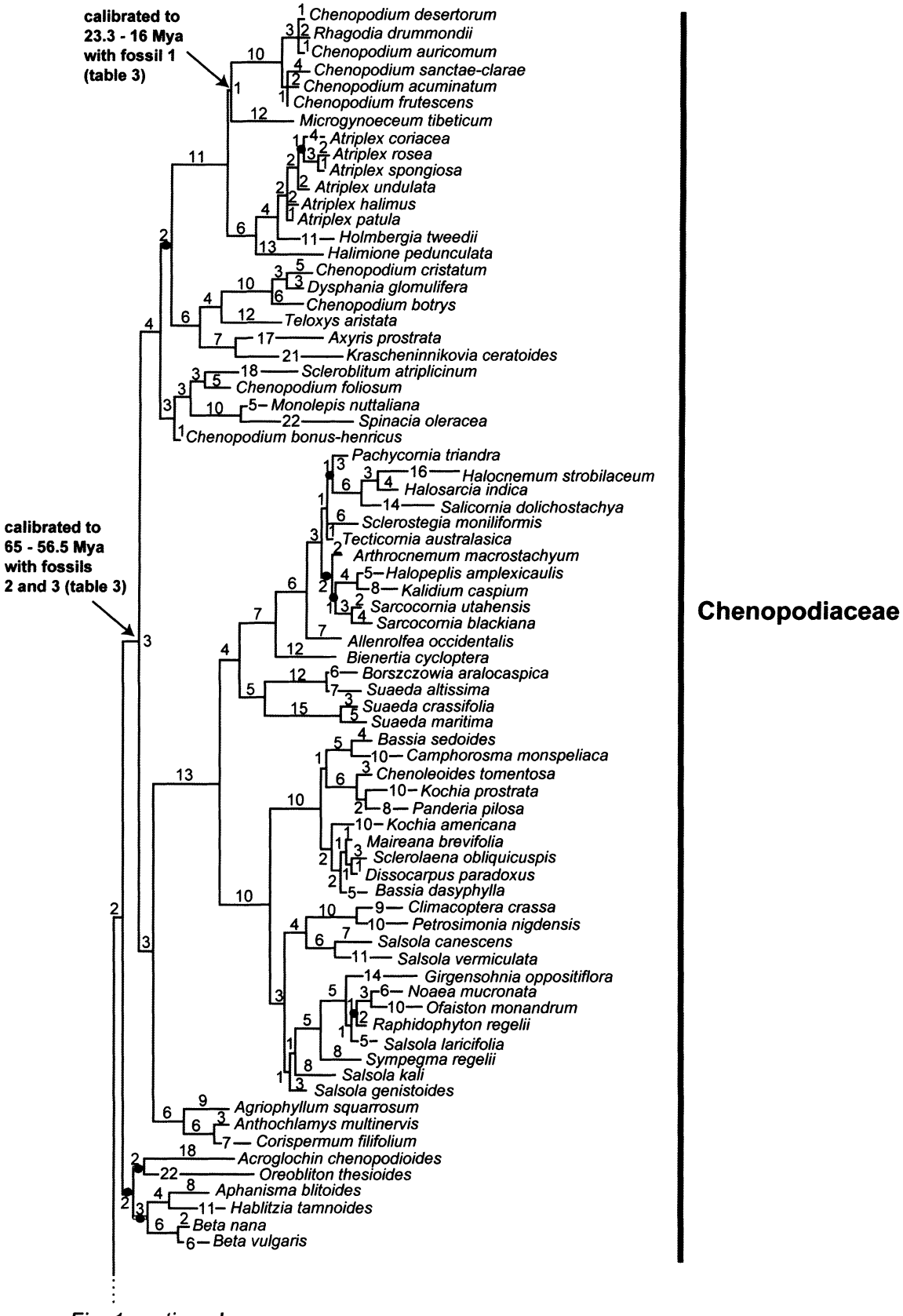

Fig. 1 continued

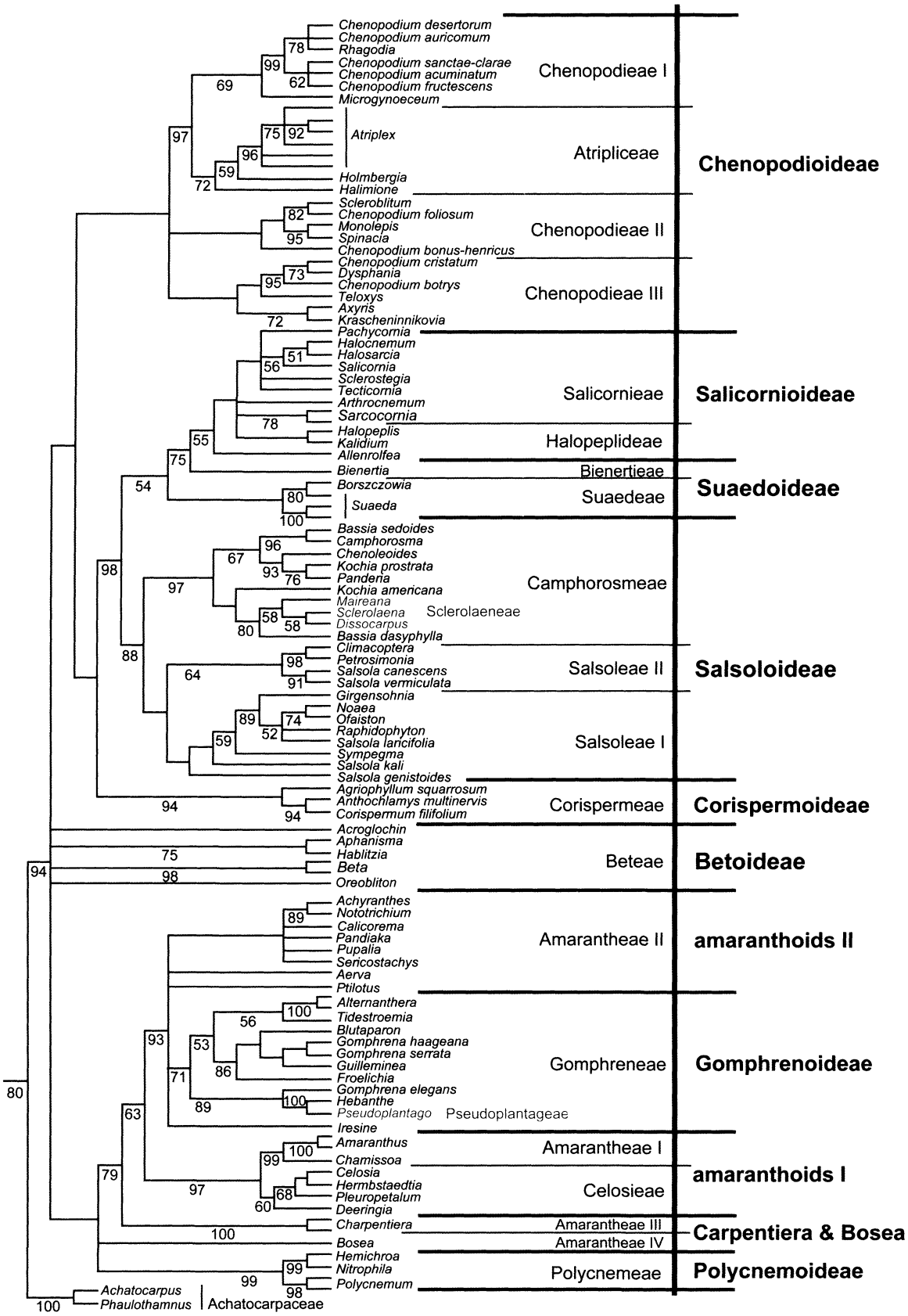

Fig. 2 Strict consensus of the Amaranthaceae/Chenopodicaeae/Achatocarpaceae (ACA)-clade retained from 18,910 equally parsimonious trees. Numbers below branches indicate bootstrap support. Subfamilies and tribes as found in this *rbc*L analysis are indicated.

Table 4

Results of the Molecular Clock Analyses

	Chenopodioideae	Salsoloideae
Excluded taxa with strongly deviating rates	*Chenopodium bonus-henricus*, *Teloxys aristata*, *Chenopodium foliosum*, *Monolepis nuttaliana*	*Kochia prostrata*, *Petrosimonia nigdensis*
Outgroup	*Acroglochin chenopodioides*	*Suaeda maritima*
No. of taxa in the ML analysis	23	21
ML settings (the best-fit model was selected by Modeltest Version 3.06)	HKY85 + I + G, nucleotide frequencies set to A = .2610, C = .1916, G = .2497, T = .2977, tr/tv ratio = 4.0222, rates = gamma, shape parameter = 1.0061, pinvar = .7042	GTR + G + I, nucleotide frequencies set to A = .2606, C = .1872, G = .2467, T = .3055, substitution rate matrix: AC 1.0, AG 3.732, AT 0.222, CG 0.222, CT 8.048, GT 1.0, shape parameter = .398
Fossils for calibration (see table 3; fig. 1)	Fossil 1: 23.3–16 Ma; fossil 2: 65 Ma; fossil 3: 65–56.5 Ma	None, age calculated with the rate of synonymous substitutions found in Chenopodioideae ($0.28–0.41 \times 10^{-9}$ per site per year)
Age of C_4 taxa	C_4 *Atriplex*; fossil 1: 11.5–7.9 Ma; fossils 2 and 3: 11.5–10.0 Ma	Salsoleae II (entirely C_4): 21.5–14.4 Ma; C_4 lineages in Salsoleae I: *Salsola kali*: 21.5–14.4 Ma; *Girgensohnia* clade: 19.6–13.4 Ma; *Noaea* clade: 12.5–8.5 Ma; C_4 lineage in Camphorosmeae: 21.6–14.5 Ma
Rate of synonymous substitutions per site per year	Fossil 1: $0.28–0.41 \times 10^{-9}$; fossil 3: $0.28–0.33 \times 10^{-9}$	

Relationships within the Amaranthaceae-Polycnemoideae Clade

The Amaranthaceae-Polycnemoideae clade as resolved with our *rbc*L data (fig. 2) consists of the three genera of Polycnemoideae and the Amaranthaceae as circumscribed by Schinz (1893, 1934) and Townsend (1993). All three genera of Polycnemoideae, *Polycnemum* (seven to eight spp.), *Nitrophila* (six to seven spp.), and *Hemichroa* (three spp.) were included in our study. They form a highly supported clade, with *Polycnemum* (Eurasia) being sister to *Nitrophila* (North America) plus *Hemichroa* (Australia). From a biogeographical point of view, such a relationship is somewhat surprising.

Within the Amaranthaceae *sensu* Schinz, only the Gomphrenoideae seem to be monophyletic (71% bootstrap). They are nested within the Amaranthoideae (figs. 1, 2). The Amaranthoideae fall into two groups, with the large tribe Amarantheae being paraphyletic. One group (here referred to as amaranthoids II) comprises several genera of subtribe Aervinae (= Achyranthinae) that are united in a highly supported clade with Gomphrenoideae. The other group (here referred to as amaranthoids I) comprises the apparently monophyletic Celosieae and *Amaranthus* and *Chamissoa*. Isolated and relatively basal positions are taken by *Bosea* and *Charpentiera*, both of which also have been classified within subtribe Amaranthineae of Amarantheae (Schinz 1893, 1934; Townsend 1993), along with *Amaranthus* and *Chamissoa*. Since the subfamilial and tribal classification as traditionally employed, in particular for the diverse subfamily Amaranthoideae, is not reflected by our *rbc*L tree, the following more detailed discussion will not be structured according to these entities.

Although *Bosea* appears in a polytomy with Polycnemoideae in the strict consensus (fig. 2), it is excluded with moderate support from all other Amaranthaceae. A basal position of *Bosea* is also indicated by combined *trn*K intron plus *mat*K data (K. Müller and T. Borsch, unpublished data) with a denser sampling in Amaranthaceae. *Charpentiera* follows in the basal grade of Amaranthaceae, which is remarkable in the light of its extant distribution restricted to a few Pacific islands (Hawaii, Austral Ridge). However, in the *ndh*F tree (Pratt 2003), *Charpentiera* branches first, and *Bosea* follows. *Bosea* and *Charpentiera* share their woody habit with many Celosieae and *Chamissoa*, and *Bosea* seems to be similar in its fleshy, berry-like capsules to *Deeringia* and *Pleuropetalum*. However, further sampling of genera of the Amaranthinae will be crucial to establish the exact branching order at the base of the Amaranthaceae because Amaranthinae as circumscribed by Schinz (1893) and subsequent authors are strongly paraphyletic.

Within amaranthoids I, Celosieae are resolved as monophyletic, albeit with low support. This is not surprising because the Celosieae have a number of unique morphological features within Amaranthaceae, such as multiovulate ovaries. Cavaco (1962) even assigned them subfamilial rank. The sister group relationship of *Amaranthus* and *Chamissoa* is remarkable and is congruently inferred with *trn*K intron plus *mat*K (Müller and Borsch, unpublished data) and *ndh*F sequence data (Pratt 2003). Earlier indications of an isolated position of *Amaranthus* outside the core of Amaranthaceae based on ORF2280 (Downie et al. 1997) were probably a result of limited sampling. Relationships within amaranthoids II (comprising all genera of Aervineae sampled in this study) are not resolved. Nevertheless, their separation from the Amaranthineae is strongly indicated, so the possibility of a common origin of

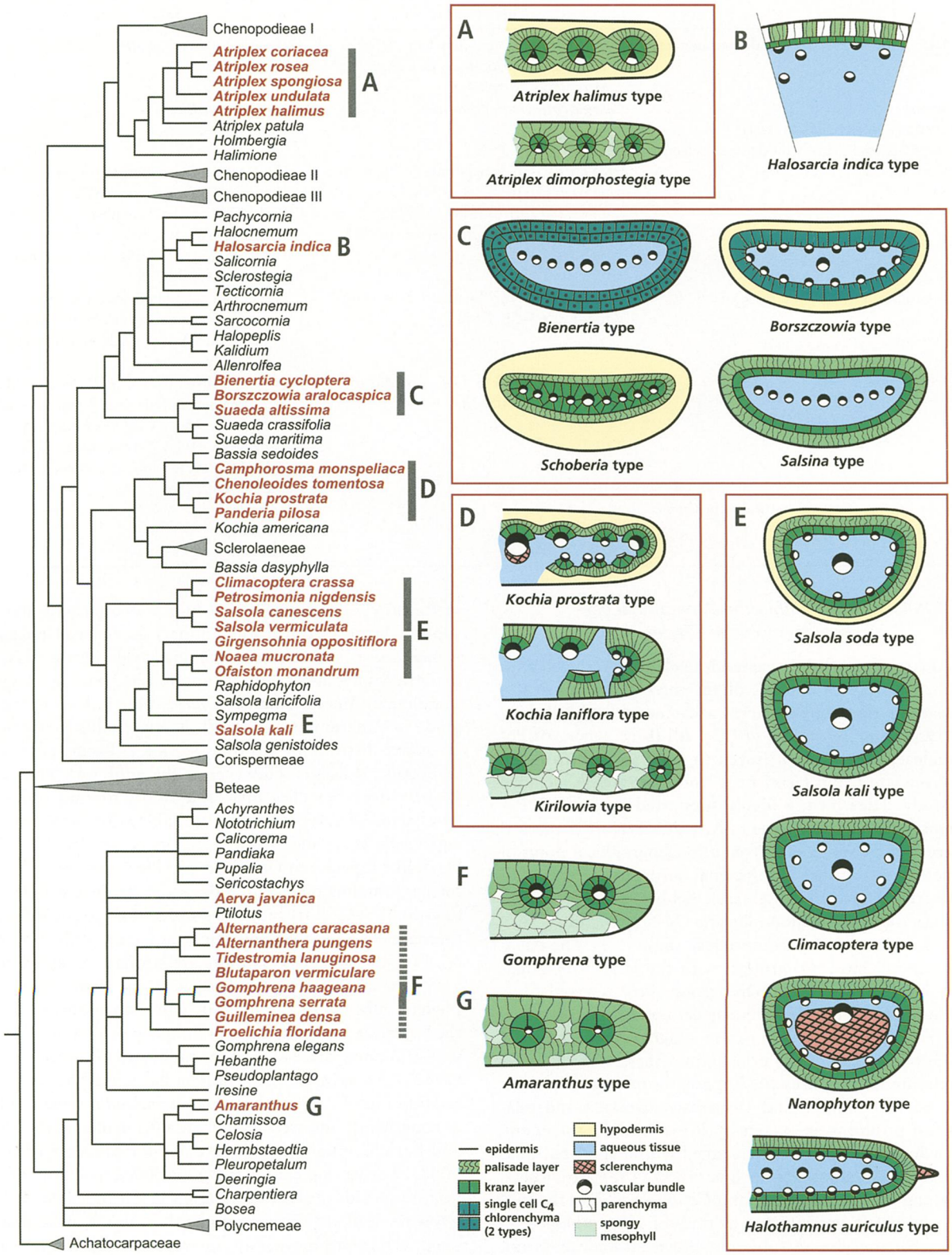

Fig. 3 Distribution of C_4 lineages (red) in Chenopodiaceae and Amaranthaceae, and major C_4 leaf types. Tree topology is identical to that in fig. 2, but large C_3 clades are reduced to gray-shadowed triangles.

Table 5

Characters Separating Chenopodiaceae and Amaranthaceae

Characters	Chenopodiaceae (excl. Betoideae, Polynemoideae)	Betoideae	Polycnemoideae	Amaranthaceae
Tepals in flower	Sepaloid (membranous, herbaceous, often succulent)	Sepaloid (herbaceous, at least along the dorsal vein)	Petaloid (scarious, white or pinkish)	Petaloid (scarious or papyraceous, often variously pigmented)
Tepals in fruit	Often conspicuously modified	Conspicuously modified only in *Beta*	Never conspicuously modified	Never conspicuously modified
Filaments	Usually inserted on a hypogynous disc or on a rim	Inserted on a rim, in a ring, or on tepal bases	Basally united into a filament tube	Basally united into a filament tube
Anthers	4-locular	4-locular	2-locular	2- and 4-locular
Distribution	Essentially temperate	Temperate	Temperate	Essentially tropical

the amaranthoids II, comprising the bulk of Old World genera, has to be further tested.

A core of Gomphrenoideae is resolved with 71% bootstrap that includes all genera except *Iresine*, which appears as a separate branch in a polytomy. The signal in *rbc*L is probably not sufficient to resolve the clade including *Iresine*, but the presumed synapomorphy of 2-locular anthers for Gomphrenoideae remains uncontradicted. The core of genera as revealed in this study is largely congruent with the occurrence of metareticulate pollen (Borsch and Barthlott 1998). The only exception is *Pseudoplantago*, which is sister to *Hebanthe*. *Pseudoplantago* shares 2-locular anthers but has a rather amaranthoid morphology, including the presence of sterile flowers not found in any other Gomphrenoideae (Covas 1939).

The subtribal level is more important in the classification of Gomphreneae. It was introduced by Schinz (1893) and largely accepted by Townsend (1993). Froelichiinae and Gomphreninae divide the large number of gomphrenoid genera into two groups. Of the genera sampled in this study, *Froelichia*, *Alternanthera*, *Guilleminea*, and *Tidestromia* were classified as Froelichiinae and the remainder as Gomphreninae. The *rbc*L tree indicates both subtribes to be polyphyletic, confirming an assumption already made on the basis of pollen characters (Borsch 1998). Statistical support and sampling coverage are not sufficient yet to draw final conclusions, but two lineages are worth mentioning: one clade containing *Froelichia*, *Blutaparon*, *Guilleminea*, and two species of *Gomphrena* (86% bootstrap) and another clade with *Gomphrena elegans*, *Hebanthe*, and *Pseudoplantago* (89% bootstrap). *Gomphrena elegans* has been anticipated to be different from the first group because of its *Pfaffia*-type pollen (Borsch 1998). Also, the status of *Hebanthe* independent from *Iresine* and other gomphrenoids as inferred by Borsch and Pedersen (1997) from morphological characters seems to be supported by *rbc*L sequences. Furthermore, *rbc*L data reveal *Guilleminea* (=*Brayulinea*) and *Tidestromia* as members of tribe Gomphreneae as defined by Schinz (1934) with some confidence. Standley (1917) described a separate tribe Brayulineae based on the presence of perigynous stamens (*Brayulinea*) and a protruding to ascending habit with flowers solitary in the axils of cauline leaves (*Brayulinea*, *Tidestromia*). This was accepted by Schinz (1934) and even raised to subfamily rank by Cavaco (1962). Townsend (1993) did not uphold the separation of Brayulineae from Gomphreneae, a view now clearly supported by *rbc*L data.

Relationships within Chenopodiaceae

Within Chenopodiaceae, the strict consensus *rbc*L tree shows several clades that are congruent with traditional tribes or subfamilies (fig. 2). Five major clades can be identified that will be ranked as subfamilies here: (1) Betoideae (Beteae); (2) Chenopodioideae embracing intermingled members of the Chenopodieae and Atripliceae; (3) Corispermoideae (Corispermeae); (4) Salicornioideae/Suaedoideae including Suaedeae, Bienertieae, and Salicornieae; and (5) Salsoloideae comprising Camphorosmeae, Sclerolaeneae, and Salsoleae. The *rbc*L data strongly support the sister group relationship of the latter two clades (98% bootstrap). Relationships among the other clades remain uncertain because of low statistical support. A separation of Salicorniaceae and Salsolaceae as revived by Scott (1977*a*, 1997*b*) is not supported by our data. The relationships of the major groups of Chenopodiaceae are discussed for each subfamily including taxonomic implications.

Betoideae Ulbr. 1934, Beteae (Moq. 1849) Volkens 1883

All five genera of the subfamily, namely *Hablitzia* (one sp.), *Oreobliton* (one sp.), *Acroglochin* (one of two spp.), *Beta* (two of ca. 13 spp.), and *Aphanisma* (one sp.), were included in the analysis. Betoideae are not monophyletic in the strict consensus tree (fig. 2). Instead, they form four clades that are part of the basal polytomy of Amaranthaceae and Chenopodiaceae. Only the Transcaucasian *Hablitzia* and the North American *Aphanisma* are sister taxa (75% bootstrap), and also the two representatives of *Beta* (the high mountain endemic *Beta nana* and the widespread *Beta vulgaris*) form a monophyletic group. Traditionally, the Betoideae were characterized by fruits that remain fused with the persistent perianth and open with a circumscissile lid. Among Betoideae, *Beta* is most similar to Chenopodiaceae in having condensed partial inflorescences, hardening tepals, an obscured lid on its mature fruit, and—with most species—is adapted to saline habitats. Additional studies are required to resolve the phylogenetic relationships of Betoideae. Their results will be crucial for retention of the two families Chenopodiaceae and Amaranthaceae. For the time being, we recommend to maintain the subfamily.

Chenopodioideae Ulbr. 1934

Of the 19 genera and ca. 500 species of this subfamily, 13 genera and 26 species were included in our analysis. The large,

widely distributed and taxonomically insufficiently known *Atriplex* and *Chenopodium* are represented by six and nine species, respectively. The remaining 11 genera are represented by one species each. *Atriplex* is estimated to contain ca. 150 (Aellen 1965–1968) to 200–300 spp. in two subgenera (Judd and Ferguson 1999; Hedge 2001) and a variable number of sections (up to 15 in Ulbrich 1934). *Chenopodium* has about 140 spp. and is subdivided into two (Scott 1978*a*) or three subgenera (Judd and Ferguson 1999) and numerous sections (e.g., 16 sections listed in Aellen 1965–1968; Scott 1978*a*).

In the *rbc*L tree, a monophyletic group is formed by all genera of Chenopodioideae as defined by Ulbrich (1934), with the exclusion of Camphorosmeae, which, according to our data, belong elsewhere. Whereas bootstrap support is below 50% in the *rbc*L tree, the same lineage received low bootstrap in a recent study based on *ndh*F sequences (Pratt 2003). Nevertheless, convincing morphological characters shared by all members of the lineage are lacking. Earlier and later circumscriptions of Chenopodioideae also included Beteae and Corispermeae, which, according to our results, are more distantly related. Within Chenopodioideae, some subclades can be identified (fig. 2). One clade comprises *Atriplex*, *Holmbergia*, and *Halimione* and is sister to Chenopodieae I. The sister group relationship of these two clades receives high statistical support (97% bootstrap). The Chenopodieae II and III clades receive less than 50% bootstrap support.

This topology is not in agreement with the traditional circumscription of tribes Chenopodieae and Atripliceae. The representatives of Atripliceae (*Atriplex*, *Halimione*, *Microgynoeceum*, *Spinacia*, *Axyris*, and *Kraschenninnikovia*; 12 spp.) and Chenopodieae (*Chenopodium*, *Holmbergia*, *Rhagodia*, *Monolepis*, *Dysphania*, *Scleroblitum*, and *Teloxys*; 14 spp.) as traditionally defined are strongly intermingled. The presence or absence of bracteoles subtending the naked female flower has been considered most important for defining the two tribes, but this character appears to be too homoplastic in Chenopodioideae (in contrast to Salsoloideae, Salicornioideae, and Suaedoideae). This is also evident from the conditions in *Atriplex* sect. *Atriplex* (=*Dichospermum*) where naked flowers with bracts occur side by side with regular chenopodioid flowers, provided with a perianth and devoid of bracteoles.

Atripliceae C.A. Mey. 1829. The sampled *Atriplex* are monophyletic, which contradicts the inference of paraphyly of *Atriplex* drawn by Flores and Davis (2001) from morphology-based cladistics. Any decision on the matter certainly requires broader sampling. Together with *Halimione*, which has often been included in *Atriplex*, the species investigated here form the nucleus of a redefined tribe Atripliceae. The monotypic South American *Holmbergia* also belongs to this clade. Hitherto, this genus was classified with Chenopodieae, and Ulbrich (1934) and Scott (1978*c*) have stressed its resemblance with the Australian *Rhagodia*. However, traditional genera of Atripliceae such as *Spinacia*, *Axyris*, and *Kraschenninnikowia* are found in other lineages of Chenopodioideae.

Chenopodieae. The three other lineages of Chenopodioideae as defined by the *rbc*L tree do not fit into the traditional tribe Chenopodieae or subtribes Chenopodiinae and Rhagodiinae (Scott 1978*c*). The distribution of the nine species of *Chenopodium* in three different clades indicates that the genus is polyphyletic, as was already suspected by Judd and Ferguson (1999). Most likely, certain subgenera and sections of *Chenopodium*, together with other genera, represent natural groups of tribal rank. The redefined Chenopodieae (= Chenopodieae I in fig. 2) include the type section of *Chenopodium* that is represented in our sampling by *Chenopodium acuminatum* (central Asia) and *Chenopodium frutescens* (central Asia). It also includes *Chenopodium sanctae-clarae*, the type of sect. *Skottsbergia* embracing four shrubby species from the Juan Fernandez Islands and Hawaii, and the Australian taxa *Rhagodia* (11 spp.), *Chenopodium* sect. *Auricoma* (two spp.), and *Chenopodium* sect. *Desertorum* (three spp.). The Australian taxa appear in a well-supported subclade (78% bootstrap) sister to the taxa from Eurasia and Juan Fernandez Islands. This position confirms subtribe Rhagodiinae Scott but only as far as the core genus is concerned (Scott 1978*c*). It is likely that all morphologically rather similar species of *Chenopodium* subgenus *Chenopodium* (ca. 100 spp.) will remain in tribe Chenopodieae. The monotypic central Asian *Microgynoeceum* is in a basal position of Chenopodieae as defined here (bootstrap support 69%).

Other tribes of Chenopodoideae may emanate from Chenopodieae II and III after an increase of taxon sampling. In Chenopodieae II, one clade unites the Australian *Scleroblitum* (monotypic) and the Eurasian *Chenopodium foliosum*, which have berry-like fruits formed by succulent accrescent tepals in common. Similar fruits, however, are also known from *Rhagodia* and *Holmbergia*. Another well-supported group is formed by *Spinacia* and *Monolepis*. In Chenopodieae III, a close relationship of the Eurasian species *Chenopodium botrys* and *Teloxys aristata*, and of the Australian species *Chenopodium cristatum* and *Dysphania glomulifera*, is supported by the presence of multicelluar glandular hairs (type 8 in Carolin 1983). By that character, they fit into subgenus *Ambrosia* (Scott 1978*a*; Simón 1996). Likewise, the subclade consisting of *Axyris* and *Kraschenninikovia* may represent a natural group that is characterized by a dense indumentum of stellate hairs (type 2 in Carolin 1983) and corresponds to the subtribe Eurotiinae (Volkens 1893). These results confirm the proposal of Mosyakin and Clemants (2002) to transfer *Chenopodium* subg. *Ambrina* to *Dysphania* and some ideas of Mosyakin (2003) for additional rearrangements in Chenopodieae, e.g., establishing the new tribe Ceratocarpeae to accommodate *Ceratocarpus*, *Axyris*, and *Kraschenninnikovia*.

Concluding remarks on Chenopodioideae. For the time being, Chenopodioideae *sensu* Ulbrich (1934), excluding Camphorosmeae, should be maintained although the support by molecular evidence is still weak and convincing morphological characters are missing.

Corispermoideae Ulbr. 1934, Corispermeae Moq. 1840

This subfamily comprises only the three genera *Corispermum* L. (60 spp.), *Agriophyllum* M. Bieb. (six spp.), and *Anthochlamys* Fenzl. (two spp.), which all were included in our study. Originally, we included two species of *Corispermum*. The *rbc*L sequence obtained from *Corispermum ladakhianum*, however, was identical to that of *Corispermum filifolium*. In the *rbc*L tree, the Corispermeae are clearly monophyletic (bootstrap 94%), but their phylogenetic relationship with other subfamilies remains somewhat elusive. Morphologically,

the genera of Corispermeae are united by a complex of morphological characters unique in Chenopodiaceae. The leaves are laminate, but, in contrast to other groups with flat leaves, always scleromorphic; the indumentum is prominent and consists of peculiar dendritic hairs (trichome type 1 in Carolin 1983; see also figs. 202*F*, 202*K* in Ulbrich 1934) somewhat similar to those in the amaranthaceous genus *Aerva*; flowers are arranged in spikes; and the tepals are not persistent. In *Anthochlamys*, the tepals strongly resemble Amaranthaceae in structure and color.

The three genera are so similar to one another in morphology that their placement in one tribe has never been questioned. The tribe consists of annual herbs distributed in arid regions of Eurasia, with all three genera occurring sympatrically in central Asia. Only *Corispermum* is also present in North America. The maintenance of subfamily Corispermoideae is recommended.

Suaedoideae and Salicornioideae

One unexpected result of the *rbc*L study presented here is that the genera of the traditional subfamilies Salicornioideae and Suaedoideae group together in one lineage, although support is limited (54% bootstrap). Within the Salicornioideae/Suaedoideae clade, the suaedoid genera *Suaeda* and *Borszczowia* are sister to the rest of the clade, which comprises *Bienertia* and a lineage of 11 salicornioid and halopeplioid genera. Because *Bienertia* traditionally belongs to Suaedoideae, this subfamily becomes paraphyletic in relation to the Salicornioideae in our *rbc*L tree. Morphologically, *Bienertia* has no synapomorphies with Salicornioideae but agrees in many morphological characters with Suaedoideae (table 6). In the study of Schütze et al. (2003), the position of *Bienertia* was ambiguous, being sister to *Suaeda* in the chloroplast *atp*B-*rbc*L and *psb*B-*psb*H trees but showing affinities to Salicornioideae in the ITS tree (Schütze et al. 2003). Finally, *Bienertia* also has three characters that are unique in the Suaedoideae/Salicornioideae clade (Freitag and Stichler 2002): (1) the small bracteoles have a fleshy, green back as is also found in Salsoleae, (2) their indumentum consists of vesicular hairs as is common in Chenopodioideae, and (3) the leaves have a special non-Kranz C_4 anatomy (fig. 3).

In respect to the overwhelming morphological and anatomical differences between the two subfamilies (summarized in table 6) and the comparative weak molecular support, we argue for maintaining Suaedoideae (incl. *Bienertia*) and Salicornioideae. The only character connecting the two groups is, as far as we know, their ecology. They both are pronounced obligate (hygro)halophytes.

Of the four genera belonging to Suaedoideae, namely *Suaeda* (ca. 80–90 spp. worldwide; see Schütze et al. 2003), *Alexandra*, *Borszczowia*, and *Bienertia* (all monotypic, central Asia), *Suaeda* is represented by three species from two of the nine sections recognized by Schenk and Ferren (2001), and only *Alexandra* was not available because of PCR amplification problems.

The *rbc*L data support the view of Volkens (1893) and Ulbrich (1934) that Suaedoideae are not closely related to Salsoloideae as was assumed by all later authors. The main argument in favor of including Suaedeae in Salsoloideae was the presence of a spirally twisted embryo in both groups. In the traditional view, first stated by Moquin-Tandon (1840), the embryo is plano-spiral in suaedoids and conical-spiral in salsoloids. However, even those characters are not strictly confined to the respective groups because in our comparative morphological studies, we also observed plano-spiral embryos in several salsoloids, e.g., in genera with vertical fruits such as *Anabasis* and *Horaninowia*. Our molecular data suggest the parallel evolution of spirally twisted embryos in both subfamilies. But as the monophyly of Salicornioideae/Suaedoideae receives only weak molecular and no morphological support, more molecular evidence is needed to understand the evolution of embryo shape within these three subfamilies.

Bienertieae Ulbr. 1934, Suaedeae Dumort. 1934

Considering the set of unique morphological characters exhibited by *Bienertia cycloptera* and its ambiguous placement by molecular data, we recommend to maintain the monotypic tribe Bienertieae in addition to Suaedeae, though with a different circumscription. The phylogeny and taxonomy of both tribes are fully discussed in the recent contribution by Schütze et al. (2003).

Halopeplideae Ulbr. 1934, Salicornieae

Salicornioideae comprise ca. 80 species and 15 genera, of which 12 species from 11 genera are represented in our sampling, which covers a significant part of the morphological diversity exhibited by the group; morphological synapomorphies of Salicornioideae are listed (table 6). Whereas monophyly of Salicornioideae is moderately supported (55% bootstrap), the relationship of Halopeplideae and Salicornieae was not resolved by our *rbc*L data. This is mainly a result of low sequence variation within this subfamily (fig. 2). Preliminary evidence from ITS sequence data indicates that Halopeplideae form a basal grade and are paraphyletic in respect to Salicornieae (Schütze et al. 2003; G. Kadereit, unpublished data). Bracts and leaves are alternate in Halopeplideae and opposite in Salicornieae. A less derived position of Halopeplideae is also supported by the occurrence of species with normal or only slightly reduced leaf blades (e.g., *Kalidiopsis*, *Kalidium foliatum*) and a stem that often is not completely covered by photosynthetic leaf tissues (see also James and Kyhos 1961).

Salsoloideae Ulbr. 1934

The Salsoloideae comprise the largest number of genera within Chenopodiaceae. In our sampling, it is represented by 16 of 49 genera. *Salsola* varies from ca. 100 spp. (Freitag 2001) to ca. 250 spp. on the basis of numbers given in the numerous papers of Botschantzev (1969, 1989). Five *Salsola* species from different sections were included in this analysis. The monophyly of the Salsoloideae clade is well supported. It comprises three major subclades: Camphorosmeae (including Sclerolaeneae) and Salsoleae I and II. Only Camphorosmeae are statistically well supported.

Like the molecular results, morphological and anatomical characters of the three clades (table 7) do not give a clear picture of their phylogenetic relationships. Presence of conspicuous bracteoles, embryo shape, and C_4 leaf type support

Table 6

Morphological Differences between Suaedoideae and Salicornioideae

Characters	Suaedoideae	Salicornioideae
Leaf lamina	Always present	Usually highly reduced
Lamina venation	One central bundle and many lateral bundles (except *Borszczowia*)	One central bundle and many peripheral bundles
Leaf base	Neither decurrent, nor amplexicaul	Amplexicaul, adnate to stem cortex
Inflorescence	Axillary cymes	Terminal club-shaped spikes
Flower position	Free in leaf axils	In hollows of inflorescence axis and ± fused with it
Bracteoles	Present	Absent
Tepals	5, usually fused at base	3–4, usually fused close to apex
Number of stamens	Usually 5	(1)2–3(4)
Albumen	Absent, or remnants of perisperm	Abundant, perisperm and (mostly) endosperm
Embryo	Spiral	Curved, horse-shoe- or ring- shaped

a sister group relationship of Salsoleae I and II, while indumentum, cotyledon anatomy, and C_4 biochemical subtype support a sister group relationship of Salsoleae II and Camphorosmeae. The problem will be dealt with in ongoing analyses with different markers (G. Kadereit and H. Freitag, unpublished manuscript).

Camphorosmeae (Endl. 1837) Moq. 1840. Our sampling included seven species belonging to five (of six) genera of Camphorosmeae and representatives of three out of 11 endemic Australian genera, which were placed in the separate tribe Sclerolaeneae by Scott (1978*b*). The molecular data clearly indicate that Camphorosmeae including Sclerolaeneae are much more closely related to Salsoleae than they are to Chenopodioideae, despite their plesiomorphic seed structure with a ringlike embryo and abundant perisperm. This was already stated by Volkens (1893), but except for Scott (1978*b*), who raised them to subfamilial rank, the section remained in Chenopodioideae in all other classifications (table 1). According to Scott (1978*b*), the fruiting perianth, with its various appendages (spines, wings, crests), provides the most important characters for the delimitation of Camphorosmeae/Sclerolaeneae from other tribes of Chenopodiaceae. However, in our opinion, the morphological difference to Salsoloideae—not discussed by Scott—is much smaller because the winglike fruit appendages of several Camphorosmeae/Sclerolaeneae (e.g., *Kochia, Maireana*) agree perfectly with those in Salsoleae I and II, and in some representatives of all three groups, such appendages are absent. Furthermore, Camphorosmeae/Sclerolaeneae have the same hair types as Salsoleae II and agree with them also in shape and structure of their cotyledons (table 7). In contrast to Salsoleae I and II, which are restricted to the Old World, Camphorosmeae have an almost worldwide distribution and include a large number of Australian genera (Sclerolaeneae).

Salsoleae I and II. Our Salsoleae I and II largely correspond to the NAD-ME and the NADP-ME clades, respectively, found by P'yankov et al. (2001) in an ITS analysis focused on species of *Salsola*. The species found in these clades are adapted most perfectly to desert conditions by the evolution of special morphological, anatomical, and physiological traits, with about 95% of the species having C_4 metabolism. The obvious polyphyly of *Salsola* had been suggested earlier on the basis of morphological studies (Freitag 1997). A more detailed analysis of the Salsoleae is in preparation and will be published elsewhere (H. Freitag and G. Kadereit, unpublished manuscript).

Distribution of C_4 Photosynthesis and Diversity of C_4 Leaf Anatomy

The C_4 leaf types are plotted on the *rbc*L tree to illustrate the diversity of C_4 photosynthesis syndromes in the two families and to indicate their distribution among the different C_4 lineages (fig. 3). Their main characteristics are summarized in table 8 (see also "Anatomical Results"). The C_4 leaf types also differ in several characters of the Kranz cells such as size, shape, wall thickness, and ultrastructural characters. A complete documentation of the C_4 leaf types will be given elsewhere, together with a full discussion of their presumable evolution from C_3 precursors, once our detailed phylogenetic analyses of the large C_4 clades are completed. Therefore, the following discussion and interpretation of evolutionary shifts from C_3 to C_4 remains somewhat incomplete.

Amaranthaceae

Amarantheae I and II. In Amarantheae I, C_4 photosynthesis is restricted to *Amaranthus*, an almost cosmopolitan genus of ca. 45 C_4 species. The *Amaranthus* leaf type (fig. 3*G*; table 8; see also fig. 2 in Carolin et al. 1978 [*Amaranthus interruptus* R. Br.] and fig. 2 in Ruthsatz and Hofmann 1984 [*Amaranthus haughtii* Standl.]) occurs in all species studied so far. It has probably evolved once from C_3 ancestors with isolateral leaves. In Amarantheae II, C_4 photosynthesis has been documented for only two species of *Aerva*, namely *Aerva javanica* and *Aerva pseudotomentosa* (R. F. Sage, unpublished data). It originated probably only once and is correlated with the switch from humid/semihumid to arid/semiarid habitats. The leaf anatomy of C_4 *Aerva* species is unknown.

Gomphrenoideae. Most C_4 taxa of Amaranthaceae are found in Gomphrenoideae. Genera of this subfamily, which are entirely C_4, are *Froelichia*, *Guilleminea*, *Blutaparon*, *Tidestroemia*, and *Lithophila* (R. F. Sage, unpublished data). *Lithophila* was not included in our analysis, but on the basis of morphological characters, a position of this genus close to *Blutaparon* can be expected. *Alternanthera* and *Gomphrena* include both C_3 and C_4 species. The large genus *Alternanthera* contains ca. 13 C_4 and ca. 72 C_3 species (R. F. Sage, unpub-

Table 7

Diagnostic Characters of Camphorosmeae, Salsoleae I, and Salsoleae II

Characters	Camphorosmeae	Salsoleae II	Salsoleae I
Bracteoles	Absent	Present	Present
Embryo	Horseshoe- or ringlike	Spiral	Spiral
Plant surface	Hairy with multicellular trichomes	Hairy with multicellular trichomes	Glabrous or hispidulous with 1-cellular papillae
Cotyledons[a]	Flat, dorsiventral	Flat, dorsiventral	Semiterete or terete, isolateral
C_4 leaf types[a]	Kochioid types *Kirilowia* type (fig. 3*D*; table 8)	Salsoloid types (fig. 3*E*; table 8)	Salsoloid types (fig. 3*E*; table 8)
C_4 biochemical subtype	NADP-ME	NAD-ME	NADP-ME

[a] H. Freitag and A. A. Butnik, unpublished data.

lished data) as well as several C_3/C_4 intermediates (Rajendrudu et al. 1986; Devi and Raghavendra 1993). The C_4 species are distributed among different sections of the genus. Therefore, C_4 photosynthesis may have originated repeatedly within this genus. The *rbc*L data indicate that *Gomphrena* is not monophyletic (fig. 2), and more detailed molecular analyses with *mat*K and ITS indicate that C_3 and C_4 species may belong to different lineages (T. Borsch and T. Ortuño, unpublished manuscript). The C_4 leaves of Gomphrenoideae studied so far resemble those of *Amaranthus* but are distinctly dorsiventral (fig. 3*F*; table 8; fig. 5 in Carolin et al. 1978 [*Gomphrena conica* Spreng.] and fig. 3 in Ruthsatz and Hofmann 1984 [*G. pallida* (Suess.) Pedersen]). The leaf anatomy described for *Alternanthera pungens* and *Froelichia* (Carolin et al. 1978) is similar, suggesting the presence of one anatomical type for Gomphrenoideae.

Chenopodiaceae

Chenopodioideae. Within Chenopodioideae, C_4 photosynthesis is known only from *Atriplex*. C_4 species occur in two subgenera and 16 (of 18) sections of this very polymorphic and almost cosmopolitan genus. The atriplicoid leaf type (Carolin et al. 1975), here called *Atriplex halimus* leaf type (fig. 3*A*; Volkens 1887, pl. 11, fig. 125), is most common among the numerous C_4 species of *Atriplex*. It varies considerably in structure of the hypodermis, the more radial or more perpendicular arrangement of the palisade layer, and the isolateral symmetry. A second origin of C_4 photosynthesis within *Atriplex* could be represented by the *Atriplex dimorphostegia* type (fig. 3*A*), which mainly differs in lacking a hypodermis. This type was first described by Khatib (1959, fig. 9) but was overlooked by all subsequent authors. It is present in a number of annual species from central and southwest Asia with thin and partially translucent leaves (e.g., *Atriplex ornata* Iljin, *Atriplex belangeri* (Moq.) Boiss.; A. Sukhorukov, personal communication and personal observation). Most likely, the two *Atriplex* C_4 leaf types have evolved from flat isolateral C_3 leaves. Any definite conclusions about the number of shifts from C_3 to C_4 in Atripliceae depends on additional molecular and leaf anatomical evidence from a sampling that includes members of all sections.

Salicornioideae. Within Salicornioideae, C_4 photosynthesis originated only once in the palaeotropical genus *Halosarcia*. The unique *Halosarcia indica* type (fig. 3*B*) is the only C_4 type that is restricted to the stem cortex. It was discovered by Wilson (1980) and described by Carolin et al. (1982, figs. 1, 2 therein). The stem anatomy of C_4 *Halosarcia* is characterized by a two-layered external chlorenchyma followed by aqueous tissue and a massive central cylinder. In tissue arrangement, it superficially resembles the *Salsola* types except for the palisade layer (unusual mosaic-like pattern of cells with and without chloroplasts) and the arrangement of conducting tissue in the peripheral bundles (internal xylem and external phloem). In contrast to leafless C_4 species of Salsoleae, the *H. indica* type probably has evolved in the stem itself because leaves are absent from Salicornieae.

Suaedoideae. Of the four fundamentally different C_4 leaf types found in Suaedoideae (fig. 3*C*; for full description and figures, see Freitag and Stichler 2000, 2002), three are represented in the *rbc*L analysis. The topology of the *rbc*L tree indicates two independent origins of C_4 photosynthesis in Suaedoideae: one each in the monotypic *Bienertia* (Bienertieae) and in Suaedeae (monotypic *Borszczowia* plus *Suaeda altissima*). On the basis of a much broader sampling, Schütze et al. (2003) nevertheless suggested that C_4 photosynthesis in Suaedeae has originated independently in three lineages, i.e., *Borszczowia*, *Suaeda* sect. *Salsina* (which includes *Suaeda altissima*), and *Suaeda* sect. *Schoberia*. Interestingly, the different anatomy of the four C_4 leaf types in Suaedoideae contrasts with the rather similar isolateral leaves of their C_3 relatives.

Salsoloideae, Camphorosmeae. In the Camphorosmeae, it is equally parsimonious to postulate two origins or one origin and one loss of C_4 photosynthesis. Either there is one shift from C_3 to C_4 photosynthesis at the base of a large C_4 clade and a secondary loss of C_4 characters in *Bassia sedoides* or there are two shifts from C_3 to C_4 photosynthesis, one in *Camphorosma* and one at the base of the clade that comprises *Panderia*, *Chenoleoides*, and *Kochia prostrata* (fig. 3). A molecular study of Camphorosmeae based on ITS sequences (G. Kadereit and H. Freitag, unpublished manuscript) supports the *rbc*L results.

Our anatomical data, however, showed at least three different C_4 leaf types that might represent independent origins or different stages of C_4 evolution inside the *Panderia*, *Chenoleoides*, and *Kochia prostrata* lineage and one in the *Camphorosma* lineage. (1) The *Kochia laniflora* type is most common (fig. 3*D*; see also fig. 23 in Monteil 1906 [*Kochia laniflora* (S.G. Gmelin) Borbás, sub *Kochia arenaria*]; fig. 1*a* in Gamaley 1985 [*Bassia hyssopifolia* (Pall.) O. Kuntze]) and identical to the kochiod type s. str. of Carolin et al. (1975). It varies mainly

Table 8

C_4 Leaf Types in Amaranthaceae and Chenopodiaceae

Leaf type	Fig.	Traditional type name[a]	Leaf shape, general anatomy	Succulence,[b] sclerophylly[b]	Indument,[b] hair type	Secondary bundles	Kranz cell arrangement	Hypodermis[b]	Biochemical type,[c] $\delta^{13}C$ values[c]
Amaranthus	3*G*	*Amaranthus*	Flat, ± isolateral	0, 0	0−++, glandular	Lateral	Closed BS	0	NAD, ?
Gomphrena	3*F*	*Gomphrena*	Flat, dorsiventral	0, 0	0−+, uniseriate	Lateral	Closed BS	0	NADP, 10.7–16.5
Atriplex halimus	3*A*	Atriplicoid	Flat, ± isolateral	+−++, 0	+++, vesicular	Lateral	Open BS	+	NAD, 11.4–14.3
Atriplex dimorphostegia	3*A*	Atriplicoid	Flat, isolateral	0, 0	+−++, vesicular	Lateral	Open BS	0	NAD, 11.8
Halosarcia indica	3*B*	Kranz halosarcoid	In stems only	+++, 0	0,–	Peripheral	Concentric	0	?, 14.2
Bienertia	3C	Bienertioid	Semiterete, isolateral	++−+++, 0	+, vesicular	Lateral	Concentric (non-Kranz)	0	NAD, 13.4–15.5
Borszczowia (=*Suaeda* sect. *Borszczowia*)	3C	Borszczowioid	Semiterete, centric	+++, 0	0, 0, vesicular	Peripheral	Concentric (non-Kranz)	+	NAD, 12.5–13.8
Schoberia	3C	Conospermoid	Semiterete, isolateral	+++, 0	0, 0	Lateral	Modified BS	+	?, 10.5–13.6
Salsina	3C	Kranz Suaedoid	Semiterete, isolateral	+−+++, 0	0, (papillate)	Lateral	Concentric	0	NAD, 9.7–14.8
Kochia prostrata	3*D*	Kochioid	Flat, isolateral	+−++, 0	+−+++, uniseriate	Peripheral	Arclike BS	+	NADP, 12.9–13.4
Kochia laniflora	3*D*	Kochioid	Flat, isolateral	+−++, 0	+−+++, uniseriate	Peripheral	Arclike BS	0	NADP, 11.4–13
Kirilowia	3*D*	Atriplicoid	Flat, isolateral dorsiventral	0, 0	+, uniseriate	Lateral	Arclike BS	0	?, 12.2
Salsola soda	3*E*	Salsoloid	Semiterete or terete, centric (or isolateral)	+++, 0	0−++, various	Peripheral (and lateral)	Concentric	+	NAD and NADP, 9.8–15.2
Salsola kali	3*E*	Salsoloid	Semiterete or terete, centric (or isolateral)	+++, 0	0−++, various	Peripheral (and lateral)	Concentric	0	NADP and NAD, 11.1–14.1
Climacoptera	3*E*	Salsoloid	Semiterete or terete, centric	+++, 0	0−++, uniseriate	Subperipheral	Concentric	0	NAD, 11.0–14.6
Nanophyton	3*E*	Salsoloid	Semiterete or terete, centric	0, +++	0, (prickles)	Peripheral	Concentric	0	?, 13.5
Halothamnus auriculus	3*E*	Salsoloid	Flat, isolateral	+−++, 0−+	0−+, (prickles)	Peripheral and lateral	Concentric	0	NADP, 11.8–13.4

[a] References are cited in the text.

[b] Symbols: 0/+ character absent/present, +−++−+++ intensity of character expression.

[c] List of references available on request.

in the amount of aqueous parenchyma in the central mesophyll and the resulting degree of succulence. (2) The *Kochia prostrata* type (fig. 3*D*) differs from the *Kochia laniflora* type in the presence of a distinct hypodermis. This type is known so far from *K. prostrata* and *Panderia pilosa* only. The leaves of *Camphorosma* (fig. 16 in Monteil 1906) exhibit a small variation of the *K. prostrata* type. They resemble the salsoloid leaf type and were classified accordingly as intermediate by Carolin et al. (1975). (3) The *Kirilowia* type (fig. 3*D*; see also fig. 17 in Monteil 1906) deviates from the others in the replacement of peripheral by lateral secondary bundles. In the almost radial arrangement of the chlorenchyma around the bundles, it resembles the *Atriplex* types. Accordingly, Carolin et al. (1975) described *Kirilowia* leaves as atriplicoid. All C_4 leaf types in Camphorosmeae may have originated from flat dorsiventral (*Kirilowia* type) or flat isolateral (*K. prostrata* type, *K. laniflora* type, and *Camphorosma* type) and moderately succulent C_3 leaves.

Salsoloideae, Salsoleae. The Salsoleae I and II contain only ca. 10 C_3 species. Four of them representing their diversity were included in our study. The molecular data (our *rbc*L tree and preliminary results of an ITS analysis (G. Kadereit and H. Freitag, unpublished manuscript) point to at least three (probably four) independent shifts to C_4 photosynthesis (fig. 3) and against the interpretation of C_3 species of Salsoleae I as reversals (Carolin et al. 1975; P'yankov et al. 1997; Voznesenskaya et al. 2001*a*). The leaves of Salsoleae I and II are comparatively uniform in the arrangement of chlorenchyma, probably because they have evolved from similar, more or less succulent C_3 leaves. They all belong to the traditional salsoloid type, which is divided here into the *Salsola soda* type, with a hypodermis (fig. 3*E*; see also pl. 12, fig. 34 in Volkens 1887 [*Halogeton* sp., *Salsola longifolia* Forssk.]) and the *Salsola kali* type, without hypodermis (fig. 3*E*; see also fig. 34 in Monteil 1906 [*Salsola tragus* (L.) L., sub *Salsola kali*]; fig. 131 in Fahn 1990 [same species]). Both types occur intermingled in Salsoleae I and II but clearly separated among genera or sections, which indicates multiple origins of these two leaf types. This is also supported by the fact that both types are correlated with different biochemical subtypes in the two tribes. *Climacoptera* (*Climacoptera crassa* and five more species studied so far) as well as all species of *Halocharis* investigated represent a remarkable variant of the *Salsola kali* type. They show a subperipheral position of the secondary bundles (fig. 3*E*). Furthermore, spectacular modifications by strong sclerophyllization (e.g., *Nanophyton* type; fig. 3*E*) or flattening of leaves (e.g., *Halothamnus auriculus* type; fig. 3*E*) occur in several subclades. These were first detected by Butnik (1984, 1995), who described the latter as laminate centric. The two types lack a hypodermis, but in other lineages, a hypodermis is present (not shown).

In most genera of Salsoleae II, the stem cortex also contains chlorenchyma, and in many taxa with reduced leaves, this is the main photosynthetic tissue, e.g., *Anabasis*, *Haloxylon*, *Girgensohnia*. In these taxa, leaf and stem chlorenchyma show identical anatomical structure, suggesting that the C_4 syndrome has evolved in the leaves that are still present in many more plesiomorphic species of the respective genera.

Shift from C_3 to C_4 Photosynthesis

The *rbc*L phylogeny presented here allows a first estimate of the number and placement of C_4 lineages in Amaranthaceae and Chenopodiaceae. For Amaranthaceae, we found molecular evidence for three independent shifts from C_3 to C_4 metabolism, and possibly at least two more may have occurred. As far as known, the multiple origins of C_4 photosynthesis in Amaranthaceae are poorly reflected in C_4 leaf anatomy, probably because the evolution of the C_4 leaf characters started from structurally ± identical isolateral and/or dorsiventral flat leaves. According to recent summaries (R. F. Sage, unpublished data), Amaranthaceae contain ca. 120 C_4 species distributed among nine genera. The delimitation of genera is uncertain in several cases, and the photosynthetic pathway is still unknown in many taxa.

In Chenopodiaceae, evidence from our *rbc*L analysis, the analysis of Schütze et al. 2003, and preliminary ITS data (G. Kadereit and H. Freitag, unpublished data) points to at least 10 origins of C_4 photosynthesis, a number far higher than suggested before (Carolin et al. 1975, 1982) and comparable to Poaceae, the largest C_4 family in monocots (Giussani et al. 2001). In Chenopodiaceae, ca. 570 C_4 species are distributed among ca. 42 genera. Diversity in leaf anatomy is higher in Chenopodiaceae than in any other family, presumably because evolution of C_4 leaf types started relatively early in the geological history and from ancestors with different C_3 leaf types. In both families, C_4 photosynthesis is absent from the basal lineages (Polycnemoideae, *Bosea*, *Charpentiera*, Betoideae) and concentrated in certain terminal groups. While some C_4 groups are very successful in terms of species diversity (e.g., *Atriplex*, Salsoleae, *Suaeda* sect. *Salsina* in chenopods, and *Amaranthus* and *Alternanthera* in amaranths), others obviously were not (e.g., *Bienertia* and *Halosarcia* in chenopods, *Aerva* in amaranths). This may be caused only in some cases by the different geological age of the respective clades. In other cases, however, the efficiency of C_4 photosynthesis may differ among anatomical leaf types and biochemical subtypes. This might apply in particular to the single-cell C_4 systems of the *Bienertia* and the *Borszczowia* types, which according to tree topology have originated early in geological history, at least in *Bienertia* (fig. 6 in Schütze et al. 2003). Another example may be the low number of species in the C_4 clade in Camphorosmeae compared to its C_3 sister clade, which was most successful in the Australian semideserts. These facts suggest that the invention of C_4 photosynthesis as such does not guarantee evolutionary success. The efficiency of C_4 photosynthesis, and its contribution to fitness, might be hampered by other anatomical, morphological, and physiological properties of the taxa concerned.

With regard to the evolution of the two biochemical subtypes of C_4 photosynthesis recorded from Chenopodiaceae (Sage and Monson 1999), our data strongly suggest independent origins of these biochemical subtypes from C_3 ancestors in this family. The NAD-ME subtype is found in C_4 species of *Amaranthus*, *Atriplex*, *Halosarcia*, Suaedoideae, and Salsoleae II, while C_4 species of Salsoleae I and Camphorosmeae show the NADP-ME subtype. As far as is known, no shifts from NAD-ME to NADP-ME or vice versa occurred.

The Age of C_4 Photosynthesis in Chenopodiaceae

After the exclusion of four taxa of Chenopodioideae and two taxa of Salsoloideae, we obtained constant substitution rates of *rbc*L among lineages within these two subfamilies. A rate of 0.28–0.41 × 10^{-9} synonymous substitutions per site per year was found for Chenopodioideae. This rate is comparable to the rates of synonymous substitutions found for *rbc*L sequences of *Cornus* (1.23 ± 0.128 × 10^{-9}; Xiang et al. 2000) and also to the estimate of 1.3 × 10^{-9} for dicots in general by Zurawski and Clegg (1987), which was based on sequence comparison between spinach and tobacco. The age of C_4 photosynthesis in *Atriplex* as the only genus with C_4 species within Chenopodioideae is here estimated to be 11.5–7.9 Ma, a period that lies within the late Miocene. The C_4 photosynthesis in Salsoloideae seems to be older; Salsoleae II, which is entirely C_4, *Salsola kali* from Salsoleae I (both 21.5–14.4 Ma), the *Girgensohnia* clade from Salsoleae I (19.6–13.4 Ma), and the C_4 Camphorosmeae (21.6–14.5 Ma) date back to the early Miocene, while the *Noaea* clade, with an age of 12.5–8.5 Ma, probably originated, like *Atriplex*, in the late Miocene. We are aware that these data are first estimates and need to be corroborated by approximations derived from different markers. Our calculations are also in some contrast to suggestions that could be derived from biogeography. The almost global distribution of C_4 lineages in *Atriplex* and in *Suaeda* indicates that these groups have a relatively higher age than C_4 lineages in Salsoloideae, which are restricted to Eurasia and Africa despite being equipped with most efficient devices for long-distance dispersal.

The oldest known paleorecords of C_4 plants have been found in sediments of the Middle Miocene. They include a grass with Kranz anatomy from California (Thomasson et al. 1986) dated to 12.5 Ma and, less reliably, grass cuticles from Kenya (Retallack et al. 1990) dated to 14 Ma. The much older reports of C_4 plants from the Cenomanian/Turonian boundary (ca. 94 Ma) by Kuypers et al. (1999), on the basis of $\delta^{13}C$ values from leaf wax *n*-alkanes embedded in oceanic sediments near northwest Africa are not convincing because the respective values (−22 to −28) are clearly in the range of C_3 plants. However, the paleorecord is sparse (for review, see Cerling 1999). Molecular clock interpretations similar to those presented here estimated the age of C_4 photosynthesis in grasses to range from ca. 17 (split of maize and sorghum) to 25 (split of maize from *Pennisetum*) million years ago (Gaut and Doebley 1997). Kellogg and Russo (GPWG 2001) estimated the origin of the C_4 grass *Danthoniopsis* to 16 Ma, which roughly agrees with our calculations. This allows us to conclude that the first origins of C_4 photosynthesis in Chenopodiaceae and Poaceae occurred in about the same geological periods of the Lower to Middle Miocene between ca. 25 and 15 Ma and possibly already in the uppermost Oligocene.

The expansion of C_4 taxa in Chenopodiaceae and Poaceae—radiation of the first C_4 lineages, repeated origin of new C_4 lines, enhanced performance in plant communities—may have happened in parallel, albeit in very different environments. Carbon isotope data from palaeosoils and palaeodiets indicate that C_4 plants, probably most of them belonging to Poaceae, expanded during the late Miocene, at about 10–6 Ma (Cerling 1999). This process is closely related to the evolution of tropical grasslands under semihumid to arid conditions with precipitation during the growing season. In contrast to this, the expansion of Chenopodiaceae took place predominantly in warm temperate desert ecosystems and xero-saline habitats with no or very little rain in the growing season. The tight linkage of today's C_4 Chenopodiaceae to regions with arid and preferably hot climates is particularly well documented for the area of the former Soviet Union (e.g., fig. 5 in P'yankov and Mokronosov 1993). The environmental and evolutionary preconditions for the origin and diversification of the C_4 photosynthetic syndrome were reviewed by Sage (2001). The hypothesis that C_4 photosynthesis evolved in response to decreasing atmospheric CO_2 in recent geological times is widely accepted. It has been postulated that the lowering of P_{CO_2} from its high level in the Cretaceous was the trigger causing first origins of C_4 plants, followed by a further drop of P_{CO_2} during the Miocene leading to a global expansion of C_4 taxa. The number and importance of C_4 plants were further increased when the P_{CO_2} minima were reached during the glacial periods of the Pleistocene. There are strong biochemical arguments and theoretical predictions in favor of this view (for reference, see Sage 2001). However, with regard to C_4 taxa in Chenopodiaceae, it underestimates the importance of aridity, light, and temperature for the distribution of species. In Chenopodiaceae, it seems that C_4 photosynthesis is an evolutionary response to a permanent shortage in water supply in combination with high temperatures and light intensities during summer. In desert and semidesert environments as well as in ecophysiologically similar hypersaline habitats, most likely the primary advantage of C_4 plants is their high water use efficiency. The CO_2-concentrating mechanism in the C_4 chlorenchyma allows a stronger reduction of stomatal aperture before photosynthesis decreases significantly compared with C_3 plants (Osmond et al. 1982; Schulze et al. 1996). By that, most C_4 taxa in chenopods surpass the xerophytic properties of their C_3 ancestors and were able not only to replace them almost completely in all suitable habitats but also to colonize niches not accessible to C_3 xerophytes. Our hypothesis concerning the importance of precipitation and temperature for the selective advantage or disadvantage of C_4 species relative to C_3 species is supported by the study of Huang et al. (2001), who compared the relative abundance of the two groups in Mesoamerican lake sediments since the last glacial maximum. They have shown that large-scale expansions of C_4 plants were triggered only by major changes in precipitation and temperature despite constant, or even increasing, P_{CO_2}. However, the C_4 syndrome is an extremely complex evolutionary achievement, and apart from anatomical and biochemical factors, its evolution might also be constrained by genetical limitations (Monson 2003).

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