

## Phylogenetic Analyses of Amaranthaceae Based on matK DNA Sequence Data with Emphasis on West African Species

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**Abstract:** Comparative sequencing of the chloroplast matK coding and non-coding regions was used to examine relationship among the species of Amaranthaceae with emphasis on the West African species and other closely related family such as Chenopodiaceae, Portulacaceae, and Caryophyllaceae. Phylogenetic analysis of the matK sequences alone and in combination using maximum parsimony methods produced monophyletic lineage of Amaranthaceae-Chenopodiaceae. Our results indicated that a polyphyletic Celosieae as sister to an Amaranthus-Chemissoa lineage. Subfamily Amaranthoideae is paraphyletic to the core Gomphrenoids. This study also shows that the polyphyly of Amaranthaceae is apparent and so is the polyphyly of Amaranthinae.

**Key Words:** Amaranthaceae, matK, Phylogenetic analyses

### Introduction

Amaranthaceae, commonly called the **Amaranth family**, contains about 65 genera and 900 species. They are widespread and cosmopolitan in distribution but most abundant in tropics, subtropics, and warm-temperate regions, evidently absent from alpine and arctic regions. Centres of diversity for Amaranthaceae are south-western North America, Central America, South America, and Africa south of the Sahara Desert. Most species occur in tropical Africa and North America (Townsend, 1993). Some species are considered weeds, but a number of others are popular garden ornamental plants, especially species of *Alternanthera* Forssk., *Amaranthus* L., and *Celosia* L. Amaranths are probably among the most promising unexploited food and fodder crops (Becker et al., 1981; Dey & Mandal, 1993).

Amaranthaceae and Chenopodiaceae are similar in their morphology, anatomy, and phytochemistry (Brown, 1810; Bentham & Hooker, 1880; Baillo, 1887; Volkens, 1893; Carolin, 1983; Kuhn et al., 1993; Townsend, 1993). Furthermore, based on serological studies, it is believed that Amaranthaceae and the related sister family Chenopodiaceae should be merged together (Baillon, 1887 & Mallingson, 1922). APG (Angiosperm Phylogeny Group) 1998 and 2003 supported this recommendation based on the molecular studies.

Giannasi et al. (1992), Rettig et al. (1992), and Cueñoud et al. (2002) in their studies included more than two taxa of Amaranthaceae and Chenopodiaceae in their sampling and both families were resolved as sister families. Manhart and Rettig (1994) and Cueñoud et al. (2002) identified Amaranthaceae as nested within

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Chenopodiaceae. Müller and Borsch (2005) using matK/trnK in their study on Amaranthaceae and Chenopodiaceae concluded that this lineage is monophyletic, with Achatocarpaceae and Caryophyllaceae being successive sisters.

Amaranthaceae have been subjected to review by several authors in the past (Martius, 1825; Endlicher, 1837; Schinz, 1934; Cavaco, 1962; Townsend, 1993). Martius (1825) recognised 28 genera in his study and Townsend (1993) carried out several treatments on members of the family. Seubert (1875) based his classification on Endlicher (1837) and recognised three tribes in Amaranthaceae in the *Flora brasiliensis*. In 1993, Townsend based on the work of Schinz (1893, 1934) proposed the currently present classification. The work of Schinz (1893, 1934) was derived from the study of Bentham and Hooker (1880). Amaranthaceae was divided into subfamilies Amaranthoideae (anthers 4-locular with two lines of dehiscence) and Gomphrenoideae Schinz (anthers 2-locular with one line of dehiscence). Some changes were later made by Schinz (1893) by distinguishing the tribes Gomphreneae (Gomphreninae and Froelichinae) and Guillemineae and later change Guillemineae to Brayulineae and introduced a new subtribe called Tidestromiinae (Schinz, 1834). Covas (1939) classified Pseudopiantogo in its own tribe because it displayed a mixture of amaranthoid and gomphrenoid floral morphology. Later the tribe Pseudopiantageae was classified within Gomphrenoideae (Townsend, 1993).

Townsend (1993) stated that the subtribe Aervinae (Amarantheae, Amaranthoideae) comprises the largest member of species within the family. Bentham and Hooker (1880) used Achyranthinae instead of Aerveae in their study but Townsend (1993) re-established the modern name.

Modern genetics-based classification schemes, considered Amaranthaceae and Chenopodiaceae as sister-groups that form the most basal branch of the order Caryophyllales (Borsch et al., 2001). Müller and Borsch (2005) reported the phylogenetics of Amaranthaceae based on matK/trnK sequence data. The Amaranthaceae-Chenopodiaceae lineage was resolved as monophyletic.

The present study examines generic-level phylogenetic relationships in Amaranthaceae using matK DNA sequence data. Recent studies have substantiated the use of matK gene for resolving phylogenetic relationships in a broad taxonomic range (Johnson & Soltis, 1995; Hilu & Liang, 1997; Meimberg et al., 2000; Hilu et al., 2003).

The *matK* gene stands out among genes used in angiosperm systematics in its substantially greater number of nucleotide substitutions, nonsynonymous mutations, and insertion/deletion events or indels (Johnson & Soltis, 1995; Hilu & Liang, 1997; Soltis & Soltis, 1998). The gene also exhibits a relatively high proportion of transversions, with the transition/transversion ratio (ti/tv) approaching unity (Hilu & Liang, 1997).

In this study, matK was used for the species and combined with the one studied by Müller and Borsch (2005). The main goal of the study was to construct phylogenetic hypotheses and the intergeneric relationships within members of Amaranthaceae. The results will be used to examine the relationship between members of Amaranthaceae with emphasis on West African species and other closely related families such as Chenopodiaceae, Portulacaceae, and Caryophyllaceae.

## Materials and Methods

**Plant Specimens** Voucher information, localities, and GenBank numbers are as listed in Table 1. We examined 11 genera with three lines of *Amaranthus hybridus* L. to obtain comparative sequences of the matK coding and non-coding regions. Sequences of several species were downloaded from GenBank and are also listed in Table.

**DNA Extraction and Amplifications.** Fresh leaf material, leaf material dried in silica gel, or recently collected herbarium specimens were used as sources of DNA. Total genomic DNA was extracted using the CTAB protocol (Doyle & Doyle, 1987). PCR reactions were performed on 50 µl reactions containing 1 mM dNTP, 0.5 Taq DNA polymerase (Promega), 5 µl of 10 × buffer (Promega), 4 µl of MgCl<sub>2</sub>, 1.5 µl of BSA, 0.7 µl of Fw + Rv matK with 32.5 µl water. Polymerase chain reaction (PCR) amplification was carried out in a GeneAmp PCR System 9700 ABI using the the primers 390 F > and 2R < for matK. Amplifications were carried out using the following programme: initial denaturation for 3 min at 94 °C followed by one cycle of denaturation for 1.00 min at 94 °C, annealing for 45 sec at 52 °C and extension for 2 min 30 sec at 72 °C followed by 35 cycles of denaturation for 1 min at 45 °C. The amplification was completed by holding the reaction mixture for 7 min at 72 °C to allow complete extension of the PCR products. PCR products were then purified. Clean PCRs were then sequenced in

Table. Taxa sampled in the present study. Vouchers and GenBank accession numbers for the sequences generated in this study are provided. Classification follows Townsend (1993) for Amaranthaceae, Kühn et al. (1993) for Chenopodiaceae, Bittrich (1993) for Caryophyllaceae, and the Angiosperm Phylogeny Group (APG, 2003) for the remaining family assignments. For sequences downloaded from GenBank, the reference instead of the voucher specimen is shown as well as the accession number. Herbarium acronyms follow *Index Herbariorum*.

Family, subfamily, tribe	Species DNA source (Garden/field origin/voucher)		GenBank accession
Amaranthaceae	<i>Celosia elegantissima</i> Hauman	Nigeria, Idanre, <i>Ogundipe</i> 2085	K21094
Amaranthoideae	<i>Celosia argentea</i> L.	Nigeria, Ijebu-ode; <i>Ogundipe</i> 2035	K21112
Celosieae	<i>Celosia leptostachya</i> Benth.	Nigeria, Lagos; <i>Ogundipe</i> 2012	K21063
	<i>Celosia trigyna</i> L.	Burkina Faso, <i>Kalheber 161</i> (FR)	AY514811
	<i>Celosia trigyna</i> L.	Nigeria, Olokemeji; <i>Ogundipe</i> 2042	K21065
	<i>Celosia insertii</i> L.	Nigeria, Olokemeji, <i>Ogundipe</i> 2055	K21066
	<i>Pleuropetalum sprucei</i> (Hook, f.) Standl.	Hilu et al. (2003)	AF542596
	<i>Hermestaedtia glauca</i> (Wendl.) Rchb. ex Steud	Namibia, <i>C.Neinhuis s.n.</i> (BONN)	AY514812
	<i>Deeringia amaranthoides</i> (Lam.) Guam, Philipp Merr.	Sea, <i>E.Moore 746</i> (BONN)	AY514814
Amaranthaceae	<i>Aerva javanica</i> (Burm. f.) Juss.	Madagascar, <i>E.Fischer s.n.</i> (BONN)	AY514793
Amaranthoideae	<i>Amaranthus spinosus</i> L.	Nigeria, Lagos, <i>Ogundipe</i> 2095	K21070
Amarantheae	<i>Amaranthus greacizans</i> L.	Nigeria, Olokemeji; <i>Ogundipe</i> 2043	K21072
	<i>Amaranthus lividus</i> L.	Nigeria, Ile-Ife; <i>Ogundipe</i> 2010	K21074
	<i>Amaranthus dubius</i> Mart. ex Thell	Nigeria, Ile-Ife; <i>Ogundipe</i> 2087	K21075
	<i>Amaranthus hybridus</i> L.	Nigeria Ibadan <i>Ogundipe</i> (NH84/457-1)	K21097
	<i>Amaranthus hybridus</i> L. (NH84/457-1E)	Nigeria, Osun, Ibadan <i>Ogundipe</i>	K21098
	<i>Amaranthus hybridus</i> L. (NH94/443-1WHITE)	Nigeria, Osun, Ibadan <i>Ogundipe</i>	K21099
	<i>Amaranthus caudatus</i> L.	Bot. Gard. <i>Mainz s.n.</i>	AY514809
	<i>Bosea yervamora</i> L. Müller 751 BONN	Canary Islands, Tenerife, <i>K.</i>	AY514810
	<i>Calicorema capitata</i> f. (Moq.)Hook.	Namibia, <i>C.Neinhuis s.n.</i> (BONN)	AY514807
	<i>Chamissoa altissima</i> (Jacq.) Kunth var. <i>altissima</i>	Paraguay, <i>E.Zardini &amp; L.Guerrero 42592</i> (BONN, MO)	AY514857
	<i>Achyranthes aspera</i> L.	Bot. Gard. Bonn, BG Bonn 03616, <i>K. Müller 900</i> (BONN)	AY514815
	<i>Charpentiera obovata</i> Gaudich.	U.S.A., Hawaii, <i>B.A.Prigge 15251</i> (LA)	AY514855
	<i>Cyathula uncinulata</i> L.	Nigeria, Oyo, <i>Ogundipe</i> 1811	K21092
	<i>Cyathula prostrata</i> (L.) Blume	Nigeria, shaki, <i>Ogundipe</i> 2060	K21068
	<i>Aerva javanica</i> (Burm.f.) Schult.	Nigeria, Kaduna, <i>Ogundipe</i> 1045	K21082
	<i>Aerva leucura</i> Moq.	Nigeria, kaduna. Olatunji 4587	K21083
	<i>Nothosaerva brachiata</i> Wight	Burkina Faso, <i>Anonymous s.n.</i> (FR)	AY514806
	<i>Nototrichium humile</i> Hillebr.	U.S.A., Hawaii, <i>B.A.Prigge 15249</i> (LA)	AY514816
	<i>Achyranthes bidentata</i> Blume	Nigeria, Shaki; <i>Ogundipe</i> 2100	K21080
	<i>Achyranthes aspera</i> L.	Nigeria, Shaki; <i>Ogundipe</i> 2099	K21081
	<i>Pandiaka angustifolia</i> (Vahl) Hepper	Burkina Faso, <i>J.Müller 324</i> (FR)	12AY514818
	<i>Pandiaka involucrata</i> Moq. Jackson	Nigeria, Lagos; <i>Ogundipe</i> 2004	K21069
	<i>Psilotrichum africanum</i> Oliver	South Africa, <i>K.Müller 878</i> (BONN, PRE)	AY514822
	<i>Ptilotus manglesii</i> (lindl.). F.Muell	ustralia, <i>Th.Borsch 3543</i> (BONN)	AY514824
	<i>Ptilotus obovatus</i> F.Muell.	[Australia], BG Bonn <i>s.n.</i>	AY514823
	<i>Pupalia lappacea</i> (L.) Juss	Nigeria, Ile-Ife; <i>Ogundipe</i> 2088	K21076
	<i>Sericostachys scandens</i> Gilg & Lopr	Nigeria, Lokoja, <i>Olatunji 3888</i>	K21086
Amaranthaceae Gomphrenoideae	<i>Pseudoplantago friesii</i> Suess	Argentina, <i>T.M.Pedersen 15792</i> (CTES, C)	AY514820
Pseudoplantageae	<i>Alternanthera caracasana</i> Kunth	Hilu et al. (2003)	AF542595
Amaranthaceae	<i>Alternanthera pungens</i> Kunth 3449 (ISC, BONN)	U.S.A., Texas, <i>Th.Borsch Pratt &amp; K.Müller</i>	AY514795
Gomphrenoideae	<i>Alternanthera pungens</i> Kunth	Nigeria, Ife; <i>Ogundipe</i> 2079	K21078
Gomphreneae	<i>Alternanthera sessilis</i> (L.) DC.	Nigeria, Ife; <i>Ogundipe</i> 2006	K21079

Table. Continued.

	<i>Blutaparon vermiculare</i> (L.) Mears	U.S.A., Texas, <i>Th.Borsch, D.Pratt &amp; K.Müller</i> 3444 (ISC, BONN)	AY514798
	<i>Froelichia floridiana</i> (Nutt.) Moq.	BG Bonn 03615-90, <i>Th.Thiel s.n</i> (BONN)	AY514799
	<i>Gomphrena celosioides</i> Mart.	<i>Nigeria, Ile-Ife; Ogundipe</i> 2095	K21077
	<i>Gomphrena haageana</i> Klotzsch	Bot. Gard. Mainz	AY514800
	<i>Gomphrena mandonii</i> R.E.Fr.	Bolivia, <i>S.Beck</i> 18720 (BONN, LPB)	AY514801
	<i>Gomphrena macrocephala</i> St.-Hil.	Paraguay, <i>E.Zardini</i> 45564 (BONN, MO)	AY514803
	<i>Hebanthe occidentalis</i> (R.E.Fr.) Borsch & Pedersen var. <i>occidentalis</i>	Paraguay, <i>E.Zardini</i> 45377 (BONN, MO)	AY514821
	<i>Iresine lindenii</i> Van Houtte	Bot. Gard. Mainz <i>s.n.</i>	AY514805
	<i>Iresine palmeri</i> S.Watson	U.S.A., Texas, <i>Th.Borsch, D.Pratt &amp; K.Müller</i> 3445 (ISC, BONN)	AY514804
	<i>Tidestromia lanuginosa</i> (Nutt.) Standl.	U.S.A., Texas, <i>Th.Borsch, D.Pratt &amp; K.Müller</i> 3439 (ISC, BONN, MEXU)	AY514797
Chenopodiaceae	<i>Atriplex elegans</i> (Moq.) D.Dietr.	U.S.A., Texas, <i>Th.Borsch, D.Pratt &amp; K.Müller</i> 3425 (ISC, BONN)	AY514830
Chenopodioideae			
Atripliceae	<i>Spinacia oleracea</i> L.	Zurawski et al. (1982)	AJ400848
Chenopodiaceae	<i>Beta vulgaris</i> L.	Germany, Baltic Sea, <i>G.Kadereit</i> 99/255 (MJG)	AY514832
Chenopodioideae	<i>Acroglochin chenopodioides</i> Schrad.	BG Kassel ex BG Lyon, <i>Anonymous s.n.</i> (KAS)	AY514826
Beteae			
	<i>Hablitzia tamnoides</i> M.Bieb.	<i>Th.Borsch</i> 3546; BG Bonn 3609- 90(BONN)	AY514825
	<i>Aphanisma blitoides</i> Nutt. ex Moq.	U.S.A., California, <i>S.Junak</i> SR- 987 (SBBG)	AY514844
Chenopodiaceae	<i>Bassia hirsuta</i> (L.) Aschers.	U.S.A., Texas, <i>Th.Borsch, D.Pratt &amp; K.Müller</i> 3430 (ISC, BONN)	AY514831
Chenopodioideae			
Camphorosmeae	<i>Camphorosma monspeliaca</i> L.	Kazakhstan, Prov. Gur'yev (Atyrau), Makhambat, <i>H.Freitag</i> 28.133 (KAS)	AY514829
Chenopodiaceae	<i>Chenopodium. acuminatum</i> Willd	Mongolia, <i>M.Azuma s.n.</i> (FR)	AY514836
Chenopodioideae	<i>Chenopodium bonushenricus</i> L.	Bot. Gard. Mainz, without voucher	AY514834
Chenopodieae	<i>Chenopodium botrys</i> L.	Germany, <i>T.Gregor &amp; S.Demuth</i> , (FR)	AY514835
Chenopodiaceae	<i>Agriophyllum squarrosum</i> (L.) Moq.	SE Russia, Prov. Astrakhan, <i>H.Freitag</i> 28.196a (KAS)	AY514827
Chenopodioideae			
Camphorosmeae	<i>Corispermum ladakhianum</i> Grey-Wilson & Wadhwa	Bot. Gard. Mainz <i>s.n.</i>	AY514837
Chenopodiaceae	<i>Nitrophila occidentalis</i> S.Watson	U.S.A., Utah, <i>D.Pratt</i> 204 (ISC, BONN)	AY514840
Polycnemoideae	<i>Polycnenum majus</i> A.Braun	Bot. Gard. Mainz <i>s.n.</i>	AY514839
Polycnemeae	<i>Polycnenum verrucosum</i> Lang	Turkey, <i>K.P.Buttler</i> 15976 (FR)	AY514838
Chenopodiaceae	<i>Allenrolfea vaginata</i> (Grisb.) Kuntze	BG Bonn 2488	AY514828
Salicornioideae			
Haloeploideae			
Chenopodiaceae	<i>Halocnenum strobilaceum</i> (Pall.) M.Bieb	Turkey, <i>H.Freitag &amp; Adigüzel</i> 28.783 (KAS, GAZI)	AY514842
Salicornioideae			
Salicornieae			
Chenopodiaceae	<i>Salsola kali</i> L.	Graswarder, Baltic Sea Coast, Germany, <i>G.Kadereit</i> 1999/211 (MJG)	AY514843
Salsoloideae			
Salsoleae			

Table. Continued.

Chenopodiaceae	<i>Suaeda cf. calceoliformis</i> (Hook.f.) Moq.	U.S.A., Texas, <i>Th.Borsch, D.Pratt &amp; K.Müller</i>	AY514841
Salsoloideae		3440 (ISC, BONN)	
Suaedeae	<i>Bienertia cycloptera</i> Bunge	Iran, Kavir protected area near Mobarakiyeh, <i>H.Akahani s.n.</i> , 16.11.2000 (Hb. Akhani, KAS)	AY514833
Achatocarpaceae	<i>Achatocarpus praecox</i> Griseb.	Argentina, <i>B. Leuenberger, Arroya-Leuenberger</i>	AY514845
		& <i>U.Eggli 4345</i> (B), Bot. Gard. Berlin (142-78-94-10)	
	<i>Phaulothamnus spinescens</i> A.Gray	Hilu et al. (2003)	AY514846
Caryophyllaceae	<i>Scleranthus perennis</i> L.	Germany, <i>Th.Borsch</i> 3389 (BONN)	AY514847
Alsinoideae			
Caryophyllaceae	<i>Illecebrum verticillatum</i> L.	Germany, <i>Th.Borsch &amp; K.Müller</i> 3541 (BONN)	AY514849
Paronychioideae			
Caryophyllaceae	<i>Silene otites</i> Sm.	Netherlands, <i>Th.Borsch</i> 3495 (BONN)	AY514848
Caryophylloideae			
Halophytaceae	<i>Halophytum ameghinoi</i> Speg.	Sukkulentensammlung Zürich <i>s.n.</i>	AY514852
Portulacaceae	<i>Anacampseros vulcanensis</i> Añon	Hilu et al. (2003)	AF542597
	<i>Portulaca oleracea</i> L.	Nigeria, Modakeke; <i>Ogundipe</i> 2080	K21087
	<i>Talinum portulacifolium</i> (Forssk.) Asch. ex Schweing	Nigeria, Odeomu; <i>Ogundipe</i> 2096	K21088
	<i>Talinum triangulare</i> L.	Nigeria, Lagos; <i>Ogundipe</i> 2000	K21089
	<i>Talinum paniculatum</i> (Jacq.) Gaertn.	Nigeria, Okuku; <i>Olatunji</i> 2687	K21090
	<i>Talinella</i> sp. indet.	<i>Röösli s.n.</i> (BONN)	AY514859
Phytolaccaceae	<i>Rivina humilis</i> L.	BG Bonn 9865, <i>Th.Borsch</i> 3542 (BONN)	AY514850
Drosophyllaceae	<i>Drosophyllum lusitanicum</i> (L.) Link	BG Bonn 09015	AY514860
Frankeniaceae	<i>Frankenia laevis</i> L.	BG Bonn <i>s.n.</i> , <i>K.Müller</i> 884 (BONN)	AY514853
Plumbaginaceae	<i>Limonium latifolium</i> (Sm.) Kuntze	BG Bonn 03678, <i>K.Müller</i> 883 (BONN)	AY514861
Simmondsiaceae	<i>Simmondsia chinensis</i> (Link) C.K.Schneid.	Bot. Gard. Bonn 310700001	AY514854

an ABI 3730 DNA Analyzer following the manufacturer (ABI) instructions.

**Sequence editing and alignment.** Electropherograms were assembled and edited with Sequencer 4.5 software. A total of 845 nucleotides were included in the matrices for phylogenetic analyses for *matK* and alignment in PAUP (Swofford, 2002).

**Phylogenetic analyses.** Cladistic analyses were performed using the parsimony algorithm of the software package PAUP\* version 4.0b10 (Swofford, 2002). Maximum parsimony search were conducted using

heuristic search methods and 1000 random addition sequence, tree bisection-reconnection (TBR) branch swapping, and MULTREES on (Keeping multiple shortest trees), but holding only 10 trees per replicate to reduce the time spent in swapping on large numbers of suboptimal trees. After the 1000 replicates, we then used the shortest trees from the first round as starting trees for a search with a tree limit of 10,000. Robustness of clades was estimated using the bootstrap (Felsenstein, 1985) involving the use of 1000 replicates with simple sequence addition and SPR branch swapping.

## Results

The matK coding and non-coding regions had an aligned length of 1387 bp of which 542 characters were excluded due to accumulation of missing data. The remaining 845 included characters and 207 of these were potentially parsimony informative. Maximum parsimony analysis resulted in 635 most parsimonious trees with Consistency Index, CI, of 0.60 and retention index, RI, of 0.78. In the matK bootstrap consensus tree (Figure), Amaranthaceae is supported by 100 bootstrap percentages (BP). Bootstrap percentages (BP) consistent with the strict consensus tree are shown above each branch, but groups with BP less than 50 are not indicated.

Clade A, Amaranthoideae, and clade B, Gomphrenoideae, form a well supported clade with BP 69. The species *Celosia*, *Gomphrena* L., and *Alternanthera* are found not to be monophyletic. *Iresine* P.Browne, *Achyranthes* L. (BP 97), *Cyathula* Lour. (BP 97), *Aerva* Forssk. (BP 88), *Ptilotus* R.br. (BP 100), and *Amaranthus* (BP 99) all form well-supported monophyletic groups. However, *Alternanthera* is sister to *Philoxerus vermicularis* Mart. ex Moq. (BP 100). The sample of *Amaranthus hybridus* are hybrids, which is presented in the result (BP 99).

## Discussion

Despite recent interest in the taxonomy and classification of the family Amaranthaceae, there is still need to study its phylogeny. In previous studies, Amaranthaceae was considered as the sister-group of Chenopodiaceae in the order Caryophyllales (Borsch et al., 2001). In this study we investigated the relationship among the genera of Amaranthaceae occurring in West Africa in relation to the other result on the Amaranthaceae and other related families like Chenopodiaceae, Portulacaceae, Caryophyllaceae, Phytolaccaceae, Halophytaceae, Drosophyllaceae, Plumbaginaceae, Frankeniaceae, and Simmondsiaceae. Our study confirmed the grouping of members of Amaranthaceae into clades Amaranthoideae and Gomphreneae. There was little difference between the report of Müller and Borsch (2005) using trnK/matK and the result we obtained using matK based on parsimony. While *Alternanthera* occurs as monophyletic with trnK/matK, it occurs as polyphyletic with only matK. The

study support the reports of Müller and Borsch (2005) that *Celosia* and *Gomphrena* are not monophyletic. The present study support previous reports (Rodman, 1994; Cueñoud et al., 2002; Kadereit et al., 2003; Pratt, 2003) and shows that the Amaranthaceae–Chenopodiaceae alliance constitutes a monophyletic group together with the Achatocarpaceae (BP 96). In this study *Amaranthus*, *Cyathula*, *Pupalia* Juss., *Ptilotus*, *Aerva*, and *Achyranthes* species are recognized as monophyletic, but *Celosia*, as currently recognized, is polyphyletic. This study supports the previous study that Chenopodiaceae should be merged with Amaranthaceae (BP 91).

Sequence information from the *matK* gene produced an angiosperm tree that is considerably more robust than any previous single gene tree. Congruence is high between our *matK* tree and those based on multiple genes representing 1, 2, or all 3 genomes (Savolainen et al., 2000a, b; Soltis et al., 2000; Zanis et al. 2002). The analyses of Zanis et al. (2002) were based on 8733 (5 genes) and over 15,000 (11 genes) nucleotides, respectively, and thus represent approximately 8 and 13 times the number of characters used here. Congruence between our *matK* phylogenies and various multigene/multigenome phylogenies of angiosperms underscores the utility of *matK* in angiosperm phylogenetics.

Furthermore, *matK* sequences provide good resolution within many angiosperm orders. Combined analyses of *matK* and other rapidly evolving DNA regions with available multigene data sets have strong potential to enhance resolution and internal support in deep level angiosperm phylogenetics and provide additional insights into angiosperm evolution (Hilu et al, 2003). In this study *Amaranthus*, *Cyathula*, *Pupalia*, *Ptilotus*, *Aerva*, and *Achyranthes* species are recognized as monophyletic, but *Celosia* as polyphyletic. This study supports the previous study that Chenopodiaceae should be merged with Amaranthaceae (BP 91)

The matK analysis resolved two clades containing the Amaranthaceae: Gomphrenoideae and Amaranthoideae. The genera from Amaranthoideae–Amaranthaceae–Amaranthinae form the basal grade of Amaranthaceae (sensu Townsend, 1993). *Bosea* had previously been identified to branch first based on five representatives and partial *matK* sequence data in a study on Caryophyllales phylogeny (Cueñoud et al., 2002).

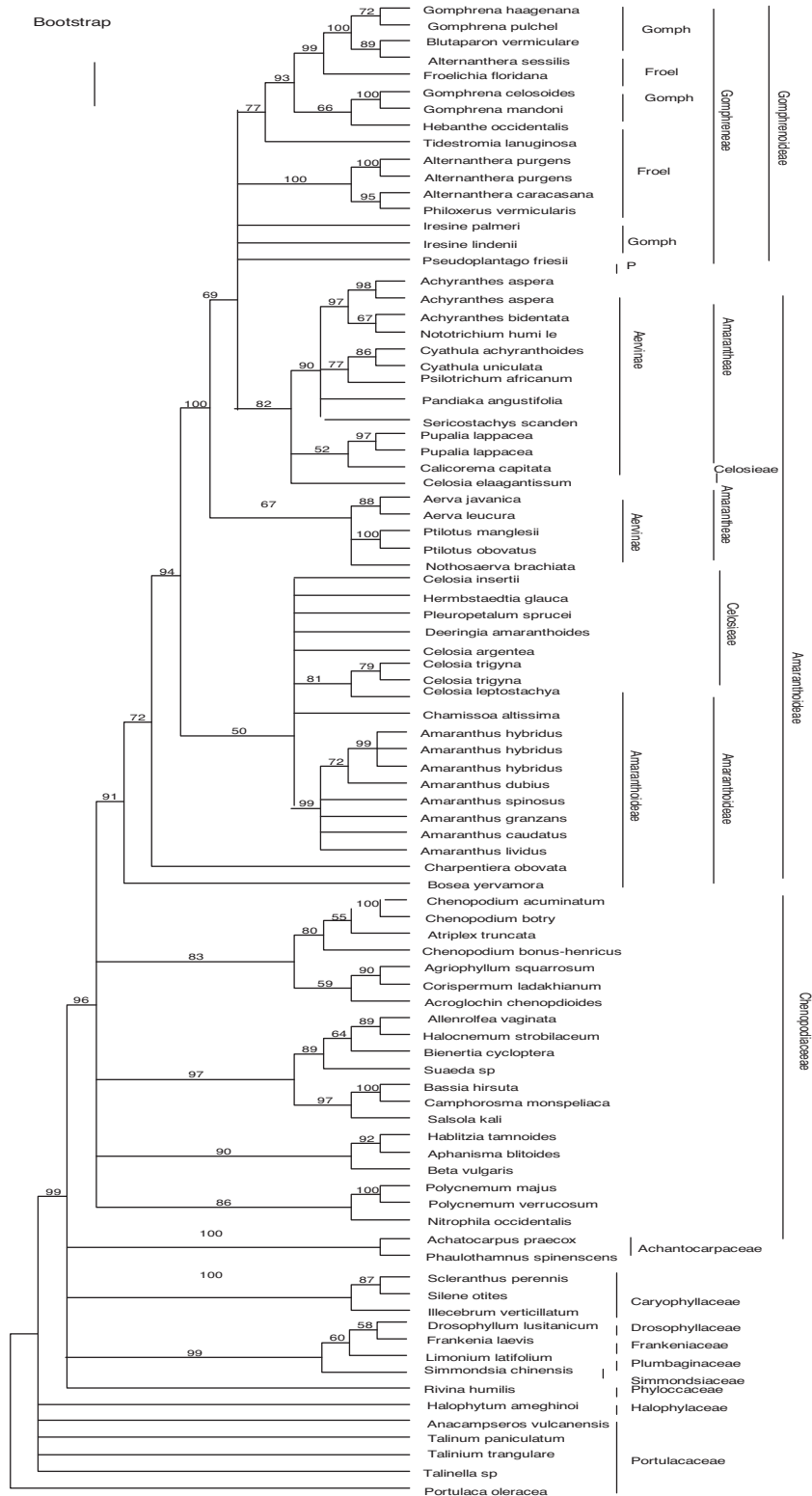


Figure. Consensus tree from the parsimony analyses. Bootstrap values are given above branches, section of the tree focusing on Amaranthaceae; the classification follows Townsend (1993). P = Pseudoplantageae; Gomph = Gomphreninae; Froel = Froelichiinae. From left to right, subtribes, tribes, and subfamilies are indicated.

A Polyphyletic Celosieae as sister to an Amaranthus–Chamissoa lineage is in agreement with this study. Subfamily Amaranthoideae is paraphyletic to the core Gomphrenoids and potentially to the traditional Gomphrenoideae depending on the first branching position and monophyly of the genus *Iresine*. The polyphyly of the tribe Amarantheae is apparent, and so is the polyphyly of Amaranthinae. Likewise the study supported the polyphyletic Aervinae (sensu Townsend 1993) comprising 2 monophyletic groups, here called Achyranthoids (*Nototrichum* Hillebr., *Achyranthes*, *Cyathula* Blume, *Pandiaka* Benth. & Hook. f.,

*Sericostachys* Gilg & Lopt. ex Lopr., *Calicorema* Hook. f., *Pupalia* Juss., *Psilotrichum* Blume) and Aervoids (*Ptilotus*, *Nothosaerva* Wight, *Aerva*).

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