Molecular Phylogeny of Atripliceae (Chenopodiaceae, Chenopodiaceae): Implications for Systematics, Biogeography, Flower and Fruit Evolution, and the Origin of C₄ Photosynthesis

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- **Premise of the study:** Atripliceae (Chenopodiaceae), including *Atriplex* (300 spp.) as the largest genus of the family, are an ecologically important group of steps and semideserts worldwide. Relationships in Atripliceae are poorly understood due to obscure and potentially convergent morphological characters.

- **Methods:** Using sequence variation of two chloroplast markers (*rbcL* gene, *apB-rbcL* spacer) and one nrDNA marker (ITS) analyzed with BEAST, we investigated the systematics and biogeography of Atripliceae. We surveyed flower morphology and fruit anatomy to study the evolution of flowers and fruits in the tribe.

- **Key results:** Female flowers with persistent foliar cover (the diagnostic character of traditional Atripliceae) evolved three times in Chenopodiaceae, in Atripliceae s.s., Axyrideae, and *Spinacia*. Atripliceae s.s. started to diversify during the Early Miocene in Eurasia, separating into the *Archatriplex* and the *Atriplex* clades. The former consists of eight species-poor, disjunct, and morphologically heterogeneous genera and is likely a relicual lineage. The *Atriplex* clade comprises the majority of species and evolved one *C₄* lineage 14.1–10.5 Ma, which diversified rapidly worldwide. The *C₄* Atriplex entered North America during the Middle/Late Miocene and spread to South America subsequently. Australia was colonized by two *C₄* lineages both arriving during the Late Miocene. One of them diversifed rapidly, giving rise to most Australian *Atriplex* species.

- **Conclusions:** Atripliceae s.s. comprise *Archatriplex*, *Atriplex*, *Exononis*, *Extriplex*, *Grayia*, *Halimione*, *Holmbergia*, *Manochlamys*, *Proatriplex*, and *Stutzia*. *Microgynoeicum* is included based on morphology but only weak molecular support. *Axyris*, *Krascheninnikovia*, and *Ceratocarpus* (here described as Axyrideae) and *Spinacia* are excluded from Atripliceae.

**Key words:** *Archatriplex*, *Atriplex*, *Cremmophyton*, *Exononis*, *Extriplex*, *Grayia*, *Halimione*, *Holmbergia*, *Manochlamys*, *Proatriplex*, and *Stutzia*. *Microgynoeicum* is included based on morphology but only weak molecular support. *Axyris*, *Krascheninnikovia*, and *Ceratocarpus* (here described as Axyrideae) and *Spinacia* are excluded from Atripliceae.

The Atripliceae traditionally belong to subf. Chenopodioidae and comprise c. 330 species in 12 genera according to the most recent comprehensive treatment by Kühn et al. (1993) (Table 1). The tribe is distributed worldwide in subtropical and temperate regions and consists of annual or perennial herbs, subshrubs or shrubs found in steppes, deserts, and coastal or ruderal habitats (Table 1). Many species can tolerate saline conditions and several species are dominant elements of arid communities worldwide, e.g., shadscaple (*Atriplex conif collo*) in the Great Basin (Sanderson et al., 1990), zampa (*A. lampa*) in northwestern Patagonia (Busso and Bonvissuto, 2009), bladder saltbush (*A. vesicaria*) in western New South Wales and northern and eastern regions of South Australia (Groves, 1994), Oldman saltbush (*A. nummularia*) in southeastern Australia (Anderson, 1967; Leigh, 1994), and *A. cana* formations in Eurasian semideserts (Korovin, 1934; Bykov, 1965).

After Meyer (1829), who included four genera in the tribe Atripliceae (Table 2), characteristic flower morphology has been considered as the key diagnostic character for this tribe. In the majority of species, all flowers are unisexual (a few species also show some bisexual flowers), and the female flowers lack a perigon but have two subtending, paired bracteoles (hereafter referred to as bracts, after Urm-König, 1981) that enclose the ovary. These bracts are either tightly attached to but free from each other or connate to various degrees along their margins. In fruit the bracts are persistent and variously modified. Often they develop structures that likely enhance fruit dispersal. These typical female flowers with a foliar cover have been viewed by most authors as a syndrome that characterizes a natural group (Standley, 1916; Ulbrich, 1934; Aellen, 1979; Kühn et al., 1993; Judd and Ferguson, 1999). The tribe has often been subdivided into two subtribes, Atriplicinae and Eurotiinae, according to differences in pubescence (e.g., Moquin-Tandon, 1849;...
Table 1. Genera of Atripliceae and Chenopodiaceae and information about species number, distribution, life form and representatives in this study.

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<tr>
<th>Taxon</th>
<th>Species number, distribution and life form</th>
<th>Number of sampled species for data set</th>
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<td>A (rbcL), B (atpB-rbcL spacer), C (ITS)</td>
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Chenopodiaceae

**Baoria** H. W. Kung & G. L. Chu

1 sp., China, annual

**Chenopodium** L.

c. 100 spp., worldwide, annual or perennial herbs, subshrubs, shrubs or small trees

**Cycloloma** Moq.

1 sp., west and central North America, annual

**Dysphania** R. Br.

c. 32 spp., worldwide, annual or short-lived perennial herbs

**Einadia** Raf.

6 spp., Australia, New Zealand, herbaceous or weakly woody perennials

**Holmbergia** Hicken

1 sp., Paraguay, Uruguay, Argentina, climbing shrub

**Micromonelepis** Ulbr.

1 sp., western North America, annual

**Monolepis** H. W. Kung & G. L. Chu

5 spp., northern Siberia, western North America, South America, annuals

**Rhagodia** R. Br.

11 spp., Australia, shrubs

**Sclerodinitis** Ulbr.

1 sp., Australia, annual

**Telosyox** Moq.*

1 sp., Central Asia, annual

Atripliceae

**Archatriplex** G. L. Chu

1 sp., China, annual

**Atriplex** L.

−300 spp., worldwide, annual or perennial herbs, subshrubs or shrubs

**Halimocnemis** Ulbr., **Morrisiella** Aellen,

perennial herbs, subshrubs or shrubs

**Pachypharynx** Aellen, **Senniella** Aellen,

**Theleophyton** (Hook. f.) Moq.

1 sp., Tibet, Himalaya, west China, annuals

**Axylis** L.

6 spp., Central Asia, Himalaya, west China, annuals

**Ceratocarpus** L.

2 spp., East Europe, West Asia, annuals

**Crennophyton** Brullo & Pavone

1 sp., Malta and Gozo, shrub

**Comexis** Fenzl ex Moq.

1 sp., South to West Africa, shrub

**Ectrílpe** E. H. Zacharias

2 spp., western North America, annual or perennial herbs

**Gravia** Hook. & Arn. (incl. *Zuckia* Standl.)

4 spp., western North America, shrubs

**Halimione** Aellen

3 spp., Europe, W Asia, annuals and shrubs

**Krascheninnikovia** Gueldenst.

8 spp.*4, Europe, Asia, North America, subshrubs or shrubs

**Manochlamys** Aellen

1 sp., South Africa, shrub

**Microgynoecium** Hook. f.

1 sp., Tibet, Sikkim, annual

**Proatriplex** (W. A. Weber) Stutz & G. L. Chu

1 sp., western North America, annual

**Spinacíla** L.

3 spp., North Africa, West Asia, annual or biennial herbs

**Stutzia** E. H. Zacharias (Endolepis)

2 spp., western North America, annual

**Torrey, nom. illeg.**


**Suckleya** A. Gray

1 sp., western North America, annual


Volkens, 1893; Ulbrich, 1934). The circumscription of Atripliceae in earlier important treatments is summarized in Table 2. Some authors, however, included the Atripliceae into a broadly circumscribed Chenopodiaceae (e.g., Blackwell, 1977; Williams and Ford-Lloyd, 1974; Wilson, 1984), the species of which generally have bisexual flowers with 4–5 small tepals that are persistent but not modified in fruits.

The core genus of the tribe is *Atriplex*, which comprises 91% of the species of the tribe in its present circumscription (Table 1; Kühn et al., 1993). Most of the genera that had been classified under Atripliceae had been in or excluded from the genus at various times by different authors. Exceptions are the Central Asian monotypic genera *Archatriplex* and *Microgynoecium*, several genera traditionally assigned to Eurotiinae (nom. illeg., now as subtrib. Axyrininae Heklau [Heklau and Röser, 2008]), viz., *Axylis, Ceratocarpus, and Krascheninnikovia, and the often overlooked genus* Cremnophyton. Endolepis Torrey (nom. illeg.), *Comexis* Fenzl, *Obione* Guernet., *Halimione* Aellen, *Blackilla* Aellen, *Haloxanthium* Ulbr., *Morrisiella* Aellen, *Neobotrydium* Moldenke, *Pachypharynx* Aellen, *Senniella* Aellen, *Theleophyton* Moq., *Manochlamys* Aellen, *Proatriplex* (W. A. Weber) Stutz & G. L. Chu, and *Gravia* Hook. and Arn. had all been considered as congeneric with *Atriplex* at some stage (Tables 1, 2). In several cases, a particular morphology of the fruiting bracts was considered an important character for delimitation at the genus level. Two examples of this are the Australian genera *Neopreissia* and *Senniella*. *Neopreissia* was separated from *Atriplex* on the basis of its thick and hard fruiting bracts with a turbinate stipe at the base (Ulbrich, 1934), and *Senniella* was excluded from *Atriplex* on the basis of its united and spongy fruiting
bracts (Aellen, 1937/1938). Because *Atriplex* is a rather polymorphic genus with fruiting bract morphology that has many transitional character states, the delimitation from its satellite genera has always been problematic [e.g., *Semiella* and *Neo-preissia* were included in *Atriplex* by Wilson (1984)]. Early in the taxonomic history of *Atriplex*, the genus *Obione* Gaertn. was separated on the basis of *O. muricata* Gaertn. (= *A. sibirica* L.; Gaertner, 1791). In contrast to other *Atriplex* species already described at that time, *Obione muricata* has crescentic and sclerified bracts in the female flowers and an embryo with the radicle pointing upward. Many authors agree with this segregation at different taxonomic levels (i.e., genus, subgenus, or section). Sukhorukov (2006) found that the position of the radicle is strongly correlated with the degree of concrescence of the two bracts. The radicle is always orientated toward the point where the crescent part passes into the free part. Furthermore, Sukhorukov (2006) found that the degree of concrescence of the two bracts can vary considerably within species.

In contrast to the cosmopolitan *Atriplex* the smaller genera have rather limited distribution areas (Table 1). *Atriplex* has greatest taxonomic diversity in Australia, North America, South America, and Eurasia. In South Africa, the genus seems to be less diverse, but in this region and also in South America, a region of high diversity, the genus has not been extensively studied so far. The evolution of *C₄* photosynthesis might have played a major role in the evolutionary success of the genus because the majority of *Atriplex* species perform *C₄* photosynthesis and *C₄* *Atriplex* are distributed worldwide. Unlike most other groups of *C₄* Chenopodiaceae, *Atriplex* has typical Kranz anatomy with a layer of bundle sheath cells surrounding each vascular bundle and radially arranged palisade cells and relatively little variation in *C₄* leaf types. This *atriplicoid* leaf type (Carolin et al., 1975) occurs in two variants, viz. the *Kranz* and the *C₄*. The latter differs mainly in *C₄* leaf types. This *atriplicoid* leaf type (Carolin et al., 1975) occurs in two variants, viz. the *Kranz* and the *C₄*. The latter differs mainly in *C₄* leaf types. This *atriplicoid* leaf type (Carolin et al., 1975) occurs in two variants, viz. the *Kranz* and the *C₄*. The latter differs mainly in *C₄* leaf types. This *atriplicoid* leaf type (Carolin et al., 1975) occurs in two variants, viz. the *Kranz* and the *C₄*. The latter differs mainly in *C₄* leaf types. This *atriplicoid* leaf type (Carolin et al., 1975) occurs in two variants, viz. the *Kranz* and the *C₄*.

### Table 2. Classifications of Atripliceae

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1 nom illeg. (incl. *Krascheninnikovia* p.p., *Halimione* p.p.); 2 nom. illeg. later corrected to *Krascheninnikovia* Gueldenst.; 3 nom. illeg. later corrected to *Stuzia* E. H. Zacharias; 4 classified in *Atripliceae* by Falkovitsh and Kovalev (2007). Note: The genera *Blackiella*, *Morrisiella* and *Pachyphyra* described by Aellen (1938b) were not accepted by Kühn et al. (1993) and Falkovitsh and Kovalev (2007) but included in *Atriplex*.
subfamily of its own (Kadereit and Freitag, 2010). Representatives of Chenopodieae and Atripliceae are somewhat intermingled in the rbcL phylogeny by Kadereit et al. (2003), and neither Atripliceae nor Chenopodieae in their present circumscription after Kühn et al. (1993) seem to represent natural lineages. The sampling and resolution in Kadereit et al. (2003), however, was only sufficient to uncover the possible polyphyly of Atripliceae and Chenopodium and the presence of a basal grade mainly of members of the Chenopodieae but insufficient to come up with a new classification of the subfamily. A detailed cladistic analysis based on 78 morphological characters (Flores Olivera and Davis, 2001) suggested that Atripliceae and also Atriplex are paraphyletic and that both subtribes of Atripliceae, Atriplicae, and Eurotini, are polyphyletic. A limited sampling within Chenopodieae and lack of additional evidence from other data sources restrained Flores Olivera and Davis (2001) from translating their results into a new classification of the tribe. Zacharias’ (2007) and Zacharias and Baldwin’s (in press) molecular analyses of Atripliceae showed that neither Atriplex, as traditionally recognized, nor the North American members of Atriplex constitute a monophyletic group.

This study aims to clarify the systematics and phylogeny of Atripliceae using a complete sampling of genera traditionally assigned to Atripliceae, extensive sampling of the large genus Atriplex and in a broad sampling of other genera of Chenopodioideae. Three molecular markers (rbcL gene, atpB-rbcL spacer, and ITS) were sequenced and analyzed with the program BEAST v1.4.8 (Drummond and Rambaut, 2007), which estimates topology and branch lengths simultaneously from the data using a Bayesian Markov chain Monte Carlo (MCMC) analysis. The results of the phylogeny by Kadereit et al. (2003), and neither Atriplex nor Chenopodium forms a well-supported clade with Atriplex placed in the tribe. Zacharias’ (2007) and Zacharias and Baldwin’s (in press) molecular analyses of Atripliceae showed that neither Atriplex, as traditionally recognized, nor the North American members of Atriplex constitute a monophyletic group.

MATERIALS AND METHODS

**Taxon sampling**—We included all genera currently recognized and traditionally assigned to Atripliceae. We used multiple samples of Atriplex to represent the biogeographical and morphological diversity of this large and widespread genus. Furthermore, we included most genera currently assigned to Chenopodieae with several samples of the large, polyphyletic genus Chenopodium.

We are in the fortunate situation that up to 50-yr-old herbarium material can be used for sequencing in Chenopodiaceae if the material was nicely dried and kept dry. Therefore, in this study, we mostly extracted DNA from herbarium material. In some cases, we used samples that were dried in silica gel, and for the Australian species of Atriplex, we used leaves preserved in saturated NaCl-CTAB solution supplemented with 200 mM sodium ascorbate (Thomson, 2002). Voucher information for all samples is listed in Appendix 1.

**DNA extraction**—For DNA extraction, the NucleoSpin plant DNA extraction kit (Macherey & Nagel, Düren, Germany) or the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) was used following the manufacturer’s specifications, or samples were extracted using the CTAB extraction protocol of Doyle and Doyle (1987).

**Amplification and sequencing**—For amplification and sequencing protocols of the rbcL gene, we refer to Kader et al. (2003) and for the atpB-rbcL spacer and ITS to Kader et al. (2005, 2006), Mavrodiev et al. (2005), and Zacharias and Baldwin (in press).

**Alignment**—All obtained chromatograms were edited in the program Sequencer version 4.8 (GeneCodes Corp., Ann Arbor, Michigan, USA) or ABI Prism Sequence Navigator software (Applied Biosystems, Foster City, California, USA), and partial sequences of the rbcL gene were assembled to a consensus sequence for each taxon. The alignment for all three markers was done manually in Sequencer 4.8 and was straightforward due to moderate sequence variation (even in the mutational hotspots of the atpB-rbcL spacer). Indels were not coded in the ITS data set but treated as missing data. In the atpB-rbcL spacer data set, only six informative indels (outside the mutational hotspots of the spacer) were coded as transversions.

**Phylogeny inference and divergence time estimation**—The three data sets were analyzed separately using BEAST (Bayesian Evolutionary Analysis by Sampling Trees v1.4.8; Drummond and Rambaut, 2007), which simultaneously estimates tree topology and divergence times. The BEAST.xml input files (available from the corresponding author upon request) were created with the Bayesian Evolutionary Analysis Utility v1.4.8 (BEAUti; implemented in BEAST; Drummond and Rambaut, 2007). For the rbcL and atpB-rbcL spacer analyses representatives of Corispermoideae were chosen as outgroup according to the results of Müller and Borsch (2005) and Kadereit and Freitag (2010). The ingroup was defined as monophyletic to set the root at the split between Chenopodioideae and Corispermoideae. The substitution model parameters were chosen based on the program MODELTEST 3.7 (Posada and Crandall, 1998). The relaxed Bayesian clock was implemented with rates for each branch drawn independently from a lognormal distribution (Drummond et al., 2006). A birth and death prior was set for branch lengths. In the rbcL and atpB-rbcL spacer analyses the root age was set at 57–55 Ma with the uniform prior. This calibration was done according to earlier branch dating of the Amaranthaceae/Chenopodiaceae alliance that included the reliable fossils available (Kadereit et al., 2003; Kadereit and Freitag, 2010). In the ITS analysis, the root age was set to 30–29 Ma with the uniform prior according to the results of the rbcL and atpB-rbcL spacer analyses. Other priors were in default settings and the Markov chain Monte Carlo (MCMC; Drummond et al., 2002) was initiated on a random starting tree. The first runs were used to examine MCMC performance, and operators were adjusted as suggested by the output analysis. The final run was performed with 1000000000 (rbcL) or 200000000 (atpB-rbcL spacer and ITS) iterations, a burn-in of 10% and a sample frequency of 1000. After assessing convergence in the program Tracer v1.4.1 (Rambaut and Drummond, 2007) as described in the BEAST manual (Drummond et al., 2007), the maximum clade credibility tree was summarized in the program TreeAnnotator v1.4.8 (Drummond and Rambaut, 2007) with a posterior probability (post. prob.) limit of 0.7 and summarizing mean node heights. The summary trees were edited in the program FigTree v1.2.2 (Rambaut, 2006).

**Morphological and anatomical studies**—Samples for morphological and anatomical studies were taken (with curatorial permission) from specimens of E, GH, K, LE, MHA, and MW or from collections made by the authors. Samples preserved in 70% ethanol from European Russia, the Mediterranean area, and Central Asia were included in the study. The fruit anatomy of numerous representatives of the tribe was studied comparatively. Anatomical sections were made by hand. Dry samples were soaked in an ethanol- glycerol-water (1:1:1) solution for several days prior to sectioning.

**RESULTS**

**rbcL**—The matrix comprised 1343 characters and 60 taxa. The three representatives of Corispermoideae were chosen as outgroup. The data set contained 229 variable sites (17.1%) of which 104 (7.7%) occurred in only one sample. The tree resulting from the analysis with BEAST is shown in Fig. 1. The rbcL analysis revealed a number of well-supported major clades within Chenopodioidae: Atripliceae, Axyriideae (trib. nov.), Chenopodiaceae I, Chenopodiaceae II, and Dysphanieae. Three species form clades on their own that cannot be assigned to any of these major clades. These are Microgynoecium tibeticum, Chenopodium urbicum, and C. coronopus. The placement of the latter two within Chenopodioidae requires further sampling because they do not group within the Chenopodioidae clade. Microgynoecium tibeticum forms a well-supported clade with...
Atripliceae and Chenopodioideae I. A sister group relationship of *M. tibeticum* and Chenopodioideae I is resolved in the *rbcL* tree, but this receives only weak support (post. prob. 0.76). Within Atripliceae a well-supported, basal split into two clades (the *Atriplex* clade and the *Archiatriplex* clade) was found. The *Archiatriplex* clade contains the representatives of *Halimione* and *Atriplex* (including *Cremnophyton*) as well-supported sister lineages. The *Archiatriplex* clade comprises *Archiatriplex*, *Exomis*, *Manochlamys*, *Extriplex* (gen. nov. ined.), *Grayia*, and *Stutzia* (gen. nov. ined.).

**atpB-rbcL spacer**—The matrix comprised 829 characters and 100 taxa. The three representatives of Corispermidaeae were chosen as outgroups. The data set contained 291 variable sites (35.1%), of which 101 (12.2%) occurred in only one sample. The tree resulting from the Bayesian analysis with BEAST (Fig. 2) is largely congruent with the *rbcL* tree (Fig. 1). Like the *rbcL* analysis, the *atpB-rbcL* spacer analysis revealed the same major clades (Atripliceae, Axyrideae, Chenopodioideae I, Chenopodioideae II, and Dysphanieae) with high statistical support. The main difference between the *rbcL* analysis and the *atpB-rbcL* spacer analysis is the position of the Dysphanieae. They are resolved as sister to Axyrideae plus *Chenopodium urbicum* in the *atpB-rbcL* spacer analysis (with moderate support) and as sister to a clade comprising Chenopodioideae I and II, Atripliceae, Microgynoecium and *Chenopodium coronopus* in the *rbcL* analysis. Again Atripliceae are most closely related to *Microgynoecium* and Chenopodioideae I. The three clades form a polytomy. Atripliceae are subdivided into *Atriplex* clade and *Archiatriplex* clade as in the *rbcL* analysis. Similar to the *rbcL* tree, the *Atriplex* clade shows a sister group relationship of *Halimione* and *Atriplex* (including *Cremnophyton*), and the *Archiatriplex* clade comprises the same genera, as well as *Holmbergia*, which was not sampled for the *rbcL* analysis. The *Archiatriplex* clade has lower support in the *atpB-rbcL* spacer tree than in the *rbcL* gene tree but has better resolution. *Archiatriplex* is sister to the remaining genera. *Exomis*, *Manochlamys*, and *Holmbergia* form a well-supported monophyletic lineage and *Extriplex*, *Stutzia* *coivilei* (comb. nov. ined.), and *Grayia* form a well-supported monophyletic lineage, but *Stutzia* is not supported. The position of *S. dioica* (comb. nov. ined.) remains unresolved.

**ITS**—The aligned ITS matrix of 122 Atripliceae and *Chenopodium frutescens* (a representative of Chenopodioideae I) as outgroup, comprised 613 base positions. Of these, 234 (38.2%) were polymorphic, and 70 (11.4%) polymorphisms occurred in only one sample. The tree resulting from the Bayesian analysis with BEAST is shown in Fig. 3. As in the cp data analyses, the same two major clades of Atripliceae are resolved, the *Archiatriplex* clade and the *Atriplex* clade. Within the *Archiatriplex* clade, *Microgynoecium* is sister to *Archiatriplex* plus the remaining genera. This sister group relationship receives only low statistical support (post. prob. 0.82). *Archiatriplex* is sister to the remaining genera. Within the latter clade, only the North American *C. Atripliceae* (*Proatriplex*, *Grayia* [including *Zuckia*], *Extriplex*, and *Stutzia*) receive moderate statistical support. The relationships of *Exomis*, *Manochlamys*, *Holmbergia*, and the North American *C. Atripliceae* remain unclear (Fig. 3). Within the *Atriplex* clade, *Halimione* is sister to *Atriplex* (including *Cremnophyton*). Within *Halimione*, the annual *H. pedunculata* is sister to the perennial *H. portulacoides* and *H. verrucifera*. *Atriplex cana* and *Cremnophyton* seem to form a basal grade within *Atriplex*. There are a number of well-supported lineages within *Atriplex* that will be described in detail in the discussion. Among these are the *C. A* species of the genus that form a well-supported monophyletic lineage (post. prob. 0.99; Fig. 3).

The molecular clock estimates for the major splits in Atripliceae gained from the three data sets are summarized in Table 3.

**Morphological and anatomical studies of flowers and fruits**—The results of our survey of flower morphology and fruit anatomy are presented in Table 4. They are mostly based on our own observations, but we also considered the relevant literature as indicated in Table 4.

**DISCUSSION**

A new circumscription of Atripliceae—The molecular data presented here show congruent results concerning the phylogenetic relationships of genera traditionally assigned to Atripliceae (Figs. 1–3). The following 10 genera are part of a well-supported Atripliceae clade: *Archiatriplex*, *Atriplex* (including *Blackiella*, *Cremnophyton*, *Haloxanthium*, *Neopreissia*, *Obione*, *Pachypharynx*, *Senniella*, *Thelephryton*), *Exomis*, *Extriplex*, *Grayia* (including *Zuckia*), *Halimione*, *Holmbergia*, *Manochlamys*, *Proatriplex*, and *Stutzia*. The assignment of the Himalayan genus *Microgynoecium* to Atripliceae is not unambiguously supported by molecular data. Later, we make a case for the inclusion of *Microgynoecium* in Atripliceae on the basis of morphological data and weak support from the *atpB-rbcL* spacer and ITS (Figs. 2, 3). Three of the four genera traditionally classified as subtribe Eurotiinae, viz. *Axyris*, *Ceratocarpus*, and *Krascheninnikovia* (Table 2), and also *Spinacia* and *Suckleya* clearly do not belong to Atripliceae as circumscribed here. *Spinacia* seems to be closely related to the American and Siberian genus *Monolepis*, the Australian genus *Sclerobulbinia*, and to representatives of Chenopodioideae subgenus *Blitum*. *Suckleya* is closely related to *Dysphania*, *Teloxys*, and *Cycloloma*. The three genera of subtribe Axyridinae (former Eurotiinae nom. illeg.) form a well-supported clade that is not closely related to any other clade of the Chenopodioideae but is part of a basal grade. Finally, *Holmbergia* is the only genus that was previously classified in Chenopodioideae but that clearly belongs to Atripliceae.

Within Atripliceae, the molecular analyses revealed that the 10 genera listed above fall into two clades, here named the *Archiatriplex* clade and the *Atriplex* clade. These results corroborate the findings of Zacharias (2007) and Zacharias and Baldwin (in press), who showed this basal split in the tribe (with sampling focused on the American genera). The *Archiatriplex* clade consists of a large number of small genera that show great morphological diversity and a widely disjunct distribution. These are *Archiatriplex*, *Exomis*, *Extriplex*, *Grayia* (including *Zuckia*),

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Fig. 1. Phylogeny of Chenopodioideae based on *rbcL* sequence data and a BEAST analysis. Posterior probabilities higher than 0.7 are indicated above branches, estimates of node ages are given behind the respective node, and the corresponding bars represent 95% confidence intervals. For the position of fossils used for calibration, see text.
Hohnbergia, Manochlamys, Proatriplex, and Stutzia (Figs. 1–3; Tables 1, 2). The Atriplex clade contains the majority of species but only two genera, Atriplex and Halimione.

Excluded genera and their position within Chenopodioidae—Spinacia—Spinacia is a small genus of annual or biennial herbs distributed in the Irano-Turanian floristic region. Spinacia oleracea is widely known as an important vegetable cultivated nearly worldwide. The placement of the genus within Atriplexia has never been doubted because it has unisexual flowers and perianth-less female flowers with two rounded, connate bracts. These bracts are stout in Spinacia, often have 2–6 teeth along their margin and have been interpreted as sepals by some authors (Eichler, 1878; Cohn, 1914; Sherry et al., 1993; Sather et al., 2005). A closer look at the anatomy of this bract or perianth cover revealed five zones (Fig. 4A): an outer epidermis, a chlorenchyma, a sclerenchymatic parenchyma oriented perpendicular to its long axis, 1–2 crystalliferous layer(s) with 1–3 rhombic monocrystals and an inner epidermis. This bract anatomy of Spinacia is more complex than the bract anatomy found in species of Atriplex so far. The bracts of Atriplex do not possess crystalliferous layer(s), the crystals are dispersed in the parenchyma and occur only as druses. Also, there is no continuous sclerenchymatic parenchyma in the bracts of Atriplex species studied so far, except for A. fera. However, in A. fera the mechanical tissue consisting of many-layered true sclereids is orientated differently indicating the parallel evolution of this feature (Fig. 4B). Interestingly, A. fera had been included in Spinacia as S. fera L. (Linné, 1764) or S. divaricata Turcz. ex Moq. [nomen] (Moquin-Tandon, 1849).

A complex bract anatomy may be one character that separates Spinacia from Atriplexia, but the bract anatomy of some Atriplexia groups is not known yet. Further morphological characters that support the exclusion of Spinacia from Atriplexia are (1) absence of bladder hairs, (2) 4–5 styles vs. 2 (or more rarely 3) as in other Atriplexia, (3) a deviating chromosome number of x = 6 instead of x = 9 (Ellis and Janick, 1960; Fedorov, 1969; Turner, 1994), (4) a differing branching pattern in the reproductive part (Urmí-König, 1981), and (5) a distinct pollen morphology (Flores Olvera et al., 2006).

In the molecular trees (Figs. 1, 2), Spinacia is nested among representatives of Monolepis, Scleroblitum, and Chenopodium subgenus Blitum. This is a rather heterogeneous clade within Chenopodioidae with a number of intercontinental disjuncts. Neither Chenopodium subgenus Blitum nor Monolepis have been sufficiently sampled in this study. Therefore, the resolution of the detailed phylogenetic relationships of Spinacia has to await further studies.

Suckleya—Suckleya is a monotypic genus with one succulent annual species that is distributed in western North America from southern Alberta to northwestern Texas. Chu et al. (1991) classified the genus within the monogenic subtribe Suckleyi- nae of Chenopodioidae based on its character combination of unisexual flowers, female flowers with four tepals, and superior radicles. This new subtribe of Chenopodioidae was not accepted in subsequent treatments (e.g., Kühn et al., 1993). The placement of the genus in Atriplexia, however, has also been questioned in a recent palynological analysis in which this genus differed from all other genera of Atriplexia in a remarkably small pollen diameter of 13–18 µm and a comparatively small number of pores of 28–36 (Flores Olvera et al., 2006). In their molecular study of North American Atriplexia Zacharias and Baldwin (in press) initially included a sample of Suckleya suckleyana but excluded it from the data set because of extensive sequence divergence. They suspected that the genus is more closely related to Chenopodium.

In our molecular analysis, Suckleya is closely related to Dysphania, Cyclocloma, and Teloxys and therefore the exclusion from Atriplexia is clearly confirmed (Fig. 1). Dysphania and Teloxys (which is sometimes included in Dysphania) are characterized by the presence of typical glandular trichomes (Mosyakin and Clemants, 2002). Suckleya, however, has inflated unicellular trichomes (Chu et al., 1991), and Cyclocloma has uniseriate trichomes (E. Zacharias and A. Sukhorukov, personal observation).

Axyridinae(Axyris, Ceratocarpus, and Krascheninnikovia)—Heklau and Röser (2008) conducted a morphological, morphometric, and molecular analysis of subtribe Axyridinae and found in a tree based on ITS sequence data that Axyris, Ceratocarpus and Krascheninnikovia form a well-supported clade in which Krascheninnikovia is sister to Axyris plus Ceratocarpus. This finding is only partly supported by our rbcL and atpB-rbcL data. Our molecular data support the monophyly of Axyris, Ceratocarpus and Krascheninnikovia, albeit Axyris is sister to Ceratocarpus plus Krascheninnikovia (Fig. 1). The Axyridinae are characterized by stellate hairs, which are missing in Grayia, a genus traditionally also classified in the genus Dysphania (Gray). This study shows that Grayia is part of the Archiatrithaceae clade and therefore remains within Atriplexia and that the Axyridinae are part of the basal grade of Chenopodioidae and not of Atriplexia. The exact placement of Axyridinae within Chenopodioidae has to be clarified in further molecular studies with an improved sampling of the large and obviously polyphyletic genus Chenopodium. Because of its isolated position within Chenopodioidae and because of its unique characters (stellate indumentum, absence of the “stalks” in the outer cell walls of the tests in contrast to Chenopodioidae and Atriplexia), this group is raised to tribal level, Axyridiaceae tribus nov. (see taxonomic section).

The split between Axyris and Ceratocarpus plus Krascheninnikovia is supported by flower and fruit morphology. The female flowers of Ceratocarpus and Krascheninnikovia lack a perianth, while in Axyris female flowers have a perianth that consists of three tepals. These tepals remain unchanged in the fruiting stage and do not develop into dispersal-enhancing structures. In Axyris, the pericarp adheres to the seed coat, and heterocarpy and heterospermy has been observed (Sukhorukov, 2005). Fruits on the same individual differ in the number of pericarp layers and the presence of sclereids, and the seeds differ

Fig. 2. Phylogeny of Chenopodioidae based on atpB-rbcL spacer sequence data and a BEAST analysis. Posterior probabilities higher than 0.7 are indicated above branches, estimates of node ages are given behind the respective node, and the corresponding bars represent 95% confidence intervals. For the position of fossils used for calibration, see text.
in thickness of the testa. In *Ceratocarpus* and *Krascheninnikovia* the female flowers are enclosed by two bracts that persist in the fruiting stage and fall off together with the fruit. Both genera have only one fruit/seed type, the pericarp is not adherent, and the testa is always thin and consists of two simple layers (Takhtajan, 1934; Butnik, 1981, 1991; A. Sukhorukov, unpublished results).

Our study clearly indicates that the typical female flower of Atripliceae with two persistent bracts arose two times independently in Chenopodioidae: (1) in the ancestor of Atripliceae (as defined here) and (2) in the common ancestor of *Ceratocarpus* and *Krascheninnikovia* (Fig. 1).

**Relationships within Atripliceae**—*Microgynoecium*—The relationships of *Microgynoecium* remained unclear in our molecular analyses. The monotypic genus is either resolved as sister to Chenopodioideae I (with low support; Fig. 1), as sister to Atripliceae (without support; Fig. 2), or within Atripliceae, as sister to the *Archiatriplex* clade (with low support; Fig. 3). The morphological data, however, point to a closer affinity to Atripliceae than to Chenopodioideae I and therefore support the topology found with the *atpB-rbcL* spacer and with ITS. Like the majority of Atripliceae, *Microgynoecium* has unisexual flowers with female flowers having two bracts and no perianth (Table 4). The bracts of the female flowers enclose the ovary in the fruiting stage and are slightly enlarged (A. Sukhorukov, personal observation). The female flowers are clustered in the axil of a subtending bract. This latter character is probably a plesiomorphic character state in the *Archiatriplex* clade (see below) and further supports the affinity of *Microgynoecium* to Atripliceae.

*Microgynoecium* grows in alpine meadows and disturbed sites in the Himalayan and Pamir mountains (Pratov, 1972; Zhu et al., 2003). Its unique fruit morphology with an irregular detachment of the pericarp from the seed coat is also known from some species of subf. Corispermoidae (Sukhorukov, 2007b). In Chenopodioidae, such detachment is found in *Avyris* and some species of *Chenopodium*; however, it is restricted to the upper part of the fruit (Sukhorukov, 2005; A. Sukhorukov, unpublished results).

**Archiatriplex clade**—The *Archiatriplex* clade consists of *Archiatriplex*, *Exomis*, *Extriplex*, *Grayia* (including *Zuckia*), *Holmbergia*, *Manochlamys*, *Proatriplex*, and *Stutzia* (Figs. 1–3; compare also the findings in Zacharias and Baldwin, in press). All these genera are either monotypic (*Archiatriplex*, *Exomis*, *Holmbergia*, *Manochlamys*, *Proatriplex*) or consist of a few species only (Table 1). The *Archiatriplex* clade shows a disjunct distribution in Central China, North and South America, and South Africa. Most genera have relatively long branches, but their relationship remains partly unsettled due to short basal branches. This disjunctly distributed, species-poor clade, that consists of relatively old and morphologically heterogeneous lineages, probably represents the remnants of a formerly widely distributed and species-rich lineage (see below). Recovering the true phylogenetic relationships within the *Archiatriplex* clade is most likely hampered by rampant extinction in this lineage.

**Holmbergia**—Our molecular analyses showed that *Holmbergia* traditionally assigned to Chenopodioideae (Table 2) belongs to Atripliceae. This placement supports previous molecular findings that were based on a smaller sample (Kadereit et al., 2003; Zacharias and Baldwin, in press). The assignment to Chenopodioidae was based on the morphology of the female flowers in *Holmbergia*. These are ebracteate and have a perianth that consists of five tepals, which do not take part in fruit development. Ulbrich (1934) suspected *Holmbergia* to be a species of *Rhagodia* introduced to South America from Australia, and Scott (1978) placed *Holmbergia* in Rhagodinae A. J. Scott, together with *Rhagodia* and *Einaidia* from Australia and New Zealand. This new subtribe of Chenopodioidae was based on the succulent pericarp and predominantly unisexual flowers. The anatomy of the pericarp is, however, different in *Rhagodia* and *Holmbergia*. While in *Rhagodia*, the pericarp has approximately three layers and is truly fleshy and spongy, the 3–5(6)-layered, undifferentiated pericarp of *Holmbergia* is dry, and the reddish appearance of the fruit is caused by the hard, dark red-brown testa (Fig. 4C). Berry-like fruits evolved several times in Chenopodioidae in rather isolated positions. This character state does not seem to be phylogenetically informative in the family. The few berry-fruited taxa of Camphorosmeae, for example, represent three independent lineages (Cabrera et al., 2009). The monophyly of *Rhagodia* and/or *Einaidia* and the relationships of these genera to Australian *Chenopodium* have not been resolved with molecular data yet. Our analyses, which included only a limited sample of these groups, indicate that *Rhagodia* and *Einaidia* might be nested among Australian species of *Chenopodium* (Figs. 1, 2).

The closest relative of *Holmbergia* within the *Archiatriplex* clade remains unresolved. The genus is part of a polytomy consisting of *Exomis*, *Manochlamys*, and the North American representatives of this clade in the ITS analysis (Fig. 3) and sister to *Exomis* and *Manochlamys* in the *atpB-rbcL* analysis (Fig. 2). Morphologically, *Holmbergia* does not show particular similarities to any of the other genera of the *Archiatriplex* clade.

**Exomis and Manochlamys**—The two monotypic South African genera *Exomis* and *Manochlamys* have been treated under *Exomis* by Kühn et al. (1993). While *Exomis microphylla* is
found as a weed in gardens and hedges, *Manochlamys* grows on rocky or sandy hillsides, sand dunes, and along roads. The sister group relationship of the two subshrubby genera is supported by the *rbcL* data (Fig. 1) with moderate statistical support. *Manochlamys* and *Exomis* differ morphologically in the following three main characters (compare Aellen, 1939b; Aellen, 1967): (1) The female flowers of *Manochlamys* consist of two unequal fleshy bracts that later cover the fruit and a 2-4-lobed, membranous, small perianth, while the female flowers of *Exomis* are aggregated (1 to several) in the axils of two narrow-lanceolate bracts. The female flowers of *Exomis* either lack a perianth or have a strongly reduced, thin, translucent ring around the ovary or two small, thin, and translucent lobes opposite the bracts. (2) The inflorescence of *Manochlamys* is not leafy, while that of *Exomis* is leafy throughout. (3) The tepals of the male and bisexual flowers are connate in *Manochlamys*, while they are only basally united in *Exomis*. The two genera also differ in fruit anatomy. *Manochlamys* has a thick pericarp consisting of several layers of inflated, isodiametrical cells (Fig. 4D), while *Exomis* has a thin pericarp (also consisting of isodiametrical cells) with papillae-shaped bladder hairs. In both genera, the cells of the exotesta have numerous drops containing the cell wall fortifications (Fig. 4E). We consider *Manochlamys* and *Exomis* as morphologically and molecularly distinct and prefer—as had been suggested by Aellen (1939b)—to recognize both genera.

**Proatriplex**—*Proatriplex* is a western North American annual with clusters of female flowers enclosed by a pair of subtending bracts, female flowers with a perianth, and radicles pointing downward. Weber (1950) initially included *P. pleiantha* within *Atriplex* and hypothesized that it was a primitive member of the genus, but Stutz and Chu (in Stutz et al., 1990) emphasized its distinctness when they treated Weber’s subgenus, *Proatriplex*, at genus rank. This view was also supported by Judd and Baldwin (1999). Zacharias and Baldwin (in press) showed it to be more closely related to the other North American genera of Atripliceae and *Holmbergia* than to *Atriplex*. In our study, the ITS data resolved *Proatriplex* as sister to the other North American members of the *Archiatrlepideae* clade with moderate support.

*Proatriplex* has a distinct fruit anatomy (Fig. 4E). The pericarp is single-layered, compressed, and adheres tightly to the testa. The outer cell walls of the pericarp are thick. In cross section, the outline of the testa appears wavy, and its thickness varies between 20 and 35 µm. Such a wavy outline of the testa is uncommon in Chenopodioideae. It has been observed in *Chenopodium hybridum* and *C. simplex* in section Grossefoveata (A. Sukhorukov, unpublished data) but not in other members of Atripliceae. The cells of the exotesta have characteristic oblique cell wall fortifications (Fig. 4E).

**Stutzia**—Zacharias and Baldwin (in press) plan to replace the name *Endolepis* Torr. (1860), which is a later homonym of *Endolepis* Schiedl. (1846), with *Stutzia* ined. These two species of *Stutzia* are western North American annuals with perianths in the female flowers, radicles pointing upward, and fused bracts; no other species in the *Archiatrlepideae* clade shares this combination of characters. However, the relationships of *S. covillei* and *S. dioica* to each other and to other members of the *Archiatrlepideae* clade are not well supported by the molecular data. In most analyses, they are part of the North American lineage within the *Archiatrlepideae* clade (with the exception of *S. dioica* in the *atpB-rbcL* analysis, which shows it as sister to the *Holmbergia*, *Exomis*, and *Manochlamys* clade but with no statistical support).

Zacharias and Baldwin (in press) showed both species to be part of a grade with other sampled members having female flowers with perianths (*Holmbergia* and *Proatriplex*), although their relationships to each other were not clear. See Zacharias and Baldwin (in press) for further discussion of the systematics of this genus.

Morphologically, *Stutzia* is very similar to *Atriplex*. One difference is the presence of a small perianth in the female flowers, which is rare in *Atriplex*. The fruit anatomy is simple (as in most species of *Atriplex*) with a thin, one-layered pericarp and a thin testa (3–5 µm).

**Extriplex**—The two species of *Extriplex* have radicles that point laterally, 4-lobed perianths in the male flowers, perianthless female flowers, and are endemic to the California Floristic Province. The relationships of *E. joaquinana* (comb. nov. ined.) and *E. californica* (comb. nov. ined.) to each other and to other members of the *Archiatrlepideae* clade are not consistent across the molecular data. The genus has high support in the *atpB-rbcL* spacer tree (Fig. 2) but no support in the ITS tree (Fig. 3). The *rbcL* analysis places *E. joaquinana* as sister to *Stutzia covillei* with moderate support and *E. californica* sister to *Grayia* with low support (Fig. 1). The combined nrDNA and cpDNA analysis of Zacharias and Baldwin (in press) showed that *Extriplex* is a well-supported clade. See Zacharias and Baldwin (in press) for discussion.

*Grayia* (including *Zuckia*)—The two sampled species of *Grayia* are shrubs from western North America with female flowers without perianth and radicles that point downward. As shown in Zacharias and Baldwin (in press), the molecular data resolve *Grayia* (including *Zuckia*) with high support. The sister relationship of *Grayia* is not clear. *Grayia* is sister to *Extriplex californica* with moderate support in the *rbcL* tree (Fig. 1). The *atpB-rbcL* spacer tree does not show the sister group relationships of *Grayia* with support (Fig. 2). The ITS tree shows *Grayia* as sister to *Extriplex* plus *S. dioica* with no support (Fig. 3). Zacharias and Baldwin (in press) showed *Grayia* as sister to *Extriplex* with low support. See Zacharias and Baldwin (in press) for further discussion.

**Atriplex clade**—*Halimione*—The distinctness of the three species today either classified as *Halimione* (Aellen, 1938a) or as *Atriplex* sect. *Halimus* and *Pedicellatae*, which is here supported by molecular evidence, had already been recognized 190 years ago. At that time, the oblong, entire, and greyish-coated leaves and the totally concrescent bracts were regarded as diagnostic characters separating *A. portulacoides* [= *Halimus portulacoides* (L.) Dumort.], *A. pedunculata* [= *H. pedunculata* (L.) Wallr.], and *A. verrucifera* M. Bieb. [= *Halimus verruciferus* (M. Bieb.) Claus] from other species of *Atriplex* (Gray, 1821; Wallroth, 1822; Meyer, 1833; Claus, 1851). Since then, a large number of *Atriplex* species have been described, and some of these show these same character states. Therefore, these characters cannot be regarded as unique in *Halimione* any longer (Sukhorukov, 2006). However, Aellen (1938a), who combined the three species under the name *Halimione*, found an excellent additional feature that separates *Halimione* from all other species of *Atriplex*. He discovered that the pericarp is tightly adherent to the adaxial side of the bracts. This diagnostic character for *Halimione* was validated by later studies, but—maybe for reasons of difficult assessment in the field—rarely used in floristic treatments (e.g., Grossheim, 1949; Skripnik, 1987; Nikitin and Geldikhanov, 1988; Romo, 2002).
In our own anatomical studies, we discovered a second synapomorphous character for *Halimione*. The seed coat of *Halimione* is thin and membranous and consists of two layers of simple, isodiametrical, translucent cells. In contrast, the seed coat of *Atriplex* consists of a hard, conspicuous testa that contains tannin and is therefore brownish and a 2–3-layered endodermis (Fig. 4 F, G). The simple seedcoat anatomy of *Halimione* is very unusual among Chenopodiaceae (compare Netolitzky, 1926; Butnik, 1981) and certainly supports its separated position within the *Atriplex* clade.

The molecular data congruently resolve *Halimione* as sister to *Atriplex* with high statistical support (Figs. 1–3). Because *Halimione* is also morphologically well defined, we recognize *Halimione* as a genus separate from *Atriplex*. The subdivision of *Halimione* into two sections as has been proposed by Sukhorukov (2006), albeit within *Atriplex* i.e., *Atriplex* sect. *Halimus* (S. F. Gray) Sukhor. (*A. portulacoides* L., *A. verrucifera* M. Bieb.) and *Atriplex* sect. *Pedicellatae* Sukhor. (*A. pedunculata* L.), is supported by the ITS tree (Fig. 3).

Former “satellite genera” of *Atriplex*—The molecular analyses presented here revealed that *Obione, Blackiella, Haloxanthium, Neopressia, Senniella,* and *Theleophyton* are best considered as congeneric with *Atriplex*. Figure 3 shows the position of these genera within *Atriplex*. For all these genera, a placement within *Atriplex* has been suggested before (Kühn et al., 1993; Table 2). *Morrisiella* (Aellen, 1937/1938; = *Atriplex* *morrissii* R. Anderson) was not available for this study, and *Pachypharynx* Aellen was described on the basis of gall-infected specimens (Wilson, 1984).

*Obione* is the largest genus segregated from *Atriplex* with c. 100 species worldwide. Its separation from *Atriplex* has been controversial (for a summary, see Flores Olvera, 2003) and is handled differently in flora treatments. However, mostly it had been recognized as a taxon either at subgenus or section level, and only a few authors have accepted genus rank (e.g., Moquin-Tandon, 1840, 1849; Ulbrich, 1934; Aellen, 1938b). *Obione* was described by Gaertner (1791) and comprised those species that show an inverted position of the radicle of the embryo (pointing upward), whereas in *Atriplex* the radicle points downward or laterally. Species classified within *Obione* (or *Atriplex* sect. *Obione*) do not form a monophyletic group in the ITS tree (Fig. 3), which clearly documents that *Obione* or sect. *Obione* does not represent a natural group and that the position of the radicle is highly variable within *Atriplex*, just like the connation of the bracts. As pointed out already, these two characters seem to be functionally connected (compare Sukhorukov, 2006).

The monotypic genus *Theleophyton* was described by Moquin-Tandon (1849) on the basis of *Atriplex* sect. *Theleophyton* Hook. f. The generic status was not accepted in recent treatments (e.g., Kühn et al., 1993; Wilson, 1984), but the separation on section level was accepted (Wilson, 1984). *Atriplex* (*Theleophyton*) *billardierei* shows the following combination of unique morphological characters that distinguishes it from all other species within the genus: (1) young leaves with watery bladder hairs, (2) male flowers with five tepals that are connate for at least half of their length, (3) connate bracts of female flowers forming an urceolate structure in fruit, and (4) a seed that is initially orientated parallel to the bracts but which twists during ripening so that it is at right angles to the bracts at maturity. The molecular data (Fig. 3) reveal that *A. (Theleophyton) billardierei* is nested among Australian *Atriplex* species of sect. *Semibaccates*. Its sister group was not resolved because of low resolution in terminal branches of the Australian clade. *Atriplex billardierei* is a prostrate herb that grows on sandy beaches just above high tide level on the coasts of Victoria, Tasmania, and New Zealand.

The three Australian genera *Senniella, Blackiella,* and *Morrisiella* were all described and classified within a new subtribe Spongicarpinae by Aellen (1937/1938). The diagnostic character of the subtribe is the connate, spongy bracts of the female flowers. All three genera were not accepted in subsequent treatments of the group (e.g., Wilson, 1984; Kühn et al., 1993). Here we sampled two representatives of *Senniella* (*S. spongiosa* var. *spongiosa* and *S. spongiosa* var. *holocarpa*) and one of *Blackiella* (*B. conduplicata*), which all resolve in different positions nested within the *C*4 clade of *Atriplex* (Fig. 3), indicating that spongy fruiting bracts evolved several times independently within the *C*4 clade of *Atriplex*.

The Australian genera *Haloxanthium* and *Neopressia* were described by Ulbrich (1934). In *Haloxanthium*, Ulbrich included two species, *H. quadralvatum* (Diels) Ulbrich and *H. fissivalve* (F. Muell.) Ulbrich, that both differ from *Atriplex* in having deeply divided and elaborately lobed bracts with basal teeth that become hard during ripening (Ulbrich, 1934; fig. 195 U–W). According to Ulbrich (1934), the entire glomerulate female inflorescence is dispersed like a burweed. Our molecular results show *H. fissivalve* (= *Atriplex fissivalvis*) clearly nested within the large clade of Australian *Atriplex* species (Fig. 3). Closely related species cannot be inferred because of the low resolution in this part of the tree. In *Neopressia*, Ulbrich included two species, *N. isatidea* (Moq.) Ulbrich and *N. cinerea* (Poir.) Ulbrich, that both have sessile bracts with a thick turbinate stipe. Both species were included in our ITS analysis. They are nested within *Atriplex* and appear closely related to each other. Together with *A. nummularia* and *A. rhagodioides* they form a second Australian lineage within *Atriplex*. Neither *Haloxanthium* nor *Neopressia* were accepted by Wilson (1984) and Kühn et al. (1993).

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**Table 3.** Results of the divergence time estimates (in Ma) calculated with program BEAST v1.4.8 (numbers given in brackets represent 95% confidence intervals)

<table>
<thead>
<tr>
<th>Node Description</th>
<th>rbcL gene</th>
<th>atpB-rbcL spacer</th>
<th>ITS</th>
<th>Geological Epoch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem age of Atripliceae excl. <em>Microgynoeicum</em></td>
<td>29.7 (37.9–21.2)</td>
<td>28.22 (38.3–18.9)</td>
<td>Crown age of Atripliceae incl. <em>Microgynoeicum</em>, set to 29 Ma</td>
<td>Late Oligocene</td>
</tr>
<tr>
<td>Crown group age of Atripliceae excl. <em>Microgynoeicum</em> (= stem age of Archiatriplex clade and Atriplex clade)</td>
<td>23.4 (31.0–16.4)</td>
<td>23.9 (32.7–14.9)</td>
<td>24.8 (29.4–19.2)</td>
<td>Early to Middle Miocene</td>
</tr>
<tr>
<td>Crown group age of the <em>Atriplex</em> clade (= stem age of <em>Atriplex</em> and <em>Halimione</em>)</td>
<td>19.7 (26.7–13.1)</td>
<td>17.8 (25.5–10.7)</td>
<td>20.4 (28.9–11.1)</td>
<td>Early to Middle Miocene</td>
</tr>
<tr>
<td>Crown group age of the <em>Archiatriplex</em> clade</td>
<td>16.8 (23.9–9.5)</td>
<td>20.4 (28.9–11.1)</td>
<td>24.9 (29.0–19.6)</td>
<td>Early to Middle Miocene</td>
</tr>
<tr>
<td>Crown group age of <em>Atriplex</em></td>
<td>12.9 (18.5–8.1)</td>
<td>12.6 (18.2–7.3)</td>
<td>19.3 (24.6–14.0)</td>
<td>Middle Miocene</td>
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<tr>
<td>Minimum age of <em>C</em>4 photosynthesis in <em>Atriplex</em></td>
<td>10.9 (15.3–6.2)</td>
<td>10.5 (15.2–6.0)</td>
<td>14.1 (18.3–9.4)</td>
<td>Middle to Late Miocene</td>
</tr>
</tbody>
</table>
Table 4. Survey of flower and fruit characters in genera of Chenopodioidae; data are mostly based on our own observations and those of Kühn et al. (1993), Ulbrich, (1934), Welsh (2003), and Wilson (1984).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Plant sex</th>
<th>Sex</th>
<th>Bisexual</th>
<th>Staminate</th>
<th>Perianth in fruit</th>
<th>Bract accrescence</th>
<th>Bract connation</th>
<th>Pericarp adherence</th>
<th>Seed position</th>
<th>Fruit inverted (radicle orientation)</th>
<th>Miscellaneous notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atriplicae</td>
<td></td>
<td>unisexual</td>
<td>n/a</td>
<td>ebracteate; 5 tepals; 5 stamens</td>
<td>unchanged in fruit</td>
<td>accrescent</td>
<td>n/a</td>
<td>free or fused</td>
<td>vertical</td>
<td>inferior radicle</td>
<td>very distinct in bract’s form, its hardness or connrescent stage</td>
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<tr>
<td>Archiatrithex</td>
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<td>G.L. Chu</td>
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<td>Atriplex L. a)</td>
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<td>Cremnophytion</td>
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<td>Brullo &amp; Pavone</td>
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<td>Exomis Fenzl ex Moq.</td>
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<td>(few bissexual flws)</td>
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<td>Extriplex E.H. Zacharias</td>
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<td>Manochlamys Aellen</td>
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<td>polygamo-monoecious</td>
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<td>Taxon</td>
<td>Plant sex</td>
<td>Sex</td>
<td>Bisexual</td>
<td>Staminate</td>
<td>Perianth in fruit</td>
<td>Pericarp adherence</td>
<td>Seed position</td>
<td>Fruit inverted (radicle orientation)</td>
<td>Miscellaneous notes</td>
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<tr>
<td><em>Proatriplex</em></td>
<td>monoecious</td>
<td>unisexual</td>
<td>n/a</td>
<td>ebracteate; 5 tepals; 5 stamens</td>
<td>2–6 bract pair; (5 or 4) hyaline tepals</td>
<td>changed in fruit</td>
<td>adherent to the seed coat</td>
<td>vertical</td>
<td>pointing downward</td>
<td>pericarp outgrowth in basal fruit part; stalarctides of outer periclinal wall of testa oblique; big air cavities in seed</td>
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<tr>
<td>(W.A. Weber) Stutz &amp; G. L. Chu)</td>
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<td><em>Stutzia</em> E.H. Zacharias</td>
<td>monoecious</td>
<td>unisexual</td>
<td>n/a</td>
<td>ebracteate; (1–)3(–5) hyaline tepals; 3–4 lobed</td>
<td>1/bract pair; 3 tepals</td>
<td>unchanged in fruit</td>
<td>not adherent</td>
<td>vertical</td>
<td>pointing upward</td>
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<tr>
<td><em>Axyridae</em> Axyris L.</td>
<td>monoecious</td>
<td>unisexual</td>
<td>n/a</td>
<td>ebracteate; 3–5 tepals; 2–5 stamens</td>
<td>1/bract pair; 3 tepals</td>
<td>unchanged in fruit</td>
<td>free adherent to the seed coat</td>
<td>vertical</td>
<td>inferior</td>
<td>heterocarpy and heterospermy; testa always thin, with 2 simple layers</td>
<td></td>
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<tr>
<td><em>Ceratocarpus</em> L.</td>
<td>monoecious</td>
<td>unisexual</td>
<td>n/a</td>
<td>ebracteate; 2 tepals; 1 stamen</td>
<td>1/bract pair; tepals absent</td>
<td>n/a persistent in fruit, not accrescent</td>
<td>not adherent</td>
<td>vertical</td>
<td>inferior</td>
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<tr>
<td><em>Krascheninnikovia</em> Gueldenst.</td>
<td>monoecious</td>
<td>unisexual</td>
<td>n/a</td>
<td>ebracteate; 4 tepals</td>
<td>1/bract pair; tepals absent</td>
<td>n/a persistent in fruit, slightly accrescent</td>
<td>not adherent</td>
<td>vertical</td>
<td>inferior</td>
<td>testa always thin, with 2 simple layers</td>
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<tr>
<td><em>Chenopodieae s.l.</em></td>
<td>polygamomonoecious</td>
<td></td>
<td>3–5 tepals; ≤5 stamens</td>
<td>ebracteate; 3–5 tepals; ≤5 stamens</td>
<td>unchanged in fruit, or sometimes fleshy</td>
<td>n/a not adherent, rarely tightly adherent</td>
<td>horizontal or vertical</td>
<td>inferior</td>
<td>pericarp membranous, mostly 1–2-layered</td>
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<td>Chenopodium L.</td>
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<tr>
<td>Taxon</td>
<td>Plant sex</td>
<td>Sex</td>
<td>Bisexual</td>
<td>Staminate</td>
<td>Pistillate: no. fls/bract or bract pair; no. tepals; no. stigmas</td>
<td>Perianth in fruit</td>
<td>Bract acrescence</td>
<td>Bract connation</td>
<td>Pericarp adherence</td>
<td>Seed position</td>
<td>Fruit inverted (radicle orientation)</td>
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<tr>
<td>Einadia Raf.</td>
<td>polygamomonoecious</td>
<td>terminal fls of cluster bisexual, lateral ones pistillate unisexual</td>
<td>ebracteate; 4–5 tepals; 1–3 stamens; 2 stigmas n/a</td>
<td>n/a</td>
<td>ebracteate; 4–5 tepals, no staminodes</td>
<td>fruit not enveloped by perianth</td>
<td>n/a</td>
<td>n/a</td>
<td>pericarp membranous or succulent; adherence not known</td>
<td>horizontal</td>
<td>embryo annular</td>
</tr>
<tr>
<td>Micromonolepis Ulbr.</td>
<td>monoecious</td>
<td>bisexual, some pistillate</td>
<td>1–3-lobed, or absent; 0–2 stamens; c)</td>
<td>n/a</td>
<td>ebracteate; (1–2)–3 tepals; 2 stigmas</td>
<td>unchanged in fruit</td>
<td>n/a</td>
<td>n/a</td>
<td>adherent to the seed coat</td>
<td>vertical</td>
<td>embryo annular, radicle lateral inferior</td>
</tr>
<tr>
<td>Monolepis Schrad. polygamomonoecious</td>
<td>bisexual, some pistillate</td>
<td>1–3-lobed, or absent; 0–2 stamens; c)</td>
<td>n/a</td>
<td>n/a</td>
<td>unchanged in fruit</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>not adherent; loose when dry</td>
<td>vertical</td>
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<tr>
<td>Rhagodia R. Br.</td>
<td>dioecious</td>
<td>unisexual</td>
<td>bracts small or absent; 5 tepals; 5 stamens n/a</td>
<td>n/a</td>
<td>perianth lobes expanded in fruit perianth cartilaginous</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>not adherent</td>
<td>horizontal</td>
<td>embryo annular</td>
</tr>
<tr>
<td>Scleroblitum Ulbr.</td>
<td>polygamomonoecious</td>
<td>terminal bisexual, others female unisexual</td>
<td>ebracteate; tepals 4, stamen 0 or 1 n/a</td>
<td>n/a</td>
<td>hardened cover accrescent</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>not adherent</td>
<td>vertical</td>
<td>inverted horsehoe-shaped</td>
</tr>
<tr>
<td>Spinacia L.</td>
<td>dioecious or monoecious</td>
<td>unisexual</td>
<td>ebracteate; 4–5 tepals; 4–5 stamens n/a</td>
<td>n/a</td>
<td>bract-like perianth cover; 4–5 stigmas</td>
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<tr>
<td>Dysphanieae Cycloloma Moq.</td>
<td>polygamomonoecious</td>
<td>bisexual, sometimes pistillate</td>
<td>ebracteate; perianth 5 lobed; stamens 5; stigmas 2–3</td>
<td>n/a</td>
<td>ebracteate; 5 tepals; 2–3 stigmas</td>
<td>perianth present in fruit, chartaceous with a broad horizontal annular ring</td>
<td>n/a</td>
<td>n/a</td>
<td>not adherent</td>
<td>horizontal</td>
<td>embryo annular</td>
</tr>
<tr>
<td>Dysphania R. Br.</td>
<td>polygamomonoecious</td>
<td>bisexual or pistillate</td>
<td>ebracteate; perianth segments (1–2)3–5; stamens 1–5; stigmas 2</td>
<td>n/a</td>
<td>ebracteate; (1–2)3–5 tepals; 2 stigmas</td>
<td>fruit enclosed by perianth</td>
<td>n/a</td>
<td>n/a</td>
<td>not adherent</td>
<td>vertical or horizontal</td>
<td>embryo annular, radicle lateral</td>
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</table>
### Table 4. Continued.

<table>
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<th>Plant size</th>
<th>Tephrit</th>
<th>Notes</th>
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<td>Ulbr.,</td>
<td>Monoecious</td>
<td>moundy</td>
<td>a</td>
<td></td>
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<tr>
<td>A. Gray</td>
<td>Monoecious</td>
<td>moundy</td>
<td>a</td>
<td></td>
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<tr>
<td>Moq.</td>
<td>Monoecious</td>
<td>moundy</td>
<td>a</td>
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</table>

#### Major lineages within Atriplex and comments on the traditional sectional division

While the resolution within *Atriplex* is low in the *rbcL* and the *atpB-rbcL* spacer tree (Figs. 1, 2), there are a number of well-supported clades in the ITS tree (Fig. 3). However, low statistical support of basal branches within the ITS tree makes it impossible to draw any definite conclusion of the relationships of these well-supported lineages. There is a basal polytomy consisting of the following clades: (1) *Atriplex* (*Cremnophyton*) lanfrancoi and *Atriplex* cana (two accessions); these two are resolved as a monophyletic group, albeit with very low support, Fig. 2, (2) *Atriplex* sect. *Atriplex* clade (4 spp.), (3) sect. *Teutilopsis* clade (17 species [18 accessions]), (4) *C*$_4$ clade (72 species [74 accessions]), (5) *Atriplex* crassifolia (Fig. 3). Although we included 100 accessions of *Atriplex* in the ITS data set, the genus is still not sufficiently sampled to infer an updated classification. Especially sections containing *C*$_4$ species are mostly polyphyletic and need further sampling (see below).

1. *Cremnophyton* and *Atriplex* cana. The molecular data sets resolve the monotypic, Malta and Gozo endemic genus *Cremnophyton* as nested among *C*$_3$ species of *Atriplex*. The species probably is most closely related to *A. cana*, a species widely distributed in semideserts from western China to the eastern part of European Russia (Figs. 2, 3). Morphologically *Cremnophyton* and *A. cana* are not obviously similar to any other Eurasian *Atriplex*. Sukhorukov (2006) assumed that *A. cana* represents an old lineage of *Atriplex*, likewise Brullo and Pavone (1987) proposed that *Cremnophyton* represents a Tertiary (Oligocene/Miocene) relict. Although clearly resolved within *Atriplex*, both species branch off early within the genus (Figs. 2, 3).

*Cremnophyton* is a shrub and grows on limestone cliffs of Malta and Gozo (Brullo and Pavone, 1987), while *A. cana* is a dominant subshrub of certain plant formations in clayey, saline soils of Kazakhstani semideserts. Leaves of *Cremnophyton* are spatulate and entire as in *A. cana* but also as in *Halimione*. *Cremnophyton* differs from all other species of *Atriplex* (including *A. cana*) by the basic chromosome number of *x* = 10 (basic number in *Atriplex* is *x* = 9; Sukhorukov, 2006 and ref. therein) and by the presence of small bracts (only 1/4–1/5 the length of the petals) in male flowers (Brullo and Pavone, 1987). We found additional morphological characteristics of *Cremnophyton*. The bracts of the female flowers are sometimes unequal, the bracts adhere to the lower part of the fruit, and the radicle always points upward although the bracts are not connate along the margin. These two characters, direction of the radicle and degree of bract concrescence, have been found to be correlated in most *Atriplex* species with the result that the radicle points to the end of the concrescent part between the bracts (Sukhorukov, 2006). Because of its morphological and ecological distinctness, we consider *Cremnophyton lanfrancoi* an unmistakable species within *Atriplex* (see taxonomic part).

Morphologically, cytologically, and anatomically *A. cana* is not very different from other Eurasian species of *Atriplex*. It should, however, be noted that *A. cana* shows—in contrast to other shrubby *Atriplex* species in Eurasia—light-brown seeds with a thin (5–10 µm) outer seed coat layer.

2. *Atriplex* sect. *Atriplex* (formerly sect. *Dichospermum* (Ulbrich, 1934)) seems to represent a natural group of annual *C*$_3$ species (Fig. 3). According to Sukhorukov (2006), it contains three species, *A. hortensis* (type species of the section), *A. sagittata*, and *A. aucheri*. *Atriplex* sect. *Atriplex* is supported here. *Atriplex oblongifolia* is classified in *Atriplex* sect. *Teutilopsis*.
relationships among the American

classes is necessary to understand the

eage. The ITS and chloroplast results are not congruent; in-

Dialysesect. Teutliopsisalso contains annual C₄ species.
The inflorescences consist of mixed female and male flowers
without a spatial separation of the sexes. There is only the peri-
anthous type of female flowers; female flowers with a perianth
occur very rarely in this group (Gustafsson, 1986; Medvedeva,
1996).

The two bracts are only connate up to half of their length
in fruit and remain herbaceous (Aellen, 1939a; Sukhorukov,
2006). Atriplex sect. Teutliopsisrepresents a well-supported,
natural group. The inclusion of A. davisi, a formerly poorly
known species, in this group is supported by morphological
data (Sukhorukov, 2007a; Sukhorukov and Dunin, 2009).

The C₄ clade is well supported and contains the majority
of Atriplex species (Fig. 3). The Eurasian species of this clade
belong to several different sections (namely sect. Scleroca-
lymma, sect. Psammophila, sect. Stylosa, sect. Pterochiton and
sect. Obione). These sections were separated, for example, by
growth form, the degree of connation of the two bracts, and
seed morphology and color. None of these sections seems to
represent a natural group.

There are two distinct Australian lineages within the C₄
cladethesear Australian Atriplex clade 1 and clade 2
(Fig. 3). Australian Atriplex clade 1 contains four species, A.
cinerea, A. isatidea, A. nummularia, and A. rhagodioides. These
are all large and widespread shrubs that belong to Atriplex sect.
Dialysese and have been considered closely related by previous
authors (Wilson, 1984, Aellen 1937/1938). Other morphologi-
cally similar species that have not been sampled here such as
Atriplex incrassata F. Muell. or A. amnicola Paul G. Wilson,
might belong to this clade, too. The Australian Atriplex clade 2
contains 27 species classified in four different sections, viz.
sect. Spongiocarpus, sect. Semibaccatae, sect. Dialysese, and
the monotypic sect. Theleophyton. Section Spongiocarpus, sect.
Semibaccatae, and sect. Dialysese are clearly polyphyletic.
Thirty-one of 57 endemic species of Australian Atriplex were
sampled for this study. Although this already represents a broad
sampling, more variable markers and an increased sampling are
needed to understand the phylogeny of Atriplex in Australia.

The American species and the Australian Atriplex clade 1 are
sister to each other in a well-supported clade within the C₄ clade
(Fig. 3). The American species belong to sect. Obione and sect.
Pterochiton. Section Pterochiton is polyphyletic. Within the
American clade, there are two North American lineages (one is
well supported) and one well-supported South American lin-
eage. The ITS and chloroplast results are not congruent; in-
creased taxonomic sampling is necessary to understand the
relationships among the American Atriplex lineages.

The molecular data suggests that the traditional sections of
the C₄ species are largely polyphyletic and that a revised
classification of the C₄ species of Atriplex is needed. Such
a revision, however, needs a better resolved and sampled
molecular tree.

(4) The C₄ clade is well supported and contains the majority
of Atriplex species (Fig. 3). The Eurasian species of this clade
belong to several different sections (namely sect. Scleroca-
lymma, sect. Psammophila, sect. Stylosa, sect. Pterochiton and
sect. Obione). These sections were separated, for example, by
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the C₄ species are largely polyphyletic and that a revised
classification of the C₄ species of Atriplex is needed. Such
a revision, however, needs a better resolved and sampled
molecular tree.

(5) Atriplex crassifolia. Aellen (1939a) classified A. crassifo-
lia with A. patula in sect. Crassifolia. Later the species was in-
cluded in sect. Teutliopsis (Sukhorukov, 2006). We are unaware
of distinct morphological characters that would justify a separa-
tion from sect. Teutliopsis.

Biogeography of Atripliceae and the evolution of C₄-photosyn-
synthesis—Like other subfamilies of Chenopodiaceae studied
with molecular data (Sualdeoideae: Schütze et al. [2003];
Betoideae: Hohmann et al. [2006]; Salicornioideae: Kadereit
et al., [2006]; Camphorsoideae: Kadereit and Freitag, 2010).
Atripliceae seem to have their roots in Eurasia. Microgynoecium
is distributed in Tibet, Himalaya, and Pamir, and the early branch-
ing clades of both major lineages of Atripliceae, the Atriplex
clade and Archiatriplex clade, are also distributed in Eurasia (Fig.
3). In the Archiatriplex clade, this is Archiatriplex, which occurs
in China, and in the Atriplex clade, these are Halimione, Atriplex
cana, and Cremnohyton, and the Atriplex C₃ clades, which are
(mostly) distributed in Eurasia. The Atripliceae started to diver-
sify during the Early Miocene (Table 3). The Atriplex clade and
the Archiatriplex clade both are distributed worldwide—in Eur-
asia, North and South America, South Africa, and Australia (the
latter only in the Atriplex clade). However, although of the same
age, the two clades differ fundamentally in terms of internal
divergence times and species diversification.

The Archiatriplex clade comprises only 12 spp., eight of them
found in North America. The North American species seem to
form a monophyletic clade (North American C₃ clade). Archia-
triplex split from the rest of the clade c. 20.4–16.8 Ma. The rela-
tionships of Exomis, Monochlamys, Holmbergia, and the North
American C₃ clade remain unresolved (see above). North Amer-
ica was either reached from South America or from South Africa,
or its closest relative in Eurasia went extinct. North America was
probably reached by the North American C₃ clade c. 12–10 Ma
(Figs. 1–3). It likely slightly predates the arrival of the North
American Atriplex C₄ lineage, which arrived c. 9 Ma (Fig. 3).

The Atriplex clade comprises c. 300 spp., the majority of
them belonging to the C₄ lineage of Atriplex. The C₄ pathway
likely arose only once in Atriplex (Fig. 3), and this C₄ lineage
diversified, spread, and radiated on different continents. Branch
dating of the molecular trees presented here shows that C₄ pho-
tosynthesis arose in Atriplex at least 14.1–10.9 Ma (Table 3). This
dating of the C₃ origin in Atriplex supports the current view
that the increased aridity and seasonality of the Miocene climate
enhanced the origin of the C₄ lineages (Osborne and Beerling,
2006; Behrensmeier et al., 2007; Huang et al., 2007; Kürschner
et al., 2008).

Australia was reached by C₄ Atriplex two times independ-
ently, both from Central Asia between 6.3 and 4.8 Ma, and
once from Eurasia or America between 9.8 and 7.8 Ma (Fig. 3).
The younger Australian Atriplex lineage (clade 2 in Fig. 3) radi-
ated after its arrival on the continent and comprises most of the
Australian species (G. Kadereit, unpublished results). The older
Australian Atriplex lineage seems to comprise only a few spe-
cies (clade 1 in Fig. 3). The arrival of Atriplex in Australia
during the late Miocene agrees with the recent finding that
the largest sthenurines kangaroo (Procopitodon goliah) was a brow-
se specialist for Atriplex (Prideaux et al., 2009). The sthenurines
kangaroos originated during the late Miocene and reached their
highest diversity during the Pliocene spreading into the newly
developing arid landscapes (Prideaux, 2004). The ITS tree pre-
sented here indicates that also the species-rich Australian Atri-
plex lineage radiated during the Pliocene (Fig. 3).
Fig. 4. Bract cover of (A) Spinacea oleracea and (B) Atriplex fera. Fruit/seed anatomy of (C) Holmbergia tweedii, (D) Manochlamys albicans, (E) Proatriplex pleiantha, and (F, G) Atriplex centralasiatica with evident heterospermy ([F] red seed, [G] yellow seed, pericarp deleted). Abbreviations: c, chlorenchyma; cl, crystalliferous layer; il, inner layer; it, integumental tepatum; ol, outer layer; p, pericarp; pe, perisperm; sc, sclerenchyma; sp, sclerenchymatic parenchyma; t, testa.
According to the ITS tree (Fig. 3), the Americas were reached by the C$_3$ Atriplex only once, either from Eurasia or Australia between 9.8 and 8.8 Ma. The tree topology does not allow us to specify the area of origin; however, we consider a northern hemispherical origin as more likely. South America may have been reached several times independently from North America (between 7.7–4.4 Ma and 6.0–5.2 Ma; Fig. 3). The American lineages radiated after their arrival on the continents.

**Evolution of flower and fruit morphology in Chenopodioidae**— Dioecy occurs in Spinacia, Rhagodia, Graviera, Exomis, and Atriplex (Table 4). From the scattered position of dioecious taxa in Chenopodioidae, mostly within clades that are predominantly monoecious or polygamo-monoecious, it seems obvious that dioecy evolved multiple times in the subfamily. The separation of sexes within the inflorescence is a common condition in Chenopodioidae, and it seems likely that the spatial differentiation of male and female inflorescence parts in monoecious taxa also evolved multiple times (cf. Kondorskaya, 1984). Many genera are either strictly monoecious or have mixed inflorescences with bisexual and unisexual flowers (Table 4). In contrast to this, species with exclusively bisexual flowers are relatively uncommon in Chenopodioidae and occur mainly in Chenopodium and Dysphania. Traditionally, Atripliceae were defined by unisexual flowers. Because this condition evolved several times in Chenopodioidae, viz. in Atripliceae, Axyrideae, Suckleya, Spinacia, and Rhagodia (compare Table 4), further characters are required to morphologically define the tribe. The second traditional character used to define Atripliceae is perianthless female flowers with a foliar cover of two bracts that persist and are modified during fruit ripening and become a constitutive part of the diasporas. There are a few species that have female flowers with a perianth (Table 4), but in Atripliceae as circumscribed here the perianthless female flowers with a foliar cover of two bracts are certainly the most common character state. However, in a few lineages, viz. Microgynoeicum, Archiatriplex, and Protoatriplex, one bract or a pair of bracts subtend a cluster of female flowers. Because these genera probably represent old, relictual groups, it is tempting to speculate that a cluster of female flowers per bract could be the plesiomorphic condition of flower morphology in Atripliceae. However, this will be difficult to prove because of the unstable position of Microgynoeicum in the trees and the rare occurrence of this character state in Chenopodioidae. Alternatively, it might represent an apomorphic condition that evolved independently in these lineages. The molecular analysis presented here revealed that female flowers with a persistent foliar cover evolved not only in Atripliceae, but also in the ancestor of Ceratocarpus and Krausennikovia and in Spinacia. These three lineages show distant positions from each other in the molecular trees, indicating that female flowers with a persistent foliar cover evolved three times independently in Chenopodioidae. Axyrideae (as defined here) are readily distinguished from Atripliceae by their characteristic indumentum of stellate hairs and by the absence of “stalactites” in the outer periclinal cell walls of the testa. A closer look at Spinacia revealed that it differs from Atripliceae in a set of prominent morphological and anatomical characters (see above) that further support its position outside the tribe.

Most representatives of Atripliceae have a 1- or few-layered and undifferentiated pericarp in the ripe fruits. However, members of the Archiatriplex clade seem to be comparatively diverse in fruit anatomy. They differ in the adherence of the pericarp to the seed coat and in the number of cell layers in the mature pericarp (Table 4). In contrast, the pericarp of the Atriplex clade is invariably simple (Fig. 4F). Within the Atriplex clade, Halimione evolved a pericarp that is tightly adherent to a thin, membranous testa (cf. Wunderlich, 1967; see above). In perennial Atriplex, the testa is normally thick and hard. In some annual species of Atriplex, heterospermy evolved. This character is found in several groups of Chenopodioidae (evident heterospermy in Axyris: Sukhorukov, 2005; latent heterospermy in Eurasian Chenopodium: A. Sukhorukov, pers. observation). In Atripliceae, a vertical orientation of the embryo is the predominant character state. A horizontal position has evolved in Atriplex (within sect. Atriplex) and in Graviera independently (Table 4). The rotation of the embryo orientation (radicle pointing upward, laterally or downward) likely arose in connection with a highly variable degree of bract connation multiple times in Atripliceae.

**TAXONOMIC CONCLUSIONS**

The results of this study made the following three taxonomic conclusions necessary:

1. **Atripliceae C. A. Mey.**
   - The tribe Atripliceae is accepted here, albeit with a modified generic compilation. The following genera are included: Archiatriplex, Atriplex, Exomis, Exatriplex, Graviera, Halimione, Holmbergia, Manochlamys, Microgynoeicum, Protoatriplex, Stutzia. The subfamily status Atriplicioideae Falkovich and Kovalev (Falkovich and Kovalev, 2007) is not accepted here. The inclusion of Blackiella, Haloxanthium, Neopreissia, Obione, Pachypharynx, Senniella, and Theleophyton in Atriplex is supported by our results, while the inclusion of Halimione is rejected. The inclusion of Crennophyton in Atriplex is proposed.

2. **Atriplex lanfrancoi** (Brullo & Pavone) G. Kadereit et Sukhor., comb. nov.

3. **Tribe Axyrideae (Heklau)** G. Kadereit & A. Sukhor., comb. & stat. nov.
   - Included genera: *Axyris* L., Ceratocarpus L., Krausennikovia Gueldenst.

**LITERATURE CITED**


EHZ-BE2, B. Eritter 1879 f (UC, Iran, Hamadan), (2) HM587638, (3) HM005860; Atriplex leucophylla D. Dietr., sample 1, page 2062; G. Kassahun el., USA, California, San Francisco Co., (1) HM587578, sample 2; EHZ-JeGrI, J. Greenhouse s.n. (JEPS), USA, California, Santa Barbara Co., (2) HM587639, (3) HM587526; Atriplex lindleyi Moq., page 438, S. Jacobs 9099 (NSW), Australia, N. Far W. Plains, Broken Hill, (3) HM587527; Atriplex littoralis L., EM314, N. Shvedchikov 08.1988 (MW), Latvia, Tuyia, (3) HM587528; Atriplex micrantha C.A. Mey., EM319, A. Sukhorkov s.n. 10.1997 (MW), Russia, Primorye, Primorskiy Kray, S. Jacobs 9067 (NSW), Australia, North W. Plains, Tullibardine, (3) HM587536; Atriplex undulata (Moq.) D. Dietr., chen 167, M.E. Milgura 2005 (SI, KAS); Argentina, La Pampa, (1) AY270061, (2) HM587665, (3) HM587664; Atriplex velutinella F. Muller., chen 906, S. Jacobs 9455 (NSW), Australia, N. Far W. Plains, Cobham Lake, (3) HM587565; Atriplex vesicaria Heward ex Benth., sample 1, subsp. macrostigma Porr., sample 1, (44) S. Jacobs 9103 (NSW), Australia, S. Far W. Plains, Broken Hill, (3) HM587566; A. axyridis prostrata L., chen 118, G. & S. Miehe 96-140-04, Gobi Altai, Mongolia (HB, Miehe, KAS), (1) AY270062, (2) HM587667, Ceratocarpus areniarius L., chen 466, H. Freitag 33.017 (KAS), Russia, Tuva Rep., 15 km SSE Kyzyl (1) HM587594, Chenopodium acuminatum Willd., chen 185, G. & S. Miehe 96-666-05, Ulm Miehe, KAS, (1) HM587683, (2) HM587668, (3) Chenopodium auricomum Lindley, chen 258, S. Jacobs 8655 (NSW); Australia, New South Wales, North Western Plains (1) AY270078, (2) HM587669; Chenopodium bonus-henicus L., chen 051 and 1886, grown at Bot. Gard. Mainz, Germany (MG), (1) AY270079, (2) HM587670; Chenopodium coronopus Moq., chen 721, Bot. Garden Berlin-Dahlem seed sample 687 (MIG), leg. R. Br., Spain, Canary Is., La Palma, Puerto Naos/El Remo, (1) HM587595, (2) HM587671; Chenopodium desertorum (J. Black) J. Black subsp. anidiophyllum (Aellen) Paul. G. Wilson, chen 254, S. Jacobs 8650 (NSW); Australia, New South Wales, North Western Plains, (1) AY270042, (2) HM587672; Chenopodium foliosum Asch., sample 1, (11) chen 117, (2) AY270081, sample 2, chen 1855, W. Hilbig s.n. (HAL), W. Ukraine, Aimak, Charkhwa, w. of Ulaan, (1) HM587673, (2) Chenopodium frutescens C.A. Mey., chen 210, A. Kolyuk s.n. (NSW), 23.6.2000, Tava, Russia, (1) AY270082, (2) HM587674, (3) HM587567; Chenopodium muralle L., chen 866, W. Licht 5901 (MIG), Italy, (3) HM587675; Chenopodium nitriaricaceum (F. Muller.) Bent., chen 1860, S. Jacobs 9121 (NSW, MIG), Australia, NSW, near Balranald, (2) HM587676; Chenopodium oahuense (Meyr.) Aellen, chen 2025, S. Jacobs 9105 (NSW), Hawaii, Kamuela, (2) HM587677; Chenopodium sanctae-clarae Johow, chen 194, (1) AY270043, Chenopodium urbicum L., chen 281, Bot. Garden Berlin-Dahlem seed sample 694 leg. Rau 242 (MIG), Greece, Aegean Sea, Nomos Evviva, (1) HM587596, (2) HM587678; Cossonpermum filifolium C.A. Mey., ex A.K. Becker, chen 148, H. Freitag 28.702 (KAS), Turkey A6 Samsun prov., (2) AY270074, sample 1, (3) AY270083, sample 2, (4) AY270084, sample 1, A. Korolyuk s.n. (LE), 23.1.2001, Tava, Russia, (1) AY270085, (2) HM587679, (3) HM587569; Cycloptera atriplicifolia J.M. Coul., chen 157, Bot. Garden Kassel, seeds from Hort. Bot. Berg. Stockholm 116; 0006302 (KAS), (1) HM587598, (2) HM587681; Dysphania ambrosioides (L.) Mosyakin & Clemants, chen 822, Bot. Garden Berlin-Dahlem seed sample 683 leg. Ryll 3594 (MIG), Portugal, Azores, Sao Miguel, Riberia Grande Lagao das Fogo, (1) HM587599, (2) HM587682; Dysphania botrys (L.) Mosyakin & Clemants, chen 116, Freitag & Adig ü zel 28.769 (KAS), Turkey C4 Konya, road to Karapinar, (1) AY270080, (2) HM587683; Dysphania crisata (F. Muller.) Mosyakin & Clemants, chen 256, S. Jacobs 8653 (NSW), Australia, North Western Plains, South Western Plains, (1) AY270046, (2) HM587684, (3) HM587586; Exomis gracilis (Nees) Paul G. Wilson, chen 271, S. Jacobs 8673 (NSW); Australia, New South Wales, North Western Plains, (1) AY270086, (2) HM587685; Eridania nutans (R. Br.) A. Scott, chen 417, G.M. Toller 325 (NSW); Australia, central coast, cultivated Mt. Annan Botanical Garden (1) HM587600, (2) HM587602, (3) HM587686; Eridania microphylla (Thunb.) Aellen, chen 877, L. Mucina 0812003 (MIG), South Africa, Western Cape prov., Darling, (1) HM587601, (2) HM587650, (3) HM587669; Extripula californica (Moc.) E.H. Zacharius, sample 1, (2061, G. Kadereit 2009/37 (MJG), USA, California, Point Reyes, (1) HM587602, (2) HM587687, sample 2; EHZ-1025, E. Zacharius 1025 (JEPS), USA, California, San Mateo Co. (3) HM005850; Extripula jouquina (A. Nelson) E.H. Zacharius, EHZ-306, E. Zacharius 306, USA, California,
Contra Costa Co., (1) HM587603, (2) HM587689, (3) HM005852;  
Grayia brandegeei A. Gray, sample 1: chen 1889, J. Coles s.n. (COLO),  
USA, Colorado, Mesa, (1) HM587604, (2) HM587690, (3) HM587570,  
sample 2: EHZ-H38, N. Holmgren, J. Revol & C. Lafrenz 2133 (UC),  
USA, Utah, Garfield Co., (3) HM005845; Grayia spinosa (Hook.) Moq.,  
sample 1: chen 192, Larke s.n. (BM), USA, California, Nevada, (2)  
HM587691, (3) HM587571, sample 2: EHZ-566, E. Zacharias 566 (UC),  
USA, Utah, Tooele Co., (2) HM587692, (3) HM005843, sample 2: chen  
1888; B. Neely 4244 (COLO), USA, Colorado, Mouth of Gates of Lodore,  
(1) HM587605, (2) HM587693, (3) HM587572; Halimione pedunculata  
(L.) Aellen, sample 1: chen 034, G. Kadereit 2000/202 (MJG); Denmark,  
Kattegat, (1) AY270093, sample 2: chen 471, H. Freitag 33094 (KAS),  
Russia, Novosibirsk, 20 km north of Karasuk, (2) HM587694, (3)  
HM587573, sample 2: EM336, A. Sukhorukov 09.2002 (MW), Russia,  
prov. Volgograd, Elton, (3) HQ008274; Halimione portulacoides (L.)  
Aellen, EM385, A. Sukhorukov s.n., 11.2006 (MW), Cyprus, Larnaca, (3)  
HM587574; Halimione verrucifera (M. Bieb.) Aellen, chen 470, H.  
Freitag 33.092 (KAS), Russia, Novosibirsk, Karasuk, (1) HM587606, (2)  
HM587695, (3) HM587575; Holmbergia tweedii Speg., sample 1: chen  
2003, A. Krapovickas & C.L. Cristobal 24545 (P), Argentina, Corrientes  
prov., Saladas rio Santa Lucia, (2) HM587696, sample 2: EHZ-H17, A.  
Krapovickas & A. Schinini 39143 (UC), Bolivia, (3) HM005842;  
Krascheninnikovia centaoides (L.) Gueldenst., B. Dickoré 12752; Nanga  
Parbat area, Pakistan (Hb. Dickoré, KAS), chen 012, (1) AY270105, (2)  
HM587697; Krascheninnikovia lanata (Pursh) A. Meese & A. Smit,  
chen 1887, B. Nelson 23554 (USCH, HAL 100339), USA, New Mexico,  
San Miguel County, Santa Fe Nat. Forest, (2) HM587698, Manochlamys  
albicans (Soland. in Ait.) Aellen, chen 878, L. Mucina 200900/4 (MJG),  
South Africa, RSA, Northern Cape prov., Namaqua N. P., Kamieskroon,  
(1) HM587607, (2) HM587699, (3) HM587576; Micropogonioecium  
tibeticum Hook.f., sample 1: chen 119, B. Dickoré 4284 (Hb. Dickoré,  
KAS), China, Tibet, Quinghai, (1) AY270107, sample 2: chen 874, G. &  
S. Mielke 03-059-11 (hb. Mielke, KAS), China, Xizang (Tibet), Tso basin s  
of Raka Tsangpo Tsabasang, (2) HM587700, (3) HM587577;  
Micromonolepis pusilla (Torr. ex S. Watson) Ulbr., chen 1858, A. Tiehm  
11763 (NSW), USA, Nevada, Humboldt River, (1) HM587608, (2)  
HM587701; Monolepis nuttalliana Greene, chen 125, Bot. Garden Kassel,  
seeds from Univ. Hohenheim (KAS), (1) AY27010, (2) HM587702;  
Proatriplex pleiantha (W.A. Weber) Stutz & G.L. Chu, sample 1: chen  
1857, W. A. Weber 7651 (NSW), USA, Colorado, SW Towaoc, (3)  
HM587578, sample 2: EHZ-H32, R. Spellenberg & R. Corral 8185 (UC),  
USA, New Mexico, San Juan Co., (3) HM005836;  
Rhagodia drummondii Moq., sample 1: chen 159, N. Schmalz 194  
52 (MJG); Western Australia, Hayden, (1) AY270124, sample 2: chen  
1859, S. Jacobs 9131 (NSW), Australia, Western Australia, near Meckering, (2)  
HM587703; Rhagodia parabolica R. Br., chen 532, S. Jacobs 9208 (NSW), (2)  
HM587704; Scleroblitum atriplicinum (F. Muell.) Ulbr., cult. at Botanical  
Garden University Mainz, (2) AY588611;  
Spinacia oleracea L., (1) Zurawski et al. (1981), chen 1869, G. Kadereit  
s.n. (MJG), (3) HM587705; Statzia coccilei (Standl.) E.H. Zacharias,  
EHZ-811, E. Zacharias 811 (UC), USA, Nevada, Nye Co., (1) HM587609, (2)  
HM587706, (3) HM005839; Statzia dioica (Nutt.) E.H. Zacharias, sample 1:  
AC351, L. Welp 6269 (NY), (1) HM587610, (2) HM587707, (3) HM587579,  
sample 2:EHZ-H95, C. Porter 9071 (UC), USA, Wyoming, Weston Co., (3)  
HM005838; Suckleya suckleyana Rydb., chen 2000; J. E. Larson 6492,  
USA, New Mexico, Tres Piedras, (1) HM587611, Telosyris aristata (L.)  
Moq., chen 293, B. B. Neely & H. Hurka 11.727 (Hb. Hurka, KAS),  
Mongolia, Ulaanbaatar, (1) AY270140, (2) HM587708;