Phylogeny and Systematics of *Chenopodium* L. and its allies

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Susy Fuentes-Bazan

aus

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This work was carried out between 2007 and 2012 under the supervision of Prof. Dr. Thomas Borsch, Institut für Biologie of the Freie Universität Berlin.

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Preface and acknowledgements

In the framework of the “Evolution of Amaranthaceae-Chenopodiaceae” project and supported by the scholarship of the German Academic Exchange Service (DAAD: Deutscher Akademische Austausch Dients) the present thesis was carried out under the supervision of Prof. Dr. Thomas Borsch and Prof. Dr. Helmut Hilger.

In Bolivia, *Chenopodium quinoa* (Quinua in quechua) and *Chenopodium pallidicaule* (Cñawo in aymara) are two economically and cultural important crops. The high morphological variability observed in the cultivars from the Titicaca lake region first motivated my interest in these crops, and in Bolivia, I had the opportunity to apply molecular techniques to study the intraspecific variability within these *Ch. quinoa* varieties. During the molecular course on Plants organized by M. Sc. Ortuño from the “Herbario Nacional de Bolivia, La Paz”, in collaboration with Prof. Dr. Borsch, all participants, including myself, could exchange knowledge and experience about the study fields of phylogeny and systematics. Thus this first introduction to the molecular relationships of *Ch. quinoa,* and my curiosity to know its origin based on phylogenetic studies, was extremely inspiring for my own study.

For this PhD project, the participation of many people has been of great importance. I would like to express my gratitude to Prof. Dr. Thomas Borsch. He gave me the opportunity to move into the integrative field of plant phylogeny and systematics through his invitation to Germany and to make the present PhD project under his supervision. The opportunity to dive into the fascinating world of phylogeny as applied to *Chenopodium* was stimulated by him. Without his supervision, support and continuous encouragement for all these years the present project would not have been possible. Also, I am indebted to Prof. Dr. Hartmut Hilger, who gave me his support and wise advice at the right time during the present project.

I would like to express my gratitude to Dr. Stephan Beck (ex-director of the Herbario Nacional de Bolivia, La Paz) who introduced me to the field of botany during my universitary education, and moreover his support, given in many ways, for the accomplishment of this project. He gave the first sampling from Bolivia as present, like a

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The molecular data sets of this thesis were generated in the Molecular Systematics Laboratory at the Ness Institute for Biodiversity of Plants in Bonn, which was led by Prof. Dr. Borsch until the end of 2007, after that of the Molecular laboratories of the Botanischer Garten and Botanisches Museum and of the Molecular laboratories of the Institut für Biologie/Botanic in Berlin. I would like to express my warmest thanks to all my colleagues: Dr. Nadja Korotkova, Dr. Cornelia Löhne, Dr. Lilian Ferrufino, Dr. Ludo Muller, Dr. Elisabeth Kaltenegger, Dr. Guilhem Mansion, Dr. Elizabeth Bourne, Dr. Federico Luebert, Dr. Kassahun Tesfaye, Dr. Angélica Cervantes, Dipl. Biol. Virginia Duwe, Dipl. Biol. Vanessa Di Vincenzo, Dipl. Biol. Benedikt Braun, Dipl. Biol. Markus Ackermann, Ing. Kim Govers, Bettina Giesicke and Christine Grüber, thank you all for the

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The suggestions and comments of Dr. Guilhem Mansion and Prof. Dr. Thomas Borsch were especially constructive for the first part and published paper of this project (Chapter 2). Prof. Pertti Uotila, who broadened my horizons to the complex world of the taxonomy of *Chenopodium*, and his experience in the genus helped me to keep the determination to study these species and to significantly improve the discussion of this project, especially in Chapter 3. The interesting discussions, suggestions and the challenging questions of Dr. Federico Luebert significantly improved Chapter 4. The general suggestions and comments of Dr. Angélica Cervantes during the last writing phase, and the support, comments, suggestions and discussions of Dr. Nadja Korotkova both helped me to improve the thesis. To all of them, I would like to express my gratitude for their significant input into this thesis.

Summary

Fuentes Bazan, Susy. 2012. Phylogeny and Systematics of *Chenopodium* L. and its allies. PhD thesis, Fachbereich Biologie, Chemie, Pharmazie. Institut für Biologie – Botanik, Freie Universität Berlin, Germany.

Chenopodium L. (Chenopodiaceae) is a large and almost globally distributed genus of annual or perennial herbs. It is the second largest and one of the taxonomically most complex genera within Chenopodioideae. The economically important species are *Chenopodium quinoa* (quinoa), *Ch. berlandieri* subsp. *nuttalliae* (huauzontle) and *Ch. pallidicaule* (cañihua). Molecular phylogenetic studies so far found *Chenopodium* as polyphyletic. However, the sampling was low, considering that *Chenopodium* comprises about 150 species in three subgenera and 13 sections. The evolution of *Chenopodium* may be rather complex. Many species are polyploid, and previous studies suggested common hybridization. But there was so far no detailed molecular phylogenetic study of *Chenopodium* and the origin and relationships of the polyploids, especially of the crops, are unclear.

The principal aim of this PhD-project was therefore to infer the circumscription of *Chenopodium* s.str., to clarify the phylogenetic relationships of *Chenopodium* s.l. and its allies within the subfamily Chenopodioideae, to study species-level relationships, to detect possible hybridization and to reconstruct reticulation patterns.

The Chapter 2 focuses on the phylogeny of *Chenopodium* s. l. All the subgenera and most sections were sampled along with other genera of Chenopodieae, Atripliceae and Axyrideae. Data sets of the non-coding plastid *trnL-F* and nuclear ITS regions were generated and analysed using Maximum Parsimony and Bayesian Inference. The trees derived from *trnL-F* and ITS were incongruent, suggesting hybridization and reticulation. *Chenopodium* was found as highly paraphyletic and splits in five major clades. Consequently, a new delimitation for *Chenopodium* s s., including *Einadia* and *Rhagodia*, was proposed and the necessary taxonomic combinations under *Chenopodium* were made. The tribes *Atripliceae* and *Axyrideae* were supported as monophyletic and the *Dysphanieae*

and *Spinacieae* were found as new clades. Taxonomic changes remained necessary for three newly found lineages: the *Chenopodium rubrum*-clade, the *Ch. murale*-clade and a group of species classified under *Chenopodium* that was found sister to *Spinacea*.

The taxonomic consequences for these newly found lineages were the main subject of the analyses presented in Chapter 3. It was attempted to improve the resolution and support of the phylogenetic trees for these clades using a combined data set of *trnL-F* and *matK/trnK*. The analyses showed with maximal support the *Ch. murale*-clade as sister to the clade of *Atripliceae* and *Chenopodium* s.s and the *Ch. rubrum*-clade as sister to these three clades. The improved and well supported phylogenetic hypotheses compared to Chapter 2 gave enough evidence for suggesting taxonomic changes. For the group sister to *Spinacia*, the Linnaean genus *Blitum* is resurrected. It encloses *B. capitatum* L. (type species), *B. virgatum* L., *B. nuttallianum* Shult., *B. californicum* S. Watson, *B. bonus-henricus* (L.) C.A. Mey and *B. antriplicinum* F.Muell. New generic names for the *Ch. murale* and *Ch. rubrum*-clades are also discussed.

The main topic in Chapter 4 is the phylogeny of *Chenopodium* s.str. and their reticulation patterns. Emphasis is put on *Ch. quinoa* because it is an economically important crop. The taxon sampling for *Chenopodium* s.str. was increased and new data sets of *matK/trnK* and the *rpl16* intron were generated in order to improve the support of the internal relationships in *Chenopodium* s.str. The nuclear ITS region was sequenced additionally in order to hybridization and reticulation. The data sets were analysed using tree-building methods and network reconstruction algorithms. The increased taxon sampling improved support in the tree inferred from ITS, compared to the results of Chapter 2. Based on the plastid regions, three earlier-suggested polyploid groups were supported as monophyletic. These were the *Ch. album*-group, the *Ch. quinoa*-group and the *Ch. berlandieri* subsp. *nuttalliae*-group. Compared to the plastid data set, the tree inferred from the ITS data set showed a different topology for *Ch. ficifolium* (2x), *Ch. karoi* (4x) and *Ch, opulifolium* (6x). Hybridization is assumed as the most probable cause of this incongruence. Based on the network reconstruction, the hexaploids *Ch. album* (6x) and *Ch. opulifolium* (6x) seem to have originated from autopolyploidization. In contrast, the tetraploid species appear to have resulted from allopolyploidization. *Chenopodium ficifolium* (2x) is involved as the parent of all the tetraploid species, including *Ch. quinoa* (4x). The second putative parent involved in the hybridization of the allotetraploids is a

diploid species from North America. A preliminary molecular clock analysis suggests a divergence age of ca. 2 Mya for the allotetraploids. The origin of the allotetraploid taxa in America could therefore be older than their domestication. Most probably, *Ch. quinoa* was domesticated in the Andean highlands and *Ch. berlandieri* subsp. *nuttallieae* was independently domesticated in Mexico.

As a main result of this project, *Chenopodium* could be reliably delimited. The relationships within the genus and with its allies in Chenopodioideae could be almost fully clarified. The results suggest that a dense sampling and the use of combined sequence data sets allow inferring a robust phylogenetic tree at the species level. The molecular regions selected need to have good phylogenetic signal, this seems more important than the pure amount of data. The phylogenetic tree inference in combination with the network reconstruction from plastid and nuclear data sets allowed detecting several hybridization events. Therefore these analyses could be a case study for reconstruction of hybridization events in other rich-species genera within Chenopodiaceae. Finally, this study for first time provided a well supported hypothesis for the origin and the hybrid parents of the important crop *Chenopodium quinoa*.

Zusammenfassung

Fuentes Bazan, Susy. 2012. Phylogeny and Systematics of *Chenopodium* L. and its allies. PhD thesis, Fachbereich Biologie, Chemie, Pharmazie. Institut für Biologie – Botanik, Freie Universität Berlin, Germany.

Chenopodium L. (Chenopodiaceae) ist eine große und beinahe weltweit verbreitete Gattung von einjährigen oder ausdauernden krautigen Pflanzen. Es ist die zweitgrößte und eine der taxonomisch komplexesten Gattungen in den Chenopodiaceae. Wichtige Nutzpflanzen sind *Chenopodim quinoa* (Quinoa), *Ch. berlandieri* subsp. *nuttalliae* (Huauzontle) und *Ch. pallidicaule* (Cañihua). Bisherige molekularphylogenetische Studien haben gezeigt dass *Chenopodium* polyphyletisch ist. Jedoch waren nur wenige Arten bisher berücksichtigt worden, wenn man bedenkt dass *Chenopodium* etwa 150 Arten in 3 Untergattungen und 13 Sektionen umfasst. Die Evolution von *Chenopodium* ist vermutlich recht komplex. Viele der Arten sind polyploid und bisherige Untersuchungen lassen auf häufige Hybridisierung schließen. Jedoch gab es bisher keine detaillierte molekularphylogenetische Studie von *Chenopodium* und der Ursprung und die Verwandtschaft der polyploiden Arten, insbesondere der Nutzpflanzen, sind unklar.

Das wichtigste Ziel dieser Doktorarbeit was es deshalb, die Umgrenzung von *Chenopodium* im engeren Sinne zu klären. Weiterhin ging es darum, die Verwandtschaftsverhältnisse von *Chenopodium* im weiteren Sinne und dessen Verwandten innerhalb der Chenopodioideae aufzuschlüsseln. Und schließlich war es das Ziel, die Verwandtschaft der Arten zueinander zu untersuchen, mögliche Hybridisierungs-Ereignisse zu erkennen und Muster retikulater Evolution zu rekonstruieren.

Der Schwerpunkt im Kapitel 2 liegt auf der Phylogenie von *Chenopodium* im weiteren Sinne. Alle Untergattungen und die meisten Sektionen wurden bei der Auswahl der Arten berücksichtigt. Außerdem wurden weitere Gattungen der *Chenopodieae, Atripliceae* und *Axyrideae* in die Analyse aufgenommen. Es wurden Datensätze der nicht kodierenden Chloroplasten-Region *trnL-F* und der Kern-Region ITS generiert und mit Maximum Parsimonie und Bayesianischen Verfahren analysiert. Die Stammbäume basierend auf *trnL-F* und ITS waren inkongruent, was auf Hydridisierung und Retikulation

hindeutet. *Chenopodium* zeigte sich als hochgradig polyphyletisch, es zerfällt in 5 Haupt-Clades. Als Konsequenz dieser Ergebnisse wurde eine neue Umgrenzung von *Chenopodium* im engeren Sinne, inklusive *Einadia* und *Rhagodia*, vorgeschlagen und die notwendigen Neukombinationen unter *Chenopodium* wurden erarbeitet. Die Triben *Atripliceae* und *Axyrideae* wurden als monophyletisch bestätigt und die *Dysphanieae* und *Spinacieae* wurden als neue Clades gefunden. Taxonomische Änderungen blieben notwendig für drei neu gefundene Gruppen: den *Chenopodium rubrum*-Clade, den *Ch. murale*-Clade und eine Gruppe von bisher als *Chenopodium* klassifizierten Arten die als Schwestergruppe zu *Spinacea* gefunden wurde.

Die taxonomischen Konsequenzen für diese drei neuen Gruppen sind das Hauptthema in Kapitel 3. Es wurde angestrebt, die Auflösung und die statistische Unterstützung für die Stammbäume dieser Gruppen zu verbessern, basierend auf der Kombination von *trnL-F* und *matK/trnK*. Die Analysen zeigten mit maximaler statistischer Unterstützung den *Ch. murale*-Clade als Schwester zu dem Clade bestehend aus den Atripliceae und *Chenopodium* s.str und den *Ch. rubrum*-Clade als Schwester zu diesen drei Clades. Die im Vergleich zu Kapitel 2 verbesserten phylogenetischen Hypothesen waren nun eine gute Grundlage um taxonomische Änderungen vorzuschlagen. Für die Schwestergruppe zu *Spinacea* wird Linné's Gattung *Blitum* wieder eingeführt. Diese enthält *B. capitatum* L. (die Typus-Art), *B. virgatum* L. *B. nuttallianum* Shult., *B. californicum* S. Watson, *B. bonus-henricus* (L.) C.A. Mey und *B. antriplicinum* F.Muell. Neue Gattungsnamen für die *Ch. murale*-und *Ch. rubrum*-clades werden ebenfalls diskutiert.

Im Kapitel 4 ist das hauptsächliche Thema die Phylogenie von *Chenopodium* s.str. und die Muster retikulater Evolution. Besonders hervorgehoben wird *Ch. quinoa*, da es eine wichtige Nutzpflanze ist. Das Taxon-Sampling für *Chenopodium* s.str. wurde erweitert und neue Datensätze von *trnL-F*, *matK/trnK* und des *rpl16* Introns wurden generiert um Auflösung und statistische Stützung innerhalb von *Chenopodium* s.str. zu verbessern. Zusätzlich wurde die Kern-Region ITS sequenziert um Hybridisierung und Retikulation aufzudecken. Die Datensätze wurden mit Baum-Rekonstruktionsmethoden und Netzwerk-Rekonstruktions-Algorithmen analysiert. Das erweiterte Taxon-Sampling hat die statistische Stützung von ITS gegenüber den Ergebnissen aus Kapitel 2 verbessert. Basierend auf den Plastiden-Regionen wurden drei bereits früher vermutete Gruppen von

polyploiden Arten als monophyletisch gestützt. Das waren die *Ch. album*-Gruppe, die *Ch. quinoa*-Gruppe und die *Ch. berlandieri* subsp. *nuttalliae*-Gruppe. Die ITS Region zeigte eine abweichende Topologie für *Ch. ficifolium* (2x), *Ch. karoi* (4x) und *Ch, opulifolium* (6x), verglichen mit dem Stammbaum basierend auf Plastiden-Markern. Als naheliegende Erklärung für diese Inkongruenz wird Hybridisierung angenommen. Basierend auf der Netzwerk-Rekonstruktion sind die hexaploiden Arten *Ch. album* (6x) und *Ch. opulifolium* (6x) autopolyploiden Ursprungs. Im Gegensatz dazu scheinen die hexaploiden Arten durch Allopolyploidisierung entstanden zu sein. *Chenopodium ficifolium* (2x) ist die Elternart aller tetraploiden Arten, inklusive *Ch. quinoa* (4x). Die zweite Elternart ist vermutlich eine diploide Art aus Nordamerika. Anhand einer vorläufigen Datierung mit einer molekularen Uhr lässt sich das Alter der tetraploiden Arten mit etwa 2 mya schätzen. Der Ursprung der allotetraploiden Arten scheint damit älter zu sein als deren Domestikation. Am wahrscheinlichsten erscheint dass *Ch. quinoa* im Anden-Hochland domestiziert wurde während *Ch. berlandieri* subsp. *nuttallieae* unabhängig davon in Mexiko domestiziert wurde.

Eines der wichtigsten Ergebnisse dieses Projektes ist dass *Chenopodium* zuverlässig umgrenzt werden konnte. Weiterhin konnte die Verwandtschaft innerhalb dieser Gattung und zu deren Verwandten beinahe vollständig geklärt werden. Die Ergebnisse lassen schlussfolgern dass ein ausreichend großes Taxon-Sampling und kombinierte molekulare Datensätze es erlauben, eine robuste phylogenetische Hypothese auf Artebene aufzustellen. Die verwendeten DNA-Regionen müssen dabei gutes phylogenetisches Signal liefern, das scheint wichtiger als die reine Datenmenge. Die Stammbaum-Rekonstruktion in Kombination mit der Netzwerk-Rekonstruktion erlaubte es, mehrere Hybridisierungs-Ereignisse zu erkennen. So können diese Analysen als Vorlage dienen für Rekonstruktionen von Hybridisierung in anderen artenreichen Gattungen der Chenopodiaceae. Schlussendlich lieferte diese Studie zum ersten Mal eine gut gestützte Hypothese für den Ursprung und die Hybrid-Eltern der wichtigen Nutzpflanze *Chenopodium quinoa*.

Contents

Chapter 1

General introduction

Contents

1.1 Chenopodiaceae, the goosefoot family

Chenopodiaceae is a largely herbaceous family with about 100 genera and 1700 species (Aellen, 1960; Kühn, 1993; Welsh et al., 2003) and together with the close related family Amaranthaceae constitutes the most diverse lineage of the Caryophyllales (Kadereit et al., 2003). While Chenopodiaceae is distributed predominantly in temperate and subtropical regions, especially in desert or semidesert regions, Amaranthaceae is distributed throughout the tropical and subtropical regions (Kühn, 1993; Kadereit et al., 2003; Heywood et al., 2007). The shared morphological characters (e.g. minute sessile flowers arranged in thyrse-type inflorescences, pentamerous flowers, pantoporate pollen and a single basal ovule) of both families makes their delimitation difficult (Kühn, 1993; Kadereit et al., 2003; Acosta et al., 2009). Moreover, works based on anatomy and phytochemistry supports their close relationship, suggesting the fusion of Chenopodiaceae and Amaranthaceae (e.g. the division of both families into five groups suggested by Scott in 1977). Further work, based on phylogenetic analyses, supported the close relationship of Chenopodiaceae and Amaranthaceae and, due to the non-monophyly of Chenopodiaceae (Kadereit et al., 2003), its inclusion into Amaranthaceae has been suggested by the Angiosperm Phylogenetic Group (Angiosperm Phylogeny Group II, 2003; Angiosperm Phylogeny Group III, 2009). However, most of the floras and current morphological, physiological and phylogenetic studies still consider Chenopodiaceae as a different family (Wilson, 1984; Rechinger, 1997; Uotila, 2001; Welsh, et al., 2003; Zhu et al., 2003; Müller and Borsch, 2005a; Kadereit et al., 2005; Voznesenskaya et al., 2007; Zacharias and Baldwin, 2010; Kadereit et al., 2010; Suchorukow, 2010; Flores-Olvera et al., 2011; this study). Chenopodiaceae consist of five major lineages with uncertain clade delimitations (Kadereit et al., 2003; Müller and Borsch, 2005a). Therefore, based on phylogenetic relationships Kadereit et al. (2003) suggested some taxonomic changes within Chenopodiaceae, for instance Polycnemoideae to be transferred from Chenopodiaceae to Amaranthaceae, and Sarcobateae to be excluded from Chenopodiaceae to its own family Sarcobataceae (see Figs. 1.1 and 1.2).

Chenopodiaceae enclose taxa adapted to arid and Mediterranean regions of Australia, North and South America, and South Africa. Several genera are important as major constituents of desert, semidesert and saline vegetation, and are also found in cool and high altitude areas of both hemispheres (Uotila and Suomien, 1976; Judd and

Historical Classification of Chenopodiaceae and Amaranthaceae

Moquin-Tandon 1840	Moquin-Tandon 1849	Baillon 1887	Bentham and Hooker 1880	Volkens 1893/ Schinz 1893	Ulbrich 1934/ Schinz 1934	Kühn et al.1993/ Townsend 1993	Kaderei et al. 2003
Chenopodeae	Salsolaceae	Chénopodiacées	Chenopodiaceae	Chenopodiaceae	Chenopodiaceae	Chenopodiaceae	Chenopodiaceae
Cyclolobeae	Cyclolobeae		Cyclolobeae	Cyclolobeae	Cyclolobeae		
					Chenopodioideae	Chenopodioideae	Chenopodioideae
Anserinae	Chenopodieae	Chenopodiées	Euchenopodieae	Chenopodieae	Chenopodieae	Chenopodieae	Chenopodieae I-III +
Spinaciae	Spinacieae		Atripliceae	Atripliceae	Atripliceae	Atripliceae	Atripliceae +
Camphorosmeae	Camphorosmeae		Camphorosmeae	Camphorosmeae	Camphorosmeae	Camphorosmeae	→ Salsoloideae (see below)
						Sclerolaeneae	→ Salsoloideae (see below)
					Corispermoideae		Corispermoideae
Corispermeae	Corispermeae		Corispermeae	Corispermeae	Corispermeae	Corispermeae	Corispermeae
					Betoideae		Betoideae
				Beteae	Beteae	Beteae	Beteae I-IV +
					Hablitzieae		
					Polynemoideae	Polynemoideae	→ Amaranthaceae
	(sub Amaranthaceae Polycnemeae, subtr.)	Polycnémées	Polycnemeae	Polycnemeae	Polycnemeae	Polycnemeae	
					Salicornioideae	Salicornioideae	Salicornioideae
					Halopeplideae	Halopeplideae	Halopeplideae
Salicornieae	Salicornieae	Salicorniées	Salicornieae	Salicornieae	Salicornieae	Salicornieae	Salicornieae
Spirolobeae	Spirolobeae		Spirolobeae	Spirolobeae	Spirolobeae		
					Suaedoideae	Salsoloideae	Suaedoideae
Suaedinae	Suaedeae	Salsolées	Suaedeae	Suaedeae	Suaedeae	Suaedeae	Suaedeae
					Bienertieae		Bienertieae
					Salsoloideae		Salsoloideae
Salsoleae	Salsoleae		Salsoleae	Salsoleae	Salsoleae	Salsoleae	Salsoleae I-II +
							Camphorosmeae (incl.
					Nucularieae		Sclerolaeneae) +
					Sarcobatoideae		
	(dubia sedis: Sarcobatus)	Sarcobatées	Sarcobatideae	Sarcobatideae	Sarcobateae	Sarcobateae	→ excl. as Sarcobataceae
			Eubasellea → excluded				
			Boussingaultieae → excluded				
	Amaranthaceae		Amaranthaceae	Amaranthaceae	Amaranthaceae	Amaranthaceae	Amaranthaceae
				Amaranthoideae	Amaranthoideae	Amaranthoideae	amaranthoids I+II +
	Celosieae	Célosiées	Celosieae	Celosieae	Celosieae	Celosieae	Celosieae
	Achyrantheae	Amarantées	Amarantheae	Amarantheae	Amarantheae	Amarantheae	Amarantheae I-IV +
				Gomphrenoideae	Gomphrenoideae	Gomphrenoideae	Gomphrenoideae
	Gomphreneae	Gomphrénées	Gomphreneae	Gomphreneae	Gomphreneae	Gomphreneae	Gomphreneae
							(incl. Pseudoplantageae) +
				Guillemineae			
					Brayulineae		
						Pseudoplantageae	
		Microtéées → excluded					
		Leucastérées → excluded					

+ New tribal clasiffication necessary

Fig. 1.1 – Modified table of the historical classifications of Chenopodiaceae and Amaranthaceae taken from Kadereit et al. (2003).

Ferguson, 1999; Heywood et al., 2007). All these regions usually have unfavourable climatic conditions for important crop plants (e.g. *Zea mais* or corn, *Oriza sativa* or rice), however, Chenopodiaceae include genera adapted to these extreme conditions, of which many are used as crops and fodder plants. The economically most important genera are: *Spinacia* (e.g. *Spinacia oleracea* or spinach) as an important garden vegetable, *Chenopodium* (e.g. *Chenopodium quinoa* or quinoa and *Ch. pallidicaule* or cañahua) and *Atriplex* (e.g. *Atriplex hortensis* or garden orach) (Kühn, 1993; Wiersema and León, 1999).

Most species of Chenopodiaceae are annual or perennial herbs or shrubs and, rarely, small trees. The plants are either evergreen or deciduous, and can be monoecious, dioecious or polygamous. The roots are normally taprooted and fibrous but sometimes fusiform or bulbous, or fleshy and thickened, as in *Beta*. The stems are alternate or opposite, sometimes succulent and apparently jointed or with slippery and aromatic bark, sometimes spiny. They are often farinaceous, due to the presence of inflated salt glands (trichomes) that senesce into white flakes (e.g. *Chenopodium album*). Some genera have stellate (*Axyris*, *Ceratocarpus* and *Kraschenninnikovia*) or glandular (e.g. *Dysphania*) trichomes, mostly on the leaves and also on the stems. The leaves are usually herbaceous or membranous, but can sometimes be fleshy, as in *Suaeda*, and are usually alternate, occasionally opposite, without stipules, petiolate or sessile and sometimes reduced to small scales. The blade form varies from linear to broadly triangular, with margins entire to serrate, serrate-dentate, or lobed. The inflorescences are usually condensed-spiciform, condensed-thrysoid or spiciform. Bracts are leaf-like, can be deciduous or persistent if they are present. The flowers are bisexual or unisexual, radially symmetric (*Arthrocnemum*, *Salicornia* and *Sarcocornia*) and sometimes bracteolate. The perianth is herbaceous to membranous, rarely scarious, fleshy in *Salicornia* and *Sarcocornia*; 1-5 segments or absent in *Grayia* and *Zuckia*; green and incospicuous; free or connate, strongly imbricate in *Nitrophila*. Male flowers with 1-5 stamens, epitepalous. Female flowers mostly 1-2 pistils and 1-3 styles, few enclosed by two accrescent tepal lobes (*Atriplex* and *Spinacia*, Flores-Olvera et al., 2011). The ovary is usually superior or semi-inferior in *Beta*, 1-locular with a single and basally attached ovule. The fruits are achenes or utricles, vertical or horizontal with respect to the perianth parts. The fruits are surrounded by brown, black or reddish brown bracteoles monomorphic or sometimes dimorphic. The perianth segments are deciduous or persistent in mature fruits with various shapes and ornamentation. The

pericarp is adherent or nonadherent, chartaceous or papery, sometimes reticulate, mottled or smooth. The seeds are vertical or horizontal compressed in longitudinal section; black, brown, reddish brown or a mixture of these colours (e.g. *Ch. quinoa* and its varieties); margin winged, acute or rounded; surface smooth and shiny or reticulate, rugulate, verrucate or prickly. The embryos are large, curved to annular or spirally coiled; the radicle position is medial or basal, ascending or pointing outward (Iljin, 1936; Aellen, 1960; Wilson, 1984; Kühn, 1993; Welsh et al., 2003; Bonzani et al., 2003; Shepherd et al., 2005; Heklau and Röser, 2008; Acosta et al., 2009).

The morphological characters used for the subdivisions within Chenopodiaceae are usually the plant habit and succulence, the leaf morphology, the inflorescence and the floral morphology (Kühn, 1993; Welsh et al., 2003). Hence, the subfamilies Chenopodioideae (mostly non-succulent herbs, well developed leaves, inflorescence richly flowered), Salicornioideae (succulent herbs or shrubs, reduced leaves, flowers in groups of three sunken into cavities), Salsoloideae (herbaceous or succulent, leaves mostly linear, flowers in groups of 1-3) and Polycnemoideae (herbs, subshrubs or shrubs, clustered leaves, flowers solitary) have been broadly accepted prior to the phylogenetic analyses of Chenopodiaceae (Fig. 1.1; Kühn, 1993; Heywood et al., 2007). The morphological hypotheses underlying this subdivision within Chenopodiaceae were first tested in a phylogenetic context by Kadereit et al. in 2003. This phylogenetic work based on the plastid marker *rbcL* had depicted five major clades each ranked at subfamily level, delimitation supported later by Müller and Borsch in 2005 based on *matK/trnK* (see Fig. 1.2). Therefore, based on these phylogenetic results a new tribal reorganization was necessary. In this sense, Polycnemoideae (subfamily under Chenopodiaceae) was recovered as a differentiate lineage without a clear relationship to Chenopodiaceae or Amaranthaceae. But based on on their *rbcL* data Kadereit et al. (2003) transferred Polycnemoideae to Amaranthaceae (Fig. 1.2). The tribe Beteae (under Chenopodioideae based on morphology) was raised into the subfamily Betoideae based on *rbcL* (Kadereit et al., 2003). But based on *matK/trnK* Betoideae was split into two lineages: Betoideae I encompassing *Beta*, *Hablitzia*, and *Aphanisma* and Betoideae II encompassing only *Acroglochin* (Müller and Borsch, 2005a; Fig. 1.2). The tribe Corispermeae under Chenopodioideae (sensu Kühn, 1993) was raised to the subfamily Corispermoideae. Similar to the previous case, the tribe Suaedeae under Salsoloideae (sensu Kühn, 1993)

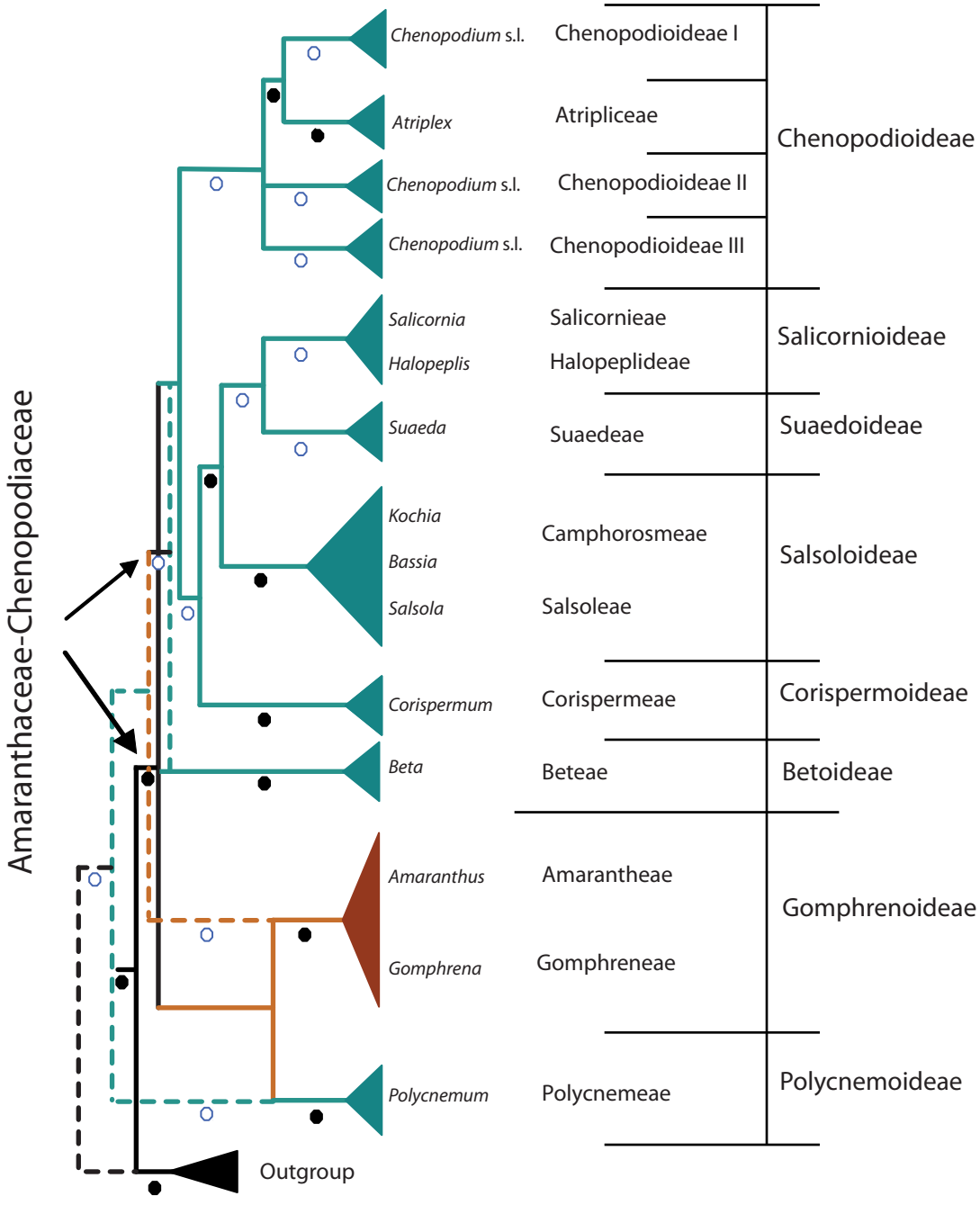


Fig. 1.2 – Simplified cladogram of Chenopodiaceae-Amaranthaceae, bold lines are based on Kadereit et al. (2003) and dotted linea are based on Müller and Borsch (2005a). Green clades correspond to Chenopodiaceae, orange clade correspond to Amaranthaceae. Only the high supports are ploted as black circles based on the *rbcL* inferred tree of Kadereit et al., (2003) and white circles are based on *matK/trnK* inferred tree of Müller and Borsch (2005a).

was suggested to be raised to the subfamily Suaedoideae (Fig. 1.2). The subfamily Salsoloideae was redefined with the inclusion of the tribe Camphorosmeae, which encompassed the tribe Sclerolaeneae, both tribes classified under Chenopodioideae sensu Kühn (1993). Salicornideae is the unique subfamily which is supported encompassing the tribes Halopeplideae and Salicornieae sensu Kühn (1993). Finally, Chenopodioideae was redefined to encompass the tribe Atripliceae and the tribe Chenopodieae. While Atripliceae is monophyletic, Chenopodieae was split into three clades: Chenopodieae I-III (Kadereit et al., 2003).These broad scale phylogenetic studies showed the well defined monophyletic lineages within Chenopodiaceae (Kadereit et al., 2003; Müller and Borsch, 2005a). The resolved lineages within Chenopodioideae are: Chenopodieae I encompassing *Chenopodium* sensu lato, *Rhagodia*, and *Microgynoecium*, Chenopodieae II enclosing *Chenopodium* s.l., *Scleroblitum*, *Monolepis* and *Spinacia*, Chenopodieae III encompassing *Dysphania*, *Chenopodium* s.l., *Teloxys*, *Axyris* and *Kraschenninnikovia* and Atripliceae enclosing *Atriplex*, *Holmbergia* and *Halimione* (Kadereit et al., 2003). The studies leading to these phylogenetic relationships showed that *Chenopodium* s.l. is polyphyletic with unclear relationships. An obvious and important next step was to clarify the internal relationships within the clades of the subfamily Chenopodioideae at both, generic and species levels. In this respect, this thesis explores the relationships of *Chenopodium* s.l and related genera within the monophyletic subfamily Chenopodioideae (Chapter 2 published as Fuentes-Bazan et al., 2012). The dense sampling of *Chenopodium* s.l. and related genera (e.g. *Atriplex*, *Spinacia*, *Mycrogynoecium*) in Chapter 2 of the present study clarify the tribal delimitations within Chenopodioideae and support the delimitations of other studies (Kadereit, et al., 2010; Zacharias and Baldwin, 2010).

1.2 The genus *Chenopodium* L.

Chenopodium sensu lato is a cosmopolitan genus reported from dry habitats of temperate and subtropical regions, and is considered the second biggest genus (after *Atriplex*) in Chenopodiaceae. Earlier works reported about 150 species for *Chenopodium* (e.g. Kühn, 1993). However, presently, *Chenopodium* s.l. comprises about 115 species due to intraspecific rearrangements, as for example the transference of the former *Chenopodium* subg. *Ambrosida* with about 32 species (Scott, 1978a) to *Dysphania* (Mosyakin and Clemants, 2002 and 2008). Whereas, the leaf shape of *Chenopodium* s.l.

commonly resembles a goosefoot (Greek: *chen*=goose; *pous*=foot), its characters has been commonly used for delimitation species within the genus. For this purpose, the most important taxonomic characters are: leaf shape, leaf base and margins, type of indumentum, type of trichomes, presence of aroma, inflorescence architecture, presence or absence of bract and bracteoles, number and fusion of the perianth parts, seeds surface morphology and their position respect to the perianth (Aellen and Just, 1943; Aellen, 1960; Uotila, 2001; Clemants and Mosyakin, 2003).

The economically important species of *Chenopodium* s.l. are *Ch. berlandieri* subsp. *nuttalliae*, which is cultivated in Mexico and named "Huauzontle" by the Aztecs, and the two cultivars of the Andean regions: *Ch. pallidicaule* named "cañihua" and *Ch. quinoa* named "quinua" by the Quechuas. The inflorescence and seeds of these crops are usually consumed as food on the American continent (Wiersema and León, 1999; Bonifacio, 2003). Other species of *Chenopodium* are also reported to be consumed, for example *Ch. murale* or *Ch. bonus-henricus*, both used as vegetables in Europe (Wiersema and León, 1999).

1.2.1 Taxonomic treatments

Taxa with angular leaves "*foliis angulofis*" and taxa with entire leaves "*foliis integris"* were the first subdivision within *Chenopodium*, suggested by Linnaeus (1753). Later, *Chenopodium* was divided into sections and subsections based on the described morphological characters and its states (e.g. sect. *Botryois* with aromatic and farinose plants described by Moquin-Tandon in 1849). The most comprehensive treatments are: i) the treatment for the American *Chenopodium* where the genus was divided in 10 sections and 4 subsections, as described by Aellen and Just (1943), and ii) the treatment in "Illustrierte Flora von Mittel Europa" where the sections of *Chenopodium* s.l. increase to 13, with 12 subsections published by Aellen (1960). The more recently taxonomic treatments of Scott (1978a) and Mosyakin and Clemants (1996, 2002) are still largely based on the sections and subsections of the previous treatments of Aellen and Just (1943) and Aellen (1960), with the main differences of recognizing the subg. *Blitum* and the subg. *Chenopodium*, and transferring the subg. *Ambrosia* to *Dysphania* based on the aromatic character of the species under subg. *Ambrosia* (the complete historical overview of the classification systems in *Chenopodium* is exposed in Chapter 2).

The species delimitation within *Chenopodium* s.l. is difficult because their high morphological variability. Currently treatments of *Chenopodium* s.l. are mostly regional, for example in Flora of Australia (Wilson, 1984); Flora of Pakistan (Freitag, et al., 2001); Flora Nordica (Uotila, 2001), Flora of North America (Clemants and Mosyakin, 2003). All these regional treatments do not consider the ample morphological variability of the species within its geographic distribution like the cosmopolite species *Ch. album* or *Ch. murale*. For example *Ch. album* is one of the world's most widespread weed (Uotila, 2001). Beyond that *Ch. album* is the type specimen of *Chenopodium* s.l. (Fig. 1.3), the cosmopolitan *Ch. album* in a wide sense is extremely variable in size, branching habit, leaf shape, inflorescence architecture, tepal and seed characters (Uotila, 2001). Moreover, in the vegetative state *Ch. album* is easily confused with several other species (e.g. *Ch. berlandieri*, *Ch. strictum*). The insufficiently understood forms of *Ch. album* are arranged in a loosely aggregate (Clemants and Mosyakin, 2003). For example part of this aggregate are: *Ch. album*, *Ch. giganteum*, *Ch. probstii* and *Ch. opulifolium*, *Ch. striatiforme*, *Ch. strictum* and *Ch. berlandieri*, *Ch. suecium* and *Ch. ficifolium* (Dvořák and Grüll, 1983; Clemants and Mosyakin, 2003; Rahiminejad and Gornall, 2004).

1.2.2 The Andean crop *Chenopodium quinoa* Willd.

Louis Éconches Feuillée in 1725 described the crop observed in Peru named "Quinoa" as *Chenopodium folio sinuato saturo virente* (Fig. 1.4). This author mentioned that the natives consumed the seeds cooked like rice as in Europe. Later, Willdenow in 1797 published in Linnaeus, "Species Plantarum" the Feuillée's specimen as *Chenopodium quinoa* Willd. from Chile. However, in the original description of Feuillée (1725) it was mentioned that the plant was observed and probably collected in Peru. At the present time these crops still grow in both Peru and Bolivia.

Quinoa and its putative wild allies occur throughout the Andes from Southern Colombia to the Highlands (Altiplano) of Northern Chile and Western Argentina. The Altiplano is a mountain plateau that has an average height of 3500 to 4000 m above sea level. The average temperature during the day ranges from about 20°C in the summer to about 15°C in the winter. During the nights, the temperature falls to about 4°C in the summer and about -10°C in the winter. In Bolivia, quinoa is distributed in the Altiplano and in the interandine valleys. The highest morphological variability of quinoa is found

A B

Fig. 1.3 – *Chenopodium album* L. A) Drawing of Carl Axel Magnus Lindam published in *Bilder ur Nordes Flora* (1901-1905), download from: http://caliban.mpiz-koeln.mpg.de/lindman. B) Type specimen from the Linnean herbarium, download from: http://plants.jstor.org.

around the Titicaca Lake between Bolivia and Peru. In Bolivia the Titicaca Lake region is part of the northern Altiplano. The elevation of the Titicaca region is 3809 m above sea level and the annual precipitation is about 800 mm (Argollo and Mourguiart, 2000). The commercial variety “Quinoa Real” is cultivated in the central and southern Altiplano of Bolivia, at more than 3600 m above sea level, with an annual precipitation of only about 200 mm and on soil with a high salt concentration (Gandarillas, 1968; Argollo and Mourguiart, 2000).

Quinoa is an important grain crop in Bolivia and Peru; its seeds are an important pseudocereal containing a higher amount of protein (13% to 22.5%) and essential amino acids compared to soy beans, wheat and meat (Ayala et al., 2001; Jellen et al., 2011). Additionally, the native people of the Andes also use the plant as a vegetable, in addition to use as animal feed and commonly as an ornamental plant. The people usually cook the seeds in soups and salads and use them as cereal mixed with dry fruits and milk for breakfast. The seeds can also be grounded and converted into flour for baking; quinoa flour is often also mixed with chocolate for children’s breakfast. It is also possible to extract vegetable oil from these seeds, comparable to corn oil, for human consumption (Feuillée, 1725; Tapia et al., 1979; Koziol, 1993). Quinoa has also been tested for use in the manufacturing of cardboard based on the extraction of its cellulose and pectin. Quinoa contains saponins which can be used for insecticides, poison, or for the manufacturing of beer and shampoo. The adaptive and nutritive qualities of quinoa have led to an increase in importance of this vital crop over the last 50 years, reflected also in the spread of quinoa crops to other countries and continents (e. g. USA and Europe). In 1982, the production of quinoa, as reported by the Food and Agriculture Organisation of the United Nations (FAO), was about 15.000 t. In 1991 the production increased to about 25.000 t with a price set at around 0.40 USD per kg (Mujica et al., 2001; FAOSTAT, 2011). More recently, the production has remained constant but the price has increased from about 0.80 USD per kg in 1999 to 1.32 USD per kg in 2009 (FAOSTAT, 2011).

Archaeological evidence of pre-Columbian cultures of South America reported the use of quinoa between 5000 or 3000 B.C. (Bruno and Whitehead, 2003). This period corresponds to the Tiahuanaco culture, which is an important pre-Columbian culture distributed from Southern Peru until Northern Chile. It is likely that quinoa seeds were at first only collected from wild or semi-wild plants, commonly with black seeds by the

people of the Tiahuanaco culture (Kolata, 1986). Bruno and Whitehead (2003) suggested that the domestication period of quinoa was developed between 1500 B.C. and 100 A.C. Based on the analysis of seed frequencies in the south of Titicaca Lake it was proved that the domestication of quinoa occurred through the selection of a specific seed form (Bruno and Whitehead, 2003). Bruno and Whitehead (2003) found two kinds of seeds in similar proportions dated to 1500 B.C. and classified the wild type with black seeds as *Ch. quinoa* var. *melanospermum* and the cultivate type with white seeds as *Ch. quinoa*. Around 800 B.C. the increased proportions of "white seeds" related to the "black seeds" suggested that the farmers became more thorough cultivators of the domesticated quinoa (Bruno and Whitehead, 2003). Later, the expansive Inca culture also used the quinoa crop as an important element for consumption and religious ceremonies. Quinoa seeds were found with human remains in small tombs called "Chulpas" suggesting that the crop was an important element for their funerary rites (Mujica et al., 2001). During the Spanish colonization, Pedro de Valdivia reported in 1540 for the first time the use of quinoa as a crop in Concepción Chile. Subsequently, quinoa was reported in Peru in 1560 by Cieza de León and Feuillée (1725), in Bolivia in 1964 by Patiño and in Colombia by Humboldt (for a detailed citation of the historical records see Mujica, et al., 2001). Although the colonizers knew about the use of quinoa from the local people they were not interested in it (Mujica et al., 2001) and instead introduced crops like rice and wheat into the Andean region (Mujica et al., 2001). After the colonization, the Aymara, Quechua and other ethnic groups from South America returned to the original use of quinoa, because this crop had been used by their ancestors as an important part of their culture (Mujica et al., 2001).

Fourty-two varieties of quinoa were reported by Rojas (2001) in his "Catalogue of the collection of Quinoa", distinguished on the habit of the plant, inflorescence pigmentation, and seed morphology and pigmentation (Gandarillas, 1968; Rojas, 2001). Agronomic evaluations of quinoa and its putative relatives described that the "wild type" of quinoa has black seeds of about 1 mm diameter with dormancy. Additionally, the "wild type" plants are branched and grow to about 1 m, the inflorescence is not compact and the plant is green (Gandarillas, 1968; Mujica et al., 2001; Rojas, 2001). In the taxonomic treatment of Aellen and Just (1943) the "wild type" is formally classified and named as *Ch. quinoa* subsp. *milleanum* (Aellen and Just, 1943). The cultivated form classified as *Ch. quinoa* has white seeds of about 3 mm diameter without dormancy, the plants have a high

Fig. 1.4 – *Chenopodium quinoa* Willd. A) Drawing of *Chenopodium folio sinuato sature virente*, vulgo Quinoa by Louis Feuillée, modified from Plate X in Feuillée (1725). B) Specimen from Bouché studied by Willdenow, deposited in BGBM Herbarium (B).

variability of pigmentation pattern, are mostly not branched and grow to about 2 m and the inflorescence is compact (Gandarillas, 1968; Planchuelo, 1975; Rojas, 2001). The biggest collection of varieties of quinoa seeds are in the seed bank of Bolivia (PROINPA). This seed collection contains 2701 accessions from Bolivia, Peru, Ecuador, Chile, Argentina, Mexico and Europe (Rojas, 2001).

1.2.3 The North American crop *Chenopodium berlandieri* subsp. *nuttalliae* (Saff.) Wilson & Heiser

The distinguished archaeologist Zelia Nuttal reported and collected for the first time the Mexican cultivar "Huautzontli" in 1917. The common name of this plant came from the náhuatl language. "Huautzontli" was widely cultivated and used by the Aztecs to pay tributes to the Emperor Moctezuma and also used for religious practices (Safford, 1918; Wilson and Heiser, 1979; Cruz Torres et al., 2009). Archaeological evidence for the origin of *Ch. berlandieri* subsp. *nuttalliae* was founded in eastern North America (Wilson, 1990; Jellen et al., 2011). Based on DNA ancient phylogenetic analysis of the archaeological North American chenopods has been suggested that the domestication of *Ch. berlandieri* forms was previous to the maize domestication and consequently pre-Columbian (Kistler and Shapiro, 2011).

In 1918 Safford published the taxonomic description of the "Huautzontli" under *Chenopodium nuttalliae* in honour of her discoverer (Fig. 1.5). Later *Ch. nuttallieae* was transferred under *Ch. berlanderi* as subspecies *nuttalliae,* based on its morphological and biochemical characters (Wilson and Heiser, 1979). *Ch. berlandieri* subsp. *nuttallieae* grows on the high regions in the Valley of Mexico, Michoacán, Oaxaca, Veracruz and Tamaulipas, around 2100 to 2600 m above sea level. The average temperature is more than 17°C in the summer and about 15°C in the winter with an annual rainfall from 1000 to 1200 mm (Safford, 1918; Rzedowski et al., 2005; Cruz Torres et al., 2009). The nutritional studies of *Ch. berlandieri* subsp. *nuttallieae* are not yet completed although current knowledge suggests protein content ranging from 13.15% to 17.95% (Cruz Torres et al., 2009). The common use of "Huautzontli" by the local people is to boil or fry the immature inflorescence as soups, stews, tortillas and salads as reported by the Secretaría de Desarrollo Rural of Puebla state. In some regions of the Michoacán state the variety called "chía roja" is cooked as tamales called "chapatas" which is a lump of corn dough prepared

Fig. 1.5 – *Chenopodium berlandieri* subsp. *nuttalliae* Wilson & Heiser. A) Photograph of specimens collected by Mrs. Zelia Nuttall, taken from Safford (1918). B) Type specimen deposited in the United States National Herbarium (US), download from:http://plants.jstor.org.

by mixing corn meal with equal parts of ground “chía roja” seeds (Cruz Torres et al., 2009). Although this crop is not exported the internal consumption shows the economic importance for the rural regions of Mexico where the price per kg ranges from 25 USD to 35 USD (Cruz Torres et al., 2009).

1.2.4 The hypothesis on the origin of the *Chenopodium* cultivars

The hypotheses on the origin for the domesticated forms of *Chenopodium* were broadly based on morphological and biochemical comparative analyses. Wilson (1990) suggested three centers of domestication of *Chenopodium*: i) a North American center with a putative ancient crop; ii) a Mexican center with the crop *Ch. berlandieri* subsp. *nuttalliae* and iii) a South American center where the domestication involves the Andean crop *Ch. quinoa* (Wilson, 1988a; Wilson, 1990).

Heiser and Nelson (1974) in their first morphological analysis about the relationships of the domesticated forms *Ch. quinoa*, *Ch. berlandieri* subsp. *nuttallieae* and *Ch. pallidicaule*. The results suggested that *Ch. quinoa* is closely related to the Mexican crop *Ch. berlandieri* subsp. *nuttalliae*, and that *Ch. pallidicaule* is not related to the other two cultivated species. Further studies based on morphology, allozyme frequences, molecular markers like Random Amplification Polymorphic DNA (RAPD) and finally plastid and nuclear region sequences, largely support the close relationship of *Ch. quinoa* to *Ch. berlandieri* subsp. *nuttalliae* (Wilson and Heiser, 1979; Wilson 1988a; Wilson 1988b; Wilson, 1990; Wilson and Manhart, 1993; Ruas et al., 1999; Rojas, 2001; Kistler and Shapiro, 2011 and this study). Additionally, the first hypothesis about the origin of the domesticated forms *Ch. quinoa* and *Ch. berlandieri* subsp. *nuttalliae* suggested that migrations from South America to North America or migrations from North America to South America may play an important role in this process (Heiser and Nelson, 1974). Later, allozyme frequencies supported the suggestion that the origin of the domesticated group from South America (*Ch. quinoa* cultivar and their “wild type”) was is in North America (Wilson, 1990). However, Wilson and Heiser (1979) suggested that the domestication of both, the South American and North American groups, should have occurred independently because the domesticated forms are more closely related to their sympatric “wild type” forms than to each other. In this sense *Ch. berlandieri* subsp. *nuttalliae* is more closely related to *Ch. berlandieri* var. *zschackei* while *Ch. quinoa* is

more closely related to *Ch. hircinum*. This hypothesis has been recently confirmed by Kistler and Shapiro (2011), based on the molecular analysis of the ancient DNA of the group of *Ch. berlandieri* subsp. *nuttalliae*, *Ch. berlandieri* var. *zschackei* and *Ch. berlandieri* var. *sinutaum*. Wilson (1990) in his monographic work on quinoa and relatives suggested, based on the morphology, that the cultivars of *Ch. quinoa* and *Ch. berlandieri* subsp. *nuttallieae* may be derived from diploids. Studies on the origin of domesticated forms to date have focussed only on the cultivated taxa of *Chenopodium* (Wilson and Heiser, 1979; Wilson 1988a; Wilson 1988b; Wilson, 1990; Wilson and Manhart, 1993; Ruas et al., 1999; Rojas, 2001; Kistler and Shapiro, 2011).

1.3 Molecular approaches towards phylogenetic reconstruction and understanding the evolution of species-rich genera

The major goals of the Systematics are i) to define species' limits and ii) to understand their phylogenetic relationships (Wiens, 2007). In taxonomic studies, morphological characters have been, and still are commonly used in order to define limits of the species in line with the morpho-species concept. However, the use of molecular data for inferring species' limits, represented by clades generated and displayed in phylogenetic trees, has gained in popularity over the use of morphological characters alone (Vandamme, 2009; Korotkova et al., 2011). Considering that all organisms evolved from a common ancestor, phylogenetic methods assume that the "homology" represented by the similarity among the orthologous genes. Therefore, evolution rates of the genes are critical in the phylogenetic reconstructions based on homology, this is especially the case at species level or in resolving relationships between closely related species. In this sense, the genes (e.g. *rbcL*) which have a low rate of evolution could result in a non-resolved tree (polytomy), whereas the noncoding regions or the third-codon position have a faster evolution rate (e.g. *trnL-F* intron, *matK* protein) and could result in a resolved dichotomic tree (Olmstead et al., 1993; Kadereit et al., 2003; Erixon and Oxelman, 2008; Korotkova et al., 2011).

Evolutionary forces favoring speciation are those that decrease variation within a population and increase the variation between populations. If speciation resulted in two reproductively isolated species due to geographical isolation, in the dichotomic terminal tree each clade represents one species and should show the limits of this species in line with the phylogenetic species concept (Nixon, 1990). On the other hand, if speciation and

the reproductive isolation were not complete the analysis could result in a non-dichotomic tree (Rieseberg and Willis, 2007). For instance, in the case of two young populations which are not reproductively isolated, they could have a second contact due to migration where events of hybridization (or reticulation) could happen. This pattern of evolution could be detected by a non-resolved tree because the studied species are recently formed by reticulation events followed by recent adaptation to new regions as one cause (see section 1.4.2; Huson and Bryant, 2006; Vandamme, 2009; Morrison, 2010). Currently, the comparison of the phylogenetic tree of the plastid signal and the phylogenetic tree of the nuclear signal is also used to detect the possible patterns of speciation as reticulation in closely related species (see section 1.5; Fuertes Aguilar and Nieto Feliner, 2003; Erixon and Oxelman, 2008; Löhne et al., 2008; Blöch et al., 2009; Chapter 2 and Chapter 4).

1.3.1 Molecular markers currently used for species level phylogeny reconstruction

The reconstruction of the phylogenetic relationships in species-rich genera requires a broad taxon sampling and a high amount of sequence data to provide enough information. The exact amount of sequence information needed appears to be lineage-specific. Young recently diverged or slowly evolving lineages will require more sequence information. But generally, the combinations of different markers from the same genome show an improved resolution of the phylogenetic relationships (e. g. Murakeözy et al., 2007; Schäferhoff et al., 2010). Currently available studies lead to the conclusion that about 5000 nucleotides per taxon are needed for full species-level resolution (e.g. Barfuss et al., 2005; Löhne et al., 2007; Tesfaye et al., 2007; Erixon & Oxelman, 2008; Korotkova et al., 2011). Because generating multiple sequence data sets for large genera will require large laboratory effort, efficient markers are needed. In angiosperms rapidly evolving plastid markers are usually used for species level phylogenies. These are mostly noncoding regions: plastid spacers, introns, genes and nuclear genes and spacers (Álvarez and Wendel, 2003; Borsch and Quandt, 2009; Calonje et al., 2009). Müller et al. (2006) quantified the hierarchical phylogenetic structure and thus the performance of the *rbcL* gene, the *matK* gene and noncoding parts of *trnT-F* in a taxon set of basal angiosperms, and showed that both quantity and quality of phylogenetic signal considerably differ among genomic regions (*rbcL* < *matK* < *trnT-trnF*). These authors underline that the use of efficient markers is a much better strategy than compiling many sequence data. An

efficient marker for phylogenetic reconstruction needs to have both a high percentage of potentially informative characters and a hierarchically structured historical distribution of mutations in order to allow the reconstruction of robust phylogenetic trees (Müller et al., 2006, Korotkova et al., 2011). Among the most frequently used markers at species level are the *trnL-F* region, the *matK/trnK* region, the intron in *rpl16* and the nuclear ITS region.

The *trnL-F* region is one of the most frequently used phylogenetic markers for the inference of intraspecific and infrageneric relationships (e.g. Worberg et al., 2007, Tank and Olmsead, 2008; Sánchez del-Pino et al., 2009). The region consists of a group I intron in *trnL* $_{(UAA)}$ and a transcribed spacer between *trnL* and *trnF* $_{(GAA)}$. Earlier investigations applied the complete *trnT-trnF* region (Taberlet et al., 1991), but due to the difficulty to amplify the *trnT-trnL* spacer, only the *trnL* intron and *trnL-F* spacer became frequently used. Compared with the *petB-D* spacer, the *petD* intron, and the *matK* gene, *trnL-F* had the highest percentage of variable and informative characters in a phylogenetic study of basal eudicots (Worberg et al., 2007). The good performance of *trnL-F* at family level was also supported in the phylogenetic reconstruction of Amaranthaceae (Sánchez del-Pino et al., 2009).

The *matK/trnK* region consists of a group II intron in *trnK* which hosts an open reading frame (ORF) *matK* encoding the maturase K. The *matK* gene is the only intact ORF within a plastid group II intron (Hausner et al., 2006) and has the highest variability among plastid genes. It is applied for phylogenetic inference either alone or in combination with the *trnK* intron (e.g. Müller and Borsch, 2005a; Müller and Borsch 2005b; Wanke et al., 2007). It was successfully used for resolving relationships at high and low taxonomic levels, for instance *Sonchus* (Kim et al., 2007), *Calceolaria* (Andersson, 2006), *Gilia* (Johnson and Soltis, 1995), *Utricularia* (Müller and Borsch, 2005a), and even for the angiosperms as a whole (Hilu et al., 2003). Its high variability is unusual for coding regions. The *matK* gene was shown to have a much better phylogenetic performance than the *rbcL* gene in basal angiosperms (Müller et al., 2006) and Cactaceae (Korotkova et al., 2011). The *matK/trnK* region was successfully used in several clades of the Caryophyllales including the Amaranthaceae-Chenopodiaceae alliance (Müller and Borsch, 2005a).

The *rpl16* plastid marker is a gene with 2 exons and one group II intron. The *rpl16* gene is usually flanked by *rps3* and *rpl14*. Most families of angiosperms have the *rpl16*

intron except for Geraniaceae, Goodeniaceae and Plumbaginaceae (Campagna and Downie, 1998). The group II *rpl16* intron is one of the most variable noncoding regions compared with other group II introns (Borsch and Quandt, 2009). The phylogenetic utility of *rpl16* intron was for the first time tested in *Chusquea* and other genera of Bambusoideae (Kelchner and Clark, 1997). In this study it was found that the *rpl16* intron is phylogenetically informative at the inter and intrageneric levels in bamboos. The authors consequently suggested the use of this noncoding region in other plant lineages. The generated data sets of *rpl16* intron in Nymphaeales supported that *rpl16* intron is better than *trnK* intron (Löhne et al., 2007). The utility of *rpl16* intron was also useful resolving in the phylogeny of Gomphrenoideae (Amaranthaceae) (Sánchez del-Pino et al., 2009) and of Rhipsalideae (Cactaceae) (Korotkova et al., 2011).

The nuclear ITS marker comprises the two internal transcribed spaces ITS1 (between 18S and 5.8 rDNA) and ITS 2 (between 5.8 rDNA and 26S) of the nuclear ribosomal cistron. This nuclear marker became popular because it is universally present in all angiosperms and the sequences vary from about 500-700 nt (Álvarez and Wendel, 2003). The high variability of the nrITS marker at generic and infrageneric levels was reported by many authors (e.g. Baldwin et al., 1995; Álvarez and Wendel, 2003; Andersson, 2006; Löhne et al., 2008). Despite the popularity and efficiency of ITS, the use of this marker also carries a number of potential problems. One of them is multiple rDNA arrays which result in erroneous phylogenetic reconstructions based on possible paralogous genes. Concerted evolution is another potential problem because the sequences or copies of ITS are complete or nearly completely homogenized. The possibility to have pseudogenes or non functional copies is also a problem in ITS. The secondary structure in ITS can obscure the phylogenetic signal because unpaired bases of the secondary structure may persist sometime as a mutation. The difficulty to align sequences across more distantly related species can be another problem for ITS. All of these problems can result in an incorrect phylogenetic tree (Álvarez and Wendel, 2003; Nieto Feliner and Rosselló, 2007). In order to avoid these problems the use of low-copy nuclear genes has been proposed as an alternative to ITS (Álvarez and Wendel, 2003; Nieto Feliner and Roselló, 2007).

1.4 Effects of reticulation and incomplete lineage sorting in the diversification of angiosperm genera

The extraordinary diversity of angiosperms encompassing a vast morphological, functional, and ecological versatility is triggered by speciation (Magallón and Castillo, 2009). Reticulation or reticulate evolution plays an important role in plant speciation (Soltis et al., 2003). Reticulate evolution involves hybridization or introgression of different genomes resulting in high or different levels of ploidy and heterozygosity (Wehrt et al., 1985; Soltis et al., 2003). These patterns of reticulation through hybridization are well documented in angiosperms, based on incongruent phylogenetic signals from different genome organelle compartments (Wendel and Doyle, 1998; Sang and Zhong, 2000, Löhne et al., 2008). On the other hand, the incongruence reflected in the topology of trees inferred by phylogenetic analysis could be also due to the "incomplete lineage sorting" on the used region or gen. While a "gen tree" is inferred based on a genomic regions data set (e.g. *trnL-F* and ITS) a "species tree" is the representation of the pattern of branching of species lineages through speciation. In angiosperm plants lineage sorting is recognised because the relationship of genes from different lineages is closer than between genes within a single population of one species (Wendel and Doyle, 1998). Young species in particular should be affected by incomplete linage sorting because the linage-specific genes are not fixed. Within species, ancestral polymorphic genes could be generated through mutation events and lost via selection (Carstens and Knowles, 2007). Therefore, some genes could be older than their respective species, some lost via selection, and other genes within a population are polymorphic and all of them are not necessary expected to be monophyletic or represent the same pattern of speciation than the species. Due to this process, the "gen trees" will be different to the "species tree" (Wendel and Doyle, 1998; Jakob and Blattner, 2006). Moreover, the possibility to have a complex scenario with hybridization is very common in the populations of young species which had not yet fixed its lineages and reproductive system (Jakob and Blattner, 2006). Reticulation and incomplete lineage sorting are considered two indistinguishable biological causes of topological incongruence (Wendel and Doyle, 1998; Sanderson et al., 2000; Madison and Knowles, 2006).

1.5 Polyploidy in flowering plant genera and current approaches to their study

Most studies of speciation focus on how lineages or species diverge; however speciation is not always about divergence (Rieseberg and Willis, 2007). In flowering plants the duplication of the entire genome is frequent. About 70-80% of all angiosperms are supposed to be polyploids (Soltis et al., 2003). The polyploid speciation in plants can be categorized as *autopolyploid* and *allopolyploid*. While the autopolyploid speciation results in chromosome duplication within a single species, the allopolyploid speciation involves the reunion of divergent genes and genomes, where the chromosome number is duplicated through hybridization (Rieseberg and Willis, 2007; Soltis et al., 2007). The polyploid speciation in plants may be a success strategy for their adaptation to new environments. In higher latitudes and altitudes the common presence of polyploids was well documented, for instance the high frequency of polyploids in the arctic region (Guggisberg, et al., 2009), was also found in the high altitudes of the Andes (Soltis et al., 2003, Lee et al., 2002, Soltis and Soltis, 1999). Moreover, the allopolyploidy in cultivated plants is also commonly observed, for example, in *Coffea arabica* (Lashermes et al., 1996; Tesfaye et al., 2007) and in *Oriza sativa* (Ge et al., 1999). Several studies based on morphology, crosses, chromosome accounts and allozyme patterns have demonstrated that in *Chenopodium* the cultivated *Ch. berlandieri* subsp. *nuttalliae* and *Ch. quinoa* within *Chenopodium* are allotetraploids and the non-cultivated *Ch. album*, *Ch. opulifolium* and *Ch. giganteum* are allohexaploids (Wilson, 1983; Wilson and Manhart, 1993; Bhargava et al., 2006).

Polyploid speciation can be inferred by phylogenetic reconstruction based on plastid and nuclear sequences. The nuclear ITS marker is one of the most popular markers for inferring reticulation patterns in flowering plants at species level (Álvarez and Wendel, 2003; Nieto Feliner and Rosselló, 2007). In ITS sequences both parental ribotypes can be present and detected by polymorphic sites (Fuertes Aguilar and Feliner, 2003; Löhne et al., 2008). It is expected that the biparental phylogenetic signal of the ITS marker will differs from the maternal phylogenetic signal of the plastid marker in the tree inference (Sang and Zhong, 2000; Löhne et al., 2008). Conflictive tree topologies from each region are used to reconstruct the reticulate nature of the hybrid speciation (Soltis, 1995; Sang and Zhong, 2000; Albach and Chase 2004; Löhne et al., 2008). Even using highly informative regions (e.g. *trnL-F*, *matK/trnK*, *rpl16 K intron* for the plastid genome, ITS, ETS or low copy regions) the phylogenetic tree could result in a non-resolved tree, especially in species-rich

genera. Then the non-resolved tree (polytomy) show that the relationships and the evolution of the studied taxa do not fit in a dichotomic tree-like model. Thus, some reticulate signal could be lost when the reconstruction of this evolution is only based on phylogenetic trees (Morrison, 2010; Huson et al., 2011). Currently phylogenetic networks are proposed in order to explore the data sets and to generate biological hybridization hypotheses (Linder and Rieseberg, 2004; Huson and Bryant, 2006; Morrison, 2010; Huson et al., 2011). Where the Phylogenetic network is defined as any graph used to represent evolutionary relationships (either abstractly or explicity) between a set of taxa that labels some of its nodes (usually the leaves) (Huson et al., 2011). For example, Morrison (2010), based on networks analyses of different data sets from other studies, showed that one reticulation event of *Viburnum* (Adoxaceae) were not detected by the original authors. This study also showed how the split networks could represent patterns of incomplete lineage sorting and hybridization.

1.6 Aims of this study and project design

The principal aim of this PhD-project is to clarify the phylogenetic relationships of the species-rich genus *Chenopodium* L. and its allies within the subfamily Chenopodioideae in order to give a clear framework for further studies on the evolutionary patterns of the delimited lineages as *Chenopodium* s.str. To outline the species delimitations and to reconstruct putative evolutionary events a combination phylogeny reconstruction, taxonomy, and network reconstruction are needed. In order to address these targets is necessary to: i) construct a well supported tree of *Chenopodium* s.l. based on suitable markers and broad sampling, ii) define species delimitations and taxonomic implications based on phylogenetic reconstruction of *Chenopodium* s.l. within Chenopodioideae and iii) reconstruct the relationships and evolutionary patterns of *Chenopodium* s.str. using phylogenetic and network reconstruction.

The success of the phylogenetic study on species-rich genera is strongly dependent on the sampling and the phylogenetic quality signal of the markers. The selected marker should have enough variability and high phylogenetic signal in order to make inferences at species level. This is particularly true for delimitations made at species level where the divergence is commonly recent and low variable markers could result in poorly resolved

trees. Therefore, for the present study the plastid regions selected are *trnL-F*, *matK/trnK* and *rpl16* intron and the nuclear region ITS.

In the first part of this study a broad sampling within the polyphyletic genus *Chenopodium* includes: 9 from 13 sections defined in the treatment of Aellen (1960) and also representative genera within Chenopodioideae (*Atriplex, Axyris, Ceratocarpus, Dysphania, Einadia, Grayia, Kraschenninikovia, Mycroginoecium, Rhagodia, Suckleya, Stutzia* and *Teloxys*). The phylogenetic analysis is based on the highly variable noncoding plastid *trnL-F* and the noncoding nuclear region ITS. The taxonomic delimitations within *Chenopodium* L. are tested and *Chenopodium* s.str. is defined with taxonomic implications within Chenopodioideae (Chapter 2). Together with the *matK/trnK,* the phylogenetic reconstruction, on a representative sampling of the *Chenopodium* s.l. lineages is presented. Well defined and supported lineages of the taxa suggest the delimitation of three genera within Chenopodioideae. The discussion based on the taxonomic changes of each taxon, and finally the conclusive delimitation based on the phylogenetic reconstruction is discussed (Chapter 3). The combined data set of three highly performing noncoding *trnL-F*, *matK/trnK* and *rpl16* intron plastid markers was applied to resolve the intraspecific relationships of *Chenopodium* s.str. The reconstruction of the evolutionary patterns is based on the comparative analyses between the plastid combined data set and the ITS nuclear region. Phylogenetic tree topologies of each plastid and nuclear partition are tested and only the significantly incongruent positions are analyzed. Exploratory network analyses are presented for each the plastid data set and the nuclear data set used to test the congruence of the lineages. Finally, the biological hybridization hypotheses, based on the hybridization network reconstruction for the allopolyploid taxa are done. Therefore, new biological hypothesis and possible scenarios for the hybridization and domestication for *Ch. quinoa* and *Ch. berlandieri* subsp. *nuttalliae* are discussed (Chapter 4).

1.7 Dissertation structure

The structure of this dissertation follows the accumulative format. In this sense each chapter is a manuscript, either published or in preparation to be submitted. Thus, Chapter 2 is structured as a journal article, and Chapters 3 and Chapter 4 are in preparation to be submitted. Each chapter includes a separate material, methods and appendixes

sections. Full references are given after Chapter 5 and a general appendix with the data sets from each chapter and its analyses is provided in a DVD.

Chapter 1. General introduction.

Chapter 2. Fuentes-Bazan, S., Mansion, G. and Borsch, T. (2012) Towards a species level tree of the globally diverse genus *Chenopodium* (Chenopodiaceae). Mol. Phylogenet. Evol. 62, 359-374.

Chapter 3. Fuentes-Bazan, S., Uotila, P. and Borsch, T. Resurrecting the Linnean genus *Blitum* and recovering two new genera for Chenopodioideae (Chenopodiaceae) based on phylogenetic reconstruction. In preparation for Willdenowia.

Chapter 4. Fuentes-Bazan, S. and Borsch, T. Phylogeny of *Chenopodium* sensu stricto, patterns of reticulate evolution, and possible origins of Quinoa. In preparation for Molecular Phylogenetics and Evolution.

Chapter 5. General conclusions and outlook.

Chapter 2

Towards a species level tree of the globally diverse genus *Chenopodium* (Chenopodiaceae)

Contents

2.1. Introduction

The genus *Chenopodium* sensu lato has been estimated to comprise some 150 species (Kühn, 1993). Most of them are annual herbs growing in arid or semi arid regions, and also on salt-rich soils. Compared to other plants of dry environments they lack typical adaptations to such ecological conditions, such as the Kranz type leaf anatomy and the C_4 photosynthetic pathway - both frequent in other Chenopodiaceae (Carolin et al., 1975, Jacobs, 2001) - and succulence. Morphologically, *Chenopodium* shows great variability in leaf shape and indumentum, floral structures, inflorescence architecture, and seed morphology (Aellen and Just, 1943; Kühn, 1993; Clemants and Mosyakin, 2003). While a large number of different species and intraspecific taxa have been described, the latest most comprehensive synopsis dates back from around 60 years ago (Aellen and Just, 1943), despite the fact that several species are economically important either as crops (e.g., *Chenopodium berlandieri* Moq. subsp. *nuttalliae* (Saff.) H.D. Wilson and Heiser, 1979 [“Huauzontle”]; *Ch. pallidicaule* Aellen [“Cañihua”]; and *Ch. quinoa* Willd. [“Quinoa”]) or weeds (*Ch. ambrosioides* L.; *Ch. murale* L.; Wiersema and León, 1999). An integrative approach to a modern systematic treatment is therefore needed.

Chenopodium belongs to the subfamily Chenopodioideae, within the goosefoot family Chenopodiaceae (Caryophyllales). Chenopodiaceae contain approximately 100 genera and 1700 species, mainly distributed in temperate and subtropical regions of both hemispheres (Aellen, 1960; Kühn, 1993; Welsh et al., 2003). Results of recent molecular phylogenetic analyses (e.g., Kadereit et al., 2003) are in line with earlier classification systems with regard to this placement of *Chenopodium* (see Kühn, 1993). Although phylogenetic relationships of major lineages within Chenopodiaceae still remain poorly understood, the subfamily Chenopodioideae is considered to be monophyletic, based on sequence data of chloroplast *rbcL* (Kadereit et al., 2003) and *matK*/*trnK* (Müller and Borsch, 2005).

While ongoing multigene analysis confirms the monophyly of the Chenopodioideae (Borsch et al., unpubl. data) all phylogenetic studies hitherto carried out, indicate that *Chenopodium* is polyphyletic. Species of *Chenopodium* were found in three different clades within the Chenopodioideae. These clades were initially named Chenopodieae I-III (Kadereit et al., 2003; Müller and Borsch, 2005) and constitute the subfamily

Chenopodioideae together with the tribe *Atripliceae*. Recent progress has been made in elucidating the evolutionary history of the *Atripliceae* based on DNA sequence data (Kadereit et al., 2010; Zacharias and Baldwin, 2010), in the context of which a distinct status of *Axyrideae* and *Dysphanieae* (both formerly Chenopodieae III) was also recognized. Nevertheless, taxon sampling and tree resolution remain insufficient for a reliable circumscription of *Chenopodium*. The aromatic species are the only group of *Chenopodium* species that have been better characterized phylogenetically. The first *rbcL* tree of Kadereit et al. (2003) revealed relationships between *Dysphania glomulifera* and other aromatic taxa within Chenopodieae III, but these lacked statistical support. More recently Kadereit et al. (2010) included four aromatic species of *Chenopodium* along with *Cycloloma*, *Suckleya* and *Teloxys*, providing greater confidence for a clade for which the tribal name *Dysphanieae* was resurrected.

The complex taxonomic history of *Chenopodium* is summarized in Table 2.1, and shows over time large differences in the number of sections (between two and 13) and subsections that were recognized (Moquin-Tandon, 1849; Bentham and Hooker, 1880; Ulbrich, 1934; Aellen and Just, 1943; Aellen, 1960; Scott, 1978a; Wilson, 1983; Mosyakin and Clemants, 1996; Judd and Ferguson, 1999; Clemants and Mosyakin, 2003). The most comprehensive treatments remain those of Aellen and Just (1943) and Aellen (1960), upon which the morphology-based classification system of Mosyakin and Clemants (1996; 2002) is largely based. Compared to the previous classification system, these authors recognized the subg. *Blitum* within *Chenopodium*, and the distinct genus *Dysphania* (Table 2.1). Mosyakin and Clemants (2002; 2008) pointed out that *Dysphania* is the oldest name for this group and consequently re-classified the subgenus *Ambrosia* with all its sections under the generic name *Dysphania*. Although there is now even increased phylogenetic support for the aromatic species to be a distinct group (Kadereit et al., 2010), the majority of aromatic species has never been included into any molecular phylogenetic analysis.

Chromosome counts in different species of *Chenopodium* show a great extent of valences, from diploid (2n=2x=18) to hexaploid (2n=6x=54; e.g. Aellen and Just, 1943; Uotila, 1973; Rahiminejad and Gornall, 2004; Bhargava et al., 2006). Based on these counts, a base number for *Chenopodium* of x=9 was suggested (Aellen and Just, 1943; Bhargava et al., 2006). However 2n=2x=16 chromosomes were reported for *Ch. ambrosioides* (Uotila, 1973; Palomino et al., 1990), while *Spinacia oleracea* was reported

Table 2.1 – Historical overview on classification systems in *Chenopodium* L.

Moquin-Tandon 1849	Bentham and Hooker 1880/ Volkens 1893	Ulbrich 1934	Aellen and Just 1943	Aellen 1960	Scott 1978	P.G. Wilson 1983	Mosyakin and Clemants 1996
				***Chenopodium* L.**			
					Subg. Ambrosia	**Subg. Ambrosida**	
Sect. *Botryois*	Sect. *Botrydium*	Sect. *Botryoides*	Sect. *Botryoides*	Sect. *Botryoides*	Sect. *Botryoides*		
			Subsect. *Botrys*	Subsect. *Botrys* **(3)**	Subsect. *Botrys*		
			Subsect. *Teloxys*	Subsect. *Teloxys* **(3)**	Subsect. *Teloxys*		
	Sect. *Ambrina*	Sect. *Ambrina*	Sect. *Ambrina*	Sect. *Ambrina* **(4)**	Sect. *Ambrina*	Sect. *Ambrina*	
	Sect. *Orthosporum*	Sect. *Orthosporum*	Sect. *Orthosporum*	Sect. *Orthosporum* **(4)**	Sect. *Orthosporum*	Sect. *Orthosporum*	
							Subg. Blitum
	Sect. *Blitum*						Sect. *Blitum*
	Sect. *Pseudoblitum*	Sect. *Pseudoblitum*	Sect. *Pseudoblitum*	Sect. *Pseudoblitum*			
				Subsect. *Viridia* **(4)**			
				Subsect. *Glauca* **(2)**			
		Sect. *Eublitum*	Sect. *Eublitum*	Sect. *Eublitum*			
				Subsect. *Capitata* **(2)**			Subsect. *Capitata*
				Subsect. *Foliosa* **(2)**			Subsect. *Foliosa*
					Subg. Chenopodium	**Subg. Chenopodium**	**Subg. Chenopodium**
	Sect. *Agathophyton*	Sect. *Agathophyton*	Sect. *Agathophyton*	Sect. *Agathophyton* **(2)**	Sect. *Agathophyton*[o]		
	Sect. *Degenia*	Sect. *Degenia*	Sect. *Degenia*	Sect. *Degenia* **(1)**	Sect. *Degenia*	Sect. *Degenia*[o]	
						Sect. *Desertorum* **(1)**	
	Sect. *Rhagodioides*	Sect. *Rhagodioides*			Sect. *Rhagodioides*	Sect. *Rhagodiodes*	
		Sect. *Roubieva*	Sect. *Roubieva*	Sect. *Roubieva* **(1)**	Sect. *Roubieva*		
		Sect. *Thellungia*	Sect. *Thellungia*	Sect. *Thellungia*	Sect. *Thellungia*		
		Sect. *Skottsbergia*			Sect. *Skottsbergia*		
		Sect. *Tetrasepala*		Sect. *Tetrasepala*			
				Sect. *Auricoma*	Sect. *Auricoma*	Sect. *Auricoma*	
Sect. *Chenopodiastrum*	Sect. *Chenopodiastrum*	Sect. *Euchenopodium*	Sect. *Chenopodia*	Sect. *Chenopodium*	Sect. *Chenopodium*	Sect. *Chenopodium*	Sect. *Chenopodium*
					Subsect. *Chenopodium*		Subsect. *Chenopodium*
					Subsect. *Glauca*[o]		
					Sect. *Leprophyllum*	Sect. *Leprophylum*	
			Subsect. *Undata*	Subsect. *Undata* **(14)**			Subsect. *Undata*
							Subsect. *Leptophylla*
							Subsect. *Urbica*
							Subsect. *Fremontiana*
							Subsect. *Favosa*
							Subsect. *Standleyana*
				Subsect. *Polysperma*			Subsect. *Polysperma*
					Sect. *Atriplicina*		
					Sect. *Margaritaria*		
					Sect. *Meiomeria*		
			Subsect. *Lejosperma*	Subsect. *Lejosperma* **(43)**			
			Subsect. *Cellulata*	Subsect. *Cellulata* **(21)**			
				Ser. *Foveasa*			
				Ser. *Cicatricosa*			Subsect. *Cicraticosa*
				Subsect. *Acuminata*			
				Subsect. *Grossefoveata* **(4)**			Sect. *Grossefoveata*
				Sect. *Polygonoidea*	Sect. *Polygonoidea*		

[o] Indicates sections which have been placed in different subgenera by the authors. Numbers in parentheses indicate the respective number of taxa sampled here with respect to the classification of Aellen (1960).

to deviate by 2n=2x=12 chromosomes (Ellis and Janick, 1960). Karyological data were also not conclusive in improving the overall classification of *Chenopodium* or in understanding relationships within the Chenopodioideae.

The degree of polyploidization encountered in *Chenopodium* has been associated with hybridization processes (Rahiminejad and Gornall, 2004; Bhargava et al., 2006). Hybrid speciation has been suggested to play an important role in *Chenopodium*, largely based on morphological observations, chromosome counts, hybridization experiments, allozyme and flavonoid analyses, (Wilson, 1988; Wilson and Manhart, 1993; Uotila, 2001; Rahiminejad and Gornall, 2004; Bhargava et al., 2006). The well-known and economically important species *Ch. quinoa* (2n=4x=36) and *Ch. berlandieri* subsp. *nuttalliae* (2n=4x=36) are both tetraploids of putative allopolyploid origin (Wilson and Manhart, 1993). Another case of morphologically allied species is the so-called *Chenopodium album* complex, members of which are reported as diploid, tetraploid or hexaploid but so far no hybrid origin has been shown. For the origin of polyploidy in *Chenopodium album*, endopolyploidy was reported and autopolyploidy may also be involved (Kolano et al., 2008).

Understanding the origin and evolution of these crop plants as of all other polyploids requires a species level phylogenetic framework of *Chenopodium* using organellar and nuclear genomic partitions in order to detect putative parental taxa. Currently there is no phylogenetic framework at all for *Chenopodium* taking into account the extensive taxonomic, morphological, and biogeographic diversity within the group. This study aims to clarify the phylogeny of *Chenopodium* based on both cpDNA (*trnL-F*) and nrDNA (ITS), using extensive sampling within the genus and broad sampling across other genera of Chenopodioideae, and also to examine whether distinct subclades possess certain chromosome numbers as synapomorphies and how ploidy levels are distributed in the group.

2.2. Materials and methods

2.2.1 Taxon sampling

All three subgenera, and nine from 13 sections of *Chenopodium* sensu lato, were sampled, overall representing c. 50% of the species. The sampling followed the most comprehensive treatments of Aellen, (1960), Table 2.2. Missing samples include only sect. *Thellungia* (1 sp. in Patagonia), sect. *Polygnoidea* (about 5 spp. in Australia), sect. *Tetrasepala* (1 sp. in Australia) that clearly belongs to *Dysphania* (Scott, 1978a), and sect. *Auricoma* that was covered by the *rbcL* analysis of Kadereit et al. (2003) and shows close affinity to both *Ch. desertorum* and the Australian genera *Einadia* and *Rhagodia*. The inclusion of the last two sections will therefore only be relevant at species level within the respective subclades. We tried to represent species from various parts of the world within these infrageneric entities, covering pronounced morphological differences between species as much as possible. Also, several individuals from very widespread species (e.g., occurring on different continents) were sampled in order to get an idea if such morphology-based taxa correlate with molecular lineages.

We further sampled potentially close relatives of *Chenopodium* within the Chenopodioideae: genera of the Chenopodieae I (*Einadia* and *Rhagodia*; not included in Kadereit et al. (2003)), the Chenopodieae II (*Monolepis*, *Spinacia*), the genus *Suckleya* (in *Atripliceae* sensu Kühn, 1993; but in *Dysphanieae* according to Kadereit et al., 2010), along with representatives of the tribes *Atripliceae* (*Atriplex*, *Grayia*, *Microgynoecium* and *Stutzia*) and *Axyrideae* (*Axyris*, *Ceratocarpus*, *Krascheninnikovia*). Several taxa from Betoideae (*Beta* and *Hablitzia*) and Salsoloideae tribe *Camphorosmeae* (*Bassia*) (Table 2.2) were used as outgroups based on the tree of Müller and Borsch (2005).

2.2.2 DNA isolation, amplification and sequencing

Genomic DNA was isolated from silica gel dried leaf tissue and herbarium specimens, using either a modified CTAB method (Borsch et al., 2003) or the Nucleo Spin Plant II extraction kit (Macherey Nagel, Düren, Germany). The quantity and quality of each DNA sample were measured by NanoDrop spectrophotometer (ND-1000, PeqLab, Erlangen, Germany).

Table 2.2 – Samples included in this study

Taxon	Field/Garden Origin	Voucher	Code	*trnL-F* Acc.	ITS Acc.
	Subfamily Chenopodioideae				
Tribe Atripliceae C. A. Meyer					
Atriplex hortensis L.	Estonia, Tallin	Gawe 41350 (B)	**AC516**	HE577500	HE577360
Atriplex patula L.	Germany, Brandenburg	R. & E. Willing 20.836 (B)	**AC605**	HE577498	HE577358
Atriplex sagittata Borkh.	Berlin Bot. Gard. No: 063119110 [Germany]	S. Fuentes 021 (B)	**AC533**	HE577499	HE577359
Atriplex nitens Schkuhr	Germany, Brandenburg	R. & E. Willing 10.701 D (B)	**AC573**	HE577501	HE577361
Stutzia dioica (Nutt.) E.H. Zacharias	USA	L. Welp 6269 (NY)	**AC351**	HE577502	HE577362
Grayia spinosa (Hook.) Moq.	USA ARS GRIN W626763 [USA, California]	S. Fuentes 177 (B)	**AC625**	HE577496	HE577356
Grayia brandegeei A. Gray	USA ARS GRIN W630044 [USA, Colorado]	S. Fuentes 179 (B)	**AC627**	HE577497	HE577357
Microgynoecium tibeticum Hook. f.	China	B. Dickoré 4284 (B)	**AC656**	HE577503	HE577363
Tribe Axyrideae G. Kadereit & Sukhor					
Axyris amaranthoides L.	Russia	L. Martins 2346 (B)	**AC647**	HE577510	HE577370
Axyris hybrida L.	Russia	L. Martins 2417 (B)	**AC648**	HE577511	HE577371
Axyris prostrata L.	Russia	E. v. Raab-Straube 020232a (B)	**AC529**	HE577509	HE577369
Ceratocarpus arenarius L.	Romania, Navodari	A. Romanovsch (B)	**AC531**	HE577504	HE577364
Ceratocarpus arenarius L.	Russia	L. Martins 2447 (B)	**AC649**	HE577505	HE577365
Krascheninnikovia ceratoides (L.) Gueldenst.	Russia	L. Martins 2500 (B)	**AC608**	HE577506	HE577366
Krascheninnikovia ceratoides (L.) Gueldenst.	Russia	R. Hand 1536 (B)	**AC532**	HE577507	HE577367
Krascheninnikovia lanata (Pursch) A. Meeuse & A. Smit	USA ARS GRIN W629970 [USA, Colorado]	S. Fuentes 178 (B)	**AC626**	HE577508	HE577368
Tribe Dysphanieae Pax					
Suckleya suckleyana (Torr.) Rydb.	USA	R. Darn 5373 (NY)	**AC350**	HE577484	HE577347
sect. Ambrina (Spach.) Hook					
Chenopodium ambrosioides L.	Bolivia, Beni	I. Guareco 420 (B, LPB)	**AC420**	HE577492	HE577352
Chenopodium ambrosioides L.	Bolivia, La Paz	S. G. Beck 31178 (B, LPB)	**AC425**	HE577493	HE577353
Chenopodium ambrosioides L.	Berlin Bot. Gard. No: 10095019310 [Italy]	S. Fuentes 024 (B)	**AC527**	HE577491	HE577351
Chenopodium ambrosioides L.	Ethiopia	M. Wondafrash 2223 (B, ETH)	**AC386**	HE577488	HE577350
[=*Dysphania ambrosioides* (L.) Mosyakin & Clemants]					
sect. Botryoides C. A. Meyer					
subsect. Botrys (Koch) Aellen et Iljin					
Chenopodium schraderianum Schult.	Ethiopia	M. Wondafrash 2255 (B, ETH)	**AC387**	HE577490	HE577349
Chenopodium graveolens Willd.	Bolivia	E. Thomas 258 (B, LPB)	**AC419**	HE577495	HE577355
[=*Dysphania graveolens* (Wlld.) Mosyakin & Clemants]					
subsect. Teloxys (Moq.) Aellen et Iljin					
Chenopodium aristatum L.	Berlin Bot. Gard. No: 309669170 [Germany]	S. Fuentes 025 (B)	**AC528**	HE577480	HE577340
Chenopodium aristatum L.	Russia	L. Martins 2377 (B)	**AC610**	HE577479	HE577339
Chenopodium aristatum L.	USA ARS GRIN Ames 25314 [Mongolia]	S. Fuentes 183 (B)	**AC654**	HE577481	HE577341
[=*Teloxys aristata* (L.) Moq.]					
sect. Orthosporum R. Br.					
Chenopodium melanocarpum (J.M. Black) J.M. Black.	Australia	C. R. Michael & J. Risles 1921 (B)	**AC429**	HE577487	HE577344
[=*Dysphania melanocarpa* (J.M. Black) Mosyakin & Clemants]					
Chenopodium pumilio R. Br.	Germany	T. Borsch (B)	**AC524**	HE577486	HE577343
Chenopodium pumilio R. Br.	Greece	R. & E. Willing 85.571 (B)	**AC604**	HE577485	HE577342
Chenopodium pumilio R. Br.	Mexico	T. Borsch (B)	**AC615**	HE577489	HE577348
[=*Dysphania pumilio* (R. Br.) Mosyakin & Clemants]					

Table 2.2 (continued)

Taxon	Field/Garden Origin	Voucher	Code	*trnL-F* Acc.	ITS Acc.
Tribe Chenopodieae					
Einadia nutans (R. Br.) A. J. Scott.	Berlin Bot. Gard. No: 187199 [Australia]	S. Fuentes 019 (B)	**AC525**	HE577553	HE577415
Monolepis nuttalliana (Schult.) Greene	USA, Utah	R. C. Holmgren 317 (B)	**AC621**	HE577515	HE577375
Rhagodia triandra (G.Forst.) Aellen	New Zealand	P. Hein 12560 (B, CHR)	**AC522**	HE577554	HE577416
	***Chenopodium* L.**				
	Sugen. Ambrosida				
sect. Botryoides C. A. Meyer					
subsect. Botrys (Koch) Aellen et Iljin					
Chenopodium coronopus Moq.	Spain, La Palma	Royl 6823 (B)	**AC570**	HE577543	HE577403
	Subgen. Blitum				
sect. Degenia Aellen					
Chenopodium chenopodioides (L.) Aellen	USA, Montana	P. C. Lesica 5792 (NY)	**AC543**	HE577519	HE577379
sect. Pseudoblitum Hook					
subsect. Glaucum Aellen					
Chenopodium glaucum L.	USA ARS GRIN PI612859 [USA]	S. Fuentes 184 (B)	**AC652**	HE577526	HE577386
Chenopodium glaucum L.	Spain	T. Borsch 3931 (B)	**AC417**	HE577527	HE577387
subsect. Viride Aellen					
Chenopodium rubrum L.	Germany, North See	T. Borsch [08.07] (B)	**AC411**	HE577520	HE577380
Chenopodium rubrum L.	Germany	E. Willing 10.931D (B)	**AC564**	HE577522	HE577382
Chenopodium rubrum L.	USA ARS GRIN Ames 23860 [Poland]	S. Fuentes 182 (B)	**AC653**	HE577521	HE577381
Chenopodium rubrum L.	USA	T. Borsch 3448 (B)	**AC385**	HE577525	HE577385
	Subgen. Chenopodium				
sect. Chenopodium Aellen					
subsect. Cellulata Aellen					
Chenopodium berlandieri Moq.	USA, Nevada	J. C. Beatley 11698 (NY)	**AC541**	HE577561	HE577423
Chenopodium berlandieri Moq.	USA , Colorado	G. Rink 2527 (NY)	**AC599**	HE577567	HE577429
Chenopodium berlandieri var. *boscianum* (Moq.) Wahl	USA, Lousiana	D. M. Ferguson 1072 (NY)	**AC545**	HE577564	HE577426
Chenopodium berlandieri subsp. *nuttalliae* (Saff.) H.Dan. Wilson & Heiser	Mexico	T. Borsch & H. Flores Olvera (B, MEXU)	**AC616**	HE577565	HE577427
Chenopodium berlandieri var. *zschackei* (Murr) Murr ex Graebn.	USA, Colorado	C. C. Freeman 16479 (NY)	**AC542**	HE577563	HE577425
Chenopodium berlandieri var. *zschackei* (Murr) Murr ex Graebn.	USA, Wyoming	A. J. Roderick 2286 (NY)	**AC600**	HE577569	HE577431
Chenopodium ficifolium Sm.	Germany, Berlin	R. & E. Willing 12.260 D (B)	**AC854**	HE577606	HE577466
Chenopodium quinoa Willd.	USA ARS GRIN Ames 13214 [Bolivia]	S. Fuentes 013 (B)	**AC401**	HE577580	HE577445
Chenopodium quinoa Willd.	USA ARS GRIN Ames 13228 [Ecuador]	S. Fuentes 017 (B)	**AC402**	HE577576	HE577441
Chenopodium quinoa Willd.	USA ARS GRIN PI568155 [Mexico]	S. Fuentes 015 (B)	**AC403**	HE577581	HE577446
Chenopodium quinoa Willd.	USA ARS GRIN PI510551 [Peru]	S. Fuentes 009 (B)	**AC404**	HE577579	HE577444
Chenopodium quinoa Willd.	USA ARS GRIN PI587173 [Argentina]	S. Fuentes 012 (B)	**AC405**	HE577577	HE577442
Chenopodium quinoa Willd.	USA ARS GRIN PI596498 [Peru]	S. Fuentes 008 (B)	**AC406**	HE577578	HE577443
Chenopodium quinoa Willd.	USA ARS GRIN PI614880 [Chile]	S. Fuentes 010 (B)	**AC407**	HE577582	HE577447
Chenopodium quinoa Willd.	USA ARS GRIN PI614914 [Bolivia]	S. Fuentes 011 (B)	**AC408**	HE577583	HE577448
Chenopodium quinoa Willd.	USA ARS GRIN PI568155 [Mexico]	S. Fuentes 016 (B)	**AC394**	HE577571	HE577433
Chenopodium neomexicanum Standl.	USA, New Mexico	R. D. Worthington 13394 (NY)	**AC555**	HE577611	HE577471
Chenopodium neomexicanum Standl.	USA, Arizona	S. Fuentes 172 (B)	**AC598**	HE577601	HE577461
Chenopodium pallescens Standl.	USA, Missouri	G. Yatskievych 03-93 (MO)	**AC594**	HE577547	HE577409
Chenopodium pallescens Standl.	USA, Illinois	T. G. Lammers 10336 (NY)	**AC557**	HE577604	HE577464
Chenopodium watsonii A. Nelson	USA, Arizona	D. H. Goldman 2095 (NY)	**AC561**	HE577602	HE577462

Table 2.2 (continued)

Taxon	Field/Garden Origin	Voucher	Code	*trnL-F* Acc.	ITS Acc.
subsect. Grossefoveata Aellen					
Chenopodium hybridum L.	Germany	T. Borsch 3897 (B)	**AC380**	HE577530	HE577390
Chenopodium hybridum L.	Russia, Altay Republic	L. Martins 2329 (B)	**AC609**	HE577528	HE577388
Chenopodium hybridum L.	Germany, Brandenburg	R. & E. Willing 20.856 D (B)	**AC521**	HE577529	HE577389
Chenopodium gigantospermum var. *standleyanum* Aellen	USA, Kansas	C. A. Morse 10855 (NY)	**AC550**	HE577551	HE577413
subsect. Lejosperma Aellen					
Chenopodium album L.	Greece, Messinia	R. & E. Willing 122.544 (B)	**AC571**	HE577558	HE577420
Chenopodium album L.	Germany, Usedom	Weber (B)	**AC602**	HE577559	HE577421
Chenopodium album L.	Russia, Altay Republic	E. v. Raab-Straube 020350 (B)	**AC575**	HE577609	HE577469
Chenopodium album L.	Germany, Bonn	S. Fuentes 001 (B)	**AC388**	HE577557	HE577419
Chenopodium album L.	Spain	T. Borsch 3921 (B)	**AC414**	HE577592	HE577453
Chenopodium album L.	USA ARS GRIN PI608030 [USA]	S. Fuentes 007 (B)	**AC395**	HE577568	HE577430
Chenopodium album L.	USA ARS GRIN Ames 27372 [USA]	S. Fuentes 006 (B)	**AC396**	HE577570	HE577432
Chenopodium album L.	Spain	T. Borsch 3921 (B)	**AC427**	HE577593	HE577456
Chenopodium album L.	Russia, Altay Republic	L. Martins 2423 (B)	**AC614**	HE577552	HE577414
Chenopodium album L.	USA, Arizona	H. D. Hammond 11926 (MO)	**AC591**	HE577596	HE577457
Chenopodium album L.	USA, Wisconsin	N. J. Holmberg 1976 (MO)	**AC590**	HE577556	HE577418
Chenopodium atrovirens Rydb.	Bolivia, La Paz	S. G. Beck 11328 (B, KAS, LPB)	**AC363**	HE577586	HE577450
Chenopodium atrovirens Rydb.	Bolivia, La Paz	S. G. Beck 8377 (B, LPB)	**AC421**	HE577587	HE577452
Chenopodium atrovirens Rydb.	USA, Utha	M. Madsen 40772 (MO)	**AC586**	HE577584	HE577449
Chenopodium atrovirens Rydb.	USA, Colorado	T. G. Lammers et al. 11321 (NY)	**AC540**	HE577585	HE577451
Chenopodium cycloides A. Nelson	USA	T. Borsch, Müller and Pratt 3452 (B)	**AC384**	HE577598	HE577459
Chenopodium cycloides A. Nelson	USA, Kansas	C.C. Freeman 2549 (NY)	**AC544**	HE577599	HE577460
Chenopodium desiccatum A. Nelson	USA, Missouri	B. Summers & Harris 9813 (MO)	**AC588**	HE577550	HE577412
Chenopodium fremontii S. Watson	USA, California	G. Schoolcraft 2206 (UC)	**AC579**	HE577546	HE577408
Chenopodium fremontii S. Watson	USA. Utha	S. Fuentes 185 (B)	**AC597**	HE577572	HE577436
Chenopodium giganteum D. Don	Bonn Bot. Gart. No: 21397 [India]	S. Fuentes 014 (B)	**AC428**	HE577597	HE577458
Chenopodium hians Standl.	USA, Wyoming	S. Stephens 70636 (NY)	**AC551**	HE577610	HE577470
Chenopodium incanum (S. Watson) A. Heller	USA, New Mexico	R. D. Worthington 17439 (NY)	**AC553**	HE577548	HE577410
Chenopodium iljinii Golosk.	Russia, Altay Republic	L. Martins 2490 (B)	**AC611**	HE577608	HE577468
Chenopodium iljinii Golosk.	Russia, Altay Republic	L. Martins 2424 (B)	**AC613**	HE577607	HE577467
Chenopodium leptophyllum (Moq.) Nutt. ex S. Watson	USA, Montana	P. C. Lesica 8846 (NY)	**AC554**	HE577566	HE577428
Chenopodium nevadense Standl.	USA, Nevada	A. Tiehm 13320 (NY)	**AC556**	HE577549	HE577411
Chenopodium opulifolium Schrad. ex W.D.J. Koch & Ziz	Slovakia	T. Borsch 3899 (B)	**AC410**	HE577595	HE577455
Chenopodium opulifolium Schrad. ex W.D.J. Koch & Ziz	Spain	T. Borsch 3926 (B)	**AC416**	HE577594	HE577454
Chenopodium pallidicaule Aellen	USA ARS GRIN PI478406 [Bolivia]	No Voucher	**AC398**	HE577574	HE577439
Chenopodium pallidicaule Aellen	USA ARS GRIN PI510525 [Peru]	No Voucher	**AC399**	HE577573	HE577438
Chenopodium pallidicaule Aellen	USA ARS GRIN PI 510530 [Peru]	No Voucher	**AC400**	HE577575	HE577440
Chenopodium pallidicaule Aellen	Bolivia, Tarija	S. G. Beck 31939 (B, LPB)	**AC426**	HE577600	HE577437
Chenopodium pratericola Rydb.	USA, Wyoming	K. H. Dueholm 10922 (B, LPB)	**AC558**	HE577562	HE577424
Chenopodium petiolare Kunth	Bolivia, Oruro	R. de Michel 2873 (B, KAS, LPB)	**AC359**	HE577588	HE577434
Chenopodium petiolare Kunth	Bolivia, La Paz	S. G. Beck 22972 (B, LPB)	**AC423**	HE577589	HE577435
Chenopodium standleyanum Aellen	USA, Missouri	A. E. Brant & R. Jefferson 4450 (MO)	**AC595**	HE577560	HE577422
Chenopodium standleyanum Aellen	USA, Missouri	N. J. Holmberg 554 (MO)	**AC596**	HE577603	HE577463
Chenopodium subglabrum (S. Watson) A. Nelson	USA, Wyoming	R. D. Dorn 5434 (NY)	**AC559**	HE577605	HE577465
Chenopodium urbicum L.	Greece, Fthiotis	R. & E. Willing 146.1979 (B)	**AC576**	HE577524	HE577384
Chenopodium urbicum L.	Berlin Bot. Gard. No: 269400010 [Greece]	S. Fuentes 026 (B)	**AC536**	HE577523	HE577383

Table 2.2 (continued)

Taxon	Field/Garden Origin	Voucher	Code	*trnL-F* Acc.	ITS Acc.
Chenopodium vulvaria L.	Spain	T. Borsch 3918 (B)	**AC412**	HE577591	HE577407
Chenopodium vulvaria L.	Greece, Evrytania	R. & E. Willing 148.759 (B)	**AC562**	HE577590	HE577406
subsect. Undata Aellen					
Chenopodium murale L.	Bolivia, La Paz	S. G. Beck 22970 (B, KAS, LPB)	**AC360**	HE577538	HE577398
Chenopodium murale L.	Chile	T. Borsch 3097 (B)	**AC383**	HE577539	HE577400
Chenopodium murale L.	USA ARS GRIN Ames 26140 [USA]	S. Fuentes 005 (B)	**AC397**	HE577534	HE577394
Chenopodium murale L.	Spain	T. Borsch 3919 (B)	**AC413**	HE577535	HE577395
Chenopodium murale L.	Spain	T. Borsch 3924 (B)	**AC415**	HE577536	HE577396
Chenopodium murale L.	Bolivia, La Paz	S. G. Beck 145PG94 (B, LPB)	**AC424**	HE577537	HE577397
Chenopodium murale L.	Slovakia	T. Borsch 3915 (B)	**AC409**	HE577533	HE577391
Chenopodium murale L.	Greece, Korinthias	R. & E. Willing 143.462 (B)	**AC430**	HE577540	HE577399
Chenopodium murale L	Mexico, Ixtapan	T. Borsch & H. Flores Olvera 3871 (B, MEXU)	**AC382**	HE577541	HE577401
Chenopodium murale L.	USA, California	C. Dietrich et al. 32 (MO)	**AC589**	HE577531	HE577392
Chenopodium murale L.	Greece, Evvia	R. & E. Willing 145.733 (B)	**AC566**	HE577532	HE577393
Chenopodium murale L.	Greece	R. & E. Willing 145.592 (B)	**AC565**	HE577542	HE577402
Chenopodium murale L.	USA, California	T. Ross 4084 (UC)	**AC581**	HE577544	HE577404
Chenopodium murale L.	USA, California	G. Gust & L. Nyle 476 (MO)	**AC587**	HE577545	HE577405
sect. Desertorum Wilson					
Chenopodium desertorum subsp. *anidiophyllum* (Aellen) P.G. Wilson	Australia	C. Michaell & J.Risler 1773 (B, NT)	**AC519**	HE577555	HE577417
sect. Roubieva Rouy in Rouy et Foucauld					
Chenopodium multifidum L. [=*Dysphania multifida* (L.) Mosyakin & Clemants]	Greece, Florina	R. & E. Willing 85631 (B)	**AC574**	HE577494	HE577354
Tribe Spinaceae Moq. (this study)					
sect. Agathophyton Hook					
Chenopodium bonus-henricus L.	Austria	T. Borsch 3821 (B)	**AC381**	HE577512	HE577372
Chenopodium californicum (S. Watson) S. Watson.	USA, California	P. Davis & D. Lightowless 66504 (B)	**AC431**	HE577516	HE577376
sect. Eublitum (Moq.) Aellen					
subsect. Capitata Kowal ex Mosyakin and Clemants					
Chenopodium capitatum (L.) Ambrosi	Bonn Bot. Gart. No: 19116	S. Fuentes 004 (B)	**AC391**	HE577513	HE577373
Chenopodium capitatum var. *parvicapitatum* S.L. Welsh	USA, Utha	K. Moon et al. 1993 (NY)	**AC547**	HE577514	HE577374
Spinacia oleracea L.				AJ400848.1	
Spinacia oleracea L.					EU606218.1
Spinacia tetrandra Steven ex M. Bieb.	USA ARS GRIN Ames 23664 [Asia]	S. Fuentes 180 (B)	**AC650**	HE577482	HE577345
Spinacia turkestanica Iljin	USA ARS GRIN Ames 23666 [Asia]	S. Fuentes 181 (B)	**AC651**	HE577483	HE577346
subsect. Foliosa Kowal ex Mosyakin and Clemants					
Chenopodium foliosum Asch.	Bonn Bot Gart No: 19117 [Germany]	S. Fuentes 003 (B)	**AC392**	HE577517	HE577377
Chenopodium foliosum Asch.	Kirgistan, Central Asia	Cubr 42389 (B)	**AC520**	HE577518	HE577378
OUTGROUPS	**Subfamily Betoideae Ulbr.**				
Beta vulgaris subsp. *maritima* (L.) Thell.	Denmark, Jylland	Cubr 39900 (B)	**AC530**	HE577473	HE577334
Hablitzia tamnoides M. Bieb.	Germany, Bonn Bot.Gard No: 03609-90	No Voucher	**AC018**	HE577475	-
Hablitzia tamnoides M. Bieb.	Germany, Berlin Bot Gard No: 16611	S. Fuentes 018 (B)	**AC523**	HE577474	HE577335
Hablitzia tamnoides M. Bieb.					AY858590.1
	Subfamily Camphorosmoideae Luersss.				
Bassia laniflora (S.G. Gmel.) A.J. Scott	Germany, Berlin Bot Gard No: 17809970	S. Fuentes 022 (B)	**AC534**	HE577476	HE577336
Bassia scoparia (L.) A.J. Scott	Russia	L. Martins 2295 (B)	**AC607**	HE577477	HE577337
Bassia prostrata (L.) A.J. Scott	Russia	L. Martins 2429 (B)	**AC606**	HE577478	HE577338
	Subfamily Salicornideae Ulbr.				
Allenrolfea vaginata Kuntze	Germany, Bonn Bot Gard No: 2488		**AC017**	HE577472	-
Allenrolfea occidentalis Kuntze					AY181875.1

Note: The circumscription of subfamilies in Chenopodiaceae follows the tree annotations in Kadereit et al. (2003); within Chenopodioideae the tribes *Atripliceae, Axyrideae* and *Dysphanieae* are recognized based on Kadereit et al. (2010); *Spinacieae* are listed as resurrected here; remaining genera are included into *Chenopodieae* (Kühn 1993) using the infrageneric classification of *Chenopodium* by Aellen (1960) except the members of *Dysphanieae* and Wilson (1983) for sect. *Desertorum* of *Chenopodium*. USA, ARS, GRIN refers to USDA, ARS, National Genetic Resources Program. *Germplasm Resources Information Network* - (GRIN). [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland.

The *trnL-F* region was amplified and sequenced using the forward primer trnTAC2 (5'-CATTTTTCGGTATAGTAABCC-3'), specifically designed for the Amaranthaceae-Chenopodiaceae clade (this study) and the standard reverse primer trnTf (5'-ATTTGAACTGGTGACACGAG-3'; Taberlet et al., 1991). For some samples the standard forward primer trnTc (5'-CGAAATCGGTAGACGCTACG-3'; Taberlet et al., 1991) was used for both amplification and sequencing. The internal sequencing primers used were: trnL-460F (5'-GAGAATAAAGATAGAGTCC-3'; Worberg et al., 2007) and trnTd (5'-GGGGATAGAGGGACTTGAAC-3'; Taberlet et al., 1991). The ITS region was amplified and sequenced with a specific Amaranthaceae-Chenopodiaceae forward primer designed in this study: AC-ITS5 (5'-GGAAGGAGAAGTCGWAACARGG-3'), and the universal reverse primer ITS4 (5'- TCCTCCGCTTATTGATATGC-3'; White et al., 1990).

PCR amplification was performed using the following reaction mix: 1.5 mM $MgCl_2$, 1X PeqLab Taq Buffer S (including $MgCl_2$), 0.25 mM each dNTP, 0.8 pmol primer, 0.03 U/ul Taq polymerase (PeqLab, Erlangen Germany) and 0.8 ng/ul DNA template. For difficult templates (e.g. DNA isolated from herbarium material), betaine was added to a final concentration of 1 M. The PCR was performed in a T3 Thermocycler (Biometra, Göttingen, Germany) or a Mastercycler (Eppendorf, Hamburg, Germany). The PCR program used for the chloroplast region *trnL-F* was: 30 cycles of denaturation (60 s at 94°C), annealing (60 s at 52°C), extension (120 s at 72°C) and a final extension step (15 min at 72°C). The PCR program for the ITS region was: 35 cycles of denaturation (60s at 97°C), annealing (60 s at 48°C), extension (45 s at 72°C) and a final extension step (7 min at 72°C). Primer dimers and secondary banding patterns were separated from the requested bands using a 1.5% NEEO agarose gel (Carl Roth, Germany) running for 3 hours at 100 volts. Gel extraction was performed using the AveGene Gel /PCR DNA Fragments Extraction Kit (AveGene life science Corporation). The quality and quantity of the purified PCR product were measured with a NanoDrop spectrophotometer. Cycle sequencing, fragment purification, and direct automated sequencing was performed by Macrogen Inc. (Seoul, South Korea).

2.2.3 Alignment and coding of length mutational events

Sequences were edited and aligned manually using PhyDE (Phylogenetic Data Editor) version 0.995 (Müller et al., 2007), following the rules outlined in Löhne and

Borsch (2005). Regions of uncertain homology (mutational hotspots) were excluded from the analysis (see Appendices A, B and D). Hypothesized microstructural mutations that explain the length variability patterns of sequences in the aligned partition are listed in Appendices A and B, as suggested by Borsch et al. (2007), Morrison (2009) and Ochoterena (2009). The inversions were re-inverted and coded as mutational event in the indel matrix following Löhne and Borsch (2005). Indels were then coded automatically using the Simple Indel Coding method (Simmons and Ochoterena, 2000) as implemented in SeqState 1.40 (Müller, 2005a). The alignments are available in TreeBase (Submission 11780).

2.2.4 Phylogenetic analyses

Maximum Parsimony (MP) analyses were performed using the Parsimony Ratchet (Nixon, 1999) using the software PRAP (Müller, 2004) in combination with PAUP* v. 4.0b10 (Swofford, 1998). Ratchet settings were 200 ratchet iterations with 25% of the positions randomly up weighted (weight =2) during each replicate and 10 random addition cycles.

The command files generated with PRAP were then run in PAUP, using the heuristic search with the following parameters: all characters have equal weight, gaps are treated as “missing”, TBR branch swapping, initial swapping on 1 tree already in memory, Maxtrees set to 100 (auto increased by 100) and branches collapsed actively if branch length is zero. The Jackknife (JK) support for branches was also performed in PAUP with 10,000 replicates, using a TBR branch swapping algorithm with 36.788% of characters deleted and one tree held during each replicate, following Müller (2005b).

Bayesian inference (BI) was carried out using MrBayes 3.1 (Huelsenbeck and Ronquist, 2001). Optimal nucleotide substitution models for the respective *trnL-F* (GTR + G) and ITS (GTR + G + I) data sets were chosen following the Akaike Information criterion (AIC) in Modeltest 3.7 (Posada and Crandall, 1998). A binary (restriction site) model was implemented for the coded indels. All analyses were performed with four independent runs of Markov Chains Monte Carlo (MCMC) each with four parallel chains. Each chain was performed for 1 million generations, saving one random tree every 100th

generation. The burn in was set to 100,000, and a majority consensus tree was computed with the remaining trees.

To test for congruence between the respective data sets, we ran the Incongruence Length Difference (ILD) test (Farris et al., 1994), implemented in PAUP* as the Partition Homogeneity Test, and using the following parameters: 10,000 replicates with 50 Random Addition Searches, holding only two trees each step and saving no more than 5 trees. The test was conducted for (i) the complete data set (140 taxa), (ii) a reduced data set including only diploid taxa, and (iii) for each of the well-supported major clades.

2.3. Results

2.3.1 The non-coding *trnL-F* chloroplast region

Sequence lengths varied from 304-643 nt in the intron and 137-386 nt in the spacer. The aligned data set comprised 1240 characters including 345 (27%) that were parsimony informative. Seven areas classified as “hotspots” (HS) sensu Borsch et al. (2003) were excluded from the analyses (Appendix D). One inversion was found in the *trnL* intron in all samples of *Krascheninnikovia* (Appendix A). The final matrix, including coded indels, comprised 1402 characters of which 461 (33%) were parsimony informative. The MP search resulted in 307 shortest trees (L= 1027, CI= 0.702, a RI= 0.933 and a RC= 0.655). The resulting strict consensus tree was identical in topology with the Bayesian majority-rule consensus tree (see Fig. 2.1).

2.3.2 The nuclear ITS region

Sequence lengths varied from 149-174 nt in ITS1 and 188-205 nt in ITS2. Both spacers were surprisingly well aligned except some sequence parts excluded as mutational hotspots (Appendix D). The hotspot in ITS1 was on average 60 nt in length and the two hotspots in ITS2 were 6 and 19 nt in length, respectively. Hypothesized microstructural mutations are listed in Appendix B. Of all characters, 35% were parsimony informative, after indels were coded as binary characters and added to the matrix (687 characters in total), the percentage of parsimony informative characters increased to 39%. Parsimony analyses of the ITS region resulted in 1633 shortest trees (L= 939, CI= 0.502, RI= 0.890,

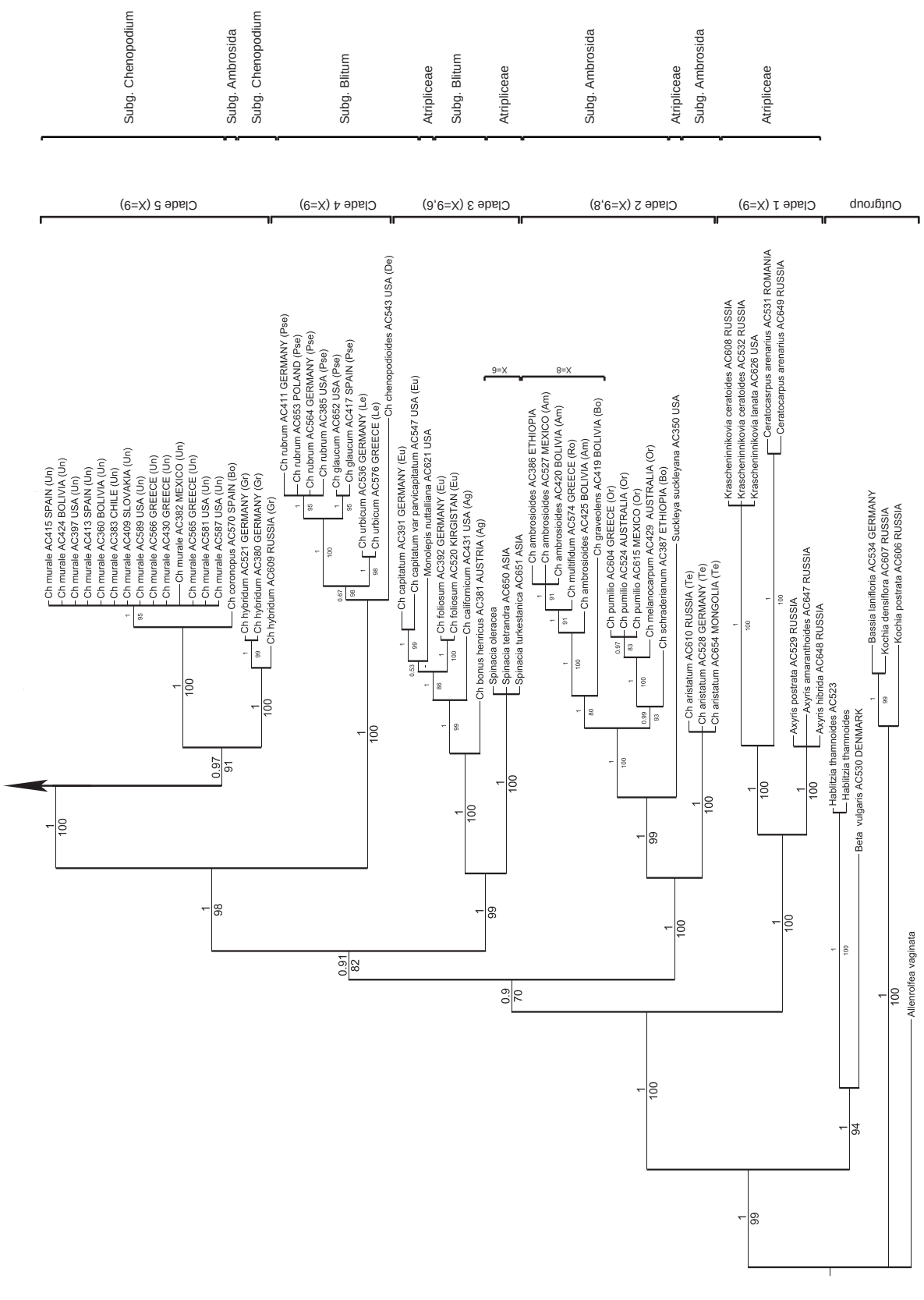


Fig. 2.1 – Bayesian majority rule tree based on the sequence dataset of *trnL-F* including coded indels. Bayesian posterior probabilities (PP) are given above and Jackknife (JK) values below branches. The abbreviations following species names in parentheses refer to the sections and subsections proposed for *Chenopodium* by Aellen (1960); (Ag) = Sect. *Agathophyton*, (Am) = sect. *Ambrina*, (Bo) = sect. *Botryoides*; the sect. *Chenopodium* is represented by (Ce) = subsect. *Cellulata*, (Gr) = subsect. *Grossefoveata*, (Le) = subsect. *Lejosperma*, and (Un) = subsect. *Undata*; further sections are (De) = sect. *Degenia*, (Eu) = sect. *Eublitum*, (Or) = sect. *Orthosporum*, (Pse) = sect. *Pseudoblitum*, and (Ro) = sect. *Roubieva*. The second column of clade annotations refers to the accepted subgenera of *Chenopodium* (Judd and Ferguson, 1999), and the third column to tribe names accepted in this study as explained in Fig. 2.2.

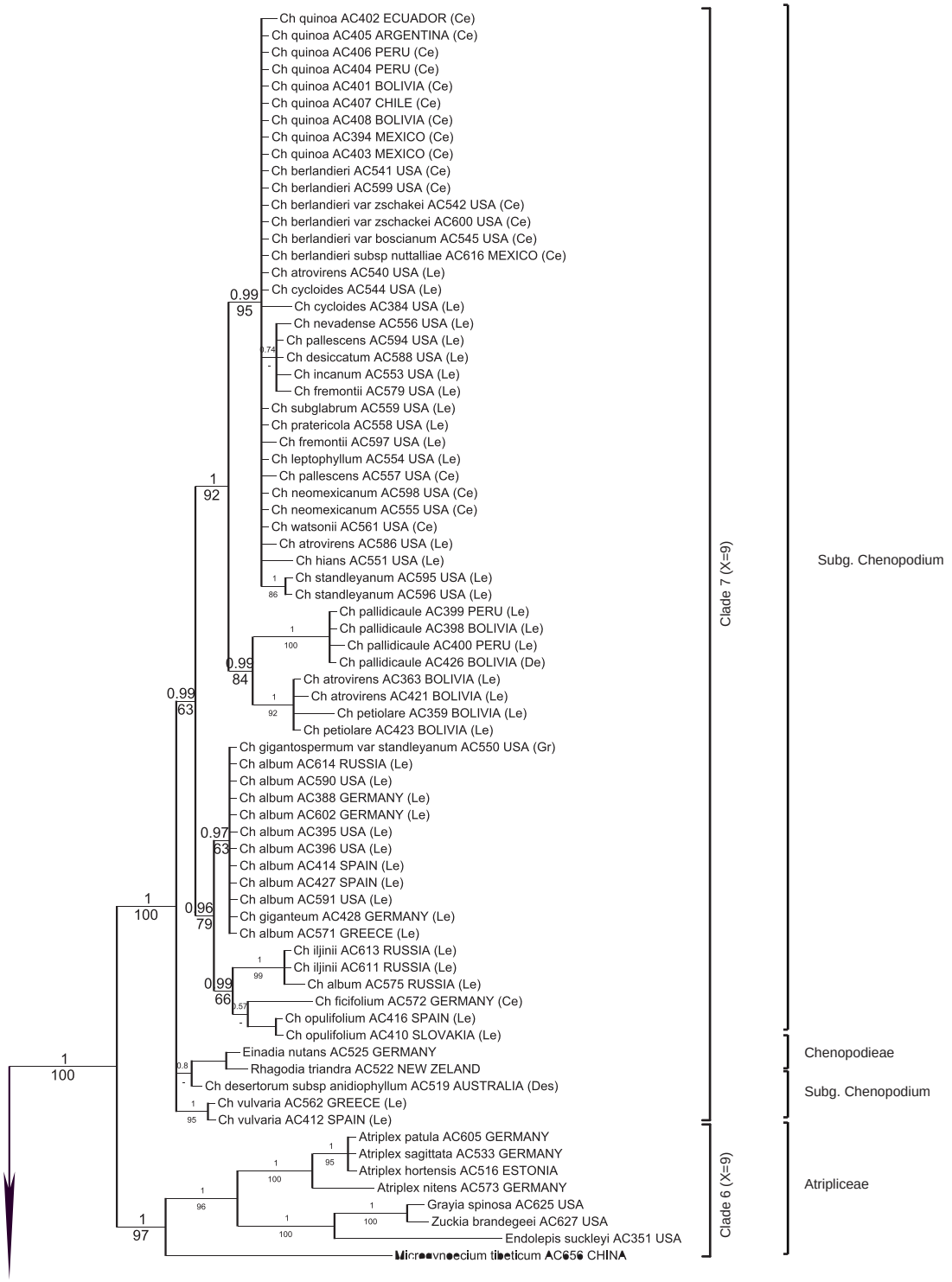


Fig. 2.1 – Continued. Bayesian posterior probabilities (PP) are given above and Jackknife (JK) values below branches. The abbreviations following species names in parentheses refer to the sections and subsections proposed for *Chenopodium* by Aellen (1960); (Ag) = Sect. *Agathophyton*, (Am) = sect. *Ambrina*, (Bo) = sect. *Botryoides*; the sect. *Chenopodium* is represented by (Ce) = subsect. *Cellulata*, (Gr) = subsect. *Grossefoveata*, (Le) = subsect. *Lejosperma*, and (Un) = subsect. *Undata*; further sections are (De) = sect. *Degenia*, (Eu) = sect. *Eublitum*, (Or) = sect. *Orthosporum*, (Pse) = sect. *Pseudoblitum*, and (Ro) = sect. *Roubieva*. The second column of clade annotations refers to the accepted subgenera of *Chenopodium* (Judd and Ferguson, 1999), and the third column to tribe names accepted in this study as explained in Fig. 2.2.

RC= 0.446) with indels coded. Both MP and Bayesian analyses gave consensus trees with identical topology (Fig. 2.2).

2.3.3 Phylogenetic relationships

MP and Bayesian analyses of the respective *trnL-F* and ITS data sets depict seven strongly supported clades (clades 1-7; Figs. 2.1 and 2.2), encompassing both the *Chenopodieae* (clades 2-5, 7) and the *Atripliceae* sensu stricto (clade 6). Clade 1 contains *Axyris, Ceratocarpus* and *Kraschenninnikovia* (maximum support in all trees) and either appears sister to the remaining Chenopodioideae (*trnL-F*; Fig. 2.1) or is inconsistently resolved among the early branching lineages of the Chenopodioideae (ITS). The genus *Chenopodium* itself is highly paraphyletic to nearly all other genera of the subfamily and its species are distributed in five different well defined lineages (clades 2-5 and 7; Figs. 2.1 and 2.2). Clade 2 (*trnL-F* 100% JK / 1 PP, ITS 98% JK / 1 PP) encompasses *Ch. ambrosioides* and a number of other aromatic species as well as the monotypic genus *Suckleya*. Clade 3 receives high support with *trnL-F* (99% JK /1 PP) but only moderate support in the ITS tree (67% JK /1 PP). It comprises *Chenopodium bonus-henricus* and relatives and *Monolepis* in one subclade and all species of *Spinacia* in another. The monophyly of *Spinacia* (*trnL-F* 100% JK / 1 PP, ITS 100% JK / 1 PP) is supported here for the first time. Clade 4 (*trnL-F* 100% JK / 1 PP, ITS 100% JK / 1 PP) is composed of *Ch. rubrum* and a number of other species (*Ch. rubrum*-clade) and clade 5 (*trnL-F* 100% JK / 1 PP, ITS 83% JK / 1 PP) contains *Chenopodium murale, Ch. hybridum* and relatives (*Ch. murale*-clade). Finally, clade 7 (*trnL-F* 100% JK / 1 PP, ITS 88% JK / 1 PP) embraces most of the other *Chenopodium* species, along with the genera *Einadia* and *Rhagodia*. The remaining clades 1 and 6 only include taxa from the former *Atripliceae* sensu lato. While clade 6 (*trnL-F* 97% JK / 1 PP, ITS 50% JK / 0.65 PP) encompasses *Atriplex*, *Stutzia*, *Grayia* and *Microgynoecium*, the maximally supported clade 1 consists of *Axyris*, *Ceratocarpus* and *Kraschenninnikovia*.

2.3.4 Phylogenetic incongruence

The ILD test showed strong incongruence between the respective partitions of the data sets, even in the absence of polyploid taxa (P<0.001). When clades were compared

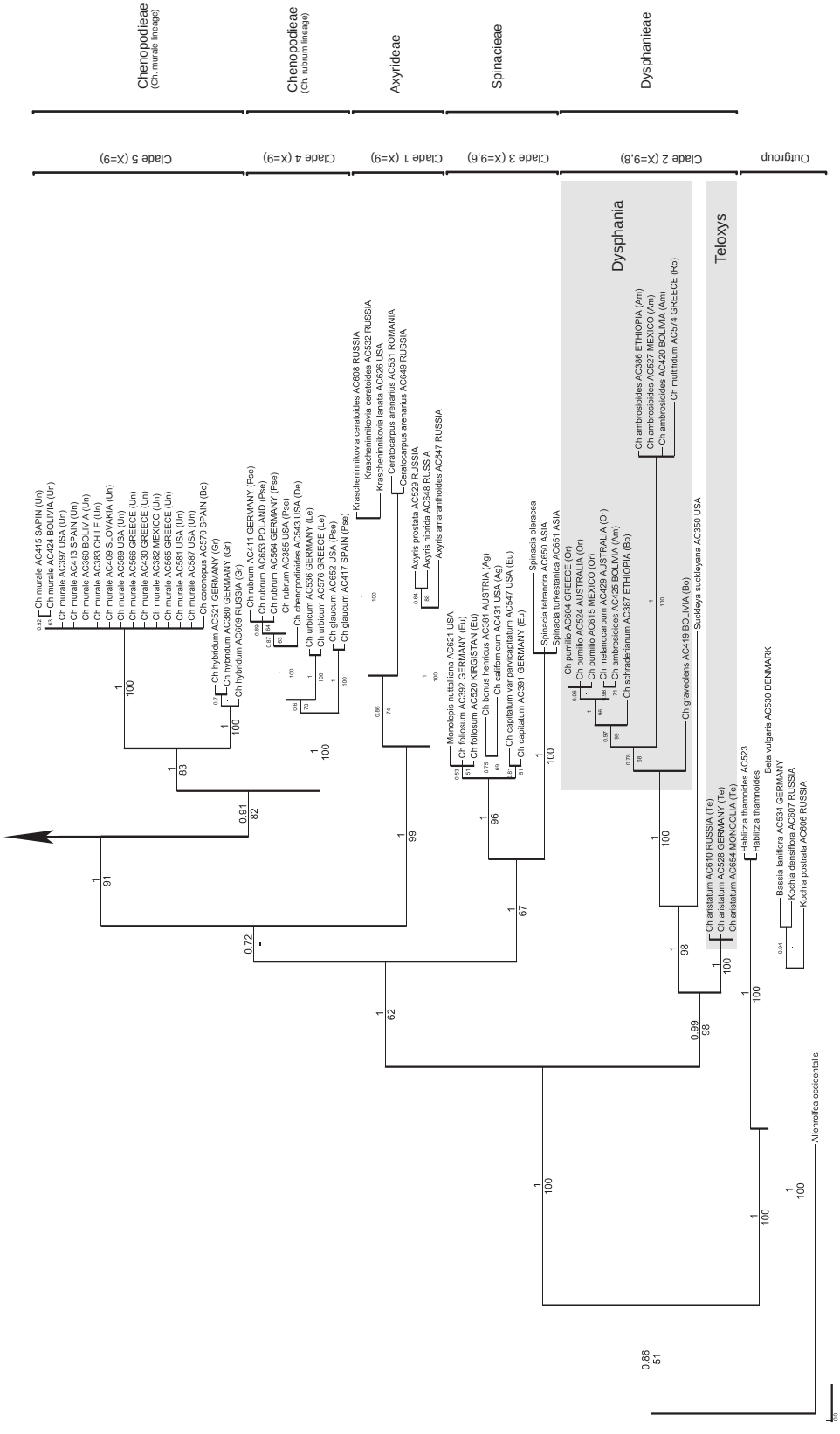


Fig. 2.2 – Bayesian majority rule tree based on the sequence dataset of nrITS including coded indels. Bayesian posterior probabilities (PP) are given above and Jackknife (JK) values below branches. Boxes in gray mark the genera *Dysphania* and *Teloxys* from the Dysphanieae that are now accepted as genera distinct from the former *Chenopodium* sensu lato, and the representatives of the former genera *Einadia* and *Rhagodia* that are included into *Chenopodium* s.str. (clade 7) in this study. The second column refers to tribe names Spinacieae (newly resolved in this study), Atripliceae, Axyrideae and Dysphanieae (sensu Kadereit et al. 2010) and Zacharias and Baldwin (2010); supported in this study); and for Chenopodieae the newly *Chenopodium* s.str, *Ch. murale* lineage and *Ch. rubrum* lineage found in this study.

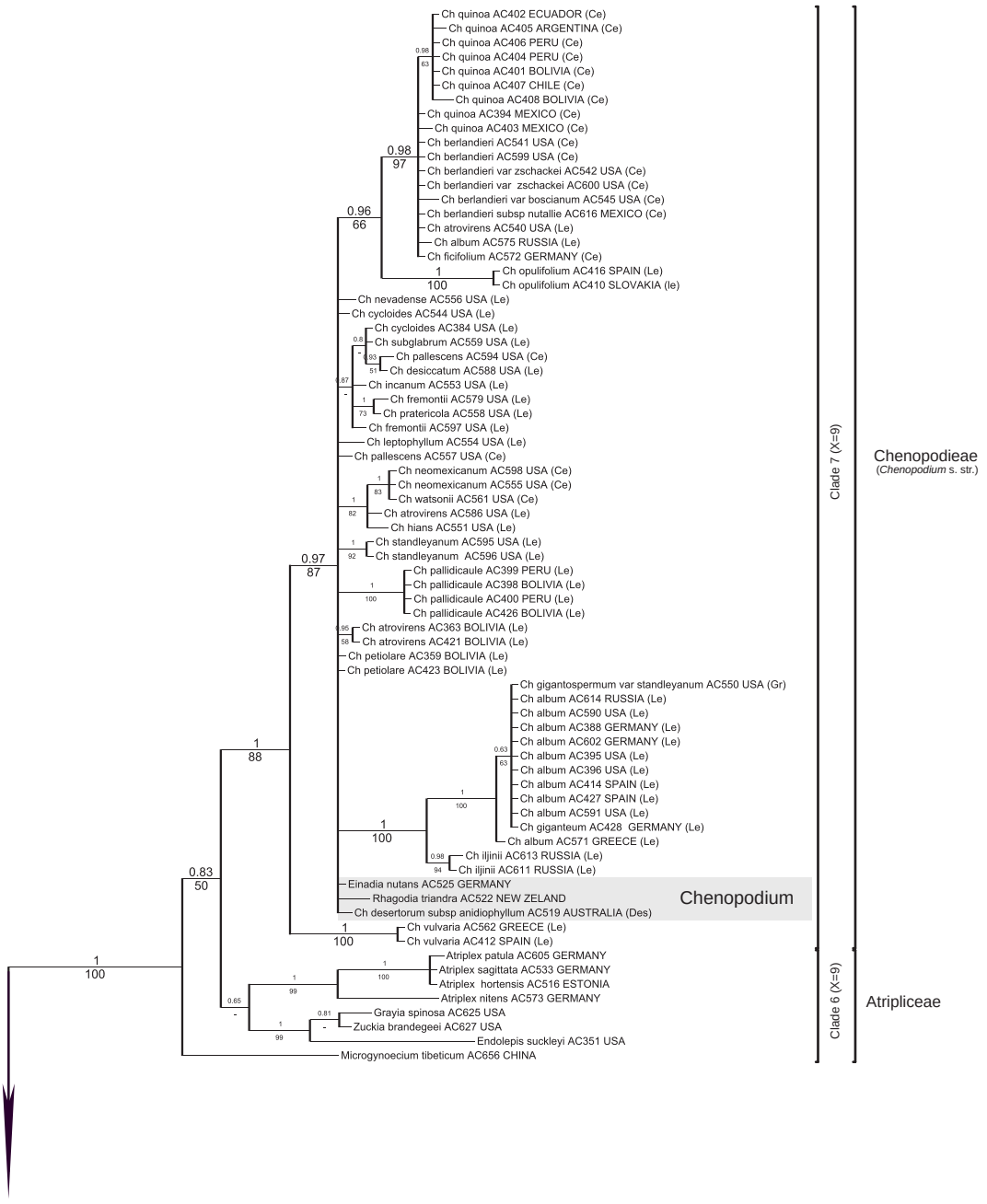


Fig. 2.2 – Continued. Bayesian posterior probabilities (PP) are given above and Jackknife (JK) values below branches. Boxes in gray mark the genera *Dysphania* and *Teloxys* from the Dysphanieae that are now accepted as genera distinct from the former *Chenopodium* sensu lato, and the representatives of the former genera *Einadia* and *Rhagodia* that are included into *Chenopodium* s.str. (clade 7) in this study. The second column refers to tribe names Spinacieae (newly resolved in this study), Atripliceae, Axyrideae and Dysphanieae (sensu Kadereit et al. 2010) and Zacharias and Baldwin (2010); supported in this study); and for Chenopodieae the newly *Chenopodium* s.str, *Ch. murale* lineage and *Ch. rubrum* lineage found in this study.

separately, topological incongruence was also detected within clades 2, 3, 4 and 7 (P<0.001), but not for clades 1, 5, and 6 (P=1, excluding *Microgynoecium* due to its unclear phylogenetic position). The position of the three first branching lineages in Chenopodioideae differs between ITS and *trnL-F*. In the ITS tree, deep nodes are unsupported in MP and only clade 1 as third branching is supported by a PP of 1. Clade 4 and 5 are resolved either as sister lineages (ITS 82% JK / 0.91 PP) or in a grade (*trnL-F*). Within clade 7, resolution is poor but individual samples (e.g. *Ch. ficifolium*) are inferred incongruently in the chloroplast and nuclear trees.

2.4. Discussion

This study is based on the most extensive sampling of *Chenopodium* species to date. By using the highly variable non-coding *trnL-F* (cpDNA) and ITS (nrDNA) regions, we provide the first comprehensive phylogeny of this controverted large genus. Overall, we support the highly paraphyletic status of *Chenopodium*, as suggested by Kadereit et al. (2003) and Müller and Borsch (2005), and reveal new well-supported lineages, resolved with high confidence (Figs. 2.1 and 2.2). Because of this paraphyly, our results also affect the picture of the subfamily Chenopodioideae. Whereas the studies by Kadereit et al. (2003; 2010) suggested three of the five lineages of *Chenopodium* s.l. (Chenopodieae I, II, III; Chenopodieae III were already called *Dysphanieae* in Kadereit et al., 2010), our results offer further support and resolution of these lineages and identify two novel major clades (*Ch. murale* and relatives, *Ch. rubrum* and relatives) out of the Chenopodieae I. Our data also provide statistical support for the Chenopodieae II, which we recognize as *Spinacieae*.

2.4.1 Congruence of data partitions

Different combinations on the ILD test reveal some topological incongruence between the respective data partitions, as exemplified by the respective positions of clades 1, 2 and 3 (*Ayridae*, *Dysphanieae*, *Spinacieae*; Figs. 2.1 and 2.2) or the position of *Ch. opulifolium* within clade 7. In the first example incongruence is soft (no statistic confidence in deviating topologies) whereas it is hard (well supported nodes differ) in the second case. Causes for incongruence are manifold, and can be either of non-biological (e.g. insufficient taxon sampling, long-branch attraction, etc.) or biological (e.g.

incomplete linage sorting, orthology/paralogy conflation, or hybridization) origin (Wendel and Doyle, 1998; Sanderson et al., 2000).

On the one hand, the extent of our current taxon sampling (more than 135 taxa representing the diversity of *Chenopodium* sensu lato) and the similar topologies obtained from both MP and Bayesian analyses of the respective markers allow us to reject with confidence most analytical causes of topological incongruence. Indeed, it has been recently demonstrated that an increase of taxon sampling in a Bayesian context tends to decrease the risk of erroneous topologies due to long-branch attraction (Van der Niet and Linder, 2008). Furthermore, incomplete lineage sorting of the ITS alleles can be excluded as a cause of topological conflict due to the absence of polymorphic sites in all direct sequences investigated. Yet, this lack of ITS polymorphism does not allow us to identify potential additive polymorphic sites, which would support past hybridization events (Nieto Feliner et al., 2001; Mansion et al., 2005; Guggisberg et al., 2009).

We are aware that we cannot clearly discriminate between orthology/paralogy conflation and reticulation patterns in the absence of extensive cloning of the ITS region, especially in clades with low interspecific resolution (e.g. clade 7, Figs. 2.1 and 2.2). A more detailed study, taking into account the current limitations of our current molecular data set, and using non-molecular evidence based on caryology, morphology, and phytochemistry is underway.

Overall, we feel that it is more appropriate to individually discuss evolutionary and taxonomic implications of trees inferred from organellar and nuclear genomic compartments, especially when sources of potential incongruence remain unclear. Furthermore, such an approach allows us to compare the phylogenetic utility of the respective data partitions.

2.4.2 Phylogenetic utility of the *trnL-F* and ITS regions

The use of non-coding and rapidly evolving genomic regions from the chloroplast genome in angiosperm phylogenetics has been accelerating during recent years. Following initial proposals (e.g. Taberlet et al., 1991), it has been demonstrated that not only the percentage of variable sites, and thus the quantity of information, but also the quality of

phylogenetic signal of non-coding regions outperforms more conserved coding genes such as *rbcL* (Borsch et al., 2003; Müller et al., 2006). One of the major insights is that chloroplast DNA mutational dynamics follows certain principles across genomic regions and taxa (see Borsch and Quandt, 2009 for a summary). As a consequence, motif based alignment allows more precise homology statements (Morrison, 2009; Ochoterena, 2009), although, on the other hand mutational hotspots of unclear homology have to be excluded even in data sets representing species level diversity within genera. This study provides a further example for this (seven mutational hotspots in the *trnL-F* region of *Chenopodium* s.l.). The current *trnL-F* data set is one of the largest so far generated for a family of Caryophyllales. Previous workers used the *trnL-F* region, due to its high variability compared to other chloroplast markers in *Suaeda*, *Salicornia* and allies, or *Beta* and allies (Kapralov et al., 2006; Murakeözy et al., 2007; Hohmann et al., 2006). The first molecular analysis of the Amaranthaceae-Chenopodiaceae alliance by Kadereit et al. (2003) was based on sequences of the *rbcL* gene, but the resulting trees were largely unresolved at deeper nodes and lacked statistical support in many parts. A subsequent analysis by Müller and Borsch (2005) used *trnK/matK* plastid data, and generated a much improved phylogenetic hypothesis for Amaranthaceae and Chenopodiaceae. Remarkably, the *trnL-F* region (composed of the *trnL* gene with its group I intron and the *trnL-trnF* intergentic spacer; e.g. Quandt et al., 2004) is about half the size of *matK/trnK* and yields the so far best resolved and supported tree of a major Chenopodiaceae lineage. This is paralleled by *trnL-F* trees from the speciose subfamily Gomphrenoideae of the Amaranthaceae (Sánchez del Pino et al., 2009), suggesting that *trnL-F* should be employed as a standard marker in Amaranthaceae-Chenopodiaceae.

The nuclear, biparentally inherited internal transcribed spacer region (ITS1, 5.8S, ITS2) yields trees that essentially show the same seven major lineages. Only some nodes, especially at deeper parts of the tree, are weakly supported and inconsistently resolved (see Clades 1, 2 and 3, Fig. 2.2). This pattern may be explained because ITS evolves differently to chloroplast regions. Motifs for microstructural changes are less evident. In fact, most mutations (see indel character list; Appendix B) appear to be mostly deletions or insertions of single nucleotides. Occurring repeatedly at greater distances, this may obscure sequence divergence caused by substitutions, leading to less robust homology assessments, in addition to potential effects of concerted evolution of many ITS copies (see Álvarez and

Wendel, 2003; Nieto Feliner and Rosselló, 2007). Some parts of the ITS tree are clearly incongruent (see Fig. 2.2) to the chloroplast tree. This suggests reticulate patterns which could be explained by patterns of speciation. The deep topological differences are rather inconsistent (low support) and this can be explained by intrinsic patterns of molecular evolution.

2.4.3 Paraphyly of *Chenopodium* L.

Both MP and Bayesian analyses based on DNA markers of different genomic compartments (cp- and nrDNA) support the paraphyly of the genus as currently described, and further the inclusion of all the investigated species of *Chenopodium* into five different clades (Figs. 2.1 and 2.2). For clarity, the name *Chenopodium* is used throughout for all species it has been applied to over time (Figs. 2.1 and 2.2).

A clade containing aromatic species of *Chenopodium* (clade 2, see below "*Dysphanieae"*), is highly supported (Figs. 2.1 and 2.2), and is either resolved as a first or second diverging lineage of *Chenopodium* s.l., depending on whether ITS or *trnL-F* are analysed. It comprises *Ch. ambrosioides*, *Ch. graveolens*, *Ch. melanocarpum*, *Ch. multifidum*, *Ch. pumilio*, *Ch. schraderianum*, and *Ch. aristatum*, which all share the presence of specialized aromatic glandular hairs ("type 8"; Carolin, 1983; Bonzani et al., 2003), as well as *Suckleya suckleyana* which has inflated unicellular trichomes (Chu et al., 1991). Our data support the previous proposal by Carolin (1983) and Mosyakin and Clemants (1996) to separate aromatic chenopods with glandular hairs under the generic name *Dysphania* (including *Teloxys* in this genus) from the remaining ones (possessing bladder or sub-stellate hairs), and denote the importance of hair types as characters for the systematics of Chenopodioideae. Within the aromatic clade, *Suckleya suckleyana*, previously placed in the *Atripliceae* sensu lato (Kühn et al., 1993), is resolved with high confidence (99% JK / 1 PP) as sister group to all other species of aromatic *Chenopodium* except *Ch. aristatum (= Teloxys aristata*).

Our results corroborate a recent tree based on *rbcL* (Kadereit et al., 2010) and also find *Suckleya* in a position sister to a number of *Dysphania* species, clearly apart of the *Atripliceae* clade. *Suckleya* shares ebracteolate flowers with the aromatic chenopods, but differs by being monoecious and by its female perianth which becomes winged when

mature (Chu et al., 1991). It is thus justified to maintain it as a separate genus distinct from the other aromatic species of *Chenopodium* (= *Dysphania spp.*). To confirm the presence of glandular trichomes as a putative synapomorphy for clade 2, it will be necessary to revisit the fine structure of *Suckleya* trichomes and to confirm their glandular nature. Nevertheless, available data (Chu et al., 1991) show that the inflated unicellular trichomes of *Suckleya* appear to be similar to the Type I trichomes found in other aromatic species of *Chenopodium* (= *Dysphania spp.*; Bonzani et al., 2003). The three samples representing geographically different populations of *Ch. aristatum* appear as sister group (100% JK/ 1 PP) to the remainder of the species in clade 2. This species also differs morphologically by having dichasial inflorescences (Moquin-Tandon, 1840; Weber, 1985) and bristle-tipped terminal inflorescence branches (Mosyakin and Clemants 2002; 2008) that would support its recognition as a distinct genus *Teloxys*. It should be noted that this circumscription of *Teloxys* corresponds to the concept of the genus held by Ulbrich (1934) or of subsect. *Teloxys* (of *Dysphania* sect. *Dysphania*) proposed by Mosyakin and Clemants (2002), respectively.

Clade 3 is strongly supported by *trnL-F* (Fig. 2.1; 99% JK / 1 PP) to comprise several *Chenopodium spp.*, *Monolepis* and *Spinacia* but has only moderate support in the ITS tree (67% JK, Fig. 2.2). It corresponds to "Chenopodieae II" sensu Kadereit et al. (2003; 2010) that, however, was not recovered with statistic confidence in their previously published *rbcL* tree. "Chenopodieae II" as depicted here (Figs. 2.1 and 2.2) lacks clear morphological synapomorphies except the presence of dense, head-like glomerules on terminal or axillary branches. Within this clade, a *Spinacia* lineage is highly supported by both DNA markers (100 JK, Figs. 2.1 and 2.2) as sister to the remaining taxa, and comprises all *Spinacia* species described so far (Kühn et al., 1993; Welsh et al., 2003). The *Spinacia* subclade is further characterized by the presence of unisexual flowers without perianth, not found in the sister lineage comprising *Ch. capitatum, Ch. californicum*, *Ch. bonus-henricus*, *Ch. foliosum* and *Monolepis nuttalliana*. The latter all have bisexual flowers and a differentiated perianth. Our data strongly refute a previous hypothesis of a sister group relationship between *Spinacia oleracea* and *Monolepis nuttaliana* that was found based on *rbcL* sequences (Kadereit et al., 2003), but agree with the recently suggested exclusion of *Spinacia* from *Atripliceae* (Kadereit et al., 2010).

The *Chenopodium rubrum*-clade (Clade 4; Figs. 2.1 and 2.2) is newly resolved here based on both *trnL-F* and ITS with maximum confidence. It encompasses *Chenopodium chenopodioides, Ch. glaucum, Ch. rubrum* and *Ch. urbicum*. Whereas chloroplast sequences suggest its placement in a grade branching after the Chenopodieae II (= Spinacieae; see Fig. 2.1), nuclear ITS data provide some evidence for the clade being sister to a *Ch. murale*-clade (Clade 4; Fig. 2.2). Based on the available morphological data, morphological synapomorphies for this clade are not clear at this point. Flowers with 3-4 perianth segments are shared by *Ch. rubrum* and *Ch. glaucum*, whereas flowers of *Ch. chenopodioides* consistently present only three segments, and those of *Ch. urbicum* have five segments. Moreover, a reddish seed coat is shared by *Ch. rubrum, Ch. glaucum* and *Ch. urbicum*, whereas *Ch. chenopodioides* has seeds with a black coat. Inflorescences with subglobose glomerules are present in all these taxa and seem to be a synapomorphy. Nevertheless, this character is homoplastic within Chenopodioideae (Iljin, 1936; Aellen, 1960; Welsh et al., 2003). Looking at the sectional level within *Chenopodium*, (based on Aellen and Just, 1943 and Aellen, 1960) this clade is composed of members of section *Pseudoblitum* (*Ch. rubrum* and *Ch. glaucum*) that is morphologically similar to section *Degenia* (Aellen and Just, 1943), here represented by *Ch. chenopodioides.* Our data confirm this, although the type species of the latter section, *Ch. macrospermum*, remains to be included in a molecular analysis. *Chenopodium urbicum* of section *Chenopodia* subsection *Lejosperma* is also part of this clade (Aellen and Just, 1943). This means that smooth or nearly smooth seeds and an indistinctly ridged or slightly pitted testa must have evolved twice, once in *Ch. urbicum* and second in the ancestor of the remaining, largely North American species of subsect. *Lejosperma*, which thus is clearly polyphyletic.

As currently depicted, the *Chenopodium rubrum*-clade comprises annual herbs with triangular, narrowly triangular, rhombic or lanceolate leaf blades, and sinuate, dentate or serrate leaf margins. The inflorescences are composed of subglobose glomerules. The flowers have 3-5 tepals, and uniseriate trichomes are only found on axillary (flower or leaf) buds. The seeds have a rounded margin and are smooth or rugulate.

The *Chenopodium murale*-clade (Clade 5; 91% JK with *trnL-F* and 83% JK with ITS; Fig. 2.1) contains three species (*Ch. murale*, *Ch. coronopus* and *Ch. hybridum*), all characterized by rounded, compressed and rugose seeds (Iljin, 1936). This feature, not present in all other species sampled so far, seems to be a synapormorphy for clade 5. The

Chenopodium murale-clade is further divided into two well-supported subclades (each 100% JK *trnL-F* and ITS; Figs. 2.1 and 2.2): one containing all accessions of *Ch. Hybridum*, the other all accessions of *Ch. murale* and *Ch. coronopus*. Morphologically, *Ch. hybridum* differs from the two latter species by having seeds without conspicuously flattened margins (Iljin, 1936). The *Chenopodium murale*-clade corresponds to the subsection *Unduata* as formally recognized by Mosyakin and Clemants (1996). The description of this subsection was based on *Ch. murale* as type species (Mosyakin and Clemants, 1996).

The large clade shown at the top of Figs. 2.1 and 2.2 (clade 7) is the clade of *Chenopodium* s.str., and corresponds to the "Chenopodieae I" of Kadereit et al. (2003), but excluding *Microgynoecium*. It is sister to the phylogenetically defined *Atripliceae* (Kadereit et al., 2010; clade 6). The position of *Microgynoecium* was inferred with only weak support as sister to the remaining Chenopodieae I using *rbcL*. There is now increasing evidence that it rather belongs to the *Atripliceae* (Fig. 2.1; see below). The large *Chenopodium* s.str. clade (clade 7) encloses most species of *Chenopodium* sampled in this study (> 40%), including *Chenopodium album*, which is the type species of *Chenopodium* (Mosyakin and Clemants, 1996), along with the genera *Einadia* and *Rhagodia* (Figs. 2.1 and 2.2). Overall, the phylogenetic relationships within this core *Chenopodium* clade are not well resolved. There is also some difficulty in finding morphological synapomorphies for the entire clade. Nevertheless, some characters seem diagnostic to particular subclades. *Chenopodium vulvaria* is characterized by a particular fetid odour due to its trimethylamine compounds (Croemwell, 1950). It forms an isolated lineage (100% JK / 1 PP) in both the ITS and *trnL-F* trees. *Einadia* and *Rhagodia*, which differ from *Chenopodium* by their perennial habit and fleshy fruits (Wilson, 1983), form a well-supported lineage in the *trnL-F* tree, which also includes the Australian *Ch. desertorum* (92% JK / 1 PP). The chloroplast tree (Fig. 2.1) further depicts three major subclades, one comprising the polyploid *Ch. album* and relatives (the "*Ch. album* complex"), then a lineage of South American diploid species (*Ch. atrovirens*, *Ch. frigidum*, *Ch. pallidicaule* and *Ch. petiolare*), and the biggest one with the allotetraploid *Ch. quinoa*, and *Ch. berlandieri* together with numerous North American diploid species. Nonetheless, resolution within this major *Chenopodium* clade in the ITS tree is even worse than in the chloroplast tree (Fig. 2.2).

2.4.4 Circumscription and phylogenetic position of the tribes *Atripliceae* and *Axyrideae*

Within the *Atripliceae* and the *Axyrideae*, our analyses reveal two highly supported lineages of *Chenopodium* sensu lato that have been recognized at tribal levels (Heklau and Röser, 2008; Kadereit et al., 2010; clades 1 and 6, respectively). The *Axyrideae* might be sister to all remaining Chenopodioideae (Fig. 2.1; chloroplast data), also found by Kadereit et al. (2010), or constitute a third branch (ITS data; Fig. 2.2). As indicated before, additional nuclear data are needed to test this hypothesis. The *Atripliceae* are congruently inferred to be nested within *Chenopodium* sensu lato. Looking at a refined tribal classification within Chenopodioideae, they are also nested within Chenopodieae.

The *Atripliceae* clade including *Microgynoecium* receives high support (97% JK/ 1 PP) based on *trnL-F* sequence data. The position of *Microgynoecium tibeticum* as sister to all remaining taxa of *Atripliceae* (96% JK / 1 PP *trnL-F*; Fig. 2.1) is also depicted here. This is congruent to the *atpB-rbcL* topology in Kadereit et al. (2010), albeit the tree shown in the latter study lacks significant posterior probabilities for the respective nodes. However, our nuclear ITS tree, the position of *Microgynoecium* is inconsistently resolved as sister to the remaining Atripliceae plus the *Chenopodium* s.str. clade (50% JK / 0.83 PP; Fig. 2.2). The BEAST summary tree based on ITS of Kadereit et al. (2010) depicts *Microgynoecium* in yet another position, as sister to the *Archiatriplex* clade, but again lacking statistical confidence. The broad scale analysis of Caryophyllales using *petD* intron sequences (Schäferhoff et al., 2009) also indicates a close affinity of *Microgynoecium* to the *Atripliceae*, although their taxon sampling of Chenopodiaceae is low. The flowers of *Microgynoecium* are similar to *Archiatriplex* (Flores Olvera and Davis, 2001), a fact supporting close affinities between these two taxa. Pollen morphology of *Microgynoecium* rather stands out from most other taxa of *Atripliceae*. Together with *Manochlamys*, the genus *Archiatriplex* has very large pollen grains but a high pore number with few ektexinous bodies (Flores Olvera et al., 2006). Overall, our results do not support a relationship between *Axyris* and *Microgynoecium*, as suggested by Flores Olvera and Davis (2001), based on flower morphology. Instead, *Microgynoecium* most likely belongs to the *Atripliceae*, although the nuclear-based phylogenies require further testing through additional genomic regions. Internally, the *Atripliceae* are composed of two major lineages: one encompassing *Atriplex*, the other *Grayia brandegeei*, *G. spinosa* and *Stutzia*

dioica (Figs. 2.1 and 2.2). This corresponds to the *Atriplex* clade and the *Archiatriplex* clade in line with the denser sampled analyses of the *Atripliceae* by Kadereit et al. (2010) and Zacharias and Baldwin (2010). *Graya* and *Stutzia* share morphological features, such as the presence of carnose leaves, characteristic inflorescences in glomerules and with non-foliose bracts, and fruits with short bracteoles of less than half the length of the leaves (Flores Olvera and Davis, 2001; Flores Olvera et al., 2006; Kadereit et al., 2010).

The *Axyrideae* appear as an isolated lineage and include *Axyris*, *Ceratocarpus*, and *Krascheninnikovia*. The genera *Ceratocarpus* and *Krascheninnikovia* are characterized by the presence of sub-stellate dendroid hairs and form a strongly supported subclade, which is sister to *Axyris* (*trnL-F* 100% JK / 1 PP; ITS 74% JK / 0.86 PP), the latter having sub-stellate branched hairs (Kühn, 1993; Flores Olvera and Davis, 2001; Welsh et al., 2003; Zhu et al., 2003; Heklau and Röser, 2008). Their close relationship was also suggested based on pollen morphology because these three genera share the highest density of microspines (Flores Olvera et al., 2006). Our results support the monophyly and relationships of *Axyris*, *Ceratocarpus*, and *Krascheninnikovia* as reported by Heklau and Röser (2008) and Kadereit et al. (2010). However, considering the branching sequence of major clades of the Chenopodioideae, which are all recognized at tribal level, the subtribe *Axyridinae* should also be classified as an own tribe as suggested by Kadereit et al. (2010).

2.4.5 Chromosome evolution in Chenopodioideae

Differences in chromosome numbers have long been known in *Chenopodium* and relatives, but so far no attempt has been made to study chromosome evolution in a phylogenetic context (Appendix C). In addition to genome duplications that result in higher ploidy level, as reported from *Atriplex* (Kühn et al., 1993; Welsh et al., 2003) and *Chenopodium* s.str. (this study), dysploid changes in chromosome number were anticipated (Aellen and Just, 1943). The base chromosome numbers in angiosperms can either correlate with lineages (Schneeweiss et al., 2004, Hidalgo et al., 2007; Blöch et al., 2009) or evolve independently (e.g., Baldwin and Wessa, 2000; Ellison et al., 2006). Chenopodioideae provide another case for lineage specific dyploid chromosome number changes, as suggested by our results. Whereas a base number of x=9 can be unambiguously inferred for Chenopodioideae, thus corroborating earlier ideas of Turner (1994), our tree topology suggests independent dysploid chromosome loss in two derived lineages. One is

found in the subclade of *Ch. ambrosioides, Ch. multifidum* and *Ch. graveolens* (x=8; IPCN 1986-2003; Fig. 2.1). Also, the *Spinacia* subclade is characterized by the unusual chromosome number of x=6, that only can be explained by a reduction from x=9 (Fig. 2.1). Polyploidy, on the other hand, seems to be less characteristic for lineages. It rather seems to occur within some lineages, such as the *Atripliceae* and *Chenopodium* s.str. (clade 7), where speciation may be triggered by polyploid formation. For *Chenopodium*, further analysis of clade 7 will be needed to unravel putative events of reticulation and allopolyploid speciation. Additional chloroplast markers are necessary to improve tree resolution and sequences of low copy nuclear genes are needed for testing the ITS topology.

2.4.6 Towards a new tribal and generic classification of Chenopodioideae

Our results support the subdivision of *Chenopodium* into five separate, well-supported clades (Figs. 2.1 and 2.2) within Chenopodioideae. These clades themselves are paraphyletic to other genera. The necessary taxonomic changes should be oriented at a compromise to conserve traditional use of generic names and to implement new molecular results that allow classifying only monophyletic groups. Keeping a large genus *Chenopodium* (Aellen and Just, 1943; Aellen, 1960; Kühn, 1993; Judd and Ferguson, 1999; Welsh et al., 2003) would dramatically underestimate the morphological diversity in this group. It would also result in the inclusion of well-known genera, such as *Atriplex* or *Spinacia*, in *Chenopodium*. Giving a new name for each clade found by molecular data without any other evidence, would add to the current taxonomic confusion in the group.

In this study, we found that the highly paraphyletic genus *Chenopodium* comprises five lineages which could be recognised at generic level (corresponding to clades 2, 3, 4, 5 and 7; Fig. 2.2). However, the situation is complex and further work, including a larger taxon sampling, are needed before the genus can finally be re-classified. For some clades, the situation is clearer. e.g. *Dysphania* should be accepted as a genus for the most diverse sublineage of clade 2 (see Figs. 2.1 and 2.2), following the suggestion of Kadereit et al. (2010). The same applies to the genus *Teloxys* (see above) which so far is only sometimes accepted in more recent treatments. All names for the respective genera are already available. Another clear situation exists for the well supported clade of *Chenopodium* sensu stricto (clade 7) that also contains the type species of the genus, *Ch. album* L.

(lectotypified by Mosyakin and Clemants, 1996). As a consequence of this study, *Einadia* Raf. and *Rhagodia* R. Br. should be included in *Chenopodium* L. (the necessary new names are provided below). The subtribe *Rhagodiinae*, proposed by Scott (1978b) to subdivide the *Chenopodieae*, can therefore not be upheld. Its diagnostic features, such as a succulent pericarp and predominantly unisexual flowers (Scott 1978b), now rather appear as homoplastic derived states that arose independently in several lineages of the subfamily Chenopodioideae. *Holmbergia*, which was also included in *Rhagodiinae* by Scott (1978b) based on its spongy and inflated berries, was shown to belong to the *Archiatriplex* clade of *Atripliceae* by Kadereit et al. (2010) and Zacharias and Baldwin (2010).

Within Chenopodioideae, two additional major clades deserve recognition at tribal level. One are the *Dysphanieae* (Fig. 2.2). Based on molecular markers and morphological characters (trichomes), the monophyly and isolated position of *Dysphania*, *Suckleya*, and *Teloxys* is evident (Clade 2, Figs. 2.1 and 2.2). This tribe *Dysphanieae* was already proposed by Pax (1889) but to accommodate the three Australian species of *Dysphania* within Caryophyllaceae-Alsinoideae. Pax thereby had followed the view of Bentham (1870) who placed *Dysphania* rather as an isolated genus in Chenopodiaceae than a somewhat abnormal genus of Illecebraceae (based on *Illecebrum*, a member of Caryophyllaceae-Alsinoideae-Paronychieae; Pax, 1889). Almost half a century later, Pax (1927) created an own family Dysphaniaceae for the genus *Dysphania*, which was based on the valvate perianth and pedicelled perianth parts. He considered Dysphaniaceae to be intermediate between Chenopodiaceae and Caryophyllaceae, a view upheld in the second edition of the Natürlichen Pflanzenfamilien (Pax and Hoffmann, 1934). Aellen (1960) included *Dysphania* in *Chenopodium* as an own section. Eckardt (1967) corroborated this view by his comparative anatomical study, in which he found the floral architecture and gynoecium of *Dysphania* to strongly differ from Illecebraceae. Scott (1978a), however, classified an own subg. *Ambrosia* of *Chenopodium*, based on *Ch. ambrosioides* as type species, but on the other hand kept *Dysphania* as a separate genus. As indicated above, our phylogenetic data finally show that all the aromatic species that were shuffled in these pre-cladistic classification systems, in fact belong to a single clade that is best named *Dysphania*. On a higher level, *Dyphania*, *Suckleya* and *Teloxys* compose the *Dysphanieae*. In line with this, and recent molecular findings by Kadereit et al. (2010), our results also

support the inclusion of the subtribe *Suckleyinae* in *Dysphanieae* and not in *Chenopodieae* as originally proposed by Chu et al. (1991).

The other lineage that should be recognized at tribal level is the *Spinacieae* (clade 3). The tribe *Spinacieae* was originally described by Moquin-Tandon (1840) and included *Atriplex* along with a number of further genera. The earlier published *Atripliceae* (Meyer, 1829) were then used by most other authors in a circumscription that included *Spinacia*.

In this study, we newly define *Spinaceae* as different from *Atripliceae* and to include the genera *Monolepis* and *Spinacia*, along with a group of *Chenopodium* species related to *Chenopodium capitatum* and *C. foliosum* (Figs. 2.1 and 2.2) and *Scleroblitum* (not sampled here but closely related to *Ch. foliosum* based on *rbcL*; Kadereit et al. 2003). However, relationships within *Spinacieae* require further study. An issue will be to test, by inclusion of more taxa and sequence characters, if the respective *Chenopodium* species within this larger clade are monophyletic. Such a monophyletic assemblage would then correspond to the Linnaean genus *Blitum* (*Blitum capitatum* = *Ch. capitatum*; lectotypified by Mosyakin and Clemants, 1996).

Even with these realignments, the tribe *Chenopodieae* remains paraphyletic to the *Atripliceae*. Right now, our trees depict two clades which are composed of *Chenopodium rubrum* and relatives (clade 4, Figs. 2.1 and 2.2), and of *Chenopodium murale* and relatives (clade 5; Figs. 2.1 and 2.2), with unclear relationships to each other. Further characters, both molecular and morphological, are needed to resolve this part of the *Chenopodieae* and to move towards a stable generic classification. Finally, phylogenetic classification will either require establishing one or two additional tribes or merging *Atripliceae* and *Chenopodieae*.

In summary, our study showed that the current delimitations of *Chenopodium* need to be redefined. We suggest, based on our phylogenetic reconstruction, that the clade 7 (Figs. 2.1 and 2.2) may best represent the monophyletic *Chenopodium* s.str.

2.5. Taxonomic conclusions

For several species of the genera *Einadia* and *Rhagodia*, no names under *Chenopodium* exist. These are validated in the following treatment. However, names under

Chenopodium do already exist for a number of species that so far have been treated under *Einadia* and *Rhagodia* (Wilson, 1983). These are: *Chenopodium allanii* Aellen; *Chenopodium baccatum* Labill.; *Chenopodium polygonoides* (Murr.) Aellen; *Chenopodium preissii* (Moq.) Diels; *Chenopodium triandrum* G. Forster; *Chenopodium trigonon* Roem. et Schult.; *Chenopodium ulicinum* Gand.

1) *Chenopodium nutans* (R. Br.) S. Fuentes & Borsch, **comb. nov.** Basionym: *Rhagodia nutans* R. Br., Prodr. Fl. Nov. Holland. 408. 1810. ≡ *Einadia nutans* (R. Br.) A. J. Scott, Feddes Repert. 89: 3. 1978.

1a) *Chenopodium nutans* (R. Br.) S. Fuentes & Borsch subsp. *nutans.*

1b) *Chenopodium nutans* subsp. *oxycarpa* (Gauba) S. Fuentes & Borsch, **comb. nov.** Basionym: *Einadia nutans* subsp. *oxycarpa* (Gauba) Paul G. Wilson, Nuytsia 4(2): 203. 1983. ≡ *Rhagodia nutans* var. *oxycarpa* Gauba, Vict. Nat. 65: 167. 1948.

1c) *Chenopodium nutans* subsp. *linifolia* (R. Br.) S. Fuentes & Borsch, **comb. nov.** Basionym: *Einadia nutans* subsp. *linifolia* (R. Br.) Paul G. Wilson, Nuytsia 4(2): 204. 1983. ≡ *Rhagodia linifolia* R. Br., Prodr. Fl. Nov. Holland. 408. 1810. ≡ *Einadia linifolia* (R. Br.) Raf. Fl. Tellur. 4: 121. 1838; Ulbrich in Engler et Pratl. Nat. Pflanzenfam. ed. 2, 16c: 558. 1934 pro. syn. sub *Suaeda linifolia* Pall. ≡ *Einadia nutans* var. *linifolia* (R. Br.) A. J. Scott, Feddes Repert. 89: 4. 1978.

1d) *Chenopodium nutans* subsp. *eremaea* (Paul G. Wilson) S. Fuentes & Borsch, **comb. nov.** Basionym: *Einadia nutans* subsp. *eremaea* Paul G. Wilson, Nuytsia 4: 204. 1983.

2) Infraspecific taxa of *Chenopodium trigonon* Roem. et Schult., Syst. Veg. 6: 275. 1820.

2a) *Chenopodium trigonon* subsp. *stellulatum* (Benth.) S. Fuentes & Borsch, **comb. nov.** Basionym: *Einadia trigonos* subsp. *stellulata* (Benth.) Paul G. Wilson, Nuytsia 4(2): 208. 1983. ≡ *Chenopodium triangulare* var. *stellulatum* Benth. Fl. Austral. 5: 161.1870. ≡ *Ch. stellulatum* (Benth.) Aellen, Verh. Naturf. Ges. Basel 41: 93. 1931. nom. illeg., non Aellen, 1928.

2b) *Chenopodium trigonon* subsp. *leiocarpa* (Paul G. Wilson) S. Fuentes & Borsch, **comb. nov.** Basionym: *Einadia trigonos* subsp. *leiocarpa* Paul G. Wilson, Nuytsia 4(2): 209. 1983.

3) *Chenopodium hastata* (R. Br.) S. Fuentes & Borsch, **comb. nov.** Basionym: *Rhagodia hastata* R. Br., Prodr. Fl. Nov. Holland. 408. 1810. ≡ *Einadia hastata* (R. Br.) A. J. Scott, Feddes Repert. 89: 4 (1978).

4) Infraspecific taxa of *Chenopodium baccatum* Labill.

4a) *Chenopodium baccatum* subsp. *dioicum* (Nees) S. Fuentes & Borsch, **comb. nov.** Basionym: *Rhagodia baccata* subsp. *dioica* (Nees) Paul G. Wilson, Nuytsia 4(2): 225. 1983. ≡ *Rhagodia dioica* Nees, Pl. Preiss. 1: 636. 1845.

5) *Chenopodium candolleanum* (Moq.) S. Fuentes & Borsch, **comb. nov.** Basionym: *Rhagodia candolleana* Moq., Chenop. Monogr. Enum. 10. 1840. ≡ *Rhagodia baccata* var. *candolleana* (Moq.) Moq., Prod. (DC.) 13(2): 50 (1849).

5a) *Chenopodium candolleanum* (Moq.) S. Fuentes & Borsch, subsp. *candolleanum.*

5b) *Chenopodium candolleanum* subsp. *argenteum* (Paul G. Wilson) S. Fuentes & Borsch, **comb. nov.** Basionym: *Rhagodia candolleana* subsp. *argentea* Paul G. Wilson, Nuytsia 4(2): 215. 1983.

6) *Chenopodium crassifolium* (R. Br.) S. Fuentes & Borsch, **comb. nov.** Basionym: *Rhagodia crassifolia* R. Br., Prodr. Fl. Nov. Holland.: 408. 1810.

7) *Chenopodium acicularis* (Paul G. Wilson) S. Fuentes & Borsch, **comb. nov.** Basionym: *Rhagodia acicularis* Paul G. Wilson, Nuytsia 4(1): 51. 1982.

8) Infraspecific taxa of *Chenopodium preissii* (Moq.) Diels.

8a) *Chenopodium preissii* subsp. *obovatum* (Moq.) S. Fuentes & Borsch, **comb. nov.** Basionym: *Rhagodia preisii* subsp. *obovata* (Moq.) Paul G. Wilson, Nuytsia 4(2): 222. 1983. ≡ *Rhagodia obovata* Moq., Chenop. Monogr. Enum.: 10 (1840).

9) *Chenopodium latifolium* (Benth.) S. Fuentes & Borsch, **comb. nov.** Basionym: *Rhagodia latifolia* (Benth.) Paul G. Wilson, Nuytsia 4(2): 228. 1983. ≡ *Rhagodia crassifolia* var. *latifolia* Benth., Fl. Austral. 5: 155. 1870.

9a) *Chenopodium latifolium* subsp. *rectum* (Paul G. Wilson) S. Fuentes & Borsch, **comb. nov**. Basionym: *Rhagodia latifolia* subsp. *recta* Paul G. Wilson, Nuytsia 4(2): 228. 1983.

10) *Chenopodium drummondii* (Moq.) S. Fuentes & Borsch, **comb. nov**. Basionym: *Rhagodia drummondii* Moq., Prod. (DC.) 13(2): 52. 1849.

11) *Chenopodium spinescens* (R. Br.) S. Fuentes & Borsch, **comb. nov.** Basionym: *Rhagodia spinescens* R. Br., Prodr. Fl. Nov. Holland.: 408. 1810.

12) *Chenopodium eremaea* (Paul G. Wilson) S. Fuentes & Borsch, **comb. nov.** Basionym: *Rhagodia eremaea* Paul G. Wilson, Nuytsia 4(2): 232. 1983.

13) *Chenopodium parabolicum* (R. Br.) S. Fuentes & Borsch, **comb. nov.** Basionym: *Rhagodia parabolica* R. Br., Prodr. Fl. Nov. Holland.: 408. 1810.

Appendix 2.A – List of indels found in the *trnL-F* region

	trnL-F intron		
No.	**Extension**	**Length**	**Sequence motif**
1	72-72	1	Gap in *Microgynoecium tibeticum*, probably deletion.
2	78-78	1	Gap in *Ch. atrovirens*.
3	106-110	5	Gap in *Bassia*, probably deletion.
4	107-110	4	"TTTT" SSR in *Hablitzia* and "TTTG" insertion in *Kraschenninikovia*.
5	108-110	3	Gap in *Ch. chenopodioides* overlaping with indel no. 4.
6	120-147	28	Insertion of "TACTCAAAAGAAAAAAATAATAAAAAAG" in clade 5 (except *Ch. hybridum*).
7	120-150	31	Gap in *Kraschenninikovia*.
8	120-166	47	Gap in clade 3 and *Bassia prostrata*, probably deletion.
9	120-169	50	Gap in *Hablitzia*, probably deletion.
10	120-175	56	Gap in *Bassia lanifloria*, *Bassia scoparia* and *Allenrolfea vaginata*, probably deletion.
11	148-163	16	Gap in clade 5 (except *Ch. hybridum*), probably deletion.
12	151-175	25	Gap in *Suckleya suckleyana*, probably deletion.
13	163-163	1	Insertion of "T" in *Ceratocarpus* and *Kraschenninikovia*.
14	171-175	5	Insertion of "GGAAA" in *Ch. hybridum*.
15	215-524	310	Gap in *Spinacia*, probably deletion.
16	222-226	5	Insertion of "GGGTT" in *Allenrolfea*, *Bassia*.
17	232-241	10	Gap in *Bassia*, probably deletion.
18	234-238	5	Gap in *Allenrolfea*, clade 2 (except *Ch. aristatum*), *Ch. fremontii* sample AC579, *Ch. pallescens* sample AC594, *Ch. incanum*, *Ch. nevadense* and *Ch. desiccatum*.
19	235-239	5	Gap in *Ceratocarpus arenarius*.
20	256-256	1	Insertion of "A" in *Kraschenninikovia*.
21	259-264	6	Insertion of "GCTTCC" SSR in *Microgynoecium tibeticum*.
22	275-275	1	Gap in *Allenrolfea*.
23	277-301	25	Gap in clade 4.
24	286-286	1	Insertion of "A" in clade 3 (except *Spinacia*).
25	300-301	2	"AT" SSR in *Bassia prostrata*.
26	308-310	3	"AAA" SSR in *Ceratocarpus arenarius*.
27	315-498	184	Gap in clade 2, probably deletion.
28	339-339	1	Insertion of "T" in *Allenrolfea*, *Beta*, *Hablitzia*, *Bassia*.
29	340-346	7	Gap in *Allenrofea* and *Beta*.
30	346-379	34	Gap in *Hablitzia*.
31	347-457	111	Gap in *Ceratocarpus arenarius* sample AC531.
32	347-460	114	Gap in *Ceratocarpus arenarius* sample AC649.
33	347-495	149	Gap in *Microgynoecium tibeticum*.
34	347-556	210	Gap in *Grayia*, *Atriplex* and *Stutzia*, probably large deletion.
35	371-375	5	Gap in *Ch. pallidicaule*, probably deletion.
36	372-375	4	Gap in clade 4, clade 5, clade 7, clade 3 (except *Ceratocarpus*), clade 1 (except *Spinacia*).
37	373-375	3	Gap in *Ch. opulifolium*, *Ch. iljinii* and *Ch. album* sample AC575.
38	383-391	9	Insertion of "AATATAGAA" in *Ch. foliosum*.
39	397-407	11	Insertion of "TTCGAATATGA" in *Ch. hybridum*.
40	410-457	48	Gap in clade 4.
41	413-422	10	Gap in *Bassia*, probably deletion.
42	415-416	2	Gap in *Kraschenninikovia*.
43	416-422	7	Gap in *Allenrolfea* and *Bassia*.
44	417-420	4	Gap in clade 3 except *Spinacia*.
45	417-422	6	Gap in *Beta*.
46	418-470	53	Gap in clade 5 and clade 7.
47	424-425	2	Gap in *Axyris* and clade 3 (except *Spinacia*).

Appendix 2.A – List of indels found in the *trnL-F* region (continued)

***trnL-F* intron (continued)**			
No.	**Extension**	**Length**	**Sequence motif**
48	427-427	1	Gap in *Allenrolfea*, *Beta*, *Hablitzia*, *Bassia*.
49	427-443	17	Gap in *Krascheninnikovia* and *Axyris*.
50	430-443	14	Gap in *Beta*, *Hablitzia* and *Bassia*.
51	434-435	2	Gap in *Ch. capitatum* var. *parvicapitatum*.
52	434-457	24	Gap in *Monolepis nuttalliana*.
53	435-450	16	Gap in *Ch. californicum* and *Ch. foliosum*.
54	436-443	8	Gap in *Allenrolfea*.
55	444-450	7	Gap in *Ch. capitatum* var. *parvicapitatum*.
56	445-457	13	Gap in *Bassia*.
57	447-450	4	Gap in *Allenrolfea*, *Beta* and *Hablitzia*.
58	457-457	1	Insertion of "T" in *Ch. capitatum* var. *parvicapitatum*.
59	465-470	6	Gap in clade 4.
60	468-470	3	Insertion of "AAA" in *Bassia*.
61	470-470	1	Gap in *Bassia scoparia*.
62	484-490	7	Gap in clade 5 (except *Ch. hybridum*).
63	486-495	10	Gap in *Allenrolfea*, *Beta*, *Hablitzia*, *Bassia*, *Ceratocarpus*, *Krascheninnikovia* and *Axyris*.
64	487-495	9	Gap in *Ch. hybridum* and clade 7.
65	491-495	5	"ATTTA" SSR in clade 5 (except *Ch. hybridum*).
66	513-516	4	Insertion of "GAAA" in *Bassia lanifloria* and *Bassia scoparia*.
67	531-546	16	Gap in *Beta*.
68	550-550	1	Insertion of "C" in clade 3 (except *Spinacia*).
69	550-555	6	Gap in all taxa (except *Bassia prostrata*, *Ch. bonus-henricus*, *Ch. capitatum*, *Ch. foliosum*, *Ch. californicum and Monolepis nuttalliana*).
70	551-555	5	Insertion of "CATAA" in *Bassia prostrata*.
71	557-605	49	Gap in *Allenrolfea*.
72	560-569	10	Gap in *Hablitzia*.
73	562-564	3	Insertion of "GAA" in *Ch. rubrum*.
74	562-565	4	Gap in *Bassia*.
75	565-565	1	Gap in *Ch. rubrum* sample AC385, probably deletion of "C".
76	585-585	1	Insertion of "A" in *Spinacia*.
77	589-595	7	"AATTAAT" SSR in *Spinacia*
78	608-610	3	"AGA" SSR in *Ceratocarpus*, *Krascheninnikovia*, *Axyris*

***trnL-F* spacer**			
No.	**Extension**	**Length**	**Sequence motif**
79	735-736	2	Gap in *Beta*, *Bassia*.
80	736-736	1	Gap in clade 2 (except *Ch. aristatum*), in clade 1, *Atriplex*, *Microgynoecium tibeticum* and *Ch. hybridum*.
81	737-760	24	Gap in *Hablitzia*.
82	744-749	6	Insertion of "ATCCCT" in *Grayia*.
83	761-761	1	Gap in *Ceratocarpus*.
84	761-793	33	Gap *Ch. urbicum*.
85	765-765	1	Insertion of "C" in *Ch. atrovirens* sample AC540.
86	771-1116	346	Large deletion in *Stutzia dioica*.
87	778-1072	295	Large deletion in *Ch. petiolare* sample AC359.
88	789-790	2	Gap in clade 4, clade 5, clade 6 and clade 7 (except *Ch. petiolare* sample AC359).
89	790-793	4	Gap in *Ceratocarpus*.

Appendix 2.A – List of indels found in the *trnL-F* region (continued)

trnL-F spacer (continued)			
No.	**Extension**	**Length**	**Sequence motif**
90	800-845	51	Gap in *Spinacia*, probably deletion.
91	804-819	21	Gap in *Ch. graveolens*.
92	808-808	1	Insertion of "C" in *Ch. aristatum*.
93	808-813	6	Gap in all taxa except *Ch. aristatum* and *Ch. foliosum*.
94	809-813	5	"TATGA" SSR in *Ch. foliosum*.
95	819-825	7	Gap in clade 4.
96	828-828	1	Gap in *Ch. californicum*.
97	833-852	20	Gap in *Ch. pumilio*, *Ch. melanocarpum* and *Ch. schraderianum*.
98	838-842	5	"ATCCC" SSR in *Hablitzia* and "ATCAC" SSR in *Ceratocarpus* and *Krascheninnikovia*.
99	852-877	26	Gap in *Beta*.
100	863-871	9	Gap in clade 2.
101	864-864	1	Gap, probably "A" deleted in *Bassia*.
102	865-871	7	Gap in *Bassia*, clade 1 (except *Ceratocarpus*), clade 3 and clade 6.
103	865-875	11	Gap in *Ceratocarpus*.
104	867-869	3	Gap in *Ch. hians*.
105	867-871	5	"ATATA" SSR in *Ch. iljinii* and *Ch. album* sample AC575.
106	869-871	3	Gap in *Allenrolfea*.
107	874-875	2	Insertion of "GT" in *Bassia*.
108	878-882	5	Gap in clade 7 except *Ch. petiolare* sample AC359.
109	900-904	4	Insertion of "AAAAA" in *Ch. graveolens*.
110	914-918	5	Insertion of "ATAGA" in *Ceratocarpus* and *Krascheninnikovia*.
111	928-928	1	Insertion of "A" in *Einadia nutans*.
112	945-949	5	"AATAT" SSR in *Ch.rubrum* and *Ch. glaucum*.
113	949-949	1	Gap in clade 1.
114	952-956	5	Insertion of "TATCA" in *Beta*.
115	952-964	13	Gap in *Ceratocarpus* and clade 2 (except *Ch. aristatum*).
116	952-972	21	Gap in *Allenrolfea*, *Hablitzia*, *Bassia*, *Ch. aristatum*, clade 3 (except *Ch. chenopodioides*), clade 5 (except *Ch. hybridum*), clade 6 and clade 7.
117	952-976	25	Gap in *Ch. chenopodioides*.
118	957-972	16	Gap in *Beta*.
119	960-964	5	Gap in *Krascheninnikovia*.
120	960-972	13	Gap in *Axyris*.
121	962-972	11	Gap in *Ch. hybridum*.
122	963-972	10	Gap in *Ch. rubrum* and *Ch. glaucum*.
123	978-980	3	Gap in clade 2 (except *Ch. aristatum*).
124	992-1013	22	Gap in *Suckleya suckleyana*.
125	993-1001	9	Insertion of "GTTAAATAA" in *Ch. aristatum*.
126	993-1004	12	Gap in *Grayia spinosa*.
127	993-1012	20	Gap in *Ceratocarpus*.
128	993-1044	52	Gap in *Beta*.
129	1007-1043	37	Gap in *Ch. graveolens*.
130	1008-1013	6	Insertion of "ATKAAA" in *Ch. hybridum*.
131	1018-1021	4	Insertion of "CTTA" in clade 7.
132	1018-1025	8	Gap in *Ch. aristatum*.
133	1018-1032	15	Gap in clade 6 and in *Microgynoecium tibeticum*.
134	1018-1036	19	Gap in *Monolepis nuttalliana*.

Appendix 2.A – List of indels found in the *trnL-F* region (continued)

trnL-F spacer (continued)			
No.	**Extension**	**Length**	**Sequence motif**
135	1018-1041	24	Gap in *Ch. ambrosoides* AC425.
136	1018-1042	24	Gap in *Allenrolfea*, *Beta*, *Bassia*, clade 1, clade 2 (except *Ch. ambrosioides* sample AC425, *Ch. graveolens* and *Ch. aristatum*), clade 3 (except *Monolepis nuttalliana*), clade 4 and clade 5.
137	1022-1042	21	Gap in clade 7 except *Ch. petiolare* sample AC359.
138	1026-1042	17	Gap in *Hablitzia*.
139	1033-1042	10	Gap in *Ch. aristatum*.
140	1037-1042	6	Gap in clade 6.
141	1054-1059	6	Gap in *Hablitzia*.
142	1055-1059	5	Insertion of "TTTTW" in *Ch. capitatum*, *Ch. californicum*, *Ch. foliosum* and *Monolepis nuttalliana*.
143	1065-1069	5	Gap in *Ch. rubrum* and *Ch. glaucum*.
144	1078-1093	16	Insertion of "TTAAGGAATTAAGGAA" in *Ch. desertorum*.
145	1086-1093	8	Gap in *Rhagodia* and *Einadia*.
146	1101-1108	8	Insertion of "ACGCATAT" in *Ch. bonus-henricus*, *Ch. capitatum*, *Monolepis nuttalliana* and *Ch. foliosum*.
147	1121-1126	6	Insertion of "YTAAMA" in clade 4.
148	1121-1131	11	Gap in all taxa (except clade 4 and *Bassia*).
149	1127-1131	5	Insertion of "TCAAA" in *Bassia lanifloria* and *Bassia scoparia* and of "GAAAA" in *Bassia prostrata*.
150	1136-1136	1	Insertion of "A" in *Beta*.
151	1138-1143	6	Gap in *Ch. capitatum*, *Ch. californicum*, *Ch. foliosum* and *Monolepis nuttalliana*.
152	1152-1162	11	Gap in *Krascheninnikovia*.
153	1157-1161	5	Insertion of "HTTYT" in *Allenrolfea*, *Beta*, *Hablitzia*, *Bassia*.
154	1161-1161	1	Gap in *Ch. ambrosioides*.
155	1195-1204	10	Gap in *Allenrolfea*, *Beta*, *Hablitzia*, *Bassia*.
156	1200-1204	5	Insertion of "AAATT" in clade 5.
157	1207-1217	11	Gap in *Ch. capitatum* sample AC391.
158	1212-1212	1	Gap in *Ch. bonus-henricus*, *Ch. californicum*, *Ch. foliosum* and *Monolepis nuttalliana*.
159	1213-1215	3	Gap in *Bassia*.
160	1224-1228	5	Gap in clade 6 and in *Microgynoecium tibeticum*.
161	1225-1227	3	Gap in *Ch. chenopodioides*.
162	1225-1228	4	"AAAA" SSR in *Allenrolfea*.

Appendix 2.B – List of indels foun in the ITS region

ITS 1			
No.	**Extension**	**Length**	**Sequence motif**
1	6-6	1	Gap in *Beta*.
2	29-29	1	Gap in *Spinacia oleracea*.
3	39-39	1	Insertion of "T" in *Ceratocarpus arenarius*.
4	53-53	1	Insertion of "G" in *Ch. opulifolium*.
5	64-64	1	Insertion of "A" in *Beta* and *Hablitzia*.
6	71-71	1	Gap in *Ch. urbicum*, insertion of "A" in all other taxa.
7	75-75	1	Gap in *Ch. vulvaria*, insertion of "C" in all other taxa.
8	99-104	6	Insertion of "TGAAAA" in *Beta* and *Hablitzia*.
9	105-105	1	Gap in *Beta*.
10	109-113	5	Gap in *Beta*.
11	111-113	3	Gap in *Hablitzia*.
12	113-113	1	Gap in *Bassia*.
13	116-117	2	Insertion of "GC" in *Allenrolfea*.
14	120-121	2	Insertion of "GC" in *Bassia*.
15	128-128	1	Gap in clade 2 (except *Ch. aristatum*), *Krascheninnikovia* and *Ceratocarpus*.
16	136-136	1	Insertion of "C" in *Bassia*.
17	136-138	3	Gap in most of the taxa except *Allenrolfea*, *Beta*, *Bassia*.
18	137-138	2	Insertion of "TC" in *Beta*.
19	138-138	1	Gap in *Allenrolfea*.
20	141-141	1	Insertion of "C" in *Stutzia dioica*.
21	150-150	1	Insertion of "G" in *Axyris*.
22	150-151	2	Gap in all taxa (except *Allenrolfea*, *Beta*, *Hablitzia*, *Bassia* and *Axyris*).
23	151-151	1	Insertion of "T" in *Bassia* and *Beta*. Insertion of "C" in *Hablitzia* and insertion of "A" in *Allenrolfea*.
24	158-158	1	Gap in *Axyris*.
25	159-161	3	Gap in *Bassia*.
26	168-170	3	Gap in *Allenrolfea*, *Beta* and *Hablitzia*.
27	172-173	2	Insertion of "CC" in *Bassia*.
28	178-178	1	Insertion of "C" in *Hablitzia* and insertion of "T" in *Beta*.
29	178-185	8	Gap in *Allenrolfea*.
30	184-185	2	Insertion of "AA" in *Krascheninnikovia*.
31	185-185	1	Gap in *Ch. multifidum*.
32	187-187	1	Gap in *Bassia*.
33	190-195	6	Insertion of "ATATTA" in *Allenrolfea*.

ITS 2			
No.	**Extension**	**Length**	**Sequence motif**
34	368-368	1	Gap in *Ch. pallidicaule*.
35	375-380	6	Gap in *Ch. atrovirens* sample AC586, *Ch. neomexicanum*, *Ch. watsonii* and *Ch. hians*.
36	376-380	5	Gap in clade 3 (except *Spinacia*).
37	378-380	3	Insertion of "AAG" in *Ceratocarpus*.
38	386-386	1	Gap in clade 2, *Spinacia*, *Beta*, *Hablitzia*, *Bassia*.
39	388-388	1	Gap in *Beta*.
40	390-391	2	Gap in *Microgynoecium tibeticum*, *Krascheninnikovia*, *Axyris*, and clade 3 (except *Spinacia*).

Appendix 2.B – List of indels foun in the ITS region (continued)

ITS 2 (continued)			
No.	**Extension**	**Length**	**Sequence motif**
41	391-391	1	Insertion of "G" in *Stutzia dioica*.
42	450-450	1	Gap in *Microgynoecium tibeticum*.
43	463-463	1	Gap in clade 5 (except *Ch. hybridum*).
44	464-464	1	Gap in *Bassia prostrata*.
45	466-466	1	Gap in clade 5 (except *Ch. hybridum*).
46	470-470	1	Gap in *Ch. album*, *Ch. gigantospermum* and *Ch. giganteum*.
47	478-507	30	Gap in clade 5 (except *Ch. hybridum*).
48	484-484	1	Insertion of "C" in *Ch. ambrosioides* and *Ch. multifidum*.
49	489-491	3	Insertion of "CTT" in *Suckleya suckleyana*.
50	494-495	2	Insertion of "TT" in *Axyris*.
51	497-507	11	Gap in *Krascheninnikovia*.
52	498-498	1	Gap in *Ceratocarpus*.
53	498-500	3	Gap in *Hablitzia*, *Bassia*, *Ch. pumilio*, *Ch. schraderianum* and *Ch. opulifolium*.
54	498-502	5	Gap in *Spinacia*.
55	498-507	10	Gap in all taxa (except *Ceratocarpus*, *Bassia*, *Hablitzia*, *Spinacia*, *Ch. pumilio*, *Ch. schraderianum*, *Ch. opulifolium*, *Ch. bonus-henricus*, *Ch. capitatum*, *Ch. californicum*, *Ch. foliosum* and *Monolepis nuttalliana*).
56	499-507	9	Gap in *Ch. bonus-henricus*, *Ch. capitatum*, *Ch. californicum*, *Ch. foliosum* and *Monolepis nuttalliana*.
57	501-507	7	Gap in *Ceratocarpus*.
58	502-507	6	Gap in *Hablitzia*, *Bassia*.
59	503-507	5	Insertion of "AAAAA" in *Spinacia*.
60	512-516	5	Insertion of "AATAT" in clade 5 (except *Ch. hybridum*).
61	525-527	3	Insertion of "TGC" in *Ch. hybridum*.
62	526-527	2	Gap in *Atriplex*, *Bassia*.
63	530-530	1	Insertion of "C" in *Beta*.
64	530-532	3	Gap in clade 7.
65	530-547	18	Gap in all taxa (except *Beta*, *Bassia* and clade 7).
66	531-547	17	Gap in *Beta vulgaris*.
67	533-547	15	Gap in *Bassia*.
68	548-562	15	Gap in clade 7.
69	550-550	1	Gap in all taxa (except *Grayia*, *Microgynoecium tibeticum*, *Stutzia dioica* and *Atriplex*).
70	569-570	2	Gap in *Beta*.
71	584-585	2	Gap in *Ch. coronopus*.
72	585-585	1	Insertion of "G" in *Ch. murale*.
73	591-591	1	Gap in *Ch. desertorum*.
74	592-603	12	Gap in *Stutzia dioica*.
75	596-597	2	Gap in most of the taxa (except clade 2 and outgroups).
76	597-597	1	Insertion of "A" in *Suckleya*.
77	601-601	1	Gap in *Ch. glaucum*.

Appendix 2.C – Chromosome number list

Species	Gam.	Spor.	Reference
Atrilex sagittata		18	Goldblatt, P., Johnson, D.E. (Eds.), 1998. Index to plant chromosome numbers 1994-1995. Monogr. Syst. Bot. Missouri Bot. Gard. 69. Missouri Botanical Garden, St. Louis.
Atrilex sagittata		18	Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Atrilex sagittata		18	Goldblatt, P., Johnson, D.E. (Eds.), 2000. Index to plant chromosome numbers 1996-1997. Monogr. Syst. Bot. Missouri Bot. Gard. 81. Missouri Botanical Garden, St. Louis.
Atrilex sagittata		18	Goldblatt, P., Johnson, D.E. (Eds.), 2000. Index to plant chromosome numbers 1996-1997. Monogr. Syst. Bot. Missouri Bot. Gard. 81. Missouri Botanical Garden, St. Louis.
Atriplex hortensis	9		Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Atriplex hortensis		18	Moore, R.J. (Ed.), 1970. Index to plant chromosome numbers for 1968. Regnum Vegetabile. vol 68. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Atriplex hortensis	9	18	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Atriplex hortensis	9		Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Atriplex hortensis		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Atriplex hortensis		18	Goldblatt, P., Johnson, D.E. (Eds.), 1990. Index to plant chromosome numbers 1986-1987. Monogr. Syst. Bot. Missouri Bot. Gard. 30 Missouri Botanical Garden, St. Louis
Atriplex hortensis		18	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Atriplex hortensis		18	Goldblatt, P., Johnson, D.E. (Eds.), 1998. Index to plant chromosome numbers 1994-1995. Monogr. Syst. Bot. Missouri Bot. Gard. 69. Missouri Botanical Garden, St. Louis.
Atriplex hortensis		18	Goldblatt, P., Johnson, D.E. (Eds.), 2006. Index to plant chromosome numbers 2001-2003. Monogr. Syst. Bot. Missouri Bot. Gard. 106. Missouri Botanical Garden, St. Louis
Atriplex nitens		18	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Atriplex nitens		18	Goldblatt, P., Johnson, D.E. (Eds.), 1994. Index to plant chromosome numbers 1990-1991. Monogr. Syst. Bot. Missouri Bot. Gard. 51. Missouri Botanical Garden, St. Louis.
Atriplex nitens		18	Goldblatt, P., Johnson, D.E. (Eds.), 2006. Index to plant chromosome numbers 2001-2003. Monogr. Syst. Bot. Missouri Bot. Gard. 106. Missouri Botanical Garden, St. Louis
Atriplex nitens		18	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Atriplex patula		36	Cave, M.S. (Ed.), 1960. Index to Plant Chromosome Numbers. Volume I. Comprising Numbers 1-4 and Suplement, and covering the years 1956, 1957, 1958, 1959. University of North California Press. Chapel Hill.
Atriplex patula	18	36	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Atriplex patula		36	Moore, R.J. (Ed.), 1974. Index to plant chromosome numbers for 1972. Regnum Vegetabile. vol 91. International Bureau for Plant Taxonomy and Nomenclature, Utrecht
Atriplex patula		18	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Atriplex patula		36	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Atriplex patula	18		Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Atriplex patula		36	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Atriplex patula		36 (72)	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Atriplex patula	18		Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Atriplex patula		36	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Atriplex patula		36	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Atriplex patula		36	Goldblatt, P. (Ed.), 1988. Index to plant chromosome numbers 1984-1985. Monogr. Syst. Bot. Missouri Bot. Gard. 23 Missouri Botanical Garden, St. Louis.
Atriplex patula		18.36	Goldblatt, P., Johnson, D.E. (Eds.), 1994. Index to plant chromosome numbers 1990-1991. Monogr. Syst. Bot. Missouri Bot. Gard. 51. Missouri Botanical Garden, St. Louis.
Atriplex patula		36	Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Atriplex patula		36	Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Atriplex patula		18, 36	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Axyris amaranthoides		18	Cave, M.S. (Ed.), 1960. Index to Plant Chromosome Numbers. Volume I. Comprising Numbers 1-4 and Suplement, and covering the years 1956, 1957, 1958, 1959. University of North California Press. Chapel Hill.
Axyris amaranthoides		18	Moore, R.J. (Ed.), 1973. Index to plant chromosome numbers 1967-1971. Regnum Vegetabile. vol. 90. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Axyris amaranthoides		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Axyris amaranthoides		18	Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Axyris hybrida		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Axyris hybrida		18	Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Axyris prostrata		36	Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Ceratocarpus arenarius		18	Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Chenopodium hircinum		36	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.

Appendix 2.C – Chromosome number list (continued)

Species	Gam.	Spor.	Reference
Chenopodium melanocarpum	no data		
Chenopodium album		18	Cave, M.S. (Ed.), 1960. Index to Plant Chromosome Numbers. Volume I. Comprising Numbers 1-4 and Suplement, and covering the years 1956, 1957, 1958, 1959. University of North California Press. Chapel Hill.
Chenopodium album	9	18	Cave, M.S. (Ed.), 1960. Index to Plant Chromosome Numbers. Volume I. Comprising Numbers 1-4 and Suplement, and covering the years 1956, 1957, 1958, 1959. University of North California Press. Chapel Hill.
Chenopodium album	27	54	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium album	9, 18, 27		Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium album	18		Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium album	27	54	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium album		54	Moore, R.J. (Ed.), 1970. Index to plant chromosome numbers for 1968. Regnum Vegetabile. vol 68. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium album	9, 27	18, 54	Moore, R.J. (Ed.), 1973. Index to plant chromosome numbers 1967-1971. Regnum Vegetabile. vol. 90. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium album	27		Moore, R.J. (Ed.), 1974. Index to plant chromosome numbers for 1972. Regnum Vegetabile. vol 91. International Bureau for Plant Taxonomy and Nomenclature, Utrecht
Chenopodium album	27	ca.54	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium album		54	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium album		36	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium album	27	54	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium album		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium album	27	54	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium album	27		Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P. (Ed.), 1988. Index to plant chromosome numbers 1984-1985. Monogr. Syst. Bot. Missouri Bot. Gard. 23 Missouri Botanical Garden, St. Louis.
Chenopodium album		36	Goldblatt, P. (Ed.), 1988. Index to plant chromosome numbers 1984-1985. Monogr. Syst. Bot. Missouri Bot. Gard. 23 Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P. (Ed.), 1988. Index to plant chromosome numbers 1984-1985. Monogr. Syst. Bot. Missouri Bot. Gard. 23 Missouri Botanical Garden, St. Louis.
Chenopodium album		18	Ornduff, R. (Ed.), 1969. Index to plant chromosome numbers for 1967. Regnum Vegetabile. vol 59. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium album	9, 27		Goldblatt, P., Johnson, D.E. (Eds.), 1990. Index to plant chromosome numbers 1986-1987. Monogr. Syst. Bot. Missouri Bot. Gard. 30 Missouri Botanical Garden, St. Louis
Chenopodium album		54	Goldblatt, P., Johnson, D.E. (Eds.), 1990. Index to plant chromosome numbers 1986-1987. Monogr. Syst. Bot. Missouri Bot. Gard. 30 Missouri Botanical Garden, St. Louis
Chenopodium album		54	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P., Johnson, D.E. (Eds.), 1990. Index to plant chromosome numbers 1986-1987. Monogr. Syst. Bot. Missouri Bot. Gard. 30 Missouri Botanical Garden, St. Louis
Chenopodium album		18	Goldblatt, P., Johnson, D.E. (Eds.), 1994. Index to plant chromosome numbers 1990-1991. Monogr. Syst. Bot. Missouri Bot. Gard. 51. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P., Johnson, D.E. (Eds.), 1994. Index to plant chromosome numbers 1990-1991. Monogr. Syst. Bot. Missouri Bot. Gard. 51. Missouri Botanical Garden, St. Louis.

Appendix 2.C – Chromosome number list (continued)

Species	Gam.	Spor.	Reference
Chenopodium album		54	Goldblatt, P., Johnson, D.E. (Eds.), 1990. Index to plant chromosome numbers 1986-1987. Monogr. Syst. Bot. Missouri Bot. Gard. 30 Missouri Botanical Garden, St. Louis
Chenopodium album		54	Goldblatt, P., Johnson, D.E. (Eds.), 1998. Index to plant chromosome numbers 1994-1995. Monogr. Syst. Bot. Missouri Bot. Gard. 69. Missouri Botanical Garden, St. Louis.
Chenopodium album		36	Goldblatt, P., Johnson, D.E. (Eds.), 2000. Index to plant chromosome numbers 1996-1997. Monogr. Syst. Bot. Missouri Bot. Gard. 81. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium album		54, ca.54	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Goldblatt, P., Johnson, D.E. (Eds.), 2006. Index to plant chromosome numbers 2001-2003. Monogr. Syst. Bot. Missouri Bot. Gard. 106. Missouri Botanical Garden, St. Louis
Chenopodium album		54	Goldblatt, P., Johnson, D.E. (Eds.), 2006. Index to plant chromosome numbers 2001-2003. Monogr. Syst. Bot. Missouri Bot. Gard. 106. Missouri Botanical Garden, St. Louis
Chenopodium album		54	Goldblatt, P., Johnson, D.E. (Eds.), 2006. Index to plant chromosome numbers 2001-2003. Monogr. Syst. Bot. Missouri Bot. Gard. 106. Missouri Botanical Garden, St. Louis
Chenopodium album		18,36,54	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium album		54	Rahiminejad, M.R., 2006. Chenopodiaceae, in: Marhold, K., (Ed.), IAPT/IOPB chromosome data 1. Taxon 55 (2), 443.
Chenopodium album s.l.		18	Moore, R.J. (Ed.), 1973. Index to plant chromosome numbers 1967-1971. Regnum Vegetabile. vol. 90. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium album s.l.		36,54 ca. 108	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium album s.l.		36.54	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium ambrosioides	16	32	Cave, M.S. (Ed.), 1960. Index to Plant Chromosome Numbers. Volume I. Comprising Numbers 1-4 and Suplement, and covering the years 1956, 1957, 1958, 1959. University of North California Press. Chapel Hill.
Chenopodium ambrosioides		32	Cave, M.S. (Ed.), 1960. Index to Plant Chromosome Numbers. Volume I. Comprising Numbers 1-4 and Suplement, and covering the years 1956, 1957, 1958, 1959. University of North California Press. Chapel Hill.
Chenopodium ambrosioides	16		Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium ambrosioides		32	Moore, R.J. (Ed.), 1971. Index to plant chromosome numbers for 1969. Regnum Vegetabile. vol 77. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium ambrosioides	16		Moore, R.J. (Ed.), 1970. Index to plant chromosome numbers for 1968. Regnum Vegetabile. vol 68. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium ambrosioides		32	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium ambrosioides	16		Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium ambrosioides		32	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium ambrosioides		32	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium ambrosioides		32	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium ambrosioides	16		Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium ambrosioides		32	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium ambrosioides	16		Goldblatt, P. (Ed.), 1988. Index to plant chromosome numbers 1984-1985. Monogr. Syst. Bot. Missouri Bot. Gard. 23 Missouri Botanical Garden, St. Louis.
Chenopodium ambrosioides		32	Ornduff, R. (Ed.), 1969. Index to plant chromosome numbers for 1967. Regnum Vegetabile. vol 59. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium ambrosioides		32	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium ambrosioides		32	Goldblatt, P., Johnson, D.E. (Eds.), 1990. Index to plant chromosome numbers 1986-1987. Monogr. Syst. Bot. Missouri Bot. Gard. 30 Missouri Botanical Garden, St. Louis
Chenopodium ambrosioides		32	Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Chenopodium ambrosioides		32	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium ambrosioides	16		Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Chenopodium ambrosioides		32	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium aristatum		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium aristatum		18	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium aristatum		18	Goldblatt, P., Johnson, D.E. (Eds.), 1998. Index to plant chromosome numbers 1994-1995. Monogr. Syst. Bot. Missouri Bot. Gard. 69. Missouri Botanical Garden, St. Louis.
Chenopodium aristatum		18	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium atrovirens		18	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.

Appendix 2.C – Chromosome number list (continued)

Species	Gam.	Spor.	Reference
Chenopodium berlandieri		36	Rahiminejad, M.R., 2006. Chenopodiaceae, in: Marhold, K., (Ed.), IAPT/IOPB chromosome data 1. Taxon 55 (2), 443.
Chenopodium berlandieri		36	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium berlandieri	18		Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium berlandieri var. *boscianum*		36	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium berlandieri var. *bushianum*		36	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium berlandieri var. *sinuatum*		36	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium berlanideri subsp. *nuttalliae*		36	Goldblatt, P., Johnson, D.E. (Eds.), 1994. Index to plant chromosome numbers 1990-1991. Monogr. Syst. Bot. Missouri Bot. Gard. 51. Missouri Botanical Garden, St. Louis.
Chenopodium berlandieri var. *zschackei*		36	Moore, R.J. (Ed.), 1973. Index to plant chromosome numbers 1967-1971. Regnum Vegetabile. vol. 90. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium berlandieri subsp. *zschackei*		36	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium berlandieri subsp. *zschackei*	18		Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium bonus-henricus	18		Cave, M.S. (Ed.), 1960. Index to Plant Chromosome Numbers. Volume I. Comprising Numbers 1-4 and Supplement, and covering the years 1956, 1957, 1958, 1959. University of North California Press. Chapel Hill.
Chenopodium bonus-henricus		36	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Supplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium bonus-henricus		36	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium bonus-henricus		36	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium bonus-henricus		36	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium bonus-henricus		36	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium bonus-henricus		36	Goldblatt, P. (Ed.), 1988. Index to plant chromosome numbers 1984-1985. Monogr. Syst. Bot. Missouri Bot. Gard. 23 Missouri Botanical Garden, St. Louis.
Chenopodium bonus-henricus		36	Goldblatt, P., Johnson, D.E. (Eds.), 1994. Index to plant chromosome numbers 1990-1991. Monogr. Syst. Bot. Missouri Bot. Gard. 51. Missouri Botanical Garden, St. Louis.
Chenopodium bonus-henricus		36	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium botrys		18	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium botrys	18		Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium botrys		18	Moore, R.J. (Ed.), 1971. Index to plant chromosome numbers for 1969. Regnum Vegetabile. vol 77. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium botrys		18	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium botrys		18	Moore, R.J. (Ed.), 1973. Index to plant chromosome numbers 1967-1971. Regnum Vegetabile. vol. 90. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium botrys	9		Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium botrys		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium botrys		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium botrys		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium botrys		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium botrys		18	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium botrys		18	Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Chenopodium botrys	9		Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Chenopodium botrys		18	Goldblatt, P., Johnson, D.E. (Eds.), 2006. Index to plant chromosome numbers 2001-2003. Monogr. Syst. Bot. Missouri Bot. Gard. 106. Missouri Botanical Garden, St. Louis

Appendix 2.C – Chromosome number list (continued)

Species	Gam.	Spor.	Reference
Chenopodium californicum		18	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium capitatum		16	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium capitatum		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium capitatum		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium capitatum		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium capitatum		18	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium capitatum	9	18	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium capitatum		18	Cave, M.S. (Ed.), 1960. Index to Plant Chromosome Numbers. Volume I. Comprising Numbers 1-4 and Suplement, and covering the years 1956, 1957, 1958, 1959. University of North California Press. Chapel Hill.
Chenopodium capitatum	9		Cave, M.S. (Ed.), 1960. Index to Plant Chromosome Numbers. Volume I. Comprising Numbers 1-4 and Suplement, and covering the years 1956, 1957, 1958, 1959. University of North California Press. Chapel Hill.
Chenopodium iljinii		18	Goldblatt, P., Johnson, D.E. (Eds.), 1998. Index to plant chromosome numbers 1994-1995. Monogr. Syst. Bot. Missouri Bot. Gard. 69. Missouri Botanical Garden, St. Louis.
Chenopodium chenopodioides		18	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium coronopus	no data		
Chenopodium cycloides	no data		
Chenopodium desertorum subsp. *anidiophyllum*	no data		
Chenopodium desiccatum		18	Moore, R.J. (Ed.), 1973. Index to plant chromosome numbers 1967-1971. Regnum Vegetabile. vol. 90. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium desiccatum	18		Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium desiccatum	9	18	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium ficifolium	9		Cave, M.S. (Ed.), 1960. Index to Plant Chromosome Numbers. Volume I. Comprising Numbers 1-4 and Suplement, and covering the years 1956, 1957, 1958, 1959. University of North California Press. Chapel Hill.
Chenopodium ficifolium		18	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium ficifolium		18, 36	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium ficifolium	9	18	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium ficifolium		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium ficifolium		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium ficifolium		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium ficifolium		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium ficifolium		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium ficifolium		18	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium ficifolium		18.36	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium ficifolium		18	Goldblatt, P., Johnson, D.E. (Eds.), 1994. Index to plant chromosome numbers 1990-1991. Monogr. Syst. Bot. Missouri Bot. Gard. 51. Missouri Botanical Garden, St. Louis.
Chenopodium ficifolium		18	Goldblatt, P., Johnson, D.E. (Eds.), 1998. Index to plant chromosome numbers 1994-1995. Monogr. Syst. Bot. Missouri Bot. Gard. 69. Missouri Botanical Garden, St. Louis.
Chenopodium ficifolium		18	Goldblatt, P., Johnson, D.E. (Eds.), 2000. Index to plant chromosome numbers 1996-1997. Monogr. Syst. Bot. Missouri Bot. Gard. 81. Missouri Botanical Garden, St. Louis.
Chenopodium ficifolium		18	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium ficifolium		18	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium ficifolium		18	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium ficifolium		18	Rahiminejad, M.R., 2006. Chenopodiaceae, in: Marhold, K., (Ed.), IAPT/IOPB chromosome data 1. Taxon 55 (2), 443.
Chenopodium ficifolium		18	Probatova, N.S., Seledets, V.P., Gnutikov, A.A., Shatokhina, A.V., 2008. Chenopodiaceae, in: Marhold, K., (Ed.), IAPT/IOPB chromosome data 6. Taxon 57 (4), 1272.

Appendix 2.C – Chromosome number list (continued)

Species	Gam.	Spor.	Reference
Chenopodium foliosum		18	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium foliosum		18	Goldblatt, P. (Ed.), 1988. Index to plant chromosome numbers 1984-1985. Monogr. Syst. Bot. Missouri Bot. Gard. 23 Missouri Botanical Garden, St. Louis.
Chenopodium foliosum		18	Goldblatt, P., Johnson, D.E. (Ed.), 1998. Index to plant chromosome numbers 1994-1995. Monogr. Syst. Bot. Missouri Bot. Gard. 69. Missouri Botanical Garden, St. Louis.
Chenopodium foliosum		18	Moore, R.J. (Ed.), 1971. Index to plant chromosome numbers for 1969. Regnum Vegetabile. vol 77. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium foliosum		18	Goldblatt, P., Johnson, D.E. (Eds.), 1990. Index to plant chromosome numbers 1986-1987. Monogr. Syst. Bot. Missouri Bot. Gard. 30 Missouri Botanical Garden, St. Louis
Chenopodium foliosum		18	Goldblatt, P., Johnson, D.E. (Eds.), 2006. Index to plant chromosome numbers 2001-2003. Monogr. Syst. Bot. Missouri Bot. Gard. 106. Missouri Botanical Garden, St. Louis
Chenopodium fremontii		18	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium fremontii		18	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium fremontii	9	18	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium giganteum		54	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium giganteum		54	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium giganteum		54	Rahiminejad, M.R., 2006. Chenopodiaceae, in: Marhold, K., (Ed.), IAPT/IOPB chromosome data 1. Taxon 55 (2), 443.
Chenopodium gigantospermum		36	Ornduff, R. (Ed.), 1967. Index to plant chromosome numbers for 1965. Regnum Vegetabile. vol 50. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium glaucum	9		Cave, M.S. (Ed.), 1960. Index to Plant Chromosome Numbers. Volume I. Comprising Numbers 1-4 and Suplement, and covering the years 1956, 1957, 1958, 1959. University of North California Press. Chapel Hill.
Chenopodium glaucum		18	Moore, R.J. (Ed.), 1971. Index to plant chromosome numbers for 1969. Regnum Vegetabile. vol 77. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium glaucum		18	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium glaucum		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium glaucum		18C	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium glaucum		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium glaucum		36	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium glaucum		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium glaucum	9	18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium glaucum		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium glaucum		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium glaucum	9		Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium glaucum		18	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium glaucum		18	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium glaucum		18	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium glaucum		18	Goldblatt, P., Johnson, D.E. (Eds.), 1998. Index to plant chromosome numbers 1994-1995. Monogr. Syst. Bot. Missouri Bot. Gard. 69. Missouri Botanical Garden, St. Louis.
Chenopodium glaucum		18	Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Chenopodium glaucum		18	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium glaucum	9		Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Chenopodium glaucum		18	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium glaucum		18	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium glaucum		18	Goldblatt, P., Johnson, D.E. (Eds.), 2006. Index to plant chromosome numbers 2001-2003. Monogr. Syst. Bot. Missouri Bot. Gard. 106. Missouri Botanical Garden, St. Louis
Chenopodium glaucum		18	Rahiminejad, M.R., 2006. Chenopodiaceae, in: Marhold, K., (Ed.), IAPT/IOPB chromosome data 1. Taxon 55 (2), 443.
Chenopodium glaucum		18	Probatova, N.S., Seledets, V.P., 2008. Chenopodiaceae, in: Marhold, K., (Ed.), IAPT/IOPB chromosome data 5. Taxon 57 (2), 556.

Appendix 2.C – Chromosome number list (continued)

Species	Gam.	Spor.	Reference
Chenopodium graveolens		32	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium hybridum		18	Ornduff, R. (Ed.), 1968. Index to plant chromosome numbers for 1966. Regnum Vegetabile. vol 55. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium hybridum		18	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium hybridum		18	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium hybridum		18	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium hybridum		18	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium hybridum		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium hybridum		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium hybridum		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium hybridum		18	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium hybridum		18	Goldblatt, P., Johnson, D.E. (Eds.), 1998. Index to plant chromosome numbers 1994-1995. Monogr. Syst. Bot. Missouri Bot. Gard. 69. Missouri Botanical Garden, St. Louis.
Chenopodium hybridum		18	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium hybridum		18	Rahiminejad, M.R., 2006. Chenopodiaceae, in: Marhold, K., (Ed.), IAPT/IOPB chromosome data 1. Taxon 55 (2), 443.
Chenopodium hybridum		18	Probatova, N.S., Seledets, V.P., 2008. Chenopodiaceae, in: Marhold, K., (Ed.), IAPT/IOPB chromosome data 5. Taxon 57 (2), 556.
Chenopodium incanum		18	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium leptophyllum		18	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium leptophyllum		18	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium leptophyllum		18	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium multifidum		36	Moore, R.J. (Ed.), 1970. Index to plant chromosome numbers for 1968. Regnum Vegetabile. vol 68. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium multifidum		32	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium multifidum	16		Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium multifidum		32	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium multifidum		32	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium murale		18	Cave, M.S. (Ed.), 1960. Index to Plant Chromosome Numbers. Volume I. Comprising Numbers 1-4 and Suplement, and covering the years 1956, 1957, 1958, 1959. University of North California Press. Chapel Hill.
Chenopodium murale		18	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium murale		18	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium murale	9	18	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium murale	9		Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium murale		18	Moore, R.J. (Ed.), 1974. Index to plant chromosome numbers for 1972. Regnum Vegetabile. vol 91. International Bureau for Plant Taxonomy and Nomenclature, Utrecht
Chenopodium murale	9	18	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium murale		18	Moore, R.J. (Ed.), 1973. Index to plant chromosome numbers 1967-1971. Regnum Vegetabile. vol. 90. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium murale		18	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium murale		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.

Appendix 2.C – Chromosome number list (continued)

Species	Gam.	Spor.	Reference
Chenopodium murale		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium murale		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium murale		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium murale	9		Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium murale		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium murale		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium murale		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium murale		18	Goldblatt, P. (Ed.), 1988. Index to plant chromosome numbers 1984-1985. Monogr. Syst. Bot. Missouri Bot. Gard. 23 Missouri Botanical Garden, St. Louis.
Chenopodium murale		18	Goldblatt, P., Johnson, D.E. (Eds.), 1990. Index to plant chromosome numbers 1986-1987. Monogr. Syst. Bot. Missouri Bot. Gard. 30 Missouri Botanical Garden, St. Louis
Chenopodium murale		18	Goldblatt, P., Johnson, D.E. (Eds.), 1990. Index to plant chromosome numbers 1986-1987. Monogr. Syst. Bot. Missouri Bot. Gard. 30 Missouri Botanical Garden, St. Louis
Chenopodium murale		18	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium murale		18	Goldblatt, P., Johnson, D.E. (Eds.), 2000. Index to plant chromosome numbers 1996-1997. Monogr. Syst. Bot. Missouri Bot. Gard. 81. Missouri Botanical Garden, St. Louis.
Chenopodium murale		18	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium murale		18	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium murale		18	Rahiminejad, M.R., 2006. Chenopodiaceae, in: Marhold, K., (Ed.), IAPT/IOPB chromosome data 1. Taxon 55 (2), 443.
Chenopodium neomexicanum		18	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium neomexicanum		18	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium nevadense	no data		
Chenopodium opulifolium		27	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium opulifolium	27		Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium opulifolium	27		Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium opulifolium	9		Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium opulifolium		54	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium opulifolium		54	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium opulifolium		54	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium opulifolium		54	Goldblatt, P. (Ed.), 1988. Index to plant chromosome numbers 1984-1985. Monogr. Syst. Bot. Missouri Bot. Gard. 23 Missouri Botanical Garden, St. Louis.
Chenopodium opulifolium		54	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium opulifolium		54	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium opulifolium		54	Goldblatt, P., Johnson, D.E. (Eds.), 1990. Index to plant chromosome numbers 1986-1987. Monogr. Syst. Bot. Missouri Bot. Gard. 30 Missouri Botanical Garden, St. Louis
Chenopodium opulifolium		54	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium opulifolium		54	Goldblatt, P., Johnson, D.E. (Eds.), 2000. Index to plant chromosome numbers 1996-1997. Monogr. Syst. Bot. Missouri Bot. Gard. 81. Missouri Botanical Garden, St. Louis.
Chenopodium opulifolium		54	Goldblatt, P., Johnson, D.E. (Eds.), 2000. Index to plant chromosome numbers 1996-1997. Monogr. Syst. Bot. Missouri Bot. Gard. 81. Missouri Botanical Garden, St. Louis.
Chenopodium opulifolium		54	Goldblatt, P., Johnson, D.E. (Eds.), 2006. Index to plant chromosome numbers 2001-2003. Monogr. Syst. Bot. Missouri Bot. Gard. 106. Missouri Botanical Garden, St. Louis
Chenopodium opulifolium		54	Rahiminejad, M.R., 2006. Chenopodiaceae, in: Marhold, K., (Ed.), IAPT/IOPB chromosome data 1. Taxon 55 (2), 443.
Chenopodium pallescens		18	Moore, R.J. (Ed.), 1973. Index to plant chromosome numbers 1967-1971. Regnum Vegetabile. vol. 90. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium pallidicaule		18	Moore, R.J. (Ed.), 1973. Index to plant chromosome numbers 1967-1971. Regnum Vegetabile. vol. 90. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium pallidicaule	9	18, 36	Moore, R.J. (Ed.), 1974. Index to plant chromosome numbers for 1972. Regnum Vegetabile. vol 91. International Bureau for Plant Taxonomy and Nomenclature, Utrecht

Appendix 2.C – Chromosome number list (continued)

Species	Gam.	Spor.	Reference
Chenopodium petiolare	18		Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium polyspermum		18	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium polyspermum		18	Ornduff, R. (Ed.), 1968. Index to plant chromosome numbers for 1966. Regnum Vegetabile. vol 55. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium polyspermum		18	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium polyspermum	9		Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium polyspermum		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium polyspermum		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium polyspermum		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium polyspermum	9		Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium polyspermum		18	Goldblatt, P. (Ed.), 1988. Index to plant chromosome numbers 1984-1985. Monogr. Syst. Bot. Missouri Bot. Gard. 23 Missouri Botanical Garden, St. Louis.
Chenopodium polyspermum		18	Goldblatt, P., Johnson, D.E. (Eds.), 1990. Index to plant chromosome numbers 1986-1987. Monogr. Syst. Bot. Missouri Bot. Gard. 30 Missouri Botanical Garden, St. Louis
Chenopodium polyspermum		18	Goldblatt, P., Johnson, D.E. (Eds.), 1994. Index to plant chromosome numbers 1990-1991. Monogr. Syst. Bot. Missouri Bot. Gard. 51. Missouri Botanical Garden, St. Louis.
Chenopodium polyspermum		18	Goldblatt, P., Johnson, D.E. (Eds.), 1998. Index to plant chromosome numbers 1994-1995. Monogr. Syst. Bot. Missouri Bot. Gard. 69. Missouri Botanical Garden, St. Louis.
Chenopodium polyspermum		18	Goldblatt, P., Johnson, D.E. (Eds.), 2000. Index to plant chromosome numbers 1996-1997. Monogr. Syst. Bot. Missouri Bot. Gard. 81. Missouri Botanical Garden, St. Louis.
Chenopodium polyspermum		18	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium polyspermum		18	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium polyspermum		18	Rahiminejad, M.R., 2006. Chenopodiaceae, in: Marhold, K., (Ed.), IAPT/IOPB chromosome data 1. Taxon 55 (2), 443.
Chenopodium pratericola		18	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium pratericola	9	18	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium pratericola		18	Goldblatt, P., Johnson, D.E. (Eds.), 1998. Index to plant chromosome numbers 1994-1995. Monogr. Syst. Bot. Missouri Bot. Gard. 69. Missouri Botanical Garden, St. Louis.
Chenopodium pumilio		16	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium pumilio		16	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium pumilio		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium pumilio		18	Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Chenopodium pumilio		18	Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Chenopodium quinoa	18		Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium quinoa	18	18, 27, 36, 45	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium quinoa		36	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium rubrum		36	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium rubrum		36	Moore, R.J. (Ed.), 1973. Index to plant chromosome numbers 1967-1971. Regnum Vegetabile. vol. 90. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium rubrum		36	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium rubrum		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium rubrum		36	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium rubrum		18	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.

Appendix 2.C – Chromosome number list (continued)

Species	Gam.	Spor.	Reference
Chenopodium rubrum		18	Goldblatt, P. (Ed.), 1988. Index to plant chromosome numbers 1984-1985. Monogr. Syst. Bot. Missouri Bot. Gard. 23 Missouri Botanical Garden, St. Louis.
Chenopodium rubrum		36	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium rubrum		36	Goldblatt, P., Johnson, D.E. (Eds.), 1998. Index to plant chromosome numbers 1994-1995. Monogr. Syst. Bot. Missouri Bot. Gard. 69. Missouri Botanical Garden, St. Louis.
Chenopodium rubrum		36	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium rubrum		18	Probatova, N.S., Seledets, V.P., 2008. Chenopodiaceae, in: Marhold, K., (Ed.), IAPT/IOPB chromosome data 5. Taxon 57 (2), 556.
Chenopodium schraderianum		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium standleyanum		18	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium subglabrum		18	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium urbicum		36	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Chenopodium urbicum		18	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium urbicum	9		Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium urbicum		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium urbicum		18	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium urbicum		18	Goldblatt, P., Johnson, D.E. (Eds.), 1998. Index to plant chromosome numbers 1994-1995. Monogr. Syst. Bot. Missouri Bot. Gard. 69. Missouri Botanical Garden, St. Louis.
Chenopodium urbicum		18	Goldblatt, P., Johnson, D.E. (Eds.), 2000. Index to plant chromosome numbers 1996-1997. Monogr. Syst. Bot. Missouri Bot. Gard. 81. Missouri Botanical Garden, St. Louis.
Chenopodium urbicum		18	Goldblatt, P., Johnson, D.E. (Eds.), 2006. Index to plant chromosome numbers 2001-2003. Monogr. Syst. Bot. Missouri Bot. Gard. 106. Missouri Botanical Garden, St. Louis
Chenopodium vulvaria		18	Cave, M.S. (Ed.), 1960. Index to Plant Chromosome Numbers. Volume I. Comprising Numbers 1-4 and Suplement, and covering the years 1956, 1957, 1958, 1959. University of North California Press. Chapel Hill.
Chenopodium vulvaria		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Chenopodium vulvaria		18	Goldblatt, P. (Ed.), 1984. Index to plant chromosome numbers 1979-1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8. Missouri Botanical Garden, St. Louis.
Chenopodium vulvaria		18	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium vulvaria		18.36	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Chenopodium vulvaria		18	Goldblatt, P., Johnson, D.E. (Eds.), 1998. Index to plant chromosome numbers 1994-1995. Monogr. Syst. Bot. Missouri Bot. Gard. 69. Missouri Botanical Garden, St. Louis.
Chenopodium vulvaria		18	Goldblatt, P., Johnson, D.E. (Eds.), 1998. Index to plant chromosome numbers 1994-1995. Monogr. Syst. Bot. Missouri Bot. Gard. 69. Missouri Botanical Garden, St. Louis.
Chenopodium vulvaria		18	Goldblatt, P., Johnson, D.E. (Eds.), 2000. Index to plant chromosome numbers 1996-1997. Monogr. Syst. Bot. Missouri Bot. Gard. 81. Missouri Botanical Garden, St. Louis.
Chenopodium vulvaria		18	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Chenopodium vulvaria		18	Goldblatt, P., Johnson, D.E. (Eds.), 2006. Index to plant chromosome numbers 2001-2003. Monogr. Syst. Bot. Missouri Bot. Gard. 106. Missouri Botanical Garden, St. Louis
Chenopodium watsonii		18	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium watsonii		18	Moore, R.J. (Ed.), 1973. Index to plant chromosome numbers 1967-1971. Regnum Vegetabile. vol. 90. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium watsonii		18	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodium watsonii		18	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.
Chenopodium watsonii		18	Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodiun hians		18	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Chenopodiun hians	9	18	Goldblatt, P. (Ed.), 1985. Index to plant chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden, St. Louis.

Appendix 2.C – Chromosome number list (continued)

Species	Gam.	Spor.	Reference
Grayia spinosa		36	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Grayia spinosa		36	Goldblatt, P. (Ed.), 1988. Index to plant chromosome numbers 1984-1985. Monogr. Syst. Bot. Missouri Bot. Gard. 23 Missouri Botanical Garden, St. Louis.
Bassia scorparia (*Kochia densiflora*)		18	Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Bassia scorparia (*Kochia densiflora*)		18	Goldblatt, P., Johnson, D.E. (Eds.), 2006. Index to plant chromosome numbers 2001-2003. Monogr. Syst. Bot. Missouri Bot. Gard. 106. Missouri Botanical Garden, St. Louis
Bassia prostrata (*Kochia prostrata*)	9		Moore, R.J. (Ed.), 1977. Index to plant chromosome numbers for 1973/74. Regnum Vegetabile. vol 96. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Bassia prostrata (*Kochia prostrata*)		18	Goldblatt, P. (Ed.), 1981. Index to plant chromosome numbers 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5. Missouri Botanical Garden, St. Louis.
Bassia prostrata (*Kochia prostrata*)	8		Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Bassia prostrata (*Kochia prostrata*)		18	Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Bassia prostrata (*Kochia prostrata*)		18	Goldblatt, P., Johnson, D.E. (Eds.), 1998. Index to plant chromosome numbers 1994-1995. Monogr. Syst. Bot. Missouri Bot. Gard. 69. Missouri Botanical Garden, St. Louis.
Bassia prostrata (*Kochia prostrata*)		18	Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Bassia prostrata (*Kochia prostrata*)		18.36	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Krascheninnikovia ceratoides		36	Goldblatt, P., Johnson, D.E. (Eds.), 2000. Index to plant chromosome numbers 1996-1997. Monogr. Syst. Bot. Missouri Bot. Gard. 81. Missouri Botanical Garden, St. Louis.
Krascheninnikovia lanata	no data		
Microgynoecium tibeticum	no data		
Monolepis nutalliana		18	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Rhagodia triandra	no data		
Spinacia oleracea	6	12	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Spinacia oleracea		12	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Spinacia oleracea	6II, 6III, 6IV, 6II+1III	12, 18, 24, 13	Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Spinacia oleracea		12	Ornduff, R. (Ed.), 1969. Index to plant chromosome numbers for 1967. Regnum Vegetabile. vol 59. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Spinacia oleracea		12	Goldblatt, P., Johnson, D.E. (Eds.), 1991. Index to plant chromosome numbers 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40 Missouri Botanical Garden, St. Louis.
Spinacia oleracea		12	Goldblatt, P., Johnson, D.E. (Eds.), 1996. Index to plant chromosome numbers 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58. Missouri Botanical Garden, St. Louis.
Spinacia oleracea		12	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Spinacia oleracea		12	Goldblatt, P., Johnson, D.E. (Eds.), 2003. Index to plant chromosome numbers 1998-2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94. Missouri Botanical Garden, St. Louis.
Spinacia oleracea	6		Cave, M.S. (Ed.), 1964. Index to Plant Chromosome Numbers. Volume II. Comprising Numbers 5-9 and Suplement, and covering the years 1959-1964. University of North California Press. Chapel Hill
Spinacia tetrandra	no data		
Spinacia turkestanica	no data		
Stutzia dioica	no data		
Suckleya suckleyana		18	Moore, R.J. (Ed.), 1972. Index to plant chromosome numbers for 1970. Regnum Vegetabile. vol. 84. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
Zuckia brandegeei	no data		

Appendix 2.D – List of sequence parts excluded as mutational hotspots in *trnL-F* and ITS for each individual sequence

Taxon	Code	*trnL*	Position HS1 *trnL*	*trnL*	*trnL*	*trnL*	*trnL-F* spacer	Position HS1 *trnL-F*	Position HS2 *trnL-F*	ITS 1	ITS 1	ITS 2	Position HS1 ITS 2	Position HS2 ITS 2
Allenrolfea vaginata	**AC017**	534	114-121	121-121	266-321	429-434								
Allenrolfea vaginata	**AY181875.1**						378	30-39	71-81	247	54-129	221	14-21	194-208
Atriplex hortensis	**AC516**	414	114-118	140-147		299-310	361	29-35	65-69	219	43-102	227	14-20	195-214
Atriplex nitens	**AC573**	413	114-118	140-147		299-310	361	29-35	65-69	219	54-102	230	14-20	195-217
Atriplex patula	**AC605**	414	114-118	140-147		299-310	361	29-35	65-69	230	54-113	227	14-20	195-214
Atriplex sagittata	**AC533**	414	114-118	140-147		299-310	361	29-35	65-69	230	54-113	227	14-20	195-214
Axyris amaranthoides	**AC647**	626	114-120	142-149	301-370	479-485	372	29-37	69-76	242	54-125	226	14-21	195-213
Axyris hybrida	**AC648**	625	114-120	142-149	301-369	478-484	372	29-37	69-76	242	54-125	227	14-22	196-214
Axyris prostrata	**AC529**	625	114-120	142-149	301-369	478-484	372	29-37	69-76	242	54-125	226	14-21	195-213
Bassia laniflora	**AC534**	574	113-118	118-118	266-329	427-433	386	28-39	71-92	242	54-124	225	14-21	197-211
Bassia prostrata	**AC606**	581	113-118	122-125	275-338	433-439	371	28-39	71-78	242	54-124	224	14-21	196-210
Bassia scoparia	**AC607**	597	113-118	118-118	266-353	450-456	384	28-38	70-91	242	54-124	225	14-21	197-211
Beta vulgaris	**AC530**	509	51-56	78-87	233-278	381-387	342	28-36	68-75	248	53-129	220	14-20	190-206
Ceratocarpus arenarius	**AC531**	508	114-121	144-151	301-321	360-367	379	29-36	63-75	237	46-121	229	14-20	198-216
Ceratocarpus arenarius	**AC649**	508	114-120	143-150	300-321	360-367	379	29-36	63-75	247	55-131	230	14-20	198-217
Chenopodium album	**AC571**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	227	14-21	194-214
Chenopodium album	**AC602**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	227	14-21	194-214
Chenopodium album	**AC575**	566	114-118	140-147	299-341	422-428	364	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium album	**AC388**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	220	45-103	227	14-21	194-214
Chenopodium album	**AC414**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	227	14-21	194-214
Chenopodium album	**AC395**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	211	36-94	227	14-21	194-214
Chenopodium album	**AC396**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	211	36-94	227	14-21	194-214
Chenopodium album	**AC427**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	221	46-104	227	14-21	194-214
Chenopodium album	**AC614**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	227	14-21	194-214
Chenopodium album	**AC591**	564	114-118	140-147	299-340	420-426	354	30-35	65-69	229	54-112	227	14-21	194-214
Chenopodium album	**AC590**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	227	14-21	194-214
Chenopodium ambrosioides	**AC420**	419	114-119	141-145		275-281	370	29-36	68-74	241	44-125	228	14-21	194-214
Chenopodium ambrosioides	**AC425**	419	114-119	141-145		275-281	367	29-36	68-74	218	43-101	215	14-21	183-202
Chenopodium ambrosioides	**AC527**	419	114-119	141-145		275-281	370	29-36	68-74	251	54-135	228	14-21	194-214
Chenopodium ambrosioides	**AC386**	419	114-119	141-145		275-281	370	29-36	68-74	251	54-135	228	14-21	194-214
Chenopodium aristatum	**AC610**	429	114-120	142-150		285-291	382	30-39	71-78	251	54-134	225	14-21	193-211
Chenopodium aristatum	**AC654**	429	114-120	142-150		285-291	382	30-39	71-78	251	54-134	225	14-21	193-211
Chenopodium aristatum	**AC528**	429	114-120	142-150		285-291	382	30-39	71-78	251	54-134	225	14-21	193-211
Chenopodium atrovirens	**AC363**	563	114-118	140-147	299-339	419-425	359	30-35	65-69	211	36-94	228	14-21	195-215
Chenopodium atrovirens	**AC421**	564	115-119	141-148	300-340	420-426	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium atrovirens	**AC586**	564	114-118	140-147	299-340	420-426	360	30-35	66-70	229	54-112	225	14-21	192-212
Chenopodium atrovirens	**AC540**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium berlandieri	**AC541**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium berlandieri	**AC599**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium berlandieri subsp. *nuttalliae*	**AC616**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium berlandieri var. *boscianum*	**AC545**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium berlandieri var. *zschackei*	**AC542**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium berlandieri var. *zschackei*	**AC600**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium bonus-henricus	**AC381**	591		118-126	279-340	446-452	372	30-38	70-76	235	36-118	223	14-21	192-210
Chenopodium californicum	**AC431**	592		118-128	281-341	447-453	363	30-39	71-77	236	36-119	223	14-21	192-210
Chenopodium capitatum	**AC391**	590		118-127	280-339	445-451	362	30-40	72-77	253	54-136	223	14-21	192-210
Chenopodium capitatum var. *parvicapitatum*	**AC547**	598		118-127	280-339	453-459	353	30-40	72-77	253	54-136	223	14-21	192-210

Appendix 2.D – List of sequence parts excluded as mutational hotspots in *trnL-F* and ITS for each individual sequence (continued)

Taxon	Code	*trnL* intron	*trnL*	*trnL*	*trnL*	*trnL*	*trnL-F* spacer	*trnL-F*	*trnL-F*	ITS 1	ITS 1	ITS 2	Position HS1 ITS 2	Position HS2 ITS 2
Chenopodium chenopodioides	**AC543**	559	115-118	140-149	279-332	415-421	359	30-39	69-74	229	54-112	226	14-21	194-213
Chenopodium coronopus	**AC570**	580	110-114	149-155	307-354	436-442	367	30-37	67-71	229	54-112	214	14-21	182-201
Chenopodium cycloides	**AC384**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	221	46-104	228	14-21	195-215
Chenopodium cycloides	**AC544**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium desertorum subsp. *anidiophyllum*	**AC519**	565	114-118	140-147	299-341	421-427	375	30-35	65-69	229	54-112	227	14-21	194-214
Chenopodium desiccatum	**AC588**	559	114-118	140-147	294-335	415-421	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium ficifolium	**AC854**	566	114-118	140-147	299-342	423-428	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium foliosum	**AC392**	599		118-127	280-339	454-460	376	30-39	71-76	245	46-128	223	14-21	192-210
Chenopodium foliosum	**AC520**	599		118-127	280-339	454-460	377	30-40	72-77	253	54-136	223	14-21	192-210
Chenopodium fremontii	**AC579**	559	114-118	140-147	294-335	415-421	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium fremontii	**AC597**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium giganteum	**AC428**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	221	46-104	227	14-21	194-214
Chenopodium gigantospermum var. *standleyanum*	**AC550**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	227	14-21	194-214
Chenopodium glaucum	**AC652**	557	114-117	139-147	277-330	413-419	369	30-40	70-73	229	54-112	225	14-21	194-213
Chenopodium glaucum	**AC417**	557	114-117	139-147	277-330	413-419	369	30-40	70-73	221	46-104	225	14-21	194-213
Chenopodium graveolens	**AC419**	426	114-120	142-148		283-289	353	29-36	68-74	251	54-135	225	14-21	193-211
Chenopodium hians	**AC551**	544	94-98	120-127	279-320	400-406	361	30-35	65-69	230	54-113	225	14-21	192-212
Chenopodium hybridum	**AC380**	599	114-118	140-150	307-364	307-364	375	29-39	69-73	220	45-103	229	14-21	197-216
Chenopodium hybridum	**AC609**	601	114-118	140-150	307-366	457-463	375	29-39	69-73	229	54-112	229	14-21	197-216
Chenopodium hybridum	**AC521**	599	114-118	140-150	307-364	455-461	375	29-39	69-73	222	47-105	229	14-21	197-216
Chenopodium iljinii	**AC611**	566	114-118	140-147	299-341	422-428	364	30-35	65-69	229	54-112	227	14-21	194-214
Chenopodium iljinii	**AC613**	566	114-118	140-147	299-341	422-428	364	30-35	65-69	229	54-112	227	14-21	194-214
Chenopodium incanum	**AC553**	559	114-118	140-147	294-335	415-421	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium leptophyllum	**AC554**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium melanocarpum	**AC429**	397	114-119			252-259	351	29-36	68-74	245	47-129	227	14-21	195-213
Chenopodium multifidum	**AC574**	419	114-119	141-145	261-261	275-281	366	29-36	68-74	254	54-137	228	14-21	194-214
Chenopodium murale	**AC382**	584	114-118	153-159	311-358	440-446	367	30-37	67-71	217	42-100	215	14-21	183-202
Chenopodium murale	**AC360**	584	114-118	153-159	311-358	440-446	367	30-37	67-71	220	45-103	215	14-21	183-202
Chenopodium murale	**AC383**	584	114-118	153-159	311-358	440-446	367	30-37	67-71	220	45-103	215	14-21	183-202
Chenopodium murale	**AC397**	584	114-118	153-159	311-358	440-446	367	30-37	67-71	211	36-94	215	14-21	183-202
Chenopodium murale	**AC413**	584	114-118	153-159	311-358	440-446	367	30-37	67-71	221	46-104	215	14-21	183-202
Chenopodium murale	**AC415**	584	114-118	153-159	311-358	440-446	367	30-37	67-71	229	54-112	216	14-21	184-203
Chenopodium murale	**AC424**	584	114-118	153-159	311-358	440-446	367	30-37	67-71	220	45-103	216	14-21	184-203
Chenopodium murale	**AC409**	584	114-118	153-159	311-358	440-446	367	30-37	67-71	213	38-96	215	14-21	183-202
Chenopodium murale	**AC430**	584	114-118	153-159	311-358	440-446	367	30-37	67-71	231	33-115	227	14-21	195-213
Chenopodium murale	**AC589**	564	94-98	133-139	291-338	420-426	367	30-37	67-71	229	54-112	215	14-21	183-202
Chenopodium murale	**AC566**	584	114-118	153-159	311-358	440-446	367	30-37	67-71	229	54-112	215	14-21	183-202
Chenopodium murale	**AC565**	584	114-118	153-159	311-358	440-446	367	30-37	67-71	229	54-112	215	14-21	183-202
Chenopodium murale	**AC581**	584	114-118	153-159	311-358	440-446	367	30-37	67-71	229	54-112	215	14-21	183-202
Chenopodium murale	**AC587**	584	114-118	153-159	311-358	440-446	367	30-37	67-71	229	54-112	215	14-21	183-202
Chenopodium neomexicanum	**AC555**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	225	14-21	192-212
Chenopodium neomexicanum	**AC598**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	225	14-21	192-212
Chenopodium nevadense	**AC556**	559	114-118	140-147	294-335	415-421	359	30-35	65-69	229	54-112	244	14-21	195-231
Chenopodium opulifolium	**AC410**	565	114-118	140-147	299-340	421-427	359	30-35	65-69	221	43-104	228	14-21	195-215
Chenopodium opulifolium	**AC416**	565	114-118	140-147	299-340	421-427	359	30-35	65-69	221	43-104	228	14-21	195-215
Chenopodium pallescens	**AC594**	559	114-118	140-147	294-335	415-421	359	30-35	65-69	229	54-112	225	14-21	192-212
Chenopodium pallescens	**AC557**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	225	14-21	192-212

Appendix 2.D – List of sequence parts excluded as mutational hotspots in *trnL-F* and ITS for each individual sequence (continued)

Taxon	Code	*trnL* intron	*trnL*	*trnL*	*trnL*	*trnL*	*trnL-F* spacer	*trnL-F*	*trnL-F*	ITS 1	ITS 1	ITS 2	Position HS1 ITS 2	Position HS2 ITS 2
Chenopodium pallidicaule	**AC398**	562	114-118	140-147	299-339	418-424	359	30-35	65-69	219	44-102	227	13-20	194-214
Chenopodium pallidicaule	**AC399**	562	114-118	140-147	299-339	418-424	359	30-35	65-69	211	36-94	227	13-20	194-214
Chenopodium pallidicaule	**AC400**	562	114-118	140-147	299-339	418-424	359	30-35	65-69	220	45-103	227	13-20	194-214
Chenopodium pallidicaule	**AC426**	562	114-118	140-147	299-339	418-424	359	30-35	65-69	229	54-112	227	13-20	194-214
Chenopodium petiolare	**AC359**	563	114-118	140-147	299-339	419-425	164	30-35		208	33-91	228	14-21	195-215
Chenopodium petiolare	**AC423**	563	114-118	140-147	299-339	419-425	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium pratericola	**AC558**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium pumilio	**AC524**	421	114-119	141-146		276-283	350	29-35	67-73	252	54-136	227	14-21	195-213
Chenopodium pumilio	**AC604**	421	114-119	141-146		276-283	350	29-35	67-73	252	54-136	227	14-21	195-213
Chenopodium pumilio	**AC615**	421	114-119	141-146		276-283	350	29-35	67-73	252	54-136	227	14-21	195-213
Chenopodium quinoa	**AC401**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	211	36-94	228	14-21	195-215
Chenopodium quinoa	**AC402**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	220	45-103	228	14-21	195-215
Chenopodium quinoa	**AC403**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	211	36-94	228	14-21	195-215
Chenopodium quinoa	**AC404**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	213	38-96	228	14-21	195-215
Chenopodium quinoa	**AC405**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	220	45-103	228	14-21	195-215
Chenopodium quinoa	**AC406**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	211	36-94	228	14-21	195-215
Chenopodium quinoa	**AC407**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	211	36-94	228	14-21	195-215
Chenopodium quinoa	**AC408**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	213	38-96	228	14-21	195-215
Chenopodium quinoa	**AC394**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	211	36-94	228	14-21	195-215
Chenopodium rubrum	**AC411**	559	114-117	139-147	277-332	415-421	369	30-39	69-73	229	54-112	226	14-21	194-213
Chenopodium rubrum	**AC564**	559	114-117	139-147	277-332	415-421	369	30-39	69-73	229	54-112	226	14-21	194-213
Chenopodium rubrum	**AC653**	559	114-117	139-147	277-332	415-421	369	30-39	69-73	229	54-112	226	14-21	194-213
Chenopodium rubrum	**AC385**	561	114-117	139-147	277-332	415-421	369	30-39	69-73	221	46-104	226	14-21	194-213
Chenopodium schraderianum	**AC387**	422	114-120	142-148		278-284	352	29-37	69-75	249	54-133	227	14-21	195-213
Chenopodium standleyanum	**AC595**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium standleyanum	**AC596**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium subglabrum	**AC559**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	228	14-21	195-215
Chenopodium urbicum	**AC576**	558	114-117	139-147	277-331	414-420	332	30-36		228	54-112	226	14-21	194-213
Chenopodium urbicum	**AC536**	558	114-117	139-147	277-331	414-420	332	30-36		228	54-112	226	14-21	194-213
Chenopodium vulvaria	**AC412**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	228	54-112	228	14-22	196-215
Chenopodium vulvaria	**AC562**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	228	54-112	228	14-22	196-215
Chenopodium watsonii	**AC561**	564	114-118	140-147	299-340	420-426	359	30-35	65-69	229	54-112	225	14-21	192-212
Einadia nutans	**AC525**	564	112-118	140-147	299-340	420-426	368	30-35	65-69	229	54-112	228	14-21	195-215
Grayia brandegeei	**AC627**	411	114-118	140-146	298-307		368	36-42	72-76	229	54-112	228	14-22	196-215
Grayia spinosa	**AC625**	411	114-118	140-146	298-307		368	36-41	71-76	229	54-112	227	14-22	196-214
Hablitzia tamnoides	**AC523**	523	118-123	124-126	278-313	386-392	353	12-12	44-54	244	47-124	224	14-21	194-210
Hablitzia tamnoides	**AC018**	523	118-123	124-126	278-313	386-392	353	12-12	44-54	251	54-131	224	14-21	194-210
Kraschenninnikovia ceratoides	**AC608**	642	117-123	143-150	303-383	492-501	386	29-39	71-78	246	54-128	223	14-21	192-210
Kraschenninnikovia ceratoides	**AC532**	630	99-105	125-132	285-370	479-489	386	29-39	71-78	246	54-128	223	14-21	192-210
Kraschenninnikovia lanata	**AC626**	643	119-125	145-152	305-385	494-502	386	29-39	71-78	246	54-128	223	14-21	192-210
Microgynoecium tibeticum	**AC656**	476	113-117	139-146	304-3015	332-338	363	29-35	65-69	229	54-112	225	14-21	193-212
Monolepis nuttalliana	**AC621**	574		118-126	279-330	429-435	377	30-39	71-76	247	46-130	223	14-21	192-210
Rhagodia triandra	**AC522**	565	114-118	140-147	299-341	421-427	367	30-35	65-69	229	54-112	228	14-21	195-215
Spinacia oleracea	**AJ400848.1**	357		118-127			330	30-38	70-76					
Spinacia oleracea	**EU606218.1**									245	53-128	233	14-21	198-220
Spinacia tetrandra	**AC650**	304		118-127			330	30-38	70-76	246	54-129	232	14-21	198-219
Spinacia turkestanica	**AC651**	304		118-127			330	30-38	70-76	246	54-129	232	14-21	198-219
Stutzia dioica	**AC351**	415	114-121	144-150		302-311	137	30-36		207	54-89	208	14-22	197-205
Suckleya suckleyana	**AC350**	395	114-118			251-257	362	29-37	69-77	253	54-137	228	14-20	195-213

Chapter 3

Resurrecting the Linnaean genus *Blitum* and recovering two new genera for Chenopodioideae (Chenopodiaceae) based on phylogenetic reconstruction

Contents

3.1 Introduction

In Chenopodioideae *Chenopodium* sensu lato with about 150 species encloses a high morphological variability, which has led to various generic and intrageneric groupings. Aellen (1960) in the treatment of *Chenopodium* s.l. in the "Illustrierte Flora von Mitteleuropa" divided the genus in 13 sections. Later these sections were organized in three subgenera: the subg. *Ambrosia* and the subg. *Chenopodium* by Scott (1978a), and the subg. *Blitum* by Hiitonen (1933) (Chapter 2). Some morphological characters have shown to be useful for the intrageneric differentiation and delimitation. For instance, the presence of glandular hairs and aroma in the species of the subg. *Ambrosia*, were used for transfer this subgenera to *Dysphania* (Carolin, 1983; Mosyakin and Clemants, 1996). However, most of the morphological characters in *Chenopodium* s.l. are very variable and make difficult its intrageneric delimitations. The differentiation between *Chenopodium* and other genera like *Blitum*, *Rhagodia* or *Einadia* has been problematic in the different taxonomic treatments (Ambrosi, 1857; Meyer, 1929; Aellen; 1960; Wilson, 1983).

Linnaeus described *Chenopodium* and *Blitum* as two different genera in "Species Plantarum" (1753). But the delimitation between *Chenopodium* and *Blitum* based on morphology was changing through the years (see Table 3.1). *Blitum* was also accepted as a different genus from *Chenopodium* by e.g., Meyer (1829), Schur (1866) and Scott (1978a). But other authors included *Blitum* within *Chenopodium* s.l., first as a section by Ambrosi (1857) and then as a subgenus by Hiitonen (1933). Currently, the subg. *Blitum* is accepted containing five sections: sect. *Blitum*, sect. *Pseudoblitum*, sect. *Glauca*, sect. *Agatophytum* and sect. *Degenia* (Mosyakin and Clemants, 1996; Judd and Ferguson, 1999; Clemants and Mosyakin, 2003; Heywood et al., 2007).

Chenopodium s.l. based on phylogenetic analyses is not monophyletic. The phylogenetic reconstruction for the alliance Chenopodiaceae-Amaranthaceae showed that within Chenopodioideae *Chenopodium* s.l. depict three clades (Kadereit et al., 2003, Müller and Borsch, 2005a). In Chapter 2 the incremented sampling of *Chenopodium* s.l. depict five differentiate and supported lineages based on the plastid region *trnL-F* data set. Therefore, one of the lineages represents the *Chenopodium* s.str. enclosing *Ch. album* which is the lectotype specimen for the genus designed by Mosyakin and Clemants (1996). *Einadia* and *Rhagodia* were merged into *Chenopodium* s.str. and new generic

Table 3.1 – Taxonomic changes within *Blitum* L.

Linnaeus (1753/1771)	Meyer (1829)	Moquin-Tandon (1849)	Ambrosi (1857)	Schur (1866)	Bentham and Hooker (1880)	Hiitonen (1933)	Ulbrich (1934)	Aellen (1960)	Scott (1978a)	Mosyakin and Clemants (1996)	Clemants and Mosyakin (2003)
***Blitum* L.**	***Blitum* L.**	***Blitum* L.**	***Chenopodium* L.**	***Blitum* L.**	***Chenopodium* L.**	***Chenopodium* L.**	***Chenopodium* L.**	***Chenopodium* L.**	***Blitum* L.**	***Chenopodium* L.**	***Chenopodium* L.**
						Subg. *Blitum*				**Subg. *Blitum***	**Subg. *Blitum***
	Sect. *Blitum*	**Sect. *Morocarpus***			**Sect. *Blitum***		**Sect. *Eublitum***	**Sect. *Eublitum***		**Sect. *Blitum***	**Sect. *Blitum***
								Subsect. *Capitata*		**Subsect. *Capitata***	
B. capitatum	*B. capitatum*	*B. capitatum*	*Ch. capitatum*		*Ch. capitatum*	*Ch. capitatum*	*Ch. capitatum*	*Ch. capitatum*	*B. capitatum*	*Ch. capitatum*	*Ch. capitatum*
								Subsect. *Foliosa*		**Subsect. *Foliosa***	
B. virgatum	*B. virgatum*	*B. virgatum*	*Ch. virgatum*		*Ch. foliosum*	*Ch. foliosum*	*Ch. foliosum*	*Ch. foliosum*	*B. virgatum*	*Ch. foliosum*	*Ch. foliosum*
	B. petiolare	*B. petiolare*									
		B. maritimum									
		B. rubrum									
		B. antarticum									
									B. hastatum		
	Sect. *Orthosporum*	**Sect. *Orthosporum***		**Subg. *Pseudoblitum***	**Sect. *Pseudoblitum***		**Sect. *Pseudoblitum***	**Sect. *Pseudoblitum***			**Sect. *Pseudoblitum***
											Ch. chenopodioides
B. chenopodioides											
	B. polimorphum										
	B. carinatum	*B. carinatum*									
	B. pumilo	*B. pumilo*									
	B. maritimum										
	B. nuttallianum										
								Subsect. *Viridia*			
	B. rubrum		*Ch. rubrum*	*B. rubrum*	*Ch. rubrum*		*Ch. rubrum*	*Ch. rubrum*			*Ch. rubrum*
		Sect. *Agatophytum*									**Sect. *Agatophytum***
	B. bonus-henricus	*B. bonus-henricus*	*Ch. bonus-henricus*	*B. bonus-henricus*	*Ch. bonus-henricus*						*Ch. bonus-henricus*
											Ch. californicum
				B. acuminatum							
				B. crassifolium							
								Subsect. *Glauca*			**Sect. *Glauca***
			Ch. glaucum	*B. glaucum*	*Ch. glaucum*			*Ch. glaucum*			*Ch. glaucum*
					Ch. antarticum						
		B. tenue									
		B. glandulosum									
											Sect. *Degenia*
											Ch. macrospermum

combinations were done (Chapter 2). The aromatic species of *Chenopodium* s.l. subg. *Ambrosia* were enclosed in another lineage, hence the transference of these species to *Dysphania* is supported (Chapter 2; Kadereit et al., 2010). Moreover the aromatic lineage is included within the tribe *Dysphanieae* as *Dysphania,* genus related to *Teloxys* and *Suckleya* (Chapter 2).

The remaining three lineages of *Chenopodium* s.l. within Chenopodioideae were not taxonomically defined because the main objective of the Chapter 2 was to resolve the phylogeny of *Chenopodium* s.l. Therefore, several species of *Chenopodium* s.l. subg. *Blitum* (e.g. *Ch. capitatum*) related to *Monolepis* and *Scleroblitum* are enclosed in a monophyletic clade within the *Blitum* lineage. This lineage of *Chenopodium* s.l. is sister to the *Spinacia* lineage and both are enclosed in the monophyletic clade of *Spinacieae*. The *Ch. rubrum* lineage encompassed the rest of species classified under the *Chenopodium* s.l. subg. *Blitum* (e.g. *Ch. glaucum*) and several species classified on the subg. *Chenopodium* (e.g. *Ch. urbicum*). Finally, the *Ch. murale* lineage enclosed also species of the subg. *Chenopodium* (e.g. *Ch. hybridum*). Whereas *Chenopodium* s.str. is sister to Atripliceae, the *Blitum* lineage is included within *Spinacieae* and the aromatic lineage of *Dysphania* is included within *Dysphanieae*, the positions of the *Ch. murale* lineage and *Ch. rubrum* lineage within Chenopodioideae are not yet clear (see Chapter 2). Adding further characters from the *matK/trnK* region will allow to test the relationships showed in the tree inferred based on *trnL-F* (Chapter 2).

In this sense, the objectives of this paper are: i) to test the respective positions of the *Ch. rubrum* and *Ch. murale*-lineages within Chenopodioideae based on a combined data set of *trnL-F* and *matK/trnK* plastid regions, and ii) based on the phylogenetic reconstruction discuss the taxonomic status of the Linnean genus *Blitum* L. within *Spinacieae* and to elaborate the correct formal taxonomy for the *Ch. rubrum* and *Ch. murale* lineages.

3.2 Materials and methods

3.2.1 Taxon sampling

Species of *Chenopodium* s.str. *Ch. rubrum*-clade, *Ch. murale*-clade and also species of the tribes *Atripliceae*, *Axyrideae*, *Dysphanieae* and *Spinacieae*, were sampled. The sampling represents the most distant branches of each clade founded in Chapter 2. New samples were incremented for *Dysphanieae* (*D. ambrosioides* and *D. graveolens*) and for *Chenopodium* s.l. (*Ch. polyspermum*). Taxa from Betoideae (*Beta* and *Hablitzia*) and Salicornioideae (*Allenrolfea*) were used as outgroups based on the tree of Müller and Borsch (2005a) (Chapter 2). All the samples are listed in Appendix 3.A.

3.2.2 DNA isolation, amplification and sequencing

Genomic DNA of the new samples was isolated from silica gel dried leaf tissue using the modified CTAB method (Borsch et al., 2003). The rest of samples were isolated for the analyses of Chapter 2 and used for this chapter. The *trnL-F* region was amplified and sequenced following the methodology described in Chapter 2. The *matK/trnK* region was amplified and sequenced in two overlapping halves, or with amplification in four overlapping halves for herbarium specimens and other difficult samples, using internal primers as described by Müller and Borsch (2005a). All primer names and sequences are given in Table 3.2. PCR amplification was performed using the following reaction mix: 1.5 mM $MgCl_2$, 1X PeqLab Taq Buffer S (including $MgCl_2$), 0.25 mM each dNTP, 0.8 pmol primer, 0.03 U/ul Taq polymerase (PeqLab, Erlangen Germany) and 0.8 ng/ul DNA template. For difficult templates (e.g. DNA isolated from herbarium material), betaine was added to a final concentration of 1 M. The PCR was performed in a peqStart Thermocycler (peqLab Biotechnologic, Germany). The PCR program used for the *trnL-F* region was: 30 cycles of denaturation (60 s at 94°C), annealing (60 s at 52°C), extension (120 s at 72°C) and a final extension step (15 min at 72°C). The PCR program for *matK/trnK* region was: 34 cycles of denaturation (60 s at 94°C), annealing (60 s at 50°C), extension (120 s at 72°C) and a final extension step (15 min at 72°C).

Table 3.2 – List of primers used in this study

Primer Name	Sequence	Source
For amplification of the *trnL-F* region		
trnTAC2	5'-CATTTTTCGGTATAGTAABCC-3'	Fuentes-Bazan et al. (2011)
trnTC	5'-CGAAATCGGTAGACGCTACG-3'	Taberlet et al. (1991)
trnTf	5'-ATTTGAACTGGTGACACGAG-3'	Taberlet et al. (1991)
For sequencing of the *trnL-F* region		
trnL-460F	5'-GAGAATAAAGATAGAGTCC-3'	Worberg et al. (2007)
trnTd	5'-GGGGATAGAGGGACTTGAAC-3'	Taberlet et al. (1991)
For amplification and sequencing of the *matK/trnK* region		
First halve		
trnKbry	5'-ATCATGGGGTTGCTAACTC-3'	Müller and Borsch (2005a)
ACmatK1401R	5′-ATGGATTCGTATTCACATAC-3'	Borsch, unpublished
Internal primers		
ACmatK 200R	5'-GCAGTCATTGTGGAAATTC-3'	Müller and Borsch (2005a)
ACmatK 100F	5'-CTCGACTGTATCAACAGAAT-3'	Müller and Borsch (2005a)
Second halve		
ACmatK 490F	5′-CTGGTTGAAAGATGCTTCKT-3′	Müller and Borsch (2005a)
trnK 2R	5′-AACTAGTCGGATGGAGTAG-3′	Johnson and Soltis (1995)
Internal primers		
ACmatK 1300R	5'-GTGCTAGAACTTTGTCTCGYA-3'	Müller and Borsch (2005a)
ACmatK 1250F	5'-CTCATTATTATAGTGGCTCYT-3'	Müller and Borsch (2005a)

3.2.3 Alignment and coding of length mutational events

Sequences were edited and aligned manually using PhyDE (Phylogenetic Data Editor) version 0.995 (Müller et al., 2007), following the rules outlined in Löhne and Borsch (2005). The inversions were re-inverted and coded as mutational events in the indel matrix following Löhne and Borsch (2005). Regions of uncertain homology (mutational hotspots) were excluded from the analysis (Borsch et al., 2003; Müller and Borsch 2005a). The *trnL-F* and *matK/trnK* data sets were combined for phylogenetic analysis. Indels were coded automatically using the Simple Indel Coding method (Simmons and Ochoterena, 2000) as implemented in SeqState 1.40 (Müller, 2005a).

3.2.4 Phylogenetic analyses

Maximum Parsimony (MP) analyses were performed using Parsimony Ratchet (Nixon, 1999) implemented in the software PRAP (Müller, 2004) in combination with PAUP* v. 4.0b10 (Swofford, 1998). Ratchet settings were 200 ratchet iterations with 25% of the positions randomly up weighted (weight =2) during each replicate and 10 random addition cycles.

The command files generated with PRAP were then run in PAUP, using the heuristic search with the following parameters: all characters have equal weight, gaps are treated as “missing”, TBR branch swapping, initial swapping on 1 tree already in memory, Maxtrees set to 100 (auto increased by 100) and branches collapsed actively if branch length is zero. The Jackknife (JK) support for branches was also performed in PAUP with 10,000 replicates, using a TBR branch swapping algorithm with 36.788% of characters deleted and one tree held during each replicate, following Müller (2005b).

Bayesian inference (BI) was carried out using MrBayes 3.1 (Huelsenbeck and Ronquist, 2001). Optimal nucleotide substitution models for the combined data set of *trnL-F- matK/trnK* was GTR+G based on the AIC criteria calculated by JModeltest 0.1 (Posada, 2008). A binary (restriction site) model was implemented for the coded indels. All analyses were performed with four independent runs of Markov Chains Monte Carlo (MCMC) each with four parallel chains. Each chain was performed for 1 million generations; saving one

random tree every 100th generation. The burn in was set to 200,000, and a majority consensus tree was computed with the remaining trees.

3.3 Results

The PCR amplification of *trnL-F* was successful for all samples except for *Ch. polyspermum*. Although specific Amaranthaceae-Chenopodiaceae primers were used, it seems that the variability of *Ch. polypermum* for *trnL-F* makes it difficult to amplify. Work underway is the design of new primers for *trnL-F*. For *matK/trnK* the amplification was successful, the tree reconstruction with only this region including *Ch. polyspermum* is in Appendix 3.C.

3.3.1 The combined *trnL-F* and *matK/trnK* data set

The aligned combined data set, without the areas classified as "hotspots" (HS), comprised 3772 characters, including 822 characters that were parsimony informative. In the *trnL-F* region seven HS were excluded (Chapter 2) and in the *matK/trnK* region three HS were excluded. The statistics of the regions including and excluding HS are in Appendix 3.2. One inversion was found in the *trnL* intron in *Kraschenninikovia* (Chapter 2). The final matrix, including coded indels, comprised 3992 characters, of which 948 characters were parsimony informative. The MP search resulted in 128 shortest trees (L=2415, CI=0.720, RI= 0.918 and RC= 0.661). The resulting strict consensus tree for MP was identical in topology with the Bayesian (BI) majority-rule consensus tree (Fig. 3.1).

3.3.2 Phylogenetic relationships

Both reconstructions (MP and BI) supported the seven founded lineages within Chenopodioideae (Fig. 3.1). *Chenopodium* s.str. is highly supported as monophyletic (100% JK/ 1 PP). The tribe Atripliceae (100% JK/ 1 PP) represented by *Atriplex* and *Microgynoecium* is supported as the sister clade to *Chenopodium* s.str. The sister clade to Atripliceae and *Chenopodium* s.str. is the *Ch. murale*-clade. The *Chenopodium murale*-clade (100 % JK/ 1 PP) includes the close related *Ch. murale* and *Ch. coronopus* (100% JK/ 1 PP), and the sister to these two taxa is the clade of *Ch. hybridum* and *Ch. badachschanicum* (100% JK/ 1 PP, Fig. 3.1). The sister clade to all the previous clades is

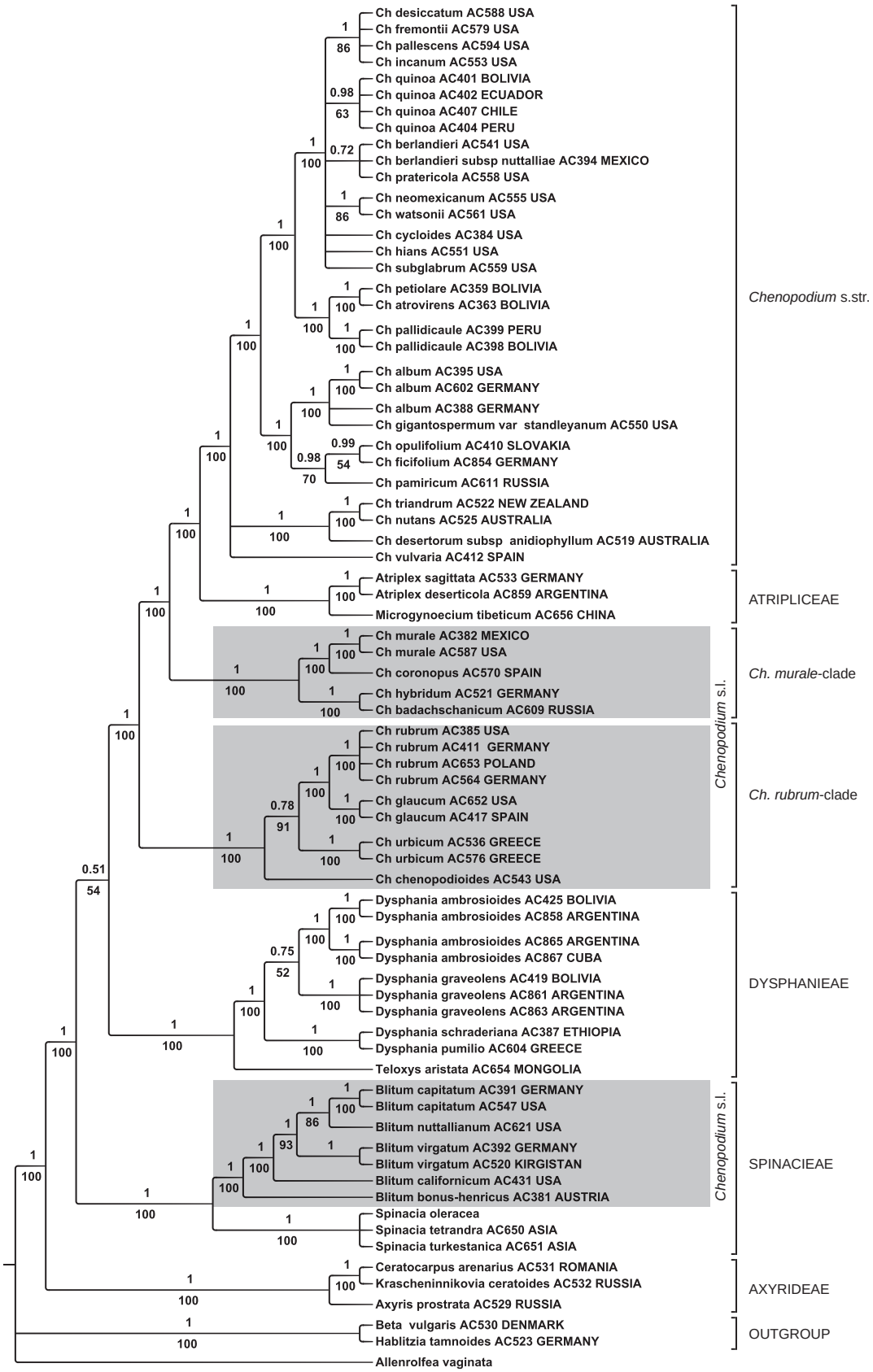


Fig. 3.1 – Strict consensus tree based on the *trnL-F* and *matK/trnK* data sets. Jackknife values (JK) are given below and Bayesian posterior probabilities (PP) above branches. Boxes in gray mark the species of *Chenopodium* s.l. taxonomically discussed in this study.

the *Chenopodium rubrum*-clade (100% JK/ 1 PP) encompassing the close related *Ch. rubrum* and *Ch. glaucum* (91% JK/ 1 PP). *Ch. urbicum* is the sister to these two taxa (100% JK/ 1 PP) and *Ch. chenopodioides* is the sister to all of them (100% JK/ 1 PP) (Fig. 3.1). Based only in the plastid region *matK/trnK*, *Ch. polyspermum* results in a low supported (56% JK) differentiate lineage sister to the monophyletic group composed by *Ch. rubrum*-clade, *Ch. murale*-clade, Atripliceae and *Chenopodium* s.str. (Appendix 3.C).

The tribe *Spinacieae* (100% JK/ 1 PP) is highly supported encompassing two defined lineages: the *Spinacia* lineage (100% JK/ 1 PP) encompassing *Spinacia oleracea*, *S. tetrandra* and *S. turkestanika*, and their sister *Blitum* lineage (100% JK/ 1 PP, Fig. 3.1). Within the *Blitum* lineage *Ch. capitatum*, *Monolepis nuttalliana* and *Ch. foliosum* (100% JK / 1 PP) are close related, and the next sister to them is *Ch. californicum* (100% JK/ 1 PP); *Ch. bonus-henricus* is the sister to all the previous taxa within the *Blitum* lineage.

The tribe *Dysphanieae* is highly supported (100% JK/ 1 PP) encompassing *Dysphania* and *Teloxys*. Although the increment of the characters in the combined data set, *Dysphanieae* is still showing an unclear position within Chenopodioideae (see Fig. 3.1). Finally, the tribe *Axyrideae* (100% JK/ 1 PP) represented by *Axyris*, *Ceratocarpus* and *Krascheninnikovia* is high supported and is the sister group to all the previously described tribes and clades within Chenopodioideae (Fig. 3.1).

3.4 Discussion

Based on the combined data set of *trnL-F* and *matK/trnK*, the phylogenetic tree reconstruction recovers five highly supported lineages of *Chenopodium* s.l. within Chenopodioideae. The delimitation of *Chenopodium* s.str. as monophyletic is highly supported. Atripliceae is still the sister group of *Chenopodium* s.str. The next sister group of the two previous clades with maximally support is the *Ch. murale*-clade resolved in this study. Finally *Ch. rubrum*-clade is the next sister also maximally supported to all of the previous clades (Fig. 3.1). A new differentiate lineage of *Ch. polyspermum* is detected as next sister to all of them, but low supported (Appendix 3.C). The pollen, seeds and inflorescence characters of *Ch. polyspermum* are different from the rest of chenopods and consequently no close morphologic relatives are reported (Uotila, 2001). The low supported relationship of *Ch. polyspermum* is probably because the phylogenetic

reconstruction was possible only with *matK/trnK*. In this sense the suggestion of some taxonomic changes for *Ch. polyspermum* is not yet possible because it is necessary to test its position based on a combined data set within Chenopodioideae.

The maximally supported *Blitum* lineage as the sister group of *Spinacia* within the tribe Spinacieae shows that the genus *Blitum* can be resurrected as different from *Chenopodium* s.str. This result is in agreement with the original description and delimitation done by Linnaeus in 1753. Due to the *Ch. rubrum*-clade and *Ch. murale*-clade are two lineages differentiated from each other and from the *Blitum* lineage and also from the *Chenopodium* s.str., new generics names for each one will be discussed in this study[1].

The following discussion is structured clade by clade in order to evaluate the possible generic names of the sampled members enclosed in each clade or lineage. Each sampled member is presented with its chronological taxonomical changes. Finally, for each clade the choice of the generic name is based on an integrative discussion of all possible generic names on line with the rules of the ICNB.

3.4.1 The tribe *Spinacieae*

The tribe *Spinacieae* is a monophyletic clade showing the newly sister relationship between the *Spinacia* and the *Blitum* lineages recently defined in Chapter 2 and high supported by the current study. The *Blitum* lineage encloses the closely related *Ch. capitatum, Ch. foliosum, Monolepis nuttalliana, Ch. californicum* and *Ch. bonus-henricus* and the *Spinacia* lineage encloses all the species of the genus (Fig. 3.1).

3.4.1.1 Resurrecting the Linnaean genus Blitum

Chenopodium capitatum (L.) Ambrosi, was described by Linnaeus (1753) as *Blitum capitatum*. Later, Ambrosi in “Flora del Tirolo Meridionale” (1857) includes *Blitum* (L.) within *Chenopodium* L. and also made the necessary combination *Chenopodium capitatum*. Ascherson (1864) again made the same combination for the “Flora der Provinz Brandenburg” as *Chenopodium capitatum* (L.) Asch. However, Britton and Brown (1913)

[1] The final decision of the new names are still in consideration by the authors of this paper and the new combinations will be prepared for the submission of the paper to Willdenowia.

accepted the genus *Blitum* L. and designated *Blitum capitatum* L. as the lectotype specimen for *Blitum*. But the inclusion of *Blitum* within *Chenopodium* made by Ambrosi (1857) is currently accepted (Aellen, 1960; Scott 1978a, Uotila, 2001; Clemants and Mosyakin, 2003).

Chenopodium foliosum (Moench) Asch., was treated by Moench in 1794 under the genus *Monocarpus*. Moench (1794) described the genus *Monocarpus* and *Blitum* L. was transferred under this one. Therefore, *Blitum virgatum* L. was a synonym of *Monocarpus foliosus*. Later Ascherson in "Flora der Provinz Brandenburg" (1864) modified the generic concept of Moench, and included the genus *Monocarpus* under *Chenopodium*, using *Monorcapus foliosus* as basionym for *Chenopodium foliosum.*

Monolepis nuttalliana (Schult.) Greene, was first described as *Blitum nuttalianum* by Schultes (1822). Schultes argued that the reported *Blitum chenopodioides* with a question mark "?" annotated by Nuttal (1818) should be understood as different from the described *Blitum chenopodioides* by Linnaeus (1753). Due to Nuttal (1818) reported *Blitum chenopodioides?* for the Flora of North America as a doubtful native, Schultes (1822) renamed it as *Blitum nuttalianum*. This last name was not aware by Treviranus, who based on "*Blitum chenopodioides?* Nutt." described *Chenopodium trifidum* in 1829. Later, Schrader (1830) recognized that "*Chenopodium trifidum* Trev." was different from *Chenopodium* and renamed this one as *Monolepis trifida*, which is the type specimen used by Schrader for the description of the genus *Monolepis* (Meyer, 1843). Finally, Greene in "Flora Franciscana" (1891) transferred *Blitum nuttalianum* Schult. to *Monolepis nuttalliana*.

Chenopodium californicum (S. Watson) S. Watson, was first described as *Blitum californicum* by Watson (1874) following the classification of Chenopodiaceae by Moquin-Tandon (1849), where *Blitum* was considered different from *Chenopodium*. Later, Watson in "Geological Survey of California, Botany" (1880) explained that the limits of *Chenopodium* as distinct from *Blitum* were very vague and he included all the *Blitum* species from its previous revision into *Chenopodium*. In this context he also made the combination *Chenopodium californicum*.

Chenopodium bonus-henricus L. was originally described under *Chenopodium* by Linneaus (1753). Later, Du Mortier (1827) described the genus *Anserina* based on only *Ch.*

bonus-henricus. However, the genus *Anserina* was neither accepted by Meyer (1829) in "Flora Altaica" nor by Moquin-Tandon (1834) in "Descriptions de plusieurs nouveaux generes de Chenopodees". Meyer (1829) rather described the new section *Orthosporum* section under *Blitum* L., to accommodate *Blitum bonus-henricus* (L.) C.A. Mey. (see Table 3.1). In 1834 Moquin-Tandon described yet another genus, called *Agatophytum* that only included *Ch. bonus-henricus*. But again Meyer (1835) in "Genera plantarum florae germanicae" raised his section *Orthosporum* to a genus level, including the previously described *Agatophytum* Moq. Finally, Moquin-Tandon (1849) revised the delimitation of *Agatophytum* and changes its taxonomical status as a section under *Blitum*, hence the name *Blitum bonus-henricus* is again used. However as explained before, *Blitum* was once again included within *Chenopodium* in posterior works using the already existing original name *Chenopodium bonus-henricus* L. (Table 3.1; Ambrosi, 1857; Bentham and Hooker, 1880; Hiitonen, 1933; Ulbrich, 1934; Aellen, 1960; Scott, 1978a; Mosyakin and Clemants, 1996).

The definition of *Blitum* by Linnaeus (1753) was based on *B. capitatum* and *B. virgatum* (see original description in Fig. 3.2). Later, Linnaeus in "Mantissa Plantarum (1771)" described *Blitum chenopodioides*. While *Blitum* was considered as different from *Chenopodium*, intrageneric divisions were suggested. For example, Meyer (1829) suggested two sections: the sect. *Blitum* encompassing *Blitum virgatum* L., *B. capitatum* L. and *B. petiolare* Link., and the sect. *Orthosporum* based on an older delimitation by Brown (1810) within *Chenopodium* considering *Ch. carinatum* R. Br., *Ch. pumilio* R. Br., and adding *B. maritimum* Nutt., *B. nuttalianum* Schult., *Ch. rubrum* L. and *Ch. bonus-henricus* L. Moquin-Tandon (1849) maintained the sect. *Orthosporum* R. Br. under *Blitum* with *B. carinatum* and *B. pumilio*; additionally this author delimited the sect. *Morocarpus* including *B. capitatum, B. virgatum, B. rubrum, B. petiolare, B. maritimum* and *B. antarticum*, and the sect. *Agatophytum* with only *B. bonus-henricus*. In 1866 Schur divided *Blitum* in subgenera and described the subgen. *Pseudoblitum* encompassing *B. bonus-henricus, B. rubrum, B. glaucum, B. acuminatum* and *B. crassifolium*. Finally, Scott (1978a) considered that *Blitum* should be maintained encompassing *B. capitatum, B. hastatum* and *B. virgatum*. The original delimitation of *Blitum* based on the morphological characters proposed by Linnaeus and latter re-evaluated by the authors was left because the morphological characters were ambiguous for its delimitation (Ambrosi, 1857; Watson,

1880; Aellen, 1960; Clemants and Mosyakin, 2003). Actually all the Linnaean *Blitum* species are included within *Chenopodium* subg. *Blitum* and the designed type specimen is *Chenopodium capitatum* (L.) Ambrosi (Mosyakin and Clemants, 1996).

In this study the close relationship of *Ch. capitatum* (≡ *B. capitatum*), *Ch. foliosum* (≡ *B. virgattum*) as a different lineage from *Chenopodium* s.str. support the existence of the Linnaean genus *Blitum* (Fig. 3.1). Moreover, the phylogenetic reconstruction shows a clear delimitation for *Blitum* where *Monolepis nuttalliana* should be transferred to *Blitum* and named as *B. nuttalianum* Schult. The same nomenclatural change was made for *Ch. californicum* as *Blitum californicum* S. Watson, and *Ch. bonus-henricus* should be accepted as *B. bonus-henricus* as suggested by Meyer (1829). Moreover, based on the phylogenetic reconstruction of Kadereit et al. (2003), the monotypic genus *Scleroblitum* Ulbr. should be also part of *Blitum* as *Blitum atriplicinum* F. Muell., because its inclusion within the *Blitum* lineage as sister to *Ch. foliosum*. On the other hand, this study also indicates that *Blitum chenopodioides* L. (≡ *Ch. chenopodioides*) described in 1771 should be interpreted as a specimen related to *Chenopodium rubrum* and not to *Blitum* (Uotila, 2001).

This study does not support the intrageneric sections and subgenera suggested within *Blitum*, showing for example that under the sect. *Orthosporum*, *Ch. pumilio* and *Ch. carinatum* actually belongs to *Dysphania* based on molecular and morphological studies (Chapter 2; Mosyakin and Clemants, 2002). Another example is *Ch. rubrum* (≡*B. rubrum*) which in this study belongs to the independent *Ch. rubrum* lineage (Fig. 3.1, Chapter 2, discussion below).

3.4.1.2 The genus Spinacia

The genus *Spinacia* was also described by Linnaeus (1753) including only *Spinacia oleracea*. Modern treatments recognize three species within *Spinacia* (sampled in Chapter 2 and this study; Fig. 3.1): *Spinacia oleracea* L., *Spinacia tetrandra* Steven and *Spinacia turkestanica* Iljin (Iljin, 1936; Shults, 2003; Kühn, 1993). While the morphology and taxonomic delimitation of the genus *Spinacia* is generally well understood (Shults, 2003), *Spinacia* were considered part of Atripliceae because their unisexual flowers (Kühn, 1993).

Nevertheless, phylogenetic studies showed that *Spinacia* does not belong to Atripliceae (Kadereit et al., 2010), but it is within the newly tribe *Spinacieae*.

Blitum and *Spinacia* are herbaceous plants with ascending stems, and mostly glabrous and globose paraclades of the inflorescence. Thus, these morphological characters should used for the characterization of *Spinacieae*. *Spinacia* could be easily separated from *Blitum* because they are perennial and monoecious plants and the pistillate flowers are enclosed by two opposite acrescent tepal lobes (newly described by Flores-Olvera et al., 2011). In addition, *Spinacia* has a base chromosome number of six (Schmitz-Linneweber et al., 2001). In contrast, *Blitum* may be characterized by the annual or biannual habit, dioecious plants, not enclosed flowers and a base chromosome number of nine.

3.4.2 Taxonomy of the *Ch. rubrum*-clade

The combined data set of *trnL-F* and *matK/trnK* in this study resolves a highly supported *Ch. rubrum*-clade enclosing *Ch. rubrum*, *Ch. glaucum*, *Ch. chenopodioides* and *Ch. urbicum* (Fig. 3.1). The *Ch. rubrum*-clade is maximally supported as sister to the monophyletic *Ch. murale*-clade, Atripliceae and *Chenopodium* s.str.

Chenopodium rubrum was described by Linnaeus (1753) within *Chenopodium*. Later, Meyer (1829) included *Ch. rubrum* within *Blitum* as *B. rubrum* in his delimitation of the sect. *Orthosporum*. The same author in 1835 described the genus *Orthosporum* and consequently made the combination of *Orthosporum rubrum*. However, Schur (1866) accepted the treatment of Meyer (1829) and he transferred *B. rubrum* this species from the sect. *Orthosporum* to the subg. *Pseudoblitum*. When Ambrosi (1857) again included *Blitum* under *Chenopodium* he also treated *Chenopodium rubrum* in this group. Currently, *Ch. rubrum* is accepted under the section *Pseudoblitum* subg. *Blitum* within the genus *Chenopodium* (Aellen, 1960; Mosyakin and Clemants, 1996; Clemants and Mosyakin, 2003).

Chenopodium glaucum was described by Linnaeus in 1753. Later, Koch (1837) included *Ch. glaucum* under *Blitum*. Then this species was treated together with *Ch. rubrum* and this name suffer the same changes explained before. Finally, *Ch. glaucum* is classified under the section *Glauca* subg. *Blitum* within the genus *Chenopodium* as

currently accepted (Aellen, 1960; Mosyakin and Clemants, 1996; Clemants and Mosyakin, 2003).

Chenopodium chenopodioides (L.) Aellen, was originally described by Linnaeus (1771) as *Blitum chenopodioides*. Later, Aellen (1933) and Aellen and Just (1943) included *B. chenopodioides* within *Chenopodium* sect. *Degenia*. The neotypification by Uotila (2001) of *Ch. chenopodioides* clarified the interpretation of the original description of *B. chenopodioides* by Aellen (1933). Uotila (2001) explained that the three connate tepals close to the apex ("Calyx triphyllus, concavus, conivens", original description) is the most important character to differentiate *Ch. chenopodioides* from other taxa. Nowadays, *Ch. chenopodioides* is included within *Chenopodium* subg. *Blitum*, sect. *Pseudoblitum* (Aellen, 1960; Scott, 1978a; Mosyakin and Clemants, 1996; Clemants and Mosyakin, 2003).

Chenopodium urbicum was described by Linnaeus in 1753. In 1856 Montandon published the combination of *Ch. urbicum* under *Anserina* Dumortier based on the horizontal position of the seeds. Nevertheless, this change was not accepted by Aellen (1960), who included *Ch. urbicum* within subg. *Chenopodium* sect. *Lejosperma*. Currently, Mosyakin and Clemants (1996) suggested the monotypic subsection *Urbica* where *Ch. urbicum* is the designed type species.

The morphological characters such as leaf surface and form, architecture of the inflorescence, perianth number, seed position and testa surface are commonly used in the delimitation of the subgenera and sections within *Chenopodium* s.l. but their morphological states are very homoplastic. Therefore the use of some selected characters by the authors has created different conflicting classifications system through the taxonomic history of *Chenopodium* s.l. (see Chapter 2). However, it seems that the characters used for the description of the sect. *Pseudoblitum* are good candidates for the characterization of the *Ch. rubrum-clade*. Bentham and Hooker (1880) described the sect. *Pseudoblitum* including *Ch. rubrum* and *Ch, glaucum* based on the non-succulence of the fruit perianth. Moreover, Aellen (1960) considered other characters for the sect. *Pseudoblitum* such as the hermaphrodite flowers which have 3, 4, or 5 perianth elements with horizontal seeds, and the presence of vertical seeds with free perianth in the lateral flowers. The reduced number of perianth elements and the presence of vertical seed are also observed in *Ch. chenopodioides* which in current treatments is included in sect.

Pseudoblitum (Aellen, 1960; Uotila, 2001; Clemants and Mosyakin, 2003). The inclusion of *Ch. urbicum* within the sect. *Chenopodium* seems even to be artificial based on its morphology. *Ch. urbicum* is highlighted in the description of the subsect. *Lejorperma* (sect. *Chenopodium*) because the rare fine foveate brands on the seed surface and the also rare vertical position of the seeds (Aellen, 1960). Due to these morphological differences of *Ch. urbicum* Mosyakin and Clemants in 1996 described the monotypic subsect. *Urbica* within the subgen. *Chenopodium*, however Clemants and Mosyakin (2003) mention that *Ch. urbicum* should be transferred to the subg. *Blitum*. Hence, this study supports only the exclusion of *Ch. urbicum* from the subg. *Chenopodium*. The close relationship between *Ch. rubrum*, *Ch. glaucum*, *Ch. urbicum* and *Ch. chenopodioides* based on phylogeny in this study supports the morphological observations made by Bentham and Hooker (1880) and Uotila (2001).

Based on the phylogenetic reconstruction *Ch. rubrum-clade* showed to be a differentiate lineage from *Chenopodium* s.str., thus previous generic names or a new generic name should be proposed for these taxa, following the rules of the International Code of Botanical Nomenclature (McNeill et al., 2006). After Linnaeus in the 19th century, *Ch. rubrum* and *Ch. glaucum* were treated under *Blitum* L. by Meyer in 1829, under *Agatophytum* by Moquin-Tandon in 1834 and under *Orthosporum* by Meyer in 1835. However, the generic name *Blitum* as described before is defined and can not be used for this lineage. The generic name *Agatophytum* described by Moquin-Tandon (1834) and the later synonym *Orthosporum* described by Meyer (1835) are also useless for the *Ch. rubrum*-clade, because the type specimen of both genera is *Ch. bonus-henricus* which is clearly part of the *Blitum* lineage (Fig. 3.1).

On the other hand, the species of the *Ch. rubrum*-clade were treated under illegitimate generic names like *Anserina* published by Montandon in 1856 or *Botrys* by Nieuwland in 1914. The generic name *Anserina* was described based on *Chenopodium bonus-henricus* L. by Du Mortier (1827). However, in the treatment of Montandon (1856) the genus *Anserina* did not include *Ch. bonus-henricus*. *Ch. bonus-henricus* was included by the same Montandon (1856) under *Orthosporum*. Additionally, *Anserina* is also a generic name under Ascobolaceae, a fungi family described by Velenovský (1934). The generic name *Botrys* described by Nieuwland (1914) was synonym of *Chenopodium* L. and its type should be *Ch. album*. Moreover, *Botrys* Nieuwl. is an homonym of the earlier

Botrys described by Fourreau (1869) under Lamiaceae and the type is *B. chamaedryoides* Fourr. Finally *Botrys* D. I. Schirschova (1985) is also a generic name under Chlorophyceae an algae family and the type is the fossil *Botrys compacta*. Thus, none of these names is appropriate for naming this new taxon. The species within this clade have a reduced number of perianth parts (3) in lateral flowers, and the presence of mostly horizontal but also vertical seeds. These morphological characters could be useful to propose the generic name for this lineage (Uotila, com. pers.).

3.4.3 Taxonomy of the *Ch. murale*-clade

The combined data set of *trnL-F* and *matK/trnK* supports the *Ch. murale*-clade as sister to the monophyletic *Chenopodium* s.str. and Atripliceae (Fig. 3.1). This clade encompasses *Ch. murale*, *Ch. coronopus, Ch. hybridum* and *Ch. badachschanicum* (Fig. 3.1).

Ch. murale was described by Linnaeus in 1753. *Ch. murale* was included within *Atriplex* by Crantz (1766) and later within *Anserina* by Montandon (1856). Nevertheless, the follow treatments did not accept the inclusion of *Ch. murale* either under *Atriplex* nor under *Anserina* and accept the original Linnaean classification. In the current widely used taxonomic treatments, *Ch. murale* is included within the monotypic subsect. *Unduata*, sect. *Chenopodium* under the subg. *Chenopodium* (Aellen, 1960; Clemants and Mosyakin, 2003).

Ch. coronopus was described by Moquin-Tandon (1849) within the sect. *Botryois*. *Ch. coronopus* originally was collected by Eugene Bourgeau in the Canary Islands and its endemic to Atlantic Islands.

Ch. hybridum was described by Linnaeus in 1753. *Ch. hybridum* was included within *Atriplex* (Crantz, 1766) and *Anserina* (Montandon, 1856), and together with other taxa under *Chenopodium* (e.g. *Ch. murale, Ch. rubrum*). However, actually *Ch. hybridum* is included within the sect. *Grosseofoveata* subg. *Chenopodium* (Mosyakin and Clemants, 1996).

Ch. badachschanicum was described by Tzvelev in 1960. This is a Central Asiatic species, related to *Ch. hybridum* (Uotila, 1997; Freitag et al., 2001).

The *Ch. murale*-clade is well differentiated from *Chenopodium* s.str., the *Ch. rubrum* lineage and the *Blitum* lineage based on the phylogenetic reconstruction (Fig. 3.1). As well as the *Ch. rubrum-clade*, the *Ch. murale-clade* should be named. The older generic names *Anserina* Dumontier (1827) and *Botrys* Nieuwland (1914) are illegitimate as explained in the previous discussion for *Ch. rubrum-clade*. As well as the generic names *Atriplex* Crantz (1766) and *Vulvaria* Bubani (1897) are synonyms to *Chenopodium* L. and *Ch. album* is not the designed type species of these genera is inappropriate the use of this generic names. In this study *Pseudochenopodium* is the new proposed generic name for this lineage, because the included taxa resemble *Chenopodium*. Moreover based on observations of the included taxa, the trichome morphology, and the morphology and ornamentation of the seeds could help to characterize this lineage and to decide a final new generic name.

3.5 Taxonomic synopsis for *Blitum* L.

Blitum L. Sp. Pl.: 4 (1753).

Type: *Blitum capitatum* L.

1. *Blitum capitatum* L. Sp. Pl.: 4 (1753).

LT: Linnean herbarium 14.1 (LINN). Designed by Jonsell and Jarvis, Regnum Veg. 127:25 (1993).

2. *Blitum virgatum* L. Sp. Pl.: 4 (1753).

LT: Linnaean herbarium 14.2 (LINN). Designed by Jafri and Rateeb in Jafri and El-Gadi, Fl: Libya 58: 11 (1978).

3. *Blitum nuttallianum* Shult. Schultes in: Roemer and Schultes. Systema Vegetabilium 1: 65 (1822).

T: not designed[1] (Note: 1 the designation will be ready for the submission of the paper to Willdenowia)

4. *Blitum californicum* S.Watson. Proc. Amer. Acad. 9: 101 (1874).

T: not designed. One syntype is deposited in NY which could be designed as type if the other mentioned vouchers by Watson (1874) are not available.

5. *Blitum bonus-henricus* (L.) C.A.Mey. Flora Altaica 1:11 (1829).

Chenopodium bonus-henricus L. Sp. Pl.: 218 (1753).

LT: Linnaean herbarium 313.1 (LINN). Designed by Jonsell & Jarvis, Nordic J. Bot. 14: 155 (1994).

6. *Blitum atriplicinum* F. Muell. Trans. & Proc. Victorian Inst. Advancem. Sci. for 1845-55 133 (1855).
LT: F. Mueller s/n. Cudnaka. Oct. 1850 (MEL). Designed by Wilson, P.G., Fl. Australia 4:197 (1983).

Note: The synopsis includes only the synonyms of those species not described originally under *Blitum*.

Appendix 3.A - Samples included in this study

Taxon	Field/Garden Origin	Voucher	Code	*trn* L-F Acc.	*matK/trnK* Acc.
	Subfamily Chenopodioideae				
Tribe Atripliceae C. A. Meyer					
Atriplex deserticola Phil.	Argentina	Z. Noaga F O 12057 (B)	**AC859**	this study	this study
Atriplex sagittata Borkh.	Berlin Bot. Gard. No: 063119110 [Germany]	S. Fuentes 021 (B)	**AC533**	HE577499	this study
Microgynoecium tibeticum Hook. f.	China	B. Dickoré 4284 (B)	**AC656**	HE577503	this study
Tribe Axyrideae (Heklau) G. Kadereit & Sukhor.					
Axyris prostrata L.	Russia	E. v. Raab-Straube 020232a (B)	**AC529**	HE577509	this study
Ceratocarpus arenarius L.	Romania, Navodari	A. Romanovsch (B)	**AC531**	HE577504	this study
Krascheninnikovia ceratoides (L.) Gueldenst.	Russia	R. Hand 1536 (B)	**AC532**	HE577507	this study
Tribe Chenopodieae					
	***Chenopodium* s. str.**				
Chenopodium album L.	Germany, Bonn	S. Fuentes 001 (B)	**AC388**	HE577557	this study
Chenopodium album L.	USA ARS GRIN PI608030 [USA]	S. Fuentes 007 (B)	**AC395**	HE577568	this study
Chenopodium album L.	Germany, Usedom	Weber (B)	**AC602**	HE577559	this study
Chenopodium atrovirens Rydb.	Bolivia, La Paz	S. G. Beck 11328 (B, KAS, LPB)	**AC363**	HE577586	this study
Chenopodium berlandieri Moq.	USA, Nevada	J. C. Beatley 11698 (NY)	**AC541**	HE577561	this study
Chenopodium cycloides A. Nelson	USA	T. Borsch, Müller and Pratt 3452 (B)	**AC384**	HE577598	this study
Chenopodium desertorum subsp. *anidiophyllum* (Aellen) P.G. Wilson	Australia	C. Michaell & J.Risler 1773 (B, NT)	**AC519**	HE577555	this study
Chenopodium desiccatum A. Nelson	USA, Missouri	B. Summers & Harris 9813 (MO)	**AC588**	HE577550	this study
Chenopodium ficifolium Sm.	Germany, Berlin	R. & E. Willing 12.260 D (B)	**AC854**	HE577606	this study
Chenopodium fremontii S. Watson	USA, California	G. Schoolcraft 2206 (UC)	**AC579**	HE577546	this study
Chenopodium gigantospermum var. *standleyanum* Aellen	USA, Kansas	C. A. Morse 10855 (NY)	**AC550**	HE577551	this study
Chenopodium hians Standl.	USA, Wyoming	S. Stephens 70636 (NY)	**AC551**	HE577610	this study
Chenopodium pamiricum Iljin	Russia, Altay Republic	L. Martins 2490 (B)	**AC611**	HE577608	this study
Chenopodium incanum (S. Watson) A. Heller	USA, New Mexico	R. D. Worthington 17439 (NY)	**AC553**	HE577548	this study
Chenopodium neomexicanum Standl.	USA, New Mexico	R. D. Worthington 13394 (NY)	**AC555**	HE577611	this study
Chenopodium nutans (R. Br.) S. Fuentes & Borsch	Berlin Bot. Gard. No: 187199 [Australia]	S. Fuentes 019 (B)	**AC525**	HE577553	this study
Chenopodium opulifolium Schrad. ex W.D.J. Koch & Ziz	Slovakia	T. Borsch 3899 (B)	**AC410**	HE577595	this study
Chenopodium pallescens Standl.	USA, Missouri	G. Yatskievych 03-93 (MO)	**AC594**	HE577547	this study
Chenopodium pallidicaule Aellen	USA ARS GRIN PI478406 [Bolivia]	No Voucher	**AC398**	HE577574	this study
Chenopodium pallidicaule Aellen	USA ARS GRIN PI510525 [Peru]	No Voucher	**AC399**	HE577573	this study
Chenopodium petiolare Kunth	Bolivia, Oruro	R. de Michel 2873 (B, KAS, LPB)	**AC359**	HE577588	this study
Chenopodium pratericola Rydb.	USA, Wyoming	K. H. Dueholm 10922 (B, LPB)	**AC558**	HE577562	this study
Chenopodium quinoa Willd.	USA ARS GRIN Ames 13214 [Bolivia]	S. Fuentes 013 (B)	**AC401**	HE577580	this study
Chenopodium quinoa Willd.	USA ARS GRIN Ames 13228 [Ecuador]	S. Fuentes 017 (B)	**AC402**	HE577576	this study
Chenopodium quinoa Willd.	USA ARS GRIN PI510551 [Peru]	S. Fuentes 009 (B)	**AC404**	HE577579	this study
Chenopodium quinoa Willd.	USA ARS GRIN PI614880 [Chile]	S. Fuentes 010 (B)	**AC407**	HE577582	this study
Chenopodium berlandieri subsp. *nuttalliae* (Saff.) H.Dan. Wilson & Heiser	USA ARS GRIN PI568155 [Mexico]	S. Fuentes 016 (B)	**AC394**	HE577571	this study
Chenopodium subglabrum (S. Watson) A. Nelson	USA, Wyoming	R. D. Dorn 5434 (NY)	**AC559**	HE577605	this study
Chenopodium triandrum G.Forst.	New Zealand	P. Hein 12560 (B, CHR)	**AC522**	HE577554	this study
Chenopodium vulvaria L.	Spain	T. Borsch 3918 (B)	**AC412**	HE577591	this study
Chenopodium watsonii A. Nelson	USA, Arizona	D. H. Goldman 2095 (NY)	**AC561**	HE577602	this study

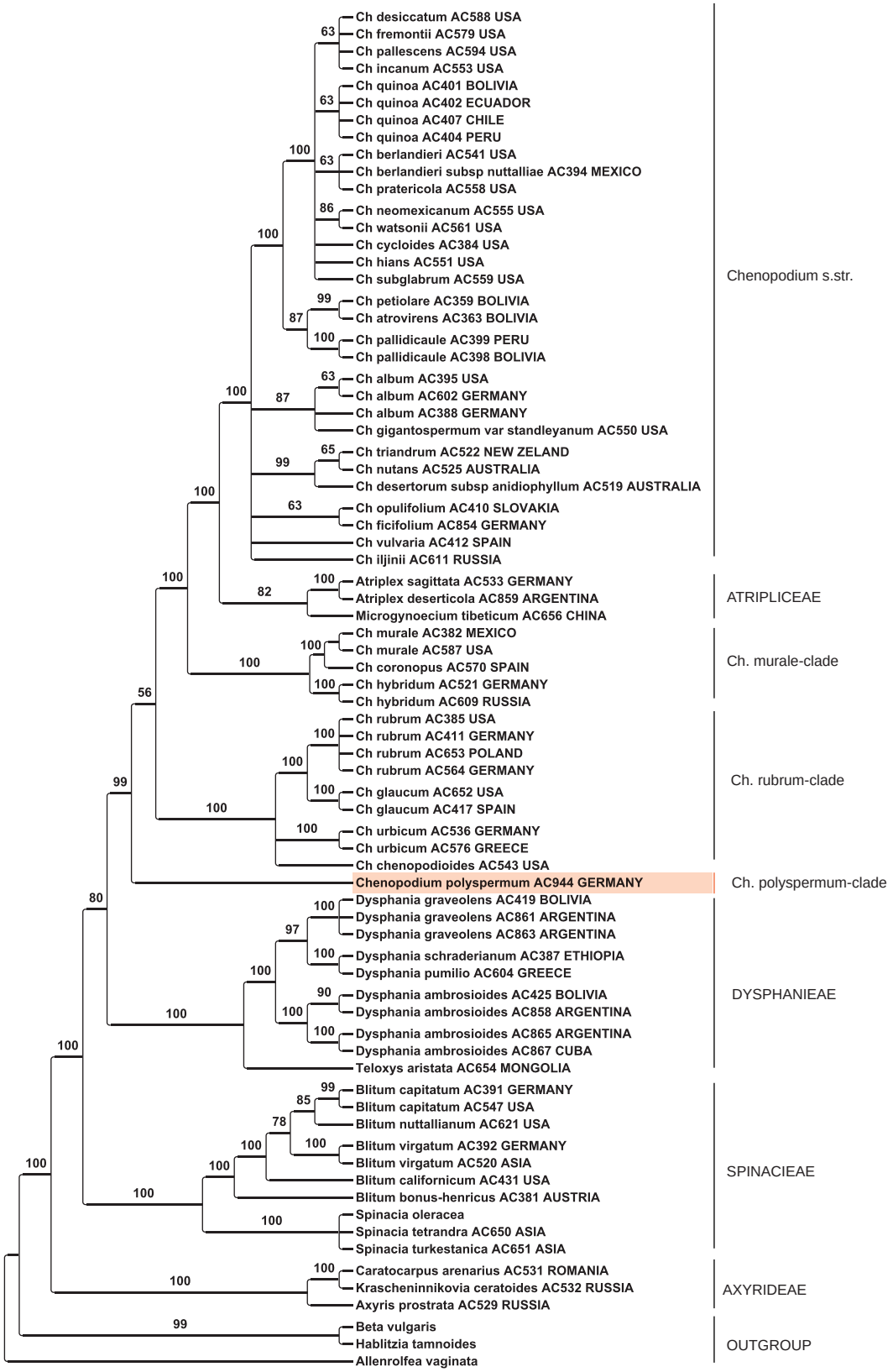
Appendix 3.A - Samples included in this study (continued)

Taxon	Field/Garden Origin	Voucher	Code	*trn* L-F Acc.	*matK/trnK* Acc.
Tribe Dysphanieae Pax					
Dysphania ambrosioides (L.) Mosyakin & Clemants	Bolivia, La Paz	S. G. Beck 31178 (B, LPB)	**AC425**	HE577493	this study
Dysphania ambrosioides (L.) Mosyakin & Clemants	Argentina	Z Noaga F O 11806 (B)	**AC858**	this study	this study
Dysphania ambrosioides (L.) Mosyakin & Clemants	Argentina	Z Noaga F O 11603 (B)	**AC865**	this study	this study
Dysphania ambrosioides (L.) Mosyakin & Clemants	Cuba	T. Borsch et al. 4397 (B)	**AC867**	this study	this study
Dysphania graveolens (Wlld.) Mosyakin & Clemants	Argentina	Z Noaga F O 11911 (B)	**AC861**	this study	this study
Dysphania graveolens (Wlld.) Mosyakin & Clemants	Argentina	Z Noaga F O 11913 (B)	**AC863**	this study	this study
Dysphania graveolens (Wlld.) Mosyakin & Clemants	Bolivia	E. Thomas 258 (B, LPB)	**AC419**	HE577495	this study
Dysphania pumilio (R. Br.) Mosyakin & Clemants	Greece	R. & E. Willing 85.571 (B)	**AC604**	HE577485	this study
Dysphania schraderianum (Schult.) Mosyakin & Clemants	Ethiopia	M. Wondafrash 2255 (B, ETH)	**AC387**	HE577490	this study
Teloxys aristata (L.) Moq.	USA ARS GRIN Ames 25314 [Mongolia]	S. Fuentes 183 (B)	**AC654**	HE577481	this study
Tribe Spinaceae Moq.					
Chenopodium bonus-henricus L.	Austria	T. Borsch 3821 (B)	**AC381**	HE577512	this study
Chenopodium californicum (S. Watson) S. Watson.	USA, California	P. Davis & D. Lightowless 66504 (B)	**AC431**	HE577516	this study
Chenopodium capitatum (L.) Ambrosi	Bonn Bot. Gart. No: 19116	S. Fuentes 004 (B)	**AC391**	HE577513	this study
Chenopodium capitatum (L.) Ambrosi	USA, Utha	K. Moon et al. 1993 (NY)	**AC547**	HE577514	this study
Chenopodium foliosum Asch.	Bonn Bot Gart No: 19117 [Germany]	S. Fuentes 003 (B)	**AC392**	HE577517	this study
Chenopodium foliosum Asch.	Kirgistan, Central Asia	Cubr 42389 (B)	**AC520**	HE577518	this study
Monolepis nuttalliana (Schult.) Greene	USA, Utha	R. C. Holmgren 317 (B)	**AC621**	HE577515	this study
Spinacia oleracea L.				AJ400848.1	AJ400848.1
Spinacia tetrandra Steven ex M. Bieb.	USA ARS GRIN Ames 23664 [Asia]	S. Fuentes 180 (B)	**AC650**	HE577482	this study
Spinacia turkestanica Iljin	USA ARS GRIN Ames 23666 [Asia]	S. Fuentes 181 (B)	**AC651**	HE577483	this study
	Chenopodium s. l.				
Chenopdoium polyspermum L.	Germany	T.Borsch s/n (B)	**AC944**	this study	this study
***Ch. murale*-clade**					
Chenopodium murale L	Mexico, Ixtapan	T. Borsch & H. Flores Olvera 3871 (B, MEXU	**AC382**	HE577541	this study
Chenopodium murale L.	USA, California	G. Gust & L. Nyle 476 (MO)	**AC587**	HE577545	this study
Chenopodium hybridum L.	Germany, Brandenburg	R. & E. Willing 20.856 D (B)	**AC521**	HE577529	this study
Chenopodium badachschanicum Tzvelev	Russia, Altay Republic	L. Martins 2329 (B)	**AC609**	HE577528	this study
Chenopodium coronopus Moq.	Spain, La Palma	Royl 6823 (B)	**AC570**	HE577543	this study
***Ch. rubrum*-clade**					
Chenopodium chenopodioides (L.) Aellen	USA, Montana	P. C. Lesica 5792 (NY)	**AC543**	HE577519	this study
Chenopodium glaucum L.	USA ARS GRIN PI612859 [USA]	S. Fuentes 184 (B)	**AC652**	HE577526	this study
Chenopodium glaucum L.	Spain	T. Borsch 3931 (B)	**AC417**	HE577527	this study
Chenopodium rubrum L.	Germany, North See	T. Borsch [08.07] (B)	**AC411**	HE577520	this study
Chenopodium rubrum L.	Germany	E. Willing 10.931D (B)	**AC564**	HE577522	this study
Chenopodium rubrum L.	USA ARS GRIN Ames 23860 [Poland]	S. Fuentes 182 (B)	**AC653**	HE577521	this study
Chenopodium rubrum L.	USA	T. Borsch 3448 (B)	**AC385**	HE577525	this study
Chenopodium urbicum L.	Greece, Fthiotis	R. & E. Willing 146.1979 (B)	**AC576**	HE577524	this study
Chenopodium urbicum L.	Berlin Bot. Gard. No: 269400010 [Greece]	S. Fuentes 026 (B)	**AC536**	HE577523	this study
OUTGROUPS					
Beta vulgaris subsp. *maritima* (L.) Thell.	Denmark, Jylland	Cubr 39900 (B)	**AC530**	HE577473	AY514832.1
Hablitzia tamnoides M. Bieb.	Germany, Berlin Bot Gard No: 16611	S. Fuentes 018 (B)	**AC523**	HE577474	AY514825.1
Allenrolfea vaginata Kuntze	Germany, Bonn Bot Gard No: 2488		**AC017**	HE577472	AY514828.1

Note: The circumscription of tribes in Chenopodioideae are recognized based on Kadereit et al. (2010) and Fuentes-Bazan et al. (2012). USA ARS GRIN refers to USDA, ARS, National Genetic Resources Program. *Germplasm Resources Information Network* - (GRIN). [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland. The *trnL-F* acc. are published by Fuentes-Bazan et al. (2012) and the *matK/trnK* acc. are published by Müller and Borsch (2005).

Appendix 3.B – Sequence statistics of individual regions and the combined data set for *Chenopodium* s.l.

	trnL intron	*trnL* 3' exon	*trnL-F* spacer	*trnK* 5' intron	*matK*	*trnK* 3′ intron	Combined
Dataset with hotspots							
Length range	304-630	50	164-386	672-750	1493-1536	195-229	
Mean length (SD)	531(73)	50	358(25)	706(12)	1525(6)	210(7)	
% GC	31.8	30.3	44	31.3	32.3	33	
Inversions	1	0	0	0	0	0	
Dataset without hotspots							
Length range	295-538	50	159-369	651-722	1493-1536	178-212	3053-3347
Mean length (SD)	478(54)	50	347(24)	680(11)	1525(6)	197(7)	3277(6)
% variable characters	24.7	2	42.3	28.9	33.1	41.3	32.1
% informative characters	16.3	2	27.2	19.8	23.4	25.6	21.8
Number of coded indels	63	0	74	44	6	30	217



Appendix 3.C – Strict consensus tree based on *matK/trnK* data set. Jackknife values (JK) are given above branches. Box in orange mark *Chenopodium polyspermum* as a new clade for *Chenopodium* s.l. within Chenopodioideae.

Chapter 4

Phylogeny of *Chenopodium* sensu stricto, patterns of reticulate evolution, and possible origins of Quinoa

Contents

4.1 Introduction

Chenopodium sensu lato is a cosmopolite species-rich genus within the subfamily Chenopodioideae; Kühn (1993) listed about 150 species. The distribution of the genus is in temperate and subtropical dry regions and comprises annual, biannual or perennial plants. *Chenopodium* s.l. has a high morphological variability which has been the cause of different intrageneric delimitations like subgenera, sections and subsections (see Table 2.1 in Chapter 2). For instance, the differentiation between *Blitum* and *Chenopodium* s.l. was not clear based on their morphological characters. Consequently *Blitum* was included as subgenera under *Chenopodium* (see Chapter 3). Currently phylogenetic analyses of *Chenopodium* s.l. and other genera resolve five independent lineages within Chenopodioideae (Chapter 2, Chapter 3). Thus, the defined *Chenopodium* sensu stricto (as the first lineage of *Chenopodium* s.l) is sister to Atripliceae and the next sister to these two lineages is the second lineage *Ch. murale-clade*. Directly related to all these three clades is *Ch. rubrum-clade,* as the third lineage of *Chenopodium* s.l. The fourth lineage defined as *Blitum* is sister to *Spinacia* both within the monophyletic tribe *Spinacieae*. The position of the Spinacieae-clade is not resolved and shares the basal position with the Dysphanieae-clade. Dysphanieae encloses *Teloxys*, *Suckleya* and the last depict lineage of the aromatic species of *Chenopodium* s.l, which has been transferred to *Dysphania* (Mosyakin and Clemants, 2002; Chapter 2 and Chapter3).

Even in its new delimitation, *Chenopodium* s.str. is still a species-rich genus enclosing more than 100 species and maintains its high morphological variability and cosmopolitan distributions as described before. Moreover, *Chenopodium* s.str. encloses species with different ploidy levels like diploids (e.g. *Ch. vulvaria*; *Ch. ficifolium* and *Ch. pallidicaule*), tetraploids (e.g. *Ch. quinoa*, *Ch. berlandieri* and *Ch. karoi*) and hexaploids (e.g. *Ch. album* and *Ch. opulifolium*) (Aellen and Just 1943; Uotila, 1973; Jellen et al., 2011). Soltis et al. (2003) mentioned that about 70% of the angiosperm species are polyploids. The polyploidization process is believed to trigger the adaptation of plants to new environments (Soltis et al., 2003; Rieseberg and Willis, 2007). Soltis et al. (2004) reported that the polyploids may be more common at higher latitudes and altitudes than diploids. In plants there are two main kinds of polyploidization: autopolyploidization (the ploidy level increases without hybridization) and allopolyploidization (hybridization causes the increase of ploidy). In *Chenopodium* s.str. is suggested that their polyploid

species are allopolyploids (Wilson, 1988a; Wilson 1988b; Wilson, 1990; Rahiminejad and Gornall, 2004; Kolano et al., 2008). In this sense several polyploid groups have been described within this genus: the *Ch. album*-group, the *Ch. quinoa*-group and the *Ch. berlandieri* subsp. *nuttalliae*-group.

The *Chenopodium album*-group (or aggregate) has been defined based on morphology and encompasses the hexaploids *Ch. album*, *Ch. giganteum, Ch. probstii* and *Ch. opulifolium*; the tetraploids *Ch. striatiforme*, *Ch. strictum* and *Ch. berlandieri*; and the diploids *Ch. suecium* and *Ch. ficifolium* (Clemants and Mosyakin, 2003; Rahiminejad and Gornall, 2004). Based on the morphological characters, flavonoid patterns and the chromosome number have been suggested that the diploids *Ch. suecium* and *Ch. ficifolium* and the tetraploid *Ch. strictum* are involved in the ancestry of the hexaploids within the *Ch. album*-group (Dvořák et al., 1983; Rahiminejad and Gornall, 2004).

The *Chenopodium quinoa*-group encompasses only tetraploid species: the crop *Ch. quinoa* (white seeds) which is closely related to its sympatric weed *Ch. quinoa* subsp. *milleanum* (black seeds), both of the Andean region; the Chilean lowland variety of *Ch. quinoa* called “qingua” distributed along the coast; and the weed *Ch. hircinum* distributed in the Argentinean lowlands (Wilson, 1988a; Wilson, 1988b). Wilson (1990) based on the comparative studies of leaf morphology and allozyme frequencies suggested the origin of the Andean tetraploids as an independent crop/weed complex. This hypothesis refers that the Andean tetraploids are originated from the Argentinean lowlands where *Ch. hircinum* and allies (not specified by Wilson) are involved. Additionally, he suggested that after the differentiation of the Andean tetraploids a subsequent dispersal to north (until Colombia) and south (until the Chilean coast) had happened (Wilson, 1990).

Similar studies of morphology and allozyme frequencies were carried out for the crop *Chenopodium berlandieri* subsp. *nuttalliae* distributed in Mexico. These studies showed that *Ch. berlandieri* subsp. *nuttalliae* is also a group of related tetraploids distributed in Mexico and U.S. This group encloses the crop *Ch. berlandieri* subsp. *nuttalliae* close related to the sympatric weeds *Ch. berlandieri* subsp. *berlandieri* and *Ch. berlandieri* var. *sinuatum*. The next closest relative to these Mexican taxa is the weed *Ch. berlandieri* var. *zschackei* widely distributed through western Mexico, southern California, the Rocky Mountains and Central Plains in U.S. to Alaska. The weed *Ch. berlandieri* var.

bushianum of the northeastern U.S. is also part of this group and showed to be a remnant of a crop/weed complex distributed in eastern of North America based on archaeological material (Wilson and Heiser, 1979; Wilson, 1990). The recent phylogenetic analyses based on archaeological material supports that the origin of the North American tetraploids was in the eastern North American region (Kistler and Shapiro, 2011). Finally a generalized hypothesis indicates that the tetraploids of America derived from diploid forms similar to *Ch. neomexicanum* and *Ch. watsonni* both natives of western North America and then dispersed to the continent. Kistler and Shapiro (2011) suggested that three independent events of domestication take place in: i) eastern of North America, ii) in Mexico and iii) in the Andes of South America. However the studies used by Wilson (1990) were done independently and with a small sampling. For example, Wilson and Heiser (1979) and Kistler and Shapiro (2011) included only species of the *Ch. berlandieri* subsp. *nuttalliae*-group; and Wilson (1981 and 1988a) consider only species of the *Ch. quinoa*-group.

In order to detect patterns of reticulation at the species level in plants is necessary to reconstruct the phylogeny from plastid and nuclear regions. If the phylogenetic signal from plastid and nuclear non-coding regions differs, this point out to a biological causes like reticulation events caused by hybridization, recombination, gene conversion or horizontal gene transfer (Fuertes Aguilar and Nieto Feliner, 2003; Löhne et al., 2008; Blöch et al., 2009; Huson et al., 2011). The differentiate signal from plastid and nuclear regions are detected in the inferred tree topology, where the incongruent position of some species are observed. Non-biological causes (e.g. insufficient sampling or long branch attraction) could also generate the incongruent topology of the inferred trees. Hence, is necessary to test if the different signal from the plastid regions and the nuclear regions is due to the mentioned biological causes. Phylogenetic incongruence between plastid and nuclear markers has been detected in *Chenopodium* s.str. (Chapter 2). Pervasive hybridization can be put forward as a hypothesis to explain this incongruence, and allopolyploidization as the mechanism to explain the presence of several polyploids in *Chenopodium* s.str. (see above).

On the other hand in species-rich genera especially in young species, the genome evolutionary history may not be dichotomus and the phylogenetic tree reconstruction could fail because the region studied do not evolve according to a tree-like manner. Currently the phylogenetic networks showed to be useful to understand the evolutionary patterns in these

taxa (Linder and Riesenberg, 2004; Jakob and Blattner, 2006; Huson and Bryant, 2006). Morrison (2010) suggested the use of the phylogenetic network display in order to detect possible non-biological and biological causes for conflictive data sets. Moreover the reconstruction of biological hybridization events based on networks is also suggested for generating biological hypothesis (Huson and Klöpper, 2007; Morrison, 2010).

In the previous phylogenetic reconstruction *Chenopodium* s.str. showed a signal of non-dichotomy in its inferred trees. This pattern could be because *Chenopodium* s.str. is a rich genera with evidence of hybridization process (Chapter 2). In this sense the use of the phylogenetic network display for explore the data sets and generate hypothesis of hybridization will give more evidence for the origin of the allopolyploids as the interesting case of the crop *Ch. quinoa*.

In order to resolve the phylogeny at species level and its deep relationships the use of non-coding regions is broadly used in angiosperms. The evaluation of the existing plastid introns and spacers performance with respect to resolution and support of the inferred trees remarks the utility of these regions (Borsch and Quant, 2009; Borsch et al., 2009; Korotkova et al., 2011). For instance, the best phylogenetic signal was reported for the plastid *rpl16* and *trnK* introns (Korotkova et al., 2011). Due to the internal relationships in *Chenopodium* s.str. are not well supported based on the phylogenetic analysis of *trnL-F* (Chapter 2), the present study combined the plastid regions *trnL-F*, *matK/trnK* and *rpl16* intron for improve the supports of their internal relationships. Moreover for the reconstruction of the reticulate patterns within *Chenopodium* s.str. the sampling especially for *Ch. quinoa* is incremented for the plastid regions and for the nuclear ITS region. In this sense the objectives of the present study are: i) test the relationships within *Chenopodium* s.str., ii) test the causes of the incongruent signal from the nuclear and the plastid regions; and iii) apply network reconstruction to infer possible reticulate evolutionary history.

4.2 Material and methods

4.2.1 Taxon sampling

Thirty-one species of *Chenopodium* s.str. were analysed, representing among others the *Ch. album*-group with *Ch. album*, *Ch. ficifolium*, *Ch. giganteum*, *Ch. opulifolium* and

Ch. strictum. The *Ch. quinoa*-group with the crop *Ch. quinoa* (white seeds) and its sympatric weed *Ch. quinoa* subsp. *milleanum* (black seeds) and their relatives *Ch. quinoa* (variety quingua from Chile) and *Ch. hircinum* (from Argentina). Finally, the *Ch. berlandieri* subsp. *nuttalliae*-group with the crop *Ch. berlandieri* subsp. *nuttalliae* and the sympatric weeds *Ch. berlandieri* var. *sinuatum* and *Ch. berlandieri* var. *zschackei*. The complete data set analysed had 90 ingroup samples. The outgroup is selected based on the phylogenetic reconstruction of Chapter 2 and Chapter 3. Therefore the outgroup are the differentiate lineage of Atripliceae which is sister to *Chenopodium* s.str. and the *Ch. murale* lineage which is the next sister to both clades. The reported ploidy levels by the Index to plant chromosome number (1981-2006) and by Aellen and Just 1943, Uotila, 1973, Dvořák, et al., 1983, Bhragava et al., 2006 and Jellen et al. (2011) from each taxon included in this study are listed in Appendix 4.A.

4.2.2 DNA isolation, amplification and sequencing

Genomic DNA was isolated from silica gel dried leaf tissue and herbarium specimens, using either a modified CTAB method (Borsch et al., 2003) or the Nucleo Spin Plant II extraction kit (Macherey Nagel, Düren, Germany). The quantity and purity of each DNA sample was measured by the NanoDrop spectrophotometer (ND- 1000, PeqLab, Erlangen, Germany).

The PCR amplification and the programs used for *trnL-F*, *matK/trnK* and ITS were the same described in Chapter 2 and Chapter 3.

In the case of the newly used marker *rpl16* intron was amplified and sequenced using the forward universal primer rpl16F (5’ -CTATGCTTAGTGTGTGACTC -3’, Campagna and Downie, 1998) and the reverse universal primer rpl16R (5’ -TCTTCCTCTATGTTGTTTACG -3’, Campagna and Downie, 1998). The PCR program for *rpl16* intron was: an initial denaturation step (94°C 3 min), 30 cycles of denaturation (60 s at 94°C), annealing (60 s at 54°C), extension (120 s at 72°C) and a final extension step (15 min at 72°C). Primer dimers and secondary banding patterns were separated from the requested bands using a 1.5% NEEO agarose gel (Carl Roth, Germany) running for 3 hours at 100 volts. Gel extraction was performed using the AveGene Gel /PCR DNA Fragments Extraction Kit (AveGene Life Science Corporation). The quality and quantity of

the purified PCR product were measured with a NanoDrop spectrophotometer. Cycle sequencing, fragment purification, and direct automated sequencing was performed by Macrogen Inc. (Seoul, South Korea).

4.2.3 Alignment and coding of length mutational events

Sequences were edited and aligned manually using PhyDE (Phylogenetic data editor) version 0.995 (Müller et al., 2007), following the rules outlined in Löhne and Borsch (2005). Regions of uncertain homology (mutational hotspots) were excluded from the analysis (Borsch et al., 2003). Hypothesized microstructural mutations that explain the length variability patterns of sequences in the aligned according to the guidelines by Borsch et al., 2007, Morrison, 2009 and Ochoterena, 2009 are listed (see Appendixes 4.B for *trnL-F*, 4.C for *matK/trnK*, 4.D for the *rpl16* intron and 4.E for ITS). Indels were then coded automatically using the Simple Indel Coding method (Simmons and Ochoterena, 2000) as implemented in SeqState 1.40 (Müller, 2005a).

4.2.4 Phylogenetic analyses

Maximum Parsimony (MP) analyses were performed using the Parsimony Ratchet (Nixon, 1999) using the software PRAP (Müller, 2004) in combination with PAUP* v. 4.0b10 (Swofford, 1998). Ratchet settings were 200 ratchet iterations with 25% of the positions randomly up weighted (weight =2) during each replicate and 10 random addition cycles. The command files generated with PRAP were then run in PAUP, using the heuristic search with the following parameters: all characters have equal weight, gaps are treated as “missing”, TBR branch swapping, initial swapping on 1 tree already in memory, Maxtrees set to 100 (auto increased by 100) and branches collapsed actively if branch length is zero. The Jackknife (JK) support for branches was also performed in PAUP with 10,000 replicates, using a TBR branch swapping algorithm with 36.788% of characters deleted and one tree held during each replicate, following Müller (2005b).

Bayesian inference (BI) was carried out using MrBayes 3.1 (Huelsenbeck and Ronquist, 2001). Optimal nucleotide substitution models for the combined data set of *trnL-F*, *matK/trnK* and *rpl16* (TIM2 + G) and ITS (GTR + G) data sets were chosen following the Akaike Information criterion (AIC) in JModeltest (Posada, 2008). A binary (restriction

site) model was implemented for the coded indels. All analyses were performed with four independent runs of Markov Chains Monte Carlo (MCMC) each with four parallel chains. Each chain was performed for 1 million generations, saving one random tree every 100th generation. The burn in was set to 100,000, and a majority consensus tree was computed with the remaining trees.

To test for congruence between the respective data sets, the Incongruence Length Difference (ILD) test (Farris et al., 1994) was implemented in PAUP* as the Partition Homogeneity Test. The following parameters were set: 1,000 replicates with 50 Random Addition Searches, holding only two trees each step and saving no more than 5 trees. The test was conducted for: i) the complete data set and ii) a reduced data set without incongruent taxa (see results).

The Shimodaira and Hasegawa (1999) nonparametric test, implemented in PAUP* as SH test, was conducted to asses incongruence in the topology of the trees generated from the plastid and nuclear data sets. The tree topology generated from the plastid data set was therefore manipulated in order to reflect the nuclear topology in those clades where they differ: i) *Ch. karoi* nested within the clade of *Ch. quinoa*, *Ch. hircinum* and *Ch. berlandieri*; ii) *Ch. ficifolium* nested within the clade of *Ch. quinoa*, *Ch. hircinum* and *Ch. berlandieri*; iii) *Ch. opulifolium* sister to the clade of *Ch. quinoa*, *Ch. hircinum* and *Ch. berlandieri*. Likewise, the tree topology generated from the nuclear data set was manipulated in order to reflect the plastid topology in those clades where they differ: i) *Ch. karoi* sister to *Ch. pamiricum*, ii) *Ch. ficifolium* nested within the clade of *Ch. pamiricum* and iii) *Ch. opulifolium* sister to *Ch. pamiricum* constrained to the nuclear data set. The SH test was carried out to test one by one each manipulated clade leaving the rest of the tree with the original topology.

Network analysis were carried out for: i) making exploratory split trees with the program Neighbour Net from each the combined plastid data set and nuclear data set excluding gaps, parsimony uninformative sites and constant sites, running Bootstrap (BS) with 1000 repeats as implemented in the software SplitsTree in order to detect causes of the conflictive data sets and ii) for exploring reticulation patterns with the Hybridization-Network from the MP trees with the RECOMB2007 method as implemented in the software SplitsTree4 version 4.11.3 (Huson and Bryant, 2006).

A preliminary molecular clock analysis based on the plastid regions using the package BEAST V. 1.4. (Drummond and Rambaut, 2007) is carried out in order to infer the divergence time of the polyploids species within *Chenopodium* s.str. (Further details of the analysis in Appendix 4.G).

4.3 Results

4.3.1 The combined *trnL-F*, *matK/trnK* and *rpl16* intron data set

The aligned combined data set, without the areas classified as "hotspots" (HS), comprised 4596 characters, including 453 characters that were parsimony informative. Alignment was straightforward for all regions. No inversions were detected. Three hotspots (HS) were excluded from the *trnL-F* region, two from the *matK/trnK* region and six from the *rpl16* intron. The final matrix, including coded indels, comprised 4690 characters, of which 509 characters were parsimony informative. The statistics values for each region partition are in Appendix 4.F. Within *Chenopodium* s.str. the *trnK* 3' intron showed the highest informative characters percentage (12%), followed by the *trnL-F* spacer (11.9%) and by *rpl16* intron (11.4%). Mutational HS from each region are restricted to poly A or Poly T stretches. The MP search resulted in 31 shortest trees (L=917, CI=0.876, RI= 0.961 and RC= 0.842).

The resulting strict consensus tree for MP was identical in topology with the Bayesian (BI) phylogram (Fig. 4.1). Based on the combined plastid data set, five highly supported clades are resolved (clades 1-5, Fig. 4.1). Clade 1(100% JK/1 PP) encompasses only *Ch. vulvaria*. Clade 2 (100% JK/1 PP) encompasses the Australian taxa *Ch. nutans* and *Ch. desertorum* and *Ch. triandrum* from New Zealand. Clade 3 (100% JK/0.95 PP) encompasses *Ch. album*, *Ch. giganteum*, *Ch. gigantospermum*, *Ch. strictum*, *Ch. opulifolium*, *Ch. ficifolium*, *Ch. pamiricum* and *Ch. karoi*, all of them part of the *Ch. album*-group. Two internal subclades are well resolved within the clade 3: the "Album I" (100% JK/ 0.99 PP) enclosed the hexaploids *Ch. album* and *Ch. giganteum* and the tetraploids *Ch. strictum* and *Ch. gigantospermum* (Fig. 4.1) and the "Album II" (88% JK/0.75 PP) that shows the relationship between *Ch. opulifolium* (6x) and *Ch. ficifolium* (2x) with low support (67% JK/0.82 PP) and the relationship between *Ch. karoi* (4x) and *Ch. pamiricum* (?x) with high support (100% JK/1 PP, Fig. 4.1). Clade 4 (99% JK/0.8 PP)

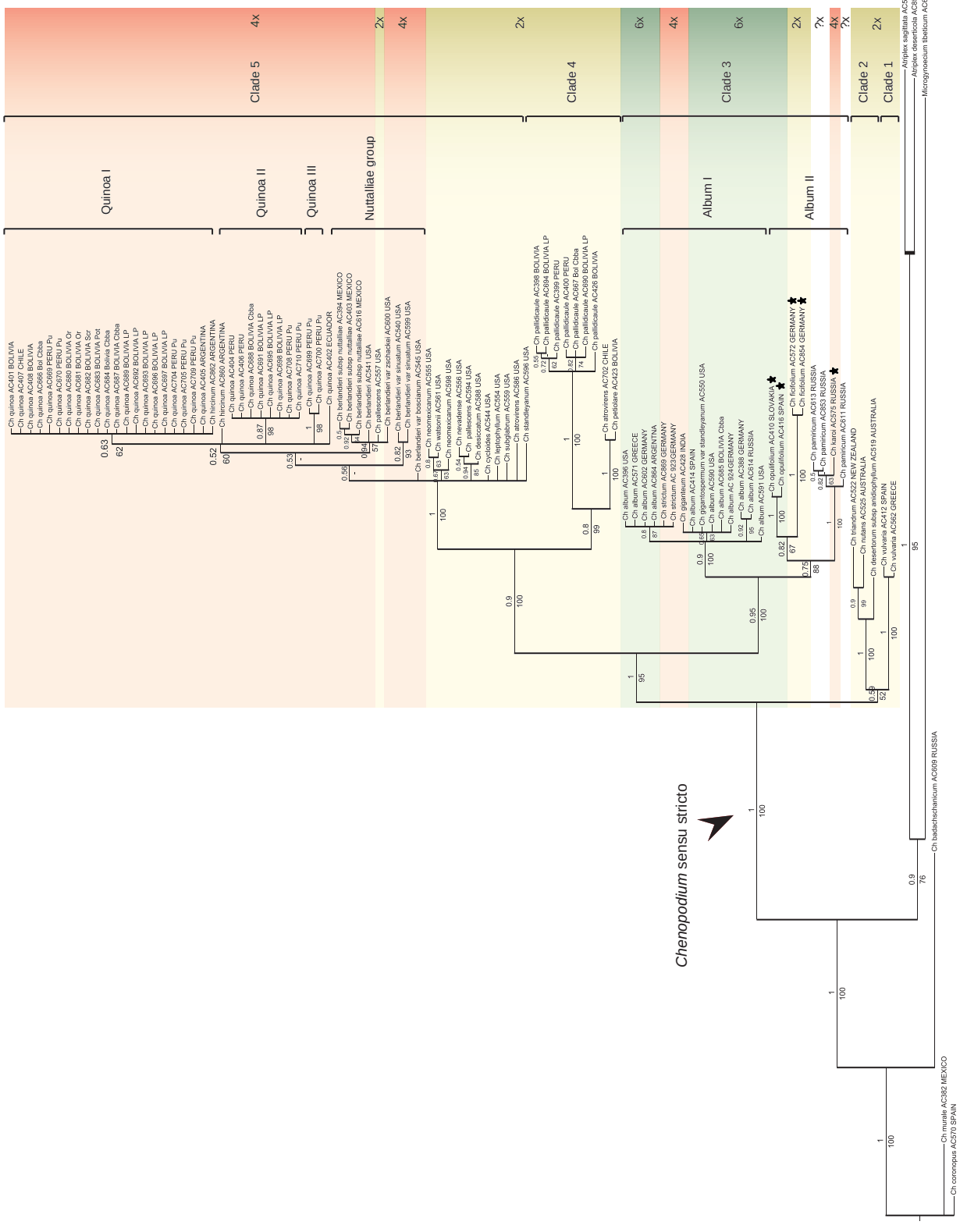


Fig. 4.1 – Bayesian phylogram based on the combined *trnL-F*, *matK/trnK*, *rpl16* intron data set. Bayesian posterior probabilities (PP) are given above and Jackknife (JK) values are below branches. The ploidy is represented by boxes in green for 6x, red for 4x and yellow for 2x. Taxa highlighted with stars are significantly incongruent.

encompasses the South American taxa *Ch. pallidicaule*, *Ch. atrovirens* and *Ch. petiolare*. The close relationship between *Ch. atrovirens* (2x) and *Ch. petiolare* (2x) is highly supported (100% JK/1 PP), and the next relative to these two taxa is *Ch. pallidicaule* (2x, 100% JK/1 PP see Fig 4.1). Clade 5 (100% JK/1 PP) is the biggest clade which encompasses the South American *Ch. quinoa* and *Ch. hircinum* (*Ch. quinoa*-group) and the North American *Ch. berlandieri* as well as *Ch. berlandieri* subsp. *nuttalliae*-group, *Ch. cycloides*, *Ch. pallescens*, *Ch. neomexicanum*, *Ch. watsonii*, *Ch. subglabrum*, *Ch. leptophyllum*, *Ch. standleyanum*, *Ch. nevadense*, *Ch. desiccatum* (see Appendix 4.A for the geographical origin of the included specimens). Some internal relationships without a defined position are the relationship of *Ch. neomexicanum* to *Ch. watsonii* (63% JK/ 0.67 PP) and the relationship of *Ch. nevadense* to *Ch. desiccatum* (85% JK/0.95 PP, Fig. 4.1). Although the low supports within the clade 5 the *Ch. quinoa*-group lineage which represents the Andean cultivar (60% JK/0.52 PP) is differentiated from the *Ch. berlandieri* subsp. *nuttalliae*-group which is the Mexican cultivar (57% JK/0.94 PP annotated as "Nuttalliae group" in Fig. 4.1). Additionally, three internal subclades are shown within the *Ch. quinoa*-group: Quinoa I (62% JK/0.63 PP), Quinoa II (98% JK/0.87 PP) and Quinoa III (96% JK/1 PP) (Fig 4.1).

The MP tree reconstructions for each partition are shown in Appendix 4.H. The MP tree of each partition shows in general the same topology as the combined data set (Fig. 4.1), but some internal nodes have lower support. In the case of *trnL-F*, internal nodes within the clade 5 are not resolved except for *Ch. quinoa* from Peru (samples AC699 and AC700 see Appendix 4.H, Fig. A). The support for the clades 2, 3 and 4 is low and clade 2 encompasses only *Ch. nutans* and *Ch. triandrum* (Appendix 4.H, Fig. A). For *matK/trnK* the described clade 5 is high supported (100% JK) and some internal nodes are resolved (Appendix 4.H, Fig. B). For example within *Ch. quinoa* the samples AC699 and AC700 are still related as in *trnL-F* (Appendix 4.H, Fig. B). The clade 4 as sister to clade 5 is higher supported compared to *trnL-F* alone (from 78% JK *trnL-F* to 100% JK *matK/trnK*). The clade 3 is not found based on the *matK/trnK* data set and the subclades form a polytomy to the rest of the clades. However, the clade 2 including *Ch. desertorum*, *Ch. nutans* and *Ch. triandrum* is highly supported (100% JK, Appendix 4G, Fig. B). Based on the *rpl16* intron, the clade 5 is still present, but within *Ch. quinoa* the samples AC699 and AC700 are in a polytomy with the rest of the *Ch. quinoa* samples (Appendix 4.H, Fig. C).

The clade 4 is recovered only with *Ch. pallidicaule* and form a polytomy with the clade 5, *Ch. atrovirens* and *Ch. petiolare*. The clade 3 is recovered with improved support (94% JK) compared to *trnL-F*; the clade 2 is still present as described (Appendix 4.H, Fig. C) and the clade 1 encompassing only *Ch. vulvaria* is resolved in each partition (Appendix 4.H).

4.3.2 The ITS data set

The ITS data set without the three HS comprised 580 characters, including 97 characters that were parsimony informative. The final matrix with coded indels comprised 598 characters of which 110 were parsimony informative. The statistics for each partition are provided in Appendix 4.F. Within *Chenopodium* s.str., ITS1 and ITS2 both showed a high parsimony informative percentage (23.9% and 23.4% respectively). The MP search resulted in 256 shortest trees (L=0.241, CI=0.751, RI=0.940 and RC=0.706).

The resulting strict consensus tree for MP was identical in topology with the Bayesian (BI) majority-rule consensus tree and contrary to the plastid data set the ITS depict a polytomy (see Fig. 4.2). The ITS tree showed a different tree topology because the nuclear phylogenetic signal is different compared to the plastid phylogenetic signal (Figs. 4.1 and 4.2). Therefore, for practical reasons in the ITS tree the annotation *pro part* (p.p.) in the clades refers to the described clades based on the plastid inferred tree which are not supported by the ITS data set (see above, Figs. 4.1 and 4.2).

The clade 1 included only *Ch. vulvaria* is high supported (100% JK/1 PP) was still present as a distinct lineage. The clade 3 p.p. (100% JK/1 PP) encompasses the Album I subclade with *Ch. album*, *Ch. giganteum*, *Ch. gigantospermum*, *Ch. strictum* related to *Ch. pamiricum* (Album II subclade p.p.). The clade 4 p.p. (100% JK/1 PP) encompasses only *Ch. pallidicaule*. The clade 5 p.p. (98% JK/1 PP) resolved the new relationship of *Ch. karoi* (4x) and *Ch. ficifolium* (2x) (Album II subclade p.p.) are part of the polytomy with *Ch. quinoa, Ch. hircinum* and *Ch. berlandieri*, being *Ch. opulifolium* (6x) (Album II p.p) the highly supported (100% JK/1 PP) next sister of the clade 5 p.p. (Fig. 4.2).The rest of clades and taxa are part of a polytomy (Fig. 4.2).

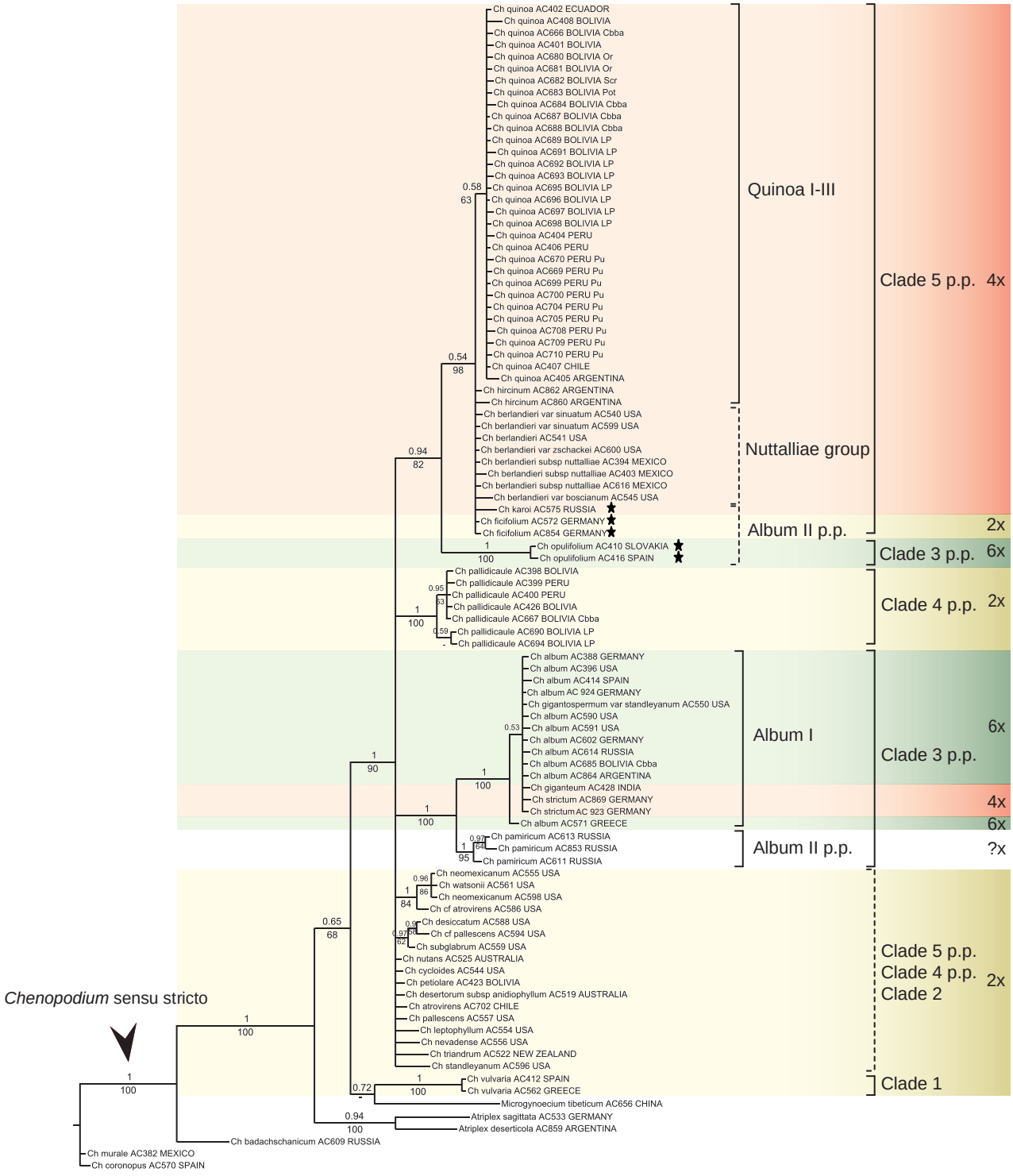


Fig. 4.2 – Bayesian phylogram based on the nuclear ITS sequence data set. Bayesian posterior probabilities (PP) are given above and Jackknife (JK) values are below branches. The ploidy is represented by boxes in green for 6x, red for 4x and yellow for 2x. Taxa highlighted with stars are significantly incongruent. Brackets with dotted lines and annotation p.p. shows the species from the clades described in Fig. 4.1. which changes their position.

4.3.3 The ILD and SH test

Plastid and nuclear data sets were found to be significantly incongruent (P< 0.001) based on the ILD test. The exclusion of those taxa resolved in different positions of plastid and nuclear topologies (*Ch. opulifolium*, *Ch. ficifolium*, *Ch. karoi* and *Microgynoecium*, see Figs. 4.1 and 4.2) does not change the results of the ILD test (P< 0.001).

The SH test conducted for the plastid constraint topologies: i) *Ch. karoi* nested within the clade of *Ch. quinoa*, *Ch. hircinum* and *Ch. berlandieri*; ii) *Ch. ficifolium* nested within the clade of *Ch. quinoa*, *Ch. hircinum* and *Ch. berlandieri*; iii) *Ch. opulifolium* sister to the clade of *Ch. quinoa*, *Ch. hircinum* and *Ch. berlandieri* resulted significantly incongruent (P<0.05) for each hypothesis. The same result (P<0.05) was found for the nuclear constrains topologies: i) *Ch. karoi* sister to *Ch. pamiricum*; ii) *Ch. ficifolium* nested within the clade of *Ch. pamiricum* and iii) *Ch. opulifolium* sister to *Ch. pamiricum*.

4.3.4 The network reconstruction

The split graph based on the combined plastid data set reveals three well defined splits corresponding to clade 5 (100% BS), clade 4 (96% BS) and clade 3 (89% BS) (Fig. 4.4). However, the split of clade 1 and 2 is not conclusive and shows to be fused with the outgroup (88% BS). This observation is supported by the phylogenetic reconstruction (compare Figs. 4.1 and 4.3). The split graph based on the nuclear data set shows two clear splits corresponding to the clade 3 p.p. (99% BS) and clade 5 p.p. (96% BS) described in the ITS phylogenetic reconstruction (compare Figs. 4.2 and 4.4). The rest of taxa present with low BS support a non-conclusive split position (Fig. 4.4).

The network reconstruction used was the galled networks implemented as RECOM2007 for computing reticulate or hybridization networks from trees (Huson and Klöpper, 2007). The reticulation or hybridization hypothesis is the “gall”, which is showed in the network tree as a cycle where the involved taxa are connected (see Fig 4.5 and Huson and Klöpper, 2007). The computed galled network based on the MP trees of the plastid and the nuclear data set suggested three galls of possible hybridizations scenarios represented by *Ch. karoi* (annotated with “HI” in Fig. 4.5). The second (“HII”) had two internal galls represented by *Ch. quinoa* (“HIIa”) and by *Ch. berlandieri* (HIIb) and the

third scenario is represented by *Ch. berlandieri* subsp. *nuttalliae* (“HIII”) and two galls of possible inconsistencies: one shows by *Ch. vulvaria* and the second shows by the related taxa *Ch. album* and *Ch. strictum* (Fig. 4.5).

4.4 Discussion

The present study addresses the phylogenetic relationships and the possible reticulation events within a more densely sampled clade of *Chenopodium* s.str. including multiple individuals from several species representing different geographical areas, especially from *Ch. quinoa*. Based on the combined data set of highly variable plastid markers (*trnL-F*, *matK/trnK* and *rpl16*) and the data set of the nuclear ITS, the monophyly of *Chenopodium* s.str. is highly supported as found in Chapter 2 (100% JK/1 PP for both plastid and nuclear regions, Figs. 4.1 and 4.2). Additionally, the internal five lineages are highly supported due to the combined plastid data set, whereas these lineages had low support based on *trnL-F* alone (see Figs. 4.1 and 2.1, Chapter 2). For ITS the recovered lineages improve their supports compared to the results of Chapter 2 (See Figs. 4.2 and 1.2, Chapter 2). In the phylogenetic tree reconstruction had been demonstrated that the sampling plays an important role (Lecointre et al, 1993; Pollock et al. 2002; Rydin and Källersjö, 2002; Zwickl and Hillis, 2002; Álvarez and Wendel, 2003; Towsend and Leuenberger, 2011). Rydin and Källersjö (2002) compared the effect of the tree inference based on 38 taxon matrices and the tree inference based on 80 taxon matrices and concluded that the incremented sampling improved the phylogeny inference for seed plants. The increased sampling within *Chenopodium* s.str. totalling 90 samples improved the tree inference for the genus in agreement with those previous studies.

In the ITS nuclear region the presence of polymorphic sites are valuable for the inference of reticulation events trough hybridization in plant speciation (Fuertes Aguilar, 2003; Löhne et al., 2008). However the concerted evolution of the ITS nuclear regions could also tend to homogenize the copies into one parental type and this polymorphic sites could be lost as well as the hybridization signal (Álvarez and Wendel, 2003). This effect was detected in *Chenopodium* s.str. in the previous phylogenetic inference based on ITS and it is still present in this study. Although this homogenization, evidence of speciation through hybridization within *Chenopodium* s.str. is detected due to the significant incongruence of the tree topologies from the plastid regions and from the nuclear region

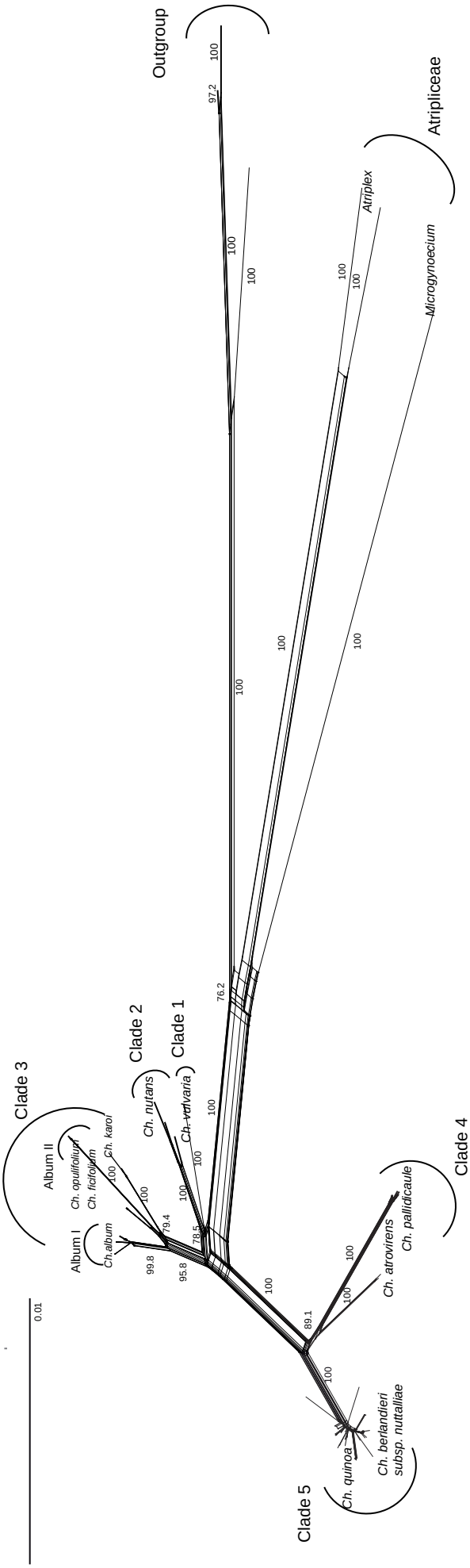


Fig. 4.3 – Split graph resulting from Neighbour Net analysis based on the combined *trnL-F*, *matK/trnK* and *rpL16* intron data set. Numbers are Bootstrap (BS) values. Clades names are according with Fig. 4.1.

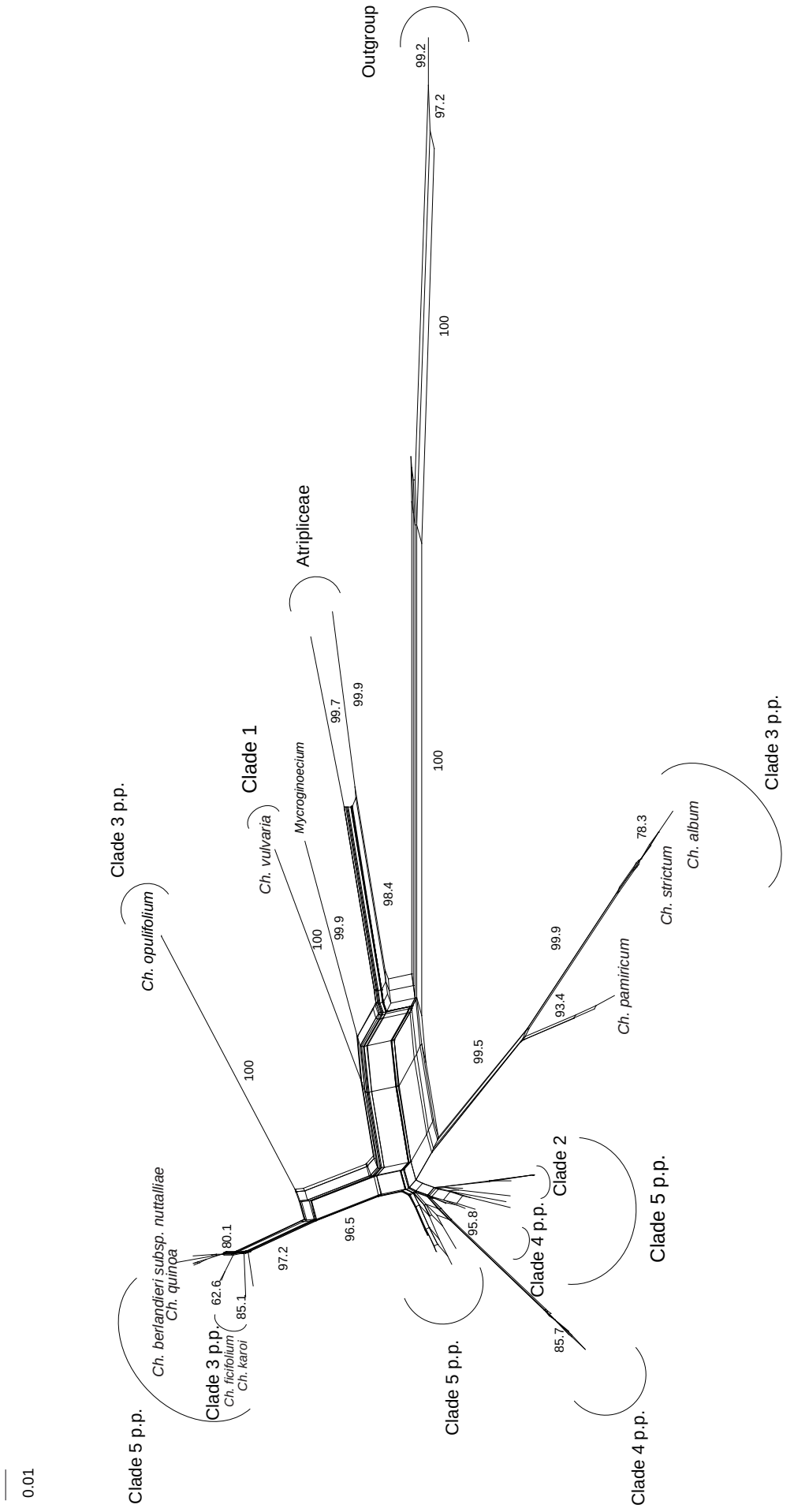


Fig. 4.4 – Split graph resulting from Neighbour Net analysis based on the nuclear ITS sequence data set. Numbers are Bootstrap (BS) values. Clades names are according with the Fig. 4.2.

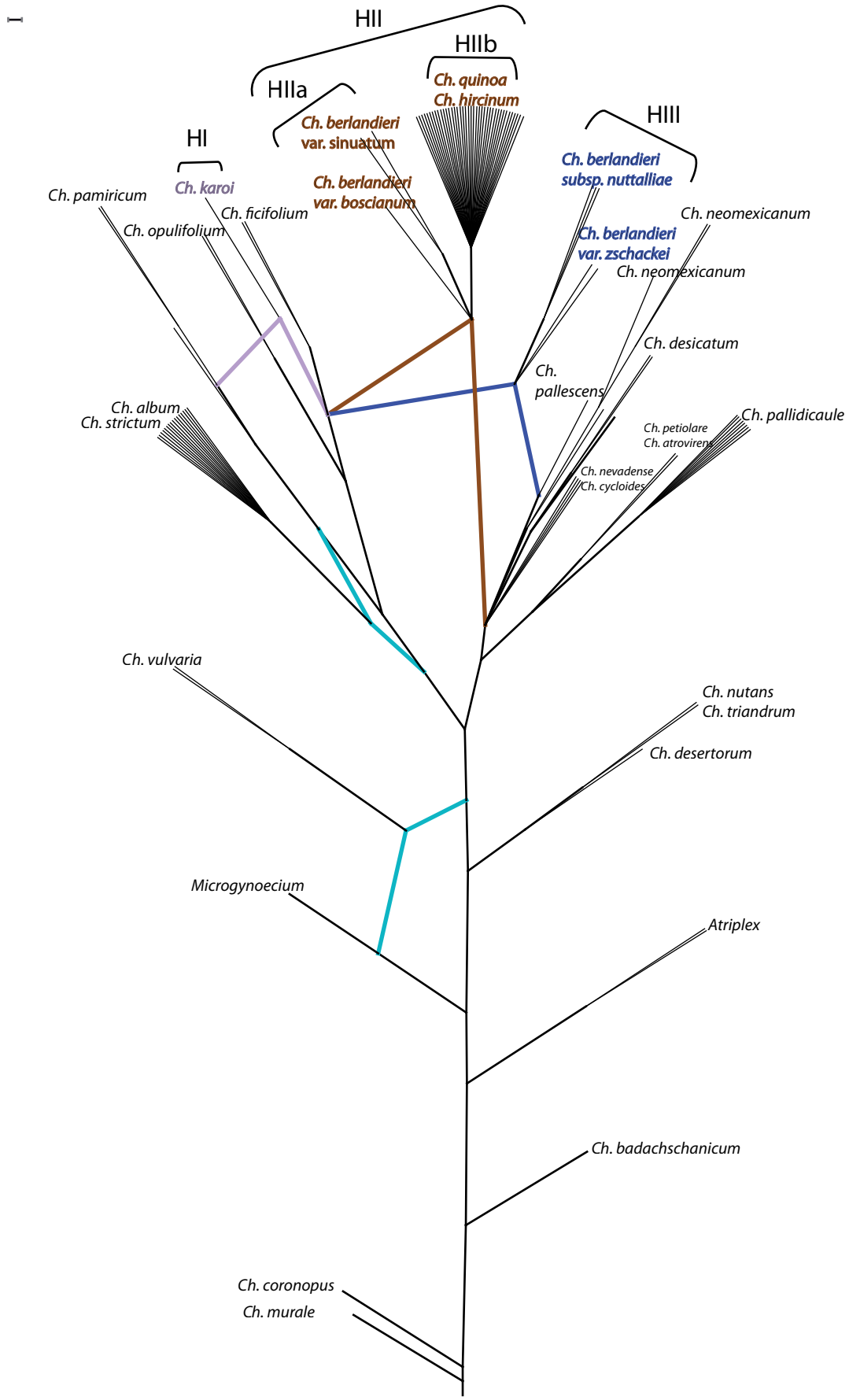


Fig. 4.6 – Hybridization Network based on concatenated MP trees of the plastid regions and the ITS region. Lila gall shows the hybridization hypothesis for *Ch. karoi* (4x); brown gall shows the hybridization hypothesis for i) *Ch. berlandieri* var. *boscianum* and *Ch. berlandieri* var. *sinuatum* both (4x) and ii) *Ch. quinoa* (4x); blue gall shows the hybridization hypothesis for *Ch. berlandieri* subsp. *nuttalliae* (4x). Sky-blue gall shows a false hybridization hypothesis.

(Figs. 4.1 and 4.2). These results are in line with previous studies on allopolyploids where the incongruent signal of the nuclear and plastid regions showed the hybridization evolution of the species involved (Sang and Zhong, 2000; Kim and Donoghue, 2008).

4.4.1 The improved phylogenetic signal of combined non-coding markers at species level

Non-coding chloroplast regions are broadly used for phylogenetic reconstruction at species level (Borsch and Quandt, 2009). These regions such as introns and spacers are highly variable and have higher mutational rates compared to coding regions (Borsch and Quandt, 2009). Combined data sets of non-coding plastid and nuclear markers appear to be the best strategy for reconstructing a supported phylogenetic tree at species level (Korotkova et al., 2011). The robustness of the phylogenetic tree can be evaluated based on the statistic "R" (Müller et al., 2006). The phylogenetic structure R resulting of this evaluation shows that the performance of one selected region or regions not only depend on its quantity. Müller et al. (2006) showed that the quality represented by the phylogenetic structure R of the evaluated region will result in a robust inferred tree. Hence, this author reported that the rapid evolving regions have a higher phylogenetic structure than the slow evolving regions. Between the rapid evolving regions analysed by Müller et al. (2006) the noncoding intron *trnT-F* region showed better phylogenetic structure than the protein *matK*. Korotkova et al. (2011) evaluated the phylogenetic structure R of seven noncoding regions including *matK/trnK* and *rpl16* intron. They reported that the *rpl16* and *trnK* introns yielded the best phylogenetic structure. Based on this evidence, the present phylogenetic analyses of *Chenopodium* s.str. combined the *matK/trnK*, *rpl16* intron plastid regions with the earlier generated *trnL-F* data set (Chapter 2). In agreement with the reported quality of the choose regions the inferred phylogenetic tree for *Chenopodium* s.str. shows for the internal clades an improved resolution and supports, compared with the inferred tree of single partitions.

In order to test the resolution of each region a tree inference for each partition was done. The best resolved tree for *Chenopodium* s.str. is from the single partition of *rpl16* intron (Appendix 4.G) on line with the results reported by Korotkova et al. (2011). However some statistical supports based on *rpl16* intron and on the other two partitions are low and the resolution of the relationships is poor. For instance, based on the *trnL-F*

partition or the *matK/trnK* partition the clade 2 is supported encompassing *Ch. pallidicaule*, *Ch. atrovirens* and *Ch. petiolare*, but based on the *rpl16* intron partition the clade *Ch. pallidicaule*, *Ch. atrovirens* and *Ch. petiolare* is not supported (see section 4.3.1 for more examples and Appendix 4.G). The combined data set notably improve these two factors (see Appendix 4.G and Fig. 4.1). This result is in agreement with the suggestion of Müller et al. (2006) in the sense that combine regions with high phylogenetic structure allow infer a robust phylogenetic tree at species level.

The ITS nuclear region encloses a high percentage of informative characters (23% for each ITS1 and ITS2, Appendix 4.F) within *Chenopodium* s.str., comparable to results reported in other studies at species level (Fuertes Aguilar and Nieto Feliner, 2003; Löhne et al., 2008). ITS has a different phylogenetic signal and different patterns of evolution compared to chloroplast regions. The length variability in ITS mostly results from deletions or insertions of single nucleotides observed in the data set generated for *Chenopodium* s.str. The data set of *Chenopodium* s.str. still showing a high homogenization degree of the ITS copies as reported in Chapter 2 due to the concerted evolution of the ITS region reported by Álvarez and Wendel (2003). The sampling for *Chenopodium* s.str. in this study is larger, in total 90 samples are analysed where 53 are from the data set of Chapter 2. The new included samples are 31 samples of *Ch. quinoa*, two samples of *Ch. hircinum*, two samples of *Ch. ficifolium*, three samples of *Ch. pamiricum* and two samples of *Ch. strictum*. As explained above this incremented sampling resolves an improved inferred tree (Fig. 4.2).

4.4.2 Phylogenetic relationships of *Chenopodium* s.str.

4.4.2.1 The Ch. vulvaria *lineage*

The clade 1 with the only member *Ch. vulvaria* is highly supported by plastid and nuclear regions as showed in this study and Chapter 2. The characteristic fish-like odour and the shortly lobed perianth which encloses the fruit of *Ch. vulvaria* make it easily differentiable from the other *Chenopodium* (Wilson, 1983). The fish-like odour was also described for *Ch. detestans* (not sampled) and for *Ch. desertorum* subsp. *virosum* (not sampled) as a possible character to suggest the close relationships of this taxa (Wilson, 1983). Based on the combined data set the relationships between *Ch. desertorum* and allies

(clade2) and *Ch. vulvaria* (clade 1) is close but low supported (52% JK/0.52 PP, Fig. 4.1), and this relationship is not found based on ITS (Fig. 4.2). The fish-like odour characteristic of *Ch. vulvaria* is due to the high concentrations of trimethylamine in the leaves of this plant (Cromwell, 1950). The presence of this secondary compound especially in leaves of *Ch. vulvaria* could be a good candidate for the synapomorphy of this clade, but in order to test this hypothesis is necessary to include other taxa that share this character.

4.4.2.2 The Australian lineage

The clade 2 encompasses the endemic Australian species *Ch. nutans, Ch. triandrum* and *Ch. desertorum* and was resolved with low support based on *trnL-F* (Chapter 2). The combined analysis with further chloroplast markers provides now high support for the inclusion of *Einadia nutans* and *Rhagodia triandra* into *Chenopodium* s.str. (Figs. 4.1 and 4.2). The highly supported (99% JK/0.9 PP) close relationship between *Ch. triandrum* and *Ch. nutans* is improved based on the combined plastid data set and *Ch. desertorum* is placed as sister to these two taxa (Fig. 4.1). Based on ITS, the clade *Ch. nutans, Ch. triandrum* and *Ch. desertorum* are not supported and shares a non-resolved position with the rest of the diploid species (Fig. 4.2). In his treatment of the genera *Einadia* and *Rhagodia* for the Flora of Australia, Wilson (1983) mentioned that these genera are endemic for Australia. He differentiated these two genera from *Chenopodium* based on their particular morphological features. *Einadia* has only 2 stamens and a dry or succulent pericarp (Fig. 4.6) and *Rhagodia* has 5 stamens and a fleshy pericarp in *Rhagodia,* while *Chenopodium* has 1 to 5 stamens and a membranous or rarely succulent pericarp. However, in the same treatment it was mentioned that other authors confused *Ch. desertorum* with *Rhagodia* and sometimes *Ch. desertorum* was erroneously placed in *Rhagodia* because its shrubby habit and its succulent fruit. Additionally to this observation, Wilson (1983) also mentioned that it was difficult to distinguish *Einadia* from *Chenopodium* and pointed out that *Einadia* was close to *Ch. desertorum*. Finally, the delimitation between *Einadia* and *Rhagodia* was not clear which was reflected in the continuous changing of names: for example *Einadia nutans* was originally described as *Rhagodia nutans* and is synonymous to *R. linifolia* and *Ch. australasicum* (Wilson, 1983). Results of this work supports the close relationship suggested by Wilson (1983) based on morphological characters showing a possible lineage of Australian and New Zealand taxa.

However, due to the high morphological variability within *Chenopodium* s.str., it is necessary to increment the sampling of the Australian and New Zealand taxa (e.g. *Ch. baccatum*, *Ch.trigonon*) in order to test the existence of an exclusive lineage.

4.4.2.3 The Ch. album*-group lineage*

The clade 3 encompasses the taxa of the *Ch. album*-group suggested based on morphology (Clemants and Mosyakin, 2003). While *Ch. berlandieri* was proposed as part of the *Ch. album*-group this study shows that it should be excluded and *Ch. gigantospermum* must be included. *Ch. album*-group based on morphological characters not only shows a great morphological variability, this group also encompasses taxa with different ploidy (see Appendix 4.A and Appendix 2.C in Chapter 2). Based on the combined plastid regions the *Ch. album* lineage depict two subclades: Album I and Album II. The subclade Album I encloses the hexaploids *Ch. album* and *Ch. gigantospermum* and the tetraploids *Ch. strictum* and *Ch. giganteum* (Fig.4.1 and 4.6). These relationships support that *Ch. strictum* is close related to the *Ch. album* as suggested by Dvořák and Grüll in 1983. Whereas the diploid *Ch. ficifolium* was suggested to be also related to the hexaploids of the *Ch. album*-group (Rahiminejad and Gronall, 2004), the phylogenetic reconstruction supports that *Ch. ficifolium* is close related to the hexaploid *Ch. oupulifolium* (within the subclade Album II, Fig. 4.1) and not directly related to the hexaploid *Ch. album*. Within the subclade Album II *Ch. ficifolium* (2x) and *Ch. opulifolium* (6x) are the sister clade of the clade of *Ch. pamiricum* and *Ch. karoi* (4x). Based on ITS *Ch. karoi* (4x) and *Ch. ficifolium* (2x) (Album II subclade p.p.) are part of a polytomy with *Ch. quinoa*, *Ch. hircinum* and *Ch. berlandieri*, being *Ch. opulifolium* (6x) (Album II p.p) the next sister of all these taxa (Fig. 4.2). The supported relationships of *Ch. karoi* (4x), *Ch. ficifolium* (2x) and *Ch. opulifolium* (6X) are significantly incongruent based on incongruence tests ILD and SH. Possible causes of this incongruence will be discussed later.

4.4.2.4 The South American lineage

The clade 4 reported as the South American lineage in Chapter 2 is highly supported in this study based on the combined plastid signal. The close relationship

between *Ch. atrovirens* (2x) and *Ch. petiolare* (2x) is highly supported and the next relative to these two taxa is *Ch. pallidicaule* (2x) (Fig. 4.1). Moreover, the plastid signal supports (100% JK/1 PP, Fig. 4.1) the sister relationship of the South American lineage (clade 4) to the clade 5. The nuclear signal resolves a different tree topology, where all diploids are in a hard polytomy (Fig. 4.2, Chapter 2).

Due to the high morphological variability within *Chenopodium* s.str. is difficult to define morphological characters to characterize the *Ch. pallidicaule, Ch. petiolare* and *Ch. atrovirens* assemblage. Some common but not exclusive morphological characters are the small size (< 50 cm) and branched habit of the plants with pronounced three-nerved leaves (Bonpland et al., 1817; Rydberg, 1900; Aellen, 1929).

Ch. pallidicaule is a crop exclusively from the Andean region and *Ch. petiolare* was originally described from Ecuador (Fig. 4.6, Bonpland et al., 1817). *Ch. atrovirens* was originally described based on a specimen collected in Montana U.S. by Rydberg, in 1900. However, the last version of Flora of North America states that they have seen no reliable North American records of *Ch. atrovirens*, a species considered typical from southern South America (Clemants and Mosyakin, 2003). At the same time, Fosberg (1941) mentioned that the morphological characters of the North American *Ch. atrovirens* are non-conclusive for its differentiation and suggested its transference under *Chenopodium fremontii* (North American diploid) as var. *atrovirens*,butthis taxonomic change was not accepted in the last treatment for the Flora of North America (Clemants and Mosyakin, 2003). Based on the phylogenetic reconstruction the South American *Ch. atrovirens* is not related to the North American *Ch. atrovirens* (Fig. 4.1). This result shows either that two different species can be included under the ample morphological concept of *Ch. atrovirens* or that *Ch. atrovirens* is exclusive of South America. In this latter case *Ch. atrovirens* from North America need to be considered as *Ch. fremontii* var, *atrovirens* as suggested by Fosberg in 1941. In order to test if *Ch. atrovirens* belons to this South American lineage is necessary to analyse an incremented sampling.

4.4.2.5 The allotetraploids and allies lineage

The clade 5 encompasses the tetraploids *Ch. quinoa*, *Ch. hircinum* (*Ch. quinoa*-group), *Ch. berlandieri* subsp. *nuttalliae Ch. berlandieri*, *Ch. berlandieri* var. *boscianum*,

Ch. berlandieri var. *zschackei* (*Ch. berlandieri* subsp. *nuttalliae*-group) together with the North American diploids *Ch. neomexicanum, Ch. desiccatum , Ch. nevadense, Ch. watsonii, Ch. pallescens, Ch. cycloides, Ch. leptophylum, Ch. subglabrum, Ch.* cf. *atrovirens* and *Ch. standleyanum* based on the combined plastid regions (Fig. 4.1). Although the deep nodes within clade 5 are low supported, this study recovers some lineages not yet found with information from the *trnL-F* region alone (Chapter 2). Based on the combined plastid regions, the *Ch. quinoa* lineage which represents the Andean cultivar, is distinguished from the *Ch. berlandieri* subsp. *nuttalliae* lineage which represents the Mexican cultivar. Additionally, three internal subclades are shown within the *Ch. quinoa*-group (Quinoa I, II and III, Fig 4.1). These three subclades enclose among others the *Ch. quinoa* samples from La Paz, Bolivia (BOLIVIA LP in Figs. 4.1 and 4.2) and Puno or Cuzco Peru (PERU Pu or Cu in Figs. 4.1 and 4.2) which were collected from the Titicaca lake region. The cultivars around the Titicaca lake usually have a high morphological variability (Rojas, 2001). This variability seems to be reflected in this study because the split of the samples from Titicaca lake (LP and Pu, Fig. 4.1) in the three subclades Quinoa I-III. The Quinoa I encompasses the taxa from Titicaca lake (LP and Pu), the taxa from the interandine valleys (Cochabamba=Cbba and Sucre=Scr Bolivia), the taxa from central and south Altiplano regions (Oruro=Or and Potosi=Pot), the Chilean *Ch. quinoa* ("quingua", AC407) and *Ch. hircinum* from Argentina (wild type with black seeds, AC862, Fig. 4.1). Moreover, Quinoa I encloses intermediate individuals as the so-called "Pepino" variety with two colours in the whole plant (Fig. 4.6). The Quinoa II subclade encloses almost only samples from the Titicaca lake except for the sample AC688 which is from the interandine valley of Cochabamba. The *Ch. quinoa* AC688 with black seeds was founded as weed in the middle of a cultivated area in an interandine valley. *Ch. quinoa* AC691 is another sample with black seeds and may represent the weedy type of *Ch. quinoa* collected in an abandoned cultivate area of the Titicaca lake region. The black seeds and weedy type of *Ch. quinoa* are two morphological characters for the "wild type" and putative parental of the crop. However, the presence of black seeds also shows the natural capacity of the crop to returns to the weedy type character phenomena called by the agronomists "crop escape", because they are dominant characters in the studied trait inheritance (Gandarillas, 1968). The rest of taxa within Quinoa II subclade have white seeds. The close relationship between crop (white seeds) and its sympatric "wild type" (black seeds) suggests that these two states are constantly changing within the *Ch. quinoa*

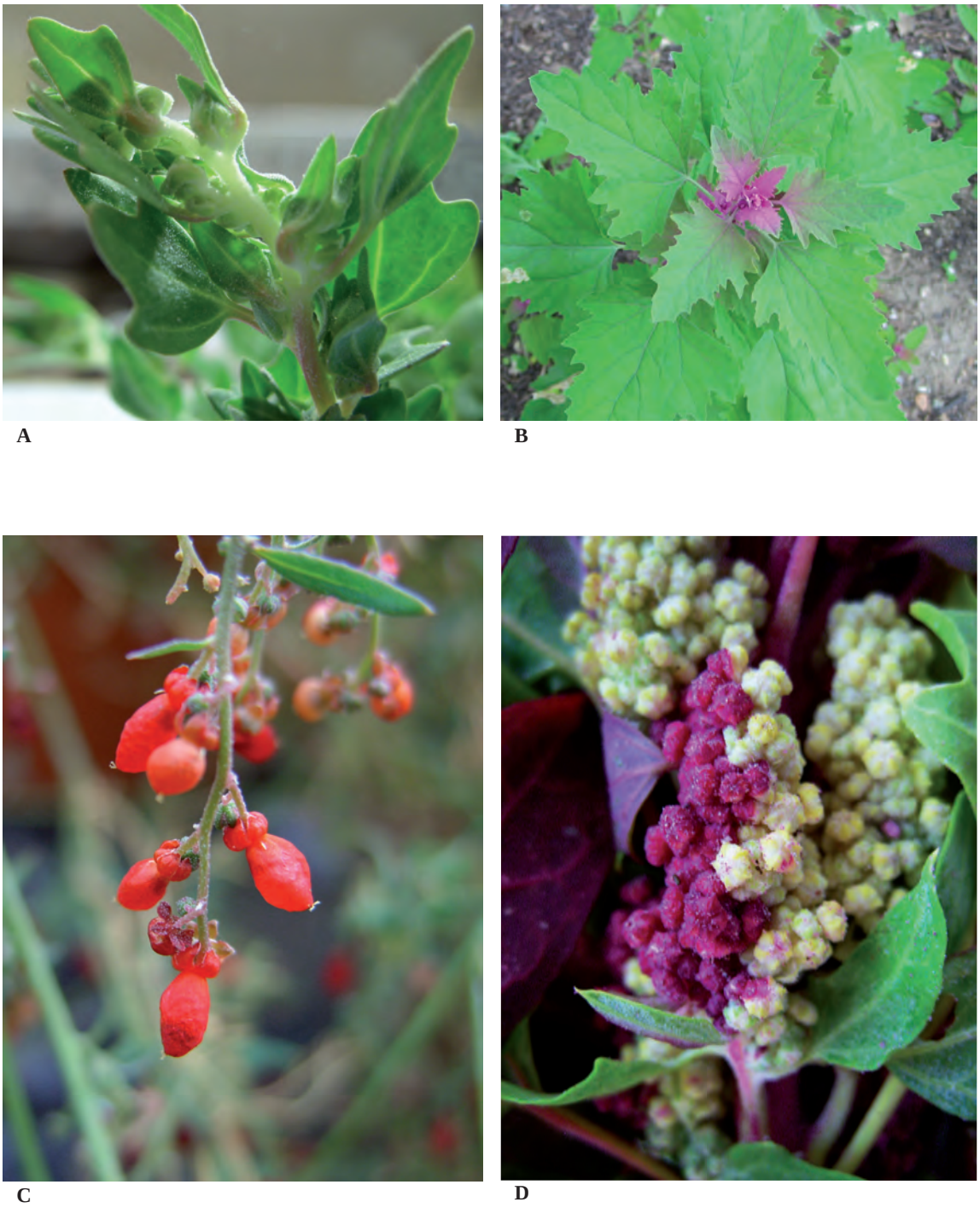


Fig. 4.6 – Species of the *Chenopodium* s.str. lineage. A) *Ch. pallidicaule* (Clade 4), B) *Ch. giganteum* (Clade 3), C) *Ch. nutans* (= *Einadia nutans*, Clade 2) and D) *Ch. quinoa* variety " Pepino" (Clade 5).

population. Finally, the Quinoa III subclade encloses two Peruvian samples (AC699 and AC700) collected from crops on the shore of the lake (See Figs. 4.1). Both samples in this subclade have white seeds and a crop form similar to the rest of taxa with white seeds, however this subclade is well differentiated from the rest based on plastid regions (Fig. 2.1 Chapter 2, Fig. 4.2). To understand the genetic variability within the *Ch. quinoa* varieties, it is necessary to carry out population analyses.

The *Ch. berlandieri* subsp. *nuttalliae* Mexican group is differentiated from the *Ch. quinoa*-group based on the combined plastid data set. Within this clade the close relationship of *Ch. berlandieri* subsp. *nuttalliae* and *Ch. berlandieri* var. *zschackei* confirm the previous hypothesis about their relationships based on morphology (Wilson and Heiser, 1979). Based on ITS, this clades are not supported and the inferred tree depict a polytomy (Fig. 4.2).

Additionally, the plastid data in this study suggest the inclusion of *Ch. pallescens* (2x) within the *Ch. berlandieri* subsp. *nuttalliae*-group which has not been suggested in prior studies (Fig. 4.1). Finally, based on the combined plastid regions, some relationships between the North American diploid taxa are shown (Fig. 4.1). For instance, the relationships of *Ch. neomexicanum* to *Ch. watsonii* and the relationship of *Ch. nevadense* to *Ch. desiccatum* (Fig. 4.1). The relationships of the diploid taxa within the clade 5 based on the plastid signal are not supported by the nuclear signal. In the tree topology of the nuclear regions all diploids (except *Ch. ficifolium*) taxa are in a polytomy (Fig. 4.2). However, based on the nuclear region the clade 5 p.p. encloses the tetraploids *Ch. quinoa*, *Ch. berlandieri* (and its subspecies and varieties), *Ch. hircinum*, *Ch. karoi*, and the diploid *Ch. ficifolium* (98% JK/0.54 PP, Fig. 4.2). Additionally all the *Ch. quinoa* samples are resolved in a low supported subclade (63% JK/0.58 PP, Fig. 4.2).

4.4.3 The concerted evolution of ITS nuclear rDNA region in allotetraploids of *Chenopodium* s.str.

The inconsistent topology (except for the incongruent position of *Ch. karoi*, *Ch. ficifolium* and *Ch.opulifolium*) and the polytomy shown by the ITS region obscure the organism relationships within *Chenopodium* s.str. (Fig. 4.2). The ITS region in *Chenopodium* s.str. has a high degree of concerted evolution especially in its

allopolyploids members. Previous studies of concerted evolution in *Nicotinana* allotetraploids suggested that this process is due first to a rDNA homogenization which is accelerated either by inbreeding or multiple origins of the allotetraploid *Nicotiana tabacum* (Kovarik et al., 2004). The concerted evolution due to intralocus or interlocus gene conversion was also reported for the allopolyploids of *Gossypium* (Wendel et al., 1995) and *Trapogon* (Kovarik et al., 2005). *Ch. quinoa* and *Ch. berlandieri* subsp. *nuttalliae* both allotetraploids are under selection for human consume, show any additive polymorphic pattern. The homogenizations of the ITS copies in this two allotetraploids with a subsequent inbreeding due to human selection could be explain the concerted evolution detected in this study. In order to test this hypothesis will be necessary to detect the origin of the ITS nuclear ribosomal DNA gen families and its genomic location as was done for *Nicotiana* by Kovarik et al. (2004).

On the other hand, the use of the low-copy regions was suggested in order to reconstruct the reticulate evolution in plants (Álvarez and Wendel, 2003; Archambault and Bruneau, 2004; Schlüter, et al., 2007; Kim et al., 2008). The LEAFY orthologs copies are present in angiosperms in a single copy for diploids and two or more copies for polyploids, thus the non-coding sequences of LEAFY introns could be used at low taxonomic levels (Archambault and Bruneau, 2004). The use of low-copy nuclear regions can provide more information when these copies are linked to genes from the maternal and paternal lineages (Kim et al., 2008). In the study of the allopolyploid speciation in *Persicacia* (Polygonaceae) based on the phylogenetic reconstruction of LEAFY showed that the diploid species *P. lapathifolia* has been involved in at least six cases of allopolyploid speciation (Kim et al., 2008). Veit et al. (2004) isolated a putative LEAFY orthologous from *Ch. rubrum*. Based on this sequences primers were designed and preliminary tests of this putative low copy *LFY* region in *Chenopodium* s.l. was carried out for the present study (results not shown). The putative *LFY* first intron region showed two copies for the diploids *Ch. cycloides* and *Ch. rubrum* and three copies for the allotetraploids *Ch. quinoa* and *Ch. berlandieri*. The high variability of some of these copies makes their alignment very difficult and therefore this marker was not further used for *Chenopodium* s.l. However, in order to solve the relationships and evolution patterns within *Chenopodium* s.str. these putative *LFY* region is still a good candidate to be tested.

4.4.4 Hybridization and allopolyploidization as possible explanation for incongruent topologies

The similar topologies for each plastid and nuclear regions obtained from both MP and Bayesian reconstructions and the dense taxon sampling (Figs. 4.1 and 4.2) allow to reject non-biological causes such as long branch attraction or insufficient taxon sampling as causes of the observed incongruence (Chapter 2; Van der Niet and Linder, 2008). The combined plastid regions showed a different tree topology than the nuclear region (Figs. 4.1 and 4.2). This pattern was already observed in Chapter 2, but this study recovers an improved support due to more data from the combined plastid regions. The hard incongruence topology is characterized by the well to high supports of the relationships and by the statistic significance of the ILD and SH tests (Van der Niet and Linder, 2008; Chapter 2). In this study the alternative topologies of *Ch. opulifolium*, *Ch. ficifolium* and *Ch. karoi* represent a significant incongruence (Figs. 4.1 and 4.2). Incomplete lineage sorting, hybridization, horizontal-transfer are biological causes for the incongruence topology (Wendel and Doyle, 1998; Van der Niet and Linder, 2008; Willyard et al., 2009). All of these biological causes are difficult to differentiate from each other, but some evidence could hint to a prevailing importance of one of them as is the case in *Chenopodium* s.str. In this study the ITS region did not present any of additive polymorphic sites because the high level of concerted evolution for the allopolyploids members of *Chenopodium* s.str. giving evidence of the hybridization process after homogenization (see above and also for *Chenopodium* s.l. in Chapter 2).

Extensive studies based on morphology, allozymes, flavonoids and chromosome counts reported evidence for a hybrid origin of the polyploids within *Chenopodium* s.str. (Wilson and Heiser, 1979, Wilson, 1983; Wilson 1988a; Wilson 1988b; Clemants and Mosyakin, 2003; Rahiminejad and Gornall, 2004). Based on seed sizes and chromosome numbers, Dvořák et al. (1983) suggested that the diploid *Ch. suecium* and the tetraploid *Ch. strictum* could be the parents of the allohexaploid *Ch. album*. Rahiminejad and Gornall (2004), based on flavonoid profiles and chromosome numbers, suggested that the hexaploids *Ch. album*, *Ch. opulifolium* and the tetraploid *Ch. giganteum* could be hybrids of the diploids *Ch. suecium* (not sampled) and *Ch. ficifolium*. Additionally, it was reported that *Ch. ficifolium* from Europe occasionally hybridizes with other species, for example with *Ch. album* and *Ch. suecium* (Clemants and Mosyakin, 2003). The inconsistent

position of *Ch. opulifolium* (6x) in this study seems to be in agreement with the hypothesis of its hybrid origin (Figs. 4.1 and 4.2). In this sense the possible event of hybridization could involve the close related diploid *Ch. ficifolium* based on the plastid tree (Fig. 4.1) and any of the tetraploid taxa within the clade 5 p.p. based on the nuclear tree (Fig. 4.2).

The allopolyploid origin of the crops *Ch. quinoa* and *Ch. berlandieri* subsp. *nuttalliae* has been also well studied using methods such as morphological comparison, allozyme variation and flavonoid profiles (Wilson, 1976; Wilson and Heiser, 1979; Wilson, 1981; Wilson, 1990; Wilson 1993; Ward, 2000; Rahiminejad and Gornall, 2004; Bhargava et al., 2006). However, most of these studies only sampled tetraploid species from limited geographic regions (e.g South America; Wilson, 1981), or simply discussed relationships within populations of *Ch. quinoa* and *Ch. berlandieri* subsp. *nuttalliae* (Wilson and Heiser, 1979; Wilson, 1990; Wilson 1993). While the close relationship of *Ch. quinoa*-group to *Ch. berlandieri* subsp. *nuttalliae*-group is well supported (Chapter 2 and this chapter; Wilson and Heiser, 1979; Wilson, 1990; Wilson 1993), this study shows that these groups are also close related to the North American diploids based on the plastid signal (Fig 4.1). On the other hand, the ITS nuclear signal, shows the incongruent topology of *Ch. karoi* (4x) and *Ch. ficifolium* (2x) both taxa related to the *Ch. quinoa* and *Ch. berlandieri* subsp. *nuttalliae* groups (Fig 4.2). The polytomy showed by both plastid and nuclear regions for these relationships could be due to the complex pattern of speciation through hybridization of these groups within *Chenopodium* s.str. Therefore, the phylogenetic reconstruction could fail for the analysis of the hybridization patterns (see next section).

4.4.5 Phylogenetic network reconstruction

The hybrid speciation in plants is a common phenomenon generating polyploid complexes especially within species-rich genera (Soltis and Soltis 1999; Albach and Chase, 2004; Mansion, 2005; Blöch et al., 2009). The traditional phylogenetic reconstruction could fail in the reconstruction of the hybridization patterns because the tree-like model does not fit with a reticulate pattern, resulting for example in a polytomy (Linder and Rieseberg 2004; Huson, 2006; Chapter 2 and this study). Currently, in order to infer reticulate patterns in plants and to explore the putative non-biological causes of

incongruence in the data sets, the network reconstruction methods have recently been developed (Linder and Rieseberg, 2004; Morrison, 2010).

4.4.5.1 The Split Neighbour Net reconstruction

In this study, the Split Neighbour Net reconstructed for the combined plastid data set and for the ITS nuclear data set showed in the splits the same clades as found by the tree reconstruction methods (Figs. 4.3 and 4.4). Some of the inconsistently resolved nodes (e.g. *Ch. vulvaria, Microgynoecium*) showed by ITS (Fig. 4.2) resulted in an inconclusive split without bootstrap support (Figs.4.3 and 4.4). The split neighbour based on the plastid data set showed in the splits the same five clades within *Chenopodium* s.str. as found with the phylogenetic reconstruction (Fig. 4.3). Similar to the ITS split neighbour net, low supported relationships like between clade 1 (*Ch. vulvaria*) and clade 2 (*Ch. desertorum*, *Ch. nutans* and *Ch. triandrum*) are also reflected in non-conclusive splits (see Figs. 4.2 and 4.4).

The congruent results showed by the network reconstructions and the phylogenetic reconstructions in this study suggest that the different signal of the plastid and nuclear regions is due to biological causes. Additionally, evidence of putative hybrid origin of the polyploid groups within *Chenopodium* s.str. seems to be the major cause of the significant incongruence and the polytomies showed in this study.

4.4.5.2 The hybridization network reconstruction in Chenopodium *s.str.*

Possible hybridization hypothesis for the allotetraploids of Chenopodium *s.str.* – This study applied the hybridization network reconstruction within *Chenopodium*. s.str. in order to address some plausible hybridization scenarios. This methodology only shows biological hypothesis of hybridization which could explain the incongruent or polytomic patterns showed by the phylogenetic reconstruction (Huson and Klöpper, 2007; Morrison, 2010; Huson com. pers.). The hybridization network reconstruction from the MP trees shows three possible hybridization hypotheses for the tetraploid taxa within *Chenopodium* s.str. (Fig. 4.5). The geographic distribution of the *Chenopodium* s.str. from North America (Clemants and Mosyakin, 2003) could be useful to understand these events as follows.

HI- The hybridization hypothesis for the tetraploid Ch. karoi – The first suggested hybridization hypothesis involves *Ch. ficifolium* (2x) and *Ch. pamiricum* (?x) as possible parentals for the tetraploid *Ch. karoi* (HI in Fig. 4.5). There are no chromosome counts for *Ch. pamiricum*, so it could be either diploid or tetraploid. The hypothesis of a hybrid origin for *Ch. karoi* is likely due to its distribution. *Ch. ficifolium* and *Ch. pamiricum* are elements reported for Europe and Asia where *Ch. karoi* shares its distribution with *Ch. ficifolium* in the Siberian region and *Ch. pamiricum* shares its distribution with *Ch. karoi* in Central Asia (Freitag et al., 2001; Uotila, 2001).

HII- The hybridization hypothesis for the tetraploids Ch. berlandieri *from North America and for* Ch. quinoa *from the Andean regions of South America* – The second hybridization hypothesis involves two events: HIIa) the hybridization event of *Ch. berlandieri* var. *bosianum* (4x) and *Ch. berlandieri* (4x) from North America (Fig. 4.5), and HIIb) the hybridization event of *Ch. quinoa* (4x) and *Ch. hircinum* (4x) from South America (Fig. 4.5). In this two hybridization events one putative parental is *Ch. ficifolium* (2x) and the second putative parental should be one of the diploids of North America: *Ch. neomexicanum*, *Ch. nevadense*, *Ch. watsonii*, *Ch. cycloides*, *Ch. subglabrum* or *Ch. pallescens*. The first event (HIIa)-which involves the North American tetraploids *Ch. berlandieri* var. *bosianum* and *Ch. berlandieri* var. *sinuatum* could be happened in three geographic regions (Fig. 4.5). One possible region for *Ch. berlandieri* var. *sinuatum* (4x) is in Oregon (eastern North America). *Ch. ficifolium* (2x) has been reported in this region where also occur the *Ch. atrovirens* (2x), *Ch. leptophyllum* (2x) and *Ch. nevadense* (2x). The second possible region for the hybridization event of *Ch. berlandieri* var. *boscianum* (4x) is in Missouri, Florida and Pennsylvania. In this region occur *Ch. ficifolium* (2x), *Ch. desiccatum* (2x) and *Ch. standleyanum* (2x). The third possible region for the hybridization event of *Ch. berlandieri* var. *boscianum* is in Louisiana, Florida, Alabama, Mississippi, Texas and Pennsylvania (south-eastern). In this region also occur *Ch. ficifolium* (2x), *Ch. cycloides* (2x) and *Ch. neomexicanum* (2x). The second event (HIIb) of the South American tetraploids *Ch. quinoa* and *Ch. hircinum* (*Ch. quinoa*-group) involves the European diploid *Ch. ficifolium* and the North American diploids as *Ch. neomexicanum*, *Ch. desiccatum* and *Ch. cycloides* (Fig. 4.5). This putative event supports the suggested North American origin of *Ch. quinoa*-group. This hypothesis states that the diversification and independent domestication of the *Ch. quinoa*-group in the Andean region was

happened after the hybridization in the North American region (Wilson and Heiser, 1979; Wilson, 1988a; Wilson and Manhart, 1993; Jellen et al., 2011; Kistler and Shapiro, 2011). Moreover based on this study it has been demonstrated that the South American diploids (*Ch. atrovirens* and *Ch. petiolare*) are not part of the hybridization of the South American tetraploids (Fig. 4.5).

HIII- The hybridization hypothesis for Ch. berlandieri *subsp.* nuttalliae*-group from Mexico* – The third suggested hybridization hypothesis is for the *Ch. berlandieri* subsp. *nuttalliae*-group (Fig. 4.5). The hybridization network of this study supports the suggested ancient hybridization of *Ch. berlandieri* subsp. *nuttalliae*-group in the eastern region of North America (Wilson, 1990; Kistler and Shapiro, 2011).This study suggest for the first time that for *Ch. berlandieri* subsp. *nuttalliae* the European diploid *Ch. ficifolium* is one of its putative parent and the second putative parent is the North American diploid *Ch. pallescens*. These two diploids are distributed in the eastern region of U.S., where the hybridization process for the putative ancestral allotetraploid has been suggested (Kistler and Shapiro, 2011).

Patterns of putative autopolyploidization for the hexaploids of Chenopodium *s.str.* – The hybridization network reconstruction showed that the hexaploids *Ch. album* and *Ch. giganteum* could be cases of autopolyploidization (Fig. 4.5). The gall showed for *Ch. album* and allies shows that the descendent would be hibridizing with is own ancestor (Morrison, 2010). The close relationship between the hexaploids *Ch. album*, *Ch. giganteum* to the tetraploids *Ch. strictum* and *Ch. gigantospermum* based on plastid and nuclear regions (Figs. 4.1, 4.2 and 4.5) suggests the hypothesis of autopolyploidy from tetraploid to hexaploid. However, evidence of the allopolyploidy in *Ch. album* has been well documented (Dvořák et al. 1983; Rahiminejad and Gornall, 2004). In this sense is necessary to include the putative parental *Ch. suecium* in future analyses in order to test the allopolyploidization of *Ch. album*. Based on the hybridization network, the second putative case of autopolyplodization is the hexaploid *Ch. opulifolium*. This species is significantly incongruent because its alternative positions based on the phylogenetic reconstruction from each region (plastid Fig. 4.1, nuclear Fig. 4.2). But in the hybridization network did not showed any gall that suggest pattern of hybridization (Fig. 4.5). This result suggests that the hexaploid *Ch. opulifolium* could have originated by autopolyploidization and an

independent lineage. However, it would be useful to generate more evidence for this hypothesis.

Patterns of inconsistence in Microgynoecium – Finally, based on the hybridization network, *Ch. vulvaria* is an hypothetic hybrid between *Microgynoecium* and some ancestor of the rest of *Chenopodium* s.str. (Fig. 4.5). This could be explained by the inconsistent position of *Microgynoecium* based on the plastid and nuclear regions and the phylogenetic analyses (Figs. 4.1, 4.2, 4.3 and 4.4).

4.4.6 Divergence time of *Ch. quinoa* and *Ch. berlandieri* subsp. *nuttallieae*

A preliminary molecular clock analyses based on the plastid regions was conducted using the minimal of 16 Ma to calibrate the crown node of *Chenopodium* s.str. The age for the calibration is from the fossil *Pavangula randeckenesis* seeds used by Kadereit et al. (2003) for calibrate the crown node of the clade Chenopodioideae I which is defined in this study as *Chenopodium* s.str. The results (Fig. 4.7) suggested a divergence time of the *Ch. quinoa* lineage and *Ch. berlandieri* subsp. *nuttalliae* lineage to be ca. 2 Ma in each of them, a divergence age of the stem node of *Ch. ficifolium* is estimated to be ca. 5Ma while the age of the North American diploids lineage is estimated to be ca. 3 Ma.

All the evidence shows that the formation of the ancestral American tetraploids appears to be much older than its domestication. Considering that the domestication in North America began around 6000 B.C. in eastern North America and around 1500 B.C. in the Andean region of South America (Bruno and Whitehead, 2003; Kistler and Shapiro, 2011). The hypothesis of independent domestications in Mexico and Mesoamerica and then in the Andean region from an ancestral eastern North American allotetraploid is most probable (Wilson and Heiser, 1979, Wilson 1988a; Wilson 1993; Bruno and Whitehead, 2003; Jellen et al., 2011; Kistler and Shapiro, 2011).

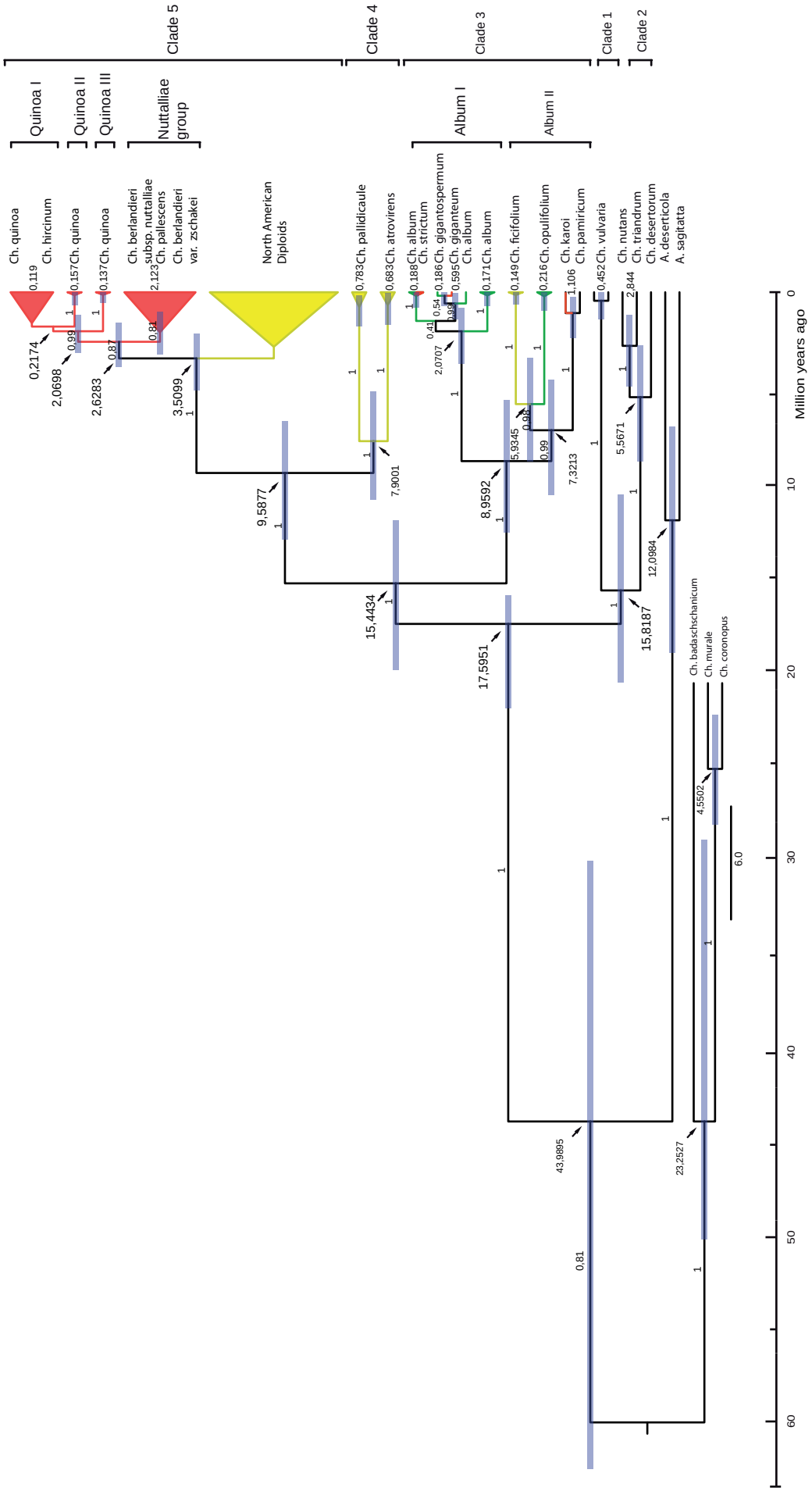


Fig. 4.7 – Bayesian maximum credibility chronogram median ages of *Chenopodium* sensu stricto obtained from Beast analysis with one calibration point. Colors correspond to the polyploidy level. Red for allotetraploids, yellow for diploids, green for hexaploids. Bayesian post probabilities (PP) are above the branches. Divergence ages are in Millon years ago.

4.5 Conclusions and future work

The high phylogenetic structure reported for the regions (plastid, *trnL-F*, *matK/trnK* and *rpl16*; nuclear, ITS) improved the supports and the relationships within *Chenopodium* s.str. Furthermore, in agreement with Korotkova et al. (2011) this study supported that the *trnK* intron and the *rpl16* intron showed good phylogenetic signal at species level. No additive polymorphic sites were observed in the ITS region, suggesting that ITS has a high degree of concerted evolution in *Chenopodium* s.str. In *Chenopodium* s.str. three significant incongruent positions (*Ch. opulifolium*, *Ch. ficifolium* and *Ch. karoi*) was probed to be due to biological causes. Exploratory hybridization network reconstruction suggested that the biological cause of the topological incongruence could be hybridization.

Chromosome counts of the species sampled in this study and more species of *Chenopodium* s.s are needed in order to complete and confirm the reported counts. Further works based on new nuclear markers (e.g. *LFY* low copy regions) and morphological data sets are needed to infer precisely relationships within *Chenopodium* s.str. In order to test the hybridization hypotheses within *Chenopodium* s.str., it is necessary to do further studies including so far missing diploid taxa (e.g. *Ch. suecium* for testing the allopolyploid origin of *Ch. album*). Finally, molecular clock analyses needs to be improved in order to address the age of the hybridization events and the lineages within *Chenopodium* s.str.

Appendix 4.A – Sampling of *Chenopodium* sensu stricto

Taxon	Field/Garden Origin	Voucher	Code	*trnL-F* Acc.	ITS Acc.	*matK-trnK* Acc.	*rpl16* Acc.	Ploidy level
Chenopodium album L.	Germany, Bonn	S. Fuentes 001 (B)	**AC388**	HE577557	HE577419	this study	this study	6x
Chenopodium album L.	USA ARS GRIN Ames 27372 [USA]	S. Fuentes 006 (B)	**AC396**	HE577570	HE577432	this study	this study	6x
Chenopodium album L.	Spain	T. Borsch 3921 (B)	**AC414**	HE577592	HE577453	this study	this study	6x
Chenopodium album L.	Greece, Messinia	R. & E. Willing 122.544 (B)	**AC571**	HE577558	HE577420	this study	this study	6x
Chenopodium album L.	USA, Wisconsin	N. J. Holmberg 1976 (MO)	**AC590**	HE577556	HE577418	this study	this study	6x
Chenopodium album L.	USA, Arizona	H. D. Hammond 11926 (MO)	**AC591**	HE577596	HE577457	this study	this study	6x
Chenopodium album L.	Germany, Usedom	Weber (B)	**AC602**	HE577559	HE577421	this study	this study	6x
Chenopodium album L.	Russia, Altay Republic	L. Martins 2423 (B)	**AC614**	HE577552	HE577414	this study	this study	6x
Chenopodium album L.	Bolivia, CBBA	S. Fuentes 033 (B)	**AC685**	this study	this study	this study	this study	6x
Chenopodium album L.	Argentina	Z. Noaga F O 11865 (B)	**AC864**	this study	this study	this study	this study	6x
Chenopodium album L.	Germany	S. Fuentes 187 (B)	**AC924**	this study	this study	this study	this study	6x
Chenopodium atrovirens Rydb.	Chile, Antofagasta	F. Luebert & A. Moreira 2992 (SGO)	**AC702**	this study	this study	this study	this study	2x
Chenopodium berlandieri Moq.	USA, Nevada	J. C. Beatley 11698 (NY)	**AC541**	HE577561	HE577423	this study	this study	4x
Chenopodium berlandieri subsp. *nuttalliae* (Saff.) H.Dan. Wilson & Heiser	Mexico	T. Borsch & H. Flores Olvera (B, MEXU)	**AC616**	HE577565	HE577427	this study	this study	4x
Chenopodium berlandieri subsp. *nuttalliae* (Saff.) H.Dan. Wilson & Heiser	USA ARS GRIN PI568155 [Mexico]	S. Fuentes 016 (B)	**AC394**	HE577571	HE577433	this study	this study	4x
Chenopodium berlandieri subsp. *nuttalliae* (Saff.) H.Dan. Wilson & Heiser	USA ARS GRIN PI568155 [Mexico]	S. Fuentes 015 (B)	**AC403**	HE577581	HE577446	this study	this study	4x
Chenopodium berlandieri var. *boscianum* (Moq.) Wahl	USA, Lousiana	D. M. Ferguson 1072 (NY)	**AC545**	HE577564	HE577426	this study	this study	4x
Chenopodium berlandieri var. *sinuatum* (Murr) Wahl	USA, Colorado	T. G. Lammers et al. 11321 (NY)	**AC540**	HE577585	HE577451	this study	this study	2x
Chenopodium berlandieri var. *sinuatum* (Murr) Wahl	USA , Colorado	G. Rink 2527 (NY)	**AC599**	HE577567	HE577429	this study	this study	4x
Chenopodium berlandieri var. *zschackei* (Murr) Murr ex Graebn.	USA, Wyoming	A. J. Roderick 2286 (NY)	**AC600**	HE577569	HE577431	this study	this study	4x
Chenopodium cf. *atrovirens* Rydb.	USA, Utha	M. Madsen 40772 (MO)	**AC586**	HE577584	HE577449	this study	this study	2x
Chenopodium cf. *pallescens* Standl.	USA, Missouri	G. Yatskievych 03-93 (MO)	**AC594**	HE577547	HE577409	this study	this study	2x
Chenopodium cycloides A. Nelson	USA, Kansas	C.C. Freeman 2549 (NY)	**AC544**	HE577599	HE577460	this study	this study	-
Chenopodium desertorum subsp. *anidiophyllum* (Aellen) P.G. Wilson	Australia	C. Michaell & J.Risler 1773 (B, NT)	**AC519**	HE577555	HE577417	this study	this study	-
Chenopodium desiccatum A. Nelson	USA, Missouri	B. Summers & Harris 9813 (MO)	**AC588**	HE577550	HE577412	this study	this study	2x
Chenopodium ficifolium Sm.	Germany, Berlin	H. Scholz 21.07.1997 (B)	**AC572**	this study	this study	this study	this study	2x
Chenopodium ficifolium Sm.	Germany, Berlin	R. & E. Willing 12.260 D (B)	**AC854**	HE577606	HE577466	this study	this study	2x
Chenopodium giganteum D. Don	Bonn Bot. Gart. No: 21397 [India]	S. Fuentes 014 (B)	**AC428**	HE577597	HE577458	this study	this study	6x
Chenopodium gigantospermum var. *standleyanum* Aellen	USA, Kansas	C. A. Morse 10855 (NY)	**AC550**	HE577551	HE577413	this study	this study	4x
Chenopodium hircinum Schard.	Argentina	Z. Noaga F O 11945 (B)	**AC860**	this study	this study	this study	this study	4x
Chenopodium hircinum Schard.	Argentina	Z. Noaga F O 11862 (B)	**AC862**	this study	this study	this study	this study	4x
Chenopodium karoi (Murr) Aellen	Russia, Altay Republic	E. v. Raab-Straube 020350 (B)	**AC575**	HE577609	HE577469	this study	this study	4x
Chenopodium leptophyllum (Moq.) Nutt. ex S. Watson	USA, Montana	P. C. Lesica 8846 (NY)	**AC554**	HE577566	HE577428	this study	this study	2x
Chenopodium neomexicanum Standl.	USA, New Mexico	R. D. Worthington 13394 (NY)	**AC555**	HE577611	HE577471	this study	this study	2x
Chenopodium neomexicanum Standl.	USA, Arizona	S. Fuentes 172 (B)	**AC598**	HE577601	HE577461	this study	this study	2x
Chenopodium nevadense Standl.	USA, Nevada	A. Tiehm 13320 (NY)	**AC556**	HE577549	HE577411	this study	this study	-
Chenopodium nutans (R. Br.) S.Fuentes & Borsch	Berlin Bot. Gard. No: 187199 [Australia]	S. Fuentes 019 (B)	**AC525**	HE577553	HE577415	this study	this study	-
Chenopodium opulifolium Schrad. ex W.D.J. Koch & Ziz	Slovakia	T. Borsch 3899 (B)	**AC410**	HE577595	HE577455	this study	this study	6x
Chenopodium opulifolium Schrad. ex W.D.J. Koch & Ziz	Spain	T. Borsch 3926 (B)	**AC416**	HE577594	HE577454	this study	this study	6x
Chenopodium pallescens Standl.	USA, Illinois	T. G. Lammers 10336 (NY)	**AC557**	HE577604	HE577464	this study	this study	2x
Chenopodium pallidicaule Aellen	USA ARS GRIN PI478406 [Bolivia]	No Voucher	**AC398**	HE577574	HE577439	this study	this study	2x
Chenopodium pallidicaule Aellen	USA ARS GRIN PI510525 [Peru]	No Voucher	**AC399**	HE577573	HE577438	this study	this study	2x

Appendix 4.A – Sampling of *Chenopodium* sensu stricto (continued)

Taxon	Field/Garden Origin	Voucher	Code	*trnL-F* Acc.	ITS Acc.	*matK-trnK* Acc.	*rpl16* Acc.	Ploidy level
Chenopodium pallidicaule Aellen	USA ARS GRIN PI 510530 [Peru]	No Voucher	**AC400**	HE577575	HE577440	this study	this study	2x
Chenopodium pallidicaule Aellen	Bolivia, Tarija	S. G. Beck 31939 (B, LPB)	**AC426**	HE577600	HE577437	this study	this study	2x
Chenopodium pallidicaule Aellen	Bolivia, Cochabamba	S. Fuentes 041 (B)	**AC667**	this study	this study	this study	this study	2x
Chenopodium pallidicaule Aellen	Bolivia, La Paz	S. Fuentes 047 (B)	**AC690**	this study	this study	this study	this study	2x
Chenopodium pallidicaule Aellen	Bolivia, La Paz	S. Fuentes 051 (B)	**AC694**	this study	this study	this study	this study	2x
Chenopodium pamiricum Iljin	Russia, Altay Republic	E. V. Raab-Straube 02032 (B)	**AC853**	this study	this study	this study	this study	-
Chenopodium pamiricum Iljin	Russia, Altay Republic	L. Martins 2490 (B)	**AC611**	HE577608	HE577468	this study	this study	-
Chenopodium pamiricum Iljin	Russia, Altay Republic	L. Martins 2424 (B)	**AC613**	HE577607	HE577467	this study	this study	-
Chenopodium petiolare Kunth	Bolivia, La Paz	S. G. Beck 22972 (B, LPB)	**AC423**	HE577589	HE577435	this study	this study	2x
Chenopodium quinoa Willd.	USA ARS GRIN Ames 13214 [Bolivia. La Paz]	S. Fuentes 013 (B)	**AC401**	HE577580	HE577445	this study	this study	4x
Chenopodium quinoa Willd.	USA ARS GRIN Ames 13228 [Ecuador]	S. Fuentes 017 (B)	**AC402**	HE577576	HE577441	this study	this study	4x
Chenopodium quinoa Willd.	USA ARS GRIN PI510551 [Peru, Puno]	S. Fuentes 009 (B)	**AC404**	HE577579	HE577444	this study	this study	4x
Chenopodium quinoa Willd.	USA ARS GRIN PI587173 [Argentina]	S. Fuentes 012 (B)	**AC405**	HE577577	HE577442	this study	this study	4x
Chenopodium quinoa Willd.	USA ARS GRIN PI596498 [Peru, Cuzco]	S. Fuentes 008 (B)	**AC406**	HE577578	HE577443	this study	this study	4x
Chenopodium quinoa Willd.	USA ARS GRIN PI614880 [Chile]	S. Fuentes 010 (B)	**AC407**	HE577582	HE577447	this study	this study	4x
Chenopodium quinoa Willd.	USA ARS GRIN PI614914 [Bolivia, Oruro]	S. Fuentes 011 (B)	**AC408**	HE577583	HE577448	this study	this study	4x
Chenopodium quinoa Willd.	Bolivia, Cochabamba	S. Fuentes 040 (B)	**AC666**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Perú, Puno	S. Fuentes 063 (B)	**AC669**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Perú, Puno	S. Fuentes 069 (B)	**AC670**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Bolivia, Oruro	S. Fuentes 111 (B)	**AC680**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Bolivia, Oruro	S. Fuentes 112 (B)	**AC681**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Bolivia, Chuquisaca	S. Fuentes 147 (B)	**AC682**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Bolivia, Potosi	S. Fuentes 148 (B)	**AC683**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Bolivia, CBBA	S. Fuentes 032 (B)	**AC684**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Bolivia, CBBA	S. Fuentes 042 (B)	**AC687**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Bolivia, CBBA	S. Fuentes 043 (B)	**AC688**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Bolivia, La Paz	S. Fuentes 046 (B)	**AC689**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Bolivia, La Paz	S. Fuentes 048 (B)	**AC691**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Bolivia, La Paz	S. Fuentes 049 (B)	**AC692**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Bolivia, La Paz	S. Fuentes 050 (B)	**AC693**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Bolivia, La Paz	S. Fuentes 053 (B)	**AC695**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Bolivia, La Paz	S. Fuentes 054 (B)	**AC696**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Bolivia, La Paz	S. Fuentes 055 (B)	**AC697**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Bolivia, La Paz	S. Fuentes 056 (B)	**AC698**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Perú, Puno	S. Fuentes 058 (B)	**AC699**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Perú, Puno	S. Fuentes 061 (B)	**AC700**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Perú, Puno	S. Fuentes 070 (B)	**AC704**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Perú, Puno	S. Fuentes 071 (B)	**AC705**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Perú, Puno	S. Fuentes 074 (B)	**AC708**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Perú, Puno	S. Fuentes 075 (B)	**AC709**	this study	this study	this study	this study	4x
Chenopodium quinoa Willd.	Perú, Puno	S. Fuentes 076 (B)	**AC710**	this study	this study	this study	this study	4x
Chenopodium standleyanum Aellen	USA, Missouri	N. J. Holmberg 554 (MO)	**AC596**	HE577603	HE577463	this study	this study	2x

Appendix 4.A – Sampling of *Chenopodium* sensu stricto (continued)

Taxon	Field/Garden Origin	Voucher	Code	*trnL-F* Acc.	ITS Acc.	*matK-trnK* Acc.	*rpl16* Acc.	Ploidy level
Chenopodium strictum Roth	Germany	E. Willing 23293 D (B)	**AC869**	this study	this study	this study	this study	2x
Chenopodium strictum Roth	Germany	S. Fuentes 186 (B)	**AC923**	this study	this study	this study	this study	2x
Chenopodium subglabrum (S. Watson) A. Nelson	USA, Wyoming	R. D. Dorn 5434 (NY)	**AC559**	HE577605	HE577465	this study	this study	2x
Chenopodium triandru G.Forst.	New Zealand	P. Hein 12560 (B, CHR)	**AC522**	HE577554	HE577416	this study	this study	-
Chenopodium vulvaria L.	Spain	T. Borsch 3918 (B)	**AC412**	HE577591	HE577407	this study	this study	2x
Chenopodium vulvaria L.	Greece, Evrytania	R. & E. Willing 148.759 (B)	**AC562**	HE577590	HE577406	this study	this study	2x
Chenopodium watsonii A. Nelson	USA, Arizona	D. H. Goldman 2095 (NY)	**AC561**	HE577602	HE577462	this study	this study	2x
OUTGROUPS								
Atripliceae								
Atriplex deserticola Phil.	Argentina	Z Noaga F O 12057 (B)	**AC859**	this study	this study	this study	this study	
Atriplex sagittata Borkh.	Berlin Bot. Gard. No: 063119110 [Germany]	S. Fuentes 021 (B)	**AC533**	HE577499	HE577359	this study	this study	
Microgynoecium tibeticum Hook. f.	China	B. Dickoré 4284 (B)	**AC656**	HE577503	HE577363	this study	this study	
***Ch. murale*-clade**								
Chenopodium murale L	Mexico, Ixtapan	T. Borsch & H. Flores Olvera 3871 (B, MEXU)	**AC382**	HE577541	HE577401	this study	this study	
Chenopodium coronopus Moq.	Spain, La Palma	Royl 6823 (B)	**AC570**	HE577543	HE577403	this study	this study	
Chenopodium badachschanicum Tzvelev	Russia, Altay Republic	L. Martins 2329 (B)	**AC609**	HE577528	HE577388	this study	this study	

Note: The ploidy level of the taxa is a theorical recopilation from: The Index to plant Chromosome number (1960-2006) see complete citations in Appendix 2.C.

Appendix 4.B – List of indels found in *trnL-F* region

***trnL-F* intron**			
No.	**Extension**	**Length**	**Sequence motif**
1	72-72	1	Gap in *Microgynoecium tibeticum*, probably deletion.
2	119-146	28	Gap in *Ch. badachschanicum* and all taxa of the ingroup.
3	147-161	15	Gap in *Ch. murale* and *Ch. coronopus*.
4	175-183	9	Insertion in *Ch. badachschanicum*.
5	176-183	8	Gap in all taxa of the ingroup.
6	237-241	5	Gap in *Ch. pallescens*, *Ch. nevadense* and *Ch. desiccatum*.
7	261-266	6	Insertion of "GCTTCC" SSR in *Microgynoecium tibeticum*.
8	342-460	119	Gap in *Microgynoecium tibeticum*.
9	342-515	174	Gap in *Atriplex*.
10	347-363	17	Gap in all taxa of the ingroup.
11	351-352	2	Insertion of "TT" in *Ch. murale* and *Ch. coronopus*.
12	353-363	11	Insertion of "CGATTTTTTT" in *Ch. badachschanicum*.
13	419-429	11	Insertion of "TTCGAATATGA" in *Ch. badachschanicum*.
14	453-455	3	Insertion of "TTT" in *Ch. badachschanicum*.
15	456-460	5	Gap in *Ch. badachschanicum* and all taxa of the ingroup.

***trnL-F* spacer**			
No.	**Extension**	**Length**	**Sequence motif**
16	683-683	1	Gap in *Ch. badachschanicum*, *Atriplex* and *Microgynoecium*
17	706-706	1	Insertion of "C" in *Ch. atrovirens*
18	797-803	7	Gap in *Atriplex*.
19	798-802	5	Insertion in *Ch. karoi*, *Ch. pamiricum* and *Ch.* cf sample AC853
20	808-812	5	Gap in all taxa of the ingroup.
21	848-848	1	Insertion of "A" in *Ch. nutans*.
22	867-871	5	Insertion in *Ch. badachschanicum*.
23	898-903	6	Insertion in *Ch. badachschanicum*.
24	908-911	4	Insertion of "CTTA" in all taxa of the ingroup.
25	908-915	8	Gap in *Ch. murale*, *Ch. coronopus* and *Ch. badachshanicum*.
26	912-915	4	Insertion of "ATTA" in *Atriplex* and *Mycrogynoecium*.
27	946-961	16	Insertion of "TTAAGGAATTAAGGAA" in *Ch. desertorum*.
28	954-961	8	Gap in *Ch. triandrum* and *Ch. nutans*
29	1043-1047	5	Insertion of "AAATT" in *Ch. murale* and *Ch. coronopus*.
30	1067-1067	1	Gap in *Atriplex* and *Microgynoecium*.

Appendix 4.B – List of indels found in *trnL-F* region

***trnL-F* intron**			
No.	**Extension**	**Length**	**Sequence motif**
1	72-72	1	Gap in *Microgynoecium tibeticum*, probably deletion.
2	119-146	28	Gap in *Ch. badachschanicum* and all taxa of the ingroup.
3	147-161	15	Gap in *Ch. murale* and *Ch. coronopus.*
4	175-183	9	Insertion in *Ch. badachschanicum*.
5	176-183	8	Gap in all taxa of the ingroup.
6	237-241	5	Gap in *Ch. pallescens*, *Ch. nevadense* and *Ch. desiccatum*.
7	261-266	6	Insertion of "GCTTCC" SSR in *Microgynoecium tibeticum.*
8	342-460	119	Gap in *Microgynoecium tibeticum*.
9	342-515	174	Gap in *Atriplex.*
10	347-363	17	Gap in all taxa of the ingroup.
11	351-352	2	Insertion of "TT" in *Ch. murale* and *Ch. coronopus*.
12	353-363	11	Insertion of "CGATTTTTTT" in *Ch. badachschanicum.*
13	419-429	11	Insertion of "TTCGAATATGA" in *Ch. badachschanicum.*
14	453-455	3	Insertion of "TTT" in *Ch. badachschanicum*.
15	456-460	5	Gap in *Ch. badachschanicum* and all taxa of the ingroup.

***trnL-F* spacer**			
No.	**Extension**	**Length**	**Sequence motif**
16	683-683	1	Gap in *Ch. badachschanicum*, *Atriplex* and *Microgynoecium*
17	706-706	1	Insertion of "C" in *Ch. atrovirens*
18	797-803	7	Gap in *Atriplex.*
19	798-802	5	Insertion in *Ch. karoi*, *Ch. pamiricum* and *Ch.* cf sample AC853
20	808-812	5	Gap in all taxa of the ingroup.
21	848-848	1	Insertion of "A" in *Ch. nutans*.
22	867-871	5	Insertion in *Ch. badachschanicum*.
23	898-903	6	Insertion in *Ch. badachschanicum*.
24	908-911	4	Insertion of "CTTA" in all taxa of the ingroup.
25	908-915	8	Gap in *Ch. murale*, *Ch. coronopus* and *Ch. badachshanicum.*
26	912-915	4	Insertion of "ATTA" in *Atriplex* and *Mycrogynoecium*.
27	946-961	16	Insertion of "TTAAGGAATTAAGGAA" in *Ch. desertorum.*
28	954-961	8	Gap in *Ch. triandrum* and *Ch. nutans*
29	1043-1047	5	Insertion of "AAATT" in *Ch. murale* and *Ch. coronopus*.
30	1067-1067	1	Gap in *Atriplex* and *Microgynoecium*.

Appendix 4.D – List of indels in *rpL16* intron region

			***rpl16* intron**
No.	**Extension**	**Length**	**Sequence motif**
1	78-82	5	Insertion in *Atriplex deserticola* posible SSR with one substitution.
2	95-99	5	Insertion in *Ch. opulifolium.*
3	108-112	5	Gap in *Microgynoecium.*
4	142-143	2	Gap in *Microgynoecium.*
5	143-143	1	Insertion of "T" in *Ch.badachshanicum* and *Ch. ficifolium.*
6	162-169	8	Insertion in *Atriplex*.
7	198-206	9	Gap in *Microgynoecium.*
8	200-206	7	Gap in *Ch. murale* and *Ch. coronopus*.
9	205-206	2	Gap in *Atriplex*, Ch. badachschanicum and *Ch. pallidicaule.*
10	206-206	1	Insertion of "A" in *Ch.ficifolium.*
11	238-238	1	Gap in *Atriplex*.
12	238-240	3	Gap in *Ch. subglabrum*.
13	293-296	4	Insertion of "AAAA" in *Atriplex*.
14	351-351	1	Gap in *Ch. murale, Ch. coronopus*, *Ch. badachschanicum*, *Atriplex* and *Microgynoecium.*
15	417-418	2	Insertion of "AG" in *Ch. desertorum*.
16	435-436	2	Insertion of "AT" in *Ch. atrovirens*, *Ch. cycloides*, *Ch. berlandieri*, *Ch, leptophyllum*, *Ch. neomexicanum*, *Ch. nevadense*, *Ch. subglabrum*, *Ch. pallescens*, *Ch. watsonii*, *Ch. desiccatum*, *Ch. standleyanum*, *Ch, hircinum*, *Ch cf. album* AC860, *Ch. pallidicaule*, *Ch. petiolare* and *Ch, quinoa*.
17	512-515	4	Gap in *Microgynoecium*.
18	552-557	6	Gap in all taxa (exept in *Atriplex deserticola*)
19	556-557	2	*Insertion of "AAAA" in Atriplex sagittata*.
20	564-571	8	Gap in *Microgynoecium*.
21	568-569	2	Insertion of "TA" in *Ch.vulvaria*, *Ch. desertorum*, *Ch. triandrum* and *Ch. nutans*.
22	608-608	1	Insertion of "A" in *Microgynoecium.*
23	612-617	6	Insertion of "TAGATA" in *Ch. ficifolium*.
24	653-653	1	Insertion of "A" in *Atriplex*.
25	665-665	1	Gap in *Microgynoecium.*
26	726-772	47	Gap in *Ch. atrovierins* sample AC586.
27	740-745	6	Insertion of "ATATTA" in *Ch. murale* and *Ch. coronopus*.
28	749-763	15	Gap in *Atriplex deserticola*.
29	749-772	24	*Gap in Atriplex sagittata*.
30	759-763	5	Insertion of "CAAAA" in *Ch. murale*, *Ch. badachschanicum* and *Ch. coronopus.*
31	771-772	2	Insertion of "AA" in *Atriplex deserticola.*
32	772-772	1	Gap in all taxa (except in *Atriplex deserticola*)
33	792-827	36	Gap in *Ch. murale, Ch. badachschanicum* and *Ch. coronopus*.
34	794-797	4	Gap in *Atriplex* and *Microgynoecium*
35	802-823	22	Gap in *Microgynoecium.*
36	802-827	26	Gap in *Atriplex*.
37	804-823	20	Insertion in *Ch. vulvaria*.
38	835-845	11	Gap in *Ch. album*, *Ch. opulifolium*, *Ch. giganteum*, *Ch. gigantospermum*, *Ch. ficifolium*, *Ch. karoi*, *Ch. pamiricum* and *Ch. strictum.*
39	836-842	7	Insertion of "ATTATTA" in *Microgynoecium.*
40	853-856	4	Gap in *Atriplex*, *Microgynoecium* and all *Chenopodium* s.s. taxa.
41	904-916	13	Gap in *Ch. leptophyllum*
42	953-953	1	Gap in *Ch. badachschanicum.*
43	953-956	4	Gap in *Ch. pallidicaule*.
44	953-959	7	Gap in *Atriplex*.
45	972-972	1	*Gap in Atriplex sagittata*.
46	975-975	1	Insertion of "A" in *Atriplex deserticola*.
47	981-982	2	Gap in *Atriplex*.

Appendix 4.E – List of indels founded in the ITS region

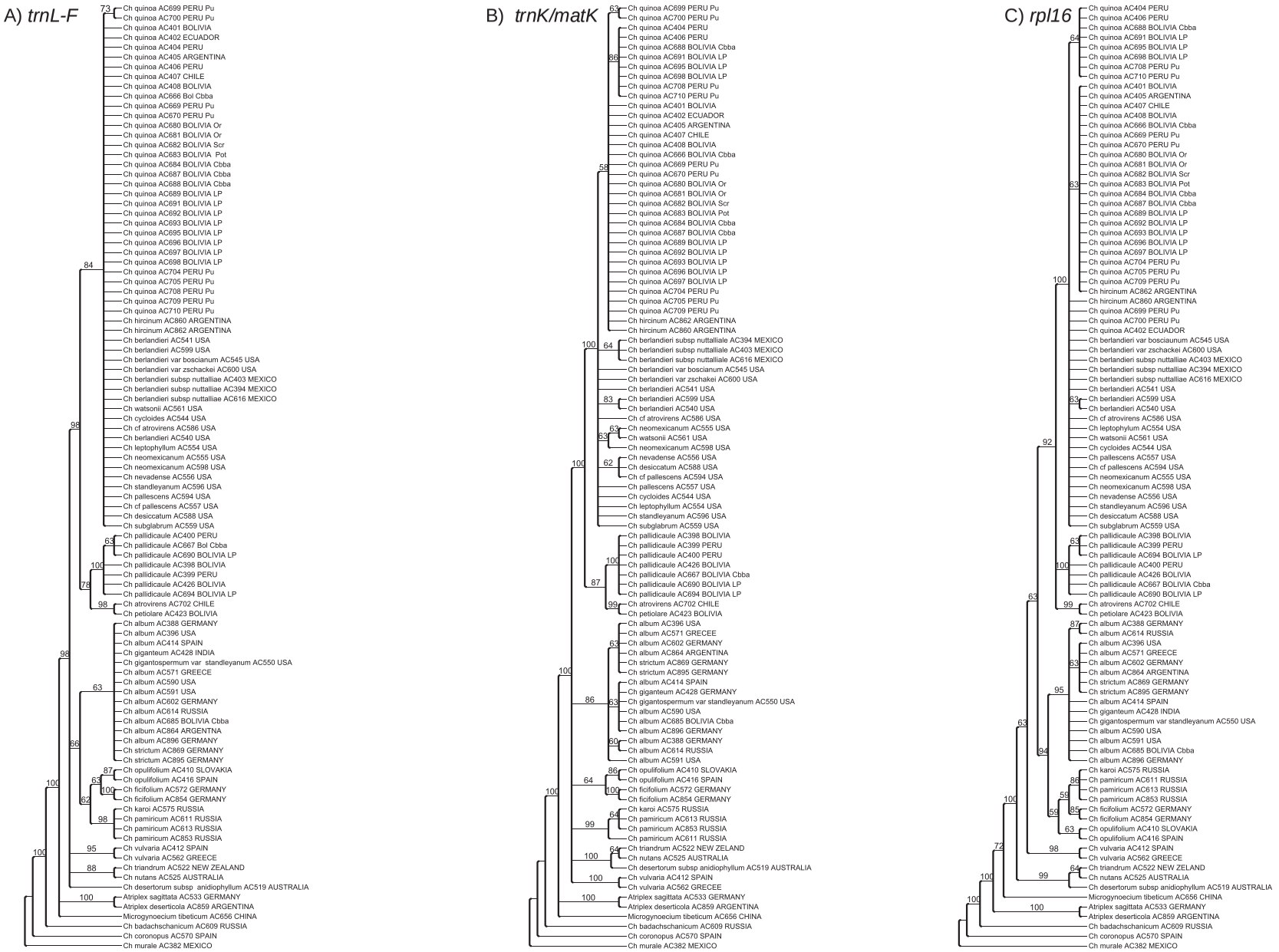
			ITS
No.	**Extension**	**Length**	**Sequence motif**
1	52-52	1	Insertion of "G" in *Ch. opulifolium*.
2	88-88	1	Gap in *Ch. vulvaria*.
3	354-354	1	Gap in *Ch.pallidicaule*.
4	361-363	3	Gap in *Ch. neomexicanum*, *Ch. watsonii*, *Ch. atrovirens* sample AC586.
5	373-373	1	Gap in *Ch. murale*.
6	432-432	1	Gap in *Ch. murale*.
7	445-445	1	Gap in *Ch. murale* and *Ch. coronopus*.
8	448-448	1	Gap in *Ch. murale* and *Ch. coronopus*.
9	452-452	1	Gap in *Ch. album*, *Ch. giganteum*, *Ch. gigantospermum*, *Ch. pamiricum* and *Ch. strictum*.
10	460-473	14	Gap in *Ch. murale and Ch. coronopus.*
11	478-482	5	Insertion of "AATAT" in *Ch. murale* and *Ch. coronopus*.
12	491-493	3	Insertion of "TGC" in *Ch. badachschanicum*.
13	492-493	2	Gap in *Atriplex*.
14	496-510	15	Gap in *Ch. murale*, *Ch. coronopus* and *Ch. badachschanicum*.
15	511-524	14	Gap in *Atriplex* and *Chenopodium* s.s.
16	546-546	1	Gap in *Ch. coronopus*.
17	552-552	1	Gap in *Ch. desertorum*.
18	558-558	1	Gap in *Ch. murale*, *Ch. coronopus*, *Ch. badachschanicum*, *Atriplex* and *Microgynoecium*.

Appendix 4.F – Sequence statistics of each partition and combined data sets for *Chenopodium* s. str.

	***trnL* intron**	***trnL* 3' exon**	***trnL-F* spacer**	***trnK* 5' intron**	***matK***	***trnK* 3' intron**	***rpl16* intron**	**Plastid combined**	**ITS1**	**5.8S**	**ITS2**
Data set with hotspots											
Length range	412-601	50-50	347-375	676-715	1527-1536	139-217	971-1037		211-230	155-155	214-244
Mean length (SD)	560 (24)	50	360 (3)	711 (5)	1529 (4)	206 (10)	1012 (8)		226 (6)	155	227 (3)
% GC	32.2	44	30.4	30.8	32.2	32.8	27.4		55	55.5	59.8
Data set without hotspots											
Length range	405-508	50-50	342-370	676-697	1527-1536	139-217	929-986	4179-4384	168-186	155-155	202-217
Mean length (SD)	543 (22)	50	355 (3)	695 (3)	1529 (4)	206 (10)	962 (8)	4339 (29)	183 (6)	155	215 (2)
% variable characters	8.4	2	18.1	12.1	14.1	17.2	17.1	14.1	29.8	1.9	30.5
% informative characters	5.6	2	11.9	9.1	10.2	12	11.4	9.8	23.9	0	23.4
Number of coded indels	15	0	15	5	1	11	47	94	2	0	16

Appendix 4.G – Parameters and priors used for the molecular clock analyses

1	The molecular clock analysis was carried out using the package BEAST V.1.4 (Drummond and Rambaut, 2007).
2	Minimal age to calibrate the crown node of *Chenopodium* s.s. is 16 Ma.
3	Substitution rate parameter = lognormal.
4	Molecular clock model for *trnL-F* and *matK/trnK* = GTR+G.
5	Molecular clock model for *rpl16* intron = GTR+G+I.
6	Tree model = Yule process.
7	MCMC run with 20 millon generations.
8	Save random trees = 1 each 1000th generation.
9	Burn in = 10%.



Appendix 4.H **–** Maximum parsimony strict consensus trees from each single plastid partition.

Chapter 5

General conclusions and outlook

Contents

5.1 The phylogeny of *Chenopodium* s.l. and its taxonomic implications within Chenopodioideae

Based on phylogenetic reconstructions, this study suggests the monophyly of three tribes under the subfamily Chenopodioideae: *Chenopodieae*, *Dysphanieae*, *Spinacieae*, and supports the monophyly of the tribes Atripliceae and Axyrideae. The subgenera and sections of *Chenopodium* s.l. are not monophyletic. *Chenopodium* s.l. splits up into six well-supported clades within Chenopodioideae (Chapters 2 and 3):

1. *Chenopodium* s.str., including most species of *Chenopodium* s.l. sampled in this study (> 40%), including *Chenopodium album,* which is the designed type species of *Chenopodium,* along with the genera *Einadia* and *Rhagodia*. Hence due to the transference of *Einadia* and *Rhagodia* under *Chenopodium* s.str. eighteen new combinations were proposed (Fig. 2.1, clade 7, Chapter 2). This lineage should represent the core Chenopodieae.

2. *Dysphania,* encompassing the aromatic chenopods (Fig. 2.1, clade 2, Chapter 2). This lineage is related to the genera *Teloxys* and *Suckleya* within the tribe *Dysphanieae*.

3. *Blitum* comprising. *B. capitatum* (designed type for the genus), *B. virgatum, B. californicum, B. bonus-henricus* and *B. nuttallianum* (Chapters 2 and 3). This lineage is the sister group of *Spinacia,* in the new circumscription of tribe *Spinacieae*.

4. The *Ch. murale* lineage, including. *Ch. murale, Ch. coronopus, Ch. badachschanicum* and *Ch. hybridum*. This clade needs a new generic name (Chapters 2 and 3).

5. The *Ch. rubrum* lineage, encompassing *Ch. rubrum*, *Ch. glaucum*, *Ch. chenopodioides* and *Ch. urbicum*. This clade also needs a new generic name (Chapters 2 and 3).

6. *Ch. polyspermum* is a differentiated lineage from all the rest. Based on *matK/trnK* data set the phylogenetic relationships of this species are not defined within Chenopodioideae (Chapter 3).

7. The relationships of *Chenopodium* s.s, Atripliceae, *Ch. murale* lineage and *Ch. rubrum* lineage are well-resolved and highly supported. Atripliceae is the sister group to *Chenopodium* s.str. *Ch. murale* lineage is the sister group to these both clades. Finally, all these lineages are nested in a monophyletic group with the *Ch. rubrum* lineage (Fig. 2.1, Chapter 2; Fig. 3.1, Chapter 3). However, the placement of *Dysphanieae* and *Spinacieae* are not resolved and share the basal position in the tree within Chenopodioideae (Chapter 2 and Chapter 3).

5.2 Phylogeny and evolution of *Chenopodium* s.str.

Chenopodium s.str. is a highly variable and species-rich genus. Phylogenetic reconstruction of *Chenopodium* s.str. based on the combination of non-coding regions with a high phylogenetic performance resulted in a well-resolved tree. Five well-supported clades are defined within *Chenopodium* s.str. based on the combined plastid data set (Chapter 4). However, these clades are not supported in the phylogenetic analyses based on the nuclear ITS data set. Based on the ITS the phylogenetic tree reconstruction shows a polytomy (Chapters 2 and 4). The following clades were recovered from the analysis (ploidy levels indicated):

1. Clade 1 includes only *Ch. vulvaria* (2x). It is well-differentiated based on both plastid and nuclear regions (Chapters 2 and 4).

2. Clade 2 composed of the Australian diploids *Ch. triandrm*, *Ch. nutans* and *Ch. desertorum* is resolved in the plastid analysis, but not supported by the nuclear analysis (Chapter 4).

3. Clade 3, comprising the *Ch. album* group, which encompasses two subclades:

   a) Album I with *Ch. album* (6x), *Ch. gigantospermum* (6x), *Ch. giganteum* (4x) and *Ch. strictum* (4x).

   b) Album II with *Ch, ficifolium* (2x), *Ch. karoi* (4x), *Ch. opulifolium* (6x) and *Ch. pamiricum* (?x).

The plastid analysis supports both Album I and II clades. Based on ITS only Album I is recovered as monophyletic (Chapter 4).

4. Clade 4 including the diploids *Ch. pallidicaule, Ch, atrovirens* and *Ch. petiolare,* all of them exclusive from South America. *Ch. pallidicaule* is a monophyletic lineage sister to the monophyletic clade formed by *Ch. petiolare* and *Ch. atrovirens.* The latter clade are not supported by the ITS tree (Chapter 4).

5. Clade 5 encompasses the allotetraploids *Ch. quinoa* and *Ch. berlandieri* subsp. *nuttallieae* and the diploid specimens of *Ch. atrovirens*, *Ch. standleyanum*, *Ch. pallescens*, *Ch. nevadense*, *Ch. leptophyllum*, *Ch. cycloides* and *Ch. neomexicanum* from North America. The monophyly of the South American Andean crop *Ch. quinoa* and the Mexican crop *Ch. berlandieri* subsp. *nuttallieae* are each low-supported. These two groups along with the rest of diploids from North America form a polytomy based on the combined plastid data set. ITS supports the polytomic relationship of the *Ch. quinoa* (4x), *Ch. hircinum* (4x), *Ch. berlandieri* including its varieties and subspecies (4x), *Ch. karoi* (4x) and only one diploid taxa, *Ch. ficifolium,* in a monophyletic clade (Chapter 4). *Ch. opulifolium* (6x), *Ch. karoi* (4x) and *Ch. ficifolium* (2x) fall into two different alternative topologies:

   a) Based on the plastid data set all three species are included in clade 3 (Fig. 4.1, Chapter 4).

   b) Based on the nuclear data set *Ch. karoi* and *Ch. ficifolium* are nested within the clade formed by the allotetraploids *Ch. quinoa*, *Ch. hircinum* and *Ch. berlandieri*, where the next sister lineage to this monophyletic group is *Ch. opulifolium* (Fig. 4.2, Chapter 4). The phylogenetic incongruence between nuclear and plastid regions showed in this study can be explained by biological causes such as hybridization.

Evidence for speciation driven by hybridization and the subsequent formation of allotetraploid taxa has been reported based on morphology, kariology and biochemical analyses. The results of the present study based on the hybridization network reconstruction (Fig. 4.5, Chapter 4) are in line with this general hypothesis. This study suggests that *Ch. ficifolium* (2x) and *Ch. pamiricum* are possible parental taxa involved in the formation of the allotetraploid *Ch. karoi*. Also, this study shows for first time that *Ch.*

ficifolium (2x) and one of the North American diploids are involved in the formation of the allotetraploid group of *Ch. quinoa* and the allotetraploid *Ch. berlandieri* var. *boscianum*. Furthermore, *Ch. ficifolium* (2x) and *Ch. pallescens* (2x) (the last one native from North America) are suggested to be involved in the formation of the allotetraploid group of *Ch. berlandieri* subsp. *nuttallieae*. Autopolyploidy is the suggested hypothesis for the formation of the hexaploids *Ch. album, Ch. gigantospermum* and *Ch. opulifolium* (Fig. 4.5, Chapter 4). Moreover the preliminary molecular clock analysis suggested that the divergence time of the origins allotetraplois within *Chenopodium* s.str. was about 2 Ma in America, suggesting that the domestication of the *Ch. quinoa* and *Ch. berlandieri* subsp. *nuttalliae* (ca. 6000 B.P.) occurred after the hybridization event and not the opposite.

5.3 Outlook

The study presented in this work leave several open questions regarding the systematics and evolution of *Chenopodium* within Chenopodioideae. The high morphological variability within *Chenopodium* s.l. and its causes needs to be further explored as showed, for example, by the non-conclusive relationships of *Ch. polyspermum*. In this sense some groups within Chenopodiodeae of *Chenopodium* s.l. still needs to be completed with the addition of samples from missing taxa (e.g. *Ch. frigidum, Ch. macrospermum*). The addition of these taxa, will contribute to a full morphological characterization of the clades, which is urgently needed to expand the presented taxonomic rearrangements for these species and for study the morphological diversification of *Chenopodium* s.l.

Hybridization patterns detected within *Chenopodium* s.str. in this study requires further investigation because this processes is difficult to assess. The currently available analyses for network hybridization reconstruction permitted the construction of biological hypotheses that need further test as explained by Morrison (2010). Missing diploid taxa such as *Ch. suecium*, which is suggested to be involved in the allopolyploidization of the hexaploids, need to be included in the analysis. Further investigation of the hybridization events of the allotetraploids as *Ch. quinoa* is also necessary. Inclusion of more American diploid taxa distributed will contribute for this purpose. Assessment of chromosome number, genome size variation and ploidy levels of the studied samples are essential in order to test the suggested hypothesis and clarify the autopolyploidization or

alloployploidization processes within *Chenopodium* s.str. So far no estimation of the ploidy level is available for *Ch. pamiricum*, and this species may be a parental of the allotetraploid *Ch. karoi*. Finally, the molecular clock analysis needs to be strengthened with the addition of more fossils for calibrating further nodes. This will improve divergence time estimations of the hybridization events.

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Contribution to chapters

Chapter 2.- Fuentes-Bazan, S., Mansion, G. and Borsch, T. (2012) Towards a species level tree of the globally diverse genus *Chenopodium* (Chenopodiaceae). Molecular Phylogenetics and Evolution 62, 359-374. http://dx.doi.org/10.1016/j.ympev.2011.10.006

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Chapter 3.- Fuentes-Bazan, S., Uotila, P. and Borsch, T. Resurrecting the Linnean genus *Blitum* and recovering two new genera for Chenopodioideae (Chenopodiaceae) based on phylogenetic reconstruction. In preparation for Willdenowia.

Own contributions: Designed work (together with T. Borsch), collected material (most), morphological determinations (together with P. Uotila), performing laboratory work and statistical analyses, and wrote the manuscript.

Chapter 4.- Fuentes-Bazan, S. and Borsch, T. Phylogeny of *Chenopodium* sensu stricto, patterns of reticulate evolution, and possible origins of Quinoa. In preparation for Molecular Phylogenetics and Evolution.

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