JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

A phylogenetic study of *Crepis* L. species sect. *Barkhausia* (Asteraceae) using low-copy nuclear genes (*gsh1*, *sqs*) and plastid genes (*rps16*, *matK1*)

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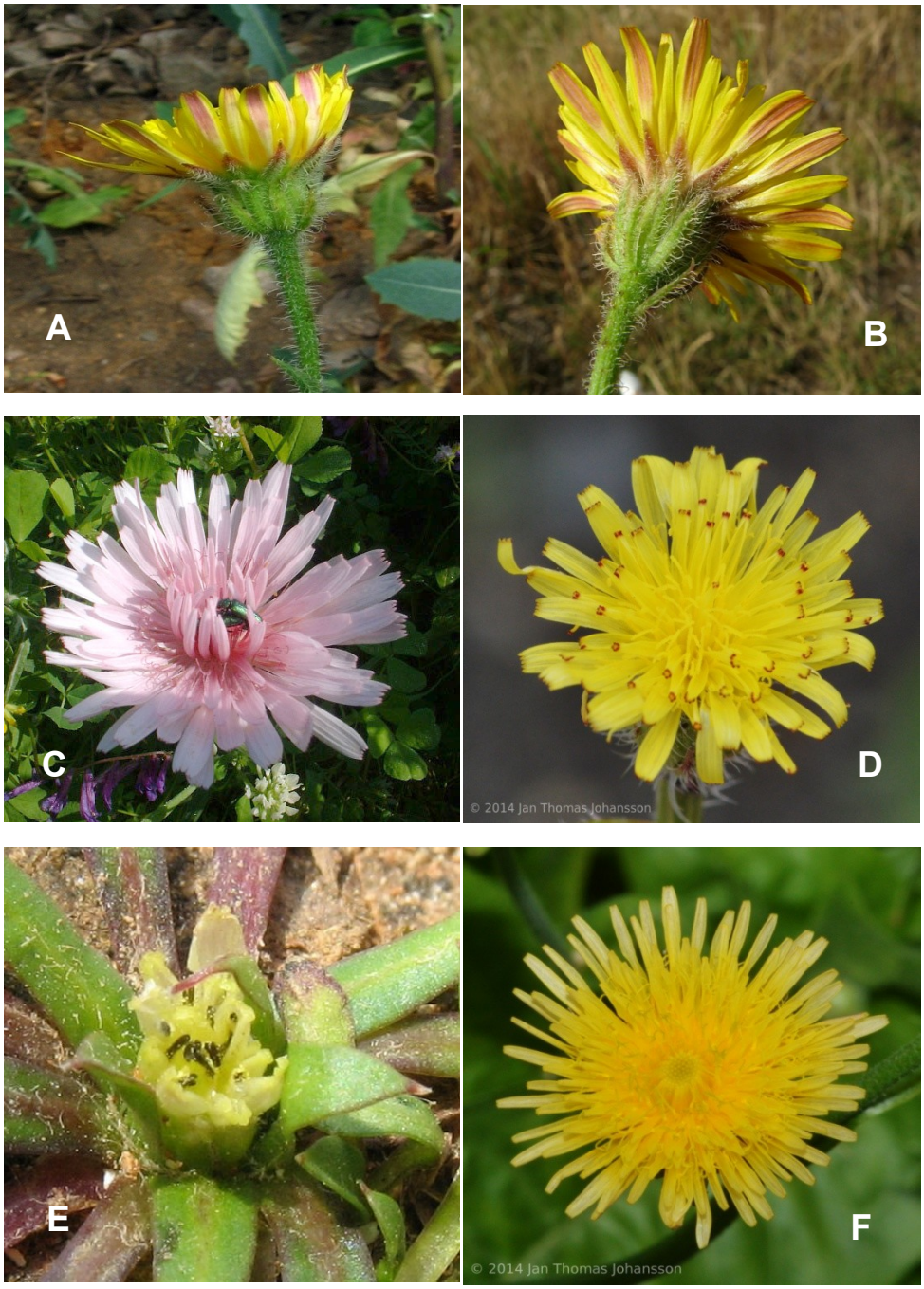
"Ignorance will come to an end when everything is presented as it actually is, and when knowledge about everything is available to each person in the manner that suits him or her."

Muammar Al-Gaddafi from The Green Book, 1976

Dedication

TO my father, mother and husband



Crepis L. species' photos: A) *C. foetida* L., Photographer: Michael Kesl, 2010 on http://www.biolib.cz; B) *C. foetida ssp rhoeadifolia* (M. Bieb.) Čelak., Photographer: Jiří Kameníček, 2011 on http://www.biolib.cz; C) *C. rubra* L., Photographer: Vojtěch Herman, 2001 on http://www.biolib.cz; D) *C. zacintha* (L.) Loisel., Photographer: Thomas Johansson, on http://angio.bergianska.se; E) *C. Pusilla* (Sommier) Merxmueller., Photographer: Mifsud S, 2010 on maltawildplants.com; F) *C. alpina* L., Photographer Jan Thomas Johansson, on http://angio.bergianska.se

Contents

Abbreviations

DNA	Deoxyribonucleic acid
°C	Degree Celsius
µl	Microgram
BGBM	Botanical Garden and Botanical Museum
bp	Base pair
BSA	Bovine Serum Albumin
CesA 1b	Cellulose Synthase
CHS	Chalcone Synthase
CNPD	Combined Nuclear and Plastid Dataset
CNPT	Combined Nuclear and Plastid Tree
CNT	Combined Nuclear Tree
cpDNA	Chloroplast DNA
ddH_2O	Double distilled water
DHS	Deoxyhypusine synthase
dNTPs	Deoxyribonucleotide triphosphate
ETS	External transcribed spacer
gsh1	Gamma-glutamylcysteine synthetase
HGT	Horizontal gene transfer
ILS	Incomplete lineage sorting
ITS	Internal transcribed spacer region
KCl	Calcium chloride
LCNGs	Low copy nuclear genes
matK	Maturase K
MCL	Maximum composite likelihood
MEGA	Molecular evolutionary genetics analysis
$MgCl_2$	Magnesium chloride
min	Minute
ML	Maximum Likelihood
mM	Mill Molar
MP	Maximum Parsimony
mtDNA	Mitochondrial DNA
NCBI	National Centre for Biotechnology Information
ndhF	NADH dehydrogenase F
nDNA	Nuclear DNA
ng	Nonogram
nrDNA	Nuclear ribosomal DNA
PCR	Polymerase Chain Reaction
PgiC	Phospho glucose isomerase
PhyC	Phytochrome C
pmol	Picomole
psbA-trnH	Photosystem II protein D1 and (tRNA-His) gene
QG8140	ADP-ribosylation factor
rbcL	Ribulose bisphosphate carboxylase large chain

rDNA	Ribosomal DNA
RNA	Ribo nucleic acid
Rpb2	RNA polymerase II
rpoB	RNA polymerase, beta subunit
rpoC1	RNA polymerase beta' chain
rps16	Ribosomal protein s16
sec	Second
shmt	Glycine hydroxymethyltransferase
SOC	Super Optimal Brot
sqs	Squalene synthase
Ta	Annealing temperature
TBR	Tree-bisection-reconnection
TOPO6	Topoisomerase VI
trnT-trnL	tRNA-Thr and tRNA-Leu (trnL) gene
U	Unit
UV	Ultra Violet

Glossary

Achene	A one-seeded, dry, indehiscent fruit with seed attached to fruit wall at one point.
Allele	Alternate forms of a gene.
Annual	Living one year or less.
Bract	Modified, usually reduced, a leaf in the inflorescence.
Capitulum	The head of the inflorescence, which forms of a disc of sessile flowers, the youngest at the centre.
Caudex	A short, thick, vertical or branched perennial stem.
Diploid	An organism or cell possess double the basic haploid number of chromosomes.
Exon	The coding sequence of a gene (in eukaryotic cells only), which translated into proteins
Floret	The small flowers are making up a composite flower head.
Fusiform	Spindle-shaped
Genotype	The genetic features that determine the structure and function of an organism.
Haploid	Having a single set of unpaired chromosomes, as in a germ cell
Herbs	Soft plant with short period growth and does not have a woody stem.
Inflorescence	The flowers arrangement on a plant.
Intron	A non-coding sequence within a gene of eukaryotes.
Involucre	A group of bracts surrounding the inflorescence.
Ligulate	Own the limb of the corolla in strap-shaped.
Locus	The specific location on the chromosome.
Pappus	Scaly or bristly calyx in the Asteraceae
Perennial	Living more than two years.
Pollen	Fine grains represent a male gametophyte
Population	A number of individuals of the same species live in a particular geographical area and have the ability of interbreeding.
Rhizome	A horizontal underground stem.
Shrub	A perennial plant with many woody branches usually without a single trunk.
Paleaceous receptacle	Palea-like
Glabrescent	Becoming devoid of hairs
Scapiform	Resemble a scape

Summary

The genus *Crepis* L. belongs to the family *Asteraceae* Martinov, tribe *Cichorieae* Lam. & DC. Babcock (1947b) divided the genus into 27 sectional ranks. A revised infrageneric classification based on molecular tools (Enke & Gemeinholzer 2008, Enke 2009) maintained 21 of Babcock's 27 sections but revised the circumscription of some of them. Section *Barkhausia* Moench with 12~14 species revealed to be monophyletic, however, incongruences between nuclear and plastid markers were recorded. The actual analysis was conducted to remove this ambiguity, detangle the evolutionary relationships of *Crepis* section *Barkhausia*, and to find out if these relationships are the result of reticulate evolution via hybridization across lineages or of incomplete lineage sorting. In most Angiosperms, the chloroplasts are maternally inherited, while the nuclear genome can be indicative of hybridization events. To reconstruct the phylogeny within section *Barkhausia*, the low-copy nuclear markers *gamma*-glutamylcysteine synthetase (*gsh*1) and squalene synthase (*sqs*) and the chloroplast markers ribosomal protein S16 (*rps*16) and maturase K (*matK*1) were analysed, also in combination with the multicopy nuclear internal transcribed spacer (ITS) of the previous studies mentioned above. 12 *Crepis* species which belong to section *Barkhausia* were analysed, and *Hispidella hispanica* L. was used as an outgroup. Maximum Parsimony and Maximum Likelihood algorithms and reticulation and network analyses were used to analyse different datasets (all markers, concatenated, and reduced).

The analysis revealed the low-copy nuclear markers *gsh1* and *sqs* to be of multi-copy origin. The different multiple copies within individuals were indicative of different evolutionary scenarios, which complicated the phylogenetic reconstruction and resulted in weak or non-resolved phylogenetic relationships. Four and five different copy types among species were identified in *sqs* and *gsh1*, respectively, and recombination analyses confirmed locus activities without recombination among the different copies. Careful analysis by hand resulted in copy sorting among individuals for phylogenetic analysis.

The comparison between the maternally inherited chloroplast markers and the nuclear markers, which are indicative for hybridization, showed topological disagreements, which confirms the earlier findings of Enke & Gemeinholzer (2008). The patterns are indicative of reticulate evolution, which can be due to incongruences resulting from gene duplication or incomplete lineage sorting (like *C. foetida spp. commutata* and *C. alpina*; *C. kotschyana* and *C. triasii*), and / or to hybridization (like *C. rubra* and *C. tybakiensis*; *C. pusilla* and *C. zacintha*). These patterns point to very recent speciation events in the *Crepis* species analysed in the current study. In combined nuclear and plastid tree analyses, *C. triasii* clustered paraphyletic to the outgroup taxon *Hispidella hispanica* and rendered *Crepis* species section *Barkhausia* in its current circumscription polyphyletic.

Zusammenfassung

Die Gattung *Crepis* L. gehört zur Familie der *Asteraceae* Martinov, Tribus *Cichorieae* Lam. & DC. Babcock (1947b) unterteilte die Gattung in 27 Sektionen. Eine revidierte infragenerische Klassifikation, die auf molekularen Werkzeugen basiert (Enke & Gemeinholzer 2008, Enke 2009), behielt 27 der 21 Sektionen von Babcock bei, veränderte aber die Abgrenzungen einiger Sektionen. Die Sektion *Barkhausia* Moench mit 12 - 14 Arten erwies sich als monophyletisch, allerdings wurden Inkongruenzen zwischen nukleären Markern und Plastidenmarkern festgestellt. Die vorliegende Analyse diente dazu, diese Unklarheiten zu beseitigen, die evolutionären Beziehungen der *Crepis*-Sektion *Barkhausia* zu entwirren und herauszufinden, ob diese Beziehungen das Ergebnis von retikulärer Evolution durch linienübergreifende Hybridisierung oder von unvollständigem lineage Sorting sind. Bei den meisten Angiospermen werden die Chloroplasten maternal vererbt, während das nukleäre Genom Hybridisierungsvorgänge anzeigen kann. Um die Phylogenie innerhalb der Sektion *Barkhausia* zu rekonstruieren, wurden die nukleären low-copy-Marker *Gamma*-Glutamylcystein-Synthetase (*gsh*1) und Squalen-Synthase (*sqs*) sowie die Chloroplastenmarker ribosomales Protein S16 (*rps*16) and Maturase K (*matk*1) analysiert, auch in Kombination mit dem nukleären multi-copy internal transcribed spacer (ITS) aus den o. g. vorhergehenden Studien. 12 zur Sektion *Barkhausia* gehörende *Crepis*-Arten wurden analysiert, und *Hispidella hispanica* L. wurde als Außengruppe verwendet. Maximum-Parsimony- und Maximum-Likelihood-Algorithmen sowie Retikulations- und Netzwerkanalysen wurden durchgeführt, um verschiedene Datensätze zu analysieren (alle Marker, verknüpft und reduziert).

Die Analyse deckte auf, dass die nukleären low-copy-Marker *gsh1* and *sqs* einen multi-copy-Ursprung haben. Die verschiedenen multiplen Kopien innerhalb der Individuen deuteten auf verschiedene evolutionäre Szenarien hin, was die phylogenetische Rekonstruktion verkomplizierte und zu schwachen oder ungeklärten phylogenetischen Beziehungen führte. Vier bzw.

fünf verschiedene Kopie-Typen zwischen den Arten wurden für *gsh1* bzw. *sqs* identifiziert, und Rekombinationsanalysen bestätigten Lokusaktivitäten ohne Rekombination zwischen den verschiedenen Kopien. Durch sorgföltige Analysen per Hand wurden die Kopien für die phylogenetische Analyse auf die Individuen verteilt.

Der Vergleich zwischen den maternal vererbten Chloroplastenmarkern und den Hybridisierung anzeigenden nukleären Markern zeigte topologische Unstimmigkeiten, was die früheren Ergebnisse von Enke & Gemeinholzer (2008) bestätigt. Die Muster deuten auf retikuläre Evolution hin, die beruhen kann auf Inkongruenzen aufgrund von Genduplikation oder unvollständigem lineage Sorting (wie *C. foetida spp. commutata* und *C. alpina*; *C. kotschyana* und *C. triasii*), und / oder auf Hybridisierung (wie *C. rubra* und *C. tybakiensis*; *C. pusilla* und *C. zacintha*). Diese Muster weisen auf sehr kürzlich erfolgte Speziationsprozesse in den in der vorliegenden Studie untersuchten *Crepis*-Arten hin. In den kombinierten Analysen von nukleärem Baum und Plastidenbaum gruppierte sich *C. triasii* paraphyletisch zur Außengruppe *Hispidella hispanica* und machte die *Crepis*-Sektion *Barkhausia* in ihrer derzeitigen Abgrenzung polyphyletisch.

1. Introduction

1.1. The Organism (*Crepis* L.)

1.1.1. Family, Tribe, Genus

The genus *Crepis* L. is assigned to the family *Asteraceae* Martinov (= *Compositae* Gisseke), which comprises herbs or shrubs, rarely trees. The family can be easily recognised by their inflorescence, which is a capitulum of radial with or without tubular florets. In total, the *Asteraceae* comprises 1.532 genera and 23.790 species, and it is considered as the largest family of flowering plants (Singh, 2010). The genus *Crepis* L. belongs to the tribe *Cichorieae* Lam. & DC (the old name is *Lactuceae* Cass.). This tribe is characterised by ligulate florets which are commonly five lobed and by milky juice, and it includes more than 98 genera and 1550 species (Kilian, 2009). Under the tribe *Cichorieae*, there are fourteen sub-tribes, one of them is *Crepidinae*, which was re-recognized by Kilian et al. (2009) with 22 genera (including *Crepis*) and approximately 360 species (Cichorieae Portal, 2016).

The genus *Crepis* L. (hawk's beard) is considered to be the second largest genus in the tribe and attracted the attention of Ernest Babcock (1939), who employed species of this genus as model plants in plant genetic investigations. The beginning was in 1905 when H. O. Juel discovered that *Crepis tectorum* L. has only four pairs of chromosomes. Afterwards, in 1909, O. Rosenberg supported Joel's discovery and found out that *Crepis capillaries* L. has just three pairs of chromosomes. Therefore, the genus *Crepis* has been intensively studied at karyological level (Dimitrova et al., 1999) based on the character of having low numbers of easily recognisable chromosomes.

Babcock classified 196 species of the genus *Crepis* into 27 sectional ranks based upon morphological, karyological and geographic distribution; Babcock went further with assumptions about evolution and phylogeny. In his sectional system, *Crepis* species were divided into three groups, namely primitive, intermediate, and advanced. He proposed primitive species to possess some of the following characters: Perennial's life cycle, rhizome, woody caudex, large lyrate-pinnatifid leaves, tall, robust stems, few large flowers-heads; the involucre poorly differentiated into outer and inner bracts, large florets, baked or fusiform achenes; and stiff pappus-bristles. In contrast, advanced species

according to Babcock are small and annual, with dissected leaves; low, slender stems; small heads; few and small involucres; small florets; and soft pappus-bristles achenes. The intermediate group shares primitive and advanced characters. Babcock's investigations indicate that the genus *Crepis* is a monophyletic group (Babcock, 1947a) based on similarity in morphological, karyological and geographical features.

Recent studies to reinvestigate Babcock's evolutionary hypotheses were carried out by Enke & Gemeinholzer (2008). In that research, molecular tools were conducted to test Babcock's evolutionary hypotheses of *Crepis*. The genus was investigated at the level of gene sequences. The Internal Transcribed Spacer region (ITS) and the Maturase K (*matK*) region were a target to reveal the relationships within the genus. The results highlight that *Crepis* is polyphyletic and can be divided into three well-supported clades. Furthermore, the molecular results disagree with most of Babcock's sectional system as well as his hypotheses about character evolution (Enke & Gemeinholzer, 2008). Also, Enke (2009) re-evaluated the available results from previous and recent studies in connection with micromorphological characteristics such as pollen, achenes, and pappus to set a limit of the classification of *Crepis*. However, this revision maintains 21 sections of Babcock's 27 sections, which are represented in Enke's 17 sections and render *Crepis* a paraphyletic taxon.

1.1.2. *Crepis* Species Section *Barkhausia* (Moench.) Enke

12~14 *Crepis* species are assigned to the *Barkhausia* section based on molecular data (Enke & Gemeinholzer, 2008; Enke, 2009). However, investigations on the Internal Transcribed Spacer (ITS) demonstrate this group is monophyletic, but the plastid gene Maturase K (*matK*) depicts at least three independent lineages of this group.

Crepis species section *Barkhausia* includes eight species from section *Hostia* (Babcock's sections), namely *C. foetida* L. *ssp. foetida*, *C*. *foetida. ssp. commutata* (Spreng.) Babc., *C. foetida. ssp*. *rhoeadifolia* (Bieb.) Celak., *C. foetida.* L. *ssp. afghanistanica* Babc., *C*. *foetida spp*. *thomsonii* Babc., *C. rubra* L., *C. tybakiensis* Vierh., *C.alpina* L., *C*. *kotschyana* Boiss. Besides, it includes two taxa from section *Zacintha* (Babcock's sections), namely *C. zacintha* (L.) Loisel., and *C. pusilla* (Sommier) Merxm., and one species from section *Berinia*, namely *C. triasii* (Cambess.) Nyman. According to ITS phylogeny tree, subspecies of *C. foetida* are assembled, while *C. rubra* seems to be a sister group, but the relationship of *C. tybakiensis* is not resolved yet. Also, the clustering of *C. alpina* with *C. kotschyana* in one subclade is not well supported, and only the sister relationships between *C. zacintha* and *C. pusilla* is well supported.

1.1.3. Geographic Distribution of *Crepis* species sect. *Barkhausia*

Crepis species section *Barkhausia* distribute everywhere in Asia, Europe, Africa, North America, and South America. The majority is found in the Mediterranean area. In particular, *C. foetida* species are native to Algeria, Morocco, Cyprus, Iran, Iraq, Lebanon, Syria, North Caucasus, Palestine, Jordan, Saudi Arabia, Turkey, Albania, Austria, Baleares, Belgium, Bulgaria, European Russia, Corsica, Czechoslovakia, France, Germany, Great Britain, Greece, Hungary, Italy, Crete, Crimea, Netherlands, Poland, Portugal, Romania, Sardinia, Sicily, South European Russia, Spain, Switzerland, Turkey-in-Europe, Ukraine, and the former Yugoslavia. However, *C. rubra*

overlaps some areas with *C. foetida*; for example Turkey, Bulgaria, France, Greece, Italy, Crete, and the former Yugoslavia. Also, species of *C. alpina* share some distribution ranges with *C. foetida* areas such as Iran, Iraq, Lebanon, Syria, North Caucasus, Italy, Turkey, Crimea, Spain, and Ukraine.

On the other hand, *C. kotschyana* is considered to be an endemic plant to temperate Asia, which represents the following areas: Afghanistan, Gulf States, Iran, Iraq, Lebanon, Syria, Palestine, Jordan, Tadzhikistan, Turkey, Uzbekistan, and Pakistan. *C. zacintha* distributes well in Cyprus, Lebanon, Syria, Palestine, Turkey, Bulgaria, Corsica, France, Germany, Greece, Italy, Crete, Crimea, Sardina, Sicily, Spain, Ukraine, and the former Yugoslavia, while the species of *C. pusilla* is restricted to Turkey, Cyprus, Greece, Baleares, Crete, but doubtful in Libya. However, the two species, *C. tybakiensis* and *C. triasii* are endemic to Crete and the Baleares subsequently (Cichorieae Portal, 2015), (Figure.1.1).

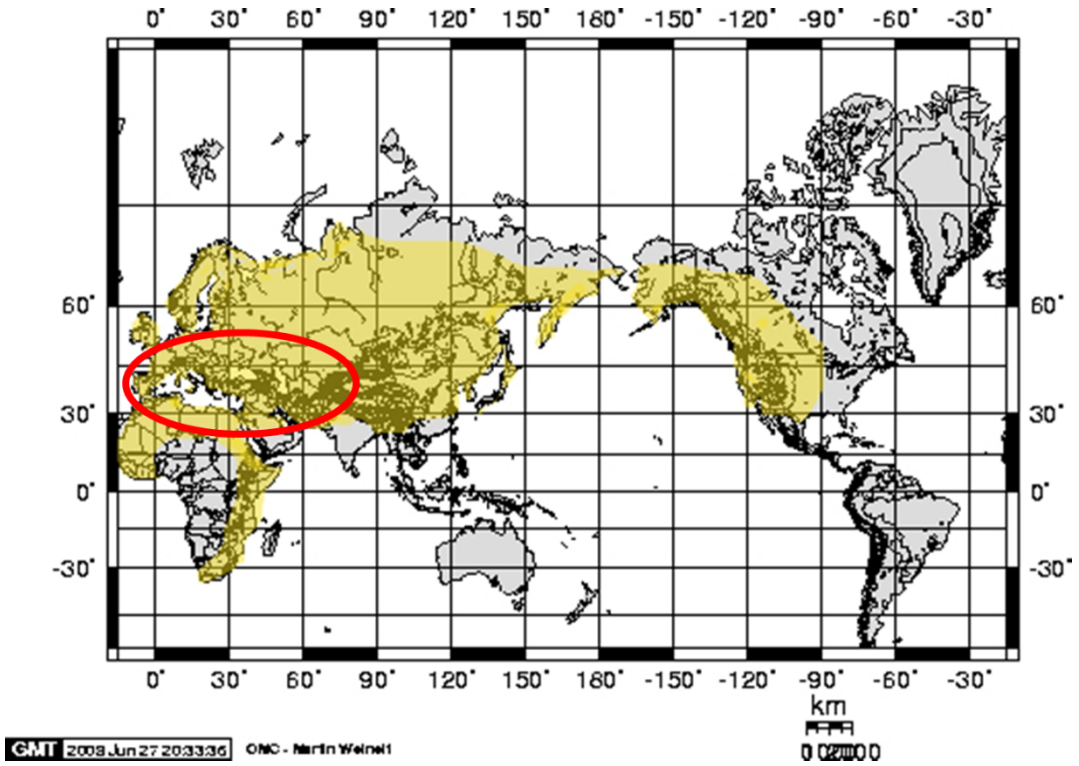


Figure1.1 Worldwide distribution of *Crepis*, red circle refers to *Crepis* species section *Barkhausia* distribution (Enke and Gemeinholzer, 2008).

1.1.4. Taxonomic Treatment of *Barkhausia* Name

The name *Barkhausia* was applied to many taxonomic ranks through revisions of the genus *Crepis.* The name of *Barkhausia* has been given to the genus, subgenus and section levels as a taxonomic rank. Earlier, Moench (1794) was the first one to describe *Barkhausia* as a genus, and it comprised *C. alpina* L. and *C. rubra* L. Then, Lessing (1832) supported Moench's findings and added *C. foetida* L. to the two mentioned species in *Barkhausia*. After that, De Candol and Alexander (1838) assigned five taxa to the *Barkhausia* genus as follows: *B. alpina* DC (= *C. alpina* L.), *B. rubra* (L.) Moench, *B. zacintha* Margot and Reut, *B. foetida* (L.) DC., and *B. rhoeadifolia* Bieb. Then, Boiss (1846) added *B. kotschyana* (=*C. kotschyana* (Boiss.) Boiss.). Later, Kock (1850) classified *B. elata* (= *C. alpina* (L.) DC.) and *B. hirta* (=*C. commutata* (Spreng.)), and *B. rhoeadifolia* var. *hispissima* (=*C. foetida* ssp. *rhoeadifolia* (M. Bieb.) Celak.) under the genus *Barkhausia*. Sequentially, Costa (1861) erected *B. balerarica* Costa (=*C. triassi* (Cambess.) Nyman).

On the other hand, Bentham and Hooker (1873) treated *Barkhausia* as a subgenus of the genus *Crepis*. They included *C. foetida* L. and *B. alpina* DC (=*C. alpina* L.) into the subgenus *Barkhausia*. However ,the name *Barkhausia* name as a section level has been suggested in many classification systems. For example, Bischoff (1851) included *C. foetida* L. and *C. rubra* L into *Crepis* section *Barkhausia*. Also, Hoffmann (1889) assigned *C. alpina* L. to section *Barkhausia* of the genus *Crepis*. Since the species under the genus *Crepis* L. do not feature many evolutionary important and morphologically discriminatory characters, they have been lumped and splitted frequently. For instance, Fiori and Giulio (1903-1904) in the Flora Analitica D Italy, recognised *Barkhausia* as a section of the genus *Crepis* which comprised *C. rubra* L., *C. foetida* L., and *C. alpina* L.

Babcock and Cameron (1934) revised the genus *Crepis* and divided it into three subgenera, namely, *Barkhausia*, *Eucrepis* and *Catonia*. According to this review, the subgenus *Barkhausia* comprised 45 Mediterranean *Crepis* species, which included 34 annuals and 11 perennials.

Recently, Enke and Gemeinholzer (2008) erected the name of *Barkhausia* to the *Crepis* species which clustered in one monophyletic clade based on the molecular data (ITS). However, the monophyletic clade comprised of 12~14 *Crepis* species, namely *C*. *foetida. ssp. foetida* L.*, C*. *foetida. ssp. commutata* (Spreng.) Babc., *C. foetida. ssp. rhoeadifolia* (Bieb.) Celak.*, C. foetida. ssp. afghanistanica* Babc., *C. foetida spp. thomsonii* Babc., *C. rubra* L., *C. tybakiensis* Vierh., *C.alpina* L., *C. kotschyana* Boiss., *C. triasii, C. zacintha* (L.) Babc., and *C. pusilla* (Sommier) Merxm. Enke (2009) suggested the name of *Barkhausia* to this clade as a section rank. However, plastid marker (*matK*) showed that at least three independent lineages made this group lack the monophyletic origin.

1.2. The Methods

Zuckerkandl and Pauling (1965) were the first scientists who draw attention to the importance of macromolecules in the evolutionary studies. In their paper, they proposed a potential phylogenetic usage of macromolecular sequence data. The differences between these sequences point to genetic variation as a result of molecular evolution (Patwardhan et al., 2014). Three different types of DNA exist in higher plant cells: chloroplast DNA (cpDNA), nuclear DNA (nDNA) and mitochondrial DNA (mtDNA).

1.2.1. Chloroplast DNA (cpDNA)

Chloroplast DNA (cpDNA) is a circular molecule which is haploid, less or non-subjected to recombination, and uniparentally inherited, mainly maternally in angiosperms. Further, it comprises coding genes and non-coding regions (intron and intergenic spacer sequences). Besides, it exists in multicopy per chloroplast (Dong et al., 2012 and small et al., 2004). Ordinarily, the evolutionary rate of the genome varies; chloroplast genome evolves at a slower rate than nuclear genome due to a low mutation rate. Besides, the genome itself exhibits an essential rate of variation; coding regions develop slower than non-coding regions (Wolfe et al., 1987). Thus, coding gene sequences (*e.g., matK, rbcL, and ndhF*) were used extensively at the family rank and higher taxonomic levels for phylogenetics (Chase et al.,1993; Soltis et al., 1999). In particular, the *rbcL* region was applied to inter and intragenic levels, and in some cases, they were beneficial (Xiang et al., 1993). However, non-coding regions like introns and intergenic spacer (*e.g. rpS16, trnK, trnL*, *trnT-trnL*, and *psbA-trnH*) were also applied to the lower taxonomic level, such as genus and species levels (Sang et al., 1997a; Small et al., 1998).

Plenty of research on the chloroplast regions (*matK, rbcL, rpoB, rpoC1, and trnH-psbA*) have been extensively applied to unveil Intra- and intergeneric relationships within the tribe *Cichorieae*. (*e.g.,* Baldwin et al .,1995; Enke & Geminholzer, 2008; Wang, Ze. et al., 2013; Wang, G. et al., 2014 and Peng et al., 2014). However, insufficient resolutions were obtained while using cpDNA to construct phylogenetic relationships of close related taxa because of the low

evolutionary rates of cpDNA. Due to its constant rate of nucleotide substitution, cpDNA is a suitable tool to infer the evolutionary history at the family level and higher taxonomic levels (Clegg et al., 1997). However, as cpDNA genome in angiosperms is maternally inherited, the information from it indicates only half the story of evolutionary history. Thus, in the state of hybridization or allopolyploidization, it cannot be a useful tool (A'varez and Wendel, 2003).

1.2.2. Mitochondrial DNA (mtDNA)

While mtDNA has been extensively employed in Zoology, in Botany, its use was limited in plant systematics (Caputo, 1997). Though, recently, the usage of mtDNA has become widely prevalent in phylogenetics and population genetic studies because of the rapid development of methods and techniques (Patwardhan et al., 2014). However, due to the slow rate of point mutations and high rates of rearrangement, it is unsuitable to infer evolutionary relationships in plants (Palmer et al., 2000).

1.2.3. Nuclear DNA (nDNA)

Based on the regions chosen, nDNA can be a useful tool to infer evolution history of the plant (Caputo, 1997). The Internal Transcribed Spacer (ITS) of the nDNA has been widely adopted in plant systematics (Small et al., 2004). It is characterised as biparentally inherited, having different rates of evolution and attributed to appear in both introns and exons within the same gene.

1.2.3.1. Nuclear Ribosomal DNA (nrDNA)

NrDNA codes for the ribosomal RNA. In eukaryotes, nrDNA consists of two elements in tandem arrays; the first component represents 5S rDNA genes and intergenic spacers while the 18S-5.8S-26S rDNA cistron forms the second element. Also, nrDNA has some features which make it a useful tool in phylogenetic reconstructions, such as biparentally inherited, simple structured, easily isolated and repeated multi-copies (Small et al., 2004).

For many years, internal transcribed spacer (ITS) has been widely used in plant systematics, in particular for phylogenetic inference at generic and infrageneric levels, due to higher evolutionary rates of ITS sequences (A'lvarez and Wended, 2003). Consequently, a single sequence of all copies and arrays will be produced by PCR and will be directly sequenced (Small et al., 2004). However, incomplete concerted evolution leads to sequence divergence inside and between arrays, and then different copies of nrDNA can be generated (Hillis and Dixon,1991; Wendel et al., 1995).

Moreover, various copies of nrDNA make it eligible to detect hybrid and allopolyploid origins (Krak et al., 2013). ITS sequences provide a significant degree of divergence and a better understanding of the evolutionary mechanism (e.g., Samuel et al., 2003; Kim et al., 2007; Enke and Gemeinholzer, 2008; Wang, Z. et al., 2013; and Peng et al., 2014). Recently, in *Compositae*, few attempts were made to involve the flanking External Transcribed Spacer (ETS) of nrDNA in order to support ITS sequences (*e.g.,* Urbatsch et al., 2003; Plovanich and Panero 2004; and Li et al., 2012).

1.2.3.2. Low-Copy Nuclear Genes (LCNGs)

Low-copy nuclear genes have the potential to enhance and resolve plant phylogenetic reconstructions at all taxonomic levels, in particular when the resolutions via cpDNA and nrDNA are unsatisfied (Sang, 2002). Currently, high numbers of contemporary literature have shed light on the significance of low-copy nuclear markers as a substitute for compensating constraints of cpDNA

and nrDNA (e.g. Sang, 2002; Álvarez and Wendel, 2003; Small et al., 2004; Steel et al., 2008; and Krak et al., 2013).

Many distinctive characters characterise LCNGs. It is biparentally inherited, exhibits a variation in sequences, and undergoes only few homogenization (concerted evolution). Herein, the use of LCNG to identify parental donors of suspected hybrids or polyploids is beneficial (Small et al., 2004). Moreover, successful achievements have been gained to reconstruct allopolyploid and hybrid origins based on LCNG in plants (*e.g.*, Brysting et al., 2007; Brassac et al., 2012; and Krak et al., 2013).

However, their phylogenetic utilities were affected by gene duplication or deletion (paralogous), incomplete lineage sorting (ILS), and genetic drift (Sang, 2002). Particularly, LCNGs are appropriate to detect the evolutionary history of a closely related group with hybridization and polyploidization events (Small et al., 1998; and Sang & Zhang, 2000).

Recently, some studies have involved the LCNG to investigate the phylogenetic relationships, whether to obtain a sufficient resolution of examined taxa or to test their utility. For example, Brassac et al. (2012) involved LCNG region *TOPO6*, to infer progenitor relationships of *Hordeum* L. (*Poaceae*) polyploids including all *Hordeum* species while covering the geographic distribution of the species. However, they were able to detect the ancestral lineages successfully. As another example, LCNGs, namely phosphoglucose isomerase (*PgiC*), phytochrome C (*PhyC*), and RNA polymerase II (*Rpb2*), were used by Russell et al. (2010) in diploid and tetraploid species of *Polystachya* Hook. (*Orchidaceae*), and they were successful to illustrate the reticulation evolution using network analyses. In the preliminary analysis for the *Senecifoneae* (*Asteraceae*), A´lvarez et al. (2008) tested the utility of four LCNGs, namely cellulose synthase (*CesA 1b*), deoxyhypusine synthase (*DHS*), putative ADP-ribosylation factor (*QG8140*), and Chalcone synthase (*CHS*). They found that the two LCNG (*DHS* and *QG8140*) were the most promising ones in phylogenetics. Furthermore, the use of *PhyC* gene in the phylogenetic reconstruction of *Hypericum* L.

(*Hypericaceae*) was beneficial, and Meseguer et al. (2014) proposed that *PhyC* marker could be an alternative to ITS.

1.3. The Evolution Factors

Evolution is a change in the genetic components of biological populations from generation to generation, which results in new species. Biological processes are involved in evolution, such as natural selection and adaptation, mutations, gene drift, gene flow, hybridization, and geographic isolation (Hall and Hallgrímsson, 2013). The latter mentioned ones leads to speciation events, and there are additional modes of speciation (homoploid / polyploidy), which are of no significance in the following work. These factors act independently or combinedly to impact the frequency of alleles among populations (Ellstrand, 2014).

1.3.1. Natural Selection and Evolution

Darwin in 1859 published his book "*The Origin of Species"* which proposed that natural selection to be the primary cause of evolutionary changes. Also, the natural selection theory suggested that all species are descended from common ancestors. However, the theory of evolution according to Charles Darwin concentrates on adaptation which consists of features that reinforce the survival or reproduction of organisms. Since that time, many scientists have conducted experiments to agree or disagree with Darwin's theory (Barton, 2010, and Futuyma, 2009).

In 1944, Babcock conducted extensive experiments on the genus *Crepis,* and he came out with some findings that agree with Darwin's view. Although natural selection is considered to be the substantial factor in biological processes such as speciation and adaptive divergence (Fisher, 1930), new views of geographic speciation have declined the importance of selection in speciation (Schneider, 2000).

1.3.2. Mutation

Mutation is commonly known as any change in the genetic material (DNA or RNA) of an organism. These changes lead to variations, and without mutation, there is no evolution (Barton et al., 2007). Because genes control all functions and development phases, mutations can give rise to changes in the structure of an encoded protein and hence lead to disorder in the organism (Lodish et al., 2000). However, mutations can result from errors during DNA replication or other types of damage to the DNA (Chen et al., 2014).

There are different systems to classify mutations; in general, the latter may occur at the chromosome, gene or molecular level. Likewise, mutations can be discovered in the nucleus, mitochondria and plastids (in plants). Nowadays, mutations can be recognised at the DNA sequence level; **Base Substitutions** and **Base Additions** or **Deletions** are types of mutation at the DNA sequence level (Griffiths et al., 1999). Therefore, the genetic variations are a result of mutations, but not all of these variations are expressed in the phenotype, namely when mutations occur in noncoding DNA regions (introns) or when silent mutations happen in coding DNA regions (exons). In the case of silent mutations, the alteration of nucleotide substitutions does not alter the amino acid sequence of the polypeptide chain (Griffiths et al., 1999).

Understanding how new species arise in nature, is a great challenge to biologists. However, many evolutionary theories have been proposed to comprehend the mechanism of evolution. For example, Hugo de Vries (1909) suggested the mutation theory of evolution; he mentioned that new species do not emerge from gradual accumulations of small variations (Darwin Theory), but rather via the existence of a permanent and abrupt change in character which is unpredictable. Hence, he explained that as a mutation; furthermore, he mentioned the importance of polyploidy and chromosomal rearrangements in plant speciation. Recently, new genomic data support his theory of the origin of species by changes in chromosomes (Nei and Nozawa, 2011).

1.3.3. Gene Drift

Genetic Drift (Allelic Drift) is a change in the genetic structure of a population; because of random events such as floods, earthquakes or fires. Which leads to variations in the allele frequencies, the frequency of an allele at a particular locus in a population, over time which influences its survival (Gillespie, 1998). The impact of genetic drift seems to be smaller in bigger populations and larger in small populations because of the last exhibits a lower number of individuals and a smaller gene pool. Consequently, it may lead to a decrease in gene variabilities and thereby limits the genetic variability (DeBenedictis, 2014).

Genetic Drift is divided into two types. The first category is the **Population Bottleneck,** which is a sudden diminution in the size of a population, usually by catastrophic environmental events, and may result in the loss of alleles and a decrease in genetic diversity (Falk and Kent,1991). The second type is the **Founder Effect,** which happens when a small group of a population splits off from the origin population and forms a new population. Moreover, the new population lacks the genetic diversity of the original one because of isolation factors. Thus, the likelihood that some genetic traits become more widespread in the population increases. Thereby, the variation in genetic frequencies between the original population and the new one may lead to a substantial divergence over the course of time, and the two populations can eventually be distinguished genetically and phenotypically (Hedrick, 2011). Founder speciation, which is a speciation event through the effects of genetic drift on reproductive isolation, has been suggested to interpret the origin of new species (Matute, 2013). For instance, recent research by Mayol et al. (2012), who studied the genetic variability in *Crepis triasii*, an endemic and isolated plant of Balearic Island, documented the importance of genetic drift in structuring the genetic variations. Noteworthy, not only genetic drift, but, also mutations, natural selection, and gene flow are involved in this divergence (Hedrick, 2011).

1.3.4. Gene Flow

Gene flow (gene migration) is a transfer of alleles from one population into another. Gene flow can happen through the movement of whole organisms or transfer of genomes from one population to another (Slatkin, 1985). However, the insertion of new alleles increases the variability within a population and allows new combinations of features. In plant communities, the movement of genes takes place via the successful fertilisation or via the migration of seeds among populations (Ellstrand and Elam, 1993). Gene flow usually occurs within species, but some studies demonstrated the movement of alleles among distant species, which is called Horizontal Gene Transfer, HGT (Ferris et al., 1983). Also, small amounts of gene flow are sufficient to prevent opposing mutation, drift, and selection in a population. Also, gene flow levels at isolation distances of hundreds to thousands of meters are repeatedly high enough to counteract genetic drift (Ellstrand, 2014)

The movement of genes can result in the homogenization of the genotype pool among populations, as a consequence preventing the genetic diversity (Slatkin, 1985). However, the significance of gene flow has been debated among evolutionists. Numerous studies of plant dispersal using pollen or seed measurements to trace the movements of individuals have been done (*e.g*. Hamilton and Miller, 2002; Hu and Ennos, 1999). Increases in population size and a decrease in the fraction of interpopulation mating can indicate gene flow rates (Ellstrand and Elam, 1993; and Ellstrand, 2014). Usually, the abiotic environment acts as a physical barrier to gene flow, such as mountains, oceans, or deserts.

1.3.5. Hybridization

Hybridization is the interbreeding of two individuals of different genotypes. It can occur between individuals of the same species, which is called **Intraspecific Hybridization**, or between members belonging to different

species (**Interspecific Hybridization**). Additionally, **Intergeneric Hybridization** was proved in the *Cichorieae* tribe (Ono, 1943; Ono and Sakai, 1952). However, the progeny produced via hybridization can be fertile, partially fertile, or sterile. Detailed researches on hybridization under natural circumstances have demonstrated that repeated back-crossing (**Introgression**) of the hybrids to one or both parents is a common phenomenon. Thereby, introgressive hybridization increases the variability in the participating species (Edgar, 1949). Mostly, interspecific hybrids cannot cross back with the parental species. Sometimes, sterile interspecific hybrids can undergo a doubling of their chromosome set and become fertile tetraploids, with four sets of chromosomes.

There are two types of origin of hybrid lineages -homoploidy (i.e., diploid derivatives) and polyploidy. Apparently, polyploidy is the most popular mode in plants (Soltis and Soltis, 1995). However, various cases of diploid level speciation were indicated for flowering plants (Rieseberg and Wendel,1993). In general, hybridization has an influence on speciation process and evolution (Abbott et al., 2013). Also, molecular phylogenetic investigations might be a convenient tool to trace fingerprints of hybridization (Linder and Rieseberg, 2004). Furthermore, contradictions between plastid and nuclear phylogenies were explained as a result of hybridization (Fehrer et al., 2007).

1.4. The Objectives

Recently, three low-copy nuclear markers, namely gamma-glutamylcysteine synthetase (*gsh1*), squalene synthase (*sqs*), and glycine hydroxymethyltransferase (*shmt*), were developed to resolve relationships at low taxonomic levels of the *Asteraceae* family with emphasis on the sub-tribe *Hieraciinae* (Krak et al., 2012).

This study aims at improving the interpretation of the evolutionary history of *Crepis* species section *Barkhausia*, thereby focusing on detecting hybrid origin within this closely related species. Low-copy nuclear markers (*gsh1* and *sqs*), and the plastid marker ribosomal protein S16 (rps16) in combination with ITS and *matK1* from the previous study will be employed to achieve the following goals:

- Investigate nuclear markers to identify species as candidates that may have contributed to the hybrid derivative lineage within *Barkhausia*.
- Examine the plastid marker ribosomal protein S16 (*rps16*) in combination with (*matK1*) to investigate if they are congruent or incongruent with nuclear markers.
- Reconstruct the phylogenetic relationships of the species to investigate if recombination events led to the difference between nuclear and chloroplast phylogenies of the section *Barkhausia*.
- Inspect the strengths and limitations of low-copy nuclear markers, *gsh1* and *sqs*, in the genus *Crepis.*

2. Materials and Methods

2.1. Plant Materials and DNA Samples

Crepis species section *Barkhausia* do not grow in Germany, however, Tutin reported in Flora Europe (1964-1993) that *C. zacintha* grows in Germany, but no evidence of collected samples has been documented yet. Silica-gel dried leaves of *C. triasii* as herbarium specimen were kindly provided by the Botanical Garden in Barcelona (Spain), while *C. kotschyana* and *C. foetida spp commutata* were supplied via the Albrecht-von-Haller-Institute for Plant Sciences, Georg August University Goettingen. However, *C. foetida spp. thomsonii and C. zacintha* were provided by the Botanical Bavarian State Collection. Besides, additional DNA samples were gained from a previous study (Enke & Gemeinholzer, 2008), and also from the DNA Bank at the Botanical Garden and Botanical Museum Berlin (BGBM; Droege et al., 2014). More information about plant materials and DNA samples are provided in the Appendix; Table 2.1.

2.2. Chemicals, Solutions, and Equipment

A list of chemicals, enzymes, and other materials is given in Table 2.3. Also, a list of buffers and solutions is shown in Table 2.4, and the instruments and laboratory materials used for laboratory analysis are presented in Table 2.5 and Table 2.6 respectively, in the Appendix.

2.3. DNA Extraction

DNeasy Plant Mini Kit was used to extract the total genomic DNA from dried herbarium specimen, and DNA extraction was carried out following standard instructions.

2.4. Agarose Gel Electrophoresis

Agarose gel electrophoresis was used to separate and estimate the size of DNA fragments and PCR products. These fragments could be easily analysed by visualising the gel with UV light and a gel imaging device. The images were

displayed on a computer connected to a camera, and then the size of the bands of interest was compared with the size of markers loaded on the same gel. In the current study, agarose gel electrophoresis was employed to check the DNA extraction, the PCR results, and the PCR cloning products (Jeppsson et al., 1979). Gel electrophoresis was prepared using 2% agarose with the standard protocol. After that, 2µl Sybr gold stain was added to 3µl of each DNA sample; then the mixture was injected into the pores. The gel was exposed to UV light to visualise the DNA bands.

2.5. PCRs Amplifications

Two low-copy nuclear genes, gamma- glutamylcysteine synthetase (*gsh1*) and squalene synthase (*sqs*), and the plastid gene, ribosomal protein S16 (*rps16*), were amplified in this study. Some missing sequences of *Crepis* species section *Barkhausia* of the regions maturase K1 (*matK*1), and Internal Transcribed Spacer (ITS) were also amplified. For all mentioned regions, Polymerase Chain Reactions (PCRs) with specific primers were performed to amplify the fragments of interest. Details about primers and their sequences are given in Table 2.2, p.23.

2.5.1. Gamma-glutamylcysteine Synthetase (*gsh1*)

The region *gsh1* was amplified using the primers GSH-4668F and GSH-6683R for PCR and GSH-4668F and GSH-HR3 for semi-nested PCRs (Krak et al., 2012). The reaction volume of each PCR was 20 µl. Containing 1 µl of DNA (12.5 - 50 ng), 2.00 µl Taq Buffer including KCl, 2.00 µl of dNTPs (each 2mM; Fermentas), 0.50 µl of each primer (10pmol/µl), and 0.10 µl Taq DNA polymerase (5U/ µl; Fermentas). The reaction mixture also contained 2.4 µl $MgCl_2$ (3 mM) and 11.50 µl ddH_2O for PCR and 1.20 µl $MgCl_2$ (1.50 mM) and 12.70 µl ddH_2O for semi-nested PCR, respectively.

2.5.2. Squalene Synthase (*sqs*)

The primers SQS-3122F, SQS-5560R, SQS-FH2, and SQS-5560R (Krak et al., 2012) were used to amplify the *sqs* region. The PCR reaction volume was 12.5 µl, containing 5.05 µl ddH_2O, 1 µl of DNA (12.5 - 50 ng), 1.25 µl 10x Dream Taq Buffer, 1.25 µl of dNTP's (each 2mM; Fermentas), 0.50 µl of each primer (10pmol/µl), 0.25 µl BSA (10ng/µl), 2.50 µl Betain Monohydrate (5mM), and 0.20 µl Dream Taq polymerase (5U/ µl; Fermentas). PCRs amplifications for both regions *gsh1* and *sqs* were carried out using Touchdown PCR *Ta* (55°C to 45°C) following the protocol of Krak et al. (2012).

2.5.3. Internal Transcribed Spacer (ITS)

ITS region of *C. foetida. spp. commutata* was amplified in two overlapping parts using the primers ITS-A and ITS-C (Blattner, 1999) for ITS 1, and ITS-D, ITS-B (Blattner, 1999) for ITS 2. PCRs were done in a reaction volume of 12.5 µl, including 5.05 µl ddH_2O, 1.0 µl of DNA, 1.25 µl 10x Dream Taq Buffer, 1.25 µl dNTP's (each 2mM; Fermentas), 0.50 µl of each primer (10pmol/µl), 0.25 µl BSA (10ng/µl), 2.50 µl Betain Monohydrate (5mM), and 0.20 µl Dream Taq polymerase (5U/ µl; Fermentas). PCRs were conducted using the following profile:

Steps	Temperature [°C]	Time
1	94	4 min
2	94	1 min
3	52	30 sec
4	72	1 min
	40 cycles	
5	72	7 min
6	8	hold

However, rest of the taxa were downloaded by NCBI centre and are provided with the GenBank number in the Appendix (Table.2.1.1).

2.5.4. Ribosomal Protein S16 Intron (*rps16*)

The primers rps-F and rps-R2 (Oxelman et al., 1997) were employed to amplify the region of the ribosomal protein S16 (*rps16*). The reaction volume was 12.5 µl, including 7.80 µl ddH2O, 1 µl of DNA, 1.25 µl 10x Dream Taq Buffer, 1.25 µl dNTP's (each 2mM; Fermentas), 0.50 µl of each primer (10pmol/µl), and 0.20 µl Dream Taq polymerase (5U/ µl Fermentas). A gradient PCR was performed under the following conditions:

Steps	Temperature [°C]	Time
1	94	4 min
2	94	30 sec
3	57-62	40 sec
4	72	50 sec
	35 cycles	
5	72	10 min
6	8	hold

2.5.5. Maturase K (*matK1*)

The region of *matK*1 was amplified using the primers *trnk-710*F (Johnson and Soltis, 1995) and *matk-IR* (Fehrer et al., 2007). The reaction volume was 12.5µl, containing 1µl DNA, 1.25µl 10x Tag buffer including KCl (Fermentas), 1.25µl dNTP's (2mM), 0. 25 µl of each primer (10pmol/ µl), 0.25µl BSA (10ng/µl), 1.20µl $MgCl_2$ (1.5mM), and 0.40 µl of Tag polymerase (Fermentas). A Touchdown PCR *Ta* (55°C to 45°C) was conducted, following Krak et al. (2012).

After getting DNA bands, the PCR products were examined on a 2.0% agarose gel to test the presence of the DNA amplification and to determine the size of the DNA bands. In most cases, the PCR process was repeated due to difficulties regarding the amplification of low-copy nuclear genes.

Region	Primer Name	Primer Sequences (5 ′ – 3 ′)	Reference
gsh1	GSH-4668-F	CCATGGAGGAGGTTATGTGCAT	Krak et al., 2012
	GSH-6683-R	GTTCCTCAAATACAGGGTCC	
gsh1-seminested PCR	GSH-4668-F	CCATGGAGGAGGTTATGTGCAT	
	GSH-HR3-R	TCCAGAAGCTTCTCTGCTGGAGTT	
sqs	SQS-3122-F	GTTCTCATGGACCAGTTCCA	
	SQS-5560-R	TGTTCCAATCGCCATGATCT	
Sqs- seminested PCR	SQS-HF2-F	CATGTTTCTGCTGCCTTTCTGGAG	
	SQS-5560-R	TGTTCCAATCGCCATGATCT	
ITS1	ITSA	GGAAGGAGAAGTCGTAACAAGG	Blattner, 1999
	ITSC	GCAATTCACACCAAGTATCGC	
ITS2	ITSD	CTCTCGGCAACGGATATCTCG	
	ITSB	CTTTTCCTCCGCTTATTGATATG	
rps16	rpsF	GTGGTAGAAAGCAACGTGCGACTT	Oxelman et al.,1997
	rpsR2	TCGGGATCGAACATCAATTGCAAC	
*matk*1	trnK-710F	GTATCGCACTATGTWTCATTTGA	Johnson and Soltis, 1995
	matK-iR	AAATGCAAAGAGGAAGCATCT	Fehrer et al., 2007

Table 2.2 Primers used for PCR amplifications and their sequences.

2.6. Large Agarose Gel

The Large Gel protocol was conducted to identify different alleles of low-copy nuclear genes (*gsh*1 and *sqs*) using standard procedures.

2.6.1. Gel Extraction

Agarose gel extraction was carried out using QIAquick Gel Extraction Kit (QIAGEN).

2.7. PCR Cloning

Cloning was done using the cloning Kit (Sticky-End Cloning Protocol (Thermo Scientific)) and was only applied to species whose DNA bands were not successfully sequenced.

2.8. PCR Products Purification

The PCR products were cleaned using ExoSAP-IT (Affymetrix) PCR Purification Kit Protocol by adding 5.0 µl of ExoSAP-IT Express reagent (1:10) to 2 µl of each PCR product sample, followed by mixing. Then the reaction mixture was incubated in a thermal cycler at 37°C for 15 min, and after that, it was heated to 80°C for 15 min to inactivate ExoSAP-IT. The purified PCR products were ready for DNA sequencing.

2.9. Sequencing

For both *gsh1* and *sqs* markers, forward and internal reverse primers were used to sequence DNA bands, while the sequencing was done with the same primers which were used to amplify the rest of the examined regions (ITS, *matK1* and *rps16*). However, sequencing was done at Starseq Company (Mainz, Germany).

2.9.1. Sequences Alignments

The sequences were adjusted with Chromas Lite 2.1 (Technelysium Pty. Ltd., Helensvale, Australia) and aligned and edited manually using BioEdit (Hall,1999). Indels were coded as single characters. The sequences of the outgroup taxa and some *Crepis* species were downloaded from NCBI (GenBank, EMBL). A list of species name and GenBank number are supplied in the Appendix; Table 2.1.1.

2.9.2. Function Assessment

Exons regions of both *gsh1* and *sqs* sequences were translated using MEGA 5.2.1 software (Tamura et al., 2011) to verify the function sequences. Also, further blast analyses were done by blasting the sequences in The National Center for Biotechnology Information (NCBI).

2.10. Phylogenetic Reconstruction Trees

Maximum Parsimony (MP) and Maximum Likelihood (ML) trees were reconstructed using MEGA 5.2.1 software (Tamura et al., 2011). All genetic regions were analysed individually. Additionally, the two plastid markers (*rps16* & *matK*1), as well as the three nuclear markers (*gsh1*, *sqs*, and ITS), were combined into one dataset. Then the combined nuclear and combined plastid datasets were concatenated into one dataset to reconstruct the combined nuclear and plastid tree (CNPT). The heuristic search was conducted with 10000 replicates of random addition, and one tree was obtained at each step by stepwise addition option. For Maximum Parsimony analyses, Tree-Bisection-Reconnection (TBR) was performed. A bootstrap analysis of 1000 replicates was done, and the initial trees were obtained by random addition of sequences (10 replicates). The Maximum Likelihood analyses were carried out by applying the General Time Reversible model with the MP tree as an initial tree. The heuristic search was performed with the Neighbor-Joining method to a matrix of pairwise distances using the Maximum Composite Likelihood (MCL) approach.

2.10.1. Phylogenetic Reconstruction Networks (Reticulation)

Reconstruction patterns of reticulate evolution were inferred from three methods based on suggestions of Linder and Rieseberg (2004). The first method is visual, whereby looking for phylogenetic signals of multiple independent loci in the combined data set. Subsequently, the concatenated combined nuclear and plastid dataset was scanned to trace the character

evolution and conflicting signals. For this purpose, an Excel format file of all informative characters was created to count the number of supported characters of taxa relationships, which were demonstrated by the combined tree, and to determine the species taxa which contribute to the conflict.

The second method via reconstructing two trees (combined nuclear and combined plastid trees), and they were reconciled if possible. In the case of reconciliation failure, discordance could be interpreted as a reticulation event, whereas the third approach was based on Splits-based methods. Hybridization Networks analyses have been reconstructed using SplitsTree V.4.14.4. (Huson and Bryant, 2006) and Dendroscope V.3.4.5 (Huson and Scornavacca, 2012). In general, a Newick format file of unrooted trees of the combined nuclear and plastid dataset (CNPD) was created by MEGA 5.2.1 software (Tamura et al., 2011) and has been used as an input file into SplitsTree and Dendroscope software. For, SplitsTree, firstly, Filtered Super-Network was reconstructed from two unrooted MP trees of CNPD, then hybridization network analysis has been done.

3. Results

3.1. Newly Designed Primers

Specific *gsh1* and *sqs* primers for the *Asteraceae* family, in particular for the sub-tribe *Hieraciinae*, were developed by Krak et al. (2012), but did not result in proper amplifications and sequencing for all *Crepis* species. So there was a need to design specific primers for some of the investigated *Crepis* species. For *gsh1,* GSH1F: 5′-TGCAAAAAGTTTTGGACATGA-3′ and GSH1R: 5′-CACCTGTTCTGACAACYTCTGTG-3′ primers were involved in amplifying the region from exon 12 to exon 14, whereas SQSF: 5′-TCGAGTTATCARGAGGCAAT-3′ and SQSR: 5′-TGWTAAACAGTCTTCAATGTGG-3′ primers were used to amplify the *sqs* region from exon 5 to exon 8. The regions lengths to be amplified with the newly designed primers (GSH1F, GSH1R for *gsh1* and SQSF, SQSR for *sqs*) were approximately 410 and 700 base pair (bp), respectively.

3.2. Sequences and Alignment

3.2.1. *Gsh1*

47 *gsh1* sequences could be obtained from 166 sequences of 26 *Crepis* taxa, representing 12 *Crepis* species of section *Barkhausia*. The amplified region ranged from exon 11 to intron 15 (with original primers, Krak et al., 2012) and from exon 12 to exon 14 (with designed primers, this study). In general, the aligned *gsh1* matrix comprised 25 sequences, which derived mainly from the original primers (Krak et al., 2012). *Gsh1* sequences had an average length of 754 bp, and the variable sites comprised on average 162 bp (21.5%). The longest *gsh1* sequence was found in *C. pusilla* with 677 bp, and the shortest one was assigned to *C. triasii* seq.4 with 582 bp (Table 3.1; Figure 3.1)

The GSH1 primers by Krak et al. (2012) and the newly designed specific primers, for *Crepis* species, resulted in sequences with different copies types and different copies number. In general, the majority of species featured more than one copy, except *C. tybakiensis* and *C. foetida ssp. commutata* (Table 3.1).

Two *gsh1* sequences of *C. foetida spp. afghanistanica* comprised 33 different nucleotide substitutions and varied by 11 indels. Quantifying similarity between both sequences by calculating pairwise identity resulted in 86% (Table 3.1). Also, two *gsh1* copies were also discovered in *C. foetida ssp. foetida*; one copy was generated by using original primers and the other by using designed ones. When only the overlapping region of both sequences was compared (from 251-551 bp), the percentage of similarity was 85.9% (see aligned *gsh1* matrix).

The same scenario repeatedly appeared in two additional copies of *gsh1* of the species *C. foetida spp. rhoeadifolia*, *C. foetida spp. thomsonii* and *C. pusilla.* Also in *C. rubra,* two full-length copies could be generated, which diverged from each other by 32 nucleotide positions and by ten indels. Even though the similarity index of both copies was 88.70%, *C. rubra* seq.2 resulted in a closer resemblance to the sequence obtained from *C. tybakiensis* than the other copy of *C. rubra* (*C. rubra* seq.1).

Only one *gsh1* full-length copy of *C. tybakiensis* was obtained. Potential other copies were not discovered in the current study. The *C. tybakiensis* sequence differed from the other *gsh1* sequences in 12 unique nucleotide sites, and it shared one indel with *C. zacintha* (seq.1, seq.2) and *C. pusilla* seq.1 from position 95 to 138 bp. The *C. tybakiensis gsh1* full-length copy shared another indel with *C. foetida spp. rhoeadifolia* seq.1 and *C. foetida spp. afghanistanica* seq.1 from 710 to 717 bp. Even though these indels were not identical, the *gsh1* sequence of *C. tybakiensis* was similar to *C. foetida rhoeadifolia* seq.1 according to identity calculation (86.7%) (Table 3.1; see aligned *gsh1* matrix).

Getting a clear *gsh1* sequence of *C. alpina* was laborious, however, three *gsh1* copies were retrieved via cloning steps. The copies were very similar to each other, but the cloned seq.1 was more characterised than the two others due to 9 single mutations and one indel event, whereas the cloned seq.2 and seq.3 were almost identical and diverged only in two nucleotide positions. Their identity index ranged between 98.40 to 99.50% (Table 3.1; see aligned *gsh1* matrix).

The cloned *gsh1* sequences of *C. kotschyana* expressed two nearly identical copies (ID=99.40%) with just four different nucleotide positions. In particular,

the sequences of *C. kotschyana* were almost identical with *C. triasii* seq.4. The sequences were very different from the ones of all other analysed *Crepis* species from which they diverged by 15 nucleotide positions, due to a deletion from bp 74 to bp 82 that was present in nearly all other *gsh1* copies of the *Crepis* species section *Barkhausia* (see aligned *gsh1* matrix).

Two completely sequenced *gsh1* copies of *C. zacintha* were almost identical (similarity index is 99.1%) and featured four point mutations and two indels. However, these two copies had two indels; the first indel from position 95 to 137 bp, which was also found in *C. pusilla* seq.1 and *C. tybakiensis*. The second one was from position 609 to 646 bp which was only found in *C. pusilla* seq.1 (Figures: .3.2. and 3.2.1).

The *gsh1* sequencing of *C. triasii* resulted in four copies, but only the third and fourth copy covered full lengths. Two received sequences were very similar to each other (seq.1 and seq.2), but quite different from seq.3 and seq.4. The similarity index ranged between 83.50-88.10% (Table 3.1; see aligned *gsh1* matrix).

As it was hard to amplify the *gsh1* region of *C. foetida. spp. commutata* with the GSH1 primers of Krak et al. (2012), the newly designed primers were used for this task, resulting in the amplification of 474 bp in total. The sequence of *C. foetida spp. commutata* was almost identical to the ones of *C. alpina* clones 1, 2, and 3, and that of *C. triasii* seq.3, with 98.3 to 100% similarity (Table 3.1).

3.2.2. *Sqs*

37 *sqs* sequences were retrieved from 81 sequences of 26 *Crepis* taxa representing 12 species of *Crepis* section *Barkhausia*. The amplified regions ranged from exon 4 to intron 8 (with original primers, Krak et al., 2012) and from exon 5 to exon 8 (with designed primer, current study). The aligned *sqs* matrix comprised 22 sequences, and the matrix covered a length of 1035 bp in total, with 293 variable sites (28.3%). *Sqs* gene sequences varied in length and nucleotide composition. The longest *sqs* sequence was found in *C. tybakiensis* seq.1 (932 bp), and the shortest one was detected in *C. triasii* seq.2 with 789 bp (Table 3.2; Figure 3.3). Similar to *gsh1*, different *sqs* copies were retrieved by using different *sqs* primers. The sequencing of *C. foetida spp. foetida* resulted in two distinct copies, with 42 variable sites and 17 indels. Pairwise identity was 84.80% for the corresponding region from 168 to 858 bp. Sequencing of *C. zacintha* and *C. foetida spp. thomsonii* resulted in two copies each, too. The similarity between the two copies was 87.21% in case of *C. zacintha* and 81.50% in case of C. *foetida spp. thomsonii*, due to 45 and 53 variable sites, respectively (Table 3.2, and see aligned *sqs* matrix).

In both *C. tybakiensis* and *C. pusilla*, the sequencing of *sqs* amplified regions yielded three different copies. For *C. tybakiensis*, the pairwise index indicated that seq.1 and seq.2 were more similar to each other than to seq.3. However, the sequence alignment matrix showed that seq.2 included nucleotide sequences, from 595 to 609 bp in intron 7, which resembled the *sqs* region of a particular *Crepis* species group (*C. triasii* seq.3, *C. foetida spp. thomsonii* seq.1, and *C. pusilla* seq.2). This resemblance indicated a PCR recombination event. On the other hand, *sqs* of *C. tybakiensis* seq.3 was expressed in the majority of *Crepis* species (see aligned *sqs* matrix).

Furthermore, the three *sqs* copies of *C. pusilla* were variable as well, and the pairwise index showed that seq.1 was more similar to seq.3 than to seq.2 (Table 3.2). However, in the intron regions, *C. pusilla* seq.1 seemed to be more similar to *C. foetida. spp. foetida* seq.1 than to *C. pusilla* seq.2 and seq.3 (see aligned *sqs* matrix).

Four different *sqs* copies were retrieved for *C. triasii*. The variability among the sequences was high, approximately 108 different nucleotide positions were variable in the final aligned *sqs* matrix. Pairwise similarity calculations indicate that seq.1 and seq.2 were more similar to each other than to any other sequence (ID=94.72%) and featured 22 variable nucleotide positions. In contrast, seq.3 and seq.4 were less analogous to each other than to other sequences (ID=77.94%) and featured 32 various nucleotide sites (Table 3.2; see aligned *sqs* matrix).

For *C. rubra* it was possible to retrieve a full-length *sqs* sequence, which resembled the one of *C. tybakiensis* seq.1 and seq.2. However, *C. foetida spp. afghanistanica*, *foetida spp. commutata, C. foetida spp. foetida* seq.2*, C. foetida spp. rhoeadifolia*, *C. foetida spp. thomsonii* seq.2*, C. kotschyana, C. pusilla* seq.3*, C. tybakiensis* seq.3*, C. triasii* seq.4 and *C. zacintha* seq.2*,* all featured only one *sqs* copy with very little variation.

3.2.3. *Rps16*

19 sequences of the *rps16* intron were obtained from 29 sequences of 25 *Crepis* taxa representing 11 species of section *Barkhausia*. Sequencing of the *rps16* region of *C. foetida spp. thomsonii* was not successful. The final *rps16* matrix comprised 11 sequences with a maximal length of 968 bp, and the variable sites were 55 bp (5.7%). Although *rps16* sequences were homogeneous within *Crepis* species section *Barkhausia*, the length and nucleotide composition between sequences of different species featured slight differences. *Rps16* matrix varied in length due to 7 indels, and the variation in nucleotide composition attributed to point mutations accounted for 55 bp (see aligned *rps16* matrix). In general, *rps16* sequence lengths among all analysed species varied between 940 bp in *C. zacintha* and 885 bp in *C. kotschyana* (Table 3.3 and Figure 3.4)

3.2.4. *MatK1*

Ten sequences of the *matK1* gene were gained for five *Crepis* taxa representing five *Crepis* species of section *Barkhausia* namely: *C. foetida spp. afghanistanica, C. foetida spp. commutata, C. foetida spp. rhoeadifolia, C. kotschyana*, and *C. triasii*. The rest of the species were provided via NCBI under GenBank numbers AJ633141.1; EU363556.1; EU363566.1; EU363575.1; EU363576.1; and EU363579.1. These sequences were partially included in a *matK1* matrix in the present study. Therefore, the *matK1* matrix had a length of 439 bp, and there were 20 variable sites (4.6%). Most of the sequences had the same length but exhibited slight differences regarding nucleotide composition (Table 3.3; Figure 3.5).

Analysed Taxa/Number of Sequence Copies		GSH1 Primers	Identity (ID)%	L in bp	A	C	G	T	G+C%	A+T%
C. foetida ssp. foetida seq.1	2	Krak et al., 2012	85.90	583*	175	78	126	204	34.99	65.01
C. foetida ssp. foetida seq.2		Designed here		253	80	34	47	90	32.02	67.19
C. foetida ssp. afghanistanica seq.1	2	Krak et al., 2012	86.20	617*	195	84	125	213	33.87	66.13
C. foetida ssp. afghanistanica seq.2		Krak et al., 2012		590	182	80	126	202	34.92	65.08
C. foetida ssp. rhoeadifolia seq.1	2	Krak et al., 2012	82.80	600*	185	79	127	209	34.33	65.67
C. foetida ssp. rhoeadifolia seq.2		Designed here		328	72	34	37	95	29.83	70.17
C. foetida ssp. thomsonii seq.1	2	Krak et al., 2012	85.90	556	173	75	113	195	33.81	66.19
C. foetida ssp. thomsonii seq.2		Designed here		314	104	42	65	103	34.08	65.92
C. rubra seq.1	2	Krak et al., 2012	88.70	614*	190	80	127	215	33.71	65.96
C. rubra seq.2		Krak et al., 2012		628*	196	82	132	218	34.08	65.92
C. alpina cloned seq.1	3	Krak et al., 2012	(1 & 2) 98.70 (1 & 3) 98.40 (2 & 3) 99.50	608*	190	80	131	207	34.70	65.30
C. alpina cloned seq.2		Krak et al., 2012		608*	189	77	131	211	34.21	65.79
C. alpina cloned seq.3		Krak et al., 2012		609*	189	78	132	210	34.48	65.52
C. kotschyana cloned seq.1	2	Krak et al., 2012	99.40	617*	81	81	136	218	35.17	64.83
C. kotschyana cloned seq.2		Krak et al., 2012		617*	183	82	134	218	35.01	64.99
C. pusilla seq.1	2	Krak et al., 2012	87.20	677*	191	97	139	250	34.86	65,14
C. pusilla seq. 2		Designed here		243	77	34	37	95	29.22	70.78
C. zacintha seq.1	2	Krak et al., 2012	99.10	663*	191	92	140	240	34.99	65.01
C. zacintha seq.2		Krak et al., 2012		665*	192	93	140	240	35.04	64.96
C. triasii seq.1	4	Krak et al., 2012	(1 & 2) 88.10 (1 & 3) 87.00 (1 & 4) 87.80 (2 & 3) 82.90 (2 & 4) 86.50 (3 & 4) 83.50	524	173	68	104	179	32.82	67.18
C. triasii seq.2		Designed here		367	123	49	78	116	34.60	65.12
C. triasii seq.3		Krak et al., 2012		606*	188	77	131	209	34.32	65.51
C. triasii seq.4		Krak et al., 2012		582*	173	76	125	208	34.54	65.46
C. foetida spp. commutata	1	Designed here	-	474	158	61	99	156	33.76	66.24
C. tybakiensis	1	Krak et al., 2012	-	646*	197	91	134	224	34.83	65.17

Table 3.1. Number, length, primers, pairwise identity, and nucleotide composition of *gsh1* sequence copies of *Crepis* section *Barkhausia*. Asterisks indicate complete sequences. ID was calculated only for corresponding regions of pairwise sequences by BioEdit V.7.1.7 (Hall, 1999).

Analysed Taxa/Number of Sequence Copies		SQS Primers	Identity %	L in bp	A	C	G	T	G+C%	A+T%
C. foetida ssp. foetida seq.1	2	Krak et al., 2012	84.80	*891	290	126	157	318	31.76	68.24
C. foetida ssp. foetida seq.2		Designed here		612	202	85	106	215	31.21	68.79
C. foetida ssp. afghanistanica	1	Designed here	-	618	208	86	109	215	31.55	68.45
C. foetida ssp. rhoeadifolia	1	Designed here	-	665	225	92	123	225	32.33	67.67
C. foetida ssp. thomsonii seq.1	2	Krak et al., 2012	81.50	693	227	97	120	249	31.31	68.69
C. foetida ssp. thomsonii seq.2		Designed here		628	211	86	116	215	32.17	67.83
C. rubra	1	Krak et al., 2012	-	*883	296	127	147	313	31.03	68.97
C. alpina	1	Designed here	-	621	202	83	113	223	31.56	68.44
C. kotschyana	1	Krak et al., 2012	-	*908	308	135	146	319	30.95	69.05
C. pusilla seq.1	3	Krak et al., 2012	(1 & 2) 80.00 (1 & 3) 84.44 (2 & 3) 79.14	*855	263	123	155	314	32.51	67.49
C. pusilla seq.2		Krak et al., 2012		460	160	63	77	160	30.43	69.57
C. pusilla seq.3		Designed here		608	204	85	105	214	31.25	68.75
C. zacintha seq.1	2	Krak et al., 2012	87.21	*852	280	128	152	292	32.86	67.14
C. zacintha seq.2		Designed here		620	208	86	111	215	31.77	68.23
C. triasii seq.1	4	Krak et al., 2012	(1 & 2) 94.72 (1 & 3) 79.65 (1 & 4) 81.41 (2 & 3) 79.65 (2 & 4) 80.80 (3 & 4) 77.94	*872	284	129	146	313	31.54	68.46
C. triasii seq.2		Krak et al., 2012		798	260	115	142	281	32.21	67.79
C. triasii seq.3		Krak et al., 2012		450	159	59	78	154	30.44	69.56
C. triasii seq.4		Designed here		644	218	89	117	220	31.99	68.01
C. foetida spp. commutata	1	Designed here	-	675	228	94	124	229	32.30	67.70
C. tybakiensis seq.1	3	Krak et al., 2012	(1 & 2) 91.8 (1 & 3) 89.10 (2 & 3) 74.10	*932	312	134	156	330	31.12	68.88
C. tybakiensis seq.2		Krak et al., 2012		822	263	119	141	299	31.63	68.37
C. tybakiensis seq.3		Designed here		655	223	91	121	220	32.37	67.63

Table 3.2. Number, length, primers, identity, and nucleotide composition of *sqs* sequence copies of *Crepis* section *Barkhausia*. Asterisks indicate complete sequences obtained. ID was calculated only for corresponded regions of pairs of DNA sequences by BioEdit V.7.1.7 (Hall, 1999).

Marker name	Analysed Taxa	L in bp	A	C	G	T	G+C	A+T
rps16	*C. foetida spp. foetida*	937	313	152	169	303	34.26	65.74
	C. foetida spp. afghanistanica	935	312	151	168	304	34.12	65.88
	C. foetida spp. rhoeadifolia	939	312	135	169	305	34.29	65.71
	C. foetida spp. commutata	936	312	152	167	305	34.08	65.92
	C. tybakiensis	933	311	150	169	303	34.19	65.81
	C. rubra	916	306	148	170	292	34.72	65.28
	C. alpina	894	308	141	162	283	33.89	66.11
	C. zacintha	940	312	153	169	305	34.26	65.64
	C. pusilla	922	311	152	167	292	34.60	65.40
	C. kotschyana	885	297	143	169	276	35.25	64.75
	C. triasii	924	310	149	161	304	33.55	66.45
matK1	Analysed Taxa	L in bp	A	C	G	T	G+C	A+T
	C. foetida spp. foetida	439	140	79	67	151	33.26	66.29
	C. foetida spp. afghanistanica	439	138	81	68	152	33.94	66.06
	C. foetida spp. rhoeadifolia	439	140	79	67	153	33.26	66.74
	C. foetida spp. commutata	439	141	78	68	152	33,26	66.74
	C. tybakiensis	439	140	78	68	153	33.26	66.74
	C. rubra	439	141	78	67	153	33.03	66.97
	C. alpina	436	141	76	65	152	32.80	67.20
	C. zacintha	439	142	79	66	152	33.03	66.97
	C. pusilla	439	141	79	67	151	33.26	66.51
	C. kotschyana	439	139	78	67	155	33.03	66.97
	C. triasii	439	143	78	68	150	33.26	66.74

Table 3.3. The length and nucleotide composition of the *rps16* intron and *matK1* sequences of the analysed *Crepis* species in section *Barkhausia*.

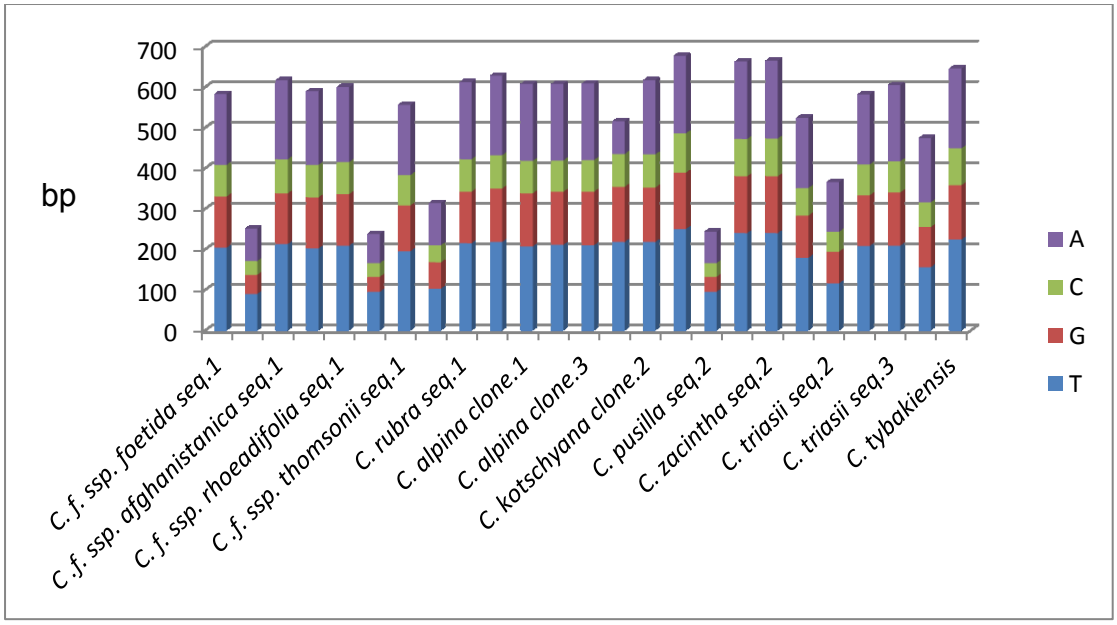


Figure 3. 1. The length and nucleotide composition of *gsh1* sequences of *Crepis* species section *Barkhausia. C. foetida spp. foetida* seq.2, *C. foetida spp. rhoeadifolia* seq.2, *C. foetida spp. thomsonii* seq.2, *C. pusilla* seq.2, and *C. triasii* seq.2 were partially amplified by using the *gsh1* primers designed in this study, while all other sequences were based on the primers by Krak et al. (2012).

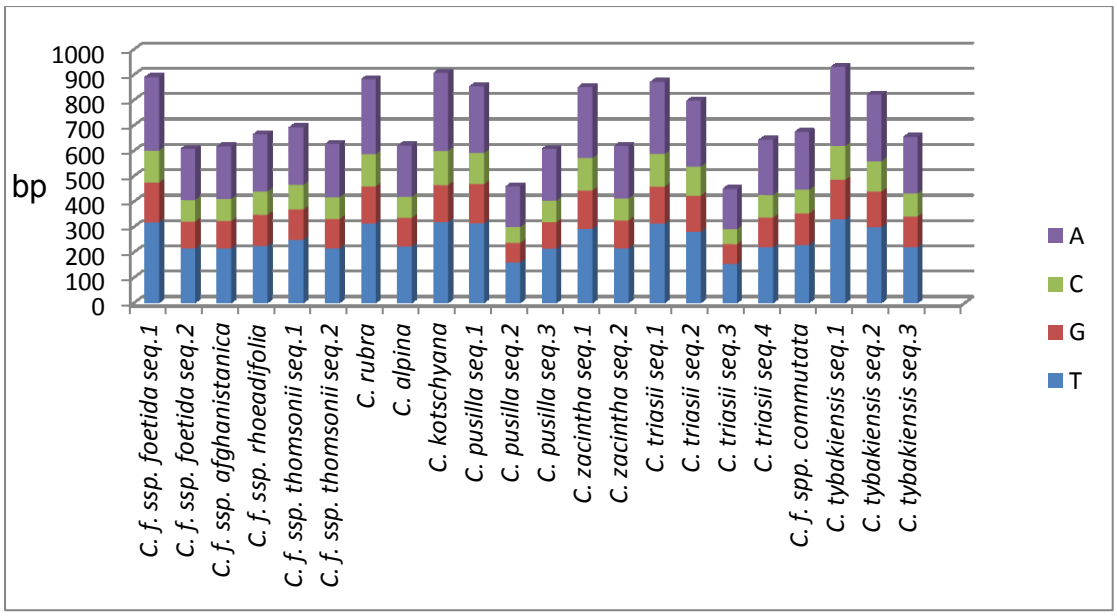


Figure 3.3. The length and nucleotide composition of *sqs* sequence of *Crepis* species section *Barkhausia*. *C. foetida spp. foetida* seq.2, *C. foetida spp. afghanistanica, C. foetida spp. rhoeadifolia*, *C. foetida spp. thomsonii* seq.2, *C. foetida spp. commutata, C. alpina*, *C. pusilla* seq.3, *C. zacintha* seq.2, *C. triasii* seq.4, and *C. tybakiensis* seq.3 were partially amplified by using the *sqs* primers designed in this study, while all other sequences were based on the primers by Krak et al. (2012).

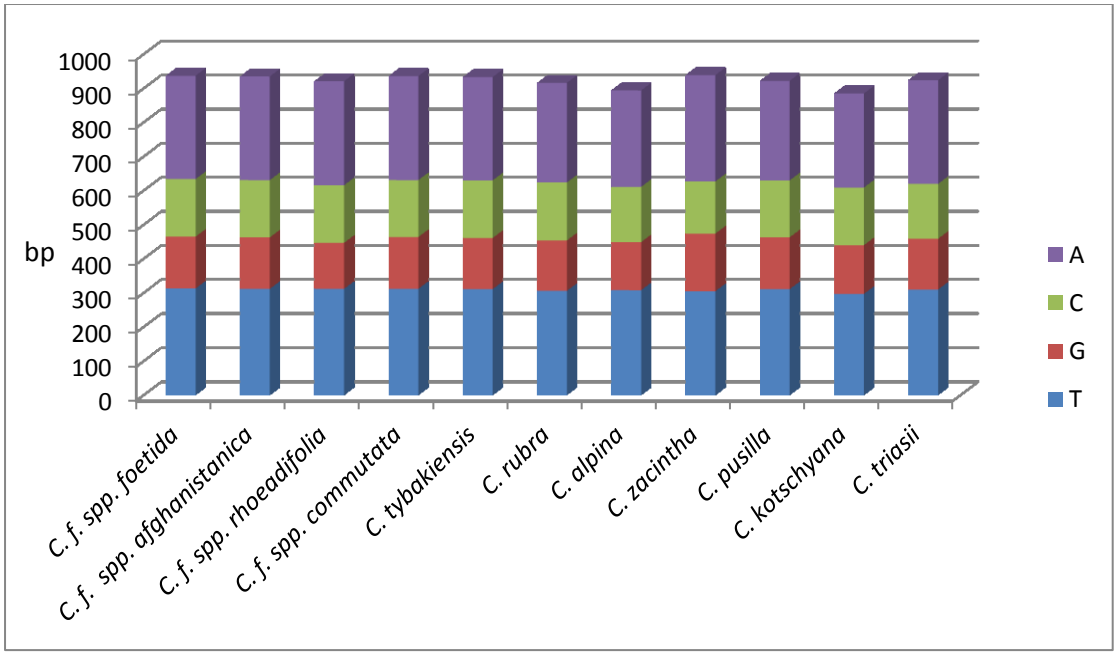


Figure 3.4. The length and nucleotide composition of *rps16* intron sequence of *Crepis* species section *Barkhausia*.

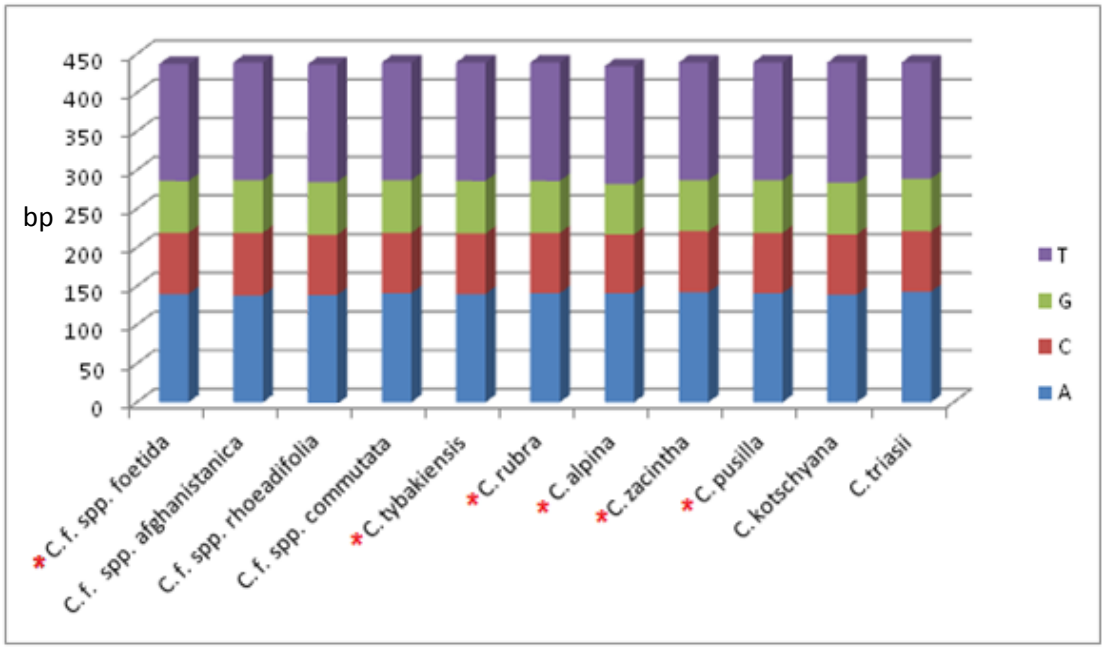


Figure 3.5. The length and nucleotide composition of *matK1* sequence of *Crepis* species section *Barkhausia*. Taxa with red asterisks refer to *matK1* sequences from Enke and Gemeinholzer (2008).

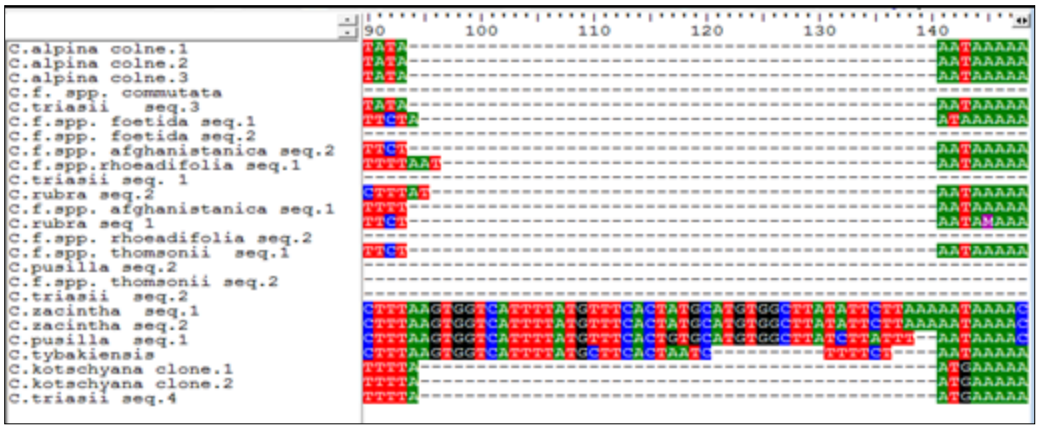


Figure 3.2. The first shared indel among *C. zacintha* seq.1 and seq.2, *C. pusilla* seq.1 and *C. tybakiensis* in the aligned *gsh1* matrix.

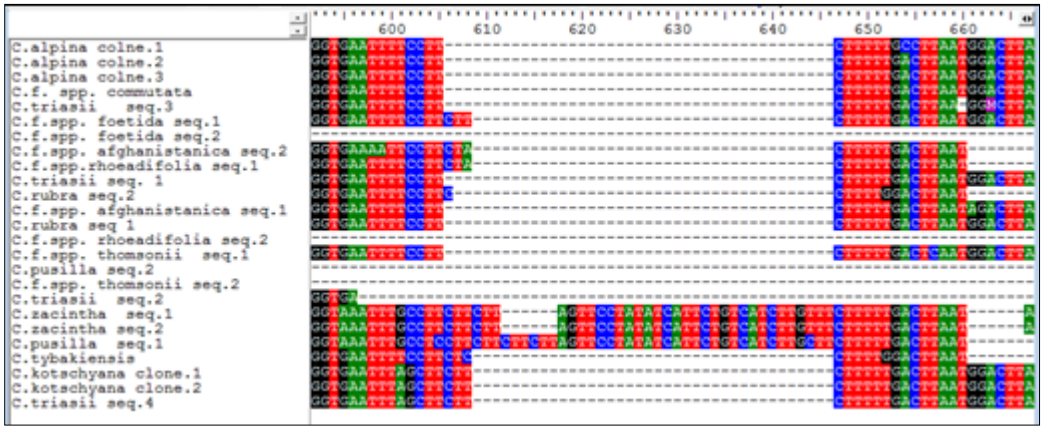


Figure 3.2.1. The second shared indel among *C. zacintha* seq.1 and seq.2, and *C. pusilla* seq.1 in the aligned *gsh1* matrix.

3.3. Phylogeny

3.3.1. *Gsh1*

3.3.1.1. Phylogeny of the Entire *Gsh1* Dataset

26 nucleotide sequences of *gsh1* could be used for the phylogenetic analyses. 25 of these sequences belonged to *Crepis,* and one was retrieved from GeneBank (GenBank Number: HQ131797.1) as an outgroup. The final matrix contained 637 characters, 363 of which were conserved, 265 were variable, and 107 (16.8%) were parsimony-informative. The trees were produced using Maximum Parsimony and Maximum Likelihood methods, and the outputs of both approaches were almost identical in their topology. The MP analysis resulted in five most parsimonious trees (MPTs) with a length of 376 steps. The consistency index was 0.735, which indicated homoplasy, the retention index was 0.871, and the composite index was 0.745 (0.641). The ML tree with bootstrap values from a bootstrap consensus tree, which was inferred from 1000 replicates of five MPTs, is shown in Figure .3.5.

The *Crepis* species clustered in five main clades (Figure 3.5), which were due to five different *gsh1* copy types. Clade 1 to clade 4 (Figure 3.5) featured a common ancestor, but their relationship lacked resolution (yellow area), whereas the fifth clade featured a different ancestor. Hence, the names of *gsh1A, gsh1B*, *gsh1C*, *gsh1D,* and *gsh1E* were given to each clustered group, respectively. However, these five copy types of *gsh1* sequences could be visually recognised in the intron 13 of the aligned *gsh1* matrix (Figure 3.7). All clusters formed a group of very similar or identical sequences of different taxa, and all species except *C. tybakiensis* and *C. foetida spp. commutata* expressed more than one copy. In some cases, these multiple copies within the same species were similar, so they gathered in the same clade, like the copies of *C. foetida spp. foetida*, *C. zacintha*, *C. alpina*, and *C. kotschyana*. In other cases, multiple copies within the same species were different, thus clustering in different clades, such as the copies of *C. foetida spp. afghanistanica*, *C. foetida spp. rhoeadifolia*, *C. foetida spp. thomsonii*, *C. pusilla, C. rubra*, and *C. triasii* (Figure 3.5).

The first clade (*gsh1A*) consisted of *C. foetida spp. foetida* (seq.1 and seq.2), *C. foetida spp. afghanistanica* (seq.2), *C. foetida spp. rhoeadifolia* (seq.1), *C. tybakiensis*, and *C. rubra* seq.2. This clade could be divided into two subclades; the first three species comprised subclade I with good support (96 BS/76ML), while the rest comprised subclade II with also good support (96BS/87ML). However, the relationship between both subclades received a week support (66BS/53ML). Despite having the same *gsh1* copy type sequence, species in this clade had some variations which made them distinguishable and, hence, their affiliations were resolved.

The second clade was *gsh1B* (Figure 3.5) that includes *C. foetida spp. afghanistanica* seq.1, *C. foetida spp. thomsonii* seq.1, *C. triasii* seq.1, *C. pusilla* seq.2, *C. foetida spp. rhoeadifolia* seq.2, and *C. rubra* seq.1. The clade gained strong support (98BS/94ML). The *gsh1* sequences of these six species were very similar to each other, but *C. foetida spp. afghanistanica* seq.1 was the most divergent one. So its linkage to the rest of the species gained a very good support (96BS/87ML). On the other hand, the last four mentioned species formed a subclade, which lacked resolution due to the low level of variations, and *C. foetida spp. thomsonii* seq.1 appeared related to this subclade.

The third clade, *gsh1C* clade, comprised *C. pusilla* seq.1, *C. zacintha* seq.2, *C. zacintha* seq.1, *C. triasii* seq.2, and *C. foetida spp. thomsonii* seq.2. All these sequences had the same *gsh1* copy type with slight differences observed in *C. pusilla* seq.1 and *C. zacintha* seq.1 and seq.2, representing 17 nucleotide substitutions. Like in the second clade, *C. pusilla* seq.1 was the most different, hence, its relationship to the other species received a strong support (99BS/90ML). Also, the lack of variation between *C. zacintha* seq.1, *C. triasii* seq.2 and *C .foetida spp. thomsonii* led to a weak link, attributed to identical sequences.

The fourth clade, *gsh1D* clade, comprised *C. alpina* clones 1, 2, 3, *C. foetida spp commutata*, and *C. triasii* seq.3. The relationships within this clade were not entirely resolved due to the low level of variations, but the clade was strong supported (100BS/99ML). Similar to *gsh1D* clade, the fifth clade,

gsh1E clade, comprised *C. kotschyana* clones 1, 2, and *C. triasii* seq.4. The three sequences were identical and lacked sufficient variation as well. Although this clade was well-supported (100BS/90ML), it appeared to have an independent lineage.

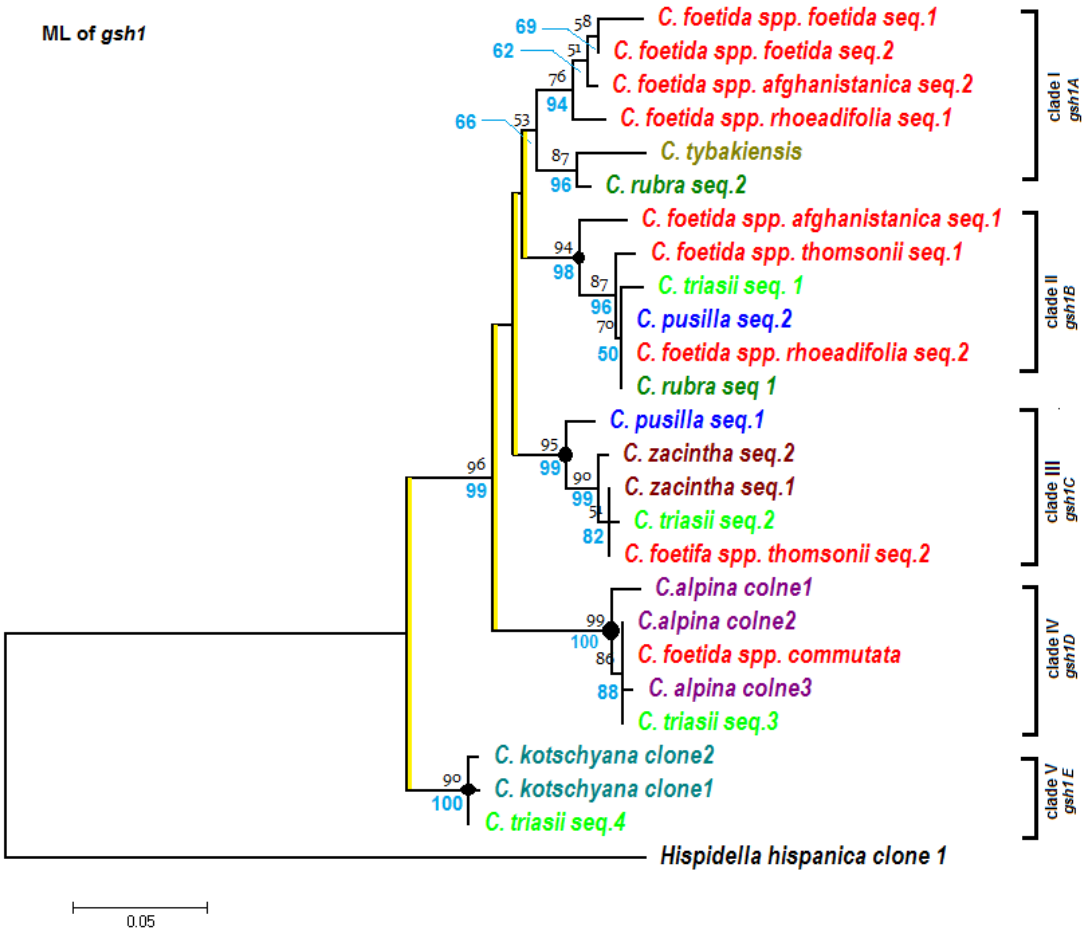


Figure 3.5. Phylogenetic analyses of the *gsh1* dataset. ML tree is presented here with ML calculation above the branches. Bootstrap values from a bootstrap consensus tree, which was inferred from 1000 replicates of five MPTs, are provided below the branches. Only values greater than 50% are shown here. Black circles indicate very similar or identical sequences of different taxa. Yellow areas indicate lack resolution.

3.3.1.2. Phylogeny of the Reduced *Gsh1* Dataset

To investigate how similar copies within a taxon and among taxa affect the topology and branch support, further analyses of the reduced *gsh1* dataset were done. The similarity was minimised as follows: 1) Similar copies within a taxon and among taxa were reduced as much as possible. 2) The copy that represented the species' sequence was chosen based on morphological traits, molecular features, or geographic distributions. For example, *C. triasii* had four different *gsh1* copy types; the *gsh1E* copy was chosen due to its morphological traits, geographical distribution and its position on the ITS tree (Enke and Gemeinholzer, 2008). The same criteria were applied to the other species with different copies. 3) in phylogenetic analysis, partial and full sequences can lead to false affiliations, so only long sequences were included in this analysis.

Hence, *gsh1* phylogenetic analyses of the reduced *gsh1* dataset included the following sequences: *C. foetida spp. foetida* seq.1, *C. foetida spp. afghanistanica* seq.2, *C. foetida spp. rhoeadifolia* seq.1, *C. foetida spp. thomsonii* seq.1, *C. foetida spp. commutata*, *C. rubra* seq.2, *C. tybakiensis*, *C. alpina* clone 3, *C. zacintha* seq.1, *C. pusilla* seq.1, *C. kotschyana* clone 1, and *C. triasii* seq.4 plus *H. hispanica.*

The aligned *gsh1* matrix of the reduced dataset comprised 632 characters, 374 of which were conserved, 253 were variable, and 90 (14.2%) were parsimony informative. MP and ML trees were identical in their overall topologies. For MP analysis, the most parsimonious tree was generated with a length of 338 steps. The consistency index was 0.773, which indicated homoplasy, the retention index was 0.777, and the composite index was 0.692 (0.601). The ML tree is shown in Figure 3.6, and it is supplied with bootstrap values from the most parsimonious tree.

The relationships within in-group were affected by deleting similar and identical *gsh1* copies of different taxa of *gsh1* sequences. However, the external nodes gained more support as well as the internal nodes, and the relationships among four first clades were also resolved, except clade V. Aalso, *C. foetida spp. thomsonii* seq.1 joined *gsh1D* subclade (Figure 3.6). In

general, *gsh1* revealed *Crepis* species section *Barkhausia* to be a polyphyletic group.

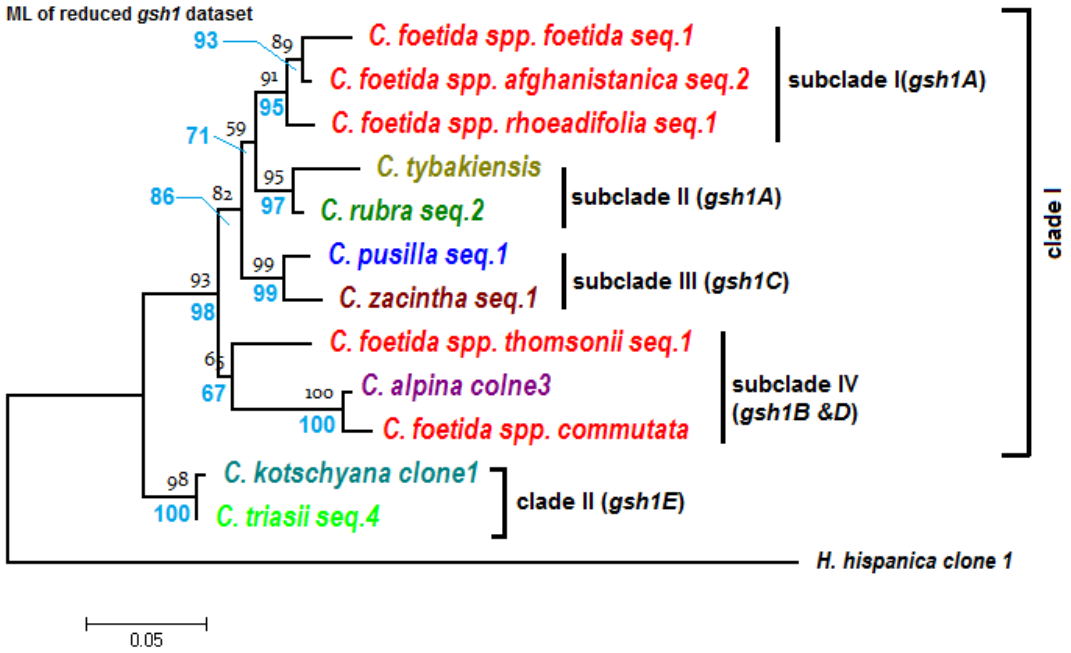


Figure 3.6. Phylogenetic analyses of the reduced *gsh1* dataset. ML tree is presented here with ML calculation above the branches. Numbers below the branches are bootstrap values of the most parsimonious tree. Only values greater than 50% are shown here.

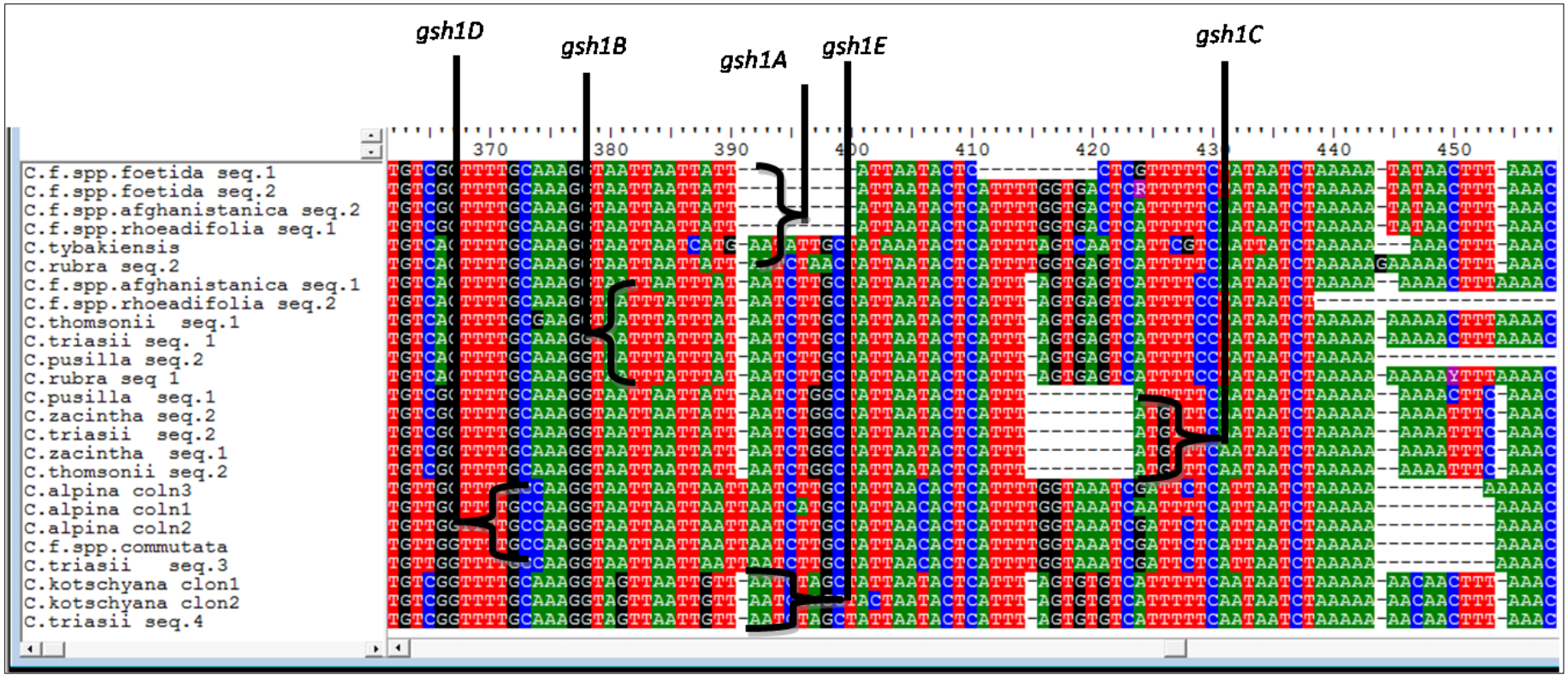


Figure 3.7. Intron 13 (from 378 to 459 bp) of the *gsh1*-alignment of 25 *gsh1* sequences representing 12 *Crepis* species sect. *Barkhausia*

3.3.2. *Sqs*

3.3.2.1. Phylogeny of the Entire *Sqs* Dataset

The analysis involved 23 *sqs* nucleotide sequences (22 of *Crepis* and one was retrieved from NCBI (GenBank Number: JX129602.1). The aligned matrix dataset contained 879 characters, of which 552 were conserved, 319 were variable, and 151 (17.2%) were parsimony-informative. The MP and ML trees for the *sqs*-analysis featured similar topologies. The MP analysis resulted in seven most parsimonious trees of equal length (507 steps). The consistency index was 0.568, which indicated homoplasy, the retention index was 0.734, and the composite index was 0.533 (0.417), all values were lower than the ones for *gsh1*. The ML tree with bootstrap values from a bootstrap consensus tree, which was inferred from 1000 replicates of seven MPTs, is presented in Figure 3.8.

The analysed species clustered in two well-supported paraphyletic clades (Figure 3.8) which were paraphyletic to the outgroup taxon. Clade I comprised four subclades which represented three different *sqs* copy types namely *sqsA*, *sqsB*, and *sqsC*. The relationships within this clade obtained a good resolution of some species, for example, *C. rubra* and *C. tybakiensis* seq.1 and seq.2. appeared related with a moderate support (76BS/83ML), whereas *C. pusilla* seq.1 and *C. foetida spp. foetida* seq.1 gained a good support (86BS/84ML).

In contrast, the second clade (Figure 3.8) contained accessions representing *sqsD* copy sequences lacked resolution. Although this clade received a strong support (100BS/96ML), the relationships of its components were not resolved (polytomy). However, unresolved accessions of *Crepis* species section *Barkhausia* were assigned to possess the same *sqs* copy, *sqsD*, with small differentiations. Intron 7 (Figure 3.10) seems to be decisive for the major branching within the phylogenetic trees.

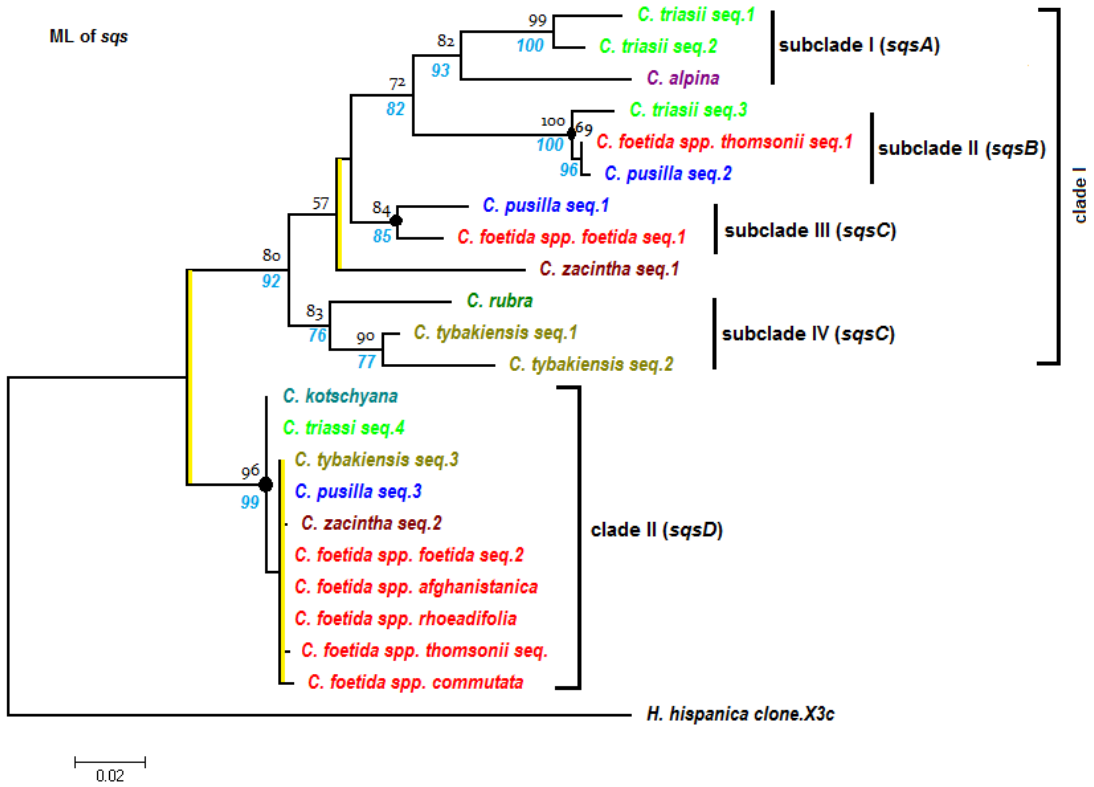


Figure 3.8. Phylogenetic analyses of the *sqs* dataset. ML tree is presented here with ML calculation above the branches. Bootstrap values from a bootstrap consensus tree, which was inferred from 1000 replicates of seven MPTs, are provided below the branches. Only values greater than 50% are shown here. Black circles indicate very similar or identical sequences of different taxa. Yellow areas indicate lack resolution.

3.3.2.2. Phylogeny of the Reduced *Sqs* Dataset

Investigating how similar copies within a taxon and among taxa affect the topology and branch support was done as described in *gsh1*. Consequently, the reduced *sqs* dataset included the following sequences: *C. triasii* seq.2, *C. alpina*, *C. foetida spp. thomsonii* seq.2, *C. pusilla* seq.1, *C. foetida spp. foetida* seq.2, *C. zacintha* seq.1, *C. tybakiensis* seq.1, *C. rubra*, *C. kotschyana*, *C. foetida spp. rhoeadifolia*, *C. foetida spp. afghanistanica*, and *C. foetida spp. commutata*. Due to missing nucleotide information in many species, nucleotide sequences from 1 to 38 and from 754 to 880 bp were excluded. The aligned matrix dataset with coding indels contained 703 characters, 72 of which (10.2%) were parsimony-informative, 204 were

variable, and 489 were conserved. Trees were generated by using MP and ML which were almost similar in their topology. The MP analysis resulted in seven most parsimonious trees (length: 291 steps). The index of consistency was 0.627, which indicated homoplasy, the one of retention was 0.665, and the composite index was 0.535 (0.417). The ML tree is presented here (Figure 3.9) and it is supplied with bootstrap values from a bootstrap consensus tree, which was inferred from 1000 replicates of seven MPTs.

Topologically, the relationships within in-group were not affected by reducing the similarity of the *sqs* copies. Also, the second clade (polytomy) remained not resolved, and its relationship to the first clade could not be identified as well (Figure 3.9). In general, *sqs* revealed *Crepis* species section *Barkhausia* to be a polyphyletic group.

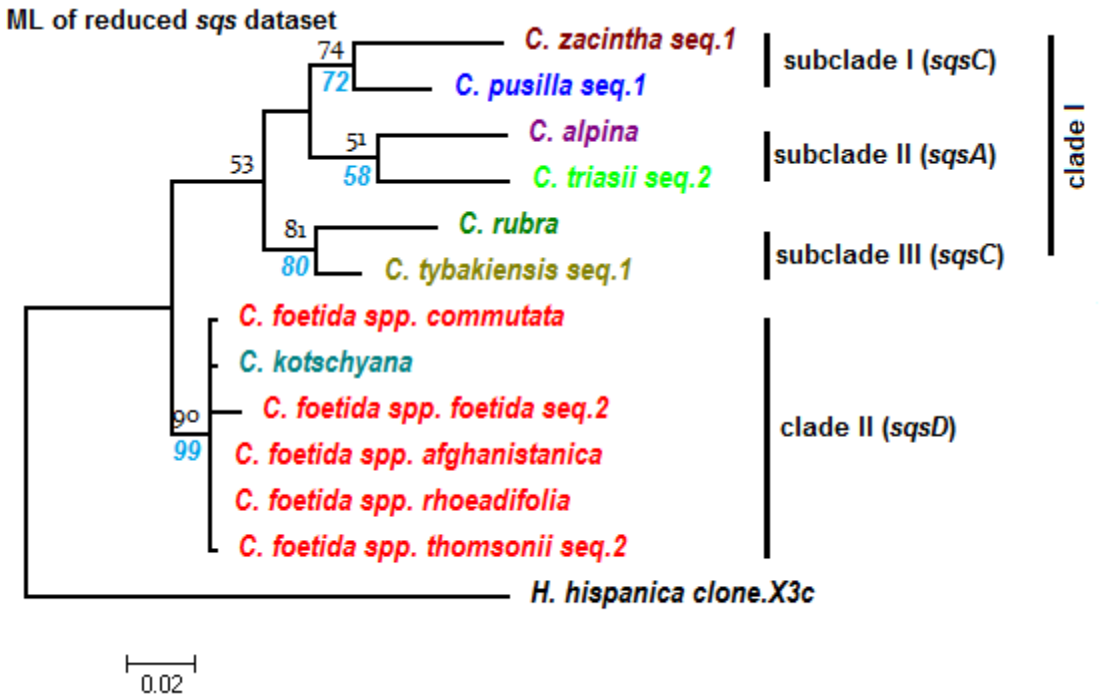


Figure 3.9. Phylogenetic analyses of the reduced *sqs* dataset. ML tree is presented here with ML calculation above the branches. Bootstrap values from a bootstrap consensus tree, which was inferred from 1000 replicates of seven MPTs, are provided below the branches. Only values greater than 50% are shown here.

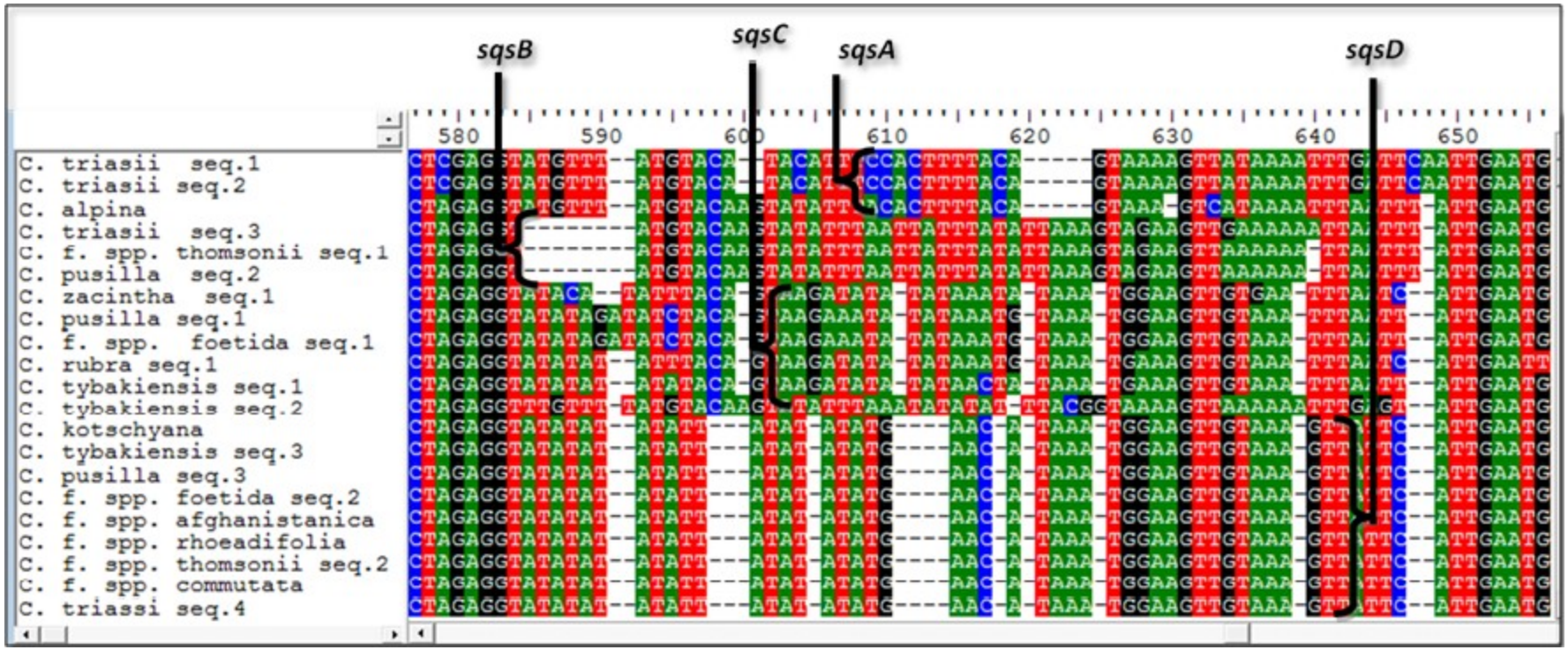


Figure 3.10. Intron 7 (from 583 to 655 bp) of the *sqs*-alignment of 22 *sqs* sequences representing 12 *Crepis* species sect. *Barkhausia.*

3.3.3. *Rps16*

19 sequences of the *rps16* intron could be obtained from 29 sequences of 25 *Crepis* taxa representing 11 species of section *Barkhausia* and one outgroup. We could not amplify *rps 16* sequences for *C. foetida spp. thomsonii*. The aligned matrix 945 characters, 692 of which were conserved, 234 were variable, and 14 (1.5%) were parsimony-informative. The trees obtained by MP and ML were similar in their topology (Figure 3.11). For MP analysis, eight most parsimonious trees with a length of 256 steps were retrieved. The index of consistency was 0.800, which indicated homoplasy, the one of retention was 0.886, and the composite measurement was 0.872 (0.709). The ML tree is presented in Figure 3.11, and it is supplied with bootstrap values from a bootstrap consensus tree, which was inferred from 1000 replicates of eight MPTs.

The tree depicted two clades; Clade I included *C. foetida spp. foetida*, *C. foetida spp. afghanistanica*, *C. foetida spp. commutata, C. tybakiensis*, *C. foetida spp. rhoeadifolia*, and *C. zacintha*. The relationship of the last two mentioned species had a moderate support (72BS/88ML), whereas the other species lacked resolution due to low informative characters. Clade I showed some statistical support (88BS/84ML) attributed to 6 out of 12 informative sites. These characters would be used to classify *rps16* sequences into two types. The first type, *rps16A*, comprising species mentioned above and the second type, *rps16B*, composed of the rest.

However, the second clade, poorly supported, contained *C. rubra*, *C. triasii*, *C. pusilla*, *C. kotschyana* and *C. alpina*. The last mentioned species obtained weak support (68BS/63ML), while the rest of species lacked resolution due to a low level of variations.

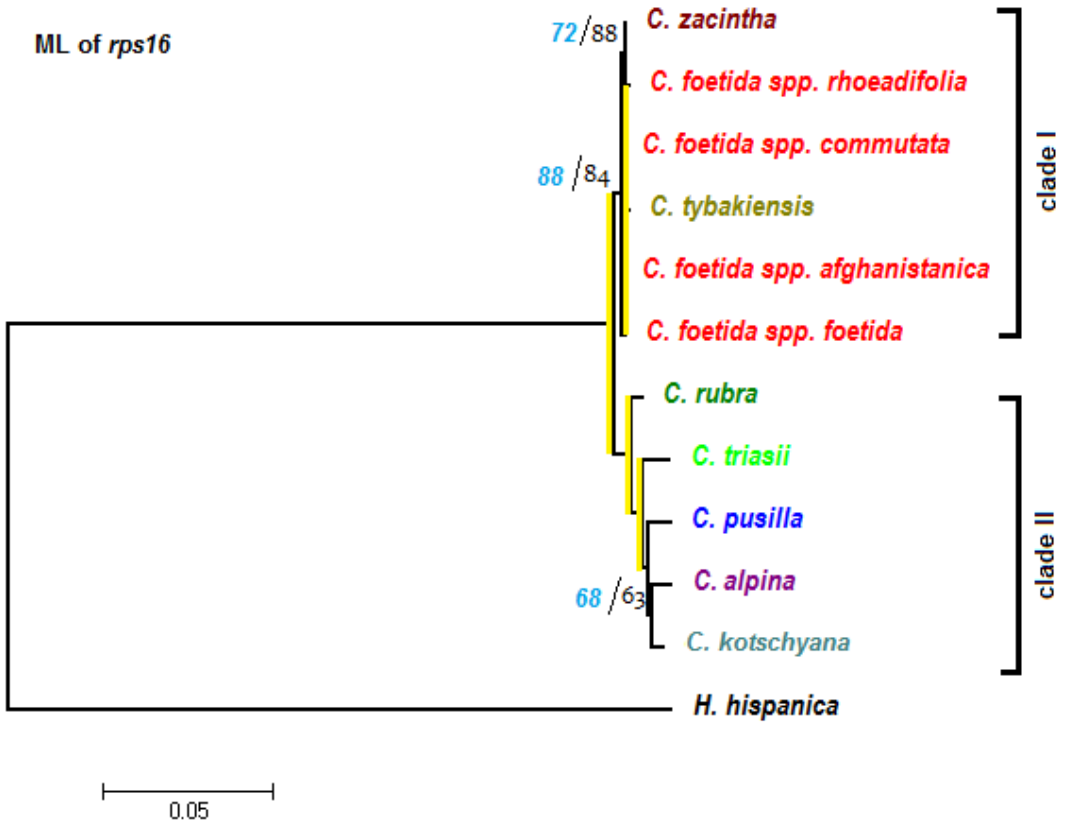


Figure 3.11. Phylogenetic analyses of the *rps16* dataset. ML tree is presented here with ML calculation above the branches. Blue numbers are bootstrap values from a bootstrap consensus tree, which was inferred from 1000 replicates of eight MPTs. Only values greater than 50% are shown here.

3.3.4. *MatK1*

Ten sequences of the chloroplast *matK1* region of five *Crepis* taxa representing five species of *Crepis* section *Barkhausia* were generated, namely: *C. foetida spp. afghanistanica*, *C. foetida spp. commutata*, *C. foetida spp. rhoeadifolia*, *C. kotschyana*, and *C. triasii*. Six additional species, namely *C. foetida spp. foetida*, *C. rubra*, *C. tybakiensis*, *C. alpina*, *C. pusilla*, and *C. zacintha* were supplied via NCBI under GenBank numbers EU363556.1, AJ633141.1, EU363566.1, EU363575.1, EU363576.1, and EU363579.1, respectively. These sequences have been included in a *matK1* matrix. The final analysis comprised 12 nucleotide sequences of *matK1* (11 of *Crepis* and one outgroup).

The aligned matrix dataset with coding indels contained 439 characters, 403 of which were conserved, 36 were variable, and 07 (1.6%) were parsimony-informative. The trees were produced using MP and ML, and they were similar in their topology (Figure 3.12). The MP analysis resulted in eight MPTs (length of 40 steps). The index of consistency was 0.778, which indicated homoplasy, the one of retention was 0.889, and the composite index was 0.843 (0.691). The ML tree is shown in Figure 3.12. Bootstrap values from a bootstrap consensus tree, which was inferred from 1000 replicates of eight MPTs are related to the branches.

The phylogenetic reconstruction resulted in many unresolved relationships. However, two clades could be detected (Figure 3.12). Clade I comprised *C. tybakiensis*, *C. foetida spp. afghanistanica*, *C. foetida spp. commutata*, *C. foetida spp. foetida* and *C. foetida spp. rhoeadifolia,* which were sorted to this clade due to 7 phylogenetic informative sites in the data matrix. *C. rubra* was basal to clade I and shared three phylogenetic informative sites with the taxa of this clade. *C. alpina* and *C. kotschyana* formed clade II, with low support (66BS/62ML). The relationships of *C. triasii*, *C. pusilla* and *C. zacintha* were not resolved.

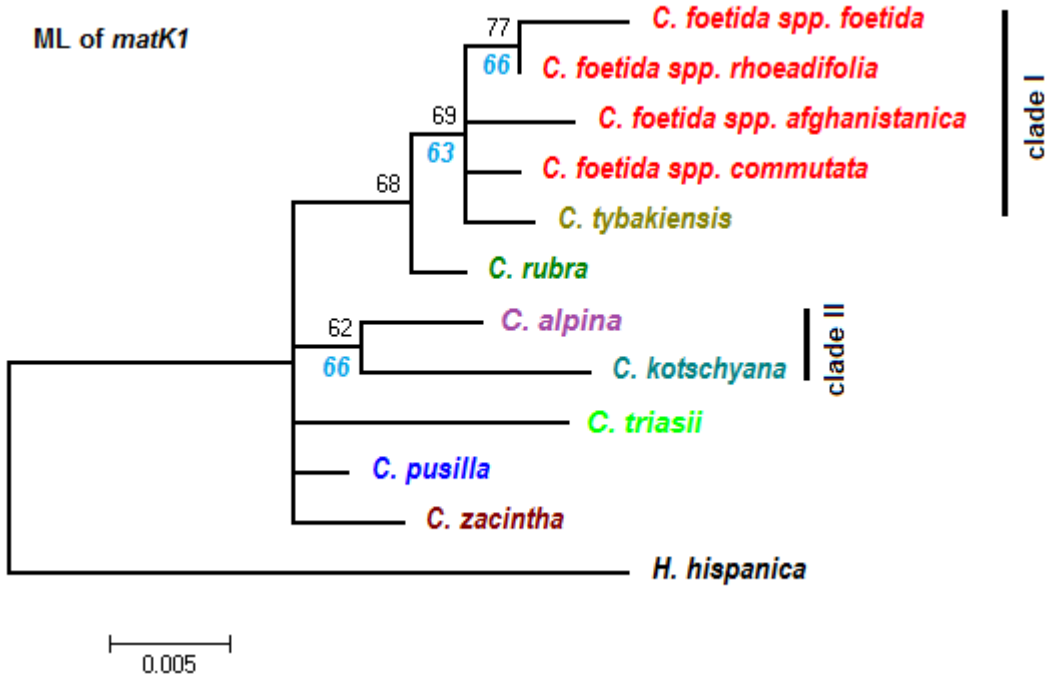


Figure 3.12. Phylogenetic analyses of the *matK1* dataset. ML tree is presented here with ML calculation above the branches. Bootstrap values from a bootstrap consensus tree, which was inferred from 1000 replicates of eight MPTs, are provided below the branches. Only values greater than 50% are shown here.

3.3.5. Concatenated Analysis of all Nuclear Sequences

The nuclear data matrix comprised the reduced data sets of *gsh1, sqs* and ITS (Enke and Gemeinholzer, 2008) and contained 13 taxa. The combined nuclear dataset matrix included 1980 characters, 1334 of which were conserved, 629 were variable, and 222 were phylogenetically informative (11.3%). The trees were produced using MP and ML methods, and they were topologically similar. For MP analysis, one most parsimonious tree with a length of 938 steps was obtained The consistency index was 0.617, which indicated homoplasy, the retention index was 0.591, and the composite index was 0.471 (0.365). The ML tree is shown in Figure 3.13, and it is supplied with bootstrap values from the most parsimonious tree.

The combined nuclear tree (CNT) featured paraphyletic groupings. However, the majority of species had one common ancestor which formed a well-supported clade (95BS/92ML). This clade consisted of three subclades: The

first subclade comprised two sister clades, namely *C. tybakiensis*, *C. rubra* (99BS/100ML) and *C. pusilla, C. zacintha* (100BS/100ML), but their relationship was not well supported (73BS/67ML). In subclade II, *C. foetida spp. commutata* and *C. alpina* were clustered together with good support (92BS/88ML).

Subclade III gained a strong support (95BS/93ML), and it contained *C. foetida spp. foetida, C. foetida spp afghanistanica, C. foetida spp. rhoeadifolia and C. foetida spp. thomsonii.* The relationships within this clade were resolved with a sufficient resolution, for example, *C. foetida spp. foetida* appeared as a sister to *C. foetida spp. afghanistanica* (100BS/96ML).

Furthermore, the tree showed that the relationship of the three subclades to each other was not resolved. However, *C. kotschyana* appeared basal to all other species, expect *C. triasii* which featured an independent lineage, rendering *Crepis* species section Barkhausia polyphyletic.

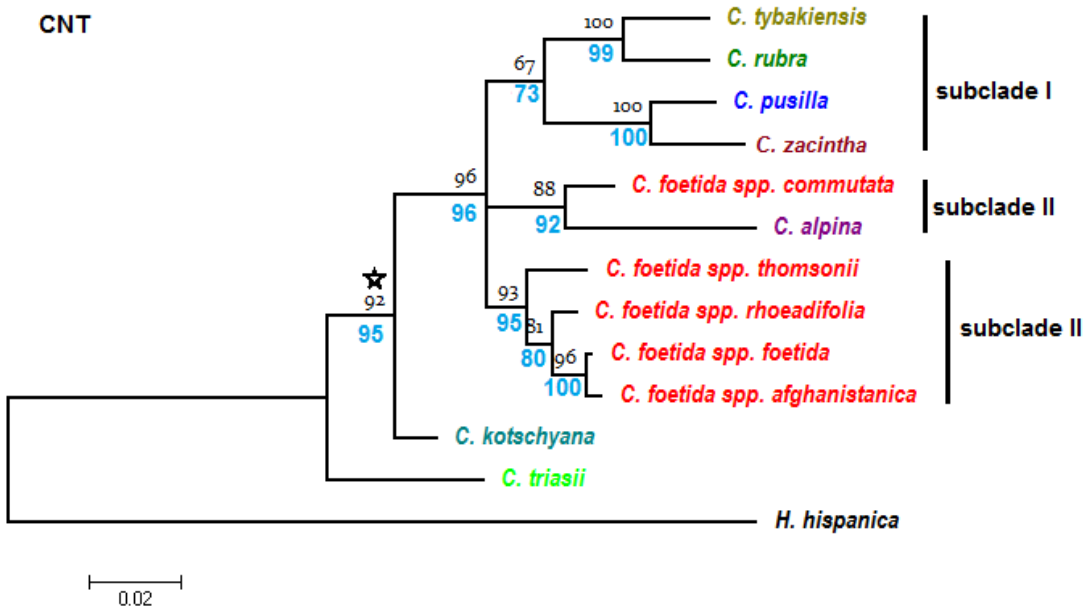


Figure 3.13. Phylogenetic analyses of the combined nuclear dataset (*gsh1, sqs* and ITS. ML tree is presented here with ML calculation above the branches. Blue numbers below the branches are bootstrap values of the most parsimonious tree. Only values greater than 50% are shown here. Asterisk indicates a common ancestor of the majority of species.

3.3.5.1. Character Evolution and Phylogenetic Signals

According to the combined nuclear tree (Figure 3.13), in subclade I, two sister groups, namely (i) *C. tybakiensis* and *C. rubra* versus (ii) *C. pusilla* and *C. zacintha*. The intermediate support of this relationship referred to 11 mutations in the *sqs* region, but this relationship had some contradictions. The sequences of *C. alpina* and *C. triasii* shared unique mutations, which were recognised as specific characters for this subclade. In the *Sqs* region, *C. alpina* had four positions (1386, 1415, 1841, 1938), and *C. triasii* had two positions (1415, 1977). Although *C. tybakiensis* and *C. rubra* shared 17 characters (the majority in *sqs*), they had a conflict with *C. alpina* and *C. triasii* in seven (1342, 1438, 1626, 1704, 1741,1813, 1868) and five (1665, 1675, 1679, 1714, 1868) sites, respectively. Similarly, *C. pusilla* and *C. zacintha* shared 30 characters in the *gsh1*, *sqs* and ITS regions, but, they also contributed to the conflict with *C. alpina* and *C. triasii* in six (1414, 1564, 1613, 1627, 1641, 1677) and five (110, 116, 1414, 1563, 1564) sites, respectively (Figure 3.14. B2).

The second subclade comprised *C. foetida spp. commutata* and *C. alpina*; the two species shared 25 specific characters, the majority of which was attributed to the *gsh1* region. However, this relationship had some discrepancies, while it shared some characters with *C. foetida spp. foetida* and *C. foetida spp. thomsonii* in the *gsh1* region at positions 856, 862, 1067 and 1032, 1108, respectively (Figure 3.14.C).

Subclade III, the relationships of *C. foetida spp. afghanistanica*, *C. foetida spp. rhoeadifolia*, *C. foetida spp. foetida and C. foetida spp. thomsonii* appeared to be well-supported. This relationship was based on 18 unique characters in the *sqs, gsh1*, and ITS regions of the combined dataset. In contrast to this, two species, namely *C. foetida spp. commutata* and *C. kotschyana*, shared seven signals in positions (1645, 1652, 1673, 1689, 1837, 1880, 1884) with the whole group referred to the *sqs* region (Figure 3.14.A).

A Position	439	143	598	1673	1880	1884	1837	1645	1652	1689	449	447	128	920	1089	1087	1002	1064
Name/gene	its	its	its	sqs	sqs	sqs	sqs	sqs	sqs	sqs	its	its	its	gsh1	gsh1	gsh1	gsh1	gsh1
C. foetida spp. afghanistaica	A	A	G	G	G	T	T	T	T	C	T	T	T	T	T	T	G	C
C. foetida spp. rhoeadifolia	A	A	G	G	G	T	T	T	T	C	T	T	T	T	T	T	G	C
C. foetida spp. foetida	A	A	G	G	G	T	T	T	T	C	T	T	T	T	T	T	G	C
C. foetida spp. thomsonii	A	A	G	G	G	T	T	T	T	C	T	T	T	C	A	A	A	G
C. tybakiensis	C	C	A	A	T	A	A	0	A	A	A	A	G	C	A	0	A	A
C. rubra	C	C	A	A	T	A	A	G	A	A	A	A	G	C	A	A	A	G
C. pusilla	A	C	A	A	T	A	A	C	A	A	A	A	G	0	A	0	A	0
C. zacintha	A	C	A	A	T	A	A	A	A	A	A	A	G	0	A	0	A	0
C. foetida spp. commutata	C	C	A	G	G	T	T	T	T	C	G	A	G	C	0	0	A	A
C. alpina	C	C	A	A	T	A	A	C	A	A	A	A		C	0	0	A	A
C. koschyana	C	T	A	G	G	T	T	T	T	C	A	A	C	G	C	A	A	G
C. triasii	C	C	A	A	T	A	A	G	A	A	C	A	G	G	C	A	A	G

B1 Position	1376	1418	1416	935	1707	1609	1665	1679	1714	1675	1868	1438	1704	1342	1741	1813	1626	1850	1844	1977	1415	1938	1841	1386	1845	1852	1858	1853
Name/gene	sqs	sqs	sqs	gsh1	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs	sqs
C. foetida spp. afghanistaica	C	G	A	A	T	0	T	A	G	A	G	G	C	C	T	T	T	G	T	A	A	A	A	A	0	0	0	0
C. foetida spp. rhoeadifolia	C	G	A	A	T	0	T	A	G	A	G	G	C	C	T	T	T	G	T	A	A	A	A	A	0	0	0	0
C. foetida spp. foetida	C	G	A	A	T	0	T	A	G	A	G	G	C	C	T	T	T	G	T	A	A	A	A	A	0	0	0	0
C. foetida spp. thomsonii	C	G	A	A	T	0	T	A	G	A	G	G	C	C	T	T	T	G	T	0	A	A	A	A	0	0	0	0
C. tybakiensis	T	C	G	G	A	A	G	C	T	T	A	A	G	T	C	C	A	A	A	G	G	G	G	T	G	T	T	A
C. rubra	T	C	G	G	A	A	G	C	T	T	A	A	G	T	C	C	A	A	A	G	G	G	G	T	G	T	T	A
C. pusilla	0	A	G	A	G	C	0	0	G	A	G	G	G	C	T	T	A	A	A	G	G	G	G	T	G	T	T	A
C. zacintha	T	A	A	A	G	A	G	A	G	A	G	G	C	C	T	C	T	A	A	G	G	G	G	T	G	T	T	A
C. foetida spp. commutata	C	G	A	A	T	0	T	A	G	A	G	G	C	C	T	T	T	G	T	A	A	A	A	A	0	0	0	0
C. alpina	A	A	A	A	G	C	T	A	G	A	A	A	G	T	C	C	A	C	T	0	G	G	G	T	A	C	C	T
C. koschyana	C	G	A	A	T	0	T	A	G	A	G	G	C	C	T	T	T	G	T	A	A	A	A	A	0	0	0	0
C. triasii	0	A	A	A	G	0	G	C	T	T	A	G	0	C	T	T	0	C	C	G	G	A	0	0	A	C	C	T

B2 Position	1123	1175	1563	116	110	1414	1564	1677	1641	1613	1627	1214	1625	1682	746	1095	863	1230	1040	427	574	211	615	119	118	103	629	617	101	497
Name/gene	gsh1	gsh1	sqs	its	its	sqs	sqs	sqs	sqs	sqs	sqs	gsh1	sqs	sqs	gsh1	gsh1	gsh1	gsh1	gsh1	its	its	its	its	its	its	its	its	its	its	its
C. foetida spp. afghanistaica	G	G	T	T	T	G	T	G	0	C	A	A	A	A	A	T	T	T	0	C	C	C	C	G	G	T	T	T	A	A
C. foetida spp. rhoeadifolia	G	G	T	T	T	G	T	G	0	C	A	A	A	A	A	T	T	T	0	C	C	C	C	G	G	T	T	T	A	A
C. foetida spp. foetida	G	G	T	T	T	G	T	G	0	C	A	A	A	A	A	T	T	0	0	C	C	C	C	G	G	T	T	T	A	A
C. foetida spp. thomsonii	A	G	T	T	T	G	T	G	0	C	A	A	A	A	A	T	T	0	T	C	C	C	C	G	G	T	T	T	A	A
C. tybakiensis	A	G	T	T	T	G	T	G	A	C	T	A	C	A	A	T	T	T	T	C	C	C	C	G	G	T	T	T	A	A
C. rubra	0	0	T	T	T	G	T	A	C	A	A	0	C	A	0	T	T	0	A	C	C	C	C	G	G	T	T	T	A	A
C. pusilla	G	A	C	A	A	T	C	T	T	T	G	G	C	C	C	C	C	G	G	T	T	T	A	A	C	A	C	G	G	G
C. zacintha	G	A	C	A	A	T	C	T	T	T	G	G	C	C	C	C	C	G	G	T	T	T	A	A	C	A	C	G	G	G
C. foetida spp. commutata	A	G	T	T	T	G	T	G	0	C	A	A	A	A	0	0	T	T	T	C	C	C	C	G	G	T	T	T	A	T
C. alpina	A	G	T	T	T	T	C	T	T	T	G	A	C	A	A	A	T	T	T	C	C	C	C	G	G	C	T	T	A	A
C. koschyana	T	G	T	T	T	G	T	G	0	C	A	A	A	A	A	T	T	T	A	C	C	C	C	G	G	T	T	T	G	A
C. triasii	T	G	C	A	A	T	C	A	C	0	0	A	0	A	A	T	T	T	A	C	C	C	C	G	T	T	A	T	A	A

C Position	1017	1071	1049	1114	1068	1062	1032	1108	881	446	879	493	859	897	1220	1242	857	911	1153	856	1067	862	477
Name/gene	gsh1	gsh1	gsh1	gsh1	gsh1	gsh1	gsh1	gsh1	gsh1	its	gsh1	its	gsh1	gsh1	gsh1	gsh1	gsh1	gsh1	gsh1	gsh1	gsh1	gsh1	its
C. foetida spp. afghanistaica	A	T	T	G	T	G	T	C	0	T	0	T	C	T	A	A	A	C	C	C	A	G	G
C. foetida spp. rhoeadifolia	A	T	T	G	T	G	T	C	0	T	0	T	C	T	A	A	A	C	C	C	A	G	G
C. foetida spp. foetida	A	T	T	G	T	0	T	C	T	T	T	T	C	0	A	A	A	C	C	G	G	T	G
C. foetida spp. thomsonii	G	T	T	G	T	G	A	A	T	T	T	T	C	T	0	0	A	C	C	C	A	G	G
C. tybakiensis	A	G	T	G	T	C	T	C	T	C	T	T	C	T	G	A	A	C	C	C	A	G	G
C. rubra	A	T	T	G	T	G	T	C	T	0	T	T	C	T	0	0	A	C	0	C	A	G	G
C. pusilla	A	T	T	G	T	0	T	C	T	T	T	T	C	T	A	A	A	C	C	C	A	G	G
C. zacintha	A	T	T	G	T	0	T	C	T	T	C	T	C	T	A	0	A	C	C	C	A	G	G
C. foetida spp. commutata	C	C	C	A	A	A	A	A	A	A	A	A	A	G	G	G	G	G	G	G	G	T	T
C. alpina	C	C	C	A	A	A	A	A	A	A	A	A	A	G	G	G	G	G	G	G	G	T	T
C. koschyana	A	T	T	G	T	G	T	C	T	T	T	A	C	T	A	G	A	C	C	C	A	G	G
C. triasii	A	T	T	G	T	G	T	C	T	0	T	T	C	T	A	0	A	C	C	C	A	G	G

Figure 3.14. Supported and conflicted characters of the relationships inferred from combined nuclear dataset. The different parts of the alignment (A, B1- B2, and C) indicate characters which support subclade I, II and III, and the conflicting signals.

3.3.6. Phylogenetic Reconstruction of the Combined Plastid Dataset

A concatenated dataset of *rps16* and *matK1* sequences was analysed. The data matrix included 12 taxa. The combined plastid dataset matrix included 1384 characters, 1096 of which were conserved, 278 were variable, and 21 were phylogenetically informative (1.5%). The trees were produced using MP and ML methods, and they were topologically similar. For the MP analysis, six most parsimonious trees with a length of 300 steps were obtained. The consistency indicator was 0.719, which indicated homoplasy, the one of retention was 0.830, and the composite indicator was 0.805 (0.597). The ML tree is presented here (Figure 3.15), and it is provided with bootstrap values from a bootstrap consensus tree, which was inferred from 1000 replicates of six MPTs.

Topologically, the combined chloroplast tree was similar to the *rps16* tree, which was indicative of the stronger influence of the *rps16* region on the combined tree. The *C. foetida* group appeared together with *C. tybakiensis* and clustered in not well-supported clade (74BS/54ML), while *C. zacintha* appeared as a sister to this clade with some support (83BS/81ML). *C. kotschyana* and *C. alpina* were also phylogenetically related and received good support (BS88/82ML). In contrast, *C. rubra* and *C. triasii* clustered between the first and second clade without support. The position of *C. pusilla* had no support either, indicating that *Crepis* species section *Barkhausia* is not monophyletic.

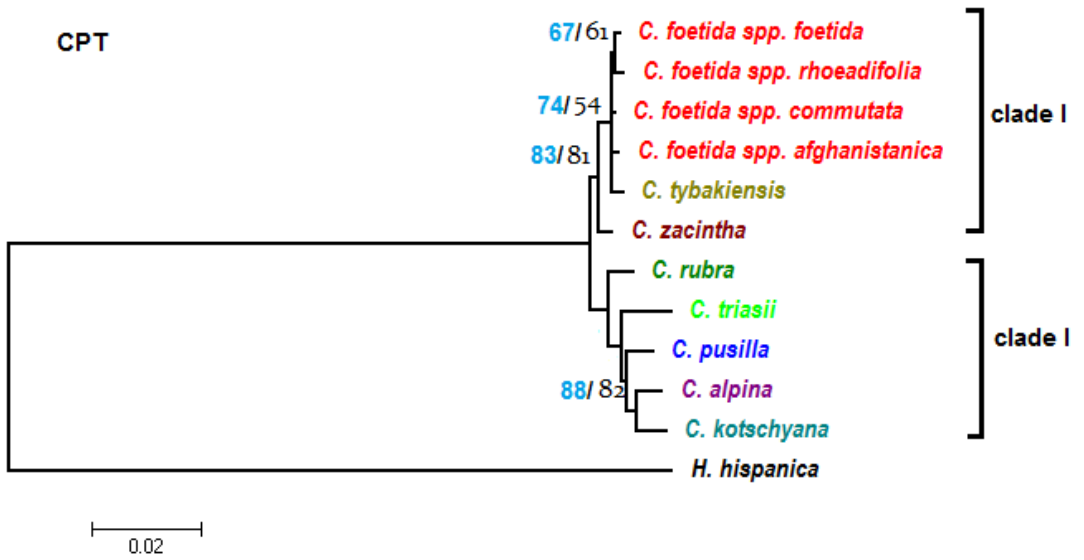


Figure 3.15. Phylogenetic analyses of the combined plastid dataset (*rps16* and *matK1*). ML tree is presented here with ML calculation above the branches. Blue numbers are bootstrap values from a bootstrap consensus tree, which was inferred from 1000 replicates of six MPTs. Only values greater than 50% are shown here.

3.3.7. Phylogenetic Reconstruction of the Combined Nuclear and Plastid Dataset

The analyses of the combined nuclear and chloroplast datasets included 12 nucleotide sequences (11 representing *Crepis* taxa and one for an outgroup). *C. foetida spp. thomsonii*, however, was excluded due to missing information in the plastid markers. In total, there were 3376 characters, 2440 of which were conserved, 905 were variable, and 245 were phylogenetically informative (7.3%). The resulting MP and ML trees were topologically similar with some differences. For the MP analysis, the most parsimonious tree with a length of 1269 steps was obtained. The consistency index was 0.583, which indicated homoplasy, the retention index was 0.529, and the composite index was 0.431 (0.309). The ML tree is presented in Figure 3.16, and it is supplied with bootstrap values from the most parsimonious tree.

Topologically, the combined nuclear and plastid tree (CNPT) was identical to the CNT due to a larger amount of informative sites in the combined nuclear dataset. Although the external and internal nodes gained less support than

the CNT, which is attributed to incongruent characters of the combined plastid dataset, the phylogenetic relationships between all analysed *Crepis* taxa remained identical.

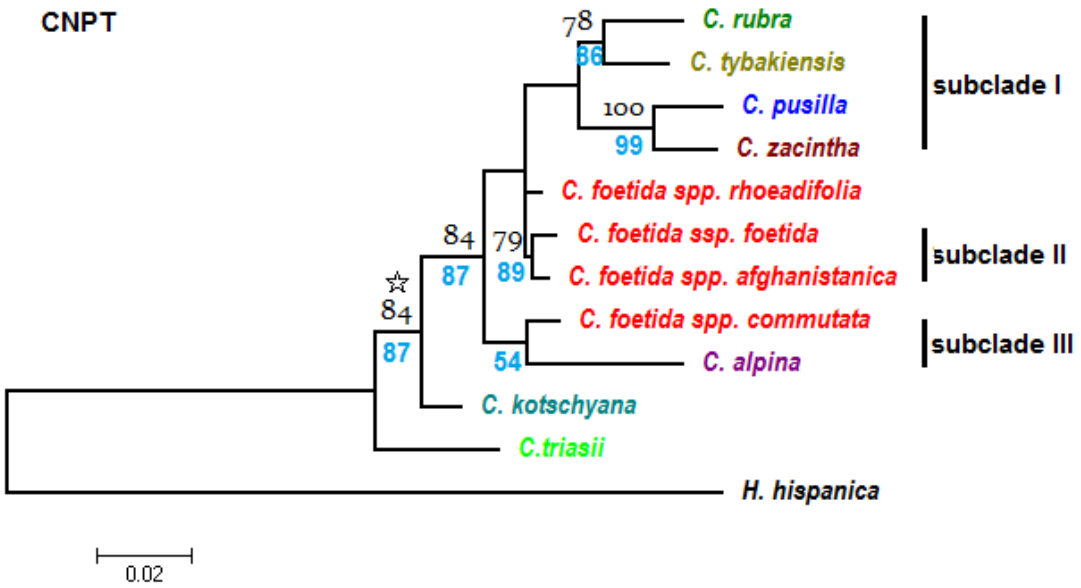


Figure 3.16. Phylogenetic analyses of the combined nuclear and plastid dataset (*gsh1*, *sqs*, ITS, *rps16* and *matK1*). ML tree is presented here with ML calculation above the branches. Blue numbers below the branches are bootstrap values of the most parsimonious tree. Only values greater than 50% are shown here. Asterisk indicates a common ancestor of the majority of species.

3.4. Combined Nuclear Tree Versus Combined Plastid Tree

As shown in Figure 3.17, all *Crepis* taxa of the combined nuclear tree clustered in different relationships in the combined plastid tree. The most closely related taxa in the nuclear tree were the most divergent ones in the plastid tree, for example, *C. zacintha* and *C.pusilla* (100BS/100ML) as well as *C. foetida spp commutata* and *C. alpina* (92BS/88ML).

The high discordances between nuclear and plastid trees could be attributed to different haplotypes in the plastid *rps16* gene and low informative sites as well. However, this contradiction of relationships within a closely related group indicated extensive reticulation patterns.

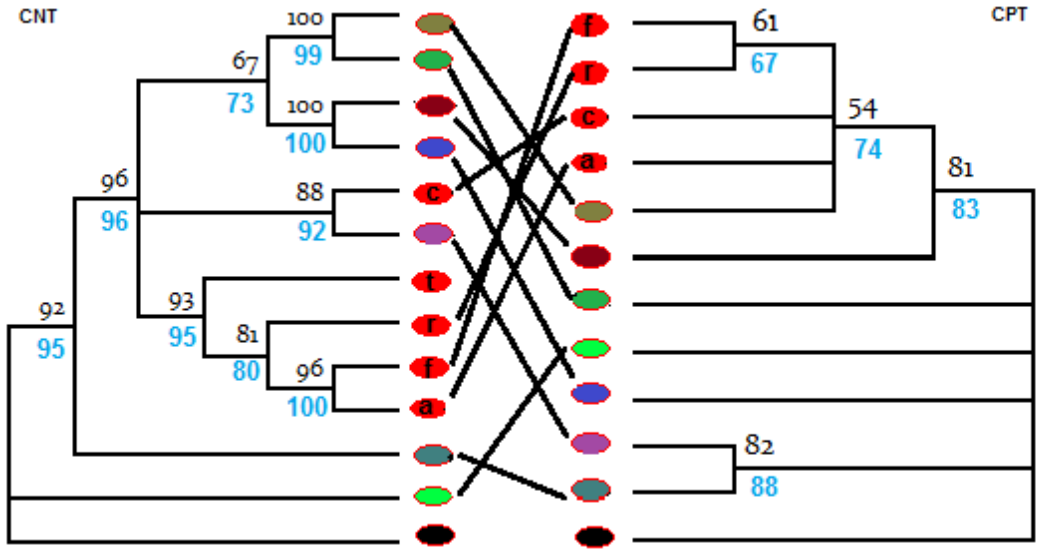


Figure 3.17. Combined nuclear tree (CNT) versus combined plastid tree (CPT). Colours indicate taxa as following: Black=outgroup; lime = *C. triasii*; teal= *C. kotschyana*; purple=*C. alpina*; blue= *C. pusilla*; maroon= *C. zacintha*; green= *C. rubra*; olive= *C. tybakiensis*; red= *C. foetida.* Small letters c, t, r, a, f: abbreviation of *commutata*, *thomsonii*, *rhoeadifolia*, *afghanistanica* and *foetida*, respectively. Numbers above the branches are ML calculation, and blue numbers are bootstrap values greater than 50%.

3.5. Hybridization Network and Reticulate Evolution

The output of Splits Tree reconstruction was a hybridization network with *C. pusilla* and *C. zacintha* as hybrids resulting from crossing between the lineage of *C. rubra* and *C. tybakiensis* and the lineage of *C. triasii*, *C. kotschyana*, *C. foetida spp. commutata*, and *C. alpina* (Figure 3.18).

On the other hand, six hybridization networks with three reticulations resulted from hybridization network analysis (Binary Trees; Huson et al., 2011) implemented in Dendroscope software (Figure 3.19). Table 3.4 summarises the hybridization outcome analysis using Dendroscope software (Huson, 2016).

In general, all six networks suggested that *C. pusilla* and *C. zacintha* are hybrids with 100% node support, while the other suggested hybrids were supported only by 50%. The networks proposed all *Crepis* taxa to be putative hybrids and derived lineages at the same time. For example, *C. kotschyana* was presented as a hybrid in the networks No.1, 3, and 5, and *C. triasii* was proposed to be one of the parents in these networks. In contrast, *C. triasii* was indicated as a hybrid in the network's Nr. 2, 5, and 6, and *C. kotschyana* was presented as one of its parents in the network's Nr. 2 and 6. Hence, the same species appeared as a hybrid in some networks and as a parent in the other networks, which indicated introgression and reticulate evolution within this section.

Putative Hybrids	Network Nr.	Node Support
C. pusilla and *C.zacintha*	1/2/3/4/5/6	100%
C. alpina and *C. foetida spp. commutata*	1/2/3	50%
C. kotschyana	1/3/5	50%
C. triasii	2/5/6	50%
C. rubra, C. tybakiensis, and *C. foetida group*	3/4/6	50%

Table 3.4. Putative hybrids within *Crepis* species section *Barkhausia* proposed by Dendroscope software V.3.4.5 (Huson and Scornavacca, 2012).

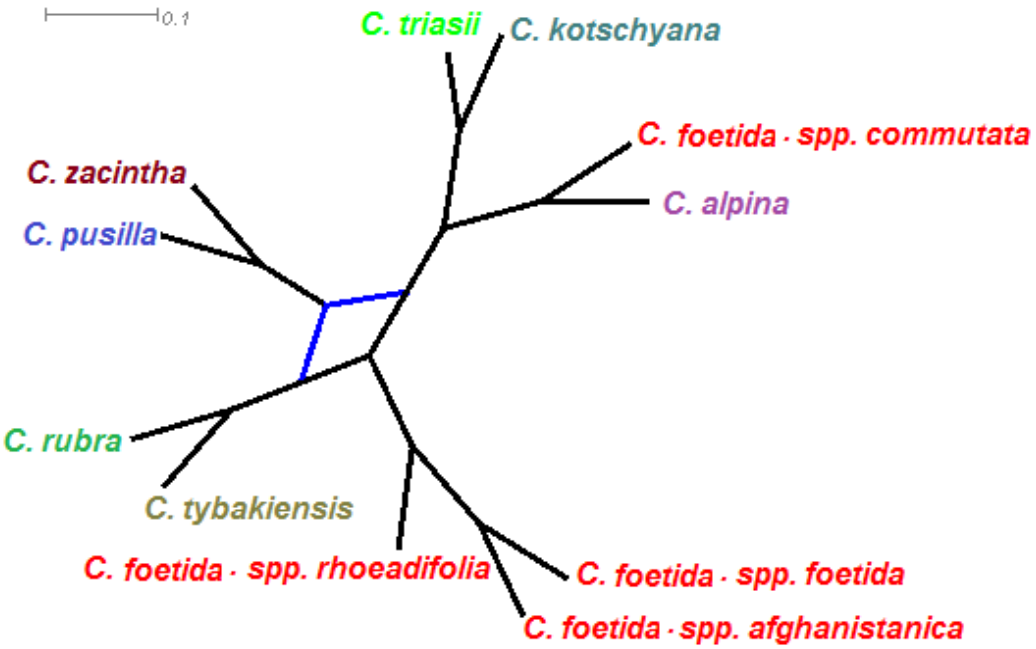


Figure 3.18. Hybridization Network, using SplitsTree V.4.14.4. (Huson and Bryant, 2006), of *Crepis* species section *Barkhausia* reconstructed from combined nuclear and plastid data sets of two unrooted trees inferred from nuclear genes *gsh1*, *sqs*, and ITS and plastid genes *matK1* and *rps16*. Blue branches represent hybridization event.

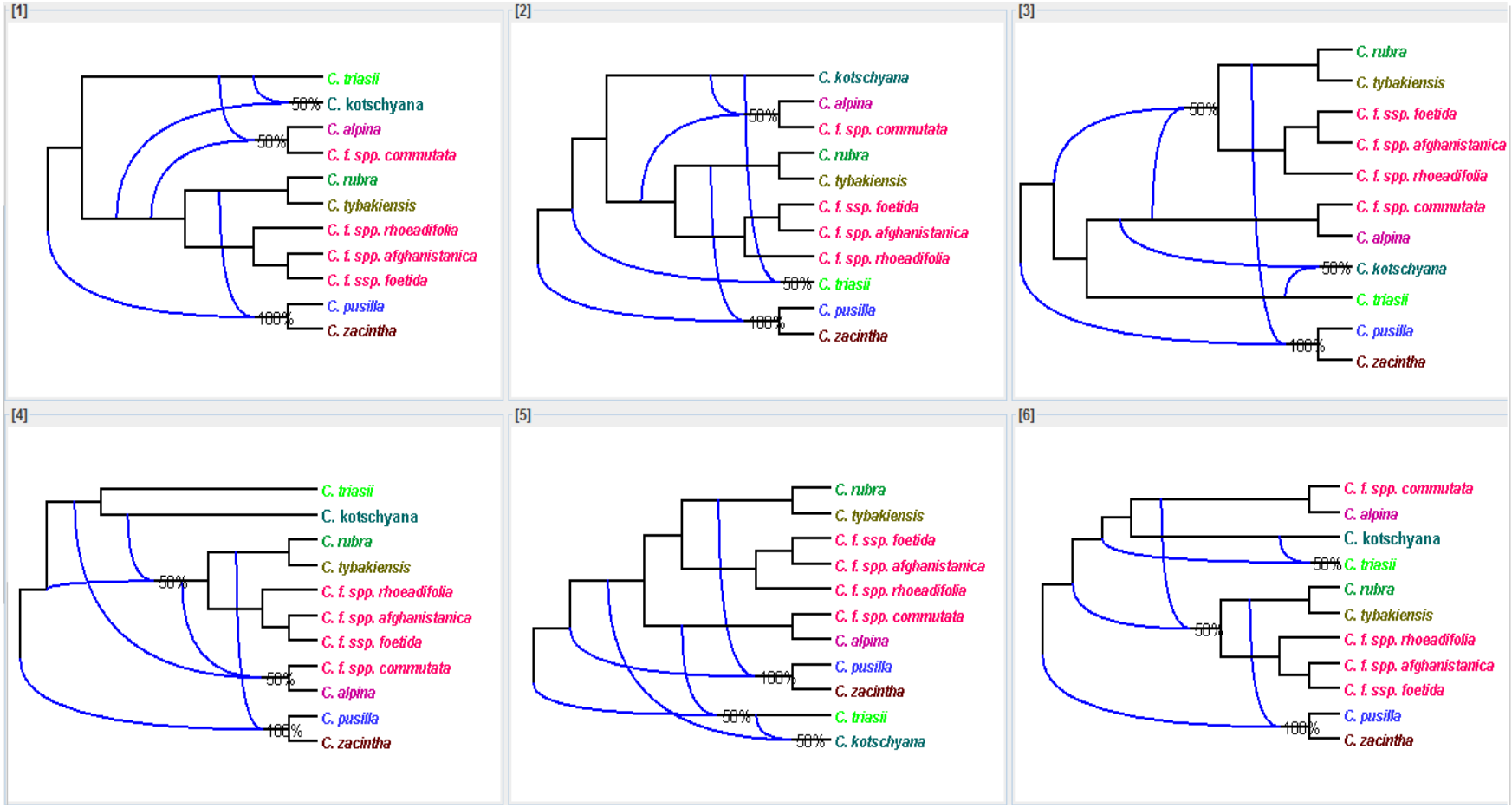


Figure 3.19. Six Hybridization Networks and three reticulations computed using Dendroscope V.3.4.5 (Husen and Scornavacca, 2012), of *Crepis* species section *Barkhausia* reconstructed from the combined nuclear data sets of two unrooted trees inferred from *gsh1*, *sqs* and ITS and plastid *matK1* and *rps16* genes. The percentage values above putative hybrids indicate node support. Blue lines across branches indicate hybridization events.

4. Discussion

4. Discussion

Four different genes of 12 *Crepis* species were analysed, namely two low-copy nuclear genes, *gsh1* and *sqs* (Krak et al., 2012), and two chloroplasts genes, *rps16* intron (Oxelman et al.,1997) and *matK1* (Johnson and Soltis, 1995; Fehrer et al., 2007). The study revealed the genes to be of multi-copy origin within the genomes of *Crepis* species section *Barkhausia*. These results are consistent with the finding of Krak et al. (2013), who found multiple sequences in the closely related group *Hieracium* s.str. and could not resolve the relationships between the species of that group.

The results found in this analysis were contradictory, but revealed *Crepis* section *Barkhausia* to be polyphyletic, which is inconsistent with the findings of Enke and Gemeinholzer (2008). Even though the combined nuclear tree, *gsh1*, *sqs*, and ITS showed strong support for external and internal nodes, the relationships within this group seemed to be sophisticated, and intensive reticulate networks were documented. Probably, duplicated *gsh1* and *sqs* genes were responsible for these complicated relationships, and the use of the newly developed markers *gsh1* and *sqs* was unhelpful to reflect the real relationships among such a closely related group.

4.1 *Gsh1* and *Sqs* Sequences

The existence of different copies of sequence copies of *gsh1* and *sqs* genes was questionable. Therefore, the *gsh1* and *sqs* alignment matrices were carefully checked to expose whether these different copies resulted from PCR recombination (Chimeras), polymerase error, or gene duplication (paralogous or orthologous sequences). The results of the investigation pointed out that there were no PCR recombinant sequences detected in *gsh1* sequences, while one case in *sqs* sequences was discovered which represents *C. tybakiensis* seq.2. In the *sqs* aligned matrix, the region of *C. tybakiensis* seq.2 from 595 to 640 bp corresponded to subclade II, while the rest of the sequence corresponded to subclade III. In addition, searching for recombination events using (Recombination Detection Program) RDP4

(Martin et al., 2015) software yielded a negative result in *gsh1*, but detected *C. tybakiensis* seq.2 as a recombinant sequence. Chimeras were also documented by investigations on *Hieracium* using *sqs* (Krak et al., 2013); a lack of resolution was recorded in that study.

It was unlikely that the production of a well-organised matrix with different copies was due to a polymerase error . Therefore, gene duplication was the most logical answer to this state, especially as these duplicated copies were found in more than one taxon. Several facts indicate that these sequences were orthologous rather than paralogous: First, the precise inspection of alignments revealed that exon regions were very similar in all different taxa among clades, while introns were vastly heterogeneous among clades, but homogeneous within clades. Second, there were no stop codons in translating data sets. Besides, all exons and exon-intron junctions were conserved, and all exons translated for the same amino acids, i.e. coded for the same protein. Third, blast analysis for checking gene function via the National Center for Biotechnology Information (NCBI) reached a positive result. Fourth, the majority of nucleotide substitutions in *gsh1* and *sqs* exons were silent mutations (non-synonymous substitutions), which confirmed the gene function of various copy sequences. All this evidence confirmed the orthology of different *gsh1* and *sqs* copy types.

4.2. Incongruence between Nuclear and Plastid Gene Trees

Topological incongruence between nuclear and plastid trees was found in the analysis presented here, which is a common phenomenon in phylogenetic studies (Degnan and Rosenberg, 2009). The incongruence can be caused by biological factors such as reticulation, incomplete lineage sorting, gene duplication, and rate heterogeneity. It could also be due to analytical factors like low informative sites and long branch attraction (Rieseberg & Soltis, 1991; Soltis & Kuzoff 1995; Soltis & al. 1996; Wendel & Doyle, 1998; Sang and Zhong, 2000).

However, long branch attraction within the group could not be found in the actual study, and probably this group has a quite young origin. In accordance with these results, Krak et al. (2013) and Liu et al. (2013) did not find long branches associated with the conflict, either. Furthermore, phylogenetic studies of *Hieracium* genus and *Faberia* (Krak et al., 2013; Liu et al., 2013) rejected the theory possibility that a low number of informative sites leads to discordant patterns, which was also rejected in the actual investigation by both *gsh1* and *sqs* markers. However, given the contradictory picture obtained from combined nuclear (*gsh1*, *sqs*, and ITS) and chloroplast datasets (*rps16* and *matK1*), gene duplication, hybridization, and incomplete lineage sorting could be feasible hypotheses (Sang, 2002; Linder and Rieseberg, 2004; Krak et al., 2013).

According to the actual research, the biotic factors are the best candidates to interpret these incongruous relationships, and the reasons for this argumentation will be presented in the following:

4.2.1 Gene Duplication

Gene duplication is defined as a duplicate in the DNA region, comprising the whole gene, several genes, or a part of the gene. Duplication is frequent in plants and can be due to unequal crossing over, retroposition, or duplication in the chromosome as well as in the genome (Zhang, 2003).

gsh1 was described as a single copy gene in *Arabidopsis thaliana* (L.) Heynh, while two copies were discovered in *Oryza sativa* L. (Fang et al., 2016). Likewise, the *sqs* gene was described as a single copy in *Euphorbia tirucalli* L. (Uchida et al., 2009), but two copies were identified in *Arabidopsis thaliana* and *Nicotiana tabacum* L. (Devarenne et al., 2002 and Kribii et al., 1997). However, most plant genes belong to gene families that comprise three or more gene members (De Grassi et al., 2008). Hence, the function similarity of analysed sequences may suggest that these copies are members of gene families. However, southern blot analysis would be necessary to determine the number of copies of the analysed genes.

Recent phylogenetic studies (Krak et al., 2012, 2013) revealed the *sqs* and *gsh1* genes to be of multi-copy origin. Krak et al. (2013) considered both copies as true allelic variations, and the excess alleles were considered as a result of polymerase error. However, in the current research, the low-copy nuclear markers, *gsh1* and *sqs*, also showed multiple forms of their sequences in *Crepis* species section *Barkhausia*. These sequences were sequentially divided into five and four copy types in *gsh1* and *sqs*. However, the number of alleles exceeded the ploidy level in only four species, namely *C. triasii* and *C. alpina* in *gsh1* sequences, and *C. triasii*, *C. pusilla*, and *C. tybakiensis* in *sqs* sequences. In agreement with these results, Krak et al. (2013) recognised surplus alleles in seven individuals in *Hieracium* as well.

In general, duplication events regarding low-copy nuclear markers were found in several phylogenetic studies (Esfeld, 2009; Babineau et al., 2013; Pillon et al., 2013). Babineau et al. (2013) defined the duplicated copies as orthology; consistently the results of the actual investigation suggest that *gsh1* and *sqs* copies were a result of orthologous gene copies instead of allelic variation. This assumption is based on the proofs mentioned above in the ***Gsh1* and *Sqs* Sequences** section.

The number of copies was unequal among *Crepis* taxa, and the high number of copies were documented in *Crepis triasii*, four copies in both *gsh1* and *sqs* analyses. The genetic variability of *C. triasii* also has been recognised by Mayol et al. (2012), in particular, for isolated species where gene flow and genetic drift, and natural selection could play important role in diversification and evolution. However, inequality of copy numbers among taxa might be indicative of the absence of copies in different taxa, but could also be the result of incomplete laboratory findings. More experimental work with specifically designed primers for each putative copy type could resolve this question. The first intron both in the *gsh1* and the *sqs* gene would be a suitable candidate to test for different copy types within different *Crepis* species. Gene duplication impedes phylogenetic reconstructions, in particular when the duplicated gene is followed by a deletion of copies in the descendant lineage, so that the gene phylogeny may reflect the evolution of the gene rather than the evolutionary history of the taxa (Sang, 2002; Linder

and Rieseberg., 2004). Nevertheless, it can be beneficial to provide insights into gene evolution and function (Doyle et., al 2003).

Finally, the presence of identical copies shared by more than two taxa in both *gsh1* and *sqs* sequences indicated a retention of ancestral polymorphism across lineages of *Crepis* species section *Barkhausia*, which agrees with the finding from the *sqs* marker in *Hieracium* s.str. (Krak et al., 2013).

4.2.2. Incomplete Lineage Sorting (ILS) and / or Hybridization

Incomplete lineage sorting occurs when there is no sufficient time to fix the ancestral polymorphic alleles in descendent taxa, while hybridization is the interbreeding of two individuals of different genotypes. In general, both mechanisms may deliver analogous phylogenetic patterns, so that it is difficult to discriminate between them (Holder et al., 2001; Buckley et al., 2006; Joly et al., 2009). There is no powerful and broadly appropriate approach to distinguish between both processes (Joly et al., 2009). However, some approaches were suggested to determine whether hybridization or ILS is the main cause of such incongruences (Holder et al., 2001; Whitfield and Lockhart, 2007; Joly et al., 2009; Pelser et al., 2010).

At inter- and intra-specific levels, ILS or deep coalescence is the most likely interpretation of incongruent gene trees (Wendel and Doyle, 1998; Sang., 2002), along with evidence of recent radiation, which means there was no adequate time for complete lineage sorting of ancestral polymorphism via genetic drift and selection (Sang, 2002; Smissen et al., 2004). However, this is only true under the assumption that short branches of the same taxa, which were obtained from different data sets, are a sign of recent rapid radiation (Whitfield and Lockhart, 2007). In the actual study, almost all *Crepis* taxa which contributed to the conflict among nuclear and plastid gene trees had short branches (0.05 in *gsh1*, 0.02 in *sqs*, 0.05 in *rps16*, and 0.005 in *matK1*). Therefore, the presence of very similar or identical sequences of different taxa seemed to indicate an ILS process, which was found out before for *Hieracium*

(Krak et al., 2013) and *Centaurea* section *Phrygia* (Lopez-Alvarado et al., 2014).

Another approach was proposed by Buckey et al. (2006), who suggested that the random nature of ILS might produce gene trees with irregular patterns of relationships among taxa, which may appear as a result of incongruent patterns. Hence, the phylogenetic analyses presented in the actual study showed that *Crepis* species, which contributed to the conflict between nuclear gene trees, were not stochastically distributed. The nuclear gene trees (subclade II and subclade III in *gsh1*; subclade I and subclade III in *sqs* (Figures 3.6 and 3.9) indicate that ILS was not responsible for discordant relationships, at least for those four *Crepis* species associated with the mentioned subclades. Hybridization could rather be the possible reason behind this conflict. This theory is supported by the fact that hybridization was described as one possible reason behind contradictory patterns in many studies (Kim and Donoghue, 2008; Soejima et al., 2008). Furthermore, hybridization within *Crepis* species recently assigned to section *Barkhausia* was suggested earlier (Babcock, 1947a,b) and recently (Enke and Gemeinholzer, 2008).

4.2.3. Reticulate Evolution

In biology, reticulation refers to the loss of independence between two lineages and leads to the unification of two or more independent evolutionary lineages at some biological levels. Reticulate evolution can occur at the gene level (recombination between genes) or species level (hybridization and introgression between lineages) (Linder et al., 2004).

The outcomes presented in the actual investigation provide insights into complex relationships within closely related *Crepis* species and shed light on the phylogenetic network, although the number of examined taxa of this study was small in comparison to the previous study done by Enke and Gemeinholzer (2008). The usage of DNA sequences from multiple loci supplied new evidence of extensive hybridization and introgression, which

indicates reticulate evolution. In addition, other scientists demonstrated reticulation events by screening datasets with multi-DNA loci including the employment of low-copy nuclear genes, for instance in the genus *Primula* L.; Primulaceae (Guggisberg et al., 2009), the genus *Pinus*.L; Pinaceae (Willyard et al., 2009), and the genus *Polystachya* Hook.; Orchidaceae (Russel et al., 2010).

4.2.3.1. Extensive Hybridization and Introgression

At low taxonomic levels (inter- and intraspecific level), the existence of evolutionary processes such as introgression and reticulation complicates the reconstruction of phylogenetic relationships (Lopez-Alvarado et al., 2014). Based on supported and conflicted characters of nuclear and plastid data sets which were used to trace the character evolution of *Crepis* species section *Barkhausia*, and on Hybridization Network analyses, more or less all analysed *Crepis* taxa were involved in hybridization and introgression processes, which pointed reticulate evolution.

The output that indicated *C. pusilla* and *C. zacintha* were hybrids resulted from hybridization network analyses by Splits Tree (Huson and Bryant, 2006) and Dendroscope (Huson and Scornavacca, 2012) softwares. However, the crossing of lineages indicated that an ancient hybridization took place across progenitors. The hypothetical parental lineages were the lineage of *C. rubra* and *C. tybakiensis*, the one of *C. foetida spp. commutata*, *C. alpina*, *C. triasii*, and *C. kotschyana* and/or the lineage of the *C. foetida* group, which was unexpected. However, the accurate inspection of the CNP dataset clarified that the conflicting signals (30 characters referring to ITS and *sqs*) of *C. pusilla* and *C. zacintha* conflicted with those species which belonged to derived lineages.

Hypothetically, in the case of *gsh1* and *sqs* copies being present in all *Crepis* taxa, these signals can be explained as ancestral polymorphism retention or an ancient hybridization if they were restricted to the examined taxa. Despite many signs that confirmed the hybridization process, relationships within

closely related species are still ambiguous until the status of the copies is verified. However, further investigations are recommended to clarify this issue.

4.3. Taxonomy in a Phylogenetic Context

C. foetida L.**:**

Babcock (1947a) mentioned in his book , that *C. foetida* is known as a polymorphic species with numerous forms which differ in quantitative and qualitative features. In both the nuclear and plastid trees of the actual analysis, in most cases, the different subspecies of *C. foetida* clustered together, and they formed a polyatomy clade due to low levels of variation. However, *C. foetida spp. commutata* (Spreng.) Babc., deviated from the group and was closer to *C. alpina* L. and *C. kotschyana* Boiss than to the other *C. foetida* subspecies in both *gsh1* and ITS analyses, however, ITS of *C. foetida spp. commutata* was obtained in the current investigation. This deviation referred to bootstrap supporting values (100) in the *gsh1* reduced dataset and was important for reconsidering taxon delimitation. Early, *C. foetida spp. commutata* was established as *Rodigia commutata* because of its paleaceous receptacle (Spreng, 1820)*;* then it was sorted into to *C. foetida* as a subspecies upon morphological and karyological resemblances (Babcock, 1947b). After that, in 1975, Greuter re-described the species and established it as an independent species. Subsequently, genome variation studies by Dimitrova et al. (1999) suggested returning this taxon to species boundaries. The results of the actual study provide additional molecular evidence which supports the opinion of Dimitrova et al. (1999) that *C. foetida spp. commutata* should return to species level as *C. commutata* (Spreng) Greuter rather than remaining *C. foetida spp. commutata* (Spreng) Babc.

C. tybakiensis Vierh. and ***C. rubra*** L.**:**

C. tybakiensis and *C. rubra* were assigned to section *Hostia* by Babcock (1947b). Babcock proposed a common progenitor for both species due to morphological similarities such as glabrescent outer involucral bracts and scapiform stems (Babcock, 1947b). Indeed, his analysis is supported by the actual phylogenetic analyses of the *gsh1* and *sqs* genes, but both species seemed to have different haplotypes in *rps16*. Similarly, some researchers also found conflicting signals in phylogeny and related this to hybridization of cross-lineages (Fehrer et al., 2009), which is supported by the findings of *rps16* of the actual study. In addition, both species also showed a close relationship to the *C. foetida* group, and especially the plastid genes revealed that this relationship to the *C. foetida* group was closer for *C. tybakiensis* than for *C. rubra.* These results support earlier findings of Enke and Gemeinholzer (2008). Geographically, *C. rubra* and *C. tybakiensis* occur on Crete island where their progenitor may have contacted.

C. zacintha (L.) Loisel., **and *C. pusilla*** (Sommier) Merxm**:**

Enke and Gemeinholzer (2008) detected a close relationship between *C. pusilla* and *C. zacintha* based on ITS and *matK* regions. The current analysis confirmed their result on a molecular basis by using the *gsh1* and *sqs* sequences (Figures 3.6; 3.9). The close relationship was already mentioned by Merxmueller (1968), who ascribed it to morphological resemblances such as pappus, marginal achene and rosette growth. Hence, the molecular results add to the finding of Maxmuller because they can play a similar role in evolution, which tells us that the two plant species had a common ancestor. In contrast, convergent evolution can also lead to similar morphological features due to similar ecological conditions, which was indicated by the high level of consistency index.

However, *C. zacintha* and *C. pusilla* did not appear to be closely related in *matK1*, which was contrarily documented by Enke and Gemeinholzer (2008) by using the whole region. It is proven by the actual study that the second part

of *matK* carried informative information that reinforces the relationship between the just-mentioned species. Hilu and Liang (1997) studied the rates and types of nucleotide substitutions in the *matK* gene, and they found that the 3' region was most useful in resolving the phylogeny. This is underscored by the results presented here. However, other scientists found that chloroplast and nuclear genes tell different evolutionary histories (Enke and Gemeinholzer, 2008; Kim and Donoghue, 2008; Petri and Oxelman, 2011) which are supported by the data of the current study.

C. alpina L. **and *C. kotschyana*** Boiss.**:**

Our findings by using *rps16* and *matK1* sequences indicated that *C. alpina* and *C. kotschyana* share the same evolutionary lineage (Figures 3.11; 3.12), which is supported by the results of Enke and Gemeinholzer (2008). In contrast, *gsh1* showed a well-supported relationship between *C. alpina* and *C. foetida spp. commutata*. However, *C. alpina, C. kotschyana*, and *C. foetida ssp. commutata* were assigned to *Crepis* section *Hostia* in Babcock's Classification. Based on the similarities in the number of chromosomes (5-paired), morphological features such as the shape of inner achenes, and geographic distribution (they occupy the Asia-Temperate region), the possibility of hybridization among the species mentioned above was proposed by Babcock (1947b). This contradiction in relationships can be interpreted as a reticulate evolution or could also be a misleading link attributed to duplication events in both *gsh1* and *sqs* genes.

C. triasii (Cambess.) Nyman**:**

The results of the actual study show that there are some close relationships with other analysed *Crepis* taxa. These findings were not supported by Babcock (1947b), who suggested that there are morphological, karyological and geographical disagreements with the analysed *Crepis* taxa. Furthermore, no overlapping area has been presented on Cichorieae Portal. Therefore, the area of contact might be difficult, and the relationships found in the current investigation are presumably due to duplication events. However, the position

of *C. triasii* in CNT is the problem; the overall picture of Enke and Gemeinholzer is proven, and the actual data support it, but the position of *C. triasii* is problematic and renders this group polyphyletic. Practically, the molecular information occupies an intermediate position between the hypotheses of Babcock (1947b) and Enke and Gemeinholzer (2008).

4.4. Strength and Limitation of Low-Copy Nuclear Markers (*gsh1* and *sqs*)

Krak et al. (2012) designed novel low-copy nuclear markers, *gsh1* and *sqs*. However, the nucleotide substitution rate of both markers was high, and it was expected to be sufficient to resolve complex relationships, especially at lower taxonomic levels (inter- and intraspecific level). In the present study, both markers were used for *Crepis* phylogenetics for the first time. However, the variability and number of phylogenetic informative sites of *gsh1* and *sqs* were higher than the ones of the rapidly evolving nuclear intergenic spacer ITS, which is consistent with the investigations done by Krak et al. (2013), but they used ETS instead of ITS. Nonetheless, the high level of variation resulting from different copy types in both markers indicated gene duplication or gene family members. The utility of such markers is restricted by the existence of very similar or identical copies among taxa due to paralogue evolution, which might lead to wrong results. As a consequence, the markers become unsuitable tools for phylogenetic studies. This limitation of low-copy nuclear markers was documented in many studies, such as Krak et al. (2013) and Babineau et al. (2013). Furthermore, both markers shed light on reticulate evolution and ancestral polymorphism retention within *Crepis* species section *Barkhausia*. Additionally, as the *gsh1* structural gene is heterogeneous among plant species (May and Lever, 1994), the *gsh1* marker would be suitable to identify the genus *Crepis* among other genera in the case of taxonomic delimitations.

5. Conclusion and outlook

Although the genus *Crepis* was a model for evolutionary studies in the first half of the twentieth century, the genus and its species obtained little scientific attention recently. However, recent investigations by Enke and Gemeinholzer (2008) based on molecular data (ITS) revealed *Crepis* section *Barkhausia* to be monophyletic. In contrast, chloroplast data (*matK*) showed more than two independent lineages, which could potentially be due to a the hybridogenic origin of this group. To unveil the phylogenetic relationships and shed light on the evolutionary history of *Crepis* species section *Barkhausia* (12~14), the implementation of molecular markers with high substitution rates was recommended, especially in case nrDNA and plastid markers provide insufficient resolutions.

This doctoral thesis represents the first attempt to reconstruct the phylogenetic relationships within *Crepis* species section *Barkhausia* by using the low-copy nuclear markers gamma-glutamyl cysteine synthetase (*gsh1*) and squalene synthase (*sqs*) and the uniparentally inherited (mainly maternally in angiosperms) markers ribosomal protein S 16 Intron (*rps16*) and maturase K (*matK1*), in combination with previous ITS results (Enke and Gemeinholzer, 2008). The Maximum Parsimony and Maximum Likelihood methods were used to reconstruct phylogenetic relationships. Reticulation patterns were inferred from incongruences between different gene trees via hybridization analyses implemented in the softwares SplitsTree and Dendroscope (Huson and Bryant, 2006; and Huson and Scornavacca, 2012).

The two low-copy nuclear markers, *gsh1* and *sqs,* expressed multi-alleles, which were very similar or identical. The evolutionary origin of the retrieved pattern is discussed in detail, but this finding is most likely due to gene duplication events. Presumably, these different copy types were orthologous rather than paralogous sequences. However, a recombination test indicated that there was a lack of recombination between all different copy types of both loci in all analysed taxa. However, sequences of both loci recorded high variability, and *sqs* was more variable than *gsh1*. The minimisation of the similarity among *gsh1* and *sqs* data sets showed different influences on

topology and branch support of the trees, which was probably due to the inequality of duplicate copies of each taxon. However, the reduced *gsh1* dataset was more successful in inferring relationships with a good resolution than the reduced *sqs* dataset.

In contrast, plastid loci, *rps16* and *matK1*, featured less phylogenetic informative mutations than the nuclear loci, while *matK1* was more informative than *rps16*. In addition, both plastid markers showed similar histories. Even though *rps16* was entirely sequenced and *matK* was only partially employed, most relationships in both regions are still not resolved due to the low level of variation.

Even the phylogenetic relationships investigated by different molecular markers revealed different evolutionary histories; some relationships were found throughout all phylogenetic reconstructions of the nuclear regions, e.g. the one of *C. rubra* and *C. tybakiensis* and the one of *C. pusilla* and *C. zacintha*. However, the chloroplast markers *rps16* and *matK1* showed conflicting signals. In general, the incongruence among nuclear and plastid markers pointed towards hybridogenic evolution within section *Barkhausia*. Hybridization analyses based on the combined nuclear and plastid data set revealed complex patterns of reticulate evolution. All *Crepis* taxa appeared to be hybrids and parental lineages, which indicated extensive ancient hybridization and introgression processes. However, these complicated relationships could also be misleading due to duplication or incomplete lineage sorting. Despite the high level of variation in both low-copy nuclear markers, *gsh1* and *sqs*, their utility in the phylogenetic study is limited by multi-copy sequences, which render these markers unsuitable for phylogenetic investigations at inter- and intraspecific levels. However, both markers might be good candidates at the generic level.

5.1. Future Work

In order to answer the remaining open questions regarding the phylogenetic relationships within *Crepis* section *Barkhausia*, it would be desirable to carry out the following investigations:

1. To elucidate whether the different copy types are present in all *Crepis* taxa or are restricted to this group, copy-specific primers should be designed. The first intron of each copy type would be a suitable region for primer design.
2. To determine the number of copies of the *gsh1* and *sqs* genes, Southern Blot or Next Generation Sequencing (NGS) analysis of *Crepis* is recommended.

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8.Appendix

8.1 DNA Samples Information and Chemicals

Species	Number	Date of DNA Extraction	Source / Barcode
C. foetida ssp foetida	DB 3454	11.02.2009	B 10 0326498
	DB 3497	12.02.2009	B 10 0326546
	Ne149	11.10.2005	Museum Botanic Berolinense / B 10 0030676
	Bg 227	unknown	unknown
C. foetida ssp afghanistanica	Ne122	10.05.2005	Herbarium of systematic botany Munich / MSB 01615
C. foetida ssp rhoeadifolia	Ne139	11.10.2005	Museum Botanic Berolinense / B 10 0120212
C. foetida ssp commutata	Ne150	11.10.2005	Museum Botanic Berolinense
	DB 540	15.02.2007	B 10 0209682
	DB 541	15.02.2007	B 10 0209666
	Gh 5	03.02.2014	Albrecht-von-Haller-Institute for Plant Sciences Georg August University Goettingen
C. *foetida ssp thomsonii*	Ne 256	20.11.2006	Royal Botanic Garden Edinburg/ E00228160
	Gh 8	03.02.2014	Botanical Bavarian State Collection
C. tybakiensis	Ne 179	13.10.2005	Museum Botanic Berolinense/ DB 18737
	Ne 180	13.10.2005	Museum Botanic Berolinense/ DB 18738
C. rubra	Bg 22	unknown	Unknown
	DB 8492	13.02.2013	B 10 0355976
C. alpina	Bg 222	unknown	Unknown
C. kotschyana	Ne 131	11.10.2005	Museum Botanic Berolinense/ 2636815
	Ne 193	13.10.2005	Museum Botanic Berolinense
	Gh 3	03.02.2014	Albrecht-von-Haller-Institute for Plant Sciences Georg August University Goettingen
C. pusilla	Ne 228	14.10.2005	Museum Botanic Berolinense
C. zacintha	Bg 123	unknown	Unknown
	DB 547	15.02.2007	B 10 0209667
	Gh 4	03.02.2014	Botanical Bavarian State Collection
C. triasii	Ne 110	10.10.205	Herbarium of systematic botany Munich / M-0088483
	Gh1	25.09.2013	Botanical Garden in Barcelona
	Gh2	25.09.2013	Botanical Garden in Barcelona

Table. 2.1 *Crepis* species section *Barkhausia* names and the DNA samples' information

Species Name	GenBank Nr.			
	matK	**ITS**	***gsh1***	***sqs***
C.foetida.spp.foetida	EU363556.1	AJ633370.1	-	-
C.foetida spp.afghanistanica	-	EU363604.1	-	-
C.foetida spp.commutata	-	-		-
C.foetida spp.rhoeadiflia	-	EU363613.1	-	-
C.foetida spp.thomsonii	-	EU363647.1	-	-
C.rubra	AJ633141.1	AJ633350.1	-	-
C.tybakiensis	EU363566.1	EU363631.1	-	-
C.alpina	EU363575.1	AJ633357.1	-	-
C.kotschyana	-	EU363635.1	-	-
C.pusilla	EU363576.1	EU363650.1	-	-
C. zacintha	EU363579.1	EU363655.1	-	-
C.triasii	-	EU363597.1	-	-
Hispedella hispanica	-	-	HQ131797.1	JX129602.1

Table 2.1.1. GenBank Numbers of Tax downloaded via NCBI

Chemicals, Enzymes	Company
6X DNA Loading Dye	Fermentas
Acetic acid	AppliChem
Agar-Agra	Roth
Agarose	Roth
Ampicillin	Roth
Betain Monohydrat	Sigma-Aldrich
Bromophenol Blue	AppliChem
Dneasy Plant Mini Kit	Qiagen
dNTP Set	Fermentas
Double Distilled Water	Roth
EDTA	AppliChem
Ethanol	AppliChem
Exosap-it	Affymetrix
Gel Extraction Kit	Qiagen
Gene Ruler DNA ladder (100 – 1000 bp)	Fermentas
Magnesium Chloride	Roth
PCR Cloning Kit	Fermentas
Pepton	Roth
Plasmid DNA Purification Kit	Qiagen
Sodium Chloride	Roth
Sodium Chloride	Roth
Sybr Gold Nucleic Acid	Invitrogen
T4 DNA Ligase	Fermentas
Taq DNA	Fermentas
Tris	AppliChem
Xylene Cyanol	AppliChem
yeast Extract	Roth

Table.2.3 The chemicals and enzymes used in the present study

Solutions	Components
TAE running Buffer (1 L. of 50 X)	60.0 g Tris-base, 14,275 ml Acetic Acid, 50,0 ml EDTA-Na_2, (pH 8), add to 250 ml dH2O
Sybr Gold Nucleic Acid Gel Stain Stock Solution Working Solution	1485 µl TE+ 15 µl Sybr gold (10000 x)= 1500 µl Sybr gold (100 x) 1350 µl H_2O + 150 µl Sybr Gold (100 x) = 1500 µl Sybr gold (10x) 1500 µl Sybr gold (10x) + 1500 µl Loading dye = for use
6 x Loading Dye for Agarose Gel	0.03% xylene cyanol FF 30 ng 0.03% bromophenol blue 30 ng 60% glycerol 60 ml 60 mM EDTA

Table.2.4 The buffer and solutions used in this study

Instruments	Company
Accurate scales	Kern
Autoclave	Tuttnauer
Biomedical freezer	Sanyo Electric Co., Ltd.
Camera	Canon
Centrifuge	Eppendorf
Electroporator	Eppendorf
Heater System	HLC
Horizontal Electrophoresis	Amersham pharmacia biotech
Ice Machine	Ziegra
Incubator	Innova
Master Cycler Gradient	Eppendorf
Microprocessor PH Meter	Hanna
Microwave	LG
Mini Centrifuge	Hermle
Nanophotometer	Implen
Refrigerator	Liebherr
Safety Cabinet	BDK
Thermomixer Compact	Eppendorf
TissueLyser Mixer	Retsch
UV surface Emitter	Benda
Vortex	Heidolph
Water Bath	GFL

Table2.5: The analytical instruments used in the present study

Laboratory Materials	Company
8 Channel Pipette	Eppendorf
96-Well PCR Plate	4titude
Centrifuge Tube Racks	Roth
Glass Tubes, Flasks, Beakers	Duran
Ice Boxes	
Laboratory Parafilm	Bemis
Magnetic Stirrer	Roth
Magnetic Stirring	Roth
Micro-centrifuge Tubes 1.5 ml	Sarstedt
Micro-centrifuge Tubes 2 ml	Sarstedt
Micropipettes Set (0.5, 10, 20, 100)	Eppendorf
Nitrile Examination Gloves	Kirchhoff / Hansa Medical 24
PCR tubes 0.2 ml, 8 strip	Sarstedt
PCR-Rack	Roth
Petri Dishes	Fermentas
Pipette Tips	Sarstedt
Tips Box	Eppendorf
Tube 50 ml	Sarstedt
Weighing Pan	Roth

Table.2.6 The Laboratory Materials used in the present study

8.2. Aligned *gsh1* DNA sequences matrix

```
                                    5         15         25         35         45         55         65         75         85         95
C.alpina clone.1                    CCATGGAGGA GGTTATGTGC ATTGCCTGCG TTTTGGGTAG GGCTATTTTG GTCATTTT-A --TCTTTCAA TATGCATGTG GCTTATCTTT ATA-------
C.alpina clone.2                    CCATGGAGGA GGTTATGTGC ATTGCCTGCG TTTTGGGTAG GGCTATTTTG GTCATTTT-A --TCTTTCAA TATGCATGTG GCTTATCTTT ATA-------
C.alpina clone.3                    CCATGGAGGA GGTTATGTGC ATTGCCTGCG TTTTGGGTAG GGCTATTTTG GTCATTTT-A --TCTTTCAA TATGCATGTG GCTTATCTTT ATA-------
C.foetida spp.commutata             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.triasii.seq.3                     CCATGGAGGA GGTTA-GTGC ATTGCCTGCG TTTTGGGTAG GGCTATTTTG GTCATTTT-A --TCTTTCAA TATGCATGTG GCTTATCTTT ATA-------
C.foetida spp.foetida.seq.1         CCATGGAGGA GGTTATGTGC ATTGCCTGCG TTTTGGGTAG GGCTATTTTG GTCATTTT-A --TGTTTCAC TATGCATGTG GCTTATCTTT TCTA------
C.foetida spp.foetida.seq.2         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.afghanistanica.seq.2  CCATGGAGGA GGTTATGTGC ATTGCCTGCG TTTTGGGTAG GGCTATTTTG GTCATTTT-A --TGTTTCAC TATGCATGTG GCTTATCTTT TCT-------
C.foetida spp.rhoeadifolia.seq.1    CCATGGAGGA GGTTATGTGC ATTGCCTGCT TTTTGGGTAG GGCTATTTTC GTCATTTT-A --TGTTTCAA TATACATGTG G-TTATCTTT TTTAAT----
C.triasii.seq.1                     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.rubra.seq.2                       CCATGGAGGA GGTTATGTGC ATTGCCTGCG TTTTGGGTAG GGCTATTTTG GTCATTTT-A --TGTTTCAA TATGCATGTG GCTTATCTTC TTTAT-----
C.foetida spp.afghanistanica.seq.1  CCATGGAGGA GGTTATGTGC ATTGCCCGCG TTTTGGGTAG GGCTATTTCG GTCATTTTTA --TGTTTCAC TATGCATGTG GCATATTTTT TTT-------
C.rubra.seq.1                       CCATGGAGGA GGTTATGTGC ATTGCCTGCG TTTTGGGTAG GGCTATTTTG GTCATTTT-A --TGTTTCAC TATGCAGGTG GCATATCTTT TCT-------
C.foetida spp.rhoeadifolia.seq.2    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.thomsonii.seq.1       CCATGGAGGA GGTTATGTGC ATTGCCTGCG TTTTGGGTAG GGCTATTTTG GTCATTTT-A --TCTTTCAA TATGCATGTG GCATATCTTT TCT-------
C.pusilla.seq.2                     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.thomsonii.seq.2       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.triasii.seq.2                     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.zacintha.seq.1                    CCATGGAGGA GGTTATGTGC ATTGCCTGCG TTTTGGGTAG GGCTATTTTG GTCATTTT-A --TGTTTTAA TATGCATGTG GCTTATCTTC TTTAAGTGGT
C.zacintha.seq.2                    CCATGGAGGA GGTTATGTGC ATTGCCTGCG TTTTGGGTAG GGCTATTTTG GTCATTTT-A TATGTTTCAC TATGCATGTG GCTTATCTTC TTTAAGTGGT
C.pusilla.seq.1                     CCATGGAGGA GGTTATGTGC ATTGCCTGCG TTTTGGGTAG GGCTATTTTG GTCATTTT-A --TGTTTCAA TATGCATGTG GCTTATCTTC TTTAAGTGGT
C.tybakiensis                       CCATGGAGGA GGTTATGTGC ATTGCCTGCG TTTTGGGTAG GGCTATTTTG GTCATTTT-A --TGTTTCAA TATGCATGTG GCTTATCTTC TTTAAGTGGT
C.kotschy.clone.1                   CCATGGAGGA GGTTATGTGC ATTGCCTGCG TTTTGGGTAG GGCTATTTTG GTCACTTT-A --TGTTTCAA TAA------- --TTATCTTT TTTA------
C.kotschy.clone.2                   CCATGGAGGA GGTTATGTGC ATTGCCTGCG TTTTGGGTAG GGCTATTTTG GTCACTTT-A --TGTTTCAA TAA------- --TTATCTTT TTTA------
C.triasii.seq.4                     CCATGGAGGA GGTTATGTGC ATTGCCTGCG TTTTGGGTAG GGCTATTTTG GTCACTTT-A --TGTTTCAA TAA------- --TTATCTTT TTTA------
```

Aligned *gsh1* DNA sequences matrix of the *Crepis* species section *Barkhausia* representing 25 DNA sequences of 12 *Crepis* taxa.↓ continue

```
                                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                     105        115        125        135        145        155        165        175        185        195
C.alpina.clone.1                  ---------- ---------- ---------- ---------- AATAAAAAAT CATCAGGTTG GTATATTGTA TGATGATGTT GCTTTGCAAA AAGTTTTGGA
C.alpina.clone.2                  ---------- ---------- ---------- ---------- AATAAAAAAT CATCAGGTTG GTATATTGTA TGATGATGTT GCTTTGCAAA AAGTTTTGGA
C.alpina.clone.3                  ---------- ---------- ---------- ---------- AATAAAAAAT CATCAGGTTG GTATATTGTA TGATGATGTT GCTTTGCAAA AAGTTTTGGA
C.foetida spp.commutata           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----TGCAAA AAGTTTTGGA
C.triasii.seq.3                   ---------- ---------- ---------- ---------- AATAAAAAAT CATCAGGTTG GTATATTGTA TGATGATGTT GCTTTGCAAA AAGTTTTGGA
C.foetida spp.fooetida.seq.1      ---------- ---------- ---------- ---------- ATAAAAAAAC CATCAGGTTG GTATATTGTA TGATGATGTT GCTTTGCAAA AAGTTTTGGA
C.foetida spp.foetida.seq.2       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.afghanistanica.seq.2 ---------- ---------- ---------- ---------- AATAAAAAAT CATCAGGTTG GTATATTGTA TGATGATGTT GCTTTGCAAA AAGTTTTGGA
C.foetida spp.rhoeadifolia.seq.1  ---------- ---------- ---------- ---------- AATAAAAAAT CATCAGGTTG GTATATTGTA TGATGATGTT GCTCTGCAAA AAGTTTTGGA
C.triasii.seq.1                   ---------- ---------- ---------- ---------- ---------- CATCAGGTTG GTATATTGTA TGAGGATGTT GCTTTGCAAA AAGTTTTGGA
C.rubra.seq.2                     ---------- ---------- ---------- ---------- AATAAAAAAT CATCAGGTTG GTATATTGTA TGATGATGTG GCTTTGCAAA AAGTTTTGGA
C.foetida spp.afghanistanica.seq.1 ---------- ---------- ---------- ---------- AATAAAAAAT CATCAGGTTG GTATATTGTA TGATGATGTT GCTTTGCAAA AAGTTTTGGA
C.rubra.seq.1                     ---------- ---------- ---------- ---------- AATAMAAAAT CATCAGGTTG GTATATTGTA TGATGATGTT GCTTTGCAAA AAGTTTTGGA
C.foetida spp.rhoeadifolia.seq.2  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.thomsonii.seq.1     ---------- ---------- ---------- ---------- AATAAAAAAT CATCAGGTTG GTATATTGTA TGATGATGTT GCTTTGCAAA AAGTTTTGGA
C.pusilla.seq.2                   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.thomsonii.seq.2     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----GCAAA AAGTTTTGGA
C.triasii.seq.2                   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---TTGCAAA AAGTTTTGGA
C.zacintha.seq.1                  CATTTTATGT TTCACTATGC ATGTGGCTTA TATTCTTAAA AATAAAACAT CATCAGGTTG GTATATTGTA TGATGATGTT GCTTTGCAAA AAGTTTTGGA
C.zacintha.seq.2                  CATTTTATGT TTCACTATGC ATGTGGCTTA TATTCTTAAA AATAAAACAT CATCAGGTTG GTATATTGTA TGATGATGTT GCTTTGCAAA AAGTTTTGGA
C.pusilla.seq.1                   CATTTTATGT TTCACTGTGC ATGTGGCTTA TCTTATTT-- AATAAAACAT CATCAGGTTG GTATATTGTA TGATGATGTT GCTTTGCAAA AAGTTTTGGA
C.tybakiensis                     CATTTTATGC TTCACTAATC ---------- TTTTCT---- AATAAAAAAT CATCAGGTTG GTATATTGTA TGATGATGTG GCTTTGCAAA AAGTTTTGGA
C.kotschya.clone.1                ---------- ---------- ---------- ---------- ATGAAAAAAT CATCAGGTTG GTATATTGTA TGATGATGTT GCTTTGCAAA AAGTTTTGGA
C.kotschya.clone.2                ---------- ---------- ---------- ---------- ATGAAAAAAT CATCAGGTTG GTATATTGTA TGATGATGTT GCTTTGCAAA AAGTTTTGGA
C.triasii.seq.4                   ---------- ---------- ---------- ---------- ATGAAAAAAT CATCAGGTTG GTATATTGTA TGATGATGTT GCTTTGCAAA AAGTTTTGGA
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Aligned *gsh1* matrix ↓ continue

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                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                      205        215        225        235        245        255        265        275        285        295
C.alpina.clone.1                CATGACGGCT GATTGGACTG CAGCTGAAAG AGAAATGTTG AGAAATAAGG TAAAAATGGT AATTTTAAAT TAGTA-TTAT AAATA----- ----------
C.alpina.clone.2                CATGACGGCT GATTGGACTG CAGCTGAAAG AGAAATGTTG AGAAATAAGG TAAAAATGGT AATTTTAAAT TAGTA-TTAT AAATA----- ----------
C.alpina.clone.3                CATGACGGCT GATTGGACTG CAGCTGAAAG AGAAATGTTG AGAAATAAGG TAAAAATGGT AATTTTAAAT TAGTA-TTAT AAATA----- ----------
C.foetida spp.commutata         CATGACGGCT GATTGGACTG CAGCTGAAAG AGAAATGTTG AGAAATAAGG TAAAAATGGT AATTTTAAAT TAGTA-TTAT AAATA----- ----------
C.triasii.seq.3                 CATGACGGCT GATTGGACTG CAGCTGAAAG AGAAATGTTG AGAAATAAGG TAAAAATGGT AATTTTAAAT TAGTA-TTAT AAATA----- ----------
C.foetida spp.foetida.seq.1     CATGACGGCT GATTGGACTA CAGCAGAAAG AGAAATGTTG AGAAATAAGG TTAAAATGAT CATTTTAAAT -AGTT-TTAT TATAATTAT- ----------
C.foetida spp.foetida.seq.2     ---------- ---------- ---------- ---------- ---------- TAAAAATCAT CATGT-AAAT -AGTAATTA- ---------- ----------
C.foetida spp.afghanistanica.seq.2 CATGACGGCT GATTGGACTA CAGCAGAAAG AGAAATGTTG AGAAATAAGG TAAAAATCAT CATGT-AAAT -AGTAATTA- ---------- ----------
C.foetida spp.rhoeadifolia.seq.1 CATGACGGCT GATTGGACTG CAGCTGAAAG AGAAATGTTG AGAAATAAGG TAAAAATCAG CATGT-AAAT -AGTAATTA- ---------- ----------
C.triasii.seq.1                 CATGACCGCT GATTGGACTA CAGCAGAAAG AGAAATGCTG AGAAATAAGG TAAAAATCAT CATGT-AAAT -AGTA-TTAT TATTATTATT ----------
C.rubra.seq.2                   CATGACGGCT GATTGGACTG CAGCCGAAAG AGAAATGTTG AGAAATAAGG TAAAAATCAT CATGT-AAAT -AGTA-TTAT TATTATTATT ATTATTATTA
C.foetida.spp.afghanistanica.seq.1 CATGACCGCT GATTGGACTA CAGCAGAAAG AGAAATGCTG AGAAATAAGG TAAAAATCAT CATGT-AAAT -AGTA-TTAT TATTATTATT ATT-------
C.rubra.seq.1                   CATGACCGCT GATTGGACTA CAGCAGAAAG AGAAATGCTG AGAAATAAGG TAAAAATCAT CATGT-AAAT -AGTA-TTAT TATTATTATT ----------
C.foetida spp.rhoeadifolia.seq.1 ---------- ---------- ---------- -----TGCTG AGAAATAAGG TAAAAATCAT CATGT-AAAT -AGTA-TTAT TATTATTATT ----------
C.foetida.spp.thomsonii.seq.1   CATGACCGCT GATTGGACTA CAGCAGAAAG AGAAATGCTG AGAAATAAGG TAAAAATCAT CATGT-AAAT -AGTA-TTAT TATTATTATT ----------
C.pusilla.seq.2                 ---------- ---------- ---------- -----TGCTG AGAAATAAGG TAAAAATCAT CATGT-AAAT -AGTA-TTAT TATTATTATT ----------
C.foetida spp.thomsonii.seq.2   CATGACGGCT GATTGGACTG CAGCTGAAAG AGAAATGTTG AGAAATAAGG TAAAAATCAT CATGC-AAAT -AGTA-TTAT CATTATTTAT T---------
C.triasii.seq.2                 CATGACGGCT GATTGGACTG CAGCTGAAAG AGAAATGTTG AGAAATAAGG TAAAAATCAT CATGC-AAAT -AGTA-TTAT CATTATTTAT T---------
C.zacintha.seq.1                CATGACGGCT GATTGGACTG CAGCTGAAAG AGAAATGTTG AGAAATAAGG TAAAAATCAT CATGC-AAAT -AGTA-TTAT CATTATTTAT T---------
C.zacintha.seq.2                CATGACGGCT GATTGGACTG CAGCTGAAAG AGAAATGTTG AGAAATAAGG TAAAAATCAT CATGC-AAAT -AGTA-TTAT CATTATTTAT T---------
C.pusilla.seq.1                 CATGACGGCT GATTGGACTG CAGCTGAAAG AGAAATGTTG AGAAATAAGG TAAAAATCAT CATGC-AAAT -AGTA-TTAT TATTATTATT ATT-------
C.tybakiensis                   CATGACGGCT GATTGGACTA CAGCAGAAAG AGAAATGTTG AGAAATAAGG TACAAATCAT CATGT-AAAT -AGTA-TTAT TGTAATTA-- ----------
C.kotschya.clone.1              CATGACGGCT GATTGGACTG CAGCCGAAAG AGAAATGTTG AGAAACAAGG TAAAAACCAC CACGT-AAAT -AGTA-TTGT TATTTATTTA TTTGTAT---
C.kotschya.clone.2              CATGACGGCT GATTGGACTG CAGCCGAAAG AGAAATGTTG AGAAACAAGG TAAAAACCAC CACGT-AAAT -TGTA-TTGT TATTTATTTA TTTGTAT---
C.triasii.seq.4                 CATGACGGCT GATTGGACTG CAGCCGAAAG AGAAATGTTG AGAAACAAGG TAAAAACCAC CACGT-AAAT -AGTA-TTGT TATTTATTTA TTTGTAT---
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Aligned *gsh1* matrix ↓ continue

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                                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                     305        315        325        335        345        355        365        375        385        395
C.alpina.clone.1                  TTTTATGCTC TTTATGAC-- --ATGATCAC TTTCATGAA- TGTTGTATTA GGTGCCTGTA ACTGGTCGGA AAACCCCATT CCGTGATGGA TTGCCGAAAC
C.alpina.clone.2                  TTTTATGCTC TTTATGAC-- --ATGATCAC TTTCATGAA- TGTTGTATTA GGTGCCTGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.alpina.clone.3                  TTTTATGCTC TTTATGAC-- --ATGATCAC TTTCATGAA- TGTTGTATTA GGTGCCTGTA ACTGGTCCGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.foetida spp.commutata           TTTTATGCTC TTTATGAC-- --ATGATCAC TTTCATGAA- TGTTGTATTA GGTGCCTGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.triasii.seq.3                   TTTTATGCTC TTTATGAC-- --ATGATCAC TTTCATGAA- TGTTGTATTA GGTGCCTGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.foetida spp.foetida.seq.1       ---------- TTTATGAC-- --ATCATCAC TATTATGAA- CGTTGTATTA GGTGCCTGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.foetida spp.foetida.seq.2       ATTTATTCTC TTTATGAC-- --ATCATCAC TATTATGAA- YGTTGTATTA GGTGCCTGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.foetida spp.afghanistanica.seq.2 ATTTATTCTC TTTATGAC-- --ATCATCAC TATTATGAA- TGTTGTATTA GGTGCCTGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.foetida spp.rhoeadifolia.seq.1  ATTTATTCTC TTTATGAC-- --ATCATCAC TATTATGAA- TGTTGTATTA GGTGCCTGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.triasii.seq.1                   TATTTTTCTC TTTATGAC-- --ATCATCAC TTTCATGAA- TGTTGTATTA GGTACCGGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.rubra.seq.2                     TTTTATTCTC TTTATGACTG ACATCATCAC TATCATGAA- TGTTGTGTTA GGTGCCTGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.foetida spp.afghanistanica.seq.1 TATTTACCTC TTTATGAC-- --ATCATCAC TTTCATGAA- TGTTGTATTA GGTGCCTGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.rubra.seq.1                     TATTTTTCTC TTTATGAC-- --ATCATCAC TTTCATGAA- TGTTGTATTA GGTACCGGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.foetida spp.rhoeadifolia.seq.2  TATTTTTCTC TTTATGAC-- --ATCATCAC TTTCATGAA- TGTTGTATTA GGTACCGGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.foetida spp.thomsonii.seq.1     TATTTTTCTC TTTATGAC-- --ATCATCAC TTTCATGAA- TGTTGTATTA GGTACCGGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.pusilla.seq.2                   TATTTTTCTC TTTATGAC-- --ATCATCAC TTTCATGAA- TGTTGTATTA GGTACCGGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.foetida spp.thomsonii.seq.2     TATTTTTCTC TTTATGAC-- --ATC----- ----ATGAA- TGTTGCATTA GGTGCCTGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAGC
C.triasii.seq.2                   TATTTTTCTC TTTATGAC-- --ATC----- ----ATGAA- TGTTGCATTA GGTGCCTGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAGC
C.zacintha.seq.1                  TATTTTTCTC TTTATGAC-- --ATC----- ----ATGAA- TGTTGCATTA GGTGCCTGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAGC
C.zacintha.seq.2                  TATTTTTCTC TTTATGAC-- --ATG----- ----ATGAA- TGTTGCATTA GGTGCCTGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.pusilla.seq.1                   TATTTTTCTC TTTATGAC-- --ATC----- ----ATGAA- TGTTGTATTA GGTGCCTGTA ACAGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.tybakiensis                     ATTTATTCTC TTTATGAC-- --ATCATCAC TATCATGAA- TGTTGTGTTA GGTGCCTGTA ACTGGTCTGA AAACCCCATT CCGTGATGGA TTGCTGAAAC
C.kotschya.clone.1                ATTTTTTCTC TTTATGAC-- --ATCATGAC TTTGATGAAA TGTTGTATTA GGTGCCTGTA ACTGGTCTGA AAACTCCATT CCGTGATGGA TTGCTGAAAC
C.kotschya.clone.2                ATTTTTTCTC TTTATGAC-- --ATCATGAC TTTGATGAAA TGTTGTATTA GGTGCCTGTA ACTGGTCTGA AAACTCCATT CCGTGATGGA TTGCTGAAAC
C.triasii.seq.4                   ATTTTTTCTC TTTATGAC-- --ATCATGAC TTTGATGAAA TGTTGTATTA GGTGCCTGTA ACTGGTCTGA AAACTCCATT CCGTGATGGA TTGCTGAAAC
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Aligned *gsh1* matrix ↓ continue

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                                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                     405        415        425        435        445        455        465        475        485        495
C.alpina.clone.1                  ATGTTGCCCA AGAAGTTGTT GGTTTTGCCA AGGTAATTAA TTAATTATCA ATCATGCTAT TAACACTCAT TTTGGTAAAT CAATTTTCAT TAATCTAAAA
C.alpina.clone.2                  ATGTTGCTCA AGAAGTTGTT GGTTTTGCCA AGGTAATTAA TTAATTATCA ATCTTGCTAT TAACACTCAT TTTGGTAAAT CGATTCTCAT TAATCTAAAA
C.alpina.clone.3                  ATGTTGCTCA AGAAGTTGTT GGTTTTGCCA AGGTAATTAA TTAATTATCA ATCTTGCTAT TAACACTCAT TTTGGTAAAT CGATTCTCAT TAATCTAAAA
C.foetida spp.commutata           ATGTTGCTCA AGAAGTTGTT GGTTTTGCCA AGGTAATTAA TTAATTATCA ATCTTGCTAT TAACACTCAT TTTGGTAAAT CGATTCTCAT TAATCTAAAA
C.triasii.seq.3                   ATGTTGCTCA AGAAGTTGTT GGTTTTGCCA AGGTAATTAA TTAATTATCA ATCTTGCTAT TAACACTCAT TTTGGTAAAT CGATTCTCAT TAATCTAAAA
C.foetida spp.foetida.seq.1       ATGTTGCTCA AGAGGTTGTC GGTTTTGCAA AGGTAATTAA TTATT----- --------AT TAATACTC-- --------CT CGTTTTTCTA TAATCTAAAA
C.foetida spp.foetida.seq.2       ATGTTGCTCA AGAGGTTGTC GGTTTTGCAA AGGTAATTAA TTATT----- --------AT TAATACTCAT TTTGGTGACT CRTTTTTCTA TAATCTAAAA
C.foetida spp.afghanistanica.seq.2 ATGTTGCTCA AGAGGTTGTC GGTTTTGCAA AGGTAATTAA TTATT----- --------AT TAATACTCAT TTTGGTGACT CATTTTTCAA TAATCTAAAA
C.foetida spp.rhoeadifolia.seq.1  ATGTTGCTCA AGAGGTTGTC GGTTTTGCAA AGGTAATTAA TTATT----- --------AT TAATACTCAT TTTGGTGACT CATTTTTCAA TAATCTAAAA
C.triasii.seq.1                   ATGTTGCTCA AGAAGTTGTC AGTTTTGCAA AGGTAATTTA TTTAT----A ATCTTGCTAT TAATACTCAT TT-AGTGAGT CATTTTCCTA TAATCTAAAA
C.rubra.seq.2                     ATGTTGCTCA AGAAGTTGTC AGTTTTGCAA AGGTAATTAA TTATT----A ATCTAACTAT TAATACTCAT TTTGGTGAGT CATTTTTCAA TAATCTAAAA
C.foetida spp.afghanistanica.seq.1 ATGTTGCTCA AGAAGTTGTC AGTTTTGCAA AGGTAATTAA TTTAT----A ATCTTGCTAT TAATACTCAT TT-AGTGAGT CATTTTCCAA TAATCTAAAA
C.rubra.seq.1                     ATGTTGCTCA AGAAGTTGTC AGTTTTGCAA AGGTAATTTA TTTAT----A ATCTTGCTAT TAATACTCAT TT-AGTGAGT CATTTTCCTA TAATCTAAAA
C.foetida spp.rhoeadifolia.seq.2  ATGTTGCTCA AGAAGTTGTC AGTTTTGCAA AGGTAATTTA TTTAT----A ATCTTGCTAT TAATACTCAT TT-AGTGAGT CATTTTCCTA TAATCT----
C.foetida spp.thomsonii.seq.1     ATGTTGCTCA AGAAGTTGTC AGTTTTGCGA AGGTAATTTA TTTAT----A ATCTTGCTAT TAATACTCAT TT-AGTGAGT CATTTTCCTA TAATCTAAAA
C.pusilla.seq.2                   ATGTTGCTCA AGAAGTTGTC AGTTTTGCAA AGGTAATTTA TTTAT----A ATCTTGCTAT TAATACTCAT TT-AGTGAGT CATTTTCCTA TAATCTAAAA
C.foetida spp.thomsonii.seq.2     ATGTTGCTCA AGAAGTTGTC GGTTTTGCAA AGGTAATTAA TTATT----A ATCTGGCTAT TAATACTCAT TT-------- -ATGTTTCAA TAATCTAAAA
C.triasii.seq.2                   ATGTTGCTCA AGAAGTTGTC GGTTTTGCAA AGGTAATTAA TTATT----A ATCTGGCTAT TAATACTCAT TT-------- -ATGTTTCAA TAATCTAAAA
C.zacintha.seq.1                  ATGTTGCTCA AGAAGTTGTC GGTTTTGCAA AGGTAATTAA TTATT----A ATCTGGCTAT TAATACTCAT TT-------- -ATGTTTCAA TAATCTAAAA
C.zacintha.seq.2                  ATGTTGCTCA AGAAGTTGTC GGTTTTGCAA AGGTAATTAA TTATT----A ATCTGGCTAT TAATACTCAT TT-------- -ATGTTTCAA TAATCTAAAA
C.pusilla.seq.1                   ATGTTGCTCA AGAAGTTGTC GGTTTTGCAA AGGTAATTAA TTATT----A ATCTGGCTAT TAATACTCAT TT-------- -ATTTTTCAA TAATCTAAAA
C.tybakiensis                     ATGTTGCTCA AGAAGTTGTC AGTTTTGCAA AGGTAATTAA TCATG----A ATATTGCTAT AAATACTCAT TTTAGTCAAT CATTCGTCAA TTATCTAAAA
C.kotschya.clone.1                ATGTTGCTCA AGAAGTTGTC GGTTTTGCAA AGGTAGTTAA TTGTT----A ATCTAGCTAT TAATACTCAT TT-AGTGTGT CATTTTTCAA TAATCTAAAA
C.kotschya.clone.2                ATGTTGCTCA AGAAGTTGTC GGTTTTGCAA AGGTAGTTAA TTGTT----A ATCTAGCTAC TAATACTCAT TT-AGTGTGT CATTTTTCAA TAATCTAAAA
C.triasii.seq.4                   ATGTTGCTCA AGAAGTTGTC GGTTTTGCAA AGGTAGTTAA TTGTT----A ATCTAGCTAT TAATACTCAT TT-AGTGTGT CATTTTTCAA TAATCTAAAA
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Aligned *gsh1* matrix ↓ continue

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                                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                     505        515        525        535        545        555        565        575        585        595
C.alpina.clone.1                  A--------- -----AAAAC AGGATGGACT TGAAAGGAGA GGATATAAGG AAACCGGATT CTTAAATGAA GTGACAGAAG TTGTCAGAAC AGGTGAATTT
C.alpina.clone.2                  A--------- -----AAAAC AGGATGGACT TGAAAGGAGA GGATATAAGG AAACCGGATT CTTAAATGAA GTGACAGAAG TTGTCAGAAC AGGTGAATTT
C.alpina.clone.3                  A--------- ----AAAAAC AGGATGGACT TGGAAGGAGA GGATATAAGG AAACCGGATT CTTAAATGAA GTGACAGAAG TTGTCAGAAC AGGTGAATTT
C.foetida spp.commutata           A--------- -----AAAAC AGGATGGACT TGAAAGGAGA GGATATAAGG AAACCGGATT CTTAAATGAA GTGACAGAAG TTGTCAGAAC AGGTGAATTT
C.triasii.seq.3                   A--------- -----AAAAC AGGATGGACT TGAAAGGAGA GGATATAAGG AAACCGGATT CTTAAATGAA GTGACAGAAG TTGTCAGAAC AGGTGAATTT
C.foetida spp.foetida.seq.1       A-----TATA ACTTT-AAAC AGGATGGCCT TGAGAGGAGA GGGTATAAGG AAACCGGATT CTTAAATGAA GTCACAGAGG TTGTCAGAAC AGGTGAATTT
C.foetida spp.foetida.seq.2       A-----TATA ACTTT-AAAC AGGATGGCCT TGAGAGGAGA GGGTATAAGG ---------- ---------- ---------- ---------- ----------
C.foetida spp.afghanistanica.seq.2 A-----TATA ACTTT-AAAC AGGATGGCCT TGAGAGGAGA GGGTATAAGG AAACCGGATT CTTAAATGAA GTCACAGAGG TTGTCAGAAC AGGTGAAAAT
C.foetida spp.rhoeadifolia.seq.1  A-----TATA ACTTT-AAAC AGGATGGCCT TGAGAGGAGA GGGTATAAGG AAACCGGATT CTTAAATGAA GTCACAGAGG TTGTCAGAAC AGGTGAATTT
C.triasii.seq.1                   A-----AAAA ACTTTAAAAC AGGATGGACT TGAGAGGAGA GGATATAAGG AAACTGGATT CTTAAATGAA GTCACAGAAG TTGTCAGAAC AGGTGAATTT
C.rubra.seq.2                     AGAAAAAAAA ACTTT-AAAC AGGATGGCCT -GAGAGGAGA GGATATAAGG AAACCGGATT CTTAAATGAA GTCACAGAGG TTGTCAGAAT AGGTGAATTT
C.foetida spp.afghanistanica.seq.1 A------AAA ACTTTAAAAC AGGATGGACT TGAAAGGAGA GGATATAAGG AAACTGGATT CTTAAATGAA GTCACAGAGG TTGTCAGAAC AGGTGAATTT
C.rubra.seq.1                     A-----AAAA AYTTTAAAAC AGGATGGACT TGAGAGGAGA GGATATAAGG AAACTGGATT CTTAAATGAA GTCACAGAAG TTGTCAGAAC AGGTGAATTT
C.foetida spp.rhoeadifolia.seq.2  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.thomsonii.seq.1     A-----AAAA ACTTTAAAAC AGGATGGACT TGAGAGGAGA GGATATAAGG AAACTGGATT CTTAAATGAA GTCACAGAAG TTGTCAGAAC AGGTGAATTT
C.pusilla.seq.2                   A--------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.thomsonii.seq.2     A------AAA ATTTC-AAAC AGGATGGCCT -GAGAGGAGA GGGTAT---- ---------- ---------- ---------- ---------- ----------
C.triasii.seq.2                   A------AAA ATTTC-AAAC AGGATGGCCT TGAGAGGAGA GGGTATAAGG AAACCGGATT CTTAAATGAA GTCACAGARG TTGTCAGAAC AGGTGA----
C.zacintha.seq.1                  A------AAA ATTTC-AAAC AGGATGGCCT TGAGAGGAGA GGGTATAAGG AAACCGGATT CTTAAATGAA GTCACAGAGG TTGTCAGAAC AGGTAAATTT
C.zacintha.seq.2                  A------AAA ATTTC-AAAC AGGATGGCCT TGAGAGGAGA GGGTATAAGG AAACCGGATT CTTAAATGAA GTCACAGAGG TTGTCAGAAC AGGTAAATTT
C.pusilla.seq.1                   A------AAA ACTTC-AAAC AGGATGGCCT TGAGAGGAGA GGGTACAAGG AAACCGGATT CTTAAATGAA GTCACAGAGG TTGTCAGAAC AGGTAAATTT
C.tybakiensis                     A-------AA ACTTT-AAAC AGGATGGCCT TGAGAGGAGA GGATATAAGG AAACCGGATT CTTAAATGAA GTCACAGAGG TTGTCAGAAT AGGTGAATTT
C.kotschya.clone.1                A-----AACA ACTTT-AAAC GGGATGGCCT TGAGAGGAGG GGTTATAAGG AAACAGGATT CTTGAATGAA GTCACAGAAG TTGTCAGAAC AGGTGAATTT
C.kotschya.clone.2                A-----AACA ACTTT-AAAC AGGATGGCCT TGAGAGGAGA GGTTATAAGG AAACAGGATT CTTGAATGAA GTCACAGAAG TTGTCAGAAC AGGTGAATTT
C.triasii.seq.4                   A-----AACA ACTTT-AAAC AGGATGGCCT TGAGAGGAGA GGTTATAAGG AAACAGGATT CTTGAATGAA GTCACAGAAG TTGTCAGAAC AGGTGAATTT
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Aligned *gsh1* matrix ↓ continue

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                                     605        615        625        635        645        655        665        675        685        695
C.alpina.clone.1                  TCCTT----- ---------- ---------- ---------- ------CTTT TTGCCTTAAT GGACTTAAT- --------GG GAACAT--GG CTTAATCCGT
C.alpina.clone.2                  TCCTT----- ---------- ---------- ---------- ------CTTT TTGACTTAAT GGACTTAAT- --------GG GAACAT--GG CTTAATCTGT
C.alpina.clone.3                  TCCTT----- ---------- ---------- ---------- ------CTTT TTGACTTAAT GGACTTAAT- --------GG GAACAT--GG CTTAATCTGT
C.foetida spp.commutata           TCCTT----- ---------- ---------- ---------- ------CTTT TTGACTTAAT GGACTTAAT- --------GG GAACAT--GG CTTAATCTGT
C.triasii.seq.3                   TCCTT----- ---------- ---------- ---------- ------CTTT TTGACTTAA- GGMCTTAAT- --------GG GAACAT--GG CTTAATCTGT
C.foetida.spp.foetida.seq.1       TCCTTCTT-- ---------- ---------- ---------- ------CTTT TTGACTTAAT GGACTTAAT- --------GG GAACAT--GA CTTAAT----
C.foetida spp.foetida.seq.2       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida.spp.afghanistanic.seq.2 TCCTTCTA-- ---------- ---------- ---------- ------CTTT TTGACTTAAT ---------- --------GG GAACAT--GA CTTAATCTGT
C.foetida.spp.rhoeadifolia.seq.1  TCCTTCTA-- ---------- ---------- ---------- ------CTTT TTGACTTAAT ---------- --------GG GAACAT--GA CTTAATCTGT
C.triasii.seq.1                   TCCTT----- ---------- ---------- ---------- ------CTTT TTGACTTAAT GGACTTAATG GACTTAATGG GAACAT--GA CTTAATCTGT
C.rubra.seq.2                     TCCTTC---- ---------- ---------- ---------- ------CTTT TGGACTTAAT ---------- --------GG GAACAT--GG CCTAATCTGT
C.foetida spp.afghanistanica.seq.1 TCCTT----- ---------- ---------- ---------- ------CTTT TTGACTTAAT AGACTTAAT- --------GG GAACATATGA CTTAATCTGG
C.rubra.seq.1                     TCCTT----- ---------- ---------- ---------- ------CTTT TTGACTTAAT GGACTTAAT- --------GG GAACAT--GA CTTAATCTGT
C.foetida spp.rhoeadifolia.seq.2  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.thomsonii.seq.1     TCCTT----- ---------- ---------- ---------- ------CTTT TTGACTCAAT GGACTTAAT- --------GG GAAC------ ----------
C.pusilla.seq.2                   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.thomsonii.seq.2     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.triasii.seq.2                   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.zacintha.seq.1                  GCCTTCTTCT T------AGT TCCTATATCA TTCTGTCATC TTGTTTCTTT TTGACTTAAT ------AAT- --------GG GGACAT--GA CTTAATCTGG
C.zacintha.seq.2                  GCCTTCTTCT T------AGT TCCTATATCA TTCTGTCATC TTGTTTCTTT TTGACTTAAT ------AAT- --------GG GGACAT--GA CTTAATCTGG
C.pusilla.seq.1                   GCCTCCTTCT TCTTCTTAGT TCCTATATCA TTCTGTCATC TTGCTTCTTT TTGACTTAAT --------T- --------GG GGACAT--GA CTTAATTTGG
C.tybakiensis                     TCCTTCTC-- ---------- ---------- ---------- ------CTTT TGGACTTAAT ---------- --------GG GAACAT--GG CCTAATCTGT
C.kotschya.clone.1                AGCTTCTT-- ---------- ---------- ---------- ------CTTT TTGACTTAAT GGACTTAAT- --------GG GAACAT--GA CTTGATCTGT
C.kotschya.clone.2                AGCTTCTT-- ---------- ---------- ---------- ------CTTT TTGACTTAAT GGACTTAAT- --------GG GAACAT--GA CTTGATCTGT
C.triasii.seq.4                   AGCTTCTT-- ---------- ---------- ---------- ------CTTT TTGACTTAAT GGACTTAAT- --------GG GAACAT--GA CTTGATCTGT
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Aligned *gsh1* matrix ↓ continue

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                                  ....|....| ....|....| ....|....| ....|....| ....|....| ....
                                     705        715        725        735        745
C.alpina.clone.1                  TAAGCTAT-- ------ATGT GTTAAT-ATG ATGCAGGTTT AACTCCAGCA GAGA
C.alpina.clone.2                  TAAGCTAT-- ------ATGT GTTAAT-ATG ATGCAGGTTT AACTCCAGCA GAGA
C.alpina.clone.3                  TAAGCTAT-- ------ATGT GTTAAT-ATG ATGCAGGTTT AACTCCAGCA GAGA
C.foetida spp.commutata           TAAGCTAT-- ------ATGT GTTAAT-ATG ATGCAGGTTT AACTCCAGCA GAGA
C.triasii.seq.3                   TAAGCTAT-- ------ATGT GTTAAT-ATG ATGCAGGTTT AACTCCAGCA GAGA
C.foetida spp.foetida.seq.1       ---GCTAT-- ------ATAT GTATAT-ATG GTGCAGGTTT AACTCCAGCA GAGA
C.foetida spp.fooetida.seq.2      ---------- ---------- ---------- ---------- ---------- ----
C.foetida spp.afghanistanica.seq.2 TAAGCTATAA TGC------- -TATAT-ATG GTACAGGTTT AACTCCAGCA GAGA
C.foetida spp.rhoeadifolia.seq.1  TAAGCTATAA TGCTATATAT GTATAT-ATG GGACAGGTTT AACTCCAGCA GAGA
C.triasii.seq.1                   TAAGTTAT-- ------ATGT GTTAAT-ATG ATGCAGGTTT AACTCCAGCA GAGA
C.rubra.seq.2                     TAAGCTAT-- -----GATAT GTATAT-ATG GTACAGGTTT AACTCCAGCA GAGA
C.foetida spp.afghanistanica.seq.1 TAGGCTACA- ---------- -TATATAATG GTGCAGGTTT AACTCCAGCA GAGA
C.rubra.seq.1                     TAAGTTAT-- ------ATGT GTATAT-A-G GTGCAGGTTT AACTCCAGCA GAGA
C.foetida spp.rhoeadifolia.seq.2  ---------- ---------- ---------- ---------- ---------- ----
C.foetida spp.thomsonii.seq.1     ---------- ---------- ---------- ---------- ---------- ----
C.pusilla.seq.2                   ---------- ---------- ---------- ---------- ---------- ----
C.foetida spp.thomsonii.seq.2     ---------- ---------- ---------- ---------- ---------- ----
C.triasii.seq.2                   ---------- ---------- ---------- ---------- ---------- ----
C.zacintha.seq.1                  TAAGCTAT-- ---------- -------ATG GTGCAGGTTT AACTCCAGCA GAGA
C.zacintha.seq.2                  TAAGCTAT-- ---------- -------ATG GTGCAGGTTT AACTCCAGCA GAGA
C.pusilla.seq1                    TAATCTAT-- ------ATAT GTTTAT-ACG GTGCAGGTTT AACTCCAGCA GAGA
C.tybakiensis                     TAAGCTATGA TACTATATAT GTATAT-ATG GTACAGGTTT AACTCCAGCA GAGA
C.kotschya.clone.1                TAAGCTCT-- ------ATGT GTTAAT-ATG ATGCAGGTTT AACTCCAGCA GAGA
C.kotschya.clone.2                TAAGCTCT-- ------ATGT GTTAAT-ATG ATGCAGGTTT AACTCCAGCA GAGA
C.triasii.seq.4                   TAAGCTCT-- ------AT-- ---------- ---------- ---------- ----
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Aligned *gsh1* matrix . The end

8.3. Aligned *sqs* DNA sequences matrix

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                                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                      5        15        25        35        45        55        65        75        85        95
C.rubra                           ---------- ---------- ---------- ---------- ---------- -AATAATTAA AACGAAAAAA GTTGACTGTT AAAGAAAGCC ATTTTTTCT-
C.pusilla. seq.1                  ---------- ---------- ---------- ---------- ---------- ---------- ---------- GATGACTGTT AAAGAAAACC ATTTTTTCT-
C.zacintha.seq.1                  ---------- ---------- ---------- ---------- ---------- ---------- ---------- GATGAGTGTT AAAGAAAGCC ATTTTTTCTA
C.foetida spp.foeitda.seq.1       ------CTAG AAGG---GTA TTTATATTTT C--ACTTATC TATTTAGCCA CATAACTATA TTAGAAAAAA GTTGACTGTT AAAGAAAACC ATTTTTCCT-
C.tybakiensis.seq.1               ---------- ---------T TACCTATTTA GCAACTTAAC TATCTTCTTT -AATAATTAA A---AAAAAA GTTGACTGTT AAAGAAAACC ATTTTTCCT-
C.pusilla.seq.2                   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.thomsonii.seq.1     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.triasii.seq.3                   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.tybakiensis.seq.2               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.alpina                          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.triasii.seq.1                   ---------- ---------T TATCTATTTA TCCACTTAAC TATCTTCTCT ACTATTTTAA AAA------- --------TT AAAGAAAACC ATTATTCCT-
C.triasii.seq.2                   ---------- ---------- ---------- ---------- ---------- ---------- ----ACTGTT AAAGAAAGCC ATTTTTTCT-
C.kotschyana                      CTCTCGCTAT TCTATTTGCT TGCCTTTTTA CCCAC-TAAC TATCTATAAT AAATTAAAAA AAAAAAAAAA AGTGACTG-T AAAGAAAACC ATTTTTACTA
C.tybakiensis.seq.3               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.pusilla.seq.3                   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.afghanistanica      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.rhoeadifolia        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.commutata           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.triasii.seq.4                   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.zacintha.seq.2                  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.foetida.seq.2       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.thomsonii.seq.2     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

                                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                     105       115       125       135       145       155       165       175       185       195
C.rubra                           AA-TTCAAAT TAAATTACAG TTAT-CAAGA GGCAATTGAG GACATAACCA TGAGAATGGG AGCAGGAATG GCAAAATTTA TCTGTAAAGA GGTAACTAAC
C.pusilla.seq.1                   AACTTAAAGT TAAATTGCAG TTAT-CAAGA GGCAATTGAG GACATAACCA TGAGAATGGG AGCAGGAATG GCAAAATTCA TCTGTAAAGA GGTAACTAAC
C.zacintha.seq.1                  AAGTTAAAGT TAAATTGCAG TTAT-CAAGA GGCAATTGAG GACATAACCA TGAGAATGGG AGCAGGAATG GCAAA-TTCA TCTGCAAAGA GGTAACTAAC
C.foetida spp.foetida.seq.1       AA-CTAAAAT TACATTGCAG TTAT-CAAGA GGCAATTGAG GACATAACCA TGAGAATGGG AGCAGGAATG GCAAAATTCA TCTGTAAAGA GGTAACTAAC
C.tybakiensis.seq.1               AA-CTAAAAT TAACTTGCAG TTAT-CAGGA GGCAATTGAG GACATAACAA TGAGAATGGG AGCAGGAATG GCAAAATTTA TCTGCAAAGA GGTAACTAAC
C.pusilla.seq.2                   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.thomsonii.seq.1     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.triasii.seq.3                   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.tybakiensis.seq.2               ---------- ------GCAG TTAT-CAAGA GGCAATTGAG GACATAACAA TGAGAATGGG AGCAGGAATG GCAAAATTTA TCTGCAAAGA GGTAACTAAC
C.alpina                          ---------- ---------- ---------- ---------- -----AACCA TGAGAATGGG TGCAGGAATG GCAAAATTTA TCTGTAAAGA GGTAACTAAC
C.triasii.seq.1                   AACTTAAAGT TAAATTGCAG TTAT-CAAGA GGCAATTGAG GACATAACCA TGAGAATGGG TGCAGGAATG GCAAAATTCA TCTGTAGAGA GGTAATTAAG
C.triasii.seq.2                   AAGTTAAAA- TAAATTGCAG TTAT-CAAGA GGCAATTGAG GACATAACCA TGAGAATGGG TGCAGGAATG GCAAAATTCA TCTGTAAAGA GGTAACTAAA
C.kotschyana                      AAGTT-AAAT TAAATTGCAG TTAT-CAAGA GGCAATTGAG GACATAACCA TGAGAATGGG AGCAGGAATG GCAAAATTCA TCTGCAAAGA GGTAACTAAC
C.tybakiensis.seq.3               ---------- ---------- -----CAGGA GGCAATTGAG GACATAACCA TGAGAATGGG AGCAGGAATG GCAAAATTCA TCTGCAAAGA GGTAACTAAC
C.pusilla.seq.3                   ---------- ---------- ---------- ---------- ---------- ---------- ---------- GCAAA-TTCA TCTGCAA-GA GGTAACTAAC
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Aligned *sqs* DNA sequences matrix of the *Crepis* species section *Barkhausia* representing 22 DNA sequences of 12 *Crepis* taxa.↓ continue.

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C.foetida spp.afghanistanica       ---------- ---------- ---------- ---------- ---------- ---------- AGCAGG-ATG GCAAA-TTCA TCTGCAAAGA GGTAACTAAC
C.foetida spp.rhoeadifolia         ---------- ----TTGCAG TTAT-CAGGA GGCAATTGAG GACATAACCA TGAGAATGGG AGCAGGAATG GCAAAATTCA TCTGCAAAGA GGTAACTAAC
C.foetida spp.commutata            ---------- ----TTGCAG TTAT-C-GGA GGCAATTGAG GACATAACCA TGAGAATGGG AGCAGGAATG GCAAAATTCA TCTGCAAAGA GGTAACTAAC
C.triasii.seq.4                    ---------- ---------- ---------- -GCAATTGAG GACATAACCA TGAGAATGGG AGCAGGAATG GCAAAATTCA TCTGCAAAGA GGTAACTAAC
C.zacintha.seq.2                   ---------- ---------- ---------- ---------- ---------- -------GAG AGCAGG-ATG GCAAA-TTCA TCTGCAA-GA GGTAACTAAC
C.foetida spp.foetida. seq.2       ---------- ---------- ---------- ---------- ---------- ---------- -------ATG GCAAA-TTCA TCTGCAAAGA GGTAACTAAC
C.foetida spp.thomsonii.seq.2      ---------- ----TTGCAG TTTTTCAGGA GGCAATTGAG GACATAACCA TGAGAATGGG AGCAGGAATG GCAAAATTCA TCTGCAAAGA GGTAACTAAC


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                                      205        215        225        235        245        255        265        275        285        295
C.rubra                            TAACTAACTA GTTATCAATT ATC---ATTA TCTGTC~TTT TT~GAGTTGT TTAAA-GGG- ACAATAAATA AATGAAATT~ ATTGTTATGA AA-CTTGCAG
C.pusilla.seq.1                    T---------T GTTAT-AATT ATC------- --TTTC~TTT ---~GAGTTGT TTGAA-TGG- AAAATGAA-- AATGAAAAT~ GTTGTTATGA TA-TTTGTAG
C.zacintha.seq.1                   T---------T GTTATCAATC ATC---ATTA TCTGTC~TTT TC~GAATTGT TTAAAGTGAA AAAGTAAATA AATGAAATC~ GTTGTAATGA TA-TTTGCAG
C.foetida spp.foetida.seq.1        T---------T GTTAT-AATT ATC------- --TTTC~TTT ---~GAGTTGT TTGAA-TGG- AAAATGAA-- AATGAAAAT~ GTTGTTATGA TA-TTTGTAG
C.tybakiensis.seq.1                T---------T GTTATCAATT ATC---ATTA TCTGTC~TTT TT~GAGTTGT TTAAA-GGG- ACAATAAATA AATGAAAAT~ ATTGTTATGA TA-TTTTCAG
C.pusilla.seq.2                    ---------- ---------- ---------- ------~--- --~-------- ---------- ---------- ---------~ ---------- ----------
C.foetida spp.thomsonii.seq.1      ---------- ---------- ---------- -----C~TTT TT~GAGTTAT TTAAA-YTG- AGAATAAATA AATGAAATT~ GTTGTAATGA TA-TTTGCAG
C.triasii.seq.3                    ---------- ---------- ---------- ------~--- --~-------- ---------- ---------- ---------~ ---------- ----------
C.tybakiensis.seq.2                T---------T GTTATCAATT ATC---ATTA TCTGTC~TTT TT~GAGTTGT TTAAA-GGG- AGAATAAATA AATGAAAAT~ ATTGTTATGA TA-TTTTCAG
C.alpina                           TAACT----T GTTATCAAAT ATC---GTTA TTTGTC~TTT TT~GAGTTGT TTAAA-GTG- AGAACAAA-- AATGAAAAT~ ATTGTTATGA TA-TATGCAG
C.triasii.seq.1                    C---------T GATATCATTA ATT--AATTA TCTGTC~TTT ---~GAGGTGT TTAAAAAGG- AGAATAAA-A AATGAAAAT~ GGTGATATGA TA-TTTTCAG
C.triasii.seq.2                    T--------- --TATCATT- ---------- -------~TTT ---~GAGTTAT TTAAA-CTG- AGAATAAATA AATGAAATT~ GTTGTAATGA TA-TTTGCAG
C.kotschyana                       T---------T GTTATCAACT ATC---ATAA CCTGTC~TTT -T~GAGTTGT TTAAA-GAA- AGAATAAATA AATGAAAAT~ GTTGTTATGA TA-TTTGCAG
C.tybakiensis.seq.3                T---------T GTTATCAACT ATC---ATAA CCTGTC~TTT TT~GAGTTGT TTAAA-GAA- AGAATAAATA AATGAAAAT~ GTTGTTATGA TA-TTTGCAG
C.pusilla.seq.3                    T---------T GTTATCAACT ATC---ATAA CCTGTC~TTT TT~GAGTTGT TTAAA-GAA- AGAATAAATA AATGAAAAT~ GTTGTTATGA TA-TTTGCAG
C.foetida spp.afghanistanica       T---------T GTTATCAACT ATC---ATAA CCTGTC~TTT TT~GAGTTGT TTAAA-GAA- AGAATAAATA AATGAAAAT~ GTTGTTATGA TA-TTTGCAG
C.foetida spp.rhoeadifolia         T---------T GTTATCAACT ATC---ATAA CCTGTC~TTT TT~GAGTTGT TTAAA-GAA- AGAATAAATA AATGAAAAT~ GTTGTTATGA TA-TTTGCAG
C.foetida spp.commutata            T---------T GTTATCAACT ATC---ATAA CCTGTC~TTT TT~GAGTTGT TTAAA-GAA- AGAATAAATA AATGAAAAT~ GTTGTTATGA TA-TTTGCAG
C.triasii.seq.4                    T---------T GTTATCAACT ATC---ATAA CCTGTC~TTT TT~GAGTTGT TTAAA-GAA- AGAATAAATA AATGAAAAT~ GTTGTTATGA TA-TTTGCAG
C.zacintha.seq.2                   T---------T GTTATCAACT ATC---ATAA CCTGTC~TTT TT~GAGTTGT TTAAA-GAA- AGAATAAATA AATGAAAAT~ GTTGTTATGA TA-TTTGCAG
C.foetida spp.foetida seq.2        T---------T GTTATCAACT ATC---ATAA CCTGTC~TTT TT~GAGTTGT TTAAA-GAA- AGAATAAATA AATGAAAAT~ GTTGTTATGA TA-TTTGCAG
C.foetida spp.thomsonii seq.2      T---------T GTTATCAACT ATC---ATAA CCTGTC~TTT TT~GAGTTGT TTAAA-GAA- AGAATAAATA AATGAAAAT~ GTTGTTATGA TA-TTTGCAG
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Aligned *sqs* matrix ↓ continue

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                                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                     305        315        325        335        345        355        365        375        385        395
C.rubra                           GTTGAGACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAAAAAC
C.pusilla.seq.1                   GTTGAGACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAAAAAC
C.zacintha.seq.1                  GTTGAAACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCTTCTGGC ACTGAAAAAC
C.foetida spp.foetida. seq.1      GTTGAGACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAAAAAC
C.tybakiensis.seq.1               GTTGAAACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAAAAAC
C.pusilla.seq.2                   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.thomsonii.seq.1     GTTGAGACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGGTTGTCAA AACTCTYCCA TGCATCAGGC ACTGAAAAAC
C.triasii.seq.3                   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.tybakiensis.seq.2               GTTGAAACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGGTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAAAAAC
C.alpina                          GTTGAGACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAGAAAC
C.triasii.seq.1                   GTTGAAACAG TTGATGATTA TGATGAGTAT TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAAAAAC
C.triasii.seq.2                   GTTGAGACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGGTTGTCAA AACTCTTCCA TGCATCTGGC ACTGAAAAAC
C.kotschyana                      GTTGAGACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAAAAAC
C.tybakiensis.seq.3               GTTGAGACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAAAAAC
C.pusilla.seq.3                   GTTGAGACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAAAAAC
C.foetida spp.afghanistanica      GTTGAGACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAAAAAC
C.foetida spp.rhoeadifolia        GTTGAGACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAAAAAC
C.foetida spp.commutata           GTTGAGACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAAAAAC
C.triasii.seq.4                   GTTGAGACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAAAAAC
C.zacintha.seq.2                  GTTGAGACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAAAAAC
C.foetida spp.foetida.seq.2       GTTGAGACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAAAAAC
C.foetida spp.thomsonii.seq.2     GTTGAGACAG TTGATGATTA TGATGAGTAC TGTCATTATG TTGCTGGACT TGTTGGATTA GGTTTGTCAA AACTCTTCCA TGCCTCTGGC ACTGAAAAAC
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                                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  405        415        425        435        445        455        465        475        485        495
C.rubra                           TCTTTTCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT TCAGGTAATT A-ATT-AAAT T---CTTCTT CAATCTTACA TCATACTTT- -------GAA
C.pusilla.seq.1                   TCTTCCCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTAATT C-ATTTCTTT TTCA------ --GTCTTACA TTATATTTTC AAATA-----
C.zacintha.seq.1                  TCTTCCCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTAATT A-ATTTCTTT TTAACTCTTA CTGTCTTACA TTATATTTAC AAAAAAAAAA
C.foetida spp.foetida.seq.1       TCTTCCCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT ACAGGTAATT A-ATT-AAAT T---CCTTTT CATTCTTACA TT-TAA---- -------AAA
C.tybakiensis.seq.1               TCTTTTCTGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTAATT A-ATT-CAAT T---CTTTTT CATTCTTACA TT-TAA---- ------AAAA
C.pusilla.seq.2                   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----AAAAA
C.foetida spp.thomsonii.seq.1     TCTTCCCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTAATT A-ATT-AATT T---CTTTTT TCAGCTTACA TTA---TTT- -AGAAAAAAA
C.triasii.seq.3                   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---AAAAAAA
C.tybakiensis.seq.2               TCTTTTCTGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTAATT A-ATT-CAAT T---CTTTTT CATTCTTACA TCATACTTT- -------GAA
C.alpina                          TCTTTCCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTAATT C-ATT-T--- ----CTTTTT CAGTCTAACA TTATATTTT- ------CAAA
C.triasii.seq.1                   TCTTCCCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTATTT ---------- ---------- -----TAACA TTATACTTT- -------GAA
C.triasii.seq.2                   TCTTCCCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTATTT ---------- ---------- -----TAACA TTATACTTT- -------GAA
C.kotschyana                      TCTTTTCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTAATT CAATT-CT-- ----TTTTTA TAAACTTACA TTATA-TTT- ----TAAAAA
C.tybakiensis.seq.3               TCTTTTCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTAATT CAATT-CT-- ----TTTTTA TAAACTTACA TTATA-TTT- ----TAAAAA
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Aligned *sqs* matrix ↓ continue

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C.pusilla.seq.3                 TCTTTTCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTAATT CAATT-CT-- ----TTTTTA TAAACTTACA TTATA-TTT- ----TAAAAA
C.foetida spp.afghanistanica    TCTTTTCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTAATT CAATT-CT-- ----TTTTTA TAAACTTACA TTATA-TTT- ----TAAAAA
C.foetida spp.rhoeadifolia      TCTTTTCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTAATT CAATT-CT-- ----TTTTTA TAAACTTACA TTATA-TTT- ----TAAAAA
C.foetida spp.commutata         TCTTTTCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTAATT CAATT-CT-- ----TTTTTA TAAACTTACA TTATA-TTT- ----TAAAAA
C.triasii.seq.4                 TCTTTTCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTAATT CAATT-CT-- ----TTTTTA TAAACTTACA TTATA-TTT- ----TAAAAA
C.zacintha.seq.2                TCTTTTCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTAATT CAATT-CT-- ----TTTTTA TAAACTTACA TTATA-TTT- ----TAAAAA
C.foetida spp.foetida.seq.2     TCTTTTCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTAATT CAATT-CT-- ----TTTTTA TAAACTTACA TTATA-TTT- ----TAAAAA
C.foetida spp.thomsonii.seq.2   TCTTTTCAGA TTCCATCTCC AATTCCATGG GTTTATTCCT CCAGGTAATT CAATT-CT-- ----TTTTTA TAAACTTACA TTATA-TTT- ----TAAAAA


                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                   505        515        525        535        545        555        565        575        585        595
C.rubra                         AAATCTTCTT AATGGTTGTA TATTTAGCTA ACATTGTATT ATTATTTAAT TGTTGATACA AATTTCAGAA AACAAACATC ATCAGAGATT ACCTGGAGGA
C.pusilla.seq.1                 ---TCTT--- ---------- TA-ATTG-TT CCA-TGTATT TATTATTAAT TGGTGAT-GT A-TTGCAGAA AACAAACATA ATTAGAGATT ATCTTGAGGA
C.zacintha.seq.1                AAATCTTTTT AAGGGTTCCA TATATTGATA CCATTGTATT ----ATTTAT TGGTCGT-GA A-TTGCAGAA AACAAACATC ATTAGAGATT ATTTGGAGGA
C.foetida spp.foetida.seq.1     -A-------- AATGGTTCCA TATTTGGCTA ACATTGTATT ----ATTTAT TGGTGAT--A AATTGCAGAA AACAAACATC ATTAGAGATT ACCTGGAGGA
C.tybakiensis.seq.1             AAATC----- AATGGTTCCA TATTTGGCTA ACATTGTATT ----ATTAAT TGGTGATACA AATTTCAGAA AACAAACATC ATTAGAGATT ACCTGGAGGA
C.pusilla.seq.2                 AAATCTTTTT AGTGGTCCCA TATTTGGCTA ACATTGTATT ----ATTAAT TGGTGGTACA AATTTCAGAA AACAAACATC ATTCGAGATT ATCTTGAGGA
C.foetida spp.thomsonii.seq1    AAATCTTTTT AGTGGTCCCA TATTTGGCTA ACATTGTATT ----ATTAAT TGGTGGTACA AATTTCAGAA AACAAACATC ATTCGAGATT ATCTTGAGGA
C.triasii.seq.3                 AAATCTTTTT AGTGGCCCCA TATTTGGCTA ACATTGTATT ----ATTAAT TGGTGGTACA AATTTCAGAA AACAAACGTC ATTCGAGATT ATCTTGAGGA
C.tybakiensis.seq.2             AAATCTTCT- ---------A TATTTAGCTA ACATTGTATT ----ATTAAT TGGTGACACA A-TATCAGAA AACAAACATC ATTAGAGATT ATCTGGAGGA
C.alpina                        TATT-TTT-- AATTGCTCCA TATATTGATA ACATTGTATT TATTATTAAT TGGTGAT-GT A-TTGCAGAA TACAAACATC ATTAGAGATT ACCTGGAGGA
C.triasii.seq.1                 AATTCTTTTC AATGGTTCCA TATTTAGCTA ACATGGTATT ----ATTAAT T------ACA AATTTTAGAA AACAAACATC ATTAGAGATT ATCTGGAGGA
C.triasii.seq.2                 AATTCTTTTC AATGGTTCCA TATTTAGCTA ACATGGTATT ----ATTAAT T------ACA AATTTTAGAA AACAAACATC ATTAGAGATT ATCTGGAGGA
C.kotschyana                    TAATCTTTT- AATTGTTCCA TGTATGGATA ACATTGTCTT ---------- ----CATTCA A-TTGCAGAA AACAAACATC ATTAGAGATT ATCTGGAGGA
C.tybakiensis.seq.3             TAATCTTTT- AATTGTTCCA TGTATGGATA ACATTGTCTT ---------- ----CATTCA A-TTGCAGAA AACAAACATC ATTAGAGATT ATCTGGAGGA
C.pusilla.seq.3                 TAATCTTTT- AATTGTTCCA TGTATGGATA ACATTGTCTT ---------- ----CATTCA A-TTGCAGAA AACAAACATC ATTAGAGATT ATCTGGAGGA
C.foetida spp.afghanistanica    TAATCTTTT- AATTGTTCCA TGTATGGATA ACATTGTCTT ---------- ----CATTCA A-TTGCAGAA AACAAACATC ATTAGAGATT ATCTGGAGGA
C.foetida spp.rheoadifolia      TAATCTTTT- AATTGTTCCA TGTATGGATA ACATTGTCTT ---------- ----CATTCA A-TTGCAGAA AACAAACATC ATTAGAGATT ATCTGGAGGA
C.foetida spp.commutata         TAATCTTTT- AATTGTTCCA TGTATGGATA ACATTGTCTT ---------- ----CATTCA A-TTGCAGAA AACAAACATC ATTAGAGATT ATCTGGAGGA
C.triasii.seq.4                 TAATCTTTT- AATTGTTCCA TGTATGGATA ACATTGTCTT ---------- ----CATTCA A-TTGCAGAA AACAAACATC ATTAGAGATT ATCTGGAGGA
C.zacintha.seq.2                TAATCTTTT- AATTGTTCCA TGTATGGATA ACATTGTCTT ---------- ----CATTCA A-TTGCAGAA AACAAACATC ATTAGAGATT ATCTGGAGGA
C.foetida spp.foetida.seq.2     TAATCTTTT- AATTGTTCCA TGTATGGATA ACATTGTCTT ---------- ----CATTCA A-TTGCAGAA AACAAACATC ATTAGAGATT ATCTGGAGGA
C.foetida spp.thomsonii.seq.2   TAATCTTTT- AATTGTTCCA TGTATGGATA ACATTGTCTT ---------- ----CATTCA A-TTGCAGAA AACAAACATC ATTAGAGATT ATCTGGAGGA
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Aligned *sqs* matrix ↓ continue

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                                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                     605        615        625        635        645        655        665        675        685        695
C.rubra                           CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAACAAACTA GAGGTATATA T--ATTTACA -GTAAGATAT
C.pusilla.seq.1                   CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTA GAGGTATATA GATATCTACA -GTAAGAAAT
C.zacintha.seq.1                  CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCCCGT GAAATCTGGA GTAAATATGT CAACAAACTA GAGGTATAC- -ATATTTACA -GTAAGATAT
C.foetida spp.foetida.seq.1       CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTA GAGGTATATA GATATCTACA -GTAAGAAAT
C.tybakiensis.seq.1               CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAACAAACTA GAGGTATATA T--ATATACA -GTAAGATAT
C.pusilla.seq.2                   CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTA GAGGT----- ---ATGTACA AGTATATTTA
C.foetida spp.thomsonii.seq.1     CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTA GAGGT----- ---ATGTACA AGTATATTTA
C.triasii.seq.3                   CATAAACGAG ATTCCCAAGT CACGTATGTC TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTA GAGGT----- ---ATGTACA AGTATATTTA
C.tybakiensis.seq.2               CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GCAAATATGT CAACAAACTA GAGGTTTGTT T-TATGTACA AGTATATTTA
C.alpina                          CATAAACGAG ATTCCCAAGT CACGCATGTT TTGGCCTCGT GAAATATGGA GTAAATATGT CAACAAACTA GAGGTATGTT T--ATGTACA AGTATATT--
C.triasii.seq.1                   CATAAATGAG ATTCCTAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTC GAGGTATGTT T--ATGTACA --TACATT--
C.triasii.seq.2                   CATAAATGAG ATTCCTAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTC GAGGTATGTT T--ATGTACA --TACATT--
C.kotschyana                      CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTA GAGGTATATA T--ATAT--- --TATATA--
C.tybakiensis.seq.3               CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTA GAGGTATATA T--ATAT--- --TATATA--
C.pusilla.seq.3                   CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTA GAGGTATATA T--ATAT--- --TATATA--
C.foetida spp.afghaniatanica      CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTA GAGGTATATA T--ATAT--- --TATATA--
C.foetida spp.rheoadifolia        CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTA GAGGTATATA T--ATAT--- --TATATA--
C.foetida spp.commutata           CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTA GAGGTATATA T--ATAT--- --TATATA--
C.triasii.seq.1                   CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTA GAGGTATATA T--ATAT--- --TATATA--
C.zacintha.seq.2                  CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTA GAGGTATATA T--ATAT--- --TATATA--
C.foetida spp.foetida.seq.2       CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTA GAGGTATATA T--ATAT--- --TATATA--
C.foetida spp.thomsonii.seq.2     CATAAACGAG ATTCCCAAGT CACGTATGTT TTGGCCTCGT GAAATCTGGA GTAAATATGT CAATAAACTA GAGGTATATA T--ATAT--- --TATATA—

                                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                     705        715        725        735        745        755        765        775        785        795
C.rubra                           A-------TA TAAATGTAAA -TGAAAGTTG TAAAT----- ----TTAATC ------ATTG AATT--ATAT GTTTCAGGAG TTGAAATATG AGGAGAACTC
C.pusilla.seq.1                   A-------TA TAAATGTAAA -TGGAAGTTG TAAAT----- ----TTAATT ------ATTG AATG--ATAT GTTTCAGGAG TTGAAGTATG AAGAGAACTC
C.zacintha.seq.1                  A-------TA TAAATATAAA -TGGAAGTTG TGAAT----- ----TTAATC ------ATTG AATGTGATAT GTTTCAGGAG TTGAAATATG AAGAGAACTC
C.foetida spp.foetida.seq.1       A-------TA TAAATGTAAA -TGGAAGTTG TAAAT----- ----TTAATT ------ATTG AATG--ATAT GTTTCAGGAG TTGAAGTATG AAGAGAACTC
C.tybakiensis.seq.1               A-------TA TAACTATAAA -TGAAAGTTG TAAAT----- ----TTAATT ------ATTG AATG--ATAT GTTTCAGGAG TTGAAATATG AAGAGAACTC
C.pusilla.seq.2                   ATTATTTATA TATATTTAAA GTAGAAGTT- --AAAAAAAA ATAATTAATT T-----ATTG AATG--ATAT GTTCCAGGAG TTGAAGTATG AAGAAAACTC
C.foetida spp.thomsonii.seq.1     ATTATTTATA TATATTTAAA GTAGAAGTT- -AAAAAAAAA T-AATTAATT T-----ATTG AATG--ATAT GTTCCAGGAG TTGAAGTATG AAGAAAACTC
C.triasii.seq.3                   ATTATTTATA TATATTTAAA GTAGAAGTTG AAAAAAAAAA TTAATTAATT T-----ATTG AATG--ATAT GTTCCAGGAG TTGAAGTATG AAGAAAACTC
C.tybakiensis.seq.2               ACTATTTATA TATATTTACG GTAAAAGTTA AAAAAAAAAA TAATTTGAGT ------ATTG AATG--ATAT GTTTCAGGAG TTGAAATATG AAGAGAACTC
C.alpina                          --------TA CACTTTTACA GTA-AAGTCA TAAAGAAAA- -TATTTAATT T-----ATTG AATG--ATAT GTTTCAGTAG TTGAAGTATG AAGAGAACTC
C.triasii.seq.1                   --------TC CACTTTTACA GTAAAAGTTA TAAAGGAAAA ATATTTGATT CTGATAATTG AATG--ATAT GTTTCAGGAG TTGAAATATG AAGAGAACTC
C.triasii.seq.2                   --------TC CACTTTTACA GTAAAAGTTA TAAAGGAAAA ATATTTGATT CTGATAATTG AATG--ATAT GTTTCAGGAG TTGAAATATG AAGAGAACTC
C.kotschyana                      --------TA TGAACATAAA -TGGAAGTTG TAAAG----- ----TTATTC ------ATTG AATG--ATAT GTTTCAGGAG TTGAAATATG AAGAGAACTC
C.tybakiensis.seq.3               --------TA TGAACATAAA -TGGAAGTTG TAAAG----- ----TTATTC ------ATTG AATG--ATAT GTTTCAGGAG TTGAAATATG AAGAGAACTC
C.pusilla.seq.3                   --------TA TGAACATAAA -TGGAAGTTG TAAAG----- ----TTATTC ------ATTG AATG--ATAT GTTTCAGGAG TTGAAATATG AAGAGAACTC
C.foetida spp.afghanistanica      --------TA TGAACATAAA -TGGAAGTTG TAAAG----- ----TTATTC ------ATTG AATG--ATAT GTTTCAGGAG TTGAAATATG AAGAGAACTC
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Aligned *sqs* matrix ↓ continue

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C.foetida spp.rhoeadifolia          --------TA TGAACATAAA -TGGAAGTTG TAAAG----- ----TTATTC ------ATTG AATG--ATAT GTTTCAGGAG TTGAAATATG AAGAGAACTC

C.foetida spp.comutata              --------TA TGAACATAAA -TGGAAGTTG TAAAG----- ----TTATTC ------ATTG AATG--ATAT GTTTCAGGAG TTGAAATATG AAGAGAACTC
C.triasii.seq.4                     --------TA TGAACATAAA -TGGAAGTTG TAAAG----- ----TTATTC ------ATTG AATG--ATAT GTTTCAGGAG TTGAAATATG AAGAGAACTC
C.zacintha.seq.2                    --------TA TGAACATAAA -TGGAAGTTG TAAAG----- ----TTATTC ------ATTG AATG--ATAT GTTTCAGGAG TTGAAATATG AAGAGAACTC
C.foetida spp.foetida.seq.2         --------TA TGAACATAAA -TGGAAGTTG TAAAG----- ----TTATTC ------ATTG AATG--ATAT GTTTCAGGAG TTGAAATATG AAGAGAACTC
C.foetida spp.thomsonii.seq.2       --------TA TGAACATAAA -TGGAAGTTG TAAAG----- ----TTATTC ------ATTG AATG--ATAT GTTTCAGGAG TTGAAATATG AAGAGAACTC


                                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       805        815        825        835        845        855        865        875        885        895
C.rubra                             AGATAAGGCT GTTCAGTGTC TGAATGATAT GGTGACAAAT GCTTTGATCC ACATTGAAGA CTGTTTAACA TACATGTCTG AATTGCGTGA TCCTGCTATC
C.pusilla.seq.1                     AGATAAGGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCTTTGATCC ACATTGAAGA CTGTTTATCC TACATGGCTG ACTTGCGTGA TCCTGCTATC
C.zacintha.seq.1                    AGATAAGGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCTTTGACCC ACATTGAAGA CTGTTTATCA TACATGGCTG ACTTGCGTGA TCCAGCCATC
C.foetida spp.foetida.seq.1         AGATAAGGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCTTTGATCC ACATTGAAGA CTGTTTATCC TACATGGCTG ACTTGCGTGA TCCTGCTATC
C.tybakiensis.seq.1                 AGATAAGGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCTTTGACCC ACATTGAAGA CTGTTTAACA TACATGTCTG AATTGCGTGA TCCTGCTATC
C.pusilla.seq.2                     AGATAAGGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCCTTAATCC ACATTGAAGA CTGTTTAACA TACATGTCTG AGTTGCGTGA TCCTGCTATC
C.foetida spp.thomsonii.seq.1       AGATAAGGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCCTTAATCC ACATTGAAGA CTGTTTAACA TACATGTCTG AGTTGCGTGA TCCTGCTATC
C.triasii.seq.3                     AGATAAGGCA GTTCAATGTC TGAATGATAT GGTGACAAAT GCCTTAATCC ACATTGAAGA CTGTTTAACA TACATGTCTG AGTTGCGTGA TCCTGCTATC
C.tybakiensis.seq.2                 AGATAAGGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCTTTGACCC ACATTGAAGA CTGTTTAACA TACATGTCTG AATTGCGTGA TCCTGCTATC
C.alpina                            AGATAAGGTT GTTCAATGTC TGAA------ ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.triasii.seq.1                     AGATAAAGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCTTTGATCC ACATTGAAGA CTGTTTATCA TACATGGCTG ACTTGCGTGA TCCTGCTATC
C.triasii.seq.2                     AGATAAAGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCTTTGATCC ACATTGAAGA CTGTTTATCA TACATGGCTG ACTTGCGTGA TCCTGCTATC
C.kotschyana                        AGATAAAGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCTTTAATCC ACATTGAAGA CTGTTTAACA TACATGTCTG AATTGCGTGA TCCTGCTATC
C.tybakiensis.seq.3                 AGATAAAGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCTTTAATCC ACATTGAAGA C--------- ---------- ---------- ----------
C.pusilla.seq.3                     AGATAAAGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCTTTAATCC ACATTGAAGA C--------- ---------- ---------- ----------
C.foetida spp.afghanistanica        AGATAAAGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCTTTAATCC ACATTGAAGA C--------- ---------- ---------- ----------
C.foetida spp.rhoeadifolia          AGATAAAGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCTTTAATCC ACATTGAAGA C--------- ---------- ---------- ----------
C.foetida spp.commutata             AGATAAAGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCTTTAATCC ACATTGAACA CGGTTTAACA AT-------- ---------- ----------
C.triasii.seq.4                     AGATAAAGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCTTTAATCC ACATTG---- ---------- ---------- ---------- ----------
C.zacintha.seq.2                    AGATAAAGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCTTTAATCC ACATTGAAGA C--------- ---------- ---------- ----------
C.foetida spp.foetida.seq.2         AGATAAAGCT GTTCAATGTC TGAATGATAT GGTGACAAAT GCTTTAATCC ACATTGAAGA C--------- ---------- ---------- ----------
C.foetida spp.thomsonii.seq.2       AGATAAAGCT GT-CAATGTC TGAA------ ---------- ---------- ---------- ---------- ---------- ---------- ----------


                                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       905        915        925        935        945        955        965        975        985        995
C.rubra                             TTCAGGTTTT GTGCCATTCC TCAGGTATAT AT-TTCACTA ATTTTTTTAT CAT------- -TATAGCATC CTACTTT--- CCTTGCTTAA ATTTACCTA-
C.pusilla.seq.1                     TTCAGGTTTT GTGCCATTCC TCAGGTATAT ---TTCTTTA ATTTATTTAT CATA------ --ATAGCATC CTACTTT--- CCTTTTTCAA ATTTACCTAT
C.zacintha.seq.1                    TTCAGATTCT GTGCCATTCC TCAGGTATAT ---TTCTTTA ------TTA- CA-------- --ATAGCATC CTACTTT--- CATT-CTTAA ATTTAC----
C.foetida spp.foetida.seq.1         TTCAGGTTTT GTGCCATTCC TCAGGTATAT ---TTCATTT ATTTGTTTAT TATTTAATAT TTATAGCATC CTACTTT--- CCTT-CTTAA ATTTAC-TA-
C.tybakiensis.seq.1                 TTCAGGTTTT GTGCCATTCC TCAGGTATAT AT-TTCTTTG ATTTCTTTAT TAT------- -AATAGCATC CTACTTT--- CCTTTCT-AA ATTTACCTAT
C.pusilla.seq.2                     TTCAAGTTTT GTGCTATTCC ACAGGTATAT AATTTCATTG ATTTGTTTAT TAGA------ --ACAGCATC CTACTT---- CC-TTCT-AA A---------
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Aligned *sqs* matrix ↓ continue

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C.foetida spp.thomsonii.seq.1       TTCAAGTTTT GTGCTATTCC ACAGGTATAT AATTTCATTG ATTTGTTTAT TAGA------ --ATAGCATC CTAC------ ---------- ----------
C.triasii.seq.2                     TTCAAGTTTT GTGCTATTCC ACAGGTATAT AATTTCATTG ATTTGTTTAT TATA------ --ATAGCATC ---------- ---------- ----------
C.tybakiensis.seq.2                 TTCAGGTTTT GTGCCATTCC TCAGGTATAT ATTTTCTTTG ATTTCTTTAT TAT------- -AATAGCATC CTACTTT--- CCTTTCT-AA ATTTACCTAT
C.alpina                            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.triasii.seq.1                     TTCAGGTTTT GTGCCATTCC TCAGGTATAT ---TTCTTAT T--------- ---------- --ATAGCATC CTACTTTAA- --TTTCTTAA ATTTACCTTT
C.triasii.seq.2                     TTCAGGTTTT GTGCCATTCC TCAGGTATAT ---TTCTTAT T--------- ---------- --ATAGCATC CTACTTTAA- --TTTCTTAA ATTTACC---
C.kotschyana                        TTCAGGTTTT GTGCCATTCC TCAGGTATAT AT-CTCATCA ATATCTGTAT TAATGTATTA TAATAGCATC CTACTTTTT- CTTTTTCAAA ATTTAC----
C.tybakiensis.seq.3                 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.pusilla.seq.3                     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.afghanistanica        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.rhoeadifolia          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.commutata             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.triasii.seq.4                     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.zacintha.seq.2                    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.foetida.seq.2         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C.foetida spp.thomsonii.seq.2       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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                                    ....|....| ....|....| ....|....| ....|....| .
                                       1005       1015       1025       1035
C.rubra                             ---------- ---------- ---------- ---------- -
C.pusilla.seq.1                     TTCACCCTGG TGGTTTTTTC TTAGATCATG ---------- -
C.zacintha.seq.1                    ---------- ---------- ---------- ---------- -
C.foetida spp.foetida.seq.1         ---------- ---------- ---------- ---------- -
C.tybakiensis.seq.1                 TTTTTTTTAT AAGGATGAGA GGAGCAAGAA TGTGGAGACA C
C.pusilla.seq.2                     ---------- ---------- ---------- ---------- -
C.foetida spp.thomsonii.seq.1       ---------- ---------- ---------- ---------- -
C.triasii.seq.3                     ---------- ---------- ---------- ---------- -
C.tybakiensis.seq.2                 TTTTT----- ---------- ---------- ---------- -
C.alpina                            ---------- ---------- ---------- ---------- -
C.triasii.seq.1                     TTGACCATAC TA-------- ---------- ---------- -
C.triasii.seq.2                     ---------- ---------- ---------- ---------- -
C.kotschyana                        ---------- ---------- ---------- ---------- -
C.tybakiensis.seq.3                 ---------- ---------- ---------- ---------- -
C.pusilla.seq.3                     ---------- ---------- ---------- ---------- -
C.foetida spp.afghanistanica        ---------- ---------- ---------- ---------- -
C.foetida spp.rhoeadifolia          ---------- ---------- ---------- ---------- -
C.foetida spp.commutata             ---------- ---------- ---------- ---------- -
C.triasii.seq.4                     ---------- ---------- ---------- ---------- -
C.zacintha.seq.2                    ---------- ---------- ---------- ---------- -
C.foetida spp.foetida.seq.2         ---------- ---------- ---------- ---------- -
C.foetida spp.thomsonii.seq.2       ---------- ---------- ---------- ----------
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Aligned *sqs* matrix .The end

8.4. Aligned *rps16* DNA sequences matrix

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                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                     5         15         25         35         45         55         65         75         85         95
C.foetida spp.foetida           GTGGTAGAAA GCAACGTGCG ACTTGAAGGA CACGATCCGG TGTGGATTTT TATTCTTACA TCCACCATTT TATTTTATAT AGGAATGAAG GTGCTCTTGG
C.foetida spp.afghanistanica    GTGGTAGAAA GCAACGTGCG ACTTGAAGGA CACGATCCGG TGTGGATTTT TATTCTTACA TCCACCATTT TATTTTATAT AGGAATGAAG GTGCTCTTGG
C.foetida spp.commutata         GTGGTAGAAA GCAACGTGCG ACTTGAAGGA CACGATCCGG TGTGGATTTT TATTCTTACA TCCACCATTT TATTTTATAT AGGAATGAAG GTGCTCTTGG
C.tybakiensis                   GTGGTAGAAA GCAACGTGCG ACTTGAAG-A CACGATCCGG TGTGGATTTT TATTCTTACA TC-A-CATTT TATTTTATAT AGGAATGAAG GTGCTCTTGG
C.foetida spp.rhoeadifolia      GTGGTAGAAA GCAACGTGCG ACTTGAAGGA CACGATCCGG TGTGGATTTT TATTCCTACA TCCACCATTT TATTTTATAT AGGAATGAAG GTGCTCTTGG
C.zacintha                      GTGGTAGAAA GCAACGTGCG ACTTGAAGGA CACGATCCGG TGTGGATTTT TATTCTTACA TCCACCATTT TATTTTATAT AGGAATGAAG GTGCTCTTGG
C.rubra                         GTGGTAGAAA GCAACGTGCG ACTTGAAGGA CACGATCCGG TGTGGATTTT TATTCTTACA TCCACCATTT TATTTTATAT AGGAATGAAG GTGCTCTTGG
C.kotschyana                    GTGGTAGAAA GCAACGTGCG ACTTGAAGGA CACGATCCGG TGTGGATTTT TATTCTTACA TCCACCATTT T-TTTTATAT AGGAATGAAG GTGCTCTTGG
C.alpina                        GTGGTAGAAA GCAACGTGCG ACTTGAAGGA CACGATCCGG TGTGGATTTT TATTCTTACA TCCACCATTT TATTTTATAT AGGAATGAAG GTGCTCTTGG
C.pusilla                       GTGGTAGAAA GCAACGTGCG ACTTGAAGGA CACGATCCGG TGTGGATTTT TATTCTTACA TCCACCATTT TCTTTTATAT AGGAATGAAG GTGCTCTTGG
C.triasii                       GTGGTAGAAA GCAACGTGCG ACTTGAAGGA CACGATCCGG TGTGGATTTT TATTCTTACA TCCACCATTT TATTTTATAT AGGAATGAAG GTGCTCTTGG

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                   105        115        125        135        145        155        165        175        185        195
C.foetida spp.foetida           CTCGACGTCA TTTGTTCTAT TCTACTAGAA CCCTTCTTTT TTTATTGGGT TGTAATGTAA ATAGTTCATG ATGGAGCTCG AGTAGAAAGT CTTTTTT-AT
C.foetida spp.afghanistanica    CTCGACGTCA TTTGTTCTAT TCTACTAGAA CCCTTCTTTT TTTATTGGGT TGTAATGTAA ATAGTTCATG ATGGAGCTCG AGTAGAAAGT CTTTTTT-AT
C.foetida spp.commutata         CTCGACGTCA TTTGTTCTAT TCTACTAGAA CCCTTCTTTT TTTATTGGGT TGTAATGTAA ATAGTTCATG ATGGAGCTCG AGTAGAAAGT CTTTTTTTAT
C.tybakiensis                   CTCGACGTCA TTTGTTCTAT TCTACTAGAA CCCTTCTTTT TTTATTGGGT TGTAATGTAA ATAGTTCATG ATGGAGCTCG AGTAGAAAGT CTTTTTTTAT
C.feotida spp.rhoeadifolia      CTCGACGTCA TTTGTTCTAT TCTACTAGAA CCCTTCTTTT TTTATTGGGT TGTAATGTAA ATAGTTCATG ATGGAGCTCG AGTAGAAAGT CTTTTTTTAT
C.zacintha                      CTCGACGTCA TTTGTTCTAT TCTACTAGAA CCCTTCTTTT TTTATTGGGT TGTAATGTAA ATAGTTCATG ATGGAGCTCG AGTAGAAAGT CTTTTTTTAT
C.rubra                         CTCGACGTCA TTTGTTCTAT TCTACTAGAA CCCTTCTTTT TTTATTGGGT TGTAATGTAA ATAGTTCATG ATGGAGCTCG AGTAGAAAGT CTTTTTTAAT
C.kotschyana                    CTCGACGTCA TTTGTTCTAT TCTGCTAGAA CCCTTCTTTT TTTATTGGGT TGTAATGTAA ATAGTTCATG ATGGAGCTCG AGTAGAAAGT CTTGATTAAT
C.alpina                        CTCGACGTCA TTTGTTCTAT TCTACTAGAA CCCTTCTTTT TTTATTGGGT TGTAATGTAA ATAGTTCATG ATGGAGCTCG AGTAGAAAGT CTTGATTAAT
C.pusilla                       CTCGACGTCA TTTGTTCTAT TCTACTAGAA CCCTTCTTTT TTTATTAGGT TGTAATGTAA ATAGTTCATG ATGGAGCTCG AGTAGAAAGT CTTGATTAAT
C.triasii                       CTCGACGTCA TTTGTTCTAT TCTACTAGAA CCCTTCTTTT TTTATTGGGT TGTAATGTAA ATAGTTCATG ATGGAGCTCG AGTAGAAAGT CTTTATTAAT

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                   205        215        225        235        245        255        265        275        285        295
C.foetida spp.foetida           TTCTCAGGGG CAACGATCTA GGGTTAATGC CAATCAATAA ATTGGAACAA CTTCGTAAGT ATATCTTCGA TATAGAAATC AAAAAGATCC GATTCGAGCA
C.foetida spp.afghanistanica    TTCTCAGGGG CAACGATCTA GGGTTAATGC CAATCAATAA ATTGGAACAA CTTCGTAAGT ATATCTTCGA TATAGAAATC AAAAAGATCC GATTCGAGCA
C.foetida spp.commutata         TTCTCAGGGG CAACGATCTA GGGTTAATGC CAATCAATAA ATTGGAACAA CTTCGTAAGT ATATCTTCGA TATAGAAATC AAAAAGATCC GATTCGAGCA
C.tybakiensis                   TTCTCAGGGG CAACGATCTA GGGTTAATGC CAATCAATAA ATTGGAACAA CTTCGTAAGT ATATCTTCGA TATAGAAATC AAAAAGATCC GATTCGAGCA
C.foetida spp.rhoeadifolia      TTCTCAGGGG CAACGATCTA GGGTTAATGC CAATCAATAA ATTGGAACAA CTTCGTAAGT ATATCTTCGA TATAGAAATC AAAAAGATCC GATTCGAGCA
C.zacintha                      TTCTCAGGGG CAACGATCTA GGGTTAATGC CAATCAATAA ATTGGAACAA CTTCGTAAGT ATATCTTCGA TATAGAAATC AAAAAGATCC GATTCGAGCA
C.rubra                         TTCTCAGGGG CAACGATCTA GGGTTAATGC CAATCAATAA ATTGGAACAA CTTCGTAAGT ATATCTTCGA TATAGAAATC AAAAAGATCC GATTCGAGCA
C.kotschya                      TTCTCAGGGG CAACGATCTA GGGTTAATGC CAATCAATAA ATTGGAACAA CTTCGTAAGT ATATCTTCGA TATAGAAATC AAAAAGATCC GATTCGAGCA
C.alpina                        TTCTCAGGGG CAACGATCTA GGGTTAATGC CAATCAATAA ATTGTAACAA CTTCGTAAGT ATATCTTCAA TATAGAAATC AAAAAGATCC GATTCGAGCA
C.pusilla                       TTCTCAGGGG CAACGATCTA GGGTTAATGC CAATCAATAA ATTGGAACAA CTTCGTAAGT ATATCTTCGA TATAGAAATC AAAAAGATCC GATTCGAGCA
C.triasii                       TTCTCAGGGG CAACGATCTA GGGTTAATGC CAATCAATAA ATTGGAACAA CTTCGTAAGT ATATCTTCGA TATAGAAATC AAAAAGATCC GATTCGAGCA
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Aligned *rps16* DNA sequences matrix of the *Crepis* species section *Barkhausia* representing 12 DNA sequences of 12 *Crepis* taxa.↓ continue

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                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                   305        315        325        335        345        355        365        375        385        395
C.foetida spp.foetida           AATTTTCAAT TCAAAAAAAT TGTTGGAACC GCAAAAACTT TTTCGATCCA CAGTGGATCG CGCACGAATC AACCGTCGGT ATGATTCTTT GATAGAAAGA
C.foetida spp.afghanistanica    AATTTTCAAT TCAAAAAAAT TGTTGGAACC GCAAAAACTT TTTCGATCCA CAGTGTATCG CGCACGAATC AACCGTCGGT ATGATTCTTT GATAGAAAGA
C.foetida spp.commutata         AATTTTCAAT TCAAAAAAAT TGTTGGAACC GCAAAAACTT TTTCGATCCA CAGTGTATCG CGCACGAATC AACCGTCGGT ATGATTCTTT GATAGAAAGA
C.tybakiensis                   AATTTTCAAT TCAAAAAAAT TGTTGGAACC GCAAAAACTT TTTCGATCCA CAGTGTATCG CGCACGAATC AACCGTCGGT ATGATTCTTT GATAGAAAGA
C.foetida spp.rhoeadifolia      AATTTTCAAT TCAAAAAAAT TGTTGGAACC GCAAAAACTT TTTCGATCCA CAGTGTATCG CGCACGAATC AACCGTCGGT ATGATTCTTT GATAGAAAGA
C.zacintha                      AATTTTCAAT TCAAAAAAAT TGTTGGAACC GCAAAAACTT TTTCGATCCA CAGTGTATCG CGCACGAATC AACCGTCGGT ATGATTCTTT GATAGAAAGA
C.rubra                         AATTTTCAAT TCAAAAAAAT TGTTGGAACC GCAAAAACTT TTTCGATCCA CAGTGTATCG CGCACGAATC AACCGTAGGT ATGATTCTTT GATAGAAAGA
C.kotschyana                    AATATTCAAT TCAAAAAATT TGTTGGAACC GCAAAAACTT TTTCGATCCA CAGTGTATCG CGCACGAATC AACCGTAGGT ATGATTCTTT GATAGAAAGA
C.alpina                        AATTTTCAAT TCAAAAAAAT TGTTGGAATC GCAAAAACTT TTTCGATCCA CAGTGTATCG CGCACGAATC AACCGTAGGT ATGATTCTTT GATAGAAAGA
C.pusilla                       AATTTTCAAT TCAAAAAAAT TGTTGGAACC GCAAAAACTT TTTCGATCCA CAGTGTATCG CGCACGAATC AACCGTAGGT ATGATTCTTT GATAGAAAGA
C.triasii                       AATTTTCAAT TCAAAAAAAT TGTTGGAACC GCAAAAACCT TTTCGATCCA CAGTGTATCG CGCACGAATC AACCGTAGTT ATGATTCTTT GATAGAAAGA


                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                   405        415        425        435        445        455        465        475        485        495
C.foetida spp.foetida           AATCACAAAA GGGGTATGTT GCTACCATTT TGAAAGGATT AAGAAGCACC GAAGTAATGT CTAAACCCAA TGATTTAAAA CAAAGATAAA GGATCCCAGA
C.foetida spp.afghanistanica    AATCACAAAA GGGGTATGTT GCTACCATTT TGAAAGGATT AAGAAGCACC GAAGTAATGT CTAAACCCAA TGATTTAAAA CAAAGATAAA GGATCCCAGA
C.foetida spp.commutata         AATCACAAAA GGGGTATGTT GCTACCATTT TGAAAGGATT AAGAAGCACC GAAGTAATGT CTAAACCCAA TGATTTAAAA CAAAGATAAA GGATCCCAGA
C.tybakiensis                   AATCACAAAA GGGGTATGTT GCTACCATTT TGAAAGGATT AAGAAGCACC GAAGTAATGT CTAAACCCAA TGATTTAAAA CAAAGATAAA GGATCCCAGA
C.foetida spp.rhoeadifolia      AATCACAAAA GGGGTATGTT GCTACCATTT TGAAAGGATT AAGAAGCACC GAAGTAATGT CTAAACCCAA TGATTTAAAA CAAATATAAA GGATCCCAGA
C.zacintha                      AATCACAAAA GGGGTATGTT GCTACCATTT TGAAAGGATT AAGAAGCACC GAAGTAATGT CTAAACCCAA NGATTTAAAA CAAATATAAA GGATCCCAGA
C.rubra                         AATCACAAAA GGGGTATGTT GCTACCATTT TGAAAGGATT AAGAAGCACC GAAGTAATGT CTAAACCCAA TGATTTAAAA CAAAGATAAA GGATCCCAGA
C.kotschyana                    AATCACAAAA GGGGTATGTT GCTACCATTT TGAAAGGATT AAGAAGCACC GAAGTAATGT CTAAACCCAA TGATTTAAAA CAAAGATAAA GGATCCCAGA
C.alpina                        AATCACAAAA GGGGTATGTT GCTACCATTT TGAAAGGATT AAGAAGCACC GAAGTAATGT CTAAACCCAA TGATTTAAAA CAAAGATAAA GGATCCCAGA
C.pusilla                       AATCACAAAA GGGGTATGTT GCTACCATTT TGAAAGGATT AAGAAGCACC GAAGTAATGT CTAAACCCAA TGATTTAAAA CAAAGATAAA GGATCCCAGA
C.triasii                       AATCACAAAA GGGGTATGTT GCTACCATTT TGAAAGAATT AAGAAGCACC GAAGTAATGT CTAAACCCAA TGATTTAAAA CAAAGATAAA GGATCCCAGA

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                   505        515        525        535        545        555        565        575        585        595
C.foetida spp.foetida           ACAAGGAAAC ACCGCTTCCA TTGTCTCACA GGTCCGAATA AAGAATCCAA ATAGATTTTC AACGAGACAA AAAAAAGGGG GGGGTTAAAG ACCACTCAAT
C.foetida spp.afghanistanica    ACAAGGAAAC ACCGCTTCCA TTGTCTCACA GGTCCGAATA AAGAATCCAA ATAGATTTTC AACGAGACAA AAAAAAGGGG GGGGTTAAAG ACCACTCAAT
C.foetida spp.commutata         ACAAGGAAAC ACCGCTTCCA TTGTCTCACA GGTCCGAATA AAGAATCCAA ATAGATTTTC AACGAGACAA AAAAA--GGG GGGGTTAAAG ACCACTCAAT
C.tybakiensis                   ACAAGGAAAC ACCGCTTCCA TTGTCTCACA GGTCCGAATA AAGAATCCAA ATAGATTTTC AACGAGACAA AAAA---GGG GGGGTTAAAG ACCACTCAAT
C.foetida spp.rhoeadifolia      ACAAGGAAAC ACCGCTTCCA TTGTCTCACA GGTCCGAATA AAGAATCCAA ATAGATTTTC AACGAGACAA AAAAAGGGGG GGGGTTAAAG ACCACTCAAT
C.zacintha                      ACAAGGAAAC ACCGCTTCCA TTGTCTCACA GGTCCGAATA AAGAATCCAA ATAGATTTTC AACGAGACAA AAAAAGGGGG GGGGTTAAAG ACCACTCAAT
C.rubra                         ACAAGGAAAC ACCGCTTCAA TTGTCTCACA GGTCCGAATA AAGAATCCAA ATAGATTTTC AACGAGACAA AAA----GGG GGGGTTAAAG ACCACTCAAT
C.kotschyana                    ACAAGGAAAC ACCGCTTCAA TTGTTTCACA GGTCCGAATA AAGAATCCAA ATAGATTTTC AACGAGACAA AAA-----GG GGGGTTAAAG ACCACTCAAT
C.alpina                        ACAAGGAAAC ACCGCTTCAA TTGTTTCACA GGTCCGAATA AAGAATC--- ------TTTC AACGAGACAA AAAAA---GG GGGGTTAAAG ACCACTCAAT
C.pusilla                       ACAAGGAAAC ACCGCTTCAA TTGTCTCACA GGTCCGAATA AAGAATCCAA ATAGATTTTC AACGAGACAA AAAA-----G GGGGTTAAAG ACCACTCAAT
C.triasii                       ACAAGGAAAC ACCGCTTCAA TTGTCTCATA GGTCCGAATA AAGAATCCAA ATATATTTTC AACGAGACAA AAA----GGG GGGGTTAAAG ACCACTCAAT
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Aligned *rps16* matrix . The end

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                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                   605        615        625        635        645        655        665        675        685        695
C.foetida spp.foetida           AAAAAAATAT CTTAAT---- ---------- ----TTTCTT T--------- AAGATATTTG ATATTATTGA TTATTGAACT TGAGTTATGA GTACAAATGA
C.foetidaafghanis               AAAAAAATAT CTTAAT---- ---------- ----TTTCTT T--------- AAGATATTTG ATATTATTGA TTATTGAACT TGAGTTATGA GTACAAATGA
C.foetida spp.commutata         AAAAAAATAT CTTAAT---- ---------- ----TTTCTT T--------- AAGATATTTG ATATTATTGA TTATTGAACT TGAGTTATGA GTACAAATGA
C.tybakiensis                   AAAAAAAGAT CTTAAT---- ---------- ----TTTCTT T--------- AAGATATTTG ATATTATTGA TTATTGAACT TGAGTTATGA GTACAAATGA
C.foetida spp.rhoeadifolia      AAAAAAATAT CTTAAT---- ---------- ----TTTCTT T--------- AAGATATTTG ATATTATTGA TTATTGAACT TGAGTTATGA GTACAAATGA
C.zacintha                      AAAAAAATAT CTTAAT---- ---------- ----TTTCTT T--------- AAGATATTTG ATATTATTGA TTATTGAACT TGAGTTATGA GTACAAATGA
C.rubra                         AAAAAAATAT CTTAAT---- ---------- ----TTTCTT T--------- AAGATATTTT ATATTATGGA TTATTGAACT TGAGTTATGA GTACAAATGA
C.kotschyana                    AAAAAAATAT CTTAAT---- ---------- ----TTTCTT T--------- AATATATTTG ATATTATTGA TTATTGAACT TGAGTTATGA GTACAAATGA
C.alpina                        AAAAAAATAT CTTAATATAA AAAAATATCT TAATTTTCTT T--------- AATATATTTG ATATTATTTA TTATTGAACT TGAGTTATGA GTACAAATGA
C.pusilla                       AAAAAAATAT CTTAAT---- ---------- ----TTTCTT T--------- AATATATTTG ATATTATTGA TTATTGAACT TGAGTTATGA GTACAAATGA
C.triasii                       AAAAAAATAT CTTAAT---- ---------- ----TTTCTT TAATATATTT AATATATTTG ATATTATTGA TTATTGAACT TGAGTTATGA GTACAAATGA


                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                   705        715        725        735        745        755        765        775        785        795
C.foetida spp.foetida           TTTTTTAGTT TTAAGGAAGG AAGCTAAATA AAAATGACTA TGAGTTAAAT T-------AT AGGCTAATTT ATTTTATGAA TTCCTTTACT ATTTATTCTA
C.foetida spp.afghanistanica    TTTTTTAGTT TTAAGGAAGG AAGCTAAATA AAAATGACTA TGAGTTAAAT T-------AT AGGCTAATTT ATTTTATGAA TTCCTTTACT ATTTATTCTA
C.foetida spp.commutata         TTTTTTAGTT TTAAGGAAGG AAGCTAAATA AAAATGACTA TGAGTTAAAT T-------AT AGGCTAATTT ATTTTATGAA TTCCTTTACT ATTTATTCTA
C.tybakiensis                   TTTTTTAGTT TTAAGGAAGG AAGCTAAAGA AAAATGACTA TGAGTTAAAT T-------AT AGGCTAATTT ATTTTATGAA TTCCTTTACT ATTTATTCTA
C.foetida spp.rhoeadifolia      TTTTTTAGTT TTAAGGAAGG AAGCTAAATA AAAATGACTA TGAGTTAAAT T-------AT AGGCTAATTT ATTTTATGAA TTCCTTTACT ATTTATTCTA
C.zacintha                      TTTTTTAGTT TTAAGGAAGG AAGCTAAATA AAAATGACTA TGAGTTAAAT T-------AT AGGCTAATTT ATTTTATGAA TTCCTTTACT ATTTATTCTA
C.rubra                         TTTTTTAGTT TTAAGGAAGG AAGCTAAATA AAAATGACTA TGAGTTAAAT T-------AT AGGCTAATTT ATTTTACGAA TTCCTTTACT ATATTATT--
C.kotschyana                    TTTTTTAGTT TTAAGGAAGG AAGCTAAATA AAAATGACTA TGAGTTAAAT T-------AT AGGCTAATT- ---------- ---------- ----------
C.alpina                        TTTTTTAGTT TTAAGGAAGG AAGCTAAATA AAAATGACTA TGAGTTAAAT T-------AT AGGCTAATT- ---------- ---------- ----------
C.pusilla                       TTTTTTAGTT TTAAGGAAGG AAGCTAAATA AAAATGACTA TGAGTTAAAT TT-AAATTAT AGGCTAATTT CTTTTACCAA TTCCTTTACT ATTTATT---
C.triasii                       TTTTTTAGTT TTAAGGAAG- ---CTAAATA AAAATGACTA TGAGTTAAAT TTTAAATTAT AGGCTAATTT CTTTTACGAA TTCCTTTACT ATTTATTT—


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                                   805        815        825        835        845        855        865        875        885        895
C.foetida sp.foetida            ATTACTATTT ATTCTAATCT AATTTTATCC ATAGACAAAA TTTCAAATCA TTTTTTCTCG AGCCGTACGA GGAGAAAACT TCCTATACGG TTCTAGGGGG
C.foetida spp.afghanistanica    ATTACTATTT ATTCTAATCT AATTTTATCC ATAGACAAAA TTTCAAATCA TTTTTTCTCG AGCCGTACGA GGAGAAAACT TCCTATACGG TTCTAGGGGG
C.foetida spp.commutata         ATTACTATTT ATTCTAATCT AATTTTATCC ATAGACAAAA TTTCAAATCA TTTTTTCTCG AGCCGTACGA GGAGAAAACT TCCTATACGG TTCTAGGGGG
C.tybakiensis                   ATTACTATTT ATTCTAATCT AATTTTATCC ATAGACAAAA TTTCAAATCA TTTTTTCTCG AGCCGTACGA GGAGAAAACT TCCTATACGG TTCTAGGGGG
C.foetida spp.rhoeadifolia      ATTACTATTT ATTCTAATCT AATTTTATCC ATAGACAAAA TTTCAAATCA TTTTTTCTCG AGCCGTACGA GGAGAAAACT TCCTATACGG TTCTAGGGGG
C.zacintha                      ATTACTATTT ATTCTAATCT AATTT-ATCC ATAGACAAAA TTTCAAATCA TTTTTTCTCG AGCCGTACGA GGAGAAAACT TCCTATACGG TTCTAGGGGG
C.rubra                         ---------- --------CT AATTTTATCC ATAGACAAAA TTTCGAATCA TTTTTTCTCG AGCCGTACGA GGAGAAAACT TCCTATACGG TTCTAGGGGG
C.kotschyana                    ---------- --------CT AATTTTATCC ATAGACAAAA TTTCGAATCA TTTTTTCTCG AGCCGTACGA GGAGAAAACT TCCTATACGG TTCTAGGGGG
C.alpina                        ---------- --------CT AATTTTATCC ATAGACAAAA TTTCGAATCA TTTTTTCTCG AGCCGTAGGA GGAGAAAACT TCCTATACGG TTCTAGGGGG
C.pusilla                       ---------- ---------CT AATTTTATCC ATAGACAAAA TTTCGAATCA TTTTTTCTCG AGCCGTACGA GGAGAAA-CT TCCTATACGG GTCTAGGGGG
C.triasii                       ---------- ---------T CATTTTATCC ATAGACAAAA TTTCGAATCA TTTTTTCTCG AGCCGTACGA GGAGAAAACT TCCTATACGG TTCTAGGGGG
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Aligned *rps16* matrix ↓ continue

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                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|...
                                   905        915        925        935        945        955        965        975
C.foetida spp.foetida           GGG---TTCT TTTT-CATCT ACATCTATCC C-GAT-GAGC CGTCTATCGA ATCGTTGCAA TTGAATGTTC GATCCCGA
C.foetida spp.afghanistanica    GGG---TTCT TTTT-CATCT ACATCTATCC C-GAT-GAGC -GTCTATCGA -TCGTTGCAA TTGAATGTTC GATCCCGA
C.foetida spp.commutata         GGG---TTCT TTTT-CATCT ACATCTATCC C-GAT-GAGC CGTCTATCGA ATCGTTGCAA TTGAATGTTC GATCCCGA
C.tybakiensis                   GGGG--TTCT TTTT-CATCT ACATCTATCC C-GAT-GAGC CGTCTATCGA ATCGTTGCAA TTGAATGTTC GATCCCGA
C.foetida spp.rhoeadifolia      GGGG--TTCT TTTT-CATCT ACATCTATCC C-GAT-GAGC CGTCTATCGA ATCGTTGCAA TTGAATGTTC GATCCCGA
C.zacintha                      GGGG--TTCT TTTTTCATCT ACATCTATCC CCGAT-GAGC CGTCTATCGA ATCGTTGCAA TTGAATGTTC GATCCCGA
C.rubra                         GGGGG-TTCT TTTT-CATCT ACATCTATCC C-GAT-GAGC CGTCTATCGA ATCGTTGCAA TTGAATGTTC GATCCCGA
C.kotschya                      GGGGG-TTCT TTTT-CATCT ACATCTATCC C-GAT-GAGC CGTCTATCGA ATCGTTGCAA TTGAATGTTC GATCCCGA
C.alpina                        GG----TTCT TTTT-CATCT ACATCTATCC C-GAT-GAGC CGTCTATCGA ATCGTTGCAA TTGAATGTTC GATCCCGA
C.pusilla                       GGGGGGTTCC TTTTTCATCA ACATCTATCC C-AATAGAGC CGTCTATCGA ATCGTTGCAA TTGAATGTTC GATCCCGA
C.triasii                       GG----TTCT TTTT-CATCT ACATCTATCC C-GAT-GAGC CGTCTATCGA ATCGTTGCAA TTGAATGTTC GATCCCGA
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Aligned *rps16* matrix . The end

8.5. Aligned *matK1* DNA sequences matrix

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                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                   5        15        25        35        45        55        65        75        85        95
C.foetida spp.afghanistanica    GGGCTAGATA GATCTCAACA ACACTACTTC TTATATCCAC TTATCTTTCA GGAGTATATT TATGTCCTTG CTCATGATCA TGGTTTAAAT AGATCGATTT
C.foetida spp.commutata         GGGCTAGATA GATATCAACA ACACTACTTC TTATATCCAC TTATCTTTCA GGAGTATATT TATGTACTTG CTCATGATCA TGGTTTAAAT AGATCGATTT
C.foetida spp.rhoeadifolia      GGGCTAGATA GATCTCAACA ACACTACTTC TTATATCCAC TTATCTTTCA GGAGTATATT TATGTACTTG CTCATGATCA TGGTTTAAAT AGATCGATTT
C.kotschyana                    GGGCTAGATA GATCTCAACA ACACTACTTC TTATATCCAC TTATCTTTCA GGAGTATATT TATGTACTTG CTCATGATCA TGGTTTAAAT AGATCGATTT
C.triasi                        GGGCTAGATA GATCTCAACA ACACTACTTC TTATATCCAC TTATCTTTCA GGAGTATATT TATGTACTTG CTCATGATCA TGGTTTAAAT AGATCGATTT

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  105       115       125       135       145       155       165       175       185       195
C.foetida spp.afghanistanica    TGTTGGAAAA TGCGGGTTAT GACAAAAAAT CCAGCTTACT AATTGTGAAA CGTTTAATCA ATCGAATGTA TCAACAGAAC CATTTGATTC TTTCGGTTAA
C.foetida spp.commutata         TGTTGGAAAA TGCGGGTTAT GACAAAAAAT CCAGCTTACT AATTGTGAAA CGTTTAATCA ATCGAATGTA TCAACAGAAC CATTTGATTC TTTCGGTTAA
C.foetida spp.rhoeadifolia      TGTTGGAAAA TGCGGGTTAT GACAAAAAAT CCAGCTTACT AATTGTGAAA CGTTTAATCA ATCGAATGTA TCAACAGAAC CATTTTATTC TTTCGGTTAA
C.kotschyana                    TGTTGGAAAA TGCGGGTTAT GACAAAAATT TCAGCTTACT AATTGTGAAA CGTTTAATCA ATCGAATGTA TCAACAGAAC CATTCGATTC TTTCGGTTAA
C.triasi                        TGTTGGAAAA TGCGGGTTAT GACAAAAAAT ACAGCTTACT AATTGTGAAA CGTTTAATCA ATCGAATGTA TCAACAGAAC CATTTGATTC TTTCGGTTAA

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  205       215       225       235       245       255       265       275       285       295
C.foetida spp.afghanistanica    CAATTCTAAA CAGACTCCAT TTTGGGGGCA CAACAAGAAT TTTTATTCGC AAGTAATGTC AGAGGTATCT TCAATCATTA TGGAAATTCC CTTGTCTTTG
C.foetida spp.commutata         CAATTCTAAA CAGACTCCAT TTTGGGGGCA CAACAAGAAT TTTTATTCGC AAGTAATGTC AGAGGTATCT TCAATCATTA TGGAAATTCC CTTGTCTTTG
C.foetida spp.rhoeadifolia      CAATTCTAAA CAGACTCCAT TTTGGGGGCA CAACAAGAAT TTTTATTCGC AAGTAATGTC AGAGGTATCT TCAATCATTA TGGAAATTCC CTTGTCTTTG
C.kotschyana                    CAATTCTAAA CAGACTCCAT TTTGGGGGCA CAACAAGATT TTTTATTCGC AAGTAATGTC AGAGGTATCT TCAATCATTA TGGAAATTCC CTTGTCTCTG
C.triasi                        CAATTCTAAA CAGACTCCAT TTTGGGGGCA CAACAAGAAT TTTTATTCGC AAGTAATGTC AGAGGTATCT TCAATCATTA TGGAAATTCC CTTGTCTCTG

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  305       315       325       335       345       355       365       375       385       395
C.foetida spp.afghanistanica    CGATTCATAT CTTCCCTAGA AAGGAAAAGG GTAGTCAAAT CCGAGAATTT ACGCTCAATT CATTCGATAT TTTCTTTTTT AGAGGACAAC TTTTCACATT
C.foetida spp.commutata         CGATTCATAT CTTCCCTAGA AAGGAAAAGG GTAGTCAAAT CCGAGAATTT ACGATCAATT CATTCGATAT TTTCTTTTTT AGAGGACAAC TTTTCACATT
C.foetida spp.rhoeadifolia      CGATTCATAT CTTCCCTAGA AAGGAAAAGG GTAGTCAAAT CCGAGAATTT ACGATCAATT CATTCGATAT TTTCTTTTTT AGAGGACAAC TTTTCACATT
C.kotschyana                    CGATTAATAT CTTCCCTAGA AAGGAAAAGG GTAGTCAAAT CCGATAATTT ACGATCAATT CATTCGATAT TTTCTTTTTT AGAGGACAAC TTTTCACATT
C.triasi                        CGATTAATAT CTTCCCTAGA AAGGAAAAGG GTAGTCAAAT CCGATAATAT ACGCTCAATT CATTCGATAT TTTCTGTTTT AGAGGACAAC TTTTCACATT

                                ....|....| ....|....| ....|....| ....|....
                                  405       415       425       435
C.foetida spp.afghanistanica    TAAATTATGT ATTAGATATA CTAATACCTT ACCCAGCCC
C.foetida spp.commutata         TAAATTATGT ATTAGATATA CTAATACCTT ACCCAGCCC
C.foetida spp.rhoeadifolia      TAAATTATGT ATTAGATATA CTAATACCTT ACCCAGCCC
C.kotschyana                    TAAATTATGT ATTAGATATA CTAATACCTT ATCCAGCCC
C.triasi                        TAAATTATGT ATTAGATATA CTAATACCTT ACACAGCCC
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Aligned *matK1* DNA sequences matrix of the *Crepis* species section *Barkhausia* representing five DNA sequences of five *Crepis* taxa

9. Declaration

I declare that I have completed this dissertation single-handedly without the unauthorised help of a second party and only with the assistance acknowledged therein. I have appropriately acknowledged and referenced all text passages that are derived literally from or are based on the content of published or unpublished work of others, and all information that relates to verbal communications. I have abided by the principles of good scientific conduct laid down in the charter of the Justus Liebig University of Giessen in carrying out the investigations described in the dissertation.

Ghalia Esklual	03.03.2017, Albersweiler
_____	_____
Signature	Date, Place