Phylogeny and Character Evolution in the Genus *Crepis* L. (Cichorieae, Compositae)

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Introduction

1.1 THE OBJECTIVE: THE INFLUENCE OF KARYOTYPE EVOLUTION ON SPECIES FORMATION

1.1.1 Karyotype Alterations

Karyotypes are the "phenotypic appearance of the somatic chromosomes, in contrast to their genetic contents" (Levitsky, 1924, 1931). Number, shape, size and symmetry of chromosomes, the distribution of hetero- and euchromatic regions as well as the arrangement of genes and linking groups on the chromosomes define the karyotype of a given organism. Chromosomal differences account for interbreeding barriers between species leading to the formation of new species.

An important and often encountered mode of karyotypic alteration is polyploidisation, especially in plants. Approximately 70% of the angiosperms have experienced at least one episode of polyploidy in their ancestry (Masterson, 1994), whereas in animals polyploidy is comparatively rare. Two major causes of polyploidisation are observed, one results from the hybridisation of two different chromosome sets (allopolyploids) and the other is caused by a multiplication of one chromosome set (autopolyploids) (Kihara & Ono, 1926).

Series of aneuploid changes of chromosome number are found in many genera throughout the Angiosperms (e.g. *Crepis*, Babcock & Jenkins, 1943; *Brachyscome*, Watanabe et al., 1999; *Hypochaeris*, Cerbah et al., 1998). Multiple translocations can cause such aneuploid differences in chromosome number (Stebbins, 1950). Robertsonian fissions and fusions (the breaking and rejoining of two nonhomologous acrocentric chromosomes at the centromer, in which process the short arms can be lost), have also been proposed as mechanism which can influence the chromosome number of species (Robertson, 1916; Jonson, 1998). However, an increasing understanding of the genetic control of chromosome pairing (e.g. Riley et al., 1959; Schifino & Moraes Fernandes, 1987; Naranjo & Corredor, 2004) and the phylogenetic mapping of chromosome numbers (e.g. Watanabe et al., 1999; Cerbah et al., 1998) suggest, that species relationships are not well portrayed by chromosomal data (Levin, 2002).

Substantial differences in genome size between closely related species indicate possible reproductive isolation (Greilhuber & Ehrendorfer 1988; Bennett & Leitch, 2005). How genome size variation influences speciation is still unclear. High mutational rates have been reported

for the *Genlisea/Utricularia* clade of the Lentibulariaceae, which comprise species with ultrasmall genomes (e.g. 1C-value 0.065 pg in *Genlisea aurea*), whereas the sister group, *Pinguicula*, shows neither ultrasmall genomes nor accelerated mutational rates (Greilhuber et al., 2006). The "large genome constraint hypotheses" assumes that genera with large genomes are less likely to speciate as accumulation and replication of "junk" DNA is associated with evolutionary costs (Knight et al., 2005). Transposable elements, which account for a high fraction of heterochromatic DNA, compete with other DNA sequence segments for "resources", e.g. material for replication (Gregory, 2005). This leads to the hypothesis that genome size variation influences speciation.

Genome size is not correlated to the organisational level of an organism: more complex organisms do not necessarily feature more DNA ("C-value Paradox", Thomas, 1971; also e.g. Vendrely, 1955; MacLean, 1973; Gall, 1981); the actual number of genes required for normal development is similar in most plants (Flavell, 1980). Variation in genome size is mostly due to the amount of heterochromatin (e.g. Flavell et al., 1977; Flavell, 1986; Barakat et al., 1997). Questions as why there is such a considerable variation in non-coding DNA, how it is distributed among taxa and how it developed are still unanswered ("C-value enigma", Gregory, 2001). Because of the self-replicating DNA elements, genomes are often considered to have a "one way ticket to obesity" (Bennetzen & Kellogg, 1997). But recent studies suggest that genomes can also "shrink" (e.g. Wendel et al., 2002). The reasons for the variation of genome size are manifold: increases in DNA content can be due to the accumulation of retroelements (e.g. SanMiguel & Bennetzen, 1998; Bennetzen, 1996, 2000) and repeated circles of polyploidy (e.g. Soltis & Soltis, 1999; Otto & Whitton, 2000; Wendel, 2000). Decreases can be due to e.g. the loss of whole chromosomes or parts thereof (Dart et al., 2004) or through intrachromosomal homologous recombination between LTR's (long terminal repeats, Vicient et al., 1999; Ma et al., 2004).

Gregory (2005) stated that genomes represent a distinct and legitimate level of biological organisation, with their own inherent properties and unique evolutionary histories. The variety of levels on which genomic or chromosomal changes occur renders karyotype and genome evolution highly complex. Many facets of genomic evolution are up to now only poorly understood.

1.1.2 Karyosystematics and Phylogeny

Karyotype similarities and differences, especially chromosome number have often been used as criteria to infer species relationships (e.g. Babcock et al., 1937; Babcock, 1947a,b; Gokhman, 2007; Guerra, 2008). Extensive contributions in the field of karyosystematics have been made by E.B. Babcock and G.L. Stebbins through thorough cytological studies within the Compositae subtribe Cichorieae (e.g. Babcock & Stebbins, 1937; Babcock & Jenkins,

1943; Babcock, 1947a,b; Stebbins et al., 1953). The cooperation of the two botanists led to the first pre-cladistic "phylogenetic" hypotheses for confined plant groups: Based on a phenetic multi-evidence approach by considering chromosomal data, geographical distribution, and morphology, Babcock (1947a,b) postulated phylogenetic coherences within the genus *Crepis* L (Compositae), later followed by a publication on phylogenetics within the whole Compositae subtribe Cichorieae (Stebbins, 1953; Stebbins et al., 1953). Subsequently, Stebbins (e.g. 1950) became one of the architects of the Modern Evolutionary Synthesis, together with Dobzhansky (e.g. 1937, 1970), Mayr (e.g. 1942, 1997), and Simpson (e.g. 1944).

Hennig, a German entomologist, is regarded the founder of modern evolutionary studies with the publication of his "Phylogenetic Systematics" (Hennig, 1966). He proposed a cladistic approach to infer the evolutionary history of a taxon. Species are classified hierarchically based on evolutionary ancestry. Classical cladistic analyses use mainly morphological and anatomical characters, but also cytological and biogeographical data.

Since the advent of DNA sequencing species relationships in evolutionary contexts are mainly inferred by molecular data. Phylogenies based on molecular data have several advantages: In very high or very low taxonomic ranks where morphological variation is often too low or too high to reflect evolutionary patterns, a molecular approach is advantageous. Furthermore, molecular data can be generated in relatively short time; whereas it is often time consuming to assess morphological characters (Sudhaus, 2007). The virtually infinite abundance of molecular characters allows for statistical approaches to infer the reliability of a given reconstruction (Knoop & Müller, 2006). Still, other factors influence the reliability of a phylogenetic reconstruction as well: First of all, a phylogenetic gene tree reflects the history of the applied gene or marker and not necessarily that of the species (Maddison, 1997). Furthermore, DNA from different cell organelles can reflect different evolutionary histories, equivalent to the different evolutionary histories of the complete organelles. And marker loci can vary in their mutational rates; coding gene regions are in general more conserved than non coding regions.

One approach to obtain reliable phylogenetic histories for species groups is to use multiple marker loci (e.g. Qiu et al., 1999; Graham & Olmstead, 2000; Soltis et al., 2000; Zanis et al., 2002). Another important factor is the choice of the employed marker: chloroplast markers in Angiosperms are passed on mostly in the maternal lineage, while nuclear markers show biparental inheritance patterns. In plant phylogenetic studies chloroplast regions are widely amplified and analysed because they exhibit a suitable rate of evolution for phylogenetic reconstructions and are easily accessible. To trace the biparental history of taxa nuclear markers are used. Inconsistencies of chloroplast and nuclear based reconstructions can be used to identify potential reticulate evolution events (e.g. Rieseberg & Soltis, 1991). One of the most widely used nuclear markers in angiosperm phylogeny is ITS (internal transcribed

spacer) (e.g. Baldwin et al., 1995). ITS is located within the highly conserved 18S-26S nuclear ribosomal gene cluster and arranged in numerous tandem repeats. The manifold applications using ITS are due to the high number of copies and the conserved flanking regions which makes the spacer region easily accessible even with universal primers (Baldwin et al., 1995); in some plant groups different paralogues are present, which reflect complex inheritance patterns and therefore, complicate phylogenetic analysis (e.g. Álvarez & Wendel, 2003). In most plant groups, however, the ITS paralogues show high uniformity, attributed to rapid concerted evolution (e.g. Arnheim et al., 1980; Arnheim, 1983; Hillis et al., 1991). At present the use of single copy nuclear genes is time and cost consuming, so they are not yet widely used for routine analysis. Primer binding is often impeded by secondary structures of the large genome and primer binding sites must be very specific so as to not accidentally target orthologues. Furthermore, for the identification of suitable loci for phylogenetic reconstructions of certain taxa and for primer design, sequence information of closely related taxa are needed (Álvarez et al., 2008). The high potential of nuclear single copy genes to reveal phylogenetic coherences makes their application increasingly common (e.g. Strand et al., 1997; Sang, 2002; Zhang & Hewitt, 2003; Small et al., 2004; Álvarez et al., 2008).

With the “molecular revolution” and the use of DNA sequences for phylogenetic analysis the focus of karyological and morphological contributions to evolutionary studies has changed (Endress, 2002): Structural similarities can illuminate cryptic molecular groups as well as groups with a conflicting molecular and morphological background, and character progressions can be reconstructed on molecular phylogenies (Endress, 2002).

1.1.3 The Objective

The present study investigates aspects of phylogeny, character evolution and systematic classification in the genus *Crepis*. It aims to contribute towards the understanding of character evolution and speciation mechanisms in higher plants. Based on the reconstruction of a molecular phylogeny, the evolution of karyotypic changes, e.g. in chromosome number or genome size, and its influence on diversification in diploid plant genera are of special interest. Hypotheses on character evolution are postulated for the model group *Crepis*, which might prove to be significant for character evolution in higher plants.

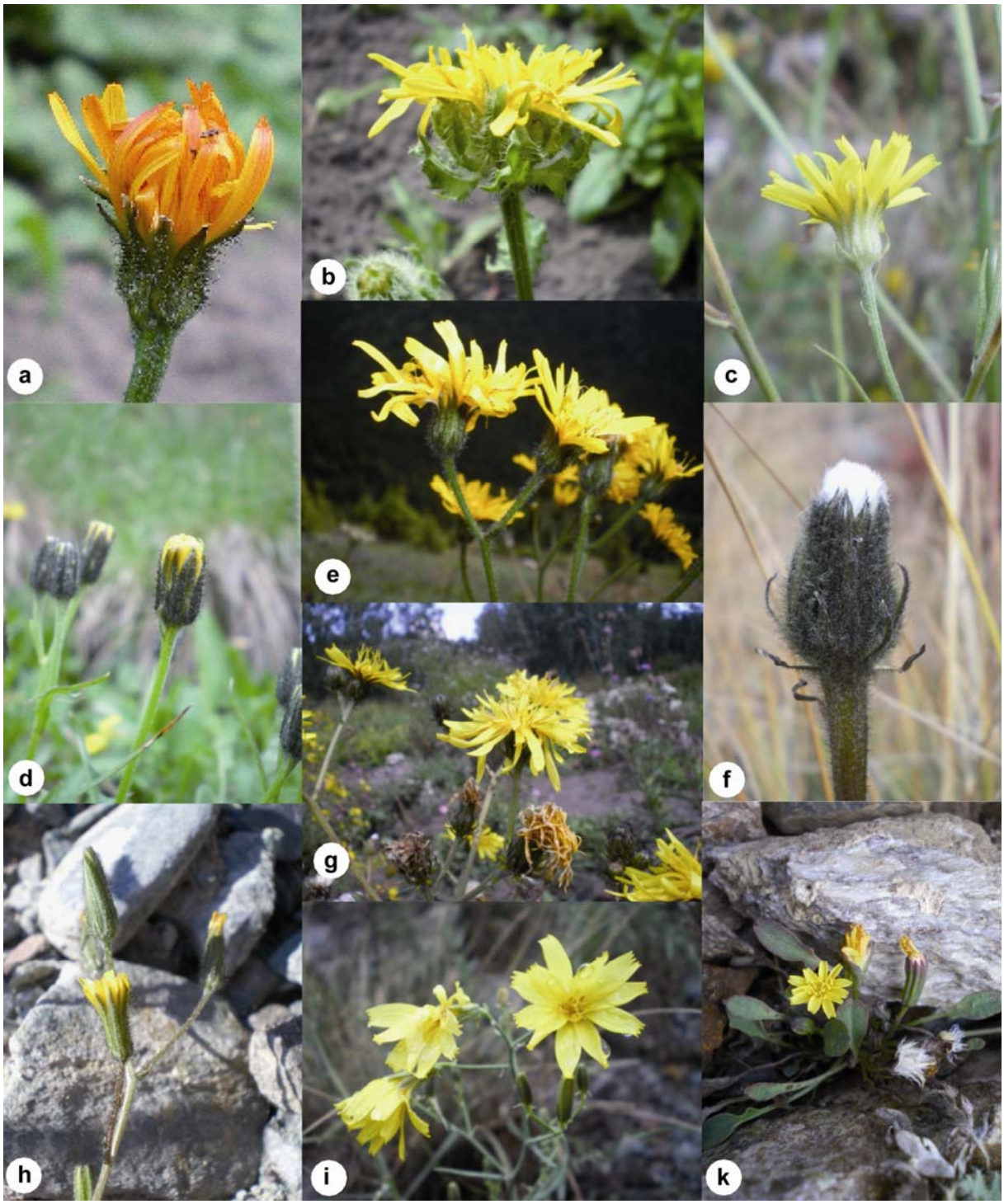

FIG. 1: Crepis and related genera. a) *Crepis aurea,* b) *C. blattarioides* c) *C. tectorum* d) *C. jacquini*, e) *C. mollis,* f) *C. chrysantha*, g) *C. sibirica*, h) *C. multicaulis*, i) *Youngia tenuifolia,* k) *Askellia nana.*

1.2 THE OBJECT: *CREPIS* AS MODEL GROUP

Crepis is a genus within the Compositae (Syn: Asteraceae) tribe Cichorieae Lam. & DC. (formerly Lactuceae Cass.) and subtribe Crepidinae. Following the traditional classification of the Crepidinae (*Ixeris*-*Youngia*-Line (Stebbins, 1953)), *Crepis* series (Jeffrey, 1966) and Crepidinae (Bremer, 1994)) the most closely related genera to *Crepis* were considered to be *Lapsana* (sensu Pak & Bremer, 1995), *Youngia* (monographed by Babcock & Stebbins, 1937, Figs.1,3-4), *Ixeris* and *Dubyaea* (treated by Stebbins, 1940). A recent revision of the Cichorieae by Kilian et al. (2008) taking molecular sequence data into account largely confirmed these relations. Due to the lack of discriminating characters for many genera within the Crepidinae some species have been classified in the past under different generic names; e.g. the species of the genus *Askellia* (Figs.1, 3-4). Babcock (1947a,b) already perceived the intermediate position of *Askellia* between *Crepis* and *Ixeris* and *Youngia* (Fig.3), even though most of the species (e.g. *A. nana*, *A. flexuosa*) were treated under *Youngia* to which they were recently reassigned (Adylov & Zuckerwanik, 1993). Other genera (e.g. *Ixeris* by Pak & Kawano, 1990a,b,c, 1992; *Youngia* by Sennikov & Illarionova, 2007) have been subdivided into new genera due to e.g. carpological and karyological characters.

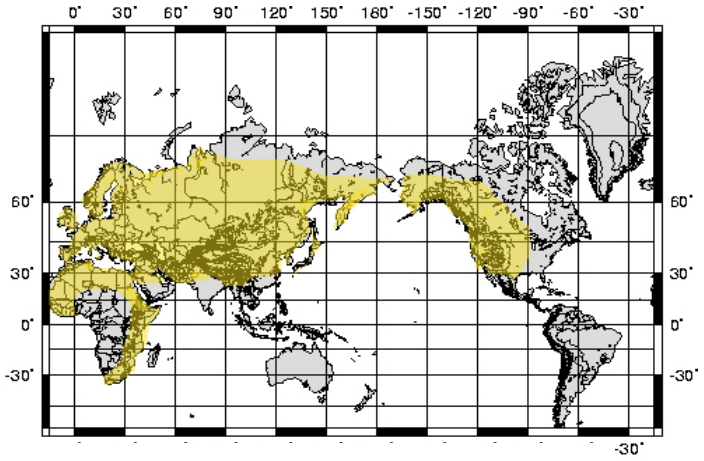

FIG. 2: Worldwide distribution of *Crepis*.

Crepis is with over 200 species (Bremer, 1994) one the largest genera of the Crepidinae and even of the Cichorieae. Species of the genus are distributed throughout the northern hemisphere with single species occurring in South East Asia (Fig.2). Some species also occur in tropical east Africa, South Africa and West Africa, as well as the Canary Islands and Madeira. The origin of *Crepis* is thought to be in the Altai/Tien Shan region in Central Asia (Babcock, 1947a). From there the genus spread north-eastward into North America, south-westward into southern Europe and northern Africa and westward across the southern end of the Ural Mountains into north-eastern Europe (Babcock, 1947a). The genus presently has its highest species diversity in the circum-Mediterranean area.

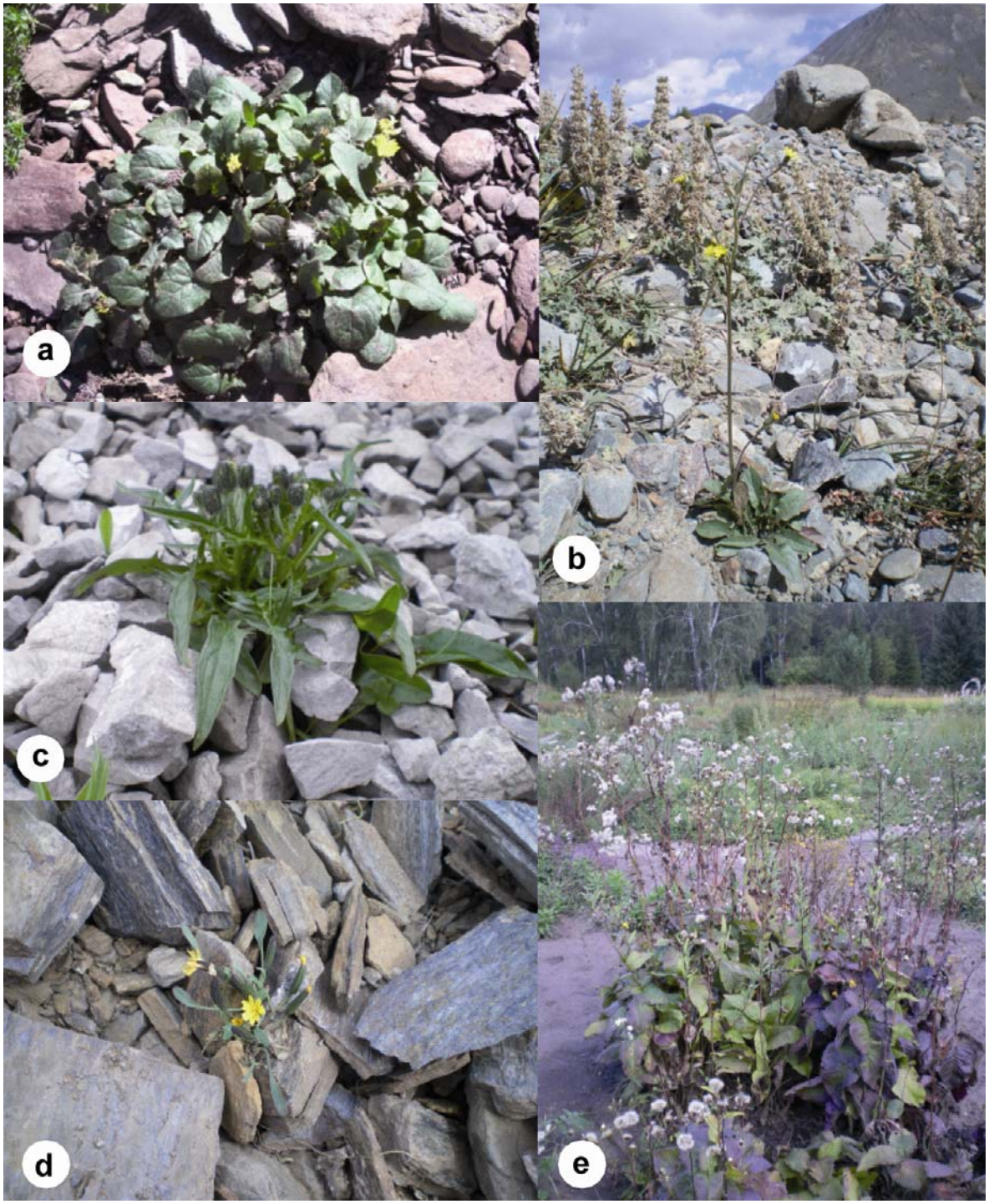

FIG. 3: Growth form and habit of *Crepis* and related genera. a) *Crepis pygmaea,* b) *C. multicaulis*, c) *C. jacquini*, d) *Askellia nana*, e) *C. sibirica.*

Crepis, as all Cichorieae, is notorious for its lack of discriminating characters. Many characters vary more within a species than between closely related species. In the past this often led to unclear specific and generic boundaries. The taxonomic history of the genus *Crepis* is a long tale of lumping and splitting natural groups on various taxonomic levels.

FIG. 4: Floral characters of *Crepis* and related genera. a) ligules, style branches (*Crepis sibirica*), b) achenes, pappus (*C. sibirica*), c) involucrum in two distinct rows, capitula (*C. sibirica*), d) achenes, pappus (*C. tectorum*), e) achenes, pappus (*Youngia tenuicaulis*), f) glabrous and cylindrical involucrum, capitula (*Y. tenuifolia*), g) glabrous and cylindrical (*Askellia nana*).

Linné (1753) assigned 13 species to *Crepis* (type species: *C. biennis*) of which 10 are still part of the genus today. Moench (1794) accepted only 3 genera (*Crepis*, *Catonia* and *Barkhausia*); where Cassini (1830) recognised 14 genera (*Crepis, Anisoderis, Barkhausia, Brachyderia, Catonia, Gatyona, Intybellia, Nemauchenes, Omalocline, Paleya, Phaecasium, Pterotheca,* and *Zacintha*). 60 years later Hoffmann (1889) assigned about 170 species to *Crepis* comprising those genera listed in Cassini (1830) and several of the 130 species mentioned in Bentham & Hooker (1873). The most recent revision of the genus *Crepis* has been published by Babcock (1947a,b). Babcock's monograph of the genus not only includes detailed descriptions of 196 species, but extensive hypotheses on origin, phylogeny, character evolution and speciation within the genus.

Crepis species occur in different types of habitats (Fig.3) ranging from alpine zones, swamps, low grasslands, forests to beaches. Size ranges from only a few centimetres in height (e.g. *C. pygmaea*) to nearly two meters in *C. sibirica* (Fig.3a,e). The capitula of *Crepis*

possess two distinct rows of involucral bracts (Fig.4c). Florets are ligulate (Fig.4a). Even though the prevailing flower colour is the typical bright yellow of composites (Fig.1), some species show other shades, like yellowy orange in *C. aurea* (Fig.1a) or whitish yellow in *C. albiflora*. *C. incarnata, C. rubra* and *C. incana* have purple flowers. Some of the yellow flowered species have a red dorsal stripe on the ligule. Corolla tubes are either pubescent or glabrous with 5 ligule teeth; style branches are usually cylindrical and attenuate at the apex (Fig.1a). The receptacle is either areolate or alveolate. Areoles can be separated by a membranous ridge which is occasionally fibrillate. The fibrillae are replaced in rare cases by palae. Colour ranges from dark green to light yellow. The achenes are narrowly terete to fusiform, more or less attenuate, and sometimes beaked (Fig.4b). Some species (e.g. *C. sancta*) have biform achenes. The simple pappus bristles are never plumose, scabrid barbellulate and often pure white, seldom dusky or yellowish (Fig.4b,d). Except for the 15 North American species of section *Psilochaenia* and very few other species, the majority of species is diploid. The basic chromosome number ranges from x=3 to x=6, respectively x=11 in the polyploid species of section *Psilochaenia*.

In his monograph, Babcock (1947a,b) defined 27 sections based on morphology, geographical distribution, chromosome number and karyotype composition and assigned the 196 *Crepis* species accordingly. He assumed that the sectional classification reflected phylogenetic coherences within the genus. He formulated several progressive character changes; e.g. that chromosome number and size would decrease during evolution, while chromosome asymmetry would increase. He furthermore postulated that tap rooted species are derived and rhizomatous species are basal. He considered large species with few, big heads, a high chromosome number, and a rhizome to be primitive (e.g. *C. sibirica*, Fig.33); advanced species are generally more fragile in habit with many small flower heads, have a lower chromosome number and are tap rooted (e.g. *C. multicaulis*, Fig.3b). In an often overlooked publication he later withdrew his theory that rhizomes are basal, as ontogenetically the taproot develops first (Babcock, 1949). This resulted in a minor rearrangement in the phylogenetic order of the sections (Babcock, 1949). Babcock (1947a) described karyotype evolution as driving force of speciation in *Crepis* L.. Following his hypotheses, it is mainly a change in chromosome number that leads to interspecific sterility and therefore to speciation. According to Babcock (1947a) hybridisation plays a minor role for explaining speciation processes in the genus.

An essay on karyotype evolution in the Cichorieae was published in 1953 by Stebbins and co-workers. The karyological studies on *Crepis* by Babcock & Jenkins (1943) contributed considerably to a phylogenetic understanding of the tribe Cichorieae. *Crepis* traditionally is important for karyological studies in higher plants. Even before Babcock (1947a,b) published his monograph on the genus, *Crepis* was often the subject of studies on plant genetics (e.g. Hollingshead, 1930a,b; Tobgy, 1943; Sherman, 1946), evolution, and speciation. Babcock

and Jenkins' (1943) work on *Crepis* karyotypes was extensive but limited by the methods available at the time (Fig.5). Since then many studies on different aspects in the field of genetics and karyotype evolution in *Crepis* have been published (B chromosomes e.g. by Maluzynska & Schweizer (1989), Maluzynska (1990), Jamilena et al. (1994); banding patterns e.g. by Siljak-Yakovlev & Cartier (1982), Dimitrova & Greilhuber (2001); chromosomal aberrations e.g. by Dimitrov (1994); or the evolution of quantitative karyotype characteristics e.g. by Jones & Brown (1976), Dimitrova & Greilhuber (2000)). With the extensive systematic treatment of the genus by Babcock (1947a,b) based on morphological, karyological and biogeographical characters a solid foundation has been laid on which additional studies on the genus *Crepis* can build. The low chromosome number, the predominant diploidy of species and the excellent response of the chromosomes to staining procedures, make chromosomal features easily accessible and comparison between species is facilitated without much effort. These features make *Crepis* an excellent model group to study speciation due to karyotype changes in (diploid) higher plants. Furthermore, *Crepis* species represent a large number of growth forms and habitat adaptations and are broadly distributed.

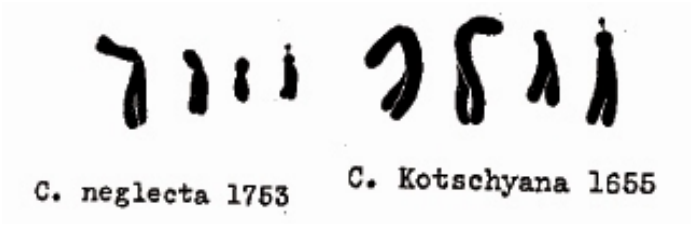

FIG. 5: Karyotypes of *C. neglecta* and *C. kotschyana* reproduced from Babcock & Jenkins, 1943.

1.3 COMMENTS ON THE STRUCTURE OF THE PRESENTED STUDY

The present study covers aspects of phylogeny, speciation, character evolution, and systematic classification in the genus *Crepis* L. (Compositae, Cichorieae). *Crepis* is used as model system representing the angiosperms; it is believed that the gained insights on character as well as karyotype evolution can illuminate evolutionary processes in other flowering plant genera.

The study is divided into two parts dealing with different aspects of evolution, speciation and phylogeny in the genus *Crepis*. The first part discusses character evolution - especially considering karyological traits as used by Babcock (1947a,b) to constitute the systematic treatment of the genus - and phylogeny in a molecular framework. In the second part the systematic classification of *Crepis* s.l. is reconsidered including molecular as well as morphological characters.

The first part with the main focus on phylogenetic investigations and its implications for karyological and morphological character evolution comprises two chapters (2,3). Chapter 2 illustrates the basic phylogenetic coherences within the genus. Molecular phylogenies based on the nuclear marker ITS (internal transcribed spacer) and the chloroplast region *matK* (maturase K) of 40% of accepted *Crepis* species are presented. General trends in character evolution and the incongruence between the natural molecular groups and the current taxonomic classification are discussed. Babcock's hypotheses on character evolution and speciation are revaluated. Chapter 3 includes a more detailed discussion of character evolution and speciation and investigates genome size evolution within *Crepis*. Genome size of 21 species is measured and the correlation to several factors (e.g. life form and geographic distribution) is statistically tested. The direction of genome size variation during evolution is reconstructed on a molecular phylogeny inferred from the nuclear marker ITS.

The second part evaluates in chapter 4 new characters for their applicability for generic and infrageneric delimitation and comparative morphological studies. These characters include achene morphology and anatomy, pappus ultrastructure and pollen grain morphology. Chapter 5 discusses the taxonomic implications of the molecular phylogeny presented in chapter 2 with a critical reassessment of the current systematic classification. Chapter 6 deals with a newly found congener of *Crepis*, former genus *Dianthoseris*, which in the past was of unclear position within the Cichorieae.

Chapters 2,3 and 6 are published or submitted papers (see appendix), so chapters 4 and 5, even though not published yet, follow in their internal structure publication requirements, viz. the chapters are divided into abstract, introduction, material/methods, results, discussion, acknowledgements, literature cited and appendix.

Chapter 7 presents perspectives on how results and data accumulated in the present study could further be interpreted. It also discusses aspects and questions of species evolution

which were brought up during the present investigation and which await further treatment. Chapter 8 summarises the insights gained from the different approaches to understand character evolution, speciation, and species relations in the genus *Crepis* as model group for higher plants.

Chapter 2

Babcock revisited: New insights into generic delimitation and character evolution in *Crepis* L. (Compositae: Cichorieae) from ITS and *mat*K sequence data

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ABSTRACT

In 1947 Babcock published his widely acknowledged monograph of the genus *Crepis* L. including a sectional classification of the species as well as extensive hypotheses about character evolution. To reinvestigate Babcock's evolutionary hypotheses and the generic delimitation of *Crepis* L. a phylogenetic analysis was conducted using ITS and chloroplast *mat*K sequence data. The results revealed *Crepis* L. to be polyphyletic. A monophyletic clade including Central Asian and North American species of *Crepis* section *Ixeridopsis* is clearly isolated from *Crepis* sensu stricto and, as also supported by additional morphological evidence, needs to be transferred to the genus *Askellia* Weber (1984).

A second clade comprising the genera *Lapsana* L. and *Rhagadiolus* Juss. as well as a statistically strongly supported clade of several C*repis* species is sister to a third clade: the monophyletic *Crepis* s.str.. Within *Crepis* s.str. the molecular data do not support Babcock's sectional delimitation which is mainly based on his hypotheses about karyotype evolution. Hence, morphological and karyological characters are re-assessed with regard to the molecular phylogeny.

KEYWORDS: *Askellia, Crepis*, ITS, matK, phylogeny.

2.1 INTRODUCTION

The genus *Crepis* L. (Cichorieae: Crepidinae) comprises about 200 species and is distributed throughout the northern hemisphere and Africa. The genus presumably originated in the Pamir/Altai region in Central Asia (Babcock, 1947a). Presently the centre of diversity is the circum-Mediterranean area.

In the first edition of *Species Plantarum,* Linné (1753) assigned 13 species to *Crepis* (Type: *Crepis biennis* L.) of which 10 are still valid today. Hoffmann (1889) included about 170 species comprising those genera listed in Cassini (1830) (*Crepis, Anisoderis, Barkhausia, Brachyderia, Catonia, Gatyona, Intybellia, Nemauchenes, Omalocline, Paleya, Phaecasium, Pterotheca, Zacintha*) and several of the 130 species mentioned in Bentham & Hooker (1873).

Babcock (1947a, b) was the last to revise the genus and assigned 196 species to 27 sections on the basis of morphological and karyological similarities. This included a priori assumptions about character evolution. According to Babcock, primitive characters are being perennial, exhibiting a rhizome, and a chromosome number of x = 6; derived features are being annual, having a taproot, and a chromosome number of less than x = 6. In a rarely cited publication Babcock (1949) withdrew the rhizome as basic character because seedlings of rhizomatous species first develop a taproot which later is lost. Therefore a taproot is to be considered the basic character state.

Babcock's interpretation of karyotype evolution is of special importance, since it tended to be cited as exemplary (e.g. Stebbins, 1950; Briggs & Walters, 1984). Babcock defined several karyomorphotypes depending on number and size of chromosomes and their symmetry, and arranged the species into sections due to these chromosomal characteristics (Babcock & Jenkins, 1943; Babcock, 1947a). He postulated karyotype rearrangements to be the driving force of speciation while hybridisation between members of different karyomorphotypes is largely inhibited (Babcock, 1947a).

To reinvestigate Babcock's evolutionary hypotheses as well as the generic and infrageneric delimitation of *Crepis* a molecular phylogeny based on ITS and *mat*K sequence data has been established. The applicability of the molecular markers ITS and *mat*K to reveal intra- and intergeneric relationships in the Cichorieae (Compositae) has already been demonstrated in several studies (e.g. Baldwin, 1993; Goertzen et al.; 2003, Samuel et al., 2003). Pre-studies on several chloroplast regions (trnS-trnR, trnS-trnFM, atpB-rbcL, trnS-trnG, psbA-trnH, trnK-trnQ) have been carried out of which *mat*K proved to be informative and universally amplifiable.

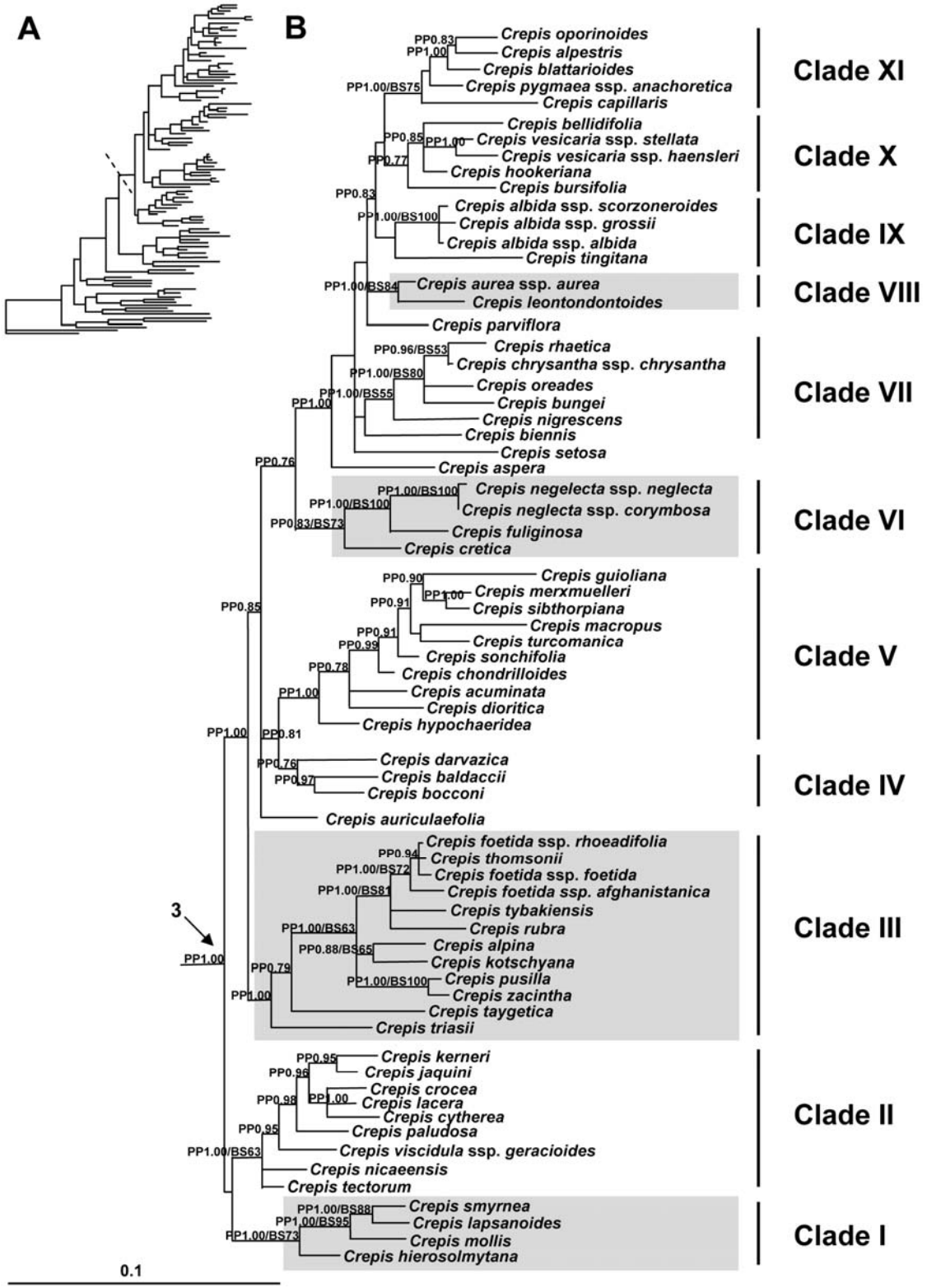

FIG. 1: Phylogram derived from the Bayesian Inference (ITS dataset). A, overview of the tree, the dashed line indicates the two parts shown in B and C. B, upper part of the tree, all clades belong to *Crepis* s.str.; C, lower part of tree, showing *Askellia* and *Lagoseris* groups as well as other Crepidinae. Bayesian posterior probabilities > 0.70 and bootstrap values >50% given above branches. Arrows with numbers indicate nodes discussed in the text; a and b denote subclades of *Lagoseris*. Clades within *Crepis* s.str. are numbered as not to reflect any infrageneric taxonomic system. Shaded clades are discussed in detail in the text.

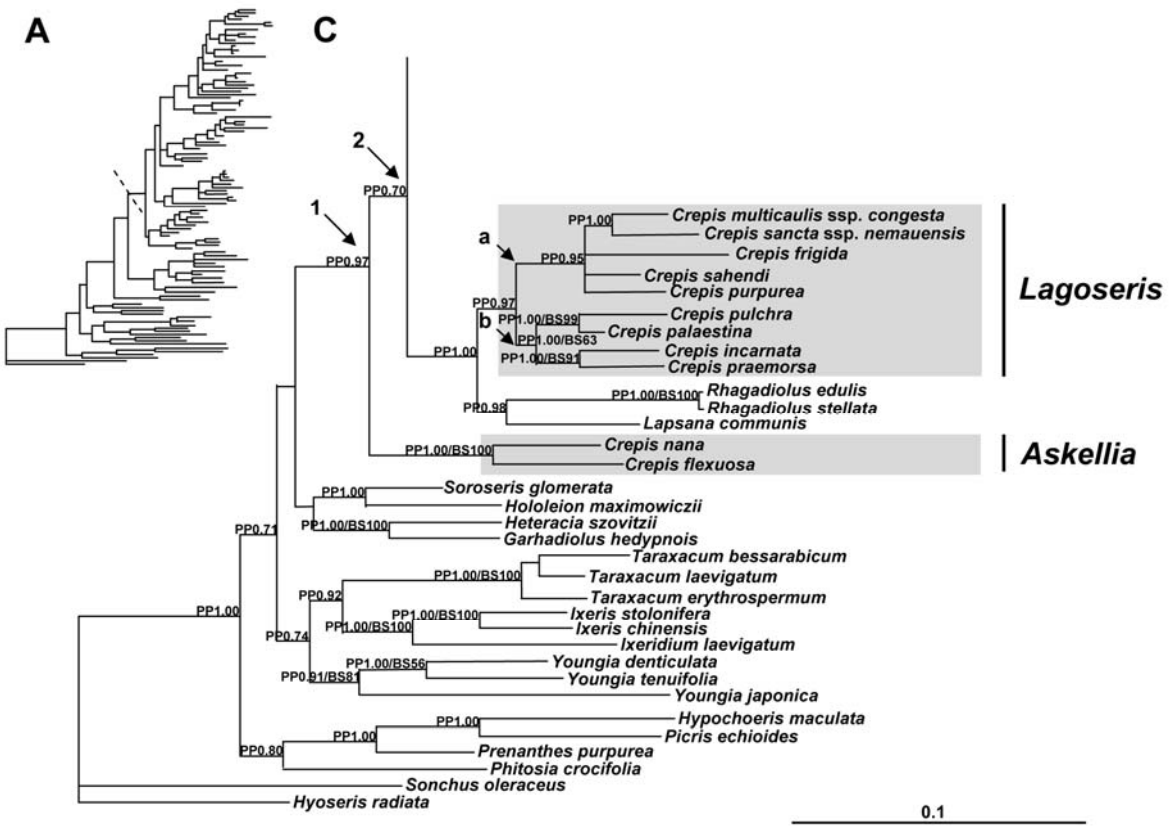

FIG.1 (continued)

2.2 MATERIAL AND METHODS

Sampling – 200 samples from 102 *Crepis* species of 26 sections were obtained. Two separate individuals (from different collections) per species were sampled whenever possible. As outgroup closely related taxa of the Cichorieae such as *Taraxacum*, *Ixeris*, *Tolpis* and *Youngia* were chosen. Samples were taken from dried herbarium specimens of various herbarium collections (B, M, MSB, E, UPS and US). The sequences of the outgroup taxa and some *Crepis* species were downloaded from NCBI (GenBank, EMBL).

A list of samples, voucher location and GenBank numbers is provided in the appendix.

DNA isolation, amplification and sequencing – Total genomic DNA was isolated from dried herbarium specimen. 24 mg per sample of mostly leaf material was taken. The samples were crushed and DNA was then extracted using Quiagen DNeasy Mini Kit and following standard procedure.

The ITS and *mat*K regions were amplified in two overlapping parts using the primers ITS-A and ITS-C (Blattner, 1999) for ITS 1, ITS2-D, ITS-B (Blattner, 1999) for ITS 2 and trnK-710f

(Johnson & Soltis, 1995) and *mat*K-iR (Fehrer et al., 2007) for *mat*K 1. For *mat*K 2 specific primers were designed: *mat*K-ifN (5'-CATTCRAYATTTTCTTTTT-3') and *mat*K-rN ('5-TTATATAAATCCTTCCTG-3). For ITS the following protocol during the Polymerase Chain Reaction (PCR) was used: initial denaturation 2 min at 94°C, denaturation 20 sec at 94°C, annealing 45 sec at 52°C, elongation 1 min at 72°C (40 cycles) and final extension 10 min at 72°C. The *mat*K region was amplified in 40 cycles under following conditions: denaturation 40 sec at 94°C, annealing 1 min 30 sec at 50°C, elongation 2 min at 72°C, preceded by initial denaturation 2 min at 94°C and followed by a final extension 15 min at 72°C. PCR was carried out with a reaction volume of 11.5µl core mix plus 1 µl DNA (1:10, 1:50 or 1:100 dilution depending on usability). The reaction volume contained 8.05 µl ddH_2O, 1.25 µl 10x buffer (Biotherm) and dNTP's (Fermentas), 0.25 µl BSA (BioLabs), 0.25 µl of each primer (10pmol/µl) and 0.04 µl 1u Taq-Polymerase (Biotherm). The PCR products were purified with Milipore DNA purification Kit (Roth) and then cycle sequencing was carried out using CEQ DCTS Quick Start Kit (Beckmann-Coulter) following the standard procedure. As sequencer a CEQ8000 (Beckmann-Coulter) was used.

Sequence Alignment – The sequences were edited in ChromasLite2000 (Technelysium Pty. Ltd., Helensvale, Australia) and aligned by hand using BioEdit (Hall, 1999) following Goertzen et al. (2003) for ITS gap-coding. ITS alignment was sometimes ambiguous. To avoid alignment ascendancies some positions were excluded (1--19, 118,222--223, 242,288--289, 372--376, 516--519, 555--556, 706--727). For *mat*K indels at positions 118--122, 353--366, 799--807 and 813--817 have been coded as one mutational step each. The alignments are available from the first author upon request.

Phylogenetic Analyses – The trees were reconstructed using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) and PAUP 4.0b10 (Swofford, 2002).

A Bayesian Analysis was performed on both datasets, using gamma distribution rate variation among sites and 10 million generations of the MCMC chains in two independent runs, trees saved every 100 generations. The first 30 000 trees were discarded as burn-in for the analysis then reached stationarity. All other trees sampled were used to calculate a strict consensus tree.

ITS and *mat*K datasets were analysed using Maximum Parsimony. All heuristic searches were conducted in Paup 4.0b10 with equal weights, 1000 closest sequence additions and tree bisection-reconnection (TBR) branch swapping, permitting 10 trees to be held at each step. An evaluation of the trees was performed by using bootstrap analysis with 1000 replicates, equal weights, TBR swapping, MulTrees option in effect and 10 trees held at each step.

The strict consensus tree of Bayesian inference was compared to the bootstrap 50% majority rule consensus tree.

Trees were drawn using TreeView (Page, 1996) and Adobe Illustrator (Adobe Systems, Inc., San Jose, California, USA).

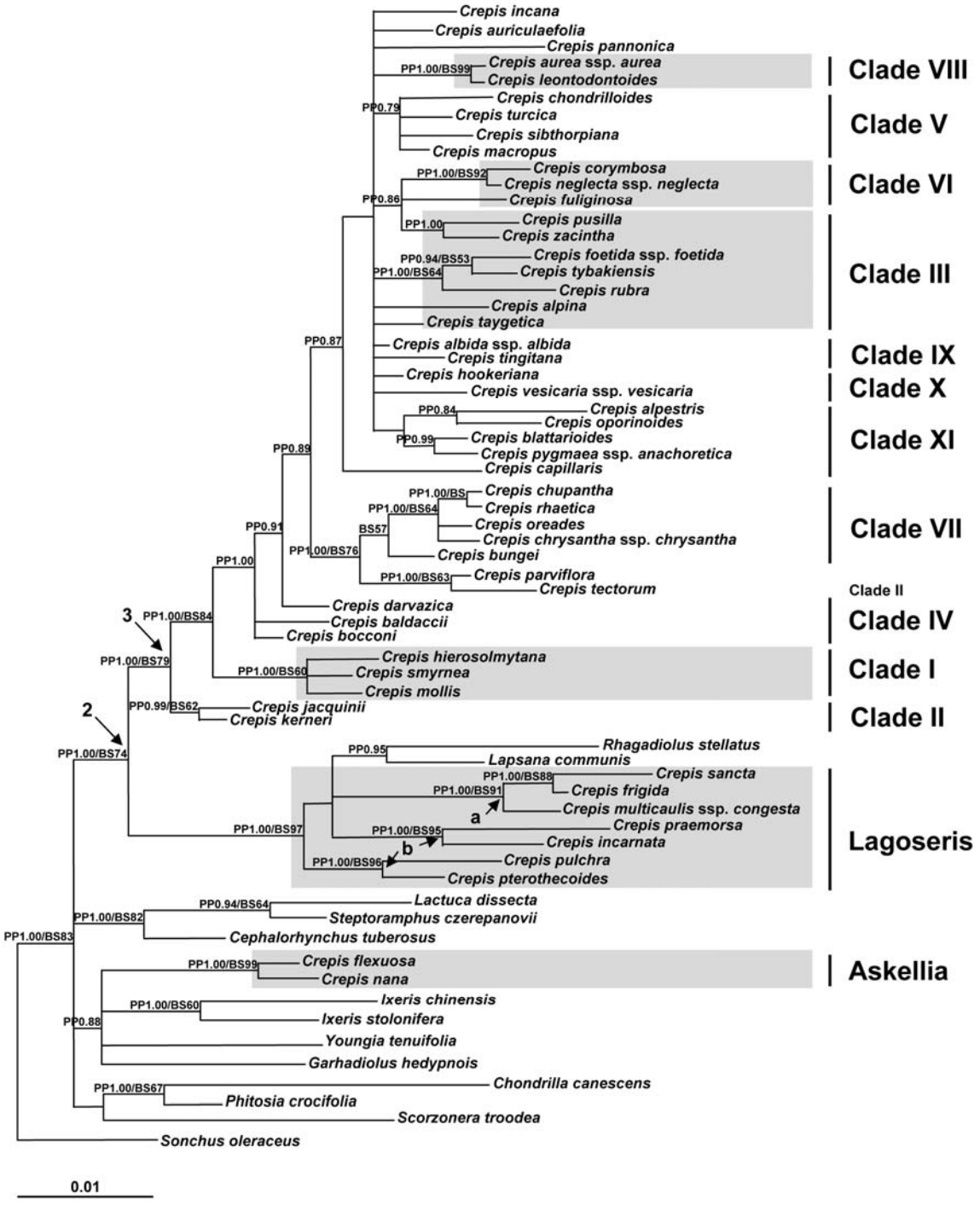

FIG.2: Phylogram derived from the Bayesian Inference (*matK* dataset). Numbering of clades according to ITS phylogeny (Fig. 1). For further explanation see Fig. 1.

2.3 Results

Phylogeny – 123/73 (ITS/*mat*K) sequences could be obtained from 78/52 *Crepis* taxa representing 24/20 of the 27 sections. 22/13 accessions including the genera *Ixeris* (ITS/*mat*K), *Taraxacum* (ITS), *Heteracia* (ITS), *Youngia* (ITS/*mat*K), *Lapsana* (ITS/*mat*K), and *Rhagadiolus* (ITS/*mat*K) represent the Crepidinae. *Sonchus* (ITS/*mat*K), and *Hyoseris* (ITS) (Hyoseridinae) were chosen as outgroup taxa. Different accessions for ITS and *mat*K are due to sequence availability.

For the ITS Maximum Parsimony analysis both spacer regions as well as the 5.8rDNA sequence have been analysed. In total 671 characters were included in the analysis, of which 327 were parsimony informative (48.7%). For *mat*K in total 943 characters were included of which 191 were phylogenetically informative (20.3%).

The trees from Bayesian and Maximum Parsimony analyses were congruent, but the basal nodes in the ITS Maximum Parsimony bootstrap analysis could not be resolved.

Fig.1 (ITS) and Fig.2 (*mat*K) depict strict consensus trees from the Bayesian Analyses. Concerning the distribution of *Crepis* both trees are congruent in their overall topology but differ in their terminal branching patterns; with the exception of the *Askellia* clade, which is sister to *Lagoseris* in the ITS tree but forms a monophyletic and unresolved group with *Ixeris*, *Youngia* and *Garhadiolus* in the chloroplast phylogeny (Figs. 1, 2). The relations of the outgroup taxa will be dealt with elsewhere.

The trees support three main clades comprising species of *Crepis* s.l. (Bayesian posterior probabilities (PP) above 70% and bootstrap values (BS) above 50% are given separately for each marker): The first clade is the above-mentioned one of unresolved relations among the outgroup taxa. It includes only species of Babcock's section *Ixeridopsis* (ITS: PP1.00/BS100; *matK*: PP1.00/BS99) and was described as genus *Askellia* by Weber (1984).

The second main clade comprises the genera *Lapsana* and *Rhagadiolus*, as well as a clade which comprises *Crepis* species from Babcock's sections *Intybellia*, *Lagoseris*, *Phaecasium*, *Microcephalum,* and *Pterotheca* (ITS: PP1.00; *matK*: PP1.00). This clade preliminary was named *Lagoseris*. The name, however, does not refer to any generic circumscription as the group needs further validation. The clade is sister to *Crepis* s.str. (ITS: PP0.70; *matK*: PP1.00/BS74 ; Node 2, Figs. 1, 2). In the ITS phylogeny the *Crepis* species of clade *Lagoseris* fall into two groups a and b (ITS: PP0.97; Fig. 1); subclade a comprises the species of sections *Lagoseris*, *Microcephalum,* and *Pterotheca*, subclade b those of sections *Intybellia* and *Phaecasium*. The latter is polyphyletic in the *mat*K phylogeny.

All other species sampled belong to *Crepis* s.str., which is monophyletic (Node 3; Figs. 1, 2) in both the ITS and the *mat*K analyses (ITS: PP1.00; *matK*: PP1.00/BS79). In the *mat*K analysis several clades lack resolution within *Crepis* s.str. due to low variation of the chloroplast marker, thus clades are defined and named following the ITS based phylogeny.

The clades are numbered as not to suggest any infrageneric taxonomic system. Within *Crepis* s.str. six out of eleven clades are supported with PP > 0.90. The clades do not coincide with Babcock's sections except for Clade I. Clades II, IV, V, VII, IX, X and XI are highly heterogeneous in respect to Babcock's sections, distribution and habit. At present, only Clades I, III, VI and VIII provide feasible results for detailed discussion, while for the other more detailed analyses are necessary.

Clade I is monophyletic in both analyses and comprises only members of section *Mesomeris* (ITS: PP1.00/BS73; *matK*: PP1.00/BS60).

Clade III (ITS: PP1.00) forms a monophyletic group in the ITS based phylogeny, but lacks a monophyletic origin in the *matK* analysis (Figs. 1, 2). It comprises mainly species of section *Hostia* joined by taxa from other sections. Within Clade III *C. zacintha* and *C. pusilla* (section *Zacintha*) form a very close relationship (ITS: PP1.00/BS100; *matK*: PP1.00).

Clade VI (ITS: PP0.83/BS73; *matK*: PP1.00/BS92) includes *C. neglecta*, *C. corymbosa*, *C. fuliginosa,* and *C. cretica,* only members of section *Phytodesia*, but other species of Babcock's section *Phytodesia* are also found elsewhere, e.g. *C. capillaris* within Clade XI and *C. nicaeënsis* in Clade II.

Clade VIII comprises *C. leontodontoides* (section *Gephyroides*) and *C. aurea* (section *Brachypodes*). Their association is statistically well supported (ITS: PP1.00/BS84; *matK*: PP1.00/BS99) by both markers.

Character Evolution – To investigate Babcock's overall hypotheses on progressive character evolution in *Crepis* L and the suitability of these characters for infrageneric delimitation three morphological characters (basic chromosome number/life history/root type) upon which Babcock's phylogenetic hypotheses are based were mapped on the molecular phylogeny of the ITS sequence data. According to Varela et al. (2004) it can be assumed that the nuclear marker reflects the evolution of morphological traits. Fig. 3 shows the distribution of all three applied characters to be variable in and between clades of the tree. Babcock (1947a) implied a direction of evolution in his hypotheses: chromosome number should always decrease and annuals should derive from perennials. Concerning the root type he withdrew his initial hypothesis that taproots are derived but still implied a directional character development (Babcock, 1949). Our molecular data do not support a unidirectional character development.

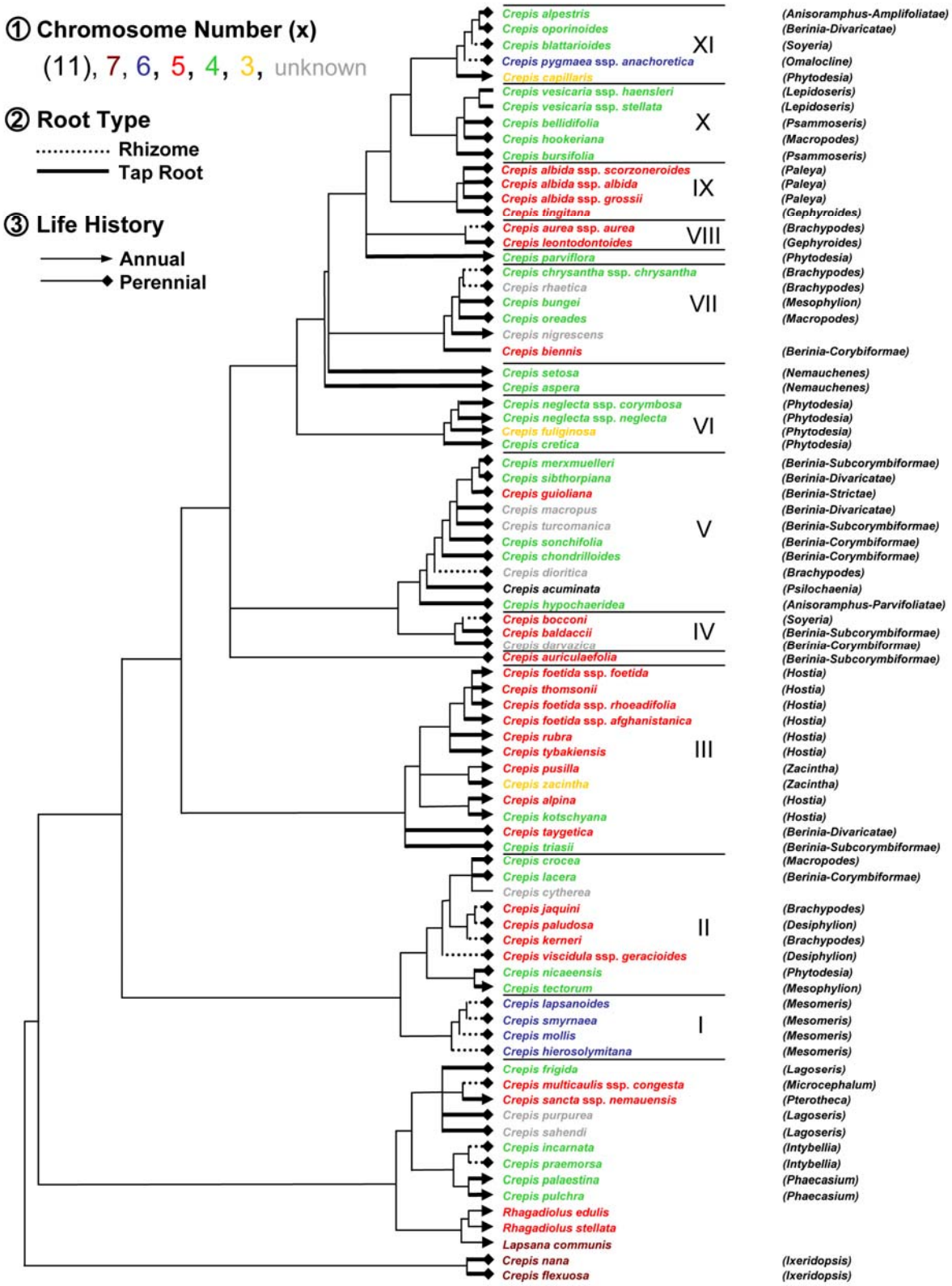

FIG.3: Characters (1, 2, 3) mapped on Bayesian strict consensus tree (ITS). Babcock's sections are given in parentheses behind each taxon. Numbers designate clades within *Crepis* s.str.

2.4 DISCUSSION

Phylogeny – The *Askellia* clade comprises only species belonging to Babcock's section *Ixeridopsis*. The section comprises seven species of which only *C. nana* and *C. flexuosa* have been investigated on a molecular base. However, all members of section *Ixeridopsis* are morphologically and cytologically very similar to each other and differ distinctly from *Crepis* s.str. in the tuft-like growth, the absence of hairs, the nearly always entire leaves, the smaller number of florets, and the chromosome number (x = 7) that is unique in *Crepis* s. l. In closely related genera x = 7 is only known for *Ixeridium* (Pak & Kawano, 1992) which is molecularly isolated from *Askellia* in the ITS phylogeny and differs in featuring compressed achenes; *Ixeris* and *Youngia* have a chromosome number of x = 8 (Pak & Kawano, 1992, Babcock & Stebbins, 1937). *Mat*K sequence data also suggests a close relationship of *Askellia* to *Ixeris*, *Youngia,* and *Garhadiolus*. The members of section *Ixeridopsis* resemble *Ixeris* in habit but differ in achene morphology (Babcock, 1947b). Achenes are terete in *Askellia* and fusiform and flattened in *Ixeris* and *Ixeridium*. *Youngia* differs in chromosome number (x = 8) and by forming compressed achenes (Babcock & Stebbins, 1937). *Garhadiolus* is characterised amongst other features by minutely hispid or scabrid achenes (Jaubert & Spach, 1847-50), which are not present in *Askellia*.

According to the molecular data *Askellia* indeed is transitional between *Ixeris*/*Ixeridium* and *Crepis* s.str. as Babcock suggested (1947a,b), but not part of *Crepis* s.str.. As *Lapsana* and *Rhagadiolus* differ in their generic circumscription substantially from all of these genera (see discussion below), it is not reasonable to include them into *Crepis* s.l.. So following the molecular, karyological, and morphological evidence, it is necessary to reassign the seven species of *Crepis* section *Ixeridopsis* to *Askellia*, a genus which was defined by Weber (1984) based on the unique number of chromosomes, including all species of section *Ixeridopsis*.

The second main clade comprises *Lapsana* and *Rhagadiolus* as well as *Crepis* species from sections *Intybellia*, *Lagoseris*, *Phaecasium*, *Microcephalum,* and *Pterotheca*. In both analyses all sampled species of these five sections appear in this clade, indicating a common monophyletic origin.

Lapsana is traditionally considered to be closely related to *Crepis* (*Youngia*-*Ixeris*-Line in Stebbins (1953), *Crepis* series by Jeffrey (1966) and Crepidinae in Bremer (1994)). Presently, European *L. communis* is considered to be the only species of *Lapsana*, while the East Asian species have been separated as *Lapsanastrum* by Pak & Bremer (1995). *Lapsana* is far more closely related to *Crepis* than to *Lapsanastrum* (Pak & Bremer, 1995).

Rhagadiolus has been treated as member of the Leontodontinae (Stebbins, 1953) or Hypochaerinidae (Bremer, 1994), different terms for the same generic combination (comprising e.g. *Hypochaeris*, *Picris*, *Leontodon*). Due to molecular evidence *Rhagadiolus*

was included in the Crepidinae close to *Lapsana* as sister clade to *Crepis* (Whitton et al., 1995; Gemeinholzer et al., 2006). The basic chromosome number of *Lapsana communis* is x = 7 (Pak & Bremer, 1995), that of *Rhagadiolus* is x = 5 (Meikle, 1979).

The group of *Crepis* species in the *Lagoseris* clade fall into two subgroups (a and b; Figs.1, 2).

Subclade a comprises sections *Lagoseris*, *Microcephalum*, and *Pterotheca*. These species have a chromosome number of x = 5. The karyotype of *C. sancta* shows strong resemblances to the one of *C. multicaulis* even though Babcock (1947b) placed them into two different sections: *Pterotheca* and *Microcephalum*. In the Flora of the USSR (Bobrov & Tzelelev, 2000) *Lagoseris* and *Pterotheca* are treated as subgenera of genus *Lagoseris* M.Bieb.*,* amongst other features for their peculiar bristles on the receptacle which can exceed the achenes.

Subclade b comprises the species of the rhizomatous section *Intybellia* and the tap rooted section *Phaecasium*. All of these species possess a chromosome number of x = 4 (Babcock, 1947b) and their karyotypes are highly similar. The karyotype of these species is distinct from those of other species of *Crepis* s.l., e.g. in the A chromosome which has a proximal arm which almost equals the distal one, a feature that does not occur in any other *Crepis* species. Babcock considered combining these two sections were it not for their different root habit (Babcock & Jenkins, 1943).

The putative close relation of *Lapsana* and *Rhagadiolus* to the five sections of *Crepis* is unexpected as they are both distinct in fruit morphology. The most obvious difference between the genera is the absence of a pappus in *Lapsana* and *Rhagadiolus*. In *Crepis* s.l. the pappus might be much reduced (e.g. *C. patula*) but is always present. Traditionally this has been an important trait in genus delimitation (Cassini, 1830; Hoffmann, 1889). Nevertheless, recent studies show that this character can vary within genera or even within species (e.g. *Lasthenia*; Chan et al., 2002). Furthermore, the achene of *Lapsana* is strongly compressed and the fruits of *Rhagadiolus* are only negligibly ribbed and densely hispidulous (Meikle, 1979), all features not known from *Crepis* s.l.. The chromosome number of *Lapsana* (x = 7) is unique within this clade, whereas *Rhagadiolus* features the same number as some of the *Crepis* taxa (x = 5).

Morphologically the whole *Lapsana*/*Rhagadiolus*/*Lagoseris* clade is far more variable and less easy to define than the *Askellia* clade and a formal taxonomic revision will require additional studies. At the present stage it seems appropriate to conserve the genera *Lapsana* and *Rhagadiolus* due to their morphological distinctness and combine the *Crepis* sections *Intybellia*, *Lagoseris*, *Phaecasium*, *Microcephalum,* and *Pterotheca* as new genus *Lagoseris* M.Bieb..

The third main clade, *Crepis* s.str., comprises all other *Crepis* species sampled. Out of eleven monophyletic clades of the ITS analysis, only one coincides with the sections defined by Babcock (1947a,b). This is Clade I, recognized both in the ITS and the *mat*K trees, that corresponds to Babcock's section *Mesomeris*. The species of section *Mesomeris* are rhizomatous, have a basic chromosome number of x = 6, and are distributed in the east Mediterranean, except *C. mollis*, that occurs throughout middle Europe, *C. willementioides* in the Far East, and *C. lyrata* in Southern Siberia.

Clade III is monophyletic in the ITS phylogeny but forms two clades in the chloroplast based tree. It comprises mainly species from section *Hostia* joined by taxa from sections *Zacintha* (*C. zacintha*, *C. pusilla*) and *Berinia* (*C. taygetica*, *C. triasii*). The species of section *Hostia* are annual and monocarp as are *C. zacintha* and *C. pusilla*. The typically carinate involucral bracts of section *Hostia* can also be found in the other species. The close relationship between *C. zacintha* and *C. pusilla*, as suggested by Merxmueller (1968) due to morphological similarity, is corroborated by the molecular results. These two species not only form a molecular and morphological subgroup within Clade III, but are sister to Clade VI in the chloroplast phylogeny, exhibiting a genetic as well as morphological isolation from their presumed closest relatives (Figs.1, 2).

Even though other members of section *Phytodesia* are found elsewhere in the tree (Clades II (*C. nicaeënsis*), XI (*C. capillaris*)), Clade VI partly reflects Babcock's assumptions about the relationships within section *Phytodesia*. He placed *C. neglecta*, *C. corymbosa*, *C. fuliginosa,* and *C. cretica* in one of four subgroups of section *Phytodesia* due to their peculiar narrow chromosomes which are distinct from those of the other members of this section (Babcock, 1947b).

The close molecular relationship between *C. leontodontoides* and *C. aurea* of Clade VIII mirrors their similarity in karyotype and morphology. Babcock already recognised these similarities but assigned these two species to different sections (*Gephyroides* and *Brachypodes*) due to their different root types.

Character Evolution – Fig. 3 depicts the three main morphological characters on which Babcock's classification (Babcock, 1947a) is based in relation to the molecular phylogenetic reconstruction (ITS). Babcock's hypotheses allow for multiple evolutionary character state emergences which, once developed, only change in a directional manner such as chromosome numbers only increase, tap rooted plants derive from rhizomatous ones, or changes occur only from annual to perennial life history. Babcock (1947a) rejected hybridisation as main cause of speciation in the genus whereas in his view karyotypic changes were the driving force of evolution. His understanding of phylogenetic coherence

was strongly influenced by his time and predated the publication of Hennig's pioneering Phylogenetic Systematics (Hennig, 1966).

The basic chromosome number, upon which all of Babcock's hypotheses about character evolution are based, is highly variable in and between the molecular clades and thus contradicts Babcock's hypotheses on progressive character evolution and speciation in the genus (Fig.3). An increase of chromosome number occurred several times during evolution (e.g. from x = 4 (*C. nicaeënsis, C. tectorum*) to x = 6 (e.g. *C. jaquinii, C. paludosa*) in Clade II and from x = 4 (e.g. *C. sonchifolia*) to x = 5 (*C. guioliana*) in Clade V; Fig.3). Both reduction and rise in chromosome number throughout evolution are known from other genera of the Compositae, e.g. *Hypochaeris* (Cerbah et al., 1998). The chromosome number can also vary between closely related species like *C. pusilla* (x = 5) and *C. zacintha* (x = 3) (Merxmueller, 1968). The only clade being characterised by its chromosome number is *Askellia* (Weber, 1984). The fact that chromosome number is highly variable within *Crepis* s.str., even among closely related species, suggests a broad capacity of this genus to undergo karyotype changes.

Chromosome number alone does not seem to indicate phylogenetic relationships. The suitability of chromosome number as delimiting character for infrageneric groups has been rejected for other Compositae as well, e.g. *Artemisia* (Torrell et al., 1999) and even for closely related genera, e.g. the genus *Hypochaeris* (Parker, 1975). In general, variation in chromosome number is known from several groups throughout herbaceous Angiosperms (Levin, 2002; e.g. *Brachyscome* Cass. (Compositae, Watanabe et al., 1999) *Lopezia* Cav. (Onagraceae, Plitmann et al. 1975; O'Kane et Schaal, 1998), *Clarkia* Pursh (Onagraceae, Lewis, 1952; Gottlieb & Ford, 1996,), as well as in the centrospermous (Ehrendorfer, 1976) and basal angiosperms (Grant, 1982)). Karyotypes are characterised not only by the number of chromosomes but genome size and the distribution of gene loci on the chromosomes. Chromosomal rearrangements generally exhibit a low level of homoplasy and it is thought unlikely that the same chromosomal patterns appear in different phylogenetic lineages (Lysak et al. 2006). Furthermore, it is known that different karyotypic traits are not mandatorily linked to each other; e.g. DNA content, which depends on the genome size, and chromosome number (Levin, 2002). According to Jones & Brown (1976) *Crepis* species with divergent chromosome numbers can have a similar DNA content (e.g. *C. neglecta* and *C. rubra*), while other species exhibit the same chromosome number but differ in DNA content (e.g. *C. capillaris* and *C. zacintha*). *Crepis* species with identical chromosome numbers might exhibit a different distributional pattern of gene loci. Therefore, an ostensible homoplasy of chromosome number should be doubted. Given the complexity of karyotypic traits more detailed analyses of chromosome morphology and structure are necessary to explain the evolutionary pattern of the development of karyotypes within *Crepis*.

The varying chromosome numbers within the *Crepis* s.str. clades and the different phylogenetic relationships of taxa in the nuclear and plastid phylogeny suggest hybridisation between species, maybe even species with different chromosome numbers. Presumably similar chromosome morphology and structure could allow for hybridisation across taxa and might therefore be more indicative of phylogenetic relations than chromosome number alone. For a detailed analysis of reticulate evolution in the genus, however, more data on chloroplast relationships is needed.

Rhizomes are most common within Clades I and II, but can also be found in other clades (IV, V, VII, VIII, and XI). Rhizomes most likely evolved as environmental adaptation to humid habitats and have arisen several times within *Crepis* s.str. (Fig.3). Annual life history has been developed mainly in two clades (Clade III and Clade VI) but can also sporadically be found elsewhere (Clades II, VII, XI) (Fig.3). According to the molecular reconstruction multiple origins for life history and root type as well as changes from rhizome to tap root (e.g. rhizomatous *C. jaquinii* to tap rooted *C. lacera*, Clade II) and from annual to perennial life history (e.g. annual *C. capillaris* to perennial *C. pygmaea*, Clade XI) can be demonstrated (Fig.3).

Studies showed that edaphic heterogeneity has been crucial for the evolution of growth form and life history in grass species (Verboom et al., 2004). The close association of root type and life history to environmental influences could imply that these characters only exhibit a weak phylogenetic signal and might therefore be inapt for the analysis of phylogenetic relationships. Summarising the above, based on our analysis the examined character variability within the clades does not allow for hypotheses about progressive character changes.

2.5 SUMMARY AND CONCLUSIONS

The ITS phylogeny revealed *Crepis* L. sensu Babcock to be polyphyletic, which is also confirmed by the chloroplast marker *mat*K. Three main groups comprising species of *Crepis* s.l. can be deduced from the molecular data. The first one includes the species of *Crepis* section *Ixeridopsis* and corresponds to the genus *Askellia* (Weber, 1984); the second one comprises the genera *Lapsana* and *Rhagadiolus* as well as *Crepis* species from sections *Intybellia*, *Lagoseris*, *Phaecasium*, *Microcephalum,* and *Pterotheca*. In case further morphological studies confirm the outcomes of the presented molecular data it seems most reasonable to transfer the *Crepis* species of this clade to the genus *Lagoseris*, rather than to fuse them with *Lapsana* and *Rhagadiolus*, which feature differences in achene and karyotype morphology. The third and largest group comprises the majority of sampled *Crepis* species

as *Crepis* s.str.. Within this group the composition of clades deviates from Babcock's sectional concept.

The results of the molecular analyses confirm only parts of Babcock's sectional arrangement but contradict his hypotheses about character evolution in the genus. His assumption of absent infrageneric hybridisation cannot be maintained as the differences between the nuclear and chloroplast marker hint on crossings between taxa of different sections, even between taxa featuring distinct chromosome numbers.

According to the here presented data karyotype evolution in the genus is far more complex than Babcock assumed. Yet, chromosomal similarities in karyomorphotypes, which could explain hybridisation between taxa of different base chromosome numbers, might provide promising evidence for subsequent analyses. To shed more light on both infrageneric delimitation and karyotype evolution further investigations into karyotype morphology and hybridisation are essential, potentially leading to a refined concept of infrageneric delimitation within *Crepis*.

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2.8 APPENDIX

Taxa sampled for DNA.

GENUS, ***Section***, *Taxon*, Voucher, Origin, GeneBank Accesion No. (ITS/*mat*K)

CREPIS, ***Desiphylion,*** *C. paludosa* Moench, P. Brückner 478/76 (B), Germany: Brandenburg, ITS EU366428; *C. viscidula* Froel. ssp. *geracioides* (Hausskn.) Kamari; K.H. Rechinger 20933 (B), Greece: Mt.Smolika, ITS EU363629; ***Omalocline,*** *C. pygmaea* L. ssp. *anachoretica* Babc, B. Valdes et al. It1968/88 (B), Spain: Granada, ITS EU363638/*mat*K EU363569; ***Brachypodes,*** *C. aurea* (L.) Cass. ssp. *aurea,* K.Faber AU87 (B), Austria: Tauern, ITS EU363627/*mat*K EU363564; *C. chrysantha* (Ledeb.) Turcz. ssp. *chrysantha,* E. Raab-Straube 020201 (B), Russia: Altay Rep., ITS EU363622/*mat*K EU363560; *C. dioritica* Schott et Kotschy ex Boiss., H.Kehl 8/II-C (B), Turkey: Antalya, ITS EU363620; *C. jaquini* Tausch spp. *kerneri* Merxm., R. Vogt et al. RV 15954 (B), Italy: Friaul, *mat*K EU363568; *C. jaquini* Tausch, ITS AJ633378; *C. kerneri* Rech. f., T. Wraber 9748/4 (B), Slowenia, ITS EU363636; *C. kerneri* Rech. f., *mat*k AJ633152; *C. rhaetica* Hegetschw., ITS AJ633379;*C. rhaetica* Hegetschw., A. Kuhns & Ch. Zidorn 98-00204 (priv.), Austria, *mat*K EU363537; ***Mesomeris,*** *C. hierosolymitana* Boiss., A. Liston 7-82-30/2 (M), Palaestina: Nahal Bezet, *mat*K EU363543; *C. hierosolymitana* Boiss., H. Roessler 5317 (M), Libanon: Jabal Barouk, ITS EU363602; *C. lapsanoides* (Gouan) Tausch, H. Kalheber 88-2748 (M), France: Dpt.Hts.Pyr., ITS EU363599; *C. mollis* (Jacq.) Asch., ITS AJ633380; *C. mollis* (Jacq.) Asch., A. Zidorn, Ch. Zidorn 99-00383 (priv.), Austria: Aschland, *mat*K EU363538; *C. smyrnaea* DC., K. P. Buttler 23431 (M), Turkey: Bursa, ITS EU363598/*mat*k EU363546; ***Soyeria,*** *C. blattarioides* (L.) Vill., T. Eckhardt 1555 (B), Switzerland: Valais, ITS EU363624/ *mat*k EU363561; *C. bocconi* P.D. Sell, ITS AJ633375/ *mat*k AJ633146; ***Paleya,*** *C. albida* Vill. ssp. *albida,* O. Angerer M-0088570 (M), France: Dep. Lozère, ITS EU363606/*mat*k EU363550; *C. albida* Vill. ssp. *grossi* (Pau) Babc., E. Bayer & J. Grau B.G.79 (M), Spain: Andalucia, ITS EU363594; *C. albida* Vill. ssp. *scorzoneroides* (Rouy) Babc., A. Segua 16.113 (M), Spain: Soria, ITS EU363595; ***Anisoramphus,*** *C. alpestris* (Jacq.) Tausch, ITS AJ633373/ *mat*k AJ633153; *C. hypochaeridea* (DC.) Thell., N.J. Devenish 915 (B), S. Africa: Transvaal, ITS EU363617; ***Gephryoides,*** *C. leontodontoides* All., Vogt 10/1992 (B), France: Corsica, ITS EU363592/ *mat*k EU363542; *C. tingitana* Ball., Ch. Zidorn 970526a (priv.), Spain: Andalusia, ITS EU363586; *C. tingitana* Ball., *mat*k AJ633149; ***Berinia,*** *C. auriculaefolia* Sieber ex Spreng., R. Jahn s.n. (B), Greece: Crete, ITS EU363626/ *mat*k EU363563; *C. baldaccii* Halácsy, Franzén et al. 669 (B), Greece: Ioannina, ITS EU363625; *C. baldaccii* Halácsy, K.H. Rechinger 21313 (B), Greece: Mt. Timphi, *mat*k EU363562; *C. biennis* L. ITS AJ633355; *C. chondrilloides* Jacq., J. Schimmitat M-0088551 (M), Jugoslavia: Biokovo, ITS EU363593; *C. chondrilloides* Jacq., O. Angerer M-0088547 (M), Italy: Triest, *mat*k EU363545; *C. darvazica* Krasch.; V. Goloskokov s.n. (B), Kazakhstan: Alatau, ITS EU363600/ *mat*k EU363558; *C. guioliana* Babc., W. Greuter 14533

(B), Greece: Epirus, ITS EU363618; *C. incana* Sibth. et Sm., W. Greuter et al. 14821 (B), Greece: Parnassos, *mat*k EU363554; *C. lacera* Ten., C. Ricceri 8741 (B), Italy: Umbria, ITS EU363634; *C. macropus* Boiss. et Heldr., J. & F. Bornmüller 14397 (B), Turkey: Bursa, ITS EU363589; *C. macropus* Boiss. et Heldr., Davis & Coode D37063 (E), Turkey: Bolu, *mat*k EU363577; *C. merxmuerlleri* Kamari et Hartvig, Hartvig & Seberg 5059 (B), Macedonia: Mt Smolikas, ITS EU363644; *C. oporinoides* Boiss. ex Froel., B. Valdés et al. It916/88 (B), Spain: Sierra Nevada, ITS EU363633/*mat*k EU363567; *C. pannonica* (Jacq.) K. Koch, E. Vitek 99-393 (B), Austria: Vienna, *mat*k EU363571; *C. sibthorpiana* Boiss. et Heldr., P.H. Davis 18140 (E), Crete: Mt Svowitchii, ITS EU363648/*mat*k EU363574; *C. sonchifolia* C.A. Mey., Nazarova (B), Armenia, ITS EU363637; *C. taygetica* Babc., W. Lippert 21366 (M), Greece: Peloponnes, ITS EU363603/*mat*k EU363548; *C. triasii* (Camb.) Fries, H. Merxmüller 148/57 (M), Spain: Mallorca, ITS EU363597; *C. turcica* Degen et Baldacci, *mat*k AJ633360; *C. turcomanica* H. Krasch., J.R. Edmondson 1177 (E), Iran: Khorasan, ITS EU363652; ***Macropodes,*** *C. crocea* (Lam.) Babc., Sukaczev et Poplavskaja (B), Russia: Czita, ITS EU363590; *C. hookeriana* J. Ball., D. Podlech 47476 (MSB), Marocco: Atlas, ITS EU363605/*mat*k EU363549; *C. oreades* Schrenk,Schevireva et Kojuvapova 619/1000 (B), Tajikistan: Pamir Alay, ITS EU363640/*mat*k EU363572; ***Ixeridopsis,*** *C. flexuosa* (DC.) Benth. Et Hook. F., Timokhina et Djukov M-0088593 (M), Russia: Rep. Tuva, ITS EU363596/*mat*k EU363544; *C. nana* Richards, Jonsell et Urbanska 6456 (UPS), USA: Alaska, ITS EU363591; *C. nana* Richards, O. Martensson U27 (UPS), USA: Alaska, *mat*k EU363541; ***Intybellia,*** *C. incarnata* (Wulf.) Tausch, H.&H. Doppelbaur 14684 (M), Italy: Südtirol, ITS EU363608; *C. incarnata* (Wulf.) Tausch, *mat*k AJ633151; *C. praemorsa* (L.) Tausch , G. van Buggenhout 13637 (B), Italy: Bolzano, ITS EU363654/*mat*k EU363578; ***Mesophylion,*** *C. bungei* Ledeb., ITS AJ633374/*mat*k AJ633147; *C. nigrescens* Pohle, O. Rebristaja US-329530(US), Russia: Tiumen, ITS EU363609; *C. tectorum* L., E. Willing 4.502 D (B), Germany: Berlin, ITS EU363643/*mat*k EU363536; ***Psilochenia,*** *C. acuminata* Nutt., L.S. Rose 55156 (B), USA: California, ITS EU363616; ***Lagoseris,*** *C. frigida* (Boiss.) Babc., P. Hein 74 (B), Turkey: Bolkar Daglari, IST EU363612/*mat*k EU363555; *C. purpurea* (Willd.) M. Bieb, Ivanov (B), Russia: Tauria, ITS EU363653; *C. sahendi* Boiss. et Buhse, Davis & Polunin D24074 (E), Turkey: Hakkari, ITS EU363651; ***Phaecasium,*** *C. palaestina* (Boiss.) Bornm., A. Danin et al. 53.019 (B), Israel: Galilee, ITS EU363639; *C. pterothecoides* Boiss., A. Danin (B), Israel: Negev, *mat*k EU363570; *C. pulchra* L., ITS AJ633369/*mat*k AJ633145; ***Hostia,*** *C. alpina* L., J. Trelawny 1423 (E), Turkey: Hakkari, ITS EU363649/*mat*k EU363575; *C. foetida* L. ssp. *foetida,* J. Lambinon 00/F/214bis (B), France: Ardéche, ITS EU363619/*mat*k EU363556; *C. foetida* L. ssp. *afghanistanica* Babc., D. Podlech 18361 (MSB), Afghanistan: Baghlan, ITS EU363604; *C. foetida* L. ssp. *rhoeadifolia* (Bieb.) Celak, J. Lambinon 00/214 (B), France: Ardéche, ITS EU363613; *C. kotschyana* Boiss., K.H. Rechinger 51631 (B), Persia: Khorasan, ITS EU363635; *C. rubra* L., ITS AJ633350/*mat*k AJ633141; *C. thomsonii* Babc., M. Jabcobs 6374 (E), Iran: Lorestan-Sheshom, ITS EU363647; *C. tybakiensis* Vierh., N. Böhling 5452b (B), Greece: Lasithiou, *mat*k EU363566; *C. tybakiensis* Vierh., Greuter et Matthäs 19649 (B), Greece: Crete, ITS EU363631; ***Microcephala,*** *C. multicaulis* Ledeb. ssp. *congesta* (Regel) Babc., Nüsser 422 (B), Pakistan: Nanga Parbat, ITS EU363642/*mat*k EU363573; ***Pterotheca,*** *C. sancta* (L.) Babc. ssp. *nemauensis* (Gouan) Babc., J. Lambinon 84/F/373 (B), France: dep. Gard, ITS EU363632; *C. sancta* (L.) Babc., *mat*k AJ633150; ***Zacintha,*** *C. pusilla* (Sommier) Merxm., Th. Raus 7715 (E), Greece: Kasos, ITS EU363650/*mat*k EU363576; *C. zacintha* (L.) Babc., Ralf Hand 5323 (priv.), Greece: Rhodes, ITS EU363655/*mat*k EU363579; ***Phytodesia,*** *C. capillaris* (L.) Wallr., ITS AJ633381/*mat*k AJ633142; *C. corymbosa* Ten., P. Hiepko

065 (B), Italy: Apulia, *mat*k EU363559; *C. cretica* Boiss., R. Jahn 4.4.1996/02 (B), Greece: Selinou, ITS EU363614; *C. fuliginosa* Sibth.& Sm., D. Philos 4032 (B), Greece: Euboea, ITS EU363601/*mat*k EU363547; *C. nicaeënsis* Balb., v.Ooststroom& Hennipan 23519 (B), Jugoslavia: Macedonia, ITS EU363641; *C. neglecta* L. ssp. *neglecta,* Eisenblätter & Willing 82.085 (B), Greece: Kardhitsa, ITS EU363610/*mat*k EU363553; *C. neglecta* L. ssp. *corymbosa* (Ten.) Nyman, Eisenblätter & Willing 81.622 (B), Greece: Trikala, ITS EU363611; *C. parviflora* Desf., R.D. Meikle M-0088516 (M), Cyprus: Yerosa, ITS EU363607/*mat*k EU363551; ***Lepidoseris,*** *C. vesicaria* L. ssp. *vesicaria,* R.&E. Willing 88.781 (B), Greece: Ilias, *mat*k EU363565; *C. vesicaria* L. ssp. *stellata* (Ball) Babc., R. Vogt 11757 (B), Marocco: Anti-Atlas, ITS EU363630; *C. vesicaria* L. ssp. *haensleri* (Boiss.ex DC.) P.D. Sell, ITS AJ633371; ***Nemauchenes,*** *C. aspera* L., R. Hand 3708 (B), Cyprus: Polystipos, ITS EU363628; *C. setosa* Hall.f., N. Enke 0123a (B), Italy: Pisa, ITS EU363585; ***Psammoseris,*** *C. bellidifolia* Loisel., J. Lambinon 84/Co/433 (B), France: Corsica, ITS EU363615; *C. bursifolia* L., Molina & Gavilán 15645 (B), Spain: Madrid, ITS EU363623; ***Species without sectional assignment,*** *C. chupantha,* T. Elias, Shetler & Murray US 498520 (US), Russia: Republ. Tuva, *mat*k EU363552; *C. cytherea* Kamari, R. Jahn (B), Greece: Kythira, ITS EU363646; **OTHER GENERA:** *Cephalorynchus tuberosus* (Steven) Schchian, Cho 10189 (B), *mat*k EU363582*; *Chondrilla canescens* Kar. & Kir., *mat*k AJ633349; *Garhadiolus hedypnois* Jaub. et Spach, ITS AJ633307; *Garhadiolus hedypnois* Jaub. et Spach, Fayed & El Garf DB0305 (B), Egypt: Alexandria, *mat*k EU363540; *Heteracia szovitzii* Fischer & C.A. Meyer, ITS AJ633283; *Hololeion maximoviczii* Kitam., ITS AJ633425; *Hypochoeris maculata* L., ITS AJ633311; *Hyoseris radicata* L., ITS AJ633299; *Ixeridium laevigatum* (Blume) Pak & S. Kawano, Bartholomew & Boufford 6192 (US), Taiwan: Chiayi Hysien, ITS EU363588; *Ixeris chinensis* (Thunb.) Nakai, Bartholomew, Boufford, Li 749 (US), ITS EU363587/*mat*k EU363539; *Ixeris stolonifera* A. Gray, ITS AJ633284/*mat*k AJ633156; *Lactuca dissecta* D. Don., Kilian 2547 (B), Tadshikistan: Warob, *mat*k EU363580*; *Lapsana communis* L., ITS AJ633285/*mat*k AJ633138; *Phitosia crocifolia* (Boiss. et Heldr.) Kamari & Greuter, Strid et al. 15261(B), Greece: Messinias, ITS EU363621/*mat*k EU363557; *Picris echioides* L. ITS AJ633321, *Prenanthes purpurea* L., ITS AJ633342; *Rhagadiolus edulis* Gaertner, ITS AJ633297; *Rhagadiolus stellatus* (L.) Gaertner, ITS AJ633296/*mat*k AJ633224; *Scorzonera troodea* Boiss, R. Hand 3271 (priv.), Greece: Cyprus, *mat*k EU363584*; *Sonchus oleraceus* L., ITS AY862581/*mat*k DQ840449; *Soroseris glomerata* (Decne) Stebbins, T.N. Ho 1692 (CAS), China: Quinghai, ITS EU363656*; *Steptoramphus czerepanovii* Kirp., Cho 10155 (B), *mat*k EU363581*; *Taraxacum bessarabicum* Fisch., ITS ZJ633287; *Taraxacum erythrospermum* Andrz. ex Bess., ITS AJ633291; *Taraxacum laevigatum* DC, ITS AJ633288; *Youngia denticulata,*ITS AJ633293; *Youngia japonica* (L.) DC, ITS AJ633294; *Youngia tenuifolia* (Willd.) Babc. & Stebbins, Beljaeva et al. (B), Russia: Primorje, ITS EU363645/*mat*k EU363583.

All sequences bearing numbers starting with EU produced in the present study. * Samples provided by N. Kilian.

Chapter 3

Shrinking Genomes? Evidence from Genome Size Variation in *Crepis* L. (Compositae)

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ABSTRACT

Large scale surveys of genome size evolution in angiosperms showed that the ancestral genome most likely was small with a tendency towards an increase in DNA content during evolution. Due to polyploidisation and self replicating DNA elements, angiosperm genomes were considered to have a “one way ticket to obesity” (Bennetzen & Kellogg, 1997). New findings on how organisms can lose DNA challenged the hypotheses of unidirectional evolution of genome size. The present study shows that within 30 diploid species of the genus *Crepis* there is a striking trend towards genome contraction. Genome size of 21 species has been estimated by flow cytometry. Findings were combined with additional data from literature. The directionality of genome size evolution was analysed by reconstructing ancestral character states on a molecular phylogeny based on ITS sequence data. DNA content is shown to be correlated to distributional aspects as well as life form. Genome size is significantly higher in perennials than in annuals. Within sampled species very small genomes are only present in Mediterranean or European species, whereas their Central and East Asian relatives show small or intermediate 1C-values. The only cases of well supported 1C-value increase within the genus are due to polyploidisation.

KEYWORDS: ancestral character state reconstruction, *Crepis*, Compositae, flow cytometry, genome evolution, ITS.

3.1 INTRODUCTION

Two of the most obvious and probably the most discussed attributes of karyotypes are chromosome number and size. Total chromosome size is directly linked to DNA content (given as C-value) while chromosome number is not (Levin, 2002). Both characters vary enormously throughout angiosperms. Chromosome number ranges from n=2 to n= ca.300 (Grant, 1982; Masterson, 1994). In diploid *Actinidia* species the basic chromosome number is as high as x=29 (Yan et al., 1997). Genome size varies nearly 2000fold within the angiosperms (Greilhuber et al., 2006); the smallest genome is not found in *Arabidopsis* as previously assumed but in the carnivorous genus *Genlisea* (Greilhuber et al., 2006). As within the genome the genetic constituency of each organism is encrypted and because of the heterogeneity of these traits there is a lot of interest in understanding chromosome evolution, its direction as well as its underlying mechanisms (e.g. Leitch et al., 1998; Soltis et al., 2003; Bennett & Leitch, 2005).

For the sake of unambiguousness and comparability in the discussions of genome size variation it is inevitable to distinguish between genome size and 1C-value. According to Bennett et al. (1998) genome size is the 2C value divided by ploidy level, so the genome size corresponds to the 1C-value in diploid but can be lower than the 1C-value in polyploids. Or, as Greilhuber et al. (2005) put it, 1C-value and 1Cx-value should be discriminated: 1Cx-value is the “monoploid” genome size (Greilhuber et al., 2005) whereas 1C is the unreplicated reduced chromosome complement (Bennett & Smith, 1976). In this study the 1C-value is used.

The “C-value paradox” (Thomas, 1971) refers to the phenomenon that the amount of DNA is not reflected by the complexity of an organism. More complex organisms do not necessarily feature more DNA, in fact the basic set of genetic information required for normal development is similar in most plants (Flavell, 1980). Thus, most variation in genome size is due to non-coding or repetitive DNA elements (Flavell, 1986; Kubis et al., 1998; Schmidt & Haslop-Harrison, 1998). The “C-value enigma” (Gregory, 2001) addresses the question of why there is such a considerable variation in non-coding DNA, how it is distributed among taxa and how it developed. Within a species genome size is almost always constant, whereas it can vary strongly between species (Greilhuber, 1998, 2005). As a consequence, the use of genome size to infer phylogenetic relations is questionable, but it still can be useful for species delimitation (Murray, 2005).

The remarkable variation in genome size throughout the angiosperms as well as within more confined taxa has many causes. In the context of size variation the correlation between genome size and life history often has been discussed (e.g. Bennett, 1972; Sims & Price, 1985; Bennett & Leitch, 1995; Watanabe et al., 1999). Following the nucleotype theory (Bennett, 1972) annual plants mostly have small genomes while perennials can have larger

genomes. In some cases it appears that genome size is also correlated to various environmental and physiological factors (Knight & Ackerley, 2002; Jakob et al., 2004; Ohri, 2005).

In the basic angiosperms the overall direction of genome size evolution is unambiguous: it increases (Soltis et al., 2003). It therefore complies with the hypothesis of Bennetzen & Kellog (1999) who postulated “a one way ticket to genomic obesity”: Due to the accumulation of retroelements genome size generally increases. Repeated circles of polyploidy also lead to an increase in genome size (Wendel, 2000). But within plant families or genera decreases as well as increases have been observed (e.g. Watanabe et al., 1999; Wendel et al., 2002; Jakob et al., 2004). Various explanations have been proposed about how DNA is lost (e.g. Vicient et. al., 1999; Kirik et al., 2000; Hancock, 2002; Petrov, 2002; Zuckerkandl, 2002). Differences in DNA content could be caused by the loss of whole chromosomes or parts of it (Dart et al., 2004). Vicient et al. (1999) showed that in barley the removal of *BARE-1* elements through intrachromosomal homologous recombination between LTR’s (long terminal repeats) can be responsible for a reduction of DNA content. Similar mechanisms have been found in rice (Ma et al., 2004).

So far studies on genome size variation were mostly either concerned about infraspecific variation (e.g. Greilhuber & Ebert, 1994; Turpeinen et al., 1999; Temsch & Greilhuber, 2001) or variation within families or even higher taxa (e.g. Kellogg, 1998; Wendel et al., 2002; Soltis et al., 2003; Greilhuber et al., 2006). Only few studies investigate genome size variation between species belonging to clearly confined groups (genera or subgeneric ranks) to discuss its role in the evolution of the genera, which is presented here for the Compositae genus *Crepis*.

The genus *Crepis* comprises over 200 species in the holarctic region and Africa. Over 80% of the species are diploid. Polyploidy is virtually constrained to one section being distributed in North America. The basic chromosome number varies from x = 3--6 respectively x = 11 in the 15 polyploid North American species. These rare features make it ideal to explore changes of genome size and basic chromosome number that are not connected to changes of ploidy level.

Even before Babcock published his extensive work on karyotype evolution in the genus (Babcock, 1947a,b), *Crepis* has been a popular object of cytological studies (e.g. Hollingshead, 1930; Tobgy, 1943; Sherman, 1946). Babcock scrutinized the karyotypes of over one hundred *Crepis* species and summed up the results in several hypotheses: First, chromosome number decreases during evolution, so that species with small chromosome numbers are derived. Second, short living annuals have undergone a reduction in the quantity of nuclear DNA. Furthermore, he stated chromosomal rearrangements to be the driving force of evolution in the genus. Since Babcock’s hypotheses, new methods in karyotype studies (e.g. genome size estimation) brought additional insights into the karyology

of *Crepis*. (e.g. Siljak-Yakovlev & Cartier, 1982; Kamari, 1992; Dimitrova & Greilhuber, 2000). With the establishment of a molecular phylogeny (Enke & Gemeinholzer, 2008) the findings of Babcock and his successors can be further interpreted in an evolutionary context of phylogenetic relations.

Table 1: 1C value, used standards and tested characters of the presently measured *Crepis* species. Ind = individuals, S = samples. Distr = distribution, A = North Africa, Middle East, Mediterranean or Southeast Europe, B = Europe, C = Eurasia, D = Central and East Asia. Alt = altitude, alp = alpine, mont = montane, low = lowland. end = endemic. p = perennial, a= annual.

Species	1C value (pg)	SD (+/-)	Number of Ind	Number of S	Standard	Basic chrom. number (1x)	Ploidy level	Section	Clade	Distr	Alt	Range	Life History
Crepis aurea	1.75	0.075	5	6	*G. max*	5	D	*Brachypodes*	VIII	A	alp	wide	p
C. biennis	9.51	0.492	6	8	*P. sativum*	5	O	*Berinia*	VII	B	-	-	-
C. blattarioides	3.58	0.051	3	5	*S. cereale*	4	D	*Soyeria*	XI	B	mont	wide	p
C. bungei	4.05	0.053	5	5	*S. cereale*	4	D	*Mesophylion*	VII	D	low	wide	p
C. chrysantha	4.64	0.053	5	5	*S. cereale*	4	D	*Brachypodes*	VII	D	alp	wide	p
C. conyzaefolia	5.90	0,069	7	10	*P. sativum*	4	D	*Soyeria*	-	B	mont	wide	p
C. crocea	9.61	0.135	8	10	*P. sativum*	4	T	*Macropodes*	II	D	-	-	p
C. foetida	1.46	0.034	3	4	*G. max*	5	D	*Hostia*	III	A	low	wide	a
C. kerneri	5.56	0.046	3	4	*S. cereale*	6	D	*Brachypodes*	II	A	alp	wide	p
C. leontodontoides	1.14	0.012	5	5	*R. sativus*	5	D	*Gephyroides*	VIII	A	lowl	end	p
C. lyrata	2.79	0.037	8	7	*P. sativum*	6	D	*Mesomeris*	-	D	mont	wide	p
C. multicaulis	1.78	0.018	5	5	*G. max*	5	D	*Microcephalum*	*Lagoseris*	D	mont	wide	p
C. paludosa	4.53	0.074	4	10	*S. cereale*	6	D	*Desiphylion*	II	B	mont	wide	p
C. polytricha	9.62	0.300	6	8	*P. sativum*	4	T	*Brachypodes*	-	D	-	-	p
C. pusilla	1.11	0.025	3	4	*R. sativus*	5	D	*Zacintha*	III	A	mont	wide	a
C. sibirica	6.90	0.086	4	5	*P. sativum*	5	D	*Desiphylion*	-	C	mont	wide	p
C. turcica	6.41	0.100	3	6	*P. sativum*	4	D	*Berinia*	-	A	mont	end	p
C. vesicaria	4.18	0.050	5	5	*S. cereale*	4	D	*Lepidoseris*	X	B	mont	wide	-
Askellia karelinii	6.78	0.072	3	5	*P. sativum*	7	D	*Ixeridopsis*	-	D	-	-	p
Youngia tenuicaulis	2.08	0.066	4	5	*G. max*	5	D	-	*Youngia*	D	-	-	p
Youngia tenuifolia	4.10	0.060	5	5	*S. cereale*	5	T	-	*Youngia*	D	-	-	p

The aim of the present study is to investigate variation of genome size in the genus *Crepis* and its relations to speciation and phylogeny. Therefore, it discusses genome size variation in a molecular phylogenetic framework. Estimations of the ancestral character state for the 1C-value were performed based on a phylogenetic tree inferred from the nuclear marker ITS. The chloroplast marker *matK* has also been considered for reconstruction but been discarded for two reasons: First, the tree inferred by Enke & Gemeinholzer (2008) shows very low resolution and second, sequence availability is very low. It would, however, be of interest for future analyses to use a better resolving chloroplast marker to detect possible reticulate evolution within the genus, also in correlation to genome size evolution.

3.2 MATERIAL AND METHODS

Some of the 1C-values used in the statistical analysis and the ancestral character state reconstruction have been taken from literature (table 2). These have been inferred by different methods; so the values should not be directly compared. Therefore the 1C-values were assigned to different character classes following Leitch et al. (1998) and Soltis et al. (2003), defining 1C-values ≤1.4pg as "very small", >1.4pg but ≤ 3.5pg as "small" and 1C-values >3.5pg as "intermediate". In cases where the 1C-Values of individual species differed between studies, these differences, however, never influenced the character class the species were assigned to. Whenever 1C-values are directly compared, they are always from within one study if not stated differently.

Two datasets were used throughout the study: data set (1) comprising all species measured for DNA content in the present study and data set (2) comprising all *Crepis* species with known DNA content. The composition of the data sets can vary slightly between the ancestral character state reconstruction and statistical analysis due to the availability of sequence information for a given species.

TABLE 2: 1C value and tested characters of *Crepis* species from published data. Sources of published data are given in table. Ind = individuals, S = samples. Distr = distribution, A = North Africa, Middle East, Mediterranean or Southeast Europe, B = Europe, C = Eurasia, D = Central and East Asia. Alt = altitude, alp = alpine, mont = montane, low = lowland. end = endemic. p = perennial, a= annual.

Species	1C value (pg)	Basic chrom. number (1x)	Ploidy level	Section	Clade	Distr	Alt	Range	Life History
Crepis alpina	3.0[1]	5	D	*Hostia*	III	A	mont	wide	a
C. bithynica	2.8[5]	5	D	*Macropodes*	-	A	mont	wide	p
C. fuliginosa	0.9[1]	3	D	*Phytodesia*	VI	A	mont	end	a
C. incarnata	6.0[2]	4	D	*Intybellia*	*Lagoseris*	A	mont	wide	p
C. lapsanoides	5.6[3]	6	D	*Mesomeris*	I	B	mont	end	p
C. neglecta	1.8[1]	4	D	*Phytodesia*	VI	A	mont	wide	a
C. palaestina	6.1[3]	4	D	*Phaecasium*	*Lagoseris*	A	low	end	a
C. paludosa	4.5[5]	6	D	*Desiphylion*	II	B	mont	wide	p
C. praemorsa	5.3[3]	4	D	*Intybellia*	*Lagoseris*	C	mont	wide	p
C. pontana	6.9[3]	5	D	*Soyeria*	-	B	mont	end	p
C. pulchra	5.5[5]	4	D	*Phaecasium*	*Lagoseris*	A	mont	wide	a
C. rubra	2.9[3]	5	D	*Hostia*	III	A	mont	wide	a
C. sancta	2.2[5]	5	D	*Pterotheca*	*Lagoseris*	A	mont	wide	a
C. schachtii	2.8[5]	5	D	*Macropodes*	-	A	mont	end	p
C. setosa	1.7[5]	4	D	*Nemauchenes*	-	B	low	wide	a
C. tectorum	3.4[3]	4	D	*Mesophylion*	II	C	low	wide	a
C. viscidula	4.9[5]	6	D	*Desiphylion*	II	A	alp	end	p
C. zacintha	1.1[5]	3	D	*Zacintha*	III	A	low	wide	a

[1] Wallace, 1972; [2]Marie & Brown, 1993; [3]Bennett & Smith 1976; [4] Dimitrova et al., 1999; [5]Dimitrova & Greilhuber, 2000.

Genome Size Estimation – Seed from 22 accessions of 21 *Crepis* species and two species of closely related *Youngia* (table 1) were germinated and cultivated in pots (vouchers in B). Species from several sections (Babcock, 1947a,b) as well as different clades derived from molecular data (Enke & Gemeinholzer, 2008) were chosen to represent a variety of taxonomic and molecular groups. Material was taken from leaves of seedlings except for *C. aurea*, *C. pannonica* and *C. turcica* where leaf material of adult plants grown the previous year was used. For the internal standards leaves of adult plants potted and grown in a greenhouse were used (*Glycine max* (L.) Merr. convar. *max* var. *max* 'Cina 5202' (Genbank Gatersleben, accession number: SOJA 392 (2.23 pg/2C)), *Pisum sativum* L. subsp. *sativum* convar. *sativum* var. *ponderosum* Alef. 'Viktoria, Kifejtö Borsó' (Genbank Gatersleben, accession number: PIS 630 (9.07 pg/2C)), *Secale cereale* subsp. *cereale* (Genbank Gatersleben, accession number: R 737 (16.01 pg/2C)) and *Raphanus sativus* L. convar. *sativus* Small radish group 'Voran' (Genbank Gatersleben, accession number: RA 34 (1.11 pg/2C)).

TABLE 3: Correlation of genome size and karyological, systematical and distributional factors. Correlation coefficient (r_S) and significance from Spearman rank-order correlation.

	data set (1)		data set (2)	
	r_S	p	r_S	p
Chromosome number	-0,253	0,363	-0,005	0,975
Section	-0,447	0,095	-0,310	0,074
Clade	-0,243	0,471	-0,007	0,972
Life History	**0,557***	**0,039**	**0,519****	**0,001**
Distribtuion	0,229	0,411	**0,331***	**0,046**

Leaf fragments of the sample plant and the respective standard plant (see table 1) were chopped in 1ml of modified WPB (Loureiro et al., 2007; 0.2 M Tris HCl, 4 mM $MgCl_2 \cdot 6H_2O$, 2 mM EDTA $Na_2 \cdot 2H_2O$, 86 mM NaCl, 10 mM potassium metabisulfite, 1 % PVP-30, 1 % (v/v) Triton X-100, pH 7.5) supplemented with 50µg/ml propidium iodide and 50µg/ml DNAse-free RNAse, filtered through a 35µM mesh and stored on ice until measurement. 4 to 10 samples of 3-8 individuals per taxon (see table 1) were measured on two consecutive days using a FACStar^{PLUS} flow sorter (BD Biosciences) equipped with an argon ion laser INNOVA 90C (Coherent). Usually, 10,000 nuclei per sample were analysed.

Ancestral Character State Reconstruction – The ancestral character state reconstruction was carried out for both datasets on most parsimonious trees inferred from ITS sequence data. Sequences from Enke & Gemeinholzer (2008) were analysed by addition of new accessions (see appendix). Phylogenetic histories were reconstructed using Maximum Parsimony and Bayesian inference. Maximum Parsimony analyses were conducted in Paup 4.10b* (Swofford, 2002) with equal weights, 1000 closest sequence additions and tree bisection-reconnection (TBR) branch swapping, permitting 10 trees to be held at each step. Maxtrees was set to unlimited. An evaluation of the trees was performed using bootstrap analysis with 10000 replicates, equal weights, TBR swapping, MulTrees option in effect and

10 trees held at each step. Bayesian analyses were conducted using MrBayes (Ronquist & Huelsenbeck, 2003) assuming gamma distribution rate variation among sites and applying 10 million generations of the MCMC chains in two independent runs, trees saved every 100 generations. The first 27 000 trees were discarded as burn-in for the analysis then reached stationarity. All other trees sampled were used to calculate a strict consensus tree. Trees were rooted with *Youngia* as outgroup (Enke & Gemeinholzer, 2008).

The character history was traced independently for data sets (1) and (2) on rooted trees with the Ancestral Character State Reconstruction package of Mesquite 2.5 (Maddison & Maddison, 2008). When using character classes, character states were treated as unordered categorical and a most parsimonious approach for character state reconstruction was used (Figs.1,3). Continuous character states were assumed for data set (1) when using 1C-values. In this case the character history was reconstructed with a squared parsimony model (Fig.2).

Statistical Analysis – Descriptive statistics were conducted with SPSS 16.0 including all diploid species. The correlation between genome size and chromosome number, life form, distribution (divided into geographic region, altitudinal rank and distributional range) and phylogenetic relations (clade, section) was tested on both data sets. For data set (1) direct 1C-values were used, for data set (2) character classes. Tables 1-2 show all species with 1C-values and the tested attributes for each species. Sources for 1C-values taken from literature are given in the table. Chromosome numbers were taken from Babcock (1947a,b) and Kamari (1992). Information on life form was taken also from Babcock (1947a,b) as well as distributional and habitat information. Species were assigned to be distributed in four geographic areas (following the categories used by Kilian et al. (2008)): Group A is distributed in North Africa, Middle East, Mediterranean or Southeast Europe. Group B comprises species from Europe, group C from Eurasia, and group D from Central and East Asia. The species were assigned to one of three altitudinal ranks (lowland, mountainous, alpine) and one of two distributional patterns: widespread or endemic. Sections used in tables and figures refer to the infrageneric taxonomic classification sensu Babcock (1947b) and clades to the molecular groupings found by Enke & Gemeinholzer (2008).

Correlation tests were carried out using Spearman's rank order correlation as the data were mainly categorical and/or showed no normal distribution.

To test the significance of difference in 1C-value between annuals and perennials (both data sets) a Mann-Whitney-U test was applied, appropriate to test not normally distributed data.

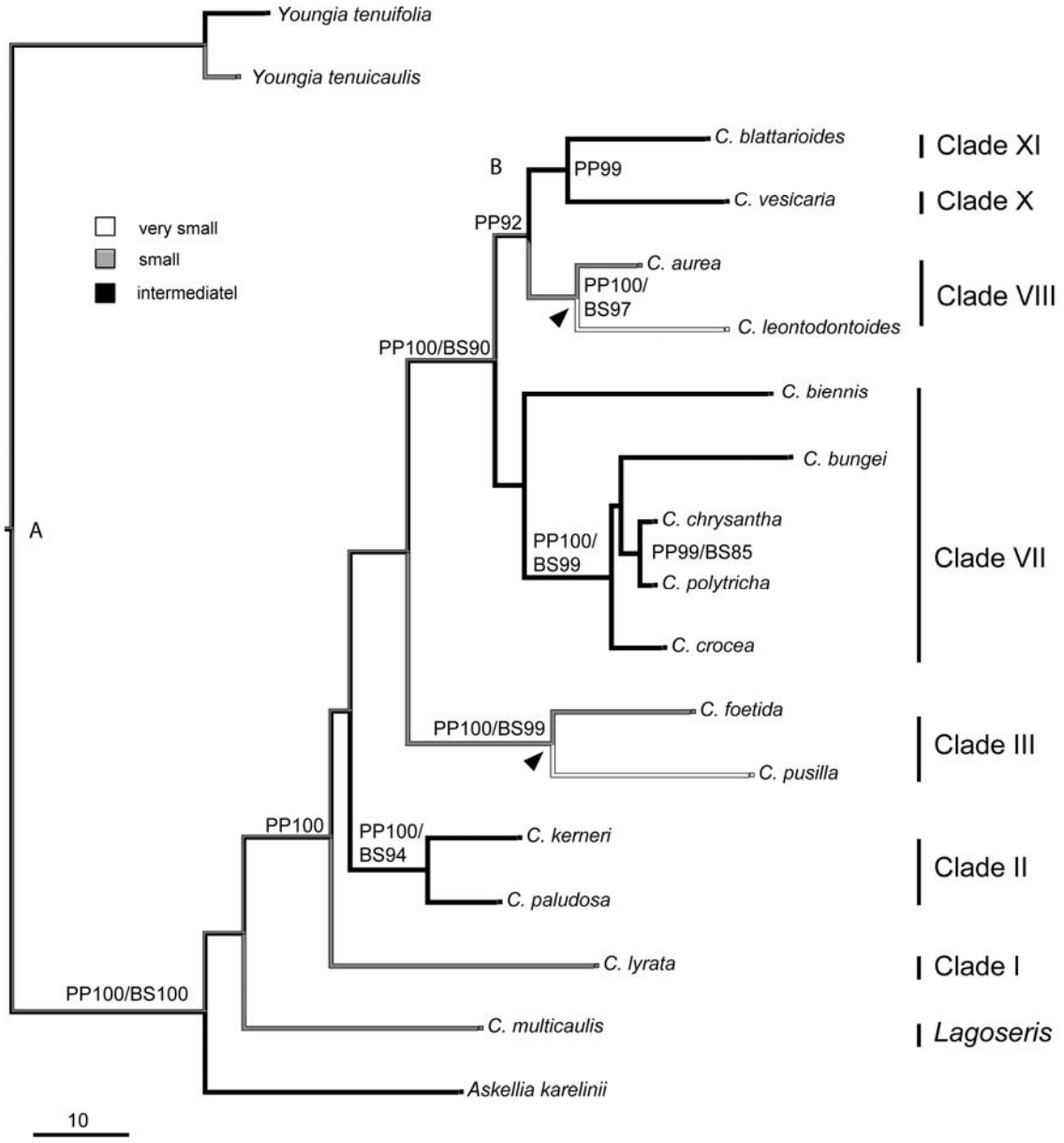

FIG.1: Ancestral character state reconstruction for data set (1) using character classes on 1 of 2 most parsimonious trees. Bayesian posterior probabilities (PP) > 90 and bootstrap values (BS) > 75 are given above branches. Arrows indicate decreases in genome size. A and B denote nodes discussed in the text. Clades named following Enke & Gemeinholzer (2008).

3.3 RESULTS

Ancestral character state reconstruction – For data set (1) two most parsimonious trees, for data set (2) three most parsimonious trees were inferred. Trees inferred by Maximum Parsimony and Bayesian analyses showed congruent topologies. One of the most parsimonious trees of each data set was used for the ancestral character state reconstruction. The trees are shown in Figs.1-3. Posterior probabilities and bootstrap values above 75 are given above branches. The clades are named following Enke & Gemeinholzer (2008). Species relationships are similar to those found by Enke & Gemeinholzer (2008),

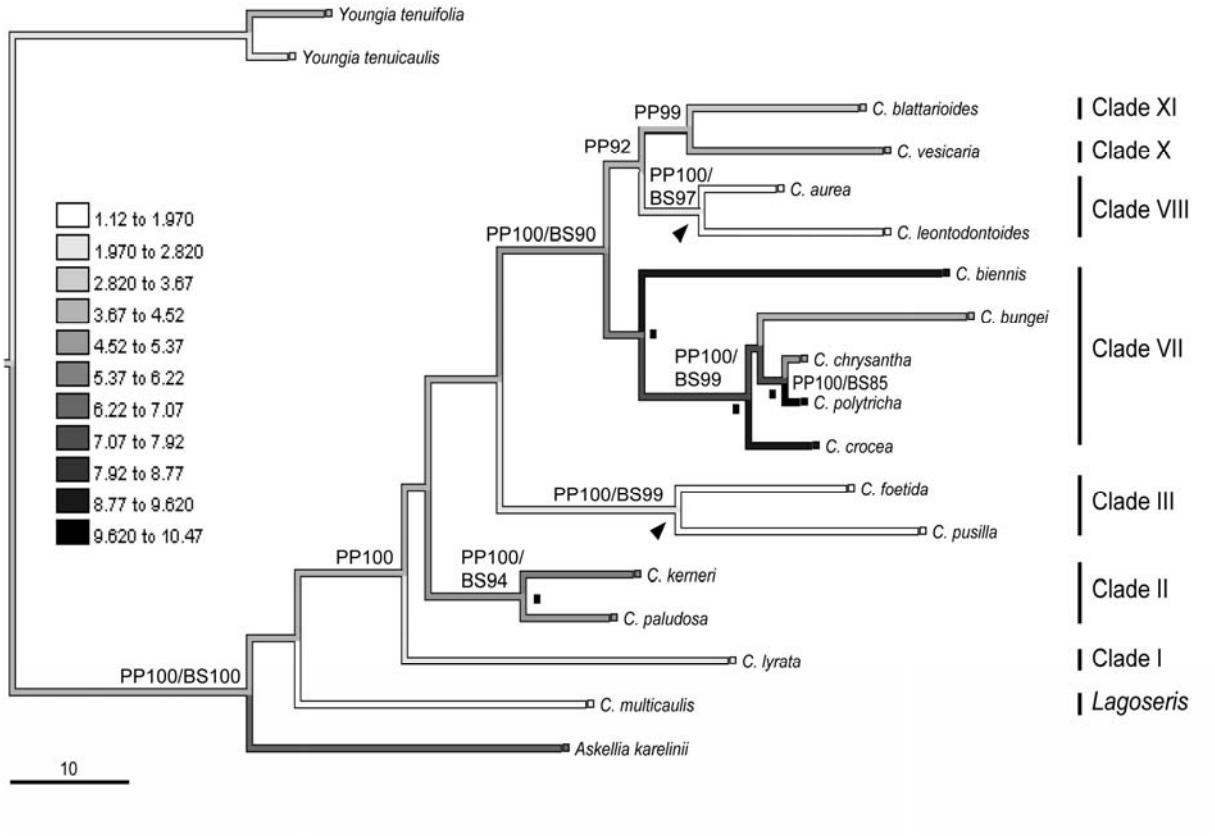

FIG.2: Ancestral character state reconstruction for data set (1) using 1C-value on 1 of 2 most parsimonious trees. Bayesian posterior probabilities (PP) > 90 and bootstrap values (BS) > 75 are given above branches. Arrows indicate decreases, squares increases in genome size. Clades named following Enke & Gemeinholzer (2008).

differences (mainly the position of *C. setosa*) are due to the considerably smaller sample size in the present study.

The ancestral character state reconstruction for data set (1) (only data obtained by the presented study and using character classes) is ambiguous (Fig.1). From node A to B the ancestral character state could be either intermediate or small. In only two cases (Fig. 1, arrows) unambiguous character state changes can be observed. In both cases a decrease from small to very small can be observed.

If 1C-values are used for ancestral state reconstruction on data set (1) the ancestral 1C-value is estimated between 2.8 and 3.7pg (Fig.2). Only in clades II and VII the overall trend towards a decline in 1C-value is reversed (Fig.2, squares). In clade VII the increases are due to the high 1C-values of the two tetraploid species *C. crocea*, *C. polytricha* and octoploid *C. biennis*.

In data set (2) representing character classes and all genome sizes analyzed here or elsewhere published (Fig.3) the basic character state for *Crepis* s.str. and the *Lagoseris* group is an intermediate 1C-value equivalent to the closest relatives to Crepis. Decrease of

genome size to either a small or very small 1C-value occurred seven times during evolution of the species within the genus (Fig.1, arrows). In three cases (Fig.1, stars) uncertain character states (small/very small) are found. If ambiguous states were treated as small the number of decreases raises to ten. Increases can only be found if uncertain states are treated as very small (3 times, Fig.1, squares).

The decreases found by the analysis of data set (1) using character classes (Fig.1, arrows) reoccur in the analysis of data set (2) (Fig.3, arrows, clades III, VIII). In the analysis of data set (1) using 1C-values these decreases are located at a deeper node (Fig.2, arrows) due to the fact that *C. leontodontoides* (1.14pg/1C) and *C. aurea* (1.75pg/1C) respectively *C. pusilla* (1.11pg/1C) and *C. foetida* (1.46g/1C) fall into different character classes (Fig.1, very small, small) but into the same categorical group (Fig.2, 1.12-1.97pg).

Fig.3 (arrows, *Lagoseris* group, Clade I) shows that the small genome sizes of *C. multicaulis* and *C. lyrata* are derived. Consequently, the ancestral character states small/2.8-3.7pg for data set (1) (Figs.1-2) might be underestimated in both analyses, as *C. multicaulis* (*Lagoseris* group) as well as *C. lyrata* (Clade I) have considerably lower 1C-values than their relatives included in data set (2).

Genome Size Variation/Statistical Analysis – The 1C-values within the screened *Crepis*-species show a 8.5fold difference between the highest (*C. crocea* 9.62 pg/1C) and lowest value (table 1). This difference, however, includes the tetraploid *C. crocea*. The difference between highest (*C. sibirica* 6.90pg/1C) and lowest diploid (*C. pusilla* 1.11 pg/1C) is a 6fold difference.

The correlation between the genome size and other factors (chromosome number, life form, distribution, and phylogenetic relations (clade, section)) is shown in table 3. In data set (1) life history and 1C-value show a significant positive correlation implying that perennial plants tend to have larger genomes than annuals. In data set (2) both life history and distribution are significantly positive correlated to genome size class. Fig.4 visualises the positive correlation between genome size classes and distribution. Very small genomes are only present in plants from the circum Mediterranean region and Europe, even though plants from these two regions are present in all three genome classes. The three analyzed species with widespread Eurasian distributions feature only intermediate genomes. Analyzed species from Central Asia have either small or intermediate genomes.

Genome sizes within annuals (data set (1) mean 1C = 1.3pg) are significantly lower than in perennials (data set (1) mean 1C = 4.1pg) (Mann-Whitney-U, data set (1) $P < 0.05$; data set (2) $P < 0.005$).

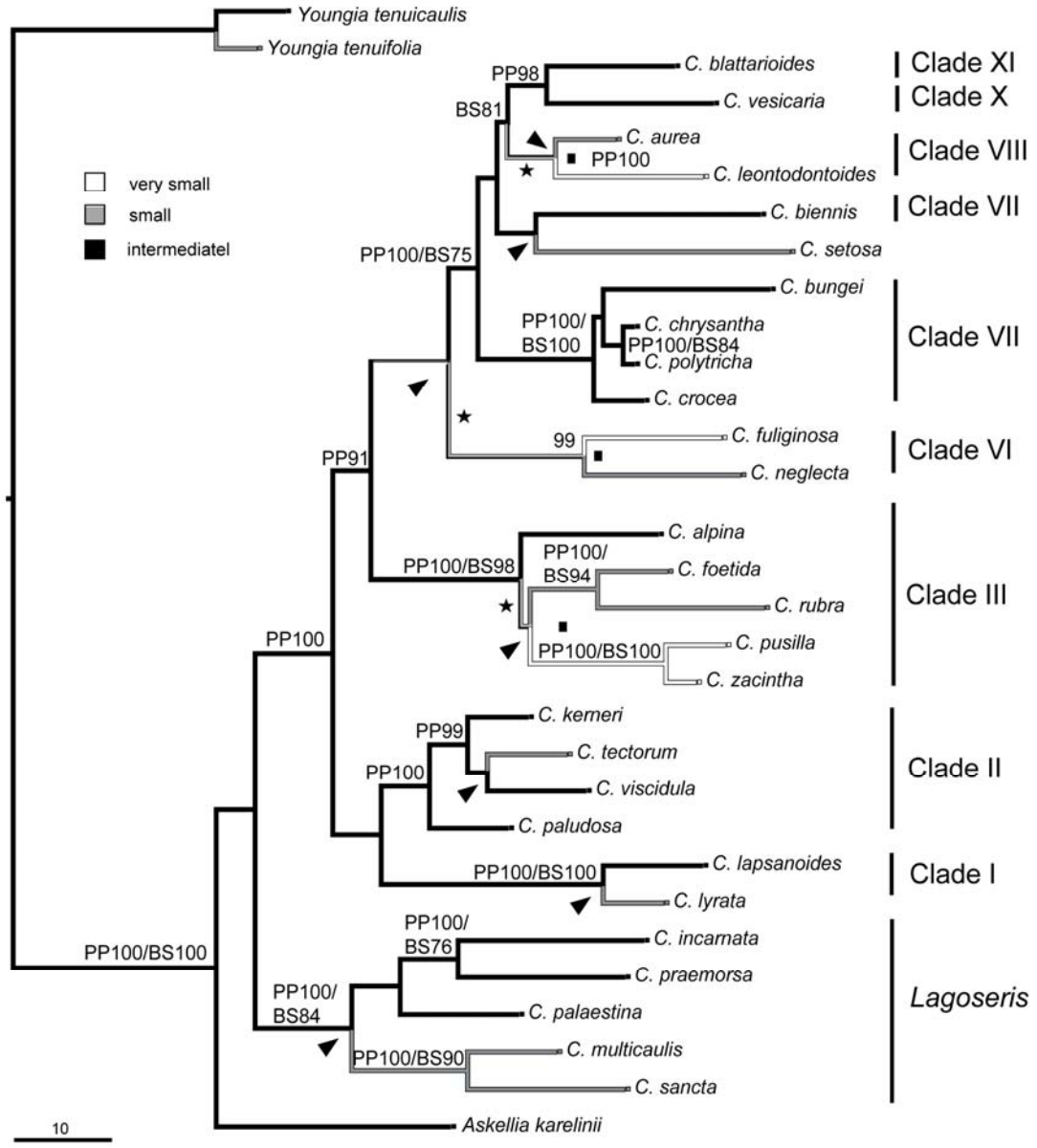

FIG.3: Ancestral character state reconstruction for data set (2) using character classes on 1 of 3 most parsimonious trees. Bayesian posterior probabilities (PP) > 90 and bootstrap values (BS) > 75 are given above branches. Arrows indicate decreases, squares increases in genome size. Stars mark ambiguous branches. Clades named following Enke & Gemeinholzer (2008).

3.4 DISCUSSION

Ancestral Character State Reconstruction – The results of the different analyses based on character classes and direct 1C-value are comparable and lead to the same conclusion that 1C-value in diploid *Crepis* species decreases. However, the ancestral character state analysis using 1C-values is more sensitive (viz. the only analysis detecting the increases

caused by polyploidisation, Fig.2, squares), but relies on the assumption that 1C-values inferred by different methods are directly comparable.

The character state reconstructions within the evolution of Crepis (Figs.1-3) show an overall trend towards decrease in genome size. In the present study, the only reliable increases in 1C-value within *Crepis* are due to polyploidisation (Fig.2, clade VII, squares). The rise of 1C-value in clade II (Fig.2, *C. kerneri*) in the analysis of data set (1) using 1C-value could be a result of the possible underestimation of ancestral genome size in data (1) and therefore has to be treated with caution.

The observed decrease in genome size confirms findings of earlier studies in *Crepis* (Jones & Brown, 1976; Dimitrova & Greilhuber, 2001). Only few studies so far used molecular phylogenetic data and direct genome size estimations such as flow cytometry or Feulgen densitometry to infer directionality of genome size evolution on a generic level. Jakob et al. (2004) analysed genome size evolution on a molecular phylogenetic background within *Hordeum* and discovered increases as well as decreases among diploid species. Wendel et al. (2002) also observed a "bidirectional" genome size evolution in diploid *Gossypium* species. Based on total chromosome length which, according to e.g. Raina & Rees (1983) or Jones & Brown (1976), is positively correlated to genome size, Watanabe et al. (1999) found decreases as well as increases in the evolutionary history of *Brachyscome* (Asteraceae). Regarding this context, the here presented trend in *Crepis* towards a decrease in genome size is remarkably striking. However, only about a fifth of all known *Crepis* species could be included in this study and the addition of further species might somewhat change the observed trend.

Mechanistically not much is known about how DNA content decreases in Crepis. Tobgy (1943) reported unequal reciprocal translocations to be the cause of a reduction from x=4 in *C. neglecta* to x = 3 in *C. fuliginosa*. The decrease in chromosome number was accompanied by a decrease in total chromosome length (Tobgy, 1943) and therefore a reduction in nuclear DNA content. Kamari (1976) found *C. fuliginosa* more closely related to *C. cretica* than to *C. neglecta*. *C. fuliginosa* also features x = 4 but its total chromosome length is not known. Furthermore, a similar decrease in chromosome number from x = 5 in *C. alpina* to x = 4 in *C. kotschyana* did not lead to a change in total chromosome length (Sherman, 1947). Even though losses of whole chromosomes can lead to a decrease of genome size (Dart et al., 2004) it can only be hypothesised at the present stage if it plays a role within *Crepis*.

As increases in 1C value within *Crepis* are mainly due to polyploidisation, the highest amount of DNA of all species is found in two tetraploid species (*C. crocea*, *C. polytricha*) and the octoploid *C. biennis*. One might expect the 1C-value of the tetraploid to be the sum of the 1C-values of its diploid ancestors, respectively to vary proportionally with the ploidy level, but this is rarely the case. Frequently genome downsizing after polyploidisation has been observed (Leitch & Bennett, 2004). *C. crocea* (9.61pg/1C), however, has nearly 15% more

DNA than twice the amount of its close relative *C. bungei* (4.05pg/1C). *C. crocea* (2n = 4x = 16) is thought to result from hybridisation between *C. bungei* (2n = 2x = 8) and *C. oreades* (2n = 2x = 8) (Babcock, 1947b). Unfortunately *C. oreades* was not available for genome size estimation. For *C. polytricha* (9.62pg/1C) the amount of DNA is slightly (5%) higher than twice the amount of its presumed closest relative *C. chrysantha* (4.64pg/1C). *C. polytricha* (2n = 4x = 16) might have originated as an amphidiploid hybrid between *C. chrysantha* (2n = 2x = 8) and some other species (Babcock, 1947b). The relation of 1C-value of polyploid species to their diploid relatives is similar in *Magnolia* (Soltis et al., 2003); however, the lack of data on one of the putative parents, in both *C. crocea* and *C. polytricha,* should be taken into account.

The observable contraction in genome size within *Crepis* contradicts the "one way ticket to obesity"-hypothesis of Bennetzen & Kellogg (1997) and the general trends within the angiosperms (Soltis et al., 2003). Increase of genome size within *Crepis* seems to be caused largely by polyploidisation. The genus *Crepis* clearly demonstrates that the DNA amount of a species reflects a dynamic balance between expansion and contraction, in terms of both mechanisms and selective consequences (Bennett & Leitch, 2005).

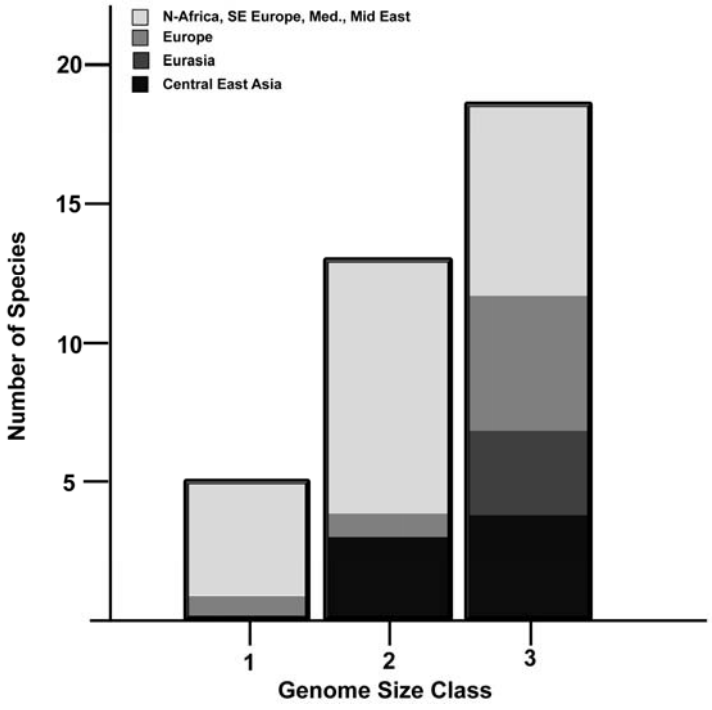

FIG.4: Geographic distribution of species frequency sorted for genome size class.

Genome Size Variation and Correlation – The 6fold variation of genome size found in the diploid species measured in this study is within the 7fold differences found by Jones & Brown (1976) and the 5.15fold variation found by Dimitrova & Greilhuber (2000) for diploid *Crepis* species. The variation is similar to those found in other genera of the Asteraceae. In *Artemisia* the genome size varies 7fold (Torrell & Vallès, 2001). In the *Echinops* group there is a 13fold variation (Garnatje et al., 2004). So far only little is known about why DNA content varies greatly between even closely related species; e.g. *C. lyrata* (2.8pg/1C) and *C .lapsanoides* (5.6pg/1C, Bennett & Smith, 1976). In genera of other families genome size can vary more widely (e.g. 24fold in *Genlisea*, Lentibulariacea (Greilhuber et al., 2006).

As already reported by Babcock (1947a), a positive correlation between genome size and life form in *Crepis* is indicated by present results. Annual species have a significantly lower genome size than perennials. But still variation within perennials is high: Both *C. leontodontoides* (1.14pg/1C) with one of the lowest and *C. sibirica* (6.90pg/1C) with the highest 1C-value are perennials. Even though this correlation is common throughout angiosperms (e.g. Bennett et al., 1998; Bennett & Leitch, 2005), some authors give an alternative explanation for the often observed correlation between life history and genome size: selfing species tend to have smaller genomes than outcrossing ones, and because selfing species are often annuals, a correlation between annuality and small genomes is apparent (Govindaraju & Cullis, 1991; Albach & Greilhuber, 2004). Not many *Crepis* species have been tested for self compatibility, and so far no obligate selfer is known. Known self compatible species are *C. tectorum*, *C. pulchra*, *C. multicaulis*, and *C. alpina* (Babcock, 1947a). Except for *C. pulchra* with an intermediate 1C-value, all of these species have small 1C-values. Except for *C. multicaulis* all of the species are also annuals. Only two species are known with no or low self compatibility, *C. foetida* (Babcock, 1947a; Hughes & Babcock, 1950) and *C. sancta* (Cheptou et al., 2000). Both have small 1C-values and are annual. Due to the small sample size of the species included in this study, it is hard to estimate how representative these species are for the whole genus, but the annual species have very small, small and only in rare cases intermediate 1C-values, whereas perennials are more variable in genome size in general.

A correlation between the geographic distribution of species and genome size has been found before: genomes were generally larger in species of temperate regions than in tropical species (Avdulov, 1932; Levin & Funderburg, 1979; Ohri, 2005). Even though this has been shown to be especially true in Asteraceae (Ohri, 2005), it is not applicable to *Crepis* as this genus is mainly distributed in temperate regions. A correlation between geographic distribution and genome size still could be found: Most species featuring very small genomes are found in the circum Mediterranean region. In Central and East Asia, the easternmost areas of the distribution range of *Crepis*, no species with very small genomes are found (Fig.4). The continental climate of Central and East Asia is characterised by hot summers and cold winters whereas in the Mediterranean climate fluctuations of temperature are considerably lower. In contrast to their continental relatives, species in the Mediterranean are not frequently subjected to frost. So, one factor to explain the absence of plants with very small genomes from Central and East Asia could be freeze tolerance: A positive correlation between freeze tolerance and genome size could be demonstrated for maize populations (McMurphy & Rayburn, 2006). That temperature can influence genome size has also been shown on population level by Turpeinen et al. (1999). *Crepis* is thought to have originated in the Central Asian Altai/Tien Shan region (Babcock, 1947a), and to have spread from there towards Europe and the Mediterranean. This means that many of the more derived species are found in the mild climate of the Mediterranean (where species are also more likely to

have a very small genome), whereas species with very small genomes are absent from Central and East Asia, where a harsher climate prevails.

The altitudinal rank of species (lowland, mountainous or alpine) and endemical status (widespread or restricted) showed no correlation to genome size. To further explore the connection between the distribution of a species and its genome size, geographic and ecological data is needed on a much finer scale (as has been used to explain infraspecific (e.g. Kalendar et al., 2000) or infrageneric (Jakob et al., 2004) genome size variation).

Genome size is often correlated to phylogeny within the Asteraceae (e.g. Cerbah et al., 1998; Torrell & Vallès, 2001; Garnatje et al., 2004), but in the present case no significant correlation between genome size and clade (respectively taxonomic section sensu Babcock, 1947(a,b)) could be found. This can be in account of the comparatively small sample size of the large genus and the heterogeneity of samples. Other chromosomal characters proved to be more informative for phylogenetic purposes even in unclear species groups, such as comparative C-band analysis in the *C. praemorsa* complex (Siljak-Yakovlev & Cartier, 1982). Genome size is fairly constant within species but varies between species, which make it useful for taxonomic purposes (Ohri, 1998; Murray, 2005). Genome size per se is not reflecting the phylogeny within *Crepis*.

How genome size is influenced by selective pressure, is largely unknown. One approach is the “large genome constraint hypotheses”: genera with large genomes are less likely to be highly specious as accumulation and replication of “junk” DNA is associated with evolutionary costs (Knight et al., 2005). Furthermore, it is now accepted that selection works on non coding DNA as well as on coding regions (Bennett et al., 2000).

3.5 Summary and Conclusion

Within *Crepis* there is a remarkably unidirectional trend towards a decrease in genome size of diploid species. The rare cases of increases in genome size within *Crepis* are always resulting from polyploidisation. Life form and genome size are correlated. Annuals have significantly lower 1C-values than perennials. This, however, could also be related to the reproductive mode (selfing/outcrossing) of species. *Crepis* species in the milder climates of the Mediterranean are also more likely to have smaller genomes than their Central Asian relatives.

The present study should be a starting point for subsequent investigations into mechanisms of genome size variation, especially mechanisms of decrease, in diploids. Comparative studies of coding/noncoding DNA ratios in Crepis could shed light on the questions of the C-value enigma. Analysis of speciation rates could improve insights on the “large genome

constraint hypothesis": If species with large genomes are not likely to speciate fast, is the reverse also true? Are plant groups with small genomes more prone to fast diversification? The comparison of chromosomal maps or chromosomal banding patterns between close relatives could elucidate how differences in chromosomal composition could be connected to genome size.

Furthermore, it would be interesting to test environmental influences on genome size; e.g. if the smaller genomes in the Mediterranean are connected to the mild climate or what other factors (such as aridity, elevation, or physiological adaption) could be linked to the observed patterns.

In conclusion this study emphasises the need to further investigate mechanisms and factors which influence the genome size of a species, how selection acts upon genome size, and to define the role genome size variation plays in speciation.

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3.8 APPENDIX:

Taxa Sampled for DNA Content

GENUS, *Taxon*, Voucher, Location.

ASKELLIA: *Askellia karelinii* (Popov & Schischk.) W.A.Weber, N. Enke, T. Dürbye & B. Gemeinholzer NE0163 (B) Russia. **CREPIS:** *Crepis aurea* (L.) Cass., N. Enke NS0051 (B), Austria; *C. biennis* L., Roy et al. 844 (B), Austria; *C. blattarioides* (L.) Vill., T. Dürbye 3195 (B), France; *C. bungei* Ledeb., N. Enke, T. Dürbye & B. Gemeinholzer NE0178 (B), Russia; *C. chrysantha* Froel., N. Enke, T. Dürbye & B. Gemeinholzer NE0196 (B), Russia; *C. conyzifolia* Dalla Torre, R. Vogt 15877 (B), Austria; *C. crocea* (Lam.) Babc., N. Enke, T. Dürbye & B. Gemeinholzer NE0177 (B), Russia; *C. foetida* L., R. Hand 5263 (B), Greece: Cyprus; *C. kerneri* Rech. f., N. Enke NS0047 (B), Italy; *C. leontodontoides* All., BG Liege 126-25-91-14 (B), France; *C. lyrata* (L.) Froel., N. Enke, T. Dürbye & B. Gemeinholzer NE0149 (B), Russia; *C. multicaulis* Ledeb., N. Enke, T. Dürbye & B. Gemeinholzer NE0164 (B), Russia; *C. multicaulis* Ledeb., E. Raab-Straube 020302 (B), Russia; *C. paludosa* Moench, BG Bratislava 136-01-06-10 (B), Slovakia; *C. polytricha* (Ledeb.) Turcz., N. Enke, T. Dürbye & B. Gemeinholzer NE0185 (B), Russia; *C. pusilla* (Sommier) Merxm., R. Hand 5130 (B), Greece: Cyprus; *C. sibirica* L., BG Halle 092-22-97-14 (B), Russia; *C. turcica* Dega & Bald, BG Paris 138-01-06-10 (B), Turkey; *C. vesicaria* T. Dürbye 1244 (B), Spain; **YOUNGIA:** *Youngia tenuicaulis* L., N. Enke, T. Dürbye & B. Gemeinholzer NE0149 (B), Russia; *Y. tenuifolia* (Willd.) Babc. & Stebbins, N. Enke, T. Dürbye & B. Gemeinholzer NE0165 (B), Russia.

Taxa Sampled for DNA Sequence

GENUS, *Taxon*, Voucher, Origin, GeneBank Accesion No. (ITS)

ASKELLIA: *Askellia karelinii* (Popov & Schischk.) W.A.Weber, N. Enke, T. Dürbye & B. Gemeinholzer NE0163 (B), Russia: Kosh-Agatsh FJ424075. **CREPIS:** *Crepis alpina* L., J. Trelawny 1423 (E), Turkey: Hakkari EU363649; *C. aurea* (L.) Cass., K. Faber AU87 (B), Austria: Tauern, EU363627; *C. biennis* L., BG Frankfurt 217 109 34/03 (GAT) AJ633355; *C. blattarioides* (L.) Vill., T. Eckhardt 1555 (B), Switzerland: Valais EU363624; *C. bungei* Ledeb., BG Uppsala/Sweden 31/113/03 (GAT), Mongolia: Tovaimag AJ633374; *C. chrysantha* Froel., N. Enke, T. Dürbye & B. Gemeinholzer, NE0170 (B), Russia: Kosh-Agatsh FJ424077; *C. crocea* (Lam.) Babc., N. Enke, T. Dürbye & B. Gemeinholzer, NE0177 (B), Russia: Kosh-Agatsh FJ424078; *C. foetida* L., J. Lambinon 00/F/214bis (B), France: Ardéche EU363619; *C. fuliginosa* Sibth. & Sm., D. Philos 4032 (B), Greece: Euboea EU363601; *C. incarnata* (Wulf.) Tausch, H. & H. Doppelbaur 14684 (M), Italy: Südtirol EU363608; *C. kerneri* Rech. f., T. Wraber, 9748/4 (B), Slowenia EU363636; *C. lapsanoides* (Gouan) Tausch, H. Kalheber 88-2748 (M), France: Dpt. Hts. Pyr. EU363599; *C. leontodontoides* All.,Vogt 10/1992 (B), France: Corsica EU363592; *C. lyrata* (L.) Froel., N. Enke, T. Dürbye & B. Gemeinholzer, NE0198 (B), Russia: Ongudai FJ424081; *C. multicaulis* Ldb., N. Enke, T. Dürbye & B. Gemeinholzer, NE0164 (B), Russia: Kosh-Agatsh FJ424076; *C. neglecta* L., Eisenblätter & Willing 82.085 (B), Greece: Kardhitsa EU363610; *C. palaestina* (Boiss.) Bornm., A. Danin & al. 53.019 (B), Israel: Galilee EU363639; *C. paludosa* Moench, P. Brückner 478/76 (B) EU366428; *C. polytricha* (Ldb.) Turcz., N. Enke, T. Dürbye & B. Gemeinholzer, NE0185 (B), Russia: Ulagan FJ424080; *C. praemorsa* (L.) Tausch, G. van Buggenhout 13637 (B), Italy: Bolzano EU363654; *C. pusilla* (Sommier) Merxm., Th. Raus 7715 (E), Greece: Kasos EU363650; *C. rubra* L., Hort. Bot. Haunensis, 301-S1948-2634*A130 (GAT) AJ633350; *C. sancta* (L.) Babc., J. Lambinon 84/F/373 (B), France: dep. Gard EU363632; *C. setosa* Hall. f., N. Enke 0123a (B), Italy: Pisa EU363585; *C. tectorum* L., E. Willing 4.502 D (B), Germany: Berlin EU363643; *C. vesicaria* L., R. Vogt 11757 (B), Marocco: Anti-Atlas EU363630; *C. viscidula* Froel., K.H. Rechinger 20933 (B), Greece: Mt. Smolika EU363629; *C. zacintha* (L.) Babc., R. Hand 5323 (priv.), Greece: Rhodes EU363655. **YOUNGIA:** *Youngia tenuicaulis* (Babc. & Stebbins) Czerep., N. Enke, T. Dürbye & B. Gemeinholzer, NE0181 (B) , Russia: Kosh-Agatsh FJ424079; *Y. tenuifolia* (Willd.) Babc. & Stebbins, Beljaeva & al. s.n. (B), Russia: Primorje EU363645.

Chapter 4

In the Search of Additional Characters Supporting Systematic Delimitation in *Crepis* L. and Related Genera in the Subtribe Crepidinae (Cichorieae/Compositae)

ABSTRACT

Recent molecular work on *Crepis* and its allied genera (e.g. *Askellia*, *Lapsana*, *Rhagadiolus*, and *Youngia*) raised several questions on generic and infrageneric classification within the Crepidinae. *Lapsana* and *Rhagadiolus* as well as *Crepis* species from sections *Intybellia*, *Phaecasium*, *Lagoseris*, *Microcephalum*, and *Pterotheca* were found to be closely related. To resolve the incongruence between clades inferred by molecular approaches and current taxonomic classification additional morphological characters need to be assessed.

Morphological characters within the genus have been intensively studied; however, extensive parallel evolution of traits complicates the recognition of natural groups. Here, a pilot study was carried out to test additional characters (fruit morphology and anatomy, pappus ultrastructure, pollen morphology, and style branch papillae) for their applicability in generic delimitation and infrageneric classification.

KEYWORDS: *Crepis*, achene morphology, achene anatomy, pappus bristles, pollen, SEM, style branch papillae.

4.1 INTRODUCTION

The uniform character combination of milky latex and capitula with 5-dentate, ligulate flowers makes the members of the predominantly holarctic Cichorieae easy to identify. However, the classification within the tribe is obstructed by the notorious lack of characters suitable for delimitation. Since Tournefort (1694) first recognised the Cichorieae until the most recent classification by Kilian and co-workers (2008), the circumscription of the Cichorieae did not change much, whereas the generic and suprageneric classification was subject to various changes. This is also true for the subtribe Crepidinae. The subtribe Crepidinae gained importance in the first half of the 20th century through the work of two American botanists, E.B. Babcock and G.L. Stebbins who studied the genera of the Crepidinae not only morphologically but also cytologically and used the results to establish new classifications and generic circumscriptions (e.g. Babcock et al., 1937; Babcock & Stebbins, 1937; Babcock & Jenkins, 1943; Babcock, 1947a,b). Among the most notable works is Babcock's (1947a,b) monograph of the genus *Crepis*, that was used as basis for recent molecular work (Enke & Gemeinholzer, 2008). The availability of molecular phylogenies (e.g. Enke & Gemeinholzer, 2008) led to new insights into generic interrelations within the Crepidinae, but also raised new questions: discrepancies between relations found by molecular inference and taxonomically recognised groups demonstrate the need to further evaluate the current taxonomic classification. Of special importance here is the delimitation of *Lapsana* L. and *Rhagadiolus* Juss. as these two genera were found to be nested within *Crepis* L. by molecular data (Enke & Gemeinholzer, 2008), even though they are easily distinguished on morphological grounds (e.g. fruit morphology). The infrageneric delimitation within *Crepis* also needs revision: The molecular analyses by Enke & Gemeinholzer (2008) could not corroborate the current taxonomic sections (Babcock, 1947b). A thorough morphological re-evaluation of species and genera, and the identification of additional discriminating characters are needed to achieve a revised classification. Jeffrey (1966) stated that microcharacters (e.g. the shape of hairs on the stigmatic surface) provide good criteria for taxonomy in the Cichorieae (Compositae). The microcharacteristic investigations by e.g. Pak & Kawano (1990), Sennikov & Illarionova (2007) and Torres & Galetto (2007) confirm the usability of a micromorphological approach. Here, a pilot study is carried out to screen possible micromorphological and anatomical traits for their applicability in generic and infrageneric classification within Crepidinae. These traits include fruit morphology and anatomy, pappus ultrastructure, pollen morphology and style branch papillae.

4.2 MATERIAL AND METHODS

Achene Habit and Surface - Dry achenes of 23 species (*Youngia tenuicaulis*, *Y. tenuifolia*, *A. elegans*, *A. flexuosa*, *A. karelinii*, *A. nana*, *Lapsana communis*, *Rhagadiolus spec*., *Crepis bocconi*, *C. bungei*, *C. chrysantha*, *C. crocea, C. foetida*, *C. lapsanoides*, *C. lyrata*, *C. multicaulis*, *C. paludosa*, *C. purpurea*, *C. pusilla*, *C. pyrenaica*, *C. tectorum*, and *C. zacintha*) were documented at the Wild M5A stereomicroscope equipped with digital camera Leica DFC 290 and Leica Application Suite software Version 2.5.0. The fruits were taken from either herbarium specimen or living plants (appendix).

Ultra Thin Sections for Light Microscopy - Achenes of 21 species (*Rhagadiolus spec*., *Askellia flexuosa*, *A. nana*, *Crepis acuminata*, *C. albida*, *C. biennis*, *C. capillaris*, *C. chondrilloides*, *C. foetida*, *C. kerneri*, *C. lapsanoides*, *C. leontodontoides*, *C. mollis*, *C. multicaulis*, *C. neglecta*, *C. paludosa*, *C. praemorsa*, *C. purpurea*, *C. sancta*, *C. tectorum*, and *C. zacintha*) for light microscopy were taken from herbarium sheets or living plant material (Appendix). Dried achenes were stored in 96% ethanol. Fruit from living material was progressively dehydrated by ascending ethanol solutions (30%, 50%, 70%, 90%, and 96%). The achenes remained 24h in each dilution. For infiltration with resin (Unicryl, BBI International) the objects were first transferred into a mix of 1:2 Unicryl and Ethanol (96%), then stepwise into 1:1, 2:1 and last into 100% Unicryl. The samples remained 3-6 days (depending on size) at each step. The objects were embedded in gelatine capsules filled with resin and dried for 3-5 days in a heating cabinet at 40°C. The ultra thin sections through the middle part of the achene were cut at a rotation microtome (Supercut 2065, Reichert/Jung). The transverse sections (3- 4 µm) were stained with toluidine blue (Serva, 0.5%, 20-35 s), mounted in corbit-balm (Kobe) and dried for 2 days at 40°C. Micrographs were taken at Zeiss microscope Standard 14 with the digital documentation system Zeiss Axio Cam MRc and Axio Vision software (release 4.4, Zeiss).

Preparation of Pollen for SEM – Pollen samples were taken from 12 species (*Crepis albida*, *C. biennis*, *C. dioscoridis*, *C. foetida*, *C. hypochaeridea*, *C. lapsanoides*, *C. leontodontoides*, *C. paludosa*, *C. pulchra*, *C. sancta*, *C. tectorum*, and *C. vesicaria*). Prior to coating samples were treated by acetolysis following Erdtman (1960) to avoid artefacts by the protoplast. After acetolysis pollen grains were suspended in ethanol and the pollen surface was cleaned from debris in an ultrasonic bath. The pollen suspension was transferred onto a 14mm cover slip mounted on an SEM stub, and left to dry. The samples were coated with gold and studied with a LEO Supra 55VP.

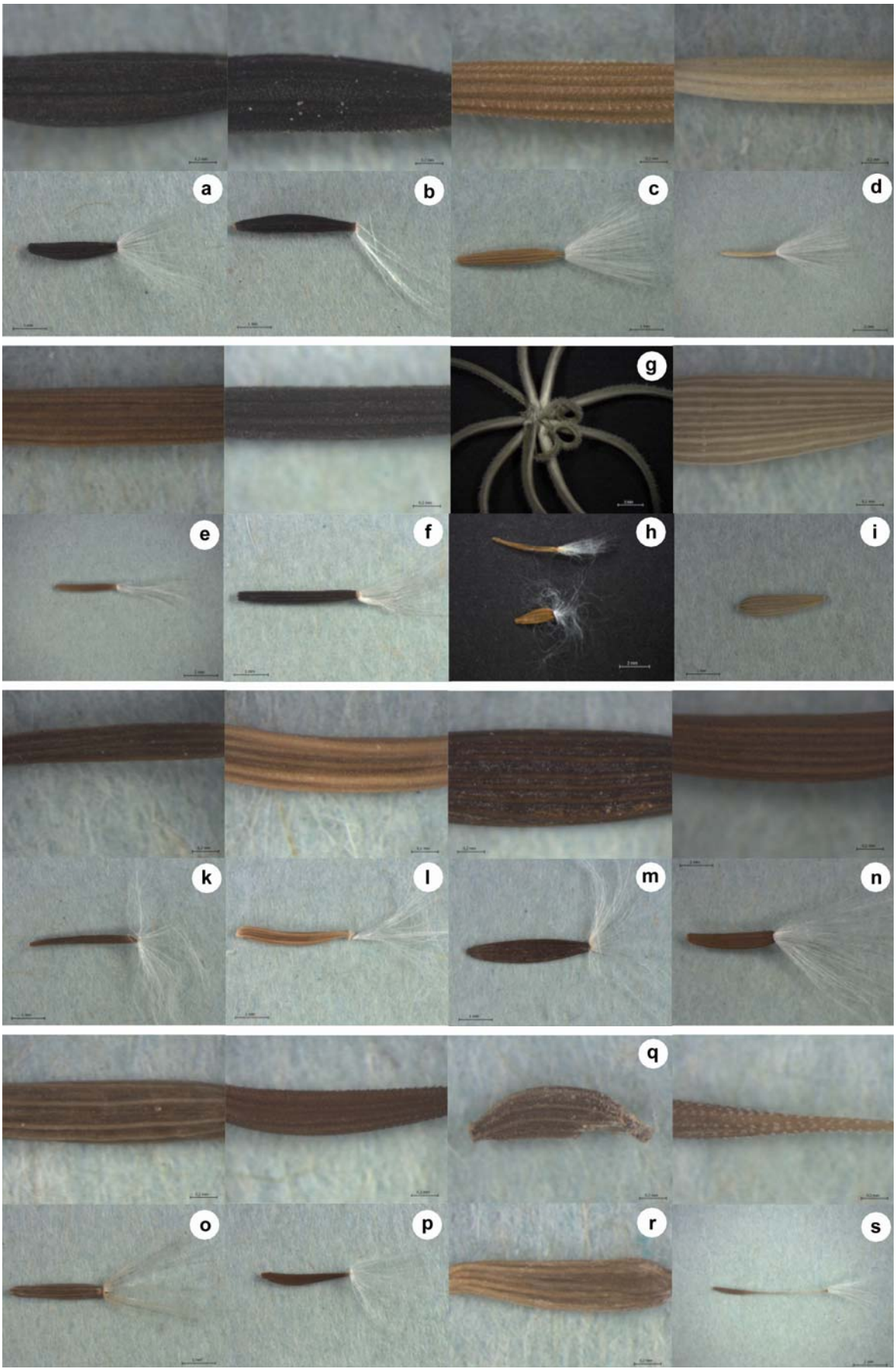

FIG.1 (continued p.60)

Preparation of Other Samples for SEM – Samples (10 species, *Askellia nana*, *Crepis bungei*, *C. capillaris*, *C. kerneri, C. lapsanoides*, *C. leontodontoides*, *C. pulchra*, *C. sancta*, *C. tectorum*, *C. zacintha*) for SEM were fixed in FAA (5 ml formaldehyde solution (min. 35%), 15 ml glacial acetic acid, 20 ml Ethanol (96%), and 60 ml Aqua dest.) for 2x 24h. Dehydration of the object was facilitated by a subsequent treatment of ascending Ethanol dilutions (70%, 80%, 90%, 96% and 2x 100%). The samples remained in each step for at least 1h. Then the samples were treated with acetone (100%) twice for 1h. The samples were transferred to the Critical Point Drier K 850 (EMITECH) for final desiccation. Then the objects were mounted on aluminium stubs and coated with gold/palladium (layer thickness 20nm) in a Low Voltage Cool Sputter Coater K 550 (EMITECH). The specimen stubs were studied with a Philips SEM 515. The objects studied were pappus bristles, papillae on the inside of style branches.

4.3 RESULTS

Results are summarised in Table 1. Missing data was complemented by values from literature as far as possible. Sources are given in Table 1.

Achene Morphology

Achenes of the 23 sampled Crepidinae species are either terete or fusiform, unbeaked, attenuate, or beaked and in most cases straight with no or 8-20 ribs. Few achenes are striate. Size ranges between 1.3 and 7mm. Colour varies from light gold brown to black (Fig.1).

***Youngia* –** Achenes are black with lighter spicules, terete and have up to 12 ribs. Size varies between 2.6 mm (*Y. tenuifolia*) and 3.7 mm in *Y. tenuicaulis*.

***Askellia* –** Achenes are fairly uniform in size (3.5-3.7 mm) and shape (terete). The fruits are smooth, and unbeaked with 8-10 ribs, except for *A. flexuosa*, where achenes are fusiform, attenuate and spicate. The prevailing achene colour is light brown, the only exception to that is *A. elegans*, which has black achenes.

***Lagoseris* group, *Lapsana,* and *Rhagadiolus* –** Within sampled genera of the Crepidinae the achenes of *Lapsana* and *Rhagadiolus* are distinct from all others by gross morphology: *Lapsana* and *Rhagadiolus* both possess no pappus in contrast to the barbellulate bristles of all *Crepis* species. The fruits of *Lapsana* are 2.6 mm in average, flattened, broadly fusiform, and light brown with twenty prominent lighter ribs with a smooth surface. The achenes of *Rhagadiolus* are fusiform and between 4.7mm and 7mm long. The outer achenes are

enclosed in the involucral bracts, stellately outspread and smooth. The inner achenes are strongly curved, yellowish white, with few shallow but strongly spiculate ribs. The fruits of the five *Crepis* species of the *Lagoseris* clade are heterogeneous: size ranges between 2.5-5 mm, the shape is terete, attenuate or biform, with colours being light brown or brown with a smooth, rugulose, or spiculate surface. Number of ribs varies between 10 and 12, except for *C. sancta* where achenes are striate. *C. multicaulis* and *C. sancta* both have conspicuously soft and bendable pappus bristles.

***Crepis* s.st. –** *C. lapsanoides* of Clade I has a broadly fusiform, unbeaked and light brown achene with ca. 17 smooth ribs. Size is 3.4-3.7mm. The other analysed species of Clade I, *C. lyrata*, has terete, slightly curved, light brown achenes of 2.6-2.8mm length with ca. 18 smooth ribs. In Clade II the achenes of *C. tectorum* and *C. paludosa* share no similarity except size (2.6-2.9mm). The first species has dark brown, fusiform, and spiculate achenes. The latter possesses strongly terete and brown achenes, with few prominent, lighter and smooth ribs. In clade III *C. pusilla* and *C. zacintha* both have comparatively small (1.3-1.8 mm), slightly curved and pubescent achenes. *C. foetida* differs from these two species in the biform achenes, which can be strongly beaked. Size is ca. 7mm. The only sampled species of clade IV, *C. bocconi*, has terete, attenuate, and brown achenes of 5.6mm length with 18 smooth ribs. The Central Asian species of Clade VII (*C. chrysantha*, *C. bungei*, and *C.*

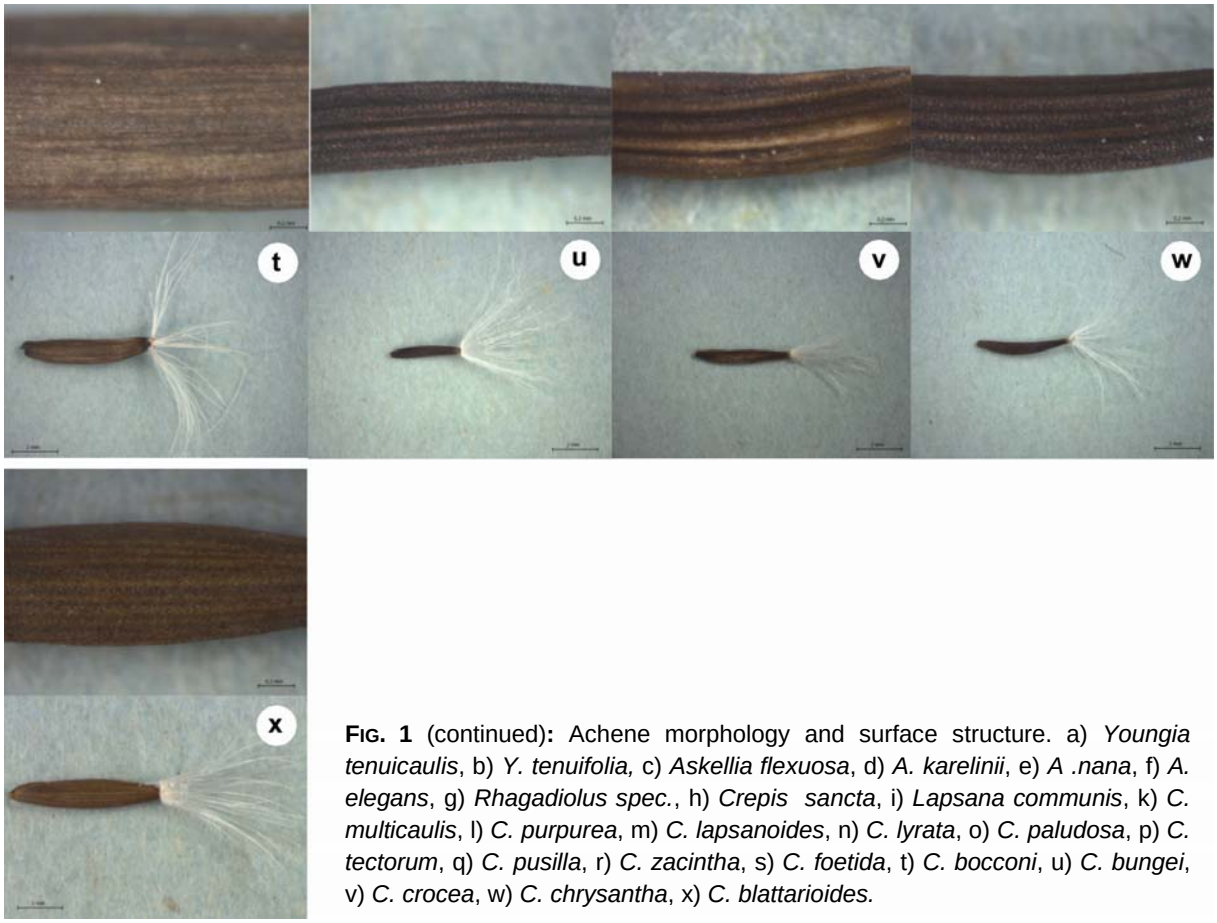

FIG. 1 (continued)**:** Achene morphology and surface structure. a) *Youngia tenuicaulis*, b) *Y. tenuifolia,* c) *Askellia flexuosa*, d) *A. karelinii*, e) *A .nana*, f) *A. elegans*, g) *Rhagadiolus spec.*, h) *Crepis sancta*, i) *Lapsana communis*, k) *C. multicaulis*, l) *C. purpurea*, m) *C. lapsanoides*, n) *C. lyrata*, o) *C. paludosa*, p) *C. tectorum*, q) *C. pusilla*, r) *C. zacintha*, s) *C. foetida*, t) *C. bocconi*, u) *C. bungei*, v) *C. crocea*, w) *C. chrysantha*, x) *C. blattarioides.*

crocea) have slender achenes of (dark) brown colour with prominent spiculate or rugulose ribs. The fruits are terete or fusiform, unbeaked to attenuate and between 3.1mm and 6.9mm long. *C. blattarioides* of Clade XI has fusiform, attenuate, and brown achenes of 3.9-4.3mm length with 18 smooth ribs.

Achene Anatomy

In anatomical contexts ribs are referred to as costae. The achenes are normally of rounded outline with (8) 10-12 (20) costae made of sclerenchymatous cell bundles. The exocarp is one-layered with a thick outer cell wall, but the cells can be collapsed. Parenchymatic regions might or might not be present in the mesocarp between costae or between costae and testa. Endocarp is two layered and collapsed (Fig.2b). Four different achene types could be found in *Crepis* s.l. (Fig.2).

Type Ia – Achenes are of a rounded outline. The cells of the exocarp have thick outer cell walls but are (partly) collapsed. The 10-12 costae are far apart with distinct intercostal areas where parenchyma cells are partly collapsed. No intercostal sclerenchymatous cells are present.

Type Ib – Similar to Ia, except that intercostal parenchyma cells are well developed. 3-6 layers of protoplastic parenchyma cells present in the mesocarp between the testa and the costae.

Type Ic – This type has no distinct costae. Sclerenchymatous islands are embedded in the parenchymatous cells of the mesocarp. No intercostal sclerenchyma is found. The outer walls of the one layered exocarp are only slightly thickened.

Type II - The achenes are of round outline, with 8-12 pointed costae. Parenchyma is well developed beneath the sclerenchyma of the costae, but often collapsed in the intercostal regions. The sclerenchyma builds a band in the intercostal areas.

Type III - Achenes are +/- circular in outline. The costae are (weakly) prominent, intercostal regions are mostly made up of 1-6 cell layers. Intercostal sclerenchymatous cells are present.

Type IV – Exocarp can be collapsed. Costae are very prominent with deep or no intercostal furrows. Sometimes 3-6 layers of protoplastic parenchymatous cells are found between testa and sclerenchyma but never between the sclerenchymatous islands of the costae. Even though the costae seem to merge in some cases, they are always separated by a layer of collapsed parenchyma cells or intercostal furrows.

***Askellia* –** Both sampled species (*A. flexuosa* and *A. nana*) have Type Ia achenes.

***Lagoseris* group and *Rhagadiolus* –** Except for *C. purpurea* (Type VI) and *C. praemorsa* (Type Ia) with deep intercostal furrows, the other 4 sampled species of the *Lagoseris* group as well as *Rhagadiolus* are of Type III. The exocarp is one layered with thick outer cell walls (intermediate in *C. sancta* and *C. multicaulis*) and bearing protruding elements, except for C. *incarnata*. *Rhagadiolus spec.* and *C. sancta* bear long cellular appendages.

***Crepis* s.str. –** Both species of Clade I feature achenes of Type I. *C. mollis* has achenes of Type Ic, whereas *C. lapsanoides* of Type Ib, the most frequent type. In Clade II *C. tectorum* is Type Ib and *C. paludosa* a Type II. *C. kerneri*, however, has to be considered intermediate between Type Ib and II, as it has slightly pointed costae but no intercostal sclerenchyma. In Clade III *C. zacintha* shows a distinct achene anatomy: it is of angular outline and a Type IV where costae are nearly merged. *C. foetida* is Type Ib. *C. acuminata* and *C. chondrilloides* of Clade V are Type II respectively Type Ia. In Clade VI only *C. neglecta* as Type Ia has been sampled. *C. biennis* (Clade VII) is Type Ib as is *C. albida* of Clade IX. *C. leontodontoides* of Clade VII is Type IV; costae are nearly merged as in *C. zacintha*. *C. capillaris* (Clade XI) is Type Ib and achene anatomy shows high similarity to *C. tectorum*.

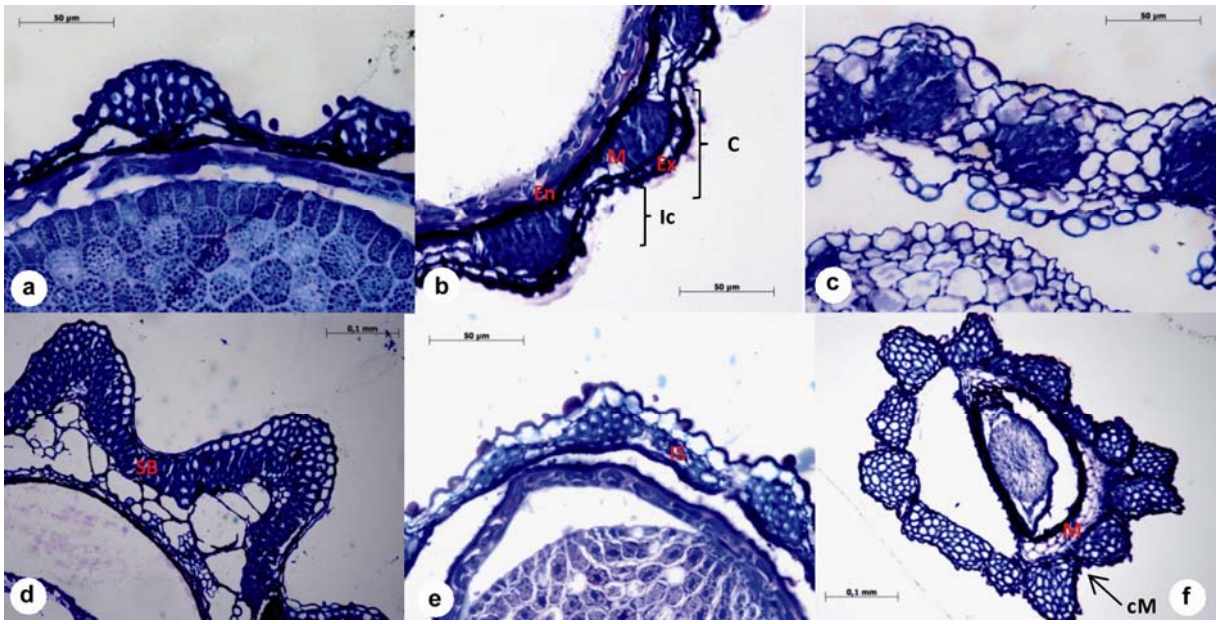

FIG.2: Achene cross sections stained with toluidine blue. LM. a) Typa Ia, *A. nana* b) Type Ib, *C. foetida* c) Type Ic, *C. mollis* d) Type II, *C. acuminata* e) Type III, *C. multicaulis* f) Type IV *C. zacintha*. cM collapsed, C costa, En endocarp, Ex exocarp, Ic intercostae, IS intercostal sclerenchyma, M mesocarp, SB sclerenchymatic band.

Pappus Bristles

Pappus bristles vary in diameter, number of cells they are composed of, and prominence and frequency of spikes. The different pappus bristles are shown in Fig.3.

***Askellia* –** *A. nana* has pappus bristles made up of 6-8 cells in diameter. The 1-4 spikes per 100µm are adherent to the bristle, thus the pappus bristles are smooth. The diameter is 30-32µm.

***Lagoseris* group –** The pappus bristles of the sampled species of the *Lagoseris* clade are fine (only 10-15µm in diameter, especially in *C. sancta*) and made up of 2-3 cells. The spikes are very far apart (0-2 per 100µm) but sticking out prominently.

***Crepis* s.str. –** *C. lapsanoides* of Clade I has very spiky pappus bristles (6-7 spikes/100µ) and the bristles are comparatively strong (19-20µm, 4-6 cells). *C. kerneri* (Clade II) has very coarse pappus bristles (35-37µm, 6-7 cells) with prominent spikes. In *C. tectorum* (Clade III) the spikes are pointed, sharp and long, the bristles are slender (14-15µm, 3-4 cells). *C. zacintha*, also of Clade III, has shorter spikes but similarly slender bristles (15-16µm, 4-5 cells). In Clade VII *C. bungei* has the strongest bristle of all sampled species (37-40µm, 6-7cells), but only comparatively few adherent spikes (3-4 spikes/100µm). In *C. leontodontoides* (Clade VII) the spiky pappus measures 20-22µm and is made up of 3-5 cells. The pappus of *C. capillaris* (Clade XI) is one of the finer ones within *Crepis* s.str. (16-17µm, 4-5 cells). It has 3-4 spikes per 100µm.

Pollen Morphology

Terminology is according to Blackmore (1984). All sampled species have echinolophate pollen of the *Cichorium intybus* type. 5 species fall into the *Cichorium intybus* subgroup (*Crepis albida*, C*. dioscoridis*, *C. lapsanoides*, *C. paludosa*, *C. pulchra*), 7 species into the *Taraxacum officinalis* subgroup (*C. biennis, C. foetida, C. hypochaeridea, C. leontodontoides, C. sancta, C. tectorum*) (Fig.4). Grain size ranges from 25-42µm.

Style Branch Papillae

The papillae showed some variation and three types could be identified (Fig.5). The first type, Type A, is a slender (7.5-10µm) papilla, the sides are nearly parallel and gradually narrow into a tip. Type B is narrow at the base, widens in the middle (11-20µm) and narrows from the widest point in a straight line to the tip. The median widening in Type C is more distinct (19-25µm) and the narrowing towards the tip is incurved rather than straight. Type B is the most frequent type (5 species: *Askellia nana*, *Crepis bungei*, *C. capillaris*, *C. kerneri*, and *C. pulchra*). Type A (*C. leontodontoides*, *C. zacintha*) and Type C (*C. lapsanoides*, *C. tectorum*) are found in 2 species each.

4.4 DISCUSSION

In achene morphology *Askellia* and both sampled *Youngia* species differ mainly in colour and outline, as the fruits of the *Youngia* species are more rounded and black, whereas the *Askellia* achenes are gold brown and columnar; except for *A. elegans*, where the achene is very similar to *Youngia* in their dark colour with spicules. *A. elegans* is restricted to North America, so no distributional overlap with the strictly Asian *Youngia* species is given. Achene anatomy in *Youngia* (sec. *Desiphylum* = *Crepidifolium*) differs from the presently studied *Askellia* species (Type Ia, Fig.2): *Youngia* features no clear furrows between costae (Sennikov & Illarionova, 2008), and therefore resembles Type Ic. The achene cross section of *A. elegans* published by Pak (1993) is of Type Ia. However, according to Pak (1993) variation within the genus *Askellia* is high and as the cross section given there for *A. nana* differs from the present findings. Character continuity within and between species should be investigated in further analyses on additional samples.

Of the here studied morphological aspects the only character uniquely found in *Askellia* is the thick pappus (Ø30-32µ) with a smooth surface (Fig.3).

Achene and pollen features of *Lapsana, Rhagadiolus,* and the five *Crepis* sections (*Intybellia*, *Phaecasium*, *Lagoseris*, *Microcephalum*, and *Pterotheca*) are by far more heterogeneous than in *Askellia*.

In fruit anatomy Type III achenes are uniquely present in the *Lagoseris* clade. It is not the only type found in the *Lagoseris* group though; Type Ia (*C. praemorsa*) and Type IV (*C. purpurea*) are found as well (table 1, Fig.2). Regarding the pollen types both *Cichorium intybus* and *Taraxacum officinale* subtypes are found in the *Lagoseris* group. The structure of the pappus bristles differ to *Crepis* s.str. Both sampled species (*C. pulchra, C. sancta*) of the *Lagoseris* group have few celled, fine (10-15µm) and very soft pappus bristles, which in its smoothness (0-2 spikes/100µ) are unique in all sampled species (Fig.3).

Within *Crepis* s.str. the significance of presently tested microcharacters varies strongly. General trends of the variability of microcharacters are discussed on exemplary groups. Species affiliation to clades and sections is given in table 1.

First are the species of section *Zacintha*, namely *C. pusilla* and *C. zacintha* (Clade III); their peculiar morphology is reflected not only in a close molecular relation (Enke & Gemeinholzer, 2008) but also in the presently studied characters: Fruit morphology, fruit anatomy and style branch papillae, which show unique characters among tested taxa of *Crepis* s.str., except the similarity to *C. leontodontoides* (table 1). *C. zacintha* and *C. leontodontoides* show resemblance in style branch papillae; both species have Type A papillae. *C. pusilla* and *C. zacintha* have by far the smallest achenes in the genus (Fig.1). In the case of section *Zacintha*, molecular results, morphology and microcharacters agree.

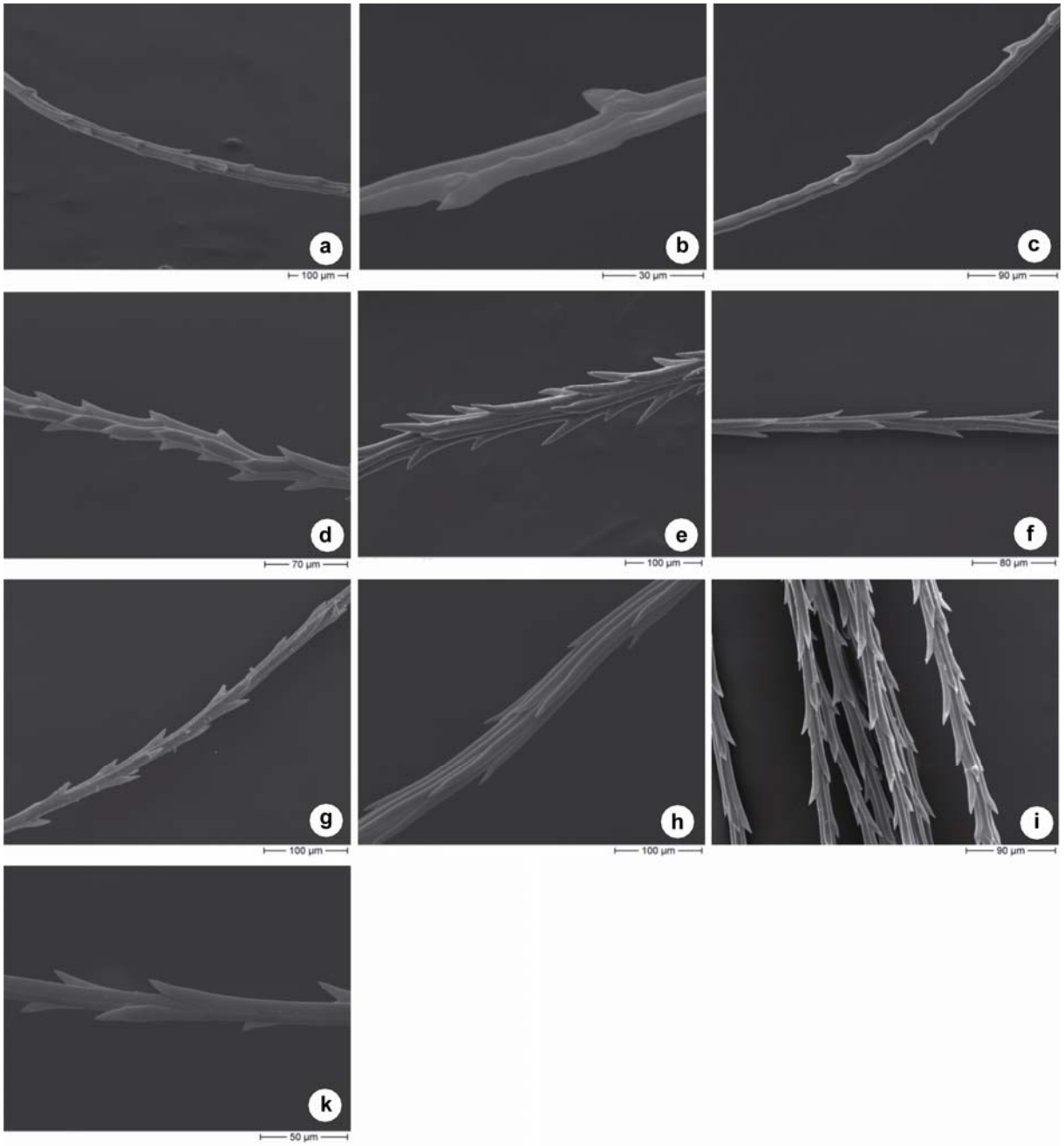

FIG. 3: Pappus bristles. SEM. a) *A. nana.* b) *C. sancta.* c) *C. pulchra.* d) *C. lapsanoides.* e) *C. kerneri.* f) *C. tectorum.* g) *C. zacintha.* h) *C. bungei.* i) *C. leontodontoides.* k) *C. capillaris.*

Second are the Central Asian species of Clade VII: *C. chrysantha*, *C. crocea*, and *C. bungei*; even though the species in clade VII are from various sections (*Brachypodes*, *Macropodes*, and *Mesophylion*, respectively) they show morphological similarity. For the three sampled species this is also reflected in their achene morphology (Fig.1). *C. bungei* and *C. oreades* are thought to be the putative diploid parents of tetraploid *C. crocea* (Babcock, 1947b). *C. chrysantha* is thought to be one of the putative parents of *C. polytricha* (Babcock, 1947b). The “hybrid” complex is obviously closely related, and needs a new systematic treatment.

Two species from different clades as well as different sections show resemblance in their fruit anatomy *C. tectorum* (Clade II, sec. *Mesophylion*) and *C. capillaris* (Clade XI, sec. *Phytodesia*), both species have highly similar Type Ib achenes.

The distinctiveness of the North American species of section *Psilochaenia* is mirrored in fruit morphology and anatomy. The ribs are very prominent and distinctly pointed in *C. acuminata* (Fig.2d), which has not been observed in any of the other species.

The presently studied *C. vesicaria* (sec. *Lepidoseris*) specimen has pollen of the *Cichorium intybus* subtype. According to Blackmore (1984) *C. vesicaria* has pollen of the *Taraxacum officinale* subtype (Table 1). As *C. vesicaria* with at least eight subspecies (Babcock, 1947b) is a highly polymorphic species, pollen type constancy throughout the (sub)species might not be given and should be further investigated.

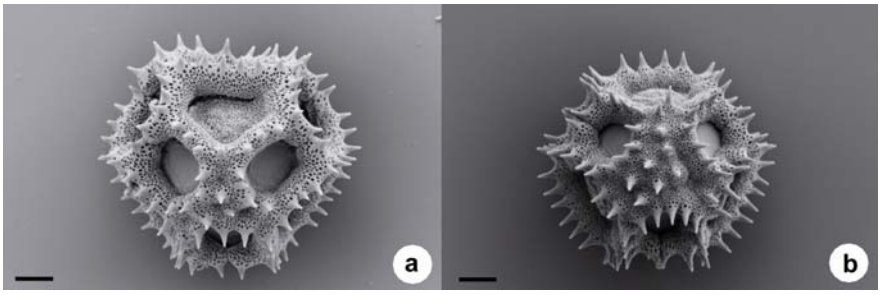

FIG.4: Pollen grain types. SEM. a) *Cichorium intybus* group, *C. foetida* b) *Taraxacum officinale* group*, C. tectorum.* Polar view. Scale 4 µm.

Systematic Usability of Tested Characters

The limitation of the presently tested characters is the sample size, which is in almost all cases too small to provide conclusive evidence for the delimitation of groups within the large and heterogeneous genus *Crepis*. However, some characters proved to be more indicative of relationships between species than others.

As studies in the Cichorieae have shown before (e.g. Pak & Kawano, 1990; Pak, 1993; Pak et al., 2001; Zhu et al., 2006; Sennikov & Illarionova, 2008) fruit anatomy is helpful to investigate infrageneric relationships. With a wider sampling of *Crepis* species a possible developmental linkage of achene types to phylogeny might provide new insights (viz. did one type of achenes derive from another). Especially for the different subtypes of Type I achenes (Fig.2) a progressive development of types could be hypothesised. Furthermore, not only cross sections of the achenes might be useful to support systematic problems but also longitudinal sections could provide additional information. Longitudinal sections of testa epidermis cells by Tegel (2002) suggested a high systematic value of this feature at generic level. The testa epidermis is less exposed to selective pressure than the pericarp and therefore more likely to carry independent characters (Grau, 1980; Tegel, 2002). However, variation within tested *Crepis* species is low (Tegel, 2002). Zarembo & Boyko (2008) came to

a similar conclusion in Cardueae where variation of testa structures is low within but helpful to distinguish between genera.

The pappus has always been an important feature to discriminate groups on all taxonomic levels in the Cichorieae (for a recent review see e.g. Kilian et al, 2008). The here presented data suggests pappus ultrastructure as possibly being discriminative at least on the generic level. Again, a larger sample size could provide evidence for a revised infrageneric classification of *Crepis* s.str.. In addition to presently tested ultrastructural characters of the pappus, other features should be included; e.g. the initiation of the pappus on the achene and the number of pappus parts per achene; the latter is genetically determined (Bachmann et al., 1981)

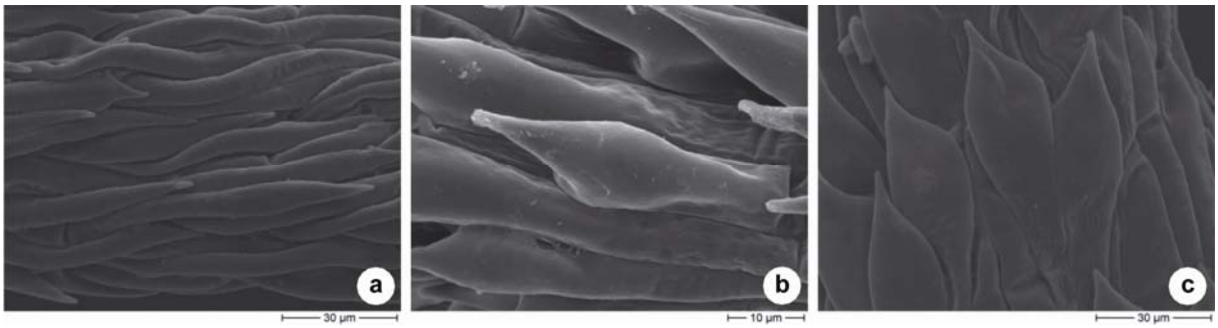

FIG. 5: Style branch papillae. SEM. a) Type A, *C. zacintha.* b) Type B*, C. pulchra.* c) Type C, *C. bungei*.

Micromorphological investigations of Torres & Galletto (2007) found stigmatic papillae within the Cichorieae to be useful characters to support systematics. Even though continuity of characters could be observed within studied species, style branch variation should be interpreted with caution. The species might not be restricted to one type of papillae. Types might vary both with location on style branches and with life stage of the plant.

Pollen analyses of the present study partly differ from those specified by Blackmore (1984) (Table 1), which formerly has been carried out with different technology and different resolution. Pollen was not informative for intergeneric delimitation within *Crepis*, at least not at the morphological level which has been assessed in the present study. Pollen ultrastructure and anatomy could provide additional information.

In conclusion, some characters feature promising variation for delimitation on sectional level but a far bigger sample size is needed in regard to species number as well as sampled individuals per species. The first is needed to test the interspecific constancy, whereas the second will give evidence on infraspecific variation respectively constancy of characters.

4.5 SUMMARY AND CONCLUSION

The microcharacters, especially the pappus, vary more between the sampled genera (*Askellia*, *Crepis*, *Lapsana*, *Rhagadiolus* and *Youngia*) than within. Within *Crepis* the variation of characters reflects either the sectional classification (Babcock, 1947b), namely in the section *Zacintha*, the groups based on molecular marker (e.g. Clade VII), or morphological and physiological similarity as in *C. tectorum* and *C. capillaris*.

The use of several achene features is considered promising for genus delimitation as well as infrageneric classification; namely anatomy and pappus ultra structure. Style branch micro morphology shows some potential as diagnostic character, but needs further investigation as does pollen morphology.

The applicability of the tested characters to the delimitation of genera and subgeneric groups is mainly impeded by sample size.

4.6 AKNOWLEDGEMENTS

The author thanks H.C. Weber, K. Dörr and M. Rath (Philipps University, Marburg), S. Blackmore, M. Watson and F. Christie (Royal Botanic Garden Edinburgh) and M. Lüchow (Botanic Garden Berlin) for technical advice, and the curators of B and E, R. Hand and B. Zimmer for providing plant material. This study is funded by the DFG (GE 1242/3-2). Synthesys provided the funding for the visit at the RBGE.

TABLE 1. Summary of Results. Species ordered for affiliation to molecular clades.

Species	Clade[1]	Section[2]	Achene Characters					Pappus		Pollen		Papilla Type
			length (mm)	color	No of Ribs	Ø	Ø (µm)	spikes/ 100µm	No of cells	Type	Ø (µm)	
Youngia tenuicaulis	*Youngia*	-	3.4-3.7	black	10-12	-	-	-	-	-	-	-
Y. tenuifolia	*Youngia*	-	2.6	black	10-12	-	-	-	-	-	-	-
Askellia flexuosa	*Askellia*	*Ixeridopsis*	3.5 (4-5.5[2])	light brown	10	Ia	-	-	-	-	-	-
A. nana	*Askellia*	*Ixeridopsis*	3.7 (4-6[2])	light brown	10	Ia	30-32	1-4	6-7	-	-	B
A. karelinii	*Askellia*	*Ixeridopsis*	3.4	light brown	10	-	-	-	-	-	-	-
A. elegans	*Askellia*	*Ixeridopsis*	3.6-3.7 (5[2])	black	10	-	-	-	-	-	-	-
Lapsana communis	*Lapsana*	-	2.6	light brown	20	-	-	-	-	-	-	-
Rhagadiolus spec.	*Rhagadiolus*	-	4.8-7.0	light brown	-	III	-	-	-	-	-	-
C. multicaulis	*Lagoseris*	*Microcephalum*	3.2-3.4 (4[2])	brown	10	III	-	-	-	-	-	-
C. pulchra	*Lagoseris*	*Phaecasium*	4-6[2]	-	striate[2]	-	14-15	0-2	2-3	C	28-34	B
C. purpurea	*Lagoseris*	*Lagoseris*	3.4 (4-5[2])	light brown	12	IV	-	-	-	-	-	-
C. praemorsa	*Lagoseris*	*Intybellia*	4.5-5[2]	light brown[2]	20[2]	Ia	-	-	-	T[3]	38-46[3]	-
C. sancta	*Lagoseris*	*Pterotheca*	2.8-4.6	light brown	3-striate[2]	III	10-11	0-2	2-3	T	28-34	-
C. lapsanoides	Clade I	*Mesomeris*	3.4-3.7 (5-6[2])	dark brown	20	Ib	19-20	6-7	4-6	C	31-37	C
C. lyrata	Clade I	*Mesomeris*	2.6-2.8 (3.5-4[2])	light brown	20	-	-	-	-	-	-	-
C. mollis	Clade I	*Mesomeris*	3-4.5[2]	brown[2]	20[2]	Ic	-	-	-	C[3]	34-38[3]	-
C. kerneri	Clade II	*Brachypodes*	-	-	-	Ib	35-37	4-6	6-7	-	-	B
C. paludosa	Clade II	*Desiphylion*	2.9 (4.5-5.5[2])	light brown	10	II	-	-	-	C	36-42	-
C. tectorum	Clade II	*Mesophylion*	2.6-2.8 (3-4[2])	dark brown	10	Ib	14-15	2-3	3-4	T(C[3])	26-32	B
C. foetida	Clade III	*Hostia*	6.9	light brown	10	Ib	-	-	-	T	26-32	-
C. pusilla	Clade III	*Zacintha*	1.3	dark brown	6	-	-	-	-	-	-	-
C. zacintha	Clade III	*Zacintha*	1.8 (2.5[2])	brown	10	IV	16-17	3-4	4-5	-	-	A
C. bocconi	Clade IV	*Soyeria*	5.6 (10-12*)	brown	18	-	-	-	-	-	-	-
C. acuminata	Clade V	*Psilochaenia*	5.5-9*	light brown*	12[2]	II	-	-	-	-	-	-
C. chondrilloides	Clade V	*Berinia*	5-7*	-	14-18[2]	Ia	-	-	-	-	-	-
C. dioscorides	-	*Brachypodes*	5.0[2]	dark brown[2]	Striate[2]	-	-	-	-	C	26-32	-
C. hypochaeridea	Clade V	*Anisoramphus*	6.5-10[2]	dark brown[2]	10-13[2]	-	-	-	-	T	31-37	-
C. neglecta	Clade VI	*Phytodesia*	2-2.5[2]	light brown	10[2]	Ia	-	-	-	-	-	-
C. biennis	Clade VII	*Berinia*	4.0-7.5[2]	light brown[2]	10-20[2]	Ib	-	-	-	T(C[3])	33-39	-
C. bungei	Clade VII	*Mesophylion*	3.1 (4-5[2])	dark brown	12	-	37-40	3-4	6-7	-	-	C
C. chrysantha	Clade VII	*Brachypodes*	4.2-6.3	dark brown	14	-	-	-	-	-	-	-
C. crocea	Clade VII (II)	*Macropus*	4.0-4.9 (5-6[2])	brown	16-(18[2])	-	-	-	-	-	-	-
C. leontodontoides	Clade VIII	*Gephyroides*	3.5-5[2]	brown[2]	10[2]	IV	13-14	2-4	2-3	T	25-31	A
C. albida	Clade IX	*Paleya*	10-17[2]	light brown[2]	15[2]	Ib	-	-	-	C	26-32	-
C. vesicaria	Clade X	*Lepidoseris*	5-9[2]	light brown[2]	10-12[2]	-	-	-	-	C(T[3])	27-33	-
C. capillaris	Clade XI	*Phytodesia*	-	light/dark brown[2]	10[2]	Ib	16-17	3-4	4-5	T[3]	34-41[3]	B
C. blattarioides	Clade XI	*Soyeria*	3.9-4.3 (5.8-8[2])	brown	18	-	-	-	-	-	-	-

[1]Enke & Gemeinholzer (2008); [2]Babcock (1947b); [3]Blackmore (1984)

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4.8 APPENDIX

Taxa Sampled for Achene Morphology

GENUS, *Taxon*, Voucher, Location.

ASKELLIA: *Askellia elegans* (Hook.) W.A. Weber, W.J. Cody, Gutteridge s.n. (B), Canada; *A. flexuosa* (Ledeb.) W.A. Weber, S. Smirnov s.n. (B), Mongolia, Altai Mountains; *A. karelinii* (M.Pop. et Schischk. ex Czer.) W.A. Weber, N. Enke, T. Dürbye & B. Gemeinholzer NE0163 (B), Russia, Kosh-Agatsh; *A. nana* (Rich.) W.A. Weber, N. Enke, T. Dürbye & B. Gemeinholzer NE0186 (B), Russia, Ulagan. **CREPIS:** *Crepis blattarioides* Vill., N. Enke NE0070 (B), France, Pyrenees; *C. bocconi* P.D. Sell, B. Zimmer 2644 (B), Austria; *C. bungei* Ledeb., N. Enke, T. Dürbye & B. Gemeinholzer NE0178 (B), Russia, Kosh-Agatsh; *C. chrysantha* (Ledeb.) Froel., N. Enke, T. Dürbye & B. Gemeinholzer NE0168 (B), Russia, Kosh-Agatsh; *C. crocea* (Lamk.) Babc., N. Enke, T. Dürbye & B. Gemeinholzer NE0167 (B), Russia, Kosh-Agatsh; *C. foetida* L., R. Hand 5263 (priv), Greece, Cyprus; *C. lapsanoides* (Gouan) Tausch, N. Enke NE0020 (B), France, Pyrenees; *C. lyrata* Froel., N. Enke, T. Dürbye, B. Gemeinholzer NE0147 (B), Russia, Ongudai; *C. multicaulis* Ledeb., N. Enke, T. Dürbye & B. Gemeinholzer NE0164 (B), Russia, Kosh-Agatsh; *C. paludosa* Moench, N. Enke NE0019 (B), France, Pyrenees; *C. purpurea* (Willd.) M. Bieb.; Steven s.n. (B), Russia; *C. pusilla* (Sommier) Merxm., R. Hand 5310 (priv), Greece, Cyprus;*C. sancta* (L.) Babc., K.H. Rechinger s.n. (B), Persia; *C. tectorum* L., N. Enke NE0076 (B), Switzerland, Vallis; *C. zacintha* (L.) Babc., R. Hand 5323 (priv), Greece, Cyprus; **LAPSANA:** *Lapsana communis* L., Willing 18929 (B), Germany; **RHAGADIOLUS:** *Rhagadiolus spec.* Juss., BG St. Gallen s.n. (B), France; **YOUNGIA:** *Youngia tenuicaulis* (Babc. & Stebbins) Czerep., N. Enke, T. Dürbye & B. Gemeinholzer NE0180(B), Russia, Kosh-Agatsh; *Y. tenuifolia* (Willd.) Babc. & Stebbins, N. Enke, T. Dürbye & B. Gemeinholzer NE0148 (B), Russia, Ulagan.

Taxa Sampled for Achene Ultra Thin Sections

GENUS, *Taxon*, Voucher, Location.

ASKELLIA: *Askellia flexuosa* (Ledeb.) W.A.Weber, Kürschner Sonnentag 01-203 (B) China, Gansu; *A. nana* (Rich.) W.A. Weber, J.A. Calder s.n. (B), Canada, Fairy Lake; **CREPIS:** *Crepis acuminata* Nutt., L.S. Rose s.n. (B), USA, California; *C. albida* Vill., R. Valdes s.n. (B), Spain, Almeria; *C. aurea* Rchb., N. Enke NE0142 (B), Austria; *C. biennis* L., N. Enke NE0143 (B), Austria; *C. capillaris* (L.) Wallr., N. Enke NE0043 (B), Spain, Pyrenees; *C. chondrilloides* Jacq., Bornmüller s.n. (B); *C. foetida* L., R. Hand 5263 (priv), Greece, Cyprus; *C. kerneri* Rech. f., N. Enke NE104 (B), Italy, Dolomites; *C. lapsanoides* (Gouan) Tausch, N. Enke NE0020 (B), France, Pyrenees; *C. leontodontoides* All., Greuter & Agababian 24457 (B), Italy, Sicily; *C. mollis* (Jacq.) Asch., Koziol s.n. (B), Poland; *C. multicaulis* Ledeb., Raab-Straube 020302 (B), Russia, Altay; *C. multicaulis* Ledeb., Dürbye s.n. (B), Kirghizia, Tien Shan; *C. neglecta* (Sm) Vierh., Nielssen s.n. (B) Greece, Etolias; *C. paludosa* Moench, N. Enke NE0019 (B), France, Pyrenees; *C. praemorsa* (L.) Tausch, van Bouggenhout s.n. (B), Italy, Bolzano; *C. purpurea* (Willd.) M. Bieb., Steven s.n. (B), Russia; *C. rubra* L., E.Willing 13378 (B), Greece, Etolia; *C. sancta* (L.) Babc., K.H. Rechinger s.n. (B), Persia; *C. sancta* (L.) Babc., J. Lambinon s.n. (B), France, Gard; *C. tectorum* L., N. Enke NE0076 (B), Switzerland, Vallis; *C. zacintha* (L.) Babc., R. Hand 5323 (priv), Greece,

Cyprus; **RHAGADIOLUS:** *Rhagadiolus stellatus* (L.) Gaertn., R. Hand 2265 (B), Cyprus; *R. stellatus.*(L.) Gaertn., W. Lang s.n. (B) Cyprus, Salamis.

Taxa Sampled for SEM (Pollen)

GENUS, *Taxon*, Voucher, Location.

CREPIS: *Crepis albida* Vill., P.F.Cannon, P.R. Crane, S.L. Jury, D.M. Moore 1023 (E), Spain, Almeria; *C. biennis* L., N.Enke NE0146 (B), Austria; *C. dioscorides* L., Raus et al. s.n. (B), Greece, Pelopones; *C. foetida ssp. Commutata* (Spreng) Babc., J.R. Edmondoson, M.A.S. McClintock E 2513 (E), Greece, Tokmakia; *C. hypochaeridea* (DC.) Thell., N.J. Devenish 1657 (E), South Africa; *C. lapsanoides* (Gouan.)Tausch, D.W. Dresser 1256a (E), Spain, Oviedo; *C. leontodontoides* All., BG Liege (B), France, Corse; *C. paludosa* (L.) Moench, M.F. Gardner, S.G. Gardner s.n. (E), Germany; *C. pulchra* L., 137-02-06-70 (B), BG Konstanz; *C. sancta* (L.) Babc., Romi s.n. (B), Italy, Siena; *C. tectorum* L. Coll., Aune Haakana s.n. (E), Finland, Nylandia; *C. vesicaria* L., N.Enke NE0016 (B), Frankreich, Pyrenees.

Taxa Sampled for SEM (Pappus Bristles, Style Branch Papillae)

GENUS, *Taxon*, Voucher, Location.

CREPIS: *Crepis albida* Vill., P.F.Cannon, P.R. Crane, S.L. Jury, D.M. Moore 1023 (E), Spain, Almeria; *C. biennis* L., N.Enke NE0146 (B), Austria; *C. dioscorides* L., Raus et al. s.n. (B), Greece, Pelopones; *C. foetida ssp. Commutata* (Spreng) Babc., J.R. Edmondoson, M.A.S. McClintock E 2513 (E), Greece, Tokmakia; *C. hypochaeridea* (DC.) Thell., N.J. Devenish 1657 (E), South Africa; *C. lapsanoides* (Gouan.)Tausch, D.W. Dresser 1256a (E), Spain, Oviedo; *C. leontodontoides* All., BG Liege (B), France, Corse; *C. paludosa* (L.) Moench, M.F. Gardner, S.G. Gardner s.n. (E), Germany; *C. pulchra* L., 137-02-06-70 (B), BG Konstanz; *C. sancta* (L.) Babc., Romi s.n. (B), Italy, Siena; *C. tectorum* L. Coll., Aune Haakana s.n. (E), Finland, Nylandia; *C. vesicaria* L., N.Enke NE0016 (B), Frankreich, Pyrenees.

Chapter 5

Guideline to a New Infrageneric System in *Crepis* L. (Compositae/Cichorieae)

ABSTRACT

The molecular analysis of the genus *Crepis* by Enke & Gemeinholzer (2008) revealed several problems concerning the systematic subdivision of the genus. The genus *Crepis* proved to be polyphyletic and split into three clades statistically well supported by molecular markers ITS and *matK*. The first group comprises most sampled species as *Crepis* s.str., the second clade species of five *Crepis* sections (*Intybellia*, *Lagoseris*, *Phaecasium*, *Microcephalum,* and *Pterotheca*) as well as the genera *Lapsana* and *Rhagadiolus*; the third group includes all species of *Crepis* section *Ixeridopsis*.

Suggestions concerning a revised infrageneric classification are made, considering both molecular and morphological evidence: the genera *Lapsana* and *Rhagadiolus* are preserved in their current generic circumscription and *Crepis* is treated as paraphyletic taxon. *Crepis* sections *Desiphylion*, *Omalocline*, *Mesomeris*, *Psilochaenia*, *Lagoseris, Hostia*, *Microcephalum*, *Pterotheca*, *Zacintha*, *Lepidoseris*, *Nemauchenes*, and *Psammoseris* are retained in their current sectional delimitation.

KEYWORDS: *Crepis*, infrageneric classification, *Lagoseris*, *Phaecasium*

5.1 INTRODUCTION

The genus *Crepis* L. with over 200 species (Bremer, 1994) is widely distributed throughout the northern hemisphere and Africa. The last revision of the genus comprises detailed descriptions of 196 species in 27 sections (Babcock, 1947a,b). Babcock was one of the first to use not only morphology and biogeography but karyotypic similarity as criterion for infrageneric relations. Babcock (1947a,b) also assumed that the sectional system reflected phylogenetic relations within the genus.

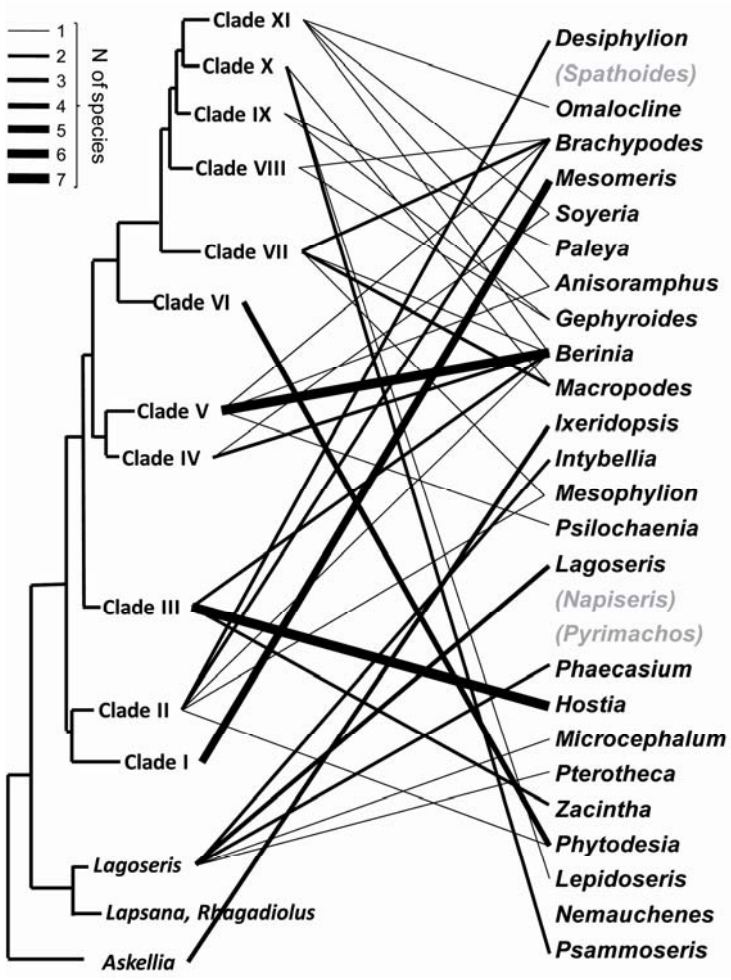

FIG.1: Distribution of Babcock's sections (right) on molecular clades (left) named following Enke & Gemeinholzer, 2008. Line width corresponds to number of species. Sections held in grey have not been sampled for molecular data.

Cichorieae, including *Crepis*, are notorious for their lack of discriminative morphological characters. Many characters vary more within a species than between closely related species and this is also true for some characters on the generic level. In the past this often led to unclear specific and generic boundaries. Molecular data can provide further insights on species relationships. But to draw taxonomic consequences from phylogenies inferred from molecular sequence data, support is needed from additional morphological, anatomical or karyological characters as names of plant species are linked to type specimen and taxa are defined by morphological character complexes.

The recent establishment of a molecular phylogeny of the genus (Enke & Gemeinholzer, 2008) based on ITS and *matK* sequence data revealed *Crepis* L. to be diphyletic. Two clades comprising *Crepis* species are statistically well supported by both nuclear and chloroplast marker (Enke & Gemeinholzer, 2008): one group comprises the (monotypic) genera *Lapsana* and *Rhagadiolus* as well as *Crepis* species from sections *Intybellia*, *Lagoseris*, *Phaecasium*, *Microcephalum,* and *Pterotheca*. The other, larger group includes the main part (ca. 80%) of

sampled *Crepis* species as monophyletic clade *Crepis* s.str. (see Enke & Gemeinholzer, 2008: Fig.1-2). The infrageneric classification of Babock (1947b) is in many cases not congruent with molecular clades, indicating that the present sections do not represent natural groups. In fact, many molecular clades comprise more than one section, whereas most sections emerge into more than one clade (Fig. 1; and Enke & Gemeinholzer, 2008: Fig.3). Some suggestions are made towards the taxonomic treatment of the *Lagoseris* clade (including the genera *Lapsana* and *Rhagadiolus*) as well as the delimitation of infrageneric groups within *Crepis* s.str. (sensu Enke & Gemeinholzer, 2008). For this Babcock's morphological, karyological and biogeographic findings are critically reassessed, and recently presented evidence) from molecular and morphological analyses are taken into account (Enke & Gemeinholzer, 2008; Enke et al., subm.; chapter 4).

5.2 DISCUSSION

A comparison of all species sampled for DNA sequence data (Enke & Gemeinholzer, 2008) in regard to Babcock's (1947b) sectional classification, the clades inferred by a molecular phylogenetic approach (Enke & Gemeinholzer, 2008), and the proposed revised sectional system are shown in table 1.

Sections *Desiphylion*, *Omalocline*, *Mesomeris*, *Psilochaenia*, *Lagoseris, Hostia*, *Microcephalum*, *Pterotheca*, *Zacintha*, *Lepidoseris*, *Nemauchenes*, and *Psammoseris* are temporarily preserved in their current sectional circumscription as neither molecular nor morphological data indicate otherwise.

Lagoseris Clade

The close relation of *Crepis* sections *Intybellia*, *Phaecasium*, *Lagoseris*, *Microcephalum*, and *Pterotheca* of the *Lagoseris* clade, and the genera *Lapsana* and *Rhagadiolus*, is statistically well supported by both nuclear and chloroplast data (Enke & Gemeinholzer, 2008). All sampled species of the five *Crepis* sections (*Intybellia*, *Phaecasium*, *Lagoseris*, *Microcephalum*, and *Pterotheca*) appear in the *Lagoseris* clade, no sectional overlap with *Crepis* s.str. could be observed (Fig.1. and Enke & Gemeinholzer, 2008). However, not only the distinct characteristics of *Lapsana* and *Rhagadiolus* but also the variation of characters within the *Crepis* species render this clade problematic.

Two of the five *Crepis* sections within the *Lagoseris* clade, namely *Lagoseris* and *Pterotheca*, are treated as genus *Lagoseris* in the Flora of the USSR (Bobrov & Tzevelev, 2000). The exclusion of both species from *Crepis* is mainly based on the presence of conspicuously long bristle-like palae on the receptacle, which can sometimes exceed the achenes (Bobrov & Tzevelev, 2000). Babcock (1947a) reported natural occurrences of individuals of *C. sancta* (sec. *Pterotheca*) lacking palae. Collins (1924) discovered that the presence and absence of palae on the receptacle is due to a very simple genetic mechanism. Furthermore, palae are

TABLE 1 (CONTINUED ON NEXT PAGE) All species sampled by Enke & Gemeinholzer (2008) ordered for sectional assignment (after Babcock, 1947b) with molecular relations and revised sectional assignment.

Species	Sectional Classification[2]	Molecular Clades[3]	Revised Classification
C. paludosa Moench	***Desiphylion***	**Clade II**	***Desiphylion***
C. viscidula Froel.			
C. pygmaea L.	***Omalocline***	**Clade XI**	***Omalocline***
C. jaquini Tausch	***Brachypodes***	**Clade II**	***Brachypodes***
C. kerneri Rech. f.			
C. dioritica Schott et Kotschy		Clade V	
C. chrysantha (Ledeb.) Turcz		**Clade VII**	*Macropodes*
C. polytricha Turcz.[1]			
C. rhaetica Hegetschw.			
C. aurea (L.) Cass		Clade VIII	***New Section II***
C. hierosolymitana Boiss.	***Mesomeris***	**Clade I**	***Mesomeris***
C. lapsanoides (Gouan) Tausch			
C. lyrata Froel.[1]			
C. mollis (Jacq.) Asch.			
C. smyrnaea DC.			
C. blattarioides (L.) Vill.	***Soyeria***	Clade XI	
C. bocconi P.D. Sell		Clade IV	
C. albida Vill.	***Paleya***	Clade IX	*Gephyorides*
C. alpestris (Jacq.) Tausch	***Anisoramphus***	Clade XI	
C. hypochaeridea (DC.) Thell.		Clade V	
C. leontodontoides All.	***Gephyroides***	Clade VIII	***New Section II***
C. tingitana Ball.		Clade IX	***Gephyroides***
C. taygetica Babc.	***Berinia***	Clade III	
C. triasii (Camb.) Fries			
C. lacera Ten.		Clade II	
C. baldaccii Halácsy		**Clade IV**	***Berinia***
C. darvazica Krasch.			
C. chondrilloides Jacq.		**Clade V**	
C. guioliana Babc.			
C. macropus Boiss. et Heldr.			
C. merxmuerlleri Kamari et Hartvig			
C. sibthorpiana Boiss. et Heldr.			
C. sonchifolia C.A. Mey.			
C. turcica Degen et Baldacci			
C. turcomanica H. Krasch.			
C. biennis L.		Clade VII	
C. oporinoides Boiss.		Clade XI	
C. auriculaefolia Sieber		?	
C. incana Sibth. et Sm.		?	
C. pannonica (Jacq.) K. Koch		?	
C. crocea (Lam.) Babc.[1]	***Macropodes***	**Clade VII**	***Macropodes***
C. oreades Schrenk			
C. hookeriana J. Ball.		Clade X	
A. flexuosa (Ledeb.) W.A. Weber	***Ixeridopsis***	***Askellia***	***Askellia***
A. karelinii (Popov. & Schischk.) W.A. Weber			
A. nana (Richards.) W.A. Weber			
C. incarnata (Wulf.) Tausch	***Intybellia***	***Lagoseris***	*Phaecasium*
C. praemorsa (L.) Tausch			
C. bungei Ledeb.	***Mesophylion***	**Clade VII**	***Mesophylion***
C. nigrescens Pohle			*?*
C. tectorum L.		Clade II	*Phytodesia*
C. acuminata Nutt.	***Psilochaenia***	**Clade V**	***Psilochaenia***
C. frigida (Boiss.) Babc.	***Lagoseris***	***Lagoseris***	***Lagoseris***
C. purpurea (Willd.) M. Bieb			
C. sahendi Boiss. et Buhse			
C. palaestina (Boiss.) Bornm.	***Phaecasium***	***Lagoseris***	***Phaecasium***
C. pterothecoides Boiss.			
C. pulchra L.			
C. alpina L.	***Hostia***	**Clade III**	***Hostia***
C. foetida L.			
C. kotschyana Boiss.			
C. rubra L.			
C. thomsonii Babc.			
C. tybakiensis Vierh.			
C. multicaulis Ledeb	***Microcephalum***	***Lagoseris***	***Microcephalum***
C. sancta (L.) Babc	***Pterotheca***		***Pterotheca***
C. pusilla (Sommier) Merxm.	***Zacintha***	**Clade III**	***Zacintha***
C. zacintha (L.) Babc.			
C. corymbosa Ten.	***Phytodesia***	**Clade VI**	***New Section***
C. cretica Boiss.			
C. fuliginosa Sibth.& Sm.			
C. neglecta L.			
C. capillaris (L.) Wallr.		**Clade XI**	***Phytodesia***
C. nicaeënsis Balb.		**Clade II**	
C. parviflora Desf.		**?**	

TABLE 1 (CONTINUED)

Species	Sectional Classification[2]	Molecular Clades[3]	Revised Classification
C. vesicaria L.	***Lepidoseris***	**Clade X**	***Lepidoseris***
C. aspera L.	***Nemauchenes***	**?**	***Nemauchenes***
C. setosa Hall.f.		**?**	
C. bellidifolia Loisel.	***Psammoseris***	**Clade X**	***Psammoseris***
C. bursifolia L.			

[1]Sequence published in Enke et al. (subm.); [2]Babcock, 1947a,b; [3]Enke & Gemeinholzer, 2008

not unique to sections *Lagoseris* and *Pterotheca*, they are also present in *C. commutata* (*Crepis* s.str., sec. *Hostia,* syn*. C. foetida* ssp*. commutata;* Babcock & Cave,1938). These findings led Babcock (1947a) to the inclusion of *Pterotheca* and *Lagoseris* into *Crepis*.

C. multicaulis (as the only representative of sec. *Microcephalum* sampled in the molecular analysis of Enke & Gemeinholzer (2008)) resembles *C. sancta* (sec. *Pterocthеca*) in some aspects of morphology and karyotype (Babcock & Jenkins, 1943), but not in the receptacular palae as they are lacking in *C. multicaulis*. *C. multicaulis* and *C. sancta* also show a close molecular relation (Enke & Gemeinholzer, 2008). However, the morphological resemblance of *C. multicaulis* to other species (e.g. the other species of sec. *Microcephalum*) is more poignant than that to *C. sancta* (Babcock, 1947b). So, to include the whole section *Microcephalum* into a putative new genus *Lagoseris* would be rather artificial, especially because all the species of *Microcephalum* lack the bristle like palae, which are one of the main delimiting characteristics of the genus *Lagoseris* sensu Bobrov & Tzevelev (2000).

C. praemorsa and *C. incarnata* of section *Intybellia* are very closely related and sometimes treated as subspecies of *C. praemorsa* (Sell, 1976). The section *Intybellia* is based on the genus *Intybellia* Monn. (non Cass.; *Intybellia* Cass. corresponds to *Crepis* section *Pterotheca* respectively the genus *Pterotheca* Cass.), and has been recombined on several taxonomic levels. There is some taxonomic overlap between the sections *Intybellia* and *Phaecasium*. Monnier (1929) included *C. pulchra* (presently in section *Phaecasium*) into *Intybellia* along with *C. praemorsa* and *C. incarnata*. Before that *C. pulchra* was included in genus *Phaecasium* as *P. lampsanoides* Cass. (Cassini, 1826) and later returned to the genus as *P. pulchrum* Benth. et Hook. by Bentham & Hooker (1873). Babcock & Jenkins (1943) would have merged the two sections into one section *Intybellia* due to their identical karyotype features if it were not for differences in root morphology. As root morphology is influenced by ecological factors (Verboom et al., 2004), it is inapt as systematically discriminating factor. The karyotypic resemblance between *C. praemorsa* and *C. pulchra* is likewise supported, if banding patterns given by Siljak-Yakovlev & Cartier (1982) for *C. praemorsa* and Dimitrova & Greilhuber (2001) for *C. pulchra* are compared. Both molecular markers (ITS, *matK*) support the close relationship of these two sections (Enke & Gemeinholzer, 2008). This would provide sufficient evidence to merge sections *Intybellia* and *Phaecasium* into *Phaecasium* Cass.

The *Crepis* species of the *Lagoseris* group differ in pappus ultrastructure from *Crepis* s.str. (chapter 4). The pollen of *Lapsana* is similar to the pollen of *Crepis* (*Cichorium intybus*

pollen, subtype *Taraxcum officinale*, chapter 4, Blackmore, 1984), but according to Osman (2006) *Rhagadiolus* has a distinct pollen type with 21 lacunae compared to only 15 in *Crepis* and *Lapsana.*

The here discussed data is ambiguous with respect to the question whether to exclude the five sections clustering within the *Lagoseris* clade from *Crepis*, or to treat *Crepis* as paraphyletic genus. The variation of characters within *Crepis* species of the *Lagoseris* clade is mostly within the range known for species of *Crepis* s.str. Chemosystematical evidence shows that *C. multicaulis* and *C. pulchra* (as representatives of the *Lagoseris* group) are very similar in the composition of their phytochemical compounds to the other 21 sampled *Crepis* species (all belonging to *Crepis* s.str.), whereas *Lapsana* differs (Zidorn, 2008). For all characters which have been used to accept the species of sections *Intybellia*, *Phaecasium*, *Lagoseris*, *Microcephalum*, and *Pterotheca* as separate genera *Lagoseris* (including *Lagoseris*, *Pterotheca* and possibly *Microcephalum*) and *Phaecasium* (including *Intybellia* and *Phaecasium*) equivalent character states could be found within *Crepis* s.str.. *Lapsana* and *Rhagadiolus* differ from all *Crepis* species in achene features and the latter distinctly in pollen type. As has been shown by Tegel (2002), the cell wall structure of the testa epidermis in the achenes is fenestrate in *Lapsana* and *Rhagadiolus*; whereas it is unstructured in all sampled *Crepis* species except for *C. biennis* (Tegel (2002) sampled *C. sancta* and *C. pulchra* of the *Lagoseris* group). Conclusively, no argument could be found to encourage an exclusion of the species of the *Lagoseris* group from *Crepis*; neither could any convincing argument be found to merge *Lapsana* and *Rhagadiolus* into *Crepis*.

As the discussed characters allow no palpable decision whether to exclude the relevant sections from *Crepis*, it is proposed to preserve the current generic circumscription of *Crepis*, even though it would be paraphyletic from a molecular point of view, until further evidence emerges. To expand the generic description of *Crepis* to include *Lapsana* and *Rhagadiolus* seems inappropriate given the morphological distinctness of both genera. Furthermore, the phylogeny of *Crepis* s.l. (Enke & Gemeinholzer, 2008) might reflect a more complex evolutionary history than can be drawn from dichotomous branching patterns of phylogenetic trees, so further analyses and investigations are still needed.

Crepis s.str.

Some of Babcock's sections are retained in their present circumscription (e.g. section *Mesomeris*), while others are partly reorganised (e.g. *Phytodesia*). Several species are relocated to other sections or left for further consideration.

The Central Asian species of Clade VII (*C. bungei, C. chrysantha*, *C. crocea*, and *C. polytricha*) are obviously related as the species are similar in morphological, karyological and molecular characteristics (Enke & Gemeinholzer, 2008, Enke et al., subm.; chapter 4). Two

additional species in Clade VII which share morphological and molecular similarities are *C. oreades* and *C. rhaetica* (Enke & Gemeinholzer, 2008). *C. bungei* and *C. oreades* are considered to be the putative diploid parents of tetraploid *C. crocea*, whereas *C. chrysantha* is suspected to be one of the parents of tetraploid *C. polytricha* (Babcock, 1947b). Popov (1957-59), on the other hand considered *C. bungei*, *C. crocea* and *C. polytricha* to have arisen from a hybridisation between *Youngia tenuifolia* and *C. chrysantha*. The species of this "hybrid complex", however, belong to different sections: *C. chrysantha*, *C. polytricha* and *C. rhaetica* belong to section *Brachypodes*, *C. bungei* to section *Mesophylion,* and *C. oreades* and *C. crocea* to section *Macropodes*. *C. bungei* and *C. oreades* are the type species for their sections.

Section *Brachypodes* is heterogeneous. Seven out of ten species in this section have been sampled for DNA sequence data; three species (namely *C. chrysantha*, *C. polytricha* and *C. rhaetica*) cluster in Clade VII, whereas the four others clustered in three different clades: *C. jacquini* and *C. kerneri* both in Clade II, *C. dioritica* in Clade V and *C. aurea* in Clade VIII (Enke & Gemeinholzer, 2008). *C. chrysantha* and *C. polytricha* have a basic chromosome number of x = 4, *C. jacquini* and *C. aurea* have x = 5. The chromosome numbers of *C. rhaetica* and *C. dioritica* are unknown.

Section *Mesophylion* includes four species, *C. bungei*, *C. ircutensis, C. nigrescens* and *C. tectorum*. In the Flora of the USSR (Bobrov & Tzevelev, 2000) *C. bungei* and *C. ircutensis* are both treated as *C. bungei*. *C. bungei* and *C. tectorum* show a distant relation in the matK analysis, but cluster in completely different clades in the nuclear phylogeny; *C. bungei* in Clade VII and C. tectorum in Clade II (Enke & Gemeinholzer, 2008). Morphologically *C. nigrescens* is very similar to *C. tectorum* but differs from it in the type of pubescence on the stem and involucrum as well as a larger and darker corolla (Bobrov & Tzevelev, 2000). In the molecular based phylogeny it is, however, sister to *C. bungei* (Enke & Gemeinholzer, 2008). *C. bungei* and *C. tectorum* share a karyotypic similarity (Babcock, 1947b). Microcharacters, however, indicate some relation of *C. tectorum* to *C. capillaris* (sec. *Phytodesia*, Clade XI; Chapter 4).

C. oreades and *C. crocea* from section *Macropodes* are the only species in the section with a Central Asian distribution, whereas all other species are of Mediterranean or African distribution. The only other member of section *Macropodes* sampled for DNA sequence data is *C. hookeriana*, a Northwest African species, found in Clade X (Enke & Gemeinholzer, 2008) and shows alliances to species centred in NW African/SE Spain, e.g. *C. dianthoseris*, *C. albida*, *C. tingitana* and *C. oporinoides*, and beyond in the Mediterranean and S Europe to species such as *C. vesicaria* and *C. alpestris* (Enke et al., 2008).

For the species of the "hybrid complex" it is proposed, with all reservations in respect to further morphological and karyological studies, to transfer *C. chrysantha*, *C. polytricha* and *C. rhaetica* to section *Macropodes* and to leave *C. bungei* in section *Mesophylion*. Section

Brachypodes needs critical reassessment, as molecular data (Enke & Gemeinholzer, 2008) indicates that section *Brachypodes* is polyphyletic (table 1).

As the relations between the species within the “hybrid complex” and the relation of the “hybrid complex” to other species such as e.g. *C. tectorum* and *C. nigrescens* are very complex they need further careful investigation. Interestingly, the tetra-/octoploid species *C. biennis* (sec. *Berinia*) clusters within Clade VII as well (Enke & Gemeinholzer, 2008), so all polyploid species sampled for ITS sequence data (*C. biennis*, *C. crocea* and *C. polytricha*) are found in clade VII the “hybrid complex”. Genetical and cytological characterisation of the species would be the method of choice as morphology in this case is hard to interpret in regard to the hybridogeneous origin of at least some of the species.

As mentioned above *C. tectorum* shows similarity to C*. capillaris*. The two species feature gross morphological similarities, but have been placed in different sections as the seed of *C. capillaris* show a longer viability (Babcock, 1947b). The genetical investigations of Hollingshead (1930a,b) suggest some relation between these two species. Fruit anatomical similarities could, however, reflect convergent evolution. These two polymorphic species share some similarity, such as their annuality, their wide distribution and some gross morphological congruencies with two other species, namely *C. nicaeënsis* and *C. parviflora* (both sec. *Phytodesia*). *C. tectorum* is sister to *C. nicaeënsis* in the ITS phylogeny (Clade II), whereas *C. tectorum* and *C. parviflora* cluster together in the chloroplast based phylogeny (Enke & Gemeinholzer, 2008). After further careful morphological consideration and the discussion of the above mentioned objections, it might appear reasonable to transfer *C. tectorum* to sec. *Phytodesia*, of which *C. nicaeënsis* is the type species.

Clade VI partly reflects the relations Babcock (1947b) assumed for section *Phytodesia*. The *C. neglecta* complex comprises in addition to *C. neglecta* (including subspecies) the species *C. fuliginosa* and *C. cretica*. The closer relation within these species than to the others of same section is also reflected in their karyotypes (Babcock & Jenkins 1943). Cytological studies by Tobgy (1943) and Kamari (1976) demonstrated the close relation within this complex. Clade VI can be considered to be equivalent to the *C. neglecta* complex. The type species of *Phytodesia*, however, is *C. nicaeënsis* of Clade II, so a new section for this group is necessary.

Most species of section *Berinia* sampled for DNA sequence data cluster in Clades IV and V (table 1 and Enke & Gemeinholzer, 2008). Section *Berinia*, the biggest section in Babcock’s (1947) monograph of the genus *Crepis*, is divided into four subsections: *Corymbiformae*, *Subcorymbiformae*, *Divaricatae* and *Strictae*, which are however not reflected by molecular data (Fig.3 in Enke & Gemeinholzer, 2008). The species of this large and heterogeneous section need careful further consideration. The type species of section Berinia is *C. chondrilloides* that clusters within Clade V, so it is assumed that Clade V and IV correspond to section Berinia.

Clade VII includes only *C. aurea* (section *Brachypodes*) and *C. leontodontoides* (section *Gephyroides*). Both species have similar and fairly small karyotypes (Babcock & Jenkins, 1943). The two species have been considered to be closely related before, but have been placed into different sections due to differing root morphology (Babcock & Jenkins, 1943; Babcock 1947b). Avery (1930) reported viable hybrids between these two species. Their close relation is likewise well supported by nuclear as well as chloroplast data (Enke & Gemeinholzer, 2008). *C. aurea* and *C. leontodontoides* should be placed into one section, not necessarily in section *Brachypodes*, as both species show considerable differences in the karyotype to other species of this section (Babcock & Jenkins, 1943). Section *Gephyroides*, however, is not suited to include *C. aurea* and *C. leontodontoides*, as *C. tingitana*, whose sister taxon in the molecular phylogeny is *C. albida* (section *Paleya*), is the type species of section *Gephyroides* and differs morphologically as well as cytologically from both *C. aurea* and *C. leontodontoides*.

Because *C. elymaitica* (type species of section *Paleya*) has to be reconsidered as member of the genus *Crepis* (unpublished molecular data) the morphologically heterogeneous section *Paleya* should not be maintained. The close association of *C. albida* (section *Palyea*) to *C. tingitana* (sec. *Gephyroides*) based on molecular data (Enke & Gemeinholzer, 2008) could, after further morphological investigation, legitimate a transfer of *C. albida* to section *Gephyroides*.

The species not discussed (no revised sectional assignment in table 1) are of unclear relations within the genus and need further consideration.

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5.3 SUMMARY AND CONCLUSIONS

In the present study and with regard to data published in various other studies the integrity of the genera *Lapsana* and *Rhagadiolus* from *Crepis* s.l. has been demonstrated. No characters could be found for the species of the five sections *Intybellia*, *Phaecasium*, *Lagoseris*, *Microcephalum*, and *Pterotheca* which would justify an exclusion from *Crepis* (as either one or several genera) at this stage. Therefore, the temporary acceptance of *Crepis* as paraphyletic taxon and the conservation of *Lapsana* and *Rhagadiolus* as separate genera are suggested.

Some reclassifications of infrageneric groups within *Crepis* s.str. are proposed: the fusion of sections *Intybellia* and *Phaecasium* and the renaming of the “core group” of section *Phytodesia*. Clades I, III, IV and V are found to be largely congruent with taxonomic sections *Mesomeris* (Clade I), *Hostia* and *Zacintha* (Clade III), and *Berinia* (Clades IV, V). Furthermore, it is proposed, to assign the species of Clade VII (the “hybrid complex”) to two sections *Mesophylion* and *Macropodes.* Further genetical and cytological analyses of the

"hybrid complex" are needed to resolve the relations within the "hybrid complex" as well as to other species.

The proposed revision of the sectional classification of the genus Crepis should be treated as provisional, because it almost exclusively considers species included into the molecular analyses by Enke & Gemeinholzer (2008). Taxonomic consequences can be drawn in future projects after careful morphological and karyological studies taking more species into account, to infer the applicability of the here presented suggestions

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Chapter 6

Afroalpine *Dianthoseris* actually a congener of *Crepis* s.str. (Compositae, Cichorieae, Crepidinae)

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ABSTRACT

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The phylogenetic relationship of *Dianthoseris* is reconstructed with Maximum Parsimony and Bayesian Inference based on both nrDNA ITS and cpDNA matK datasets. The analyses revealed that the East African afro-alpine *D. schimperi* is deeply nested within *Crepis* s.str., forming a clade with the more widespread afro-alpine *C. newii*. This clade is sister-group to a clade of species centred in NW Africa/SE Spain, viz. *C. hookeriana*, *C. albida*, *C. tingitana* and *C. oporinoides*, and beyond in the Mediterranean and S Europe, viz. *C. vesicaria* and *C. alpestris*. An emended, illustrated description of *D. schimperi*, for the first time considering mature achenes, is given, its delimitation from the afro-alpine Ethiopian local endemic *Nannoseris inopinata* is re-evaluated, its morphological and karyological affinities within *Crepis* are discussed and its distribution is mapped. Two new nomenclatural combinations in *Crepis*, *C. dianthoseris* for *D. schimperi* and *C. inopinata* for *N. inopinata*, are validated.
KEYWORDS: *Dianthoseris schimperi*, *Nannoseris inopinata*, *Crepis dianthoseris*, ITS, matK, molecular phylogeny, morphology, taxonomy.

6.1 INTRODUCTION

The acaulescent East African *Dianthoseris schimperi* A. Rich. was first collected in the alpine zone of the Ethiopian Simen Mts (Puff & Nemomissa, 2001, 2005) in 1840 by the German plant collector Wilhelm Georg Schimper (Gillett, 1972) and the name validly published in 1848 for a species in a genus of its own. Since then, it was treated as a distinct genus of the Compositae tribe Cichorieae. Although Schultz Bipontinus (1866) and Chiovenda (in Pirotta, 1904) suggested its inclusion in the genera *Omalocline* (currently part of *Crepis*) and *Sonchus,* respectively, this was not followed by others. Besides, *Launaea rueppellii* (Oliv. &Hiern) Boulos, an acaulescent rosette herb confined to the upper montane zone of Ethiopia (Kilian, 1997: 107), was sometimes considered as a close relative of *Dianthoseris* (Oliver & Hiern in Oliver, 1877: 456, Hoffmann ,1890–94: 373, Chiovenda in Pirotta, 1903-04: 200). As a result the taxonomy of *D. schimperi* remained relatively stable (Fries, 1928: 162; Hedberg, 1957: 252; Jeffrey, 1966: 433; Jeffrey & Beentje, 2000: 92; Tadesse, 2004: 52).

In the 20th century *Dianthoseris* was associated with the *Launaea-Sonchus* alliance of subtribe Crepidinae s.l. by Stebbins (1953: 77), with the Sino-Himalayan *Dubyaea-Soroseris* alliance of Crepidinae s.str. by Jeffrey (1966: 430, 433–434, “but the link is by no means a close one and it remains as an isolated and specialized plant with no close affinities”) and with Crepidinae s.str. by Bremer (1994: 180) and Lack (2006: 184).

Dianthoseris schimperi was stated by Jeffrey (1988: 434) to have “the very low somatic chromosome number 4”, citing an unpublished result communicated to him by O. Hedberg. This number, the lowest within the entire Cichorieae, was subsequently repeated by Jeffrey & Beentje (2000: 92 as “2n = 4”) and Lack (2006: 185 as “x = 2”), but is obviously erroneous, because Hedberg’s own publication (Hedberg & Hedberg, 1977: 24), hitherto being the only report of the chromosome number of *D. schimperi*, instead gives 2n = 8, counted in two provenances, from the Simen Mts in Ethiopia and Mt Kenya in Kenya.

In the course of preparing a molecular phylogeny of the tribe Cichorieae, Kilian et al. (2008, in press) have also considered the position of *Dianthoseris*. Since the analyses revealed that *Dianthoseris* is nested within *Crepis* s.str., the first author, working on a molecular phylogeny of *Crepis* (Enke & Gemeinholzer, 2008, in press), elucidated its affinities within *Crepis*. The present study has aimed at (1) clarifying the relationships of *D. schimperi* within *Crepis* based on nrDNA ITS and cpDNA matK datasets, new (micro)morphological and the published karyological data, (2) re-evaluating the status of *Nannoseris inopinata*, described as a related local endemic Ethiopian species but recently considered as conspecific with *D. schimperi*, and (3) drawing from these analyses the necessary taxonomic and nomenclatural conclusions.

6.2 MATERIAL AND METHODS

Plant material - The investigation is based on field studies by the third author in Ethiopia, on herbarium material from the herbaria B, CAS, E, ETH, MSB, STU, UPS and US (abbreviations according to Holmgren & Holmgren, 1998) and high resolution images of types and other specimens accessed online via Aluka (2007).

Molecular phylogenetic analyses - 27 taxa from 6 genera of subtribe Crepidinae (*Crepis*, *Taraxacum*, *Ixeris*, *Youngia*, *Lapsana* and *Soroseris*) and one species, *Hyoseris radiata*, of subtribe Hyoseridinae were included in the analysis. Sequences for 10 taxa were obtained from herbarium specimens, the others were downloaded from NCBI (GenBank, EMBL). The relevant data for the source of the sequences are presented in Table 1.

TABLE 1 (continued next page)

Taxon	ITS	*matK*
Crepis albida Vill. subsp. *albida*	EU363606	EU363550
Crepis alpestris Rchb.	AJ633373	AJ633153
Crepis asadbarensis Bornm.	–	Iran, Prov. Mazanderan, Distr. Nur, inter Kamarband et jugum Naftab, 3200 m, 8.8.1948, *Rechinger 6445* (B), ne025
Crepis chondrilloides Jacq.	EU363593	EU363545
Crepis dianthoseris N. Kilian & al. ≡ *Dianthoseris schimperi* Sch. Bip.	Ethiopia, Wollo, Amhara, Mt Abune Yosef, Peak Guli Bamba (4284 m), 4000-4200 m, 27.11.2001, *Ortiz & Vivero 26* (ETH 74116), NK155	id.
C. foetida subsp. *afghanistanica* Babc. ≡ *Crepis trichocephala* (Krash.) V.V. Nikitin	EU363604	Afghanistan, Prov. Baghlan, Surkhab-Tal, 6 km NO Dahane Eshpushta, 1300 m, *Podlech 18361* (MSB 01615), ne122
Crepis foetida L. subsp. *foetida*	EU363619	EU363556
Crepis hierosolymitana Boiss.	EU363602	EU363543
Crepis hookeriana Ball	EU363605	EU363549
Crepis hypochaeridea Thell.	EU363617	–
Crepis incarnata Tausch	EU363608	AJ633151
Crepis macropus Boiss. & Heldr.	EU363589	EU363577
Crepis mollis Asch.	AJ633380	EU363538
Crepis neglecta L. subsp. *neglecta*	EU363610	EU363553
Crepis newii subsp. *oliveriana* (Kuntze) C. Jeffrey & Beentje ≡ *C. oliveriana* (Kuntze) C. Jeffrey	Tanzania, Arusha region, Mt Meru, c. 3450 m, 4.9.1966, *Stein W. Bie* (UPS) [2n = 8] , ne032	id., ne032; Cameroon, Mt. Cameroon, above Buea, near upper forest limit above Mann's spring; Lat/long; 04:10N, 09:13E, Alt: 2200-2300m, *Hedberg* (UPS), ne036
Crepis oporinoides Boiss.	EU363633	EU363567
Crepis robertioides Boiss.	–	West slope of Lalaat Musa. 7800-8000ft, *Davies 9761* (E 00228145), ne259
Crepis sahendi Boiss. & Buhse	EU363651	–

TABLE 1 (CONTINUED). List of the taxa and the source of the sequences used in the molecular phylogenetic analyses. Accession numbers only are given for the sequences downloaded from GenBank/EMBL, the source of the plant material and the accession number are cited for original sequences of the present study.

Taxon	ITS	*matK*
Crepis sancta (L.) Babc.	AJ633372	AJ633150
Crepis sibthorpiana Boiss. & Heldr.	EU363648	EU363574
Crepis tectorum L.	EU363643	EU363536
Crepis tingitana Ball	EU363586	AJ633149
Crepis vesicaria subsp. *stellata* (Ball) Babc.	EU363630	–
Crepis vesicaria L. subsp. *vesicaria*	–	EU363565
Crepis zacintha (L.) Babc.	EU363655	EU363579
Hyoseris radiata L.	AJ633299	AJ633215
Ixeris chinensis (Thunb.) Nakai	EU363587	EU363539
Ixeris repens A. Gray	–	China,Yunnan Province, Dali Xian, E. side of Diancang Shan mountain range. Viciinity of Butterfly Springs. Badly disturbed hillsides & abandoned paddy fields 2550 m 25°55' N 100°05' E , *Bartholomew 6192* (US 3043724), DB243
Ixeris stolonifera A. Gray	AJ633284	AJ633156
Lapsana communis L.	AJ633285	AJ633138, AJ633138
Soroseris erysimoides (Hand.-Mazz.) C. Shih	China, Gansu, Qilian Mt, 3400-3800 m, 27.7.1991, *T.N. Ho 2812* (CAS 795834), NK160	China, Qinghai, Qilian Mt, 3400-3600 m, 4.7.1991, *T.N. Ho 1641* (CAS 791443), NK159
Soroseris glomerata (Decne.) Stebbins	EU363656	China, Qinghai, Bayan Har pass, 4700 m, 12.8.1996, *T. N. Ho 1692* (CAS 939054), NK162
Soroseris trichocarpa (Franch.) C. Shih	China, Qinghai, 3850 m, 14.8.1996, T.N. Ho & al. 1787 (CAS 939119), NK175	–
Taraxacum bessarabicum Fisch.	AJ633287	–
Taraxacum crepidiforme DC.	–	Armenia, Mt Teghenis, 40 32'N, 44 40'E, 2300 m, 16.6.2002, C. *Oberprieler CHO 10074* (B), NK084
Taraxacum erythrospermum Besser	AJ633291	–
Youngia denticulata (Houtt.) DC.	AJ633293	AJ633139
Youngia japonica (L.) DC.	AJ633294	–

Total genomic DNA was isolated from 24 mg per sample of herbarium leaf material, which was ground down. DNA was then extracted according to standard protocol using Quiagen DNeasy Plant Mini Kit. Both ITS and matK fragments were amplified in two overlapping parts using the primers ITS-A and ITS-C (Blattner, 1999) for ITS 1, ITS2-D, ITS-B (Blattner, 1999) for ITS 2 and trnK–710f (Johnson & Soltis, 1995), matK-iR (Fehrer et al., 2007), matK-ifN and matK-rN (Enke & Gemeinholzer, 2008) for matK. During the Polymerase Chain Reaction (PCR) the following protocol was used (ITS/matK): initial denaturation 2 min/2 min at 94 °C, denaturation 1 min/1 min 30 sec at 94 °C, annealing 1 min at 66 °C/ 2 min at 62 °C,

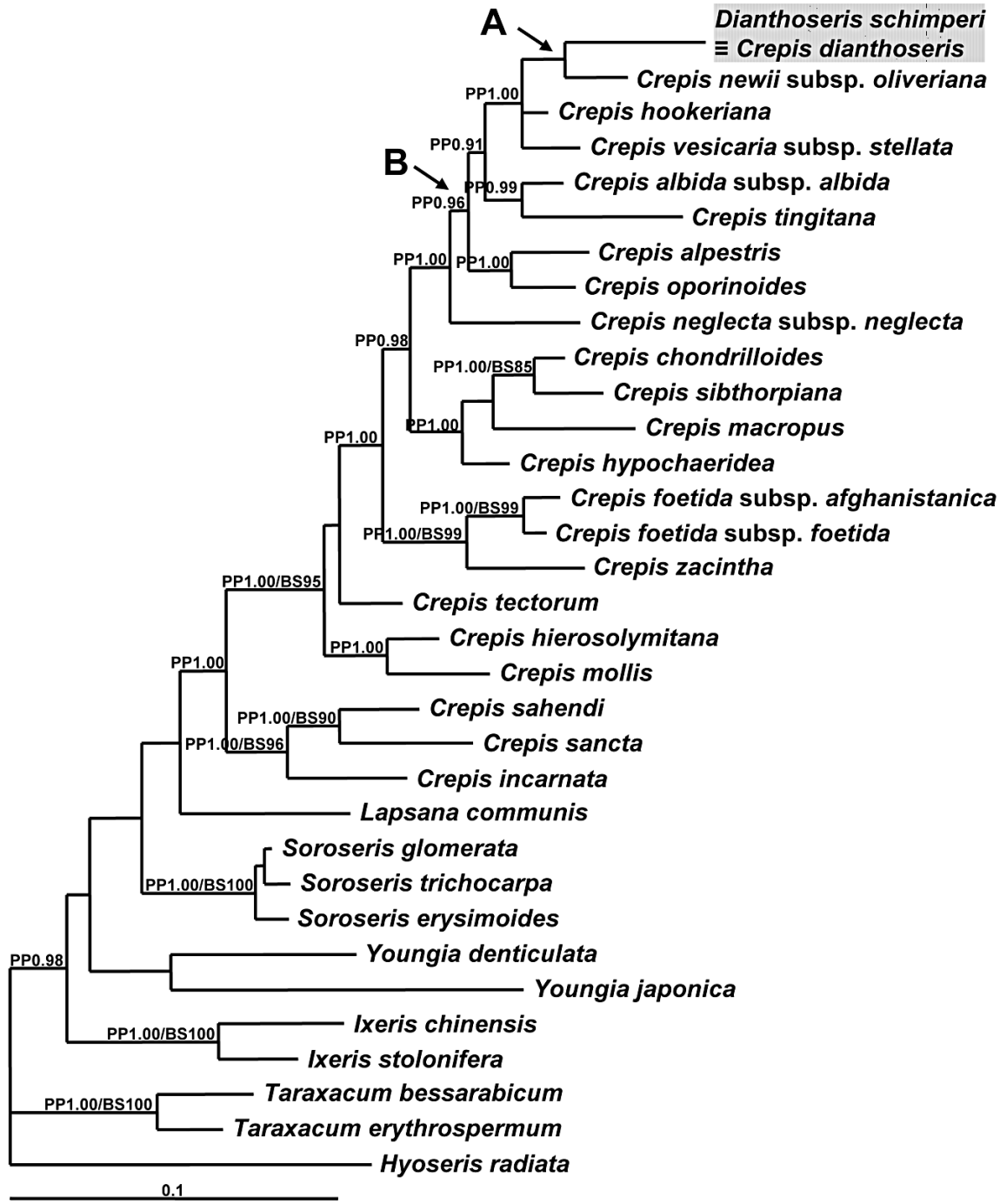

FIG. 1 : Phylogram derived from ITS sequence data by Bayesian Analysis (50% majority rule). Bayesian Posterior Probabilities (>0.90, PP) and bootstrap values (>80, BS) are given above branches. Former *Dianthoseris schimperi* ≡ *Crepis dianthoseris* highlighted. A and B indicating nodes of interest.

elongation 2 min/ 3 min at 72 °C (38 cycles) and final extension 10 min /10 min at 72°C. PCR was carried out with a reaction volume of 11.5 µl core mix plus 1 µlDNAsolution (1:10, 1:50 or 1:100 dilution depending on usability). The reaction volume contained 6.73 µl ddH2O, 2.5 µl 5x buffer (Finnzymes), 1.25 µl dNTP's (Fermentas), 0.4 µl BSA (Finnzymes), 0.25 µl of each primer (10 pmol/µl) and 0.12 µl 1u Phusion High FidelityDNAPolymerase (Finnzymes). The PCR products were purified with MSB Spin PCRapace (Invitek) and subsequently sent to StarSEQ (Mainz, Germany) for sequencing.

The sequences were edited in ChromasLite2000 (Technelysium Pty. Ltd., Helensvale, Australia) and aligned by hand using BioEdit (Hall, 1999) following Goertzen et al. (2003) for ITS gap-coding. For matK an indel at position 118–122 has been coded as one mutational step. The alignments are available from the first author upon request.

The phylogenetic relationships from both ITS and matK datasets were reconstructed with Maximum Parsimony (MP), using PAUP* 4.0b10 (Swofford, 2002), and with Bayesian Inference (BI), using Mr Bayes 3.1.2. (Ronquist & Huelsenbeck, 2003). For the BI analyses a gamma distribution rate variation among sites and 10 million generations of the MCMC chains in two independent runs were used, trees being saved every 100 generations. The first 25 000 trees were discarded as burn-in for the analysis then reached stationarity. All remaining trees sampled were used to calculate a 50 % majority rule consensus tree. For the MP analyses heuristic searches were conducted in PAUP 4.0b10 with equal weights, 1000 closest sequence additions and tree bisection-reconnection (TBR) branch swapping, permitting 10 trees to be held at each step. An evaluation of the trees was performed by using bootstrap analysis with 1000 replicates, equal weights,TBR swapping, MulTrees option in effect and 10 trees held at each step. Trees were drawn using TreeView (Page, 1996) and Adobe Illustrator (Adobe Systems, Inc., San Jose, California, USA).

Micromorphology - Flowers taken from herbarium material were shortly boiled in water, then spread on a microscope slide and photographed with aLEICADFC290 digital camera mounted on a WILDM5 optical reflected-light microscope (up to × 40 magnification). Achenes and pappus were mounted onto SEM stubs on double-sided sticky tape, coated with 20 nm Au-Pd using an Emitech K550 sputter-coater, examined using a Philips SEM 515 scanning electron microscope and documented with the Point Electronic WinDISS III digital imaging device (hard- and software).

6.3 RESULTS

Molecular Phylogeny

A total of 33/33 (ITS/matK) sequences were obtained from 22/22 Crepis species and 10/8 other taxa of the Crepidinae. *Hyoseris* (Hyoseridinae) was chosen as outgroup taxon. Minor variation in taxon sampling for ITS and matK are caused by differences in sequence availability. In total 683/974 (ITS/matK) characters were included in the Maximum Parsimony analysis, of which 198/75 were parsimony informative (29/7.7 %).

The trees inferred from the nuclear (Fig. 1) and plastid (Fig. 2) markers are similar in their topology, but the ITS tree is better resolved in the apical branches.

In both trees *Dianthoseris schimperi* is nested deeply within *Crepis* s.str. and placed far away from *Soroseris*, to which an affinity had been hypothesized by Jeffrey (1966). Furthermore, *Dianthoseris schimperi* forms a clade with *Crepis newii* subsp. *oliveriana* (Kuntze) C. Jeffrey & Beentje (Fig. 1–2, clade A), which is only in the matK tree statistically supported by Bayesian posterior probabilities (1.00) and bootstrap values (98). This clade A is part of the larger clade B (Fig. 1–2, B) in both the nuclear and the chloroplast trees, which comprises the species *C. vesicaria* L., *C. hookeriana* Ball, *C. tingitana* Ball (ITS tree only), *C. alpestris* Rchb., *C. oporinoides* Boiss. and *C. albida* Vill. Clade B is statistically supported only in the ITS tree by Bayesian posterior probability (0.96). In the ITS tree the clade comprising *C. hookeriana* and *C. vesicaria* is sister-group to *Dianthoseris* and *C. newii* subsp. *oliveriana* (Bayesian posterior probability 1.00), while in the matK tree the relations between these taxa are unresolved.

Morphology

Description - Acaulescent perennial rosette herb with thick, deepseated, lignified taproot and usually unbranched caudex. Leaves in a basal rosette appressed to the ground, glabrous, (narrowly) lanceolate to oblanceolate, 2–4(–7) × 0.2–1.2(–2) cm, with a conspicuously broad and pale midrib, tapering to a narrow base, apex obtuse and mucronulate, margin entire to remotely sinuatedentate. Capitula usually solitary, sessile, with up to approximately 100 flowers. Involucre 11–16(–18) mm long, involucral bracts in several rows, abaxially and adaxially entirely glabrous; inner involucral bracts subequal, linear-lanceolate, 10–12 × 2.5–3 mm, margins distally shortly ciliolate; outer involucral bracts similar to the inner in shape and size to even larger and grading in size and shape into the innermost rosette leaves. Flowers (fully developed marginal ones) with a 4.5–7 mm long corolla tube and a ligule varying considerably in size between different plants, of 1.5–9 × 0.5–1.8 mm, therefore being either distinctly longer (Fig. 3E) to shorter than the tube; anthertube without appendages 1.2–2.5 mm long; basal appendages up to 0.5 mm, apical appendages up to 0.3 mm long. Achenes (fully mature ones, Fig. 3A, C-D) 3.2–3.8 mm long, 0.8–1 mm in diameter, subspherical in cross-shape and apically less attenuate than basally, glabrous, with c. 20 longitudinal ribs, 5 of which being more prominent than the others, achene surface otherwise smooth. Pappus 6–10 mm long, white, of 2(–3) distinct, basally connected series of smooth to weakly barbellulate setae of equal length; setae of the outer 1(–2) series clearly thicker than those of the inner one and curved outwards at maturity, the setae of the inner series remaining straight (Fig. 3B).

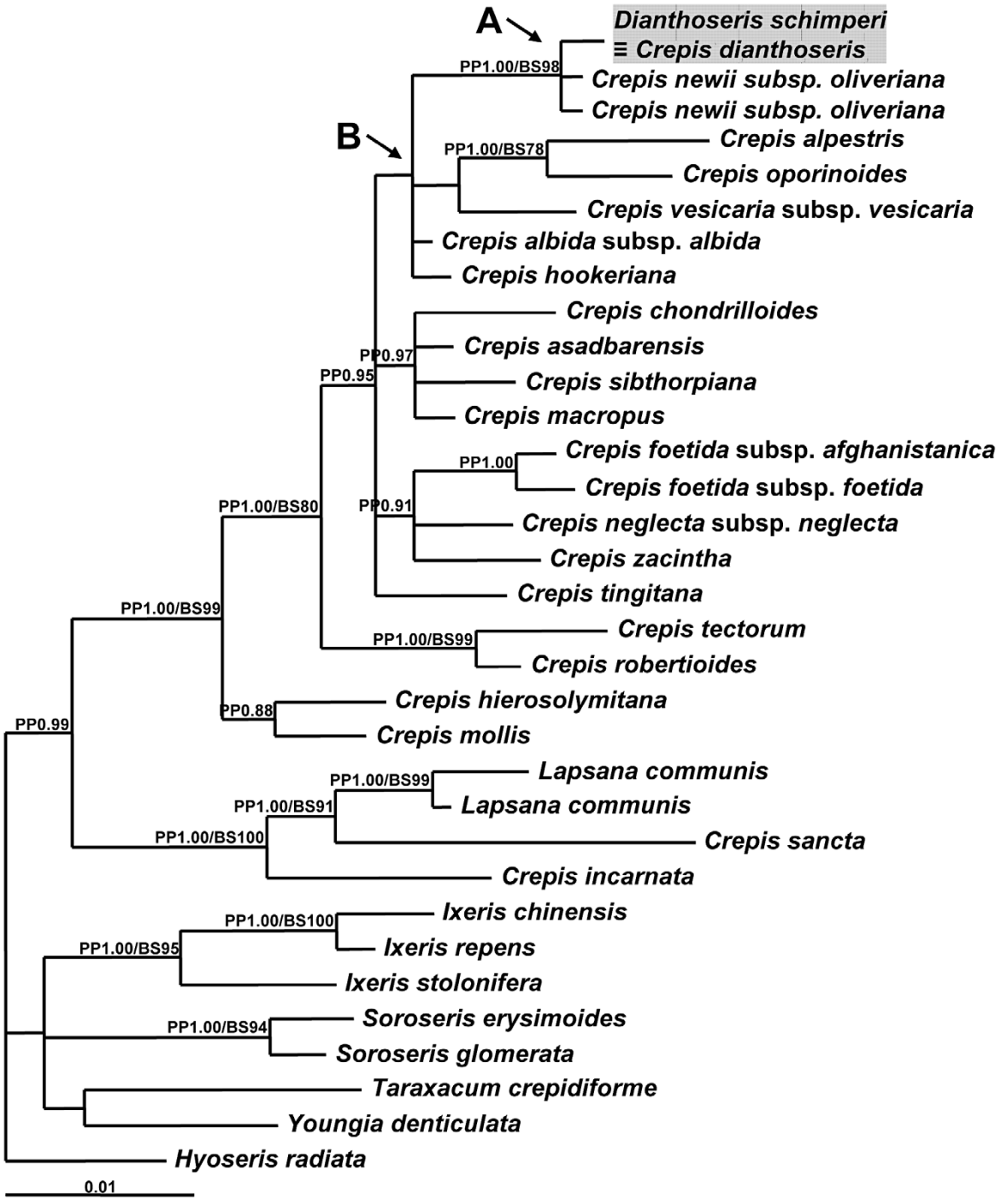

Fig. 2. Phylogram derived from *matK* sequence data by Bayesian Analysis (50% majority rule). Bayesian Posterior Probabilities (>0.90, PP) and bootstrap values (>80, BS) are given above branches. Former *Dianthoseris schimperi* ≡ *Crepis dianthoseris* highlighted. A and B indicating nodes of interest.

Notes - The taproot of *Dianthoseris schimperi* seems to bear adventitious buds, which lead to a "branching" of the taproot and the growth of new rosette shoots. Mature achenes have not been available to the authors of previously published descriptions, which explains the lower achene size of 2.5 mm given by them (Jeffrey & Beentje, 2000; Tadesse, 2004). We found that the achene length is almost 4 mm in the mature fruits of Ortiz & Vivero 26 (ETH).

Delimitation - An acaulescent species habitually very similar to *Dianthoseris schimperi* was collected in the late 1960s from one locality in the Simen Mts (Sebald, 1968) and described as *Nannoseris inopinata* Cufod. (Cufodontis, 1968). However, Tadesse (1999: 29, 2004: 52) treated *N. inopinata* as conspecific with *D. schimperi*. An inspection of the holotype (see below, Taxonomy, Note 2) as well as of a second specimen referable to this taxon with almost mature fruits from the same locality (Begemdir, prov. Simien, 13°15–16'N, 38°3–13'E, Geech, 3600 m, thin soil with short grass, 15.10.1973, O. Hedberg & G. Aweke 5360, ETH37263) revealed, however, that *N. inopinata* should be maintained as a separate species allied to *D. schimperi*. The differences between the two species in the leaves (for *N. inopinata* see Cufodontis (1968: fig. 5 [habit; b/w photograph of herbarium specimen]) and the indumentum of the involucre are conspicuous, as *Dianthoseris schimperi* is very uniform in appearance across its disjunct distribution area, as already noted by Fries (1928: 162). This, however, does not hold true for the corolla size in *D. schimperi*. Whereas Sebald 1247,STU(Fig. 3E) has large corollas with a tube of 6–7 mm and ligule of 8.5–9 mm, and thus with a ligule longer than the tube, Hedberg 5543, ETH, has a tube of c. 4.5 mm and a ligule of 3.5–4 mm, thus a ligule shorter than the tube. Still smaller corollas with a tube of c. 4.5 and a ligule of only 1.5 mm length are reported from the Kenya/Uganda/Tanzania subarea (Jeffrey& Beentje, 2000: 92). In N. inopinata (Fig. 3F) the corolla tube is always longer than in *D. schimperi*, while the ligule length is within its wide range of variation in *D. schimperi*. The involucre (apart from the indumentum), the achenes and the pappus are rather similar in both species, the latter with 10–12 mm, however, a little longer.

6.4 DISCUSSION

Molecular phylogeny - According to our molecular phylogenetic analyses, *Dianthoseris schimperi* has to be considered as a member of *Crepis*, having its closest affinities to the widespread and polymorphic, afro-montane to afro-alpine *C. newii* Oliv. & Hiern, of which subsp. *oliveriana* was included in our analyses. Treated by Jeffrey & Beentje (2000: 70) as a subspecies, the taxon was considered by Babcock (1947) as a separate species, *C. oliveriana*, and like *C. newii* as a member of *C.* sect. *Anisorhamphus*. This is one of the two largest sections of the genus. Except for its sole non-African member *C. alpestris*, the section comprises the vast majority of the tropical African species. The inclusion of *C. alpestris* in the larger clade B in both the nuclear and the chloroplast trees confirms its moderate affinity to *C. newii* subsp. *oliveriana* and *D. schimperi*. In contrast, the third and only other member of *C.* sect. *Anisorhamphus* included in our analyses, *C. hypochaeridea* (ITS tree only, see Fig. 1), is a sister-group to a clade consisting of three Mediterranean species of Babcock's *C.* sect. *Berinia*, i.e. *C. macropus* Boiss. & Heldr., *C. sibthorpiana* Boiss. & Heldr. and *C. chondrilloides* Jacq. This indicates that Babcock's section *Anisorhamphus* is polyphyletic,

and a similar result for other sections of *Crepis* was reported by Enke & Gemeinholzer (2008). The remaining six members of clade B are heterogenous in terms of their previous sectional classification, belonging to six different sections in Babcock's system of *Crepis*: *C. oporinoides* Boiss. from SE Spain belongs to the aforementioned section *Berinia* and *C. hookeriana* Ball from Morocco belongs to section *Macropodes*, which, besides others, comprises two endemics (*C. xylorrhiza* Sch. Bip. and *C. tenerrima* (Sch. Bip.) R. E. Fr.) and one more widespread species (*C. rueppellii* Sch. Bip. [Syn.*: C. abyssinica* Sch. Bip., *C. forsskalii* Babc.]) of the Abyssinian Highlands not available for our analyses. *C. tingitana* Ball (ITS tree only, see Fig. 1) from N Morocco/SE Spain belongs to section *Gephyroides* and *C. albida* Vill. from E Spain to section *Paleya*, which also includes *C. achyrophoroides* from the Abyssinian Highlands also not available for our analyses.

Finally the W European-W Mediterranean *C. vesicaria* L. belongs to section *Lepidoseris*. Since the relationships within *Crepis* are insufficiently known, according to the available molecular phylogenetic results, and since a complete revision of the taxonomy of *Crepis* is needed, some limitations to the analysis of the systematic position of *Dianthoseris* within *Crepis* exist. Our molecular analyses nevertheless give two straightforward hints: (1) *Dianthoseris schimperi* has close affinities to the afromontane-afroalpine *C. newii* group and (2) the *C. newii* group seems to be allied to species centred in NW African/SE Spain, such as *C. hookeriana*, *C. albida*, *C. tingitana* and *C. oporinoides*, and beyond in the Mediterranean and S Europe to species such as *C. vesicaria* and *C. alpestris*.

Chromosome numbers - *Dianthoseris schimperi* shares its chromosome number of n=4 with all species of clade B except *C. albida* and *C. tingitana*. These latter two species have n = 5 (for references see Watanabe 2008). The latter taxa are the sister-group to the remainder of clade B in the ITS tree (Fig. 1). A decrease in chromosome number is considered an evolutionary trend in *Crepis* (Babcock 1947). This has been confirmed by the molecular phylogenetic analyses of *Crepis* (Enke in prep.) and the Cichorieae in general (Kilian et al. 2008).

Morphology - The glabrous achenes with c. 20 ribs, the 2–3-seriate pappus and the capitulum size of *Dianthoseris schimperi* perfectly match *Crepis.* The involucre, however, appears rather odd. The complete absence of an indumentum on the involucral bracts is a rare feature in *Crepis* but, e.g., also found in *C. pulchra*. More peculiar is, however, the fact that the outer bracts are not distinctly smaller than the inner series of bracts as is usual in *Crepis*, but are of equal length even at maturity instead. This has, however, to be considered in the context of the fully acaulescent habit of the species. Whereas in caulescent species the outer involucral bracts usually grade in shape und size into the usually small bracts of the capituliferous axis, the capitulum in *D. schimperi* immediately terminates the rosette shoot and the rosette leaves next to the involucre are intermediate in size and shape between the outer involucral bracts and the rosette leaves further outside. The peculiarities of the

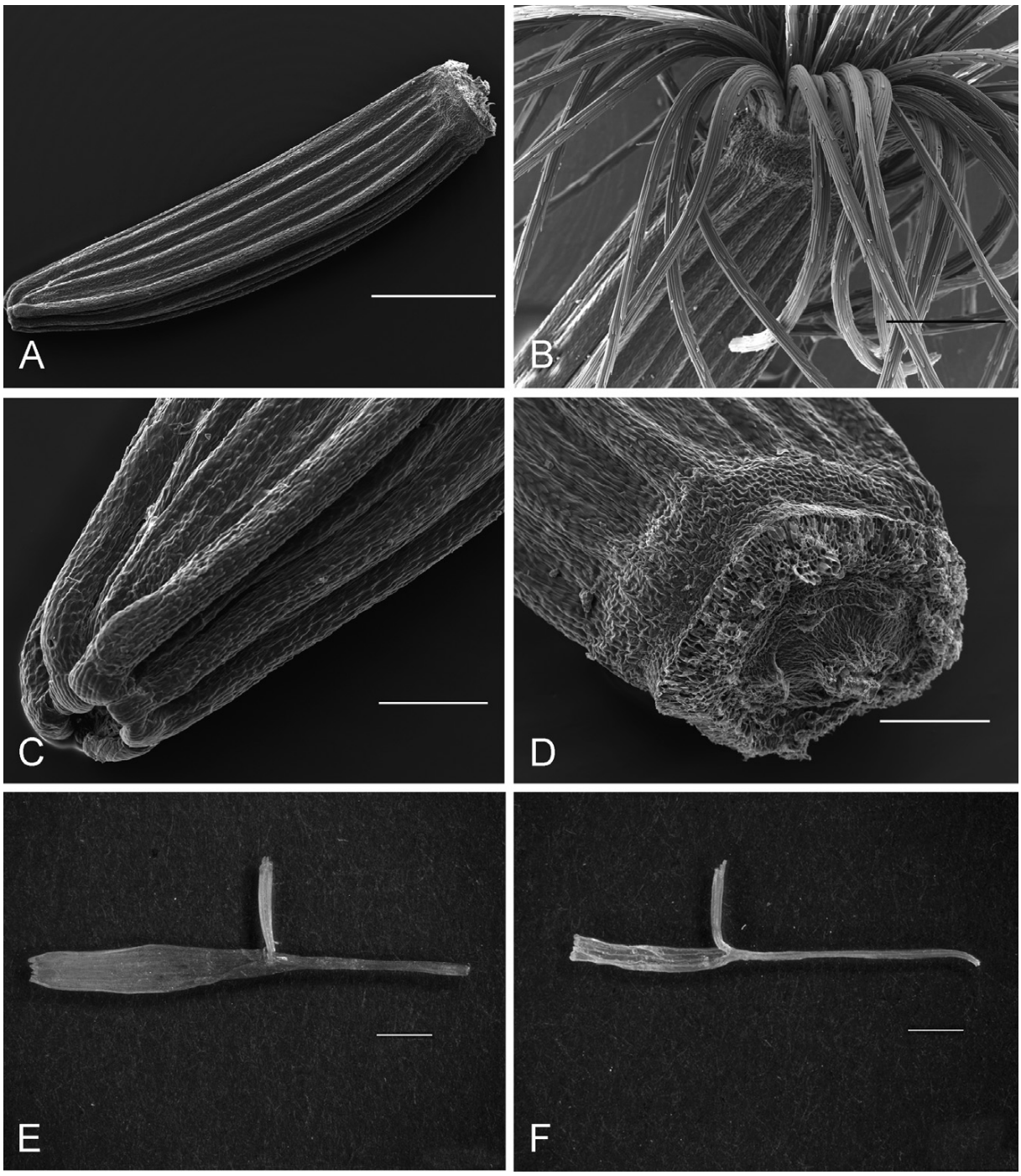

FIG. 3. A-E: *Crepis dianthoseris* (≡ *Dianthoseris schimperi*) – A: achene (pappus removed), overview; B: pappus with the 1(-2) outer series of setae curved outwards and the inner, somewhat thinner, series of setae being erect; C: achene base; D: achene apex (pappus removed); E: marginal flower; A-D from *Ortiz & Vivero 26*, ETH, E from *Sebald 1247*, STU. – F: *Crepis inopinata* (≡ *Nannoseris inopinata*), marginal flower at the same stage of anthesis as the one in E; from the holotype, *Sebald 1046*, STU. – Scale bars: A = 0.9 mm, B = 0.5 mm, C-D = 0.2 mm, E-F = 2 mm.

involucre of *D. schimperi* may thus be interpreted as a consequence of the acaulescent growth form. The acaulescent growth form and the involucre of *Nannoseris inopinata* are essentially similar, apart from minor differences in the shape of the involucral bracts and the

fact that the inner bracts are densely glandular-hairy. The acaulescent growth form as present in both species is otherwise without parallel in *Crepis*, which supports the hypothesis that the two species are closely related.

According to Blackmore & Persson (1996) *Dianthoseris* has subechinolophate pollen grains with rudimentary paraporal lacunae and rounded abporal lacunae, which is more similar to *Dubyaea* and *Soroseris* than to *Crepis*, where echinolophate pollen grains with tricolporate, ectocolpi divided into three lacunae, and somewhat angular, large abporal lacunae predominate. However, missing congruence of pollen features with taxonomic entities recognized by molecular and other morphological characters are rather frequent and make pollen features often difficult to interpret.

Table 2. Differential characters between *Nannoseris inopinata* and *Dianthoseris schimperi*. Further explanations are given in the text.

Characters	***N. inopiniata***	***D. shimperi***
Leaves	distinctly runcinate	margin entire to remotely sinuate-dentate
Inner involucral bracts	abaxially densely covered with long, pale glandular trichomes	entirely glabrous
Corolla of fully developed marginal flowers		
tube length [mm]	7.5-9	4.5-7
Ligule size [mm]	5-5.5 x c.1.2	1.5-9 x 0.5-1.8
Pappus length [mm]	10-12	6-10

Regarding the systematic position of *Dianthoseris schimperi* within *Crepis*, the morphological characters are inconclusive, even considering the species indicated by the molecular results as next allies. This is, however, not surprising, if we take into consideration (1) the poverty of features in our habitually much reduced species, and (2) that among the closest allies of *D. schimperi* are members of no less than seven of Babcock's morphology-based sections.

6.5 TAXONOMY

Crepis L. = *Dianthoseris* [Sch. Bip. in Flora 25: 439. 1842, nom. inval., ex] A. Rich., Tent. Fl. Abyss. 1: 468. 1848 = *Omalocline* subg. *Dianthoseris* (Sch. Bip.) Sch. Bip. in Jahresber. Pollichia 22–24: 321. 1866 = *Nannoseris* Hedberg in Symb. Bot. Upsal. 15: 251. 1957, nom. illeg. — Typus: *Dianthoseris schimperi* A. Rich.

Crepis dianthoseris N. Kilian, Enke, Sileshi & Gemeinholzer, nom. nov. = *Dianthoseris schimperi* [Sch. Bip. in Flora 25: 439. 1842, nom. inval., ex] A. Rich., Tent. Fl. Abyss. 1: 468. 1848 [non *Crepis schimperi* (A. Rich.) Schweinf., Beitr. Fl. Aethiop.: 144. 1867, i.e. *Crepis foetida* L.] = *Omalocline schimperi* (A. Rich.) Sch. Bip. in Jahresber. Pollichia 22–24: 321. 1866 [& Schweinf., Beitr. Fl. Aethiop.: 285, 307. 1867 as "*Homalocline schimperi*", orth. var.] = *Sonchus dianthoseris* ["*Sonchus dianthoseris* var. *schimperi*"] Chiov. in Annuario R. Ist. Bot. Roma 8 [Pirotta, Fl. Eritrea]: 200. 1904, nom. illeg. = *Nannoseris schimperi* (A. Rich.) Hedberg in Symb. Bot. Upsal. 15: 251. 1957.—Holotype: [Ethiopia], "in regione superiori

montis Bachit [Bwahit, 13°13'N, 38°13'E] 12000–13000 pedes supra", 19.8.1840, Schimper 755 [erroneously quoted as "775" in the protologue] (P015825!; isotypes: BM000924929!, BR0000008361790!, K000251829!, K000251831!, K000251832!, M0105528!, NY00167853!, P015826!, P015827!, see Aluka 2007).

Crepis nivalis Schweinf. & Asch. in Schweinfurth, Fl. Aethiop.: 284. 1867, nom. nud. (fide Babcock 1947: 924).

Notes - (1) For the invalid publication of the name *Dianthoseris* by Schultz-Bipontinus, its later validation by Richards and the resulting illegitimacy of Hedberg's generic name *Nannoseris* see Jeffrey (1966: 433).

(2) The epithet of *Dianthoseris schimperi* is not available for the corresponding combination in *Crepis*, because it would result in a later homonym of *Crepis schimperi* (A. Rich.) Schweinf., which is treated as a taxonomic synonym of *C. foetida* L. by Jeffrey (1966: 462) and as a species closely related to the latter by Babcock (1947: 705). If *Nannoseris inopinata* were actually conspecific with *Dianthoseris schimperi*, as is treated by Tadesse (1999: 29), the epithet *inopinata* would have to be taken up for the *Dianthoseris* species in Crepis. However, as shown above, *N. inopinata* is a different, although closely related species: *Crepis inopinata* (Cufod.) N. Kilian, Enke, Sileshi & Gemeinholzer, comb. nov. = *Nannoseris inopinata* Cufod. in Stuttgarter Beitr. Naturk. 195: 7. 1968.—Holotype: [Ethiopia], "Hochsemyen, beim Lagerplatz Kurbät-Mätaya zwischen Amba Ras und Buahit, 13°15.5'N, 38°11.4'E, 3600 m, in beweidetem Grasland zwischen Erica arborea-Büschen", 9.11.1966, O. Sebald 1046 (STU 000506, see Aluka 2007).

The binomial *Sonchus dianthoseris* Chiov. of 1904, to which that author designated "var. a Schimperi = *Dianthoseris schimperi* Schultz Bip. ...." as "la forma typica" (constituting definite indication of a type of that binomial according Art. 7.5 of the Code, McNeill et al., 2006) and in which he included as a second variety *Dianthoseris rueppellii* (see 3, below) was correctly qualified as illegitimate by Jeffrey (1966: 433): Chiovenda should have adopted the epithet rueppellii of the latter included species after the epithet schimperi was not available in *Sonchus* because of *S. schimperi* A. Braun & Bouche of 1857 (a synonym of *S. oleraceus*, see Boulos 1973: 155). The use of the epithet dianthoseris in *Crepis* is, however, sanctioned by Art 58.1 of the Code (McNeill et al., 2006).

(3) The second similarly acaulescent species formerly placed in *Dianthoseris* is actually a member of *Launaea* (subtribe Hyoseridinae): *D. rueppellii* [Sch. Bip. in Flora 25: 440. 1842, nom. inval. ex] Oliv. & Hiern in Oliver, Fl. Trop. Afr. 3: 456. 1877 = *Sonchus dianthoseris* var. *rueppellii* Chiov. in Annuario Ist. Bot. Roma 8 [Pirotta, Fl. Eritrea]: 200. 1904, nom. illeg. = *Sonchus rueppellii* (Oliv.&Hiern) R. E. Fr. in Acta Horti Berg. 8: 112. 1925 = *Launaea rueppellii* (Oliv. & Hiern) Boulos (see Jeffrey 1966: 467, Kilian 1997: 104).

Ic. - Fig. 3A-E; Fries 1928: t. 10,4 (habit; b/w photograph of herbarium specimen); Hedberg 1957: 252, fig. 18 = Tadesse 2004: 52, fig. 36 (habit and single flower; drawings); Jeffrey & Beentje 2000: 91, fig. 24 (habit & details; drawings); Puff & Nemomissa 2005: 155, fig. D55A-C (habit, flowering, dug out plant showing taproot; colour photographs);

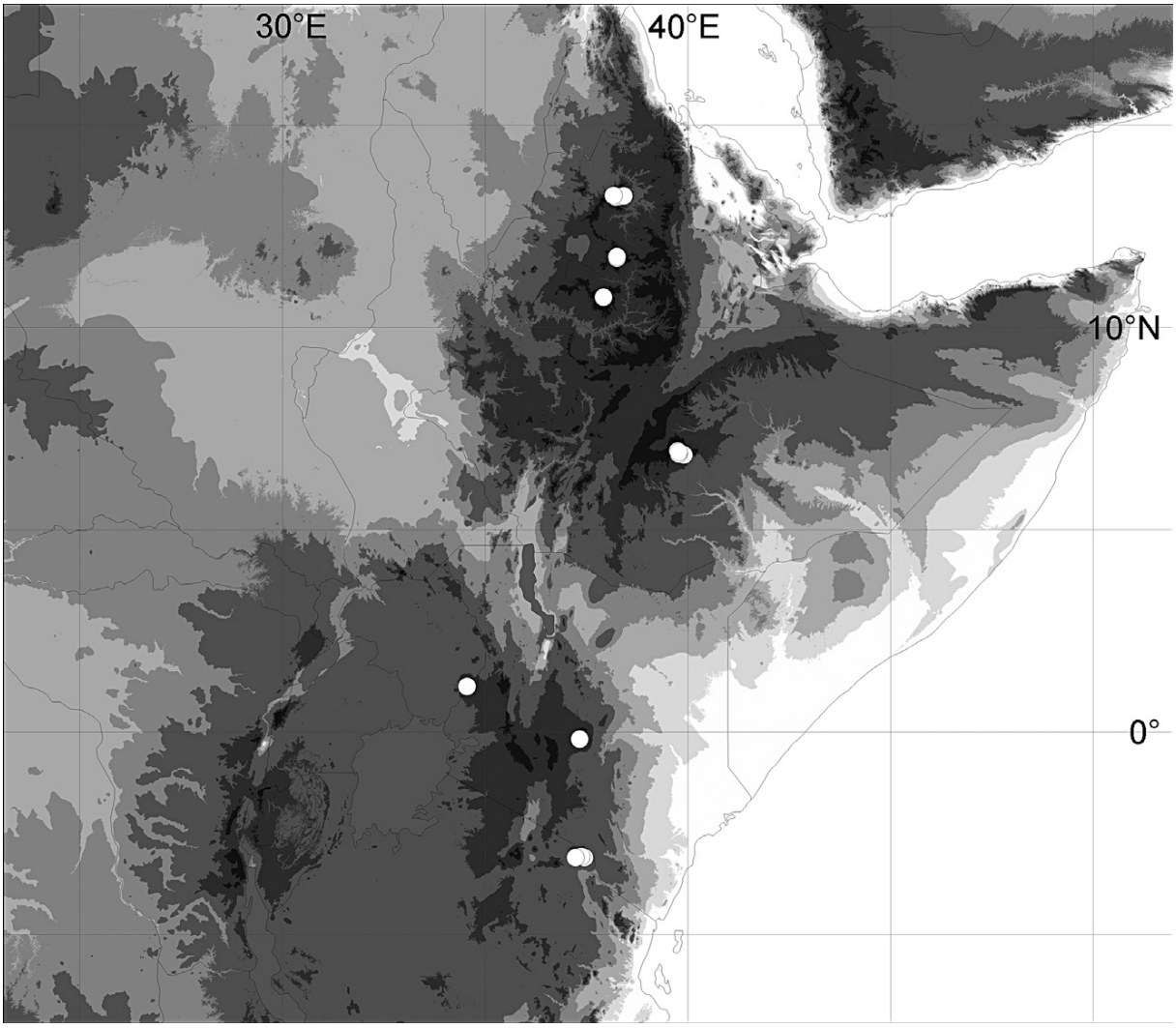

FIG. 4. Distribution of *Crepis dianthoseris* (≡ *Dianthoseris schimperi*) (circle). – Georeferenced map based on the specimens seen and literature data and generated with DIVA-GIS (Hijmans & al. 2005) using an adaptation of the SRTM 90 m digital elevation data (CGIAR-CSI 2004).

Distribution and ecology - *Crepis dianthoseris* occurs in the Eastern African countries Ethiopia (Gonder, Gojam & Bale region; Tadesse, 1999: 30, 2004: 52), Kenya, Uganda, Tanzania (Jeffrey, 1966: 433;Jeffrey & Beentje, 2000: 92), where it is restricted to elevations between 3600 and 4500m (–5800 m, on Mt Kilimanjaro, see Hedberg, 1970; up to c. 4200min Ethiopia, see Sebald, 1968: 31), see Fig. 4. It grows on moist ground in open sparse vegetation, such as afro-alpine grasland, *Helichrysum* heath and open *Erica arborea*

scrub, especially on solifluction soils and in rock crevices (Jeffrey & Beentje, 2000: 92; Tadesse, 2004: 52; Puff & Nemomissa, 2005: 154; Sebald, 1968: 31; label data).

Flowering plants are found almost throughout the year; fruiting specimens are, however, very rare in herbaria.

Additional specimens seen - Ethiopia: Bale: Bale Mts, National Park, 6°51'–7°10'N, 39°41–48'E, at Garba Goracha camp, 3950 m, on ground disturbed by Giant Mole Rat, 30.10.1973, O. Hedberg 5543 (ETH 36765); Bale Mts, Wasama, 6°55'N, 39°46'E, 4120 m, afro-alpine *Helichrysum* heath, 12.1.1990, G. & S. Miehe 958 (ETH 36753); Bale Mts, Chuofo Hadji Biftu, 6°53'N, 39°46'E, 4200 m, afro-alpine *Helichrysum* heath, open substratum, 18.1.1990, G. & H. Miehe 1175 (ETH 36760). — Gonder: Begemdir, prov. Simien, 13°15–16'N, 38°3–13'E, on the crest, 4225 m, on solifluction terraces between boulders, 18.10.1973, O. Hedberg & G. Aweke 5436 (ETH 36752); Begemder, Hochsemyen, am Südwesthang des Kiddis Ared im alpinen Grasland, 4200 m, 14.11.1966, O. Sebald 1247 (STU); c. 42 km from Debra Tabor, eastern ascent of Mt Guna, 11°45'N, 38°15'E, 3800 m, alpine meadow, moist area, 6.10.1981, C. Puff, D. Mantelli & E. Kelbessa 8110006–1/5 (ETH 36755); Amhara, Peak Guli Bamba (4284 m), afro-alpine belt close to the peak, boulder, rocky slopes, montane grassland with *Lobelia rhynchopetalum*, 4000–4200 m, 27.11.2001, S. Ortiz & J. L. Vivero 26 (ETH 74116).

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Chapter 7

Outlook

The morphological, karyological and molecular data acquired and interpreted in the course of the present analysis on *Crepis* yielded new insights into phylogeny, evolution and systematics of the genus; phylogenetic reconstructions revealed the genus *Crepis* to be polyphyletic, the measurement and reconstruction of character progression demonstrated genome size to shrink during evolution, and a survey of morphological and anatomical data helped to resolve systematic ambiguities. But with the applied methods not all questions towards an understanding of the evolutionary history of the genus could be answered: such as explaining the phylo- and biogeography of the genus, and clarifying the role of karyotype progression and hybridisation for speciation processes within the genus.

Further analyses might combine fossil records and molecular sequence data by applying molecular clock hypotheses on a phylogenetic tree. Supporting data is already available: Babcock (1947a) dated the origin of the genus into the late Tertiary (Miocene, 24-5 mya) in the Central Asian Altai/Tien Shan region. The fossil record of *Crepis* reaches back into the Middle Pliocene (Piacenzian, 3.6-2.6 mya): Reid & Reid (1916) report fossil achenes of two *Crepis* species (C*. spec*., *C. blattarioides*) for the Reuverian Beds in southern Holland; later Babcock (1947a) identified the achenes to belong to *C. tergluoensis* and *C. conyzaefolia*. The fossil pollen record is not genus specific but well documented for Compositae in general (e.g. Blackmore et al., 1986). A molecular clock analyses (r8s, Sanderson, 2002), partly based on the recent analysis of the biogeographic history of the Cichorieae (Tremetsberger et al., subm.), could further illuminate the time of origin of the genus *Crepis*. Combined with the analysis of distributional data via a dispersal-vicariance approach (Ronquist, 1997) using the program DIVA, the biogeographic history of the genus could be reconstructed. So Babcock's hypotheses that the genus originated in Central Asian Mountains and from there spread south-westward into the Mediterranean regions and Africa, westward into Europe and north-eastward into North America via the Bering land Bridge, which joined Siberia and Alaska repeatedly during the Pleistocene (1.8 my - 10.000 BP), can be re-evaluated. The evolution of the 15 polyploid *Crepis* species of sect. *Psilochaenia* also can be explored based on the phylogeographic analysis of the history of the genus *Crepis*.

The historical importance of *Crepis* as model group for karyological studies has been perpetuated by the present results (chapter 3) providing promising starting points to further

illuminate karyotype evolution within the genus. Babcock (Babcock & Cameron, 1934; Babcock et al., 1937; Babcock & Jenkins, 1943; Babcock, 1947a) postulated hypotheses on karyological evolution in the genus: chromosome size and number decrease while chromosomal asymmetry increases during evolution. But the methodological potential has increased since Babcock's time and neither present molecular results (chapter 2) nor studies by e.g. Jones & Brown (1976) or Dimitrova & Greilhuber (2001) could confirm Babcock's postulations. New hypotheses should be tested: Can monophyletic clades (ITS, chapter 2) be characterised by karyotypic similarities (karyomorphotypes)? Is hybridisation possible between species of different karyomorphotypes? To identify and characterise different karyomorphotypes the following methods should be applied as they proved to yield promising results in earlier studies on *Crepis* (Dimitrova & Greilhuber, 2000, 2001): Giemsa C-banding (Schwarzacher et al., 1980), Ag-Nor staining (Bloom & Goodpasture, 1976; Kodama et al., 1980), measurements of chromosomes (total length of chromosomes, length of short and long arms and the satellite), measurements of band positions and the calculation of short/long arm ratio, and the calculation of centromeric and asymmetry index. The data gathered could be mapped on the molecular phylogenetic reconstruction to trace character changes and might possibly be used for cladistic analyses. The distribution of certain karyomorphotypes within species could indicate possible hybridisation events within the genus, as the role of chromosomal rearrangements and hybridisation within the genus up to now remains largely obscure. Babcock (1947a) considered hybridisation as minor agent of speciation, but the incongruence of nuclear and chloroplast phylogenies (see chapter 2) hint on possible reticulation in the phylogenetic history of the genus *Crepis*. To identify signatures of hybridisation in molecular data it is important to eliminate other causes of phylogenetic incongruence (McBreen & Lockhart, 2006), so the incongruence of nuclear and chloroplast phylogenies has to be analysed, statistically assessed and taxa which are possibly responsible for the incongruence have to be identified (for methodological approaches see e.g. Johnson & Soltis, 2001). Network reconstructions (e.g. SplitsTree, Huson & Bryant, 2006) account for more complex evolutionary events than dichotomous divergence and can therefore be used to analyse and visualise reticulate evolution.

It is also recommended to further validate the phylogenetic relations within the genus by using other chloroplast markers to resolve apical branching patterns in the chloroplast phylogeny (e.g. *psbA-trnH*) and nuclear single-copy genes to sustain basal divergences and further illuminate ambiguous molecular groupings, e.g. Clade II (chapters 2,4). Possible candidates (e.g. *DHS*, *QG8140*) were recently proposed for Compositae (Álvarez et al., 2008).

As the incongruence of molecular and current taxonomic groups within *Crepis* s.str. could so far not completely be resolved, an expansion of morphological analyses (as indicated in chapter 4) is of high systematic and taxonomical value.

The here presented suggestions are all directly based on or connected to data accumulated within the present study, but other approaches are also of interest: e.g. to investigate the correlation of genome size to distribution area (chapter 3), or extending the karyotype studies using FISH (Fluorescence In-Situ Hybridisation) methodology (e.g. to clarify relations within the “hybrid complex” (Clade VII, chapters 3,4,5)). A fusion of current and future results would not only further enlighten the evolutionary history of *Crepis* and karyotype evolution in higher plants, but also contribute towards a revision and new infrageneric classification of the genus.

Chapter 8

Summary

8.1 SUMMARY

Karyotype alterations play an active role in plant speciation processes. N. Enke's dissertation "Phylogeny and Character Evolution in the genus *Crepis* L. (Cichorieae, Compositae)" contributes towards the understanding of the influence of karyotype changes on diversification in diploid plant genera using *Crepis* as model group.

The genus *Crepis* is distributed in the Holarctic and Africa with the highest diversity in the Mediterranean. Most of the species within the genus are diploid; except for the 15 species of section *Psilochaenia* and approximately five additional species. The basic chromosome number in the genus ranges from x = 3 to 6 (7), respectively x = 11 in section *Psilochaenia*.

The genus *Crepis* was revised by Babcock in 1947 (The genus *Crepis* I&II, University of California Press). He assigned 196 of the over 200 species known today to 27 sections mainly due to karyological characters, such as chromosome number and shape. Babcock also postulated hypotheses on evolution and speciation within *Crepis*: karyotype rearrangements are the driving force of speciation, while hybridisation only plays a minor role in species formation.

Based on Babcock's monograph the present study (1) postulates phylogenetic hypotheses for *Crepis*, (2) reassesses existing hypotheses on karyotype evolution and speciation mechanisms within the genus, (3) identifies morphological and anatomical characters reflecting infrageneric groups, and (4) revaluates the current infrageneric classification.

The phylogenetic relations within the genus are inferred from both nuclear (ITS) and chloroplast (*matK*) markers. Genome size is measured by flow cytometry and evaluated on a molecular phylogenetic background. Achene anatomy and morphology, pollen morphology and structure of style branch papillae are investigated via SEM and LM for their applicability to delimitate infrageneric groups.

The phylogenetic reconstructions based on 123 ITS sequences of 78 species and 73 *matK* sequences of 52 species differ in apical branching pattern but support three main clades: the first comprises approximately 80% of sampled species as *Crepis* s.str., the second includes the genera *Lapsana* and *Rhagadiolus* and all sampled taxa of five *Crepis* sections, the third corresponds to former *Crepis* section *Ixeridopsis*; now genus *Askellia*.

Combined karyological and molecular analyses show a complex pattern of karyotype evolution within the genus. Chromosome number is highly variable in and between clades, and de- and also increased during evolution. A trend toward a decrease in genome size within *Crepis* is observed. Annuals predominantly feature small genomes while in perennials genome size is variable. Species from the Mediterranean in general feature smaller genomes than species from N-Europe, Eurasia and Central/E-Asia.

Of the tested microcharacters pappus structure differs between the three clades inferred by molecular analyses. Achene anatomy, pollen morphology and style branch papillae provide no evidence for infrageneric classification, mostly due to low sample size. Achene anatomy and style branch papillae show sufficient variation for systematic use if sample size is broadened.

As taxonomic consequences of the presented study the genera *Lapsana* and *Rhagadiolus* are preserved and the genus *Crepis* is treated as paraphyletic. The former genus *Dianthoseris* is included into *Crepis*. Comments on a revised sectional classification are given.

8.2 ZUSAMMENFASSUNG

Artbildungsprozesse bei Pflanzen können auf Karyotypveränderungen zurückgehen. Die Dissertation von N. Enke “Phylogeny and Character Evolution in the Genus *Crepis* L. (Cichorieae, Compositae)” analysiert den Einfluß von chromosomalen Veränderungen auf die Artneubildung in diploiden Pflanzengruppen. Die Gattung *Crepis* wird modellhaft in diesem Rahmen untersucht.

Crepis L. ist in der gesamten Holarktis und Afrika verbreitet mit der höchsten Diversität im Mediterranraum. Die meisten Arten der Gattung sind diploid, bis auf 15 Arten der Sektion *Psilochaenia* und ca. 5 andere Arten. Die Chromosomengrundzahl variiert zwischen x=3 und x=6 (7), bzw. x=11 in der Sektion *Psilochaenia*.

Die letzte Revision der Gattung geht auf Babcock (1947, The Genus Crepis I&II, University of California Press) zurück. Er gliederte 196 der über 200 heute bekannten Arten auf Grund hauptsächlich karyologischer Ähnlichkeiten, wie z.B. Chromsomenform und – zahl, in 27 Sektionen. Babcock postulierte, daß die Hauptursache von Artbildungsereignissen Änderungen des Karyotyps seien, und Hybridisierung nur eine kleine Rolle spiele.

Aufbauend auf Babcocks Monografie postuliert die vorliegende Arbeit (1) phylogenetische Hypothesen für *Crepis*, überprüft (2) bereits bestehende Hypothesen zu Karyotypevolution und Artbildungsmechanismen in der Gattung, identifiziert (3) morphologische und anatomische Merkmale zur Definition infragenerischer Gruppen, und bewertet (4) die bestehende Gliederung in Sektionen neu.

Die phylogenetischen Zusammenhänge der Gattung Crepis wurden mit Hilfe des Kernmarkers ITS und des Chloroplastengens *matK* rekonstruiert. Genomgrößen wurden mittels Flow Cytometry gemessen und in einem phylogenetischen Zusammenhang interpretiert. Frucht- und Pollenmerkmale, sowie die Papillae der Narbenäste wurden mittels LM und REM auf eine Eignung zur Abgrenzung infragenerischer Gruppen untersucht.

Die Phylogenierekonstruktionen (ITS/*matK*: 123/73 Sequenzen von 78/52 Arten), unterscheiden sich in der Anordnung der apikalen Gruppen, aber unterstützen beide drei Hauptkladen: Die erste besteht aus ca. 80% der untersuchten Arten als *Crepis* s.str., die zweite umfasst neben den Gattungen *Lapsana* und *Rhagadiolus* alle untersuchten Arten aus fünf *Crepis* Sektionen, die dritte entspricht der früheren *Crepis* Sektion *Ixeridopsis*, mittlerweile Gattung *Askellia*.

Die gemeinsame Interpretation karyologischer und molekularer Ergebnisse ließ ein komplexes Muster der Karyotypevolution erkennen. Die Chromosomengrundzahl variiert stark sowohl innerhalb als auch zwischen den Kladen. Sowohl eine Ab- als auch eine Zunahme der Chromosomengrundzahl während der Aufspaltung rezenter Arten konnte beobachtet werden, sowie eine Abnahme der Genomgröße. Annuelle haben tendenziell kleine Genomgrößen, während ausdauernde Arten eine höhere Variation zeigen. Des

Weiteren besitzen Arten der Mediterranregion im Allgemeinen kleinere Genome als Arten aus N- und Mitteleuropa, Eurasien sowie Zentral- und O-Asien.

Von den untersuchten Mikromerkmalen unterschied die Struktur der Pappusborsten zwischen den drei Kladen. Frucht-, Pollen- und Narbenastmerkmale zeigten auf Grund der geringen Stichprobenmenge keine interpretierbaren Muster. Die behandelten Merkmale könnten jedoch bei Einbeziehung weiterer Arten eine Eignung als Unterscheidungsmerkmal infragenerischer Gruppen aufweisen.

Taxonomische Konsequenzen aus den vorliegenden Ergebnissen sind die Aufrechterhaltung der Gattungen *Lapsana* und *Rhagadiolus*, die Behandlung von *Crepis* als paraphyletisches Taxon und die Eingliederung der ehemaligen Gattung *Dianthoseris*. Anmerkungen zu einer revidierten sektionalen Gliederung der Gattung werden gemacht.

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APPENDIX

10.1 PUBLICATION LIST

10.1.1 Publications in Journals

Enke, N., Kilian, N., Nemomissa, S., & Gemeinholzer, B. 2008. Afro-alpine Dianthoseris actually a congener of Crepis s.str. (Compositae, Cichorieae, Crepidinae). *Botanische Jahrbücher* 127(3): 389-405.

Enke, N. & Gemeinholzer, B. 2008. Babcock revisited: new insights into generic delimitation and character evolution in Crepis L. (Compositae: Cichorieae) from ITS and matK sequence data. *Taxon* 57(3): 756-768.

Enke, N. Fuchs, J. & Gemeinholzer, B. Shrinking Genomes? Evidence from Genome Size Evolution in Crepis L. (Cichorieae, Compositae). *Molecular Biology and Evolution* (subm).

10.1.2 Talks and Posters at National and International Conferences

10.1.2.1 Talks

Enke, N. 2006. Character Evolution within *Crepis* L. (Compositae) – First Insights. Royal Botanic Garden Edinburgh, Scotland.

Enke, N. & Gemeinholzer, B. 2007. First Insights into Speciation Processes in *Crepis* L. (Compositae). GfBS Jahrestagung 2007, Vienna, Austria. (Award for Best Student Presentation).

Enke, N. & B. Gemeinholzer, B. 2007. Contributions Towards a New Generic and Infrageneric Classification Delimitation of *Crepis* L. (Compositae). Young Systematists Forum, London, England.

Enke, N. 2008. Molekulare Phylogenie und Morphologie: Der lange Weg zu einer Neugliederung der Gattung *Crepis* L. (Cichorieae, Compositae). Invited Talk at the Colloquium of Organismic Biology, Phillips-University, Marburg, Germany.

Enke, N. & Gemeinholzer, B. 2008. Mechanisms of Speciation: Hybridisation and Karyotype Evolution in *Crepis* L. (Cichorieae, Compositae). Systematics 2008, Göttingen, Germany.

Enke, N. & Gemeinholzer, B. 2008. Karyotype Evolution and Speciation in Higher Plants: The Genus *Crepis* L. (Cichorieae, Compositae). Botany 2008, Vancouver, Canada.

10.1.2.2 Posters

Gemeinholzer, B., **Enke, N.** & Jahn, R. 2007. Establishing DNA-Barcoding Methods in Diatoms for Diversity Assessments. Biodiversity Infomatics and the Barcode of Life, Aarhus.

Enke, N. & Gemeinholzer, B. 2008. Possible hybrid origin of Asian/American alpine genus *Askellia* W. A. Weber (Cichorieae, Compositae). Xth Symposium of the International Organisation of Plant Biosystematists, Strbske Pleso, Slovakia.

10.2 ERKLÄRUNG ÜBER DEN PERSÖNLICHEN ANTEIL AN DEN PUBLIKATIONEN

Kapitel 2 - Babcock revisited: New Insights into Generic Delimitation and Character Evolution in *Crepis* L. (Compositae: Cichorieae) from ITS and *matK* Sequence Data.

Datenerfassung:

- Eigenanteil der Autorin: Recherche nach geeigneten Herbarbelegen in Herbaren B, E, C und CGE, Nachbestimmen der Herbarbelege, Recherche für geeignete Außengruppen Sequenzen in der GeneBank, Extraktion der DNA, PCR, Aufreinigen der PCR Produkte, Sequenzierung, Editierung und Alignierung der Sequenzen.
- Bereitstellung von Herbarbelegen aus M und W, Beratung beim Primerdesign und Anpassen der PCR Protokolle durch B. Gemeinholzer.

Datenauswertung:

- Eigenanteil der Autorin: Rekonstruktion der Phylogenien basierend auf ITS und matK mittels Maximum Parsimony und Bayesischer Statistik, Interpretation der Daten.

Manuskript:

- Eigenanteil der Autorin: Erstellung des englischen Manuskripts.
- Korrekturen durch B. Gemeinholzer.

Kapitel 3 – Shrinking Genomes? Evidence from Genome Size Variation in *Crepis* L. (Compositae).

Datenerfassung:

- Eigenanteil der Autorin: Samenaufsammlungen in den Gebirgen Südeuropas, DNA Extraktion zusätzlicher Proben, PCR, Aufreinigen der PCR-Produkte, Sequenzierung, Editierung und Alignierung der Sequenzen, Recherche nach bereits veröffentlichten Genomgrößen in der C-Value Database, Auswahl geeigneter Pflanzen und Vorbereitung der Proben für die Genomgrößenmessung.
- Samenaufsammlung in Sibirien gemeinsam mit B. Gemeinholzer.
- Messung der Genomgrößen am FacStar^{PLUS} gemeinsam mit J. Fuchs.

Datenauswertung:

- Eigenanteil der Autorin: Phylogenierekonstruktion mittels Maximum Parsimony basierend auf ITS, Rekonstruktion der Ancestral Character States in Mesquite, Interpretation der Daten.

Manuskript:

- Eigenanteil der Autorin: Erstellung des englischen Manuskripts.
- Korrekturen durch B. Gemeinholzer.

Kapitel 6 - Afroalpine *Dianthoseris* actually a congener of *Crepis* s.str. (Compositae, Cichorieae, Crepidinae)

Datenerfassung:

- Eigenanteil der Autorin: Auswahl geeigneter DNA Sequenzen, DNA Extraktion zusätzlicher Proben, PCR, Aufreinigen der PCR-Produkte, Sequenzierung, Editierung und Alignierung der Sequenzen.
- Erfassen morphologischer, geographischer und karyologischer Daten durch N. Kilian, S. Nemomissa und B. Gemeinholzer

Datenauswertung:

- Eigenanteil der Autorin: Rekonstruktion der Phylogenien basierend auf ITS und matK mittels Maximum Parsimony und Bayesischer Statistik, Interpretation der molekularen Daten.
- Taxonomische Umkombinierung gemeinsam mit N. Kilian.
- Auswertung morphologischer, geographischer und karyologischer Daten durch N. Kilian, S. Nemomissa und B. Gemeinholzer.

Manuskript:

- Eigenanteil der Autorin: Erstellung und Endfassung des englischen Manuskripts (Methoden/Ergebnisse/Diskussion) für den molekularen Bereich.
- Endfassung des englischen Gesamtmanuskripts durch N. Kilian.

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