

**Molecular systematics and evolution of the
non-photosynthetic parasitic *Cistanche*
(Orobanchaceae)**

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To my parents with gratitude for your love and support and to my people in
Afghanistan

Foreword

Parasitic Orobanchaceae includes the fascinating genera with low to high degree or morphological reductions and different type of parasitism. Among fully parasitic genera, parasitic weeds like *Orobanche* and *Cistanche* are of great interest. I have been motivated to perform this research, as lack of suitable information on genus *Cistanche* in terms of its evolutionary among Orobanchaceae family. As the majority of the *Cistanche* species in the genus, has not been attended the subject of molecular analyses, our DNA sequence data associated with deeper insights into resolving phylogenetic relationships in *Cistanche* as an excellent research in the family. Building a phylogeny of the genus inferred from plastid regions and nuclear ITS was as priority issue in the study. Phylogenetic relationships provided proper information to constitute genome size, chromosome counts, systematic identities and biogeographical consequences in ongoing work. Second, the genus is of particular interest, as it possesses a high chromosome base number ($x = 20$), by far the largest chromosomes (c. 5-10 μm) and the highest genome size (8.4-10 pg per monoploid genome) among its relatives, rendering it a well-suited system for studying components of and processes leading to enlarged genomes. The specific aims included counting chromosome number for first time at least for nine species inhabiting in one of diversity center (Iran) and genome size determination in a selected set of taxa. The obtained data will be formed necessary basis for further studies on genome evolution in *Cistanche* (e.g. retroelement evolution, nuclear inserts of sequences of plastid origin). The research, however, is the first attempt towards understanding the evolutionary patterns of the species and testing usefulness of plastid and nuclear markers to infer these relationships. We hope that the research could represent the most current phylogenetic and taxonomical knowledge of the poorly understood genus in Orobanchaceae family and inspired to the further investigation.

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1 General introduction

1.1 Evolution of the Orobanchaceae

In spite of dominating autotrophic life in majority of land plants, acquisition of resources from other plants has also evolved in multiple lineages (Musselman & Press, 1995). Parasitic plants represent 1% of flowering plants with roughly 4,000 species of which c. 2,060 in 90 genera belong to Orobanchaceae (Nickrent, 2014). Current phylogenies based on organellar and nuclear markers indicate that parasitism evolved at least 12 or 13 times independently in angiosperms (Bennett & Mathews, 2006; Filipowicz & Renner, 2010; McNeal & al., 2013; Schneeweiss, 2013; The Angiosperm Phylogeny Group 2016; Westwood & al., 2010). Likewise, holoparasitic species have evolved at least 8 times independently within angiosperms (Barkman & al., 2007). Among Scrophulariaceae and Orobanchaceae, however, the evolution of parasitism occurred once; thus here parasites form a monophyletic group, as recently revealed by analyses of either plastid genes *rps2* and *rbcL* (dePamphilis & al., 1997; Nickrent & al., 1998; Wolfe & dePamphilis, 1998; Young & al., 1999) or ITS sequence data (Wolfe & al., 2005). In contrast, holoparasitism has several independent origins from hemiparasitism within Orobanchaceae. Based on the current classification of the Orobanchaceae, all genera apart from *Lindenbergia*, the non-parasitic photosynthetic sister to the parasitic Orobanchaceae (Nickrent & al., 1998; Young & al., 1999; Young & dePamphilis, 2000; Olmstead & al., 2001), are either hemiparasitic (facultative or obligate, based on level of dependence upon the host) or holoparasitic (Nickrent, 2002).

Cistanche is traditionally placed in a clade of the Orobanchaceae including members of *Orobanche* and *Phelipanche* (clade III of Bennett & Mathews, 2006), whose recent phylogenetic inference is in agreement with the traditional taxonomy, although many *Orobanche* and *Phelipanche* species are still poorly understood in terms of phylogenetic relationships, diversity and taxonomic identification assessments (Schneeweiss, 2013). Recent molecular studies of the Orobanchaceae based upon plastid markers (Figure 1.1 here; Park & al., 2008; McNeal & al., 2013) revealed that *Cistanche* is placed as sister group to the whole *Orobanche* group and to a clade including the genera *Boschniakia*, *Conopholis* and *Epifagus*.

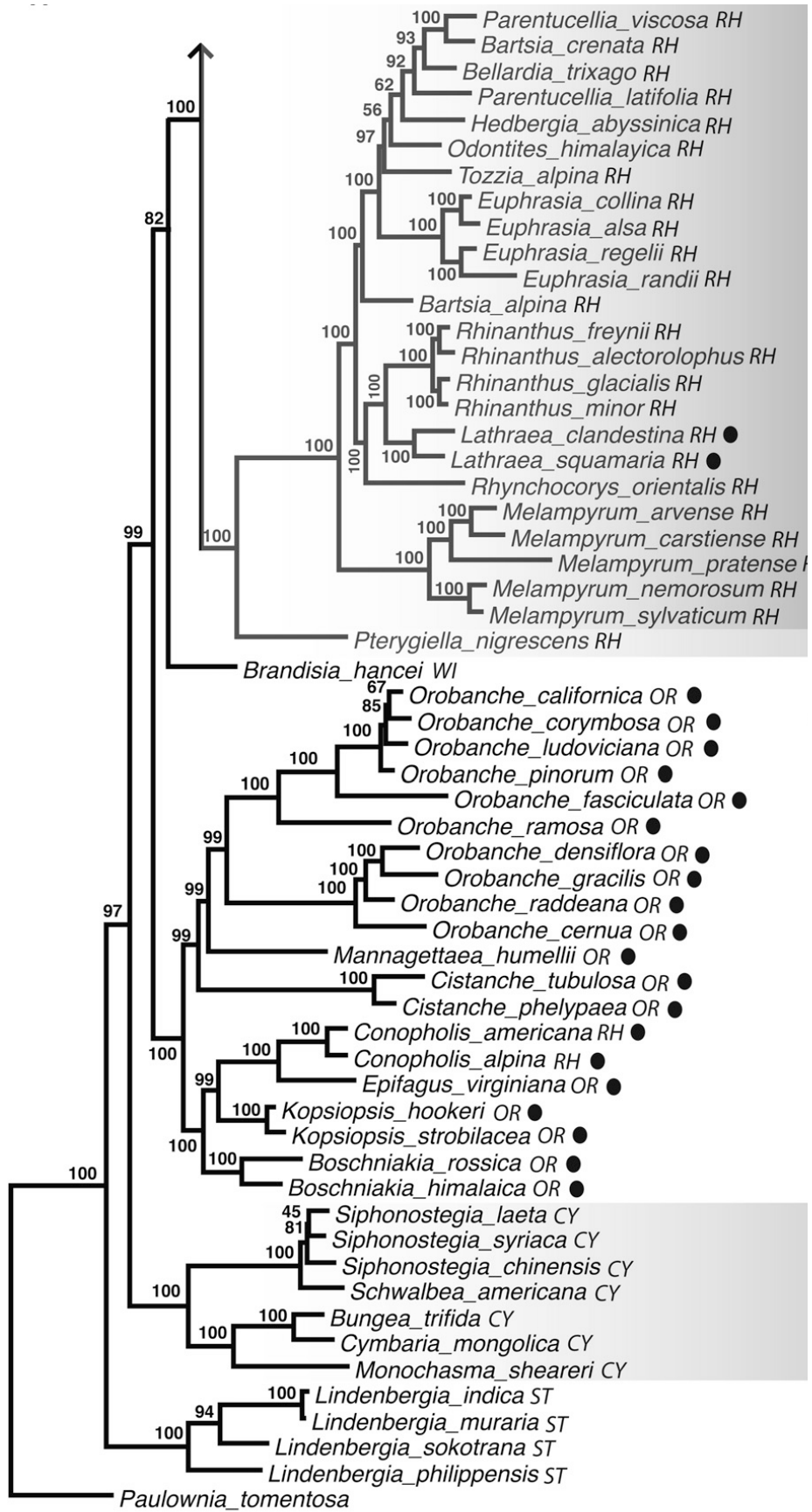


Figure 1.1 Phylogenetic relationships in Orobanchaceae in clade II, III and V inferred from maximum likelihood analysis of a combined data set of *PHYA*, *PHYB*, *ITS*, *matK*, *rps2* sequences (adopted from McNeal & al., 2013).

1.2 The genus *Cistanche*: systematics, phylogeny and biology

As common in parasitic Orobanchaceae, lack of sufficient informative vegetative characteristics in *Cistanche* emphasizes the usefulness of floral morphology which offers *per se* more subtle criteria to differentiate species and warrant the taxonomic rank of closely related entities. The genus, with a centre of highest species diversity in Southwest Asia and the Middle East (Fig. 1.2), has extremely splendid flowers with a corolla color ranging from white, pink, yellow, orange, pale to dark purple, useful for delimitation of the majority of species (e.g. Fig. 1.3). It is indicated that the species are insect pollinated (e.g. bees) as observed by Aboul-Enein & al., (2012). The latest identification key given by Beck-Mannagetta (1930) contains some ambiguity leading to questionable identifications, ever since no genus-wide treatment has been made except restricted regional floras. His exclusively morphological survey circumscribed species into four sections including 16 species (for detail, see chapter 4). Apart from studies focusing on the evolution of the plastid genome (Li & al., 2013; Wicke & al., 2013) or the relationships of species used in Traditional Chinese Medicine (using sequences of nuclear ITS2 (Sun & al., 2012), *psbA-trnH* IGS (Han & al., 2010), *rpoC2*, *rps2*, and *rpl16* (Tomari & al., 2002 & 2003)) the genus has not received any comprehensive phylogenetic attention. And very few taxonomically restricted species of *Cistanche* were included in the family level phylogeny of the Orobanchaceae. Hence, an uncertain species phylogeny pointed out the genus as excellent model for further molecular studies and taxonomic revision. Involving phylogenetic affinities may lead to accurate delimitation of mainly morphological cryptic species after Beck-Mannagetta (1930). In addition to the poorly known phylogenetic relationships and the lack of karyological data the group itself is interesting as the species are known to have relatively large genomes (Weiss-Schneeweiss & al., 2006). Moreover, in contrast to narrow host range in the majority of the non-photosynthetic *Orobanche* and *Phelipanche* (Schneeweiss, 2007) there has been no reported trend of host specialization in the parasitic *Cistanche*. The species rely on miscellaneous perennial hosts from various genera of different families. For instance, approximately 20 perennial wild woody shrubs and rarely forest trees, act as host that belong to seven families. For example, c. 20 genera of 10 different families are in the host range of *C. tubulosa*. Similarly, *C. salsa* found to parasite on c. 10 genera of 5 families (Qasem, 2009). However, frequently members of the Chenopodiaceae are reported as host for two species of *Cistanche* in Jordan.

Species of *Cistanche* also play a not to be neglected role for medicinal, biochemical and ecogeographical purposes. As an example, the fleshy stem of *C. deserticola* known as

“Cistanchis Herba” has been utilized as a tonic or remedy for many disorders like kidney pain, gynecological diseases, intestinal infections, impotence, infertility, general weakness and chronic constipation in China and Japan since years. In addition, it has been found as raising immunity, anti-age and anti-fatigue (Jiang & Tu, 2009). On the other hand, treatments can lead to impotence, infertility, menstrual disorder, and psychroalgia of the back and knees as reported by the Chinese Comprehensive Pharmaceutical Dictionary. *C. tubulosa*, for instance also used as remedy to Alzheimer's disease in Chinese herbal medicine and may act as supplements for renal function, aphrodisiac and smoothers the intestines. Furthermore, Aboul-Enein & al., (2012) revealed 100 % anticancer activity of *C. phelypaea* in water extracts determined by a 2,2 diphenyl-1-picrylhydrazyl (DPPH) assay. Due to the high demand and decreasing population counts the species has been included in the Convention on International Trade in Endangered Species (CITES) Appendix II in 2000 (endangered species scientific commission, following proposal 11.59). Solely *C. deserticola* has been officially traded as “Cistanchis Herba” in China, although other species e.g. *C. ambigua*, *C. tubulosa*, *C. salsa* and *C. sinensis* in the country were mixed and traded as “Cistanchis Herba” (IUCN/SSC & TRAFFIC, 2000). Because of overharvesting, *C. tubulosa* may also propose to follow in danger species in China, even though it is currently under sustainable harvesting.

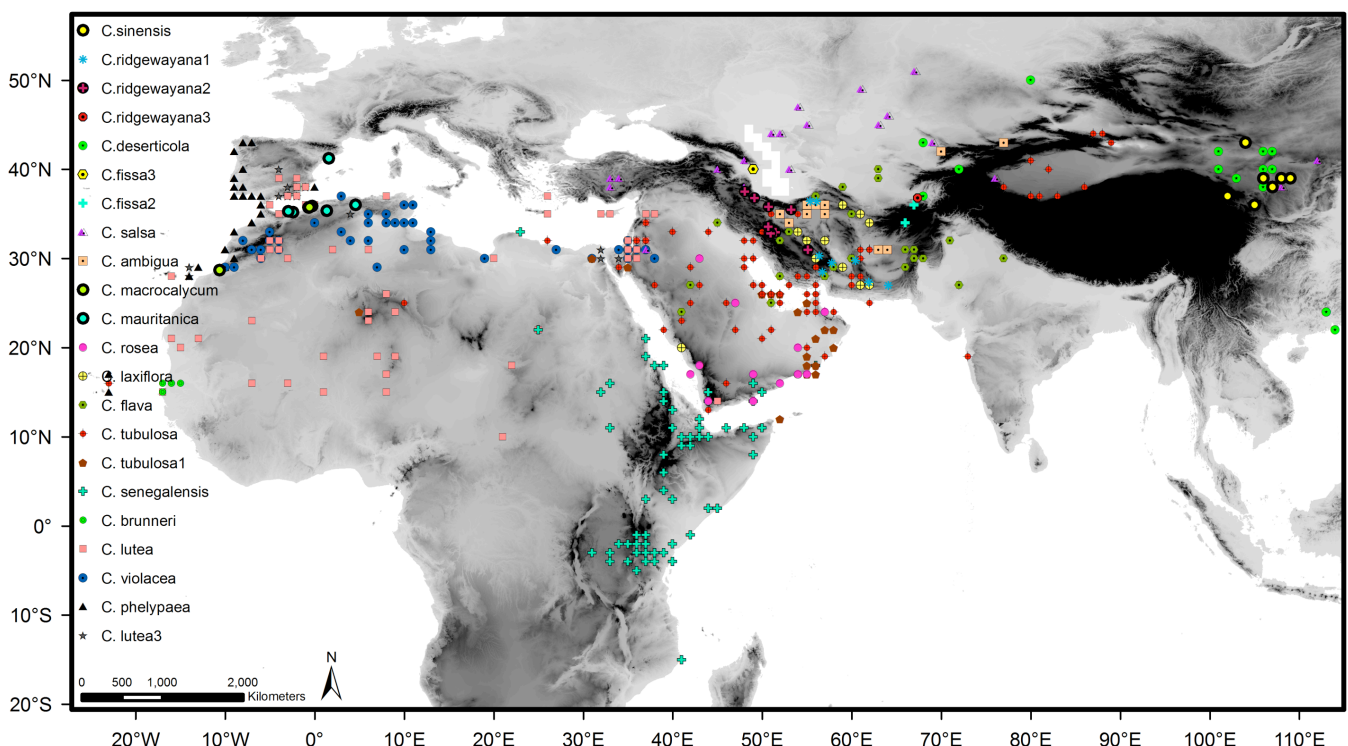


Figure 1.2 General distribution of genus *Cistanche* worldwide according to literature, herbarium and field observations.

In fact, those species with very narrow distribution range are under the high risk and should be taken more in consideration e.g. *C. fissa* reported only from Turkmanistan and Azerbaijan or *C. salsa* from certain location in Turkey and adjacent regions (for detail see chapter 4; Fig. 4.19). Affectivity of *C. deserticola* on its host (*Haloxylon ammodendron*) has been explored as reduction in relative water content and water retention capability (Li & al., 2009a), injuring protective enzyme system (Li & al., 2009b) and reducing biomass (Tan & al., 2004; Huang & al., 2009). Its negative contribution on host growth by means of reducing nutrient acquisition, inorganic ion accumulation and the photoprotection has been revealed by Li & al. (2012).



Figure 1.3 Representatives of *Cistanche* species. A) *C. violacea* from Morocco, B) *C. mauritanica* from Algeria, C) *C. senegalensis* from Mauritania (photo taken by Jeroen Onrust, personal communication), D) *C. flava* from Iran, E) *C. lutea* from Morocco, F) *C. tubulosa* from Kuwait (photo by: Khaled Al-Ghanem available at <https://www.flickr.com>), G) *C. chabaharensis* from Qatar (photo by: Dan A'Vard available at <https://www.flickr.com>), H) *C. laxiflora* subsp. *alba* from Iran, I) *C. ambigua* from Iran, J) *C. bilobata* from Azerbaijan (photo by: Holger Uhlich available at <http://orowiki.org/>), K) *C. almeriensis* from Spain (photo by: Jean-Luc Tasset available at <http://www.tela-botanica.org/>), L) *C. rosea* from Oman (photo taken by M. Schnittler available at <http://orowiki.org/>), M) *C. ridgewayana* from Turkmanistan (photo by: Jeremy Holden, personal communication). Photos A, D, E, I and H by: authors (N. Ataei & D. Quandt).

1.3 Research scopes and objectives

The essential scope was to elucidate the phylogenetic relationships within the poorly understood non-photosynthetic genus *Cistanche* (Orobanchaceae), in order to test: i) current intrageneric classifications, ii) morphological species delimitations and, iii) expanding our understanding of trends in genome size and karyology.

Chapter 2 aims at a worldwide phylogenetic analysis of all available *Cistanche* taxa, in order to test if the current infrageneric taxonomy reflects phylogenetic relationships. Comprehensive studies in this context demanded for deep sampling. Therefore, all species of *Cistanche* were sampled, generally with multiple accessions, except for a few rare taxa with very few available reports. A special focus was laid on *C. tubolosa* s.l. and *C. phelypaea* s.l. as these taxa showed a highly complex morphology with potential cryptic species and a widespread biogeographic pattern across the Mediterranean and the Arabian peninsula. The phylogenetic data guided species delimitation and assisted in the identification of cryptic species that are in need of morphological revision, as traditional morphological characteristics failed to delimit these cryptic taxa. To achieve these purposes, DNA sequences from nuclear ITS and three plastid regions (*trnL-F*, *trnS-trnfM*, *psbA-trnH*) were employed. The relationships were inferred using maximum likelihood, maximum parsimony and Bayesian inferences.

Estimating genome size and karyological studies (chromosome numbers) of the species was our next strategy (**Chapter 3**). According to previous studies the genus is of particular interest, as it possesses a high chromosome base number ($x = 20$) and it has by far the largest chromosomes (c. 5-10 μm) and the highest genome size (8.4-10 pg per monoploid genome) among its relatives, rendering it a well-suited system for upcoming studying components and processes leading to enlarged genomes. Comprehensive data on relationship between genome size and chromosome numbers are presented and discussed in a phylogenetic framework, allowing us to answer further questions as whether the genome and chromosome size variation correlates with natural groupings and propose newly taxonomic revision in ongoing studies. Exploring the 1C-value with a phylogenetic background aimed to trace any trends in genome size decrease or increase along the evolution of the species.

Our preliminary overview, exclusively based on morphology mainly from herbarium observations revealed that the species delimitations and relationships within the poorly understood genus are still controversial. The lack of a modern taxonomic treatment strongly

hampered our understanding of the phenotypic relationships among *Cistanche* species, an indispensable basis for evolutionary studies (e.g. biogeographic relationships, and genome evolution). Therefore, using the results of the phylogenetic and morphological analyses (**Chapter 2 & 3**) we examined the putative species, building towards a complete taxonomic revision of the genus (**Chapter 4**). Careful re-examinations of the genus using mainly floral morphology resulted in a dichotomous key and a new classification of the genus into two subgenera and four sections. Indeed, we defined some significant morphological characters like scale and corolla coloration which were mainly disregarded in former floristic literatures. Following this, 25 species were recognized to be involved in the genus, highlighting two subgenera, of nine new species and six new subspecies.

1.4 Contribution to chapters

Chapter 2:

Ataei, N., Schneeweiss, G.M., García, M.A., Krug, M., Lehnert, M., Valizadeh, J., Quandt, D. Phylogeny of the non-photosynthetic parasitic *Cistanche* (Orobanchaceae) inferred from nuclear and chloroplast data, reveal relationships among four major morphological distinct clades.

The chapter will be submitted to *Molecular Phylogenetics and Evolution*

Own contributions: literature research, collected material (together with Dr. Valizadeh and prof. D. Quandt in part), herbarium studies, laboratory work, phylogenetic analyses (together with M. Krug), writing manuscript (with assistance of M. Lehnert in part), revision (with Prof. Quandt, Prof. Schneeweiss)

Chapter 3:

Ataei, N., Temsch, E., Schneeweiss, G.M., Quandt, D., Valizadeh, J., Weiss-Schneeweiss, H. Evolutionary trends in the non-photosynthetic parasitic *Cistanche* (Orobanchaceae) inferred from karyological data, contrasting effects of genome size with chromosome number.

The chapter will be submitted to *Botanical Journal of Linnean Society*

Own contributions: collected material (together with Dr. Valizadeh and Prof. D. Quandt in part), laboratory work, herbarium studies, statistical analyses, writing manuscript, revision (with Dr. Weiss-Schneeweiss, Prof. GM Schneeweiss and Prof. D. Quandt)

Chapter 4:

Ataei, N., Luebert, F., Schneeweiss, G.M., Valizadeh, J., Quandt, D. Taxonomic revisions of the genus *Cistanche*, with newly northwestern African infrageneric section.

The chapter will be submitted to *Phytotaxa*

Own contributions: literature research, herbarium study, statistical analyses (together with Dr. F. Lubert), writing manuscript, revision (Dr. F. Lubert, Prof. GM Schneeweiss and Prof. D. Quandt)

2 Molecular phylogenetic analyses of the non-photosynthetic parasitic plant *Cistanche* (Orobanchaceae), inferred from three plastid markers and nuclear ITS, reveal relationships among four major morphological distinct clades

Abstract

Phylogenetic relationships of and within non-photosynthetic parasitic lineages are notoriously poorly known, which negatively affects our understanding of parasite evolution. This is also the case for *Cistanche* (Orobanchaceae), an Old-World group with about two dozen species, whose relationships have not yet been addressed using molecular phylogenetic approaches. Here we infer phylogenetic relationships within the genus, employing a taxonomically and geographically broad sampling covering all previously distinguished infrageneric groups and most of the currently recognized species. A combined matrix of three plastids (*trnL* intron and *trnL-trnF* intergenic spacer (IGS), *trnS-trnfM* IGS and *psbA-trnH* IGS) and one nuclear marker (ITS) was analyzed using maximum parsimony, maximum likelihood and Bayesian inference. *Cistanche* falls into four well-supported clades that only marginally correspond to traditionally recognized sections and rather reflect biogeographic distribution. While East Asian (EA) and NW African (NWA) clades are species-poor and geographically restricted, the widespread (W) clade and the SW Asian (SWA) clade are richer in species, some of which are rare and endangered, and more widespread in their distributions. A preliminary taxonomic re-evaluation of morphological characters and biogeography within the genus in light of the inferred phylogenies is discussed.

2.1 Introduction

Orobanchaceae is an interesting model system for studying the evolution of parasitism (Westwood & al., 2010). They also are an example of how molecular data have successfully improved our understanding of phylogenetic relationships. Based on molecular studies, the family circumscription has considerably changed and intergeneric relationships have been modified (Wolfe & al., 2005; Bennett & Mathews, 2006; Park & al., 2008; McNeal & al., 2013). The broadest and most comprehensive phylogenetic analyses of Orobanchaceae to date is by McNeal & al. (2013), who used a combined data set of five markers (nuclear ITS,

PHYA, *PHYB*; plastid *matK* and *rps2*) comprising more than 50 genera of the family. Despite this progress, many genera have not been included in molecular studies and remain unplaced (Schneeweiss, 2013). Additionally, phylogenetic relationships within genera, especially in taxonomically notoriously difficult non-photosynthetic parasitic groups are essentially unexplored.

A genus in need of a more thorough phylogenetic investigation is the non-photosynthetic root-parasitic genus *Cistanche*. It is a potentially rewarding object for studying genome evolution (much larger chromosomes and correspondingly much larger genome size than in closely related lineages: Schneeweiss & al., 2004b; Weiss-Schneeweiss & al., 2006), reductive evolution of plastid genomes (Li & al., 2013; Wicke & al., 2013), or species diversity and biogeography in arid regions, but any of these research avenues is currently hampered by our poor understanding of species relationships due to the lack of thorough phylogenetic data. The few studies that include *Cistanche* focus on the evolution of the plastid genome (Li & al., 2013; Wicke & al., 2013) or the relationships of species used in Traditional Chinese Medicine (Tomari & al., 2002 & 2003; Han & al., 2010; Sun & al., 2012), and are taxonomically very restricted. Likewise, phylogenetic studies directed at the family level only include one or few representatives of *Cistanche* (Young & al., 1999; Schneeweiss & al., 2004a; Wolfe & al., 2005; Park & al., 2008; McNeal & al., 2013; Schneeweiss, 2013). Their results show that *Cistanche* is closely related to other non-photosynthetic parasitic genera, such as *Orobanche*, *Phelipanche*, *Conopholis* or *Epifagus* (clade III of McNeal & al., 2013; *Orobanche-Phelipanche* clade of Schneeweiss, 2013), but its precise phylogenetic placement has not been fully settled yet (Schneeweiss, 2013).

The latest monograph of the entire genus is by Beck-Mannagetta (1930). Based on calyx shape and bracteole number, he distinguished four sections: (i) *C.* sect. *Cistanchella* Beck with the single species *C. ridgewayana* Aitch. & Hemsl.; (ii) *C.* sect. *Subcistanche* Beck with the single species *C. sinensis* Beck; (iii) *C.* sect. *Heterocalyx* Beck, with three species (*C. fissa* (C.A.Mey) Beck, *C. ambigua* (Bunge) Beck and *C. rosea* Backer); (iv) *C.* sect. *Cistanche* Beck containing the remaining 12 species. Later taxonomic treatments, usually in the context of national floras, add a few new species and partly reshuffle circumscription of often morphologically variable and thus taxonomically complex species already recognized by Beck-Mannagetta (1930), so that currently about 25 species are accepted (for detail, see chapter 4). The genus is widely distributed in arid regions of the Old World from the Macaronesian Islands and western Africa to central and East Asia, with the centre of highest species diversity in Southwest Asia and the Middle East (Beck-Mannagetta, 1930; chapter 4). Like other non-photosynthetic genera of Orobanchaceae (e.g. Rodrigues & al., 2011; Schneeweiss, 2013), *Cistanche* is characterized by morphological reduction especially of

vegetative characters, so that most diagnostic characters are from the inflorescence and flowers (shape and indumentums of floral bracts and bracteoles, structure and indumentum of the calyx, flower colour, shape and indumentums of the anther), some of which are poorly preserved on herbarium specimens. Lack of a comprehensive taxonomic treatment covering all currently recognized species, poor representation in collections especially from less-explored areas, and paucity of taxonomically useful morphological characters contributes to the insufficiently known and unconsolidated taxonomy of *Cistanche* species.

In this study, we want to establish phylogenetic relationships within *Cistanche* as basis for a phylogenetically predictive taxonomic system using a taxonomically and geographically comprehensive sampling. To this end, we collect sequence data from rapidly evolving and well-established plastid markers as well as nuclear ITS sequences from a taxonomically and geographically comprehensive sampling and analyze those using maximum parsimony, maximum likelihood and Bayesian methods. Specifically, we want (i) to test hypotheses of relationships implied by the classification of Beck-Mannagetta (1930), i.e. whether his sections constitute monophyletic groups, and (ii) to test whether morphologically and taxonomically complex species like *C. phelypaea* and *C. tubulosa* form natural groups.

2.2 Materials and Methods

2.2.1 Plant material

One hundred-eighty-nine samples (newly collected, herbarium material or sequences from GenBank) were included, corresponding to 17 previously identified *Cistanche* species plus seven outgroup taxa (one accession of *Conopholis americana*, *Phelipanche* cf. *iberica*, two accessions of *Orobanche cernua*, one each of *O. anatolica*, *O. densiflora* and *O. transcaucasica*). Sampling of *Cistanche* aimed at a broad geographic coverage for each species in the genus. The outgroup taxa were selected in accordance with our current knowledge on relationships within Orobanchaceae (Schneeweiss & al., 2004a; McNeal & al., 2013; Schneeweiss, 2013). Locality and voucher information is given in Appendix 2.1.

2.2.2 Isolation of genomic DNA

Extraction of genomic DNA from newly collected silica-gel dried corolla tissue followed the CTAB protocol (Doyle & Doyle, 1990). The majority of herbarium material was isolated either using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) or the NucleoSpin Plant II (Macherey-Nagel, Düren, Germany). Approximately 50 mg of dried material were homogenized using a mixer mill (Retsch MM200, Haan, Germany) at 30 Hz for 3 min followed by incubation in 700 µl extraction buffer for at least one hour at 65°C. Thereafter, the CTAB-protocol or the protocols supplied by the kit manufacturers were used.

2.2.3 Amplification and sequencing

Genomic DNA was stored at -80°C and dilutions were used for later amplifications. Three plastid markers (*trnL*(UAA) intron plus *trnL* (UAA)-*trnF* (*trnL-F*) intergenic spacer, hereinafter *trnL-F*, *trnS-trnM* and *psbA-trnH* intergenic spacers), located in the large single copy region (LSC) of the plastid genome, and the nuclear internal transcribed spacers (ITS1 and ITS2 plus the intervening 5.8S rDNA gene) were selected for phylogenetic inferences. The plastid markers have been repeatedly shown to be well-suited for phylogenetic studies at the species level (Borsch & Quandt, 2009). Additionally, *psbA-trnH* has been already used for DNA barcoding of Chinese *Cistanche* taxa (Han & al., 2010; Sun & al., 2012). The nuclear ITS region was chosen as technically readily amenable nuclear marker that despite numerous potential problems (Álvarez & Wendel, 2003) has been successfully applied in numerous phylogenetic studies with focus on species relationships (Baldwin & al., 1995; Álvarez & Wendel, 2003; Bailey & al., 2003) including *Orobanchae* and related genera (e.g. Schneeweiss & al., 2004a; McNeal & al., 2013). Furthermore, ITS2 has also been used for DNA barcoding in *Cistanche* (Han & al., 2010; Sun & al., 2012). Details of primers used in this study are given in Appendix 2.2. The *trnL-F* region was amplified using the primers *trnTC* and *trnTF* (Taberlet & al. 1991). In some cases, such as for old and supposedly degraded material, the region was amplified in two separate fragments, i.e. primers C and D (Taberlet & al., 1991) and primers *trnL460F* (Worberg & al., 2007) and *trnF* (Taberlet & al., 1991). The *trnS-trnM* (IGS) was amplified using *trnS* (UAG) and *trnM* (CAU) (Wicke, pers. comm), while amplification of the *psbA-trnH* IGS followed Kress & al. (2005). The PCR profile for the plastid markers included an initial denaturation step of 5 min at 94°C, followed by 30 cycles each with 1 min 94°C, 1 min 55°C, 90 s 72°C, and a final elongation step of 7 min at 72°C. The ITS region was amplified using the primers ITS4 and ITS5 (White & al., 1990) with an amplification profile of 5 min at 94°C followed by 40 cycles each with 1 min 94°C, 1 min 48°C with a time-increment of +4s/cycle, 45 s 68°C, and a final extension step of 7 min at 68°C. Unsuccessful PCRs were repeated using internal primers 5.8S_106 and

5.8S_32 (designed by Prof. Daniel L. Nickrent, Southern Illinois University, USA) in combination with the respective external primers ITS5 and ITS4. PCR reactions were performed in a volume of 25 µl and included 1.5 U GoTaq Flexi DNA polymerase (Promega Corporation, Madison, USA), 0-0.2 M betaine monohydrate, 0.4 µM of each forward and reverse primer, 0.15 mM dNTPs (Carl Roth, Karlsruhe, Germany), 1 mM MgCl₂ in 1x GoTaq Flexi buffer, 1 µl genomic DNA of unknown concentration, and water. For some highly degraded herbarium material Ready-To-Go PCR Beads (Amersham-Pharmacia Biotech, Amersham, UK) were used following the manufacturer's instructions. As commonly required for DNA-isolates of herbarium material, PCR additives such as 1 µl PVP-40 (10–40%) and/or 5 µl enhancer solution P (5x, PeqLab, Erlangen, Germany) were added to the reactions on the expense of water. Amplification products were gel-purified on a 1 % agarose gel using the PeqLab PCR purification kit (Peqlab) or the QIAquick PCR purification Kit (QIAGEN). For nested ITS-PCR products of less than 300 bp (amplification from herbarium material, which often is contaminated with fungi), a higher gel concentration (1.4%) for a longer period was chosen. Cleaned PCR products were sequenced by Macrogen Inc. (Seoul, Korea) with the amplification primers and the additional internal primers mentioned above, where needed.

2.2.4 Sequence alignment, indel coding and statistics

DNA sequences were assembled and aligned using PhyDE 0.97 (Müller & al., 2006a; www.phyde.de). Following Olsson & al. (2009), regions of uncertain homology (mutational hotspots) were annotated in PhyDE and removed from the analyses and inversions included as reverse-complement as suggested by Quandt & al. (2003) and Borsch & Quandt (2009). Data matrix statistics such as sequence length and range, GC-content and sequence variation were calculated using SeqState 1.25 (Müller, 2005b). Indels (Appendix 2.3) were coded using simple indel coding (SIC; Simmons & Ochoterena, 2000) as implemented in SeqState 1.25 (Müller, 2005b) and added as additional data partition. In all data matrices sequence gaps were treated as missing data and aligned positions regarded as equally weighted.

2.2.5 Phylogenetic analyses

Analyses were conducted on three sequence data sets – ITS, concatenated plastid markers, and combined (nuclear and plastid markers combined) – each with or without indels, resulting in a total of three data sets analyzed. Prior to the combined analyses the data matrix was screened for incongruences via an analysis of each single locus in MrBayes using the default settings to detect possible lab confusions (Appendices 2.7, 2.8 and 2.9). Maximum parsimony analyses (MP) were performed in PAUP* 4.0b10 (Swofford, 2003)

using a parsimony ratchet approach (Nixon, 1999) via the command files generated by PRAP2 (Müller, 2004). The following ratchet settings were used: 200 iterations with 25 % of the positions randomly up-weighted (weight = 2) in each replicate and 10 random addition cycles. Maximum likelihood (ML) analyses were conducted for both DNA and partitioned matrices using RAxML 8 (Stamatakis, 2014) using the GTRCAT model. For both MP and ML analyses support was estimated via bootstrapping (Felsenstein, 1985) using 10,000 replicates. The following support categories were used for describing the results: significant bootstrap support with at least 95%; strong bootstrap support with at least 90%, moderate support with at least 70-90%, 50-70 as weak support and <50 as no support. Bayesian inferences (BI) were performed with MrBayes 3.2.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) using the GTR+ Γ +I model for the nucleotide partition (two unlinked models in case of the combined data set) and the restriction site model (F81-like; Felsenstein, 1981) for the indel partition with default priors. Six runs with 10 million generations each and four chains each (one cold and three heated chains using the default heating) were run in parallel, sampling every 1000th generation. When summarizing the consensus tree, the first 25% were discarded as burn-in, which was well after chains had reached stationarity. Clades are considered significantly supported with a posterior probability of 0.95 or more. Support values (posteriors) were considered insignificant below 0.95. Trees were edited using TreeGraph2 (Stöver & Müller, 2010).

2.3 Results

2.3.1 Sequence parameters of the datasets

The concatenated aligned matrix contained a total of 189 sequences of *Cistanche* plus seven outgroup taxa. Out of 189 ingroup taxa, 185 were successfully amplified for *trnL-F*, 176 for *trnS-fM*, 183 for *psbA-trnH* and 163 for ITS (for nine only ITS1 and for four only ITS2 could be sequenced). Altogether, in those herbarium samples where internal primers had to be used, amplification of ITS2 was more difficult than ITS1. The average length of mutational hotspots in the whole dataset was about 10 (range 5 to 20 bp) nucleotides (Table 2.1), which were defined none in ITS, 12 in *trnL-F*, five in *trnS-fM* and two *psbA-trnH* spacers. After exclusion of hotspots, the average variability among sequences was 43.81%, of which 58%, 12.5% and 60.48% were in ITS1 (222-243 bp), 5.8S rRNA gene (129-160 bp) and ITS2 (219-241 bp), respectively. Among plastid markers *trnL-F* showed the highest sequence variability (26.04% with higher variation in *trnL-F* spacer than its intron), followed by *trnS-fM* (25.92%) and *psbA-trnH* (10.90%). Correspondingly, the highest amount of parsimony-informative positions was observed 34.42% in ITS, 18.03% in *trnS-fM* spacer, 17.80% in *trnL-F* (with a higher amount in *trnL-F* spacer than its intron) and 8.87% in *psbA-trnH* spacer. The latter region showed the minor substitution rate among plastid regions studied here. In the concatenated matrix out of 4929 total characters, 4226 were from plastid regions, of which 14.94% were parsimony-informative. Four hairpin associated inversions were observed in data matrix (compare Quandt & al. 2003, Borsch & Quandt, 2009), three in *psbA-trnH* and one in *trnS-trnfM* region (compare Table 2.1). As expected the observed hairpin associated inversions were homoplasious (compare Quandt & al. 2003). Most indels in the dataset were simple sequence repeats (SSR) of adjacent fragments, ranging from 2 to 20 nucleotides (Appendix 2.3). Additionally, a deletion of up to 1148 bp in the aligned matrix was observed in acc. ED1040 in *psbA-trnH* spacer, i.e. a reduction in sequence length to 20% of the average length (Appendix 2.3). The characteristics of three plastid regions, nuclear ITS in the combined data, position of hotspots and inversions before implementing indel coding are listed in Tables 2.1 and 2.2.

Table 2.1 List of mutational hotspots (H), including hairpin associated inversions (HAI) with their description and position in the combined data set.

Number	Region	Position in the alignment	Accession number corresponding to hairpins	Length	Comment
H1	<i>trnL</i> intron	77-81		5	Poly A
H2	<i>trnL</i> intron	108-112		5	Poly T
H3	<i>trnL</i> intron	125-132		8	Poly A (with T & G substitutions)
H4	<i>trnL</i> intron	188-199		12	Poly A (with T & G substitutions)
H5	<i>trnL</i> intron	496-504		9	Poly A
H6	<i>trnL</i> intron	572-578		7	Poly A
H7	<i>trnL-F</i> spacer	936-943		8	Poly T
H8	<i>trnL-F</i> spacer	1040-1059		20	Poly T (with A & G substitutions)
H9	<i>trnL-F</i> spacer	1071-1075		5	Poly A (with G substitution)
H10	<i>trnL-F</i> spacer	1266-1274		9	Poly T (with C substitution)
H11	<i>trnL-F</i> spacer	1297-1312		16	Poly A
H12	<i>trnL-F</i> spacer	1336-1344		9	Poly A
H13	<i>trnS-trnfM</i> spacer	1546-1558		13	Poly T
H14	<i>trnS-trnfM</i> spacer	1609-1613		5	Poly G (with A substitutions)
H15	<i>trnS-trnfM</i> spacer	1918-1925		8	Poly T
HAI I	<i>trnS-trnfM</i> spacer	2709-2733	ED1040 & ED788	25	Hairpin Associated Inversion I
H16	<i>trnS-trnfM</i> spacer	2745-2749		5	Poly T
H17	<i>trnS-trnfM</i> spacer	2759-2766		8	Poly A (with C substitution)
H18	<i>psbA-trnH</i> spacer	3298-3317		20	Poly T (with A substitutions)
HAI II	<i>psbA-trnH</i> spacer	3322-3391	ED892, ED719, ED603, ED711 & <i>C. rosea</i>	70	Hairpin Associated Inversion II
HAI III	<i>psbA-trnH</i> spacer	3392-3415	ED787 & ED511	24	Hairpin Associated Inversion III
HAI IV	<i>psbA-trnH</i> spacer	3416-3502	In all accession of clade ii but ED1040, ED481, ED437, ED444 & ED448	87	Hairpin Associated Inversion IV
H19	<i>psbA-trnH</i> spacer	3639-3653		15	Poly A (with T substitutions)

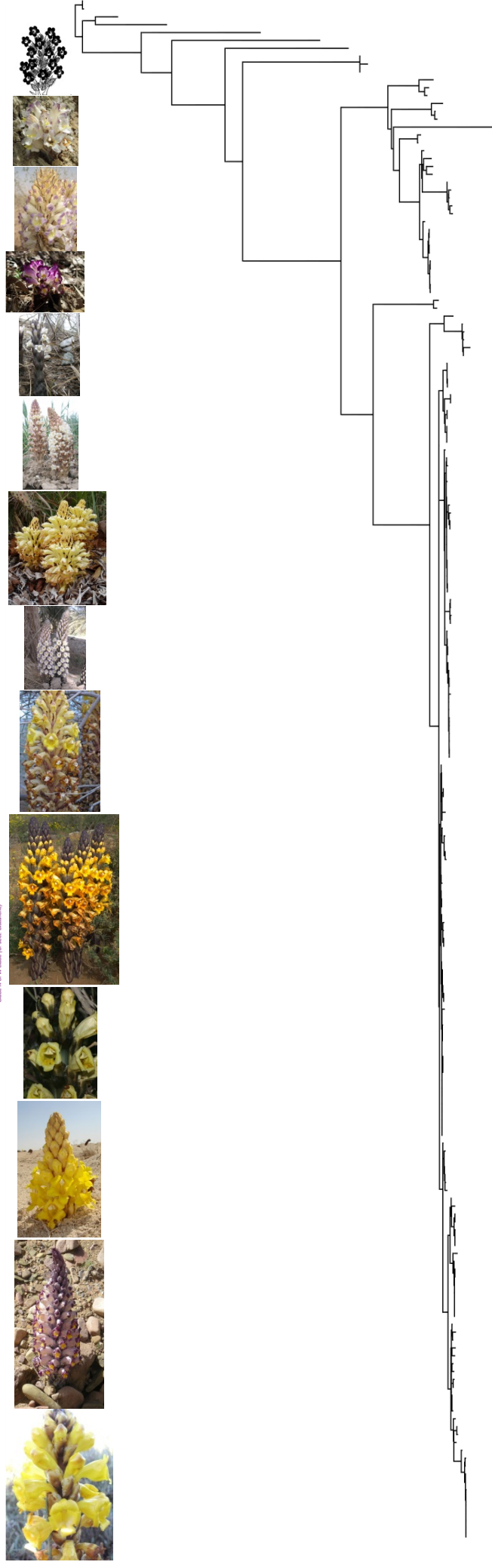
Table 2.2 Sequence statistics for the markers used. IGS: intergenic spacer

Dataset including hotspots	<i>trnL-F</i>	<i>trnL</i> (UAA intron)	<i>trnL-F</i> (IGS)	<i>trnS-fM</i> (IGS)	<i>psbA-trnH</i> (IGS)	ITS 1 & 2	Combined
Position in the alignment	1-1494	1-785	850-1476	1495-2793	2794-4226	4227-4929	1-4929
Number of characters	1494	785	627	1299	1433	703	4929
Minimum sequence length range (nt.)	880 (ED445, ED447 & ED451)	342 (ED428, outgroup) & 472 (ED1042)	306 (ED428, outgroup) & (ED445, ED447 & ED451)	559 (ED722)	72 (ED1040)	619 (AY209298)	2235
Maximum sequence length range (nt.)	996 (ED704 & ED611)	576 (ED611)	385 (ED709)	741 (ED896)	585 (ED707)	695 (ED401)	2966
% GC	30.66	29,46	28,54	35.75	22.00	54.54	36.16
Inversions	0	0	0	1	3	0	4
Dataset excluding hotspots							
Position in the alignment	1-1366	1-723	789-1348	1367-2597	2598-3826	3827-4529	1-4529
Number of characters	1366	723	560	1231	1229	703	4529
variable sites %	26.04	22.13	31.79	25.92	10.90	43.81	24.62
parsimony-informative sites %	17.80	15.91	20.54	18.03	8.87	34.42	18.00
Number of coded indels	187			127	175	63	1556

2.3.2 Phylogenetic analyses

We observed no significant incongruence among and between the plastid as well as the nuclear analyses. Generally, the plastid tree was less well resolved/supported in particular at shallower nodes than the ITS tree (Appendices 2.4 and 2.5). This was consistent with the higher proportion of parsimony informative characters in the ITS matrix (Table 2.2), which likely improved resolution at shallower nodes without having negative effects on deeper nodes. There were no significant discrepancies between trees from indel and non-indel data sets. However, using indel information not only increased the overall topological resolution but also resulted in better supported clades, specifically for first branching lineages (Appendix 2.4A).

The phylogenetic trees obtained from ML and BI based on combined plastid and nuclear markers, both with indels and without indels, were strongly congruent and yielded a well-resolved tree (Fig. 2.1). The support values for the clades in indel and non-indel analyses were separately indicated with “/” in the text, respectively, unless otherwise mentioned. These analyses identified *Cistanche* as monophyletic group, although only weakly supported (ML 61/< 50; PP 0.77/0.91). In MP analysis, the position of *C. sinensis* with respect to other *Cistanche* species and the outgroups was unresolved (Appendix 2.6). *Cistanche* falls into four major well-supported monophyletic clades i-iv (Fig. 2.1, Appendix 2.6), henceforth referred to as EA (East Asian), SWA (South West Asian), NWA (North West African) and W (Widespread) clade, respectively. The EA clade contained only *C. sinensis* (MP 100/100; ML 100/100; PP 1.00/1.00) from China and Mongolia and was significantly supported sister to the rest of *Cistanche* (MP 100/100; ML 100/100; PP 1.00/1.00). The maximally supported SWA clade was composed of eight species occurring in SW Asia (MP 100/100; ML 100/100; PP 1.00/1.00) and significantly sister to the remaining species (MP 100/99; ML 100/100; PP 1.00/1.00). The highly supported NWA clade contained one species (MP 100/100; ML 100/100; PP 1.00/1.00) exclusively from NW Africa and was placed sister to the widely distributed W clade comprising the rest of *Cistanche* species (MP 100/100; ML 100/100; PP 1.00/1.00).



Order for FW case (C. nec. Clonality) | Order for BW case (C. nec. Clonality) | Order for FW case (C. nec. nov.) | Order for BW case (C. nec. nov.)

Figure 2.1 Maximum likelihood tree (left) and phylogram (right) obtained with RaxML based on the combined data set including indels. Numbers above branches show posterior probabilities and likelihood bootstrap support from the analyses with indels included, values below the branches refer to support values without indels coded. The plant photos in clade ii (*C. fissa*) and iii taken by Y. Pirogow (<http://www.plantarium.ru/>, accessed Feb. 2016) and M. Tapia Claro (<https://www.flickr.com/>, accessed Feb. 2016), respectively. *C. senegalensis* in clade iv taken from (<http://www.wikiwand.com/>, accessed Feb. 2016).

In the SWA clade the following five groups were resolved: clade A is maximally supported *C. ambigua* (MP 98/100; ML 100/100; PP 1.00/1.00) that falls into two geographically separate groups, one (MP 79/100; ML 75/< 50; PP 1.00/< 50) containing exclusively accessions from Northeast Iran and the second (MP 75/51; ML 82/< 50; PP 1.00/0.51) with some accessions from North Iran as well as two accessions from Southwest Afghanistan. That is sister to a single accession of “ridgewayana_3” (MP 70/52; ML 69/< 50; PP 1.00/0.93) occurred in Afghanistan. The rest “ridgewayana” accessions form moderate-resolved clade B (MP 62/68; ML 75/79; PP 0.97/1.00), well-resolved sister to this previously described one (MP 98/99; ML 100/100; PP 1.00/1.00). Within clade B at least two subclades with phylogenetic structure corresponding to geography are observed as: “ridgewayana_2” in Northwest and Center Iran, “ridgewayana_1” mainly in Center and South Iran and subsequent sister groups are: monophyletic clade C containing two accessions of “fissa_2” (MP 100/100; ML 100/100; PP 1.00/1.00) from Afghanistan with maximum support, sister to the previously mentioned groups with strong support (MP 74/60; ML 96/93; PP 1.00/1.00). That placed sister to the single accession of “fissa_3” ED1040 from Azerbaijan (unsupported), which placed sister to all former groups (MP 57/100; ML 84/85; PP 0.88/0.57). *C. salsa* (MP 100/100; ML 100/100; PP 1.00/1.00) form highly supported clade D distributed in SW Asia and China, sister to the previous clades with relatively moderate support (MP 100/100; ML 80/90; PP 0.99/0.99). Subsequently, the highly supported clade D contains *C. deserticola* (MP 100/100; ML 100/100; PP 1.00/1.00) distributed in China and Mongolia, sister to all previously described clades in SWA clade.

In the W clade the following groups were resolved: monophyletic clade F contains *C. rosea* (MP 100/100; ML 100/100; PP 1.00/1.00), sister to the remaining species (MP 99/94; ML 98/99; PP 1.00/0.99), whose relationships are unresolved/poorly supported but several distinct lineages are recognizable. Two sister groups of *C. laxiflora* (MP 63/99; ML 84/54; PP 1.00/< 50) reconstructed a paraphyletic group G with accessions from Iran, Afghanistan and China. Subsequent groups in this clade are: *C. laxiflora* subsp. nov. is significantly supported (MP 99/95; ML 99/95; PP 1.00/1.00), sister to the next clade (MP 54/<50; ML 60/91; PP 1.00/1.00) containing two accessions of *C. tubulosa*. The Chinese species (GenBank accs. FJ914382 and JF915383) placed inside *C. laxiflora* in the analysis including indel (MP 98/65;

ML 99/73; PP 1.00/0.77); that is sister to *C. laxiflora* subsp. *laxiflora* (MP 85/< 50; ML < 50/50; PP < 0.50/0.74). Previously described clades all placed sister to the Asian-African paraphyletic clade H (MP unsupported; ML 97/50; PP 1.00/< 50), in which the monophyly of single lineage of *C. senegalensis* accession ED725 is unsupported. The rest of the group is only weakly supported in BI analysis (MP 95/unsupported; ML <50; PP 0.64/< 50) and falls in two clades containing the rest other specimens of *C. senegalensis* as well as some accessions of the paraphyletic “tubulosa_1” (MP 90/73; ML 86/69; PP 0.79/0.86), and entire specimens of yet unsupported clade of *C. flava* distributed in North and South Iran. The monophyly of many specimens in this subclade remains unsupported apart from a few nodes, of which the taxa mainly distributed in Kenya, Tanzania and Somalia. All above clades (G-H) placed (unsupported but ML 54 in non-indel tree) sister to the most unresolved bulk of clade I comprising *C. tubulosa* widely distributed across the Middle East, rarely North Africa to India (MP unsupported; ML 62/54; PP 1.00/< 0.50). Phylogenetic structure in this clade is mostly poorly supported. The first paraphyletic subclade comprised the species occurred in Iran, Pakistan and Saudi Arabia, including two accessions of *C. tubulosa* subsp. *nov.* from Iran. Generally, in terminal nodes the low to moderate supports are observed; its sister group to next subclade (MP 60/73; ML 69/< 50; PP 0.94/< 50) is unsupported, in which the terminal nodes received moderate supports. The species in the subclade distributed mainly in Arabian Peninsula included a single accession of *C. tubulosa* subsp. *tubulosa* (acc. ED806) placed along with “tubulosa_1” as well as one accession of *C. tubulosa* subsp. *nov.*, in which the latter two groups received moderate support particularly in the matrix including indel (MP 76/73; ML 78/67; PP 0.94/0.56). The rest comprises unresolved/poorly supported accessions from Arabian Peninsula, Cape Verde and Mali and largely Iran. Clade J contains Mediterranean and African *C. lutea*, *C. brunneri*, *C. violacea* and *C. phelypaea*, differentiated by “tubulosa” clade with the moderate/strong support (MP 92/53; ML 97/87; PP 1.00/0.99). Internal structure of this clade corresponds to some extent with species limits, as both *C. phelypaea* (MP 100/98; ML 100/100; PP 1.00/1.00) and *C. violacea* (MP 68/58; ML 93/82; PP 0.90/0.63), with the exception of a single accession (unresolved), are monophyletic. However, *C. lutea* is polyphyletic being found (i) as grade paraphyletic (MP 94/60; ML 95/69; PP 1.00/0.99) to *C. phelypaea* and thus in the same subclade (unresolved) as *C. violacea* and (ii) as paraphyletic grade at the base of the entire clade J and thus including *C. brunneri*. Subclade containing *C. violacea* with moderate/strong support (MP 68/58; ML 93/82; PP 0.90/0.63) is distinct from subclade comprising *C. phelypaea* and the rest of *C. lutea* with relatively good support (MP 90/53; ML 97/92; PP 0.96/0.89), forms two geographical distinct groups: one including the well-supported “violacea_2” (MP 92/93; ML 99/99; PP 1.00/0.95) exclusively from Morocco, and the low to moderately supported “violacea_1” (MP 53/70; ML 84/85; PP 0.92/0.59) occurring widely in North Africa. However,

both species received relatively well-supported either in basal and terminal nodes. The relationship of the species in subclade containing *C. lutea* and *C. phelypaea* (MP 55/unsupported; ML 76/67; PP 0.64/< 50) is mainly unsupported or weakly resolved. Two accessions of “violacea_1” (ED807 and ED1012 from Saudi Arabia and Jordan, respectively) are nested within *C. lutea* group. These vouchers also morphologically re-examined and the first one confirmed to be *C. violacea* and the latter shows hybrid of *C. violacea* with “lutea_3”; however, both represent incongruences in morphology and phylogeny pattern. Two other accessions of “lutea_3” remained unresolved within the next clade with low basal only in the data including indel (MP 57; ML 83; PP 1.00). *C. phelypaea* largely distributed in NW African coasts and through Mediterranean area form two sister groups, of which *C. phelypaea* subsp. nov. placed among *C. phelypaea* subsp. *phelypaea* both received maximum support at basal (MP 100/98; ML 100/100; PP 1.00/1.00). However, none to lowly resolved inside the clade.

2.4 Discussion

2.4.1 Phylogenetic relationships

Our molecular phylogenetic results of the combined data sets agree to some extent with traditional infrageneric classification based on floral characters, especially of the calyx form. However, there are several changes to the current classification that have to be accommodated. This will be done thoroughly in a subsequent taxonomic revision of the genus. In the following, we discuss our findings by comparing the retrieved clades with the traditional sections, involving geographical grouping and diagnostic morphological characters (Fig. 2.1, clade i and clades ii-iv):

2.4.2 East Asian clade (EA clade)

Cistanche sinensis is the sole species in this group and the only species with a deeply incised quadripartite calyx (vs. short incised pentamerous calyx in the other species of *Cistanche*); shorter inflorescence and shorter corolla tubes that are yellow outside and occasionally dispersed royal-blue inside in particular around lobes (vs. various usually homogeneous color inside the tube for the rest of species). It had been placed in its own section (*Subcistanche*) by Beck-Mannagetta (1930) and is supported here as distinct lineage that is sister to the rest of the genus *Cistanche*. It is the only subgroup of the genus to be restricted to East Asia (Mongolia and Northern China; Fig. 2.2), largely parasite on wide-range hosts of Amaranthaceae, Rosaceae, Tamaricaceae, Chenopodiaceae and Zygophyllaceae.

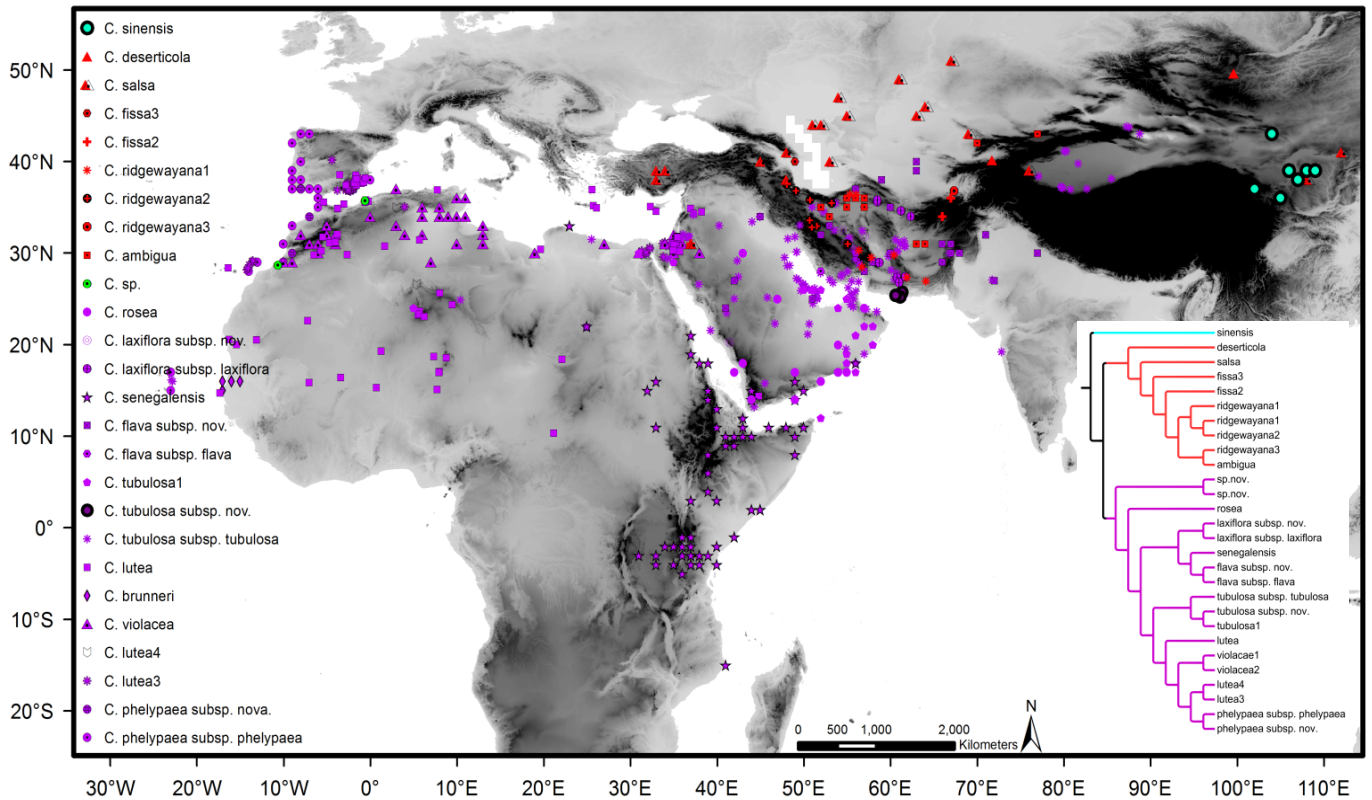


Figure 2.2 Distribution map of *Cistanche* species worldwide, according to literature, herbarium and field observations. Here the general distribution of the species is regarded in which the specimens molecularly studied is also included. Infrageneric classification have shown according to Figure 2.1.

2.4.3 SW Asian clade (SWA clade)

The floral parts of the species in this clade are partially covered by hairs. This clade corresponds to major parts of the traditionally recognized *C. sect. Heterocalyx* plus, nested within, *C. sect. Cistanchiella* and *C. salsa* from sect. *Cistanche*. Beck-Mannagetta (1930) diagnosed the species of section *Heterocalyx* (*C. fissa* (C.A.Mey.) Beck, and *C. ambigua* (Bunge) Beck) by having a pentamerous calyx, whose posterior lobe is nearly free from the others, or a tetramerous calyx with equal or unequal lobes along with pubescent floral parts. *C. sect. Cistanchiella* was formerly considered monotypic being characterized by having no or only one bracteole (vs. two bracteoles in other species).

The traditionally recognized species *C. ambigua*, *C. deserticola*, and *C. salsa* are congruent with phylogenetic groups, but *C. ridgewayana* and *C. fissa* are paraphyletic, comprising three and two, respectively, distinct lineages (Fig. 2.1). These lineages within *C. ridgewayana* and *C. fissa* differ morphologically and geographically and, therefore, deserve taxonomic recognition (a detailed taxonomic treatment will be published separately). All accessions of the “ridgewayana” group are united by having capsules that are always dehiscent with 3-valves (vs. 2-valves in all other *Cistanche* species). This means that this

typical character has to have evolved either twice independently in the “ridgewayana” paraphylum or lost once again in *C. ambigua*.

2.4.4 NW African Clade (NWA Clade)

The next clade contains only two accessions of one currently undescribed species from southern Morocco (Fig. 2.2). It is not only phylogenetically distinct enough to warrant recognition as separate section (*C. sect. nov.*), but has remarkable morphological features that support its taxonomic status. The bracteoles are slightly shorter than the calyx and are joined to it on one side but in the other side and apically free (vs. fully free-bracteole in the other species of *Cistanche*); the calyx is shallowly incised but has clearly acute lobes; floral bracts and bracteoles have arachnoid-lanuginose hairs; scales and bracts are broadly rhomboid (vs. various shape of bract and bracteoles the other species of *Cistanche*). Based on morphological similarities *C. mauritanica*, which differs from the included samples by bracteoles being free instead of laterally attached to the calyx, likely also belongs here, although we do not have any molecular data. Both species are restricted to the Atlas-region of Morocco and Algeria.

2.4.5 Widespread Clade (W clade)

This clade corresponds to *C. sect. Cistanche* in its traditional circumscription with the exception of *C. salsa*, belonging to the SWA clade. Additionally, this clade includes, as sister to the remaining species, *C. rosea*. Based on calyx structure *C. rosea* has been traditionally classified within sect. *Heterocalyx*. Evidently, an unevenly incised calyx has evolved at least twice independently within *Cistanche*. Members of this clade are characterized by the lack of hairs (vs. being hairy in the other clades). In contrast to the other clades, phylogenetic resolution in this clade is poor and several traditional species are not supported as monophyletic.

The second clade to discuss contains all samples of *C. laxiflora* and some of *C. tubulosa*. Both species are widespread, occurring mainly on the Arabian Peninsula and the Saharo-Sindian region. *C. laxiflora* is centered more to the East, stretching into the Western Himalayas and Tianshan Mountains, whereas *C. tubulosa* is the most abundant on the Persian Gulf with enclaves in Western India and the desert region of central China. It is the latter region from where the vouchers of the questionable specimens that are nested in *C. laxiflora* (GenBank accs. FJ914382 and JF915383, Fig. 2.1; Appendix 2.1) originate. The vouchers of these accessions were unavailable to us for revision but photographs of Chinese plants (web images) from adjacent areas show white corolla tubes with light purple lobes, which agree well with *C. laxiflora* subsp. *laxiflora* (vs. corolla pale to deeply yellow in

C. tubulosa). Both molecular data and the geographical proximity to *C. laxiflora*, this strongly suggest that these specimens were wrongly determined, maybe being caused by an erroneous concept of the species in China (Beck-Mannagetta, 1930). The sister group *C. laxiflora* subsp. *nov.* differs in having corolla tube yellow-whitish and lobes yellow or blue-violet (vs. white corolla lobes both abaxially and adaxially in *C. laxiflora* subsp. *laxiflora*). The corolla tube of the latter carries two or five irregular orange veins inside (vs. two yellow veins inside in the other morpho-species). Both hold whitish corolla (vs. varied corolla tube but white in the other species of W clade) and lax scales (vs. medium to dense scales in the other species of W clade). The group containing taxa of *C. laxiflora* is usually parasite on wide-range families e.g. Tamaricaceae, Polygonaceae, Fabaceae and Rosaceae. The following clade comprises all samples of *C. flava* and *C. senegalensis* and a group of “tubulosa_1” samples nested within *C. senegalensis*. Within this subclade, *C. flava* forms a monophyletic group, yet only poorly supported, whose sister to a hardly supported clade comprising all *C. senegalensis* samples except one (ED725) and *C. tubulosa* accessions. The exclusion of *C. senegalensis* (ED725) is, however, hardly supported either, rendering assessment of relationships among these putative lineages difficult. The “tubulosa_1” samples form a paraphyletic clade nested within *C. senegalensis* as well as *C. tubulosa* s. str.. Morphologically, they actually are similar to *C. tubulosa* s. str. and differs with its glabrous and acute anthers (vs. tomentose and obtuse anthers in *C. tubulosa*), its glabrous filaments at base (vs. highly tomentose filaments at base in *C. tubulosa*), and sinuate margins of scales, bracts and bracteoles (vs. straight margins of scales, bracts and bracteoles in *C. tubulosa*), so that they can be confidently excluded from that species. The morphology and phylogeny, thus, in this clade showed discrepancies. *C. flava* is differentiated by having bract twice as calyx or even longer (vs. equal as calyx even shorter or slightly longer in the other species of W clade). Groups in the *C. flava* clade are geographically separated, of which, *C. flava* subsp. *nov.* occurred in Northeast and East Iran with Fabaceae as host preference. *C. flava* subsp. *flava* containing the majority of specimens in group “flava” distributed largely in Southeast Iran towards South Afghanistan with a preference to be parasite on mainly *Calligonum bungei* (Polygonaceae). While *C. flava* is restricted to the Middle East and India exclusively North of the Tropic of Cancer, *C. senegalensis* occurs mainly along the East-African Rift formation South of the Tropic of Cancer (one record from northern Libya could not be verified; the single sample at the base of the *C. flava* subclade comes from Eritrea). *C. senegalensis* is distinguished by corolla nearly as 3-times or longer than calyx (vs. twice or slightly longer than calyx in the other species of the W clade) and calyx lobes with 3-times longer than two others (vs. usually length equal to width in the other species of the W clade). The “tubulosa_1” samples nested within *C. senegalensis* are almost exclusively found in the Southeastern Arabian Peninsula. This suggests a parapatric

speciation in the *C. flava* subclade probably from an East African ancestral stock with possibly two expansions towards Asia, from a more subtropical niche in *C. senegalensis* towards a more temperate-subtropical niche in *C. flava* and towards more pronounced aridity in “*tubulosa_1*” *sp. nov.*, respectively. The species of this clade live on Amaranthaceae, Polygonaceae, Fabaceae and occasionally on Asteraceae, whereas *C. senegalensis* is often found on *Acacia sp.* (Fabaceae). Group “*tubulosa_1*” lives usually on Chenodiaceae. The remainder of the *C. tubulosa* samples forms its own subclade. The species occurs mainly in Arabia and Iran, centered on the Persian Gulf and it is a robust, tall plant with showy pale to deep yellow flowers. The monophyly of the majority of subclades inside *C. tubulosa* are unsupported, however, they morphologically show no differentiation. All these differentiate geographically subsequently: the first and second-branching paraphyletic subclades contain the Middle East and Pakistan species, in which some members of “*tubulosa_1*” and *C. tubulosa* subsp. *nov.* observed and received moderate to rarely strong supports in the major groups. The parasite occurrences on Amaranthaceae and Asteraceae reported for these two groups. The next unsupported group comprised mainly the species from Middle East, of which two occurred in Western Africa and moderately supported in indel data. Morphologically the latter specimens relatively recognized by strong and robust stem and very long inflorescences. The all members of next group distributed in Middle East but one ambiguous record (acc. ED763) from North Africa. Host preferences for latter two groups are unknown. The last large group included the specimens distributed widely in Middle East thought Iran, Saudi Arabia and Qatar. Species of these clades live mainly on plants of the order Caryophyllales (Amaranthaceae, Chenopodiaceae, Tamaricaceae). *C. tubulosa* is sister to the last subclade containing *C. brunneri*, *C. lutea*, *C. phelypaea* and *C. violacea*, which is parapatrically distributed in Northern Africa and the Mediterranean region. Phylogenetic relationships do, however, not follow taxonomic limits (neither *C. lutea* nor *C. violacea* are monophyletic), but rather show a geographic pattern. The basal-most branch, whose sister-relationship to the remainder of this subclade is unsupported a single sample of *C. lutea* (ED726) from Nigeria. The next clade contains samples from *C. lutea* mainly from Morocco as well as the single accession of *C. brunneri*, a morphologically divergent endemic from Senegal, recent divergence resulting in morphological divergence but not yet phylogenetic differentiation (*brunneri*). Morphologically *C. brunneri* is close to *C. senegalensis* and *C. lutea* based on corolla colour and geographically close to Morocco, where the species of this clade occurred, although it does not form distinct clade in phylogeny. Morphologically *C. lutea* characterized by having highly scarious margins of scales, bracts and bracteoles (vs. none to slightly scarious in the rest of species in the W clade) and apiculate apex anther (vs. usually rounded, acute to acuminate anther in the rest of species in the W clade). They are parasite on Amaranthaceae, Chenopodiaceae and

Zygophyllaceae, with the exception of one occurrence on *Salvadora* (Salvadoraceae, Brassicales). Next follows the monophylum of NW African *C. violacea*, which itself falls into two sister clades (mainly due to differences in the ITS sequences, not the cp data): One contains all Moroccan accessions, the second includes accessions from Algeria to Libya. Morphologically they only differ in the intensity of the flower colour. This clade received strong to maximum well supports in the majority of subclades. Having corolla tube pale-lilac with lobes light to dark purple (vs. varied colour in the rest of species in W clade) distinguished *C. violacea* morphologically. Their host preferences are families Amaranthaceae, Chenopodiaceae and rarely Nitrariaceae. Next follows a paraphyletic clade containing *C. lutea* accessions (with clear morphological plasticity), mainly from the Eastern part of its distribution area (Morocco to Saudi Arabia through Egypt) including one putative hybrid between both taxa, “lutea_3”, “lutea_4”, and two accessions from *C. violacea* (from Jordania and Saudi Arabia). The latter were morphologically distinctly *C. violacea*, although the morphology and phylogeny is inconsistent. The single accession “lutea_4” (acc. ED711) nested among *C. lutea* specimens and does not form distinct group, although it differs from the latter in its long oblong-lanceolate scale (vs. shorter oblong to oblong-lanceolate scale in *C. lutea*) and bract equally long to the corolla tube (vs. bract up to half as long as the corolla tube in *C. lutea*). The group “lutea_3” did not form distinct group in phylogeny, rather remained unsupported among *C. lutea*, although morphologically recognized by gray-blackish colored scale, bract, bracteole and partially calyx (vs. yellow to brown in *C. lutea*) and the presence of yellow folds inside the corolla tube (vs. absence of folds in *C. lutea*). The latter group is parasite on Amaranthaceae widely on (*Salsola genistoides*, *Atriplex halimus* and *Haloxylon sp.*). Nested within this paraphyletic *C. lutea* is the well-supported clade of *C. phelypaea*, the only species of the genus with a pronounced Atlantic distribution (Portugal to Morocco, Canary and Cape Verde Islands. The species is distinguishable by keel-shaped scale and bract and broadly obovate-spatulate bracteoles (vs. none-keel shaped in the rest of species of W clade). This clade highly supported at basal but remained unresolved in terminal nodes. The families of Amaranthaceae and Tamaricaceae are of particular interest hosts for *C. phelypaea*. The host preferences have considerable overlapping in the clade *C. tubulosa* and *C. lutea*.

The species of the SWA clade (*C. sect. Cistanchiella*) do not show generally a well-supported ingroup-taxa phylogeny but also they show clearer morphological variability than the W clade (*C. sect. Cistanche*). For instance, various density and type of hairs in leaflets and floral bract and bracteoles may differ inter / -intra specific level particularly in the clade containing *C. ridgewayana*. The species distinguished with relatively high support even though fewer species have been sampled than the species in W clade (Appendix 2.1).

Within this SWA clade we observed a geographical divergence succession from Central Asia into Iran (Fig. 2.2), where two independent radiations occurred. However, it may be suggested that differentiation in the W clade is relatively low and younger. And above all, nearly all species in this clade are taxonomically being not easily assigned. The specie in W clade mainly morphologically recognized by varied corolla colour and type of scales/bracts/bracteoles. Some species in particular contain several distinct lineages that may be taxonomically recognized distinct but were omitted in Beck's revision (Beck-Mannagetta, 1930). In many cases in this clade, the phylogenetic differentiation corresponds with morphological/geographical differentiation, indicating that current taxonomy is underestimating species diversity in *Cistanche*.

2.4.6 Host range in phylogenetic groupings

There is no trend of host specialization for any phylogenetic groupings in the genus. Most species of *Cistanche* have wide host ranges but with a clear preference for Amaranthaceae and Chenopodiaceae, although other families like Fabaceae, Rosaceae, Tamaricaceae etc., are attacked as well. While there are only few (e.g. *C. senegalensis*) instances of narrow host range.

Similar to *Orobanche* (Manen & al., 2004), the same host may be shared by several unrelated *Cistanche* species (e.g. *Haloxylon ammodendron* is host for *C. deserticola*, and *C. phelypaea*). In the absence of a sound species phylogeny and due to the lack of sufficiently detailed data no formal testing for host specialization was done.

2.5 Conclusions

our comprehensive phylogenetic analyses inferred from plastid and ITS data robustly support four major phylogenetic clades for *Cistanche*, largely corresponding to traditional infrageneric classification (Beck-Mannagetta, 1930). The analyses show *Cistanche* monophyletic. Although species relationship is solved among major clades, however, there is low support in the major subclades within wide spread clade containing *C. sect. Cistanche*. Using phylogenetic relationships, a preliminary taxonomic revision particularly recognition of cryptic species was represented. It would be highly appropriated to use more plastid sequences for resolving unsupported taxa. Additionally, more taxon sampling specially for rare species would impact overall phylogenetic accuracy of geus *Cistanche*.

3 Evolutionary trends in the non-photosynthetic parasitic *Cistanche* (Orobanchaceae) inferred from karyological data, contrasting effects of genome size with chromosome number

Abstract

Trends in variation of genome size and chromosome number evolution in the non-photosynthetic parasitic Orobanchaceae can mirror the different aspects like hybridization, polyploidy and ancestral genome evolution. Genome size and chromosome numbers were analyzed in the holoparasitic genus *Cistanche*, one of the group of particular interest, and interpreted in a phylogenetic context. Genome size of 53 accessions of nine *Cistanche* species, nine accessions of three *Orobanche* species and four accessions of three *Phelipanche* species, the majority of which represented the group's biodiversity hotspot in Southwest Asia, was analyzed using Feulgen densitometry. Chromosome numbers were obtained for 23 accessions of *Cistanche*, seven of *Orobanche* and three of *Phelipanche*. A well-supported topology inferred from combined nuclear ITS and plastid datasets was employed to analyze the variation in genome size. The constant chromosome number in *Cistanche* ($2x = 40$) contrasted with high levels of inter- and intraspecific variation of genome sizes (nearly 4-fold and up to 2.5-fold, respectively). All analyzed accessions of *Cistanche* were diploid ($n = 20$), except for one triploid individual ($n = 30$) of *C. violacea*. Their karyotypes consisted of large meta- to submetacentric chromosomes with only a few acrocentrics. All analyzed accessions of selected species of the genus *Orobanche* were diploid with $n = 19$ with an exception of one polyploid *O. densiflora* ($n = 38$), and all possessed small meta-to submetacentric chromosomes. Chromosome numbers of all *Phelipanche* species analyzed were $n = 12$. Variation in genome size among *Cistanche* species coincided to some extent with sectional classification of the genus (sects. *Cistanchiella* and *Cistanche*), but no clear trend of genome size increase or decrease was observed that could be correlated with the inferred major phylogenetic lineages. Apart from sect. *Cistanchiella*, the remaining analyzed species were divided into five clades in which the genome size variation was high and uncorrelated with phylogenetic groupings. The large genome size of *Cistanche* is related to changes in the amounts of repetitive DNA elements rather than polyploidy ($x = 20$ in *Cistanche*, $x = 19$ in *Orobanche*). Genome size did not correlate with geographical and climatic variables. Overall, this study reveals that the genome size variation in the genus *Cistanche* neither correlates with chromosome numbers

nor with phylogenetic groups, and suggests mechanisms other than polyploidy for the large genome size in this genus.

3.1 Introduction

Plant genomes are very dynamic and experience a myriad of chromosomal, genetic and epigenetic changes in the course of their evolution. Important clues for understanding genome evolution can be obtained from genome size, chromosome number and karyotype structure data, which are the parameters most commonly used to characterize the genomes of non-model plant groups. The DNA content in plants varies 2400-fold ranging from $1C = 63$ Mb in *Genlisea aurea* and *G. margaretae* ($2n = c. 52$ and $c. 40$, respectively; Greilhuber & al., 2006), to $1C = 149\,000$ Mb in *Paris japonica* ($2n = 8x = 40$; Pellicer & al., 2010). Both genome size increase, most often resulting from the accumulation of transposable elements (Kalendar & al., 2000; Bennetzen, 2005; Grover & Wendel, 2010; Heslop-Harrison & Schwarzacher, 2011) and/or polyploidization (Leitch & Bennett, 1997; Soltis & Soltis, 2000; Heslop-Harrison & Schwarzacher, 2011), and genome downsizing have been demonstrated in various plant groups (Leitch & Bennett, 2004; Heslop-Harrison & Schwarzacher, 2011; Renny-Byfield & al., 2011). A number of studies have found correlations of nuclear DNA content with environmental and geographical variables both at inter- and intraspecific levels (Bennett, 1987; Kalendar & al., 2000; Knight & Ackerly, 2002; Jakob & al., 2004; Knight & al., 2005; Murray, 2005), but these correlations were often group specific (Šmarda & al., 2008). Additional studies from more groups are necessary to amend this idea. A group of non-photosynthetic poorly understood parasitic *Cistanche* would also be of great interest in terms of this study.

In contrast to commonly steady distribution of chromosome number across the families in gymnosperms, it varies 160-fold in eudicots ranging from $2n = 4$ to $2n = 640$ in *Sedum* family Crassulaceae (t Hart & Bleij, 2005; Heslop-Harrison & Schwarzacher, 2011). The dynamics of chromosome number changes is group specific and involves both dysploidy and polyploidy (Weiss-Schneeweiss & Schneeweiss, 2013). Polyploidy is an important phenomenon contributing to increasing genome complexity and impacting all aspects of plant development and evolution (Adams & Wendel, 2005; Moore & Purugganan, 2005). Obtaining more data of ploidy level in plant will enable us to address the genome size changes, hereby, higher rate of speciation and its importance in the origin of new plant species. This will impact on our understanding of plant evolution and function.

Among higher plants, parasitic Orobanchaceae are a remarkable case study for evolutionary process, host specialization, genome size and chromosome number evolution and diversification; however, they are relatively poorly analyzed with respect to chromosome

numbers and genome sizes, and their evolution (Bennett & Leitch, 2005; Wicke, 2013). Various holoparasitic Orobanchaceae, a comparatively well-studied group are *Orobanche* and related genera, have been shown to vary in chromosome numbers, genome sizes, and incidence of polyploidy (Schneeweiss & al., 2004b; Weiss-Schneeweiss & al., 2006). Several basic chromosome numbers were reported in several genera of the family ($x = 12, 19, 20$), which lead to hypothesis on ancestral chromosome number(s) and their changes in the related group of genera (Schneeweiss & al., 2004b). Extant polyploidy has been reported only in some genera of Orobanchaceae like *Orobanche* (Schneeweiss & al., 2004b). Despite this karyological data is still missing for some taxonomically interesting sister genera to *Orobanche* like poorly understood *Cistanche* with about two dozen widely distributed species. Only two subspecies of genus *Cistanche* (*C. phelypaea* subsp. *phelypaea* and *C. phelypaea* subsp. *lutea*), the latter is treated as a different species in a current revision (compare chapter 4), have so far been counted chromosomally and their genome sizes were estimated. One basic chromosome number of $x = 20$ has been found in both analyzed species (Weiss-Schneeweiss, 2006). Both the chromosomes and nuclear genome size were large (5–10 μm ; 16.8–19.9 pg), which represented, however, only a small fraction of the whole genus and notably only the westernmost part of the genus distribution range. Only two chromosome numbers (both $2n = 40$) were reported for one species from eastern distribution range, *C. tubulosa* from India (Hamblen, 1956) and Israel (Pazy, 1998). Chromosome number was also reported triploid ($2n = 60$) for a single individual of *C. salsa* (Pazy, 1998). Basic chromosome number of $x = 20$ in *Cistanche* was hypothesized to represent the secondary basic chromosome number resulting from polyploidization(s) of primary hypothetical ancestral chromosome number of $x = 5$ (Schneeweiss & al., 2004b), although no experimental evidence exists to support this inference (Weiss-Schneeweiss & al., 2006). Genome size of *Cistanche* was large when compared to its closest relatives, the sister groups *Orobanche* and *Phelipanche* (2.9–11.6; 4.9–10.5 vs. 16.8–19.9 pg; Weiss-Schneeweiss & al., 2006; Piednoël & al., 2012).

Genus *Cistanche* consists of about 25 currently recognized species (for detail see chapter 4), distributed along the coastline of North Africa via Mediterranean to the mountain slopes in Southwest (SW) Asia, China and Mongolia in arid and semi-arid regions (Blatter, 1921; Agrawal, 1984; Musselman, 1984). A narrow-range endemism is restricted to Middle East and South West Asia for species like *C. fissa*, *C. sinensis* and *C. deserticola* (Schiman-Czeika, 1964; Teryokhin, 1993; Zhang & Tzvelev, 1998). Classification of the genus into four sections has remained unchanged since the latest monograph by Beck-Mannagetta (1930) and species delimitation within the genus is poorly understood and controversial. The majority of *Cistanche* species placed in *C. sect. Cistanche* Beck, and the three remaining sections each contain one or two species. *C. ridgewayana* belongs to sect. *Cistanchella*

Beck, *C. sinensis* to sect. *Subcistanche* Beck, and *C. fissa* and *C. rosea* to sect. *Heterocalyx* Beck. The floral characters of *Cistanche* are the most useful taxonomic characters for species delimitation. *Cistanche* is recognized as non-host-specific largely parasite on Amaranthaceae and Chenopodiaceae. The phylogenetic relationship of *Cistanche* to other genera in Orobanchaceae is not well resolved (Park & al., 2007a; Piednoël & al., 2012), and instead a sister relationship to the lineages of *Boschniakia*, *Conopholis*, *Epifagus* and *Orobanche* was proposed (e.g. McNeal & al., 2013).

This study in particular aims to elucidate the evolution of the genome size, chromosome number and karyotype structure in genus *Cistanche* and interpret these changes in a phylogenetic context. Specifically, this study aims to: (i) determine genome size, chromosome numbers and karyotype structure of selected taxa of *Cistanche*, (ii) representing those taxa in the phylogenetic context, (iii) correlate genome size and chromosome numbers with phylogenetic relationships and ecological variables. All these data allow hypotheses on mechanisms and processes which might have shaped genomes of *Cistanche* to be tested. Usefulness of these characters for supporting novel taxonomic combinations is discussed.

3.2 Materials and Methods

3.2.1 Taxon sampling

Vouchers are deposited at the herbaria of the University of Bonn (BONN) and the University of Sistan & Baluchestan (USB) in Iran. Fifty-five accessions (of which 53 accessions for genome size and 24 for chromosome number, representing nine species of *Cistanche* were estimated) pertaining to 38 populations of *Cistanche* from Iran, six populations from Morocco and two from Spain corresponding to nine species (Appendix 3.1 and Fig. 3.1) were collected during the spring of 2010, 2011 and 2012. Additionally, the genome size of 13 accessions representing three species of *Orobanche* (*O. cernua* Loefl., *O. densiflora* Salzm. ex Reut., and *O. transcaucasica* Tzvel.) and two accessions of *Phelipanche* cf. *iberica* from Iran plus *P. ramosa* L. *P. purpurea* Jacq. were measured and included in the phylogenetic analyses as sister groups. All but one *Cistanche* and one *Phelipanche* species were represented by at least two accessions. The sampled *Cistanche* species represented the three morphologically defined sections (Beck-Mannagetta, 1930) *Cistanchiella* Beck (*C. ridgewayana*), *Heterocalyx* Beck (*C. ambigua*), and *Cistanche* Beck (the remaining species). The species identification was based on available floristic literature (e.g. Beck-Mannagetta, 1930; Schiman-Czeika, 1964) and was accompanied by own field and herbarium observations.

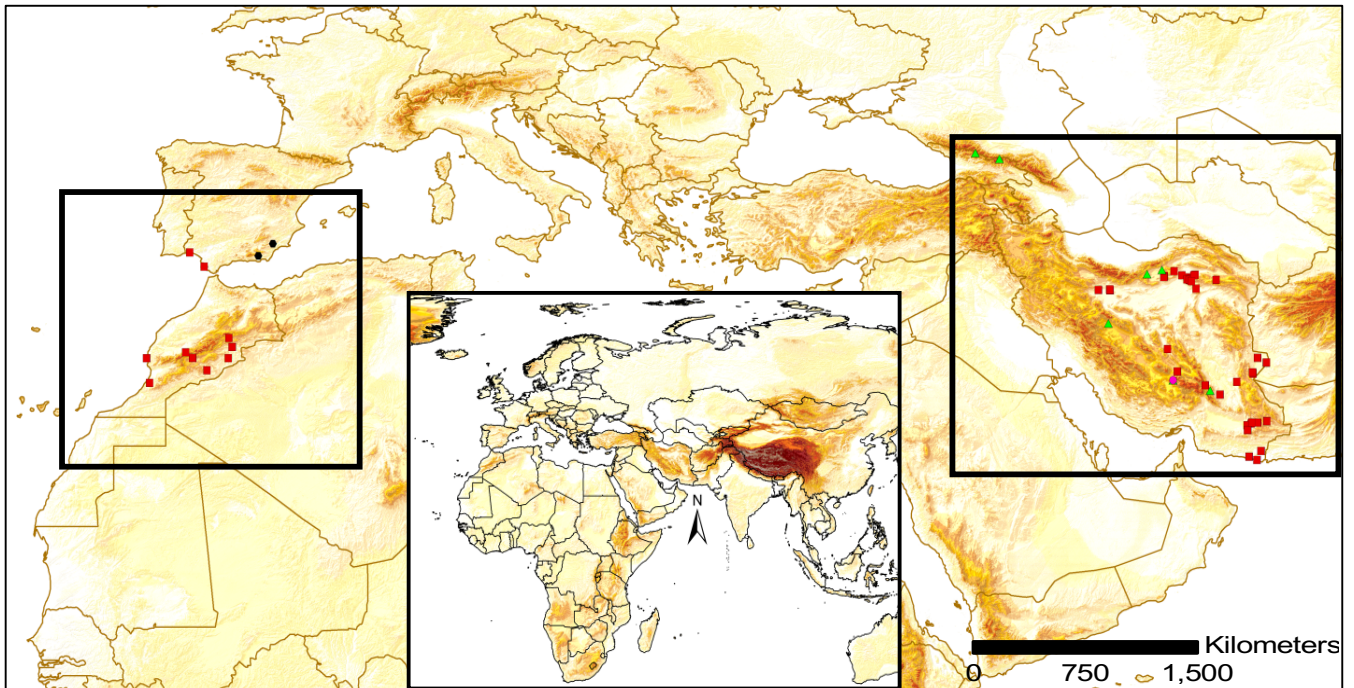


Figure 3.1 Map of the geographic distribution of the *Cistanche* (red rectangle spots), *Orobanche* and *Phelipanche* (green triangle and pink circle spots, respectively) based on our samplings. Black circle spots show *C. phelypaea* abstracted from Schneeweiss & al., (2006).

3.2.2 Feulgen densitometry

Feulgen densitometry (FDM) was employed to estimate the genome size of a total of 53 *Cistanche* populations from at least one individual per population. Young flower buds were fixed in the field in a freshly prepared fixative (ethanol : glacial acetic acid of 3 : 1) for at least 24 hrs and stored at -20°C until use. *Pisum sativum* cv. Kleine Rheinländerin ($2C = 8.84$ pg; Greilhuber & Ebert, 1994) grown in the laboratory of the University of Vienna was used as internal standard. Briefly, tissues of both object and standard were washed six times with distilled water in the same tube, hydrolyzed for 60 min in 5N HCl at 20°C in water bath, rinsed thoroughly with distilled water, and stained with Schiff's reagent (VWR, Germany) for at least 1 hr in the darkness. Stained material was washed for five times each in SO_2 -water (0.5 g potassium bisulphite in 100 mL 1N HCl), and rinsed with distilled water. Dissected cells were gently squashed in 45% acetic acid, coverslips removed at -80°C and material air-dried. DNA content was assessed using FDM via video-based image analysis with CIRES (Cell Image Retrieval and Evaluation System, version 3.1, Kontron, Munich, Germany) following Dimitrova & al. (1999) and Greilhuber & Ebert (1994). Feulgen stained DNA absorption in individual cell nuclei relevant to the spot size of each nuclei was measured as integrated optical density (IOD) using a monochromatic green filter and a 63x oil immersion objective. At least twenty intact nuclei in telophase and prophase were measured separately

for each preparation of both object and internal standard. IOD's mean values were converted into the absolute DNA amounts using $2C = 8.84$ pg as absolute genome size of the standard *Pisum sativum* (Greilhuber & Ebert, 1994). Some preparations were also used for chromosome number estimation from somatic cells in flower buds or from meiotic cells.

3.2.3 Chromosome numbers

Holoparasites lack typical root meristems, therefore chromosome numbers were established in meiotically dividing pollen mother cells (PMCs). The procedure of Feulgen staining followed Schneeweiss & al. (2004b). All species for which appropriate flower developmental stages were available were used for chromosome counting, often coinciding with FDM measurements.

3.2.4 Isolation of genomic DNA

The silica-gel dried corolla tissue collected in the field was used to isolate total genomic DNA. Approximately 50 mg of dried material was homogenized using a mixer mill (Retsch MM200, Haan, Germany) at 30 Hz for 3 min followed by incubation for at least 1 hr at 65°C in standard CTAB extraction buffer (Doyle & Doyle, 1990). The DNA was dissolved in 100 µl of TE buffer and stored at -80°C until use.

3.2.5 Amplification, purification and sequencing

Three plastid regions: *trnL* intron and *trnL-F* spacer (Taberlet & al., 1991), *trnS-trnfM* intergenic spacer (primers; *trnS* (UAG) and *trnfM* (CAU) (Wicke & Quandt, pers. comm.), and *psbA-trnH* intergenic spacer (Kress & al., 2005) as well as nuclear ribosomal internal transcribed spacer region (ITS1 and 2; White & al., 1990) were amplified for all analyzed *Cistanche* accessions (Appendix 2.2). Amplification reactions consisted of 0-0.2M betaine monohydrate, 0.4 µM of each forward and reverse primers, 0.15mM dNTPs (Carl Roth GmbH, Karlsruhe, Germany), 1mM MgCl₂, 1x GoTag Flexi buffer, 1.5U of GoTaq® flexi DNA polymerase (Promega, Madison, USA) and 1 µl of genomic DNA of unknown concentration. For difficult DNA templates PCR reactions were performed with the peqGOLD Taq DNA Polymerase Kit (PeqLab, Germany) with an addition of 0.4% PVP-40 and 1x Enhancer Solution P (PeqLab, Germany). Ready-To-Go® PCR Beads (Amersham-Pharmacia Biotech) were used for amplifications of plastid spacer *psbA-trnH* and nuclear ITS 1 and 2 in some taxa. Cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 and 7 min of final elongation at 72°C. Poorly amplifying samples were amplified multiple times (usually three times), and the

amplification products were pooled together, concentrated and visualized on 1% agarose gel. Bands were excised and purified using peqGOLD Gel Extraction Kit (peqlab, Germany) and sequenced externally at Macrogen Inc. (Korea) using appropriate primers.

3.2.6 Sequence alignment and phylogenetic analysis

The DNA sequences obtained from the three plastid regions and nuclear ITS were manually edited, assembled, and aligned using PhyDE v0.9971 (Müller & al., 2006a). Multilocus alignment was constructed by concatenating the three plastid regions and nuclear ITS. Simple indel coding (SIC) after Simmons & Ochoterena (2000) as implemented in SeqState v. 1.25 (Müller, 2005b) was applied to the concatenated data set. The data was phylogenetically analysed either with DNA sequences only (data not shown) or in addition to indels. Maximum Likelihood Analysis was conducted using RAxML 8 (Stamatakis, 2014) with 10,000 bootstrap replicates and Bayesian analyses with MrBayes v 3.2.1. (Ronquist & Huelsenbeck 2003). The data was partitioned (plastid : nuclear : indels) and analyzed under GTR+ Γ +I for the nucleotide data and F81 for indels. Each analysis was subjected to 10 million generations, with a sampling frequency of 1000 generations in six runs of four Metropolis-coupled Markov Chain Monte Carlo (MCMC) chains. Bayesian results were inspected via Tracer v.1.4 (Rambaut & Drummond, 2007), discarding the first 25% of trees as burn-in. Maximum parsimony analysis was performed using a parsimony ratchet approach (Nixon, 1999) in PAUP* v. 4.0b10 (Swofford, 2003) with command files being generated by PRAP2 (Müller, 2004). The support values are shown in the following order: first value represents bootstrap support (BS) resulting from maximum parsimony analysis, which is followed by maximum likelihood BS (> 50) and posterior probabilities (> 0.60). Final tree editions were applied in TreeGraph2 (Stöver & Müller, 2010).

3.2.7 Statistical analyses

Data were statistically tested with SPSS ver. 19 (IBM SPSS Inc., Chicago, Illinois, USA) and XLSTAT (Addinsoft, 2013). Normality of data was estimated with Shapiro-Wilkes test. As the normality assumption was not met for several accessions (*C. ambigua* acc. IRB14a, *C. ambigua* acc. IRB14 B, *C. laxiflora* subsp. *laxiflora* acc. IRBj, *C. tubulosa* subsp. *tubulosa*, acc. IRBv4, *C. tubulosa* subsp. *tubulosa*, acc. IRBd and *C. flava* subsp. *flava*, acc. IRBt) they were excluded prior to the analysis of variance. One-way ANOVA (Analysis of Variance) was employed at a significant level of $P < 0.001$ with Dunnett's C post hoc test to explore significant differences within and between populations and species. The members of *Orobanche* and *Phelipance* were excluded from the statistical analyses because of their much lower genome size in comparison to *Cistanche*. Spearman's Correlation coefficient

test was performed to test the relationship between mean nuclear DNA content and geographic position (altitude, latitude and longitude) as well as bioclimatic data obtained from WorldClim database 1.4 (Hijmans & al., 2005b; <http://worldclim.org/>) at a resolution of 2.5 arc-seconds. Data were extracted using ArcGis 9.3 (ESRI, 2008).

3.3 Results

3.3.1 Inter- and intraspecific variation in genome size

The mean values and standard deviations of 2C-values of 53 accessions of *Cistanche* from Iran, Morocco and Spain estimated with FDM are shown in Appendix 3.1 and Figure 3.2. Genome size was also measured for three accessions of *C. phelypaea* reported earlier by Weiss-Schneeweiss & al. (2006). 2C-values of *Cistanche* species showed significant inter- and intraspecific differences (Welch's ANOVA, $P = 0.001$, Dunnett's C post hoc test). Specifically, strong significance of genome size variation among each species was found with analysis of variance among normally distributed accessions of *C. ambigua* ($F = 113.61$; $P < 0.0001$), *C. tubulosa* group ($F = 622.985$; $P < 0.0001$), *C. flava* group ($F = 88.242$; $P < 0.0001$) and *C. violacea* ($F = 635.444$; $P < 0.0001$) (Appendices 3.2-3.7). Interspecific diversity in nuclear DNA content (2C-values) in *Cistanche* ranged from 8.56 pg in *C. ambigua* acc. IRB24 (sect. *Cistanchiella*) to 31.88 pg in *C. tubulosa* acc. IRBm (sect. *Cistanche*), amounting to a nearly 3.72-fold difference (Appendix 3.1, Figs. 3.2 and 3.5 clades B and G). About 2-fold intraspecific variation of genome size was encountered in two populations of *C. tubulosa* (accs. IRBm and IRBk) and of *C. ambigua* (accs. IRB14b and IRB24), despite the lack of any apparent morphological or DNA sequence differences (Appendix 3.1, Figs. 3.2 and 3.5 clades B and G). The lowest intraspecific variation was observed in *C. phelypaea* subsp. *phelypaea* (members of clade E in Fig. 3.5; Appendix 3.1; Fig. 3.2) from Morocco and Spain (1.16-fold) and *C. flava* subsp. *flava* (members of clade L in Fig. 3.5; Appendix 3.1; Fig. 3.2) from Iran (1.24-fold).

Phelipanche and *Orobanche* species displayed much lower genome size range than *Cistanche* (Appendix 3.1; Fig. 3.2). Genome size in *P. ramosa* (2C 7.92 pg) and *P. purpurea* (2C 14.20 pg) was large and overlapped with the genome size of *Cistanche* (Fig. 3.2). While genome size of *Phelipanche* cf. *iberica* (acc. IRB9; 2C 2.90 pg; Appendix 3.1; Fig. 3.2) was much lower (11-fold) than of *Cistanche* and showed 1.9-fold intraspecific variation with acc. IRB7 (Appendix 3.1; Fig. 3.2). Analysis of ANOVA showed c. 65% of significant differences ($F = 59.38$; $p < 0.0001$) on interspecific level (data not shown).

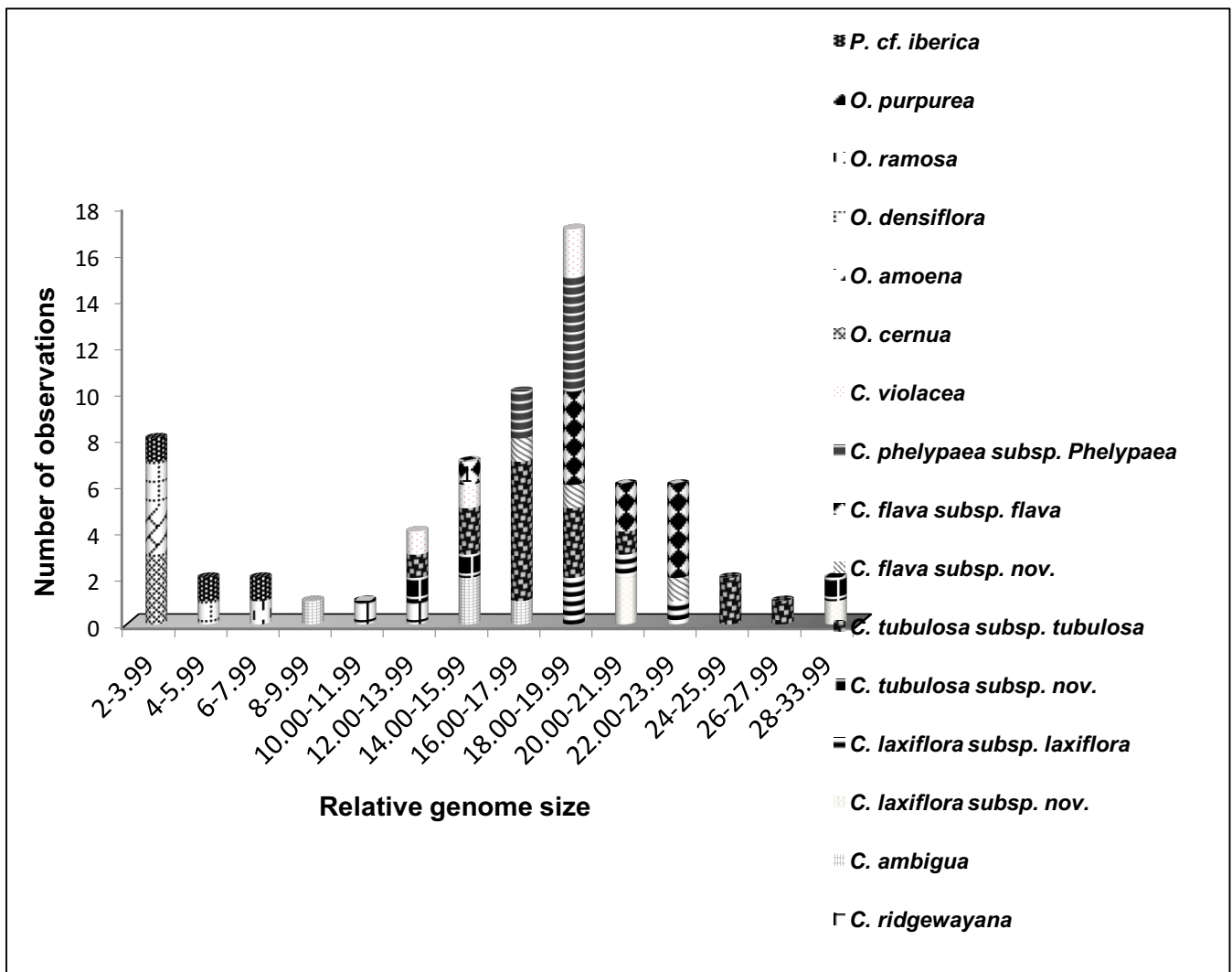


Figure 3.2 Histogram of distribution of genome size in *Cistanche* and its two relatives. The letters C., O. and P. indicate the species of *Cistanche*, *Orobanchaceae* and *Phelipanche*, respectively.

3.3.2 Chromosome number estimation

Chromosome number is shown in Table 3.1 and Figs 3.3 and 3.4. All species of *Cistanche* were diploid with $2n = 2x = 40$ except for a single triploid individual of *C. violacea* (acc. Cis4; $n = 30 \pm 1/2B$; Fig. 3.4B and R; other accessions were diploid as well (Figs. 3.4A and P; Fig. 3.4C and Q) were diploid. The karyotypes of *Cistanche* species consisted of supernumerary chromosomes predominantly of large meta- to submetacentric, and occasionally also of acrocentrics in *C. ambigua* (e.g. acc. IRB20; $n = 20$; 2C 15.31 pg; Fig. 3.3C and R) and *C. flava subsp. flava* (e.g. acc. IRB27; $n = 20$; 2C 22.15 pg; Fig. 3.3Q and Y).

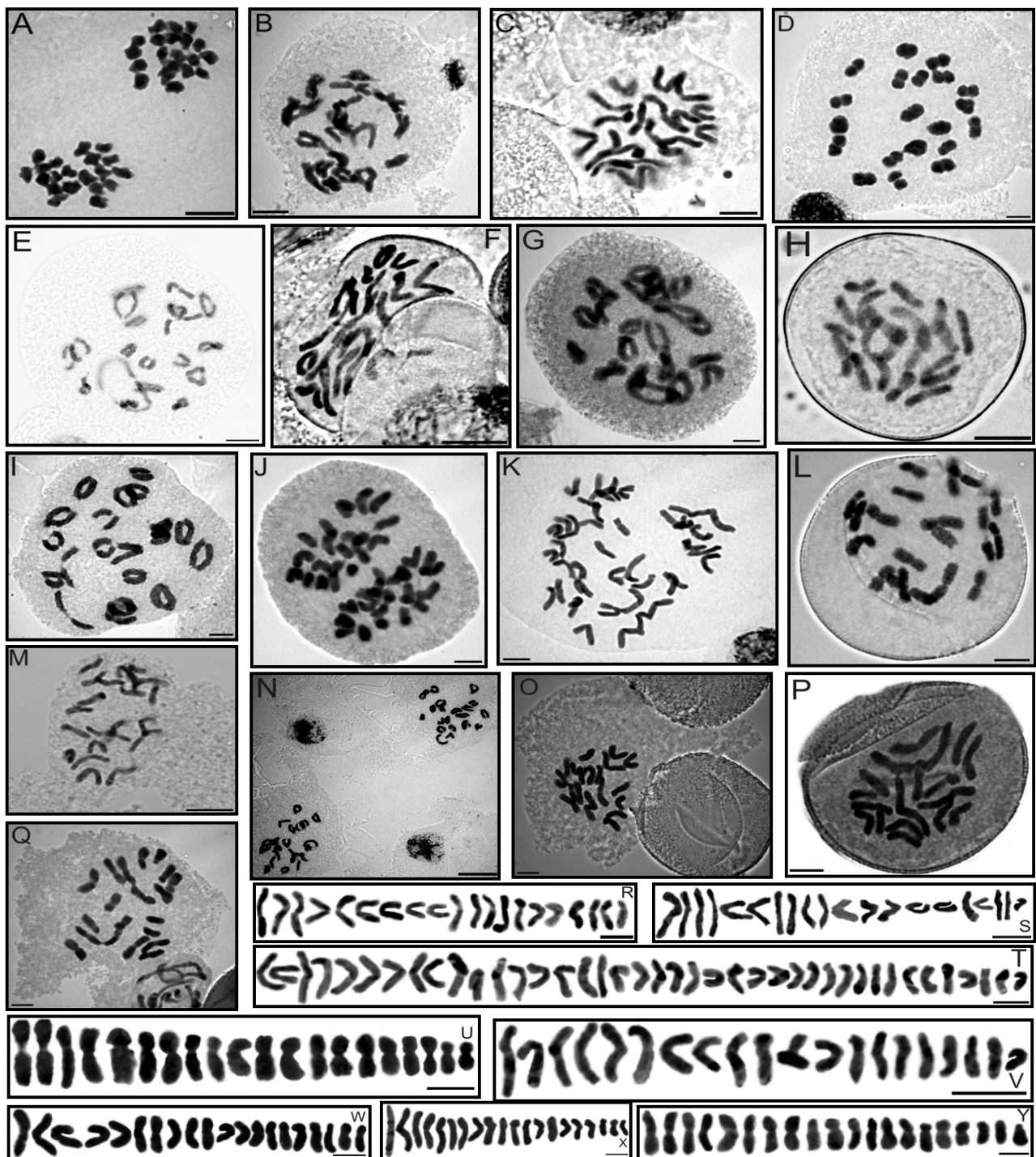


Figure 3.3 Chromosome number and karyotype of *Cistanche* from Iran: ***Cistanche* sect. *Cistanchiella***: A) *C. ridgewayana* (acc. IRB3), $2n = 40$ (AI/TI); B) *C. ambigua* (acc. IRB14a), $2n = 40$ (daikinesis); C) *C. ambigua* (acc. IRB20), $n = 20$ (MitI). ***Cistanche* sect. *Cistanche***: D) *C. laxiflora* subsp. *laxiflora* (acc. IRB6), $n = 20$ (MitI); E) *C. laxiflora* subsp. *laxiflora* (acc. IRB11), $2n = 40$ (daikinesis); F) *C. laxiflora* subsp. *laxiflora* (acc. IRB11), $n = 20$ (MitI); G) *C. laxiflora* subsp. *laxiflora* (acc. IRB15), $2n = 40$ (daikinesis); H) *C. laxiflora* subsp. *laxiflora* (acc. IRB15), $n = 20$ (MitI); I) *C. laxiflora* subsp. *nov.* (acc. IRB15), $2n = 40$ (diakinesis); J) *C. tubulosa* subsp. *tubulosa* (acc. IRBb), $2n = 40$ (AI); K) *C. tubulosa* subsp. *tubulosa* (acc. IRBd), $2n = 40$ (in somatic cell); L) *C. flava* subsp. *nov.* (acc. IRB21), $n = 20$ (MitI); M) *C. flava* subsp. *flava* (acc. IRB22), $n = 20$ (MitI); N) *C. flava* subsp. *nov.* (acc. IRB23), $2n = 40 \pm 1B$ (AI); O) *C. flava* subsp. *nov.* (acc. IRB29), $n = 20$ (MitI); P) *C. flava* subsp. *flava* (acc. IRB25), $n = 20$ (MitI); Q) *C. flava* subsp. *flava* (acc. IRB27), $n = 20$ (MitI); R) Karyotype of C; S) Karyotype of F.; T) Karyotype of K in somatic cell; U) karyotype of L; V) karyotype of M; W) karyotype of O; X) karyotype of P; Y) karyotype of Q. Abbreviations: AI, anaphase I; MI, metaphase I; MitI, metaphase of first mitotic division in microspore; TI: Telophase I; Scale = 5 μ m. Figure names were depicted in association with correspondant accessions in Appendix 3.1.

Chromosomes of most analyzed *Cistanche* species pair as regular bivalents in metaphase I of meiosis, usually with two terminal chiasmata (ring bivalents; acc. IRB31; $2n = 40$; $2C$ 19.07 pg; Fig. 3.3I and acc. IRB23; $2n = 40 \pm 1B$; $2C$ 19.25 pg; Fig. 3.3N). Putative supernumerary B chromosome was observed in the later accession of *C. flava* subsp. *nov.* (acc. IRB23; Fig. 3.3S). Two other accessions of *C. flava* subsp. *nov.* Similar to three accessions of *C. flava* subsp. *flava* were exclusively diploid (Figs. 3.3L and U, 3.3O and W, 3.3M and V, 3.3P and X, 3.3Q and Y). Meiotic counts confirmed also diploid chromosome number of $n = 20$ for *C. ridgewayana* (acc. IRB3; $2n = 40$; $2C$ 11.42 pg; Fig. 3.3A) of the sect. *Cistanchiella* and *C. ambigua* (acc. IRB14a; $2C$ 14.31 pg; Fig. 3.3B and acc. IRB20; $2C$ 15.31 pg; Figs. 3.3C and R) of sect. *Heterocalyx*. All accessions of *Orobanche* sect. *Orobanche* explored in this study were exclusively diploid $2n = 2x = 38$ (*O. cernua*, *O. transcaucasica*; Figs. 3.4F and X, and 3.4I and L, respectively), except for tetraploid $2n = 4x = 76$ for *O. densiflora* (Fig. 3.4E). The chromosomes of *Orobanche* were relatively small, meta- to submetacentric (Figs. 3.4X, U, W and V). *Phelipanche purpurea*, *P. ramosa* and *P. cf. iberica* were all diploid with $2n = 24$ (Figs. 3.4G, H, O and T).

3.3.3 Phylogenetic relationships

Well supported infrageneric phylogenetic relationships were inferred from maximum likelihood, maximum parsimony and Bayesian analyses of the combined plastid and nuclear markers (Fig. 3.5 and Appendix 3.9). The DNA sequence including indels was phylogenetically analysed and showed the better resolutions (Fig. 3.5 and Appendix 3.9). The tree topologies of all analyses were almost identical except for trichotomy at basal of sect. *Cistanche*, when maximum likelihood and Bayesian analysis considered (Fig. 3.5, clades C-L). In sect. *Cistanche* using maximum parsimony, however, medium to low-supported monophyletic, i.e. the so called, Mediterranean and African clades were observed (Appendix 3.9). All three types of analyses indicate the monophyly of *Cistanche* species with high support at basal nodes (MP 100; BS 100; PP 1.00). Besides two traditionally recognized sections: *Cistanchiella* (encompassing only eastern species) and *Cistanche* are recognized (MP 100; BS 100; PP 1.00; Fig. 3.5, clades A-B and Appendix 3.9).

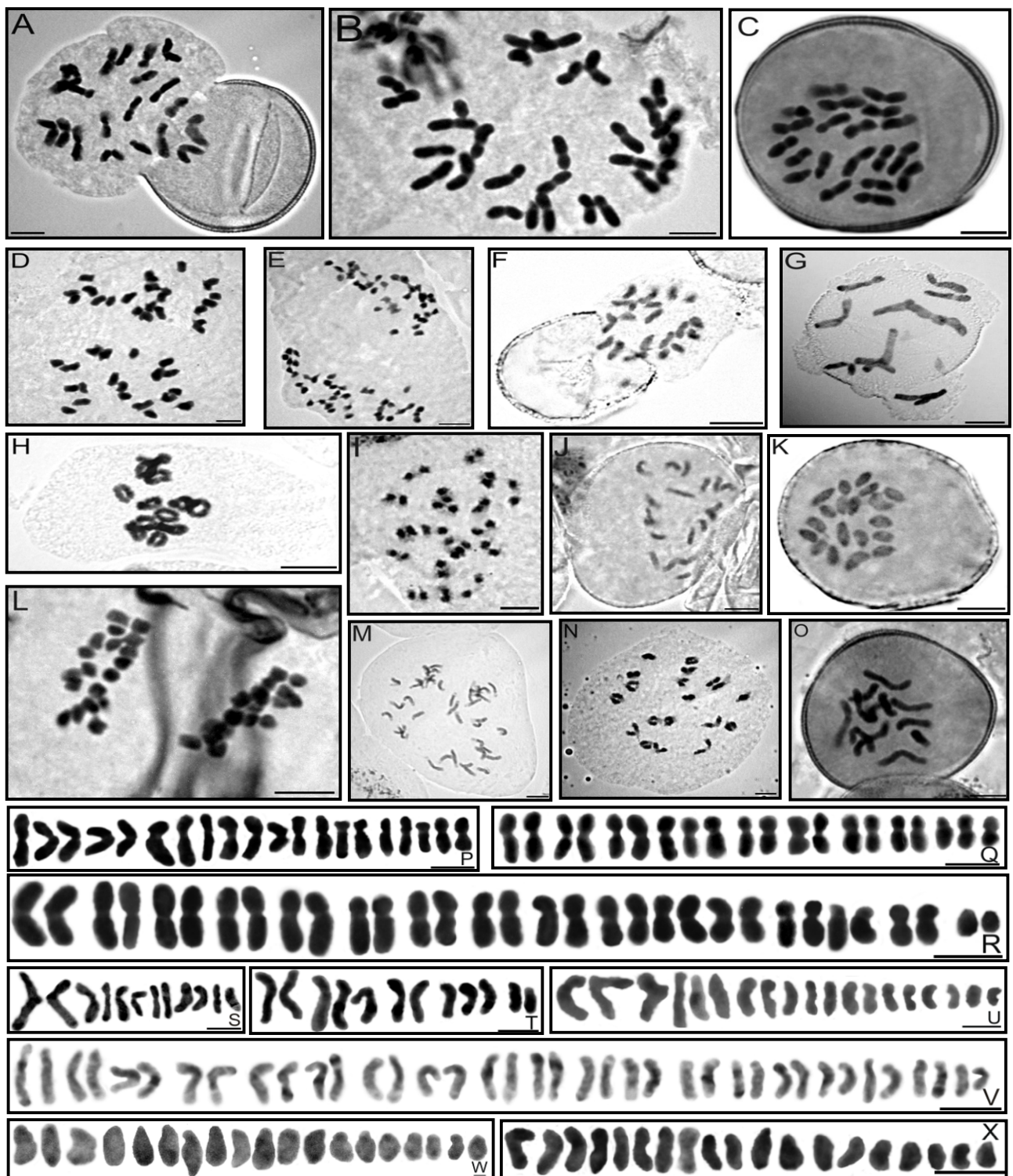


Figure 3.4 Chromosome number of *Cistanche* from Morocco and Spain plus Chromosome number of genera *Orobanche* and *Phelipanche*: ***Cistanche* sect. *Cistanche***: A) *C. violacea* (acc. Cis1), $n = 20$ (MitI); B) *C. violacea* (acc. Cis4), $n = 30 \pm 1 / 2B$ (MitI); C) *C. violacea* (acc. Cis5), $n = 20$ (MitI); D) *C. phelypaea* subsp. *phelypaea* (acc. Ova.1), $2n = 40$ (TI). ***Orobanche* sect. *Orobanche* and *Phelipanche* sect. *Phelipanche***: E) *O. densiflora* (acc. Ger.1), $2n = 76$ (TI); F) *O. transcaucasica* (acc. Hell), $n = 19$ (MitI); G) *P. purpurea* (acc. BONN2), $n = 12$ (MitI); H) *P. ramosa* (acc. BONN1), $2n = 24$ (diakinesis); I) *O. cernua* (acc. IRB2), $2n = 38$ (AI); J) *O. cernua* (acc. IRB2), $n = 19$ (MitI); K) *O. cernua* (acc. IRB12), $n = 19$ (MitI); L) *O. cernua* (acc. IRB19), $2n = 38$ (AI/TI); M) *O. cernua* (acc. IRB16), $n = 19$ (in somatic cell); N) *O. cernua* (acc. IRB18), $2n = 38$ (diakinesis); O) *P. cf. iberica* (acc. IRB7), $n = 12$; P) karyotype of A; Q) karyotype of E; R) karyotype of B; S) karyotype of G; T) karyotype of O; U) karyotype of J; V) karyotype of M; W) karyotype of K; X) karyotype of F. Abbreviation listed in Figure 3.3.

Our phylogenetic data strongly supports that *C. ambigua* traditionally belonging to sect. *Heterocalyx* (Beck-Mannagetta, 1930) merged in sect. *Cistanchiella*, (Fig. 3.5 and Appendix 3.9). Although the other sect. *Cistanche* is well-supported (MP 100; BS 100, PP 1.00; Fig. 3.5, clades C-L and Appendix 3.9), relationships within this section remain unresolved due to a polytomy including western (Moroccan and Iberian Peninsula) and eastern species (Iran). *Cistanche lutea* from Morocco forms the only western clade (MP 89; BS 72; PP 1.00; Fig. 3.5; clade F and Appendix 3.9), which splits into two monophyletic Iranian species in sect. *Cistanche* (BS 84/61, PP 1.00, Fig. 3.5 and Appendix 3.9): the Northern and Southern species form clade containing *C. flava* group (Fig. 3.5, clades K-L) and the other included *C. laxiflora* group mainly distributed in the center (Fig. 3.5, clades I-J). Altogether terminal clades have received moderate to high support in all analyses. The outgroups formed two distinct clades highly supported (BS 100, PP 1.00) including *Phelipanche* and *Orobanche* genera, respectively.

3.3.4 Genome size variation among phylogenetic groups

The 2C-values of distinct phylogenetic groups were randomly distributed and did not reveal any significant trend with phylogenetic groupings (Figs. 3.5 clades A-L). The two sections *Cistanchiella* and *Cistanche* differed slightly in mean genome size (Fig. 3.5, scatter plot). The average nuclear DNA amount in the basal sect. *Cistanchiella* (2C 13.42 pg; Appendix 3.1 and Fig. 3.5, clades A-B) was lower than genome size of sect. *Cistanche* (2C 20 pg; Table 1 and Fig. 3.5, clades C-L). Appendix 3.11 represents the box plots of variation of mean genome size among phylogenetic clades. Significant difference was observed among clades (A-L), resulting from Kruskal-Wallis tests ($\chi^2(2) = 25.63$, $df = 11$, $P < 0.007$).

3.3.5 Variation of genome size versus habitat preferences

Non-parametric Spearman test indicated that nuclear DNA amount was significantly correlated neither with bioclimatic nor geographical variables (Appendix 3.8). The only positive correlation was found between genome size and altitude in *C. phelypaea* subsp. *phelypaea* and was likely caused by low sampling density. Similarly, no significant correlation of genome size within each phylogenetic clade with habitat preferences was found. Generally, *Cistanche* species occupy mostly sub-desert areas (Appendix 3.10).

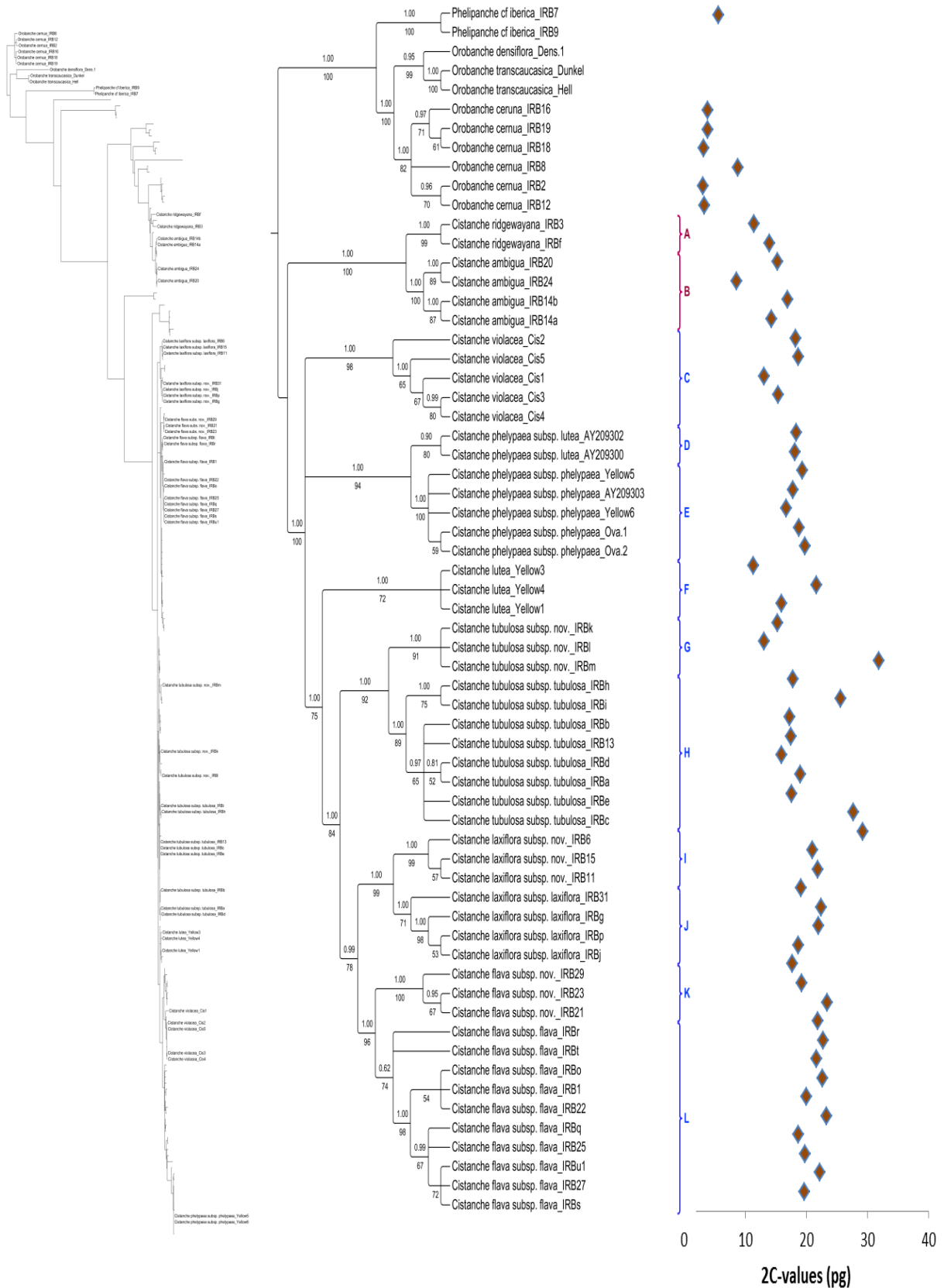


Figure 3.5 Maximum likelihood analysis tree of *Cistanche* species including outgroups inferred from combined chloroplast and nuclear DNA sequences (middle) and phylogram representing the phylogeny of all *Cistanche* molecularly analyzed (left, adopted from Chapter 2). Bayesian posterior probabilities ≥ 0.6 shown above the branches and bootstrap values ≥ 50 showd below the branches. Scatter polot of 2C-values (pg) are shown next to each specimen. Phylogenetic clades are named as (A-I) according to boxplot diagram (Appendix 3.11). Red legends show the members of *Cistanche* sect. *Cistanchiella* and blue ones indicate *Cistanche* sect. *Cistanche*.

3.4 Discussion

3.4.1 Genome size and chromosome number

The amount of karyological data for *Cistanche* has substantially increased following this study, whilst the genome sizes of taxa in genus *Cistanche* were previously only reported for two species (Weiss-Schneeweiss & al., 2006), similarly to chromosome numbers of few individuals in the genus. Whereas ploidy levels are homogenous (ignoring the occasional triploid), the variation in genome size is much larger than previously thought. Only the lowest amount of genome size of *C. phelypaea* subsp. *phelypaea* (ranging from 16.74 to 19.72 pg / 2C) supports the previous results (Weiss-Schneeweiss & al., 2006). In agreement with previous studies chromosome number in *C. phelypaea* subsp. *phelypaea* was found $2n = 40$ (Fig. 3.4D; Gardè, 1952; Talavera, 1978; Schneeweiss, 2004b). For *C. tubulosa* subsp. *tubulosa*, chromosomes in meiotic and somatic dividing cells were counted ($n = 20$ and $2n = 40$; Figs. 3.3J, 3.3K, T) and Hamblen, 1956, 1958; Pazy & Plitman, 1996; Pazy, 1998). *C. violacea* was found to be diploid ($n = 20$; Figs. 3.4A and C; Reese, 1957), with the exception of one triploid individual (Fig. 3.4B). This study clearly shows, by extending the sampling for both chromosome number and genome size estimation within the genus that increases of genome size in *Cistanche* is not associated with extent polyploidy. All analyzed species were diploid with $2n = 40$, although genome size varied considerably both within and among taxa. The dynamics of genome size changes was also not correlated with phylogenetic relationships. A significant genome size differences among individual accessions of *C. ambigua* and one of *C. lutea* from Morocco do not correlate with differences in ploidy nor with phylogenetic relationships. The most likely mechanism contributing to the expansion of genome size without involving polyploidy is the amplification and/or removal of transposable elements, particularly retroelements (e.g. Flavell & al., 1974; Grover & Wendel, 2010; Kellogg & Bennetzen, 2004).

3.4.2 Genome size in relation to phylogeny, habitat and host preferences

The variation of genome size in the genus *Cistanche* did not correlate with any habitat, geographical or environmental factors (Appendix 3.8 and 3.10), possibly because of high dependency to the host plant. If this scenario is true, then the species e.g. *C. flava* subsp. *flava* (rather with narrow-host specific, for detail see chapter 4) would also follow its host ecological niche as expected for holoparasites. Ecologically the species which occupy broader geographical range and occur in different climatic zones or altitudes (Appendix 3.8) might differ in morphology, but might not necessarily differ in genome size or incidence of polyploidy (e.g. Accs. IRBv4, IRBs and IRBu1, Appendix 3.1). Higher average 2C-values of

two distinct phylogenetic *C. flava* subsp. *flava* (robust plant with very long-narrow scale and bract in arid regions of northeastern area with c. 300 mm annual precipitation) in comparison to geographically close *C. flava* subsp. *nov.* (short and lax scales and bracts in mild southeastern sub-desert area with c. 200 mm annual precipitation) may correlate with ecological differentiations (Fig. 3.5 clades K-L and Appendix 3.11). The two-fold genome size variation between two individuals of *C. tubulosa* subsp. *nov.*, one growing in saltmarshes of southeastern Saharo-Sindian coast (acc. IRBm; Appendix 3.1) and the other located c. 50 km away from the coasts (acc. IRBk; Appendix 3.1), does not ecologically correlate. The subspecies forms a distinct phylogenetic clade sister to *C. tubulosa* subsp. *tubulosa* (Fig. 3.5 clade G). The variation of genome size was very random and uncorrelated with habitat preferences almost in all phylogenetic clades clearly observed e.g. in the clade *C. laxiflora* subsp. *laxiflora* (Fig. 3.5 clade I; Appendix 3.10), wide-host parasite growing on e.g. genera *Alhagi*, *Robus*, *Prosopis*. Phylogenetic data allow inference of ancestral lower genome sizes for the early-diverging lineages of the genus *Cistanche* (e.g. *C. sinensis* and *C. mauritanica*), and increase in their sister group sects. *Cistanchiella* and *Cistanche* (for detail see chapter 2). The constancy in chromosome numbers and high diversity of genome size, uncorrelated with phylogenetic/ecological relationships; however, offers only very limited support to resolve the species relationships. Moreover, chromosome number stability contrasts with nearly four-fold genome size variation, suggesting mechanisms other than polyploidy for the increase/decrease of the genome size (e.g. Bennetzen, 2005).

3.5 Conclusions

Apart from a few previous karyological reports on the genus, our study provides the first insight into chromosome number and genome size variation in nine species of *Cistanche*, and attempt of interpreting the data in a phylogenetic context. Distribution of genome sizes is to some extent in accordance with phylogenetic groupings when the sections are considered. However, for the majority of clades no significant correlations of genome size were obtained, although low sampling affects the conclusions. The newly obtained data contribute to further research of genome evolution of parasitic plants in general, and genus *Cistanche* in particular, provides hypotheses of genome evolution in the context of parasite specialization or host traits.

4 Taxonomic revisions of the genus *Cistanche*, with newly northwestern African infrageneric section

Abstract

Cistanche is a holoparasitic genus of about two dozen species distributed mainly from northern and central Africa to central and eastern Asia, with its diversity center in Southwest Asia. The taxonomy of this genus is, however, poorly understood and in urgent need of revision. We performed morpholometric analyses of 20 quantitative and 50 nominal characters using ordination (Principal Component Analysis, PCA) and clustering (complete linkage) techniques, of which the latter compared in the context of a recently developed molecular phylogenetic hypothesis of *Cistanche*. Morphometric analysis of joint quantitative plus nominal morphological characters separated the majority of taxa. Cluster analysis separated four major groups (corresponding to taxonomic subgenera and sections), but there are discrepancies between morphological clusters and molecular-phylogenetic groups. Based on these data, *Cistanche* is separated into the two subgenera *Subcistanche* (*C. sinensis* only) and *Cistanche*, the latter comprising three sections (*Heterocalyx*, *Macrocalycinum* sect. nov. and *Cistanche*). Altogether, 25 species are distinguished, nine of which are described as new (*C. almeriensis* sp. nov., *C. algeriensis* sp. nov., *C. somaliensis* sp. nov., *C. chabaharensis* sp. nov. in *C.* sect. *Cistanche*; *C. persica* sp. nov., *C. tomentosa* sp. nov., *C. bilobata* sp. nov., *C. bamianica* sp. nov. in *C.* sect. *Heterocalyx*; *C. macrocalycinum* sp. nov. in *C.* sect. *Macrocalycinum*). Additionally, six new subspecies are described (*C. phelypaea* subsp. *naviculata*, *C. rosea* subsp. *crimson*, *C. laxiflora* subsp. *alba*, *C. flava* subsp. *brevibracteata* and *C. tubulosa* subsp. *iranica* in *C.* sect. *Cistanche* and *C. tomentosa* subsp. *longibracteata* in *C.* sect. *Heterocalyx*). *C. tinctoria* f. *lusitanica* (Coss.) and *C. tubulosa* var. *flava* (C.A.Mey.) Beck previously recognized formae or variety are raised to specific rank (*C. phelypaea* and *C. flava*). Furthermore, *C. mongolica* was merged into *C. laxiflora* subsp. *laxiflora*. *C. christisonioides* was synonymized into *C. flava* subsp. *flava*, *C. compacta* into *C. tinctoria*, *C. trivalvis* into *C. ambigua* and *C. hesperugo* into *C. brunneri*. Taxonomic key, species description, distribution maps, botanical illustrations, host preferences and status of endangerment (according to IUCN), where appropriate, are provided for each species.

4.1 Introduction

The holoparasitic genus *Cistanche* Hoffmanns. & Link is distributed in the Old World from the Macaronesian Islands through central and northern Africa and the Mediterranean to

southwestern, central and eastern Asia. Based on morphological characters, *Cistanche* has been taxonomically placed in subtribe Orobanchinae together with *Orobanche*, *Phelipanche* and *Diphelypaea* (Teryokhin & al., 1993). Morphologically, *Cistanche* differs from *Orobanche* and *Phelipanche* by having usually unbranched and robust stem, a calyx with relative to the tube only short lobes, and less strongly zygomorphic flowers; from *Diphelypaea*, *Cistanche* additionally differs by multi-flowered inflorescences. The genus was first described in 1813 in the Flora of Portugal (Hoffmannsegg & Link, 1913), where it comprised two species, *C. lutea* (Desf.) Hoffmanns. & Link and *C. violacea* (Desf.) Hoffmanns. & Link. Later, Hooker (1885) transferred an additional species from the genus *Phelypaea* L. to *Cistanche*, (*C. tubulosa* (Schenk) R. Wight ex Hook.f.). Aitchison (1888) described a new species *C. laxiflora* Aitch. & Hemsl. and Baker (1894) *C. rosea* Baker in genus *Cistanche*. Guimarães (1904) transferred *Phelypaea lusitanica* Coss. to *Cistanche*, which Coutinho (1913) correctly placed under its correct name *C. phelypaea* Cout. Pax (1907) described *C. carnosa* Pax.

In the last taxonomic revision of *Cistanche*, Beck-Mannagetta (1930) recognized 17 species (one doubtful), mostly by transferring taxa from *Phelypaea* and *Orobanche* to *Cistanche*, three of which he described newly (*C. christisonioides*, *C. mongolica* and *C. sinensis*). He divided *Cistanche* into four sections based on morphological characters of the calyx and the bracteoles. *Cistanche* sect. *Cistanchiella* Beck, with the single species *C. ridgewayana* Aitch. & Hemsl., is characterized by having none or one bracteole only (in contrast to two in the other sections). *Cistanche* sect. *Subcistanche* Beck, with the single species *C. sinensis* Beck, possesses a deeply divided quadripartite calyx. While *C. sect. Heterocalyx*, with the three species *C. fissa* (C.A.Mey.) Beck, *C. ambigua* (Bunge) Beck, and *C. rosea* Baker, has a pentamerous calyx, whose posterior lobe is nearly free from the others, or a tetramerous calyx with equal or unequal lobes. *C. sect. Cistanche* has a pentamerous calyx with equal and nearly completely connated lobes.

Since Beck-Mannagetta (1930), taxonomic work in *Cistanche* has been restricted to regional treatments. For the Flora of USSR, Novopokrovskii & Tzvelev (1958) recognized seven species, including *C. flava* (C.A.Mey.) Korsh that was considered a variety of *C. tubulosa* (Schenk) Wight by Beck-Mannagetta (1930). In Iran, seven species have been recorded altogether by Bornmüller (1936), who described *C. eremodoxa* Bornm., and by Schiman-Czeika (1964). Later Gilli (1979) placed *C. eremodoxa* in the synonymy of *C. ambigua* (Bunge) Beck, a species considered doubtful by Schiman-Czeika (1964). Gilli (1979) also described the new species *C. afghanica* Gilli. Zhang & Tzvelev (1998) recognized five species of *Cistanche* in China, including *C. salsa* (C.A.Mey.) Beck, *C. tubulosa* (Schenk) R. Wight ex Hook.f., *C. sinensis* Beck, *C. lanzhouensis* Zhi Y.Zhang and *C. deserticola* Ma, the latter two described as new species after the publication of Beck-Mannagetta (1930). **Lobin**

(1996) described the genus in Cape Verde Island with only one species (*C. phelypaea*). The genus introduced with seven species in the latest monographic treatment from Iran (Saeidi Mehrvarz & Shahi Shavvon, 2008). Five species including *C. armena* (K.Koch) M.V.Agab. were recorded for *Cistanche* from Transcaucasia (Aghababayan, 2013). Recent molecular phylogenetic studies (chapter 2, Fig. 2.1) have shown that the infrageneric classification of Beck-Mannagetta (1930) hardly reflects phylogenetic relationships. The only exception is sect. *Subcistanche* Beck, which is confirmed as phylogenetically distinct lineage sister to the remainder of *Cistanche*. The latter falls into three clades. The first clade includes *C. ridgewayana*, the only species of sect. *Cistanchiella* Beck, some species of *C. sect. Heterocalyx* (*C. fissa* and *C. ambigua*), and some species of sect. *Cistanche* (*C. salsa* and *C. deserticola*). The second clade contains *C. mauritanica* of sect. *Cistanche* and a yet undescribed species. Finally, the third clade contains the remaining species of sect. *Cistanche* and *C. rosea* of sect. *Heterocalyx*. There are also discrepancies between molecular phylogenetically defined lineages and taxonomic species of genetically and geographically distinct lineages in single taxa (e.g. *C. phelypaea* and *C. tubulosa*).

Here, we intend to establish a sound taxonomic system of *Cistanche*. To this end, we conduct morphometric analyses of numerous quantitative and nominal characters and integrate their results with those from previous molecular-phylogenetic analyses. Based on; thus, derived phylogenetic hypotheses and using data from an extensive survey of herbarium specimens and images, we propose an infrageneric classification of *Cistanche*, where infrageneric taxa represent monophyletic groups, and provide a comprehensive species level classification (including revision of the nomenclature). For each species, we present detailed descriptions, illustrations as well as data on distribution and host preferences.

4.2 Material and methods

4.2.1 Plant material and formal taxonomy

We studied approximately 670 herbarium specimens (or photos thereof from B, BIEL, BM, BONN, BR, E, FI, G, G-DC, GB, GUM, HUJ, IRAN, JE, K, KAS, LE, M, MSB, MW, NCARTT, PEM, REG, PH, TARI, TUH, W, WU (acronyms follow Thiers (2010) [continuously updated]), Soltan Qaboos University Muscat (SQU), University of Sistan & Baluchestan (USB)) or web images. As herbarium specimens of *Cistanche* often lack plant organs in sufficient condition (e.g. floral bract, bracteole and corolla color), additionally web photos and fresh collections were used. Of the 670 herbarium specimens, 154 were included in morphometric analysis (Table 4.1). The specimens examined for each species were selected from herbarium

records, the web images or other literature, of which the latter two did not cite here. Initial determination of taxa followed Beck-Mannagetta (1930) and floristic treatments (Post, 1933; Graham, 1957; Quézel & Santa, 1963; Schiman-Czeika, 1964; Webb, 1972; Jafri & El-Gadi, 1978; Valdés & al., 1987; Teryokhin & al., 1993; Wood, 1997; Zhang & Tzvelev, 1998; Foley, 2001a; López Jiménez & Caballero de Rodas, 2004; Parker, 2006; Fennane & al., 2007; Saeidi Mehrvarz & Shahi Shavvon, 2008). Distribution and host data were collected from field observations, herbarium label information, literature, photos (pers. comm.) and web images. Delimitation of species and subspecies takes molecular-phylogenetic and geographic data into account (chapter 2).

4.2.2 Morphological characteristics and morphometric analyses

Characters for morphometric analyses were chosen based on taxonomic and floristic treatments mentioned above as well as own observations. Twenty quantitative and 50 nominal characters were documented (Table 4.2 and Appendix 4.1). Floral measurements were made at the middle or basal part of the inflorescence, where the anthetic flowers were located. Irrespective of their inclusion in a formal morphometric analysis, morphological characters were evaluated with respect to their suitability as diagnostic characters. Principal Component Analysis (PCA) was conducted on quantitative characters only. The first three principal components, which explained 99% of the total variation, were used to visualize the objects (i.e. specimens) onto scatter plots. In order to identify groups, cluster analysis was employed based on Euclidean distances between objects on the whole principal components space in the case of quantitative analysis. For analyses with nominal data and quantitative plus nominal data, the general similarity coefficient (Gordon, 1981) was calculated based on the original variables. Hierarchical cluster analysis using the complete linkage method was conducted on this similarity matrix. All analyses were implemented in R (R Development Core Team, 2013) using package cluster (Maechler & al., 2015).

Table 4.1 Taxa used in the study and voucher information of the *Cistanche* specimens examined for morphometric analyses. For full detail, refer to additional specimen examined in the text.

Taxon	Collector, voucher number and herbarium acronym	Taxon	Collector, voucher number and herbarium acronym	Taxon	Collector, voucher number and herbarium acronym
subg. <i>Subcistanche</i>		<i>ambigua</i>	Hedge & Ekberg W 7213 (E)	<i>senegalensis</i>	Friis & al. 8765 (K)
<i>sinensis</i>	Licent 3845 (P & W)	<i>ambigua</i>	Rechinger 34645 (W)	<i>senegalensis</i>	Burger 2848 (K)
subg. <i>Cistanche</i>		<i>ambigua</i>	Freitag 718 (KAS)	<i>senegalensis</i>	Ash 2572 (K)
sect. <i>Macrocalycinum</i>		<i>salsa</i>	Davis & Dodds 18709 (K)	<i>senegalensis</i>	Bally & Melville 15582 (K)
<i>macrocalycinum</i> sp. nov.	Pelry s.n. (K)	<i>salsa</i>	Davis & Dodds 18709 (BM)	<i>senegalensis</i>	Watson W1 (K)
<i>mauritanica</i>	S.col. 443 (W)	<i>salsa</i>	Boulous & al. 8625 (K)	<i>senegalensis</i>	Musselman 6238 (E)
<i>mauritanica</i>	Warion s.n. (P)	<i>salsa</i>	Beck 1541 (K)	<i>senegalensis</i>	Comm & Bent s.n. (K)
<i>mauritanica</i>	Debeaux 1886 (BM)	<i>salsa</i>	Fayvush & Tamanyan 9-2004 (P)	<i>senegalensis</i>	Wood SI72/92 (K)
sect. <i>heterocalyx</i>		<i>salsa</i>	Becker s.n. (K)	<i>senegalensis</i>	Mathew 6317 (K)
<i>persica</i>	Ataei & Heidari ED424 (BONN)	<i>salsa</i>	Bellew s.n. (K)	<i>senegalensis</i>	Greenway 8997 (K)
<i>persica</i>	Bornmüller 3907 (B)	<i>salsa</i>	Aucher-Eloy N°5079 (P).	<i>senegalensis</i>	Hepper 6940 (K)
<i>persica</i>	Freitag 15538 (KAS)	<i>salsa</i>	Aucher-Eloy N°5079 (K).	<i>senegalensis</i>	PA & WRQ Luke 5946 (K)
<i>persica</i>	Lamond 580 (W)	<i>deserticola</i>	Regel 1873 (K)	<i>senegalensis</i>	Beecker 14198 (K)
<i>tomentosa</i> subsp. <i>tomentosa</i>	Gauba 732 (B)	<i>deserticola</i>	Schipczinsky N°92 (K)	<i>senegalensis</i>	Richards 23671 (K)
<i>tomentosa</i> subsp. <i>tomentosa</i>	Rechinger 46653 (B)	<i>deserticola</i>	Ledebour s.n. (P)	<i>senegalensis</i>	Eggeling 6818 (K)
<i>tomentosa</i> subsp. <i>longibractea</i>	Freitag 15276a (KAS)	sect. <i>Cistahche</i>		<i>senegalensis</i>	Richards & Arasululu 26452 (K)
<i>ridgewayana</i>	Rechinger 34226 (E & W)	<i>laxiflora</i> subsp. <i>laxiflora</i>	Ataei ED521 (BONN & USB)	<i>senegalensis</i>	Friis & Hansen 2606 (K)
<i>fissa</i>	Beck 1864 (B)	<i>laxiflora</i> subsp. <i>laxiflora</i>	Ataei ED512 (BONN & USB)	<i>senegalensis</i>	Kiftara 12410 (K)
<i>bamianica</i>	Paine s.n. 1873 (K)	<i>laxiflora</i> subsp. <i>laxiflora</i>	Scol. s.n. (K)	<i>senegalensis</i>	Schweinfurth & Riva 1349 (K)
<i>bamianica</i>	Hedge & Wendelbo W-3427 (E)	<i>laxiflora</i> subsp. <i>laxiflora</i>	Scol. s.n. (K)	<i>senegalensis</i>	Leach 12348 (K)
<i>bilobata</i>	Sarnarovy s.n. (B)	<i>laxiflora</i> subsp. <i>alba</i>	Collenette 3566 (E)	<i>senegalensis</i>	Keiln A51 (K)
<i>ambigua</i>	Anders 8483 (MSB)	<i>senegalensis</i>	Ash 2104 (K)	<i>senegalensis</i>	Miller 581 (K)
<i>ambigua</i>	Ataei & Heidari ED437 (BONN)	<i>senegalensis</i>	Friis & al. 6680 (K)	<i>senegalensis</i>	Wood Y1132 (E)
<i>ambigua</i>	Ataei & Heidari ED481 (BONN)	<i>senegalensis</i>	Amshoff 6649 (BR)	<i>senegalensis</i>	Gillertt 4240 (K)
<i>ambigua</i>	Ataei & Heidari ED444 (BONN)	<i>senegalensis</i>	Wood 2091 (BM)	<i>senegalensis</i>	Glover & Gilliland 237 (K)

<i>ambigua</i>	Rechinger 51926 (K)	<i>senegalensis</i>	Ash 1811 (K)	<i>brunneri</i>	Kesby 17 (K)
<i>ambigua</i>	Rechinger 50654 (W)	<i>senegalensis</i>	MG & Gilbert 1075 (K)	<i>tubulosa</i> subsp. <i>tubulosa</i>	Barkley & Abbas-al-Ani 6499 (K)
<i>tubulosa</i> subsp. <i>tubulosa</i>	Dickson 704 (K)	<i>rosea</i> subsp. <i>crimson</i>	Collenette 5028 (K)	<i>violacea</i>	Collenette 3469 (K).
<i>tubulosa</i> subsp. <i>tubulosa</i>	Mandaville 1789 (BM)	<i>flava</i> subsp. <i>brevibractea</i>	Akhani, Noormohammadi & Samadi 22074 (TUH)	<i>violacea</i>	Mait land 75 (K)
<i>tubulosa</i> subsp. <i>tubulosa</i>	Sickson 182B (K)	<i>flava</i> subsp. <i>brevibractea</i>	Davis & Bokhari D.55976 (E)	<i>tinctoria</i>	Musselman 10150 (BM & E)
<i>tubulosa</i> subsp. <i>tubulosa</i>	El-Keblawy & al. TMH335 (K)	<i>flava</i> subsp. <i>flava</i>	Ataei ED522 (BONN & USB)	<i>tinctoria</i>	Musselman 10101 (E)
<i>tubulosa</i> subsp. <i>tubulosa</i>	Rashid 4650 (K)	<i>flava</i> subsp. <i>flava</i>	Ataei ED524 (BONN & USB)	<i>tinctoria</i>	Danin & al. s.n. (B)
<i>tubulosa</i> subsp. <i>tubulosa</i>	Harsuth 206/a (K)	<i>flava</i> subsp. <i>flava</i>	Ataei ED525 (BONN & USB)	<i>tinctoria</i>	Davies 4049 (K)
<i>tubulosa</i> subsp. <i>tubulosa</i>	Com RHS s.n. (K)	<i>flava</i> subsp. <i>flava</i>	Litwinow 1856 (K)	<i>tinctoria</i>	Zeller 8301 (BM)
<i>tubulosa</i> subsp. <i>tubulosa</i>	Podzorski 804 (E)	<i>flava</i> subsp. <i>flava</i>	Vvedensky 6499 (W)	<i>tinctoria</i>	Dinsmore 12903 (K)
<i>tubulosa</i> subsp. <i>tubulosa</i>	Collenette 2458 (E)	<i>flava</i> subsp. <i>flava</i>	Jachson & Taylor 6 (K)	<i>tinctoria</i>	Dmeikl 2164 (K)
<i>tubulosa</i> subsp. <i>tubulosa</i>	Shabetai 508.1239 (K)	<i>flava</i> subsp. <i>flava</i>	Drummond 23853 (K)	<i>tinctoria</i>	Rechinger 13113 (K)
<i>tubulosa</i> subsp. <i>tubulosa</i>	Boulos 11119 (K)	<i>almeriensis</i>	Davis 10.326 (K)	<i>tinctoria</i>	Podlech 52855 (MSB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	Akhani, Noormohammadi & Samadi 21965 (Iran, Khuzestan; TUH)	<i>almeriensis</i>	Van-Slageren & Henwawy MSAH992 (K)	<i>tinctoria</i>	Durieu s.n. (P)
<i>tubulosa</i> subsp. <i>tubulosa</i>	Ataei & Heidari ED506 (BONN & USB)	<i>almeriensis</i>	Dann & al. (B)	<i>tinctoria</i>	Schneeweiss & Staudinger 4224 (WU)
<i>tubulosa</i> subsp. <i>tubulosa</i>	Ataei & Heidari ED507 (BONN & USB)	<i>almeriensis</i>	Goldblatt 5017 (E)	<i>tinctoria</i>	Townsend 1136 (BM)
<i>tubulosa</i> subsp. <i>tubulosa</i>	Ataei & Heidari ED509 (BONN & USB)	<i>almeriensis</i>	Scol. 5346 (BR)	<i>tinctoria</i>	Dubuis s.n. (P)
<i>tubulosa</i> subsp. <i>tubulosa</i>	Ataei ED514 (BONN & USB)	<i>almeriensis</i>	Merxmüller & Lippert 23408 (M)	<i>tinctoria</i>	Scholz 16 (B)
<i>tubulosa</i> subsp. <i>tubulosa</i>	Assadi & Sardabi 41846 (IRAN)	<i>almeriensis</i>	Merxmüller & Gleissner 29290 (M)	<i>tinctoria</i>	Newby ZP125 (K)
<i>tubulosas</i> ubsp. <i>Chabaharensis</i>	Ataei ED516 (BONN & USB)	<i>violacea</i>	Staudinger 3219 (WU)	<i>algeriensis</i>	Davis 53288 (BM & E)
<i>tubulosas</i> ubsp. <i>Chabaharensis</i>	Ataei ED517 (BONN & USB)	<i>violacea</i>	Podlech 52705 (MSB)	<i>phelypaea</i> subsp. <i>phelypaea</i>	Ataei ED792 (BONN)
<i>tubulosas</i> ubsp. <i>Chabaharensis</i>	Ataei ED518 (BONN & USB)	<i>violacea</i>	Podlech 40705 (MSB)	<i>phelypaea</i> subsp. <i>phelypaea</i>	Smythies 252 (E)
<i>chabaharensis</i>	Willcox 200 (K)	<i>violacea</i>	Schuhwerk 90/792 (M)	<i>phelypaea</i> subsp. <i>phelypaea</i>	Markgraf s.n. (B)
<i>chabaharensis</i>	McLeish 1651 (E)	<i>violacea</i>	Merxmüller & Oberwinkler 22560 (M)	<i>phelypaea</i> subsp. <i>phelypaea</i>	Foley 2202 (E)
<i>chabaharensis</i>	Collenette 6025 (E)	<i>violacea</i>	Abulaila & Tehabshem 2006JOR4-1 (K)	<i>phelypaea</i> subsp. <i>phelypaea</i>	Foley 1007 (E)
<i>chabaharensis</i>	McLeish 966 (E)	<i>violacea</i>	Podlech 37081 (MSB)	<i>phelypaea</i> subsp. <i>naviculata</i>	Sales & Hedge 97/9 (E)
<i>chabaharensis</i>	McLeish 451 (E)	<i>violacea</i>	Davis & Lamond D.57318 (E)	<i>phelypaea</i> subsp. <i>phelypaea</i>	Olivier s.n. (P barcode P02970619)
<i>rosea</i> subsp. <i>rosea</i>	Wood JRI Y/75/1002 (BM)	<i>violacea</i>	Swann WR10 (BM)	<i>phelypaea</i> subsp. <i>phelypaea</i>	Royl 156 (B)

4.3 Results

4.3.1 Morphometric analyses

The first three principal components of the PCA (only quantitative characters) demonstrated 99% of the total variation explained by loading factor of each character on the components (Table 4.2 and Appendix 4.2). Graphical results of this analysis are presented in the scatter plot in Figure 4.1, where three groups can be identified: one corresponding to *C. mauritanica* and its morphologically sister species, one to *C. sinensis*, and one comprising all other species. Based on the PC1 and PC3 shown, the two early groups are fairly segregated from latter, in which the species remain intermixed (Fig. 4.1). Variables such as “stem length (SL)” and “florescence axis length (FAL)” are the most important in the two first PCs with 98% of total variance. The third PCs with 0.1% of total variance is mostly influenced by variables like “florescence axis diameter (FAD)”, “upper stem scale length (USSL)” and “middle stem scale length (MSSL)” (Appendix 4.2). In all three cluster analyses, two main clades were observed, but their composition differed (Fig. 4.2 and Appendices 4.3-4.4). Cluster analysis using nominal plus quantitative variables discriminates two main clusters A and B, of which cluster A contains three subclusters: I) one including *C. mauritanica* and its sister species; II) one *C. sinensis* monotypic species and; III) the other *C. sects. Cistanchiella* and *Heterocalyx*. The second large cluster B includes *C. sect. Cistanche*, which falls into two relatively geographically distinct subclusters: IV) one containing African, Arabian and Asian species; and V) comprising Nord African and Mediterranean species. Distinct species or morphologically sister groups represented from number 1-12 (Fig. 4.2). Using only nominal characters, one cluster contains *C. sinensis*, *C. sect. Cistanchiella* and *C. mauritanica*, while the second represented exclusively the species of *C. sect. Cistanche* (Appendix 4.3). Using only quantitative data species of cluster *C. mauritanica* and *C. sinensis* placed relatively sister, while the species of *C. sects. Cistanchiella*, *Heterocalyx* and *Cistanche* intermixed (Appendix 4.4).

4.3.2 Taxonomic revision

Species distribution, taxonomic keys and treatments are discussed in detail (Figs 4.3-4.31)

Table 4.2 Fifty morphological characters, their codes and ranks of 137 samples of *Cistanche* species worldwide.

Character	Abbreviation	Type	States
stem Length including inflorescence	SL	continuous	mm
lower stem scale length	LSSL	continuous	mm
lower stem scale wide at the middle	LSSW	continuous	mm
middle stem scale length	MSSL	continuous	mm
middle stem scale wide at the middle	MSSW	continuous	mm
upper stem scale length	USSL	continuous	mm
upper stem scale wide at the middle	USSW	continuous	mm
florescence axis length	FAL	continuous	mm
florescence axis diameter at the middle	FAD	continuous	mm
bract Length	BL	continuous	mm
bract wide at the middle	BW	continuous	mm
bracteole Length	BrL	continuous	mm
bracteole wide at the middle	BrW	continuous	mm
bracteole number	BN	continuous	mm
calyx Length	CaL	continuous	mm
calyx lobe length	CaLL	continuous	mm
calyx lobe wide	CLW	continuous	mm
corolla Length	CrL	continuous	mm
corolla lobe wide	CrLW	continuous	mm
anther length	AL	continuous	mm
stem height	SH	nominal	long (0), medium (1), slightly short (2)
stem diameter	SD	nominal	very thick (0), commonly slender (1), medium (2)
stem hair	SH	binary asymmetric	glabrous (0), sparsely tomentose at inflorescence axis and its underneath (1)
scale color	SC	nominal	greyish (0), light-purple (1), crimson (2), rose-pinkish (3), yellow to light-brownish (4), white-yellowish (5), brownish (6), dark-brown (7), light-yellow becoming dark-brown at apex and scarious at the margin (8)
lower scale shape	LSSh	nominal	short and broad rhomboid (0), lanceolate (1), ovate-triangle (2), linear-oblong (3), Ovate-oblong (4), long deltoid rarely hastate at base (5), ovate (6), deltoid (7)
scale margin	SM	nominal	non-scarious (0), scarious (1), sinuate (2)
lower scale density	LSD	nominal	dense (0), very dense (1), lax (2)
scale apex shape	SASh	nominal	obtuse (0), sub-acute (1), rounded (2), acute (3)
scale hair	SH	nominal	grabrous (0), underneath inflorescence scales tomentose-lanate abaxially, dense at the margin (1), underneath inflorescence scales dense arachnoid-pilose abaxially (2), underneath inflorescence scales tomentose-lanate marginally (3), middle and upper scales with long and white hairs (4)
upper scale density	USD	nominal	lax (0), dense (1), very dense (2), often arranged on the nodes (3)
pedicel at lower part of stem	P	nominal	absent (0), present at lower part (1)

middle and upper scale shape	MUSSh	nominal	lanceolate simetimes oblong-lanceolate (0),ovate-lanceolate (1), keel-shaped (2), long linner-oblong to lanceolate (3), ovate-oblong (4), broad-ovate (5), broad at its base and long lanceolate upper (6), broad-rhomboid (7), broad keel-shaped (8)
florescence shape	FSh	nominal	ovate-oblong (0), ovate (1), oblong (2)
florescence density	FD	nominal	lax (0), dense (1), very dense (2)
bract shape	BSh	nominal	ovate rarely oblong (0), broad-ovate (1), long oblong-lanceolate (2) ovate-lanceolate (3), broad at its base becoming long lanceolate upper (4), keel-shaped (5), obovate-oblong (6), short-rhomboid (7), oblong-lanceolate (8), broad rhomboid (9), broad keel-shaped (10)
bract apex shape	BASh	nominal	acute (0), obtuse (1), round (2)
bract margin shape	BMSH	nominal	roughly entire (0), irregularly highly serrate (1), slightly serrate and sinuate (2), sinuate (3)
bract margin tissue	BMT	nominal	non scarious (0), highly scarious (1), slightly scarious (2)
bract hair	BH	nominal	glabrous (0), abaxially arachnoid-lanuginose (1), marginally lanuginose (2), abaxially and specifically at the margin with long arachnoid hairs (3), at the margin very dense and abaxially sparse hairs (4)
bract color when young	BC	nominal	greyish (0), light purple (1), crimson (2), Pinkish (3), light-yellow (4), light-brown (5), dark-brown (6), brownish (7), light-yellow becoming dark-brown at scarious margin (8)
bract / calyx length ratio	BCLrat	nominal	c. equal as calyx (0), c. 1-4 mm longer than calyx (1), c. 4-9 mm exceeding than calyx (2), c. as twice or longer than calyx (3), c. 2-5 mm shorter than calyx (4)
bracteole shape	BrSh	nominal	oblong-lanceolate rarely ovate (0), lanceolate-linner (1), linear-oblong (2), ovate-lanceolate (3), bracteole absent (4), obovate-spatulate (5), obolanceolate (6)
bracteole apex shape	BrASh	binary symmetric	acute (0), obtuse (1), round (2), acute or obtuse (3), acuminate (4), bracteole absent (5)
bracteole number	BrN	nominal	2 bracteoles (0), one bracteole or in some flowers without (1), none (2)
bracteole hair	BrH	nominal	glabrous (0), marginally lanuginose particularly at 2/3 upper (1), abaxially lanuginose with long and very dense marginally hairs (2), bracteole absent (3), slightly villose marginally or without at 2/3 upper (4), abaxially and specifically at the margin with long arachnoid hairs (5)
bracteole margin	BM	nominal	non-scarious (0), slightly scarious and serrate (1), highly scarious (2), bracteole absent (3)
bracteole color when young	BrC	nominal	light-greyish (0), light-purple (1), Pinkish (2), light-yellow (3), dark-brown (4), brownish (5), light-brown (6), bracteole absent (7), crimson (8)
bracteole / calyx length ratio	BrCLrat	nominal	slightly shorter than calyx (0), equal or slightly shorter than calyx (1), 0 or 1 (2), half or shorter than calyx (3), equal or slightly longer than calyx (4), bracteole absent (5)
calyx shape	CSh	nominal	campanulate (0), tubular (1), both (2)
calyx hair	CaH	nominal	glabrous (0), abaxially with long and dense arachnoid-lanuginose (1), marginally lanuginose-pilose (2), in half upper part of calyx with long and not dense hairs abaxially and marginally (3), lobe sparsely hairy at upper margin (4), in c. half upper with dense and long hairs marginally and sparesely at backside (5), marginally with long lanuginose hairs (6), long silky hairs expanded down the calyx at joint line of calyx lobes (7), sparse hairs along the calyx abaxially expanded, in margin ciliate only (8)
calyx lobe number	CaLN	nominal	5 roughly equal (0), 2 round longer plus 2 round shorter slightly and single short acute dentate, deeply incised (1), 4 equal plus one slightly shorter (2), 4 acute equal plus one acute dentate (3), 5 equal obtuse lobes, 2 deeply incised (4), 2 broad-longer (ovate-shape) plus 3 slightly shorter, all 5 rounded (5), 4 ovate-oblong equal plus one dentate (6), 3 equal slightly longer than 2 (7), 5 acute or rounded often equal of which 2 slightly longer placed alternatively (8), 5 equal acuminate (9), 4 equal (10), 5 equal each divided into 2 acuminate parts (11)
calyx lobe shape	CaLSh	binary symmetric	roughly actinomorphic (0), zygomorph (1)
calyx lobe margin	CaLM	nominal	non-scarious (0), slightly scarious (1), broadly scarious (2), entire lobes scarious (3)

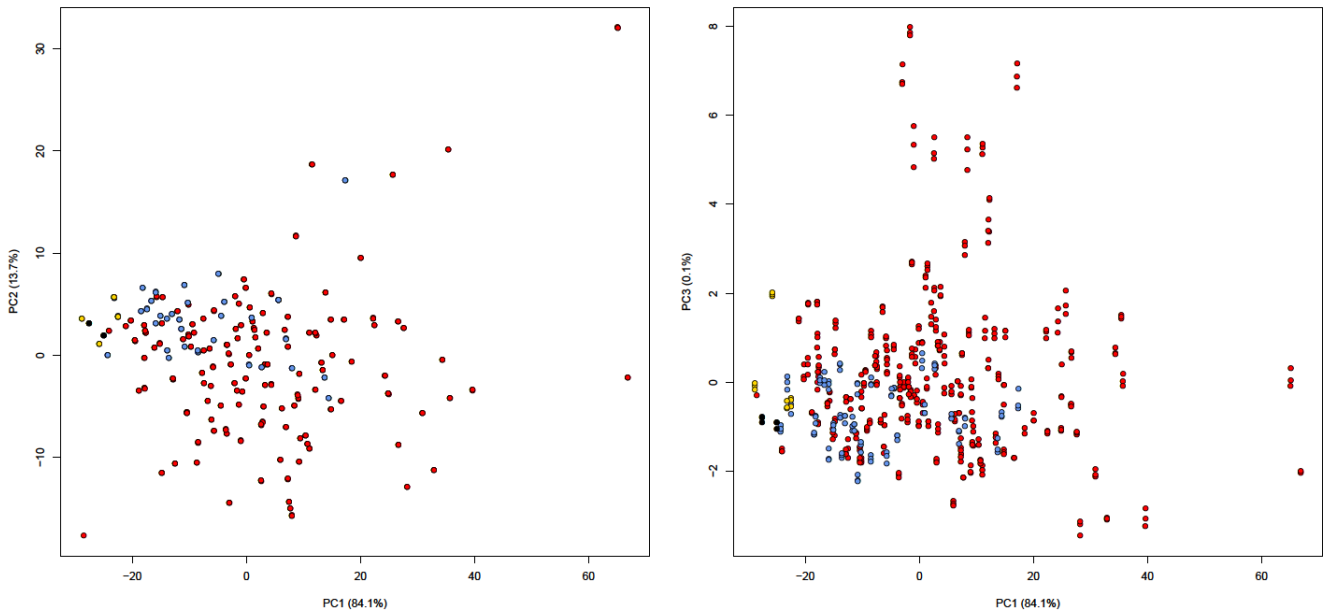


Figure 4.1 Scatter diagram of first against second shape PCs of principle component analysis (PCA) based on 20 quantitative variables. The variance explained by each shape PC is given in parentheses. The plot included 25 plant species of genus *Cistanche* belong to four sections and referred as groups A and B (similar to Fig. 4.2): group A-I indicating e.g. *C. mauritanica* (yellow circle), group A-II indicating *C. sinensis* (black circle), group A-III indicating sects. *Cistanchiella* and *Heterocalyx* (blue circle), and group B indicating sect. *Cistanche* (red circle).

4.4 Discussions

Extensive morphological and taxonomical assessments in our data revealed *Cistanche* with 25 species in two subgenera *C. subg. Subcistanche* and *C. subg. Cistanche*, of which the latter contained, three sections including *C. sect. Heterocalyx* Beck, *C. sect. Macrocalycinum nov.* and *C. sect. Cistanche* Beck. Based upon our results we merged *C. salsa*, *C. ambigua*, formerly classified in *C. sect. Cistanche*, into *C. sect. Heterocalyx*, *C. mauritanica* into *C. sect. Macrocalycinum* and maintained the majority of species in *C. sect. Cistanche*. *C. rosea* and *C. fissa* formerly defined by Beck-Mannagetta (1930) were excluded from *C. sect. Heterocalyx* and merged into *C. sects. Cistanche* and *Heterocalyx*, respectively. Already defined *C. tubulosa* from China is synonymized into *C. laxiflora* subsp. *laxiflora* based upon involving many GenBank accessions in our ITS data matrix (chapter 2, Fig. 2.1).

Table 4.3 The floral morphological characters of four major clades corresponding to traditionally recognized infrageneric in *Cistanche*. Drawings are belonging to *C. sinensis*, sole representative of subgen. *Subcistanche*; *C. brunneri*, a representative of Sect. *Cistanche*; *C. ambigua*, a representative of sect. *Heterocalyx* and; *C. macrocalycinum*, a representative of sect. *Macrocalycinum* of which three latter included in subgen. *Cistanche*.




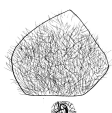














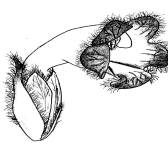

	Subgen. <i>Subcistanche</i>	Subgen. <i>Cistanche</i>		
	<i>C. sinensis</i>	Sect. <i>Cistanche</i>	Sect. <i>Heterocalyx</i>	Sect. <i>Macrocalycinum</i>
		<i>C. brunneri</i>	<i>C. ambigua</i>	<i>C. macrocalycinum</i>
Bract				
Bracteole				
Calyx				
corolla				
flower				

Table 4.4 Comparison of different diagnostic characters of *C. salsa* in different geographical areas.

Location	Diagnostic characters
Armenia	Lower scales sparsely pilose , uppers sparse, middle and upper scales with long and white densely lanuginose abaxially or marginally, entire and non-scarious at the margins; Inflorescence oblong spike; bract and bracteoles marginally with dense, white and long lanuginose hairs; calyx sparsely abaxially pilose and marginally ciliate; corolla lobes glabrous
Turkmanistan	Upper scale dense; inflorescence short-ovate spike; bract short-ovate-oblong; calyx lobes densely pilose marginally and abaxially; corolla lobes sparsely marginally pilose or entirely glabrous and sparsely pilose along the 2 yellow folds inside the tube; in half upper part of calyx long and not dense hairs abaxially and marginally
Turkey	Slightly scarious at the margins and sparsely distributed lower and upper scales, middle and upper scales with very long and white hairs; inflorescence oblong spike; bracteoles are abaxially sparsely and marginally densely pilose; corolla lobes glabrous; calyx lobe glabrous
Kazakhstan	Bract short-ovate in west country; calyx lobes densely pilose marginally and abaxially; corolla lobes sparsely marginally pilose or entirely glabrous and sparsely pilose along the 2 yellow folds inside the tube; bracts ovate-rhomboid and dorsally pilose; glabrous calyx, lobe very sparse hairy at upper margin (calyx), very sparse hair at the margin of corolla lobe
China	Upper scale dense, at lobes very sparsely hairy at upper lobe margin calyx, very sparse hairs at the margin of corolla lobe
Azerbaijan	Calyx hair glabrous with very long silky hairs expanded down the calyx at joint line of calyx lobes
Kirkystan	Calyx lobe sparsely hairy at upper margin, sparse hair at the margin of corolla lobe

According to morphological assessments vegetative characters (such as size and number are of limited use and usually characterize single species or species groups). Floral characters are more important, which includes colour, shape and indumentum of bracts, features of the bracteoles, calyx structure (especially calyx lobes), flower colour and anther shape and indumentum (Table 4.3 for comparison). Ovary and fruit characterize, however, of limited taxonomic value. Delimitation of species and subspecies takes also molecular phylogenetic and geographical data into account (chapter 2, Fig. 2.1). The species of *Cistanche* are usually non-endemic in certain area except a few new species recently introduced (this study). *Cistanche* species are generally parasite on the desert shrubs like Amaranthaceae, Chenopodiaceae, Polygonaceae and etc., and generally wide host-range is prevailed. In spite of relatively narrow distribution for the species in small sections, *C.* sect. *Cistanche* represents rather wider distributions for some species. Cluster analysis based on general similarity using nominal plus quantitative characters, did group distinct sections of *C.* subg. *Cistanche* (subclusters I and III in cluster A, and subclusters IV and V in cluster B), largely in agreement with phylogeny. *C. sinensis* (subcluster A-II) is morphometrically not as distinct as it is in molecular phylogenetic (Fig. 4.2 here; chapter 2, Fig. 2.1). Altogether, cluster analysis using quantitative plus nominal or nominal characters, resulted in a better infrageneric grouping in two distinct clusters than quantitative characters (Fig. 4.2, Appendices 3.4-4.4). Morphometrically, species relationships are mainly in consistent with phylogenetic grouping (numbers 1-12). Among them for instance, *C. rosea* shows not monophyletically distinct cluster as it is in molecular phylogenetic (Fig. 4.2 here and Fig. 2.1 chapter 2). Presumably due to a large overlapping of the quantitative variables in *Cistanche* among individuals of each species and for some extent the different species (herbarium observations, this work), PCA in consistent with cluster analysis using quantitative characters, is unable to infer the species relationships. Therefore, slight segregation has been made for *C.* subg. *Subcistanche* and *C.* sect. *Macrocalycinum* but generally failed to distinguish the infrageneric level of *Cistanche* and morphometrical grouping in *C.* sects. *Heterocalyx* and *Cistanche* (Fig. 4.1 and Appendix 4.4).

4.4.1 Taxonomic treatment and species descriptions

Cistanche Hoffmanns. & Link, Fl. Portug. [Hoffmannsegg] 1: 318, t. 63 (1813).

Type species (lectotype selected here): *Cistanche lutea* (Desf.) Hoffmanns. & Link

≡ *Phelypaea* sect. *Cistanche* (Hoffmanns. & Link) Walp., Repert. Bot. Syst. 3: 461 (1844) ≡ Reut., DC. Prodr. 11: 11 (1847), nom. illeg. ≡ Ledeb., Fl. Ross. 3: 314 (1847-1849), nom. illeg.

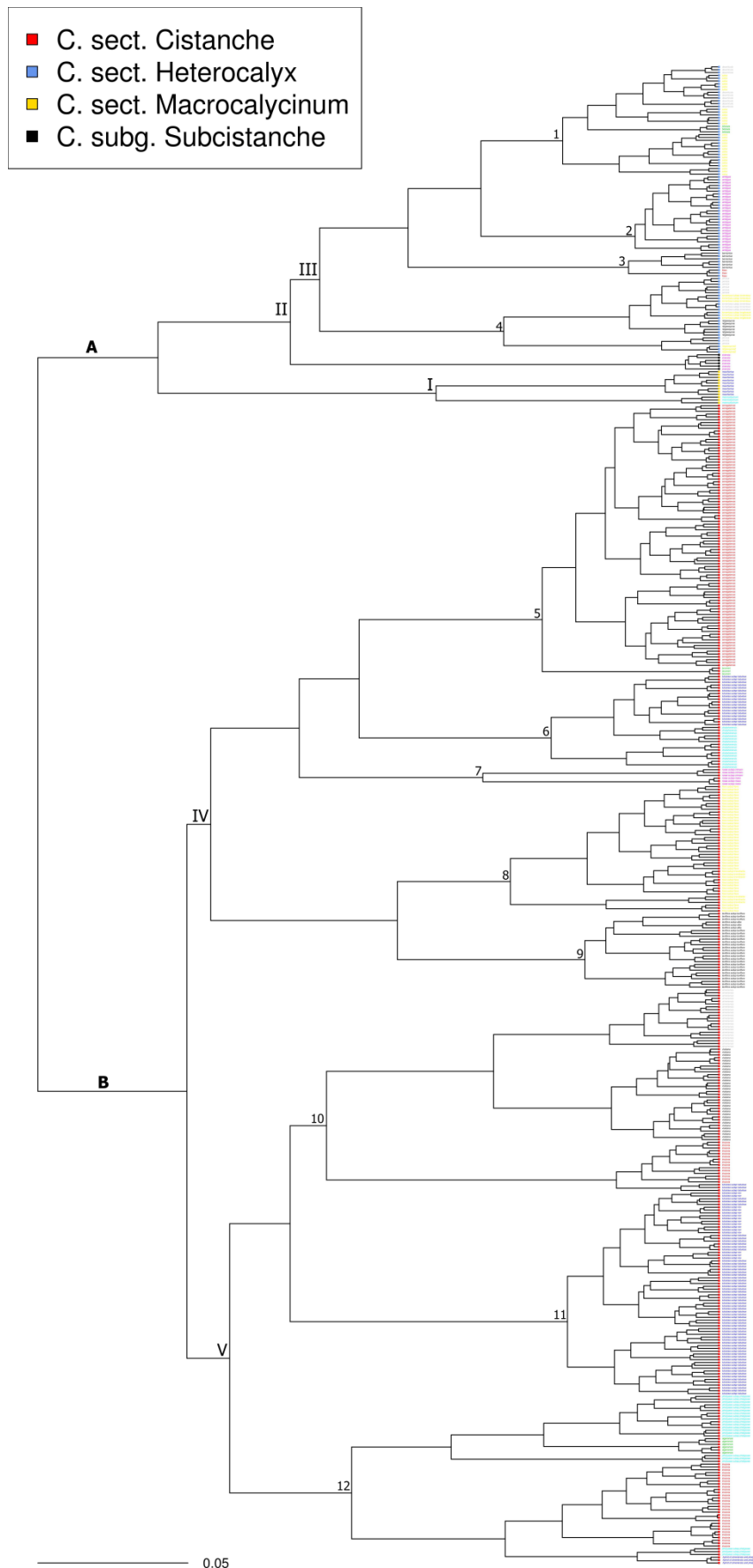


Figure 4.2 Dendrogram of hierarchical cluster analysis using the complete linkage method in a matrix using nominal plus quantitative qualitative characters. Dendrogram included 25 plant species of genus *Cistanche*. The figure legend and taxon names indicate new taxonomical ranks used in this study.

Plant perennial (rarely annual, at least in cultivation), herbaceous, holoparasitic, c. 10–150 cm high. **Stem** often swollen towards the base, fleshy, slender to thick, light yellow to grey becoming brown when old, rarely reddish to crimson, c. 7–70 cm long. **Leaves** scale-shaped, yellow-brown-grayish, light to dark-grey, dark brown-blackish, rarely reddish and white, mainly imbricate particularly at base, lower scales ovate-triangular to deltoid or short auriculate, upper ones usually dense rarely lax, oblong-ovate, ovate-lanceolate, long linear or naviculate, all scales obtuse (acuminate scales reported for *C. brunneri* by Beck-Mannagetta could not be confirmed), purple, yellow-brown blackish, glabrous or marginally on the outer side hairy, usually with scarious margins. **Inflorescence** spike rarely raceme with short pedicel, oblong-ovate, c. 4–45 cm long, with many densely packed flowers; **floral bracts** light yellow to grey or dark brown, rarely reddish or crimson, lanceolate, long linear lanceolate to naviculate, obtuse, glabrous or marginally on the outer side hairy, usually with scarious margins; **floral bracteoles** two (0–1, rarely 2, in *C. ridgewayana*), light yellow, slightly greyish to dark brown, narrowly lanceolate, linear, rarely oblanceolate, glabrous or marginally/on the outer side hairy, usually non-scarious at the margin, mainly obtuse; **flowers** 4.5–43 cm long, with heterochlamydeous perianth, zygomorphic, with different colour; **calyx** tubular or campanulate, pentamerous with equal to mostly sub-equal except in one species with 6-subequal lobes; **calyx lobes** obtuse, rarely acute or acuminate, lobes fused nearly over the entire length or with unequal lobes with the posterior lobe being only basally fused with the other four, nearly completely fused lobes, glabrous or hairy at the margins or on the outer side, entire or scarious at the margins; **corolla** pentamerous apart one gamosepalous, usually numerous; **corolla lobes** with nearly 5-equal lobes except in one species with 6-equal lobes, lobes usually of different color than the corolla tube, rarely marginally or on the outer side hairy, obtuse; **stamens** four, inserted usually at 1/3 of the corolla tube, rarely higher, at base often hairy; **anthers** glabrous, tomentose or pilose to various degrees, tip obtuse, acute, mucronate, aristate, apiculate or acuminate; **stigma** clearly two-lobed to club-shaped. **Capsule** opening with two, rarely three valves. **Seeds** numerous, usually 0.35–0.6 (–1) × 0.35–0.7 (–0.9) mm (Shahi Shavvon & Saeidi Mehrvarz, 2010), ovate-oblong, rarely oblique, light to dark-brown, glabrous, fine or thick.

Distribution: *Cistanche* is widely distributed in the Old World, from the Macaronesian Islands and the Mediterranean via northern and northeastern Africa and southwest Asia to central and Eastern Asia (China and Mongolia).

Remarks: As species descriptions used here may differ substantially from those in previous taxonomic works, we used only our own quantitative measurements for taxonomic description.

Cistanche species are classified taxonomically to two subgenera *C.* subg. *Cistnche* and *C.* subg. *Subcistanchiella* are listed here.

Key to subgenera and sections of *Cistanche*

1. Calyx with five equal or sub-equal lobes (1. **C. subg. *Cistanche***) 2
- Calyx with four equal lobes — Bracts, bracteoles and calyx on the outer side hairy; calyx lobes lanceolate, acute, often at the margin, rarely on the outer side arachnoid-villous; corolla lobes royal blue with light-blue spots dispersedly along on inner side of the tube
.....2. **C. subg. *Subcistanche*** (Beck) Ataei
2. Stem, inflorescence axis, bracts and calyx entirely glabrous 1.1. **C. sect. *Cistanche***
- Stem, inflorescence axis, bracts at least partially hairy 3
3. Bracts, bracteoles and calyx only partially pubescent, woolly or lanuginose; bract usually longer than wide — calyx always less than ½ the length of corolla 1.2. **C. sect. *Heterocalyx*** Beck
- Bracts, bracteoles and calyx densely covered with long arachnoid-lanuginose hairs; bract usually as long as wide 1.3. **C. sect. *Macrocalycinum*** Ataei

1. *Cistanche* subg. *Cistanche*

1.1. *Cistanche* sect. *Cistanche*

≡ *Cistanche* sect. *Eucistanche* Beck, Monogr. Orobanche 57 (1890).

Type species: Cistanche lutea (Desf.) Hoffmanns. & Link

Plant thick and long with fully glabrous stem; **lower scales** generally very dense, imbricate, often ovate-triangular, obtuse; **middle** and **upper scales** usually lax, rarely dense, oblong-lanceolate, obovate, oblong-linear or ovate, mainly obtuse. **Inflorescence** cylindrical, oblong, ovate-oblong spike; **floral bracts** long oblong-lanceolate, oblong-linear, broad-naviculate, ovate-lanceolate and obovate-oblong, usually obtuse, usually entire, margins non-scarious or scarious; **floral bracteoles** oblong-linear, naviculate, lanceolate, rarely linear usually obtuse, entire, margins usually non-scarious, rarely scarious; **calyx** tubular campanulate, with 5 equal to sub-equal lobes, rarely with 6 unequal lobes; **calyx lobes** rounded, obtuse to acuminate, rarely scarious at the margins; **corolla** often pentamerous, infundibuliform, corolla tube cream-colored, pale-yellow, pink-reddish, white and light-pinkish with or without 2 yellow folds or with 2 yellow veins on the inside; **corolla lobes** of varying colour; **stamens** inserted at lower rarely middle part of corolla; **anther** apiculate, acute, obtuse or sub-obtuse, apiculate rarely mucronate and acute at apex, densely tomentose rarely entirely glabrous. **Capsule** always opening with 2 valves. **Seeds** tiny, numerous, small triangular, oblique or circular.

Distribution: *Cistanche* sect. *Cistanche* contains 13 species distributed from the Macaronesian Islands and the Mediterranean via northern and northeastern Africa, Southwest Asia, China and Mongolia (Fig. 4.3).

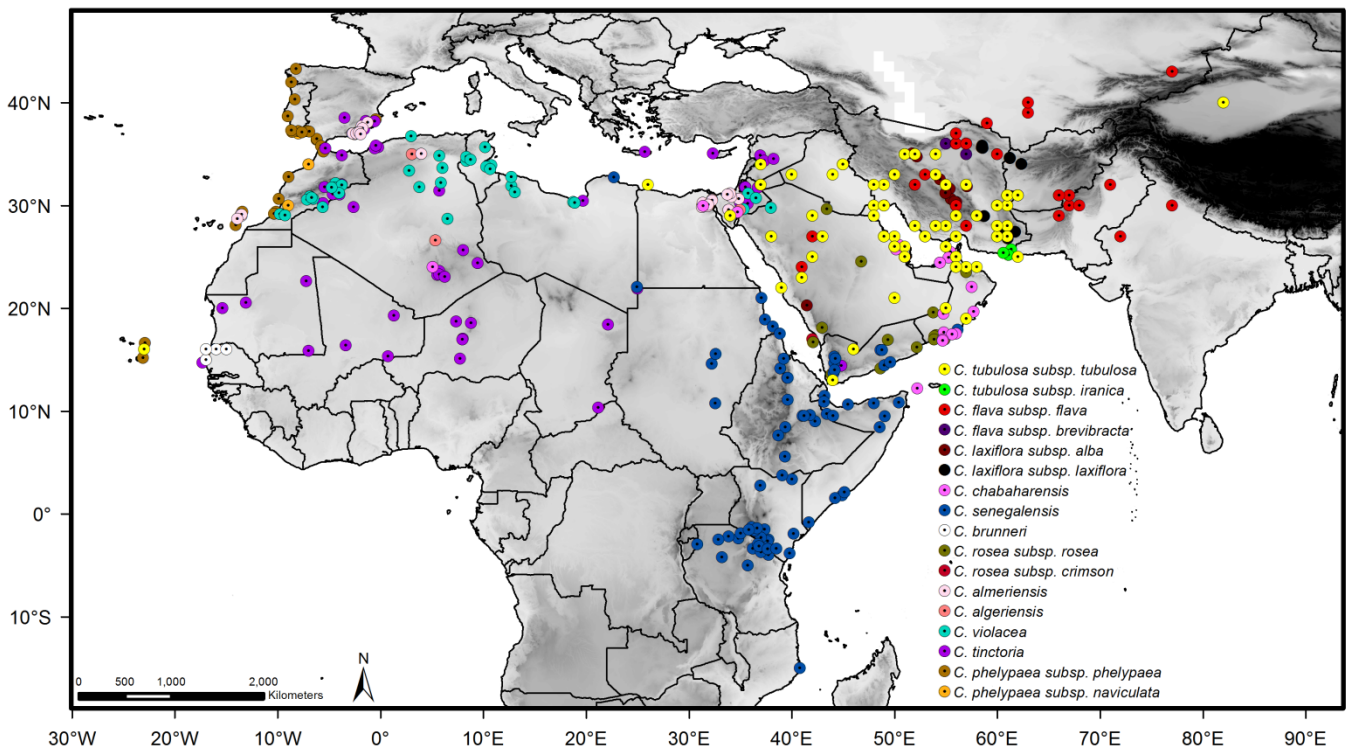


Figure 4.3 Distribution map of species of *Cistanche* sect. *Cistanche* worldwide. One species (*C. somaliensis* Ataei, *sp. nov.*) with single distribution point was not presented here.

Key to the species and subspecies of *Cistanche* sect. *Cistanche*

1. Calyx and corolla with 5 equal to sub-equal lobes; stamens inserted below the middle of the corolla tube; filaments and style usually shorter than or as long as (style rarely longer than) the corolla, stigma rarely exerted 2
 - Calyx with 6 unequal lobes and corolla with 6 equal lobes; stamens inserted at the middle of the corolla tube; filaments and style exerted. Somaliland **1.1.13 *Cistanche somaliensis***
2. Scales and bracts keel-shaped, amplexicaule, irregularly and deeply crenulate at the margins; calyx with 5 equal lobes. — corolla yellow (**1.1.1 *C. phelypaea***) 3
 - Scales and bracts not keel-shaped (ovate, oblong-lanceolate or oblong-linear), not-amplexicaule, sinuate, scarious or non-scarious at the margins; calyx with 5 equal or subequal lobes 4
3. Scales and bracts not broadly keel-shaped, bracteoles narrowly obovate-spathulate, spike usually shortened, flowers less and medium about 35 mm long. Portugal, Morocco, southern Spain, Egypt **1.1.1.1 *C. phelypaea* subsp. *phelypaea***
 - Scales and bracts broadly keel-shaped, bracteoles broadly obovate-spathulate, spike elongated, flowers many and grand about 42 mm long. Morocco, Portugal **1.1.1.2 *C. phelypaea* subsp. *naviculata***
4. Corolla tube usually yellow, white or light-lilac, corolla lobes yellow or light to dark purple, with or without 2 yellow folds inside the tube; anther at apex mucronate, apiculate or acuminate 5
 - Corolla and its lobes with variable colours, always without 2 yellow folds inside the tube; anther usually at apex sub-obtuse to acute 8

5. Scales and bracts ovate-lanceolate; corolla tube usually of different colour than the corolla lobe, with 2 yellow folds inside the tube 6
 - Scales and bracts ovate-lanceolate or oblong-lanceolate; corolla tube and lobes uniformly yellow ... 7
6. Scales, bracts and bracteoles grey-blackish, entire at their margins; calyx with 5 equal rounded lobes and scarious margins; corolla tube white or pale cream-yellow, corolla lobes tinged violet; anthers at apex long acuminate. Southeastern Spain **1.1.3 *C. almeriensis***
 - Scales, bracts and bracteoles greyish, sinuous at their margins, calyx with 5 unequal rounded lobes and scarious margins; corolla tube pale-lilac, corolla lobes light to dark purple; anthers at apex apiculate rarely mucronate. Algeria, Egypt, Israel, Jordan, Libya, Morocco, Tunisia... **1.1.5 *C. violacea***
7. Scales and bracts greyish, ovate-lanceolate; bracteoles greyish, oblong-lanceolate, bracts and bracteoles with scarious margins; calyx with 5 equal rounded lobes with scarious margins, calyx up to 17 mm long, without conspicuous veins; anthers long apiculate at apex. Algeria, Greece, Spain, Syria **1.1.2 *C. tinctoria***
 - Scales and bracts greyish, long oblong-lanceolate; bracteoles yellowish, oblong-linear; bracts, bracteoles non-scarious, and bract and calyx broadly scarious at their margins; calyx with 5 unequal oblong-rounded lobes, calyx slightly longer up to 21 mm long, with conspicuous veins; anthers acuminate at apex. Algeria **1.1.4 *C. algeriensis***
8. Scales, bracts ovate-oblong rarely obovate-rhomboid; bracteoles linear-oblong or oblanceolate, all pink-reddish or crimson (**1.1.9 *C. rosea***) 9
 - Scales, bracts and bracteoles yellowish or greyish, ovate-lanceolate or oblong-lanceolate 10
9. Scales, bracts, bracteoles, and calyx rose-pinkish, without scarious margins; bracteoles linear-oblong; calyx with 5 unequal lobes (posterior lobe smaller and less fused than remaining lobes); corolla with unequal lobes (upper ones > lateral ones > lower one), sometimes lateral lobes with short dentate segments attached, entirely rose-pinkish; stamens usually as long as the upper corolla lobe; style prominently exerted — ovary ovate. Oman, Saudi Arabia, Yemen **1.1.9.1 *C. rosea* subsp *rosea***
 - Scales, bracts and bracteoles orange, with conspicuous veins, completely scarious; bracteoles obovate-oblong; calyx with 5 equal lobes; corolla tube orange and lobes orange-crimson, like the calyx with conspicuous veins; stamens and style usually as long as the corolla. — Anthers sub-lanate. Saudi Arabia **1.1.9.2 *C. rosea* subsp *crimson***
10. Scales, bracts and bracteoles dark brown when dry; calyx with 5 equal acuminate lobes. — Spike lax; scales and bracts ovate-lanceolate, bracteoles linear-lanceolate; calyx deeply incised with broadly scarious margins; filaments glabrous at base, anthers apiculate at apex, glabrous. Senegal **1.1.12 *C. brunneri***
 - Scales, bracts and bracteoles light yellow, purple or grayish when dry; calyx with 5 unequal lobes 11
11. Corolla at least nearly 3 times as long as calyx. — Scales and bracts ovate-lanceolate, bracteoles lanceolate-linear; calyx with ovate-orbicular lobes, where 2 lateral lobes are longer than 3 others; corolla lobes crenulate; anthers acute to apiculate at apex. West southern Arabian Peninsula, Northeastern Africa Southwards Kenya to Mozambique **1.1.11 *C. senegalensis***
 - Corolla maximally twice as long as calyx 12

12. Bracts at least twice as long as calyx or bracts maximally as long as calyx, all usually light purple, usually without scarious margins (**1.1.8 C. flava**) 13
 - Bracts longer than calyx (maximally 1.5 times as long as calyx), usually without scarious margins .14
13. Scales and bracts oblong-lanceolate, at least twice as long as calyx, bracteoles oblong-linear. — Calyx partially shortly and deeply incised with 3 lobes slightly longer than the 2 others; corolla tube yellow-whitish with 2 or 5 irregular orange veins inside the tube, corolla lobes yellow or blue-violet; anthers usually sub-acute. Afghanistan, Iran **1.1.8.1 C. flava subsp. flava**
 - Scales and bracts ovate-lanceolate, maximally as long as calyx, bracteoles oblong-lanceolate, calyx with 5 equal lobes, corolla tube usually pale yellow with dispersed orange veins inside the tube. — Corolla lobes usually sinuate at the margins. Iran **1.1.8.2 C. flava subsp. brevibracteata**
- 14 Spike usually lax; scales, bracts and bracteoles oblong-lanceolate, entire. — calyx with 3 obtuse lobes longer than two others (**1.1.10. C. laxiflora**) 15
 - Spike usually dense; scales, bracts and bracteoles ovate-lanceolate 16
15. Scales and bracts light-grey; corolla entirely white with 2 orange veins inside the tube. Afghanistan, Iran **1.1.10.1 C. laxiflora subsp. laxiflora**
 - Scales and bracts dark-grey; corolla white with 2 orange veins inside the tube, corolla lobes light purple. Iran, Saudi Arabia **1.1.10.2 C. laxiflora subsp. alba**
16. Scales, bracts and bracteoles strongly sinuate at margins; corolla white-yellow or yellow; filaments densely tomentose at base, anthers obtuse, densely pilose-tomentose (**1.1.6 C. tubulosa**) 17
 - Scales, bracts and bracteoles non-sinuate at the margins; corolla yellow; filaments glabrous at base, anther acute, glabrous. — bracts usually emerged 2–5 mm down the calyx, making the inflorescence a raceme. South Middle East **1.1.7 C. chabaharensis**
17. Calyx with 4 equal entire and one dentate lobe, these without scarious margins; corolla white-yellow. — lacerate at the margins. Middle East **1.1.6.1 C. tubulosa subsp. tubulosa**
 - Calyx with 4 unequal (two lobes are longer) entire and one dentate lobes, these are slightly scarious at their margins; corolla entirely dark-yellow. — with orange veins inside the tube. South Iran
 **1.1.6.2 C. tubulosa subsp. iranica**

1.1.1.1 Cistanche phelypaea (L.) Cout. subsp. **phelypaea** Cout., Fl. Portugal 571 (1913).

≡ *Lathraea phelypaea* L., Sp. Pl. 2: 606 (1753) [basionym] ≡ *Orobanche phelypaea* (L.) Willd., Sp. pl., ed. 4, 3 (1): 352. (1800).

≡ *Phelypaea lusitanica* Coss., Notes Pl. Crit. 43 (1849) ≡ *Cistanche lusitanica* (Coss.) Guim., Brotéria 3: 190 (1904) ≡ *Cistanche tinctoria* f. *lusitanica* (Coss.) Beck, Pflanzenr. (Engler) 96, 4.261: 31 (1930).

= *Cistanche lusitanica* var. *grandiflora* Freyn ex Beck, Pflanzenr. (Engler) 96, 4.261: 31 (1930), nom. inval. in syn.

Type: Orobanche elegantissima verna, flores luteo virid. Lusit. Grisley (P-Tournefort n° 6443, barcode P00680331 seen as photo, neotype selected by Foley, 2001: 229).

Plant usually slender, 20–28 (–45) cm high. **Lower scales** very dense, imbricate, short deltoid, sub-acute, 5–7 × 5–7 mm, fleshy, amplexicaule, entire, sub-scarious at the margins, light yellow; **middle scales** dense, moderately imbricate, keel-shaped, sub-acute, 9–11 (–15) × 6–7 (–10) mm, irregularly crenulate and scarious at the margins, light yellow, becoming dark brown only at the margins at early age; **upper scales** slightly lax, keel-shaped, sub-acute, 12–18 (–27) × 8–12 (–15) mm, fleshy, amplexicaule, irregularly crenulate and scarious at the margins. **Inflorescence** a short oblong-conic spike 10–15 (–20) cm long and 5–7 cm in diameter, lax; **floral bracts** keel-shaped, obtuse, 13–16 (–23) × (7–) 11–15 (–17) mm, fleshy, amplexicaule, irregularly deeply crenulate and scarious at the margins, equal to or slightly exceeding the calyx, light yellow becoming dark brown particularly at the apex; **bracteoles** obovate-spathulate, obtuse, 8–15 (–17) × (2.5–) 4–6 (–8) mm, irregularly crenulate and scarious at the margins, slightly shorter than the calyx, light yellow; **calyx** usually tubular, pentamerous, (9–) 11–15 (–26) mm long, shortly incised, slightly longer than ½ total corolla length; **calyx lobes** 5, equal, orbicular rounded, 3–5 (–7) × 3–5 (–8) mm, non-scarious at the margins; **corolla** campanulate, pentamerous, 35–55 mm long, yellow on outer and inner side; **corolla lobes** 5, equal, rounded, (3–) 5–7 × 6–8 mm; **stamens** usually inserted shortly above the base up to 1/6 of the total corolla length, filaments 2/3 as long as the corolla, at base densely villous; **anthers** 4–5 mm long, acuminate, strongly pilose; **ovary** broad-ovate, dark brown when dry; **style** non-exserted, **stigma** bilobate, deep yellow. **Capsules** dehiscent with 2-valves. **Seeds** usually elliptic (Fig. 4.4).

Distribution: Abundantly in hot-humid Mediterranean along the Atlantic coastlines of Portugal, Spain, and Morocco, the Cape Verde and the Canary Islands; rarely along the Mediterranean coast of Eastern Spain and in Egypt (Fig. 4.3).

Additional specimens examined: **EGYPT.** *Olivier* s.n. (P barcode P02970619). **MOROCCO.** **Desembocadura Oued Noun:** SW coastline, 29RLC6324, in Dunes, 10 m, 7 April 2007, *Buira & Calva* s.n. (MA); **Doukkala-Abda:** Between Safi and Mazagan, April 1939, *L'Hermite* s.n. (P); **Souss-Massa-Daraâ:** Agadir, 30°39'00"N; 09°53'07"W, 51 m, 11 March 2010, *Quandt* ED405 (BONN); Coast line in Tiznit, Nord Sidi Ifni, 29°23'33"N, 10°10'10"W, 85 m, 17 March 2010, *Quandt* ED404 (BONN); **Tanger-Tétouan:** Tanger, *Salzmann* s.n. (P); Tangier-Tetouan, s. col. s.n. (E barcode E00029752). **PORTUGAL. Algarve:** Lagos, 25 May 1938, *Rothmaler* 13380 (B); Portimão, Alvor, 2 April 1985, s. col. 17156 (BR); **Baixo Alentejo:** Volanova de Milfontes, orilla S de la ria, c. del Punete, 29SNB2075, 0-50 m, 11 June 1988, *Gramendia & Pedrol* 3429 JP (MA); **Tavira:** Cabanas Island, salt marsh area, Sea level, 29 March 2006, *Foley* 2202 (E); **Santa Luzia:** 25 September 1994, *Pollecot* 29P (P). **Cape Verde. Coimbra:** Ponte da Mata, June 1892, *Daveau* 3082 (P); **Maio:** Terras Salgadas Salinas and dune belt N or Morrinho, 4 March 1994, *Kilian & Leyens* 3030 (B); **Sal:** SW Santa Maria, in the lighthouse beach, 3 March 1998, *Royl* 975 (B); **Santarém:** Valley Zebro, April/May 1849, *Winkler* s.n. (P). **SPAIN. Alicante:** Heriso de la route pres Saliuos del Pinet, 5 m, 10 May 1982, *Charpin & Defferaud* 16557 (MA); **Cádiz:** De l'île San Fernando, 1 March 1849, *Bourgeau* 371 (P); Chiclana de La Frontera, Santi Petri, along the coast line, 36°23'31"N, 06°12'25"W, 60 m, 23 April 2012, *Ataei* ED792 (BONN); San Fernando, March 1849, s. col. s.n. (E); North of Chiclana de la

Frontera, on saline margins of large waterway, Sea level, 12 April 1996, *Foley* 1007 (E). **Galicia:** Betanzos, on saltmarshes of Río Mandeo, 12 April 1975, *Quintana* s.n. (MA); **Huelva:** Cartaya, El Rompido, close to Golf course, 37°13'08"N, 07°07'48"W, 5 m, 23 April 2012, *Ataei* ED793 (BONN); La Rábida, salt marsh, Sea level, 24 March 1968, *Smythies* 252 (E); El Rompido, 37°13'00"N, 07°07'40"W, 12 April 2009, *Calvo & Espejo* JC3504 (MA). **Canary Islands. Las Palmas:** Lanzarote, coast E of Orzola close to El Arco, coastal sand dunes, 29°21'55"N, 13°26'56"W, 0-10 m, 03 April 2002, *Schneeweiss & Weiss-Schneeweiss* 8758 (WU); Fuerteventura, on the beach Jandia, 28°06'08"N, 14°16'19"W, 21 February 1981, *Luck* TF224 (B).

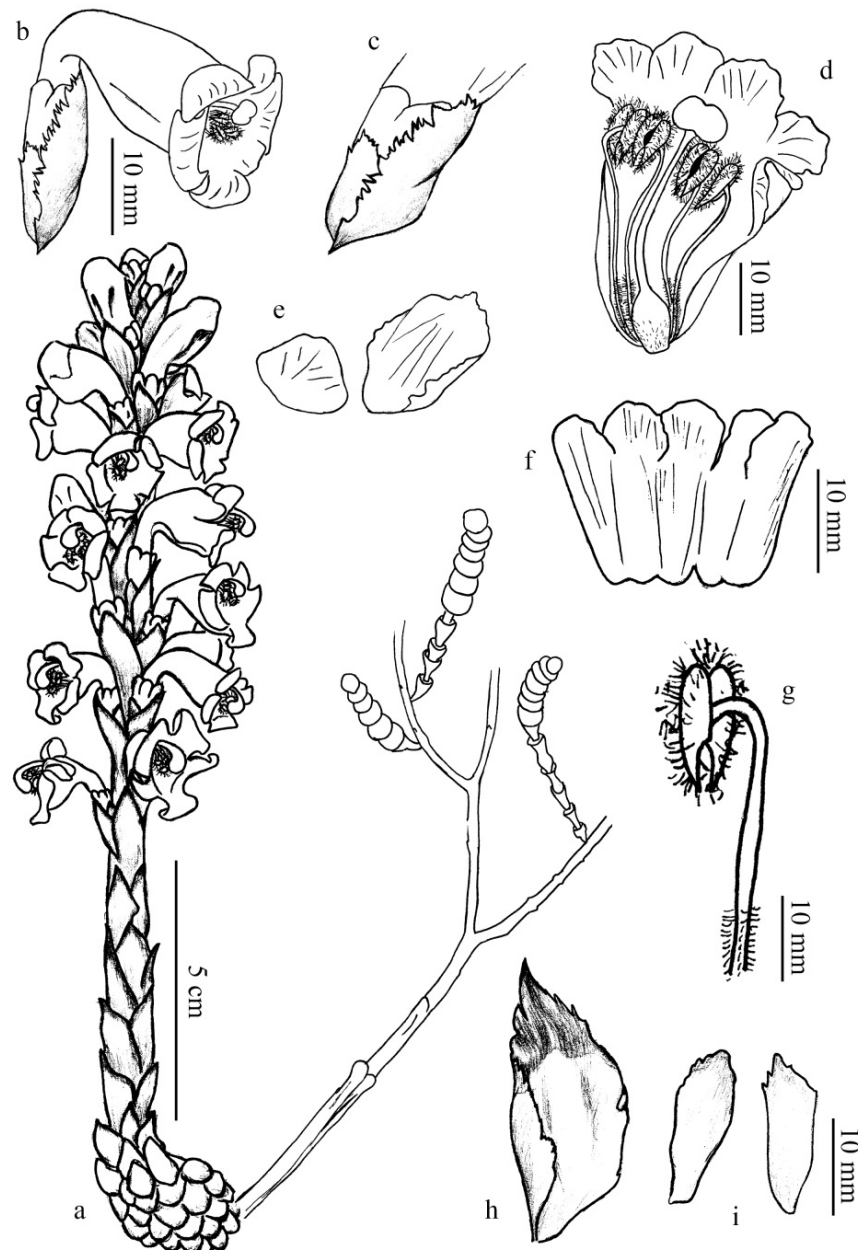


Figure 4.4 *Cistanche phelypaea* (L.) Cout. subsp. *phelypaea* Cout. a) flowering plant and partially its host (*Arthrocnemum macrostachyum* (Moric.) C. Koch), b) single flower, c) bract and one bracteole highly serrate, d) its flower inner view, e) lower and middle scale, f) abaxial calyx, g) stamen, h) bract and i) two bracteoles. The plant occurred in Southern Spain (*Ataei* ED792; BONN).

Host: Amaranthaceae: *Arthrocnemum macrostachyum* Torr. (Ataei ED792, BONN), *Salicornia ramosissima* J.Woods (photo by Sábado at <http://jardin-mundani.blogspot.de/2013/05/cistanche-phelypaea-la-reina-de-la-ria.html>, accessed 18 February 2015), *Suaeda* sp. (Roi 975, B), *Suaeda maritima* (L.) Dumort. (Quintana s.n., MA) and *Atriplex portulacoides* L. (Bourgeau 371, photo at herbarium P); **Tamaricaceae:** *Tamarix senegalensis* DC. (Kilian & Leyens 3030, B).

1.1.1.2 *Cistanche phelypaea* subsp. *naviculata* Ataei, subsp. nov.

Diagnosis: *Cistanche phelypaea* subsp. *naviculata* differs from the nominate subspecies by its very broad upper scale (width/length ratio up to 5/6); long robust stem *versus* slender and shorter, and very broad keel-shaped bract (width/length ratio up to 5/9) *versus* keel-shaped bract; flowers many and long *versus* less and shorter in the other subspecies. The subspecies has been misdetermined as *C. phelypaea*.

Type: MOROCCO. Rabat-Sale-Zemmour-Zaer: Rabat, Marais, Bouregreg, Sea level, 14 March 1980, Lewalle 9232 (holotype BM barcode BM000598617).

Plant robust, up to 37 cm high. **Lower scales** very dense, imbricate, short deltoid, obtuse, 5–7 × 5–8 mm, entire, sub-scarious at the margins; **middle scales** dense, moderately imbricate, broad, keel-shaped, obtuse, (9–) 10–15 × 6–7 (–10) mm, fleshy, broadly amplexicaule, irregularly crenulate and scarious at the margins, yellow; **upper scales** slightly lax and imbricate, broad, keel-shaped, obtuse, 12–19 × (10–) 13–15 mm, fleshy, broadly amplexicaule, irregularly crenulate and scarious at the margins. **Inflorescence** a long-oblong spike 15–20 cm long and 6–6.5 cm in diameter, dense with many flowers; **floral bracts** broad, keel-shaped, obtuse, 13–18 (–20) × 11–15 mm, broadly amplexicaule, irregularly crenulate and scarious at the margins, equal to or slightly longer than calyx, light yellow, becoming dark brown particularly at the apex; **bracteoles** obovate-spathulate, obtuse, (4–) 6–8 × (2.5–) 4–8 mm, irregularly crenulate and scarious at the margins, slightly shorter than calyx, light yellow; **calyx** usually tubular, pentamerous, shortly incised, 14–15 (–23) mm long, slightly longer than ½ total corolla length; **calyx lobes** 5, equal, orbicular rounded, 3–6 × 3–3.5 (–8) mm, non-scarious at their margins; **corolla** campanulate, pentamerous, 41–42 mm long, deep yellow on the outer and inner side; **corolla lobes** 5, equal, rounded, 3–6 × 6–10 mm; **stamens** usually inserted shortly above the base, filaments 2/3 as long as the corolla, at base densely villous; **anthers** 3.5–5 mm long, mucronate, pilose; **ovary** broad-ovate, dark brown when dry; **style** non-exserted, **stigma** bilobate, deep yellow. **Capsules** dehiscent with 2-valves. **Seeds** usually elliptic.

Distribution: Along the Atlantic coasts of Morocco and Portugal (Fig. 4.3).

Additional specimens examined (paratypes): MOROCCO. Rabat-Sale-Zemmour-Zaer: Rabat, WN, Bouregreg, 27 January 1946, *Sauvage & Vindt* n°6956 (P); **Souss-Massa-Draa:** Agadir, between Agadir and Oud Souss, 13 March 1984, *Roi* 156 (B); SW Tamri to Cap Ghir, 50 m, 19

March 1969, *P. & Davis* D.48459 (E, BM). **PORTUGAL. Tavira:** Cabanas, Ilha de Cabanas, 2 April 1997, *Sales & Hedge* 97/9 (E).

Host: Unknown.

1.1.2 *Cistanche tinctoria* (Forssk.) Beck, Bull. Herb. Boissier, ser. 2, 4: 685 (1904).

≡ *Orobanche tinctoria* Forssk., Fl. Aegypt.-Arab. 112 (1775) [basonym] ≡ *Phelypaea tinctoria* (Forssk.) Walp., Repert. Bot. Syst. (Walpers) 3: 462 (1844); Reut., DC. Prodr. 11: 13 (1847).

Type: **YEMEN. Môr:** [February 1763], *Forsskal* s.n. (holotype C barcode C10002702 seen as photo, isotype BM barcode BM000598614 seen as photo).

= *Phelypaea lutea* Desf., Fl. Atlant. 2: 61, t. 146. (1798) ≡ *Cistanche tinctoria* f. *lutea* (Desf.) Beck, Pflanzenr. (Engler) 96, 4.261: 31 (1930) ≡ *Cistanche phelypaea* (L.) Cout. subsp. *lutea* (Desf.) Fern.Casas, Anuário Soc. Brot. 39: 130 (1973). *Type:* **ALGERIA.** “in arenis humentibus ad littora fluminis Elhammah prope Mascar”, *Defontaines* (holotype P barcode P02986164 seen as photo).

= *Orobanche compacta* Viv., Fl. Libyc. Spec. 34. t. 15. f. 2. (1824) ≡ *Phelypaea compacta* (Viv.) G.Don, Gen. Hist. 4: 632 (1837); Walp., Repert. Bot. Syst. (Walpers) 3: 461 (1844) ≡ *Cistanche compacta* (Viv.) Beck, Pflanzenr. (Engler) 96, 4.261: 34 (1930). *Type:* **LIBYA.** “in littore Magnae Syrteos”, *Viviani* (GE, destroyed).

Plant usually robust, 30–65 cm tall. **Lower scales** very dense and imbricate, ovate-triangular, sub-acute, 5–8 (–13) × (2.5–) 4–5 (–12) mm, usually non-scarious at the margins, greyish; **middle and upper scales** lax, ovate-lanceolate, sometimes oblong, obtuse, 14–19 (–33) × 3–5 (–8) mm, scarious at the margins, greyish. **Inflorescence** an oblong spike, rarely lax, 10–25 (–34) cm long and 5–7.5 cm in diameter, with flowers that are slightly curved downwards; **floral bract** ovate-lanceolate, rarely oblong, sub-acute, 14–22 (–26) × 4–7 (–9) mm, irregularly crenulate (especially when dry) and scarious at the margins, as long as calyx or slightly longer, grey; **floral bracteoles** oblong, rarely lanceolate, rounded, 9–14 × 2–4 mm, crenulate or sublaciniate at the margins (when dry), shorter than calyx, grey; **calyx** campanulate-obconical, pentamerous, 12–17 mm long, slightly longer than ½ total corolla length; **calyx lobes** 5, equal, sub-orbicular, rounded, unevenly crenulate and scarious at the margins, slightly imbricate, light grey; **corolla** campanulate, pentamerous, 30–45 mm long, yellow out and inside; **corolla lobes** 5, equal, oblong-rounded, 5–8 × 5–9 mm, yellow; **stamens** inserted at about 1/8 total corolla length, filaments 2/3 as long as the corolla, at base densely pilose; **anthers** 3.5–4.5 mm long, strongly pilose along the sutures of their thecae, rounded at base and apiculate, rarely mucronate at apex; **ovary** ovate, dark brown when dry; **style** non-exserted, **stigma** bilobate, white. **Capsules** dehiscent with 2-valves. **Seeds** elliptic, sub-globose, pitted (Fig. 4.5).

Distribution: Along the Mediterranean coast to the Arabian Peninsula, and North Africa (Fig. 4.3).

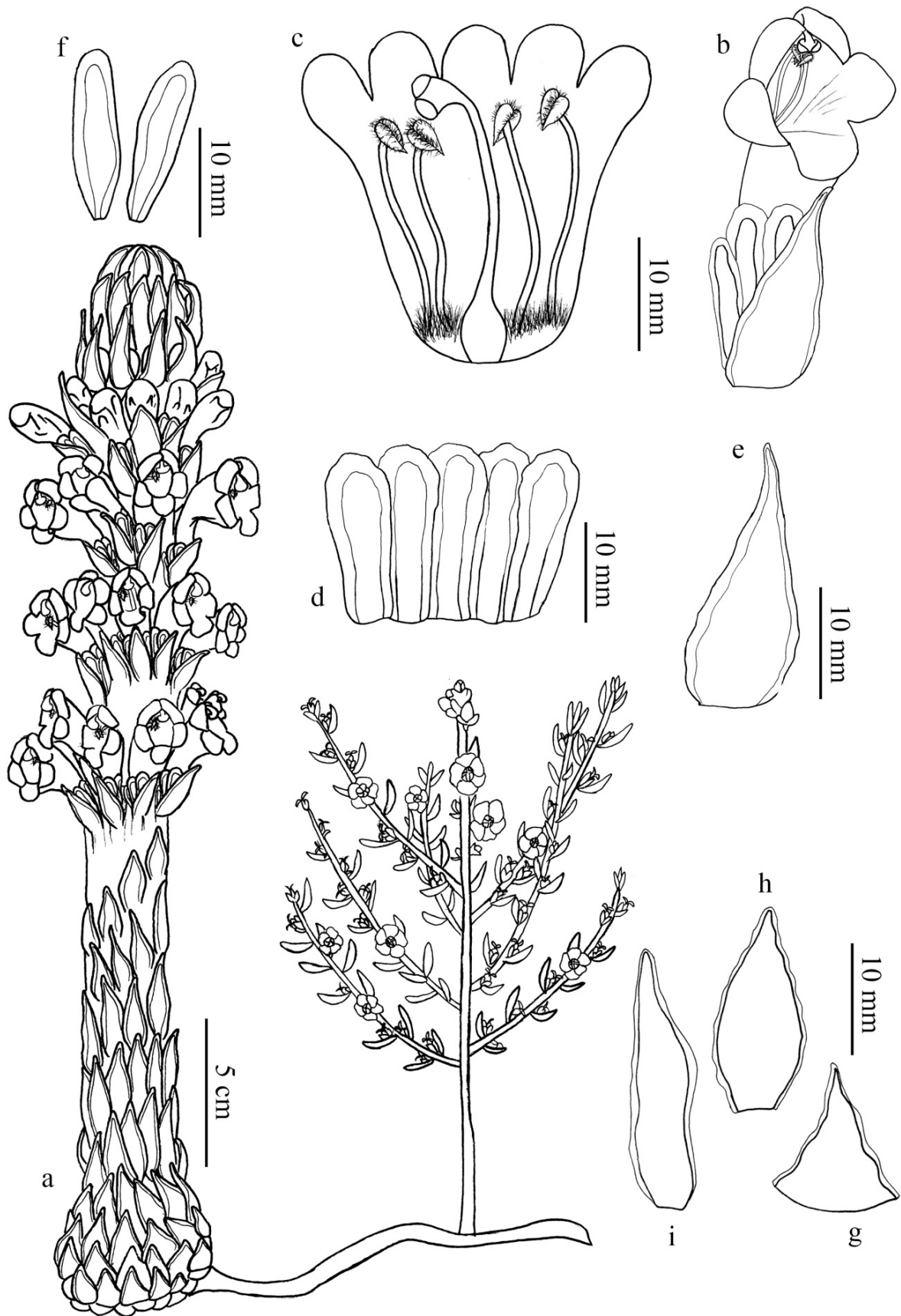


Figure 4.5 *Cistanche tinctoria* (Forssk.) Beck. a) flowering plant and partially its host (*Salsola genistoides* Juss. ex Poir), b) single flower, c) its flower inner view, d) abaxial calyx, e) bract, f) bracteoles, g) lower scales, h) middle, and i) upper scales. The plant occurred in Algeria (Podlech 33665; MSB).

Additional specimens examined: ALGERIA. Illizi: Béchar: Montagnes du Zeramra, E-Abfall der Berge 19 km SW Zeramra, c. 55 km WSW Beni Abbes, 29°51'00"N, 02°38'00"W, 1 April 1980, *Podlech* 33665 (MSB); Fort Polignac in Djanet, Oued Ilezy, 25 February 1934, s. col. s.n. (P); **Oran:** 1849, *Boissier & Reuter* s.n. (E); Falaise de Kristel, 24 April 1842, *Durieu* s.n. (P); Road to Saint Denis, Arzew, near the Gonzales farm, 4 March 1955, *Dubuis* s.n. (P); **Ouargla:** Hassi-Medjira [Mjeira], 1880, *Guyiard* s.n. (P); Tassili N'Ajjer, Tasemdjout, 25°39'00"N, 08°02'00"E, November 1976, *Ottmann* s.n. (M); **Tamanghasset:** Ahaggar, 12 February 1979, *James & al.* 10 (BM); Hirafok, west, sandy Wadi 23°39'00"N, 05°44'00"E, spring 1955, s. col. 32 (E); Hoggar-Massiv, Atakor, S of Ilamane, near the slopes of Assekrem to Outoul, 23°15'00"N, 05°31'00"E, 2290 m, 24 March 1982, *Podlech* 36743 (MSB); Oued Temorte-Haseif, Massif de l'Atakor, 23°18'00"N, 05°42'00"E, February 1976, *Lavie* 841 (P). **CYPRUS. Ayios Yeoryios Island (Akamas):** Coast, on sides of island, Sea level, 13 March 1962, *Dmeikle* 2164 (K). **GREECE. Crete island:** Lasithi, Mikronisi Island, 35°12'09"N, 25°43'55"E, 19 May 1942, *Rechinger* 13113 (K). **ISRAEL. Southern District:** Arava valley, Samar sands, 4 March 1983, *Liston* 123613 (HUJ); Ein Hosob (En-Hazeva), *Zohary* s.n. (HUJ); Dead Sea area, 15 km S Jericho, 13 February 1987, *Musselman* 123624 (HUJ); Dead Sea Valley, near Ormat's solar ponds, 2 km N of the Dead Sea northern coast, 370 m, 22.III.1989, *Danin & al.* s.n. (B); Environment of Southern Dead Sea, 23 March 1954, *Zohary* 123627 (HUJ); Environment of Massada, 7 March 1954, *D'Angelis* 123626 (HUJ); On the edge of Dead Sea, 13 February 1987, *Musselman* 123623 (HUJ); Central Negev, En-Avdat National Park, 19 March 1995, *Cohen* 123658 (HUJ); Western Negev, 10 km NW of Nizzana, Nahal Lavan, 25 March 1986, *Danin* 123612 (HUJ); Southern Negev, Nahal Barag, 25 March 1991, *Ortal* 123610 (HUJ); Between Ramon and Yotvata, 400 m, 26 March 1976, *Breckle* 3810 (W); Edom, el-Hesma, about 15 km N of Queira, about 900 m, 30 March 1930, *Eig, Zohary & Feinbrun* 123670 (HUJ). **JORDAN. Djelfa:** Zarga', Shaumari Wildlife Reserve, c. 7km W Al Azraq al Janūbī, Environment of the Visitor Centre, 31°45'00"N, 36°43'00"E, 13 April 2000, *Schneeweiss & Staudinger* 4224 (WU). **Ma'an:** Jibal al Batra', Wadi Hisman, Charsa Area, 920-950 m, 18 April 2000, *Schneeweiss* 4225 (WU); **LIBYA. Egyptian-Libyan-Sudanese border:** Jebel Uweinat (Abd el Malech), granite ground, 710 m, 12 December 1968, s. col. 4961 (B); **Gulf of Sirte:** E of Marble Arch, 23 March 1970, *Davis & Hadland* 49850 (E, K). **MALI. Kidal region:** Adrar des Iforas, 1988, *Sidiyene* 76 (P); Goundam, 12 July 1899, s. col. 5516 (P); **Gao:** Cercle de Gao, Fafa, 30 April 1937, *Wailly* 5396 (P). **MAURITANIA. Adrar:** Tayert, d' Azougui, 18 May 1934, *Monod* 649 (P); Tinioulig, Western Sahara, 28 November 1935, *Monod* N°5736 (P); El Kseïb, December 1933, s. col. s.n. (P); **Tagant:** O-d Aguelil, Touizirzit, Inchiri, 26 December 1936, *Miss. Et. Biol. Acridiens* 1755 (P). **MOROCCO. Meknès-Tafilalet:** Er Rachidia, 3 km S Tazouguerte from Boudhib to Gourrama, 32°04'00"N, 03°47'00"W, 1070 m, 10 April 1995, *Podlech* 52855 (MSB); Merzouga, sand dunes North Hotel, 31°03'08"N, 03°59'21"W, 696 m, 11 March 2010, *Quandt* ED401 (BONN); 31°03'08"N, 03°59'21"W, 696 m, 11 March 2010, *Quandt* ED402 (BONN); Ksar Es Souk, South Aït Hani, edge of a field, 31°46'00"N, 05°27'00"W, 1870 m, 17 July 1982, *Fdez, Casas, Garmendia, Susanna & Telleria* FC 7028 (B); **Souss-Massa-Daraâ:** Zagora, Zagora to M'hamid, sand dunes c. 25 km SO from Zagora, 693 m, 14 March 2010, *Quandt* ED403 (BONN); **Tanger-Tétouan:** Tetouan, Mohamed V bridge over the Oued Tzahadartz wetlands, 2 June 1981,

Casas 5538 (MA); Plaine saumatre de Talamagait (Beni-Tuzin), 28 April 1934, *Sennen & Mauricio* 9503 (BM). **NIGER REPUBLIC. Agadez:** Tafadek, 17°03'00"N, 08°00'00"E, 02 October 1965, *Popov* 94 (BM); Tin Tellous, 18°35'00"N, 08°48'00"E, 2 April 1979, *Newby* ZP125 (K); **French Niger:** Southern Aïr, Agadez, 7 February 1932, *Chevalier* 43525 (P); Road to Agadez, Arlit, 6 January 1975, *Lavie* 820 (P); **Zinder:** Barajé, 29 January 1928, s. col. 664 (P). **PALESTINA. West Bank:** Prat River (Wadi Kelt), 04 March 1942, *Davies* 4049 (K); Near Nabi Musa, 17 April 1936, *Dinsmore* 6903 (E); South Jericho at junction of Jericho and Dead Sea roads, 31°47'58"N, 35°29'26"E, 06 February 1987, *Musselman* 10085 (E); 10 km South Jericho near Dead Sea, common in saline desert, 19 February 1987, *Musselman* 10150 (BM); 15 km South Jericho on edge of Dead Sea, 13 January 1987, *Musselman* 10101 (E). **PORTUGAL.** Salvage [Savage?] Island, 6 February 1959, *Pickering* 204 (BM). **SENEGAL. Dakar:** Mbao, 1950-51, *Berhaut* Fir 51 (BR). **SPAIN. Alicante:** Jijona, South village, along the route to Alicante, on saltmarsh, 4 April 1958, s. col. 621622 (BR); Santa Pola, on saltmarshes, *Townsend* 1136 (BM). **Castille-La Mancha:** Hellín, *Polomgue* 20 (K); **Murcia:** Cieza, 200 m, 22 March 1978, *Ortiz* 168 (MA); Águilas, Cabo cope, Playa de la Cala, 37°24'04"N, 01°34'51"W, 10 m, 14 April 1993, *Aragon & Martinez* GA.0114 (MA). **SYRIA. Homs governorate:** Xeo, Qazz-Hayz (prob. Al Qusayr), 18 April 1939, *Dinsmore* 12903 (K); Al Quaryatayn, 17 km toward East, 900 m, 17 April 1965, *Roessler* 4863 (M); Palmyra, near Homs, 18 April 1943, *Wood* 5790 (K). **TCHAD. Tibesti:** North slope of Tarso Toussidé, Mosa, 1250 m, 9 February 1965, *Scholz* 16 (B); **Salamat:** Kaal [Kal], 6 December 1963, *Mosnier* 1858 (P). **YEMEN. Al Bayḍā':** Gebel Ahram, Radā', 830 m, 7 September 1975, *Ritchie* 104 (E).

Host: Amaranthaceae: *Arthrocnemum macrostachyum* Torr. (Fageer & Assubaie, 2006; Fahmy & al., 2013), *Atriplex halimus* L. (photo by *Kohlrabizirkus* at <http://www.flickr.com/photos/kohlrabizirkus/6996978156>, accessed 18 February 2015) and *Salsola genistoides* Juss. ex Poir. (photo by *Sánchez-Pedraja* at http://www.farmalierganes.com/Flora/Angiospermae/Orobanchaceae/Orobanchaceae_Checklist.htm, accessed 18 February 2015); *Anabasis articulata* (Forssk.) Moq., *Salsola baryosma* (Schult.) Dandy, *Suaeda aegyptiaca* (Hasselq.) Zohary, *Suaeda monoica* Forssk. ex J.F.Gmel., *Suaeda vermiculata* Forssk. ex J.F.Gmel.; **Chenopodiaceae:** *Seidlitzia rosmarinus* Bunge ex Boiss.; **Zygophyllaceae:** *Zygophyllum coccineum* L., *Zygophyllum simplex* L., *Zygophyllum qatariense* Hadidi (Fageer & Assubaie, 2006).

Remarks: According to Cosson (1865), the type specimen of *Orobanche compacta* was at GE, but the whole herbarium was destroyed during World War II. No isotype has been found by the authors, but the protologue clearly indicate that this name should be placed under the synonymy of *Cistanche tinctoria*.

1.1.3 *Cistanche almeriensis* Ataei, sp. nov.

Diagnosis: *Cistanche almeriensis* is similar to *C. tinctoria*, but differs by the gray-blackish scales, bracts, bracteoles and partially the calyx (versus yellow to brown in *C. tinctoria*) and the presence of two yellow folds inside the pale corolla tube (versus steady color on the inner side of the corolla in *C. tinctoria*). The species has been misdetermined as *C. phelypaea*.

Type: **SPAIN. Andalusia:** near road junction to Gergal on the Almeria to Tabernas road, in salt scrub, 27 March 2000, *Foley* 1601 (holotype E barcode E00147798)

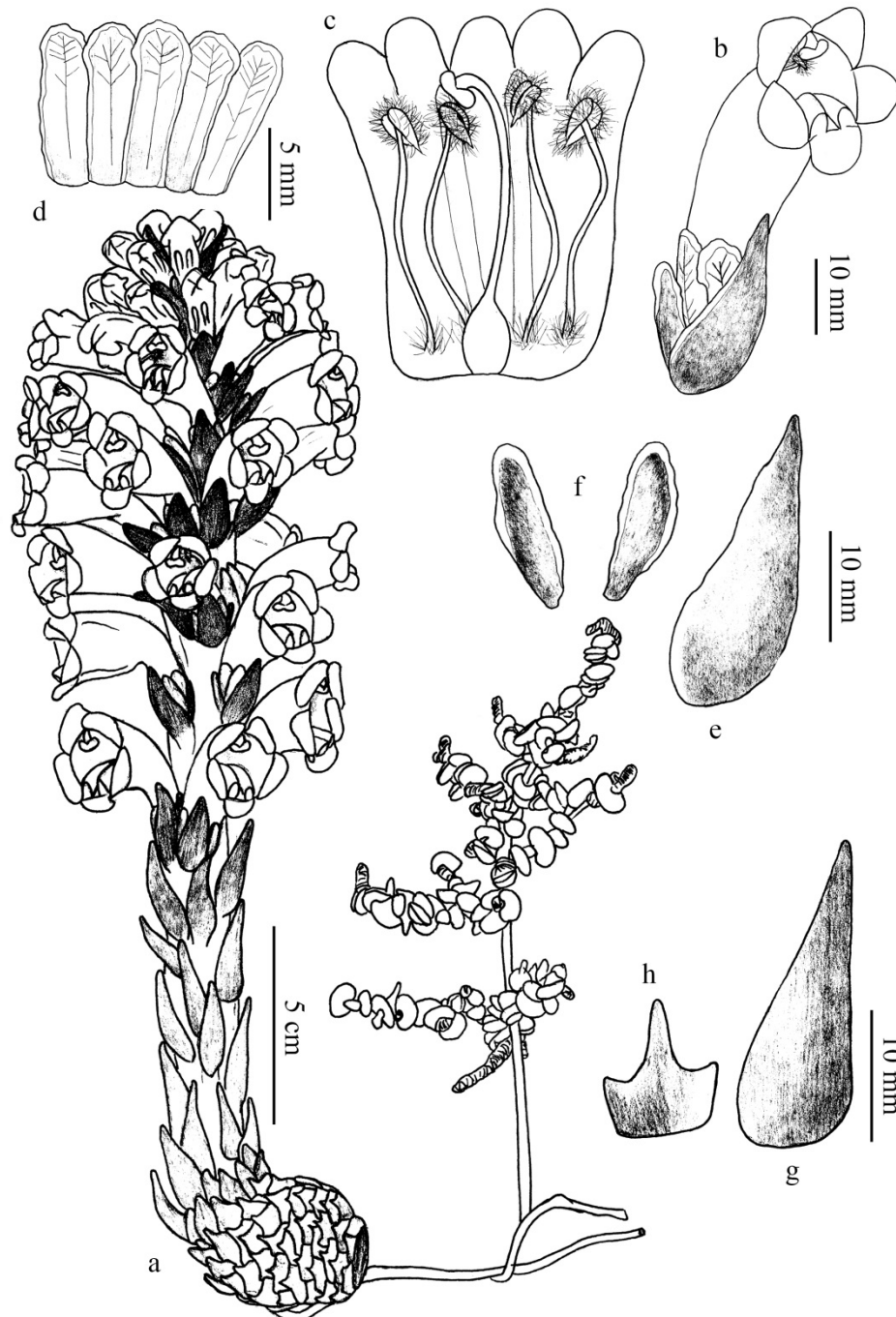


Figure 4.6 *Cistanche almeriensis* Ataei & Schneeweiss sp. nov. a) flowering plant and partially its host (*Atriplex halimus* L.), b) single flower, c) its flower inner view, d) abaxial calyx, e) bract, f) bracteoles, g) upper and h) lower scales. The plant occurred in Algeria (Davis 10.326; K).

Plant robust, rarely slender, 22–40 cm tall. **Lower scales** very dense and imbricate, ovate-triangulate to auriculate, sub-acute, 3–7 (–10) × (2–) 5–7 mm, usually non-scarious at the margins, grey-blackish; **middle and upper scales** becoming acropetally more lax upper part, ovate-lanceolate, sometimes oblong, obtuse, (8–) 10–18 (–31) × 3–5.5 (–7) mm, scarious at the margins, grey-blackish. **Inflorescence** an oblong spike, dense, 10–14 (–30) cm long and 5.5–10 cm in diameter; **floral bract** ovate-lanceolate to ovate-oblong, obtuse, 13–25 (–32) × 4.5–6 (–9) mm, almost entire and often non-scarious at the margins, equal or slightly shorter than calyx, grey-blackish; **floral bracteoles** oblong-obovate, obtuse, (7–) 10–15 (–22) × (1–) 1.5–2.5 (–4) mm, shorter than calyx, entire and non-scarious rarely slightly scarious at the margins, gray-blackish; **calyx** oblong, pentamerous, 10–15 (–21) mm long, much shorter than ½ total corolla length; **calyx lobes** 5, equal, rounded, 3.5–4.5 (–10) × 3–5.5 (–7) mm, usually entire and slightly scarious at the margins, slightly imbricate, grey; **corolla** tubular, pentamerous, 35–40 (–45) mm long, smoothly curved and widening, with two yellow folds inside the tube, white or pale cream-yellow; **corolla lobes** 5, equal, oblong-rounded, 3–5 (–8) × 5–8 (–10) mm, yellow or tinged violet; **stamens** inserted at about 1/6 total corolla length, filaments 2/3 as long as the corolla, at base densely villous; **anthers** 3.5–4.5 mm long, strongly villous, rounded at base and long acuminate at apex; **ovary** ovate, dark brown when dry; **style** non-exserted; **stigma** disk-like, white-yellowish. **Capsules** dehiscent with 2-valves. **Seeds** elliptic-circular (Fig. 4.6).

Distribution: Mostly in Southeast Spain, but also in Canary islands, Algeria, Tunisia, Egypt and Israel (Fig. 4.3).

Additional specimens examined (paratypes): ALGERIA. M'sila: Between Entu el Hafay and Bir Souid, 17 April 1896, *Murbeck* s.n. (P). **EGYPT. South Sinai:** Sinai desert, 9 April 1946, *Lupton* s.n. (BM); **Al Qāhirah:** 24 km Cairo, Suez road, 30°04'00"N, 31°52'00"E, 14 April 1945, *Davis* 10309 (E); Wadi Rished (Helwan), April 1945, *Davis* 10.326 (K); **North Sinai:** Sad Er-Rawafa, 5 km of Abu Aweigila, 30°49'47"N, 34°08'26"E, 147 m, 2 May 2009, *Van-Slageren & Henwawy* MSAH992 (K); **Shamāl Sīnā':** 22 km E Al 'Arīsh, Sandy desert, 31°11'07"N, 33°59'60"E, 18 January 1979m, *Goldblatt* 5017 (E); Al 'Arīsh desert, March 1928, *Meinertzhagen* s.n. (BM); **Al Qalyūbiyah:** On the routh of Al Khānkah dunes, 8 February 1945, *Davis* 8140 (E); **Al Ismā'īliyah:** Ismailia, 18 March 1880, *Barbey* 689 (P); Wasi el Anqalya, 3 March 1945, *Davis* 8437 (E); Isthmus of Suez, 1842, s. col. s.n. (P). **ISRAEL. Southern district:** Southern Negev, 6 km S of zihor junction, chalky ground in Eocene chalk hills, 29 March 1989, *Dann & al.* s.n. (B). **SPAIN. Andalusia:** Taha de Andarax c. 5 km W of Canjáyar, 37°00'42"N, 02°45'16"W, 750 m, 2 April 2001, *Schneeweiss & Weiss-Schneeweiss* S&T 7656 (WU); Almeria, on the road to Murcia, Western foothills of Sierra Alhamille, 14 April 1968, *Merxmüller & Lippert* 23408 (M); Alhama de Almería, 2-300 m, April 1890, s. col. 5346 (BR); 1 km E of sorbas, minor road to Lubrin, dry roadside bank, 37°05'47"N, 02°07'05"W, 400 m, 22 April 1991, *Jury, Maunder, Ross & Upson* 10381 (BM); Ost-Andalusien, parque natural Cabo de Gata-Níjar, Agua Amarga, 36°56'17"N, 01°56'18"W, 4 April 2004, *Vitek* 04-0010 (W); Sierra de Alhamilla, 2-300 m, April 1890, *Parda & Kigo* N°283 (E); Tabernas, East town, 280 m, 5 March 1992, *Lopez* 2828GL (MA); **El río Guadalentín:** valley of the río Guadalentín, 37°43'00"N, 01°48'50"W, 450 m, 15 March

2002, *Schneeweiss & Weiss-Schneeweiss* S&T 8744 (WU); **Murcia:** Lorca, ravine of Sierra del Viento, 22 April 1973, *Merxmüller & Gleissner* 29290 (M); Sierra de Gigante; 12 km from Banos Fuensante on the road to Zarcilla, 37°30'00"N, 01°45'00"W, 700 m, 24 April 1980, s. col. s.n. (BM); **Canary Islands:** Lanzarote, Teguise, SE Montana Corona, 29°01'12"N, 13°30'25"W, 80 m, 5 June 2006, *Aedo & Medina & Quintanar* AQ1732 (MA); Caleta de Famara, 29°07'00"N, 13°34'59"W, 9 April 1976, *Duvigneaud* 76 Can 160 (BR); de Lajas, February 1905, *Pitard* 1788 (P); Mountana Ganada, Haría, 29°08'00"N, 13°30'00"W, 6 April 1964, *Markgraf* s.n. (B). **TUNISIA. Al Qayrawān:** Kairouan, Rue El Bakri, in Wadi, 24 April 2001, *Ehrendorfer-Schratt* S&T 8765 (WU).

Host: Amaranthaceae: *Salsola genistoides* Juss. ex Poir. (*Jury & al.*, 10381, BM), *Atriplex halimus* L. (*Davis* 10.326, K), *Haloxyton* sp. (*Davis* 10309, E).

1.1.4 *Cistanche algeriensis* Ataei, sp. nov.

Diagnosis: *Cistanche algeriensis* is similar to *C. tinctoria*, but differs by its long (35–45 mm) oblong-lanceolate middle and upper scales (versus shorter maximally 33 mm oblong to oblong-lanceolate scales in *C. tinctoria*), bracts are twice than calyx or longer (versus bracts slightly longer than calyx in *C. tinctoria*), acuminate anther (versus apiculate rarely mucronate in *C. tinctoria*). The species has so far been misdetermined as *C. phelypaea*.

Type: **ALGERIA. Djelfa:** 10 km S of Hassi Bahbah, 800 m, 7 June 1971, *Davis* 53288 (holotype BM barcode BM000598612).

Plant high and slender, up to 60 cm tall. **Lower scales** very dense and imbricate, ovate-oblong, obtuse, 5–6 × 3.5–4 mm, non-scarious at the margins, grey; **middle and upper scales** lax, long oblong-lanceolate, obtuse, 35–45 × 3.5–4.5 (–6) mm, non- or slightly scarious at the margins, grey. **Inflorescence** a long tubular spike with many flowers, dense, 34–35 cm long and 6–11 cm in diameter, flowers slightly curved downwards; **floral bract** long oblong-lanceolate, obtuse, 39–46 × 4–7 mm, twice than calyx or longer, entire and slightly scarious at the margins, light brown; **floral bracteoles** linear-oblong, obtuse, 19–22 × 2–2.5 mm, non-scarious at the margins, slightly longer than calyx, yellow; **calyx** tubular, pentamerous, with conspicuous veins, 18–21 mm long, as long as or slightly longer than ½ total corolla length; **calyx lobes** 5, 4 equal ones longer than the lower one, oblong-ovate, obtuse, 8–9 × 3.5–6 mm, broadly scarious at the margins, yellow; **corolla** tubular, pentamerous, yellow, with brownish veins randomly distributed inside the tube, 36–40 mm long; **corolla lobes** 5, unequal 4 longer plus one slightly shorter, oblong-rounded, 6–7 × 6–9 mm, yellow; **stamens** inserted at about 1/9 total corolla length, filaments 2/3 as long as the corolla, at base densely pilose; **anthers** 4 mm long, densely villous, rounded at base and acuminate at apex; **ovary** long tubular, dark brown when dry; **style** non-exserted; **stigma** bilobate. **Capsules** dehiscent with 2-valves. **Seeds** elliptic (Fig. 4.7).

Distribution: Algeria and Jordan (Fig. 4.3).

Additional specimens examined (paratypes): ALGERIA. Tamanrasset: Wilaya Tamanrasset, 25 km N Amguid to Hassi Bel Guebbour, 26°36'00"N, 05°21'00"E, 580 m, 10 March 1982, *Podlech* 36603 (M). **JORDAN.** Aqaba: Gulf of Aqaba, Red Sea, Boarder of Israel, 10 m, 15 October 1989, *Leonard* 7484 (BR).

Host: Unknown.

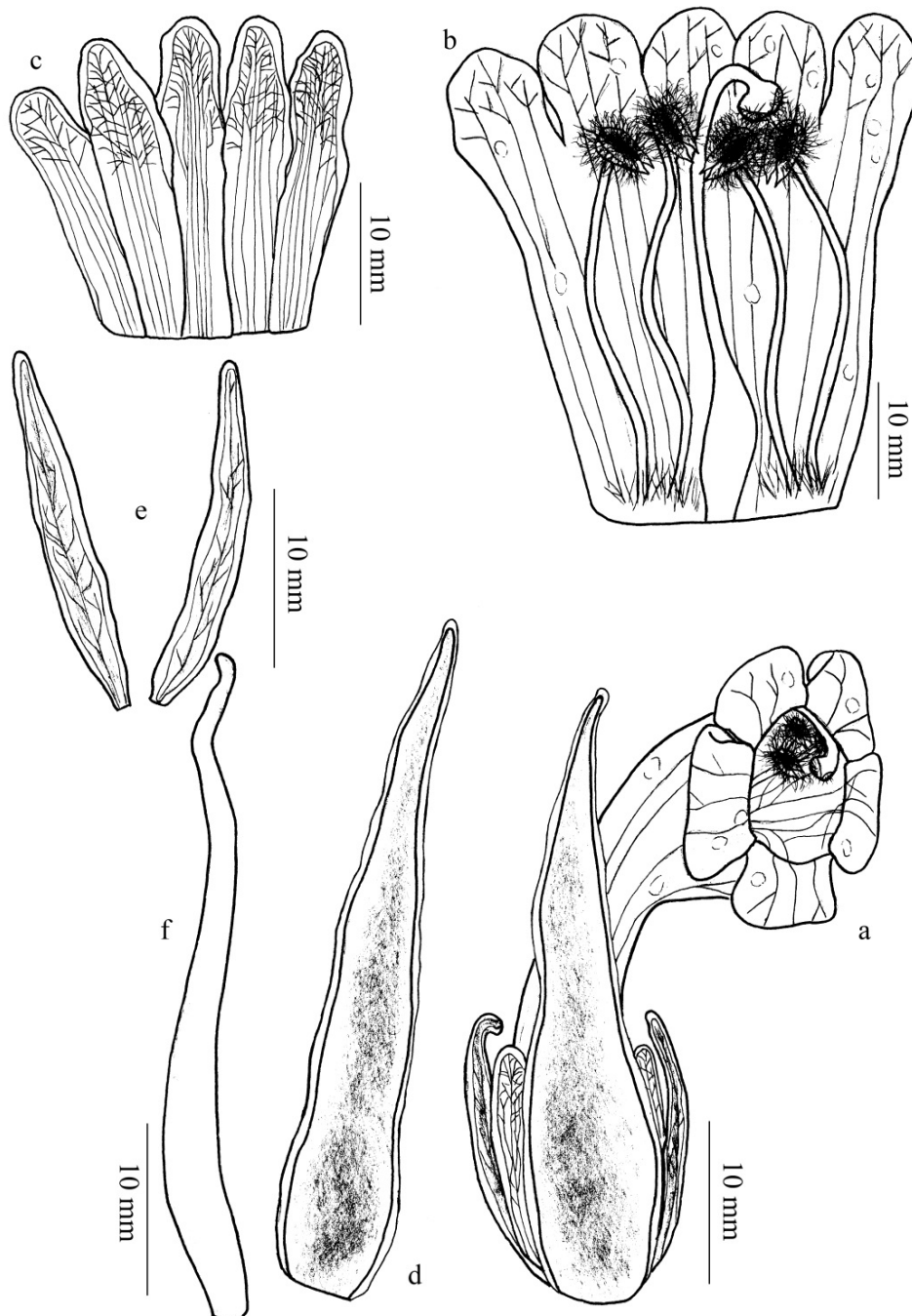


Figure 4.7 *Cistanche algeriensis* Ataei, sp. nov. a) single flower, b) its flower inner view, c) abaxial calyx d) bract, e) bracteoles, and f) upper scale. The plant occurred in Algeria (Davis 53288; BM).

1.1.5 *Cistanche violacea* (Desf.) Hoffmanns. & Link, Fl. Portug. 1: 320 (1813);

≡ *Phelypaea violacea* Desf., Fl. Atlant. 2: 60, t. 146 (1798) [basonym] ≡ *Orobanche violacea* (Desf.) Wallr., Orob. Gen. 70 (1825) ≡ *Cistanche violacea* (Desf.) Beck, Biblioth. Bot. 19: 267 (1890), comb. superfl.;

Type: **TUNISIA. Tawzar**: "in arenis deserti prope Tozzer", *Desfontaines* s.n. (G barcode G00014078 seen as photo)

= *Cistanche hybrida* Beck, Pflanzenr. (Engler) 96, 4.261:31 (1930) ≡ *Phelypaea lutea* × *violacea* Murb., Contr. Fl. Nord-Ouest Afrique 2: 29 (1898). According to original description, it is placed under the synonymy of *C. violacea*.

Plant usually low, slender, 20–25 (–35) cm tall. **Lower scales** ovate-triangular to deltoid, densely imbricate, sub-acute, (3–) 4–8 × (2.5–) 4–6 mm, grey; **middle and upper scales** increasingly lax acropetally, oblong-lanceolate, obtuse, (5–) 10–18 (–25) × (3–) 5–8 mm, sinuate and non-scarious at the margins, grey. **Inflorescence** a cylindrical to conic spike, dense, (8–) 12–20 (–25) cm long and 5–9 cm in diameter, flowers curved downwards; **floral bracts** ovate-lanceolate, obtuse, (11–) 14–19 (–28) × (3–) 4–6 (–8) mm, slightly scarious at the margins, densely serrate and sinuate, usually as long as calyx, grey; **floral bracteoles** oblong-lanceolate, rounded, 8–13 × (0.8–) 1.5–2.5 (–3) mm, slightly scarious at the margins, strongly serrate and sinuate, shorter than calyx, light grey; **calyx** usually tubular, rarely campanulate, pentamerous, 11–16 (–19) mm long, slightly longer than ½ total corolla length; **calyx lobes** 5, 3 equal slightly longer ones and 2 shorter ones, oblong, rounded-obtuse, (2.5–) 4–7 × 3–4 mm, slightly scarious and serrate at the margins, light grey; **corolla** long tubular, pentamerous, 33–45 mm long, white-pale-lilac with two yellow folds inside the tube; **corolla lobes** 5, slightly unequal (upper ones longer than lateral ones longer than lower one), 3–4 (–7) × 4–9 mm, light to dark purple; **stamens** inserted at about 1/5 total corolla length, filaments 2/3 as long as the corolla, at base densely tomentose; **anthers** 3–4.5 mm long, apiculate rarely mucronate, densely tomentose; **ovary** ovate; **style** non-exserted; **stigma** bilobate, white-cream. **Capsules** open with 2-valves. **Seeds** usually elliptic (Fig. 4.8).

Distribution: Western and Northern Africa to Arabian Peninsula, Israel and Jordan (mostly in Dead Sea valley), Saudi Arabia (previously unknown; Fig. 4.3). Foley (2001a: 227) clarifies the absence of this species in Spain.

Additional specimens examined: **ALGERIA. Laghouat**: 95 km S Ghardaia to El Golea, 31°46'00"N, 03°43'00"E, 430 m, 11 April 1982, *Podlech* 37081 (MSB); Sahara, among crops, 25 March 1931, *Meinertzhagen* s.n. (BM); **Ouargla**: 62 km N from Hassi Messaoud to Touggourt, 32°11'00"N, 05°52'00"E, 150 m, 11 March 1980, *Podlech* 32882 (MSB); Oued Rhir, close to El Arfiane, in limonistri Guyoniani, 19 March 1933, *Maire* s.n. (P); **Biskra**: Vicinity of Biskra, 3 April 1922, s. col. s.n. (BM); **Illizi**: Hassi Bel-Guebbour, March/April 1981, *Meyer* 204-51-81-10 (B); El Baadja, 28 December 1874, s. col. 250 (P). **EGYPT. Tripolitania**: Libyan desert, W of E1 Agheila,

Sea level, 20 February 1966, *Archibald* 936 (E); Wadi El Antably [?], 15 February 1945, *Davis* 8248 (E). **JORDAN. Karak:** Mujib valley, 1 km after the rest house of tourists, roadside, 6 May 2006, *Abulaila & Tehabshem* 2006JOR4-1 (K, NCARTT). **Aqaba:** Wadi Rum, sandy semi-desert, 1 April 1976, *Swann* WR10 (BM); Golfa d' Agaba, Red Sea, the border of Israel, 4144 m, 15 October 1989, s. col. 857695 (BR, E); East, October 1873, *Paine* 10 (K). **LIBYA. Az Zāwiyah:** Coast E of Zavia, open rocky ground close to Sea, 32°47'29"N, 12°43'55"E, Sea level, 17 February 1970, *Mait land* 75 (K); Jamahiriya, Gulf of Sirte, E of Marble Arch, 16 March 1970, *Davis & Hadland* 49622 (E); North of Djabal al-Soda c. 100 km NNW from south Chormat Tuzizet, 200 MH south Tripolitanian, 30 January 1955, *Meckelein* s.n. (B); **Jadu:** Gulf of Sirte, nr. Beurat el Hsun 20m As Sabkha al Kabirah, 23 March 1970, *Davis* 49831 (E). **MOROCCO. Souss-Massa-Drâa:** Agadir, 5 km E Bou-Izakarn to Akka, 29°10'00"N, 09°41'00"E, 600 m, 13 April 1986, *Podlech* 40705 (MSB); Zagora, Jebel Sarhro, between Ouarzazate and Jbl Tifernine, 30°45'57"N, 06°41'23"W, 25 March 2001, *Staudinger* 3219 (WU); East Tizi-n-Taguergoust, 9 km SE Tazenakht to Foum-Zguid, 30°33'00"N, 07°09'00"E, 1450 m, 9 April 1990, *Schuhwerk* 90/792 (M); Ouazazarte to Zagora, river valley c. 8 km SE Ouazazarte, 30°51'10"N, 06°49'56"W, 1146 m, *Quandt* ED400 (BONN); Lower Drau, tamarisk dunes at Regâbi Aït Hassou 10 km E Mhamid, 29°50'17"N, 05°37'08"W, 9 March 2001, *Staudinger* 3218 (WU); South Ouarzazate, on the road to Agdz, 1300 m, 15 April 1967, *Merxmüller & Oberwinkler* 22560 (M); Tiznit, 11 km SW Bou Izakarne to Goulimine, 29°09'00"N, 09°46'00"E, 620 m, 25 March 1990, *Schuhwerk* 90/200 (M); 11 miles Southwest Bouizakarne, near the road to Goulimine (P41), 29°09'00"N, 09°46'00"W, 1000 m, 25 March 1990, *Podlech* 48445 (MSB); **Meknès-Tafilalet:** Er Rachidia, Erg Chebbi, 35 km SE of the slope near Piste to Merzouga, 31°13'00"N, 04°01'00"W, 750 m, 8 April 1995, *Podlech* 52705 (MSB); 11 km W Boudnib on the street P32 to Er-Rachidia, 31°58'00"N, 03°45'00"W, 4 April 1993, *Podlech* 51012 (MSB); Before Er Rachidia, Oued Ziz, c. 20 km N of Stausee Hassan Addakhil, 32°08'52"N, 04°22'16"W, 45 m, 10 March 2010, *Quandt* ED395 (BONN); Oued Ziz, c. 20 km N of Stausee Hassan Addakhil, 32°08'52"N, 04°22'16"W, 1032 m, 10 March 2010, *Quandt* ED396 (BONN); After Tinejdad, Erfoud to Tinejdad, 31°44'28"N, 04°24'37"W, 792 m, *Quandt* ED399 (BONN); Before Tinejdad, 30°39'17"N, 06°33'45"W, 996 m, *Quandt* ED398 (BONN); ED, Goulmima to Ksar-es-Souk, 31°45'00"N, 04°46'00"W, 1200 m, 4 April 1969, *P. & Davis* 49060 (E); **Guelmim-Es Smara:** WD, between Bou Izakarne and Foum el Hassane, 750 m, 24 March 1972, *Davis* 53688 (E). **SAUDI ARABIA. Al Jawf:** Thanīyat Ṭurayf, Thaniyat Camp, 29°46'00"N, 37°58'00"E, 830 m, 24 March 1982, *Collenette* 3469 (K, E). **TUNISIA. Gafsa:** South, c. 2 km from Gafsa, N to Oued el Kebir, 34°27'00"N, 08°46'00"E, 400 m, 5 May 1975, *Davis & Lamond* D.57318 (E); Gobernación de gabes, Metloui, gorges de Seldja, 34°20'19"N, 08°19'45"E, 250 m, 25 March 2009, *Herreo & al.* CA3916 (MA); Oued el Kebir, 474 m, *Schratt-Ehrendorfer* S&T 8765 (WU); **Qābis:** Zerkine, 33°44'50"N, 10°16'52"E, 5 m, 24 March 2009, *Aedo & al.* CA16301 (MA); Between Medenine and Adjim, 33°31'00"N, 10°40'00"E, 4 April 1980, s. col. BR857690 (BR); **Madanīn:** Midouni, Djerba, s. col. 891908 (BR).

Host: Amaranthaceae: *Atriplex halimus* L. (*Podlech* 48445, MSB; *Davis* 49831, E), *Atriplex leucoclada* Boiss. (*Collenette* 3469, K, E), *Anabasis* sp. (*Davis & Lamond* D.57318, E), *Hammada*

scoparia (Pomel) Ilijin. (*Podlech* 52705, MSB), *Haloxylon articulatum* (Moq.) Bunge (*Davis & Hadland* 49622, E) and; **Nitrariaceae**: *Nitraria retusa* Forssk. (s. col. BR857690, BR).

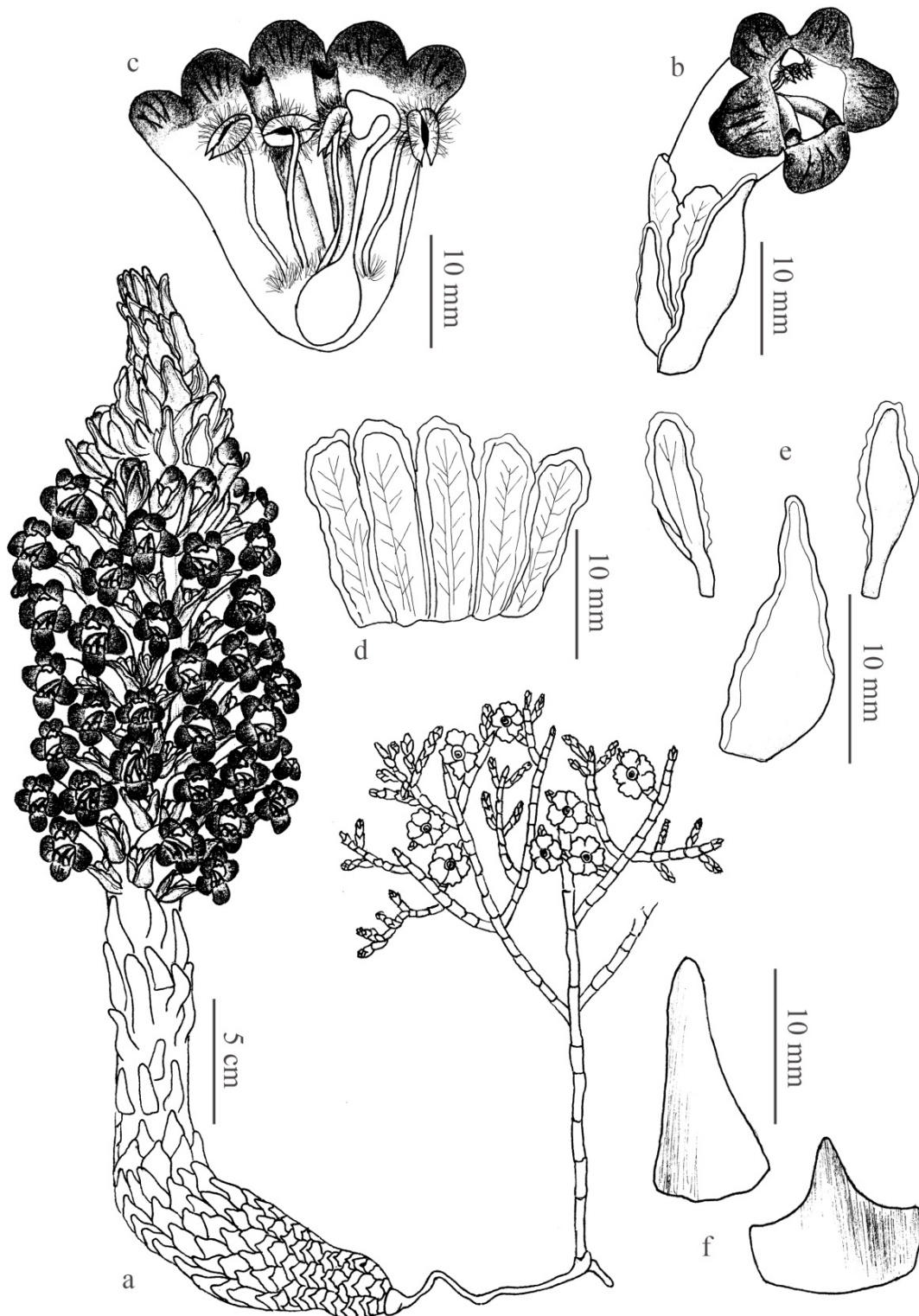


Figure 4.8 *Cistanche violacea* (Desf.) Hoffmanns. & Link. a) flowering plant and partially its host *Hammada articulata* (Moq.) O. Bolòs & Vigo. b) single flower, c) its flower inner view, d) abaxial calyx, e) bract and bracteoles, and f) lower and upper scales. The plant occurred in Morocco (Quandt ED400; BONN).

1.1.6.1 *Cistanche tubulosa* (Schenk) R. Wight ex Hook.f. subsp. ***tubulosa***, Fl. Brit. India [J. D. Hooker] 4: 324 (1885);

≡ *Phelypaea tubulosa* Schenk, Sp. Pl. Aegypt. 23-24 (October 1840) [basionym]

Type: EGYPT. South Sinai: Sinai Peninsula, “ad viam inter Suez et Tor in Arabia petraea”, February Roth & Erdl (WB?, probably destroyed).

= *Orobanche insignis* E.D. Clarke, Travels Eur. Asia & Africa 3(2): 41-42 (1814). *Type: EGYPT. AI Buḥayrah:* between Aboukir and Rosetta, April-May 1801, Clarke s.n., (BM barcode BM000606304).

= *Orobanche calotropidis* Edgew., J. Bot. (Hooker) 2: 285 (July 1840) ≡ *Phelypaea calotropidis* (Edgew.) Walp., Repert. Bot. Syst. (Walpers) 3: 462 (1844); ≡ *Cistanche calotropidis* (Edgew.) Beck, Pflanzenr. (Engler) 96, 4.261: 34 (1930). *Type: INDIA. Punjab:* Phalahi and Jhand, Edgeworth (holotype K? not seen, isotype G-DC).

Plant robust, thick, 20–50 (–150) cm tall, usually at the base tuberous up to 4 cm in diameter. **Lower scales** very dense, imbricate, ovate-triangular, rounded, 5–8 (–11) × (2.5–) 6–9 (–14) mm, sinuate and non-scarious at the margins, grey; **middle** and **upper scales** increasingly lax acropetally, ovate-lanceolate to ovate-oblong, rounded, (9–) 13–19 (–32) × (2.5–) 4–9 mm, highly sinuate, grey. **Inflorescence** ovate-oblong, compact and with many flowers, 7.5–35 cm long and 3–9 cm in diameter; **floral bracts** ovate-lanceolate, rounded, (12–) 16–24 (–30) × (3–) 6–12 (–17) mm, slightly serrate and deeply sinuate, spiny, equal or slightly longer than calyx, grey; **floral bracteoles** oblong-lanceolate sometimes spatulate, obtuse, (7–) 12–15 (–22) × (1.5–) 3–5 (–9) mm, slightly serrate and deeply sinuate, equal or shorter than calyx, grey; **calyx** usually tubular, pentamerous, (9.5–) 17–20 (–30) mm long, usually as long as ½ total corolla length; **calyx lobes** 5, 4 longer plus one shorter, oblong to oblong-ovate, rounded, (2.5–) 4–6 (–10) × 3–5 (–8) mm, slightly scarious at the margins, lacerate at the margins, light grey; **corolla** pentamerous, infundibuliform, 34–52 mm long, white to grey outside and yellow inside, rarely with orange-reddish lobes (in specimens from the Emirates and Kuwait); **corolla lobes** 5, equal, rounded, 4–7 (–10) × 5–8 (–10) mm, usually yellow, rarely orange-reddish or slightly light blue; **stamens** inserted at about 1/10 total corolla length, filaments 2/3 as long as the corolla, densely pilose-tomentose; **anthers** 2.5–4.5 mm long, cordate, rounded at base and obtuse, rarely apiculate (Emirates) at apex, densely pilose-tomentose; **ovary** ovate; **stigma** broadly bilobate, white-yellow, non-exserted. **Capsules** dehiscent with 2-valves. **Seeds** ovate, globose, rarely oblong (Fig. 4.9).

Distribution: Broadly distributed from the Cape Verde Islands, Egypt to the Arabian Peninsula, Iran, India and China (first record for China; Fig. 4.3).

Additional specimens examined: **BAHRAIN.** Unknown location, 13 September 1970, Gallagher 66 (K). **CHINA. Xinjiang Uygur Zizhiqu:** Turkestan, Takla Makan, near Kara Targaz, 4300 m, 28 April 1971, Deasy 3 (BM). **EGYPT. South Sinai:** Wadi Feiran Oasis, 17 April 1937, Shabetai

508.1239 (K). **Maṭrūḥ**: Buqbuq, Very sandy hills, 31°30'39"N, 25°32'52"E, 24 December 1989, *TTDF* 362 (E). **IRAN. Khuzestan**: Persian Gulf, Ghamar (Bone) Island, 30°08'59"N, 49°12'29"E, Sea level, 17 March 2011, *Akhani, Noormohammadi & Samadi* 21965 (TUH); Ahavaz, Albagi, 1967, *Mobayyen* 29469 (TUH); **Hormozgan**: Bandar-e-Lenghe, Tonb-e-Kochak Island, 10 m, *Mozaffarian* 63586 (IRAN); Shotur Island, *Aminirad* 33505 (IRAN); Bostano, Banifarur Island, *Terme & Karavar* 26565 (IRAN); Bandar-e-Khamir, *Shahi & Saeidi* 13229 (GIUH); Larak Island, 3 m of N coast, 26°52'00"N, 56°21'00"E, *Kunkel* 17159 (K); Mahshahr, Bone Island, 30°08'39"N, 49°10'16"E, *Tehrani & Eskandari* 33506/1 (IRAN); **Khuzestan**: Susangerd, Farhe region, 40 m, 12 April 1985, *Mozaffarian* V. 53474 (IRAN); **Bushehr**: Bushehr, Dayyer, Morghi Island, *Tehrani & Sangari* 26566 (IRAN); **Sistan & Baluchestan**: Do-Rahi Zabol Zahedan, close to water pump station, 30°11'40"N, 60°51'54"E, 552 m, 1 March 2011, *Ataei & Heidari* ED506 (BONN, USB); Zabol, 1 km Do Rahi Zabol-Zahedan, 30°15'41"N, 60°50'01"E, 469 m, 1 March 2011, *Ataei & Heidari* ED507 (BONN, USB); Zabol, Zahak, Vitis Botanical Garden, 30°50'11"N, 61°43'34"E, 478 m, 1 March 2011, *Ataei & Heidari* ED509 (BONN, USB); Zabol, Chah Nime, around South research Institute of aquatics, 30°49'45"N, 61°43'07"E, 354 m, 1 March 2011, *Ataei & Heidari* ED508 (BONN, USB); 130 km Khash to Iranshahr, Abadan village, 27°18'06"N, 60°44'27"E, 640 m, 5 March 2011, *Ataei & Heidari* ED514 (BONN, USB); Zabol, Chah Khorma, 31°06'13"N, 61°09'20"E, 451 m, 1 March 2011, *Ataei & Heidari* ED510 (BONN, USB); Khash to Iranshahr, March 2010, *Heidari* ED487 (BONN, USB); Zabol, Abgir, March 2010, *Heidari* ED486 (BONN, USB); Zabol, Chah Nime, 30°47'42"N, 61°39'04"E, March 2010, *Heidari* ED484 (BONN, USB); Hamoon lake, Chah Khorma, 31°04'37"N, 61°11'35"E, March 2010, *Heidari* ED485 (BONN, USB); Dashtak, do Rahi Zabol to Zahedan, 30°10'22"N, 60°51'43"E, March 2010, *Heidari* ED483 (BONN, USB); Iranshahr to Bampoor, Bampoor village, 27°11'55"N, 60°28'50"E, 506 m, 5 March 2011, *Ataei* ED513 (BONN, USB); Iranshahr to Bampoor, Bampoor village, 27°11'55"N, 60°28'50"E, 1186, 1 March 2009, *Valizadeh & Heidari* s.n. (USB); 97 miles E of Bam, road to Zahedan, 762 m, 13 March 1971, *Grey-Wilson & Hewer* 152 (E); Chabahar, February 2007, *Valizadeh* s.n. (USB); C. 35 km W of Zabol, 3 km W of Mile Nader, margin of Hamun lake, 31°20'36"N, 61°37'28"E, 456 m, 30 March 2011, *Akhani, Noormohammadi & Samadi* 22088 (TUH); 64 km Bazman, 640 m, *Runemark, Assadi & Sardabi* 22593 (IRAN); **Tehran**: Highway Ghom-Tehran, 60 km Ghom to Tehran, Cheshmeh Shur, 9.5 km to Hoze-Solatan lake, 35°02'51"N, 50°58'37"E, 801 m, 21 April 2010, *Ataei & Heidari* ED436 (BONN, USB); 7 km SE Karvansarai Shah Abbas, *Wendelbo & Cobham* 10905 (TARI); **Fars**: Shiraz, 43 km from Lar to Bastak, 400 m, *Assadi & Sardabi* 41846 (IRAN); 36 miles E of Lar, road to Bandar-Abbas, 564 m, 5 March 1971, *Grey-Wilson & Hewer* 88 (K); Lar, 800 m, *Iranshahr* 32311 (IRAN); **Kerman**: Bam, *Sandooghdaran* 2047 (IRAN); Khabr va Rouchun protected region, 50 km SSW of Baft, E side of Khu-e-Khabr, 12 km E of Khabr, 28°50'00"N, 56°26'00"E, 2800 m, 8 June 1977, *Asadi, Edmondson & Miller* 1677 (E); Jiroft, Maraz, 18 km SE Simjan, 750-900 m, *Tehrani & Mousavi* 32304 (IRAN); **Yazd**: Yazd, between Nain and Ardakan, c. 10 km N Aghda, 323028 M, highly saline soils, 32°30'28"N, 53°33'30"E, 1170 m, 27 March 2011, *Akhani, Noormohammadi & Samadi* 21965 (TUH); 85 km Yazd to Bafq, right side of main road, 31°38'43"N, 55°14'55"E, 969 m, 21 April 2010, *Ataei & Heidari* ED434 (BONN, USB); **Lorestan**: Horr Abad probably, 500 m, 4 March 1971, *Foroughi* 1142 (TARI); **South Khorassan**: 19 km Tabas to Yazd,

Hoze Lab-e-Kavir, 2-3 km of main road, 32°27'42"N, 56°46'37"E, 620 m, 1 May 2010, *Ataei* ED479 (BONN, USB); **Semnan**: Nezami road, toward Chah-Shirin, main road, around industrial factory, 35°27'14"N, 53°43'37"E, 1192 m, 26 April 2010, *Ataei & Heidari* ED439 (BONN, USB). **IRAQ. Mayorality of Baghdad**: Abu Ghraib, 77 km northwest of Zubair, Basra Liwa, 19 March 1964, *Barkley & Abbas-al-Ani* 6499 (K); **Anbar**: 10 km N of Rutba, 28 February 1947, *Rawi & Gillett* 6326 (K); **Maysan**: Hamrin, near Shahraban, 8 May 1958, s. col. s.n. (E). **JORDAN. Ammon**: Zarqa, 500 m SW Qasr Amra, 31°48'01"N, 36°34'53"E, 17 April 1998, *Staudinger* J10/25 (W); 80-325 m, 06 April 1980, *Frey & Kuerschner* VO 4924 (E). **KUWAIT**. Artawyeh, 28°38'00"N, 48°08'00"E, 15 November 1935, *Sickson & Ph.* 182B (K); 2 April 1946, s. col. s.n. (K); 28°38'00"N, 48°08'00"E, 15 November 1935, *Sickson* 182B (K); On the Salmi road, 137 km along from University campus at Khaldiyyah, then 6 km into the desert, 150 m, 16 March 1981, *Armet* 122 (E). **OMAN. Zufār**: Dhofar, Muqshin, 19°32'00"N, 54°54'00"E, 7 March 1993, *McLeish* 1666 (E); Central Oman, Zawrik, Huqf Depression, 19°00'00"N, 57°00'00"E, 0-250 m, 5 March 1993, *McLeish* 1640 (E); **Masqat**: Beach near Muscat Intercontinental hotel, 23°37'04"N, 58°27'52"E, 15 January 1982, *Maconochie* 3096 (BM, E); Beside Batinah road, near Sawiq, 10 m, 19 March 1978, *Whitcombe* 155 (E). **PAKISTAN. Punjab**: Darya Khan, Mianwali Rd. n. W.F.P. in sand, 31°49'14"N, 71°21'52"E, *Rashid* 4650 (K); **Baluchestan**: Saran, 22 May 1897, *Harsuth* 206/a (K); **Sindh**: Karachi, beyond the Hub River Delta, 20 miles West of Karchi, January 1985, *Anderson* s.n. (E). **QATAR**. Wadi El Eriq, 16 May 1982, *Elamin* 136 (K); Wadi Al Galaiel, toward southern end of the Qatar Peninsula, 2 April 1977, *Boulos* 11119 (K); Gulf of Arabia, in Mualla, 12 April 1949, *Com RHS* s.n. (K). **SAUDI ARABIA. Northern Borders**: Wadi Arar, 18 June 1950, *Dickson* 704 (K); NE Arabia, 13 km E Abu Hadriyyah, 23 March 1968, *Mandaville* 1789 (BM); **Eastern Province**: Eastern S-A, near Abqaiq, sand at the roadside, 25°56'00"N, 49°43'00"E, 12 April 1982, *Podzorski* 804 (E); Central track East Hail, 27°25'00"N, 42°30'00"E, 800 m, 28 March 1962, *Zeller* 8301 (BM); **Al-Qassim**: Between Uqlat Asugour and Jebel Tamiyah, off the Medina-Qassim road, 854 m, 20 April 1981, *Collenette* 2458 (E); Southern Rub al-Khali, At-Tara'tz, Rolling sand terrain, 21°09'00"N, 50°29'00"E, 16 February 1982, *Mandaville* 7612 (BM); East, 18 km N of Dammam along side road to Ras Tanura, 7 February 1982, *Naylor* 6 (E); Sabrhat al Fasl, a small sandy island in the Gulf, February 1980, *Bettioon RCF* s.n. (E); Ad-Dammam, 30 km from Dhahran, 50 m, 23 March 1981, *Frey & Kuerschner* VO6557 (E); **South-West Province**: Tawi, 30 March 1969, *Gaston* 2633 (P); **Al Madīnah**: Near Al Ula, 609 m, 24 March 1989, *Gollenette* 7023 (E); South Arabia, vicinity of Jabal Shubayyik, 28 km E Mahd ad Dhahab, 610 m, 26 January 1986, *Collenette* 5570 (E). **SYRIA**. Syrian Desert, 10 km South Al Quaryatayn, 17 April 1965, *Doppelbaur* 13407 (M). **UNITED ARAB EMIRATES**. Sharjeh, al-Hamriya, sandy plain behind dunes & beach, 25°29'17"N, 55°30'14"E, 4 m, 31 March 2010, *El-Keblawy & al.* TMH335 (K); Sharjeh, by Khawr al Khan, 2 km S of Sharjeh, saline mud-flats, 1 m, 15 February 1980, *Edmondson* 2998 (E); Road between Al-Ain and Ayn Al-Faidah, 27 February 1986, *Boulos & Al-Hassan* 15763 (E). **CAPE VERDE**: Boa vista, Curral Velho, dune belt between lagoon and the Sea, 15°59'34"N, 22°47'34"W, 9 January 1994, *Kilian & Leyens* 3085 (B). **YEMEN. Marib**: Near Marib on dunes, 4 November 1982, *Muller-Hohenstein & Deil* 1211 (E); **Ta'izz**: Wadi Ad Dabab, walls and bridges around terraced fields, 900 m, 11 March 1984, *Miller & King* 5082 (E).

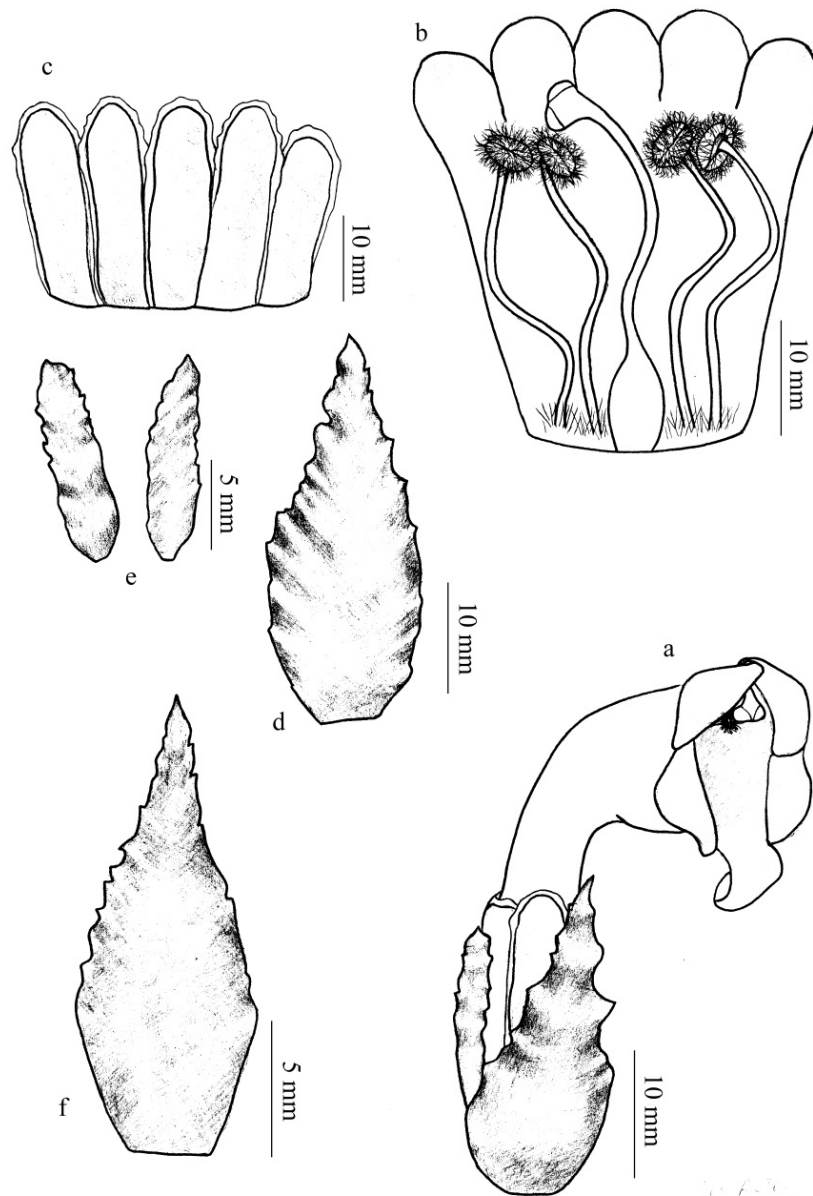


Figure 4.9 *Cistanche tubulosa* (Schenk) R. Wight ex Hook.f. subsp. *tubulosa*. a) single flower, b) its flower inner view, c) abaxial calyx, d) bract, e) bracteoles, and f) upper scale. The plant occurred in Iran (Ataei ED506; BONN).

Host: **Amaranthaceae:** *Arthrocnemum macrostachyum* Torr., (photo by Sergeev at <http://www.asergeev.com/>, accessed 18 February 2015), *Halochnemum* sp. (Edmondson 2998, E), *Haloxylon* sp. (Collenette 2458, E) and *Cornulaca arabica* Botsch. (Mandaville 7612, BM); **Zygophyllaceae:** *Zygophyllum* sp. (Rawi & Gillett 6326, K).

Remarks: The type specimen has not been seen. Most specimens of Schenk were at WB and LZ and destroyed (Stafleu & Cowan, 1976). No type specimen exists at REG (Peter Poschlod, pers. comm. 24 August 2014), W (Armin Löckher, pers. comm. 16 August 2014), BR (Sofie De Smedt, pers. comm. 25 August 2014) and WAG (Jan Wieringa, pers. comm. 05 September 2014). We refrain from designating a neotype here hoping that a type specimen still exists, because the protologue allows applying this name unequivocally. A proposal to conserve the name *Phelypaea tubulosa* Schenk (October 1840) needs to be submitted in order to be evaluated by the committee of

nomenclature because both *Orobanche insignis* E.D.Clarke (1814) and *O. calotropidis* Edgew. (July 1840) have priority. However, the latter names have never or rarely been used after their publication in contrast to the name *Cistanche tubulosa*, which is widely applied in floristic treatments and, due to its medicinal use, is frequently cited in the biomedical literature.

1.1.6.2 *Cistanche tubulosa* subsp. *iranica* Ataei, subsp. nov.

Diagnosis: *Cistanche tubulosa* subsp. *iranica* differs from the nominate subspecies by calyx structure: (two long lobes plus two medium sized ones plus one dentate versus four equally large lobes plus one smaller in subsp. *tubulosa*; lobes scarious at the margins versus slightly/or non scarious margins in subsp. *tubulosa*. The species has been misdetermined as *C. tubulosa*.

Type: **IRAN. Sistan & Baluchestan:** Chahbahar, Negoyan to Tis, 25°11'01"N, 61°06'59"E, Sea level, 7 March 2011, *Ataei* ED517 (holotype BONN, isotype USB).

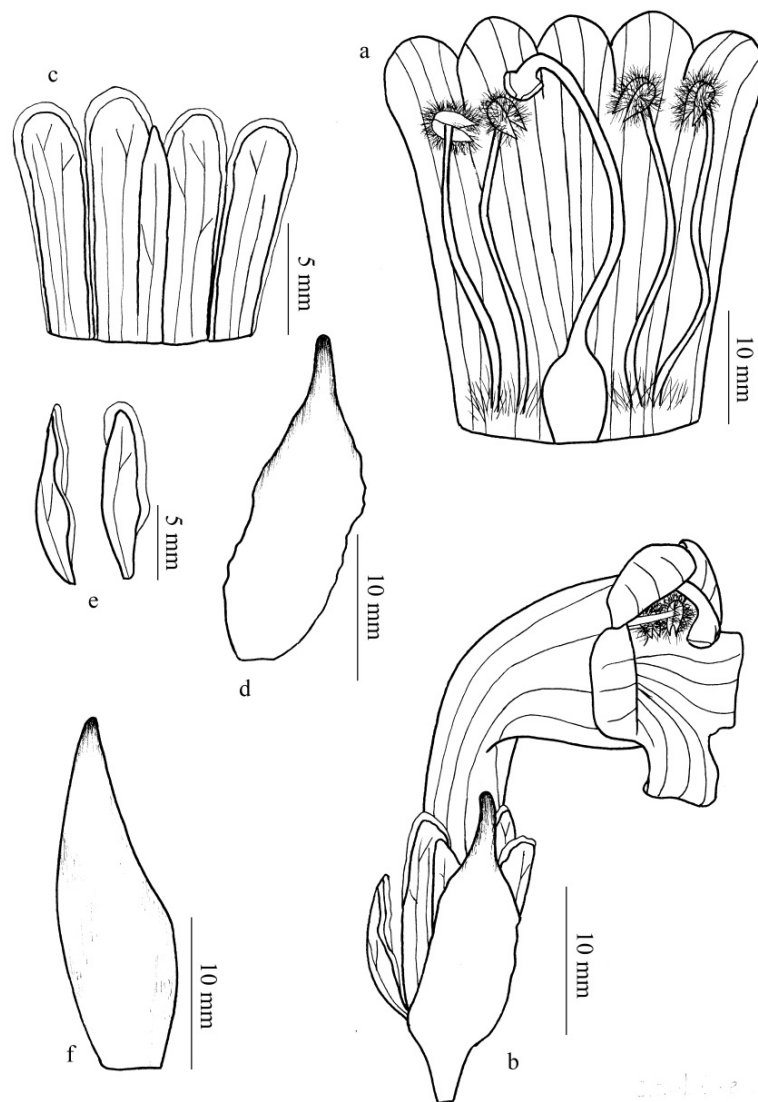


Figure 4.10 *Cistanche tubulosa* subsp. *iranica* Ataei subsp. nov. a) its flower inner view, b) single flower, c) abaxial calyx, d) bract, e) bracteoles, and f) upper scale. The plant occurred in Iran (*Ataei* ED517; BONN).

Plant robust, thick, 45–53 cm tall. **Lower scales** less dense and imbricate, ovate, rounded, 8–11 × (4–) 5–7 mm, non-scarious at the margins, yellow; **middle** and **upper scales** increasingly lax acropetally, ovate-lanceolate, rounded, (12–) 15–18 (–23) × (3–) 4–8 mm, non-scarious at the margins, yellow. **Inflorescence** an oblong spike, with many flowers, 17–35 cm long and 3–5.5 cm in diameter; **floral bracts** lanceolate, obtuse, (11–) 17–25 × (4–) 6–8 mm, usually non-scarious at the margins, slightly longer than calyx, yellow; **floral bracteoles** oblong-obolanceolate, rounded, 12–17 (–22) × (1–) 2–3 mm, slightly scarious at the margins, yellow; **calyx** usually campanulate, pentamerous, 12–18 (–22) mm long, usually longer than ½ total corolla length; **calyx lobes** 5, unequal (2 longer plus 2 medium plus one short dentate) oblong-orbicular, rounded, (3–) 5–7.5 × 4–7 mm, scarious at the margins, cream to light yellow; **corolla** pentamerous, infundibuliform, 38–42 mm long, dark-yellow, with orange veins inside the tube; **corolla lobes** 5, equal, rounded, 4–5 × 5–7 mm; **stamens** inserted at about 1/9 total corolla length, filaments 2/3 as long as the corolla, pilose-tomentose; **anthers** 3–5 mm long, cordate, rounded at base and acute at apex, pilose-tomentose; **ovary** ovate; **stigma** bilobate, white, non-exserted. **Capsules** apparently dehiscent with 2-valves. **Seeds** usually ovate (Fig. 4.10).

Distribution: Coasts of Omani Sea in South Iran (Fig. 4.3).

Additional specimens examined (paratypes): IRAN. **Sistan & Baluchestan:** Sarbaz to Chahbahar, Dempak village, roadside, 25°42'39"N, 61°22'25"E, 22 m, 6 March 2011, *Ataei* ED516 (BONN, USB); Chahbahar to Tis, Sadre Omid-e-Chahbahar, coast line, 25°22'24"N, 60°36'39"E, 15 m, *Ataei* ED518 (BONN, USB).

1.1.7 *Cistanche chabaharensis* Ataei, *sp. nov.*

Diagnosis: *Cistanche chabaharensis* is similar to *C. tubulosa*, but differs by its glabrous and acute anthers (versus tomentose and obtuse anthers in *C. tubulosa*), filaments that are glabrous at the base (versus basally highly tomentose filaments in *C. tubulosa*), and straight margins of scales, bracts and bracteoles (versus sinuate margins of scales, bracts and bracteoles in *C. tubulosa*). The species has been misdetermined as *C. tubulosa*.

Type: OMAN. **Central:** Duqm, 19°39'47"N, 57°42'10"E, Sea level, 6 March 1993, *McLeish* 1651 (holotype E barcode E00121976).

Plant thick, 20–50 (–150) cm tall, usually non-fragile when dry (in Omani specimens). **Lower scales** very dense, imbricate, ovate-triangular, rounded, 4–9 (–15) × 2.5–3.5 mm, entire and non-scarious at the margins, yellow to light grey; **middle** and **upper scales** increasingly lax acropetally, lanceolate, obtuse, (10–) 14–20 (–27) × 3–7 mm, entire and non-scarious at the margins, light grey. **Inflorescence** raceme with not many flowers, rarely lax, 9–17 cm long and 5.5–7 cm in diameter; **floral bracts** broad-lanceolate, obtuse, 13–19 (–25) × (3–) 6–10 mm, entire and non-scarious at the margins, longer than calyx by ca. 4–9 mm, usually they placed lower than calyx on the inflorescence

axis by pedicel about 2–5 mm, grey; **floral bracteoles** lanceolate to linear-oblong, obtuse, 8–14 × 1–3 mm, entire and non-scarious at the margins, slightly shorter than calyx, light grey; **calyx** usually tubular, 10–21 mm long; **calyx lobes** 5, 4 equal plus one shorter (sometimes 3 equal longer and 2 shorter), oblong-ovate rounded, (2–) 4–5.5 × 2.5–3 (–6.5) mm, slightly scarious at the margins, slightly longer than 1/3 total corolla length, yellow; **corolla** pentamerous, infundibuliform, downward curved, 34–46 mm long, pale-yellow; **corolla lobes** 5, ovate, rounded, rarely longer than wide, 4–6 × 6–7 mm, usually pinkish-mauve; **stamens** inserted at about 1/9 total corolla length, filaments 2/3 as long as the corolla, entirely glabrous; **anthers** 2.5–3 (–5.5) mm long, cordate, rounded at base and acute at apex (rarely acuminate in one specimen from Iraq: *Barkley & Abbas-Al-Ani* 6499, K or in Oman: *Al-Farsi* HA30, K), entirely glabrous; **ovary** tubular-elliptic or ovate-oblong; **stigma** bilobate, non-exserted. **Capsules** dehiscent with 2-valves. **Seeds** mostly oblong (Fig. 4.11).

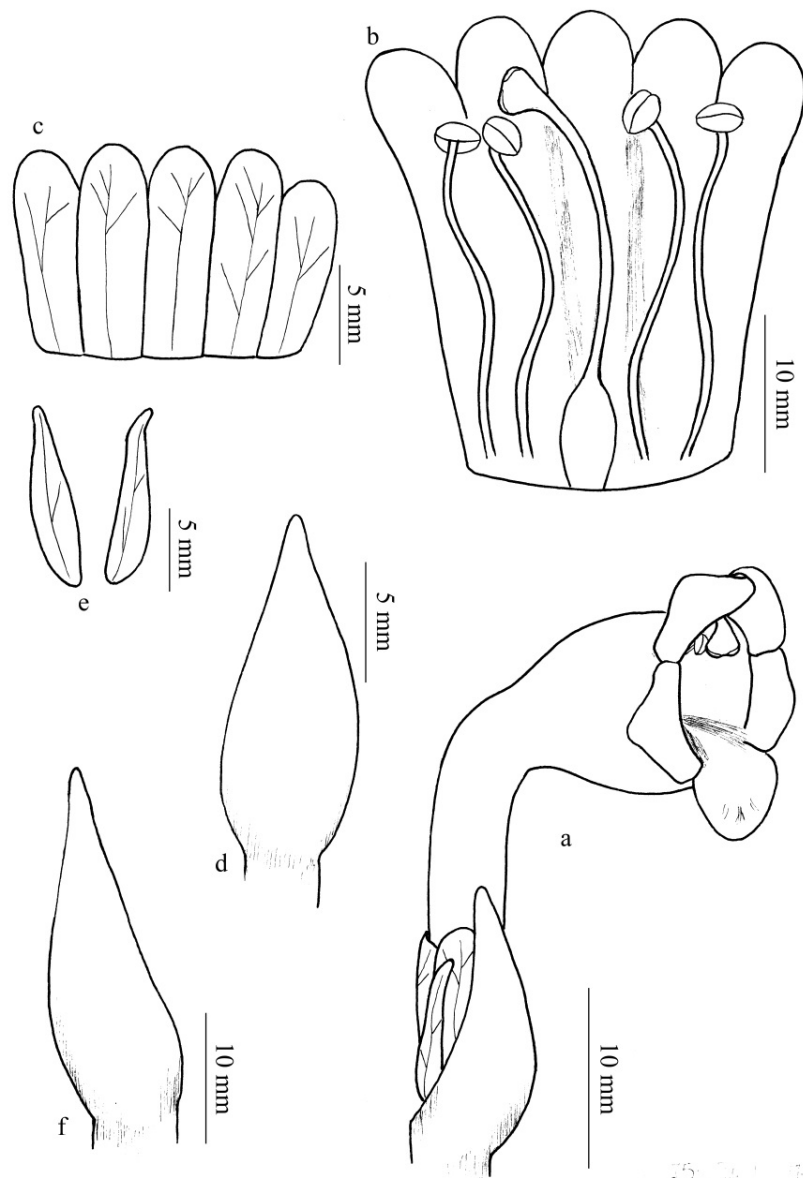


Figure 4.11 *Cistanche chabaharensis* Ataei sp. nov. a) single flower, b) its flower inner view, c) abaxial calyx, d) bract, e) bracteoles and, f) upper scale. The plant occurred in Oman (McLeish 966; E).

Distribution: From Algeria and Egypt to Central and Southern Arabian Peninsula (Fig. 4.3).

Additional specimens examined (paratypes): ALGERIA. Tamanrasset: In Ekker, 24°01'00"N, 05°05'00"E, 13 March 1981, *Podlech* 34661 (MSB). **BAHRAIN.** Sandy beach, just above high tide line, 8 February 1985, *Cornes* 235 (E). **EGYPT.** Wady Qrabah, S of Galalas, 25 March 1928, *Munipe* 5854 (K); Sinai Peninsula, Mualla, 29°21'00"N, 34°45'00"E, 5 February 1949, *Marshall* s.n. (BM). **OMAN.** **Zufār:** Dhofar, Raha beach 16 km East Mirbat, 16°57'45"N, 54°46'54"E, 13 January 1989, *McLeish* 966 (E); Kuria Muria Islands, Al Hallaniya, 16 October 1979, *Miller* 2767 (E); Mirbat, East Raha Beach, 16°51'00"N, 54°43'00"E, 0-250 m, 14 February 1993, *McLeish* 1547 (E); Around coastal lagoon 10 km E of Mirbat, 16°58'00"N, 54°42'00"E, 1 January 1985, *McLeish* 451 (E); Wadi Munloror [?], Arah, 04 March 1978, *Lawton* 2047 (BM); Al Hallaniyah Island, Dhofar beach, 2 m, 27 January 2000, *Al-Farsi* 30 (K); Central part, 100 km along roadside from Salalah to Nazwa, 22°05'00"N, 57°32'00"E, 21 January 1993, *McLeish* 1490 (E); Central part, vicinity of Mughshin, 19°28'00"N, 54°47'00"E, 150 m, 11 February 1984, *Gallagher* 6972/6 (E). **SAUDI ARABIA.** **Eastern Province:** Uqair, S of Dammam, 20 February 1987, *Collenette* 6025 (E). **UNITED ARAB EMIRATES.** **Abu Dhabi:** 25 February 1972, *Willcox* 200 (K); Old sewage farm, heavily saline, 28 February 1986, *Brown* 885 (E); Sharjeh, November 1949, *Guichard* K.G/4/Oman (BM); 9 km S Dubai, 20 February 1973, *Boulos* 5156 (BM); **YEMEN.** **Hadramawt:** Abd Al kuri island, north coast, 65 miles southwest Socotra in the Indian Ocean, 22 September 1966, *Keiln* A51 (K).

Host: Chenopodioideae: *Atriplex* sp. (*Miller* 2767, E), *Salsola* sp. (*McLeish* 1547, E), *Arthrocnemum macrostachyum* Torr. (*Brown* 885, E), *Suaeda vermiculata* Forssk. (*Cornes* 235, E).

1.1.8.1 *Cistanche flava* (C.A.Mey.) Korsh subsp. *flava*, Zap. Imp. Akad. Nauk Fiz.-Mat. Otd. 5 (1896);

≡ *Phelypaea flava* C.A.Mey., Verz. Pfl. Casp. Meer. 104 (1831) [basonym] ≡ *C. tubulosa* var. *flava* (C.A.Mey.) Beck, Bull. herb. Boissier 2. ser. 4: 686 (1904).

Type: In arena mobile versus mare Caspium prope Karvansarai. Ad Calligoni radices, 29 April 1830, *Meyer* Enum: cauc. casp. n° 899 (LE seen as photo).

= *Cistanche christisonioides* Beck, Pflanzenr. (Engler) 96, 4.261: 36 (1930). *Type: PAKISTAN. Punjab:* near Lahore, March 1857, *Schlagintweit* 10485 (lectotype W, selected here, isotype M barcode M0185673 seen as photo).

= *Cistanche flava* (C.A.Mey.) Korsh. subsp. *bicolor* Saeidi & Shahi, Iranian J. Bot. 14 (2): 96 (fig. 1) (2008). *Type: IRAN. Semnan:* Touran protected area, 3 km from Chah-jam to Toroud, 1100 m, 23 April 1978, *Freitag & Mozaffarian* 28437 (holotype TARI seen as photo).

Plant robust, 35–100 cm tall, with purple stem. **Lower scales** sparse, slightly imbricate, long deltoid hastate-lanceolate, obtuse, 9–15 × 5–12 mm, entire, non-scarious at margins, light-grey to light-

purple; **middle** and **upper scales** increasingly lax acropetally, very long-lanceolate, obtuse, 40–85 × (3–) 5–9 (11–) mm, often densely arranged on the nodes over the stem, in which the distance between nodes is about 2–3.5 cm, entire, slightly scarious at the margins, sometimes sparsely ciliate at the margins, light-grey to purple. **Inflorescence** raceme with dense and numerous flowers, (4.5–) 20–43 cm long and 6–8.5 in diameter; flowers at lower part of inflorescence with pedicels about 0.6–1 cm long; **floral bracts** oblong-lanceolate, (22–) 30–47 × (2.5–) 5–7 (–11) mm, serrate, twice as long as calyx or even as long as corolla, grey to purple; **floral bracteoles** oblong-linear, obtuse, (17–) 24–37 (–47) × 5–7 (–12) mm, slightly serrate at their margins, entire, non-scarious at the margins, slightly shorter than calyx, grey to purple; **calyx** tubular-campanulate, with 5 lobes, more deeply incised between 2 lobes than between the remaining 3, (10–) 14–20 (–27) mm long; **calyx lobes** 5, sub-equal with 3 lobes slightly longer than the 2 others or (2 longer, 2 slightly shorter plus one single shorter), rarely equal, oblong-rounded, (2.5–) 4–6.5 (–11) × 2–5 (–7) mm, entire, non-scarious at the margins; **corolla** campanulate, pentamerous, 37–60 mm long, yellow-white outside with 2 or 5 irregular orange veins inside the tube; **corolla lobes** 5, yellow or blue-violet, 5–9 × (4–) 7–10 mm, **stamens** inserted at about 1/7 of total corolla length, filaments 2/3 as long as the corolla, at base villous; **anthers** (2–) 4–4.5 mm long, densely tomentose, rounded at base and obtuse or acute, rarely apiculate or sometimes mucronate at apex; **stigma** broad club-shaped, white, non-exserted. **Capsule** opens by 2-valves. **Seeds** almost ovate (Fig. 4.12).

Distribution: Largely in South West Asia, rarely in Saudi Arabia, Iraq, Iran, Pakistan and India (Fig. 4.3).

Additional specimens examined: **AFGHANISTAN.** Kandahar: 40 km E, sand desert near Tachta Pul at Dori River, 1100 m, 22 April 1968, *Freitag* 2583 (KAS); Between Sir Cahan and Spin Buldak, 1200 m, 21 March 1969, *Freitag & Breckle* 4639 (KAS). **INDIA.** **Rajasthan:** Thar Desert, outside village of Satyaya, 27°25'00"N, 71°40'00"E, 25 February 1983, *Jachson & Taylor* 6 (K); **Punjab:** 16 March 1904, *Drummond* 23853 (K); Umballa [Ambala?], October 1917, s. col. s.n. (K). **IRAN.** **Razavi Khorasan:** Sabzevar, Norood-Abad village, Kalate-e-MirAli, 35°55'23"N, 57°09'23"E, 922 m, 29 March 2011, *Ataei* ED522 (BONN, USB); Kuh-e Patuketeh, 18 March 1970, *Monod* 14579 (P); Sabzevar to Solh-Abad, Sabri village, 35°44'06"N, 56°55'14"E, 1043 m, 30 March 2011, *Ataei* ED524 (BONN, USB); From Roodab Parand to cement factory, 35°53'26"N, 57°04'48"E, 944 m, 29 March 2011, *Ataei* ED523 (BONN, USB); Savzevar to SolhAbad, after Talkhab village, 35°33'06"N, 56°54'47"E, 1165 m, 30 March 2011, *Ataei* ED541 (BONN, USB); **Semnan:** Shahrud, 5 km after Kariz village, 35°36'08"N, 56°49'53"E, 1122 m, 30 March 2011, *Ataei* ED525 (BONN, USB); Between Semnan and Yazd, at the desert between Nain and Aqda, 1000 m, 21 April 1948, *Rechinger* 2704 (E, B); Shahrud, 5 km after Kariz village, 35°36'08"N, 56°49'53"E, 1122 m, 30 March 2011, *Ataei* ED542 (BONN, USB); 12 km Ahmad-Abad to Zaman Abad, roadside, 35°39'06"N, 56°38'39"E, 1093 m, 28 April 2010, *Ataei* ED449 (BONN, USB); Zaman Abad-Bardaskan, detour road Zaman-Abad, 2 km to Kalateh Karbalai Abbas, 35°37'24"N, 56°46'46"E, 1105 m, 29 April 2010, *Ataei & Heidari* ED450 (BONN, USB); Maimay to Biarjmand, 24 km Biarjmand, 36°07'54"N, 55°48'52"E, 1152 m, 28 April

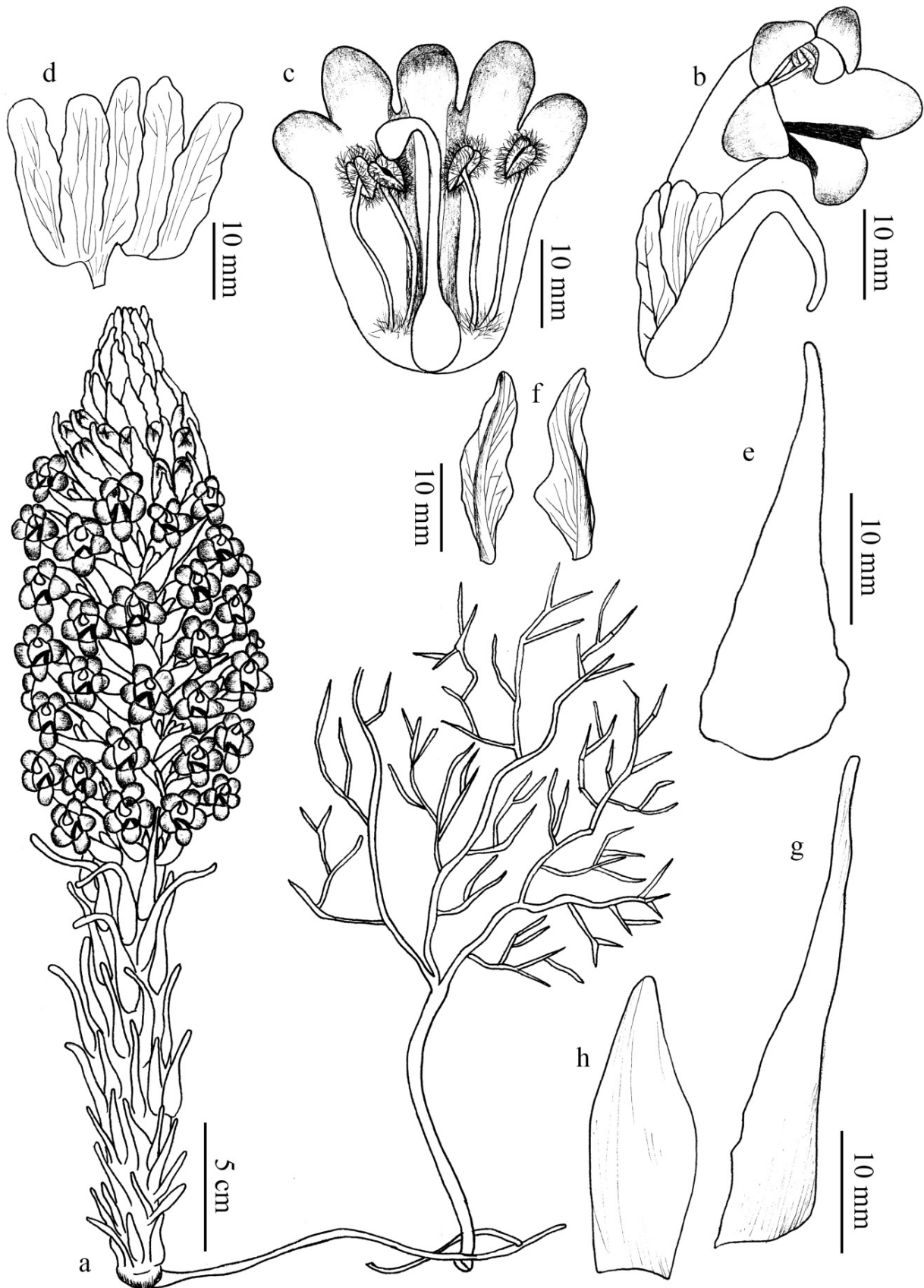


Figure 4.12 *Cistanche flava* (C.A.Mey.) Korsh subsp. *flava*. a) flowering plant and partially its host *Haloxylon persicum* Bunge ex Boiss. & Buhse. b) single flower, c) its flower inner view, d) abaxial calyx, e) bract, f) bracteoles, g) upper and h) lower scales. The plant occurred in Iran (Ataei ED519; BONN).

2010, *Ataei* ED446 (BONN, USB); **Isfahan:** Kolah Ghazi National Park (Kuh-e Kolah Qazi), 32°23'00"N, 51°49'00"E, 1700-2000 m, 29 May 1974, *Rechinger* 46697 (W); **Tehran:** Kavir National Park, Siah Kuh, Karvansarai Shah Abbas, 34°44'00"N, 52°10'00"E, 1100-1300 m, 23 April 1975, *Rechinger* 50123 (B, W); Shahrud, Kalat-e Hizomi, 35°40'12"N, 56°35'51"E, *Shahi & Saeidi* 13230 (GIUH); Shahrud, Khar Turan National Park, 36°49'00"N, 55°50'00"E, *Shahi & Saeidi* 13238 (GIUH); **Sistan & Baluchestan:** 120 km Zahedan to Bam, Nosrat Abad region, roadside, 29°43'01"N, 59°49'27"E, 1061 m, 19 March 2010, *Ataei & Heidari* ED432 (BONN, USB); In Nosratabad toward west, 29°50'00"N, 60°00'00"E, 1200 m, 24 July 1977, *Rechinger* 54650 (B); Nikshahr to Iranshahr, after Spage village, 2 km NokAbad, 26°55'03"N, 60°30'57"E, 764 m, 8 March 2011, *Ataei* ED520 (BONN, USB); 50 km Iranshahr, after Nok-Abad village, 26°55'08"N, 60°31'18"E, 847, 8 March 2011, *Ataei* ED519 (BONN, USB); **Kerman:** 78 km Kerman to Rafsanjan, Kabootarkhan to Bagherabad, the beginning of Tehran road, 30°18'10"N, 56°18'34"E, 1618 m, 20 April 2010, *Ataei & Heidari* ED426 (BONN, USB); Jiroft, Ahmad-ahad (Anbarabad!), 19 March 1971, *Iranshahr & Termé* W 1972-0002924 (W); 10 km E of Nosratabad Sipi, 27 March 1965, *Lamond* 111 (E). **IRAQ.** Shahraban, occasional on slope of large sand dunes, 11 April 1957, *Haines Wheeler* 847 (E). **PAKISTAN.** **Baluchestan:** Sibi, Februray 1889, *Lace* 3461 (E); Surkhab, May 1889, *Lace* 3387 (E). **SAUDI ARABIA.** Mahd adh dhahab probably, 23 March 1966, *Paris* s.n. (BM); Central Arabia, 1914, *E. pt. Shakespear WHI* s.n. (BM). **TURKMENISTAN.** Ashabat, July 1897-1898, *Litwinow* 1856 (K); Lebap, st. v. f. Repetek, 25 April 1925, *Vvedensky* 6499 (W, BR, BM, K). **UZBEKISTAN.** **Buchara:** Chirabad, Patta-Kum sands, 11 April 1906, *Roshewitz* 229 (P).

Host: **Amaranthaceae:** *Haloxylon persicum* Bunge ex Boiss. & Buhse (*Freitag* 14695, B) and field observations (this study); **Ephedraceae:** *Ephedra* sp. (*Lamond* 111, E); **Salvadoraceae:** *Salvadora* sp. (*Lace* 3461, E).

1.1.8.2 *Cistanche flava* subsp. *brevibracteata* Ataei, subsp. nov.

Diagnosis: *Cistanche flava* subsp. *brevibracteata* differs from the nominate subspecies by its shorter scales and bracts (versus longer in *flava*), corolla lobes with sinuate margins (versus entire lobes in *flava*) and yellow corolla with dispersed orange veins inside the tube (versus yellow-white tube with blue-violet lobes with 2 or 5 orange veins inside the tube in subspecies *flava*). The subspecies has been misdetermined as *C. flava*.

Type: **IRAN. Southern Khorassan:** 40-42 km after Nehbandan towards Zabol, 31°29'29"N, 60°36'34"E, 807-825 m, 30 March 2011, *Akhani, Noormohammadi & Samadi* 22074 (holotype TUH).

Plant robust, up to 40 cm tall, yellow. **Lower scales** dense, imbricate, deltoid with two auricles, obtuse, 9–13 × 5–6 mm, entire, non-scarious at margins, light-brown; **middle** and **upper scales** slightly lax, ovate-oblong, obtuse, 33–47 × (2.5–) 3–4 mm, non-scarious at the margins, light-grey. **Inflorescence** an oblong spike, with dense and numerous flowers, 20 cm long and 6 cm in diameter;

floral bracts oblong-lanceolate, 33–37 × 4–5.5 mm, non-scarious at the margins, exceeding than calyx by 4–9 mm; **floral bracteoles** oblong, obtuse, 18–20 × 1.5–3.5 mm, obtuse, entire, non-scarious at the margins, equal to or slightly shorter than calyx; **calyx** tubular, pentamerous, shortly incised, 19–24 mm long; **calyx lobes** 5, equal, ovate-rounded, 5–7 (–9) × 7–8 mm, entire, non-scarious at the margins; **corolla** campanulate, pentamerous, 38–43 mm long, yellow with dispersed orange veins inside the tube; **corolla lobes** pentamerous, yellow, 4–6 × 6–8 mm, **stamens** inserted at about 1/7 total corolla length, filaments 2/3 as long as the corolla, at base villous; **anthers** (2–) 3–4 mm long, densely tomentose, rounded at base and acute at apex; **stigma** broad club-shaped, white-yellowish, non-exserted. **Capsule** opens by 2-valves. **Seeds** almost ovate.

Distribution: Northern Iran (Fig. 4.3).

Additional specimens examined (paratypes): IRAN. **Bushehr:** N of Kaki, Mond river, low sand dunes on S. bank, 100 m, 27 March 1974, *Davis & Bokhari* D.55976 (E); **Semnan:** Shahrud to Torud, 2 km Chejam, roadside, 35°47'50"N, 55°09'36"E, 1102 m, 27 April 2010, *Ataei* ED445 (BONN, USB); Khar Turan National Park, Shahrud, 1.5 km SW of Chejam, 35°49'01"N, 55°06'57"E, 1100 m, 23 March 1978, *Freitag* 14695 (B); 46 km Biarjmand, 101 km Miami to Ahmad Abad, 35°53'40"N, 56°18'47"E, 769 m, 28 March 2010, *Ataei* ED447 (BONN, USB); Shahrud, Bastam, 8 km Delbar versus Ahmadabad, 35°52'00"N, 56°17'00"E, May 1975, *Iranshahr* 04409 (E, W); 78 km Zaman-Abad to Bardaskan, between Ismail Abad and Ghaleh, 2 km down the road, 35°07'01"N, 57°13'07"E, 803 m, 29 April 2010, *Ataei* ED451 (BONN, USB).

Host: Unknown.

1.1.9.1 *Cistanche rosea* Baker subsp. *rosea* Hooker, Icon. Pl. 24: 2363 (1895);

Type: YEMEN. **Hadramawt:** Tokham vicinity of Mokalla, about sea level, *Lunt* 62 (holotype K barcode K000999864, isotypes BM barcode BM000606292, P barcode P02983470).

Plant usually short and thin, (5–) 20–30 cm tall, pinkish-rose. **Lower scales** very dense, imbricate, ovate-triangular, obtuse, 7–9 × 2 mm, non-scarious at the margins, rose-pinkish; **middle** and **upper scales** ovate-oblong, obtuse, 12–14 × 3–4 mm, slightly lax, non-scarious at the margins, rose-pinkish. **Inflorescence** an ovate-oblong spike, compact, 5–13 cm long and 4–6 cm in diameter; **floral bracts** ovate-oblong, sometimes obovate-rhomboid, obtuse, 10–14 × 4 mm, slightly shorter than calyx, rose-pinkish; **floral bracteoles** linear-oblong, obtuse, 11 × 3 mm, ca. 2/3 the length of the calyx or slightly longer, rose-pinkish; **calyx** tubular rarely campanulate, pentamerous, with 2 deeper and 3 shallower incisions, 12–15 mm long, rarely scarious at the margins, rose-pinkish; **calyx lobes** 5, unequal (posterior lobe smaller and less fused than remaining lobes), oblong, rounded, 5–6 × 4 mm; **corolla** infundibuliform, curved downwards, (20–) 28–30 mm long, twice as long as calyx, rose-pinkish;

corolla lobes 5, unequal (upper longer than lateral longer than lower), lateral lobes may be accompanied by two short dentate segments, ovate, rounded, 3.5–4 × 3–3.5 mm, rose-pinkish or proximally white to pale pink; **stamens** inserted in 1/5 total corolla length, filaments as long as corolla or slightly longer, prominently exserted, slightly pilose at the base; **anthers** 2.5 mm long, sagittate at base and acute at apex, lanate, cream-yellow; **ovary** ovate; **stigma** club-shaped, red with orange ring at apex, prominently exserted. **Capsule** ovate-elliptic, dehiscent with 2 valves. **Seeds** usually triangular (Fig. 4.13).

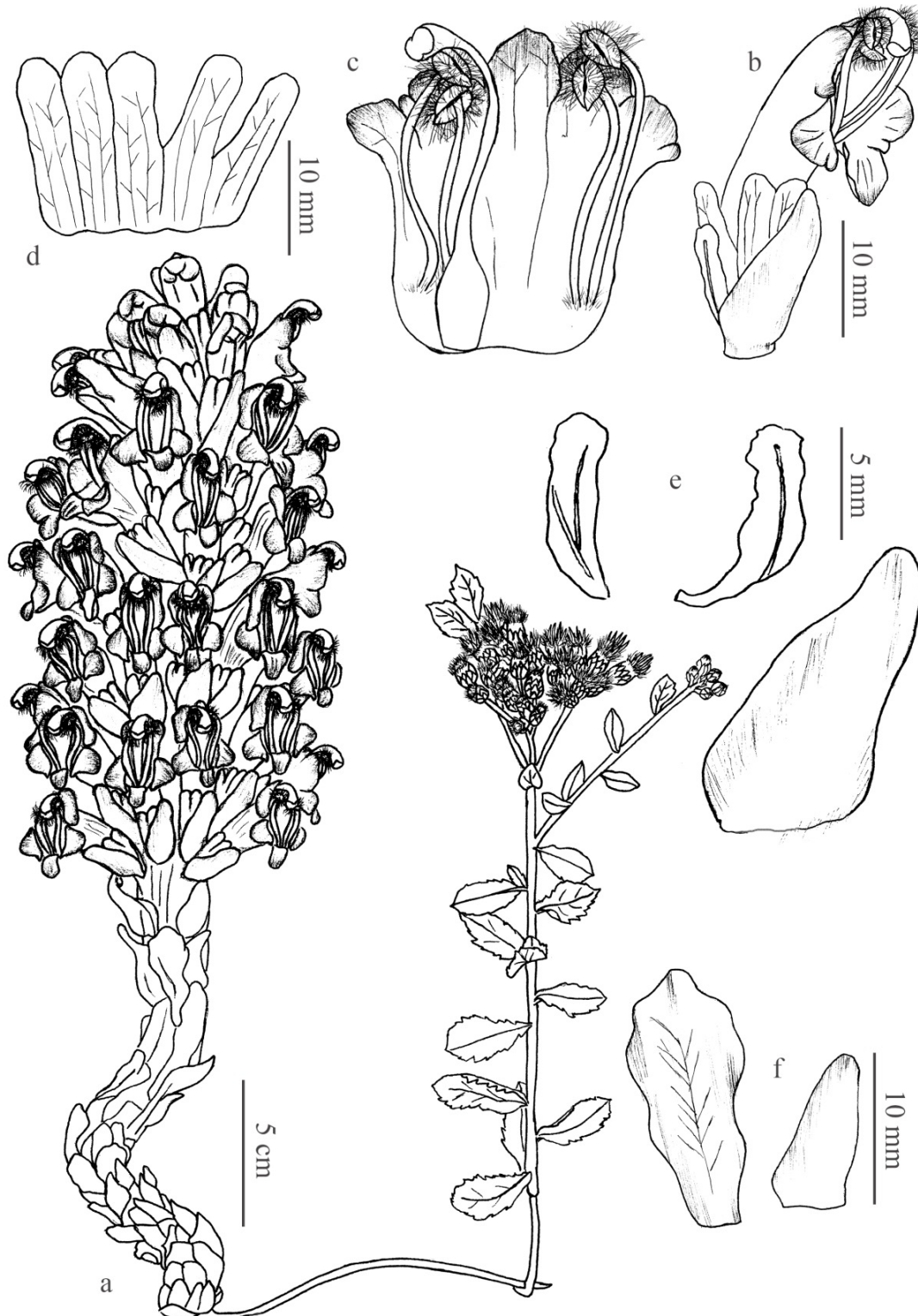


Figure 4.13 *Cistanche rosea* Baker subsp. *rosea*. a) flowering plant and partially its host *Pluchea dioscoridis* (L.) DC. b) single flower, c) its flower inner view, d) abaxial calyx, e) bract and bracteoles, f) lower and upper scales. The plant occurred in Yemen (Lunt 62; K).

Distribution: Mainly Southern Arabian Peninsula (Fig. 4.3).

Additional specimens examined: **YEMEN:** **Al Mahrah:** Wadi Dhahawn, Al Ghaydah, 16°13'10"N, 52°12'12"E, 13 March 1989, *Miller & al.* 8168 (K); **Haḍramawt:** Wadi Hajr, 100 km W of Mukalla, Mintaq, 420 m, 13 February 1989, *Miller, Guarino, Obadi, Hassan & Mohammed* M.8168 (E, K); Near Ta'izz, at the edge of cultivated land, 1300 m, 26 December 1975, *Wood* JRI Y/75/1002 (BM). **OMAN.** **Zufār:** Dhofar, half way of Jebel Qara, 11 March 1978, *Lawton* 2012 (BM); Salalah, c. 3 km beyond Ain Garziz, c. 3 km SW Ain Garziz, 17°06'35"N, 54°03'44"E, 50 m, 19 February 2005, *Schnittler & Shadwick* 24318 (SQU); Wadi Adownib, 15 km W of Raysut on Mughshahl road, wadi sides, 16°57'00"N, 53°52'00"E, 100 m, 3 October 1979, *Miller* 2491 (E); Wadi Mugshin, 19°35'00"N, 53°47'00"E, 4 March 1994, *McLeish* 3434 (E); Salalah to Thumrait road, 7 km N or Ravens Roost, 600 m, 4 October 1979, *Giller* 2521 (E); **Ash Sharqiyah:** Ayun, 17°15'13"N, 53°53'30"E, 800 m, 21 December 1988, *Whitehead* 837 (K); 2 km E of Ayn Wadi, 16°58'02"N, 54°53'00"E, 1-250 m, 23 November 1993, *McLeish* 2597 (E). **SAUDI ARABIA.** Dahmah, 5400 m, 10 November 1934, *Ingrams* 63 (BM); East road, near Raidat Elamara, 14 November 1950, *Guichard* KG/Had/419 (BM); **Northern Borders:** Dahal Abu `Araqi, 05 April 1947, *Thesiger* s.n. (BM).

Host: **Asteraceae:** *Pluchea dioscoridis* (L.) DC. (Baker, 1895; Foley 2004); **Fabaceae:** *Acacia* sp. (*Miller & al.* M.8168, E, K, UPS); **Burseraceae:** *Boswellia* sp. and *Commiphora* sp. (*Miller* 2491, E).

Remarks: In contrast to consideration heterogeneous calyx for delimitation of the *C. rosea* as determined by Beck, rose-pinkish or crimson corolla color besides oblong-rhomboid bract morphologically discriminate the subspecies.

1.1.9.2 *Cistanche rosea* subsp. *crimson* Ataei, subsp. nov.

Diagnosis: *Cistanche rosea* subsp. *crimson* differs from the nominate subspecies by its orange and translucent corolla (versus pink-reddish and not translucent corolla in subspecies *rosea*), in its crimson scales, bracts and bracteoles (versus rose-pinkish in subspecies *rosea*) and floral bracteoles longer than floral bracts and calyx (versus shorter in subspecies *rosea*).

Type: **SAUDI ARABIA.** **Jizan:** Rugged coral country N.N.E. of Farasan Island, c. 30 m, 5 February 1985, *Collenette* 5028 (holotype E barcode E00029711, isotype K).

Plant usually short and thin, up to 30 cm tall, crimson-rose. **Lower scales** very dense, imbricate, ovate-triangular, obtuse, 4–5 × 3–4 mm, non-scarious at the margins, crimson-rose; **middle** and

upper scales slightly lax, ovate-oblong, obtuse, 12–17 × 3–4 (–6.5) mm, non-scarious at the margins, crimson-rose. **Inflorescence** an ovate-oblong spike, dense, 18 cm long and 9 cm in diameter; **floral bracts** obovate-oblong or obovate-rhomboid, obtuse, 9–15 × 5–8 mm, non-scarious at the margins, slightly shorter than to sometimes as long as calyx, crimson; **floral bracteoles** oblanceolate, 15–25 × 2 mm, obtuse, entirely scarious, slightly longer than calyx, pinkish-crimson; **calyx** tubular, pentamerous, shortly incised, 14–16 mm long, entirely scarious, pinkish; **calyx lobes** 5, equal, oblong, rounded, 6–6.5 × 4.5–5 mm, veined, pinkish; **corolla** pentamerous, long tubular, abruptly infundibuliform, (20–) 28–30 mm long, very fine and membranaceous, twice as long as calyx, translucent, orange; **corolla lobes** 5, equal, overlapping, ovate, rounded, veined, 5 × 9–10 mm, orange-crimson; **stamens** inserted in 1/6 total corolla length, filaments as long as corolla or slightly longer; **anthers** 3 mm long, acute at apex, slightly lanate, orange; **ovary** long-tubular; **stigma** club-shaped, orange, prominently exserted. **Capsule** obovate, dehiscent by 2-valves. **Seeds** usually triangular (Fig. 4.14).

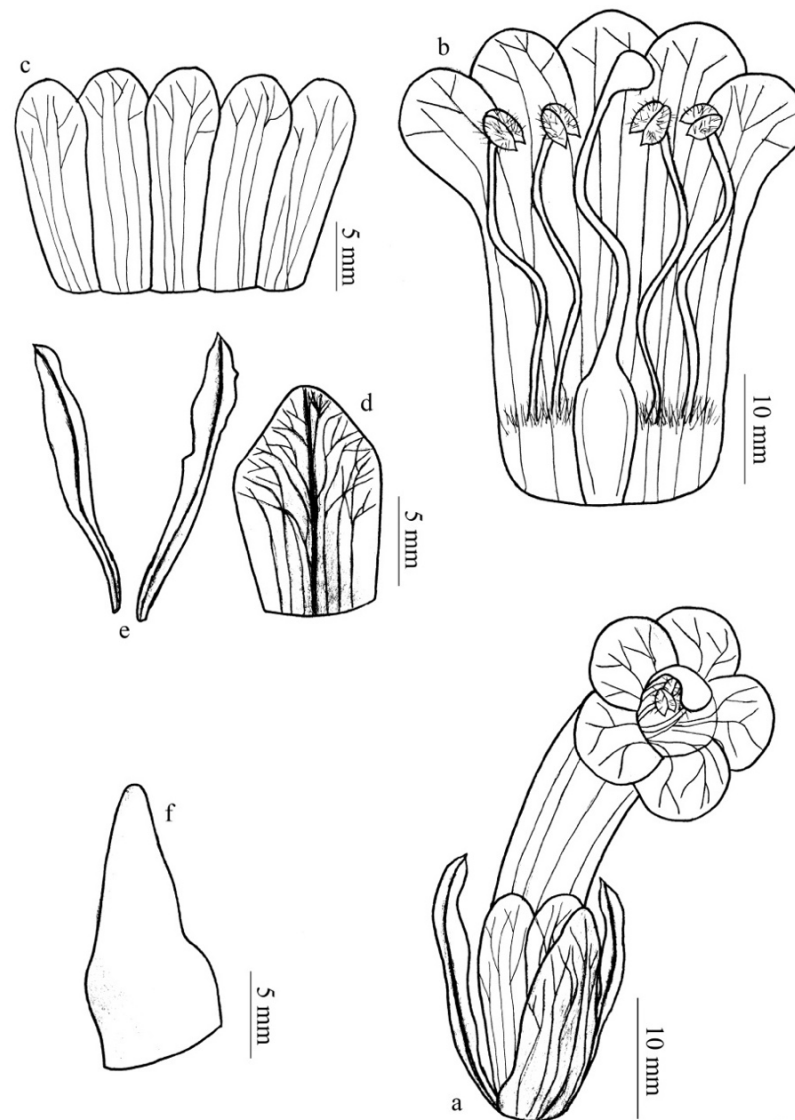


Figure 4.14 *Cistanche rosea* subsp. **crimson** Ataei subsp. nov. a) single flower, b) its flower inner view, c) abaxial calyx, c) bract, e) bracteoles, and f) upper scale. The plant occurred in Saudi Arabia (Collenette 5028).

Distribution: Only known from the type locality in western Saudi Arabia (Fig. 4.3).

Host: Burseraceae: *Commiphora gileadensis* (L.) C.Chr., or **Salvadoraceae:** *Salvadora persica* L. (*Collenette* 5028, K, E).

1.1.10.1 *Cistanche laxiflora* Aitch. & Hemsl. subsp. *laxiflora* Trans. Linn. Soc. London, Bot. 3(1): 94, t. 40. (1888);

Type: **AFGHANISTAN. Herat:** Harirud-valley, April–May 1885, *Aitchison* 1093 p.p. (K barcode K000449427).

= *Cistanche mongolica* Beck, *Pflanzenr.* (Engler) 96, 4.261: 34 (1930); *Type:* **CHINA. Xinjiang,** Kashgaria, Kuen-Lun (Kun-lun), ridge Russkii (Russkij Chrebet), *Przewalski* (lectotype LE, selected by Novopokrovskii & Tzvelev 1958: 22 not seen). Remaining syntype: **UZBEKISTAN. Buchara:** Patta Kum im Sande, 1906, *Roshewitz Pl. Buchar n.* 229 (B).

Plant usually robust, 43–110 cm tall, white to light-greyish. **Lower scales** oblong-lanceolate, sometimes deltoid, slightly imbricate, obtuse, 8–13 × 5–11 (–15) mm, entire, non-scarious at the margins, dark-grey; **middle** and **upper scales** increasingly lax acropetally, ovate-lanceolate, obtuse, 14–28 (–35) × 3–6 (–13) mm, entire, non-scarious at the margins, dark-grey. **Inflorescence** raceme with lax flowers and pedicel about 5–25 mm long at lower part, 10–30 cm long and 3.5–7 cm in diameter; **floral bracts** ovate-lanceolate, obtuse, 21–27 (–33) × 4–8 (–14) mm, very sparsely villous at the margins (in North Iran specimens, emerging at about 5 mm below the flower when mature), entire, non-scarious at the margins, exceeding the calyx, dark-grey; **floral bracteoles** oblanceolate, acute, rarely obtuse, 10–18 (–30) × (1.5–) 4–7 (–10) mm, entire, non-scarious at the margins, usually as long as calyx, sometimes shorter, greyish-purple; **calyx** tubular, 19–26 mm long, half as long or slightly longer than corolla; **calyx lobes** 5, sub-equal with 3 longer and 2 shorter ones, ovate, obtuse, 4–8 (–11) × (3–) 5–7 (–9) mm, glabrous except in North Iran, which is on the inner side very sparsely villous, slightly scarious at the margins, cream; **corolla** infundibuliform, pentamerous, tube white, with two irregular orange lines inside the tube, 40–50 mm long, sparsely hairy on the inner side in some flowers; **corolla lobes** 5, equal, with unevenly light-lilac veins, white at the youngest stage outer side mauve, longer than wide, 4–7 (–11) × 6–10 mm; **stamens** inserted at about 1/8 total corolla length, filaments 2/3 as long as the corolla, densely villous at base, sparsely hairy elsewhere; **anthers** 3–5 mm long, rounded at base and sub-obtuse at apex, densely villous; **ovary** tubular-elliptic, dark brown when dry; **stigma** disk-shaped and bilobate, glandular, white, non-exserted. **Capsules** dehiscent with 2-valves. **Seeds** oblong rarely long ovate.

Distribution: Central and Northeastern Iran, Afghanistan, Uzbekistan and China in Xinjiang (Fig. 4.3).

Additional specimens examined: AFGHANISTAN. Herat: Harirud-valley, 34°38'00"N, 61°13'00"E, May 1885, s. col. s.n. (K); C. 5 km Tirpol to Islam Ghaleh, 2 km roadside, in the riverbed of Harirud, 726 m, 8 May 2010, *Ataei* ED480 (BONN, USB); Lower Punjab [mentioned in Afghanistan, [likely India?], s. col. s.n. (K); North Adraskan, Stony slopes, 08 May 1969, *Wendelbo, Hedge & Ekberg* W 7725 (E). IRAN. Sistan & Baluchestan: Zahedan to Bam, 5 km RostamAbad, 28°58'12"N, 58°45'18"E, 704 m, 11 March 2011, *Ataei* ED521 (BONN, USB); Saravan to Suran, NaserAbad, Chah Mahmood, 27°26'16"N, 61°44'47"E, 1186 m, 1 March 2011, *Ataei* ED512 (BONN, USB); Khash to Iranshahr, Daman village, riverbed, 27°21'29"N, 60°46'59"E, 695 m, 5 March 2011, *Ataei* ED515 (BONN, USB); Iranshahr to Sarbaz, *Ghahreman & Mozaffarian* 14056 (IRAN); Razavi Khorasan: C. 70 km Nishabur to Kashmar, 2 km down detour road of Chelpo village, 1873, 35°37'29"N, 58°30'07"E, 31 April 2010, *Ataei* ED478 (BONN, USB); 70 km Nishaboor to Kashmar, 35°49'00"N, 58°32'00"E, *Assadi & Mozaffarian* 35494 (TARI).

Host: Tamaricaceae: *Tamarix ramosissima* Ledeb. (*Ataei* ED478, BONN & USB), *Tamarix aphylla* (L.) Karst. (*Ataei* ED512, BONN & USB).

1.1.10.2 *Cistanche laxiflora* subsp. *alba* *Ataei*, subsp. nov.

Diagnosis: *Cistanche laxiflora* subsp. *alba* differs from the nominate subspecies mainly by having light-grey scales and bracts (versus dark-grey in subspecies *laxiflora*), light-purple corolla lobes outside (versus white corolla lobes both on the outer and inner side in subspecies *laxiflora*) and spike inflorescence (versus raceme in subspecies *laxiflora*). The species has been misdetermined as *C. tubulosa*.

Notes: regardless of less morphological variables between these two subspecies, strong molecular data proved its differentiation.

Type: IRAN. Kerman: about 5 km on road from Rafsanjan to Mese-Sarcheshmeh, 30°19'05"N, 56°01'33"E, 1576 m, 20 April 2010, *Ataei & Heidari* ED433 (holotype BONN, isotype USB).

Plant up to 44 cm tall, white-yellowish. **Lower scales** oblong-lanceolate, obtuse, 10–12 × 7–9 mm, entire, non-scarious at the margins, light-greyish; **middle scales** lax, ovate-lanceolate, 15–16 × 6.5–7 mm, light-greyish; **upper scales** increasingly lax acropetally, lanceolate, obtuse, 25–30 × 4.5–5 mm, entire, non-scarious at the margins, light-greyish. **Inflorescence** an ovate-oblong spike, with lax flowers, up to 29 cm long and 6 cm in diameter; **floral bracts** lanceolate, obtuse, 26–27 × 5–6 mm, entire, non-scarious at the margins, exceeding the calyx, light-greyish; **floral bracteoles** oblanceolate, acute, rarely obtuse, 11 × 1.5–2 mm, entire, non-scarious at the margins, usually as long as calyx, light-greyish; **calyx** tubular, 15–17 mm long, about half long as the corolla; **calyx lobes** 5, sub-equal, 3 longer and 2 shorter ones, ovate, obtuse, 4–4.5 × 3–3.5 mm, slightly scarious at the margins, cream-greyish; **corolla** infundibuliform, pentamerous, tube white, with two irregular orange veins inside the tube, 40–42 mm long; **corolla lobes** 5, equal, spreadingly light-purple outside, usually longer than wide, 7 × 6–7 mm; **stamens** inserted at about 1/8 total corolla length, filaments

2/3 as long as the corolla, densely villous at base and sparsely villous elsewhere; **anthers** 3–5 mm long, rounded at base and sub-obtuse at apex, densely villous; **ovary** tubular-elliptic, dark brown when dry; **stigma** disk-shaped and bilobate, glandular, white, non-exserted. **Capsules** dehiscent with 2-valves. **Seeds** oblong rarely long ovate (Fig. 4.15).

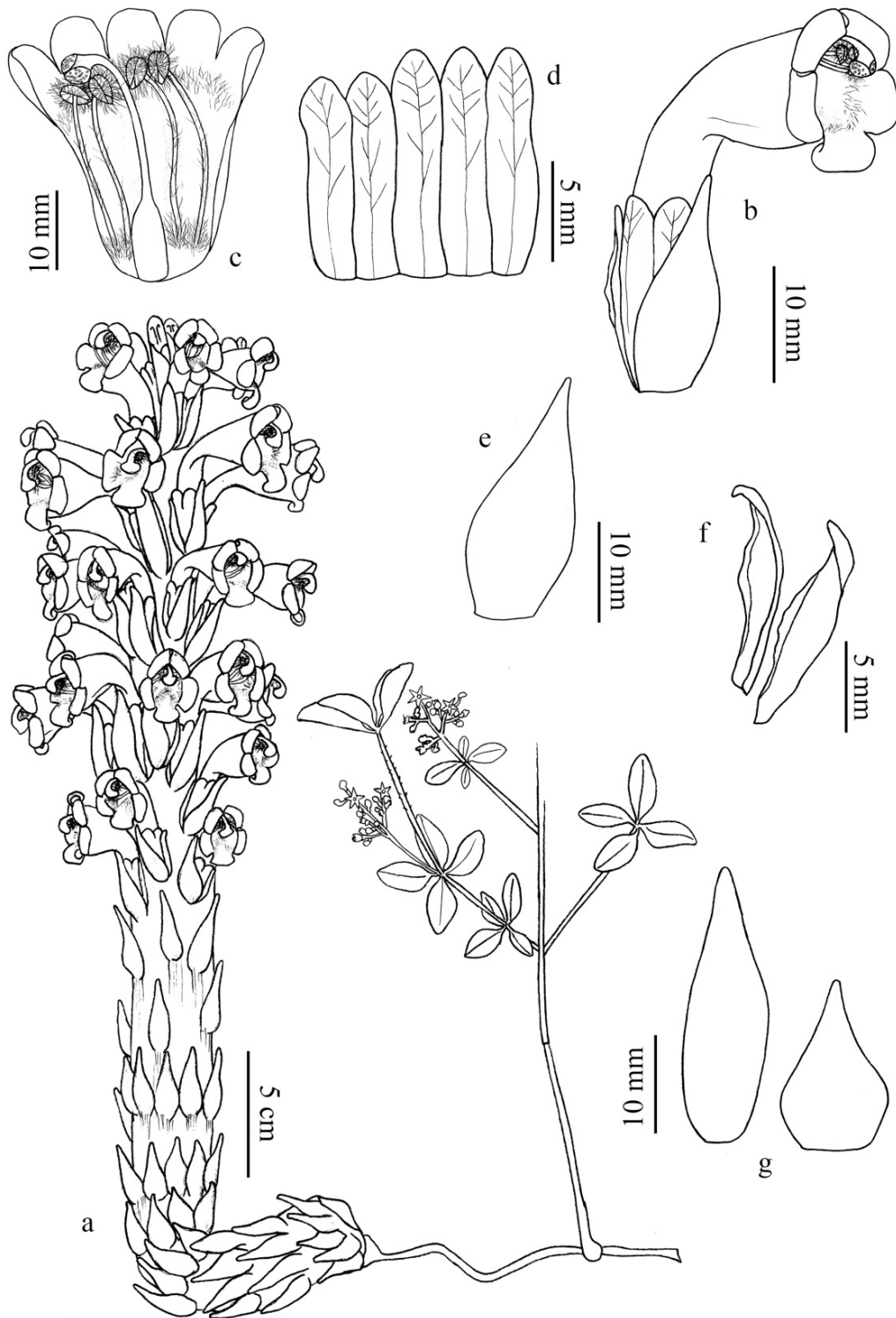


Figure 4.15 *Cistanche laxiflora* subsp. *alba* Ataei subsp. nov. a) flowering plant and partially its host *Rubus caecius* L. b) single flower, c) its flower inner view, d) abaxial calyx, e) bract f) bracteoles, and g) lower and upper scales. The plant occurred in Iran (Ataei ED433).

Distribution: Southwestern Saudi Arabia, Central and Southeastern Iran (Fig. 4.3).

Additional specimens examined (paratypes): SAUDI ARABIA. Al Bahah: Between Al Bahah and Jabal Ibrahim, off the Taif road, 1829 m, 19 April 1982, *Collenette* 3566 (E). **IRAN.** Yazd: Bafq road to Bagherabad, down the road, 31°36'53"N, 55°23'46"E, 997 m, 21 April 2010, *Ataei & Heidari* ED435 (BONN, USB); Between Yazd and Kerman, between Anar and Bahramabad (Rafsanjan), 22 April 1948, *Schiman-Ozeika* 2860 (M); Ardakan, Toot & Anjirvand, 32°31'55"N, 54°25'15"E, 800 m, *Mozaffarian* 77411 (IRAN); Between Anar and Bahramabad, 13 April 1969, *Hewer* 943 (K). **Kerman:** 100 km S of Yazd, Road to Kerman, 31°12'00"N, 54°58'00"E, 25 March 1965, *Lamond* 74 (E); Tehran, Kavir National Park, SE Waramin, close to Shokr-Abad check point, Hordeum field, 812 m, 35°04'23"N, 51°42'50"E, 24 April 2010, *Ataei & Heidari* ED438 (BONN, USB); Kavir National Park, Siah Kuh, 6 May 1976, *Runemark, Foroughi & Asadi* 19462 (TARI); Khorassan, west of Dasht-e-Lut, 750 m, *Leonard* 32319 (IRAN).

Host: Polygonaceae: *Calligonum bungei* Boiss. (*Lamond* 74, E), *Pteropyrum* sp. (*Runemark, Foroughi & Asadi* 19462, TARI); **Fabaceae:** *Prosopis farcta* (Sol. ex Russell) J.F. Macbr. (*Ataei & Heidari* ED438, BONN & USB), *Alhagi persarum* Boiss. & Buhse (*Ataei & Heidari* ED433, BONN & USB); **Rosaceae:** *Rubus caesius* L. (*Ataei & Heidari* ED435, BONN & USB).

1.1.11 *Cistanche senegalensis* (Reut.) Beck, Pflanzenr. (Engler) 96, 4.261: 33 (1930).

≡ *Phelypaea senegalensis* Reut., Prodr. [A. P. De Candolle] 11: 13 (1847) [basionym]

Type: SENEGAL. *Perrottet* 114 (lectotype P barcode P03430969 selected by Lobin & Düll 1996: 589, isotype G-DC). Remaining syntypes: **SENEGAL.** *Sieber* 53 (K barcode K000701272, M barcode M0185671 seen as photo, JE barcode JE00002306 seen as photo, G-DC); *Sparmann* (G-DC); *Roussilon* (G-DC).

= *Cistanche carnososa* Pax, Bot. Jahrb. Syst. 39: 651 (1907). **Type: ETHIOPIA.** "Prov. Ost-Schoa: Tedetschamalka, 960 m, 30 January 1905." *Rosen* (WRSL, destroyed).

Plant robust or thin, 19–60 cm tall. **Lower scales** dense, imbricate, triangular-deltoid, obtuse, 5–7 (–10) × 3–6 (–11) mm, entire, non-scarious at the margins, yellow or light-purple; **middle** and **upper scales** increasingly lax acropetally, lanceolate (sometimes oblong-lanceolate), obtuse, (10–) 13–18 (–28) mm, sometimes slightly scarious at the margins, yellow or light-purple. **Inflorescence** an ovate-oblong sometimes ovate spike, 12–40 cm long and 5.5–9 cm in diameter; **floral bract** ovate-lanceolate, obtuse, (14–) 18–25 (–37) × 3–7 (–10) mm, entire, slightly scarious at the margins, about 4–9 mm longer than the calyx, yellow or light-purple; **floral bracteoles** lanceolate-linear, acute, (8–) 12–17 × 1–3.5 mm, serrate, slightly scarious at the margins, about 2/3 as long as calyx, light-purple; **calyx** tubular, rarely campanulate, (10–) 14–18 (–22) mm long; **calyx lobes** 5, sub-equal (2 longer and 3 shorter), ovate-orbicular, 3–7 × 2.5–4.5 mm, rounded, serrate, slightly scarious at the margins;

corolla at the base tubular-angustate, distally infundibuliform, (48–) 52–60 (–69) mm long, entirely yellow or pink-purple, rarely white, at least 3 times as long as calyx; **corolla lobes** 5 orbicular-ovate, crenulate, rounded, 3.5–6 (–10) × 5–8 (–12) mm, outside yellow and inside purple; **stamens** inserted at about 1/10 down the corolla tube, filaments slightly longer than 2/3 corolla length, slightly villous at base; **anthers** 2.5–5 (–7) mm long, rounded at base and acute to apiculate at apex, sparsely villous; **ovary** ovate, dark brown when dry; **stigma** club-shaped, white. **Capsules** open with 2-valves. **Seeds** unknown (Fig. 4.16).

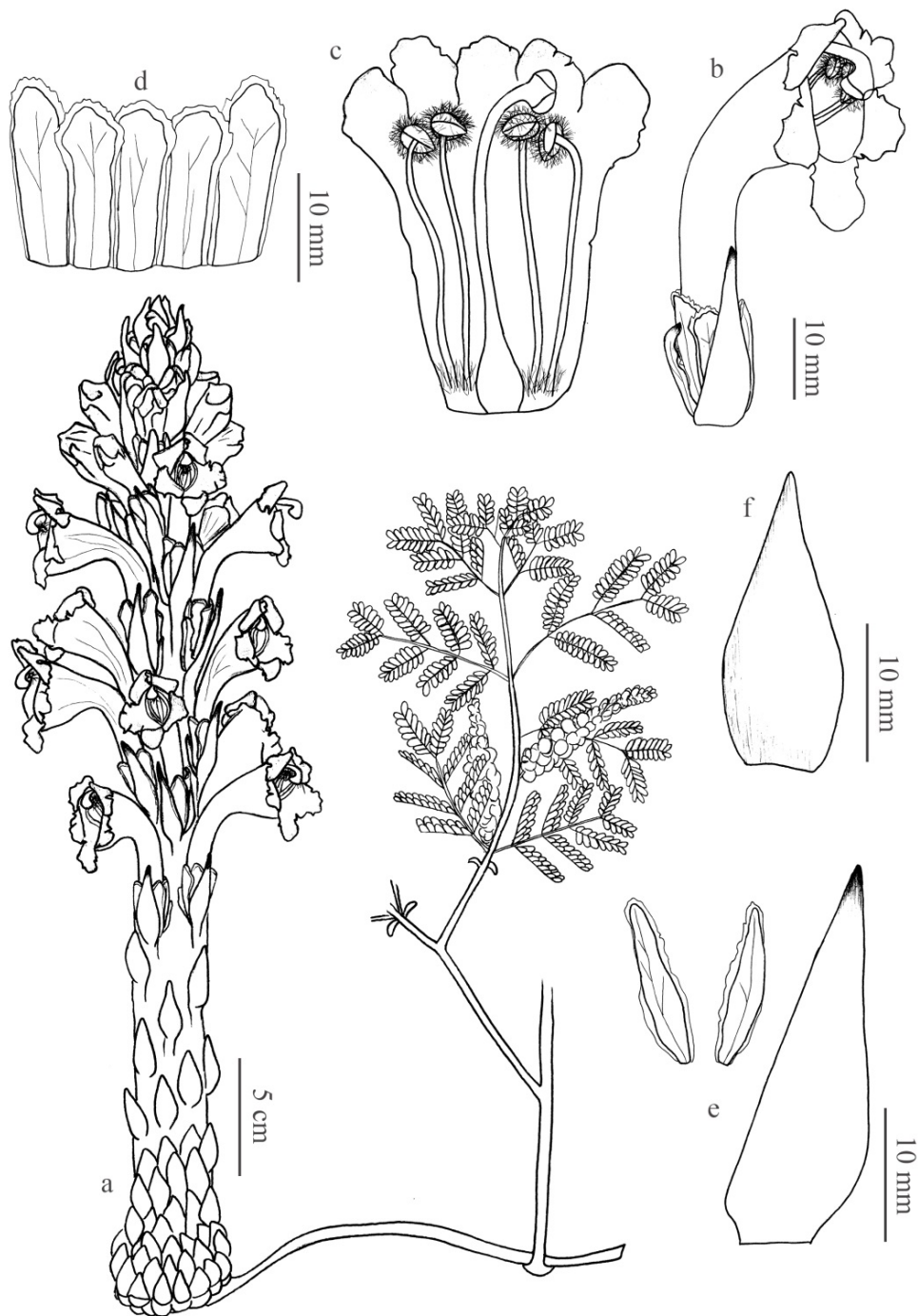


Figure 4.16 *Cistanche senegalensis* (Reut.) Beck. a) flowering plant and partially its host *Acacia* sp. L. b) single flower, c) its flower inner view, d) abaxial calyx, e) bract and bracteoles, and f) upper scale. The plant occurred in Somalia (Bally & Melville 15582; K).

Distribution: Senegal, Northeastern Africa Southwards Kenya to Mozambique in Southeastern Africa, West southern Arabian Peninsula (Fig. 4.3).

Additional specimens examined: **ERITREA.** **Debut:** Acnur, 1990 m, 16 April 1892, *Schweinfurth & Riva* 1349 (K); **Semēnawī K'eyih Bahrī:** Near Mersa Tek'lay, sandy coastal area, 17°32'36"N, 38°51'07"E, Sea level, 23 January 1957, *Hemming* 1142 (BM). **ETHIOPIA.** **Oromiya:** Gora, valley East Gora, 09°00'00"N, 42°18'00"E, 25 May 1963, *Burger* 2848 (K); Sidamo region, c. 25 km N of Moyale along the track to El Golf, 03°46'00"N, 39°05'00"E, 1300 m, 21 December 1997, *Friis & al.* 8765 (K); Shoa, Nord Bank of Awas river, c. 28 km south Nazareth, 08°25'00"N, 39°24'00"E, 1500 m, 15 September 1973, *Ash* 2104 (K); 20 km NW of Neghelli, along the road to Kebre Mengist, 05°35'00"N, 39°20'00"E, 1170 m, 19 July 1970, *Amshoff* 6649 (BR); Northwest shore of lake Langano, 07°37'00"N, 38°42'00"E, 10 November 1968, *MG & Gilbert* 1075 (K); **Tigray:** Adwa, *Quartin-Dillon* s.n. (P). **Harari:** Harar, E. of Bike, 09°31'39"N, 41°12'12"E, 27 September 1971, *Ash* 1249 (K); Near Adi Gudum, c. 30 Km south Mekelle, 13°14'35"N, 39°34'31"E, 2300 m, 10 October 1995, *Friis & al.* 6680 (K); Near Adi Gudum, South Mekelle, 13°15'00"N, 39°37'00"E, 10 October 1995, *Wondafrash & Kebede* 6680 (BR); **Amhara:** 5 km N of Woldia or 134 km N of Dessie or 3 km N of Almate, 11°07'00"N, 39°38'00"E, 1260 m, 23 December 1972, *Ash* 1811 (K); **Dire Dawa:** 136 km West of Dire Dawa on Erer to Meisso road, c. 12 km East Afdem, 09°32'00"N, 41°03'00"E, 2 September 1974, *Ash* 2572 (K); About 16 km on the road from Dire Dawa to Erer Gota, 09°37'00"N, 41°47'00"E, 1100 m, 2 January 1969, *de Wilde & Amshoff* 4334 (BR). **KENYA.** **Turkana:** (KI, Northern Province), Lokori, 7 mls, S of Kangetet, 594 m, 19 May 1970, *Mathew* 6317 (K); **Keiyo:** Chyulu Plains, 02°30'00"S, 37°45'00"E, 18 July 1999, *PA & WRQ Luke* 5946 (K); Leyenkati-Lowa, 02°39'00"S, 37°40'00"E, 1060 m, 19 February 2001, *PA & WRQ Luke & al.* 7382 (K); Magadi, Nairobi road, c. 10 miles South Ngong hills, 10 April 1956, *Greenway* 8997 (K); Near Magadi, Kajiado district, 23 January 1970, *Rajabu EAH* 14,294 (M, K); **Marsabit:** Kulal in cinder and lava below upper Gatab airstrip, 1700 m, 21 November 1978, *Hepper* 6940 (P, K); **Kilifi:** Tana river District, Kurawa, 30 ml. S. of Garsen, Tana river District, 01°56'19"S, 40°11'54"E, 5 October 1961, *Polhill & Paulo* 595 (K, P); Watmu, near Malindi, October 1950, *Tweedie* 870 (K); Vipingo, 20 miles N of Mombasa, 19 December 1953, *Verdcourt* 1100 (K); **Nairobi Area:** 32 km from Orgasalik to Nairobi, 25 August 1957, *Verdcourt* 1838 (BR); Kajiado district, Kisimus, 4 km from 46 Kisimus road, Ngong, 01°79'29"S, 36°61'04"E, 1464 m, 28 July 2008, *Omondi Kefri* 484 (K); River bank on the Magadi road about 25 miles from Nairobi, 914 m, 16 June 1962, *Glower & Samuel* 2871 (BR); **Taita Taveta:** Taveta, 915 m, 22 January 1936, *Greenway* 4482 (K); Voi-Flufs, Taita, June 1877, *Hildebrandt* 2583 (P); Narok District, Masailand, Narousura Rost Camp, 1920 m, 18 August 1961, *Glower, Gwynne, Samuel & Tucker* 2551 (BR). **LIBYA.** **Darnah:** Cirenaica, South Derna district, 17 April 1934, *Pampanini & Pichi-Sermolli* 7353 (BR); Djebel Uweinat, 700 m, 4 November 1968, s. col. 4827 (BR, K). **MOZAMBIQUE.** **Nampula:** Goa Island, 10 August 1964, *Leach* 12348 (K). **OMAN.** **Zufār:** Dhofar, Miji, 14 km W of Sharbithat, Sea level, 18 September 1989, *Miller & Nyberg* M.9431 (E). **SOMALIA.** **Bari:** 28 km S of Gardo on road to Eil, 5 January 1973, *Bally & Melville* 15582 (K); Togga Siido, 10°49'00"N, 50°27'00"E, 500 m, 14 January 1981, *Beckett* 748 (K); Galgallo, 64 km SSW of Bosaso at eastern

end of Al Mado range, 10 January 1973, *Bally & Melville* 15814 (K); **Banaadir:** Muqdisho, 3 km South Afgooye, 22 June 1982, *Watson* W1 (K); Mogadishu-Merca coastal road 15km south Gzira, 11 July 1986, *Maunder* 02 (K); **Awdal:** 20 miles from Borama, road to Hargeysa, 1220 m, May 1972, *Wood* S/72/92 (K); **Woqooyi:** Somaliland, Hargeisa, atro at Buramo, 09°33'00"N, 44°01'00"E, 1310 m, 13 October 1932, *Gillertt* 4240 (K, P); Somaliland, 13 October 1944, *Glover & Gilliland* 237 (K); 8 km S of Gzira, 11 July 1986, *Maunder* 01 (K); Djibouti, Somalian coasts, 500 m, March 1957, *Chdeville* 1798 (P); **Lower Shabeelle:** Near Afgoi, University Agriculture Faculty site, 02°06'00"N, 45°08'00"E, 70 m, 20 September 1978, *Allen & Elmi* 240 (E); Marka district, c. 5 km SE Buulo Werbe, 01°32'00"N, 44°13'00"E, 31 July 1988, *Kilian 2081 & Lobin* 6933a (B); **lower Juba:** Gobad [Goba], Somalie Fse, 14 December 1945, *Denis* N°75 (P); Somaliland, near Burama, 10°55'00"N, 43°10'00"E, 1524 m, 19 February 1932, *Billett* 4603 (K). **SUDAN. Khartoum:** East Nile, near Wauissi, 29 January 1983, *Musselman* 6238 (E); Dom Island (Umm Dom Island = Umm Dawm) south Khartoum, 6 December 1982, *Musselman* 198 #6179 (K, E); **Northern State:** Nubia, Sea-coast, 914 m, 1896, *Comm & Bent* s.n. (K); Central part, 168 m, 20 March 1930, *Chipp* s.n. (BR); **Red Sea:** Along Tokar Road, c. 20 km south Suakin, 18°53'30"N, 37°24'32"E, 28 December 1982, *Musselman* 6201 (E); **Kassala:** Aqig, 18°13'00"N, 38°11'00"E, Sea level, 17 June 1959, *Jackson* 3922 (K). **TANZANIA. Arusha:** 24 km South Longido at road to Arusha, 02°58'00"S, 30°48'00"E, August 1969, *Beecker* 14198 (K); Arusha District, 1524 m, 02 January 1971, *Richards & Arasululu* 26452 (K); 52 miles Arusha-Longido, roadside, 1220-1520, 27 June 1970, *Kabuye* 160 (K); T2, Arusha District, 10 km W of Ngare Nanyuki, 03°09'00"S, 36°50'00"E, 1600 m, 27 April 1975, *Friis & Hansen* 2606 (K); 20 miles west of Arusha, Ardai, 29 February 1960, *Hoccombe* 1416 (K); **Kilimanjaro:** Masai, 9 miles along the Moshi Track, 1219 m, 07 January 1969, *Richards* 23671 (K); **Tanga:** Lushoto District, Mombo-Moshi road, 152 m, June 1954, *Eggeling* 6818 (BR, K); **Mara:** Musoma district, Ndabaka, 12 June 1961, *Kiftara* 12410 (K); **Tabora:** Tanganika, Nzega, *Brutt* 3380 (BR); **Mwanza:** Kalemera, 1158 m, 12 July 1953, *Tanner* 1413 (BR, B); **Dodoma:** ChemChem river delta, 960 m, 21 June 1965, *Greenway & Kanuri* 11.877 (BR). **UGANDA. Northern Region:** Kenya boarder, Lotome probably, Turkana District, February 1959, *Wilson* 700 (K). **YEMEN. Sana'a:** Roadside of 20 km South Sana'a, 2400 m, 08 October 1977, *Wood* 2091 (BM); **Hadramawt:** Abd Al kuri island, 65 miles southwest Socotra in the Indian Ocean, 22 September 1966, *Keiln* A51 (K); Hadda, 4km SW of Sana'a, 15 October 1976, *Miller* 581 (K); 8 km W of Al Mukalla, 1700 m, 13 October 1978, *Miller* 509 (E); IBB district, 20 October 1975, *Hepper & Wood* 5898 (K); South, wadi Hadramaut, on the crop N or Shibani, 3 January 1978, *Monod* 16923 (P); Dunes on N or riverbed in Seiyun, 12 January 1978, *Monod* 17113 (P); South coastal sands W Ash Shihr, 25 January 1978, *Monod* 17433 (P); **Dhamār:** 3 km East Hamman Ali on road to Dhomar, 14°40'00"N, 44°10'00"E, 30 March 1979, *Wood* Y1132 (E); **Ta'izz:** North of Ta'izz, on banks roadside fields, 1500 m, 15 October 1974, *Wood* Y/74/49 (BM).

Host: Zygothylaceae: *Zygothylum* sp. (*Monod* 17113, P); **Fabaceae:** *Acacia tortilis* (Forssk.) Hayne (*Wilson* 700, K); **Solanaceae:** *Solanum* sp. (*Omondi* Kefri484, K); **Capparaceae:** *Capparis decidua* (Forssk.) Edgew. (*Musselman* 6238, E); **Salvadoraceae:** *Salvadora persica* L. (*Greenway &*

Kanuri 11.877, BR); **Amaranthaceae**: *Suaeda monoica* Forssk. ex J.F.Gmel. (Musselman 6201, E) and *Aerva* sp. (s. col. 4827, RB, K).

Remarks: Apart from the type specimen no other specimen has been reported from Senegal. No type specimen of *Cistanche carnos*a exists at WRSL, because many specimens, including Orobanchaceae, were destroyed during the World War II (Krzysztof Swierkosz, pers. comm. 25 November 2014). According to the original description of *C. carnos*a can be confidently placed in the synonymy of *C. senegalensis*.

1.1.12 *Cistanche brunneri* (Webb) Beck, Pflanzenr. (Engler) 96, 4.261: 35 (1930).

≡ *Phelypaea brunneri* Webb, Niger Fl. [W. J. Hooker]. 167 (1849) [basionym];

Type: **CAPE VERDE**. "Herbier rapporte du Portugal en 1808 par M. Geoffroy St. Hilaire" (lectotype P barcode P03004182 selected by Lobin & Düll 1996: 589). Remaining syntype: **CAPE VERDE**. Sal, *Brunner* s.n. (FI-W barcode FI000241 seen as photo).

= *Phelypaea hesperugo* Webb, Niger Fl. [W. J. Hooker]. 167 (1849); Walp., Ann. Bot. Syst. 3: 205 (1852-1853) ≡ *C. hesperugo* (Webb) Beck, Pflanzenr. (Engler) 96, 4.261: 35 (1930). *Type*: [**SENEGAL**. N. 'Boro], *Brunner* s.n. (FI-W barcode FI000240 seen as photo)

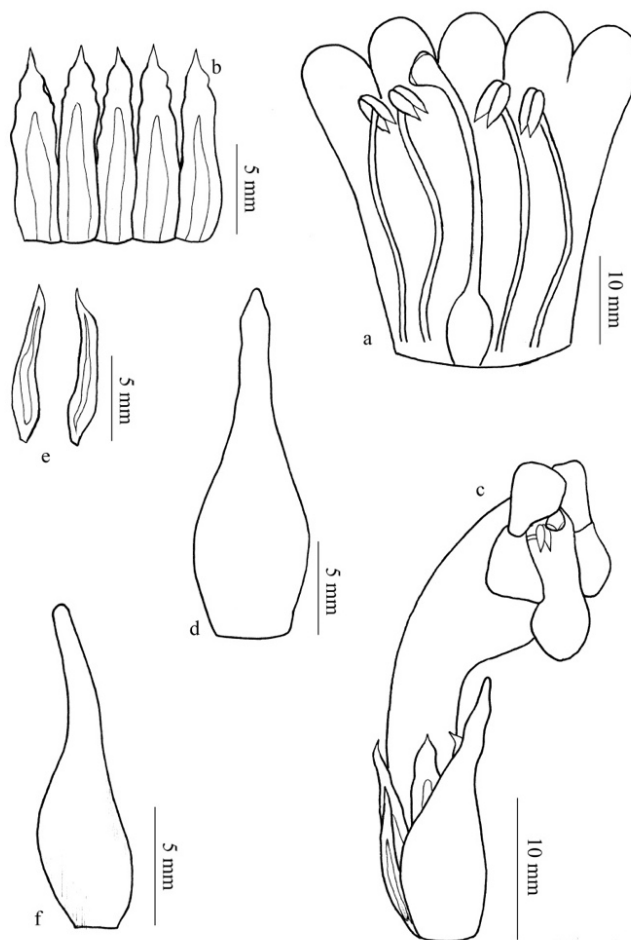


Figure 4.17 *Cistanche brunneri* (Webb) Beck. a) flower inner view, b) abaxial calyx, c) single flower, d) bract, e) bracteoles, and f) upper scale. The plant occurred in Senegal (Kesby 17; K).

Plant thin, slender, 10–40 cm tall, white-greyish. **Lower scales** very dense, imbricate, deltoid, obtuse, up to 11 × 3–4 mm, entire, non-scarious at the margins; **middle** and **upper scales** increasingly lax acropetally, lanceolate sometimes ovate-lanceolate, obtuse, 13–18 × 3–3.5 mm, entire, non-scarious at the margins, dark brown when dry. **Inflorescence** an conical-oblong lax spike, with few flowers, 12 cm long and 4.5 cm in diameter; **floral bracts** ovate-lanceolate, obtuse, 17–18 × 4 mm, with irregularly undulate margins when young, entire, non-scarious at the margins, attenuate tapering gradually at the point, about 4–9 mm longer than calyx, grayish, becoming dark-brown when dry; **floral bracteoles** linear-lanceolate, obtuse, 8–9 × 1.2 mm, entire, non-scarious at the margins, slightly sinuate when young, slightly shorter than calyx, greyish; **calyx** tubular, pentamerous, deeply incised, 11–12 mm long; **calyx lobes** 5, equal, ovate-lanceolate, acuminate, 4.5–5.5 × 2.5 mm, highly scarious at margins; **corolla** pentamerous, tubular, abruptly infundibuliform at top, 39–40 mm long, pale-yellow; **corolla lobes** 5, equal, ovate, pinkish-mauve, up to 4 × 6 mm; **stamens** inserted above the base, filaments 2/3 as long as corolla, hidden inside the tube, entirely glabrous at base; **anthers** 2.5–2.7 mm long, rounded at base and apiculate at apex, glabrous; **ovary** ovate; **stigma** club-shaped, non-exserted. **Capsules** open with 2-valves. **Seeds** unknown (Fig. 4.17).

Distribution: Cape Verde, Senegal (Fig. 4.3).

Additional specimens examined: SENEGAL. **Saint-Louis:** Ross Bethio, 30 November 1933, *Trochain* 2204 (P); **Thies:** Kayar, besides Sea, 19 June 1960, *Kesby* 17 (K, P); **Dakar:** Sangalkam, Nayar, 27 April 1954, *Berhaut* 5109 (P); **Ziguinchor:** Tilèl, 2 January 1966, *Audru* 3010 (P); Barbarie, 17 December 1933–34, *Trochain* 2125 (P).

Host: Unknown.

Remarks: In contrast to Beck-Mannagetta (1930), scales with acuminate apex (*C. brunneri*) has not been met in the species.

1.1.13 *Cistanche somaliensis* Ataei, sp. nov.

Diagnosis: *Cistanche somaliensis* is similar to *C. senegalensis*, but differs by its calyx with 5 equal obtuse lobes plus a sixth acute tooth-like (versus 5 sub-equal lobes, 2 longer and 3 shorter in *C. senegalensis*), stamens inserted at about the middle of the corolla (versus stamens inserted just above the base of the corolla in *C. senegalensis*), and stamens and style prominently exserted (versus stamens and style non-exserted in *C. senegalensis*). However, the only available specimen from Somalia, which is fragmentary and does not have specific geographical information, warrants our certain identification. Although according to strong taxonomic characters (number of calyx and corolla lobes) we refrain a new species, but new collections in Somalia and elsewhere may prove the existence of a new *Cistanche* species.

Stem, scales and Inflorescence unknown; **floral bracts** ovate-lanceolate, 22–25 × 5 mm, obtuse, entire, ca. 5 mm longer than calyx, non-scarious at the margins; **floral bracteoles** oblong, obtuse, entire, ca. 5 mm shorter than calyx, non-scarious at the margins; **calyx** tubular, up to 14 mm, deeply incised, with 5 unequal obtuse lobes plus one shorter ovate-dentate lobe; **calyx lobes** oblong, 5-rounded plus one acute, highly serrate and scarious at the margins; **corolla** tubular, at top slightly broadened, up to 40 mm long, with 6 equal ovate lobes, rounded; **stamens** inserted at about the middle of the corolla, densely villous, prominently exserted, **filaments** 2/3 corolla length; **anthers** rounded at base and acute at apex, villous, prominently exserted; **ovary** ovate-oblong; **stigma** slightly disk-like, highly exserted. **Capsules** open with 2-valves (Fig. 4.18).

Specimen examined: SOMALIA. Somaliland: 1897?, Lord Phillips s.n. (BM).

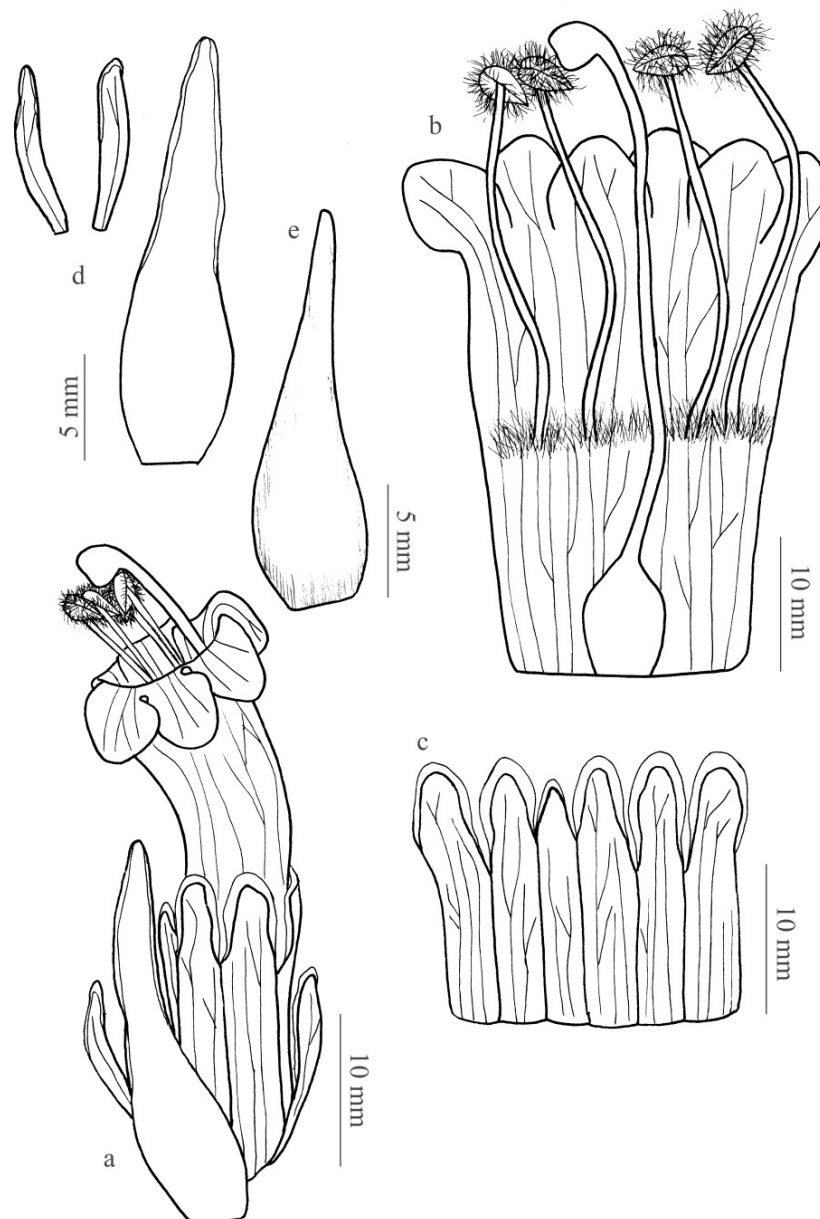


Figure 4.18 *Cistanche somaliensis* Ataei, sp. nov.. a) single flower, b) its flower inner view, c) abaxial calyx, c, d) bract and bracteoles, and e) upper scale. (Lord Phillips s.n.; BM).

1.2 *Cistanche* sect. *Heterocalyx* Beck, Monogr. Orobanche 57 (1890).

Type species: Cistanche fissa (C.A.Mey.) Beck (lectotype designated here).

≡ *Cistanche* sect. *Cistanchopsis* Beck, Pflanzenfam. 4, 3b: 129 (1895), nom. superfl.

Type species: Cistanche fissa (C.A.Mey.) Beck.

= *Cistanche* sect. *Cistanchiella* Beck, Pflanzenr. (Engler) 96, 4.261: 27 (1930).

Type species: Cistanche ridgewayana Aitch. & Hemsl.

Plant usually with short and slender rarely long stem. **Lower scales** ovate-triangular, rarely oblong, obtuse, usually non-scarious at the margins; **middle and upper scales** ovate-lanceolate, lanceolate or oblong, obtuse, usually non-scarious at the margins, fully or partially hairy on the outer side and at the margins. **Inflorescence** an ovate or oblong spike with few to many flowers; **floral bracts** ovate-lanceolate, lanceolate or oblong, obtuse, usually non-scarious at the margins, fully or partially hairy on the outer side and at the margins; **floral bracteoles** oblong-linear, lanceolate, obtuse, acute or acuminate, mainly hairy at the margins, rarely on the outer side; usually 2, but in *C. ridgewayana* rarely 0–1 (–2) tiny linear ones in few species; **calyx** usually pentamerous, either fully hairy or only on the lobes hairy (alternatively, sparsely on the outer side, which may be fully or only partially covered); **calyx lobes** 5, equal or unequal, obtuse or acute, their apices usually hairy at their margins; **corolla** commonly tubular, slightly curved down, pentamerous, often with two yellow folds inside the tube, uni- to bicolor; **corolla lobes** glabrous, marginally or on inner side hairy; **stamen** inserted at the bottom of the corolla tube, hairy; **anthers** acute, mucronate, apiculate, acuminate, cuspidate or aristate at apex; **ovary** ovate to oblong. **Capsule** opens with 2, rarely with 3-valves. **Seeds** tiny, elliptic, triangle.

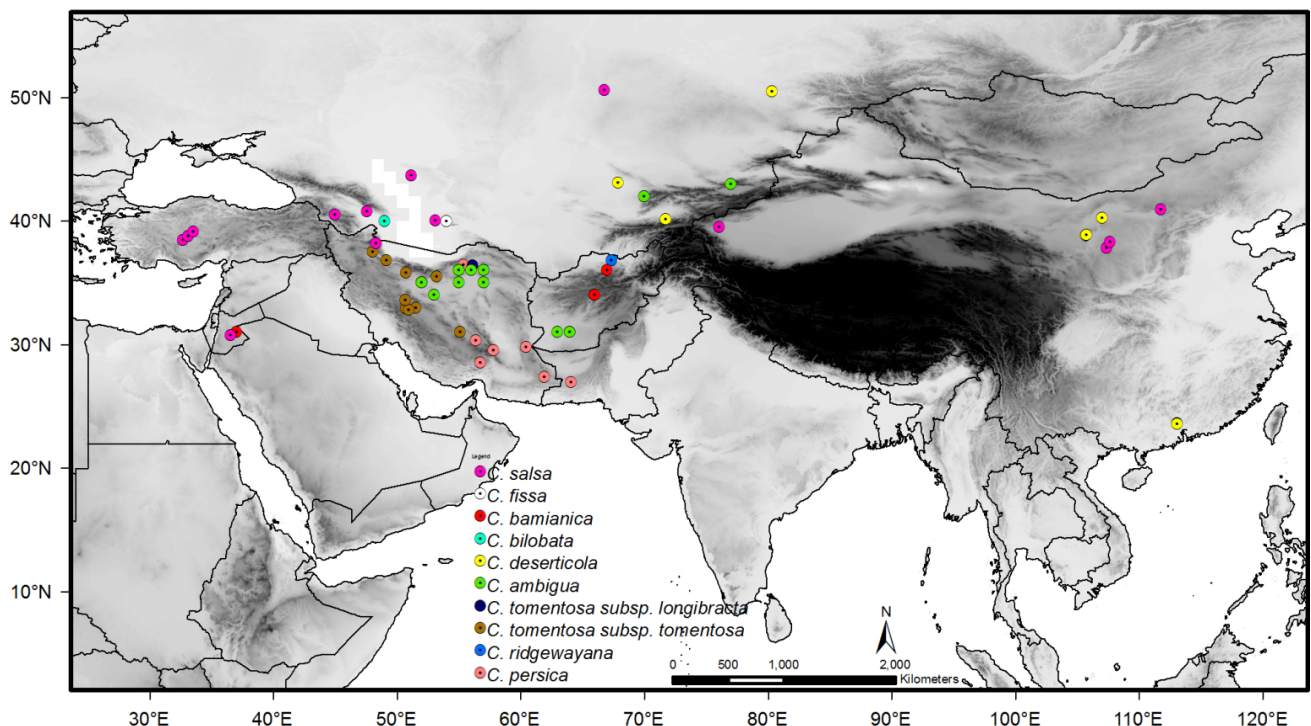


Figure 4.19 Distribution map of species of the *Cistanche* sect. *Heterocalyx* worldwide.

Distribution: *Cistanche* sect. *Heterocalyx* contains nine species distributed from Turkey, Eastwards Southwest Asia mainly in Iran, extending to China and Mongolia (Fig. 4.19).

Key to the species and subspecies of *Cistanche* sect. *Heterocalyx* Beck

- 1. Bracts, bracteoles and calyx partially hairy only at their margins 2
- Bracts, bracteoles, calyx and corolla hairy on the outer side and at their margins, more rarely also on the outer side. — Corolla with two prominent yellow ridges on the inside; capsule opening always with 2-valves 5
- 2. Bracts ovate-oblong, about twice as length of calyx; bracteoles 1 or 2, rarely absent, tiny (half as long as calyx); calyx about half as long as corolla, with 5 equal lobes, calyx lobes ciliate at their margins, rounded at the apex. — Corolla often pink-reddish 3
- Bracts long-lanceolate, about 2.5–3 times as long as calyx; bracteoles usually absent; calyx ca. 1/3 as long as corolla, with 5 unequal lobes, calyx sparsely tomentose abaxially and ciliate along its margins, usually acute at apex (1.2.3 *C. tomentosa*) 4
- 3 Scales below the inflorescence glabrous; bracteoles 1 or 2; calyx lobes ciliate at the apex underneath inflorescence scales glabrous. Southern Iran, Southwestern Pakistan **1.2.4 *C. persica***
- Scales below the inflorescence sparsely pubescent at their margins; bracteoles 1, spatulate, rarely absent; calyx lobes glabrous at apex underneath inflorescence scale covers with sparse pubescent hairs in margin. Afghanistan **1.2.2 *C. ridgewayana***
- 4- Bracts sparsely pubescent mostly at midrib; calyx sparsely pubescent abaxially and along its margins. — Bracts as long as corolla or slightly shorter. Iran in particular Isfahan, Semnan **1.2.3.2 *C. tomentosa* subsp. *longibracteata***
- Bracts pubescent only at their margins; calyx entirely glabrous. Iran in particular Azerbaijan, Karaj **1.2.3.1 *C. tomentosa* subsp. *tomentosa***
- 5. Bracts, bracteoles, calyx and corolla lobes always pilose at their margins; calyx usually with subequal lobes (3 slightly longer ones alternating with two shorter ones). — Calyx lobes acute, rarely obtuse; corolla tube white-cream, corolla lobes pink to light violet, two orange lines inside the tube lateral to the proximal corolla lobe extending on the inside. Afghanistan, Iran **1.2.5 *C. ambigua***
- Bracts always and calyx often pubescent at the margins; calyx with equal, rarely subequal lobes. — Corolla short tubular, tube on the outer side and on the inner side evenly pinkish-red colored 6
- 6. Bracts and bracteoles abaxially pilose; calyx glabrous, with 5 equal lobes 7
- Bracts, bracteoles and calyx abaxially pilose; calyx with 5 equal or sub-equal rounded lobes¹⁰
- 7- Bracts about as long as corolla. — Bracts, bracteoles, calyx and corolla lobes sparsely and mainly abaxially pubescent **1.2.1 *C. deserticola***
- Bracts about as long as or slightly longer than calyx 8
- 8- Calyx and corolla lobes at their margins slightly ciliate, sometimes corolla lobes glabrous. — Stem partially pilose from long white hairs; middle and upper scales dorsally pilose from long white hairs, rarely glabrous (except the uppermost scales), bract as length of calyx or slightly longer but never as long as corolla. Armenia, Kazakhstan, Turkey **1.2.6 *C. salsa***

- Calyx and corolla lobes on the outer side sparsely to densely woolly, folds of the corolla tube on the inside covered with long hairs 9
- 9- Calyx lobes bidentate. — Adaxially entirely covered with very long silky and villous hairs, corolla sparsely woolly on the inside. Azerbaijan **1.2.9 C. bilobata**
- Calyx lobes simple 11
- 11- Corolla lobes abaxially densely woolly, corolla tube white, lobes light violet. Turkmenistan **1.2.7 C. fissa**
- Corolla lobes woolly at their margins, sparsely woolly inside the tube and at the margins, corolla tube with two yellow ridges with almost dense hairs along inside the tube. — Bracts about 4–9 mm longer than calyx. Afghanistan, Jordan **1.2.8 C. bamianica**

1.2.1 *Cistanche deserticola* Ma, Acta Sci. Nat. Univ. Intramongol. 1(1): 63 (1960);

Type: **CHINA. Inner Mongolia:** Ālāshàn League parasite on roots of *Haloxylon ammodendron* (C.A.Mey.) Bunge, 7 May 1959, *Chen* 38 (HIMC not seen).

= *Cistanche ambigua* auct., non *Cistanche ambigua* (Bunge) Beck (Ma, 1960: 64). This name has also been mentioned in Zhang (1984: 116).

= *Cistanche deserticola* var. *flabellata* Ma & Cao, Acta Bot. Boreal.-Occid. Sin. 31 (3): 639 (2011).

Type: **CHINA. Inner Mongolia:** Alashan Azuoqi Xilingaole, 39°17.100'N, 105°26.310'E, 141 m, 14 May 2010, *Cao Rui & Chenanping* 066 (holotype HIMC seen as photo).

Plant usually robust, 10–100 (–150) cm tall, sub-glabrous, white to light-yellow. **Lower scales** dense, imbricate, ovate-triangular, obtuse, 5–6 × 2.5–3 mm, glabrous, entire, non-scarious at the margins; **middle** and **upper scales** lax, imbricate, long lanceolate-linear, obtuse, 9–13 × 3.5–5 mm, glabrous, entire, non-scarious at the margins. **Inflorescence** an ovate-oblong spike, 8–15 (–27) cm long and 4.5–6.5 cm in diameter; **floral bracts** ovate-lanceolate or lanceolate, obtuse, 13–15 × 4–6 mm, sparsely on the outer side pubescent, entire and non-scarious at margins, subequaling corolla or slightly shorter; **floral bracteoles** lanceolate or ovate-lanceolate, acute, 7–9 × 2 mm, on the outer side pubescent and at the margins denser, nearly as long as calyx or slightly longer; **calyx** campanulate, pentamerous, 10–13 mm long, sparsely pubescent on the outer side, nearly 1/3 length of corolla; **calyx lobes** 5, equal, ovate, sub-acute, 2–3 × 2–4.5 mm, with non-scarious margins, sparsely pubescent abaxially; **corolla** pentamerous, tubular to campanulate, 25–28 mm long, cream to pale-yellow or pale-purple, with two yellow folds inside the tube; **corolla lobes** 5, equal, blue to purple, usually length shorter than width, 2.5–3.8 × 3–5 (–7) mm, commonly glabrous, sometimes sparsely pubescent on the outer side; **stamens** inserted at about 1/5 total corolla length, filaments 2/3 as long as the corolla, at base villous; **anthers** 3.5–4.5 mm long, densely villous, long-ovoid, mucronate (in Mongolian and in Kazakhstan specimens aristate); **ovary** ellipsoid; **stigma** sub-globose, glabrous, non-exserted. **Capsules** ovoid-globose, open with 2-valves. **Seeds** ellipsoid or ovoid (for illustration see: Zhang & Tzvelev, 1998).

Distribution: Occurring in high elevations (up to 2700 m) from Kazakhstan, Tajikistan (photo by Davkaev at http://www.fotomontaro.com/flora/scrophularia/cistanche_salsa02.shtml, accessed 18 February 2015), Kyrgyzstan (Zhang & Tzvelev, 1998), to China in vast deserts of Taklimakan, largely in Northeast from Gansu to Inner Mongolia (Fig. 4.19).

Additional specimens examined: **KAZAKHSTAN.** Ongtüstik: Turkestan, 29 May 1877, Regel 1873 (K, BM); **East Kazakhstan:** Excursion through Semipalatinsk, 11 May 1914, Shipczinsky N°92 (K). **KYRGYZSTAN.** **Batken:** Alai Range, vicinity of Kadamzhai village, 40°07'47"N, 71°42'59"E, 18 August 2011, Laz'kov BLCKg-715 (K). **MONGOLIA.** **Govĭ-Altay:** Altaï, 1831, Ledebour s.n. (P); **Bayanhongor:**, Trans Altai Gobi, North of Tsagan, Bogdo-Ula, vicinity of Ekhiin Gol, 6 August 1978, Gubanov N3108 (MW); South Gobi, Nomgon District, 170 km SE of Dalan-Dxadgad, 27 June 1980, Gubanov N5908 (MW).

Host: Amaranthaceae: *Haloxylon ammodendron* (C.A.Mey.) Bunge, Nei Mongol, Gobi desert and China (Liu, 1992; Naran & al., 1995; Hsu & Wang, 1996) and *Haloxylon persicum* Bunge ex Boiss. & Buhse (Hsu & Wang, 1996).

Remarks: According to our herbarium observations, density and size of lower scales was often used for identification of *C. deserticola* in China, but this character is not suited to distinguish *Cistanche* species, as it frequently varies among and within species. Corolla lobes are sparsely pubescent in specimens from China and glabrous in those from Kyrgyzstan, but otherwise plants are identical. Ma & Cao (2011) described stem of *C. deserticola* var. *flabellata* fan-shaped and fleshy (i.e. it covered at base with fan-shaped and overlapped scales) with linear or lanceolate-linear scales at upper. These vegetative characters often vary depending on the age of the specimen, rendering them of limited taxonomic value. Therefore, this variety is of no taxonomic value and is placed in the synonymy of *C. deserticola*.

1.2.2 *Cistanche ridgewayana* Aitch. & Hemsl., Trans. Linn. Soc. London, Bot. 3(1): 93 (-94; pl. 39) (1888);

Type: **AFGHANISTAN.** Herat: Harirud-valley, April–May 1885, Aitchison 1093 p.p. (K).

Plant usually short, up to 24 cm tall, sub-glabrous. **Lower scales** highly imbricate, short triangular, 5–7 × 2–2.5 mm, glabrous, entire, non-scarious at margins, obtuse; **middle scales** increasingly lax acropetally, ovate-lanceolate, obtuse, 8–10 (–18) × 2–3 (–4.5) mm, glabrous, entire, non-scarious at the margins; **upper scales** very lax, ovate-lanceolate, obtuse, 11–18 (–22) × 3–4.5 mm, densely lanuginose at the margins of uppermost scales, entire, non-scarious at the margins. **Inflorescence** often a short ovoid-cylindrical spike, with many flowers, 7.5–10 cm long and 4.5–5 cm in diameter; **floral bracts** ovate, rarely ovate-lanceolate, obtuse, 13–19 × 3.5–4 mm, entire and non-scarious at

the margins, lanuginose at their margins, about 1.5 times as long as calyx; **floral bracteoles** 1, rarely 0, obovate-spathulate, obtuse, 6–8 × 1.5–2.5 mm, entire, non-scarious at the margins, slightly lanuginose in the upper two thirds at the margins or glabrous, maximally half as long as calyx; **calyx** campanulate, usually pentamerous, 7–9 (–11) mm long, ciliate at the margins, usually 1/3 corolla length; **calyx lobes** 5, equal, oblong, rounded, rarely acute, 2–3 × 2–2.5 mm, glabrous, with non-scarious margins; **corolla** short tubular, hardly widened distally, 25–29 mm long, without conspicuous folds inside the tube, pinkish; **corolla lobes** 5, broad-orbicular, glabrous, 1–2 × 1.5–2.5 mm, reddish; **stamens** inserted above the base, filaments usually short about ½ corolla length, densely villous; **anthers** 3.5–5 mm long, moderately villous, rounded at base and mucronate at apex; **ovary** tubular-elliptic; **stigma** sub-globose to club-shaped, glabrous, non-exserted. **Capsules** always dehiscent with 3-valves. **Seeds** ovate, rarely oblique oblong.

Distribution: Northern Afghanistan (Fig. 4.19).

Additional specimens examined: AFGHANISTAN. **Balkh:** Mazar-e-sharif, in the desert between Mazar-e-Sharif and Tashkurghan, 10 May 1967, *Rechinger* 34226 (B, E, M, W); 45 km NW Tashkurghan around Namak Sar, 29 September 1968, *Freitag* 3839 (KAS).

Host: Unknown.

Remarks: The type specimen of *C. ridgewayana* and the type of *C. laxiflora* (K barcode K000449427) belong to the same collection *Aitchinson* 1093 (K), and have been mounted on separate sheets (no barcode yet available for *C. ridgewayana*). The fact that *Aitchinson* 1093 contains two species has already been noted in the protologue and in Schiman-Czeika (1964: 22). *Cistanche ridgewayana* is characterized by possessing only 1, spathulate bracteole (rarely completely absent) and by glabrous calyx lobes. None of these characters has, however, been mentioned in the first description of the species (Aitchison, 1888). The reduced number of bracteoles caused Beck to distinguish a separate sect. *Cistanchiella*. Scales below the inflorescence being concealed by dense lanuginose hairs on their margins is characteristic for this species as well, but is also common in its phylogenetic sister species *C. ambigua* (see chapter 2).

1.2.3.1 *Cistanche tomentosa* Ataei, sp. nov. subsp. *tomentosa*

Diagnosis: *Cistanche tomentosa* is similar to *C. ridgewayana*, but differs from the latter in by scales below the inflorescence being ciliate (versus densely lanuginose at their margins in *C. ridgewayana*), bracts densely marginally and sparsely on the outer side tomentose (versus marginally lanuginose in *C. ridgewayana*), 0 bracteoles (versus usually 1 bracteole in *C. ridgewayana*) and triple times of corolla/calyx (versus 2.5 times range in *C. ridgewayana*). The species has so far been misdetermined as *C. ridgewayana*.

Type: IRAN. Semnan: 2-7 km Supra Sorkhe bridge, 1300-1400 m, 29 April 1975, *Rechinger* 52165 (holotype W barcode W03996, isotype B barcode B100264819).

Plant usually short, 23–25 cm tall, stem generally about 0.5–1 cm in diameter, sub-glabrous. **Lower scales** copious, highly imbricate, short triangular, sub-acute, 10–11 × 3–5 mm, glabrous, entire, non-scarious at margins; **middle scales** increasingly lax acropetally, ovate-lanceolate, obtuse, 14–15 × 4–4.5 mm, entire, non-scarious at the margins; **upper scales** very lax (usually arranged in rings along the stem), lanceolate, sub-acute, 19–21 × 3–3.5 mm, glabrous (except the uppermost ones, which are covered with ciliate hairs), entire, non-scarious at the margins. **Inflorescence** a short ovoid to cylindrical spike, with erect-tubular flowers, 6 cm long and 3.5 cm in diameter; **floral bracts** ovate, rarely ovate-lanceolate, obtuse, 20–22 × 4 mm, entire, non-scarious at the margins, densely tomentose at the margins, sparsely tomentose on the outer side, about 1.5 times as long as calyx; **floral bracteoles** none; **calyx** campanulate, usually pentamerous with 5 sub-equal lobes, 8–9 mm long, sparsely tomentose on the outer side, usually 1/3 corolla length; **calyx lobes** 5, sub-equal, oblong, rounded, rarely acute, 3.5–4 × 2–2.5 mm, non-scarious and ciliate at the margins; **corolla** short tubular, hardly widened distally, 19–20 mm long, without conspicuous folds inside the tube, yellowish; **corolla lobes** 5, broad-orbicular, glabrous, 3 × 2.5–3 mm, reddish; **stamens** inserted above the base, filaments usually short, about ½ corolla length, densely villous; **anthers** 4–4.5 mm long, villous, rounded at base and mucronate at apex; **ovary** tubular-elliptic; **stigma** sub-globose, glabrous, non-exserted. **Capsules** always dehiscent with 3-valves. **Seeds** usually ovate.

Distribution: Eastern Azerbaijan, Turkmenistan in Kara Kum (Akar-Cheshme) and Kelif Central Asia in Pamiro-Alai, and Angor (Teryokhin & al., 1993), North and Western Iran (Fig. 4.19).

Additional specimens examined (paratypes): **IRAN. Gilan:** 43 km Roodbar to Gilavan, 550 m, 5 May 1987, *Assadi, ShahMohammadi & Parsa* 60176 (TARI); **Isfahan:** Isfahan city, Djafar Abad, 20 km W Tiran Versus Daran, 32°49'00"N, 50°57'00"E, 29 May 1980, *s. col.* UI 2057 (E); 24 km W Isfahan, Murcheh Khvort, 1750 m, 27 May 1974, *Rechinger* 46653 (B); Inter Faridan & Isfahan, *Ghahreman, Sheikh Eslami & Aghustin* 8825 (THU); Kashan, Mooteh protected region, 31 May 1975, *Rechinger* 46857 (E); **East Azerbaijan:** In the river Qezel Owzan (Kizil Uzun), 10-24 km ENE Mianeh, 37°30'00"N, 48°04'00"E, 14 July 1971, *Rechinger* 43222 (W); **Tehran:** Karaj, Ravandeh, 35°49'00"N, 50°43'00"E, 3 January 1935, *Gauba* 732 (B); **Kerman:** Between Kerman and Yazd, 22 April 1948, *Rechinger* 2863 (E).

Host: Fabaceae: *Astragalus squarrosus* Bunge (*Freitag* 15538, B, KAS).

1.2.3.2 *Cistanche tomentosa* subsp. *longibracteata* Ataei, subsp. nov.

Diagnosis: *Cistanche tomentosa* subsp. *longibracteata* differs from the nominate subspecies by long-lanceolate middle and upper scales with a very dense tomentose indumentum at their margins (versus oblong-ovate scales with a loose tomentose indumentum at their margins), bracts 2.5–3 times as long as calyx (versus bracts almost equaling calyx). The species has been misdetermined as *C. ridgewayana*.

Type: **IRAN. Semnan:** Khar Turan National Park, SE of Shahrud, 25 km N of Ghazazan at road to Miandasht, 36°24'00"N, 56°06'00"E, 1340 m, 11 May 1978, Freitag 15276a (holotype KAS).

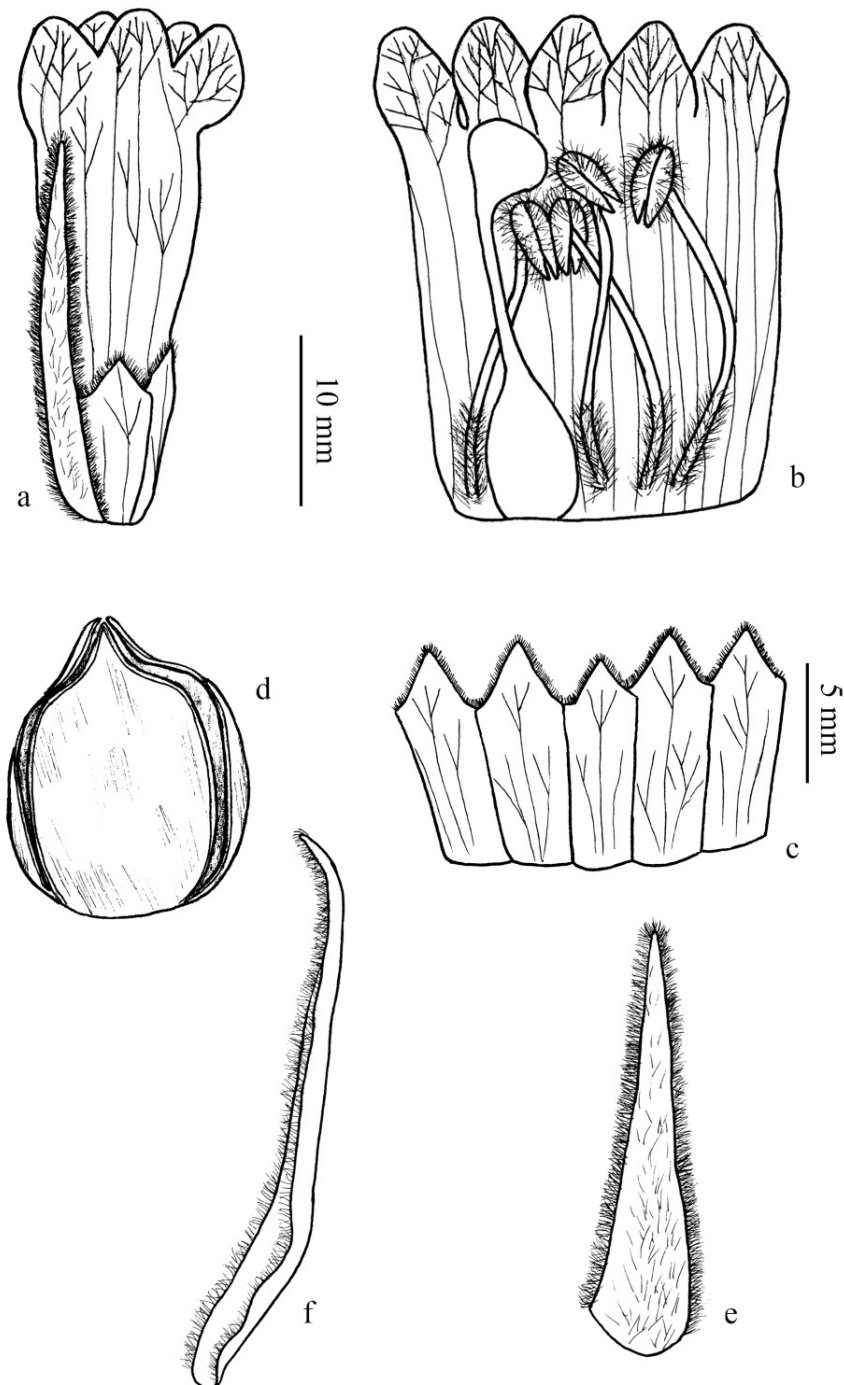


Figure 4.20 *Cistanche tomentosa* subsp. *longibracteata* Ataei subsp. nov. a) single flower, b) flower inner view, c) abaxial calyx, d) capsule, e), bract, and f) upper scale. The plant occurred in Iran (Freitag 15276a; KAS).

Plant short, 25 cm tall, sub-glabrous. **Lower scales** strongly imbricate, short ovate-triangular, sub-acute, glabrous, entire, non-scarious at margins; **middle scales** increasingly lax acropetally, long-lanceolate, obtuse, 13.5–15 × 2.5–3 mm, entire, non-scarious at the margins; **upper scales** lax, long-lanceolate, sub-acute, 22–24 × 2.5 mm, the uppermost ones with long tomentose hairs at their margins, entire, non-scarious at margins. **Inflorescence** a short ovate dense spike, with erect tubular flowers, 7 cm long and 4.5 cm in diameter; **floral bracts** long-lanceolate, obtuse, 24–25 × 2.5–3 mm, entire, non-scarious at the margins, densely tomentose at the margins, sparsely tomentose on the outer side, 2.5–3 times as long as calyx; **floral bracteoles** none; **calyx** campanulate, pentamerous, 8–10 mm long, slightly tomentose on the outer side, 1/3 corolla length; **calyx lobes** 5, unequal (four longer plus one tooth-like), acute, 3.5–4 × 3–5 mm, non-scarious and ciliate at the margins; **corolla** short tubular, 29–30 mm long, yellowish; **corolla lobes** 5, ovate, glabrous, 2.5–4 × 3–4 mm, light-orange; **stamens** inserted above the base, filaments usually short, about ½ corolla length, densely villous; **anthers** 4–4.7 mm, villous, rounded at base and acute to mucronate at apex; **ovary** tubular-elliptic; **stigma** sub-globose, glabrous, non-exserted. **Capsules** always dehiscent with 3-valves. **Seeds** tiny ovate (Fig. 4.20).

Distribution: Northern Iran (Fig. 4.19).

Host: Unknown.

1.2.4 *Cistanche persica* Ataei, sp. nov.

Diagnosis: *Cistanche persica* is similar to *C. ridgewayana*, but differs by uppermost scales being glabrous (versus lanuginose in *C. ridgewayana*), 1 (–2) tiny linear bracteoles (versus 1 spatulate bracteole in *C. ridgewayana*), calyx lobes that are glabrous on the outer side and lanuginose at their margins (versus sparsely tomentose on the outer side in *C. ridgewayana*) and a corolla tube that is sparsely hairy inside (versus glabrous corolla tube in *C. ridgewayana*). The species has been misdetermined as *C. ridgewayana*.

Type: IRAN. **Sistan & Baluchestan:** Zahedan to Kerman, 100 km Bam to Mahan, 29°31'05"N, 57°48'15"E, 1980 m, 19 April 2010, *Ataei & Heidari* ED424 (holotype BONN, isotype USB).

Plant usually short, 28–36 cm tall, sub-glabrous, light to dark-purple. **Lower scales** imbricate, short triangular, obtuse, 4–8 (–11) × 3–7 mm, glabrous, entire, non-scarious at the margins, brownish; **middle scales** increasingly lax acropetally, ovate-lanceolate, obtuse, (9–) 11–13 × 2–3.5 (–6) mm, entire, non-scarious at the margins; **upper scales** very lax, lanceolate, obtuse, (11–) 14–20 × 3–4.5 mm, glabrous, entire, non-scarious at the margins. **Inflorescence** an oblong spike, with tubular and dense flowers, 9–15 cm long and 4–6 cm in diameter; **floral bracts** ovate, obtuse, (12–) 15–18 × 3.5–4.5 mm, entire, non-scarious and lanuginose at the margins, about 1.5 times as long as calyx, brownish (rarely pink to reddish); **floral bracteoles** usually 1, rarely 2, slender oblong-linear, acute to acuminate, 4–6 (–8) × 0.5–1 (–2.5) mm, maximally half as long as calyx, entire, non-scarious at the

margins, glabrous, rarely ciliate at the margins especially in the upper part, brownish (rarely pink to reddish); **calyx** campanulate, usually pentamerous, (7–) 8–13 mm long, 2½ the corolla length or shorter; **calyx lobes** 5, equal, oblong, rounded, 2–4 (–6) × (1.5–) 2–3.5 (–5) mm, with non-scarious margins, on the outer side glabrous, lanuginose at the margins; **corolla** pentamerous, short tubular, 24–28 (–30) mm long, without conspicuous folds inside the tube, rarely sparsely hairy on the inner side, pink-reddish; **corolla lobes** 5, broad-orbicular, glabrous, 2.5–4 × 3–4.5 mm, reddish; **stamens** inserted above the base, filaments usually short, about ½ corolla length, densely villous; **anthers** 3.5–4.5 mm long, moderately villous, rounded at base and mucronate at apex; **ovary** tubular-elliptic; **stigma** sub-globose to club-shaped, glabrous. **Capsules** always dehiscent with 3-valves. **Seeds** usually ovate (Fig. 4.21).

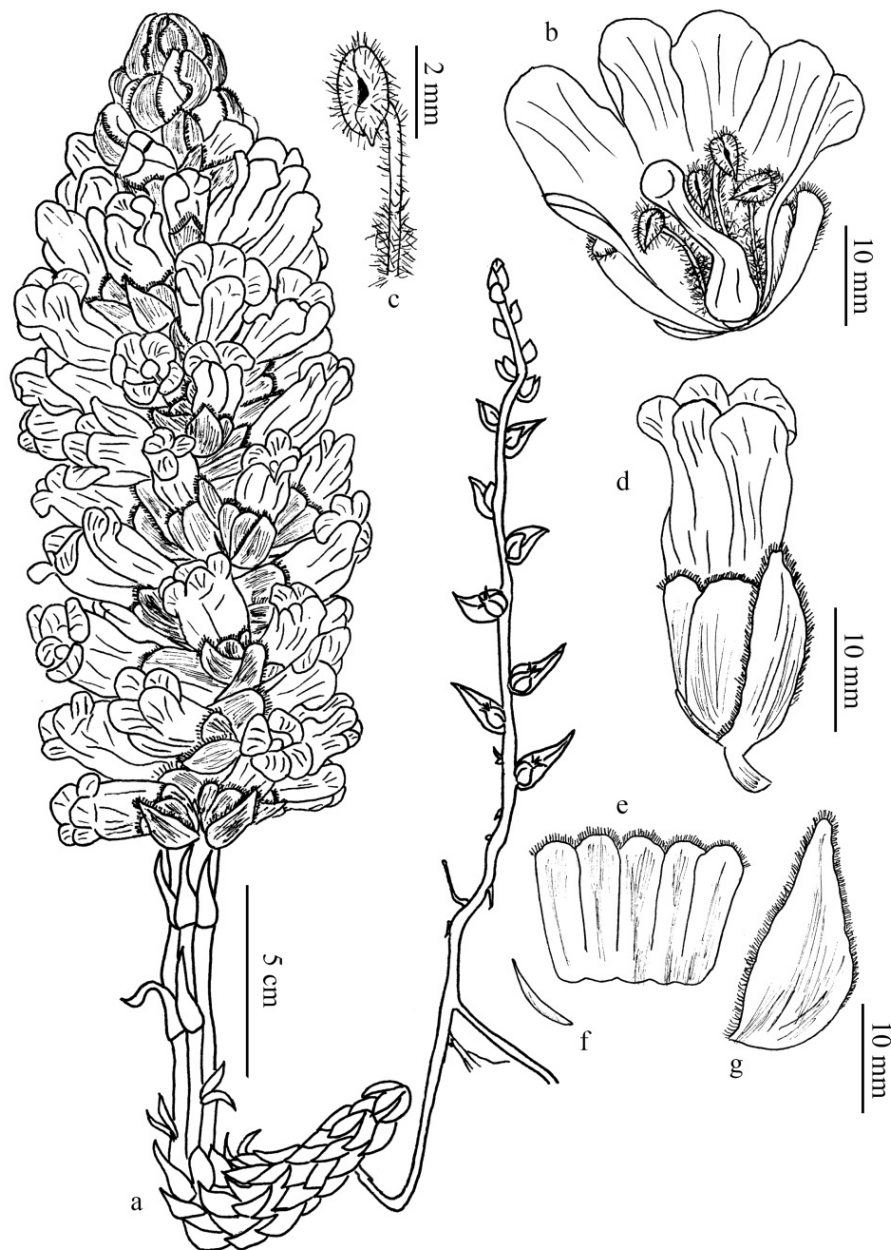


Figure 4.21 *Cistanche persica* Ataei sp. nov. a) flowering plant and partially its host (*Halothamnus auriculus* (Moq.) Botsch.), b) flower inner view, c) stamen, d) single flower, e) abaxial calyx, bract, f) one bracteole, and g) bract. The plant occurred in Iran (Ataei & Heidari ED424; BONN).

Distribution: Southern Iran, Pakistan (newly recorded) (Fig. 4.19).

Additional specimens examined (paratypes): **IRAN. Sistan & Baluchestan:** Saravan, 15 km Suran, Rigjeh village, 27°21'17"N, 61°55'53"E, 1135, 4 March 2011, *Ataei & Valizadeh* ED511 (BONN, USB); Between Zahedan and Nosratabad, 30 km Zahedan toward West, 29°47'00"N, 60°27'00"E, 1250, 24 April 1977, *Rechinger* 54614a (B); **Kerman:** BahramAbad, vicinity of Kabootarkhan, *Rechinger, Sfandary & Elen* 32301 (IRAN). **Razavi Khorasan:** Kushkuh, 1700, 16 April 1892, *Bornmüller* 3907 (B, E, P); **Semnan:** 38 km E of Shahrud at road to Miami, 36°25'00"N, 55°22'00"E, 1280, 19 May 1978, *Freitag* 15538 (B, KAS). **PAKISTAN. Balochistān:** Makran, Hoshab to Panjgur, c. 20-30 km from Panjgur, 26°58'00"N, 64°06'00"E, 20 April 1965, *Lamond* 580 (E, W).

Host: Amaranthaceae: *Halothamnus auriculus* (Moq.) Botsch. (ED424, field collection).

1.2.5 *Cistanche ambigua* (Bunge) Beck, Pflanzenr. (Engler) 96, 4.261: 40 (1930);

≡ *Phelypaea ambigua* Bunge, Mém. Acad. Imp. Sci. St.-Pétersburg Divers Savanas 7: 429 (1854) [basionym] ≡ *Orobanche salsa* var. *ambigua* (Bunge) Kuntze, Trudy Imp. S.-Peterburgsk. Bot. sada 10: 225 (1887).

Type: UZBEKISTAN. Turkestan: Kizil Kum, 20 April 1842, *Bunge* s.n. (holotype P barcode P02970944 seen as photo).

= *Cistanche eremodoxa* Bornm., Repert. Spec. Nov. Regni Veg. 40: 336 (1936); **Type: IRAN. Khorassan-e Jonubi:** Dasht-e-Kavir, "Rig-e Djinn", S Djandak-Biyabanak, 950 m, 10-12 April 1933, *Gabriel* 90 (lectotype B barcode B100264802 designated by Schiman-Czeika, 1964: 22, isolectotypes not found).

= *Cistanche trivalvis* (Trautv.) Korsh. Zap. Imp. Akad. Nauk Fiz.-Mat. Otd. 8 (4): 6 (1896) ≡ *Phelypaea trivalvis* Trautv., Trudy Imp. S.-Peterburgsk. Bot. Sada, 5:467-468 (1877). **Type: TURKMENISTAN. Krasnowodsk:** Tscheleken Island, *Becker* s.n. (holotype LE? not seen; isotype K).

Note: Morphological study on only available (almost destructed) single individual herbarium specimen of *C. trivalvis*, the specimen collected from Turkmenistan, Krasnowodsk (K) showed that it does not contain 3-valves capsules in contrast to Beck-Mannagetta (1930), who described this trait for delimitate the new species. Accordingly, based on strong morphological similarity it is here placed under the synonymy of *C. ambigua*.

= *Cistanche afghanica* Gilli, Candollea 34(2): 288 (1979). **Type: AFGHANISTAN. Helmand:** SE, 5 km N of Darweshan, 1300 m, 21 April 1969, *Hedge & Ekberg* W 7213 (holotype E barcode E00028887, isotype GB barcode GB0048287 seen as photo).

Plant commonly slender, 30–45 (–60) cm tall, sub-glabrous, white to light-brown. **Lower scales** very dense, imbricate, ovate-triangular, acute, 5–8 (–11) × 2–3.(–5) mm, glabrous, entire, non-scarious,

light-brown; **middle scales** dense, imbricate, ovate-lanceolate, obtuse, 11–15 (–21) × 1.5–3 (–5) mm, glabrous, entire, non-scarious at the margins; **upper scales** increasingly lax acropetally, ovate-lanceolate, obtuse, 16–25 (–30) × 2.5–3.5 (–6) mm, woolly or ciliate at their margins, entire, non-scarious at the margins, light-brown. **Inflorescence** an ovate-oblong spike, usually with dense downward bent flowers, (8–) 13–15 (–22) cm long and 4–8 cm in diameter; **floral bracts** ovate-lanceolate, sub-acute, (8–) 13–18 (–29) × 3–4 (–9) mm, about 4–10 mm longer than calyx, woolly at their margins, entire and non-scarious; **floral bracteoles** oblong-linear, acute, 7–12 × 0.8–2 mm, densely woolly at their margins, entire and non-scarious, either as long as calyx or slightly shorter; **calyx** usually tubular, (8–) 10–15 mm long, nearly about 1/3 as long as the corolla or slightly longer; **calyx lobes** 5, sub-equal, 2 lobes slightly longer than the 3 others alternatingly placed, ovate, acute, 3–5 × 4–6 (–9) mm, non-scarious and densely woolly at their margins; **corolla** campanulate, pentamerous, 35–39 (–42) mm long, slightly curved downward, cream, with two orange lines inside the tube, about 2.5 times as long as the calyx; **corolla lobes** 5, pink to light-purple, veined, 3–4 × (4–) 5–7 (–9) mm, densely woolly at their margins; **stamens** inserted above the base, filaments at base lanate, shorter than 2/3 corolla length; **anthers** 3–4.5 mm long, densely villous, rounded at base and apiculate at apex; **stigma** narrow club-shaped, non-exserted. **Capsules** always dehiscent with 2-valves. **Seeds** usually oblong, sometimes flat ovate (Fig. 4.22).

Distribution: mainly in Northern and Central Iran, Southern Afghanistan, Turkmenistan and Kazakhstan (Fig. 4.19).

Additional specimens examined: **AFGHANISTAN. Helmand:** O' Mohamad Rahim Kalay against Jabbar Oza Dasht, S from Darweshan, 30°41'00"N, 64°07'00"E, 710 m, 17 April 1972, *Anders* 8483 (MSB, W); **Kandahar:** Southeast, at desert Dasht-I Margo, 20 km NNW Darweshan, 20 May 1967, *Rechinger* 34710 (W); Registan-desert, vicinity of Bhaghat, 30°32'00"N, 63°52'00"E, 600 m, 19 January 1967, *Rechinger* 34645 (W); SW-border, 5 km S Bhagat, sand dunes at Hilmand valley, 19 May 1967, *Freitag* 718 (KAS). **IRAN. Tehran:** Kavir National Park, SE Waramin, 1 km after Shokr-Abad, around check point, 35°03'52"N, 51°41'54"E, 773 m, 24 April 2010, *Ataei & Heidari* ED437 (BONN, USB); SE Waramin, 5 km after Shokr-Abad, 35°01'31"N, 51°42'57"E, 923 m, 24 April 2010, *Ataei & Heidari* ED481 (BONN, USB); Kavir National Park, at desert, ± 60 km a Karavansarai Shah Abbas, 34°44'00"N, 52°10'00"E, 21 April 1975, 900 m, *Rechinger* 50176 (W); Kavir National Park, Karavansarai Shah Abbas, 900 m, 25 April 1974, *Wendelbo, Ala & Gobham* 10847 (TARI); Kavir National Park, Talheh, sand dunes, 900-1000 m, 7 May 1976, *Runemark, Foroughi & Asadi* 19539 (TARI); Kavir National Park, at the lake of Ghom (Namak Lake), 750-800 m, 8 May 1978, *Runemark, Foroughi & Assadi* 19548 (TARI); **Semnan:** Shahrud to Torud, 71 km Shahrud, roadside, 35°51'17"N, 55°11'55"E, 1062 m, 27 April 2010, *Ataei & Heidari* ED444 (BONN, USB); Shahrud, 8 Delbar versus Ahmadabad, May 1975, *Iranshahr* W 04410 (W); Zaman Abad-Bardaskan, down detour road in Zaman Abad, 2 km to Kalateh Karbalai Abbas, 35°46'36"N, 56°38'17"E, 949 m, 28 April 2010, *Ataei & Heidari* ED448 (BONN, USB); 90 km Zaman Abad to Bardaskan, between Ismail Abad and Salehieh, roadside, 35°12'36"N, 57°00'15"E, 951 m, 29 April 2010, *Ataei* ED553 (BONN, USB); Khar Turan

National Park, Chah Jam, 35°45'00"N, 55°07'00"E, 1100 m, 23 April 1978, *Freitag & Mozaffarian* 28414 (TARI); Bastam, Khar Turan National Park, NNE Ahmadabad, 35°46'00"N, 56°36'00"E, 29 April 1975, *Rechinger* 50654 (W) **Yazd**: Rig-e-Djin, 37 km Chah Malek, 800 m, 16 May 1975, *Rechinger* 51926 (W, K). **TURKMENISTAN. Balkan**: Krasnowodsk, *Beaker* s.n. (K).

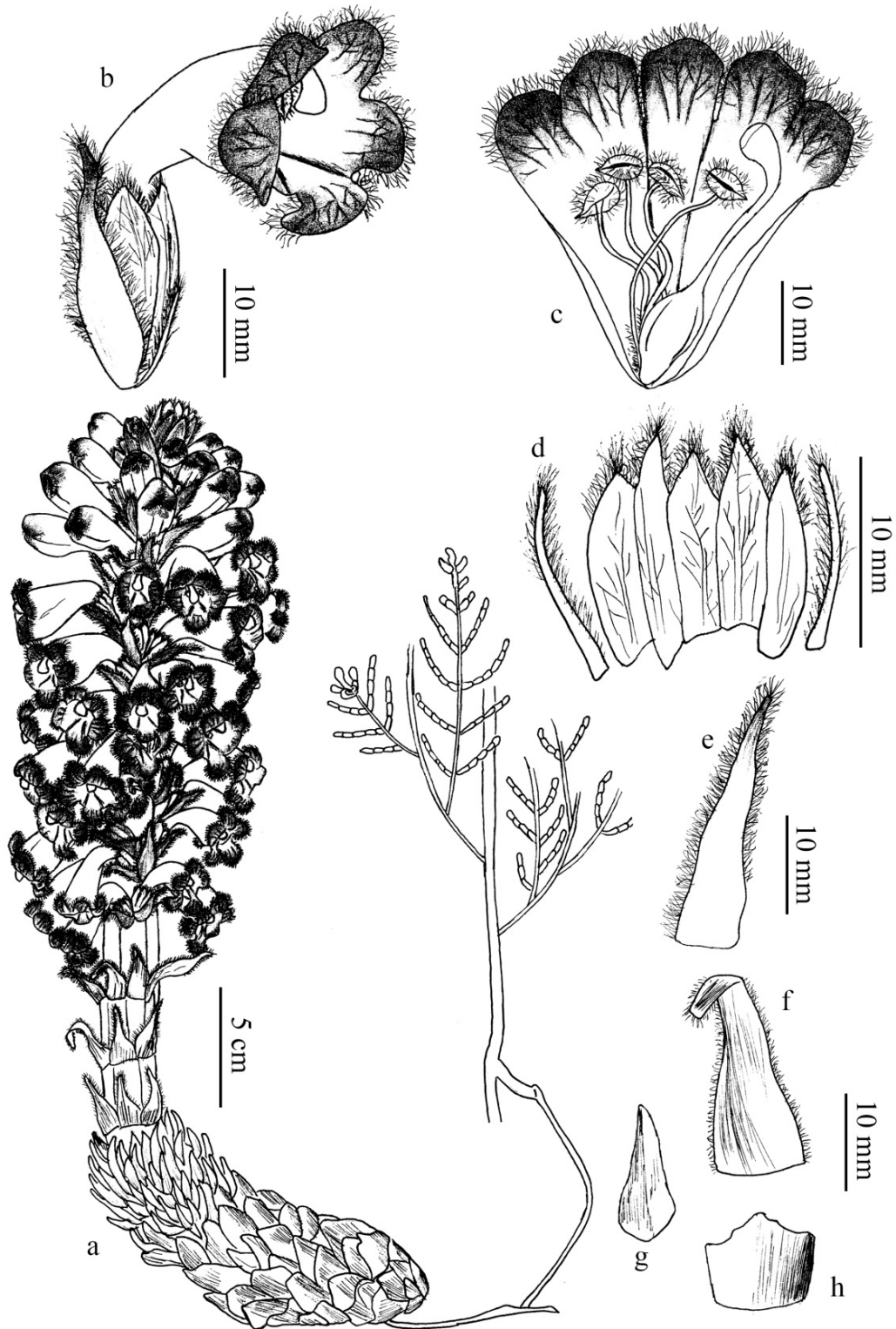


Figure 4.22 *Cistanche ambigua* (Bunge) Beck. a) flowering plant beside partially its host (*Siedlitzia rosmarinus* Ehrenb. ex Boiss.), b) single flower, c) flower inner view, d) abaxial calyx, and two bracteoles.

Host: Chenopodiaceae: *Seidlitzia rosmarinus* Bunge ex Boiss. (ED437 and ED481, field collections); **Tamaricaceae:** *Tamarix hispida* Willd. (ED553, field collection); **Amaranthaceae:** *Salsola* sp. (*Freitag* 718, KAS) in Afghanistan.

Remarks: Newly reported for Afghanistan. Beck defined *C. ambigua* as a variety of *C. fissa* while it was pointed as doubtful species in Flora Iranica (Schiman-Czeika, 1964) and considered as different species from *C. eremodoxa*. However, Gilli (1979) defined *C. eremodoxa* as a synonym of *C. ambigua*, which is accordant with our molecular data (see chapter 2 for detail) and taxonomic revision (Saeidi & Shahi, 2008; and this work). Two geographically different subclades observed in *C. ambigua*, do not show any morphological variations, while they are intermixed in cluster analysis. The species is readily differentiates from the other species in SWA clade by having bract, bracteoles, calyx and corolla on the outer side, in margins and rarely on the inner side hairy (vs. bract, bracteoles and calyx partially hairy in margins in the other species of SWA clade) (Fig. 4.2 here; Fig. 2.1 chapter 2).

1.2.6 *Cistanche salsa* (C.A.Mey.) Beck, Pflanzenfam. 4, 3b: 129 (1895); Beck, Pflanzenr. (Engler) 96, 4.261:36 (1930);

≡ *Phelypaea salsa* C.A.Mey., Fl. Altaic. [Ledebour] 2: 461 (1830) [basionym] ≡ *Orobanche salsa* (C.A.Mey.) Kuntze, Trudy Imp. S.-Peterburgsk. Bot. sada 10: 225 (1887).

Type: **KYRGYZSTAN.** “Soongoro-Kirghisici trans fl. Kurstschum oreintem versus” Meyer s.n. (holotype LE seen as photo; isotype PH barcode PH00029577, seen as photo).

= *Phelypaea armena* K.Koch, Linnaea 17: 291 (1843) ≡ *Orobanche salsa* var. *armena* (K.Koch) Kuntze, Trudy Imp. S.-Peterburgsk. Bot. sada 10: 225 (1887) ≡ *Cistanche armena* (K.Koch) M.V.Agab., Fl. Medit. 23: 135 (2013). *Type:* **ARMENIA.** “In salsis ad Araxem” [Araxes River], 1837, Koch s.n. (holotype B destroyed; lectotype LE barcode LE01010164 seen as photo, designated by Aghababayan, 2013: 135).

= *Orobanche salsa* var. *normalis* f. *breviloba* Kuntze, Trudy Imp. S.-Peterburgsk. Bot. sada 10: 225 (1887). *Type:* **TURKMENISTAN.** “Turkmenen-steppe”, May 1886, Kuntze s.n. (holotype NY barcode 00312481 seen as photo).

= *Cistanche salsa* var. *albiflora* P.F.Tu, Bull. Bot. Res. North-East. Forest. Univ. 14 (1): 32 (1994). *Type:* **CHINA. Ningxia:** Yanchi, 23 May 1992, Tu Peng-fei s.n. (holotype PEM 9252302 seen as photo).

= *Cistanche salsa* var. *longidens* Gilli, Feddes Repert. 82: 383 (1971). *Type:* **TURKEY. Konya:** Yavsa Memlehasi nr. Tuz Golu, 8 June 1952, Davis 18709 & Dodds (holotype E barcode E00028880, seen as photo).

Plant usually short and thin, 18–30 (–50) cm tall, sometimes pilose in upper part, light yellow. **Lower scales** very dense, imbricate, short ovate-triangular, obtuse, 4–7 (–10) × 3–5 (–9) mm, glabrous, entire, slightly scarious at the margins; **middle scales** increasingly lax acropetally, ovate-lanceolate

or oblong-lanceolate (in the species from Armenina), sometimes rhomboid, obtuse, 9–14 (–20) × 2.5–3.5 (–5) mm, densely white-woolly on the outer side and at the margins (in the species from Armenina), entire, rarely scarious at the margins; **upper scales** usually lax, ovate-lanceolate or oblong-lanceolate, sometimes rhomboid, obtuse, 13–20 (–30) × 3–5 mm, pilose on the outer side, rarely scarious at the margins. **Inflorescence** a short ovate-oblong spike, with usually few downward bent flowers, (5–) 8–12 (–25) cm long and 4–6 cm in diameter; **floral bracts** oblong-lanceolate, rarely ovate-oblong, obtuse, 14–18 (–27) × 3–4 (–5) mm, completely or partially pilose on the outer side, generally about 4–9 mm longer than the calyx, entire and non-scarious at the margins; **floral bracteoles** linear-oblong, acute, 8–10 (–13) × 0.9–1 (–2–2.5) mm, equal to or slightly longer than calyx, densely pilose on the outer side and at the margins, entire and non-scarious at the margins; **calyx** tubular, rarely campanulate, pentamerous, 9–12 (–14) mm long, nearly 1/3 corolla length, sparsely pilose on the outer side and at the margins; **calyx lobes** 5, equal, oblong, rounded, 2.5–4 (–6) × (1.5–) 2.5–3 mm, entire and non-scarious at the margins; **corolla** campanulate, with 5 unequal lobes (upper longer than lateral longer than lower), straight to near the apex then curved down, 25–35 (–42) mm long, pale lilac or white, with two yellow folds inside the tube; **corolla lobes** orbicular, rounded, mauve, 4–6 × 4–6 (–8) mm, glabrous, occasionally sparsely ciliate; **stamens** inserted at about 1/4 corolla length, filaments 2/3 as long as the corolla, villous at base; **anthers** 3–4 mm long, ovate-oblong, rounded at base and aristate at apex, densely villous; **ovary** ovate; **stigma** narrow club-shaped, non-exserted. **Capsules** always dehiscent with 2-valves. **Seeds** oblong, sometimes ovate (Fig. 4.23).

Distribution: Wide longitudinal distribution by occurring in high elevations (up to 2700 m) from Turkey and Caucasus region via Central Asia to Mongolia and China, also reported from Israel (Dead Sea; Post, 1933), Kazakhstan e.g. Baikonur (photo by Yuriy at http://upload.wikimedia.org/wikipedia/commons/d/d1/Cistanche_salsa%3B_Baikonur_02.jpg, accessed on February 2015). A doubtful record of *C. salsa* from Shaib al-Batin in Iraq (Jarishan (R.8766 b; Schiman-Czeika, 1964) is probably due to misidentification with *C. ambigua*, as the occurrence of *C. salsa* in Iraq is rather unlikely (Fig. 4.19).

Additional specimens examined: **ARMENIA. Ararat:** Ararat District, Khor-Virab monastery vicinity, ca. 1 km N Khor-Virab, 23 May 2004, *Fayvush & Tamanyan* 9-2004 (W, P). **AZERBAIJAN.** Qäbälä, Alp. Savalan, 1836, *Aucher-Eloy* N°5079 (P, K). **CHINA. Inner Mongolia:** 17-20 April 1923, *Ching* 40 (E); **Xinjiang Uygur Zizhiqu:** Kashgar, July 1875, *Bellew* s.n. (K). **IRAN. West Azerbaijan:** Aras, s. col. 1801 (E, K). **JORDAN. Ma'an:** Wadi Shaumari, 44 km NE EL Jafr, 1 May 1976, *Boulous & al.* 8625 (K). **KAZAKHSTAN. Qostanay:** Between Arkalyk and Derzhavinsk, 1840, *Karelin* 386 (P); Songaria [between Arganaty and Keyssyk-auss], 1868, *Schrenk* s.n. (P); Vernoie [?], *Chaffanjon* 603 (P). **TURKEY. Konya:** Yavsä Memlehasi nr. Tuz Golu, 8 June 1952, *Davis* 18719 & *Dodds* (E barcode E00028881, seen as photo); Yavsän Memlehasi nr. TuzGolu, 8 June 1952, *Davis & Dodds* 18709 (K, BM); Cihanbeyli, Yavsän Tuzlasi, 28 May 1989, *Coskun & Koyuncu* s.n. (BM).

TURKMENISTAN. Balkan: Transcaspica region Kransnowodsk, vicinity of Ufra, 21 April 1901, Beck 1541 (BM, B, K, P). **INDEFINITE.** Northern Persia ("Persia bor"), Szovits s.n. (P barcode P02983466). **Host: Chenopodiaceae:** unclear report of *Kochia* sp. (Davis & Dodds 18709, K); *Salsola passerina* Bunge, (Novopokrovskii & Tzvelev, 1958); **Amarantaceae:** *Anabasis* sp., *A. cretacea* Pall., *Atriplex* sp., *A. cana* C.A.Mey., *A. verrucifera* M.Beb., *Kalidium caspicum* (L.) Ung.-Sternb., *K. foliatum* (Pall.) Moq., *K. gracile* Fenzl., and *K. cuspidatum* (Ung.-Sternb.) Grubov (herbarium observations).

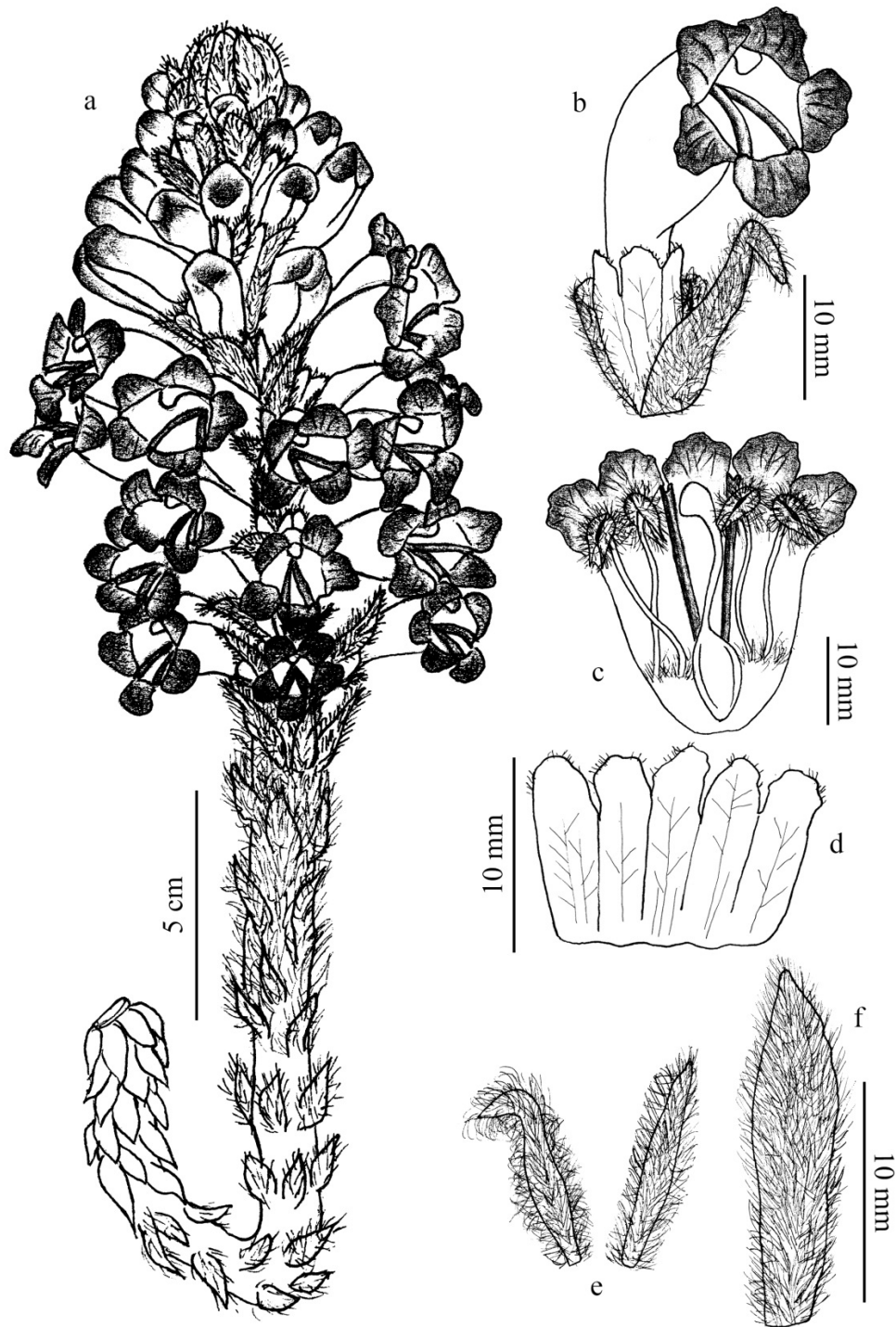


Figure 4.23 *Cistanche salsa* (C.A.Mey.) Beck. a) flowering plant, b) single flower, c) flower inner view, d) abaxial calyx, e) two bracteoles, and f) bract. The plant occurred in Armenia (Fayvush & Tamanyan 9-2004; W).

Remarks: The specimens show the most distinctive morphological differences according to locality in comparison to other species of *Cistanche*, however, lack of molecular evidence and sampling warrants any infraspecific classification (Table 4.4).

1.2.7 *Cistanche fissa* (C.A.Mey.) Beck, Pflanzenfam. 4, 3b: 129 (1895);

≡ *Phelypaea fissa* C.A.Mey., Verz. Pfl. Casp. Meer. (C.A. von Meyer). 104 (1831) [basionym] ≡ *Orobanche salsa* Kunth var. *ambigua* f. *fissa* (C.A.Mey.) Kuntze, Trudy Imp. S.-Peterburgsk. Bot. sada 10: 225 (1887) ≡ *Cistanche fissa* var. *genuina* Beck, Pflanzenr. (Engler) 96, 4.261: 39 (1930), nom. inval.

Type: **AZERBAIJAN. Baku:** near Baku, Meyer s.n. (holotype LE not seen, isotype P barcode P02970922 seen as photo).

= *Cistanche sintenisii* Beck in Bornm., Bull. Herb. Boissier ser. II. 4: 686 (1904); ≡ *Cistanche fissa* (C.A.Mey.) var. *sintenisii* (Beck) Beck, Pflanzenr. (Engler) 96, 4.261: 40 (1930). Type: **TURKMENISTAN. Transcaspica region:** Chodschakala, 14 April 1901, *Sintenis* 1864 (holotype B barcode B100264823, isotypes G barcode G00383269 seen as photo, WU barcode WU066010).

= *Cistanche lanzhouensis* Zhi Y.Zhang Bull. Bot. Res., Harbin 4 (4): 114 (1984). Type: **CHINA. Gansu:** Lanzhou, Liu & Sun 215 (LZU not seen).

= *Cistanche ningxiaensis* D. Z. Ma & J. A. Duan, Acta Bot. Boreal.-Occid. Sin. 13(1): 75 (1993). Type: **CHINA. Ningxia:** Lingwu, Ciyaobu, 1 June 1991, Duan & Wan 9161 (NXAC not seen).

= *Orobanche salsa* var. *ambigua* f. *longiloba* Kuntze, Trudy Imp. S.-Peterburgsk. Bot. sada 10: 225 (1887). Type: **TURKMENISTAN.** “Turkmenen-steppe”, May 1886, Kuntze s.n. (holotype NY barcode 00312480, seen as photo).

Plant commonly short and thin, up to 28 cm tall, sub-glabrous, white to pale purple. **Lower scales** dense, imbricate, ovate-triangular, obtuse, 5–7 × 2.5–3 mm, glabrous, entire, non-scarious at the margins; **middle scales** increasingly lax acropetally, lanceolate, sub-acute, 15–16 × 3.5–4 mm, glabrous, entire, non-scarious at the margins; **upper scales** very lax, lanceolate, sub-acute, 17–18 × 3.5–4 mm, glabrous except for the uppermost scales that are densely villous at the margins and sparsely villous on the outer side, entire, non-scarious at the margins. **Inflorescence** an ovate-oblong spike, with dense, curved down flowers, 8–12 cm long and 4.5 cm in diameter; **floral bracts** ovate to oblong-lanceolate, sub-acute, 16–17 × 5–5.5 mm, densely woolly on the outer side, about 4–9 mm longer than calyx, entire and non-scarious; **floral bracteoles** oblong-linear, acute, as long as calyx or slightly longer, 9–10 × 0.8–0.9 mm, woolly at their margins and slightly woolly on the outer side in their upper fourth, glabrous elsewhere, entire and non-scarious; **calyx** short campanulate, shortly incised, 8–10 mm long, usually half as long as the corolla, loosely woolly on the outer side and densely woolly at the margins, often entire and non-scarious; **calyx lobes** 5, unequal, 4 ovate and obtuse-rounded ones plus one tooth-like, acute, 2 × 1.5 mm; **corolla** tubular, slightly broadened at

throat, 28–30 mm long, cream to pale purple with 2 yellow folds inside the tube, about 2.5 times as long as calyx, glabrous inside the tube; **corolla lobes** 5, equal, rounded, light-pinkish or purple, on the outer side glabrous, 3.5–4 × 3–4 mm, densely woolly inside and at the margins; **stamens** inserted at about 1/6 above the base, filaments 2/3 as long as the corolla, at base lanate; **anthers** 3–3.5 mm long, oblong, rounded at base and aristate at apex, densely lanate; **ovary** ovate; **stigma** narrow club-shaped, non-exserted. **Capsules** always dehiscent with 2-valves. **Seeds** oblong, sometimes ovate (Fig. 4.24).

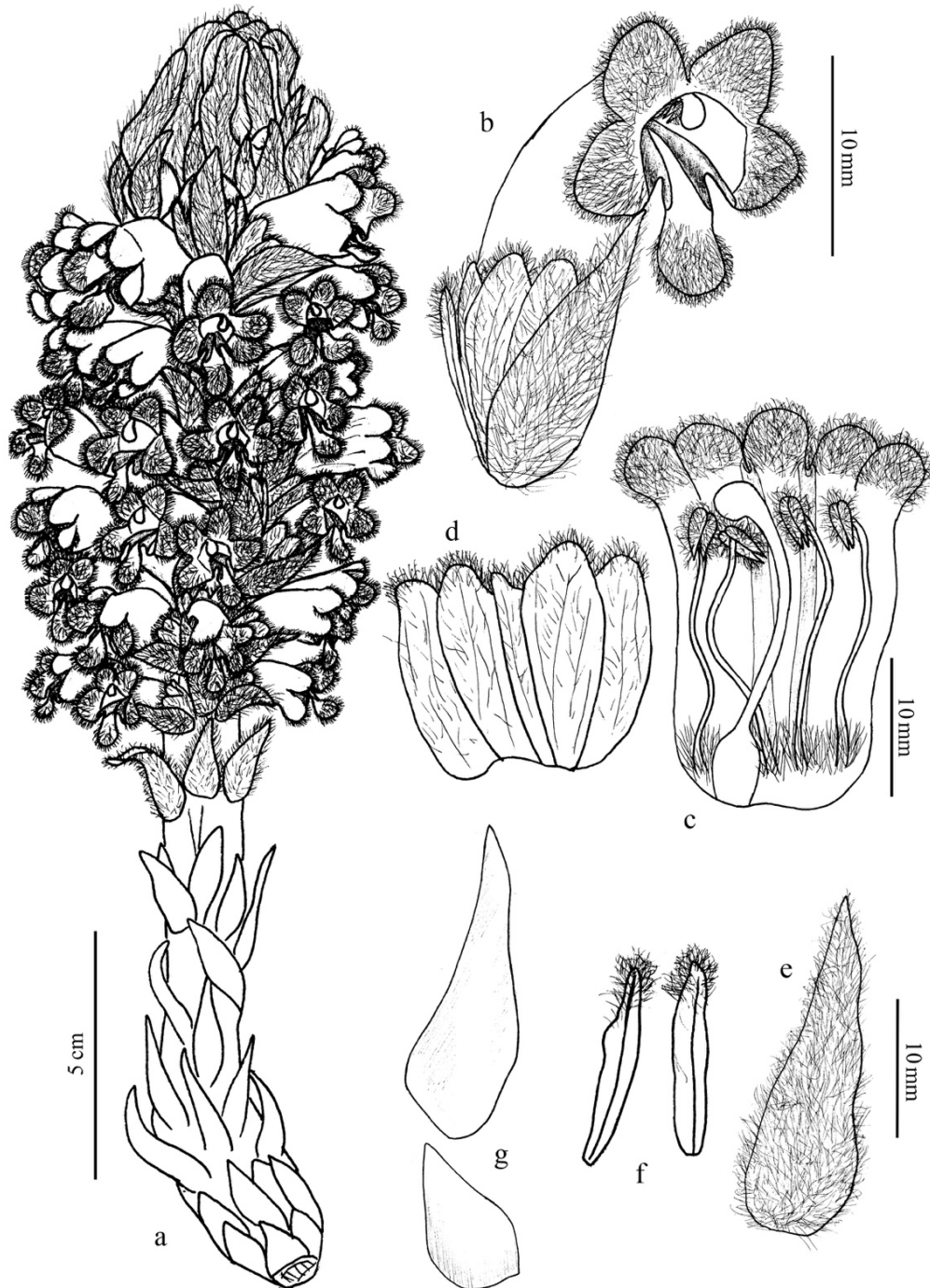


Figure 4.24 *Cistanche fissa* (C.A.M.) Beck. a) flowering plant, b) single flower, c) flower inner view, d) abaxial calyx, e) bract, f) two bracteoles, g) basal, and median scales. The plant occurred in Azerbaijan (Sintenis 1864; B).

Distribution: Azerbaijan, Turkmenistan, China (Fig. 4.19).

Host: Two suspicious reports of **Amaranthaceae:** *Salsola aucheri* (Moq.) Bunge ex Iljin.; **Asteraceae:** *Artemisia* sp. (Beck-Mannagetta, 1930; Novopokrovskii & Tzvelev, 1958).

Remarks: We did not see the type specimens of *Cistanche lanzhouensis* and *C. ningxiaensis*, but original descriptions and illustrations allow confident placement of these names in the synonymy of *C. fissa*. The corolla colour in the original description is yellow according to dry material, which interrupts the conclusions. This is, therefore, the first report of *C. fissa* for China. In the Flora of China (Zhang & Tzvelev, 1998), only *Cistanche lanzhouensis* was accepted, while *C. ningxiaensis* was placed under its synonymy. Beck differentiated *Cistanche sintenisii* as variety for *C. fissa* by having lanceolate and acuminate scales, and lax but round scales at the base; 2 oblong bracteoles shorter than calyx and vilous at the margins; calyx with 4-lobes, rounded and corolla with vilous on the outer side. Both specimens were morphologically examined and according to isotype specimen, the only different is the density of hairs on corolla lobes than in *C. fissa*, which could be affected by environmental conditions. According to very narrow distribution and unavailable current collection, it is proposed to be included in IUCN list.

1.2.8 *Cistanche bamianica* Ataei, sp. nov.

Diagnosis: *Cistanche bamianica* is similar to *C. fissa*, but differs by densely hairy yellow folds inside the corolla (versus glabrous in *C. fissa*), corolla lobes pilose at their margins, sparsely pilose on the inner side (versus densely pilose on the inner side in *C. fissa*), and bracts that are as long as or slightly shorter than the corolla (versus shorter than the corolla in *C. fissa*). The species has been misdetermined as *C. salsa*.

Type: **AFGHANISTAN. Bamian:** Doab, ca. 15 km West on the roadside, 21 March 1962, Hedge & Wendelbo W 3427 (holotype E barcode E00028886).

Plant short and thin, 25–26 cm tall, sub-glabrous, yellowish. **Lower scales** dense, imbricate, ovate-triangular, obtuse, 8–9 × 5–6 mm, glabrous, entire, non-scarious at the margins; **middle scales** lax, ovate-lanceolate, obtuse, 12–16 × 3.5–4.5 mm, glabrous, entire, non-scarious at the margins; **upper scales** very lax, ovate-lanceolate, obtuse, (13–) 17–20 × (2.5–) 4–6 mm, glabrous except uppermost scales that are densely villous at the margins and distally sparsely villous on the outer side, entire, non-scarious at the margins. **Inflorescence** an ovate spike, 10–13 cm long and 5.5–6 cm in diameter; **floral bracts** ovate-lanceolate, acute, 19–24 × (2.5–) 6–8 mm, densely woolly on the outer side, about 4–9 mm longer than calyx, entire and non-scarious; **floral bracteoles** oblong-linear, acute, longer than calyx, 12–17 (–20) × 1–1.5 (–2) mm, very densely woolly at the margins and slightly woolly on the outer side, entire and non-scarious at the margins; **calyx** short campanulate, deeply incised about ½ its length, 12–15 mm long, usually less than half as long as the corolla, densely

woolly at the margins, entire and non-scarious at the margins; **calyx lobes** 5, unequal, 4 oblong-rounded plus one slightly shorter, ovate-acute, 4.5–6 × 3–4 mm; **corolla** tubular, 30–40 mm long, about 2.5 times as long as calyx, cream to pale purple with 2 yellow folds inside the tube, sparsely hairy inside the tube, hairs extending to the two yellow folds dense at lower and sparse upper part; **corolla lobes** 5, equal, reticulate veined, rounded, light-pinkish or purple, outside glabrous, 5–9 × 7–11 mm, densely woolly on the inner side and at the margins; **stamens** inserted at about the base, filaments 2/3 as long as the corolla, at base densely lanate; **anthers** 4.5–5 mm long, oblong, rounded at base and aristate at apex, densely lanate; **ovary** ovate; **stigma** narrow club-shaped, non-exserted. **Capsules** always dehiscent with 2-valves. **Seeds** ovate (Fig. 4.25).

Distribution: Disjunctly distributed in Jordan and Afghanistan (Fig. 4.19).

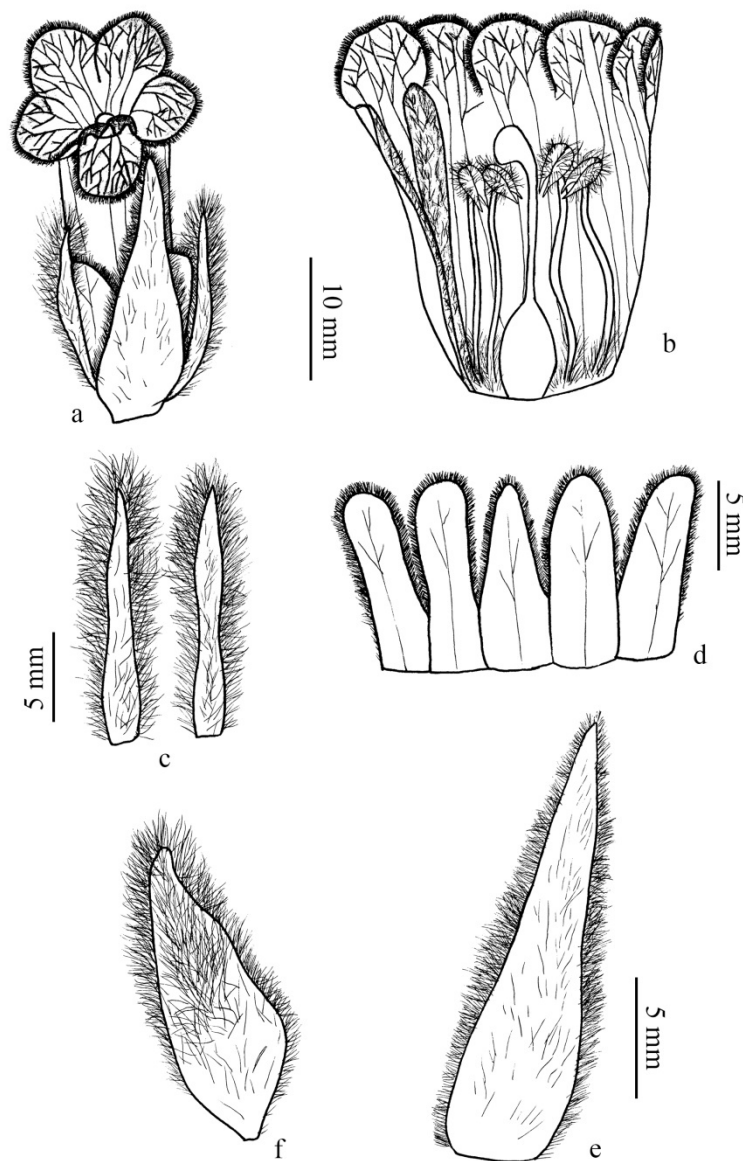


Figure 4.25 *Cistanche bamianica* Ataei, sp. nov. a) single flower, b), flower inner view, c) two bracteoles, d) abaxial calyx e) bract, and f) median scale. The plant occurred in Afghanistan (Meinertzhagen s.n.; BM).

Additional specimens examined (paratypes): JORDAN. East, October 1947, Paine 1873 (K). AFGHANISTAN. North, 1 May 1937, Meinertzhagen s.n. (BM).

Host: Unknown.

Remarks: According to very narrow distribution and unavailable current collection, it is proposed to be included in IUCN list.

1.2.9 *Cistanche bilobata* Ataei, sp. nov.

Diagnosis: *Cistanche bilobata* is similar to *C. fissa*, but differs by the calyx possessing very long silky and villous hairs and bidentate acuminate lobes (versus possessing short hairs and simple, rounded lobes in *C. fissa*). The species has been misdetermined as *C. salsa*.

Type: AZERBAIJAN. Mughan: 1929, Sarnarovy s.n. (holotype B barcode B100390223).

Plant short, slender, up to 23 cm tall, sub-glabrous, brownish. **Lower scales** very dense, imbricate, ovate-triangular, obtuse, 9–10 × 2.5–3 mm, glabrous, entire, non-scarious at the margins; **middle scales** dense, less imbricate, lanceolate, sub-acute, 14–16 × 3–4 mm, glabrous, entire, non-scarious at the margins; **upper scales** very lax, long lanceolate, subacute, 22–23 × 4–6 mm, glabrous except the uppermost ones, which are densely silky on the outer side, entire, non-scarious at the margins. **Inflorescence** an ovate-oblong spike, with a few curved down flowers, 8 cm long and 4.5 cm in diameter; **floral bracts** long lanceolate, acute-subacuminate, 20–22 × 3–4 mm, densely silky on the outer side, entire and non-scarious at the margins, about 4–9 mm longer than calyx; **floral bracteoles** linear-cylindrical, acuminate, 13 × 0.9 mm, with long silky hairs at the margins, entire and non-scarious at the margins, as long as calyx; **calyx** short tubular, 12–13 mm long, usually half as long as the corolla, covered outside with very long silky and villous hairs up to 5 mm long, but non or less hairs at the suture of the sepals; **calyx lobes** 5, equal, bidentate, 2.5–3 × 3–3.5 mm; **corolla** tubular, slightly curved downwards at apex, 31–35 mm long, about 2.5 times as long as calyx, cream-colored with 2 yellow folds inside the tube; **corolla lobes** 5, equal, rounded, 5–6 × 7–8 mm, silky on inner side of the margins of lobes, light-purple; **stamens** inserted at about 1/9 above the base, filaments 2/3 as long as the corolla, at base densely covered with silky hairs; **anthers** 3–3.5 mm long, oblong, rounded at base and long-aristate at apex, densely lanate; **ovary** oblong; **stigma** narrow club-shaped, non-exserted. **Capsules** always dehiscent with 2-valves. **Seeds** unknown (Fig. 4.26).

Distribution: Only known of the type specimen and endemic in Azerbaijan (Yevlakh district, Aharbahar ridge and Gobustan) (Fig. 4.19).

Host: Unknown.

Remarks: According to very narrow distribution and unavailable current collection, it is proposed to be included in IUCN list.

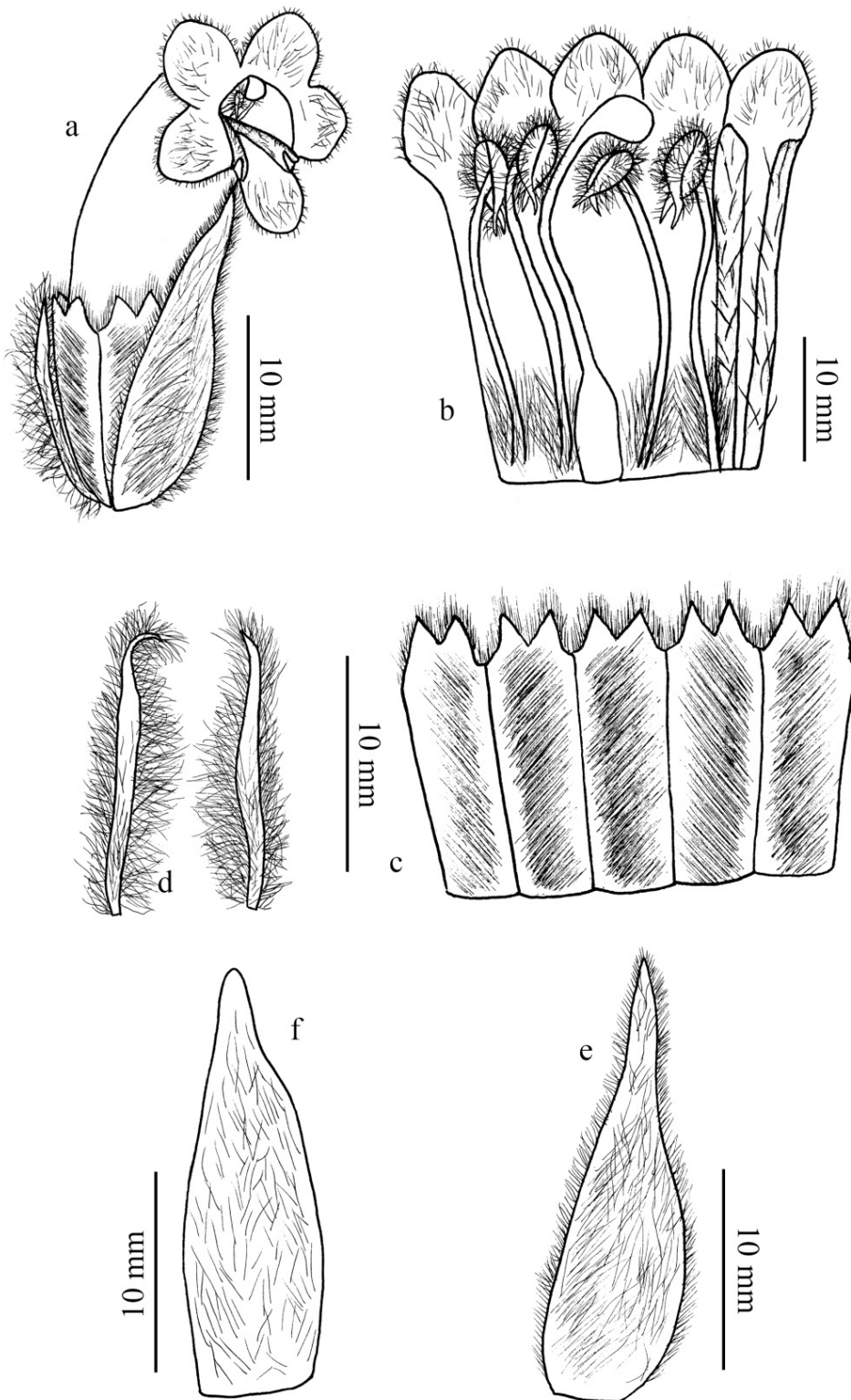


Figure 4.26 *Cistanche bilobata* Ataei, sp. nov. a) single flower, b), flower inner view, c) abaxial calyx, d) two bracteoles e) bract, and f) upper scale. The plant occurred in Azerbaijan (Sarnarovy s.n.; B).

1.3 *Cistanche* sect. *Macrocalycinum* Ataei, sect. nov.

Type species: *Cistanche macrocalycinum* Ataei

Plant usually stout, with usually short and slender stem. **Lower scales** ovate-triangular, rounded, orbicular, non-scarious at the margins; **middle and upper scales** ovate-rhomboid, rounded, orbicular, non-scarious at the margins, densely arachnoid-lanuginose on the outer side. **Inflorescence** an ovate spike usually with few flowers; **floral bracts** ovate-rhomboid, obtuse, non-scarious at the margins, densely arachnoid-lanuginose on the outer side and at the margins; **floral bracteoles** oblong, obtuse, acute, hairy mainly at the margins; **calyx** usually campanulate, shortly incised, hairy on the outer side; **calyx lobes** often 5, unequal, acute, hairy at the margins; **corolla** tubular, slightly curved downwards, pentamerous, mainly with two yellow folds inside the tube; **corolla lobes** 5, equal, entirely glabrous; **stamen** inserted at the base of corolla tube, hairy; **anthers** acute, mucronate, apiculate, acuminate, cuspidate or aristate at apex, non-exserted; **ovary** ovate to oblong. **Capsule** opens with 2-valves. **Seeds** tiny, elliptic.

Distribution: *Cistanche* sect. *Macrocalycinum* has two species distributed in Algeria and Morocco (Fig. 4.27).

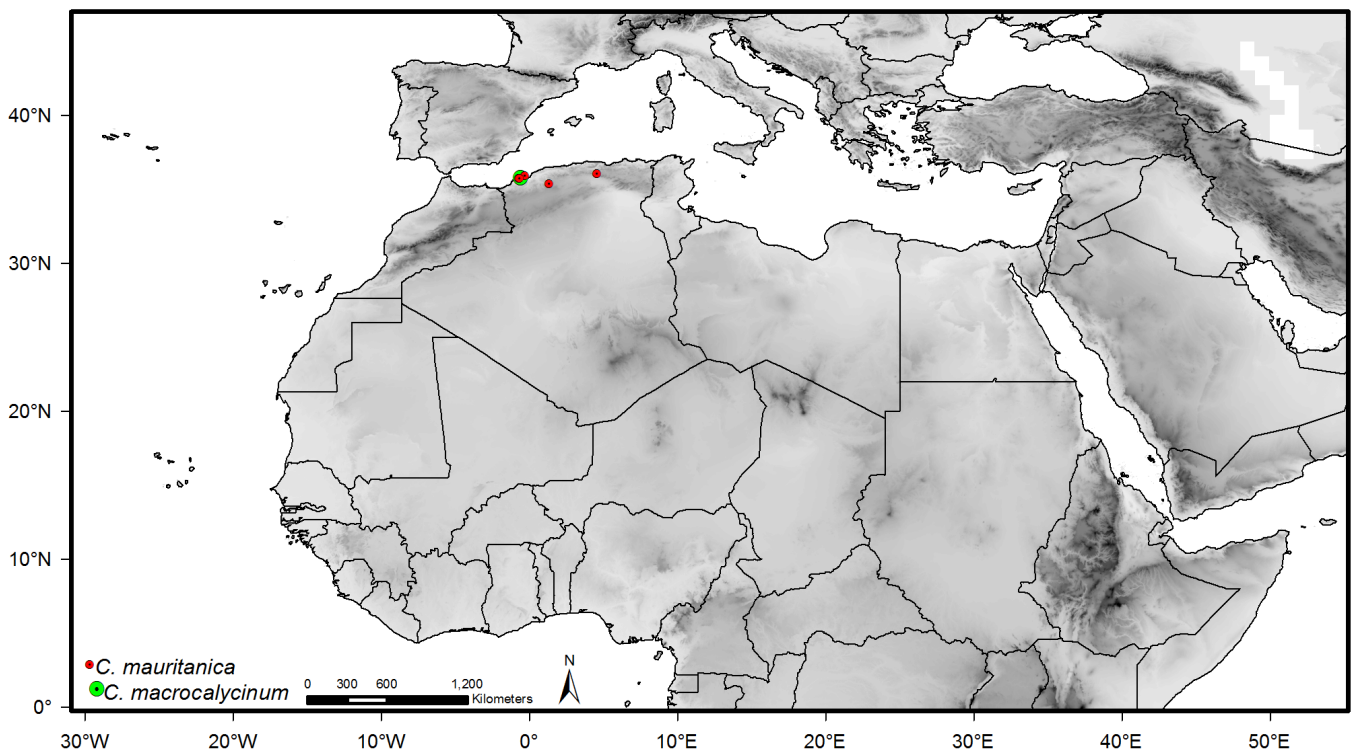


Figure 4.27 Distribution map of species of the *Cistanche* sect. *Macrocalycinum* worldwide.

Key to the species of *Cistanche* sect. *Macrocalycinum* Ataei

1. Scales and bracts ovate; bracteoles 2, oblong-linear, entirely free; calyx lobes 5, unequal, two with acute to acuminate and three with obtuse apices, one of these three is shorter than the others, with prominent midrib, calyx slightly longer than floral bracts. Inflorescence raceme; flower with short pedicel. Algeria **1.3.1 *C. mauritanica***
- Scales and bracts broadly rhomboid, often equal in length and width, embracing ca. 2/3 of calyx diameter (semi-amplexicaule); bracteoles 2, oblong, fused with calyx by means of one margin, free at the other margin and tip; calyx lobes 5, unequal, rounded, 4 longer than one, with non-prominent midrib, shorter than floral bracts; Inflorescence spike. Algeria, Morocco **1.3.2 *C. macrocalycinum***

1.3.1 *Cistanche mauritanica* (Coss. & Durieu) Beck, Pflanzenr. (Engler) 96, 4.261: 37 (1930);

≡ *Phelypaea mauritanica* Coss. & Durieu, Bull. Soc. Bot. France 4: 409 (1857) [basionym]

Type: **ALGERIA. Oran**: "Terrain salés aux environs de la Sénia", 7 February 1852, *Balansa* 413 (holotype P barcode P00214516, isotypes E barcode E00029743 seen as photo, W).

Plant usually thin and short, 5–15 rarely up to 40 cm tall, light to dark brown. **Lower scales** very dense, imbricate, broad-ovate to broad-rhomboid, acute, 3.5–7 (–8.5) × 3.5–4 (–7) mm, glabrous, entire, non-scarious at the margins; **middle** and **upper scales** dense, broad-ovate, acute, 7–11 × 4–7 (–9) mm, glabrous except for the uppermost scales (below the inflorescence) that are densely long arachnoid-lanuginose on the outer side, sometimes glabrous at the margins, entire, non-scarious at the margins, usually brownish (upper ones appearing white because of the indumentum). **Inflorescence** raceme, a few flowers possessing a short pedicel up to ca. 2 mm long, 5–7 cm long and 4.5–6 cm in diameter; **floral bracts** ovate, acute, 11–14 (–17) × 4.5–8 (–12) mm, equal to or slightly shorter than calyx, with long white arachnoid-lanuginose hairs on the outer side and particularly at the margins, entire and non-scarious at the margins; **floral bracteoles** oblong-linear, sub-acute, 12–14 (–17) × 1.5–2 mm, about as long as calyx or slightly shorter, with white arachnoid-lanuginose hairs on the outer side and at the margins except the upper sixth that is glabrous, entire and non-scarious at the margins; **calyx** campanulate- ovoid, 13–16 (–20) mm, abundantly arachnoid-lanuginose on the outer side, with prominent midrib; **calyx lobes** 5, sub-equal, 4 longer than one, 2 acute to acuminate plus 3 more rounded, 3.4–4 × 2.5–3 mm, with non-scarious margins at the margins; **corolla** tubular, at top slightly broadened, 33–38 mm long, on average twice as long as calyx, light-purple, with two yellow folds inside the tube; **corolla lobes** 5, unequal (upper longer than lateral longer than lower), overlapping, rounded, 3.5–5 × 3–6 mm, glabrous, dark-purple; **stamens** inserted at about 1/6 corolla length, filaments 2/3 as long as the corolla, at base villous; **anthers** 2.5–4 mm long, oblong, rounded at base and cuspidate to aristate at apex; **ovary** ovate, dark-brown; **stigma** narrow club-shaped, glabrous, non-exserted. **Capsules** dehiscent with 2-valves. **Seeds** unknown (Fig. 4.28).

Distribution: Endemic to North Africa. In 1998, a small population of the species was recorded from Spain [doubtful record], Islas Chafarinas off the coast of Morocco (López Jiménez & Caballero de Rodas, 2004) and Morocco (Fennane & al., 2007). Material from Morocco formerly assigned to this species (*Meauricio* s.n., BM) belongs to *C. macrocalycinum* (Fig. 4.27).

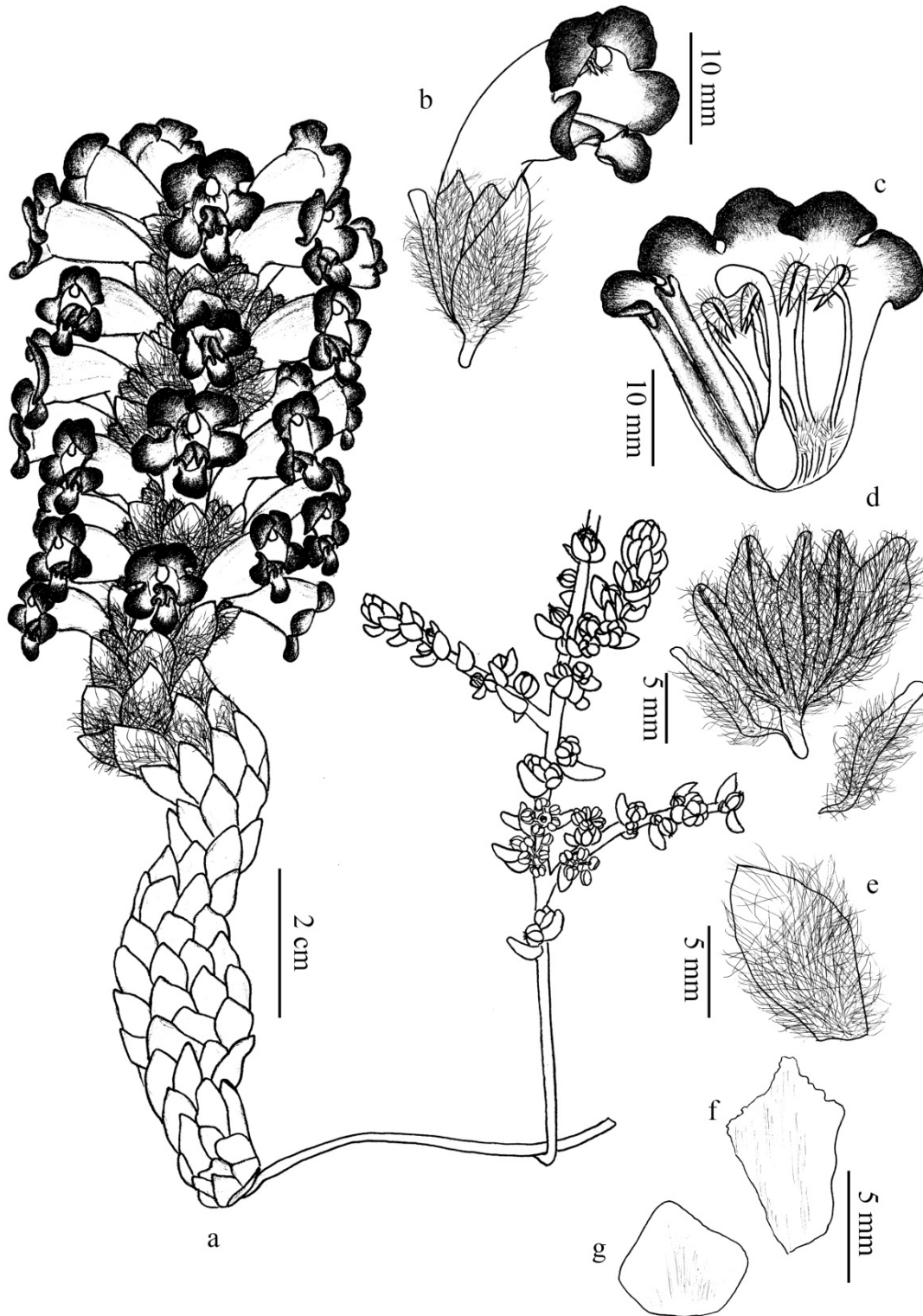


Figure 4.28 *Cistanche mauritanica* (Coss. & Durieu) Beck. a) flowering plant and its host (*Suaeda vera* Forssk ex J.F.Gmel), b) single flower, c) flower inner view, d) abaxial calyx plus two bracteoles, e) bract, f) median scale, and g) basal scale. The plant occurred in Algeria (Warion s.n.; BM).

Additional specimens examined: ALGERIA. Oran: Ravin Blanc, February 1874, *Warion* s.n. (P, BM); 13 April 1883, *Debeaux* 4993 (BM, P); Machta beldj, 1940, *Maire* s.n. (P); Arzew, *Bravais* s.n. (P).

Host: **Amaranthaceae:** *Chenopodium frutescens* C.A.Mey. (*Balansa* 413, E, P, W), *Atriplex glauca* L., (Jahandiez & Maire, 1934), *A. halimus* L. (*Maire* s.n., P); **Solanaceae:** *Lycium intricatum* Boiss. (López Jiménez & Caballero de Rodas, 2004).

Remarks: According to very narrow distribution and unavailable current collection, it is proposed to be included in IUCN list.

1.3.2 *Cistanche macrocalycinum* Ataei, sp. nov.

Diagnosis: *Cistanche macrocalycinum* is similar to *C. mauritanica*, but differs by bracteoles being fused with the calyx tissue and the broad rhomboid bracts (versus free bracteoles and rhomboid bracts in *C. mauritanica*). The species has so far been misdetermined as *C. mauritanica*.

Type: ALGERIA. Oran: February 1857, *Pelry* s.n. (holotype K).

Plant light to dark brown, thin and short, 14 cm tall, sub-glabrous. **Lower scales** very dense, imbricate, broad semi-orbicular, rounded, 5–6 × 5–6 mm, glabrous, entire, non-scarious; **middle** and **upper scales** slightly less dense, less imbricate, semi-orbicular to broad-rhomboid, acute, sometimes truncate, 8–15 × 10–14 mm, semi-amplexicaule, dark-brown when dry, often glabrous except for uppermost scales (4–5 rings below the inflorescence) that are sparsely covered with long hairs, entire and non-scarious. **Inflorescence** an ovate spike, with densely arranged flowers, 8 cm in either length or diameter; **floral bracts** amplexicaule, broad-rhomboid, acute, 28–30 × 18–20 mm, semi-amplexicaule, white when young due to the compact indumentum of long-arachnoid hairs on the outer side, about 2–5 mm shorter than calyx, entire and non-scarious at the margins, dark-brown when dry; **floral bracteoles** oblong, acute, 21–22 × 1.5–2 mm, fused by means of one margin with calyx while free in other margin and tips, equal to or slightly shorter than calyx, densely arachnoid either at the distal 2–4 mm or at the margins, entire and non-scarious; **calyx** tubular-campanulate, up to 35 mm long, shorter than floral bracts, long-arachnoid on the outer side; **calyx lobes** 5, unequal, 4 slightly longer plus one slightly shorter, rounded, 4–5 × 3–4 mm, densely long-arachnoid on the outer side and at the margins, entire and non-scarious; **corolla** tubular, straight, slightly curved downwards at the apex, 50–60 mm long, tube light purple and uniformly pale purple on the inner side (judging from dry material); **corolla lobes** 5, unequal (upper longer than lateral longer than lower), slightly overlapping, rounded, 5 × 9 mm, dark purple; **stamens** inserted at about 1/7 corolla length, filaments 2/3 as long as the corolla, almost glabrous (only a few hairs may be present); **anthers** up to 5.5 mm long, ovate, slightly villous along the sutures of their thecae, rounded at base and long-acuminate at apex; **ovary** tubular-elliptic, dark-brown; **stigma** glabrous, narrowly club-shaped, hidden inside the

tube, non-exserted. **Capsules** likely dehiscent with 2-valves (unclear according to dry material). **Seeds** dehiscent with 2-valves (Fig. 4.29).

Distribution: Morocco and Algeria (Fig. 4.27).

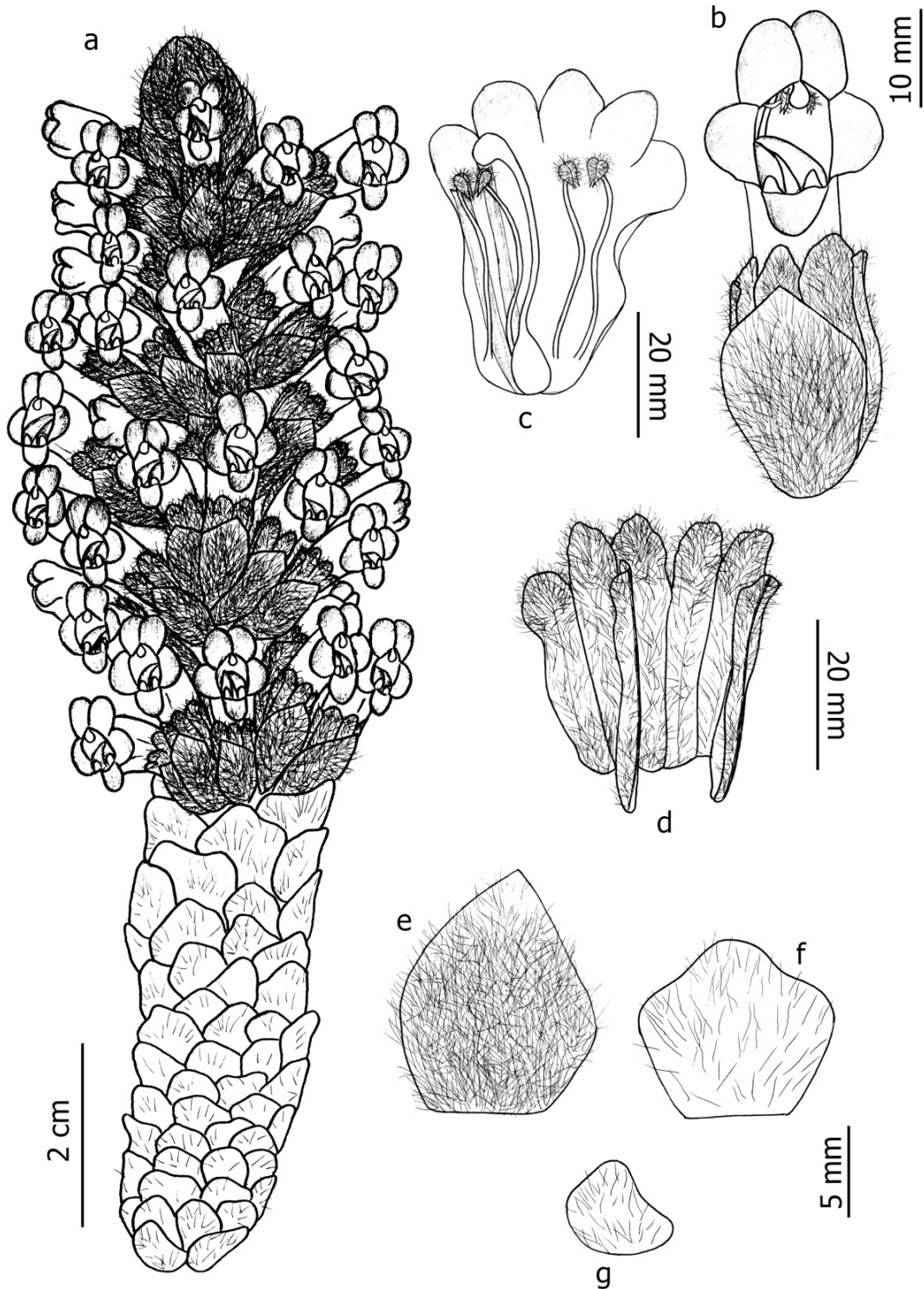


Figure 4.29 *Cistanche macrocalycinum* Ataei sp. nov. a) flowering plant, b) single flower, c) flower inner view, d) abaxial calyx plus two bracteoles stuck to the calyx e) bract, f) upper scale, and g) basal scale. The plant occurred in Algeria (Pelry s.n.; K).

Additional specimen examined (paratype): MOROCCO. 1 April 1921, *Meauricio* s.n. (BM).

Host: Unknown.

Remarks: According to very narrow distribution and unavailable current collection, it is proposed to be included in IUCN list.

2. *Cistanche* subg. *Subcistanche* (Beck) Ataei, *comb. nov.*

Type species: Cistanche sinensis Beck.

2.1 *Cistanche sinensis* Beck, Pflanzenr. (Engler) 96, 4.261: 38 (1930);

Type: CHINA. Gansu: North, septentrional, Wei tschou, 7 June 1918, *Licent* 3845 (lectotype P barcode P02983456 designated here, isotypes K barcode K000852791, W).

= *Cistanche feddeana* K.S.Hao, *Repert. Spec. Nov. Regni Veg.* 36: 222 (1934);. *Type: CHINA.*

Szechuan: Chaohua Hsien, 450 m, 25 May 1930, *Hao* 282 (CHN not seen).

Plant usually thin, 15–70 cm tall, sub-glabrous. **Lower scales** very dense, imbricate, short ovate, obtuse, 7–10 × 3–4 mm, glabrous, entire and non-scarious at the margins; **middle scales** increasingly lax acropetally, oblong-lanceolate, obtuse, 10–12 × 3–6 mm, glabrous, entire, non-scarious at the margins; **upper scales** very lax, rarely lacking (in Mongolian specimen), lanceolate, sub-acute, 13 × 3–3.5 mm, entire, non-scarious at the margins. **Inflorescence** a short ovate spike, usually with 8–14 sub-erect flowers, 6–8 cm long and 4–4.5 cm in diameter; **floral bracts** oblong-lanceolate, sub-acute, 14–16 × 3.5–5 (–6) mm, about as long as calyx, loosely covered with long arachnoid-villous hairs on the outer side (in dry state usually the outer side may be mistaken for being glabrous), densely covered with long arachnoid-villous hairs at the margins, these hairs up to 2.5 mm long; **floral bracteoles** oblong, slightly broader at the lower third, sub-acute, 9–11 (–15) × 1.5–2 (–2.5) mm, as long as or slightly longer than calyx, proximally 2–3 mm adnate to the calyx, the free part on the outer side sparsely and at the margins densely arachnoid-villous; **calyx** tubular, gamosepalous, (11–) 15–16 mm long, slightly longer than ½ the corolla length; **calyx lobes** 4, oblong-linear, acute, 7–10 × 1–2 mm, with non-scarious margins, on the outer side sparsely and at the margins densely arachnoid-villous; **corolla** short tubular-campanulate, reticulate veined, up to 24 mm long, pale yellow outside, occasionally light blue at the inside (based on dry herbarium material and Beck-Mannagetta, 1930), no folds inside the tube; **corolla lobes** 5, unequal (upper longer than lateral ones and the latter longer than lower one), broadly elliptic or orbicular, rounded, 2.5–4 × 3.5–4 mm, occasionally covered with sparse hairs inside and glabrous outside, royal blue when dry; **stamens** inserted at about 1/5 total corolla length, filaments 2/3 as long as the corolla, at base densely covered with 2–3 mm long silky hairs, otherwise glabrous; **anthers** 2–2.5 mm long, sub-

villous, rounded and glabrous at base and mucronate at apex; **ovary** broad-obovate, dark brown; **stigma** club-shaped, sub-glandular at apex, non-exserted. **Capsules** dehiscent with 2-valves. **Seeds** usually elliptic (Fig. 4.30).

Distribution: China (North Gansu and Yanchi, Ningxia Autonomous region) and Mongolia (South Gobi Aitmak) (Fig. 4.31).

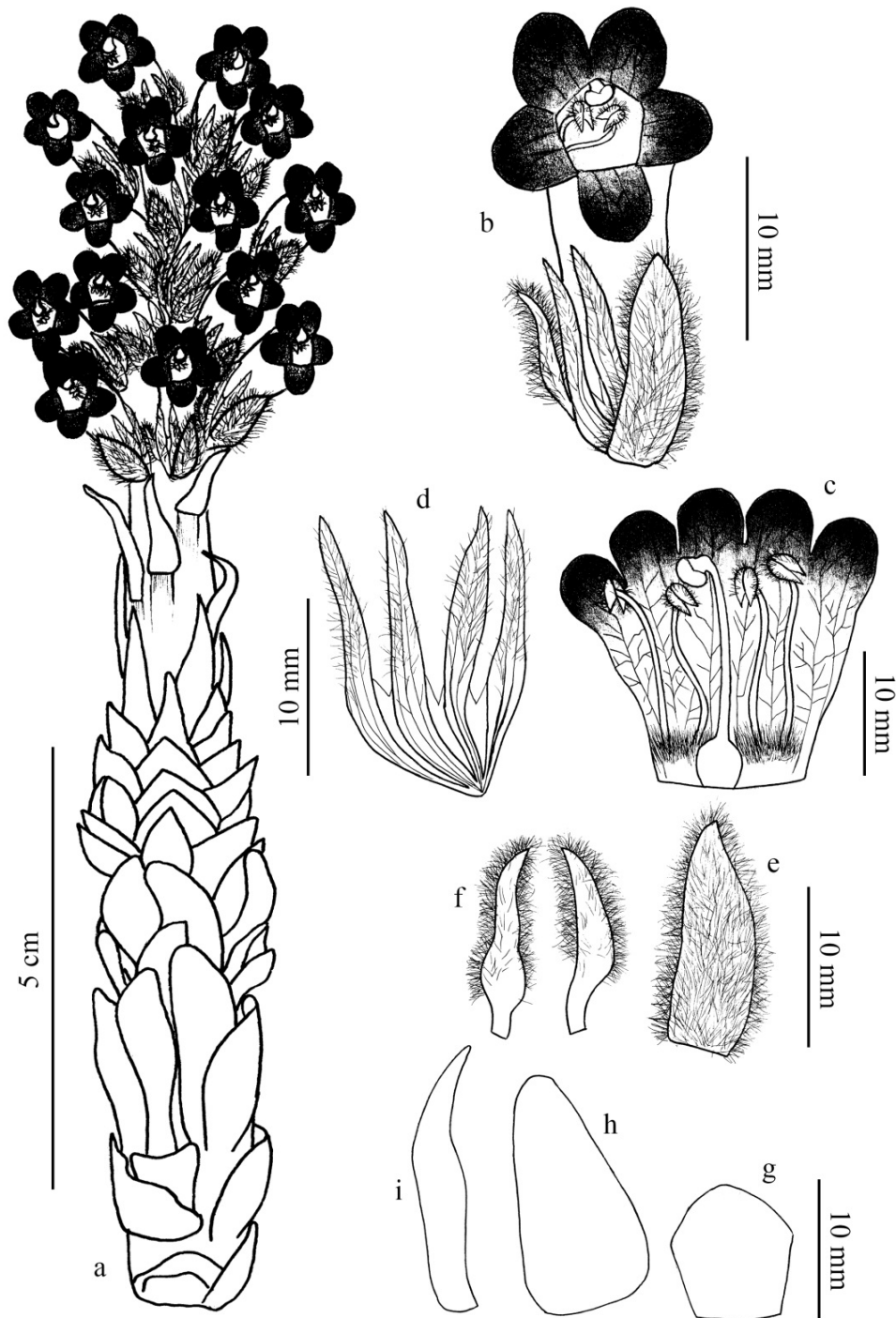


Figure 4.30 *Cistanche sinensis* Beck. a) flowering plant, b) single flower, c) its flower inner view, d) abaxial calyx, e) bract, f) two bracteoles, g) basal scale, h) median scale, and i) upper scale. The plant occurred in China (Licent 3845; P).

Additional specimens examined: MONGOLIA. Aimak: East Gobi Province, 280 km SSW from Sain-Shand, 22 July 1980, *Gubanov* N5642 (MW); Sukhe-Batar (Aimak), 15 km NW of Ongon Settlement, 1 July 1978, *Ogureeva* s.n. (MW).

Host: **Amaranthaceae:** *Haloxylon ammodendron* (C.A.Mey.) Bunge (*Tycho-Norlindh* & *Teuvo-htin* 29414, BM). **Fabaceae:** *Ammopiptanthus mongolicus* (Maxim. ex Kom.) Cheng f. (Dharmananda, 2004) and *Caragana tibetica* Kom. (Liu, 1992); **Rosaceae:** *Potaninia mongolica* Maxim. (Liu, 1992); **Tamaricaceae:** *Reaumuria soongarica* (Pall.) Maxim. (Liu, 1992; Hsu & Wang, 1996); **Chenopodiaceae:** *Salsola passerina* Bunge (Liu, 1992; Hsu & Wang, 1996); **Zygophyllaceae:** *Tetraena mongolica* Maxim. and *Zygophyllum xanthoxylum* (Bunge) Maxim. (Liu, 1992; Hsu & Wang, 1996).

Remarks: The species was previously considered endemic to northern China. Its occurrence in Mongolia is reported here for the first time.

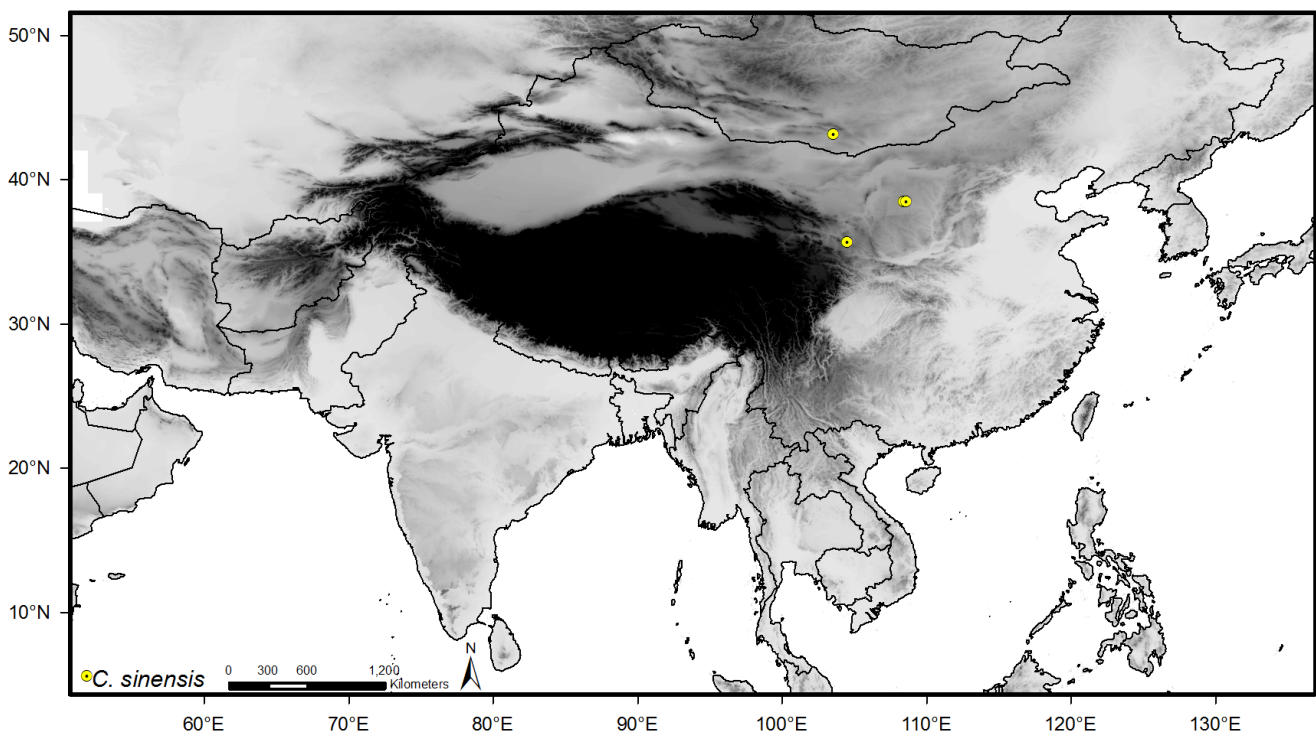


Figure 4.31 Distribution map of the *C. sinensis* in subg. *Subcistanche* (Beck) Ataei & Schneeweiss, comb. nov.

4.4.2 Excluded, doubtful and combinations under *Cistanche* that do not belong to *Cistanche*

The list includes the species for which descriptions insufficient, mainly type material lost:

Orobanche salsa var. *glabrescens* Kuntze, Trudy Imp. S.-Peterburgsk. Bot. sada 10: 225 (1887).

Cistanche allochroa Chiov. Res. Sci. Somalia Ital. 1: 131 (1916).

Cistanche stenostachya Butkov, Fl. Uzbekist. v. 508, 641 (1961).

Cistanche speciosa Butkov, Fl. Uzbekist. v. 509, 641 (1961).

Phelypaea gigantea Kar., Bull. Soc. Imp. Naturalistes Moscou 165 (1839), nomen nudum. Type is unknown where deposited. [it is synonymy under *C. salsa* however warrants to conclude as type not seen]

5 General conclusions

The present study so far reveals the most extensive study on the genus *Cistanche* and expanded largely our understanding of the species phylogeny and taxonomy worldwide. A highly resolved phylogenetic tree was obtained with maximum supports in major clades, revealing a basis for further cytogenetic, taxonomic and biogeographic studies.

The research has contributed to address: the phylogenetic relationships in *Cistanche*; evolution of genome size and chromosome number in the species and; comprehensive taxonomic revision of the genus. One limitation our research encountered was that for some inaccessible geographical regions none to less sampling of plant specimens could be performed, thus we relied on highly degraded herbarium materials that limited the amplification and sequence quality of a limited number of specimens.

Chapter 2. Molecular phylogenetic analyses of the non-photosynthetic parasitic plant *Cistanche* (Orobanchaceae), inferred from three plastid markers and nuclear ITS, reveal relationships among four major morphological distinct clades

The chapter extensively investigates the phylogenetic infrageneric and inter-/intraspecific relationships via congruent tree topologies obtained from the MP, ML and BI analyses of the combined data obtained from three plastid markers and nuclear DNA. Monophyly of *Cistanche* is highly supported by our data. *Cistanche* was subdivided into four major distinct groups: the first lineage included the monotypic *C. sinensis* belonging to previously known *C. sect. Subcistanche*. The other clade contains the rest of the species, which includes three infrageneric groups corresponding to traditionally (Beck-Mannagetta, 1930) known sects. *Cistanchiella*, *nov.* and *Cistanche*. As short coming of the analysis is the largely unresolved latter clade (sect. *Cistanche*), which is still somehow ambiguous concerning the species relationships, in particular for *C. lutea*, *C. phelypaea* and *C. tubulosa*, although the taxon sampling was based on a broad geographical distribution. Taxonomical complex and unidentified species with insufficient phylogenetic resolution in sect. *Cistanche* were subjected for further study in a taxonomic survey (chapter 4). In contrast to the combined data set, the single plastid markers or nuclear ITS were not useful for inferring species relationships on its own. No significant incongruence was observed among the trees obtained from plastid sequences and nuclear ITS. Although the present study included the most extensive sampling of *Cistanche* species, it will be considerably to increase the sampling of different morphotypes of the species e.g. *C. fissa*, *C. ridgewayana*, and *C.*

sinensis, *C. macrocalycinum* and *C. mauritanica* and to include further fast evolving markers in order to eliminate phylogenetic uncertainties in future studies. Altogether, the robust phylogenetic studies provided a basis for cytogenetic, taxonomical reappraisal and biogeography of the genus.

Chapter 3. Evolutionary trends in the non-photosynthetic parasitic *Cistanche* (Orobanchaceae) inferred from karyological data, contrasting effects of genome size with chromosome number

Following previous studies, large chromosomes and relatively high amount of genome size made the genus ideal to explore more broadly the intraspecific variation. The aim of this study was to determine the basic chromosome number, karyotype and genome size evolution. Here, we present the first detailed of karyological study of the genus *Cistanche*. Inter/intraspecific genome size varies enormously throughout the species from nearly 4-fold and up to 2.5-fold, respectively. The evolution of genome size variation was illustrated in a phylogenetic context using plastid and nuclear ITS markers inferred from maximum likelihood and Bayesian analyses showing its random distribution. Consequently, the genome size is unable to infer phylogenetic and taxonomic relations between the species. Distribution of genome size may only be informative in the differentiation of sect. *Cistanchiella* with a relatively lower genome size than sect. *Cistanche*. It might be concluded that the species with shorter stem (*C. ambigua*) from sect. *Cistanchiella* have undergone a reduction in quantity of nuclear DNA, as they represented the lowest genome content among the species studied. All species investigated showed that their chromosome numbers are constant apart from one triploid observed in *C. violacea* and there is no correlation between chromosome number and variation in genome size among the species. Their karyotypes consisted of large meta- to submetacentric and a few acrocentric chromosomes. The amplification and/or removal of transposable elements, particularly retroelements is assumed to be the main factor for variation in genome size of the *Cistanche* species, and not polyploidization. Neither chromosome numbers nor genome size in our data does not contribute in taxonomical species delimitation. The lack of chromosome counts and genome content measurements; however, warrant any conclusions for the missing species. Therefore, comprehensive field studies including the majority of species will be necessitated in order to achieve a better understanding of chromosome number and genome amount size for further investigations.

Chapter 4. Taxonomic revisions of the genus *Cistanche*, with newly northwestern African infrageneric section

Availability the latest molecular phylogenies in this study led us to get new insight into taxonomical relationships among the species, which were largely coincided with previously recognized infrageneric taxonomy. Uncertainties of some cryptic species identity was better resolved using reassessing taxonomical classification within the genus in the phylogenetic frame inferred from molecular data. Our through taxonomic survey currently circumscribed 25 species of *Cistanche*. The present revision revealed nine newly described species as well as six new subspecies. The first comprehensive nomenclatural revision, synonymy and typification were also performed in comparison with a previous taxonomic treatment (Beck-Mannagetta, 1930). Both molecular and taxonomic studies confirmed that *Cistanche* is subdivided to two subgenera, of which subg. *Cistanche* is subdivided into three different sections and subg. *Subcistanche* with one section. Apart from two traditionally known sections *Heterocalyx* and *Cistanche*, a newly described section (*C. sect. Macrocalycinum*) included only two species with distinctive morphological characters such as rhomboid lanuginose bract and bracteoles. Due to the unavailability of recent material, only one species in the section has been molecularly studied. Section *Cistanchiella* contains nine species morphologically distinguished by having hairs on the flower component surface and narrower distribution. In comparison, the 13 species in sect. *Cistanche* show always glabrous floral traits and occupy broader distribution areas, forming unresolved group in the phylogenetic trees. It is usually not easy to differentiate the species of this section morphologically, although, our molecular studies provided answers specifically for the delimitation of the cryptic species in the section. Our infrageneric classification was consistent in both molecular and morphological analyses. Apart from few newly described species, the other species are reported non-endemic. More detailed field and subsequently molecular studies may be more useful for distinguish the morphologically difficult taxon (*C. somaliensis* sp. nov.). No host specialization in *Cistanche* species has been observed so far. In contrast to local taxonomical survey of a few species in previous studies, the vegetative variation has led to unclear species classifications as the characters vary abundantly between the species and individuals, depending on climatic suitability and host conditions. Our current sectional and subgeneric classification reflected largely phylogenetic affinities in the genus. The species of western Africa was found to be closely related in terms of robust stems and huge flowers. Apart from a few discrepancies found between phylogenetic clades, the main groups showed congruent data with current taxonomic classification.

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Summary

The parasitic Orobanchaceae have been the focus of few phylogenetic and systematic studies. While previous studies targeted on the genera *Pedicularis*, *Orobanche* and *Euphrasia*, here the phylogenetic relationships, systematics and taxonomy of the genus *Cistanche* are in the focus. Published studies so far involved only a couple of *Cistanche* species along with other genera, e.g. to infer the backbone phylogeny of the family. In order to address the enigmatic species, the research was intended to investigate the molecular phylogenetic relationships among the species, inferring a thorough taxonomic revision, defining basic chromosome number in the genus, estimating genome sizes and biogeography of the genus.

A large scale molecular data set based on the rapidly evolving plastid regions (*trnL-F*, *trnS-fM*, *psbA-trnH*) and nuclear ITS was undertaken using many populations of almost all species distributed worldwide. The phylogenetic trees obtained from MP, ML and BI are relatively consistent in the major clades and confirmed the monophyly of the species. *Cistanche* was found to contain four distinct lineages. Two subgenera *Subcistanche* and *Cistanche* can be recognized, with the latter splitting into three clades that represent the three different sections. Subgenus *Subcistanche* is placed as first branching lineage, followed by the species of section *Heterocalyx*. Thus the remaining sections *Macrocalycinum* and *Cistanche* share a sister group relationship. The combined data set of plastid markers and nuclear ITS, however, could not fully resolve the widespread complex sect. *Cistanche* and failed to phylogenetically group the distinct morphological taxa.

In chapter three, genome size were measured for some representative species of which some chromosome counts were analyzed and presented in a phylogenetic context. The difference between the highest (31.88 pg / 2C in *C. tubulosa* subsp. *nov.*) and lowest (8.56 pg / 2C in *C. ambigua*) genome content was estimated at about nearly 4-fold. Chromosome counts showed that all analyzed accessions of *Cistanche* were diploid ($n = 20$), with only one exception among *C. violacea* vouchers that was triploid ($n = 30$). Following this study, no correlation was found between the genome size of *Cistanche* and environmental including climatic factors. Similarly the no phylogenetic trend was indicated. The results further indicated the

variation in genome size did not arise from polyploidization. Therefore, a possible mechanism to explain the high variation in genome size argues for differences in the amounts of repetitive DNA, e.g. the accumulation of transposable elements.

The fourth aspect of the study concerned the re-appraisal taxonomic level of the genus. The considerable purposes of the study were the determination of intraspecific relationships in the genus. The species of *Cistanche* have been hardly identified due to reduction of morphological characteristics commonly seen in holoparasitic lineages in the family. According to our latest taxonomical survey *Cistanche* falls into two subgenera based on morphological features of the calyx of which subgen. *Subcistanche* distributed in China and Mongolia and subgen. *Cistanche* contains all other species widely dispersed in the Old World. The monotypic species *C. sinensis* from the first subgenus includes quadricalyx and the second group with 5-equal to subequal lobes. The genus has not treated comprehensively since c. 80 years ago (Beck-Mannagetta, 1930 and regional floras); therefore, this is the first attempt for a through monographic treatment. As a consequence, **nine new species/subspecies** (four new species in sect. *Cistanche*, four in sect. *Heterocalyx* and one in sect. *Macrocalycinum*) and several new records are introduced to the 16 previously recognized species by Beck-Mannagetta (1930). A diagnostic key, species description, nomenclatural corrections and sectional distributions are provided.

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Supplementary data

A) Appendices to Chapter 2

Appendix 2. 1 Locality data and voucher information for the plant material used in the phylogenetic analyses of the genus *Cistanche*. Abbreviations: *: indicates approximated GIS data (google adopted) of those vouchers whose distributions is not precise; TARI: National Herbarium of Iran, Research Institute of Forests and Rangelands; BM: The Natural History Museum; IRAN: Plant Pests and Diseases Research Institute; BONN: Botanical Garden Bonn; USB: Univeristy of Sistan & Baluchistan; P: Muséum National d'Histoire Naturelle; TUH: Tehran University; E: Royal Botanical Garden Edinburgh; W: Naturhistorisches Museum Wien; KAS: Universität Gesamthochschule Kassel; MSB: Ludwig-Maximilians-Universität; K: Royal Botanic Gardens Kew; B: Botanischer Garten und Botanisches Museum Berlin-Dahlem; G: Conservatoire et Jardin botaniques de la Ville de Genève; PEY: the Center of Traditional Chinese Medicine, Peking University; M: Botanische Staatssammlung München; UG: University of Greifswald; BR: National Botanic Garden of Belgium; GUH: Gilan University Herbarium; MA: Real Jardín Botánico; WU: University of Vienna.

Taxon	Accession or isolation number	Locality; coordinates; m a.s.l. (m)	Voucher number: (Herbarium acronym)
<i>Cistanche</i>			
Sect. Eastern Asia			
<i>sinensis</i>	ED722	Mongolia: South Gobi Aitmak, Bayandalay Somon, 10 km S of Dzuramtay; (43°10'08"N; 103°33'05"E*); 110	<i>Tycho Norlindh & Teuvo</i> 29414 (BM)
<i>sinensis</i>	JF915388	China: Yanchi, Ningxia Autonomou region; (38°30'05"N; 108°25'56"E*)	<i>s. col.</i> PS2005MT01 (unknown)
<i>sinensis</i>	ED1098	Mongolia: East Gobi Province (Aimatk), 280 km SSW from Sain-Shand	<i>Gubanov</i> N5642 (MW)
Sect. NW Africa			
<i>sp. nov.</i>	ED719	Morocco: (28°42'00"N; 10°37'00"W*)	<i>Meauricio</i> s.n. (BM)
<i>sp. nov.</i>	ED892	Algeria: Oran; (35°44'09"N; 0°35'09"W*)	<i>Pelry</i> s.n (K)
Sect. SW Asia			
<i>ridgewayana_1</i>	ED1045	Iran: Semnan, Khar Turan National Park, SE of Shahrud, 25 km N of Ghazazan; (36°24'00"N; 56°06'00"E); 1340	<i>Freitag</i> 15276a (KAS)
<i>ridgewayana_1</i>	ED424	Iran: Zahedan to Kerman, near Mahan, 100 km Bam to Mahan; (29°31'05"N; 57°48'15"E); 1980	<i>Ataei & Heidari</i> ED424 (BONN, USB)
<i>ridgewayana_1</i>	ED511	Iran: Sistan & Baluchestan, Saravan, 15 km Suran, Rigjeh village; (27°21'17"N; 61°55'53"E); 1135	<i>Ataei & Valizadeh</i> ED511 (BONN, USB)
<i>ridgewayana_1</i>	ED549	Iran: Kerman, BahramAbad, close to Kabootarkhan; (30°18'00"N; 56°23'00"E*)	<i>Rechinger, Sfandary & Elen</i> 32301 (IRAN)
<i>ridgewayana_2</i>	ED559	Iran: Gilan, 43 km Roodbar to Gilavan; (36°47'00"N; 49°08'00"E*); 550	<i>Assadi, ShahMohammadi & Parsa</i> 60176 (TARI)
<i>ridgewayana_2</i>	ED781	Iran: Isfahan, Djafar Abad, 20 km W Tiran Versus Daran; (32°49'00"N; 50°57'00"E)	<i>s. col.</i> UI 2057 (E)
<i>ridgewayana_2</i>	ED788	Iran: Azerbaijan East, Qezel Owzan (Kizil Uzun), 10-24 km ENE Mianeh; (37°30'00"N; 48°04'00"E)	<i>Rechinger</i> 43222 (W)
<i>ridgewayana_2</i>	ED630	Iran: 24 km W Esfahan, Murcheh Khvort; (32°57'19"N; 51°30'28"E*); 1750	<i>Rechinger</i> 46653 (B)
<i>ridgewayana_2</i>	ED546	Iran: Isfahan, Inter Faridan & Isfahan; (32°55'00"N; 50°46'00"E*)	<i>Ghahreman, Sheikh Eslami & Aghustin</i> 8825 (TUH)
<i>ridgewayana_3</i>	ED596	Afghanistan: Mazar-e-Sharif, In the desert between Mazar-e- Sharif and Tashkurghan; (36°47'00"N; 67°24'00"E*)	<i>Rechinger</i> 34226 (E, W, B, M)
<i>fissa_2</i>	ED701	Afghanistan: North; (36°10'00"N; 67°10'00"E*)	<i>Meinertzhagen</i> s.n. (BM)
<i>fissa_2</i>	ED758	Afghanistan: Bamian, Doab, ca. 15 km West on roadside; (34°06'00"N; 66°22'00"E*)	<i>Hedge & Wendelbo</i> W 3427 (E)
<i>fissa_3</i>	ED1040	Azerbaijan: in Steppe Mughan; (40°06'00"N; 48°48'00"E*)	<i>Sarnarovy</i> [?] s.n. (B)
<i>ambigua</i>	ED604	Afghanistan: Helmand: O' Mohamad Rahim Kalay against Jabbar Oza Dasht, S Darweshan; (30°41'00"N; 64°07'00"E); 710	<i>Anders</i> 8483 (MSB & W)
<i>ambigua</i>	ED437	Iran: Tehran, Kavir National Park, SE Waramin, 1 km after Shokr-Abad; (35°03'52"N; 51°41'54"E); 773	<i>Ataei & Heidari</i> ED437 (BONN, USB)
<i>Ambigua</i>	ED481	Iran: Tehran, Kavir National Park, SE Waramin, 5 km after Shokr-Abad; (35°01'31"N; 51°42'57"E) ;923	<i>Ataei & Heidari</i> ED481 (BONN, USB)

<i>ambigua</i>	ED444	Iran: Shahrud to Torud, 71 km Shahrud, roadside; (35°51'17"N; 55°11'55"E); 1062	<i>Ataei & Heidari</i> ED444 (BONN, USB)
<i>ambigua</i>	ED448	Iran: Zaman Abad-Bardaskan, Zaman Abad, 2 km to Kalateh Karbalai Abbas; (35°46'36"N; 56°38'17"E); 949	<i>Ataei & Heidari</i> ED448 (BONN, USB)
<i>ambigua</i>	ED553	Iran: 90 km Zaman Abad to Bardaskan, between Ismail Abad and Salehieh; (35°12'36"N; 57°00'15"E); 951	<i>Ataei & Heidari</i> ED553 (BONN, USB)
<i>ambigua</i>	ED644	Iran: Dasht-e-Kavir; (34°58'03"N; 55°16'22"E*)	<i>Bornmüller</i> 90 (B)
<i>ambigua</i>	ED748	Iran: Shahrud, Bastam, Khar Turan National Park, NNE Ahmadabad; (35°46'00"N; 56°36'00"E)	<i>Rechinger</i> 50654 (W)
<i>ambigua</i>	ED1046	Afghanistan: Registan-desert, SW-border, 5 km S Bhagat, sand dunes at Hilmand valley; (30°30'19"N; 63°49'33"E*)	<i>Freitag</i> 718 (KAS)
<i>salsa</i>	ED585	Turkey: Konya, Yavsan Memlehasi nr. Tuz Golu; (38°26'18"N; 32°42'33"E*)	<i>Davis & Dodds</i> 18709 (K)
<i>salsa</i>	ED703	Turkey: Konya, Cihanbeyli, Yavsan Tuzlasi; (38°45'35"N; 33°06'58"E*)	<i>Coskun & Koyuncu</i> s.n. (BM)
<i>salsa</i>	ED787	Armenia: Ararat State, Ararat District, Khor-Virab monastery vicinity, ca. 1 km N Khor-Virab; (39°89'00"N; 44°58'00"E*)	<i>Fayvush & Tamanyan</i> 9-2004 (W, P)
<i>deserticola</i>	KC128846	China	<i>s. col.</i> s.n. (unknown)
<i>deserticola</i>	ED1042	Kyrgyzstan: Batkern province, Alai Range, near Kadamzhai village; (40°07'47"N; 71°42'59"E); 1058	<i>Laz'kov</i> BLCKG-715 (K)
<i>deserticola</i>	ED1097	Mongolia: Bayan, Khangor, Trans Altai Gobi, North of Tsagan, Bogdo-Ula, vicinity of Ekhiin Gol; (49°37'00"N; 99°35'00"E*)	<i>Gubanor</i> N3108 (MW)
Sect. widespread			
<i>rosea</i>	ED705	Yemen: Wadi Hedhraan, near Ta'izz, at the edge of cultivated land; (13°34'43"N; 43°53'15"E*); 1300	<i>Wood</i> Y/75/1002 (BM)
<i>rosea</i>	ED707	Oman: Wadi, half way of Gebel Ara; (23°30'00"N; 56°59'00"E*)	<i>Lawton</i> 2012 (BM)
<i>rosea</i>	ED611	Oman: Dhofar, Jebel Qara, Salalah, ca. 3 km beyond Ain Garziz ca. 3 km SW Ain Garziz; (17°06'35"N; 54°03'44"E); 50	<i>Schnittler & Shadwick</i> 24318 (UG)
<i>rosea</i>	ED704	Saudi Arabia: East road, near Raidat Elamara; (18°07'00"N; 42°58'00"E*)	<i>Guichard</i> KG/Had/419 (BM)
<i>rosea</i>	ED782	Oman: Dhofar, Wadi Mugshin; (19°35'00"N; 53°47'00"E)	<i>McLeish</i> 3434 (E)
<i>rosea</i>	ED708	South Arabia: Dahmah, stony plateau; (24°31'37"N; 46°45'41"E*); 5400	<i>Ingrams</i> 63 (BM)
<i>laxiflora</i> subsp. <i>nov.</i>	ED433	Iran: Ca 5 km Rafsanjan to Mese-Sarcheshmeh; (30°19'05"N; 56°01'33"E); 1576	<i>Ataei & Heidari</i> ED433 (BONN, USB)
<i>laxiflora</i> subsp. <i>nov.</i>	ED435	Iran: Bafq road to Bagherabad, down the road; (31°36'53"N; 55°23'46"E); 997	<i>Ataei & Heidari</i> ED435 (BONN, USB)
<i>laxiflora</i> subsp. <i>nov.</i>	ED438	Iran: Tehran, Kavir National Park, SE Waramin, close to Shokr-Abad check point; (35°04'23"N; 51°42'50"E); 812	<i>Ataei & Heidari</i> ED438 (BONN, USB)
<i>laxiflora</i> subsp. <i>nov.</i>	ED779	Saudi Arabia: Between Al Bahah and Jabal Ibrahim, off the Taif road; (20°16'42"N; 41°28'11"E*); 1829	<i>Collenette</i> 3566 (E)
<i>laxiflora</i> subsp. <i>laxiflora</i>	ED515	Iran: Sistan & Baluchestan, Khash to Iranshahr, Daman village, riverbed; (27°21'29"N; 60°46'59"E); 695	<i>Ataei</i> ED515 (BONN, USB)
<i>laxiflora</i> subsp. <i>laxiflora</i>	ED512	Iran: Sistan & Baluchestan, Saravan to Suran, NaserAbad, after Chah Mahmood; (27°26'16"N; 61°44'47"E); 1186	<i>Ataei</i> ED512 (BONN, USB)
<i>laxiflora</i> subsp. <i>laxiflora</i>	ED521	Iran: Sistan & Baluchestan, Zahedan to Bam, 5 km RostamAbad; (28°58'12"N; 58°45'18"E); 704	<i>Ataei</i> ED521 (BONN, USB)
<i>laxiflora</i> subsp. <i>laxiflora</i>	ED480	Afghanistan: Heart, ca. 5 km Tirpol to Islam Ghaleh, in the riverbed of Harirud; (34°36'49"N; 61°15'19"E); 726	<i>Ataei</i> ED480 (BONN, USB)
<i>laxiflora</i> subsp. <i>laxiflora</i>	ED478	Iran: ca 70 km Nishabur to Kashmar, 2 km down detour road of Chelpo vilage; (35°37'29"N; 58°30'07"E); 1873	<i>Ataei</i> ED478 (BONN, USB)
<i>tubulosa</i> _1	ED753	Oman: Dhofar, Raha beach 16 km East of Mirbat; (16°57'45"N; 54°46'54"E)	<i>McLeish</i> 966 (E)
<i>tubulosa</i> _1	ED578	Oman: Al Hallaniyah Island, Dhofar Beach; (17°31'32"N; 56°00'38"E*); 2	<i>Al-Farsi</i> HA 30 (K)
<i>tubulosa</i> _1	ED766	Saudi Arabia: Uqair, S of Dammam; (25°42'28"N; 50°11'06"E*)	<i>Collenette</i> 6025 (E)
<i>tubulosa</i> _1	ED757	Oman: Central Oman, Duqm; (19°39'47"N; 57°42'10"E); 0	<i>McLeish</i> 1651 (E)
<i>tubulosa</i> _1	ED805	United Arab Emirates: Abu Dhabi; (24°27'00"N; 54°25'00"E*)	<i>Willcox</i> 200 (K)
<i>tubulosa</i> _1	ED1052	Yemen: Abd Al kuri island, North coast, 65 miles SW of Socotra in the Indian Ocean; (12°12'00"N; 52°14'00"E*)	<i>Keiln</i> A51 (K)
<i>tubulosa</i> _1	ED772	Bahrain: Sandy beach, just above high tide line near; (26°07'49"N; 50°27'33"E*)	<i>Cornes</i> 235 (E)
<i>senegalensis</i>	ED581	Ethiopia: Tigray Region, Near Adi Gudum, c. 30 Km south of Mekelle; (13°14'35"N; 39°34'31"E); 2300	<i>Friis, Bidgood, Host, Wondafrash & Kebede</i> 6680 (K)
<i>senegalensis</i>	ED452	Ethiopia: Tigray Region, Near Adi Gudum, South of Mekelle; (13°15'00"N; 39°37'00"E)	<i>Wondafrash & Shigulte Kebede</i> 6680 (BR)

<i>senegalensis</i>	ED800	Kenya: Watmu, near Malindi; (03°21'20"S; 40°01'24"E*)	<i>Tweedie</i> 870 (K)
<i>senegalensis</i>	ED784	Yemen: 3 km East of Hamman Ali on road to Dhomar; (14°40'00"N; 44°10'00"E)	<i>Wood</i> Y1132 (E)
<i>senegalensis</i>	ED571	Yemen: IBB district; (13°59'24"N; 44°10'29"E*)	<i>Hepper & Wood</i> 5898 (K)
<i>senegalensis</i>	ED592	Kenya: Near Magadi, Kajiado district; (01°50'00"S; 36°47'00"E*)	<i>Rajabu EAH</i> 14,294 (M, K)
<i>senegalensis</i>	ED900	Kenya: Chyulu Plains; (02°30'00"S; 37°45'00"E); 1150	<i>PA & WRQ Luke</i> 5946 (EA, K)
<i>senegalensis</i>	ED893	Uganda: Kenya boarder, Lotome probably, Turkana District; (02°22'26"S; 34°49'12"E*)	<i>Wilson</i> 700 (K)
<i>senegalensis</i>	ED899	Tanzania: Arusha District; (03°42'15"S; 37°02'01"E*); 1524	<i>Richards & Arasululu</i> 26452 (K)
<i>senegalensis</i>	ED896	Tanzania: T2, Arusha District, 10 km W. of Ngare Nanyuki; (03°09'00"S; 36°50'00"E); 1600	<i>Friis & Hansen</i> 2606 (K)
<i>senegalensis</i>	ED901	Kenya: Tana river District. Kurawa, 30 ml. S. of Garsen, Tana river District; (01°56'19"S; 40°11'54"E)	<i>Polhill & Paulo</i> 595 (K, P)
<i>senegalensis</i>	ED897	Kenya: South Turkana, Lokori, 7 mls. S. of Kangetet sandy soils in riverine forest; (01°16'00"S; 36°07'00"E*); 594	<i>Mathew</i> 6317 (K)
<i>senegalensis</i>	ED894	Somalia: Muqdisho, 3 km South of Afgooye probably; (02°06'00"N; 45°08'00"E*)	<i>Watson</i> W1 (K)
<i>senegalensis</i>	ED725	Eritrea: near Mersa Teklay, sandy coastal area; (17°32'36"N; 38°51'07"E); 0	<i>Hemming</i> [?] 1142 (BM)
<i>senegalensis</i>	ED1096	Somalia: Somaliland	<i>Phillips</i> s.n. (BM)
<i>brunneri</i>	ED1094	Senegal: Kayar, besides Sea; (14°54'58"N; 17°07'17"W)	<i>Kesby</i> 17 (K, P)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED891	Pakistan: Darya Khan, Mianwali Rd. n. W. F. P. In sand; (31°49'14"N; 71°21'52"E)	<i>Rashid</i> 4650 (K)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED809	Gulf of Arabia, in Mualla?	<i>Com RHS</i> s.n. (K)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED1090	Mali: Adrar des Iforas; (19°17'00"N; 01°17'00"E*)	<i>Sidiyene</i> 76 (P)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED506	Iran: Sistan & Baluchestan, Do-Rahi Zabol Zahedan, close to Water pump station; (30°11'40"N; 60°51'54"E); 552	<i>Ataei & Heidari</i> ED506 (BONN, USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED507	Iran: Sistan & Baluchestan, Zabol, 1 k km Do Rahi Zabol-Zahedan; (30°15'41"N; 60°50'01"E); 469	<i>Ataei & Heidari</i> ED507 (BONN, USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED508	Iran: Sistan & Baluchestan, Zabol, Chah Nime, South research Institute of aquatics; (30°49'45"N; 61°43'07"E); 354	<i>Ataei & Heidari</i> ED508 (BONN, USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED509	Iran: Sistan & Baluchestan, Zabol, Zahak, close to Vitis Botanical Gatrden; (30°50'11"N; 61°43'34"E); 478	<i>Ataei & Heidari</i> ED509 (BONN, USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED510	Iran: Sistan & Baluchestan, Zabol, Chah Khorma; (31°06'13"N; 61°09'20"E); 451	<i>Ataei & Heidari</i> ED510 (BONN, USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED487	Iran: Sistan & Baluchestan, Khash to Iranshahr; (27°46'00"N; 60°47'00"E*)	<i>Heidari</i> s.n. (USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED486	Iran: Sistan, Zabol, Abgir; (30°45'00"N; 61°42'00"E*)	<i>Heidari</i> s.n. (USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED484	Iran: Sistan, Zabol, Chah Nnime; (30°47'42"N; 61°39'04"E)	<i>Heidari</i> s.n. (USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED485	Iran: Sistan, Hamoon lake, Chah Khorma; (31°04'37"N; 61°11'35"E)	<i>Heidari</i> s.n. (USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED483	Iran: Baluchestan, Dashtak, do Rahi Zabol to Zahedan; (30°10'22"N; 60°51'43"E)	<i>Heidari</i> s.n. (USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED513	Iran: Sistan & Baluchestan, Iranshahr to Bam-poor, Bam-poor village; (27°11'55"N; 60°28'50"E); 506	<i>Ataei</i> ED513 (BONN, USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED514	Iran: Sistan & Baluchestan, 130 km Khash to Iranshahr, after Abadan village; (27°18'06"N; 60°44'27"E); 640	<i>Ataei</i> ED514 (BONN, USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED436	Iran: Highway Ghom-Tehran, 60 km Ghom to Tehran, Cheshmeh Shur, 9.5 km to Hoze-Solatan lake; (35°02'51"N; 50°58'37"E); 801	<i>Ataei & Heidari</i> ED436 (BONN, USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED640	West Africa: Cape Verde Islands, Boa vista. Currel Velho; (15°59'34"N; 22°47'34"W)	<i>Kilian & Leyens</i> 3085 (B)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED801	Qatar: Wadi El Ereq; (26°02'06"N; 51°02'54"E*)	<i>Elamin</i> 136 (K)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED889	Qatar: Wadi Al Galaiel, toward southern ende of the Qatar Peninsula (Miocene); (24°31'00"N; 51°05'00"E*)	<i>Boulos</i> 11119 (K)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED771	Yemen: Near Marib on dunes; (15°47'00"N; 45°32'29"E*)	<i>Muller-Hohenstein & Deil</i> 1211 (E)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED482	Iran: Baluchestan, Chabahar; (25°12'00"N; 61°31'00"E*)	<i>Valizadeh</i> s.n. (USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED760	Oman: Dhofar, Muqshin; (19°32'00"N; 54°54'00"E)	<i>McLeish</i> 1666 (E)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED777	Oman: Central Oman, Zawrik, Huqf Depression; (19°00'00"N; 57°00'00"E); 0-250	<i>McLeish</i> 1640 (E)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED764	Saudi Arabia: Eastern S-A, near Abqaiq, Sand at the roadside; (25°56'00"N; 49°43'00"E)	<i>Podzorski</i> 804 (E)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED434	Iran: 85 km Yazd to Bafq, right side of main road; (31°38'43"N; 55°14'55"E); 969	<i>Ataei & Heidari</i> ED434 (BONN, USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED439	Iran: Semnan, Nezami road, toward Chah-Shirin, around industrial factory; (35°27'14"N; 53°43'37"E); 1192	<i>Ataei & Heidari</i> ED439 (BONN, USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED763	Egypt probably: Buqbuq?, Very sandy hills; (31°30'39"N; 25°32'52"E)	<i>TTDF</i> 362 (E)

<i>tubulosa</i> subsp. <i>tubulosa</i>	ED479	Iran: 19 km Tabas to Yazd, Hoze Lab-e-Kavir, 2-3 km of main road; (32°27'42"N; 56°46'37"E); 620	<i>Ataei</i> ED479 (BONN, USB)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED577	Kuwait; (29°31'16"N; 47°48'17"E*)	<i>s. col.</i> s.n. (K)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED907	United Arab Emirates: Sharjah, al-Hamriya, sandy plain behind small dunes; (25°29'17"N; 55°30'14"E); 4	<i>El-Keblawy, Borosova, Wilson & al.</i> TMH335 (K)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED1053	United Arab Emirates: Road between Al-Ain and Ayn Al-Faidah; (24°09'00"N; 55°44'00"E*)	<i>Boulos & Al-Hassan</i> 15763 (E)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED558	Iran: Khuzestan, Ahavaz, Albagi; (31°47'06"N; 48°60'13"E*)	<i>Mobayyen</i> 29469 (TUH)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED679	Iran: Sistan & Baluchestan, ca. 35 km W of Zabol, 3 km W of Mile Nader, Hamun lake; (31°20'36"N; 61°37'28"E); 456	<i>Akhani, Noormohammadi & Samadi</i> 22088 (TUH)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED680	Iran: Yazd, between Nain and Ardakan, ca. 10 km N Aghda; (32°30'28"N; 53°33'30"E); 1170	<i>Akhani, Noormohammadi & Samadi</i> 21965 (TUH)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED695	Saudi Arabia: Central track east of Hail; (27°25'00"N; 42°30'00"E); 800	<i>Zeller</i> 8301 (BM)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED774	Saudi Arabia: Between Uqlat Asugour and Jebel Tamiyah, Medina-Qassim; (25°00'42"N; 42°00'03"E*); 854	<i>Collenette</i> 2458 (E)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED694	Saudi Arabia: Southern Rub al-Khali, At-Tara'tz, Rolling sand terrain; (21°09'00"N; 50°29'00"E)	<i>Mandaville</i> 7612 (BM)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED697	Saudi Arabia: NE Arabia, 13 km E Abu Hadriyyah; (26°22'34"N; 50°12'55"E*)	<i>Mandaville</i> 1789 (BM)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED1011	Iran: Fars, 43 km from Lar to Bastak; (27°30'31"N; 54°20'07"E*); 400	<i>Assadi & Sardabi</i> 41846 (IRAN)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED806	Yemen: Hadda, 4km SW of Sanaa; (15°18'27"N; 44°08'33"E*)	<i>Miller</i> 581 (K)
<i>tubulosa</i> subsp. <i>tubulosa</i>	ED759	Iraq: Shahraban, occasional on slope of large sand dunes; (34°03'49"N; 44°59'21"E*)	<i>Haines Wheeler</i> 847 (E)
<i>tubulosa</i> subsp. <i>tubulosa</i>	JF915383	China	<i>s. col.</i> PS0811MT03 (unknown)
<i>tubulosa</i> subsp. <i>tubulosa</i>	FJ914382	China	<i>s. col.</i> PS0811MT02 (unknown)
<i>tubulosa</i> subsp. <i>nov.</i>	ED516	Iran: Sistan & Baluchestan, Sarbaz to Chahbahar, Dempak village, roadside; (25°42'39"N; 61°22'25"E); 22	<i>Ataei</i> ED516 (BONN, USB)
<i>tubulosa</i> subsp. <i>nov.</i>	ED518	Iran: Sistan & Baluchestan, Chahbahar to Tis, Sadre Omid-e-Chahbahar, coast line; (25°22'24"N; 60°36'39"E); 15	<i>Ataei</i> ED518 (BONN, USB)
<i>tubulosa</i> subsp. <i>nov.</i>	ED517	Iran: Sistan & Baluchestan, Chahbahar, Negoyan to Tis, coast line; (25°11'01"N; 61°06'59"E); 0	<i>Ataei</i> ED517 (BONN, USB)
<i>flava</i> subsp. <i>nov.</i>	ED445	Iran: Shahrud to Torud, 2 km Chejam, roadside; (35°47'50"N; 55°09'36"E); 1102	<i>Ataei</i> ED445 (BONN, USB)
<i>flava</i> subsp. <i>nov.</i>	ED447	Iran: Semnan, 46 km Biarjmand, 101 km Miami to Ahmad Abad; (35°53'40"N; 56°18'47"E); 769	<i>Ataei</i> ED447 (BONN, USB)
<i>flava</i> subsp. <i>nov.</i>	ED678	Iran: Southern Khorassan 40-42 km after Nehbandan towards Zabol, roadside; (31°29'29"N; 60°36'34"E); 807-825	<i>Akhani, Noormohammadi & Samadi</i> 22074 (TUH)
<i>flava</i> subsp. <i>nov.</i>	ED451	Iran: 78 km Zaman-Abad to Bardaskan, between Ismail Abad and Ghaleh, 2 km roadside; (35°07'01"N; 57°13'07"E); 803	<i>Ataei</i> ED451 (BONN, USB)
<i>flava</i> subsp. <i>flava</i>	ED432	Iran: 120 km Zahedan to Bam, Nosrat Abad region, roadside; (29°43'01"N; 59°49'27"E); 1061	<i>Ataei & Heidari</i> ED432 (BONN, USB)
<i>flava</i> subsp. <i>flava</i>	ED426	Iran: 78 km Kerman to Rafsanjan, Kabootarkhan to Bagherabad, Tehran road; (30°18'10"N; 56°18'34"E); 1618	<i>Ataei & Heidari</i> ED426 (BONN, USB)
<i>flava</i> subsp. <i>flava</i>	ED520	Iran: Sistan & Baluchestan, Nikshahr to Iranshahr, after Spage village, 2 km NokAbad; (26°55'03"N; 60°30'57"E); 764	<i>Ataei</i> ED520 (BONN, USB)
<i>flava</i> subsp. <i>flava</i>	ED519	Iran: Sistan & Baluchestan, Nikshahr to Iranshahr, 50 km Iranshahr, after NokAbad village; (26°55'08"N; 60°31'18"E); 847	<i>Ataei</i> ED519 (BONN, USB)
<i>flava</i> subsp. <i>flava</i>	ED522	Iran: Khorassan-e-Razavi , Sabzevar, Norood-Abad village, Parand mountain, Kalate-e-MirAli; (35°55'23"N; 57°09'23"E); 922	<i>Ataei</i> ED522 (BONN, USB)
<i>flava</i> subsp. <i>flava</i>	ED524	Iran: Khorassan-e-Razavi , Khora, Sabzevar to Solh-Abad, Sabri village; (35°44'06"N; 56°55'14"E); 1043	<i>Ataei</i> ED524 (BONN, USB)
<i>flava</i> subsp. <i>flava</i>	ED525	Iran: Semnan, Shahrud, 5 km after Kariz village; (35°36'08"N; 56°49'53"E); 1122	<i>Ataei</i> ED525 (BONN, USB)
<i>flava</i> subsp. <i>flava</i>	ED542	Iran: Semnan, Shahrud, 5 km after Kariz village; (35°36'08"N; 56°49'53"E); 1122	<i>Ataei</i> ED542 (BONN, USB)
<i>flava</i> subsp. <i>flava</i>	ED449	Iran: 12 km Ahmad-Abad to Zaman Abad, roadside; (35°39'06"N; 56°38'39"E); 1093	<i>Ataei</i> ED449 (BONN, USB)
<i>flava</i> subsp. <i>flava</i>	ED450	Iran: Zaman Abad-Bardaskan, detour road Zaman-Abad, 2 km to Kalateh Karbalai Abbas; (35°37'24"N; 56°46'46"E); 1105	BONN & USB: <i>Ataei N. & Heidari</i> ED450
<i>flava</i> subsp. <i>flava</i>	ED523	Iran: Khorassan-e-Razavi, Sabzevar to Sebri village, Roodab Parand to cement factory; (35°53'26"N; 57°04'48"E); 944	<i>Ataei</i> ED523 (BONN, USB)
<i>flava</i> subsp. <i>flava</i>	ED541	Iran: Khorassan-e-Razavi, Savzevar to SolhAbad, after Talkhab village; (35°33'06"N; 56°54'47"E); 1165	<i>Ataei</i> ED541 (BONN, USB)
<i>flava</i> subsp. <i>flava</i>	ED446	Iran: Semnan, Maimay to Biarjmand, 24 km Biarjmand; (36°07'54"N; 55°48'52"E) ; 1152	<i>Ataei</i> ED446 (BONN, USB)

<i>flava</i> subsp. <i>flava</i>	ED647	Iran: Tehran, Kavir National Park, Siah Kuh, Karvansarai Shah Abbas; (34°44'00"N; 52°10'00"E); 1100-1300	<i>Rechinger</i> 50123 (B, W)
<i>flava</i> subsp. <i>flava</i>	ED548	Iran: Semanan, Shahrud, Kalat-e- Hizomi; (35°40'12"N; 56°35'51"E)	<i>Shahi & Saeidi</i> 13230 (GUH)
<i>flava</i> subsp. <i>flava</i>	ED890	India: Rajasthan, Thar Desert, outside village of Satyaya; (27°25'00"N; 71°40'00"E)	<i>Jachson & Taylor</i> 7 (K)
<i>flava</i> subsp. <i>flava</i>	ED1051	Afghanistan: Kandahar, 40 km E, sand desert near Tachta Pul at Dori River; (31°19'00"N; 66°36'00"E*); 1100	<i>Freitag</i> 2583 (KAS)
<i>violacea_2</i>	ED395	Morocco: Before Ar-Rachidia, Meknès-Tafilalet, Oued Ziz, ca. 20 km N of Stausee Hassan Addakhil; (32°08'52"N; 04°22'16"W); 45	<i>Quandt</i> ED395 (BONN)
<i>violacea_2</i>	ED396	Morocco: Before Ar-Rachidia, Meknès-Tafilalet, Oued Ziz, ca. 20 km N of Stausee Hassan Addakhil; (32°08'52"N; 04°22'16"W); 1032	<i>Quandt</i> ED396 (BONN)
<i>violacea_2</i>	ED398	Morocco: before Tinejda; (30°39'17"N; 06°33'45"W); 996	<i>Quandt</i> ED398 (BONN)
<i>violacea_2</i>	ED399	Morocco: After Tinejda, Meknès-Tafilalet, Erfoud to Tinejda; (31°44'28"N; 04°24'37"W); 792	<i>Quandt</i> ED399 (BONN)
<i>violacea_2</i>	ED400	Morocco: Souss-Massa-Daraâ, Ouazazarte to Zagora, river valley ca. 8 km SE Ouazazarte; (30°51'10"N; 06°49'56"W); 1146	<i>Quandt</i> ED400 (BONN)
<i>violacea_2</i>	ED590	Morocco: Quarzazate: E Tizi-n-Taguergoust, 9 km SE Tazenakht to Fom-Zguid; (30°33'00"N; 07°09'00"E); 1450	<i>Schuhwerk</i> 90/792 (M)
<i>violacea_2</i>	ED602	Morocco: Agadir, 5 km E Bou-Izakarn to Akka; (29°10'00"N; 09°41'00"E); 600	<i>Podlech</i> 40705 (MSB)
<i>violacea_2</i>	ED586	Morocco: Tiznit, 11 km SW Bou Izakarne to Goulimine; (29°09'00"N; 09°46'00"E); 620	<i>Schuhwerk</i> 90/200 (M)
<i>violacea_2</i>	ED607	Morocco: Tiznit 11 miles Southwest Bouizakarne, near the road to Goulimine (P41); (29°09'00"N; 09°46'00"W); 1000	<i>Podlech</i> 48445 (MSB)
<i>violacea_1</i>	ED477	Tunisia: Midouni, Djerba; (33°53'46"N; 10°47'39"E*)	<i>s. col.</i> 891908 (BR)
<i>violacea_1</i>	ED762	Libya: Jamahiriya, Gulf of Sirte, E. of Marble Arch; (31°54'00"N; 12°43'00"E*)	<i>Davis & Hadland</i> 49622 (E)
<i>violacea_1</i>	ED902	Libya: Coast E of Zavia, open rocky ground close to Sea, Sea level; (32°47'29"N; 12°43'55"E)	<i>Mait land</i> 75 (K)
<i>violacea_1</i>	ED675	Tunisia: Gobernación de gabes, Metloui, gorges de Seldja; (34°20'19"N; 08°19'45"E); 250	<i>Herreo & al.</i> CA3916 (MA)
<i>violacea_1</i>	ED676	Tunisia: Gobernación de gabes, Zerkine; (33°44'50"N; 10°16'52"E); 5	<i>Aedo & al.</i> CA16301 (MA)
<i>violacea_1</i>	ED601	Algeria: Wilaya Ouargla, 62 km N from Hassi Messaoud to Touggourt; (32°11'00"N; 05°52'00"E); 150	<i>Podlech</i> 32882 (MSB)
<i>violacea_1</i>	ED1012	Jordan: Karak, Mujib valley, 1 km after the rest house of tourists, roadside; (31°09'54"N; 35°45'51"E*)	<i>Abulaila & Tehabshem</i> 2006JOR4-1 (NCARTT)
<i>violacea_1</i>	ED603	Algeria: Wilaya Laghouat: 95 km S Ghardaia an der Strasse nach El Golea; (31°46'00"N; 03°43'00"E); 430	<i>Podlech</i> 37081 (MSB)
<i>violacea_1</i>	ED807	Saudi Arabia: Thaniyat Turayf, Thaniyat Camp; (29°46'00"N; 37°58'00"E); 830	<i>Collenette</i> 3469 (K, E)
<i>lutea_3</i>	ED671	Spain: De Gran Canaria, Lanzarote, Teguisse, SE Montana Corona; (29°01'12"N; 13°30'25"W); 80	<i>Aedo, Medina & Quintanar</i> AQ1732 (MA)
<i>lutea_3</i>	ED674	Spain: Almeria, Tabernas, East of town; (37°03'19"N; 02°22'32"W*); 280	<i>Lopez</i> 2828GL (MA)
<i>lutea_3</i>	ED888	Egypt: North Sinai, Sad Er Rawafa 5 km of Abu Aweigila; (30°49'47"N; 34°08'26"E); 147	<i>Van Slageren & Henwawy</i> MSAH992 (K)
<i>lutea_3</i>	ED885	Egypt: Wadi Rished (Helwan); (29°50'27"N; 31°17'25"E*)	<i>Davis</i> 10.326 (K)
<i>Hybrid of lutea_3&violacea_1</i>	ED686	Jordan: Wadi Rum, sandy semi-desert; (29°38'00"N; 35°21'00"E*)	<i>Swann</i> WR10 (BM)
<i>lutea_4</i>	ED711	Algeria: H1, 10 km S of Hassi Bahbah; (34°58'08"N; 03°03'09"E*); 800	<i>Davis</i> 53288 (BM)
<i>phelypaea</i> subsp. <i>phelypaea</i>	Ova.1	Spain: Chiclana de La Frontera, Santi Petri, along the coast line; (36°23'31"N; 06°12'25"W); 60	<i>Ataei</i> ED792 (BONN, P)
<i>phelypaea</i> subsp. <i>phelypaea</i>	Ova.1	Spain: Province of Huelva, Cartaya, El Rompido, close to Golf course; (37°13'08"N; 07°07'48"W); 5	<i>Ataei</i> ED793 (BONN, P)
<i>phelypaea</i> subsp. <i>phelypaea</i>	ED669	Spain: Huelva, Cartaya, El Rompido; (37°13'00"N; 07°07'40"W)	<i>Calvo & Espejo</i> JC3504 (MA)
<i>phelypaea</i> subsp. <i>phelypaea</i>	ED387	Spain: Canary Islands, Lanzarote, coast E of Orzola close to El Arco; (29°21'55"N; 13°26'56"W); 0-10	<i>Schneeweiss & Weiss-Schneeweiss</i> 8758 (WU)
<i>phelypaea</i> subsp. <i>phelypaea</i>	ED670	Portugal: Baixo Alentejo, Volanova de Milfontes, orilla S de la ria, c. del Punete; (41°59'42"N; 08°40'20"W*); 0-50	<i>Gramendia & Pedrol</i> 3429 JP (MA)
<i>phelypaea</i> subsp. <i>phelypaea</i>	Yellow5	Morocco: coast line in Tiznit, Souss-Massa-Daraâ, Nord Sidi Ifni; (29°23'33"N; 10°10'10"W); 85	<i>Quandt</i> ED404 (BONN)

<i>phelypaea</i> subsp.	Yellow6	Morocco: Souss-Massa-Daraâ, Agadir; (30°39'00"N; 09°53'07"W); 51	<i>Quandt</i> ED405 (BONN)
<i>phelypaea</i>			
<i>phelypaea</i> subsp.	ED673	Morocco: SW coastline, Desembocadura Oued Noum, 29RLC6324, in Dunes; (29°11'00"N; 10°21'00"W*); 10	<i>Buira & Calva</i> 169 (MA)
<i>phelypaea</i>			
<i>phelypaea</i> subsp.	ED667	Spain: Alicante, heriso de la route pres Saliuos del Pinet; (38°21'16"N; 00°28'02"W*); 5	<i>Charpin & Defferaud</i> 16557 (MA)
<i>phelypaea</i>			
<i>phelypaea</i> subsp.	ED641	Portugal: Cape Verde, Sal, SW Santa Maria, in the lighthouse beach; (16°37'14"N; 22°55'46"W*)	<i>Royl</i> 975 (B)
<i>pheylapea</i>			
<i>phelypaea</i> subsp. nov.	ED709	Morocco: Rabat, staition Marais, Bouregreg; (34°04'00"N; 06°40'00"W*); 0	<i>Lewalle</i> 9232 (BM)
<i>phelypaea</i> subsp. nov.	ED785	Purtugal: Tavira, Cabanas, Ilha de Cabanas; (37°07'31"N; 08°36'30"W*)	<i>Sales & Hedge</i> 97/9 (E)
<i>lutea</i>	ED726	Niger republic: Tafadek; (17°03'00"N; 08°00'00"E)	<i>Popov</i> 94 (BM)
<i>lutea</i>	ED606	Algeria: Wilaya Bechar, E-Abfall der Berge 19 km SW Zeramra, ca. 55 km WSW Beni Abbes; (29°51'00"N; 02°38'00"W)	<i>Podlech</i> 33665 (MSB)
<i>lutea</i>	ED622	Tchad: North slope of Tarso Tousside, Mosa; (18°24'15"N; 22°08'44"E*); 1250	<i>Scholz</i> 16 (B)
<i>lutea</i>	ED401	Morocco: Merzouga, Meknès-Tafilalet, sand dunes North Hotel; (31°03'08"N; 03°59'21"W); 696	<i>Quandt</i> ED401 (BONN)
<i>lutea</i>	ED402	Morocco: Merzouga, Meknès-Tafilalet, sand dunes North Hotel; (31°05'23"N; 03°98'93"W); 696	<i>Quandt</i> ED402 (BONN)
<i>lutea</i>	ED403	Morocco: Souss-Massa-Daraâ, Zagora to M'hamid, sand dunes ca. 25 km SO Zagora; (30°13'49"N; 05°36'42"W); 693	<i>Quandt</i> ED403 (BONN)
<i>lutea</i>	ED672	Morocco: Tetouan, Mohamed V bridge over the Oued Tzahadartz wetlands; (35°33'51"N; 05°21'01"W*)	<i>Casas</i> 5538 (MA)
<i>lutea</i>	ED668	Spain: Murcia, Aguilas, Cabo cope, Playa de la Cala; (37°24'04"N; 01°34'51"W); 10	<i>Aragon & Martinez</i> GA.0114 (MA)
<i>lutea</i>	ED629	Algeria: Wilaya Tamanrasset, Hoggar-Massiv, Atakor, S of Ilamane; (23°15'00"N; 05°31'00"E); 2290	<i>Podlech</i> 36743 (MSB)
<i>lutea</i>	ED1050	Cyprus: Ayios Yeoryios Island (Akamas); coast, on sides of island; (35°04'27"N; 32°20'06"E*); 0	<i>Dmeikle</i> 2164 (K)
<i>lutea</i>	ED608	Morocco: Er Rachidia, 3 km S Tazouguerte from Boudnib to Gourrama; (32°04'00"N; 03°47'00"W); 1070	<i>Podlech</i> 52855 (MSB)
<i>lutea</i>	ED752	Libya: Gulf of Sirte, E of Marble Arch; 30°26'00"N; 19°41'00"E*	<i>Davis & Hadland</i> 49850 (E, K)
<i>lutea</i>	AY209298	Jordan: Ma'an, Jibal al Batra', Wadi Hisman, Charsa Area; (30°11'00"N; 35°44'00"E*)	<i>Schneeweiss</i> 4225 (WU)
<i>lutea</i>	ED627	Israel: Dead Sea Valley, near Ormat's solar ponds, 2 km N of the Dead Sea northern coast; (30°46'41"N; 35°35'24"E*); 370	<i>Danin & al.</i> s.n. (B)
Outgroups			
Orobanche			
<i>anatolica</i>	ED555	Iran: Azerbaijan, Jolfa-Aras; (38°56'52"N; 45°40'04"E*)	<i>Foroughi</i> 5701 (TARI)
<i>cernua</i>	ED423	Iran: 20 km Bam to Kerman, roadside, dunes; (29°11'55"N; 58°07'13"E); 1337	<i>Ataei & Heidari</i> ED423 (BONN, USB)
<i>cernua</i>	ED740	Iran: Esfahan to Kashan (towards Tiran), ca 76 km Tiran to Kashan; (33°06'02"N; 51°36'46"E); 1706	<i>Ataei & Heidari</i> ED740 (BONN, USB)
<i>transcaucasica</i>	ED750	Georgia: Caucasus, shkhara	<i>Reich</i> ED750 (BONN)
<i>densiflora</i>	ED746	Spain: Chiclana de La Frontera, before Santi Petri, along the coast line; (37°13'13"N; 07°10'01"W); 57	<i>Ataei</i> ED746 (BONN, P)
Phelipanche			
<i>cf. iberica?</i>	ED428	Iran: Kerman, Sarcheshmeh to Sirjan, 10 km after Pariz; (29°49'07"N; 55°44'43"E); 2135	<i>Ataei & Heidari</i> ED428 (BONN, USB)
Conopholis			
<i>C. americana</i>	AY911220	North and Central America	<i>Colwell</i> s. n. (MO)

Appendix 2. 2 Primers detail used for this study.

Region	primer name	Sequence (5'-3')	Reference
<i>psbA-trnH</i> IGS			
	<i>psbA-trnH</i> -F	GTTATGCATGAACGTAATGCTC	Kress & al., 2005
	<i>psbA-trnH</i> -R	CGCGCATGGTGGATTACAATCC	Kress & al., 2005
<i>trnL-F</i>			
	<i>trnL</i> (UAA)-F	CGAAATCGGTAGACGCTACG	Taberlet & al., 1991
	<i>trnL</i> (GAA)-R	ATTTGAACTGGTGACACGAG	Taberlet & al., 1991
Internal primers in <i>trnL-F</i>			
	<i>trnL</i> -460-F	GAGAATAAAGATAGAGTCC	Worberg & al., 2007
	<i>trnL</i> -D-R	GGGGATAGAGGGACTTGAAC	Taberlet & al., 1991
<i>trnS-trnM</i> IGS			
	<i>trnS</i> (UAG)-pF1	ACTATACCGGTTTTCAAGGCCG	Wicke; pers. comm.
	<i>trnM</i> (CAU)-pR1	TAGAGCAGTTTGGTAGCTCGCA	Wicke; pers. comm.
ITS1 & 2			
	ITS4-R	TCCTCCGCTTATTGATATGC	White & al., 1990
	ITS5-R	GGAAGTAAAAGTCGTAACAAGG	White & al., 1990
Internal primers 5.8S			
	5.8S106-R	AGGCGCAACTTGCGTTCAAA	Nickrent, pers. comm.
	5.8s32-F	GCATCGATGAAGAACGTAGC	Nickrent, pers. comm.

Appendix 2. 3 List of indels coded from the combined *Cistanche* dataset.

Number	Extension	Length	Sequence motif
trnL intron			
1	83_84	2	Insertion "CT" in <i>Cistanche lutea</i> (ED672)
2	92_92	1	Gap in <i>Phelipanche cf iberica</i> (ED428)
3	92_94	3	Gap in <i>C. deserticola</i> (ED1042)
4	97_99	3	Gap in <i>C. deserticola</i> (ED1042)
5	99_99	1	Gap in <i>C. deserticola</i> (ED1097)
6	101_108	8	Insertion "AAAGGGG" in <i>C. sp.</i> (ED719) and "GGGGGGG" in <i>C. sp.</i> (ED892)
7	116_120	5	Insertion "AAAAC" in <i>C. rosea</i> (ED705, ED704, ED611, ED708, ED707 & ED782)
8	122_130	9	SSR "AAACAAATT" in <i>C. sp.</i> (ED719 & ED892)
9	138_144	7	Gap in <i>lutea_3</i> (ED671)
10	139_152	14	Gap in whole outgroups, taxa of sects. <i>Cistanchiella</i> and <i>nov.</i>
11	146_152	7	SSR "ATCAAAAT" in <i>C. rosea</i> (ED704, ED611, ED708, ED707 & ED782)
12	192_510	318	Gap in outgroup: <i>P. cf iberica</i> (ED428)
13	206_208	3	SSR "TGC" in <i>C. salsa</i> (ED703, ED585 & ED787)
14	206_210	5	Gap in <i>C. sinensis</i> (ED722)
15	212_215	4	Insertion "TGGT" in outgroups <i>Orobanche cernua</i> (ED423) and <i>O. anatolica</i> (ED555)
16	222_225	4	SSR "ATCT" in <i>C. ambigua</i> (ED448, ED444, ED644 & ED553)
17	234_238	5	SSR "TTTCC" in <i>C. sinensis</i> (ED722)
18	234_241	8	Gap in <i>O. anatolica</i> (ED555)
19	255_259	5	SSR "AGAAA" in <i>C. deserticola</i> (ED1042)
20	264_270	7	Gap in <i>C. rosea</i> (ED708), <i>C. tubulosa</i> subsp. <i>nov.</i> (ED518), <i>tubulosa_1</i> (ED805, ED766, ED772), <i>C. flava</i> subsp. <i>flava</i> (ED548) and all taxa of <i>C. tubulosa</i> subsp. <i>tubulosa</i>
21	274_295	22	Gap in all taxa of <i>C. deserticola</i>
22	276_290	15	SSR "TATCTATTAATAACT" in <i>C. rosea</i> (ED704) with "A" substitution and SSR "TATCTATTGAATACT" in <i>C. rosea</i> (ED611, ED708, ED707 & ED782)
23	293_294	2	SSR "TC" in all taxa of <i>C. ambigua</i> and <i>ridgewayana_3</i>
24	296_300	5	Gap in <i>C. lutea</i> (ED403)
25	305_311	7	SSR "ACTATAT" in <i>O. cernua</i> (ED423)
26	313_326	14	SSR "CTATAAATAAAATA" in <i>C. laxiflora</i> subsp. <i>laxiflora</i> (ED480 & ED478)
27	313_327	15	Gap all taxa but <i>C. laxiflora</i> subsp. <i>laxiflora</i> (ED480 & ED478) and <i>C. phelypaea</i> subsp. <i>phelypaea</i> (ED387)
28	327_327	1	Inseterd "A" in <i>C. phelypaea</i> subsp. <i>phelypaea</i> (ED387)
29	330_334	5	SSR "ATAAA" in <i>C. sp.</i> (ED892)
30	336_339	4	SSR "TAAA" in <i>C. sp.</i> (ED719) and <i>C. tubulosa</i> subsp. <i>tubulosa</i> (ED680)
31	342_344	3	Insertion "TCA" in outgroups <i>O. cernua</i> (ED423), <i>O. anatolica</i> (ED555) and <i>C. sinensis</i> (ED722)
32	342_375	34	Gap in <i>C. tubulosa</i> subsp. <i>nov.</i> (ED518)
33	346_383	38	Gap in all taxa of <i>C. rosea</i> but ED705
34	347_357	11	SSR "TAAAAAATAAA" in <i>C. lutea</i> (ED629)
35	347_362	16	Gap in all taxa but <i>C. lutea</i> (ED629) and <i>violacea_1</i> (ED807)
36	347_382	36	Gap in <i>C. rosea</i> (ED705)
37	347_422	76	Gap in outgroups, sects. <i>Cistanchiella</i> and <i>nov.</i>
38	358_362	5	SSR "TAAAA" in <i>violacea_1</i> (ED807)
39	368_375	8	SSR "AATAAAAA" in <i>lutea_3</i> (ED885)
40	378_378	1	Insertion "A" in <i>lutea_3</i> (ED674)
41	378_379	2	Insertion "AA" in <i>lutea_3</i> (ED674)
42	378_380	3	Insertion "AAA" in ED674, "A" in <i>violacea_2</i> (ED396), <i>C. laxiflora</i> subsp. <i>nov.</i> (ED779), <i>C. senegalensis</i> (ED784 & ED725) and "AA" in <i>C. flava</i> subsp. <i>flava</i> (ED890)
43	385_391	7	Insertion "AAAATAA" in <i>C. lutea</i> (ED1050)
44	393_398	6	Insertion "TATAAA" in <i>violacea_1</i> (ED807 & ED686)
45	403_422	20	SSR "TATATATATATATATATA" in <i>C. rosea</i> (ED707)
46	407_422	16	SSR "TATA" in <i>C. rosea</i> (ED707)

47	409_422	14	SSR "TATATA" in <i>C. rosea</i> (ED704)
48	411_422	12	SSR "TATATATA" in <i>C. rosea</i> (ED708)
49	415_422	8	SSR "TATATATATA" in <i>C. rosea</i> (ED611)
50	417_422	6	SSR "TATATATATATATA" in <i>C. rosea</i> (ED782)
51	426_434	9	Gap in outgroups <i>O. cernua</i> (ED423), <i>O. anatolica</i> (ED555) and <i>C. sinensis</i> (ED722)
52	428_434	7	Inserterd "AATGAAA" in all <i>C. ambigua</i> and <i>C. ridgewayana</i>
53	449_449	1	Gap in <i>O. cernua</i> (ED423) and all taxa of <i>C. phelypaea</i> subsp. <i>phelypaea</i> but ED387
54	450_509	60	Gap in <i>C. rosea</i> (ED782)
55	456_480	25	Gap in <i>fissa_2</i> (ED758 & ED701) and <i>lutea_3</i> (ED885)
56	462_480	19	SSR "ATTTGTATTTTTGAATATA" in <i>C. rosea</i> (ED611)
57	462_486	25	Insertion "ATTTGT" in sect. <i>Cistanchiella</i> but <i>C. salsa</i> and <i>C. deserticola</i> and sect. <i>Cistanche</i> but <i>violacea_2</i> (ED807 & ED1012), <i>C. rosea</i> (ED705) and all taxa of <i>C. flava</i> subsp. <i>nov.</i>
58	482_493	12	Gap in <i>C. lutea</i> (ED1050)
59	488_493	6	SSR "TTTTTT" in <i>ridgewayana_1</i> (ED511)
60	504_509	6	Insertion "TTAAAA" in sect. <i>Cistanchiella</i> but in <i>C. deserticola</i>
61	506_510	5	Gap in <i>O. cernua</i> (ED423), <i>O. anatolica</i> (ED555) and <i>C. sinensis</i> (ED722)
62	507_509	3	Gap in <i>C. deserticola</i> (KC128846 & ED1097)
63	516_516	1	Gap in outgroups, sect. <i>Cistanchiella</i> and <i>C. sp.</i> (ED719)
64	516_518	3	Gap in <i>P. cf. iberica</i> (ED428)
65	527_535	9	Gap in <i>O. anatolica</i> (ED555)
66	532_532	1	insertion "T" in <i>ridgewayana_2</i> (ED559)
67	545_550	6	Gap in <i>C. flava</i> subsp. <i>nov.</i>
68	546_546	1	Insertion "T" in <i>C. sp.</i> (ED719 & ED892)
69	555_556	2	Gap in <i>P. cf. iberica</i> (ED428)
70	561_564	4	Gap in <i>P. cf. iberica</i> (ED428)
71	593_596	4	Gap in <i>O. cernua</i> (ED423) and <i>O. anatolica</i> (ED555), <i>P. cf. iberica</i> (ED428) and <i>C. sinensis</i> (ED722)
72	608_608	1	Insertion "G" in all taxa of <i>C. ambigua</i> and <i>C. ridegewayana</i>
73	611_614	4	Insertion "TTAA" in <i>C. sinensis</i> (ED722)
74	624_628	5	SSR "TTAG" in <i>C. sinensis</i> (ED722)
75	646_649	4	SSR "GAAT" in <i>C. sinensis</i> (ED722)
76	646_650	5	Insertion "A" in <i>C. sinensis</i> (ED722) and <i>fissa_3</i>
77	668_678	11	Gap in <i>O. anatolica</i> (ED555)
78	669_678	10	SSR "ACATGTCAAT" in <i>O. cernua</i> (ED423)
79	712_715	4	SSR "TAAG" in <i>C. flava</i> subsp. <i>flava</i> (ED523)
80	739_739	1	Insertion "T" in <i>fissa_3</i>
trnL	745_745	1	Insertion "A" in <i>C. sp.</i> (ED719 & ED892) and <i>C. flava</i> subsp. <i>flava</i> (ED445 & ED447)
3'exon			
82	764_775	12	SSR "CTCTATCCCCAA" in <i>C. flava</i> subsp. <i>flava</i> (ED541)
83	777_777	1	Insertion "C" in <i>C. sinensis</i> (ED1097)
84	787_787	1	Insertion "C" in <i>O. cernua</i> (ED423) and <i>C. sinensis</i> (ED722 & ED1098); insertion "A" in <i>O. anatolica</i> (ED555)
85	789_793	5	Insertion "CCCCAA" in <i>O. cf. iberica</i> (ED428)
trnL-trnF	804_808	5	SSR "TTTAA" in <i>C. rosea</i> (ED708)
IGS			
86	812_812	1	Insertion "A" in <i>C. lutea</i> (ED672) and all taxa of <i>C. rosea</i>
87	815_818	4	SSR "TTTC" in <i>C. phelypaea</i> subsp. <i>phelypaeabut</i> (ED387)
88	820_825	6	SSR "TTTACT" in <i>fissa_3</i>
89	829_834	6	Insertion "AAAATA" in <i>O. cernua</i> (ED423) and "AAAATC" in <i>O. anatolica</i> (ED555)
90	844_845	2	Gap in <i>C. lutea</i> (ED668 & ED672)
91	845_845	1	Insertion "A" in all <i>C. senegalensis</i> , <i>tubulosa_1</i> , <i>C. flava</i> subsp. <i>flava</i> and <i>C. flava</i> subsp. <i>nov.</i>
92	845_846	2	Gap in <i>C. sinensis</i> (ED722 & ED1098)
93	849_854	6	SSR "TATACT" in <i>C. sp.</i> (ED719 & ED892)
94	858_862	5	SSR "ACTCT" in <i>C. rosea</i> (ED708)

95	864_864	1	Insertion "C" in <i>O. anatolica</i> (ED555)
96	864_865	2	Insertion "C" in <i>O. cernua</i> (ED423) and <i>O. anatolica</i> (ED555); insertion "A" in sect. <i>Cistanchiella</i> but <i>fissa_3</i> and <i>C. deserticola</i>
97	864_866	3	Gap in <i>c. sinensis</i> (ED772 & ED1098)
98	874_889	16	Insertion "CATTATTCATTTTTTTT" in <i>O. anatolica</i> (ED555)
99	874_969	96	Gap in all <i>C. flava</i> subsp. <i>nov.</i>
100	874_975	102	Gap in <i>C. laxiflora</i> subsp. <i>laxiflora</i> (ED515, ED521 & ED512)
101	894_898	5	SSR "TCTTT" in <i>P. cf. iberica</i> (ED428)
102	894_901	8	Gap in <i>C. sinensis</i> (ED1098)
103	906_912	7	SSR "ATTGGTT" in <i>C. flava</i> subsp. <i>flava</i> (ED446)
104	915_915	1	Gap in <i>Phelipanche cf. iberica</i> (ED428)
105	921_940	20	SSR "ATTCATTATTGGATTGGTTC" in <i>C. laxiflora</i> subsp. <i>nov.</i> (ED433, ED435 & ED438) and <i>C. laxiflora</i> subsp. <i>laxiflora</i> (ED779, 480 & 478)
106	933_937	5	SSR "ATTGG" in <i>C. laxiflora</i> subsp. <i>nov.</i> (ED433, ED435, ED438 & ED779)
107	944_946	3	Insertion "AAA" in <i>C. sinensis</i> (ED722 & ED1098)
108	944_950	7	Insertion "AAA" in <i>C. sinensis</i> (ED722 & 1098) and "ATTA" in <i>C. salsa</i> (ED703)
109	947_950	4	Insertion "ATTA" in <i>C. salsa</i> (ED703)
110	954_960	7	Gap in <i>O. cernua</i> (ED423)
111	956_956	1	Gap in all taxa of <i>C. rosea</i>
112	961_961	1	Insertion "C" in <i>O. cernua</i> (ED423) and <i>O. anatolica</i> (ED555)
113	964_964	1	Gap in <i>P. cf. iberica</i> (ED428)
114	970_970	1	Gap in <i>C. salsa</i> (ED585 and ED787)
115	970_973	4	Gap in <i>C. lutea</i> (ED627)
116	970_975	6	Gap in <i>C. laxiflora</i> subsp. <i>laxiflora</i> (ED480 & ED478)
117	972_973	2	Insertion "AA" in <i>fissa_3</i>
118	978_980	3	Insertion "G" in <i>O. anatolica</i> (ED555) and Gap in <i>P. cf. iberica</i> (ED428) and <i>C. sinensis</i> (ED1098)
119	979_979	1	Insertion "A" in <i>C. deserticola</i> (ED1097), <i>fissa_3</i> , <i>violacea_1</i> (ED477, ED902 & ED676)
120	979_980	2	Gap in <i>O. anatolica</i> (ED555)
121	985_1002	18	Insertion "TGGGACGTAAAAATGCAT" in <i>O. cernua</i> (ED423) and "TGGGGTGTA AAAATGCAT" in <i>O. anatolica</i> (ED555)
122	985_1005	21	Gap in <i>fissa_3</i>
123	985_1030	46	Gap in <i>C. sinensis</i> (ED722 & ED1098)
124	1016_1016	1	Insertion "G" in <i>P. cf. iberica</i> (ED428)
125	1023_1023	1	Gap in <i>O. anatolica</i> (ED555)
126	1029_1033	5	Gap in <i>C. senegalensis</i> (ED725)
127	1038_1043	6	Insertion "TTTTGA" in <i>P. cf. iberica</i> (ED428)
128	1045_1045	1	Insertion "T" in <i>tubulosa</i> subsp. <i>tubulosa</i> (ED640, ED1090, ED806), <i>tubulosa</i> subsp. <i>nov.</i> (ED518) and <i>tubulosa_1</i> (ED805, ED766 & ED772)
129	1053_1053	1	Insertion "A" in <i>O. cernua</i> (ED423)
130	1058_1058	1	Gap in <i>O. cernua</i> (ED423)
131	1061_1061	1	Gap in <i>O. cernua</i> (ED423)
132	1063_1065	3	SSR "TCC" in <i>P. cf. iberica</i> (ED428)
133	1069_1079	11	Gap in <i>P. cf. iberica</i> (ED428)
134	1083_1088	6	SSR "CTATTT" in <i>C. brunneri</i> (ED1094)
135	1090_1101	12	Gap in all <i>C. ambigua</i> and <i>C. ridgewayana</i>
136	1092_1107	16	Gap in <i>C. deserticola</i>
137	1095_1098	4	SSR "GAAT" in <i>C. sinensis</i> (ED722 & ED1098)
138	1103_1107	5	SSR "TTAAT" in <i>P. cf. iberica</i> (ED428)
139	1122_1122	1	Insertion "T" in <i>ridgewayana_1</i> (ED549 & ED424)
140	1124_1128	5	Gap in <i>C. sinensis</i>
141	1134_1135	2	Gap in outgroups (ED423, ED555 & ED428) and <i>C. sinensis</i>
142	1135_1135	1	Insertion "A" in <i>C. sp.</i> (ED719 & ED892)
143	1140_1147	8	SSR "TTAATACT" in <i>C. laxiflora</i> subsp. <i>laxiflora</i> (ED515, 521 & 512)
144	1150_1153	4	Gap in <i>C. sinensis</i>

145	1160_1162	3	Insertion "TAA" in <i>P. cf. iberica</i> (ED428)
146	1167_1172	6	Gap in <i>C. rosea</i> (ED705)
147	1169_1171	3	Insertion "GGA" in majority of <i>C. flava</i> subsp. <i>flava</i>
148	1169_1172	4	Insertion "GGAG" in majority of <i>C. flava</i> subsp. <i>flava</i>
149	1169_1175	7	Gap in <i>tubulosa_1</i> (ED772)
150	1169_1210	42	Gap in <i>P. cf. iberica</i> (ED428)
151	1174_1175	2	SSR "TG" in <i>C. phelypaea</i> subsp. <i>phelypaea</i> but ED387
152	1174_1190	17	Gap in <i>violacea_1</i> (ED477, ED902, ED762, ED675 & ED676)
153	1178_1180	3	SSR "TAA" in <i>fissa_3</i>
154	1178_1181	4	Gap in <i>O. anatolica</i> (ED555)
155	1178_1182	5	Gap in all taxa but <i>O. anatolica</i> (ED555), <i>fissa_3</i> , <i>C. lutea</i> (ED668 & ED672)
156	1181_1182	2	Insertion "AA" in <i>C. lutea</i> (ED668 & 672) and Insetion "A" in <i>O. anatolica</i> (ED555)
157	1196_1196	1	Inseted "A" in <i>c. flava</i> subsp. <i>nov.</i>
158	1196_1197	2	Inseted "AA" in <i>c. flava</i> subsp. <i>nov.</i>
159	1196_1198	3	Gap in sects. <i>Cistanchiella</i> , <i>nov.</i> , <i>C. rosea</i> and outgroups
160	1206_1211	6	Gap in <i>tubulosa_1</i> (ED1052) and <i>C. flava</i> subsp. <i>flava</i> (ED1051)
161	1208_1209	2	Inseted "GG" in <i>C. sp.</i> (ED719 & ED892)
162	1231_1232	2	Gap in <i>P. cf iberica</i> (ED428) & <i>C. sinensis</i>
163	1232_1232	2	Insertion "C" in <i>violacea</i> (ED601) and <i>C. sp.</i> (ED719)
164	1235_1244	10	SSR "GTATCTTTAA" in <i>ridgewayana_2</i> (ED559, ED781, ED788, ED546 & ED630)
165	1247_1247	1	Insertion "T" in <i>O. cernua</i> (ED423) and insertion "C" in <i>O. anatolica</i> (ED555)
166	1256_1256	1	Insertion "A" in <i>C. sinensis</i> (ED1098) and <i>Conopholis americana</i>
167	1267_1281	15	Gap in <i>P. cf iberica</i> (ED428)
168	1271_1280	10	Gap in <i>C. sp.</i> (ED719)
169	1271_1281	11	Gap in <i>C. sp.</i> (ED892)
170	1273_1280	8	SSR "TCCTCTAA" in <i>c. phelypaea</i> subsp. <i>phelypaea</i> (ED641)
171	1283_1296	14	Gap in <i>C. flava</i> subsp. <i>nov.</i>
172	1287_1294	8	SSR "AATAAAAT" in <i>violacea_1</i> (ED477)
173	1287_1295	9	Gap in <i>C. rosea</i> but ED705
174	1287_1316	30	Gap in <i>P. cf iberica</i> (ED428)
175	1302_1308	7	Insertion "TAAAAA" in <i>O. anatolica</i> (ED555)
176	1324_1324	1	Insertion "T" in <i>fissa_3</i>
177	1326_1326	1	Insertion "C" in <i>O. anatolica</i> (ED555), <i>P. cf iberica</i> (ED428), <i>C. sinensis</i> , sects. <i>Cistanchiella</i> and <i>nov.</i> , and <i>C. rosea</i>
178	1328_1334	7	SSR "TTGGTAA" in <i>C. phelypaea</i> subsp. <i>phelypaea</i> , majority of <i>C. senegalensis</i> , <i>C. tubulosa</i> and <i>C. flava</i>
179	1336_1342	7	Gap in <i>C. flava</i> subsp. <i>flava</i> (ED1051)
180	1338_1342	5	Gap in <i>violacea_1</i> (ED601)
181	1356_1357	2	Gap in <i>P. cf iberica</i> (ED428)
trnS-trnFM			
IGS			
182	1399_1399	1	Gap in <i>fissa_3</i>
183	1404_1406	3	Insertion "TTC" in <i>violacea_1</i> (ED807)
184	1410_1420	3	Gap in <i>O. cernua</i> (ED423) and <i>P. cf iberica</i> (ED428)
185	1412_1412	1	Gap in outgroups, <i>C. sinensis</i> and majority of species in sect. <i>Cistanchiella</i>
186	1416_1420	5	Insertion "TTATT" in sect. <i>Cistanchiella</i> but <i>fissa_2</i> (ED758 & ED701)
187	1416_1421	6	Gap in <i>C. rosea</i> (ED705)
188	1424_1430	7	Gap in <i>C. phelypaea</i>
189	1424_1437	14	Gap in <i>C. rosea</i>
190	1426_1428	3	SSR "CCT" in <i>c. senegalensis</i> (ED592)
191	1432_1432	1	Insertion "A" in <i>P. cf iberica</i> (ED428)
192	1439_1439	1	Insertion "C" in <i>P. cf iberica</i> (ED428)
193	1442_1443	2	Insertion "GG" in <i>O. cernua</i> (ED423) and <i>P. cf iberica</i> (ED428)
194	1446_1448	3	Insertion "CCT" in <i>lutea_3</i> (ED674)
195	1446_1453	8	Insertion in <i>lutea_3</i> (ED674) and <i>tubulosa</i> subsp. <i>tubulosa</i> (ED640 & ED1090)

196	1446_1454	9	Gap in <i>ridgewayana_2</i> (ED559, ED781, ED788, ED546 & ED630)
197	1449_1453	5	SSR "CCTTA" in <i>tubulosa</i> subsp. <i>tubulosa</i> (ED640 & ED1090)
198	1458_1458	1	Insertion "T" in <i>C. phelypaea</i>
199	1458_1462	5	Gap in <i>O. cernua</i> (ED423), <i>P. cf iberica</i> (ED428) and <i>ridgewayana_2</i> (ED559, ED781, ED788, ED546 & ED630)
200	1458_1468	11	Gap in <i>P. cf iberica</i> (ED428)
201	1460_1462	3	Insertion "CAT" in sect. <i>Cistanchiella</i> but <i>ridgewayana_2</i> (ED559, ED781, ED788, ED546 & ED630)
202	1460_1466	7	Gap in <i>C. laxiflora</i> subsp. <i>laxiflora</i> (ED521)
203	1464_1466	3	Insertion "TTA" in <i>C. lutea</i> (ED402)
204	1474_1475	2	Insertion "AA" in <i>violacea_2</i> (ED396)
205	1478_1480	3	insertion "AAA" in sect. <i>Cistanchiella</i>
206	1483_1486	4	SSR "ATAC" in <i>C. violacea</i>
207	1488_1490	3	Insertion "CGC" in <i>P. cf iberica</i> (ED428) and "CAC" in <i>O. cernua</i> (ED423)
208	1488_1493	6	Gap in all taxa but <i>P. cf iberica</i> (ED428), <i>O. cernua</i> (ED423) and <i>fissa_3</i>
209	1491_1493	3	SSR "TAC" in <i>fissa_3</i>
210	1497_1518	22	Gap in outgroups, <i>C. sinensis</i> and sect. <i>Cistanchiella</i>
211	1500_1518	19	SSR "GGGGGTGAATAACTGC" in <i>C. laxiflora</i> subsp. <i>laxiflora</i> (ED480 & ED478)
212	1523_1526	4	SSR "TATT" in <i>C. deserticola</i> (ED1042) and <i>fissa_3</i>
213	1523_1529	7	SSR "TATT" in <i>C. deserticola</i> (ED1042) and <i>fissa_3</i> , and insertion "TTT" in <i>ridgewayana_2</i> (ED559, ED781, ED788, ED546 & ED630)
214	1527_1529	3	Insertion "TTT" in <i>ridgewayana_2</i> (ED559, 781, 788, 546 & 630)
215	1546_1549	4	SSR "GTAT" in sect. <i>Cistanche</i>
216	1565_1643	79	Gap in <i>C. phelypaea</i> subps. <i>phelypaea</i> (ED668 & ED672)
217	1567_1570	4	Insertion "TATT" in <i>O. cernua</i> (ED423)
218	1575_1591	17	Gap in <i>O. cernua</i> (ED423), <i>P. cf iberica</i> (ED428) and sect. <i>Cistanche</i>
219	1579_1591	13	Gap in <i>C. salsa</i> and <i>C. deserticola</i>
220	1580_1584	5	SSR "TATCT" in <i>fissa_3</i>
221	1588_1591	4	Insertion "TATT" in <i>ridgewayana_2</i> (ED559, ED781, ED788, ED546 & ED630)
222	1596_1607	12	Gap in <i>c. rosea</i> (ED705)
223	1599_1604	6	Gap in <i>C. senegalensis</i> (ED800 & ED894)
224	1602_1607	6	SSR "TATAGA" in all sect. <i>Cistanchiella</i> and majority of sect. <i>Cistanche</i>
225	1602_1608	7	Gap in <i>C. rosea</i> (ED611)
226	1619_1623	5	Gap in <i>C. rosea</i> (ED704)
227	1619_1624	6	Gap in <i>C. rosea</i> (ED611, ED708, ED707 & ED782)
228	1619_1625	7	Gap in <i>C. rosea</i> (ED705)
229	1620_1620	1	Insertion "T" in <i>lutea_3</i> (ED888), <i>tubulosa</i> subsp. <i>tubulosa</i> (ED518, ED516, ED517) and <i>tubulosa</i> subsp. <i>tubulosa</i> (ED1011, ED482, ED577, ED695 & ED891)
230	1626_1626	1	Gap in <i>ridgewayana_2</i> (ED559)
231	1636_1650	15	Gap in <i>C. brunneri</i> (ED1094), <i>C. lutea</i> (ED401, ED402, ED403, ED606, ED622) and <i>violacea_2</i> (ED395, ED398, ED399, ED607, ED400, ED590, ED602 & ED586)
232	1637_1638	2	Insertion "A" in <i>C. phelypaea</i> subsp. <i>phelypaea</i> (ED641)
233	1637_1642	6	Gap in <i>O. cernua</i> (ED423) and <i>P. cf iberica</i> (ED428)
234	1642_1642	1	Insertion "C" in <i>C. rosea</i> (ED707)
235	1647_1647	1	Insertion "T" in <i>C. rosea</i> (ED704, ED611, ED708, ED707 & ED782)
236	1647_1649	3	Insertion "TTA" in <i>C. rosea</i>
237	1662_1665	4	Gap in <i>C. rosea</i>
238	1665_1668	4	Gap in outgroup, <i>C. sinensis</i> and sect. <i>Cistanchiella</i>
239	1685_1685	1	Gap in <i>ridgewayana_1</i> (ED511)
240	1688_1688	1	Gap in <i>ridgewayana_3</i>
241	1689_1689	1	Insertion "A" in <i>C. salsa</i> (ED703) and "G" in the rest of sect. <i>Cistanchiella</i> and outgroups
242	1692_1693	2	SSR "TA" in <i>C. flava</i> and majority of <i>C. senegalensis</i>
243	1722_1722	1	Insertion "A" in <i>O. cernua</i> (ED423) and all sect. <i>Cistanchiella</i>
244	1726_1733	8	Gap in <i>C. brunneri</i> (ED1094)
245	1732_1733	2	Insertion "AT" in <i>C. phelypaea</i> subsp. <i>phelypaea</i> (ED670)

246	1735_1735	1	insertion "T" in outgroup
247	1735_1736	2	Gap in <i>C. brunneri</i> (ED1094)
248	1748_1755	8	Gap in sect. <i>Cistanche</i>
249	1750_1754	5	Gap in the majority of sect. <i>Cistanchiella</i>
250	1751_1754	4	Insertion "TTAG" in outgroups
251	1757_1758	2	Insertion "TA" in <i>C. rosea</i> but ED705
252	1770_1773	4	Insertion "AATT" in sect. <i>Cistanhiella</i>
253	1775_1775	1	Gap in <i>violacea_2</i> (ED396, ED400) and <i>C. lutea</i> (ED403)
254	1783_1785	3	Gap in sect. <i>Cistanchiella</i>
255	1799_1802	4	SSR "ATAT" in <i>C. lutea</i> (ED402)
256	1804_1816	13	SSR "TGTTGGTATATTA" in <i>C. senegalensis</i> (ED592, ED897, ED893, ED896 & ED899)
257	1829_1833	5	SSR "ATATT" in <i>C. senegalensis</i> and <i>C. flava</i>
258	1829_1836	8	Gap in sect. <i>Cistanchiella</i>
259	1845_1848	4	Gap in outgroups
260	1851_1851	1	Gap in <i>O. cernua</i> (ED423)
261	1852_2215	364	Insertion "unknown long nucleotides" in outgroups
262	1856_2215	360	Gap in <i>C. salsa</i> (ED703 & ED585)
263	1865_1866	2	Gap in <i>C. rosea</i>
264	1866_1866	1	Insertion "T" in <i>C. seneglaneis</i> (ED725)
265	1872_1872	1	Gap in <i>violacea_1</i> (ED807)
266	1875_1877	3	Gap in outgroups
267	1877_1877	1	Gap in <i>lutea_3</i> (ED671)
268	1883_1885	3	Gap in outgroups
269	1884_1884	1	Gap in <i>C. rosea</i> (ED705)
270	1904_1909	6	SSR "ATATCA" in <i>lutea_3</i> (ED885)
271	1904_1915	12	Gap in <i>C. phelypaea</i> subsp. <i>phelypaea</i> (ED668 & ED672)
272	1904_1919	16	Gap in <i>P. cf iberica</i> (ED428)
273	1931_2215	285	Gap in sect. <i>Cistanche</i>
274	1931_2217	287	Gap in <i>C. rosea</i>
275	2120_2120	1	Gap in <i>O. cernua</i> (ED423)
276	2125_2129	5	Gap in <i>P. cf iberica</i> (ED428)
277	2156_2156	1	Gap in <i>O. cernua</i> (ED423)
278	2225_2226	2	Gap in <i>ridgewayna_2</i> (ED559, ED781, ED788, ED546 & ED630)
279	2237_2241	5	Gap in <i>lutea_3</i> (ED671)
280	2242_2245	4	Gap in <i>O. cernua</i> (ED423) and <i>P. cf iberica</i> (ED428)
281	2253_2253	1	Gap in <i>C. rosea</i> but ED705
282	2254_2258	5	Gap in <i>C. laxiflora</i> subsp. <i>nov.</i>
283	2255_2255	1	Gap in <i>C. phelypaea</i>
284	2258_2271	14	Gap in <i>fissa_3</i>
285	2263_2271	9	Insertion "AATAAAAA" in <i>O. cernua</i> (ED423) and <i>P. cf iberica</i> (ED428)
286	2294_2297	4	SSR "AATT" in <i>violacea_1</i> (ED902)
287	2349_2488	140	Gap in sect. <i>Cistanchiella</i>
288	2359_2359	1	Insertion "T" in <i>C. lutea</i> (ED608)
289	2362_2364	3	Insertion "AAA" in <i>C. rosea</i> (ED704, ED611, ED707 & ED782)
290	2363_2364	2	Insertion "AA" in <i>C. rosea</i> (ED708)
291	2364_2364	1	Insertion "A" in <i>C. rosea</i> (ED705)
292	2367_2379	13	SSR "GTAATATTTTGAA" in <i>C. phelypaea</i> subsp. <i>nov.</i> (ED709)
293	2388_2391	4	SSR "TTGA" in <i>C. rosea</i> (ED705)
294	2396_2396	1	Insertion "T" in <i>tubulosa_1</i> (ED766 & ED772) and <i>C. tubulosa</i>
295	2421_2433	13	Insertion "TGGTCGTGATAGT" in <i>C. rosea</i> (ED705) and "TGGTCGTGATAAT" in the rest of <i>C. rosea</i>
296	2432_2432	1	Gap in <i>P. cf iberica</i> (ED428)
297	2454_2458	5	Gap in <i>O. cernua</i> (ED423) and <i>P. cf iberica</i> (ED428)
298	2458_2458	1	Insertion "T" in <i>C. brunneri</i> (ED1094), <i>C. lutea</i> (ED401, ED402, ED403, ED606,

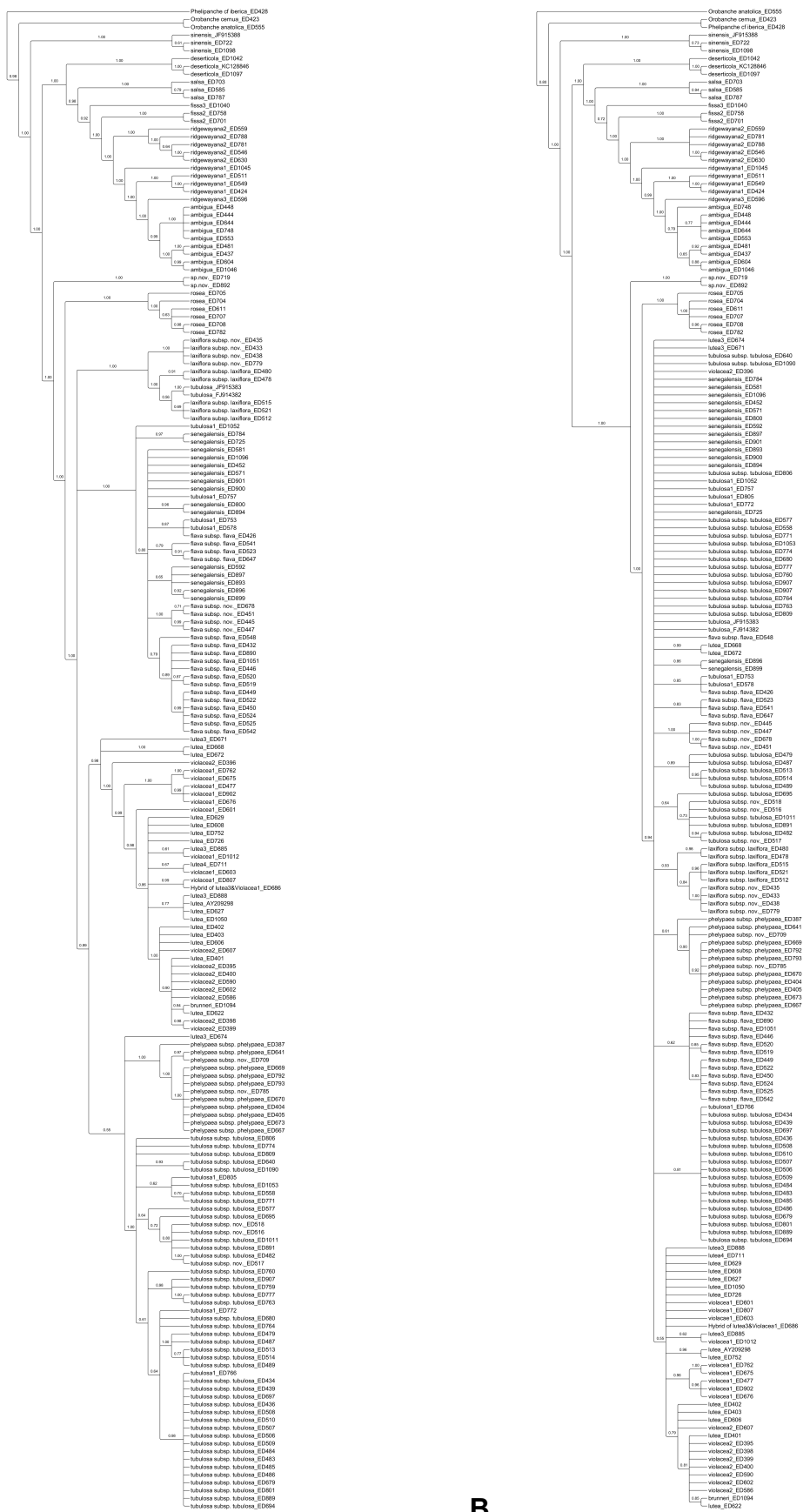
			ED622) and <i>violacea_2</i> (ED395, ED398, ED399, ED607, ED400, ED590, ED602 & ED586, ED396)
299	2466_2466	1	Gap in <i>C. tubulosa</i> subsp. <i>tubulosa</i> (ED907)
300	2469_2475	7	Gap in <i>O. cernua</i> (ED423)
301	2469_2482	14	Insertion "TCTCTTTACTACCA" in <i>P. cf iberica</i> (ED428)
302	2490_2490	1	Insertion "T" in <i>tubulosa_1</i> (ED805) and <i>tubulosa</i> subsp. <i>tubulosa</i> (ED558, ED771 & ED1053)
303	2493_2495	3	SSR "CTG" in <i>ridgewanaya_2</i> (ED781)
304	2499_2499	1	Gap in <i>C. rosea</i> (ED708)
305	2501_2504	4	Insertion "ATAT" in <i>O. cernua</i> (ED423) and <i>P. cf iberica</i> (ED428)
306	2514_2524	11	Insertion "TAATTACTTAC" in <i>fissa_3</i>
307	2556_2557	2	Gap in <i>P. cf iberica</i> (ED428)
308	2558_2558	1	Gap in <i>C. deserticola</i> (ED1042) and <i>fissa_3</i>
psbA-trnH			
IGS			
309	2601_2607	7	Insertion "TCTACAA" in <i>C. sinensis</i> and partially in outgroups
310	2602_2602	1	Gap in <i>O. cernua</i> (ED423) and <i>P. cf iberica</i> (ED428)
311	2611_2611	1	Insertion "A" in <i>ridgewayana_2</i> (ED559, ED781, ED788, ED546 & ED630)
312	2614_2789	176	Insertion "unknown long nucleotides" in outgroups
313	2614_3265	652	Gap in <i>C. sinensis</i>
314	2628_2676	49	Gap in <i>O. cernua</i> (ED423)
315	2692_2692	1	Gap in <i>O. cernua</i> (ED423)
316	2790_3416	627	Gap in <i>O. cernua</i> (ED423) and <i>P. cf iberica</i> (ED428)
317	2792_2801	10	Gap in <i>C. salsa</i> (ED585 & ED703)
318	2793_2798	6	Gap in <i>C. deserticola</i> (ED1097)
319	2793_2799	7	Gap in <i>C. deserticola</i> (KC128846)
320	2793_2800	8	Gap in <i>C. deserticola</i> (ED1042)
321	2793_2801	9	Insertion "TTAATTATA" in <i>C. lutea</i> (ED629)
322	2793_3096	304	Gap in <i>C. fissa</i>
323	2793_3099	307	Gap in <i>fissa_2</i> (ED758)
324	2793_3736	944	Gap in <i>fissa_3</i>
325	2809_2851	43	Gap in sect. <i>Cistanchiella</i> but <i>C. fissa</i>
326	2812_2815	4	Gap in sect. <i>nov.</i>
327	2814_2815	2	Insertion "TT" in <i>C. senegalensis</i> and <i>C. flava</i> but ED891
328	2818_2851	34	Insertion SSR in <i>C. sp.</i> (ED892)
329	2832_2842	11	Gap in <i>C. sp.</i> (ED719)
330	2853_3095	243	Gap in <i>C. ambigua</i> , <i>ridgewayana_3</i> and <i>ridgewayana_1</i> (ED549, ED424, ED511 & ED1045)
331	2853_3096	244	Gap in <i>ridgewayana_2</i> (ED559, ED781, ED788, ED546 & ED630)
332	2866_2884	19	Gap in <i>C. salsa</i> (ED787)
333	2867_2871	5	SSR "TTCAG" in <i>tubulosa</i> subsp. <i>tubulosa</i> (JF915383 & FJ914382)
334	2874_2874	1	Insertion "T" in <i>C. salsa</i> (ED703 & 585)
335	2880_2884	5	SSR "CAGTA" in <i>C. tubulosa</i> subsp. <i>tubulosa</i> (ED763 & ED777)
336	2889_2893	5	Gap in <i>C. laxiflora</i> subsp. <i>laxiflora</i> and <i>C. tubulosa</i> subsp. <i>tubulosa</i> (JF915383 & FJ914382)
337	2890_3095	206	Gap in <i>C. salsa</i> and <i>C. deserticola</i>
338	2893_2893	1	Insertion "A" in <i>C. lutea</i> (ED726)
339	2895_2900	6	Gap in <i>C. rosea</i> but ED705
340	2895_2922	28	SSR "TTATTATTATTAAATTATTACTATAAAAA" in <i>violacea_1</i> (ED902, ED762, ED675 & ED676)
341	2906_2919	14	Gap in <i>C. rosea</i> (ED708)
342	2926_2928	3	SSR "TTA" in <i>lutea_3</i> (ED674)
343	2932_2937	6	SSR "TTATTA" in <i>violacea_1</i> (ED477)
344	2932_2954	23	Gap in <i>C. rosea</i> (ED708)
345	2940_2948	9	SSR "ATTATTA" in sect. <i>nov.</i> and <i>C. phelypaea</i> subsp. <i>phelypaea</i>
346	2956_2958	3	SSR "TAT" in <i>tubulosa_1</i> (ED757)

347	2968_2969	2	Gap in <i>C. lutea</i> , <i>lutea_3</i> , partially <i>violacea_1</i> and <i>violacea_2</i>
348	2969_2969	1	Insertion "A" in <i>tubulosa_1</i> (ED757) and <i>C. tubulosa</i> subsp. <i>tubulosa</i> (ED697)
349	2990_3035	46	SSR "TATTATTAATTACTATAAAAAATTGTAAGACAAAAGATTTAAA" in <i>C. lutea</i> (ED403)
350	3089_3095	7	Insertion "TGTATAA" in <i>C. lutea</i> , <i>lutea_3</i> , <i>violacea_2</i> , partially <i>violacea_1</i> and <i>C. tubulosa</i> subsp. <i>tubulosa</i> (ED482 & ED517)
351	3097_3100	4	Gap in <i>C. phelypaea</i> subsp. <i>phelypaea</i> (ED670)
352	3098_3099	2	SSR "TC" in <i>violacea_2</i> (ED398 & ED399)
353	3101_3156	56	Gap in <i>C. salsa</i> , <i>C. deserticola</i> , <i>C. ambigua</i> , <i>ridgewayana_3</i> and <i>ridgewayana_1</i>
354	3101_3158	58	Gap in <i>ridgewayana_2</i>
355	3104_3118	15	SSR "TCATTATATTTCTTG" including one substitution in <i>violacea_1</i> (ED762 & ED675)
356	3125_3132	8	Gap in <i>tubulosa_1</i> (ED772)
357	3136_3154	19	SSR "AAAAGGTTGGGAAGTTTTT" in <i>C. lutea</i> (ED1050)
358	3169_3169	1	Gap in <i>C. salsa</i> (ED703 & ED585) and <i>C. deserticola</i>
359	3177_3177	1	Gap in <i>C. salsa</i> (ED787)
360	3178_3178	1	Gap in <i>C. flava</i> subsp. <i>flava</i> (ED1051)
361	3181_3181	1	Gap in <i>C. salsa</i> (ED787)
362	3184_3184	1	Insertion "T" in <i>C. deserticola</i>
363	3184_3185	2	Gap in <i>ridgewayana_1</i> (ED549)
364	3184_3186	3	Gap in <i>ridgewayana_1</i> (ED1045) and <i>ridgewayana_2</i> (ED559)
365	3184_3187	4	Gap in <i>C. salsa</i> (ED585), <i>C. ambigua</i> (ED481, ED604, 1046) and <i>ridgewayana_2</i> (ED781, ED788 & ED630)
366	3184_3188	5	Gap in <i>fissa_2</i> , <i>C. ambigua</i> (ED437), <i>ridgewayana_1</i> (ED424, ED511) and <i>ridgewayana_2</i> (ED546)
367	3184_3189	6	Gap in <i>C. ambigua</i> but (ED437, ED481, ED604 & ED1046) and <i>ridgewayana_3</i>
368	3184_3190	7	Gap in sects. <i>nov.</i> and <i>Cistanche</i>
369	3188_3188	1	Gap in <i>C. deserticola</i> (ED1042)
370	3197_3243	47	Gap in sects. <i>nov.</i> and <i>Cistanche</i>
371	3197_3257	61	Gap in <i>C. salsa</i> (ED585)
372	3202_3217	16	Gap in <i>C. salsa</i> , <i>C. deserticola</i> , <i>fissa_2</i> , <i>C. ambigua</i> , <i>ridgewayana_3</i> , <i>ridgewayana_1</i> but ED511 and <i>ridgewayana_2</i> but ED781
373	3205_3210	6	Gap in <i>ridgewayana_1</i> (ED511)
374	3223_3257	35	Gap in <i>C. deserticola</i>
375	3223_3310	88	Gap in <i>C. salsa</i>
376	3232_3313	82	Gap in <i>ridgewayana_2</i>
377	3235_3310	76	Gap in <i>ridgewayana_1</i> (ED511)
378	3239_3310	72	Gap in <i>fissa_2</i> , <i>C. ambigua</i> , <i>ridgewayana_3</i> and <i>ridgewayana_1</i> (ED1045)
379	3244_3310	67	Gap in <i>ridgewayana_1</i> (ED549 & ED424)
380	3245_3250	6	Gap in <i>C. rosea</i> (ED705)
381	3250_3250	1	Gap in <i>C. rosea</i>
382	3250_3257	8	Gap in sect. <i>nov.</i> and sect. <i>Cistanche</i> but (ED885, ED629, <i>C. rosea</i> and <i>C. flava</i> subsp. <i>flava</i>)
383	3250_3258	9	Gap in <i>C. lutea</i> (ED629)
384	3251_3257	7	Gap in <i>C. flava</i> subsp. <i>flava</i>
385	3262_3265	4	Insertion "CAAG" in <i>C. deserticola</i>
386	3268_3334	67	Gap in <i>C. sinensis</i>
387	3270_3274	5	Gap in <i>C. lutea</i> (ED672) and <i>C. tubulosa</i> subsp. <i>tubulosa</i> (ED509 & ED489)
388	3270_3280	11	Gap in <i>C. deserticola</i> , sect. <i>nov.</i> and sect. <i>Cistanche</i> but <i>C. lutea</i> (ED672 & AY209298) and <i>C. tubulosa</i> subsp. <i>tubulosa</i> (ED509 & ED489)
389	3275_3280	6	Gap in all taxa but <i>C. lutea</i> (AY209298)
390	3286_3292	7	SSR "AAATTAT" in <i>violacea_1</i> (ED1012), <i>C. laxiflora</i> subsp. <i>laxiflora</i> and <i>C. tubulosa</i> subsp. <i>tubulosa</i> (JF915383 & FJ914382)
391	3294_3301	8	SSR "AAATTAT" <i>C. laxiflora</i> subsp. <i>alba</i> , <i>C. senegalensis</i> and <i>C. flava</i>
392	3309_3313	5	Gap in <i>C. lutea</i> (ED402)
393	3323_3327	5	Insertion "AACTC" in <i>fissa_2</i> , <i>C. ambigua</i> and <i>C. ridgewayana</i>
394	3352_3352	1	Insertion "TT" in sect. <i>nov.</i>

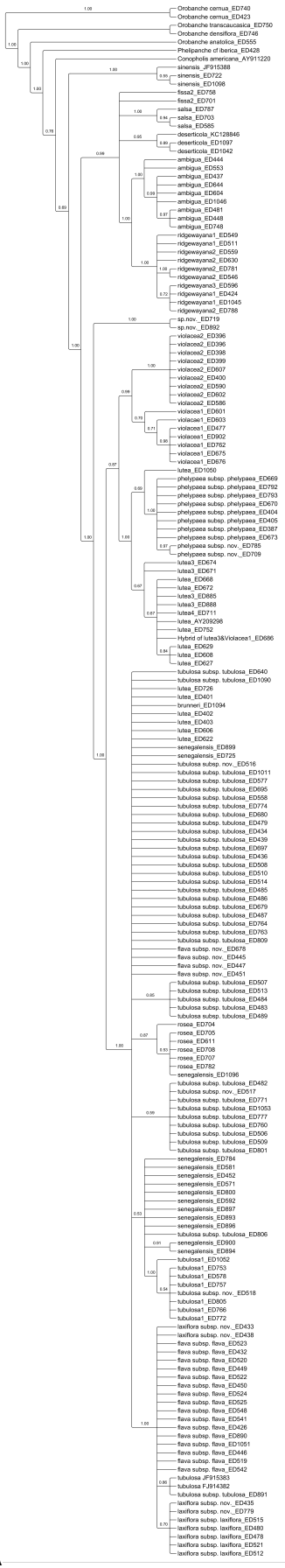
395	3352_3353	2	Gap in <i>C. sinensis</i>
396	3352_3357	6	Gap in <i>C. deserticola</i> and sect. <i>Cistanche</i>
397	3352_3486	135	Gap in <i>ridgewayana_2</i>
398	3363_3371	9	SSR "GAAATAATA" in <i>C. phelypaea</i> subsp. <i>phelypaea</i> (ED641) and <i>C. phelypaea</i> subsp. <i>nov.</i> (ED709)
399	3376_3378	3	Insertion "TTT" in <i>C. sinensis</i>
400	3380_3381	2	Gap in <i>C. sinensis</i> and sect. <i>Cistanchiella</i> but <i>ridgewayana_2</i>
401	3381_3381	1	Insertion "T" in <i>lutea_4</i> (ED711) and <i>violacea_1</i> (ED807 & ED686)
402	3386_3391	6	Insertion "TTTTTT" in <i>C. sinensis</i>
403	3398_3400	3	Insertion "TTT" in <i>C. deserticola</i> (ED1042)
404	3398_3401	4	Gap in all taxa but <i>C. deserticola</i>
405	3403_3430	28	Insertion "AATAATGAAAAGAAAAAGTCTAGACAA" in <i>C. sinensis</i>
406	3427_3663	237	Gap in <i>O. cernua</i> (ED423) and <i>P. cf iberica</i> (ED428)
407	3445_3488	44	Gap in <i>C. phelypaea</i> subsp. <i>phelypaea</i> (ED670)
408	3446_3464	19	Gap in <i>ridgewayana_3</i> and sect. <i>nov.</i>
409	3446_3469	24	Gap in all taxa but <i>C. sinensis</i> , <i>fissa-3</i> , <i>ridgewayana_3</i> , <i>ridgewayana_2</i> , sect. <i>nov.</i> and <i>C. phelypaea</i> subsp. <i>phelypaea</i> (ED670)
410	3465_3469	5	Gap in <i>C. sinensis</i>
411	3471_3481	11	Gap in <i>C. ambigua</i> , <i>ridgewayana_3</i> , <i>ridgewayana_1</i> and sect. <i>nov.</i>
412	3471_3486	16	SSR "TTAATTAATAATAATA" in <i>C. salsa</i> (ED703 & ED585)
413	3471_3487	17	Gap in <i>fissa_2</i>
414	3471_3489	19	Gap in <i>C. deserticola</i>
415	3475_3486	12	Gap in <i>C. salsa</i> (ED787)
416	3488_3496	9	Gap in <i>C. lutea</i> (ED1050)
417	3490_3496	7	Gap in <i>C. sinensis</i>
418	3491_3495	5	SSR "TTTAA" in <i>C. tubulosa</i> subsp. <i>tubulosa</i> (JF915383 & FJ914382)
419	3501_3507	7	Insertion "TAATATA" in <i>C. lutea</i> (ED1050)
420	3512_3514	3	Gap in <i>C. sinensis</i>
421	3513_3513	1	Insertion "G" in <i>C. salsa</i> , <i>C. deserticola</i> and <i>fissa_2</i>
422	3519_3626	108	Gap in <i>C. sinensis</i>
423	3520_3523	4	SSR "ATAA" in <i>ridgewayana_2</i> (ED788)
424	3527_3527	1	Gap in <i>C. ambigua</i> (ED448, ED444, ED644, ED748 & ED553)
425	3565_3569	5	SSR "TATAT" in <i>violacea_1</i> (ED601)
426	3565_3625	61	Gap in <i>C. salsa</i>
427	3565_3626	62	Gap in sect. <i>Cistanchiella</i> but <i>fissa_3</i> and <i>C. salsa</i>
428	3572_3580	9	Gap in <i>lutea_4</i> (ED711) and <i>violacea_1</i> (ED603)
429	3572_3587	16	Gap in sects. <i>nov.</i> and sect. <i>Cistanche</i> but (ED711, ED603 & ED890)
430	3581_3587	7	Gap in <i>C. flava</i> subsp. <i>flava</i> (ED890)
431	3590_3590	1	Insertion "T" in sect. <i>nov.</i>
432	3592_3604	13	Gap in <i>C. rosea</i> (ED705)
433	3592_3608	17	Gap in <i>C. rosea</i> but ED705 and ED707
434	3592_3622	31	Gap in <i>C. flava</i> subsp. <i>flava</i> but (ED523, ED541, ED426 & ED647)
435	3592_3625	34	SSR "TATTATAAATATTATATTATTATAAATAAATAT" in <i>C. rosea</i> (ED707)
436	3615_3623	9	Gap in <i>C. rosea</i> (ED705)
437	3629_3663	35	Gap in <i>C. sinensis</i>
438	3630_3641	12	SSR "ATAATTATATTT" in <i>C. tubulosa</i> subsp. <i>tubulosa</i> (ED479, ED513, ED514 and ED487)
439	3630_3663	34	Gap in sect. <i>Cistanchiella</i> but <i>fissa_3</i>
440	3645_3661	17	SSR "ATTATATTTAATATTAT" in <i>C. rosea</i> (ED708)
441	3645_3662	18	Gap in sect. <i>nov.</i>
442	3667_3674	8	Gap in <i>C. deserticola</i>
443	3668_3674	7	SSR "AATTATA" in <i>C. rosea</i> (ED705)
444	3668_3730	63	Gap in <i>O. cernua</i> (ED423) and <i>P. cf iberica</i> (ED428)
445	3677_3677	1	Gap in <i>C. deserticola</i> (ED1042)
446	3679_3683	5	SSR "ATATT" in <i>C. rosea</i> (ED705)

447	3679_3685	7	Gap in <i>C. sinensis</i>
448	3679_3703	25	Gap in <i>C. tubulosa</i> subsp. <i>tubulosa</i> (ED889)
449	3689_3699	11	Gap in <i>ridgewayana_2</i> (ED788)
450	3689_3700	12	Gap in <i>ridgewayana_2</i> (ED781, ED546 & ED630)
451	3689_3701	13	Gap in all taxa but outgroups, <i>C. sinensis</i> , <i>fissa_3</i> , <i>ridgewayana_1</i> (ED549, ED424), <i>ridgewayana_2</i> (ED781, ED788, ED546, ED630), <i>C. rosea</i> (but ED705) and <i>C. tubulosa</i> subsp. <i>tubulosa</i> (ED889)
452	3689_3707	19	Gap in <i>C. sinensis</i>
453	3689_3732	44	Gap in <i>ridgewayana_1</i> (ED424 & ED549)
454	3700_3701	2	Gap in <i>C. rosea</i> but in ED705
455	3707_3707	1	Gap in <i>C. deserticola</i> , <i>C. ambigua</i> , <i>ridgewayana_3</i> and <i>ridgewayana_1</i> (ED511)
456	3707_3708	2	Gap in <i>C. deserticola</i> (ED1097)
457	3711_3711	1	Insertion "T" in <i>ridgewayana_2</i> (ED781)
458	3713_3716	4	Gap in outgroups, <i>C. sinensis</i> , sect. <i>Cistanchiella</i> but ED781
459	3715_3716	2	Insertion "TA" in <i>ridgewayana_2</i> (ED781)
460	3715_3729	15	Gap in <i>lutea_3</i> (ED888) and <i>C. lutea</i> (AY209298, ED627 & ED1050)
461	3719_3730	12	Gap in <i>C. sinensis</i> (ED722 & ED1098)
462	3719_3731	13	Gap in <i>C. sinensis</i> (JF915388)
463	3721_3728	8	Gap in sects. <i>nov.</i> and <i>Cistanche</i>
464	3721_3736	16	Gap in <i>C. deserticola</i> (ED1042)
465	3726_3726	1	Insertion "T" in <i>C. phelypaea</i> subsp. <i>phelypaea</i> (ED667)
466	3727_3728	2	Gap in <i>C. deserticola</i> (KC128846 & ED1097)
467	3730_3730	1	Gap in sect. <i>nov.</i>
468	3736_3736	1	Insertion "T" in <i>C. phelypaea</i> subsp. <i>phelypaea</i> (ED667)
469	3739_3740	2	Gap in <i>C. deserticola</i> (ED1042)
470	3740_3740	1	Gap in sect. <i>Cistanchiella</i>
471	3747_3760	14	Gap in all taxa but <i>C. salsa</i>
472	3751_3760	10	Gap in <i>C. salsa</i> (ED703)
473	3757_3760	4	Gap in <i>C. salsa</i> (ED787)
474	3764_3770	7	Gap in sect. <i>Cistanche</i> but <i>C. rosea</i>
475	3766_3767	2	Insertion "TA" in <i>ridgewayana_2</i> (ED781)
476	3782_3785	4	SSR "TACC" in <i>ridgewayana_1</i> (ED549)
477	3789_3797	9	Gap in <i>O. cernua</i> (ED423) and <i>P. cf iberica</i> (ED428)
478	3791_3794	4	SSR "ATTT" in <i>C. ambigua</i>
479	3798_3798	1	Gap in <i>C. senegalensis</i> (ED894) and <i>C. tubulosa</i> subsp. <i>tubulosa</i> (ED558, ED777, ED907, ED759 & ED763)
480	3801_3805	5	Gap in <i>ridgewayana_1</i> (ED549), <i>C. laxiflora</i> subsp. <i>nov.</i> (ED779), <i>C. senegalensis</i> (ED894) and <i>C. tubulosa</i> subsp. <i>tubulosa</i> (ED558, ED771, ED777, ED907, ED759 & ED763)
481	3802_3805	4	SSR "TATT" in <i>ridgewayana_3</i>
482	3802_3806	5	Gap in <i>O. cernua</i> (ED423) and <i>P. cf iberica</i> (ED428)
483	3812_3812	1	Gap in <i>C. tubulosa</i> subsp. <i>tubulosa</i> (JF915383 & FJ914382)
ITS1 & ITS2			
484	3869_3869	1	Gap in <i>O. transcaucasica</i> and <i>O. densiflora</i>
485	3880_3880	1	Gap in all taxa of <i>Orobanche</i>
486	3898_3898	1	Gap in all taxa of <i>Orobanche</i> but <i>O. anatolica</i> (ED555)
487	3899_3899	1	Gap in <i>O. anatolica</i> (ED555)
488	3907_3907	1	Gap in <i>Orobanche</i> and <i>C. sinensis</i>
489	3911_3913	3	Insertion "ATT" in <i>P. cf iberica</i> (ED428)
490	3922_3922	1	Insertion "G" in <i>P. cf iberica</i> (ED428)
491	3925_3934	10	Gap in <i>Orobanche</i>
492	3927_3927	1	Insertion "G" in <i>C. americana</i> , sect. <i>Cistanchiella</i> but <i>C. ambigua</i> and <i>C. ridgewayana</i> and all taxa of sects. <i>nov.</i> and <i>Cistanche</i>
493	3929_3929	1	Insertion "A" in <i>C. americana</i>
494	3933_3933	1	Insertion "A" in <i>C. americana</i> and <i>C. sinensis</i> and insertion "C" in <i>P. cf iberica</i> (ED428)

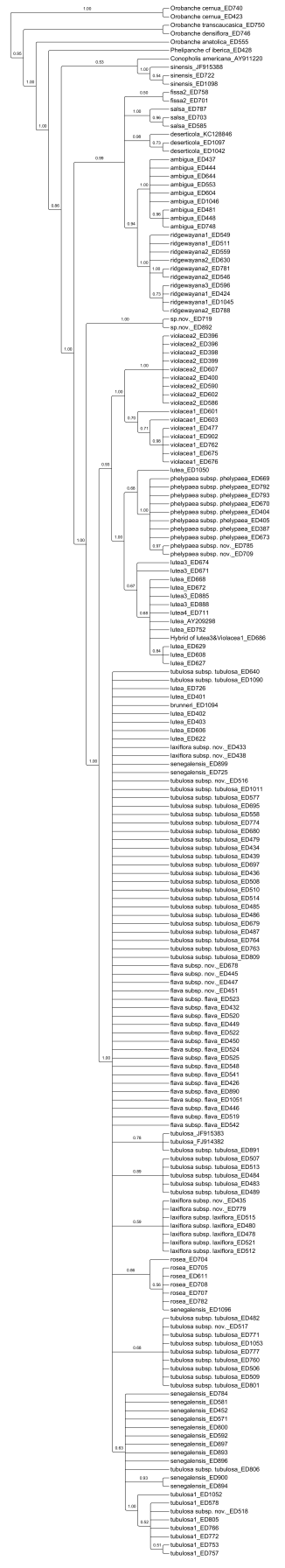
495	3950_3950	1	Gap in sect. <i>nov.</i>
496	3951_3951	1	Insertion "C" in all taxa but <i>C. salsa</i> , <i>C. laxiflora</i> and <i>C. flava</i> subsp. <i>flava</i>
497	3956_3957	2	Gap in <i>Orobanche</i> and <i>C. americana</i>
498	3957_3957	1	Insertion "C" in <i>P. cf iberica</i> (ED428)
499	3963_3963	1	Insertion "G" in <i>O. cernua</i> (ED740 & ED423)
500	3963_3965	3	Gap in <i>P. cf iberica</i> (ED428)
501	3970_3977	8	Gap in <i>C. americana</i>
502	3982_3982	1	Gap in <i>C. americana</i>
503	4031_4031	1	Insertion "A" in outgroups and <i>C. sinensis</i>
504	4033_4033	1	Insertion "A" in <i>C.s americana</i>
505	4049_4051	3	Gap in <i>O. transcaucasica</i> (ED750) and <i>O. densiflora</i> (ED746)
506	4050_4051	2	Insertion "GA" in <i>C. americana</i>
507	4053_4053	1	Insertion "T" and "C" in outgroups and <i>C. sinensis</i>
508	4068_4069	2	Gap in <i>O. anatolica</i> (ED555)
509	4075_4078	4	Insertion "GCGG" in <i>O. anatolica</i> (ED555)
510	4075_4079	5	Gap in all taxa but outgroups, <i>C. sinensis</i> , <i>ridgewayana_1</i> (ED511, ED1045), <i>ridgewayana_2</i> (ED788, ED546) and sect. <i>nov.</i>
511	4075_4080	6	Gap in <i>ridgewayana_1</i> (ED1045) and sect. <i>nov.</i>
512	4075_4081	7	Gap in <i>ridgewayana_2</i> (ED788 & ED546)
513	4096_4097	2	Gap in <i>C. ambigua</i> (ED748)
514	4238_4238	1	Insertion "T" in <i>ridgewayana_1</i> (ED549)
515	4281_4284	4	Insertion "CTCA" in <i>O. transcaucasica</i> (ED750)
516	4281_4286	6	Gap in <i>O. anatolica</i> (ED555) and <i>P. cf iberica</i> (ED428)
517	4286_4286	1	Insertion "T" in <i>O. transcaucasica</i> (ED750) and <i>O. densiflora</i> (ED746) and insertion "C" in other <i>Orobanche</i> , <i>C. americana</i> and sect. <i>Cistanche</i>
518	4292_4296	5	Gap in <i>O. anatolica</i> (ED555)
519	4293_4296	4	Gap in <i>c. deserticola</i> (ED1042)
520	4294_4296	3	Insertion "CTC" in <i>P. cf iberica</i> (ED428)
521	4303_4303	1	Gap in <i>O. densiflora</i> (ED746)
522	4308_4308	1	Insertion either "G" or "A" in all taxa but <i>C. sinensis</i> and sect. <i>nov.</i>
523	4309_4319	11	Gap in <i>O. anatolica</i> (ED555)
524	4315_4315	1	Gap in <i>C. deserticola</i> (ED1042)
525	4321_4321	1	Gap in <i>O. densiflora</i> (ED746) and <i>P. cf iberica</i> (ED428)
526	4324_4325	2	Gap in <i>C. deserticola</i> (ED1042)
527	4330_4330	1	Gap in <i>C. americana</i>
528	4348_4350	3	Gap in <i>P. cf iberica</i> (ED428)
529	4350_4350	1	Gap in sect. <i>Cistanchiella</i> but <i>C. salsa</i>
530	4351_4351	1	Gap in <i>fissa_2</i> (ED758)
531	4378_4378	1	Gap in <i>fissa_2</i> (ED758)
532	4417_4423	7	Gap in <i>P. cf iberica</i> (ED428)
533	4419_4423	5	SSR "AACTC" in all taxa of sect. <i>Cistanche</i> but <i>C. phelypaea</i> , <i>C. lutea</i> and <i>C. violacea</i>
534	4429_4430	2	Gap in <i>C. salsa</i> (ED787)
535	4433_4433	1	Gap in <i>Orobanche</i> but <i>O. anatolica</i> (ED555)
536	4442_4444	3	Gap in <i>C. sinensis</i>
537	4448_4448	1	Insertion "T" in sect. <i>nov.</i>
538	4462_4462	1	Insertion "G" or "A" in all taxa but sect. <i>Cistanche</i>
539	4470_4471	2	Gap in <i>P. cf iberica</i> (ED428)
540	4474_4474	1	Insertion "A" in <i>C. sinensis</i>
541	4478_4480	3	Insertion "TTA" in sect. <i>nov.</i>
542	4495_4498	4	Gap in <i>P. cf iberica</i> (ED428)
543	4497_4498	2	SSR "AT" in <i>C. ambigua</i> (ED481, ED437, ED448, ED644, ED748, ED604 & ED1046)
544	4502_4502	1	Gap in all taxa but sect. <i>Cistanche</i>
545	4506_4506	1	Gap in <i>O. densiflora</i> (ED746)
546	4515_4515	1	Gap in <i>C. laxiflora</i> , <i>C. flava</i> subsp. <i>flava</i> and <i>C. tubulosa</i> subsp. <i>tubulosa</i> (JF915383 and FJ914382)



Appendix 2. 4 Bayesian tree of combined plastid data involving indels (A) and DNA (B). Number above branches show posterior probabilities.

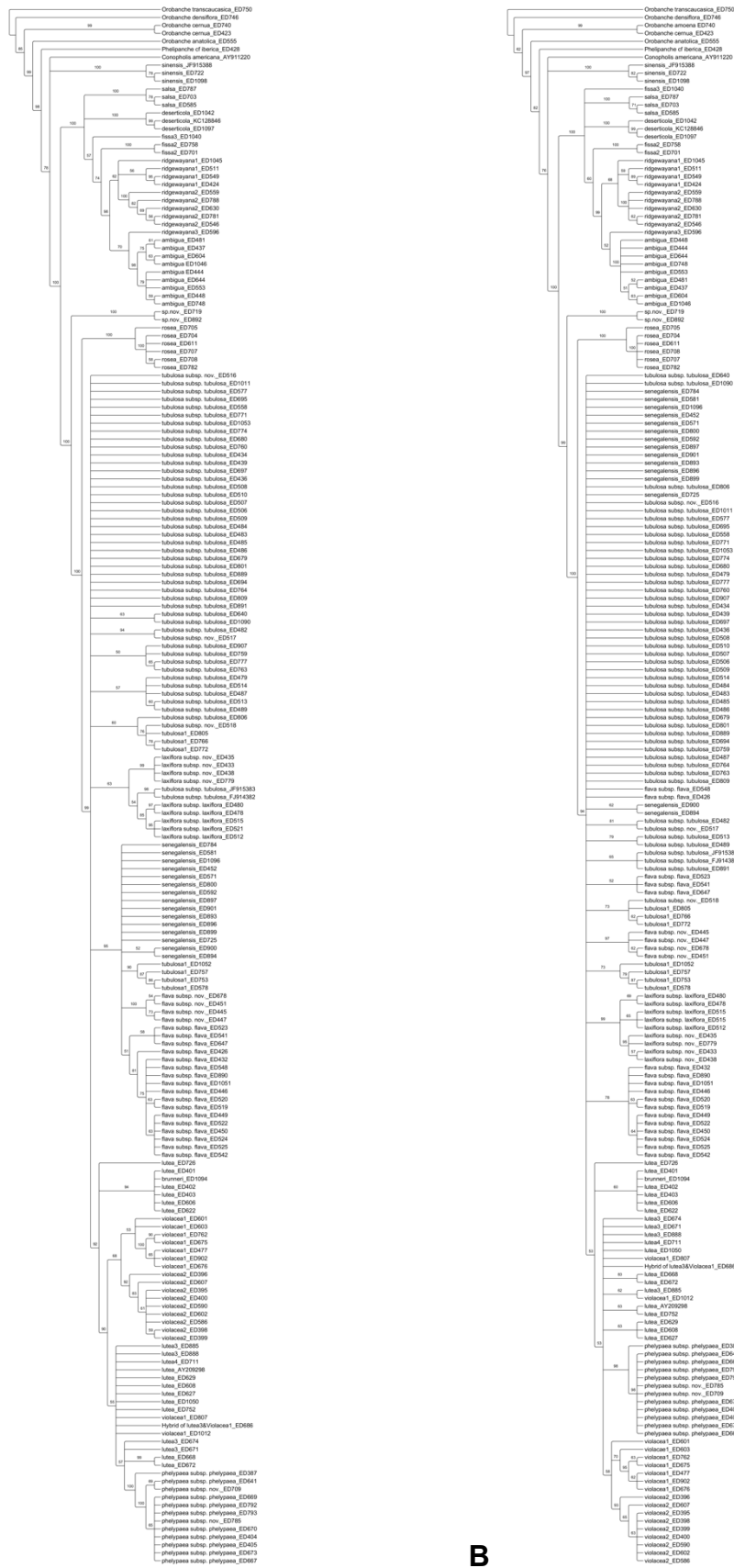


A

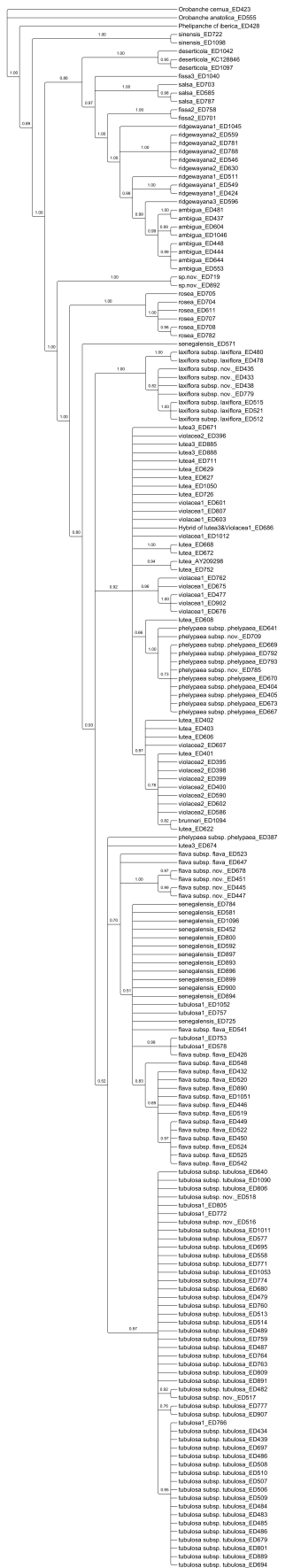


B

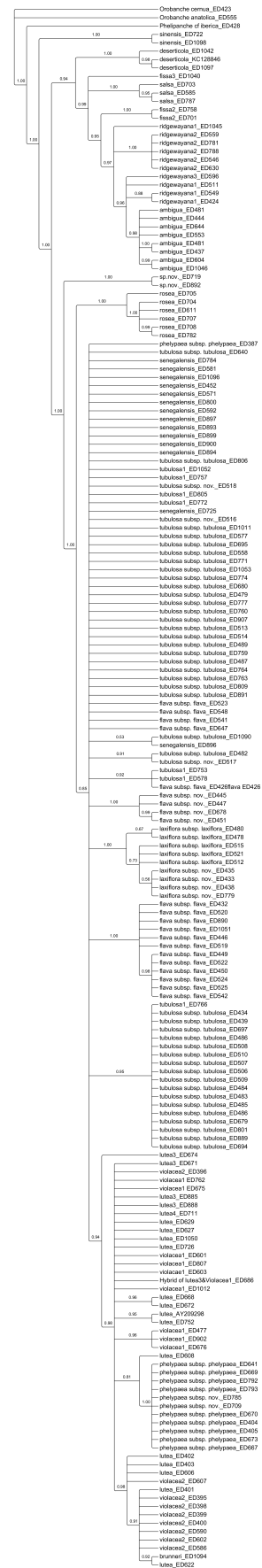
Appendix 2. 5 Bayesian tree of nuclear ITS data involving indels (A) and DNA (B). Number above branches show posterior probabilities.



Appendix 2. 6 Maximum parsimony analysis based on the combined data set involving indels (A) and only DNA (B). Values above the branches show bootstrap support above 50. The analysis of the combined analysis (nucleotides only) resulted in 197 maximum parsimony trees (MPTs) with length of 2029 steps (consistency index [CI]: 0.698, retention index [RI]: 0.934, rescaled consistency index [RC]: 0.652). The analysis including indels resulted in 193 MPTs trees with length of 2734 steps (CI: 0.719, RI: 0.935, RC: 0.672).

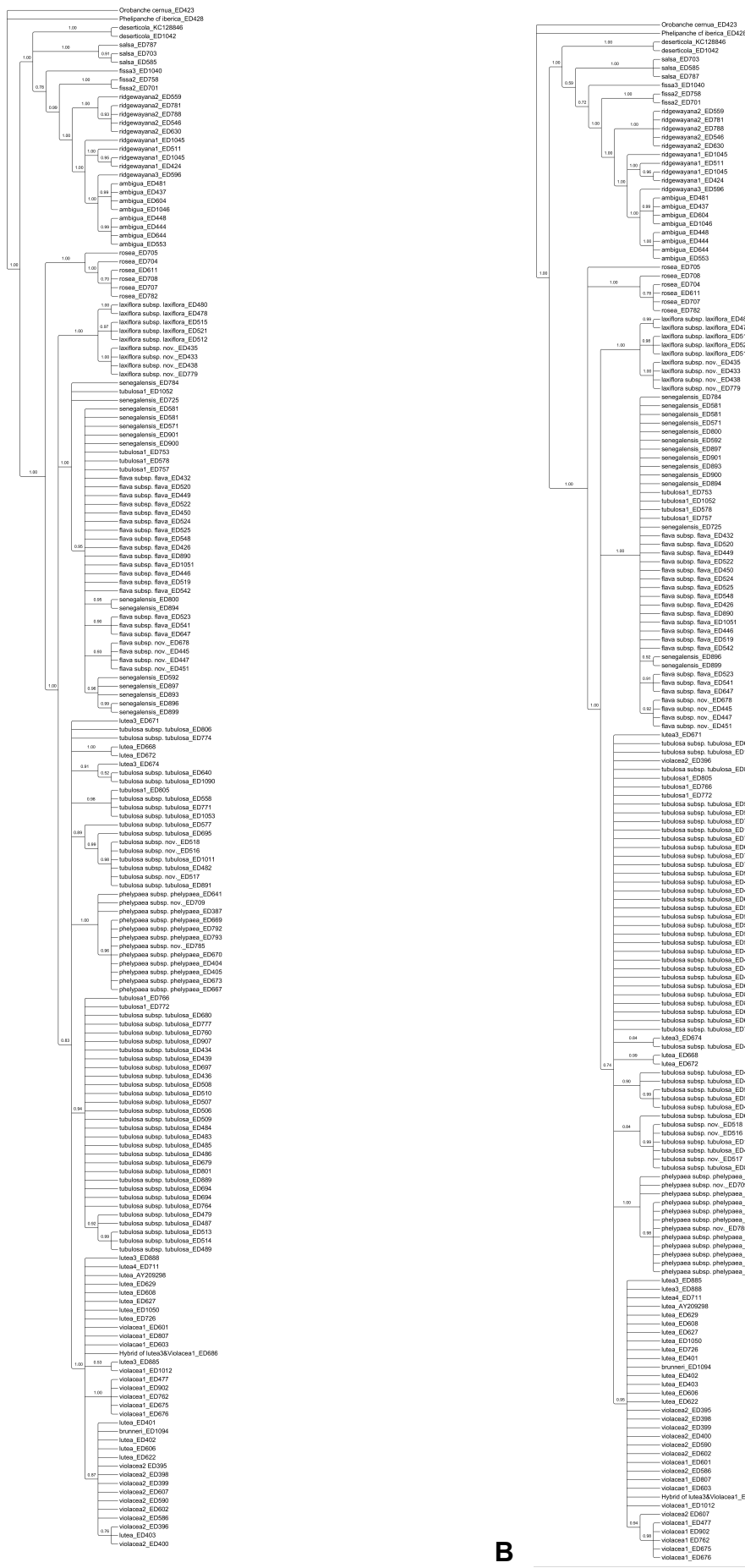


A

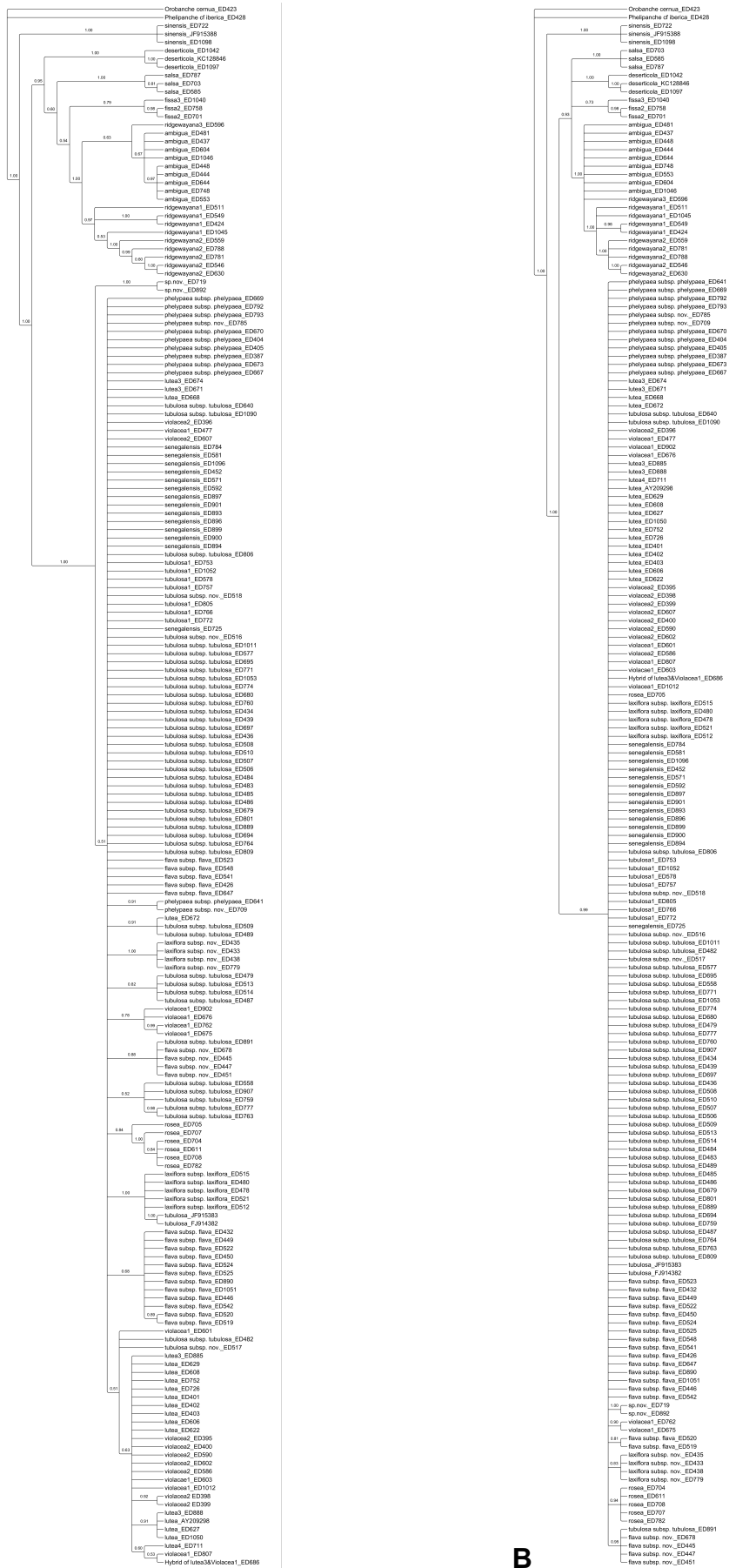


B

Appendix 2. 7 Bayesian tree of *trnL-F* region involving indels, in which infrageneric classification in the region varies from maximum support (A) and to moderate support in DNA matrix (B). Number above branches show posterior probabilities. Apart from *C. rosea*, lowly supported clades in W-clade represented by unresolved trichomy and polytomy in indel and non-indel data, respectively (A & B).



Appendix 2. 8 Bayesian tree of *trnS-fM* region involving indels (A) and DNA (B). Infrageneric conclusion cannot be fully made with this marker as the data for EA and NWA-clades were missing. However, maximum support appeared for the other two clades (SWA and W-clades) in both analyses (A & B). Number above branches show posterior probabilities.



Appendix 2. 9 Bayesian tree of *psbA-trnH* region involving indels, in which infragenic classification can be maximally or lowly supported (A) and DNA, in which NWA-clade placed inside the polytomies of W-clade (B). Number above branches show posterior probability.

B) Appendices to chapter 3

Appendix 3. 1 Nuclear DNA content of 9 species of *Cistanche*, and three and three species of its close relatives *Phelipanche* and *Orobanche*, respectively. Voucher information, mean 2C-value \pm SD and available chromosome numbers are presented. The same individuals used either to measure the genome size or chromosome number, in the case both analyses were conducted. 1 pg = 978 Mbp (Dolezel & al. 2003). ^{GB} indicates sequences and nuclear DNA content abstracted from Schneeweiss (2004a) and Weiss-Schneeweiss & al. (2006), respectively. Abbreviations: BONN: Herbarium University of Bonn; USB: Herbarium University of sistan and Baluchestan; S&T: Herbarium Schönschwetter & Tribsch; WU: Herbarium University of Vienna.

Taxon	Accession and isolation number	Locality	Coordinates	Voucher number	m a.s.l.	n (Chromosome number)/Figure	2C DNA (pg/2C) \pm SD	1C DNA content (Mbp/1C)
<i>Cistanche</i>								
Sect. <i>Cistanchiella</i>								
<i>ridgewayana</i>	IRB3	Iran: Zahedan to Kerman, near Mahan, 100 km Bam to Mahan	29°31'05"N; 57°48'15"E	BONN & USB: Ataei N. & Heidari ED424; 19.IV.2010	1980	20 / 3A	11.4236 \pm 0.652	11172.2808
<i>ridgewayana</i>	IRBf	Iran: Sistan & Baluchestan, Saravan, 15 km Suran, Rigjeh village, toward water pump station	27°21'17"N; 61°55'53"E	BONN & USB: Ataei N. & Valizadeh M. ED511; 04.III.2011	1135		13.928 \pm 0.493	13621.5840
<i>ambigua</i>	IRB14a	Iran: Tehran, Kavir protect region, SE Waramin, 1 km after Shokr-Abad, around check point	35°03'52"N; 51°41'54"E	BONN & USB: Ataei N. & Heidari ED437; 24.IV.2010	773	20 / 3B	14.31 \pm 0.697	13995.1800
<i>ambigua</i>	IRB14b	Iran: Tehran, Kavir protect region, SE Waramin, 5 km after Shokr-Abad, S of check point	35°01'31"N; 51°42'57"E	BONN & USB: Ataei N. & Heidari ED481; 24.IV.2010	923	20 (not shown)	16.956 \pm 0.483	16582.9680
<i>ambigua</i>	IRB20	Iran: Shahrud to Torud, 71 km Shahrud, roadside	35°51'17"N; 55°11'55"E	BONN & USB: Ataei N. & Heidari ED444; 27.IV.2010	1062	20 / 3C & R	15.3056 \pm 0.692	14968.8768
<i>ambigua</i>	IRB24	Iran: Zaman Abad-Bardaskan, down detour road in Zaman Abad, 2 km to Kalateh Karbalai Abbas	35°46'36"N; 56°38'17"E	BONN & USB: Ataei N. ED448; 28.IV.2010	949	20 (not shown)	8.56 \pm 0.512	8371.6800
Sect. <i>Cistanche</i>								
<i>phelypaea</i> subsp. <i>phelypaea</i>	Yellow5	Morocco: coase line in Tiznit, Souss-Massa-Daraâ, Nord Sidi Ifni	29°38'89"N; 10°17'28"W	BONN: Quandt D. ED404; 17.III.2010	85		16.7368 \pm 0.591	16368.5904
<i>phelypaea</i> subsp. <i>phelypaea</i>	Yellow6	Morocco: Souss-Massa-Daraâ, Agadir	30°65'01"N; 09°88'53"W	BONN: Quandt D. ED405; 11.III.2010	51		19.3828 \pm 0.579	18956.3784
<i>phelypaea</i> subsp. <i>phelypaea</i>	Ova.1	Spain: Chiclana de La Frontera, Santi Petri, along the coast line	36°23'31"N; 06°12'24"W	BONN: Ataei N. ED792; 23.IV.2012	60	20 / 4D	18.765 \pm 0.820	18352.1700
<i>phelypaea</i> subsp. <i>phelypaea</i>	Ova.2	Spain: Province of Huelva, Cartaya, El Rompido, close to Golf course	37°13'13"N; 07°07'40"W	BONN: Ataei N. ED793; 23.IV.2012	5		19.7218 \pm 0.740	19287.9204
<i>phelypaea</i> subsp. <i>phelypaea</i>	AY209303.1 ^{GB}	Spain: Andalucía, province of Cádiz, coast near Conil de la Frontera	36°23'14"N; 06°12'22"W	WU: S&T 8734 (11.III.2002: GS & HW)	0–5	20	17.851 \pm 0.173	17458.2780
<i>violacea</i>	Cis1	Morocco: before Ar-Rachidia, Meknès-Tafilalet, Oued Ziz, ca. 20 km N of Stausee Hassan Addakhil	32°14'82"N; 04°37'11"W	BONN: Quandt D. ED395; 10.III.2010	45	20 / 4A & P	13.0694 \pm 1.36	12781.8732
<i>violacea</i>	Cis2	Morocco: before Ar-Rachidia, Meknès-Tafilalet, Oued Ziz, ca. 20 km N of Stausee Hassan Addakhil	32°14'82"N; 04°37'11"W	BONN: Quandt D. ED396; 10.III.2010	1032		18.2462 \pm 0.800	17844.7836
<i>violacea</i>	Cis3	Morocco: before Tinejdad	30°65'49"N; 06°56'25"W	BONN: Quandt D. ED398; 12.III.2010	996		15.411 \pm 0.849	15071.9580
<i>violacea</i>	Cis4	Morocco, after Tinejdad, Meknès-Tafilalet, Erfoud to Tinejdad	31°43'88"N; 04°24'37"W	BONN: Quandt D. ED399; 12.III.2010	792	30 \pm 1/2B / 4B & R	???	
<i>violacea</i>	Cis5	Morocco: Quarzazate, Souss-Massa-Daraâ, from	30°79'84"N;	BONN: Quandt D. ED400;	1146	20 / 4C & Q	18.6456 \pm 1.220	18235.3968

		Ouazazarte to Zagora, river valley ca. 8 km SO of Ouazazarte	06°77'30"W	13.III.2010				
<i>lutea</i>	Yellow1	Morocco: Merzouga, Meknès-Tafilalet, sand dunes North Hotel	31°05'23"N; 03°98'93"W	BONN: Quandt D. ED401; 11.III.2010	696		15.9046 ± 0.824	15554.6988
<i>lutea</i>	Yellow2	Morocco: Merzouga, Meknès-Tafilalet, sand dunes North Hotel	31°05'23"N; 03°98'93"W	BONN: Quandt D. ED401; 11.III.2010	696		16.963 ± 0.811	16589.8140
<i>lutea</i>	Yellow3	Morocco: Merzouga, Meknès-Tafilalet, sand dunes North Hotel	31°05'23"N; 03°98'93"W	BONN: Quandt D. ED402; 11.III.2010	696		11.3382 ± 0.50	11088.7596
<i>lutea</i>	Yellow4	Morocco: Zagora, Souss-Massa-Daraâ, from Zagora to M'hamid, sand dunes ca. 25 km SO from Zagora	30°23'04"N; 05°01'17"W	BONN: Quandt D. ED403; 14.III.2010	693		21.624 ± 0.640	21148.2720
<i>phelypaea</i> subsp. <i>lutea</i>	AY209300.1 ^{GB}	Spain: Taha de Andarax ca. 5 km W of Canjáyar	37°00'42"N; 02°45'16"W	WU: S&T 7656 (2.IV.2001: GS & HW)	750	20	18.108 ± 0.544	17709.6240
<i>phelypaea</i> subsp. <i>lutea</i>	AY209302.1 ^{GB}	Spain: Región de Murcia, valley of the Rio Guadalentín	37°43'00"N; 01°48'50"W	WU: S&T 8744 (15.III.2002: GS & HW)	450	20	18.372 ± 0.210	17967.8160
<i>laxiflora</i> subsp. <i>nov.</i>	IRB6	Iran: ca 5 km Rafsanjan to Mese-Sarcheshmeh, roadside	30°19'05"N; 56°01'33"E	BONN & USB: Ataei N. & Heidari ED433; 20.IV.2010	1576	20 / 3D	29.2336 ± 0.510	28590.4608
<i>laxiflora</i> subsp. <i>nov.</i>	IRB11	Iran: Bafq road to Bagherabad, down the road	31°36'53"N; 55°23'46"E	BONN & USB: Ataei N. & Heidari ED435; 21.IV.2010	997	20 / 3E, F & S	21.8562 ± 0.656	21375.3636
<i>laxiflora</i> subsp. <i>nov.</i>	IRB15	Iran: Tehran, Kavir protect region, SE Waramin, close to Shokr-Abad check point, Hordeum field	35°04'23"N; 51°42'50"E	BONN & USB: Ataei N. & Heidari ED438; 24.IV.2010	812	20 / 3G & H	21.0344 ± 0.771	20571.6432
<i>laxiflora</i> subsp. <i>laxiflora</i>	IRB31	Iran: ca 70 km Nishapur to Kashmar, 2 km down detour road of Chelpo vilage	35°37'29"N; 58°30'07"E	BONN & USB: Ataei N. ED478; 31.IV.2010	1873	20 / 3I	19.0686 ± 1.277	18649.0908
<i>laxiflora</i> subsp. <i>laxiflora</i>	IRBj	Iran: Sistan & Baluchestan, Khash to Iranshahr, Daman village, riverbed	27°21'29"N; 60°46'59"E	BONN & USB: Ataei N. ED515; 05.III.2011	695		18.6646 ± 0.881	18253.9788
<i>laxiflora</i> subsp. <i>laxiflora</i>	IRBg	Iran: Sistan & Baluchestan, Saravan to Suran, NaserAbad, after Chah Mahmood, roadside	27°26'16"N; 61°44'47"E	BONN & USB: Ataei N. ED512; 01.III.2011	1186		22.4014 ± 0.741	21908.5692
<i>laxiflora</i> subsp. <i>laxiflora</i>	IRBp	Iran: Sistan & Baluchestan, Zahedan to Bam, Inter Fahraj & RostamAbad, 5 km RostamAbad	28°58'12"N; 58°45'18"E	BONN & USB: Ataei N. ED521; 11.III.2011	704		21.979 ± 0.705	21495.4620
<i>tubulosa</i> subsp. <i>nov.</i>	IRBm	Iran: Sistan & Baluchestan, Chahbahar to Tis, Sadre Omid-e-Chahbahar, coast line	25°22'24"N; 60°36'39"E	BONN & USB: Ataei N. ED518; 07.III.2011	15		31.8772 ± 1.747	31175.9016
<i>tubulosa</i> subsp. <i>nov.</i>	IRBk	Iran: Sistan & Baluchestan, Sarbaz to Chahbahar, Dempak village, roadside	25°42'39"N; 61°22'25"E	BONN & USB: Ataei N. ED516; 06.III.2011	22		15.2406 ± 0.666	14905.3068
<i>tubulosa</i> subsp. <i>nov.</i>	IRBl	Iran: Sistan & Baluchestan, Chahbahar, Negoyan to Tis, coast line, near Electrical power company	25°11'01"N; 61°06'59"E	BONN & USB: Ataei N. ED517; 07.III.2011	0		13.0394 ± 0.567	12752.5332
<i>tubulosa</i> subsp. <i>tubulosa</i>	IRBa	Iran: Sistan & Baluchestan, Do-Rahi Zabol Zahedan, close to Water pump station	30°11'40"N; 60°51'54"E	BONN & USB: Ataei N. & Heidari ED506; 01.III.2011	552		19.051 ± 0.508	18631.8780
<i>tubulosa</i> subsp. <i>tubulosa</i>	IRBb	Iran: Sistan & Baluchestan, Zabol, 1 k km Do Rahi Zabol-Zahedan	30°15'41"N; 60°50'01"E	BONN & USB: Ataei N. & Heidari ED507; 01.III.2011	469	20 / 3J	17.3078 ± 0.475	16927.0284
<i>tubulosa</i> subsp. <i>tubulosa</i>	IRBc	Iran: Sistan & Baluchestan, Zabol, Chah Nime, around South research Institute of aquatics	30°49'45"N; 61°43'07"E	BONN & USB: Ataei N. & Heidari ED508; 01.III.2011	354		27.7216 ± 0.436	27111.7248
<i>tubulosa</i> subsp. <i>tubulosa</i>	IRBd	Iran: Sistan & Baluchestan, Zabol, Zahak, close to Vitis Botanical Gatrden	30°50'11"N; 61°43'34"E	BONN & USB: Ataei N. & Heidari ED509; 01.III.2011	478	20 / 3K & T	15.9632 ± 0.454	15612.0096
<i>tubulosa</i> subsp. <i>tubulosa</i>	IRBe	Iran: Sistan & Baluchestan, Zabol, Chah Khorma	31°06'13"N; 61°09'20"E	BONN & USB: Ataei N. & Heidari ED510; 01.III.2011	451		17.5676 ± 0.597	17181.1128
<i>tubulosa</i> subsp. <i>tubulosa</i>	IRBh	Iran: Sistan & Baluchestan, Iranshahr to Bampoor, Bampoor village	27°11'55"N; 60°28'50"E	BONN & USB: Ataei N. ED513; 05.III.2011	506		17.7674 ± 0.535	17376.5172
<i>tubulosa</i> subsp. <i>tubulosa</i>	IRBi	Iran: Sistan & Baluchestan, 130 km Khash to Iranshahr, after Abadan village	27°18'06"N; 60°44'27"E	BONN & USB: Ataei N. ED514; 05.III.2011	640		25.5462 ± 0.589	24984.1836
<i>tubulosa</i> subsp. <i>tubulosa</i>	IRBv1	Iran: Sistan & Baluchestan, Iranshahr to Bampoor, Bampoor village	27°11'55"N; 60°28'50"E	USB: Valizadeh J. & Heidari; 01.III.2009	1186		24.3746 ± 0.575	23838.3588
<i>tubulosa</i> subsp. <i>tubulosa</i>	IRBv2	Iran: Sistan & Baluchestan, Iranshahr to Bampoor,	27°11'55"N;	USB: Valizadeh J. & Heidari;	1186		19.5394 ± 0.668	19109.5332

<i>tubulosa subsp. tubulosa</i>	IRBv3	Bampoor village Iran: Sistan & Baluchestan, Iranshahr to Bampoor, Bampoor village	60°28'50"E 27°11'55"N; 60°28'50"E	01.III.2009 USB: Valizadeh J. & Heidari; 01.III.2009	1186		16.4284 ± 0.684	16066.9752	
<i>tubulosa subsp. tubulosa</i>	IRBv4	Iran: Sistan & Baluchestan, Iranshahr to Bampoor, Bampoor village	27°11'55"N; 60°28'50"E	USB: Valizadeh J. & Heidari; 01.III.2009	1186		19.2034 ± 0.943	18780.9252	
<i>tubulosa subsp. tubulosa</i>	IRB13	Iran: Highway Ghom-Tehran, 60 km Ghom to Tehran, Cheshmeh Shur, 9.5 km to Hoze-Solatan lake	35°02'51"N; 50°58'37"E	BONN & USB: Ataei N. & Heidari ED436; 21.IV.2010	801		17.5204 ± 0.649	17134.9512	
<i>flava subsp. nov.</i>	IRB21	Iran: Shahrud to Torud, 2 km Chah-Jam, roadside	35°47'50"N; 55°09'36"E	BONN & USB: Ataei N. ED445; 27.IV.2010	1102	20 / 3L & U	23.4438 ± 0.839	22928.0364	
<i>flava subsp. nov.</i>	IRB23	Iran: Semnan, 46 km Biarjmand, 101 km Miamay to Ahmad Abad	35°53'40"N; 56°18'47"E	BONN & USB: Ataei N. ED447; 28.IV.2010	769	20±1B / 3N	19.2502 ± 0.484	18826.6956	
<i>flava subsp. nov.</i>	IRB29	Iran: 78 km Zaman-Abad to Bardaskan detour road, between Ismail Abad and Ghaleh, 2 km roadside, in dunes	35°07'01"N; 57°13'07"E	BONN & USB: Ataei N. ED451; 29.IV.2010	803	20 / 3O & W	17.6606 ± 0.697	17272.0668	
<i>flava subsp. flava</i>	IRB1	Iran: 120 km Zahedan to Bam, Nosrat Abad region, roadside	29°43'01"N; 59°49'27"E	BONN & USB: Ataei N. & Heidari ED432; 19.IV.2010	1061		22.6414 ± 0.997	22143.2892	
<i>flava subsp. flava</i>	IRBo	Iran: Sistan & Baluchestan, Nikshahr to Iranshahr, after Spage village, 2 km NokAbad.	26°55'03"N; 60°30'57"E	BONN & USB: Ataei N. ED520; 08.III.2011	764		21.5918 ± 0.674	21116.7804	
<i>flava subsp. flava</i>	IRBq	Iran: Khorassan-e-Razavi , Sabzevar, Norood-Abad village, Parand mountain, Kalate-e-MirAli, dunes	35°55'23"N; 57°09'23"E	BONN & USB: Ataei N. ED522; 29.III.2011	922		23.2588 ± 0.529	22747.1064	
<i>flava subsp. flava</i>	IRBs	Iran: Khorassan-e-Razavi , Khora, Sabzevar to Solh- Abad, Sabri village, dunes	35°44'06"N; 56°55'14"E	BONN & USB: Ataei N. ED524; 30.III.2011	1043		19.7214 ± 0.900	19287.5292	
<i>flava subsp. flava</i>	IRBu1	Iran: Semnan, Shahrud, 5 km after Kariz village	35°36'08"N; 56°49'53"E	BONN & USB: Ataei N. ED525; 30.III.2011	1122		19.7682 ± 0.403	19333.2996	
<i>flava subsp. flava</i>	IRB25	Iran: 12 km Ahmad-Abad to Zaman Abad, roadside, dunes	35°39'06"N; 56°38'39"E	BONN & USB: Ataei N. ED449; 28.IV.2010	1093	20 / 3P & X	18.7134 ± 1.190	18301.7052	
<i>flava subsp. flava</i>	IRB27	Iran: Zaman Abad-Bardaskan, detour road Zaman- Abad, 2 km to Kalateh Karbalai Abbas, dunes	35°37'24"N; 56°46'46"E	BONN & USB: Ataei N. & Heidari ED450; 29.IV.2010	1105	20 / 3Q & Y	22.1512 ± 0.774	21663.8736	
<i>flava subsp. flava</i>	IRBr	Iran: Khorassan-e-Razavi, Sabzevar to Sebri village, from Roodab Parand to cement factory	35°53'26"N; 57°04'48"E	BONN & USB: Ataei N. ED523; 29.III.2011	944		21.9106 ± 0.546	21428.5668	
<i>flava subsp. flava</i>	IRBt	Iran: Khorassan-e-Razavi, Savzevar to SolhAbad, after Talkhab village, dunes	35°33'06"N; 56°54'47"E	BONN & USB: Ataei N. ED541; 30.III.2011	1165		22.742 ± 0.555	22241.6760	
<i>flava subsp. flava</i>	IRB22	Iran: Semnan, Maimay to Biarjmand, 24 km Biarjmand	36°07'54"N; 55°48'52"E	BONN & USB: Ataei N. ED446; 28.IV.2010	1152	20 / 3V & M	19.9418 ± 0.660	19503.0804	
Orobanche									
sect. Orobanche									
<i>cernua</i>	IRB2	Iran: 20 km Bam to Kerman, roadside, dunes	29°11'55"N; 58°07'13"E	BONN & USB: Ataei N. & Heidari ED423; 19.IV.2010	1337	19 / 4I, J & U	3.1048 ± 0.112	3036.4944	
<i>cernua</i>	IRB16	Iran: Semnan, Nezami path, towards Chah-Shirin, 200 m roadside	35°27'16"N; 53°43'38"E	BONN & USB: Ataei N. & Heidari ED741; 26.IV.2010	1192	19 / 4M & V	3.8632 ± 0.179	3778.2096	
<i>cernua</i>	IRB18	Iran: Semnan-Damghan road, about 4.5 km Ghooshe to Emravan, down the road to Damghan	35°58'02"N; 54°04'44"E	BONN & USB: Ataei N. & Heidari ED742; 27.IV.2010	1248	19 / 4N	3.2436 ± 0.118	3172.2408	
<i>cernua</i>	IRB8	Iran: Kerman, Sarcheshmeh to Sirjan, 10 km after Pariz	29°49'07"N; 55°44'43"E	BONN & USB: Ataei N. & Heidari ED738; 21.IV.2010	2135		8.8194 ± 0.597	8625.3732	
<i>cernua</i>	IRB12	Iran: Esfahan to Kashan (towards Tiran), ca 76 km Tiran to Kashan	33°06'02"N; 51°36'46"E	BONN & USB: Ataei N. & Heidari ED740; 22.IV.2010	1706	12 / 4K & W	3.3356 ± 0.330	3262.2168	
<i>cernua</i>	IRB19	Iran: Shahrud to Torud, 25 km Chah-Jam, roadside	36°12'14"N; 55°02'03"E	BONN & USB: Ataei N. & Heidari ED743; 27.IV.2010	1348	19 / 4L	3.8402 ± 0.410	3755.7156	
<i>densiflora</i>	Dens.1	Spain: Chiclana de La Frontera, before Santi Petri, along the coast line	37°13'13"N; 07°10'01"W	BONN: Ataei N. ED745; 23.IV.2012	57		3.9926 ± 0.151	3904.7628	
<i>densiflora</i>	Ger.1	???	???	???	???	38 / 4E	4.0826 ± 0.261	3992.7828	

<i>densiflora</i>	Ger.2	???	???	???	???		3.865 ± 0.158	3779.9700
<i>transcaucasica</i>	Dunkel	Georgia, Caucasus, stepantsminda	42°39'N; 44°39'E	WU: Reich. D & ? ED749; 30.07.2012	1785		???	
<i>transcaucasica</i>	Hell	Georgia, Caucasus, shkhara	42°59'24"N; 43°06'E	WU: Reich. D & ? ED750; 26.07.2012	5063	19 / 4F & J	???	
sect. <i>Trionychon</i>								
<i>Phelipanche</i>								
<i>ramosa</i>	Bonn1	Germany, Botanic Garden BONN	50°43'30"N; 07°05'24"E	BONN	58	12 / 4H	7.9284 ± 0.310	7753.9752
<i>purpurea</i>	Bonn2	Germany, Botanic Garden BONN	50°43'30"N; 07°05'24"E	BONN	58	12 / 4G & S	14.2006 ± 0.497	13888.1868
<i>cf. iberica?</i>	IRB7	Iran: Kerman, Sarcheshmeh to Sirjan, 10 km after Pariz	29°49'07"N; 55°44'43"E	BONN & USB: Ataei N. & Heidari ED428; 21.IV.2010	2135	12 / 4O & T	5.5928 ± 0.279	5469.7584
<i>cf. iberica?</i>	IRB9	Iran: Kerman, Sarcheshmeh to Sirjan, 10 km after Pariz	29°49'07"N; 55°44'43"E	BONN & USB: Ataei N. & Heidari ED739; 21.IV.2010	2135		2.901 ± 0.410	2837.1780

Appendix 3. 3 A nuclear DNA content comparison of *Cistanche* interspecies using Welch's ANOVA (Levene test $P = 0.001$): $P < 0.0001$. Significant (+) or non-significant (-) results achieved with Dunnett' C post Hoc test.

	<i>ridgewayana</i>	<i>ambigua</i>	<i>laxiflora</i> subsp. <i>laxiflora</i>	<i>laxiflora</i> subsp. <i>nov.</i>	<i>tubulosa</i> subsp. <i>tubulosa</i>	<i>lutea</i>	<i>tubulosa</i> subsp. <i>nov.</i>	<i>flava</i> subsp. <i>flava</i>	<i>flava</i> subsp. <i>nov.</i>	<i>phelypaea</i> subsp. <i>phelypaea</i>	<i>violacea</i>
<i>ridgewayana</i>	-										
<i>ambigua</i>	+	-									
<i>laxiflora</i> subsp. <i>laxiflora</i>	+	+	-								
<i>laxiflora</i> subsp. <i>nov.</i>	+	+	+	+							
<i>tubulosa</i> subsp. <i>tubulosa</i>	+	-	+	+	-						
<i>lutea</i>	+	-	+	+	-	-					
<i>tubulosa</i> subsp. <i>nov.</i>	-	-	+	+	-	-	-				
<i>flava</i> subsp. <i>flava</i>	+	+	-	+	+	+	+	+			
<i>flava</i> subsp. <i>nov.</i>	+	+	-	-	+	+	+	-	-		
<i>phelypaea</i> subsp. <i>phelypaea</i>	+	+	+	-	+	+	-	+	+	+	
<i>violacea</i>	+	+	-	-	+	+	+	-	-	-	-

Appendix 3. 4 A nuclear DNA content comparison of intrapopulation in *C. tubulosa* using Welch's ANOVA (Levene test $\alpha = 0.001$): $P = 0.000$. Significant (+) or non-significant (-) results achieved with Bonferoni post Hoc test. Codes refer to accessions in Appendix 3.1.

	IRBa	IRBb	IRBc	IRBd	IRBe	IRBh	IRBi	IRBk	IRBv1	IRBv2	IRBv3	IRBv4
IRBa												
IRBb	+											
IRBc	+	+										
IRBd	+	+	+									
IRBe	+	-	+	+								
IRBh	-	+	+	+	+							
IRBi	+	+	+	+	+	+						
IRBk	+	+	+	-	+	+	+					
IRBv1	-	+	+	+	+	-	+	+				
IRBv2	+	+	+	-	+	+	+	-	+			
IRBv3	+	+	+	-	+	+	+	+	+	-		
IRBv4	+	+	-	+	+	+	+	+	+	+	+	

Appendix 3. 5 A nuclear DNA content comparison of intrapopulation in *C. flava* using Welch's ANOVA (Levene test $\alpha = 0.001$): $P = 0.0001$. Significant (+) or non-significant (-) results achieved with Bonferoni post Hoc test. Codes refer to accessions in Appendix 3.1.

	IRB1	IRBo	IRBq	IRBs	IRBu1	IRB25	IRB27	IRBr	IRBt	IRB22
IRB1										
IRBo	+									
IRBq	+	+								
IRBs	-	+	+							
IRBu1	+	-	+	-						
IRB25	+	+	+	+	+					
IRB27	-	-	+	-	-	+				
IRBr	+	+	-	+	+	+	+			
IRBt	+	+	-	+	+	+	+	-		
IRB22	+	-	-	+	-	+	-	+	+	

Appendix 3. 6 A nuclear DNA content comparison of intrapopulation in *C. violacea* using Welch's ANOVA (Levene test $\alpha = 0.001$): $P = 0.0001$. Significant (+) or non-significant (-) results achieved with Bonferoni post Hoc test. Codes refer to accessions in Appendix 3.1.

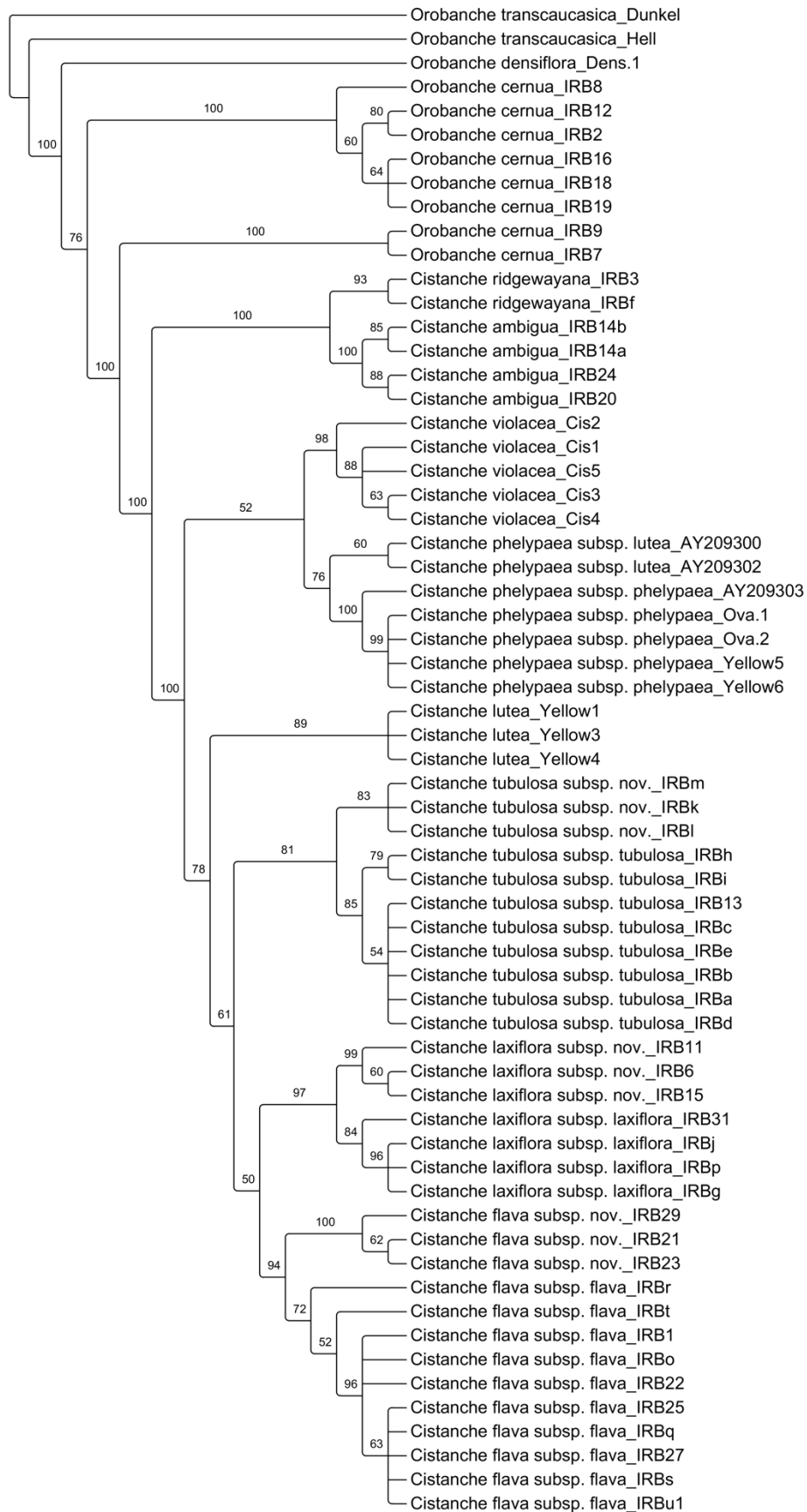
	Cis1	Cis2	Cis3	Cis5
Cis1				
Cis2	+			
Cis3	-	+		
Cis5	+	+	+	

Appendix 3. 7 A nuclear DNA content Comparison of intrapopulation in *C. laxiflora* using Welch's ANOVA (Levene test $\alpha = 0.001$): $P = 0.0001$. Significant (+) or non-significant (-) results achieved with Bonferoni post Hoc test. Codes refer to accessions in Appendix 3.1.

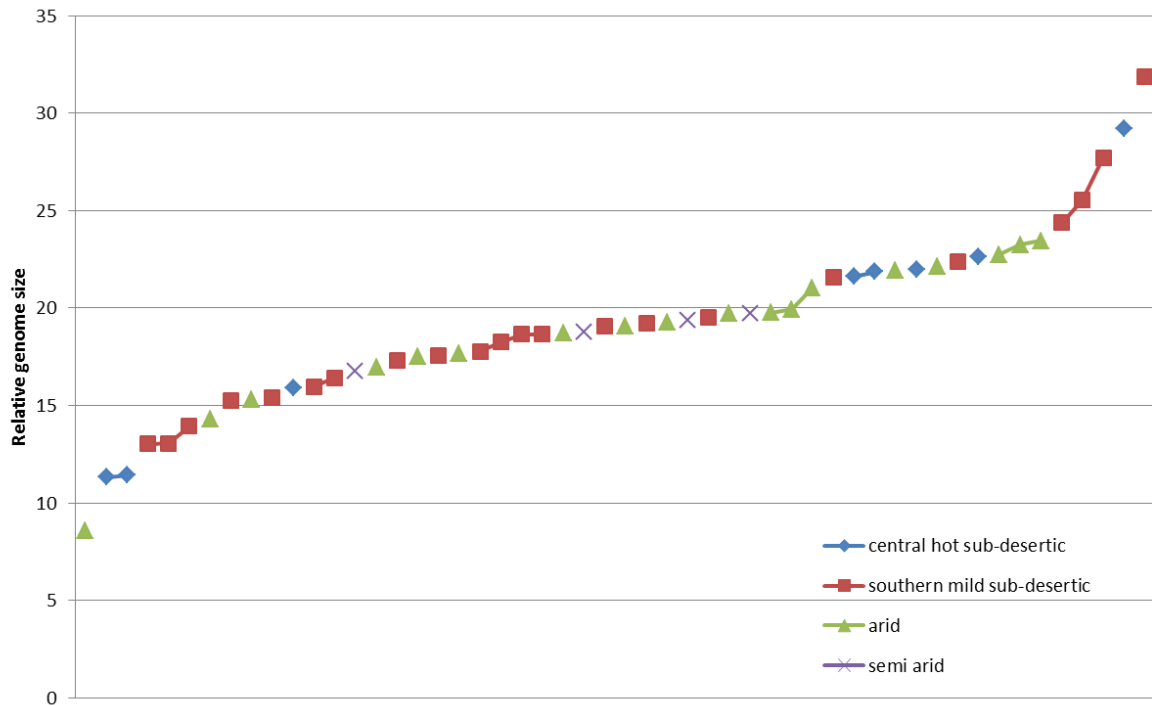
	IRB6	IRB11	IRB15	IRB31	IRBg	IRBp
IRB6						
IRB11	+					
IRB15	+	+				
IRB31	+	-	-			
IRBg	+	+	+	+		
IRBp	+	-	-	-	+	

Appendix 3. 8 A Spearman correlation coefficient (r_s) was run to evaluate the relationship between nuclear DNA content of different *Cistanche* species and geographic and climatic variables.

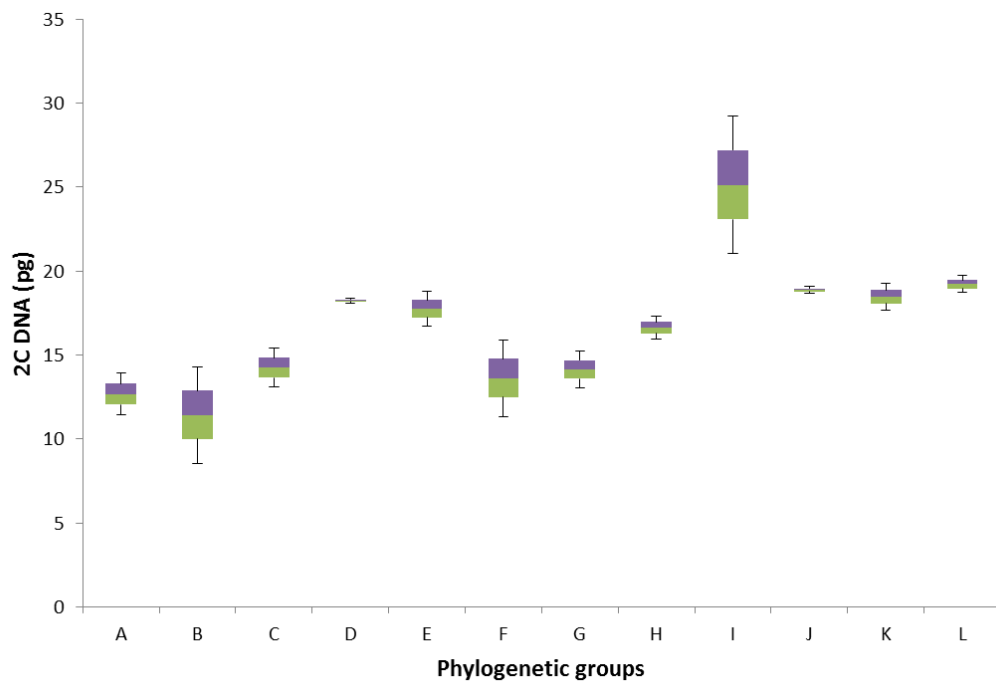
variables	Nuclear DNA content (2C-Values)				
	<i>C. ambigua</i>	<i>C. laxiflora</i>	<i>C. tubulosa</i>	<i>C. flava</i>	<i>C. phelypaea</i>
Altitude (m)	-0.429	0.357	0.096	-0.079	-0.100
<i>p</i>	0.419	0.444	0.762	0.838	0.083
X-Coordinates (°E)	-0.600	0.036	-0.036	0.455	0.400
<i>p</i>	0.242	0.963	0.918	0.185	0.750
Y-Coordinates (°N)	0.143	-0.143	-0.305	-0.115	0.800
<i>p</i>	0.803	0.783	0.336	0.759	0.333
Annual mean temperature (°C)	0.200	-0.143	0.283	0.159	-0.800
<i>p</i>	0.714	0.783	0.367	0.654	0.333
Mean diurnal range (°C)	-0.200	0.214	0.134	0.463	-0.400
<i>p</i>	0.714	0.662	0.672	0.176	0.750
Isothermality	-0.698	0.182	0.324	0.089	-0.211
<i>p</i>	0.136	0.713	0.299	0.802	0.750
Temperature of seasonality	0.486	-0.429	-0.218	0.285	0.400
<i>p</i>	0.356	0.354	0.498	0.417	0.750
Temperature of the warmest month (°C)	0.543	-0.321	0.469	0.335	0.200
<i>p</i>	0.297	0.498	0.124	0.337	0.917
Temperature of the coldest month (°C)	-0.029	0.000	0.283	0.031	0.000
<i>p</i>	1.000	1.000	0.367	0.931	1.000
Temperature annual range (°C)	0.314	-0.393	-0.232	0.365	-0.200
<i>p</i>	0.564	0.396	0.468	0.294	0.917
Mean temperature of the wettest quarter (°C)	-0.600	-0.144	0.283	0.134	-0.600
<i>p</i>	0.242	0.713	0.367	0.705	0.417
Mean temperature of driest quarter (°C)	0.429	-0.286	0.265	0.220	0.400
<i>p</i>	0.419	0.556	0.399	0.534	0.750
Mean temperature of the warmest quarter (°C)	0.200	-0.180	0.309	0.188	0.211
<i>p</i>	0.714	0.662	0.323	0.594	0.917
Mean temperature of the coldest quarter (°C)	-0.029	0.000	0.283	0.176	-0.800
<i>p</i>	0.919	1.000	0.367	0.619	0.333
Annual precipitation (mm)	-0.371	-0.179	0.142	-0.152	0.400
<i>p</i>	0.497	0.713	0.655	0.682	0.750
Precipitation of the wettest month (mm)	0.174	-0.536	0.133	-0.006	0.400
<i>p</i>	0.714	0.236	0.674	0.999	0.750
Precipitation of the driest month (mm)	-0.247	0.000	-0.218	-0.225	0.258
<i>p</i>	0.658	1.000	0.496	0.536	0.917
Precipitation seasonality	-0.257	0.000	0.393	0.280	-0.632
<i>p</i>	0.658	1.000	0.204	0.426	0.333
Precipitation of the wettest quarter (mm)	-0.543	-0.500	0.274	-0.171	0.400
<i>p</i>	0.297	0.267	0.383	0.641	0.750
Precipitation of the driest quarter (mm)	-0.203	0.073	0.124	-0.311	0.800
<i>p</i>	0.658	0.906	0.695	0.383	0.333
Precipitation of the warmest quarter (mm)	-0.206	0.073	0.124	-0.529	0.477
<i>p</i>	0.658	0.906	0.695	0.121	0.750
Precipitation of the coldest quarter (mm)	-0.257	-0.429	0.264	-0.080	0.400
<i>p</i>	0.658	0.354	0.401	0.835	0.750



Appendix 3. 9 Phylogenetic tree inferred from maximum parsimony analysis of the genus *Cistanche*. Bootstrap values ≥ 50 showed above the branches. The consensus tree was selected among 39 maximum parsimony trees (MPTs) with 1243 steps (CI: 0.892, RI: 0.964, RC: 0.860), 666 characters' parsimony informative and 3000 constant characters in a total of 3992.



Appendix 3. 10 Variation in genome size of different accessions of genus *Cistanche* versus habitat preferences.



Appendix 3. 11 Box plot distribution of 2C DNA values (Y-axis) among phylogenetic clades (X-axis) based on phylogenetic tree in Fig. 3.5.

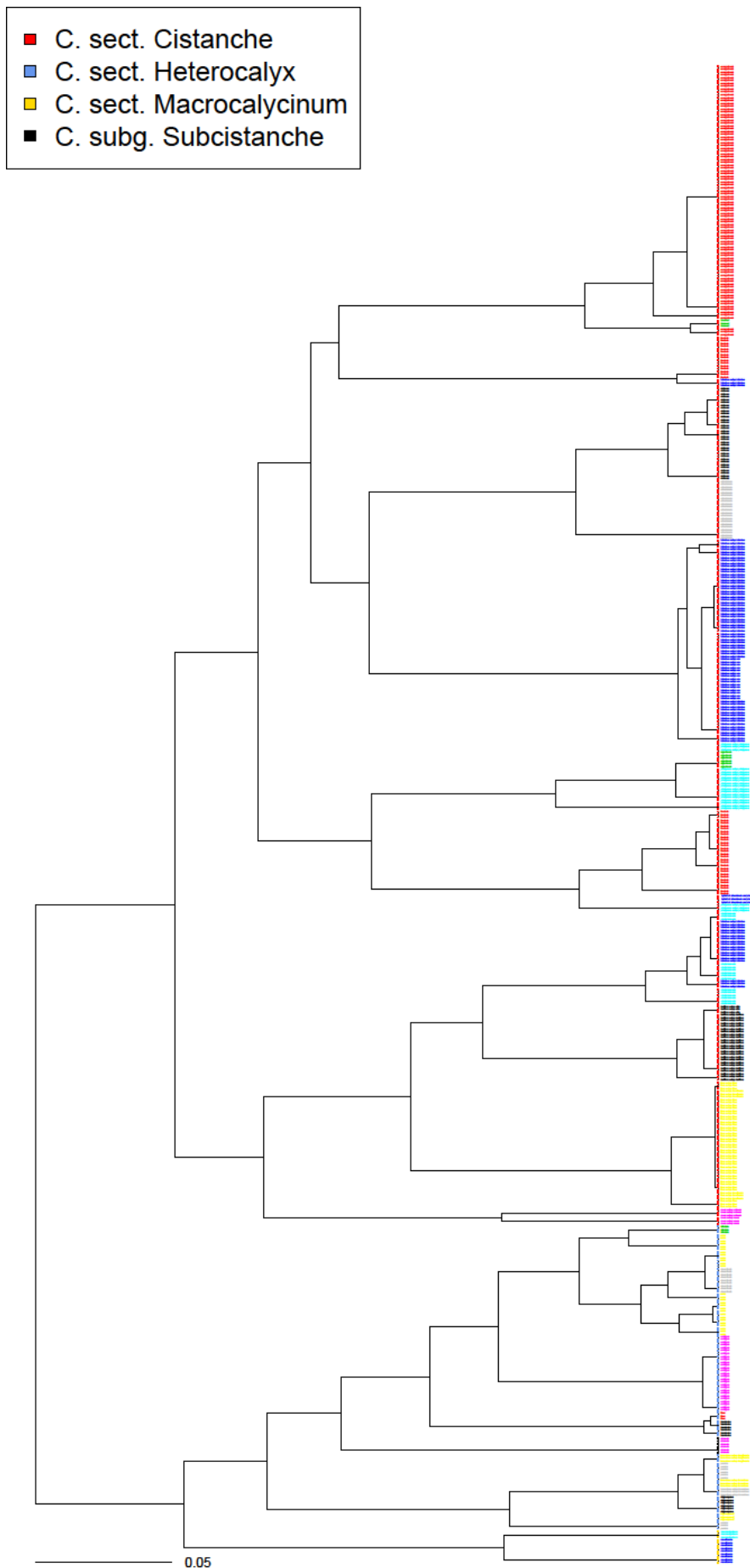
C) Appendices to chapter 4

Appendix 4. 1 The morphological measurement data used for morphological measurements in *Cistanche*.

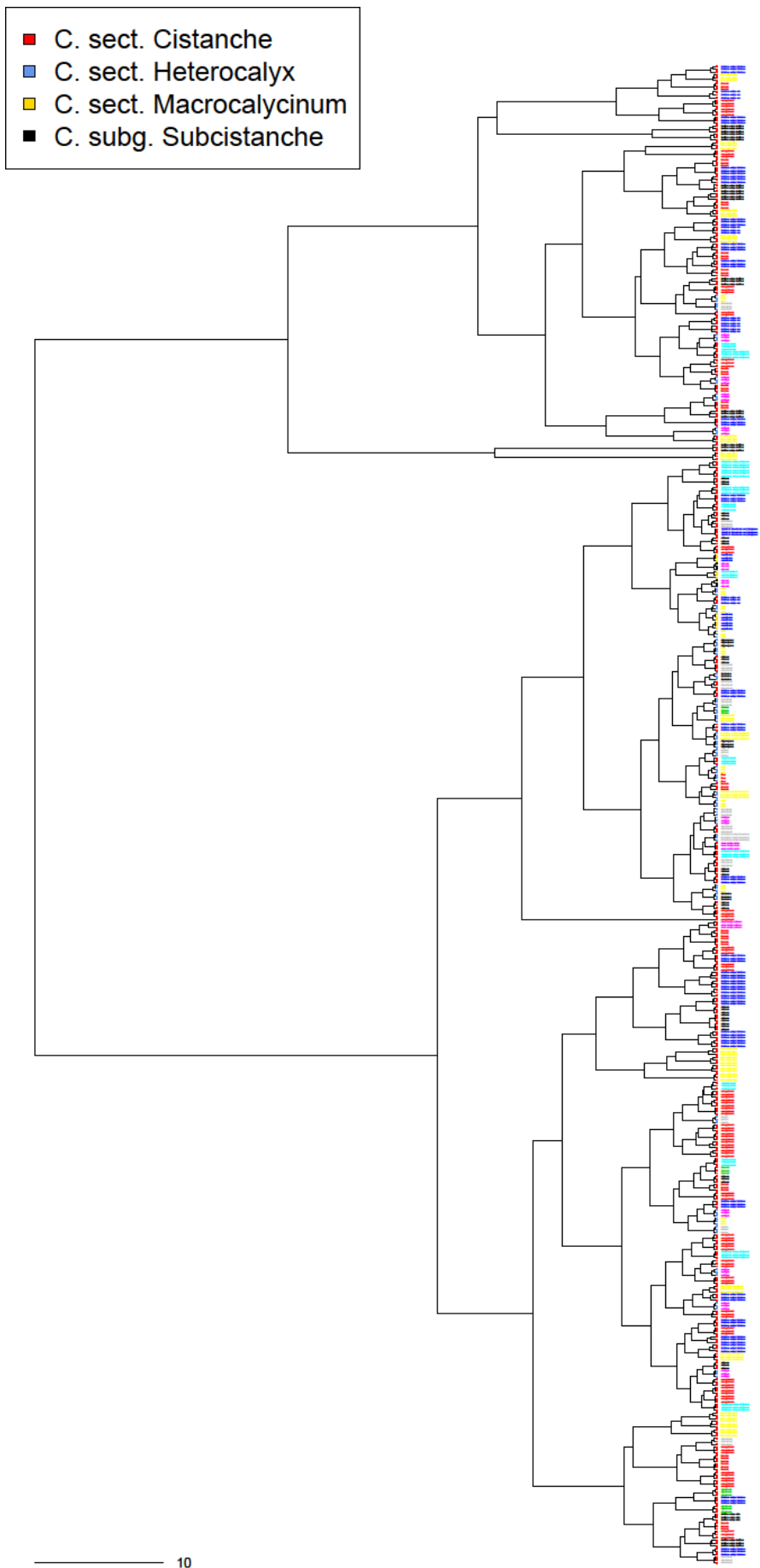
Plant	Part	Measurement	Value
Cistanche	Stem	Stem length	1.2
		Stem diameter	0.5
		Stem thickness	0.3
		Stem width	0.4
		Stem height	1.1
		Stem depth	0.6
		Stem volume	0.8
		Stem mass	0.7
		Stem density	0.9
		Stem surface area	1.0
Cistanche	Leaves	Leaf length	1.5
		Leaf width	0.8
		Leaf thickness	0.4
		Leaf area	1.2
		Leaf volume	1.0
		Leaf mass	0.9
		Leaf density	1.1
		Leaf surface area	1.3
		Leaf perimeter	1.4
		Leaf curvature	1.5
Cistanche	Flowers	Flower length	1.8
		Flower width	1.0
		Flower thickness	0.5
		Flower area	1.5
		Flower volume	1.2
		Flower mass	1.1
		Flower density	1.3
		Flower surface area	1.6
		Flower perimeter	1.7
		Flower curvature	1.8
Cistanche	Fruit	Fruit length	2.0
		Fruit width	1.2
		Fruit thickness	0.6
		Fruit area	1.8
		Fruit volume	1.4
		Fruit mass	1.3
		Fruit density	1.5
		Fruit surface area	1.9
		Fruit perimeter	2.0
		Fruit curvature	2.1
Cistanche	Seed	Seed length	0.5
		Seed width	0.3
		Seed thickness	0.2
		Seed area	0.4
		Seed volume	0.3
		Seed mass	0.2
		Seed density	0.4
		Seed surface area	0.5
		Seed perimeter	0.6
		Seed curvature	0.7

Appendix 4. 2 Importance of components in Principle Component Analysis of 20 quantitative characters.

Principal Components	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16	PC17	PC18	PC19	PC20
Standard deviation	16,05175	6,47686	1,741344	1,405957	0,756498	0,570838	0,460346	0,324366	0,283978	0,235151	0,223642	1,75E+	1,63E+	1,49E+	1,33E+	1,13E+	1,09E+	9,45E+	8,60E+	6,94E+
Proportion of Variance	85	34	868	994	45	07	599	466	017	204	398	05	05	05	05	05	05	04	04	04
Cumulative Proportion	0,841577	0,13701	0,009904	0,006456	0,001869	0,001064	0,000692	0,000343	0,000263	0,000180	0,000163	9,97E+	8,72E+	7,26E+	5,75E+	4,20E+	3,89E+	2,91E+	2,41E+	1,57E+
SL	0,94	0,34	-0,02	0,01	-0,01	0,00	0,00	0,00	0,00	-0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
LSSL	0,00	0,00	0,04	-0,07	0,01	-0,01	-0,02	0,13	-0,13	0,06	-0,01	-0,94	0,17	0,04	-0,17	-0,10	-0,02	0,02	-0,03	-0,01
LSSW	0,00	-0,01	0,03	-0,02	0,11	-0,08	-0,22	-0,13	-0,10	-0,65	0,54	0,04	0,26	0,17	-0,14	-0,08	0,24	0,09	-0,03	-0,02
MSSL	0,02	-0,03	0,54	-0,37	-0,12	0,08	-0,16	0,68	0,15	-0,11	0,00	0,11	-0,07	0,02	-0,02	0,01	0,01	0,02	0,03	0,01
MSSW	0,00	-0,01	0,02	-0,01	0,09	-0,08	-0,26	-0,10	0,05	-0,37	0,05	-0,15	-0,33	-0,37	0,22	0,10	-0,53	-0,37	-0,09	-0,08
USSL	0,03	-0,04	0,62	-0,37	-0,07	0,11	0,05	-0,64	-0,12	0,16	0,01	-0,01	0,06	-0,01	0,03	-0,02	0,01	-0,06	-0,03	-0,01
USSW	0,00	0,00	0,03	0,01	0,10	-0,07	-0,31	-0,12	0,15	-0,17	-0,41	-0,10	-0,12	-0,38	0,25	-0,14	0,42	0,46	-0,05	0,10
FAL	0,34	-0,93	-0,11	-0,05	-0,01	0,01	0,00	0,00	0,01	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
FAD	0,02	-0,08	0,43	0,79	-0,41	-0,04	-0,07	0,00	-0,02	-0,03	0,02	-0,04	-0,01	0,01	0,00	0,00	-0,01	0,01	0,00	0,00
BL	0,02	-0,03	0,20	0,00	0,18	-0,60	0,66	0,02	0,18	-0,26	-0,13	-0,04	-0,01	0,00	0,02	0,02	-0,02	0,03	0,02	0,05
BW	0,00	0,00	0,02	0,04	0,14	-0,17	-0,37	-0,13	0,48	0,00	-0,38	0,06	0,21	0,44	-0,24	-0,21	-0,16	-0,23	-0,09	0,01
BrL	0,01	-0,02	0,09	0,02	0,21	-0,40	-0,24	0,11	-0,76	0,08	-0,26	0,16	-0,05	0,07	-0,13	-0,01	0,01	-0,07	-0,07	-0,04
BrW	0,00	-0,01	0,02	0,00	0,06	-0,08	-0,18	-0,09	0,01	-0,03	-0,06	-0,06	0,07	0,12	0,04	0,44	-0,15	0,19	0,82	0,04
BN	0,01	-0,01	0,11	0,08	0,32	-0,36	-0,21	0,01	0,25	0,51	0,51	-0,02	-0,23	-0,18	-0,12	-0,05	0,12	0,03	0,04	-0,01
CaL	0,00	0,00	0,02	0,02	0,07	-0,14	-0,08	0,11	-0,03	0,16	0,12	-0,03	0,39	0,20	0,84	-0,08	-0,02	-0,09	-0,05	-0,03
CaLL	0,00	0,00	0,02	0,01	0,06	-0,04	-0,08	-0,01	0,09	0,05	0,00	-0,03	0,12	0,10	-0,04	0,76	-0,08	0,29	-0,54	0,00
CLW	0,02	-0,04	0,24	0,29	0,75	0,50	0,15	0,07	-0,04	-0,05	-0,06	-0,02	-0,06	0,10	0,04	0,00	-0,01	-0,01	0,01	0,01
CrL	0,00	0,00	0,04	0,01	0,06	0,01	0,01	0,04	-0,04	0,06	0,06	0,15	0,39	-0,28	-0,13	-0,31	-0,60	0,51	-0,03	0,04
CrLW	0,00	-0,01	0,02	0,05	0,07	0,02	0,00	0,08	0,05	0,03	-0,09	0,07	0,58	-0,56	-0,16	0,20	0,22	-0,44	0,07	0,08
AL	0,00	0,00	0,01	0,01	0,02	0,00	0,03	0,01	0,06	0,00	-0,08	0,01	0,07	-0,06	-0,03	0,00	0,06	0,07	0,05	-0,98



Appendix 4. 3 Dendrogram of Hierarchical cluster analysis using the complete linkage method in a matrix using only nominal characters. Dendrogram included 25 plant species of genus *Cistanche*. The figure legend and taxon names indicate new taxonomical ranks used in this study.



Appendix 4. 4 Dendrogram of Hierarchical cluster analysis using the complete linkage method in a matrix using only quantitative characters. Dendrogram included 25 plant species of genus *Cistanche*. The figure legend and taxon names indicate new taxonomical ranks used in this study.