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Research article

Atriplex canescens: A new host for *Cistanche deserticola*

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ABSTRACT

Cistanche deserticola has been historically used in traditional Chinese medicine for supplementing kidney (yang) function, benefiting blood and essence, and moistening intestines in order to pass stool. Its host, *Haloxylon ammodendron*, is an important pioneer plant used for windbreaks and sand dune fixation, which are strategies used for the control desertification. For a long time, it has been considered that *C. deserticola* can only parasitize *H. ammodendron*. In this study, morphological identification, gene barcoding identification and inoculation experiment were carried out, we finally found that *C. deserticola* can also parasitize *Atriplex canescens*. *A. canescens* is a species of Chenopodiaceae with a wide range of adaptability. Compared with *H. ammodendron*, it has more biomass and a wider range of ecological adaptability, making it more suitable for the industrial production of *C. deserticola*. In addition, we also found that the concentration of active components was higher in *C. deserticola* parasitized on *A. canescens* than in those parasitized on *H. ammodendron*; this finding further suggests that the application of *C. deserticola* on a larger scale warrants further exploration.

1. Introduction

The use of *Cistanche* in traditional Chinese medicine was first recorded in the Shennong Herbal Scripture, for its effects of toning kidney yang, boosting the essence of blood, and moistening the intestines to facilitate stool passage. It has also been recorded in works of ancient herbal medicine as 'desert ginseng'. The dry fleshy stems and scaly leaves of *Cistanche deserticola* Y. C. Ma and *Cistanche tubulosa* (Schenk) Wight have been first joint described since 2005 in the Chinese Pharmacopoeia. *Cistanche* is mainly grown in Xingjiang, Inner Mongolia, and Gansu of China, and globally, it is found in semi-arid and arid areas throughout the European Iberian Peninsula, North Africa, Arabia, Iran, Afghanistan, Pakistan, North India, Mongolia et al. [1]. It is resistant to harsh environmental conditions, such as extremely arid climates, severe variation of temperatures, and depauperate soils [2]. According to the Taxonomical Index of Chinese Higher Plants, there are six *Cistanche* species in China. However, a further study confirmed the existence of only four species and one variant of *Cistanche*, namely, *C. deserticola* Y.C. Ma, *C. tubulosa* (Schenk) R. Wight, *C. salsa* (C.A. Mey.) G. Beck, *C. sinensis* G. Beck and *C. salsa* var. albiflora P.F. Tu et al., [3].

C. deserticola is regarded as the only traditional source of *Cistanche* and has a long history of use in medicine, since the Eastern Han Dynasty (25 AD–220 AD) [4]. In the Compendium of Materia Medica (written by Li Shizhen, Ming Dynasty), it was documented to smoothly tonify yang (in contrast to other herbs that have a more vigorous action). An array of efficacious chemical constituents, including phenylethanoid glycosides, iridoids, lignans, alditols, oligosaccharides, polysaccharides, and alkaloids, have been isolated from *C. deserticola* by modern phytochemical methods [5]. Pharmacological studies have shown that phenethyl glycoside is the main active component, and it has been reported to improve sexual function, exert neuroprotective effects, improve learning and memory, and protect the liver. It also exerts therapeutic effects against dementia, Alzheimer's disease, Parkinson's disease, fatigue, and tumors along exhibits anti-inflammatory and immunomodulatory properties [6, 7].

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C. deserticola is an obligatory parasitic plant that lives exclusively on the roots of *Haloxylon ammodendron* [8]. A study reported that *C. deserticola* is not even found on *Haloxylon persicum* [9]. In recent years, an increasing amount of attention has been paid to *C. deserticola*, since it not only is a source of components of medicinal value, but also contributes greatly to the control of desertification [10]. *H. ammodendron* is the only host that has been used in studies involving *C. deserticola*. In April 2017, Wang Shuai, an employee of the Zhejiang Quheng Public Welfare Fund, inoculated seeds of *C. deserticola* on *Atriplex canescens* in the Desert Botanical Garden of Minqin, Gansu Province, and *C. deserticola* was found to bloom in May 2018 and continued to flower till May 2019. However, the seeds were purchased from the market, and it is doubtful whether they were truly seeds of *C. deserticola*. In addition, this phenomenon breaks traditional knowledge and needs to be studied further.

A. canescens is a C_4 perennial shrub native to the deserts of Southwestern America and rapidly adapts to conditions of salinity, heavy metals, drought, and high temperatures [11]. Since it is highly palatable and rich in nutrients, it is used as fodder for most livestock and large animals [12]. Moreover, it is especially useful for erosion control and reclamation of marginal lands on account of its excellent adaptability and extensive root system. It was first introduced in China from the United States of America in 1989 and has been widely used for soil and water conservation, sand-fixing, and saline land restoration [13]. Although, the study reporting the growth of *C. deserticola* on *A. canescens* overturns the exclusive parasitic understanding of *C. deserticola*, this might turn out to be revolutionary finding, since *A. canescens* is more suitable for the growth of *C. deserticola* because it has more biomass and a wider range of ecological adaptability compared with *H. ammodendron*.

In order to ensure the accuracy of the accidental discovery, plant identification and artificial inoculation experiments were carried out. Traditional plant identification includes organoleptic evaluation (such as touch, smell, sight, and taste), analysis of morphological characteristics (such as microscopic and macroscopic), and chemical profiling (such as high-performance liquid chromatography, thin layer chromatography, and gas chromatography) [14]. It is relatively simple to exclude *C. tubulosa* and *C. sinensis* due to the difference in size, color, and arrangement of vascular bundles in the stem. The real challenge is to distinguish between *C. deserticola* and *C. salsa*. According to the Flora of China, the length of the bract of *C. salsa* is about 1/3 of the corolla, while it is equal in *C. deserticola*. The transection of the fleshy stems is similar between *C. deserticola* and *C. salsa* and is composed of the epidermis, cortex, vascular bundles, and pith. The main difference is in the vascular bundle sheath, since it is caudate for *C. deserticola* and triangular or semi-circular for *C. salsa*.

In recent years, DNA barcoding technology has frequently been used for species identification. It is a process that uses a short DNA sequence from the standard genome, which is generally conserved and is not affected by external factors, such as age and type of plant tissue. The popular candidate sequences for plant DNA barcodes are *rbcL*, *matK*, *psbA-trnH*, *ITS*, and *ITS2* [15]. Several studies have indicated that *ITS/ITS2* is the most effective identification tool for plants. It has also been suggested that the *ITS2* region should be incorporated into the core barcodes because of its higher discriminatory power than that of plastid barcodes. It has been accepted that *ITS2* could be used as a novel universal barcode for the identification of a broad range of plant taxa [16, 17, 18, 19, 20, 21]. Although many studies have tried to identify a universal plant barcode, none of the available loci work across all species, so a multi-locus method is necessary to discriminate between plant species [22, 23, 24, 25, 26, 27, 28]. In this study, *ITS2*, *rbcL*, *psbA-trnL* were used as barcodes.

In addition to morphological and molecular identification techniques, direct evidence comes from inoculation experiments. Inoculation experiments need to be carried out in order to demonstrate that *C. deserticola* can parasitize *A. canescens*. In addition to identification, quality control becomes a primary consideration. Further investigations are needed in order to ascertain the difference between the quality of *C. deserticola* parasitized on *H. ammodendron* root and that parasitized on *A. canescens*.

2. Materials and methods

2.1. Plant materials

Cistanche grows on soft sandy soils with mild salinization, generally parasiting on 30–100 cm deep side roots of the host. The climate in the suitable growing area is arid, less rainy, large evaporation, long sunshine hours and large temperature difference between day and night. Minqin county and Baiying city are the collection locations for these samples, They are geographicaly close, and have temperate continental arid climate, with an average annual precipitation of 113.2mm and an average annual relative humidity of 44%. Specific and detailed sample collection information is shown in Table 1. All samples were frozen and preserved at -20 °C in the State Key Laboratory of Natural and Biomimetic Drugs Lab, Beijing, China.

2.2. Tissue staining and observation

Fresh samples were obtained and stored in a solution consisting of 70% ethanol, glacial acetic acid, and formaldehyde in a ratio of 90:5:5 and were dehydrated using an ethanol gradient (75%, 95%, 100%, 100%) for 1 h. The dehydrated sections were subjected to a xylene gradient (25%, 50%, 75%, 100%, 100%) for 1 h in order to obtain transparent sections. The transparent sections were subjected to paraffin infiltration, wherein a volume of paraffin equal to the volume of xylene was added to the xylene containing the sample, half of the resultant solution was then aspired out, and an equal volume of paraffin was added again. This process was repeated 10 times, and finally, all of the solution was aspired out and replaced with an equal volume of paraffin; this final step was repeated twice, and the resulting solution after each step was incubated for 1 h at 75 °C. After paraffin infiltration, the sections were subjected to embedding, wherein samples were placed in an iron tank containing liquid paraffin and additional liquid paraffin was rapidly added to fill the whole tank and left to solidify. The resultant wax block was trimmed and sectioned. Embedded sections were placed in warm water, fished out, placed on a slide, and incubated at 45 °C for 30 min. Sections on the slide were dewaxed by serially soaking in 100% xylene, 100% xylene, 50% xylene, 50% xylene, 100% ethanol, 100% ethanol, 95% ethanol, and 75% ethanol and then soaked in safranine O for 40 min. This was followed by another round of serial, rapid soaking in 75% ethanol and 95% ethanol, and then the slides are submerged in fast green for 1 min. Finally, the sections were subjected to one last serial soak in 95% ethanol, 95% ethanol, 100% ethanol, 100% ethanol, 50% xylene, 50% xylene, and 100% xylene. After the sections were stained, a drop of resin glue was placed on the slide and a cover glass was placed over it. The slides were left undisturbed for a week, and the tissue sections were observed using a Olympus optical microscope and imaged.

2.3. DNA extraction and PCR amplification

Total genomic DNA was extracted from flower specimens using a plant genomic DNA extraction kit (Solarbio Science & Technology Co., Ltd., Beijing, China) according to the manufacturer's protocols. The primers for gene amplification and sequencing and the reaction conditions are shown in Table 2. Each gene amplification was repeated thrice for each specimen.

Table 1. Details of sample collection.

Collection Number	Host	Collection Time	Collection Location
AC20190512-1	*Atriplex canescens*	2019.05.12	Sand Botanical Garden, Minqin Desert Control Station, Gansu, China
AC20190512-2	*Atriplex canescens*	2019.05.12	Sand Botanical Garden, Minqin Desert Control Station, Gansu, China
AC20190512-3	*Atriplex canescens*	2019.05.12	Sand Botanical Garden, Minqin Desert Control Station, Gansu, China
AC20199512-4	*Atriplex canescens*	2019.05.12	Sand Botanical Garden, Minqin Desert Control Station, Gansu, China
SR20190520-1	*Sympegma regelii*	2019.05.20	Baoji Town, Pingchuan District, Baiyin City, Gansu, China
SR20190520-2	*Sympegma regelii*	2019.05.20	Baoji Town, Pingchuan District, Baiyin City, Gansu, China
SR20190520-3	*Sympegma regelii*	2019.05.20	Baoji Town, Pingchuan District, Baiyin City, Gansu, China
SR20190520-4	*Sympegma regelii*	2019.05.20	Baoji Town, Pingchuan District, Baiyin City, Gansu, China
SP20190523-1	*Salsola passerina*	2019.05.23	Baoji Town, Pingchuan District, Baiyin City, Gansu, China
SP20190523-2	*Salsola passerine*	2019.05.23	Baoji Town, Pingchuan District, Baiyin City, Gansu, China
SP20190523-3	*Salsola passerine*	2019.05.23	Baoji Town, Pingchuan District, Baiyin City, Gansu, China
SP20190523-4	*Salsola passerina*	2019.05.23	Baoji Town, Pingchuan District, Baiyin City, Gansu, China
HA20190512-1	*Haloxylon ammodendron*	2019.05.12	*Cistanche* Planting Base in Qingtu Lake, Minqin County, Gansu, China
HA20190512-2	*Haloxylon ammodendron*	2019.05.12	*Cistanche* Planting Base in Qingtu Lake, Minqin County, Gansu, China
HA20190512-3	*Haloxylon ammodendron*	2019.05.12	*Cistanche* Planting Base in Qingtu Lake, Minqin County, Gansu, China
HA20190512-4	*Haloxylon ammodendron*	2019.05.12	*Cistanche* Planting Base in Qingtu Lake, Minqin County, Gansu, China

2.4. Sequencing analysis

In order to obtain accurate sequences, the final PCR products, after purification using the Transgene Quick Gel Extraction Kits, were cloned separately into pEASY®-Blunt Cloning Vectors, according to the manufacturer's instructions. After cloning, they were transformed into chemically competent Trans5α cells. Three colonies of each sample were randomly selected and sequenced using M13 primers. These colonies were sequenced bi-directionally by Sanger sequencing, using the BigDye Terminator V3.1 Cycle Sequencing Kits on the ABI Prism 3700 DNA Analyzers. Obtained sequences were aligned using Clustal X (v1.8.7) [29] and synchronized manually in BioEdit (v7.1.3.0) [30]. Utilizing the aligned sequence data, we reconstructed the phylogeny using the MEGA 7 software using the neighbor-joining (NJ) method, Kimura 2-parameter (K2P) model was used and the bootstrap was 1000 repetitions [31].

2.5. Inoculation of C. deserticola

Three grams of *C. deserticola* seeds were added to the pots (diameter × height × bottom diameter = 20 cm × 20 cm × 12 cm) containing sandy soil and stirred to ensure an even spread. The control group consisted of 3 g of *C. salsa* seeds added to similar pots containing sandy soil. Finally, *A. canescens* was planted in each pot, and the pots were placed outdoors. When soil moisture content is less than 13% (g/g), the pots were watered. The experiment was conducted at the Zhongguancun Life Science Park, Beijing, China (latitude 39°56′N, longitude 116°20′ E; 20m above sea level) from May to July. Day temperature varied between 16 and 35 °C, night temperature between 12 and 16 °C. The relative humidity of the air is greater than 50%. Sunlight is abundant. Approximately 80 days later, the soil was removed from the pots and the inoculation rate was determined.

2.6. Determination of concentration of medicinal components

The determination of concentration of medicinal components includes two parts, one is the procedure for liquid chromatography, and the other is the preparation of reference substance and test substance, more details are as follows:

i). Determination of echinacoside and verbascoside

Echinacoside and verbascoside were weighed and added in 50% methanol to yield a 0.2 mg/ml solution, which was used as the reference solution. The first is to grind dry *C. deserticola* into powder, then powder was mixed into 50 ml of 50% methanol in a 100 ml brown volumetric flask, and the test liquid was obtained after subjecting the mixture to shaking, soaking, sonication, standing, and filtration. The chromatographic column was Agilent ZORBAX SB-C18 column (4.6 mm × 150 mm, 5μm), with Methanol (A) - 0.1% Formic acid solution (B) as mobile phase, gradient elution (0–17 min, 26.5% A; 17–20 min, 26.5% → 29.5% A; 20–27 min, 29.5% A), flow rate was 1.0 mL/min, column temperature was 35 °C, detection wavelength was 330 nm, injection volume was 10μl.

ii). Determination of betaine, mannitol, fructose, glucose and sucrose

Betaine, mannitol, fructose, glucose, and sucrose were weighed precisely and added to water to make a solution of 0.25 mg/ml, which was used as reference solution. Five milliliters of the aforementioned *Cistanche* test solution was mixed with 50% methanol in a 25 ml volumetric flask, shaken well, and filtered with 0.2 μm microporous membrane. The chromatographic column was SHODEXASHAIPAK NH2P-50 4E polymerized gel column (250 mm × 4.6 mm, 5μm), the mobile phase was

Table 2. Gene amplification primers and reaction conditions.

Gene name	Primer sequences	Reaction conditions
ITS2	F: ATGCGATACTTGGTGTGAAT	Initial denaturation: 95 °C 5 minDenaturation: 95 °C 30 sAnnealing: 56 °C 30 sExtention: 72 °C 45 s,10 min on last cycleNumber of cycles: 35 cycles
	R: GACGCTTCTCCAGACTACAAT	
rbcL	F: CCAAAGATACTGATATCTTGGCAGCAT	
	R: AGACATTCATAAACAGCTCTACCGT	
psbA-trnL	F: CGAAATCGGTAGACGCTACG	
	R: GGGGATAGAGGGACTTGAAC	

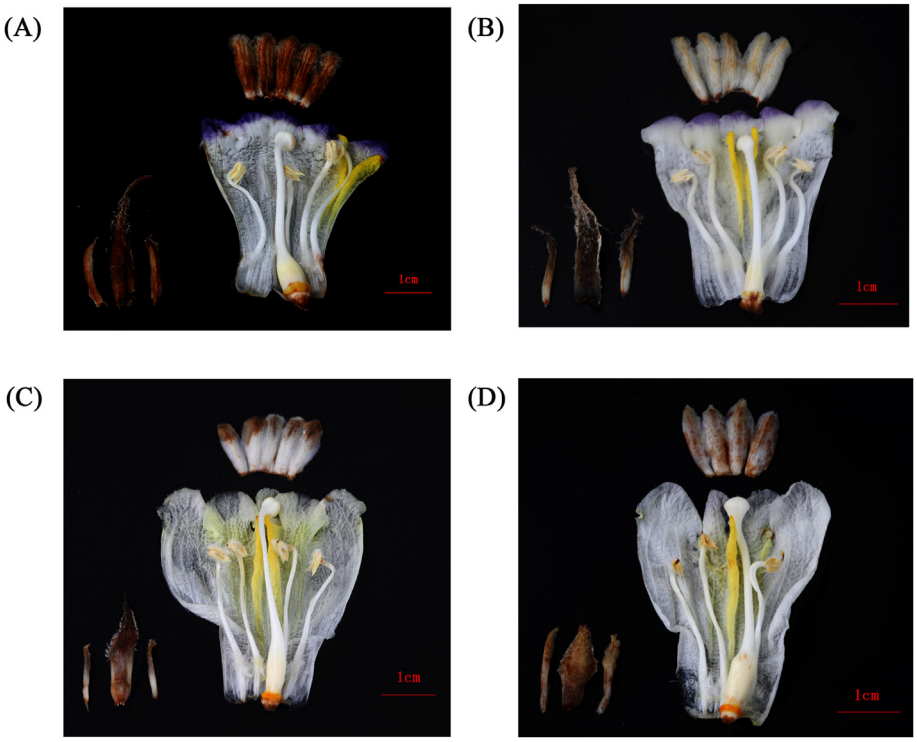

Figure 1. Morphological features of *Cistanche* flowers. (A) *Cistanche deserticola* (host: *Haloxylon ammodendron*); (B) *Cistanche* (host: *Atriplex canescens*); (C) *Cistanche salsa* (host: *Sympegma regelii*); (D) *Cistanche salsa* (host: *Salsola passerina*).

acetonitrile -water (77:23), the flow rate was 0.7 mL/min, the column temperature was 25 °C, Using evaporative light scattering detector (ELSD), the drift tube temperature was 100 °C, the carrier gas flow rate was 3 L/min, the injection volume of reference substance and sample were 5μl.

3. Results

3.1. Morphological identification of flowers

To confirm the species of *Cistanche* that parasitizes *A. canescens*, morphological analysis of flower specimens was carried out (Figure 1 and Figure S1). The overall morphology of flowers of the parasitic plant was similar to that of *C. deserticola*. Further, the corolla was thicker than that of *C. salsa* on different hosts.

According to Flora of China, *C. deserticola* and *C. salsa* have obvious differences in the bract of flowers. In *C. deserticola*, bracts sub-equal the corolla, while the bract of *C. salsa* is 1/3 the length of the corolla. Based on our statistical analysis, the bracts of *Cistanche* parasitized on *A. canescens* and that of *C. deserticola* sub-equaled the corolla (Figure S2). The *Cistanche* on *A. canescens* exhibited morphological features of *C. deserticola*, suggesting that *C. deserticola* may be the parasite on *A. canescens*.

3.2. Microscopic identification of stained tissue specimens

The transection of the fleshy stem of *C. deserticola* is very similar to that of *C. salsa*, and both of them are composed of the epidermis, cortex, vascular bundle, and pith. The vascular bundles of both plants are arranged in wavy or deep wavy rings, and the piths are obviously visible. The main difference lies in the lateral shape of the vascular bundle sheath; it is caudate in *C. deserticola* and triangular or semi-circular in *C. salsa*. Upon performing a microstructure analysis on the *Cistanche* parasitized on *A. canescens*, we found that it had a caudate shaped vascular bundle sheath, resembling that of *C. deserticola* (Figure 2).

3.3. Molecular identification

In addition to morphological identification, we also carried out a molecular identification and selected three gene fragments, namely, *ITS2*, *rbcL*, and *psbA-trnL*. An evolutionary tree was constructed using the sequence information of each fragment (Figure 3), and all three phylogenetic trees displayed that the *Cistanche* parasitized on *A. canescens* had a close phylogenetic relationship with *C. deserticola*. These results indicate that *C. deserticola* may be the parasite on *A. canescens*.

Detailed gene divergences among the different *Cistanche* species were observed upon multiple sequence alignment (Figure 4). We found three single nucleotide polymorphisms (SNPs) in the *ITS2* gene body between *C. deserticola* and *C. salsa*, at bases 139, 295, and 472. In the *rbcL* gene body, there were four gene divergences between *C. deserticola* and *C. salsa*, containing two SNPs and two insertion and deletion (InDel) mutations. Compared with *ITS2* and *rbcL*, the differences in the *psbA-trnL* gene body between *C. deserticola* and *C. salsa* were more obvious, with seven sequence divergences, wherein four were SNPs and three were InDel mutations. In particular, a series of thymine repeats, starting at base 414 of the aligned sequence, could be used to develop simple sequence repeat (SSR) markers for distinguishing *C. deserticola* and *C. salsa*.

3.4. Inoculation of C. deserticola

To test whether *C. deserticola* or *C. salsa* could parasitize *A. canescens*, an inoculation experiment was carried out, and we found evidence of parasitism in all *C. deserticola*-inoculated pots with an inoculation rate of almost 100% (Figure 5). No parasitism was observed in the control

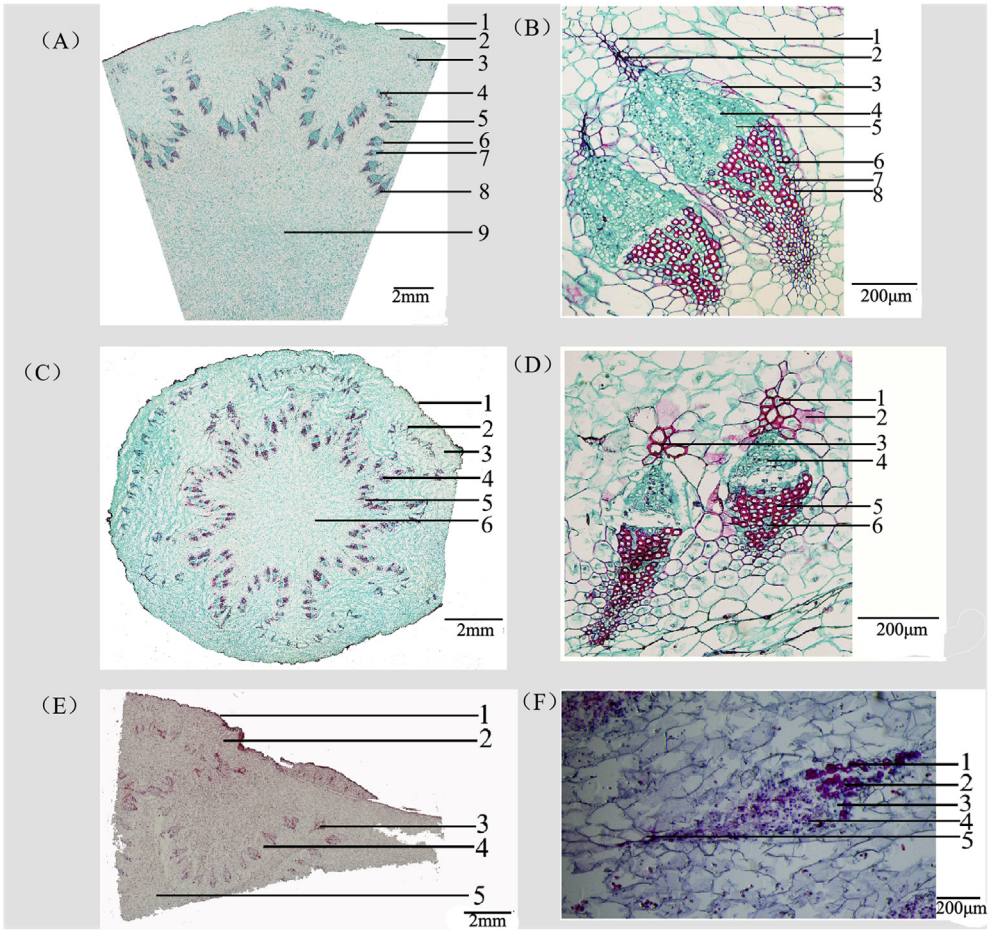

Figure 2. Microscopic characteristics of the fleshy stem in different *Cistanche* species. (A) Microscopic characteristics of fleshy stem of *Cistanche deserticola*: 1. epidermis, 2. cortex, 3. leaf trace bundle, 4. vascular bundle, 5. medullary ray, 6. vascular bundle sheath, 7. phloem, 8. xylem, 9. pith. (B) Enlarged view of the vascular bundle in *Cistanche deserticola*: 1. vascular bundle sheath, 2. fiber, 3. poreline cells, 4. bast fiber, 5. phloem, 6. xylem, 7. vessel, 8. xylon. (C) Microscopic characteristics of fleshy stem of *Cistanche salsa*: 1. epidermis, 2. leaf trace bundle, 3. cortex, 4. vascular bundle, 5. medullary ray, 6. pith. (D) Enlarged view of the vascular bundle of *Cistanche salsa*: 1. vascular bundle sheath, 2. poreline cells, 3. Fiber, 4. phloem, 5. vessel, 6. xylem. (E) Microscopic characteristics of the fleshy stem of *Cistanche* parasitized on *Atriplex canescens*: 1. epidermis, 2. cortex, 3. vascular bundle, 4. medullary ray, 5. pith. (F) Enlarged view of the vascular bundle of *Cistanche* parasitized on *Atriplex canescens*: 1. vessel, 2. xylem, 3. cambium, 4. phloem, 5. vascular bundle sheath.

groups. This result directly proves that *C. deserticola* easily parasitized *A. canescens*, whereas *C. salsa* could not.

3.5. Determination of concentration of significant medicinal components

We estimated the concentration of important medicinal components in *C. deserticola* parasitized on *A. canescens*. The specific chromatogram is shown in the supplementary material. To obtain accurate results, four independent experiments were set up. Based on our measurements (Table 3), we found that the concentration of verbascoside and echinacoside were 20 times higher than that reported in the Chinese Pharmacopoeia (according to the Chinese Pharmacopoeia, the percentage of sum of concentrations of echinacoside and verbascoside in *C. deserticola* should be less than 0.30%). The concentrations were also significantly higher than that in *C. deserticola* parasitized on *H. ammodendron* (generally 0.2–1.5%) [32]. The concentration of mannitol, betaine, fructose, and other carbohydrate components was also very high, and the overall quality was better than that in *C. deserticola* parasitized on *H. ammodendron*. Thus, these results indicate that *A. canescens* can be used to grow *C. deserticola* at an industrial level and protect endangered wild resources.

4. Discussion

Previously, *C. deserticola* has been considered to exclusively parasitize *H. ammodendron*. However, in this study, using morphological and molecular identification techniques, we demonstrated that *C. deserticola* can also parasitize *A. canescens*. Although *H. ammodendron*, *A. canescens*, and *H. persicum* all belong to the family Chenopodiaceae, it is interesting and peculiar that *C. deserticola* has species selectivity, possibly governed by the signaling molecules secreted by the host. *A. canescens*, originating from the Unites States of America, exhibits strong resistance to environmental perturbations and has a relatively large biomass. *A. canescens* is a viable host for *C. deserticola* for a variety of reasons. First, it can survive in a wide range of environmental conditions. Second, the biomass and growth rate of *C. deserticola* may be larger and more rapid, respectively, on *A. canescens* than on *H. ammodendron*. Third, due to the wide range of adaptability of *A. canescens*, the planting area can be further expanded. Thus, *A. canescens* has distinctive advantages over *H. ammodendron* as the host and will aid industrial production of *C. deserticola*.

C. deserticola and *C. salsa* are difficult to distinguish, and morphological identification in the past has produced confounding results. With advances in the field of molecular biology, molecule-based identification techniques have been widely used in Chinese herbal medicine. Since most Chinese herbal medicines offer little genomic information, DNA barcoding technology has emerged as a breakthrough identification technique. In this study, morphological and DNA barcoding technology were comprehensively applied for identifying the unknown *Cistanche* species; this has not been attempted before, and our results demonstrate that this approach is feasible.

Since *C. deserticola* parasitizes *A. canescens*, it is important to determine differences in quality of *C. deserticola* on the roots of *A. canescens* and that on the roots of *H. ammodendron*. According to our results, the

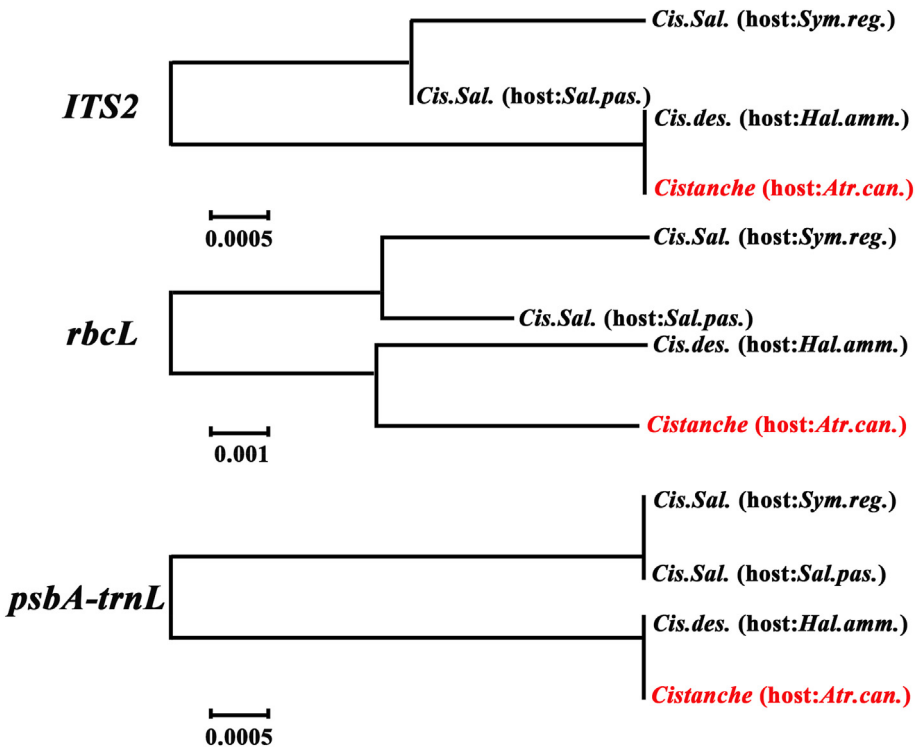

Figure 3. Phylogenetic analysis of *Cistanche* species.

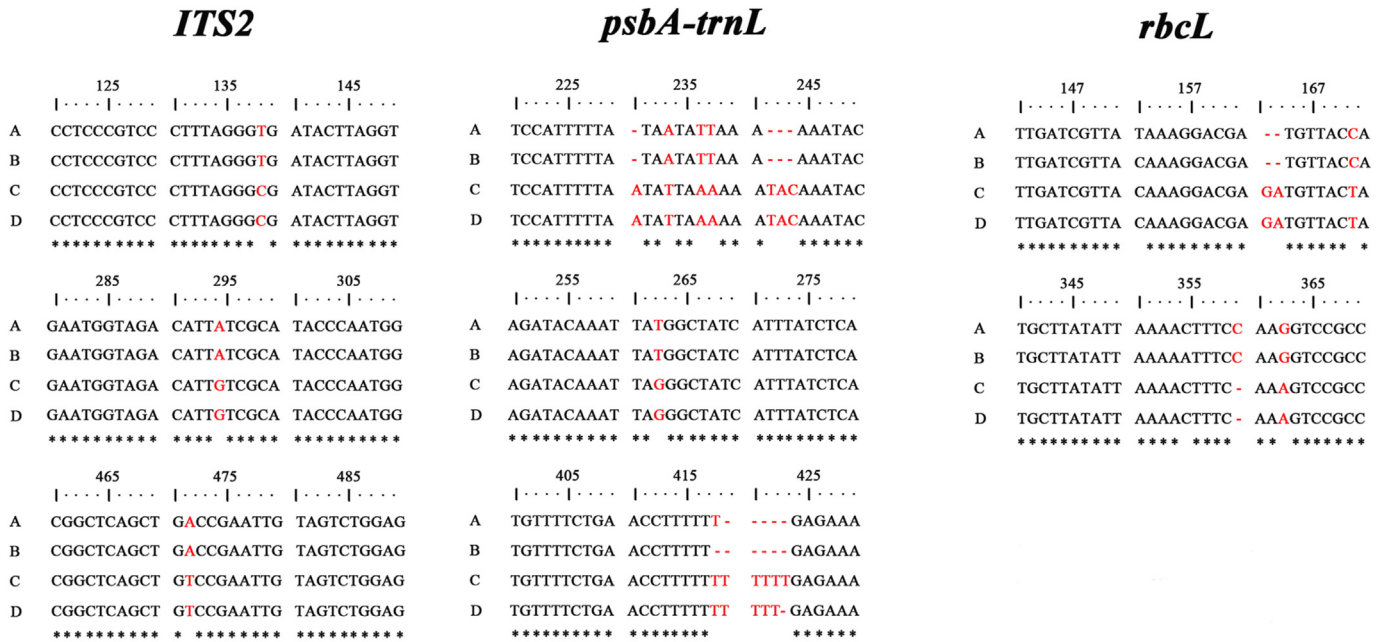

Figure 4. Major gene divergences among *Cistanche* species. Letter A represents *Cistanche deserticola* (host: *Haloxylon ammodendron*); Letter B represents *Cistanche* (host: *Atriplex canescens*); Letter C represents *Cistanche salsa* (host: *Sympegma regelii*); Letter D represents *Cistanche salsa* (host: *Salsola passerina*).

concentration of active components was higher in *C. deserticola* parasitized on *A. canescens* than in that parasitized on *H. ammodendron*. Thus, our results lay a solid theoretical foundation for the large-scale production of *C. deserticola* parasitized on *A. canescens*.

5. Conclusions

For a long time, *C. deserticola* has been considered to exclusively parasitize *H. ammodendron*. Previously, it was found that *C. deserticola* seeds purchased from the market can parasitize *A. canescens*, another

Figure 5. Inoculation experiment. (A) *Atriplex canescens*; (B) *Atriplex canescens* inoculated with *Cistanche deserticola*; (C) detailed picture of *Atriplex canescens* inoculated with *Cistanche deserticola*.

Table 3. Concentration of important medicinal components.

Batch	Echinacoside	Verbascoside	Betaine	Mannitol	Fructose	Glucose	Sucrose
1	4.32%	0.23%	2.64%	5.02%	16.86%	5.80%	17.18%
2	4.42%	0.20%	2.64%	5.88%	16.66%	5.49%	17.58%
3	4.77%	0.16%	2.18%	5.79%	16.29%	5.14%	17.04%
4	4.46%	0.14%	2.51%	5.18%	16.63%	6.80%	17.65%

Chenopodiaceae plant. Using morphological and molecular identification methods, we confirmed that the *Cistanche* species parasitizing *A. canescens* was *C. deserticola*. This result was further confirmed by the inoculation experiment. We determined the concentration of significant medicinal components, and our results suggest that the concentration and quality of components were greater in *C. deserticola* parasitized on *A. canescens* than in that parasitized on *H. ammodendron*. The discovery of new hosts can promote the industrial production of *C. deserticola*, and it can also effectively protect wild resources and the ecological environment.

Declarations

Author contribution statement

Fangming Wang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Bingyu Zhuo, Yuan Zhang, Qingliang Chen, Ziyi Shi & Yuelin Song: Performed the experiments.

Shuai Wang & Jin Lou: Contributed reagents, materials, analysis tools or data.

Pengfei Tu: Conceived and designed the experiments.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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