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The discovery of *Artemisia annua* L. in the Shengjindian cemetery, Xinjiang, China and its implications for early uses of traditional Chinese herbal medicine *qinghao*

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ABSTRACT

Ethnopharmacological relevance: *Artemisia annua* L., with the ancient name of *qinghao*, is a traditional Chinese herbal medicine. It has appeared in many ancient Chinese medical manuscripts, which describe its uses to include treatment of wounds, alleviating intermittent fevers, as well as enhancing the brightness of eyes and even improving longevity.

Materials and methods: A sheaf of plant remains, including stalks and inflorescence intentionally placed in the corner of a tomb, have been recovered from the Shengjindian cemetery (about 2400–2000 BP on the basis of ^{14}C dating), Turpan, Xinjiang, China. The morphology of these materials was examined using a stereomicroscope and a scanning electron microscope. Ancient DNA was also extracted from these remains.

Results: By comparing the morphological and DNA characteristics with modern specimens, these plant remains were identified to belong to *Artemisia annua* L. Owing to its strong fragrance, these plant remains are suggested as serving to disguise the odor of the deceased.

Conclusions: This is the first material archaeological evidence to date despite numerous records of *A. annua* in ancient Chinese texts as herbal medicine *qinghao*, though it seems to have been employed as odor suppressant, not for medical purpose.

1. Introduction

Artemisia annua L. (Sweet wormwood) is an annual, erect herb with a characteristically strong fragrance, which belongs to the genus *Artemisia* of the family Compositae. It is believed to originate in Asia and Eastern Europe, but now is widely distributed throughout the temperate region. *A. annua* has been utilized as a traditional Chinese medicine, a flavoring for spirits such as vermouth, a source of essential oils, in the crafting of aromatic wreaths (Simon et al., 1990; CFCCAS, 1991; Ferreira et al., 1997, 2006; Ferreira, 2004; Ferreira and Janick, 2009). However, *A. annua* has become well known throughout the world since it was found to be the source of the antimalarial lactone artemisinin in 1970s. Currently, artemisinin and its derivatives are widely promoted as part of combination therapies for treating drug-resistant malaria (WHO, 2006; Hsu, 2006a; Liao, 2009).

A. annua has appeared in ancient Chinese medical manuscripts since very early times under the name of *qinghao* (CFCCAS, 1991; Hsu, 2009; CPC, 2010). The earliest documentation of *qinghao* can be traced to the Warring States period (about 400–300 BC) in the 〈*Shennong ben cao jing*〉 (*Shennong's Canon of Materia Medica*), which is the foundation text of Chinese herbal medicine (Ma, 1995). It reads as follows: "The herbaceous *hao* [*caohao*] is also called *qinghao* or *fangkui*. Its flavor is bitter and cold. No poison. It treats *jie* itches, *jia* itches, and wounds. It kills lice and lingering heat in the joints. It brightens eyes. It grows in waste lands along the riverside." (Ma, 1995; Hsu, 2010).

Another early record of *qinghao* comes from the Han Tomb No.3 at Mawangdui, from which some manuscripts written on silks have been unearthed. One recipe about *qinghao* from these writings advocates urine-boiled *qinghao* for the treatment of female hemorrhoids (*pin zhi*). Researchers claim that these texts were copied on the silks between the late Warring States period (about 400–300 BC) and 168 BC (Ma, 1992; Hsu, 2010).

A. annua has been then regularly recorded as a herbal medicine in many ancient Chinese *materia medica* and writings, including 〈*Shang Han Lun*〉 (On Cold Damage, 200–205), 〈*Zhou hou bei ji fang*〉 (Handbook of Prescriptions for Emergency Treatment, 4th century), 〈*Shi liao ben cao*〉 (*Materia Medica* for Successful Dietary Therapy, 721–739), 〈*Zheng lei ben cao*〉 (*Materia Medica* Corrected and Arranged into Categories, 1082), 〈*Meng xi bi tan*〉

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(Dream Pool Essays, 1086), 〈*Ben cao gang mu*〉 (Classified Materia Medica, 1596), etc. (Willcox et al., 2004; Hsu, 2006b, 2010). On the basis of these documents, the herbal medicine *qinghao* has been recommended for treating *re lao* (exhaustion due to heat/fevers), *mie gu zheng lao re* (eliminating bone steaming and heat/fevers arising from exhaustion), treating *nüe* (intermittent fevers) and *shi zhu*, *gui qi*, *fu lian* (disorders with acute convulsions, which were related to pollution through contact with the dead and possession by demons in China). In Hsu's view, all of these effects, including the use of treating lingering heat in the joints in the 〈*Shennong ben cao jing*〉 (*Shennong's Canon of Materia Medica*), are implicitly suggestions of *qinghao*'s potency against malaria. Meanwhile, *A. annua* was associated with *ming mu* (brightening the eyes), *zhang fa* (hair growth) and enhancing longevity, which are probably some indirect manifestations of its antimalarial effects (Hsu, 2006b). In some documents, *qinghao* is also recorded as food supplement (Hsu, 2009). Research on *A. annua* continues today, mainly focused on its function against malaria and cancer, as an antioxidant, etc. (Ferreira et al., 1995, 2005, 2010; Willcox et al., 2004, 2009; Wright et al., 2010).

Despite these ancient literature citations, no archaeological occurrence of *A. annua* has been recorded in China until now. However, recent excavations performed in the Turpan area provided the first archaeological *A. annua* remains to us. Due to the dry environment, plant remains were well preserved, and systematical archaeobotanical studies were conducted in the Turpan area of Xinjiang, China. Cereal (*Panicum miliaceum*, *Triticum aestivum*, *Hordeum vulgare* var. *coeleste*), fruit (*Vitis vinifera*), and many wild plants (*Cannabis sativa*, *Capparis spinosa*, *Lithospermum officinale*, etc.) have been accordingly investigated until now (Jiang et al., 2006, 2007a, 2007b, 2007c, 2009). In the present study, we report on stalks and inflorescences of *A. annua* that have been discovered in the Shengjindian cemetery of Turpan, Xinjiang, which can give us further understanding about the plant utilizations by the indigenous ancient people of Xinjiang.

2. Site description

The Shengjindian cemetery is located in the Turpan area of Xinjiang, 40 km east of Turpan City (Fig. 1). It lies on the north slope of the Flaming Mountains (*Huoyan Shan*), opposite to a large swampy area. Characterized by a typical continental desert climate and surrounded by many mountains, it is extremely arid with little or no precipitation during the whole year, which is highly favorable for the preservation of archaeological remains.

The Shengjindian cemetery was discovered during the construction of the G312 Highway. In 2007–2008, a rescue excavation was conducted under the direction of Prof. Yongbing Zhang, one of the authors of the present paper. Thirty-one tombs of three types, i.e., rectangular earthen pit tombs, rectangular earthen tomb with a side chamber and tombs with a racking platform, were excavated (Fig. 2). Many artifacts, including pottery, wooden wares, leather products, etc., were unearthed.

On basis of the artifacts and plant remains in tombs, people of the Shengjindian cemetery seem to have led a semi-agricultural and semi-pastoral life. They mainly planted *Panicum miliaceum* L., *Hordeum vulgare* var. *coeleste* Linnaeus, *Triticum aestivum* L. and *Vitis vinifera* L. (Jiang et al., 2009). The natural vegetation around them included *Capparis spinosa* L., *Alhagi sparsifolia* Shaparenko ex Keller and Shaparenko, *Typha* sp., *Lycium ruthenicum* Murr., *Leymus secalinum* (Trin.) Tzvel., *Sophora alopecuroides* L., *Sphaerophysa salsula* (Pall.) DC., *Phragmites australis* (Cav.) Trin. ex Steud., *Aeluropus pungens* (M. Bieb.) C. Koch, *Seteria viridis* (L.) Beauv, *Echinochloa crusgali* (L.) Beauv., etc., which indicates that there were wet lands around aborigines of the Shengjindian cemetery, as well as some weeds occurred around wet lands, and some others came from the farmland (Jiang, 2010).

The tomb M4, containing the sweet wormwood (*Artemisia annua* L.) remains investigated in the present study, is located at the southwest part of the unit T7 (Fig. 2). It is a rectangular earthen pit tomb covered by some grasses. Three bodies, two men and one woman, were discovered in this tomb; the bodies had been placed on a special bed lined with felt. However, the skulls and other human bones were found scattered around the tomb, indicating that this tomb probably has been disturbed. Twelve artifacts were recovered and the sweet wormwood remains were found in a corner (Fig. 3). Sweet wormwood remains have also been discovered in tomb M14 of unit T10, where they had been spread over the entire tomb.

3. Materials and methods

3.1. Materials

Archaeological material in the present study is the stalk sheaf with inflorescence from tomb M4 of the Shengjindian cemetery (Fig. 4). Modern reference specimen was collected from the Institute of Botany, Chinese Academy of Sciences.

3.2. Morphological examination and comparisons

The materials were examined and identified using a Nikon SMZ1000 stereomicroscope with a magnification of 15 × –40 ×. The detailed structures were observed under a Quanta 200 scanning electron microscope (SEM) at an accelerating voltage of 30 kv.

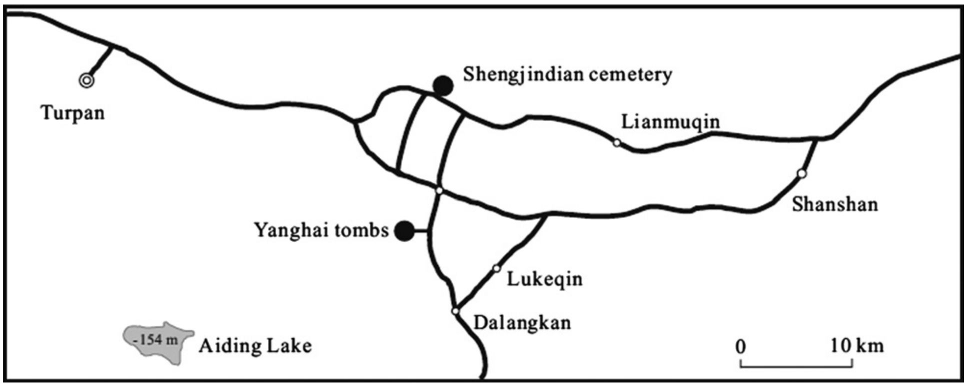

Fig. 1. Location of the Shengjindian cemetery.

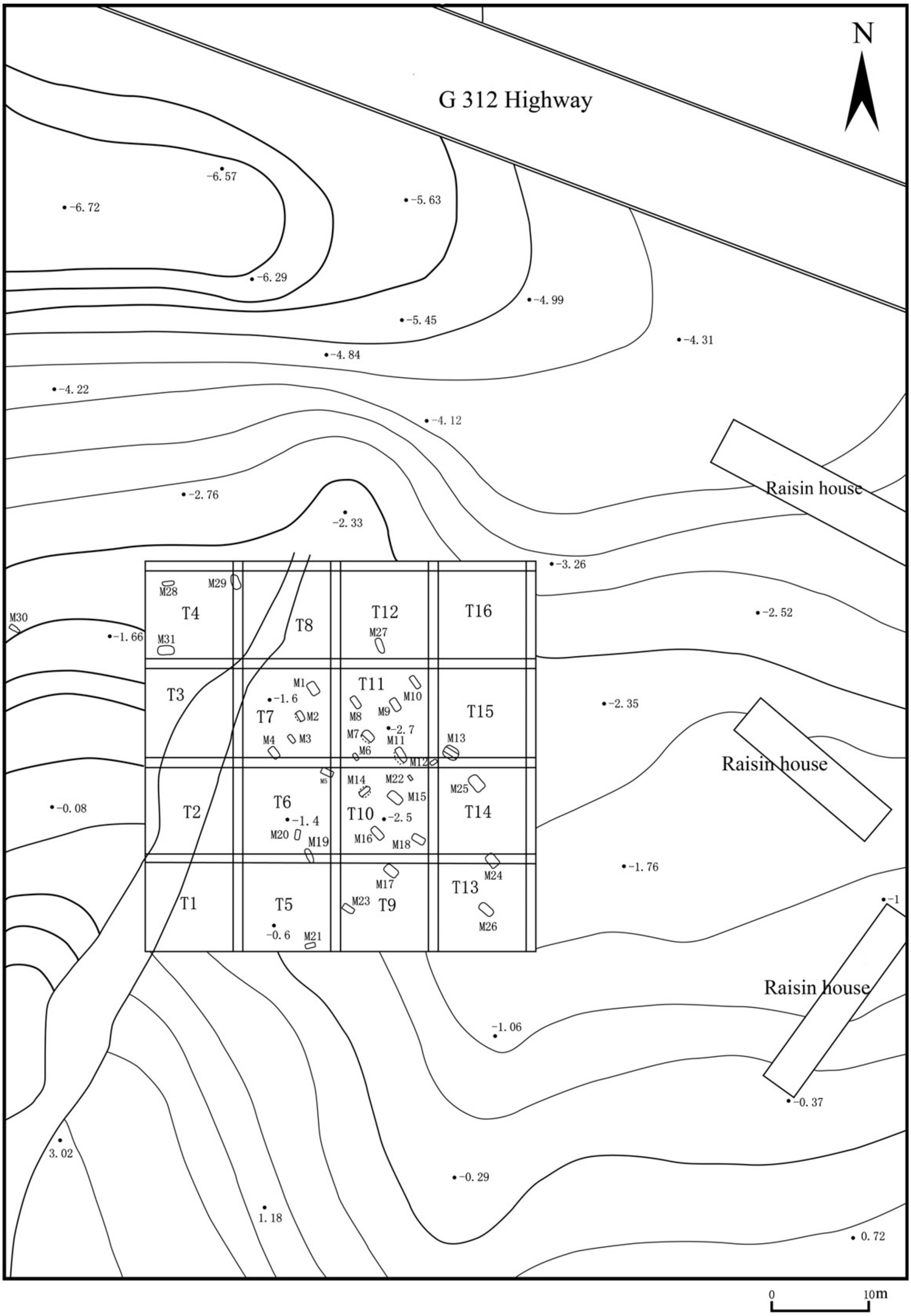

Fig. 2. Distribution of tombs in the Shengjindian cemetery.

3.3. *DNA extraction and PCR amplification*

Experiments were performed to try to extract ancient DNA from the archaeological materials. 0.05 g of the inflorescence sample was quickly ground with 0.1 g sterilized silica sand and a sterilized plastic pestle after frozen by liquid nitrogen. Then the milled powder was mixed with 0.8 ml CTAB solution, vortically vibrated for 1 min and incubated at 60 °C for 2 h with gentle agitation every 10 min (White et al. 1990). Subsequently, the mixture was centrifuged at 12,000 rpm for 10 min, to get the

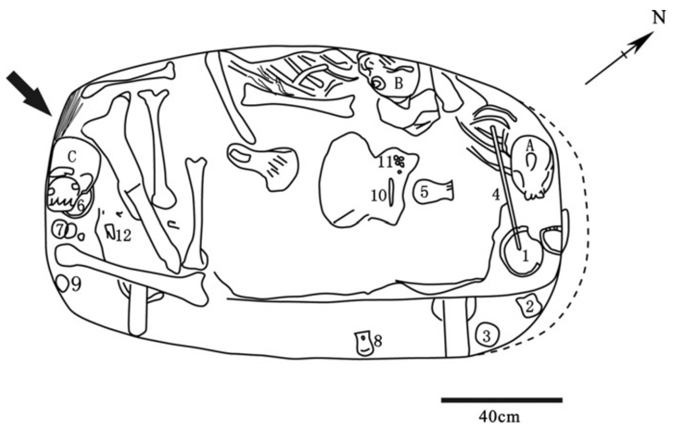

Fig. 3. Line drawing of tomb M4 with the *A. annua* remains in a corner (the black arrow). A, B, C Human skulls, 1. Pottery bowl 2. Pottery cup 3. Pottery cup 4. Spindle 5. Leather bag 6. Wooden bowl 7. Pottery cup 8. Wooden buckle 9. Wooden bowl 10. Wooden hairpin 11. Stone beads 12. Bronze fragment.

supernate. 0.7 ml chloroform/isoamylol solution (24:1, v/v) was added to the supernate. Then the mixture of supernate and solution was vortically vibrated for 10 s and centrifuged at 12,000 rpm for 10 min to get the supernate. 0.7 ml chloroform/isoamylol solution (24:1, v/v) was added to the new supernate again, and the vortically vibrating and centrifuging were repeated to get the supernate. Subsequently, 0.5 ml isopropanol was added to the double-extracted supernate and the mixture was stored at −20 °C for 24 h. Then the mixture was centrifuged at 12,000 rpm for 20 min to get the supernate. The supernate was washed twice with cold ethanol/water solution (7:3, v/v) and air dried to get the DNA precipitate. Finally, the DNA precipitate was dissolved in 30 μl TE buffer for PCR amplification.

PCR amplification was carried out by using a 50 μl-system which consists of 3 μl DNA solution, 5 μl 10X PCR buffer (with 50 mM $MgCl_2$, Tiangen Co., Ltd., China), 1.5 μl Taq polymerase (20 U/μl, Tiangen Co., Ltd., China), 5 μl dNTPs (5mM, Shanghai Sangon Co., Ltd., China), 2 μl BSA solution (50mM, Shanghai Sangon Co., Ltd., China) and 5 μl mixture of ITS1 and ITS4 primer (10 mM, Shanghai Sangon Co., Ltd., China) (White et al., 1990). Samples were amplified in a Veriti^{TM} 96 DNA thermal cycler (Applied Biosystems, USA) with program loaded as follows: 1) 94 °C for 5 min; 2) 35 cycles of 94 °C for 30 s, 59 °C for 50 s and 72 °C for 60 s; 3) 72 °C for 10 min. A negative control (DNA-free sample) was also used in the amplification.

3.4. DNA sequencing and sequence analysis

After purified by the UNIQ-10 Column Kit (Shanghai Sangon Co., Ltd.), the PCR products were sequenced on an ABI 3730 sequencer with ITS1 and ITS4 primers in Beijing BioTeke Co., Ltd. BLASTN was employed to determine the similarity (%) among the sequences acquired in this study and those from the GenBank (http://www.ncbi.nlm.nih.gov/nuccore).

3.5. Sequence alignment and phylogenetic analysis

In order to further confirm the first discovery of ancient sweet wormwood in Turpan, a phylogenetic analysis is conducted. The ITS sequences of 13 species in the genus *Artemisia*, including 6 local species in the county of the Shengjindian cemetery (*A. sericea* Web. ex Stechm., *A. rutifolia* Steph.ex Spreng, *A. annua* L., *A. vulgaris* L., *A. lavandulaefolia* DC., *A. dracunculus* L.) and 7 other Xinjiang-distributed species morphologically close to sweet wormwood (*A. macrantha* Ledeb., *A. pontica* L., *A. gmelinii* Web. ex Stechm., *A. phaeolepis* Krasch., *A. tanacetifolia* L., *A. tournefortiana* Reichb., *A. caespitosa* Ledeb.) were acquired from the Genbank. Together with the sequences of both the modern *A. annua* sample and the ancient specimen obtained in the 3.4 section, phylogenetic relationships were re-built among these 15 samples by employing the ITS1 and ITS2 sequences of their rDNA. DNA sequences were edited and aligned with Bioedit software (version 7.1.3.0) based on the following principles: 1) Ambiguously aligned positions were excluded from the matrix. 2) Gaps were treated as missing data. 3) All unambiguous characters and character transformations were weighted equally (Hall, 1999). The final dataset was analyzed with PAUP* (version 4.0 for MAC) according to the maximum parsimony principle (Swofford, 2000). 485 bases were included in each sequence. Maximum parsimony trees were generated and bootstrap was performed to affirm the consistency of these trees.

3.6. ^{14}C dating and terminology

Samples from the Shengjindian cemetery were ^{14}C-dated with an accelerator mass spectrometer (AMS) at Peking University, then calibrated using IntCal04 (Reimer et al., 2004) and OxCal v3.10 (Ramsey, 2005).

Plant nomenclature follows the revised English edition of the Flora of China (http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200023164). The botanical terms used for structural descriptions are based on Ferreira and Janick (1995, 2009).

4. Results

4.1. Morphological description

The stalks are erect, yellow, cylindrical, single-stemmed but with many branches at the upper part, 1.1–8.2 mm in diameter, and with longitudinal ridges. They are slightly hard but easily broken. Roots still adhere to some stalks. The inflorescence is a compound raceme, bearing many yellow, nodding capitula, of which only the receptacle, involucre and pedicel can be observed (Figs. 4d, 5a). The capitula vary in size with the diameter ranging from 1.2 mm to 2.5 mm. The receptacle is convex, yellow in color, and distributed with many glandular trichomes. The involucre is imbricated with 2–3 layers of bracts, which are oval in shape, lustrous, glabrous at the rearward side, some broken, some with scarious edges.

The SEM results show that both the receptacle and bracts bear abundant biseriate heart-shaped glandular trichomes. Some trichomes appear shrunken probably due to the long duration of more than 2000 years or being not fully developed originally (Fig. 5c). However, some are too blurred and deformed for identification (Fig. 5e). Many trichomes occur on the exterior surface of bracts while they are rare on the interior surface.

4.2. Highly similar sequences search

DNA was successfully extracted from the inflorescence of the ancient sample in tomb M4. Its internal transcribed spacer region (ITS) of nuclear ribosomal DNA was amplified and sequenced by using the ITS1 and ITS4 primers. At the same time, the ITS of a modern *A. annua* specimen was also amplified and sequenced as a compare. Both of their PCR products were 707 bps in length. The BLAST search of these sequences against the many thousands of data in the GenBank demonstrates that there were significant close matches (98%–99% identity) between the ancient sequences and those of *A. annua* in the GenBank. Likewise, the modern *A. annua* specimen in the present study has the same high-similarity sequences (i.e., GU724282.1, FJ980328.1, FJ980327.1, AY548201.1)

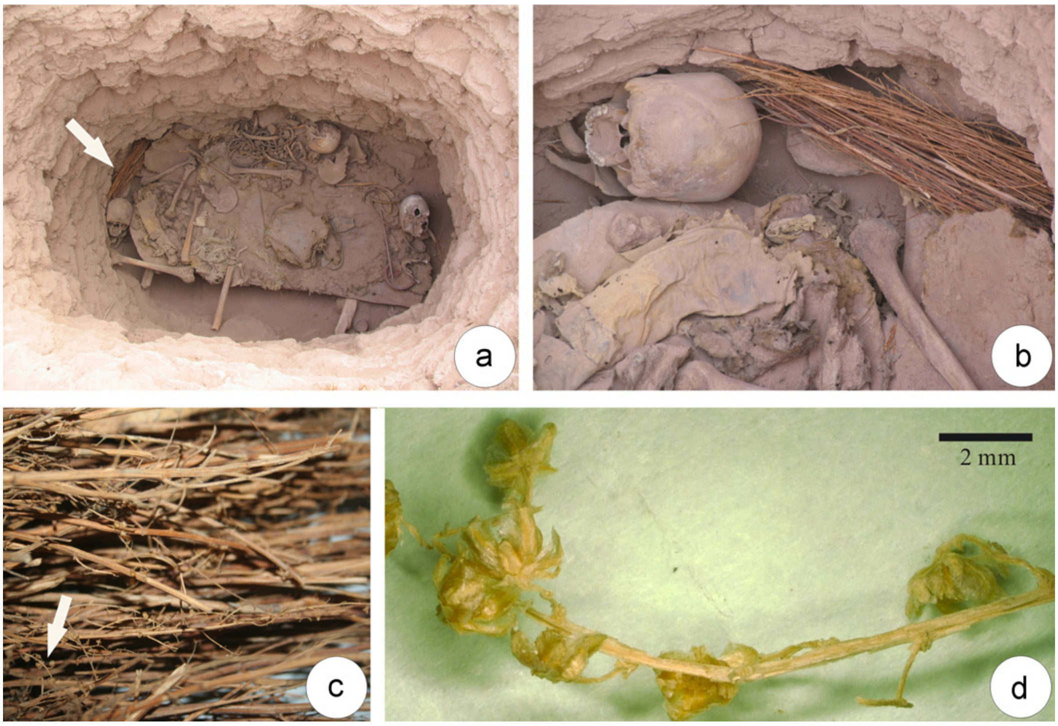

Fig. 4. (a) Tomb M4 with the stalk sheaf neatly placed in the corner (the white arrow). (b) Magnification of the corner with the stalk sheaf in Tomb M4. (c) The stalk sheaf with inflorescence in Tomb M4 (the white arrow). (d) Magnification of the inflorescence from the stalk sheaf in Tomb M4.

as the ancient sample. These results reveal that the most closely-matching species of the ancient sample is *A. annua*.

4.3. Phylogenetic analysis

2 maximum parsimony trees were built and their consensus tree was generated by repeating bootstrapping for 1000 times in heuristic search. The consistency index (CI) is 0.705. Branches with a bootstrap proportion greater than 95%, which was considered as the significant confidence interval, were shown (Fig. 6). As is shown in Fig. 6, the ancient sample was topologically gathered with two *A. annua* isolates and they formed a group (Group I). This grouping was strongly supported by 100% bootstrap proportion. It revealed that the ancient sample has the closest phylogenetic relationship with *A. annua* compared to other *Artemisia* species. Additionally, the average phylogenetic distance within the Group I was only 1 change, less than the average distance of larger than 5 changes among the *Artemisia* species. Therefore, the 3 samples in Group I were demonstrated to belong to the same species, i.e., *A. annua*.

4.4. Identification

All the morphological features of the ancient sample in tomb M4, i.e., the longitudinal ridges on the yellow stalk, the compound raceme, the yellow nodding capitula, the imbricated involucres, as well as the heart-shaped glandular trichomes on the receptacle and bracts, are identical to those of the control modern specimen of *A. annua*. Furthermore, DNA sequence analysis also supports this result. Consequently, the plant remains in M4 can be safely assigned to *A. annua* L.

4.5. Dating result

According to the tomb types and artifacts from tombs, the Shengjindian cemetery is considered belonging to the Subeixi culture. The ^{14}C dating of plant, leather and stomach content samples from this cemetery reveals a result of about 2400–2000 BP (Table 1), which is the late phase of the Subeixi culture. It is documented in *Han-shu* (History of the Han Dynasty) that the Gushi people, one of aboriginal inhabitants of Xinjiang, lived around Turpan before 2100 BP (Ban, 1st Century). Accordingly, the deceased of the Shengjindian cemetery are deduced to be ancient Gushi people.

5. Discussion

5.1. Ancient DNA research of plant remains

Ancient DNA research has received much attention since the mid-1980s. In the past several decades, it has developed significantly and contributed greatly in many substantial problems, for instance, human origin and evolution, population movement and history, agriculture origin and spread, etc. (Cann et al., 1987; Brown, 1999; Höss, 2000; Jones and Brown, 2000; Kaestle and Smith, 2001; Ke et al., 2001; Gugerli et al., 2005; Baca and Molak, 2008). Though not so widely investigated as for animals and humans, ancient DNA of several crops, i.e., wheat, maize, soybean, bottle gourd, has been explored (Rollo et al., 1991; Brown et al., 1993; Jaenicke-Despres et al., 2003; Yano et al., 2004; Erickson et al., 2005; Emshwiller, 2006). Meanwhile, recovering of ancient DNA from some non-crop plants, consisting of seagrass, plants from a mat, etc., has also been reported (Rollo et al., 1994; Raniello and Procaccini, 2002; Gould et al., 2010). Coupling with morphological analysis, ancient DNA research for the *Cannabis sativa* remains found in the Yanghai tombs, Turpan, Xinjiang, was also conducted (Jiang et al., 2006; Mukherjee et al., 2008; Russo et al., 2008). The methodology of the present study parallels that of the cannabis case.

DNA has been retrieved from carbonized and waterlogged plant remains (Brown et al., 1994; Pollmann et al., 2005). Preservation of

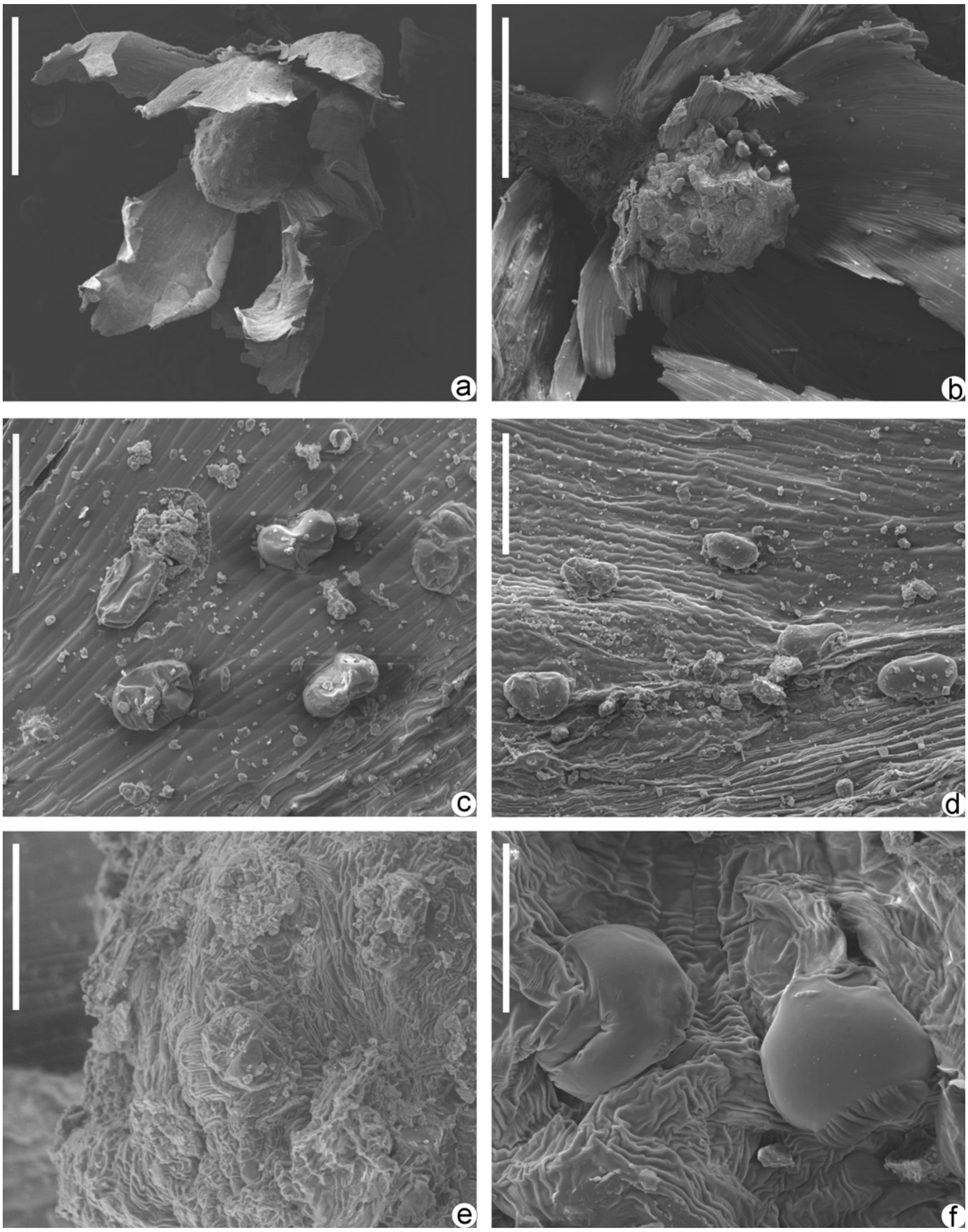

Fig. 5. (a) Inflorescence of *A. annua* remains in tomb M4. Scale bar=1.0 mm. (b) Inflorescence of modern *A. annua* sample. Scale bar=0.5 mm. (c) Glandular trichomes on the exterior surface of bracts from the *A. annua* remains in tomb M4. Scale bar=0.1 mm. (d) Glandular trichomes on the exterior surface of bracts from modern *A. annua* sample. Scale bar=0.1 mm. (e) Glandular trichomes on the receptacle from the *A. annua* remains in tomb M4. Scale bar=0.1 mm. (f) Glandular trichomes on the receptacle from modern *A. annua* sample. Scale bar=50 μm.

ancient plant DNA in sites with hot and dry climate has also been reported (O'Donoghue et al., 1996; Mukherjee et al., 2008; Russo et al., 2008). In the present Shengjindian cemetery with the same context, positive results have also been presented from the desiccated *A. annua* remains. However, cases with low success rate exist and the preservation limit and the authenticity of ancient DNA are still controversial (Marota et al., 2002). The marker in this paper, internal transcribed spacer region (ITS) of nuclear ribosomal DNA, has been widely used to explore the phylogenic relationships of species in Asteraceae (Torrell et al., 1999). It has also been used in ancient DNA research of sorghum, wheat and *Prunus* (Pollmann et al., 2005; Schlumbaum et al., 2008).

5.2. Utilization of A. annua in the Shengjindian cemetery

The *A. annua* remains were neatly and separately placed in a corner of tomb M4, which represents distinctly an intentional placement (Fig. 4). Moreover, they have also been recovered from M14. It is probably best explained as some deliberate use of this material. So, for what use did people of the Shengjindian cemetery place the stalk sheaf of *A. annua* in the tombs? Though *A. annua* is a traditional Chinese herbal medicine, *A. annua* remains in some containers, not the stalk and inflorescence coarsely piled in a corner, should most likely be placed in tombs if it is for medicinal use. As a highly aromatic herb, its purpose for disguising the tomb smell and prolonging the preservation of the deceased is more convincing than medicinal use. Biseriate glandular trichomes on the capitula of *A. annua* consist of two columns of five cells each, and sequester highly aromatic volatile oils, which are composed primarily of terpenoids with some additional phenylpropanoids and nonvolatile fatty acids, mainly including artemisia ketone, camphor, germacrene D, camphene hydrate, artemisia alcohol, pinene, linalool, camphene, cuminal, etc. (Simon et al., 1990; Ferreira and Janick, 1995, 2009; Ferreira et al., 1997; WHO, 2006). Actually, a thin perfume can still be perceptible in the immediate vicinity of the *A. annua* remains even after more than 2000 years. Moreover, they probably can also function against parasites or as repellent of tombs according to the record of *qinghao*'s usage of treating *jie* itches, *jia* itches, wounds, hemorrhoids, and bee stings as well as killing lice.

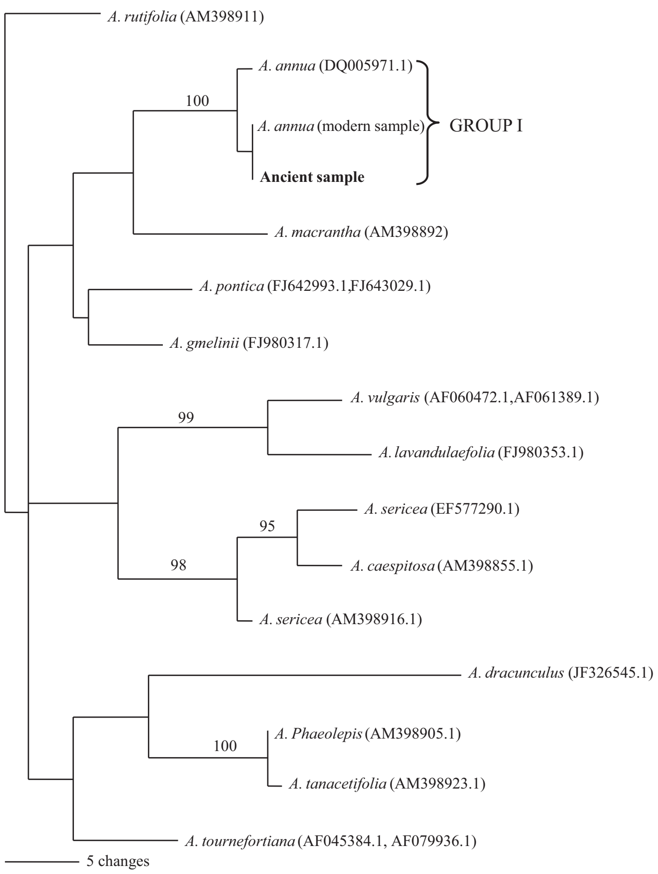

Fig. 6. One of the 2 maximum parsimony phylogenetic trees of *Artemisia* species based on the ITS1+ITS2 dataset (CI=0.705). Bootstrap proportions greater than 95% were displayed on the corresponding branches.

A significant amount of aromatic plants, like *Cinnamomum chekiangense* Nakai, *Hierochloe odorata* (L.) Beauv, *Eupatorium fortunei* Turcz., etc., have been unearthed from the famous Han tombs of Mawangdui aged about 2100 BP, Hunan Province, China, which have been considered acting the same way as the sweet wormwood remains in the present study, though some or all of them were additionally utilized as medicine (ECPC et al., 1978).

Another similar example is from the famous "Siberian Ice Maiden" unearthed from the Pazyryk Valley of the Ukok plateau in the Altai Mountains, Siberia. Some coriander seeds have been discovered in a stone dish of this Pazyryk grave dated about 2400 BP, which probably had been burned to mask the putrid odors of the deceased according to the excavators, as the corpse had not been inhumed until months after death. Similar dishes with coriander seeds have also been found in other Pazyryk tombs (Rudenko, 1970; Polosmak, 1998).

This funerary custom occurred in some areas of modern China before the practice of cremation and regionally diverse materials were employed. Alcohol was sprayed on the body before interment in some areas of northern China while tea was scattered at the bottom of coffin and on the corpse in Sangzhi, Yongshun and some other counties of Hunan Province, China. Besides, in Shaoyang city of Hunan, when coffin burial was still employed, the pillow for the deceased was usually packed with tea, which both implied the drinking tea for the departed in the afterworld and also served to eliminate the smell of the corpse (Zhu, 2005).

5.3. *Utilization of wild plant resources by ancient Subeixi aboriginals in Turpan*

The utilization of sweet wormwood in the Subeixi culture indicates a sound knowledge of its fragrance and utility among the Subeixi aborigines. Previous studies showed that the indigenous people of the Subeixi culture had significantly exploited wild plants in their vicinity around 3000–2000 BP. On top of the *A. annua* in the present study, they utilized *Cannabis sativa* L. and *Capparis spinosa* L. as medical plants, as well as *Phragmites australis* and some species of *Typha* sp. as sources of fiber (Jiang et al., 2006, 2007a; Jiang, 2010). Fruits of *Lithospermum officinale* L. were utilized by them for decorative purpose. Besides, some wild shrubs, i.e., *Lycium ruthenicum* and *Aeluropus pungens*, etc., were employed to shelter or pack tombs. The abundance of utilized wild plant species and varied use of these plants imply a deep understanding towards local wild plants of the Subeixi aborigines and a significant level of exploitation on plant resources.

6. Conclusion

The sweet wormwood remains recovered from the Shengjindian cemetery are the first record for ancient utilization of *qinghao*

Table 1
^{14}C-dating of plant, leather and stomach content samples from the Shengjindian cemetery.

Lab. no.	Sample	Tomb no.	^{14}C age (BP) ($T_{1/2}$=5568)	Dendrocalibrated, 1δ age ranges (68.2%)	Dendrocalibrated, 2δ age ranges (95.4%)
BA08016	Stomach content	M15	2080 ± 30	160BC(16.0%)130BC 120BC(52.2%)40BC	200BC(94.3%)20BC 10BC(1.1%)AD
BA08017	Wheat stalk	M10	2145 ± 35	350BC(17.0%)310BC 210BC(51.2%)110BC	360(25.6%)280BC 260BC(69.8%)50BC
BA08018	Leather artifact	M26	2115 ± 35	200BC(68.2%)90BC	350BC(5.2%)310BC 210BC(90.2%)40BC
BA08019	Wheat stalk	M13	2115 ± 35	200BC(68.2%)90BC	350BC(5.2%)310BC 210BC(90.2%)40BC
BA08020	Leather artifact	M20	2170 ± 35	360BC(37.3%)290BC 240BC(30.9%)170BC	370BC(95.4%)110BC

to date. However, they seem not to have been employed for medical purpose, but as a type of odor suppressant, providing a new perspective on the early use of this medical plant in ancient China. Meanwhile, the discovery of these *A. annua* remains further increases our knowledge about the significant utilization of local wild plants by the indigenous people of the Subeixi culture, which stands out distinctly as a typical feature of this culture.

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