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A new processing method for the pollen samples from Palaeogene red beds in the Liguanqiao Basin, Hubei Province, and Pleistocene loess from the Chinese Loess Plateau

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ABSTRACT

Over the past decade, palynoflora investigation of Palaeogene and Neogene red beds and Pleistocene loess has been the focus of an increasing number of studies. Pollen extraction from sediments is a prerequisite for palynoflora investigation. This study explored a new pollen processing method and successfully extracted a large number of pollen grains from the Palaeogene red beds in the Liguanqiao Basin, Hubei Province and the Pleistocene loess from the southern Chinese Loess Plateau. There are several key steps in this new method. (1) Samples from arid to semi-arid regions are usually well cemented and should be gently crushed and sieved with 30-mesh screen for pollen analysis to maximize the number of pollen grains recovered from sediments. (2) In order to remove organic matter most effectively, samples should be heated until just boiling in ~3% NaOH solution for not more than 5 min (3) The residue after acid–alkali treatment should be dried at 85 °C for 7–9 h to ensure that density of the heavy liquid is not diluted during the next step. (4) KI heavy liquid, with a density of 1.74–1.76, should be used to concentrate the pollen. (5) Sieving with a 7-μm stainless steel mesh resulted in the loss of few pollen grains. In contrast, sieving with a 10-μm nylon mesh resulted in loss of many small pollen grains. Importantly, this study extended the first appearance of *Artemisia* in China back to the Late Palaeocene, and is significant for vegetation reconstruction in the arid to semi-arid regions during the Cenozoic Era.

1. Introduction

Since the middle of the last century, studies of palynoflora have gradually become one of significant methods for vegetation and environment reconstruction. Pollen analysis is a key prerequisite for palynoflora investigations. Nevertheless, during the past several decades, Cenozoic palynoflora investigations were mostly focused on Late Quaternary lacustrine sediments in humid to semi-humid regions, because these sediments usually contain abundant pollen grains that are easily extracted (Fægri and Iversen, 1989). Thus, the pollen processing method for Late Quaternary sediments became relatively mature.

In recent years, however, studies on palaeoclimatic evolution shifted from humid to arid regions and extended from the Quaternary to the Palaeogene, such as tracking the Cenozoic history of desertification in Central Asia (e.g. Guo et al., 2002; Jiang and Ding, 2008, 2010; Jiang et al., 2008). This shift in depositional environment brings new challenges to pollen workers. Neogene and Palaeogene red beds are distributed widely around the world, especially in the Northern Hemisphere, and reflect significant oxidizing environments during sedimentation, which is not ideal for the preservation of pollen grains. Accordingly, a few pollen workers attempted to separate pollen grains from the sediments of red beds (e.g. Wei et al., 2001; Yu et al., 2003) but did not achieve satisfactory results (e.g. Jiang and Ding, 2008; Tang et al., 2011), which has limited vegetation reconstruction in East Asia to a great degree (Jiang and Ding, 2005, 2009) and even around the globe (Utescher and Mosbrugger, 2007).

Most published works are centered on Neogene coal beds and gray-greenish lacustrine sediments (Fig. 1). In contrast, palynoflora investigations on Palaeogene red beds are few. Therefore, new methods were explored to extract pollen from the Palaeogene red beds in the Liguanqiao Basin in Hubei Province and Pleistocene loess in the southern Chinese Loess Plateau, with the goal to separate as many pollen grains as possible from a quantitative sample. The results are not only helpful for creating a more realistic

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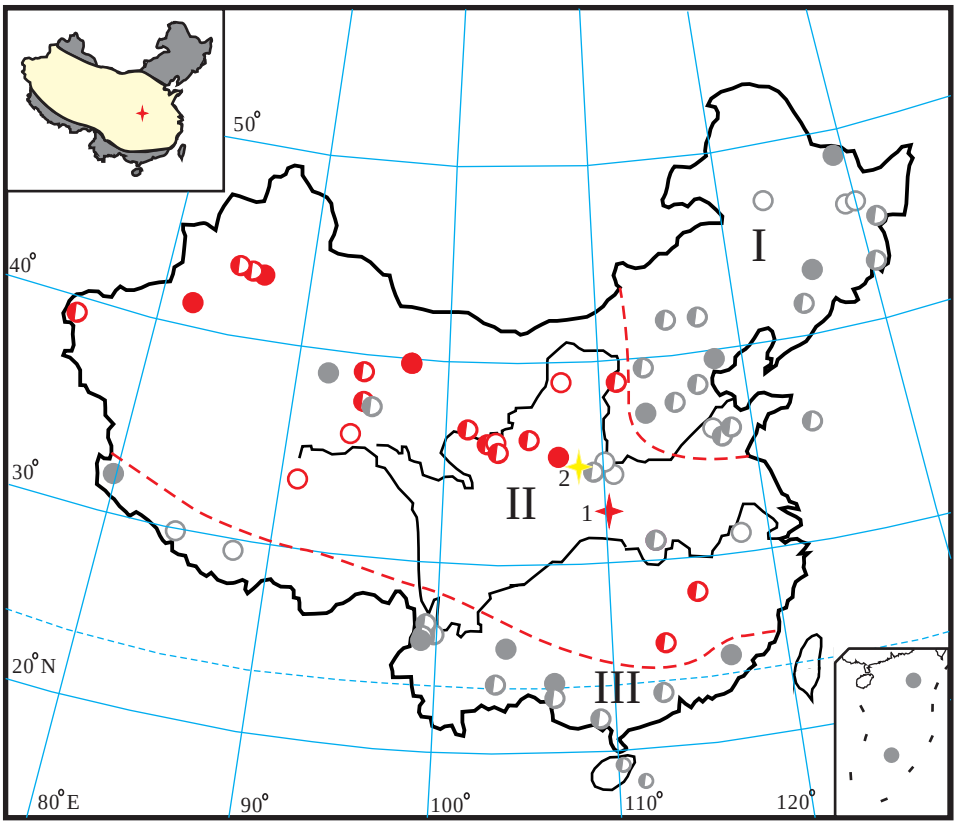

Fig. 1. Distribution of 64 Neogene and Paleogene pollen sites collected in China. Zones I and III indicate relatively humid zones while II indicates a relatively arid to semi-arid zone under the control of the planetary wind system during the Palaeogene (Liu et al., 1998; Sun and Wang, 2005). Gray and red circles represent grayish to black sediments and brown to red sediments, respectively. Solid and semi-open circles represent abundant and few to no pollen grains extracted, respectively. Red (Xijiadian section in the Liguanqiao Basin, Hubei Province) and yellow (Lintong site in the southern Chinese Loess Plateau) stars indicate both localities in this study. This map displays the few investigations of Neogene and Palaeogene palynoflora in China, especially for red sediments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pollen diagram, but also have great significance for investigations on the AMS ^{14}C dating method with pollen concentrates.

2. Study site and material

The Palaeogene Xijiadian section is located in the western Liguanqiao Basin, northwestern Hubei Province, China. The exact latitude and longitude (GPS data) of the bottom and top of the sampled section are 32°46.008′N, 111°11.276′E and 32°48.570′N, 111°12.642′E, respectively. The local elevation ranges from 205 to 418 m. The lithology of the section is mainly lacustrine shore sediments, consisting of grayish and reddish mudstone, muddy siltstone, siltstone, and occasionally interbedded with coarse sandstone and conglomerate (Fig. 2). Pollen samples were collected from mudstone and siltstone. In addition, Pleistocene loess sediments from the southern Chinese Loess Plateau (34.2°N, 109°E) were also sampled for comparison.

3. New method of pollen analysis

Though red beds represent a kind of oxidizing environment and are commonly called ‘dumb strata’ (Song, 1958), recently a few studies have made some useful explorations (e.g. Ma et al., 2005; Jiang and Ding, 2008; Miao et al., 2008; Tang et al., 2011). The processing methods can be roughly divided into two types: heavy liquid separation and sieving (Table 1). Unfortunately, most of the methods have not produced satisfactory results. For example, Song (1958) extracted some pollen grains from 21 of 150 total samples from red beds in the Jiuquan Basin, NW China. Recently, Ma et al. (2005) used sieving in place of heavy liquid separation, and were able to count more than 100 pollen grains from 597 of 615 total samples.

As previously mentioned, the methods for extracting pollen grains from Late Quaternary lacustrine sediments are relatively mature. Typically, different investigators treat 1–30 g of sediments using conventional acid-alkali treatment and heavy liquid separation/sieving, and achieve satisfactory results (Table 2). In recent years, pollen separation methods for Pleistocene loess sediments in the Chinese Loess Plateau also received extensive attention (e.g. Ke, 1994; Li and Du, 1999; Li et al., 1999; Li et al., 2003; Li and He, 2004; Li et al., 2006). Li and He (2004) suggested that loess samples should be treated repeatedly with HCl and HF during preparation until the silicate and carbonate was entirely dissolved. The residue was then sieved with a 10-μm nylon mesh, resulting in the separation of many pollen grains. In contrast, Ke (1994) chose routine acid-alkali treatment and heavy liquid separation with a specific gravity of 2.2. Although these methods were successful, they are relatively complicated and require a larger sample volume, encouraging the search for a more effective method.

Based on many experiments and a detailed comparison with previous methods, a new pollen processing method was developed (Fig. 3 and Table 3). Samples from the Palaeogene red beds from the Xijiadian section are usually well cemented by carbonate. First, the samples are gently crushed and then sieved with 30-mesh screen.

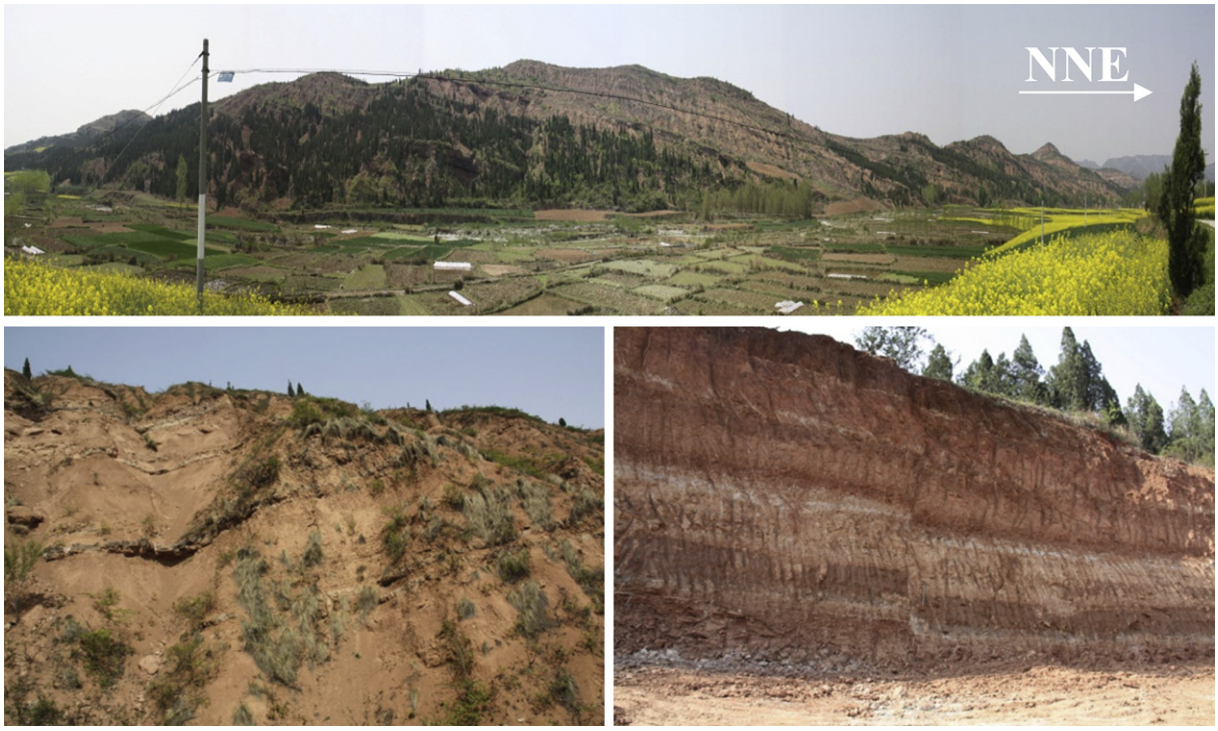

Fig. 2. Three representative pictures of the Xijiadian section in the Liguanqiao Basin, Hubei Province.

In contrast, Pleistocene loess is often uncemented and does not need to be crushed. Approximately 80 g is used for analysis from each sample. Next, 15% HCl was added to digest carbonate, and was added until samples no longer had a reaction. Third, in order to remove organic matter effectively, the samples were heated until boiling in ~3% NaOH solution for not more than 5 min. Fourth, the remaining residue after the acid-alkali treatment was dried at 85 °C for 7–9 h (Fig. 3 and Table 3) to ensure the exact density of the heavy liquid used in the next step. Fifth, KI heavy liquid separation was used with a specific gravity of 1.74–1.76 to concentrate the pollen, followed by a 40% HF bath to digest the silicate for 2 h. The samples were then immersed in a 15% HCl bath for 2 h to remove calcium fluoride that may have formed during the HF bath. All samples were centrifuged and washed with distilled water following each step. Finally, the residue was visually examined under a light microscope to ensure that the residue did not contain abundant non-pollen particles. If non-pollen particles were present, the residue was cleaned by sieving with a 7-μm stainless steel mesh (Fig. 3 and Table 3).

4. Results

One hundred and thirty-seven samples were collected from the Xijiadian section in the Liguanqiao Basin, Hubei Province and thirty-five samples were collected from the southern Chinese Loess Plateau. All of the 172 samples were processed following the above method, and each sample contained pollen grains. Further observation under a light microscope revealed abundant pollen grains with clear textures in most samples, such that they could easily be compared with officially published pollen plates and modern pollen slides preserved in the Institute of Geology, China Earthquake Administration (Appendixes A and B). Accordingly, natural nomenclature was temporarily applied to most pollen grains separated from the Palaeogene red beds of the Xijiadian section (Kong et al., 1976).

4.1. Palaeogene red beds

Though no *Lycopodium* marker-grains were added as a spike to the samples before processing, about 200–400 pollen grains could

Table 1
Two parallel methods for extracting pollen from red beds. Most studies produced unsatisfactory results.

Method	Site	Age	Weight (g)	Procedure	Result (pollen sum/pollen-bearing samples/total samples)	Reference
Heavy liquid flotation (HLF)	Jiuquan Basin	Tertiary		Acid–alkali, HF, HLF	A small quantity/18/120	Song, 1958
	Northern Tibetan Plateau	Tertiary	120	Acid–alkali, HLF (sg 2.24, 5–6 times in the volume of the sample), HF	>20 pollen grains in each slide	Wei et al., 2001
	Sikouzi Basin	20.13–0.07 Ma	100	HCl, K_2CO_3, HF, HCl, HLF (sg 2.0)	>100/104/364	Jiang and Ding, 2008
HF and sieving	Jiuquan Basin	40.2–33.4 Ma	50–400	HCl, HF, sieving (10 μm)	>100/122/259	Miao et al., 2008
	Jiuxi Basin	13–2.21 Ma	100–150	HCl, HF, sieving	>100/597/615	Ma et al., 2005
	Northern Tian Shan	28–4.2 Ma	100	HCl, HF, sieving (10 μm) and/or HLF (sg 2.0)	>100/154/292	Tang et al., 2011
	Jidong Basin	Mid-to-Late Miocene	30–50	HCl, NaOH, HF, HCl, sieving (10 μm)	>150/68/72	Shu et al., 2008

Table 2
Conventional methods for extracting pollen grains from Late Pleistocene and modern sediments.

Age	Weight (g)	Lithology	Method	Specific gravity of heavy liquid	Reference
Modern	6–15	Surface sediment	HCl, sieving (350 μm), HF, sieving (7 μm)		Pan et al., 2008
Modern	10–15	Surface lake sediment	HCl, KOH, HF, sieving (7 μm)		Huang et al., 2004
16.7 ka BP	1	Borehole lake sediment	HCl, KOH, acetolysis, sieving (160 μm, 7 μm)		Tao et al., 2010
7.0 ka BP	5	Borehole sediment	HCl, HF, HLF, acetolysis	2.0	Yi et al., 2005
4.5 ka BP	30	Peat	HCl, NaOH, HLF, acetolysis	2.0	Zhang et al., 2009
30 ka BP	3 ± 0.3	Marine sediment	HCl, HF, KOH, sieving (7 μm)		Xu et al., 2010
24 ka BP	4–8	Marine sediment	HCl, HF, HLF	2.0	Xu et al., 2009
30 ka BP	~3	Marine sediment	HCl, HF, HLF	2.2	Li et al., 2008

be counted from half a piece of cover glass (22 × 22 mm) under a light microscope with 20 × 10 times. The pollen assemblages were dominated by arboreal plant taxa. Usually present taxa included *Pinus*, *Abies*, *Picea*, *Tsuga*, *Quercus*, *Castanea-Castanopsis*, *Betula*, *Carpinus*, *Alnus*, *Juglans*, *Ulmus*, *Corylus*, Ericaceae, *Artemisia*, Cyperaceae, Chenopodiaceae, Gramineae, *Polygonum*, *Polypodium*, and *Sphagnum* (Appendix A).

The origin and development of *Artemisia* (Asteraceae) in Asia was tracked and discussed during the past decade (e.g. Song et al., 2004; Miao et al., 2011). The first appearance of *Aster* pollen was in the upper part of the Lulehe Formation in the Qaidam Basin during the Palaeocene-Eocene (Song et al., 2004). Recently, a detailed comparison of many pollen records indicated that *Artemisia* was first present in the arid to semi-arid Central Asia in the Late Eocene and became abundant since the Pliocene (Miao et al., 2011). In this study, *Artemisia* was commonly observed at the bottom of the Xijiadian section. Mammal fossils of *Asiocoryphodon conicus*, *Rhombomylus* cf. *turpanensis*, *Rhombomylus* sp. and *Advenimus hubeiensis* were discovered at the Qingtangling and Dajian sites in previous studies, suggesting the bottom part of the Xijiadian section was late Early Eocene (Du et al., 1991; Ma and Cheng, 1991). A preliminary palaeomagnetic study suggests that the Xijiadian section was deposited since the Late Palaeocene (Jiang et al., submitted for publication). Previous studies suggested that a wide arid to semi-arid zone dominated Asia during the Palaeogene (e.g. Liu et al., 1998; Sun and Wang, 2005), and probably provided favorable conditions for the Late Palaeocene development of *Artemisia* in East Asia.

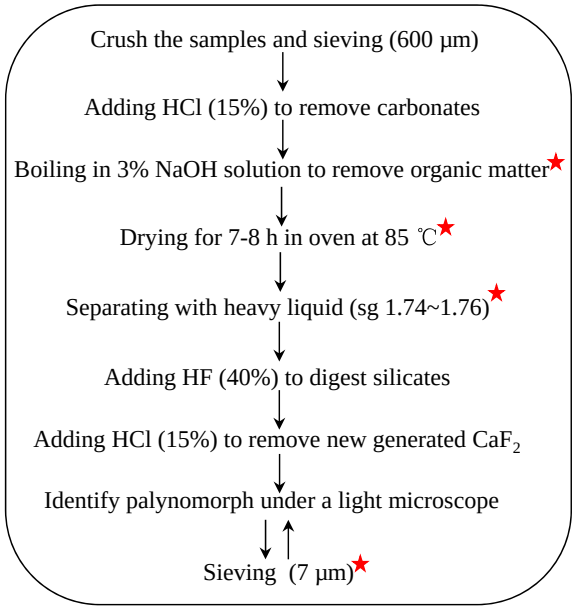

Fig. 3. Procedures for extracting palynomorphs (pollen and spores) from red sediments and Quaternary loess. Red stars denote key steps. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.2. Pleistocene loess sediments

Each sample from the Pleistocene loess sediments, of the 35 samples analyzed, had enough pollen grains (>200) for a reasonable statistical analysis. In general, the pollen assemblages are dominated by deciduous broadleaved trees and herbs. Main taxa included *Pinus*, *Picea*, *Abies*, *Tsuga*, *Quercus*, *Castanea*, *Betula*, *Carpinus*, *Juglans*, *Ulmus*, *Tilia*, *Corylus*, *Ephedra*, Ericaceae, *Artemisia*, Cyperaceae, Umbelliferae, Gramineae, Chenopodiaceae, Compositae, Polygonaceae, *Sanguisorba*, *Thalictrum*, *Myriophyllum*, *Typha*, *Polypodium*, *Selaginella*, *Pediastrum*, *Zygnema*, and *Concentricystes* (Appendix B).

5. Discussion

As noted by Lignum et al. (2008), the standard palynological processing and separation methods are still highly variable in terms of initial sample sizes, type and concentration of acids, sieve material and mesh size, use of heavy liquids, and different sequences of sample treatment. Thus, many pollen workers explored different processing methods for different samples to produce the best results (e.g. Li and Du, 1999; Li et al., 1999, 2006; Ma et al., 2005; Riding and Kyffin-Hughes, 2006, 2010; Riding et al., 2007). In this study, a heavy liquid separation with a specific gravity of 1.74–1.76 was combined with sieving with a 7-μm stainless steel mesh to extract large amounts of pollen grains from both Palaeogene red beds in the Liguanqiao Basin, Hubei Province and Pleistocene loess from the southern Chinese Loess Plateau (Appendixes A and B). Among these pollen grains, some are larger in size, such as *Pinus* and *Abies*, whereas others are smaller such as *Artemisia* and *Castanea*. These small pollen grains are easily lost during conventional processing courses. Compared with previous results, this processing method made some effective and important changes.

5.1. Crush samples

Samples from arid to semi-arid regions are usually well cemented and should be gently crushed and sieved with a 30-mesh screen (Li et al., 1995). The goal during this step is to maximize the surface area in contact with HCl, and to minimize any mechanical damage to pollen grains. Specifically, if a pollen sample is crushed too fine, extracted pollen grains are usually too broken and incomplete for proper identification. On the contrary, if a pollen sample is crushed too coarse, a long time will be required for routine acid-alkali treatment. In addition, the acid reaction of coarse pollen samples is commonly incomplete, even if bubbles are no longer observed.

Table 3
Detailed steps used in the heavy liquid separation protocol in this paper.

1a	Crush samples and sieving (600 μm)
1b	Weigh about 80 g of sediment for each sample and place in a 1000 ml beaker
2a	Remove carbonates – gradually add HCl (15%) to beakers and stir
2b	Periodcally stir and add further HCl (15%) and stir
2c	Allow to stand, perferably overnight after reaction stops to make sure complete removal of carbonate
2d	Top up with distilled water and stir, then allow to stand no less than 7 h
2e	Suck the supernatant with rubber hoses, 1–2 cm supernatant left
2f	Repeat 2d–2e to it's chemically neutral
3a	Remove humics – add NaOH to make sure the concentration of 3% and stir
3b	Boiling on the electrothermal oven and stir
3c	Top up with distilled water and stir, then allow to stand no less than 7 h
3d	Suck the supernatant with rubber hoses, 1–2 cm supernatant left
3f	Repeat 3c–3d to it's chemically neutral
4a	Decant supernatant and transfer residue to 400 ml centrifuge tube, centrifuge at 2500 rpm for 10 min, decant supernatant
4b	Drying samples, needing about 7–9 h in oven at 85 °C
5	Heavy liquid flotation twice (sg 1.74–1.76, double in the volume of the sample)
5a	Add a little KI heavy liquid to soak sample and stir
5b	Add further heavy liquid and stir carefully until double in the volume of the sample
5c	This was followed by ultrasonic for several minutes under 80–100 W
5d	Centrifuge at 2500 rpm for 15 min until impurities deposit
5e	Transfer the supernatant to a clean 1000 ml beaker
5f	Repeat 5a–5d and transfer the supernatant to the corresponding beaker
5g	Add 1% acetic solution and stir
5h	Centrifuge at 2500 rpm for 10 min and transfer the supernatant to the corresponding beaker
5i	Add more acetic solution until 2–3 times in the volume of the heavy liquid to lower the specific gravity to <1.3 and stir
5j	Allow to stand more than 20 h
6a	Suck the supernatant with a rubber hose, transfer the residue to a 400 ml centrifuge tube, centrifuge at 2500 rpm for 10 min
6b	Decant supernatant and transfer the residue to a 15 ml centrifuge tube, centrifuge at 2500 rpm for 10 min and decant supernatant
7a	Add HF (40%), stir carefully, cold treatment for 2 h
7b	Top up with distilled water, centrifuge at 2500 rpm for 10 min and decant supernatant
7c	Add HCl (15%), stir carefully, cold treatment for 2 h
7d	Top up centrifuge tubes with distilled water, centrifuge at 2500 rpm for 10 min and decant supernatant
7e	Repeat last step to it's chemically neutral
8a	Sieve (7 μm, stainless steel sieve) with ultrasonic for several minutes under 80–100 W
8b	Transfer the residue to a 15 ml centrifuge tube, centrifuge at 2500 rpm for 10 min and decant supernatant
9	Add glycerol, remove some of the sample with a glass stick to slide and identify palynomorph

Table 4
Counts of pollen grains of the <7 μm and 7–10 μm factions.

Sample	XJD105		XJD116	
Sieve	<7	7–10	<7	7–10
Artemisia	1	40	6	35
Chenopodiaceae		9		6
Gramineae		3		2
Cyperaceae		3		6
Leguminosae		3		
Compositae		1		3
Umbelliferae		1		2
Thalictrum				1
Balsaminaceae				1
Loranthaceae				1
Solanaceae				1
Myriophyllum				3
Tilia				1
Castanea	7	20	1	15
Quercus		13		15
Alnus		3		2
Betula		15		25
Corylus				4
Ephedra		1		1
Pinus		2		10
Cupressaceae				2
Picea				3
Abies				1
Polypodium				3
Zygnema		2		1
Concentricystes				1
Sphagnum				7
Total	8	116	7	152

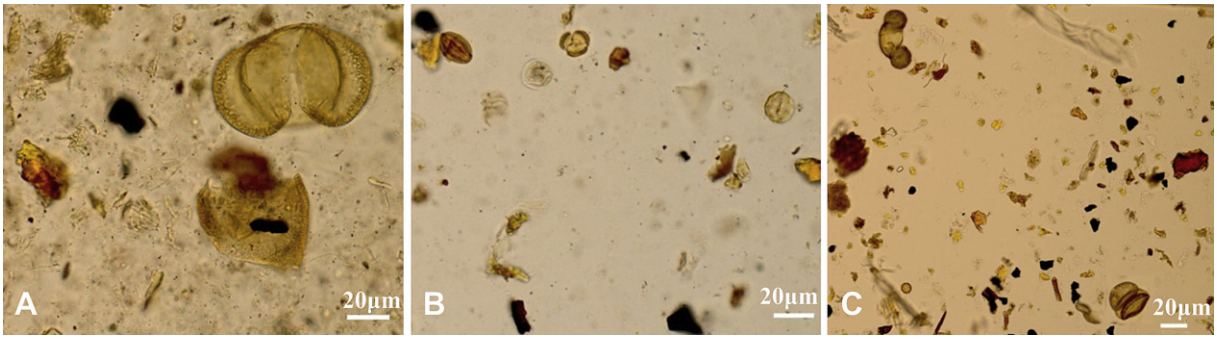

Fig. 4. Comparison of three representative pictures with different sieving. (A) No sieving with all kinds of pollen grains and impurities, (B) Sieving with a 7-μm stainless steel mesh and a 10-μm nylon mesh and the 7–10 μm fraction, (C) Sieving with a 10-μm nylon mesh and the >10 μm fraction.

5.2. Acid–alkali treatment

The acid-alkali treatment is the second key step following crushing of the sample. It is essential to complete the acid-alkali treatment properly because it directly determines whether pollen grains could be extracted from a sample. In this study, some comparative experiments were conducted following previous experimental procedures (e.g. Wang and Xu, 1988; Li et al., 1995) and found several important aspects. (1) The purpose of the alkali treatment is to remove organic matter and newly-generated humic acid, but NaOH treatment easily destroys pollen grains (e.g. Havinga, 1967, 1984; Zhu, 1982; Lignum et al., 2008). Some pollen processing methods for Quaternary peat samples added 10% NaOH and boiled for 5–15 min (e.g. Lentfer and Boyd, 2000; Vandergoes and Prior, 2003; Yi et al., 2003). When this step was adopted in the processing method for the Palaeogene red beds and the Pleistocene loess, the pollen grains were almost completely destroyed. Repeated experiments indicated that pollen grains were properly achieved by using NaOH solution with a low concentration (~3%) and heating until the sample just begins to boil (≤5 min) for samples from the Palaeogene red beds and Pleistocene loess. (2) After the alkali treatment, some samples were turbid for more than 10 h, which was probably caused by the formation of colloids. Quartz, alumina and some other minerals easily form mineral colloids in a weak alkaline environment (Xia, 1995). On the other hand, dense humus in a sample reacts with the alkali solution to form organic colloids (Li and Du, 1999). In most cases, a small amount of calcium acetate solution could destroy the colloids (Li and Du, 1999). In this study, the upper turbid liquid, after precipitation for 7 h, was sieved with a 7-μm stainless steel mesh, and no pollen grains were found. Thus, continuous turbid colloids in some samples of loess or red beds after alkali treatment did not influence the precipitation of pollen grains. (3) Compared to peat samples containing abundant pollen grains, samples from Pleistocene loess and Palaeogene red beds should not be treated with HF, because the large sample volumes require high concentrations of HF to digest. HF creates smaller silica particles at first, but repeated treatment with HCl and HF would dissolve all silica particles entirely but at the cost of destroying pollen grains (Li, 1984). Finally, the remaining residue after HF treatment is often so much that it takes longer to sieve, resulting in further loss of pollen grains.

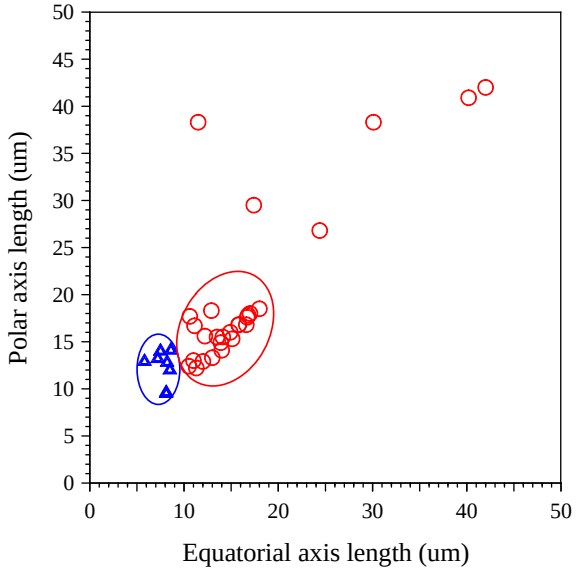

Fig. 5. Polar axis length plotted against equatorial axis length of pollen grains for the <7 μm (blue triangles) and 7–10 μm (red circles) fractions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

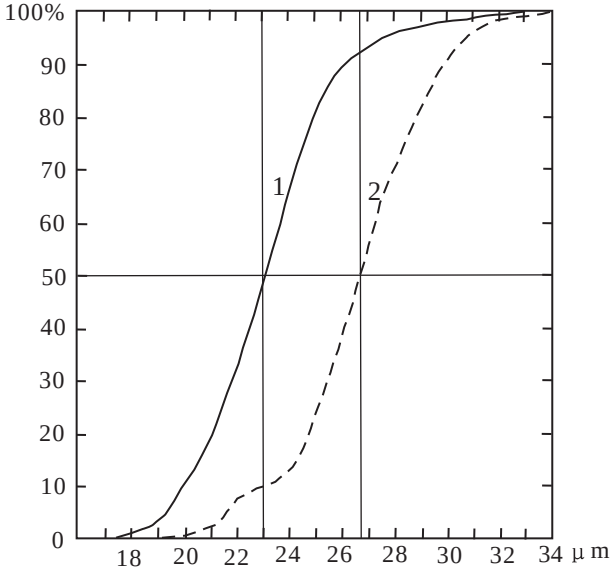

Fig. 6. Cumulative curves on size frequency showing shrinkage of fossil pollen (*Alnus*) owing to HF treatment. Curve 1: with HF treatment; Curve 2: without HF treatment (modified from Sato, 1963). Note that the size of fossil pollen grains treated with HF is 15% smaller than those untreated.

5.3. Specific gravity of heavy liquid

Funkhouser and Evitt (1959) reported that density of pollen grains varied between 1.3 and 1.7. Thirty years later, Fægri and Iversen (1989) found that pollen density fluctuated from 1.42 to 1.70. Thus, pollen density is generally less than 1.7 and in theory, a heavy liquid with a specific gravity of >1.7 could separate pollen grains from samples. It is puzzling, however, that the specific gravity of heavy liquid used by many pollen researchers is often much greater than 1.7, often as high as 2.0 (e.g. Jiang and Ding, 2005; Xu et al., 2009; Zhang et al., 2009) and even 2.2–2.4 (e.g. Ke, 1994; Wei et al., 2001; Li et al., 2008; Kezina, 2011; Yi et al., 2012). The main reason a denser heavy liquid is used is because the sample, after routine acid-alkali treatment, usually contains so much water that direct separation with heavy liquids would significantly dilute the heavy liquid. The previously mentioned researchers used a heavy liquid with a greater specific gravity (≥2.0) in order to ensure that actual density of the heavy liquid mixed with a sample is not less than the pollen density (1.7). Nevertheless, the greater the density difference between the heavy liquid and the pollen, the more non-pollen particles are extracted from the sample. The addition of non-pollen particles would significantly influence both the identification of pollen grains under a microscope and the AMS ^{14}C dating with pollen concentrates due to contamination. Therefore, using the correct specific gravity is pivotal for a successful heavy liquid separation.

In this study, the residue after routine acid-alkali treatment was dried at 85 °C for 7–9 h. Then KI heavy liquid, with a specific gravity of 1.74–1.76, was added to the dry residue for pollen separation. Under a light microscope, 215 pollen grains were counted from one cover glass after the first separation and only 3 pollen grains from two cover glasses after the second separation. Hence, the first separation is the most effective and the second is supplementary. For comparison, a heavy liquid with a specific gravity of 1.82–1.84 was used. Abundant non-pollen particles were found in the final pollen sample.

5.4. Sieving

Samples from Palaeogene red beds and Pleistocene loess usually contain large amounts of clay minerals and non-pollen particles that often become smaller after HF treatment, which can then be removed through sieving. Most pollen researchers currently use a nylon sieve with a 10-μm mesh (Table 1) (e.g. Yi et al., 2003, 2005; Miao et al., 2008; Shu et al., 2008; Tang et al., 2011). Others also use a sieve with a 7-μm mesh (e.g. Huang et al., 2004; Pan et al., 2008; Tao et al., 2010; Xu et al., 2010) and sometimes as small as a 5-μm mesh (e.g. Scourse et al., 2005). This study experimented with two kinds of sieves. One kind of sieve is stainless steel with a 7-μm mesh and the other is nylon with a 10-μm mesh. Two pollen samples were sieved from Palaeogene red beds in the Liguanqiao Basin, Hubei Province, and then counted the pollen grains of the 7–10 μm and <7 μm fractions, respectively (Table 4 and Fig. 4).

In the <7 μm fraction, *Castanea* and *Artemisia* were uncommonly found from two cover glasses. Their polar axis and equatorial axis varied in length from 9.5 to 14.1 μm and from 5.8 to 8.6 μm, respectively (Fig. 5). In the 7–10 μm fraction, many pollen grains were counted from one cover glass, including *Artemisia*, *Castanea*, *Betula*, *Quercus* and others (Fig. 4B). Mean values of the polar axis and equatorial axis for these pollen grains centered around 12–19 μm and 10–18 μm, respectively (Fig. 5). Individual pollen grains that were larger than these dimensions were rare. This experiment suggests that sieving with a stainless steel sieve with a 7-μm mesh resulted in almost no loss of pollen grains. By comparison, there was a significant loss of small pollen grains when sieving with a nylon sieve with a 10-μm mesh, resulting in decrease of the percentage of small pollen grains and a distortion of pollen diagram (Fig. 4B and C).

There are probably several reasons for the loss of small pollen grains. First, two pictures were taken, under a light microscope, of the stainless steel sieve with a 7-μm mesh and the nylon sieve with a 10-μm mesh, respectively (Appendix C). The mesh of the stainless steel sieve has a uniform diagonal length of ~9.9 μm and is distributed regularly. The mesh of the nylon sieve has an average diagonal length of ~14.1 μm, but is distributed irregularly (Appendix C). Thus, the mesh size of nylon sieve would be greater than 10 μm when used in an ultrasonic oscillator (Scourse et al., 2005). Second, previous studies indicated that pollen grains would shrink ~15% in volume after HF treatment owing to removal of inorganic matter (Sato, 1963, Fig. 6), and increasing the loss of small pollen grains. Finally, sieving for an extended period of time gradually corrodes the screen cloth and the mesh size would gradually become larger, resulting in the loss of larger pollen grains as well (Lignum et al., 2008) (Fig. 5). Therefore, sieving with a nylon sieve with a 10-μm mesh likely causes a significant loss of small pollen grains, and stainless steel sieves should be used during the pollen processing method.

6. Conclusion

Based on these experiments and a detailed comparison with previous methods, several important steps are proposed to extract pollen grains from Palaeogene red beds in the Liguanqiao Basin, Hubei Province and Pleistocene loess in the southern Chinese Loess Plateau. (1) Samples from arid to semi-arid regions should be gently crushed and sieved with 30-mesh screen. (2) ~3% NaOH solution should be added to the sample, and then heated until just boiling (≤5 min). (3) The residue after acid-alkali treatment should be dried at 85 °C for 7–9 h (4) KI heavy liquid, with a density of 1.74–1.76, should be used to concentrate pollen. (5) Sieving with a 7-μm stainless steel mesh results in almost no loss of pollen grains. This modified method is widely applicable for red beds and Pleistocene loess, and thus is significant for environmental reconstruction of arid to semi-arid regions in Asia.

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Appendix A

Representative photographs of pollen grains separated from Paleogene red sediments of the Xijiadian section in the Liguanqiao Basin. 1. *Tsuga*; 2. *Picea*; 3. *Pinus*; 4. *Ulmus*; 5. *Liquidambar*; 6, 35. *Quercus*; 7. *Elaeagnus*; 8. *Carya*; 9. *Betula*; 10. *Corylus*; 11. Ericaceae; 12–13. *Ephedra*; 14. *Aster*; 15, 28–29. Chenopodiaceae; 16, 30. *Myriophyllum*; 17. Cyperaceae; 18. Gramineae; 19. Polypodiaceae; 20. *Selaginella sinensis*; 21. *Sphagnum*; 22, 31–33. *Artemisia*; 23–27. *Catanea*; 34. *Typha*; 36. *Alnus*; 37. Compositae. All scale bars are 10 μm. Note that small pollen grains from 23 to 37 are even <10 μm in length.


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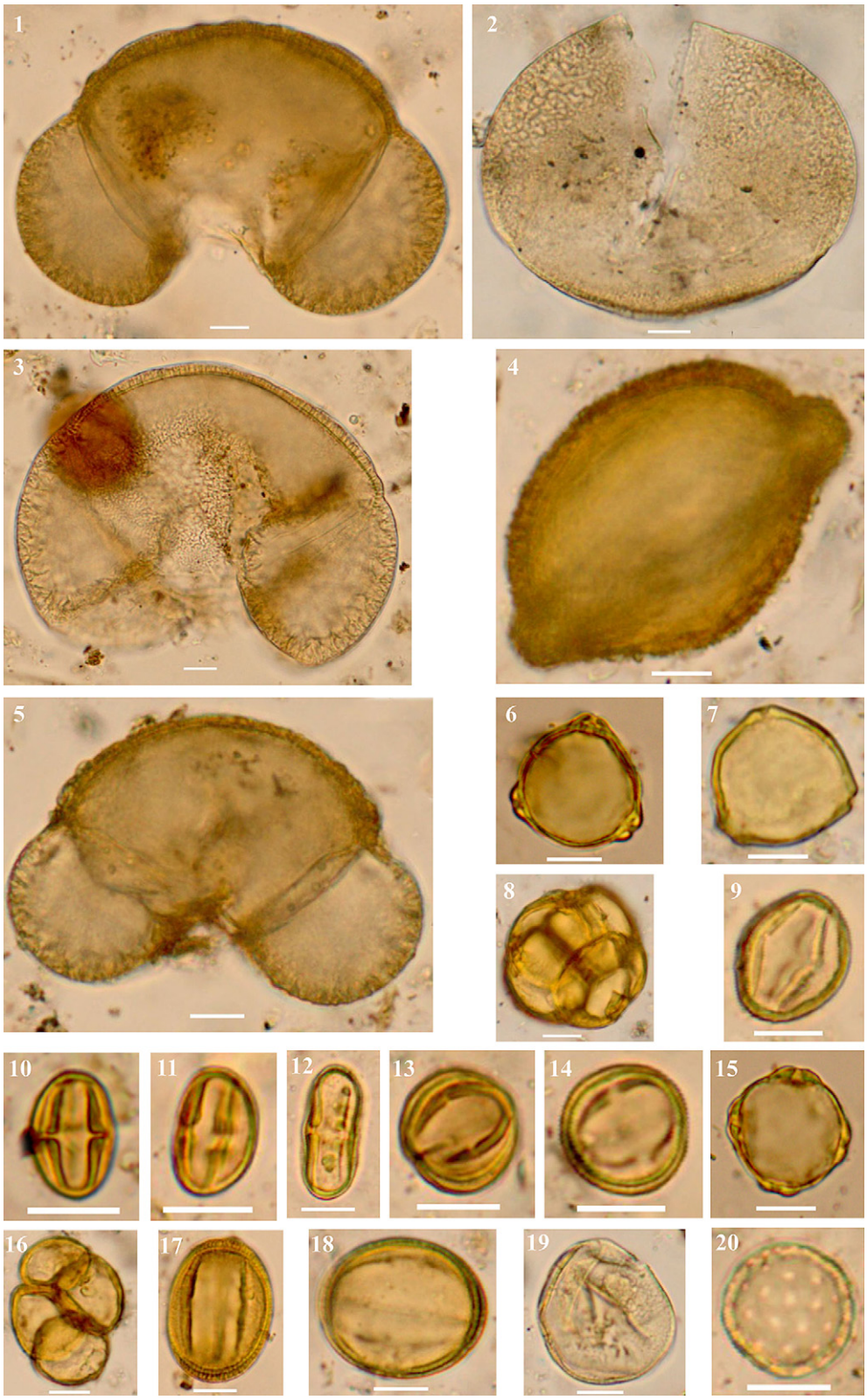
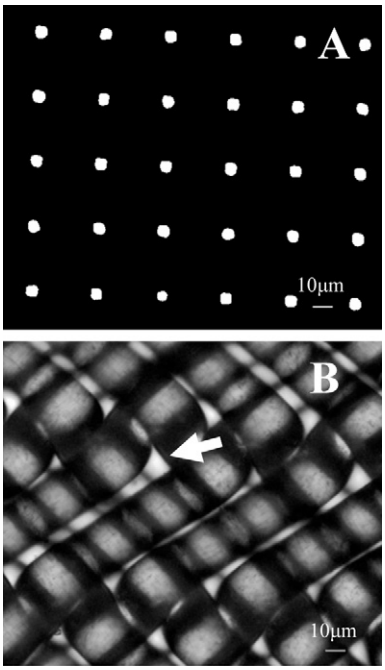
Appendix B

Representative photographs of pollen grains from Quaternary loess in the southern Chinese Loess Plateau. 1. *Abies*; 2–3. *Picea*; 4. *Tsuga*; 5. *Pinus*; 6. *Betula*; 7. *Corylus*; 8. Ericaceae; 9. *Quercus*; 10–11. *Castanea*; 12. Umbelliferae; 13–14. *Artemisia*; 15. *Myriophyllum*; 16. *Typha*; 17. *Polygonum*; 18. *Fagopyrum*; 19. Cyperaceae; 20. Chenopodiaceae. All scale bars are 10 μm.



Appendix C

Light microscope images of a 7-μm stainless steel mesh (A) and a 10-μm nylon mesh (B). The arrow denotes that the 10-μm nylon mesh has uneven apertures.



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