

DOI: 10.19615/j.cnki.1000-3118.190327

A preliminary study of serial stable isotope analysis tracks foraging ecology of fossil Asian elephants in South China

MA Jiao^{1,2} WANG Yuan^{1,3} JIN Chang-Zhu¹ ZHANG Han-Wen^{4,5} HU Yao-Wu^{1,2*}

(1 Key Laboratory of Vertebrate Evolution and Human Origins of Chinese Academy of Sciences, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences Beijing 100044, China

* Corresponding author: ywhu@ucas.ac.cn)

(2 Department of Archaeology and Anthropology, University of Chinese Academy of Sciences Beijing 100049, China)

(3 CAS Center for Excellence in Life and Paleoenvironment Beijing 100044, China)

(4 School of Earth Sciences, University of Bristol Bristol BS8 1RJ, UK)

(5 Earth Sciences Department, Natural History Museum London SW7 5BD, UK)

Abstract Until now, feeding ecology has been found to play a significant role in the evolution of Asian elephant *Elephas maximus*. As the most widely-applied method in this field, bulk stable isotope analysis on tooth enamel had revealed important evidence on their paleodiet and paleoecology. However, it might be not skilled at reflecting the overview of the paleoecology of elephants, considering their huge tooth morphology and long dental ontogeny process. A newly-developing serial sampling strategy on tooth enamel sections could provide an effective approach to reconstruct the long-term individual life history of mammals covering the whole tooth formation time with higher precision. In this study, serial sampling isotope analysis was firstly undertaken on tooth enamel of Asian elephants from Baxian Cave, South China during the Late Pleistocene. The within-tooth isotopic variations of three teeth (one DP4 and two M1s) are all surprisingly subtle (standard deviations of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values are all less than 0.6‰), though some obvious variations might be caused by weaning and/or possible migration. No seasonal variation was observed, possibly indicating that these elephants had a stable foraging ecology. Back to our previous bulk tooth enamel isotope analysis on this same site, we could confirm that the varied bulk isotope results of Asian elephants factually represent their flexible foraging ecology. We may thereby conclude that the increasing bulk isotopic analysis in this region can provide a reliable paleoecological proxy for Pleistocene proboscidea in the warm regions of South and Southeast Asia.

Key words South China, Late Pleistocene, *Elephas maximus*, stable isotopes, serial sampling, tooth enamel, foraging ecology

Citation Ma J, Wang Y, Jin C Z et al., 2019. A preliminary study of serial stable isotope analysis tracks foraging ecology of fossil Asian elephants in South China. *Vertebrata Palasiatica*, 57(3): 225–240

国家自然科学基金(批准号: 41773008, 143109, 41872022)和国家重点基础研究发展规划项目(编号: 2015CB953803)资助。

收稿日期: 2019-01-04

1 Introduction

The Asian elephant is one of the largest terrestrial animals in the world today, and is now an endangered species sporadically distributed in South and Southeast Asia (Maglio, 1973; Shoshani and Eisenberg, 1982; Shoshani and Tassy, 1996). They possess highly-evolved hypsodont molars with strong chewing ability, which are suggestive of adapting to a diet dominated by abrasive vegetation (Maglio, 1973; Sukumar, 2006; Sanders, 2018). It is generally believed that modern *Elephas maximus* is broadly speaking, a mixed browser and grazer, with a large dietary breadth depending on seasonality and geography (Sukumar and Ramesh, 1995; Cerling et al., 1999; Pradhan et al., 2008; Ahrestani et al., 2016; Baskaran et al., 2018a). However, based on the previous stable isotopic data, their diet had varied greatly from C₄ to a C₃-dominated vegetation during their evolutionary history (Cerling et al., 1999; Patnaik et al., 2014; Ma et al., 2017; Patnaik, 2017).

Even though the bulk tooth enamel isotopic analysis has been effectively applied on fossil mammals for several decades, it still has some intrinsic limitations on megaherbivores. Of particular note here is the fact that elephantid molars are morphologically huge and formed during a long and continuous process of dental ontogeny (Laws, 1966; Roth and Shoshani, 1988; Hillson, 2005; Sanders, 2018). Some researchers suggested to obtain bulk samples covering the whole range of tooth height to average the different results (Pederzani and Britton, 2019), but it is not practical for elephants considering the damage to the specimen and the contaminations on the whole surface. Therefore, it is possible that the stable isotopic ratios obtained from a small portion of bulk tooth enamel could be influenced by the different sampling loci on a tooth, which may cause misleading dietary interpretations, also as mentioned in some case studies (Feranec and MacFadden, 2000; Hoppe and Koch, 2006; Pederzani and Britton, 2019).

By contrast, serial sampling of tooth enamel provides an effective approach to reconstruct the long-term dietary and ecological use of mammals, during the time of tooth growth with higher precision (Fox and Fisher, 2004; Zazzo et al., 2006; Fox et al., 2007). This method is suitable for the huge molars of proboscidea and has been successfully applied on extinct mammoths and mastodons to track individual life history for signatures related to climate change, seasonal dietary shifts, and tooth enamel growth rate among other scientific questions (Koch et al., 1998; Feranec and MacFadden, 2000; Hoppe and Koch, 2006; Metcalfe and Longstaffe, 2012, 2014).

During the Late Pleistocene, *E. maximus* was widespread across South and Southeast Asia (Shoshani and Eisenberg, 1982; Shoshani, 1998), and also was the characteristic mammal in South China with increasing materials in recent years (Wang et al., 2017a, b; Tong et al., 2018). In our previous study (Ma et al., 2017), bulk stable isotope (C, O) analysis on Asian elephants fauna during that time from the Baxian Cave, Guangxi, China, supplied the missing link of Asian elephants foraging ecology during the Late Pleistocene. The isotope results of

Asian elephants were widely distributed among the whole fauna in pure C₃ environment, indicating a varied diet. However, based on the consideration aforementioned, these variations could also be influenced by weaning, dietary seasonality, or intraspecific difference due to the bulk sampling strategy (Ma et al., 2017).

Herein, we present a first case study of serial sampling on the tooth enamel of fossil Asian elephants from Baxian Cave. Serial stable isotope (C, O) analysis was preliminarily undertaken with the aim to further understand the isotopic variability during the tooth growth period covering the premolar and molars. Some important information on nursing, dietary seasonality, and intraspecific difference of elephants will be discussed, alongside other potential factors which may explain better about their long-term foraging ecology.

2 Methodology

Species of the Elephantidae are different to all other animals in having high and thick molar crown, composed of numerous tooth plates (Maglio, 1973; Sanders, 2018). Like other derived elephantids, *Elephas maximus* has six teeth in each quadrant and 24 teeth in total (Roth and Shoshani, 1988; Sanders, 2018). The molars are horizontally developed from the back of the jaw and successively replaced one by one, two of which at most could be in use synchronously in each quadrant (Shoshani and Eisenberg, 1982; Roth and Shoshani, 1988; Sanders, 2018). In general, the six teeth in one quadrant of whole tooth row include three deciduous premolars (DP2, DP3, and DP4) and three molars (M1, M2, and M3) (Laws, 1966; Shoshani and Tassy, 1996).

Currently, there has been no study focusing on the determination of Asian elephant tooth formation age and enamel growth rate reported yet. However, given the substantial dental morphological similarity and close phylogenetic relatedness between *E. maximus* and Late Pleistocene mammoths (*Mammuthus* spp.) (Shoshani and Eisenberg, 1982; Shoshani and Tassy, 1996; Roca et al., 2015; Sanders, 2018), we may hereby reasonably assume that the data summarized from modern African elephants (Laws, 1966) provide suitable background references for previous study on mammoth (Metcalf, 2011; Metcalfe and Longstaffe, 2012), and also *E. maximus* in this study. Here, we infer the DP4 and M1 as being roughly developed during the time of pre-birth to 3 years old and 3–15 years old respectively (Metcalf et al., 2010; Metcalfe, 2011), and the enamel extension rate of teeth plates following the height direction is the 13–14 mm/yr (Metcalf and Longstaffe, 2012). This fundamental basis is quite vital to estimate the approximate age of the animal during any apparent dietary or ecological change as recorded in the enamel serial sections.

The individual plates which make up an elephant tooth develop vertically to tooth occlusal plane, with the oldest dental tissue located towards the occlusal surface and the youngest towards the root. The stable isotope (C, O) ratios of serial sections sampled on the tooth enamel thus record the specific foraging behavior of the individual during the formation

time of the different tooth sections. Therefore, the isotopic profile of the serial sections of tooth enamel may indicate dietary and habitat shifts of individual elephants during different ontogenetic phases of each tooth. In combination with the aforementioned growing parameters of tooth enamel, a lot of important information, such as those which may elucidate climatic change and seasonal dietary shifts, possibly be recorded through the isotopic signatures of proboscidean teeth (Koch et al., 1998; Feranec and MacFadden, 2000; Hoppe and Koch, 2006; Metcalfe and Longstaffe, 2012, 2014). Therefore, this first preliminary serial sampling isotope work on *E. maximus* dentition will have the potential to effectively elucidate shifts in their foraging ecology through life history, and also help recheck the results from different sampling strategies.

3 Materials and methods

3.1 Geological setting of fossils and sample selection

Baxian Cave (22°34'31.6"N, 107°21'0.2"E) was systematically excavated by the Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences (IVPP). It is located at the town of Zuozhou, Chongzuo, Guangxi in South China. Baxian Cave and its adjacent areas are characterized by a bare karst landscape, with a northern tropical climate. The sediments from Baxian Cave are approximately 5 m thick, and can be divided into five layers from top to bottom. Three elephant fossils in this study and other associated mammals in previous study were all unearthed from the third layer, indicating they are contemporary (Ma et al., 2017).

After systematic excavation, a large variety and number of vertebrate fossils belonging to at least 40 large-mammalian species were unearthed from the deposits in Baxian Cave, including extremely abundant fossil teeth of the Asian elephant and other associated mammals. Based on the similarity with those nearby faunal assemblages and deposits, which had already obtained the accurate dating age, such as Zhiren Cave, Chongzuo, dated to 100–113 ka (Jin et al., 2009; Liu et al., 2010) and Fuyan Cave, Daoxian, Hunan, dated to 80–120 ka (Liu et al., 2015), the geological age of Baxian Cave possibly also belong to the early Late Pleistocene. The dating of U-series and Electron Spin Resonance Dating (ESR) to determine the absolute date of fossil occupation will be published elsewhere with other taxonomy materials.

Three *Elephas maximus* teeth, including one upper right DP4 (IVPP V 22700.01) and two M1s (V 22700.02 with only the median portions preserved; and V 22700.03 with natural front end preserved as demarcated by the presence of the anterior talon), were selected here for serial sampling. These three teeth belong to different individuals. The tooth plate with the longest in length and well-preserved condition of each elephant tooth was chosen for sequential sampling. These are the 8th preserved lamella on V 22700.01 (labial side), the 3rd preserved lamella on V 22700.02 (labial side), and the natural 8th lamella on V 22700.03 (labial side). All three sampled teeth are well preserved based on the preservation assessment of teeth apatite by the

analyses of X-ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) (Ma et al., 2017).

3.2 Sequential sampling of teeth and stable isotope measurements

The selected tooth plates and the sequential sampling positions are illustrated in Fig. 1. First, contaminants adhering to the surfaces of the chosen lamellae were carefully cleaned off with a diamond-tipped dental burr. Second, the drill lines were perpendicular to crown height, the intervals moved sequentially up the crown height at intervals of approximate 1–2 mm, measured by vernier calliper. The sampling strategy was designed to cover the entire crown height of each sampled lamella as much as possible. However, some upper and lower parts of the tooth plate were not sampled to avoid the possible contamination caused by the frequent contacts of the crown and cervix with the sediments (Fig. 1). Finally, 24, 46, and 54 serial samples from V 22700.01, 02, and 03, were obtained respectively, covering the lengths of 41.2, 92.8, and 72.1 mm respectively between the apex of the tooth crown and the cervix.

Protocols for the preparation of collected enamel powders followed Lee-Thorp et al. (1989) with some modifications, same to the protocol in Ma et al., 2017. Given the fact of weight losses to the enamel powders after the preparation procedures, two or three consecutive serial samples were combined into one in order to reach the requirement of sample weight for stable isotope measurements. After the reorganization, 13, 35, and 23 samples for V 22700.01, 02, and 03 were obtained.

Here, an Isoprime 100 Isotope Ratio Mass Spectrometer (IRMS), coupled with a multi-

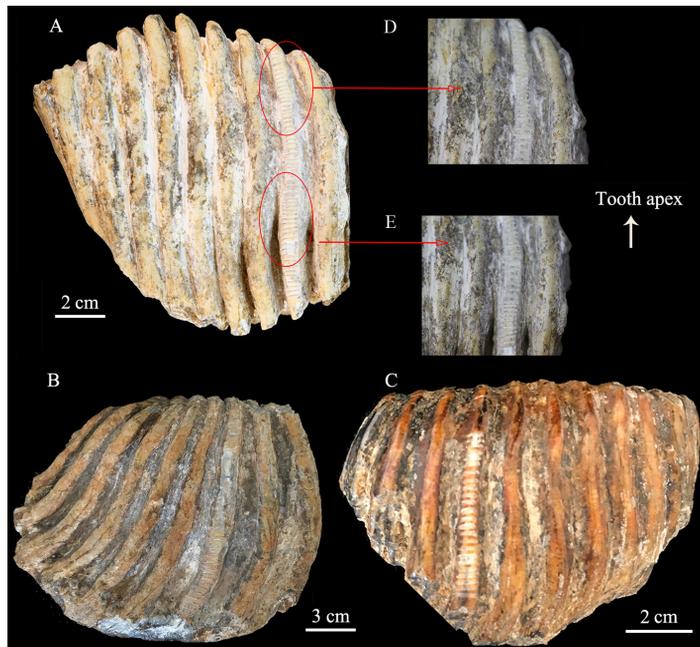


Fig. 1 Diagrams of serial sampling on tooth enamel of *Elephas maximus*
 A. IVPP V 22700.03; B. V 22700.02; C. V 22700.01;
 D, E. the partially enlarged sampling details from V 22700.03

flow system, was used to measure carbon and oxygen isotopic ratios at the Archaeology Stable Isotope Laboratory in the Department of Archaeology and Anthropology, University of Chinese Academy of Sciences. The bioapatite powder of each sample with the weight of about 2 mg was packed into sealed glass tubes and flushed with high-purity helium. Then 0.6 ml of ultrapure phosphoric acid (H_3PO_4) at 70°C was injected into every tube using a disposable medical injector. After the one-hour reaction maintained at 80°C, the carbon dioxide released in the tube was eventually conveyed by helium as carrier gas to the IRMS. International standards, IAEA CO-8 (standard $\delta^{13}\text{C}$ value: -5.8‰ ; standard $\delta^{18}\text{O}$ value: -22.7‰) and IAEA-603 (standard $\delta^{13}\text{C}$ value: 2.5‰ ; standard $\delta^{18}\text{O}$ value: -2.4‰), were used for isotopic calibration and inserted after every ten samples. In addition, another international standard of NBS18 (standard $\delta^{13}\text{C}$ value: -5‰ ; standard $\delta^{18}\text{O}$ value: -23.2‰) was inserted as well randomly as reference for monitoring the measurement stability. The long-term measurement precisions were better than $\pm 0.2\text{‰}$ for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values. Their isotopic data were listed in Supplementary Tables 1–3.

4 Results

The isotopic profiles of the sampled three elephant teeth are showed in Fig. 2. In summary, the $\delta^{13}\text{C}$ values of the whole isotopic data range from -18.3‰ to -16.3‰ , while the $\delta^{18}\text{O}$ values range from -7.9‰ to -4.2‰ . According to the $\delta^{13}\text{C}$ enrichment (14.1‰) from diets to bone or tooth apatite for large herbivores (Cerling and Harris, 1999), the $\delta^{13}\text{C}$ values of their diets range from -32.4‰ to 30.4‰ , indicating a consumption of entire C_3 plants. The $\Delta^{13}\text{C}$ values for V 22700.01, 02, and 03, representing the isotopic difference between the maximum and minimum, are 1.3‰ , 1.6‰ , and 1.0‰ respectively; and the respective $\Delta^{18}\text{O}$ values are 1.7‰ , 2.0‰ , and 2.0‰ . Therefore, the within-tooth isotopic variation in each tooth is small. The details on the isotopic profiles of each tooth are presented as follows.

The $\delta^{13}\text{C}$ values of V 22700.01 (DP4) range from -18.3‰ to -17.0‰ , yielding an average of $(-17.8 \pm 0.4)\text{‰}$ ($n=13$), while the $\delta^{18}\text{O}$ values range from -6.6‰ to -4.9‰ with the mean of $(-5.9 \pm 0.5)\text{‰}$ ($n=13$). As illustrated in Fig. 2A, the $\delta^{13}\text{C}$ values remain relatively stable, reach the highest at the eighth point and fluctuate to some extent in the profile of $\delta^{13}\text{C}$ values. For the $\delta^{18}\text{O}$ values, the highest peak is observed at the fourth point and then decrease gradually.

The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of V 22700.02 (M1) range between -17.9‰ to -16.3‰ and -6.2‰ to -4.2‰ , averaging at $(-16.9 \pm 0.4)\text{‰}$ ($n=35$) and $(-5.2 \pm 0.6)\text{‰}$ ($n=35$) respectively. The $\delta^{13}\text{C}$ values reach the lowest at the third point, then gradually rise towards a higher level from the seventh point to the peak at the 29th point, and then decline slightly afterwards. The $\delta^{18}\text{O}$ values exhibit a more complex pattern than $\delta^{13}\text{C}$ values. Higher $\delta^{18}\text{O}$ values are seen during the range of first six points, then drop sharply and keep relatively constant from the seventh to 22nd point. The rapid increase of $\delta^{18}\text{O}$ values can be seen from the 21st to 23rd loci and maintain higher level afterwards until the peak at 32nd point.

For molar V 22700.03 (M1), the sampled lamella is measured between 2–68.7 mm from tooth crown, corresponding to more than half length of this tooth plate (~110 mm). The $\delta^{13}\text{C}$ values range from -18.0‰ to -17.0‰ , with the average of $(-17.5 \pm 0.3)\text{‰}$ ($n=23$) while the $\delta^{18}\text{O}$ values range from -7.9‰ to -5.9‰ , on an average of $(-6.8 \pm 0.5)\text{‰}$ ($n=23$). In brief, the

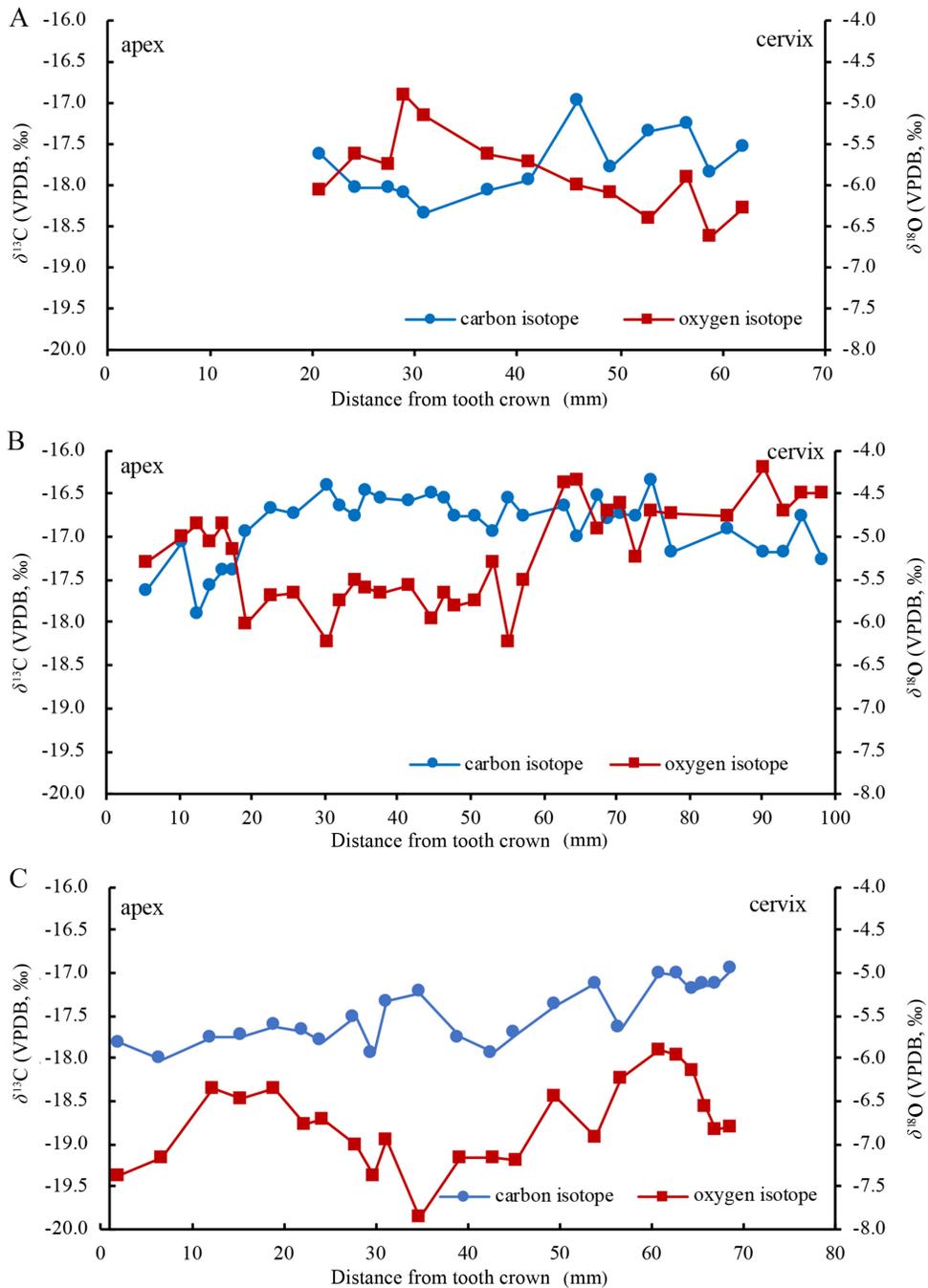


Fig. 2 Serial analysis results of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of the sampled three elephant teeth of *Elephas maximus* A. IVPP V 22700.01; B. V 22700.02; C. V 22700.03

$\delta^{13}\text{C}$ values fluctuate to small scale and increase gradually, while the lowest $\delta^{18}\text{O}$ values are observed in the middle point while the high values are around the two ends.

5 Discussions

The $\delta^{13}\text{C}$ average of the three individual samples are quite similar as -17.8‰ , -16.9‰ , and -17.5‰ , well corresponding to the previous bulk sampling isotope results (Ma et al., 2017). Nevertheless, we found there are indeed some notable isotope variations in the profiles of tooth sections for each sampled tooth (Fig. 2). Several possible factors could account for these within-tooth variations, such as weaning and seasonal effects.

The oxygen isotopic values of infant mammalian tissue samples during the milk-feeding stage would be increased compared to their mothers, due to their consumption of milk, which contains higher $\delta^{18}\text{O}$ values (Wright and Schwarcz, 1998, 1999; Renou et al., 2004; Britton et al., 2015; Tsutaya and Yoneda, 2015). Once weaning takes place and eventually milk consumption stops, the $\delta^{18}\text{O}$ values of infants will drop gradually and reach to the same values as the mothers (Wright and Schwarcz, 1998; Britton et al., 2015). For modern Asian and African elephants, the nursing period may at least last two years to meet the nutritional demand of infant elephants, even last up to six to eight years long until the birth of a sibling (Lee and Moss, 1986; Lee, 1996; Sukumar, 2003; Wittemyer et al., 2007a). Based on the aforementioned tooth formation age (Metcalf et al., 2010; Metcalfe, 2011), tooth enamel growth rate (Metcalf and Longstaffe, 2012), and the tooth enamel wear on crown surface, the decline potential of $\delta^{18}\text{O}$ values on the three samples (these three decline locus are respectively 29, 16.1, and 19 mm away from their tooth crown) seemingly all fall into the range of their weaning age, thus could be possibly influenced by weaning effect. Nevertheless, future research on the tusk samples of fossil elephantids would probably provide more accurate evidence on nursing and weaning than from check teeth samples, as indicated by prior works done on Late Pleistocene mammoths (Rountrey et al., 2007; Cherney, 2016).

Seasonal foraging preferences on different plants during wet and dry seasons have been widely found in the modern Asian elephant (Sukumar, 1989, 2003, 2006; Sukumar and Ramesh, 1995; Chen et al., 2006; Baskaran et al., 2010; Roy, 2010; Mumby et al., 2013) and African elephant (Barnes, 1982; Koch et al., 1995; Cerling et al., 2004, 2006, 2009; Codron, 2004; Wittemyer et al., 2009; Codron et al., 2012, 2013; Forrer, 2017) living in tropical regions. However, no regular significant fluctuations of the isotopic data are found in present study (Fig. 2). In the pure C_3 environments, the slightly seasonal variation of the vegetation ingested by herbivores might be hard to detect by $\delta^{13}\text{C}$ values. However, the $\delta^{18}\text{O}$ values of the large mammals in this region could be potentially influenced by seasonality, where the monsoon effect cause big seasonal variations on $\delta^{18}\text{O}$ values of local precipitation and then the drinking water of the large-sized obligate drinkers (Bryant and Froelich, 1995; Dutton et al., 2005; Biasatti et al., 2010). However, no seasonal variations were observed on $\delta^{18}\text{O}$ values of

all these three samples, it might be because that these elephants drank from the standing water bodies, such as lakes, where the seasonal $\delta^{18}\text{O}$ values are largely buffered due to long water residence times (Pederzani and Britton, 2019). Therefore, the other sharp increase or decrease of isotopic signatures (except for the aforementioned $\delta^{18}\text{O}$ values declines possibly suggestive of weaning) (Fig. 2), is much likely caused by possible movements to new habitats, where elephants ingested plants or water of different stable isotopic properties.

All in all, the isotopic variations restricted from 1‰ to 2‰ among each elephant tooth sections found in this study could be explained, in theory, by the weaning and/or migrational effects. Nevertheless, these isotopic variations within each of the three sampled tooth enamel sections are still generally small. The standard deviations for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ sequential values of each tooth are overall less than 0.6‰. It may thus be concluded that, these Asian elephants all inhabited in a relatively dense and stable forested environment during the Late Pleistocene, without obvious age and seasonal difference.

Several case studies of stable isotope analysis on modern Asian elephants in India and Thailand displayed a wide range of $\delta^{13}\text{C}$ values, representing both C_3 and C_4 food ingestion (Sukumar and Ramesh, 1992; Pushkina et al., 2010; Roy, 2010). Whereas, plenty of isotope analysis had been applied on modern African elephants for the purpose of tracing ivory trade and conservation biology, which also displayed mixed C_3 and C_4 diet, and higher seasonal variabilities among their isotopic profiles of tusks and hair sections (van der Merwe et al., 1990; Vogel et al., 1990; Cerling et al., 2004, 2006, 2009; Wittemyer et al., 2009; Codron et al., 2013). This further highlights the feeding flexibility and opportunism of elephantids from an evolutionary perspective (Cerling et al., 1999; Sukumar, 2003; Ma et al., 2017; Wang et al., 2017a; b; Zhang et al., 2017; Wu et al., 2018), and may demand more comparable evidence from their modern and fossil counterparts, and also from more regions, to better understand the interaction between ecological evolution and extinction/survival.

Back to the aforementioned consideration about possible bias caused by different bulk sampling loci on elephant teeth, this study could now exclude the seasonal effects. Based on the limited data variations, it is also reasonable to look beyond within-tooth isotopic variations caused by weaning and possible migration. Considering the increasing number of stable isotopic studies on bulk enamel from mammalian remains in South and Southeast Asia (Qu et al., 2014; Ji et al., 2016; Bocherens et al., 2017; Li et al., 2017; Suraprasit et al., 2018; Bacon et al., 2018a, b), especially our continuing exploration on foraging ecology of other proboscideans in Quzai Cave, South China (Ma et al., in review), this serial isotopic work provides significant evidence that the widely applied procedure can provide reliable inference of foraging ecology from the duration of mammalian tooth growth in warm temperate regions, unlike the evidence from high-latitude regions (Feranec and MacFadden, 2000). More similar studies on *E. maximus* fossils from wider area will be useful to reveal the foraging complexity and flexibility of *Elephas maximus* in this region, and would also be a reference to conserve this highly endangered animal today.

Finally, as the typically gregarious mammal (Jayantha et al., 2009), modern Asian elephants in India was surprisingly found that, their social structure played a significant role in habitat selection except for extrinsic environmental factors (Baskaran et al., 2018b). This phenomenon was also found previously on African elephants (Wittemyer et al., 2007b), in combination with genetic study on mammoth (Pečnerová et al., 2017), suggesting the significance of their social structure, also as warned by Fisher in the review article about the paleobiology of Pleistocene proboscideans in 2018. Our previous bulk isotope analysis of Asian elephants had disparity as two different groups. Even though weaning, seasonality, and other possible factors had been excluded by this study, it is still unconvincing to say this disparity was influenced by different ecological occupation of different elephant herds. However, at least it emphasizes and reminds us again to take social structure into consideration when reconstructing the paleoecology of fossil proboscideans. Furthermore, this provides a novel independent line of evidence which may potentially provide further corroboration on the complex metapopulation phylogeographic history of *E. maximus*, as highlighted by previous mitochondrial DNA studies (Fernando et al., 2000; Vidya et al., 2005, 2009; Yang and Zhang, 2012; Girdland-Flink et al., 2018; Kusza et al., 2018), and opens up a new window for reconstructing Late Pleistocene proboscidean paleobiology in South Asia.

6 Conclusions

In this study, serial sections of tooth enamel from three Asian elephant specimens from the Late Pleistocene deposits of Baxian Cave, Guangxi, China, were conducted for stable isotopic analysis (C, O). Their average $\delta^{13}\text{C}$ values are $(-17.8 \pm 0.4)\%$ (n=13), $(-16.9 \pm 0.4)\%$ (n=35), and $(-17.5 \pm 0.3)\%$ (n=23), respectively; whereas their respective mean of $\delta^{18}\text{O}$ values are $(-5.9 \pm 0.5)\%$ (n=13), $(-5.2 \pm 0.6)\%$ (n=35), and $(-6.8 \pm 0.5)\%$ (n=23). The isotopic profiles of three elephant teeth thus fluctuate to some degree, possibly caused by weaning and/or migration. The most important finding here is that the isotopic variability existing within the tooth profiles are highly constrained without notable signal from seasonal effect and other potential factors, suggesting a stable foraging ecology for the Asian elephants in the presently studied area during the Late Pleistocene. This isotopic pattern is different from other elephantids, such as the extinct mammoth *Mammuthus* spp., and the extant African elephant *Loxodonta africana*. Besides, this study here confirms that the widely-used bulk stable isotope analysis could supply reliable reference about their foraging ecology in this region without obvious interference from several possible factors. It also evidences the ecological flexibility of Asian elephants revealed by our previous bulk isotope analysis and emphasizes again the ecological significance of their social structure. More similar studies on both modern and fossil Asian elephants from wider area in South and Southeast Asia in the future will be useful to reveal if this apparent past and present pattern of foraging complexity and flexibility in Asian elephants continue to hold true.

Acknowledgements We are very grateful for the hard work of LIU Yi-Hong, ZHANG Ying-Qi, and ZHU Min from IVPP who attended the fieldwork at Baxian Cave. Additionally, we appreciate all the help from the bioarchaeology research group in the Department of Archaeology and Anthropology in University of Chinese Academy of Sciences. We are particularly grateful for the technical assistance on the Mass Spectrometer from Dr. WANG Ting-Ting in Sun Yat-sen University and the valuable suggestions and revisions from the reviewer Prof. DENG Tao and the editor Dr. ZHOU Shuang.

Supplementary material can be found on the website of Vertebrata PalAsiatica (http://english.ivpp.cas.cn/sp/PalAsiatica/vp_list/) in Vol. 57.

序列取样的稳定同位素研究 示踪中国晚更新世亚洲象的摄食行为

马 姣^{1,2} 王 元^{1,3} 金昌柱¹ 张瀚文^{4,5} 胡耀武^{1,2}

(1 中国科学院古脊椎动物与古人类研究所, 中国科学院脊椎动物演化与人类起源重点实验室 北京 100044)

(2 中国科学院大学考古学与人类学系 北京 100049)

(3 中国科学院生物演化与环境卓越创新中心 北京 100044)

(4 英国布里斯托大学地球科学学院 布里斯托 BS8 1RJ)

(5 英国伦敦自然历史博物馆地球科学部 伦敦 SW7 5BD)

摘要: 为了进一步探索亚洲象的摄食行为, 运用稳定同位素的序列取样(serial/sequential sampling)新方法, 首次对晚更新世筓仙洞遗址的三个亚洲象臼齿牙釉质(1个DP4, 2个M1)进行研究。结果表明, 3个亚洲象个体的 $\delta^{13}\text{C}$ 和 $\delta^{18}\text{O}$ 内部差异均很小, 未见季节性变化, 虽然可能存在断奶及迁徙导致的数据波动, 但总体来看依然表现出在牙釉质形成的长期过程中较为稳定的摄食行为。之前筓仙洞亚洲象动物群的整体取样(bulk sampling)同位素研究结果中, 亚洲象的数据分布较为分散。而本次研究中较小的个体内部差异, 则反向证实了宽泛分布的数据确实代表了灵活的摄食行为, 并非取样位置的不同所致。这也进一步证明在气候温暖的东南亚地区, 长鼻类动物的牙釉质整体取样工作可以提供可靠的古摄食行为及古生态信息。

关键词: 中国南部, 晚更新世, 亚洲象, 稳定同位素, 序列取样牙釉质, 摄食行为

中国法分类号: Q915.878 文献标识码: A 文章编号: 1000-3118(2019)03-0225-16

References

- Ahrestani F S, Heitkönig I M A, Matsubayashi H et al., 2016. Grazing and browsing by large herbivores in South and Southeast Asia. In: Ahrestani F S, Sankaran M eds. *The Ecology of Large Herbivores in South and Southeast Asia*. Dordrecht: Springer Netherlands. 99–120
- Bacon A M, Bourgon N, Dufour E et al., 2018a. Nam Lot (MIS 5) and Duoi U’Oi (MIS 4) Southeast Asian sites revisited:

- zooarchaeological and isotopic evidences. *Palaeogeogr Palaeoclimatol Palaeoecol*, 512: 132–144
- Bacon A M, Durringer P, Westaway K et al., 2018b. Testing the savannah corridor hypothesis during MIS2: the Boh Dambang hyena site in southern Cambodia. *Quat Int*, 464: 417–439
- Barnes R F W, 1982. Elephant feeding behaviour in Ruaha National Park, Tanzania. *Afr J Ecol*, 20: 123–136
- Baskaran N, Balasubramanian M, Swaminathan S et al., 2010. Feeding ecology of the Asian elephant *Elephas maximus* Linnaeus in the Nilgiri Biosphere Reserve, southern India. *J Bombay Nat Hist Soc*, 107: 3–13
- Baskaran N, Kanakasabai R, Desai A A, 2018a. Ranging and spacing behaviour of Asian elephant (*Elephas maximus* Linnaeus) in the tropical forests of Southern India. In: Sivaperuman C, Venkataraman K eds. *Indian Hotspots*. Singapore: Springer Singapore. 295–315
- Baskaran N, Kanakasabai R, Desai A A, 2018b. Influence of ranging and hierarchy on the habitat use pattern by Asian elephant (*Elephas maximus*) in the tropical forests of Southern India. In: Sivaperuman C, Venkataraman K eds. *Indian Hotspots*. Singapore: Springer Singapore. 345–358
- Biasatti D, Wang Y, Deng T, 2010. Strengthening of the East Asian summer monsoon revealed by a shift in seasonal patterns in diet and climate after 2–3 Ma in northwest China. *Palaeogeogr Palaeoclimatol Palaeoecol*, 297: 12–25
- Bocherens H, Schrenk F, Chaimanee Y et al., 2017. Flexibility of diet and habitat in Pleistocene South Asian mammals: implications for the fate of the giant fossil ape *Gigantopithecus*. *Quat Int*, 434: 148–155
- Britton K, Fuller B T, Tütken T et al., 2015. Oxygen isotope analysis of human bone phosphate evidences weaning age in archaeological populations. *Am J Phys Anthropol*, 157: 226–241
- Bryant J D, Froelich P N, 1995. A model of oxygen isotope fractionation in body water of large mammals. *Geochim Cosmochim Acta*, 59: 4523–4537
- Cerling T E, Harris J M, 1999. Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. *Oecologia*, 120: 347–363
- Cerling T E, Harris J M, Leakey M G, 1999. Browsing and grazing in elephants: the isotope record of modern and fossil proboscideans. *Oecologia*, 120: 364–374
- Cerling T E, Passey B H, Ayliffe L K et al., 2004. Orphans' tales: seasonal dietary changes in elephants from Tsavo National Park, Kenya. *Palaeogeogr Palaeoclimatol Palaeoecol*, 206: 367–376
- Cerling T E, Wittemyer G, Rasmussen H B et al., 2006. Stable isotopes in elephant hair document migration patterns and diet changes. *Proc Natl Acad Sci USA*, 103: 371–373
- Cerling T E, Wittemyer G, Ehleringer J R et al., 2009. History of animals using isotope records (HAIR): a 6-year dietary history of one family of African elephants. *Proc Natl Acad Sci USA*, 106: 8093–8100
- Chen J, Deng X, Zhang L et al., 2006. Diet composition and foraging ecology of Asian elephants in Shangyong, Xishuangbanna, China. *Acta Ecol Sin*, 26: 309–316
- Cherney M D, 2016. Records of growth and weaning in fossil proboscidean tusks as tests of Pleistocene extinction mechanisms. Ph. D thesis. Michigan: University of Michigan. 1–480
- Codron J, 2004. An isotope comparison of elephant (*Loxodonta africana*) diets in the Kruger National Park and Welgevonden Game Reserve. M. S. thesis. Cape Town: University of Cape Town. 1–177
- Codron J, Codron D, Sponheimer M et al., 2012. Stable isotope series from elephant ivory reveal lifetime histories of a true dietary generalist. *Proc R Soc B*, 279: 2433–2441
- Codron J, Kirkman K, Duffy K J et al., 2013. Stable isotope turnover and variability in tail hairs of captive and free-ranging

- African elephants (*Loxodonta africana*) reveal dietary niche differences within populations. *Can J Zool*, 91: 124–134
- Dutton A, Wilkinson B H, Welker J M et al., 2005. Spatial distribution and seasonal variation in $^{18}\text{O}/^{16}\text{O}$ of modern precipitation and river water across the conterminous USA. *Hydrol Proc*, 19: 4121–4146
- Feranec R S, MacFadden B J, 2000. Evolution of the grazing niche in Pleistocene mammals from Florida: evidence from stable isotopes. *Palaeogeogr Palaeoclimatol Palaeoecol*, 162: 155–169
- Fernando P, Pfrender M E, Encalada S E et al., 2000. Mitochondrial DNA variation, phylogeography and population structure of the Asian elephant. *Heredity*, 84: 362–372
- Fisher D C, 2018. Paleobiology of Pleistocene Proboscideans. *Annu Rev Earth Planet Sci*, 46: 229–260
- Forrer F A, 2017. The population status, habitat use and seasonal diet of African elephant (*Loxodonta africana*) in Majete Wildlife Reserve, Malawi. Ph. D thesis. Western Cape: Stellenbosch University. 1–123
- Fox D L, Fisher D C, 2004. Dietary reconstruction of Miocene *Gomphotherium* (Mammalia, Proboscidea) from the Great Plains region, USA, based on the carbon isotope composition of tusk and molar enamel. *Palaeogeogr Palaeoclimatol Palaeoecol*, 206: 311–335
- Fox D L, Fisher D C, Vartanyan S et al., 2007. Paleoclimatic implications of oxygen isotopic variation in Late Pleistocene and Holocene tusks of *Mammuthus primigenius* from northern Eurasia. *Quat Int*, 169-170: 154–165
- Girdland-Flink L, Albayrak E, Lister A M, 2018. Genetic insight into an extinct population of Asian elephants (*Elephas maximus*) in the Near East. *Open Quat*, 4: 1–9
- Hillson S, 2005. *Teeth*. 2nd ed. Cambridge: Cambridge University Press. 1–388
- Hoppe K A, Koch P L, 2006. The biogeochemistry of the Aucilla River Fauna. In: Webb S D ed. *First Floridians and Last Mastodons: the Page-Ladson Site in the Aucilla River*. Dordrecht: Springer Netherlands. 379–401
- Jayantha D, Dayawansa P N, Padmalal U et al., 2009. Social relationships of wild juvenile Asian elephants *Elephas maximus* in the Udawalawa National Park, Sri Lanka. *J Threat Taxa*, 1: 211–214
- Ji X, Curnoe D, Taçon P S C et al., 2016. Cave use and palaeoecology at Maludong (Red Deer Cave), Yunnan, China. *J Archaeol Sci Rep*, 8: 277–283
- Jin C, Pan W, Zhang Y et al., 2009. The *Homo sapiens* Cave hominin site of Mulan Mountain, Jiangzhou District, Chongzuo, Guangxi with emphasis on its age. *Chin Sci Bull*, 54: 3848–3856
- Koch P L, Heisinger J, Moss C et al., 1995. Isotopic tracking of change in diet and habitat use in African elephants. *Science*, 267: 1340–1343
- Koch P L, Hoppe K A, Webb S D, 1998. The isotopic ecology of Late Pleistocene mammals in North America: Part 1. Florida. *Chem Geol*, 152: 119–138
- Kusza S, Suchentrunk F, Pucher H et al., 2018. High levels of mitochondrial genetic diversity in Asian elephants (*Elephas maximus*) from Myanmar. *Hystrix Ital J Mammal*, 29: 152–154
- Laws R M, 1966. Age criteria for the African elephant: *Loxodonta a. africana*. *Afr J Ecol*, 4: 1–37
- Lee P C, 1996. The meanings of weaning: growth, lactation, and life history. *Evol Anthropol Issues News Rev*, 5: 87–98
- Lee P C, Moss C J, 1986. Early maternal investment in male and female African elephant calves. *Behav Ecol Sociobiol*, 18: 353–361
- Lee-Thorp J A, Sealy J C, van der Merwe N J, 1989. Stable carbon isotope ratio differences between bone collagen and bone

- apatite, and their relationship to diet. *J Archaeol Sci*, 16: 585–599
- Li D, Hu C, Wang W et al., 2017. The stable isotope record in cervid tooth enamel from Tantang Cave, Guangxi: implications for the Quaternary East Asian monsoon. *Quat Int*, 434: 156–162
- Liu W, Jin C, Zhang Y et al., 2010. Human remains from Zhirendong, South China, and modern human emergence in East Asia. *Proc Natl Acad Sci USA*, 107: 19201–19206
- Liu W, Martínón-Torres M, Cai Y et al., 2015. The earliest unequivocally modern humans in southern China. *Nature*, 526: 696–699
- Ma J, Wang Y, Jin C et al., 2017. Isotopic evidence of foraging ecology of Asian elephant (*Elephas maximus*) in South China during the Late Pleistocene. *Quat Int*, 443: 160–167
- Maglio V J, 1973. Origin and evolution of the Elephantidae. *Trans Am Philos Soc*, 63: 1–149
- Metcalf J Z, 2011. Late Pleistocene climate and proboscidean paleoecology in North America: insights from stable isotope compositions of skeletal remains. Ph. D thesis. London (Ontario): The University of Western Ontario. 1–297
- Metcalf J Z, Longstaffe F J, 2012. Mammoth tooth enamel growth rates inferred from stable isotope analysis and histology. *Quat Res*, 77: 424–432
- Metcalf J Z, Longstaffe F J, 2014. Environmental change and seasonal behavior of mastodons in the Great Lakes region inferred from stable isotope analysis. *Quat Res*, 82: 366–377
- Metcalf J Z, Longstaffe F J, Zazula G D, 2010. Nursing, weaning, and tooth development in woolly mammoths from Old Crow, Yukon, Canada: implications for Pleistocene extinctions. *Palaeogeogr Palaeoclimatol Palaeoecol*, 298: 257–270
- Mumby H S, Courtiol A, Mar K U et al., 2013. Birth seasonality and calf mortality in a large population of Asian elephants. *Ecol Evol*, 3: 3794–3803
- Patnaik R, 2017. Stable isotope based dietary reconstruction of some Plio-Pleistocene Siwalik and Narmada Valley elephant species of India. In: Abstracts Book of VII International Conference of Mammoths and Their Relatives. Taichung: National Museum of Natural Science. AF1-1
- Patnaik R, Cerling T E, Uno K T et al., 2014. Diet and habitat of Siwalik Primates *Indopithecus*, *Sivaladapis* and *Theropithecus*. *Ann Zool Fenn*, 51: 123–142
- Pečnerová P, Diez-del-Molino D, Dussex N et al., 2017. Genome-based sexing provides clues about behavior and social structure in the woolly mammoth. *Curr Biol*, 27: 3505–3510
- Pederzani S, Britton K, 2019. Oxygen isotopes in bioarchaeology: principles and applications, challenges and opportunities. *Earth-Sci Rev*, 188: 77–107
- Pradhan N M, Wegge P, Moe S R et al., 2008. Feeding ecology of two endangered sympatric megaherbivores: Asian elephant *Elephas maximus* and greater one-horned rhinoceros *Rhinoceros unicornis* in lowland Nepal. *Wildl Biol*, 14: 147–154
- Pushkina D, Bocherens H, Chaimanee Y et al., 2010. Stable carbon isotope reconstructions of diet and paleoenvironment from the late Middle Pleistocene Snake Cave in Northeastern Thailand. *Naturwissenschaften*, 97: 299–309
- Qu Y, Jin C, Zhang Y et al., 2014. Preservation assessments and carbon and oxygen isotopes analysis of tooth enamel of *Gigantopithecus blacki* and contemporary animals from Sanhe Cave, Chongzuo, South China during the Early Pleistocene. *Quat Int*, 354: 52–58
- Renou J P, Deponge C, Gachon P et al., 2004. Characterization of animal products according to geographic origin and

- feeding diet using nuclear magnetic resonance and isotope ratio mass spectrometry: cow milk. *Food Chem*, 85: 63–66
- Roca A L, Ishida Y, Brandt A L et al., 2015. Elephant natural history: a genomic perspective. *Annu Rev Anim Biosci*, 3: 139–167
- Roth V L, Shoshani J, 1988. Dental identification and age determination in *Elephas maximus*. *J Zool*, 214: 567–588
- Rountrey A N, Fisher D C, Vartanyan S et al., 2007. Carbon and nitrogen isotope analyses of a juvenile woolly mammoth tusk: evidence of weaning. *Quat Int*, 169-170: 166–173
- Roy M, 2010. Habitat use and foraging ecology of the Asian elephant (*Elephas maximus*) in Buxa Tiger Reserve and adjoining areas of northern West Bengal. Ph. D thesis. West Bengal: Vidyasagar University. 1–132
- Sanders W J, 2018. Horizontal tooth displacement and premolar occurrence in elephants and other elephantiform proboscideans. *Hist Biol*, 30: 137–156
- Shoshani J, 1998. Understanding proboscidean evolution: a formidable task. *Trends Ecol Evol*, 13: 480–487
- Shoshani J, Eisenberg J F, 1982. *Elephas maximus*. *Mamm Species Archive*, 182: 1–8
- Shoshani J, Tassy P, 1996. *The Proboscidea: Evolution and Palaeoecology of Elephants and Their Relatives*. Oxford: Oxford University Press. 1–502
- Sukumar R, 1989. Ecology of the Asian elephant in southern India. I. Movement and habitat utilization patterns. *J Trop Ecol*, 5: 1–18
- Sukumar R, 2003. *The Living Elephants: Evolutionary Ecology, Behaviour, and Conservation*. Oxford: Oxford University Press. 1–495
- Sukumar R, 2006. A brief review of the status, distribution and biology of wild Asian elephants *Elephas maximus*. *Int Zoo Yearb*, 40: 1–8
- Sukumar R, Ramesh R, 1992. Stable carbon isotope ratios in Asian elephant collagen: implications for dietary studies. *Oecologia*, 91: 536–539
- Sukumar R, Ramesh R, 1995. Elephant foraging: is browse or grass more important? In: Daniel J C, Datye H eds. *A Week with Elephants*. Oxford: Oxford University Press. 368–374
- Suraprasit K, Bocherens H, Chaimanee Y et al., 2018. Late Middle Pleistocene ecology and climate in Northeastern Thailand inferred from the stable isotope analysis of Khok Sung herbivore tooth enamel and the land mammal cenogram. *Quat Sci Rev*, 193: 24–42
- Tong H, Deng L, Chen X, 2018. Late Pleistocene proboscideans from Yangjiawan caves in Pingxiang of Jiangxi, with discussions on the *Stegodon orientalis*–*Elephas maximus* assemblage. *Vert Palasiat*, 56: 306–326
- Tsutaya T, Yoneda M, 2015. Reconstruction of breastfeeding and weaning practices using stable isotope and trace element analyses: a review. *Am J Phys Anthropol*, 156: 2–21
- van der Merwe N J, Lee-Thorp J A, Thackeray J F et al., 1990. Source-area determination of elephant ivory by isotopic analysis. *Nature*, 346: 744–746
- Vidya T N C, Fernando P, Melnick D J et al., 2005. Population differentiation within and among Asian elephant (*Elephas maximus*) populations in southern India. *Heredity*, 94: 71–80
- Vidya T N C, Sukumar R, Melnick D J, 2009. Range-wide mtDNA phylogeography yields insights into the origins of Asian elephants. *Proc R Soc B*, 276: 893–902
- Vogel J C, Eglinton B, Auret J M, 1990. Isotope fingerprints in elephant bone and ivory. *Nature*, 346: 747–749

- Wang Y, Qin D, Jin C, 2017a. New *Elephas* remains from the Zhiren Cave of Mulan Mountain, Chongzuo, Guangxi with discussion on Quaternary proboscidean evolution in southern China. *Quat Sci*, 37: 853–859
- Wang Y, Zhao L, Du B et al., 2017b. New proboscidean remains associated with *Homo sapiens* from the Mawokou Cave in Bijie, Guizhou Province of south-western China. *Acta Anthropol Sin*, 36: 414–425
- Wittemyer G, Rasmussen H B, Douglas-Hamilton I, 2007a. Breeding phenology in relation to NDVI variability in free-ranging African elephant. *Ecography*, 30: 42–50
- Wittemyer G, Getz W M, Vollrath F et al., 2007b. Social dominance, seasonal movements, and spatial segregation in African elephants: a contribution to conservation behavior. *Behav Ecol Sociobiol*, 61: 1919–1931
- Wittemyer G, Cerling T E, Douglas-Hamilton I, 2009. Establishing chronologies from isotopic profiles in serially collected animal tissues: an example using tail hairs from African elephants. *Chem Geol*, 267: 3–11
- Wright L E, Schwarcz H P, 1998. Stable carbon and oxygen isotopes in human tooth enamel: identifying breastfeeding and weaning in prehistory. *Am J Phys Anthropol*, 106: 1–18
- Wright L E, Schwarcz H P, 1999. Correspondence between stable carbon, oxygen and nitrogen isotopes in human tooth enamel and dentine: infant diets at Kaminaljuyu. *J Archaeol Sci*, 26: 1159–1170
- Wu Y, Deng T, Hu Y et al., 2018. A grazing *Gomphotherium* in Middle Miocene Central Asia, 10 million years prior to the origin of the Elephantidae. *Sci Rep*, 8: 7640
- Yang F, Zhang L, 2012. Population genetic structure and population genetic diversity analysis based on mitochondrial DNA of Asian elephants (*Elephas maximus*) in China. *Acta Theriol Sin*, 32: 90–100
- Zazzo A, Balasse M, Patterson W P, 2006. The reconstruction of mammal individual history: refining high-resolution isotope record in bovine tooth dentine. *J Archaeol Sci*, 33: 1177–1187
- Zhang H, Wang Y, Janis C M et al., 2017. An examination of feeding ecology in Pleistocene proboscideans from southern China (*Sinomastodon*, *Stegodon*, *Elephas*), by means of dental microwear texture analysis. *Quat Int*, 445: 60–70