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Abstract

Tam Pà Ling, a cave site in northeastern Laos, has yielded the earliest skeletal evidence of *Homo sapiens* in mainland Southeast Asia. The reliance of Pleistocene humans in rainforest settings on plant or animal resources is still largely unstudied, mainly due to poor collagen preservation in fossils from tropical environments precluding stable nitrogen isotope analysis, the classical trophic level proxy. However, isotopic ratios of zinc (Zn) in bioapatite constitute a promising proxy to infer trophic and dietary information from fossil vertebrates, even under adverse tropical taphonomic conditions. Here, we analyzed the zinc isotope composition (^{66}Zn/^{64}Zn expressed as δ^{66}Zn value) in the enamel of two teeth of the Late Pleistocene (63–46 ka) *H. sapiens* individual (TPL1) from Tam Pà Ling, as well as 76 mammal teeth from the same site and the nearby Nam Lot cave. The human individual exhibits relatively low enamel

δ^{66}Zn values (+0.24‰) consistent with an omnivorous diet, suggesting a dietary reliance on both plant and animal matter. These findings offer direct evidence of the broad utilization of resources from tropical rainforests by one of the earliest known anatomically modern humans in Southeast Asia.

1. Introduction

Southeast Asia is a key region for understanding patterns of human dispersal into Asia and Australasia (Bae et al., 2017; Perry and Verdu, 2017; Rabett, 2018; Louys and Roberts, 2020). While traditional assumptions have often viewed tropical rainforest habitats as an obstacle to prehistoric human dispersal and occupation (Hutterer, 1983; Bailey et al., 1989; Bailey and Headland, 1991; Wurster and Bird, 2016), increasing evidence and novel analytic approaches have demonstrated human occupation and adaptation to rainforest habitats in Southeast Asia (Barton, 2005; Barker et al., 2007; Summerhayes et al., 2010; Roberts et al., 2015; Demeter et al., 2017; Westaway et al., 2017; Wedage et al., 2019; Langley et al., 2020). Indeed, it was suggested that adaptive plasticity allowed our species to remain in Southeast Asia through ever-increasing climatic instability in the Late Pleistocene and became the last surviving hominin, as opposed to *Homo erectus* and *Homo floresiensis,* for example (Roberts and Amano, 2019; Louys and Roberts, 2020).

The site of Tam Pà Ling (TPL), at Pà Hang Mountain in the Annamite mountain range of northeastern Laos (Huà Pan Province), has shown increasing significance in anthropological and archaeological studies in Southeast Asia (Bae et al., 2017; Martinón-Torres et al.,

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2017; McColl et al., 2018), with the oldest known and richest fossil record of our species in this region (Demeter et al., 2012, 2015; Shackelford et al., 2018). Alongside faunal remains, skeletal remains from at least five human individuals have been recovered to date from a chronologically well-constrained Late Pleistocene stratigraphic context (Demeter et al., 2012, 2015, 2017; Shackelford et al., 2018), providing direct evidence of long-term human occupation of the region. Located at the top of the Pà Hang mountain, TPL cave has one main chamber, excavated at the east end of a gallery, at the base of the entrance's steep 65-m slope. The site's stratigraphy suggests a formation by periodic slope wash deposition coming from the cave's entrance (Demeter et al., 2012, 2015; Shackelford et al., 2018), with no evidence of human occupation on the surface. While the source of TPL fossils remains unknown, current evidence suggests that they originated at or near the cave's entrance before slope wash transport and burial. Various dating methods yielded an age range for the human fossil-bearing deposit at TPL of 63–46 ka (Demeter et al., 2012) for the TPL1 individual (a cranium with frontal, occipital, maxillae, and dentition showing derived modern human morphology), providing the earliest skeletal evidence of anatomically modern humans in mainland Southeast Asia.

Despite evidence of rainforest occupation by our species from at least 70 ka (Demeter et al., 2017; Westaway et al., 2017; Shackelford et al., 2018), poor organic matter preservation in tropical latitudes generally hinders our understanding of human dietary reliance on these habitats and their resources (Tappen, 1994). Occasionally, insights can be obtained when archaeological material is preserved in cave and rock shelter sedimentary sequences (Barker et al., 2007; Summerhayes et al., 2010; Perera et al., 2011). However, such depositional contexts are rare in Southeast Asia, and archaeological material (in particular faunal and plant remains) is seldom found in pre-Holocene contexts. As such, evaluating the overall dietary importance of tropical forest resources based on archaeological material alone has proven to be difficult.

Stable isotope analysis of bones and teeth can provide direct evidence of dietary resources consumed in this tropical rainforest environment. Such methods have successfully highlighted reliance on forest resources for Late Pleistocene tropical rainforest forager populations from Sri Lanka (Roberts et al., 2015, 2017). However, no information on the exact nature of these resources—whether animal or plant—can be obtained from stable carbon isotopes. In addition, nitrogen stable isotope ($\delta^{15}N$) analysis of bone or dentine collagen—an established method for investigating trophic relations (Deniro and Epstein, 1981)—is mostly unsuccessful in tropical environments due to generally poor protein preservation (van Klinken, 1999; Krigbaum, 2005; Clarkson et al., 2009).

Recently, trophic level assessment from enamel using different 'non-traditional' stable isotope systems (calcium, magnesium, zinc, and strontium) has become possible through the use of multicollector inductively coupled plasma mass spectrometry (Chu et al., 2006; Knudson et al., 2010; Costas-Rodríguez et al., 2014; Martin et al., 2014), using amounts of material similar to or lower than traditional carbon and oxygen stable isotope analysis. These methods have been successfully applied to fossil and archaeological specimens (Knudson et al., 2010; Martin et al., 2015, 2018; Jaouen et al., 2016a; Balter et al., 2019), bypassing the taphonomic limitations of collagen-bound nitrogen isotopes. In particular, the stable isotope ratios of the trace element zinc (including the $^{66}Zn/^{64}Zn$, expressed as $\delta^{66}Zn$ value) constitute a promising dietary indicator among these non-traditional isotope systems (Jaouen et al., 2013, 2016a, 2016b, 2018; Bourgon et al., 2020; McCormack et al., 2021).

Incorporated as a trace element in enamel bioapatite, Zn has better long-term preservation potential than collagen-bound nitrogen (Dean et al., 2018). Indeed, tooth enamel is more resistant to diagenetic alteration (Kohn et al., 1999; Trueman and Tuross, 2002; Dauphin and Williams, 2004), and systematic variations in $\delta^{66}Zn$ values show promise for dietary reconstructions in archeology and paleontology, as recently demonstrated (Bourgon et al.,

2020). Two key factors are currently known to control the variability of δ^{66}Zn values in animals. First, the δ^{66}Zn values of consumer body tissues depend on the Zn isotopic composition of the source intake, i.e., mainly the food ingested (Jaouen et al., 2013; Costas-Rodríguez et al., 2014). Second, biological Zn isotope fractionation occurring within an organism results in variation in δ^{66}Zn values between different plant parts or body tissues (Balter et al., 2013; Moynier et al., 2013). As such, a consumer's δ^{66}Zn value will ultimately be impacted by what plant or animal species were fed upon, as well as which parts of said food source. Other physiological factors such as metabolism and body size could also result in δ^{66}Zn variability, although such mechanisms are currently poorly understood or investigated. Given that plant material typically has higher δ^{66}Zn values relative to animal matter (including insects; Evans et al., 2016), the resulting δ^{66}Zn values of a terrestrial trophic chain follow an opposite trend to the classic trophic level tracer δ^{15}N$_{collagen}$ values (Schoeninger and DeNiro, 1984). Therefore, higher trophic levels are associated with lower tissue δ^{66}Zn values (Jaouen et al., 2013, 2016a, b, 2018; Costas-Rodríguez et al., 2014).

Here, we apply a multi-isotope approach (δ^{66}Zn, ^{87}Sr/^{86}Sr, δ^{13}C$_{apatite}$, δ^{18}O$_{apatite}$) to the TPL1 individual from TPL to determine the diet and ecological adaptations of the oldest modern human individual found in Southeast Asia (Demeter et al., 2012). Specifically, the association at TPL of a rich human fossil record and fauna with taxa of known ecological preferences for tropical forests allows us to apply methodologies such as stable Zn isotope measurements. This association is crucially important in sites such as TPL where detailed archaeological material evidence is absent (e.g., lithic technology, hearth features, plant macroremains, cut marks on bones), making isotopic approaches the only way to gain insight into past dietary reliance. We compared the variability in enamel δ^{66}Zn values to ^{87}Sr/^{86}Sr measurements from the same specimens to account for any potential δ^{66}Zn variability introduced by feeding on different bedrock (Graustein, 1989; Bentley, 2006; Bourgon et al., 2020). In addition, we used stable

carbon isotope measurements ($\delta^{13}C_{apatite}$) as a complementary dietary tracer (Cerling and Harris, 1999; Cerling et al., 2015). The $\delta^{13}C$ values of bioapatite reflect the relative proportion in a consumer's diet of ingested carbon derived from the food web's primary sources, namely plants using either C_3 (trees, bushes, shrubs, and grasses) or C_4 (grasses, sedges) photosynthetic pathways (Smith and Epstein, 1971; O'Leary, 1988; Farquhar et al., 1989). In tropical and subtropical regions, forest and woodland habitats are associated with C_3 plants that exhibit low $\delta^{13}C$ values, whereas drier and open environments are characterized by C_4 plants with high $\delta^{13}C$ values (Zazzo et al., 2000; Sponheimer et al., 2006; Kohn, 2010; Cerling et al., 2015; Bacon et al., 2018). In addition, the lowest $\delta^{13}C$ values reflect densely forested conditions resulting from a 'canopy effect' (van der Merwe and Medina, 1991) and can thus be used to further differentiate between open and forested C_3 environments (i.e., the degree of tree cover). Using $\delta^{13}C_{apatite}$ values and diet enamel spacing (see Supplementary Online Material [SOM] S1), we estimated the average $\delta^{13}C$ value of the diet ($\delta^{13}C_{diet}$) from each animal specimen. Stable oxygen isotope analysis ($\delta^{18}O_{apatite}$) was used to offer possible insights into physiology, water source, and diet (Pederzani and Britton, 2019). A description of the different stable isotope systems in a tropical context used in this study can be found in SOM S1.

2. Material and Methods

The two *Homo sapiens* tooth samples analyzed in this study come from the lateral enamel of a first and second permanent upper left molar (M^1 and M^2) of the same TPL1 individual, dated to 63–46 ka (Demeter et al., 2012). Faunal remains from TPL are scarce and excavated from a chronologically long deposit (70–1.1 ka; Shackelford et al., 2018), rendering trophic relations among the taxa equivocal. To address this uncertainty, the Late Pleistocene fossiliferous assemblage from Nam Lot cave (NL; 86 to 72 ka; Bacon et al., 2015, 2018), a

nearby cave site in the Pà Hang Mountain (≈150 m distance to TPL; SOM Fig. S1), was also analyzed to assess consistency between trophic positions of similar fauna from the two different sites and to provide a more comprehensive ecological baseline for TPL1. In total, the dental enamel of 76 mammal specimens belonging to 18 taxa was analyzed (SOM Table S1), whereby 24 specimens come from TPL (70–1.1 ka) and 52 from NL (86–72 ka). The spotted hyena (*Crocuta crocuta*) was considered separately from other carnivorous taxa (herein assigned the 'bone-eating carnivore' dietary category), as it has been found to exhibit different δ^{66}Zn values from other sympatric carnivores (Jaouen et al., 2016a). Collagen extraction was attempted in the context of this study on a subselection of specimens from NL but was not successful (collagen yield <0.05%; SOM Table S2). Previously published enamel isotope data (δ^{66}Zn, ^{87}Sr/^{86}Sr, δ^{13}C$_{apatite}$, and δ^{18}O$_{apatite}$; $n = 72$) from the Late Pleistocene fauna of Tam Hay Marklot (THM; 38.4 to 13.5 ka), northeastern Laos (Huà Pan Province; Bourgon et al., 2020), were also used for comparison throughout this study to further enlarge the faunal dataset.

The tooth enamel samples used for Zn and Sr isotopic analysis were prepared following the protocols described in SOM S2 and measured using Thermo Scientific Neptune multicollector inductively coupled plasma mass spectrometry at the Max Planck Institute for Evolutionary Anthropology in Leipzig. The stable C and O isotope composition of samples analyzed in this study was measured with a Thermo Delta V Advantage isotopic mass spectrometer coupled to a Thermo Kiel IV Carbonate Device chemical preparer, at the Service de Spectrométrie de Masse Isotopique du Muséum in Paris, using the protocol described in SOM S2.

Statistical analyses were performed in R v. 4.0.2 (R Core Team, 2018), using an alpha level for significance of 0.05. To explore sources of variability of δ^{66}Zn values in dental enamel, we fitted a linear mixed model (LMM; Baayen, 2008; similar to Bourgon et al., 2020) with a Gaussian error structure and identity link (McCullagh and Nelder, 1989) using the R package

'lme4' version 1.1–17 (Bates et al., 2015; SOM S2). Predictors included in the model were dietary category (carnivore, bone-eating carnivore, omnivore, and herbivore), site (THM, NL and TPL), $^{87}Sr/^{86}Sr$, $\delta^{13}C_{apatite}$, $\delta^{18}O_{apatite}$, zinc concentration, and body mass. One-way analysis of variance (ANOVA) was also performed to determine statistical differences in $\delta^{66}Zn$ values between dietary categories. Where differences were found to be significant, Tukey's post-hoc pair-wise comparisons were carried out to determine which dietary categories were significantly different from each other. Isotopic niches were estimated using stable isotope Bayesian ellipses (Jackson et al., 2011), where the shape and size of the ellipses are defined by the covariance matrix of $\delta^{13}C$ and $\delta^{66}Zn$ values, while the position is defined by the means of both variables. All data discussed in the paper are provided in SOM Tables S1–S8.

3. Results

All measured $\delta^{66}Zn$, $^{87}Sr/^{86}Sr$, $\delta^{13}C$ (apatite and diet), and $\delta^{18}O_{apatite}$ values from TPL and NL can be found in SOM Tables S3 and S4 and all values from reference materials in SOM Table S7. Most values for $\delta^{13}C_{apatite}$ and $\delta^{18}O_{apatite}$ values from NL specimens ($n = 47$) are from previously published work (Bacon et al., 2018).

The $\delta^{66}Zn$ values obtained from tooth enamel from TPL and NL caves range from −0.11 to +1.07‰ (Figure 1, Figure 2, Figure 3). The absence of a mixing line between Zn concentration and $\delta^{66}Zn$ values (Kendall's correlation test for TPL and NL, respectively: $\tau b = 0.13$, $p = 0.34$, and $\tau b = 0.15$, $p = 0.13$; SOM Fig. S2) suggests no significant postmortem Zn uptake. Carnivores exhibit the lowest $\delta^{66}Zn$ values ($\delta^{66}Zn = +0.04 \pm 0.13$‰ 1σ, $n = 4$), herbivores the highest ($\delta^{66}Zn = +0.72 \pm 0.21$‰ 1σ, $n = 45$), while $\delta^{66}Zn$ values from omnivores are intermediate ($\delta^{66}Zn = +0.40 \pm 0.22$‰ 1σ, $n = 21$; Fig. 2; SOM Table S3). Hyenas display higher values than those of other sympatric carnivores ($\delta^{66}Zn = +0.42 \pm 0.13$‰

1σ, $n = 6$). Most notably, the TPL1 *H. sapiens* individual exhibits an identical $\delta^{66}Zn$ value of +0.24‰ for both M^1 and M^2, corresponding to an intermediate value between carnivorous and herbivorous taxa (Fig. 1).

Seventy-eight specimens were analyzed for $^{87}Sr/^{86}Sr$, displaying a broad range of values from 0.7103 to 0.7319, with TPL1 exhibiting a value of 0.7200. Most faunal specimens exhibits ratios between 0.7127 and 0.7179 (51%, $n = 40$; SOM Table S3). These ratios are similar to what was observed for THM (0.7135–0.7173; 52%, $n = 36$; Bourgon et al., 2020) and consistent with the complex local geology composed of Late Carboniferous (Moscovian) to Permian limestone, Silurian sandstone, and Paleozoic granite (Bacon et al., 2011; Duringer et al., 2012).

Stable carbon and oxygen isotope analyses were conducted on 32 specimens, the remaining having been previously analyzed and published (Bacon et al., 2018). The enamel $\delta^{13}C_{diet}$ values of the fauna, ranging from −30.2 to −14.1‰ ($n = 32$), are typical of a wide range of feeding behaviors from pure subcanopy to open woodlandC_3 plant feeders and C_4 plant feeders (i.e., grazers; Cerling and Harris, 1999; Cerling et al., 2004, 2015; Tejada et al., 2020; Fig. 3; SOM Table S4), while the $\delta^{18}O_{apatite}$values range from −9.4 to −0.6‰. TPL1 *H. sapiens* individual exhibits $\delta^{13}C_{diet}$ and $\delta^{18}O_{apatite}$ values of −26.4‰ and −6.4‰, respectively. While a C_4 grass component is present in the area surrounding the sites (as exemplified by $\delta^{13}C_{apatite}$ values for some herbivorous specimens at NL and TPL that are typical of C_4 feeders; Cerling and Harris, 1999; Cerling et al., 2015), the prevalence of low $\delta^{13}C_{apatite}$ values seen from the fossil mammal assemblage suggests a predominant C_3 environment (average $\delta^{13}C_{apatite} = -25.0 \pm 3.7‰$ 1σ, $n = 52$ and −24.9 ± 4.3‰ 1σ, $n = 28$, respectively, for NL and TPL).

The full-null LMM comparison was significant (likelihood ratio test: $X^2 = 30.72$, df = 3, $p < 0.001$), allowing us to securely assess which tested predictors were associated with

δ^{66}Zn variability. The $\delta^{13}C_{apatite}$ and $\delta^{18}O_{apatite}$ values, site and zinc concentration show no significant relationship ($p > 0.05$) to the variability of δ^{66}Zn (SOM Table S5). Contrary to what was seen in the THM fauna (Bourgon et al., 2020), body mass was not significantly associated with δ^{66}Zn variability (likelihood ratio test: $X^2 = 2.96$, df = 1, $p = 0.35$; SOM Table S5). Finally, significant relationships were observed between δ^{66}Zn variability and ^{87}Sr/^{86}Sr (likelihood ratio test: $X^2 = 19.40$, df = 1, $p < 0.001$; SOM Table S5), as well as with dietary categories whereby omnivores, bone-eating carnivores and herbivores all have higher values than carnivores (likelihood ratio test: $X^2 = 35.84$, df = 3, $p < 0.001$; SOM Table S5). ANOVA (F [3,144] = 52.75, $p < 0.001$) revealed that only the δ^{66}Zn values of omnivores and bone-eating carnivores are not significantly different ($p = 0.99$), whereas all other dietary categories are statistically distinct (SOM Table S6).

4. Discussion

4.1. Variability in tooth enamel δ^{66}Zn and trophic level

As at THM, the preservation of enamel-bound diet-related δ^{66}Zn signals is also demonstrated at NL and TPL (SOM Fig. S2). No collagen could be successfully extracted from the dentine of the tooth samples (SOM Table S2), reinforcing the usefulness of Zn isotope analysis to assess trophic levels in a tropical context where generally poor preservation of organics makes the application of traditional collagen-bound stable nitrogen isotope analysis unfeasible. The LMM conducted on the THM, NL, and TPL datasets revealed that the faunal enamel δ^{66}Zn values associated with each trophic level were in agreement between sites (SOM Table S5), thus ensuring valid trophic relation comparisons. Just as for THM, the range of δ^{66}Zn values for TPL and NL are, however, slightly smaller (1.11‰ and 1.13‰, respectively) than those from the Koobi Fora region of Turkana Basin in Kenya (1.24‰; Jaouen et al., 2016a), and the absolute δ^{66}Zn values of the whole food web are also lower (~0.50‰). While

more work is necessary to ascertain regional variability in δ^{66}Zn values—especially in other settings or parts of the world—these results are promising for paleodietary reconstruction as they suggest a certain degree of homogeneity in Zn isotopic compositions among taxa, at least on a regional scale.

ANOVA results demonstrate that δ^{66}Zn values are significantly different between all dietary categories except omnivores and bone-eating carnivores, whereby carnivores display the lowest mean δ^{66}Zn value and herbivores the highest, while omnivores and bone-eating carnivores fall in between (Fig. 2). Indeed, the δ^{66}Zn values of hyenas are significantly higher than those of other sympatric carnivores, as also observed for the Koobi Fora region in Kenya (Jaouen et al., 2016a). Bone tissue generally exhibits higher δ^{66}Zn values than muscle tissues (Balter et al., 2013; Moynier et al., 2013), thus likely resulting in higher δ^{66}Zn values in taxa that regularly consume bones such as the spotted hyena (van Valkenburgh et al., 1990; van Valkenburgh, 1996).

Omnivores display a large range of δ^{66}Zn values, with the lower end of values likely representing a predominantly carnivorous and the upper range a primarily herbivorous diet. This distinction is supported by values from fossil orangutans (*Pongo* sp.) and wild boars (*Sus* sp.), whose modern diets are predominantly composed respectively of fruits (Fox et al., 2004; Wich et al., 2006; Kanamori et al., 2010; Hardus et al., 2012) and plant matter (Barrios-Garcia and Ballari, 2012; Ballari and Barrios-García, 2014). Indeed, while both orangutans and wild boars exhibit variability in their δ^{66}Zn values as expected from taxa that display omnivorous behaviors at times (Wich et al., 2006; Barrios-Garcia and Ballari, 2012; Hardus et al., 2012; Ballari and Barrios-García, 2014), they still fall well within the range of values characteristic for herbivores (due to insufficient data to accurately determine a frugivorous range of δ^{66}Zn values, orangutans are herein considered as herbivores in terms of their δ^{66}Zn values as fruits are plant matter). Moreover, the predictive Bayesian ellipse of

omnivores does not overlap with that of carnivores and only minimally with that of herbivores, further supporting isotopically distinct δ^{66}Zn values for an omnivorous diet (Fig. 3). Conversely, the convex hulls (comprising 100% of the data) show substantial overlap between omnivores and both herbivores and carnivores, suggesting that the lower end of δ^{66}Zn values in omnivores represent a predominantly carnivorous diet, while the upper range values represent a largely herbivorous diet. The δ^{66}Zn variability of herbivorous taxa across all sites is considerable (Δ^{66}Zn$_{max-min}$ = 0.8‰; average δ^{66}Zn = +0.70 ± 0.20‰ 1σ, n = 86), although identifying dietary trends associated with distinct δ^{66}Zn values is challenging. Moreover, no apparent differences in δ^{66}Zn can be seen between animals associated with C_3 and C_4 diets (i.e., browsers and grazers) according to the LMM, as was the case in the nearby site of THM (Bourgon et al., 2020; see SOM S3for further discussion). Thus, while δ^{66}Zn variability observed in herbivores shows potential to investigate dietary preferences of sympatric taxa, further work is needed to characterize and distinguish herbivorous dietary behaviors.

Finally, the estimates obtained from the LMM for ^{87}Sr/^{86}Sr (SOM Table S5) are in agreement with what was already highlighted for THM cave, whereby lower δ^{66}Zn values are associated with higher trophic levels and higher ^{87}Sr/^{86}Sr values. While δ^{66}Zn values recorded in tooth enamel are determined mainly by diet, local geology seems to also induce some variability in δ^{66}Zn values, albeit likely to a limited degree considering the good agreement between the different dietary categories across all three sites. Conversely, the LMM found no significant relation associated with average body mass on δ^{66}Zn variability. It is possible that larger mammals were coincidentally associated with higher δ^{66}Zn values in THM (Bourgon et al., 2020). In contrast, the current LMM—using a larger sample size with a more diverse faunal assemblage—likely better explained a relationship of δ^{66}Zn variability (here the lack thereof) to body mass. As was the case for THM (Bourgon et al., 2020), no clear differences in δ^{66}Zn can be seen between animals associated with C_3 and C_4 diets according to the LMM

(see SOM S3 and SOM Figs. S3–S6 for further discussion on herbivores' δ^{66}Zn variability). Similarly, no relationship was drawn between variability in δ^{66}Zn values and zinc concentration, as demonstrated by the absence of a mixing line between variables (SOM Fig. S2). This absence of a relationship further argues for the preservation of enamel-bound diet-related δ^{66}Zn signals, as opposed to higher Zn concentrations seen in diagenetically altered dental tissues compared to modern ones (Bourgon et al., 2020).

4.2. Stable zinc isotope measurements of the Tam Pà Ling 1 Homo sapiens individual

Owing to generally poor collagen preservation in fossils from tropical contexts, direct trophic assessment of pre-Holocene humans living in rainforests has been impossible using the traditional collagen-bound stable nitrogen isotope measurements (Krigbaum, 2005). Using Zn isotope measurements, a direct trophic assessment was successfully obtained for the TPL1 individual, allowing a better understanding of Late Pleistocene rainforest human dietary reliance. Additionally, this approach offers a unique opportunity to directly assess the diet of this individual in the absence of associated archaeological evidence at TPL.

The δ^{66}Zn values obtained for the M^1 and M^2 of the TPL1 individual are identical (δ^{66}Zn = +0.24‰) and argue for a post-weaning dietary signal when considering the typical development time of the M2 (Hillson, 1996; AlQahtani et al., 2010). When compared to δ^{66}Zn values from the fossil faunal assemblages, the Zn isotope composition of the TPL1 individual falls within the range of omnivorous taxa (δ^{66}Zn between −0.04 and 0.80‰; Fig. 1) and, more importantly, within omnivores' isotopically distinct Bayesian ellipse (Fig. 3). This strongly suggests that the diet of this individual contained both animal and plant material. Alternatively, a strictly carnivorous diet with the inclusion of bone marrow could induce similar omnivore-like values, as observed for the bone-eating carnivore spotted hyena. However, no evidence of

bone marrow extraction was observed in Late Pleistocene Southeast Asia archeological records, although younger archeological records from terminal Late Pleistocene and Holocene Hoabinian period sites suggest the utilization of this resource (Forestier et al., 2015; Zeitoun et al., 2019). The δ^{66}Zn values of TPL1 also fall outside the range of bone-eating carnivores (Fig. 3) and could further suggest an omnivorous diet. However, a smaller proportion of bone marrow to the diet of TPL1 compared with that of the spotted hyenas could perhaps produce intermediate values to the carnivores' and bone-eating carnivores' dietary categories. Further work using other isotopic approaches such as stable calcium isotopes (Martin et al., 2018, 2020) could help to further address this question and more fully distinguish a bone-eating carnivore diet from an omnivorous one.

The position at the lower end of δ^{66}Zn values for omnivorous taxa suggests that a higher proportion of animal matter was included in its diet compared with most other sympatric omnivorous mammalian species. This is supported by the higher δ^{66}Zn values seen in orangutans and wild boars, which provide a frame of reference for values reflecting mostly frugivorous (i.e., herbivorous in the larger sense) and herbivorous diets with some varying degrees of animal matter consumption (Wich et al., 2006; Barrios-Garcia and Ballari, 2012; Hardus et al., 2012; Ballari and Barrios-García, 2014). Conversely, the relatively low δ^{66}Zn values of macaques (*Macaca* sp.) support the higher consumption of animal matter as a likely driving factor to lower values in omnivores. This includes but is not limited to insects, as current data suggest depleted ^{66}Zn isotopic compositions (Evans et al., 2016) similar to what is observed for other animal matter such as meat. Indeed, while macaque species show considerable flexibility in their dietary habits (Heesen et al., 2013; Kato et al., 2014; Huang et al., 2015; Sengupta and Radhakrishna, 2016; Ruslin et al., 2019; Khatiwada et al., 2020), fruits and leaves are often a staple component of their diet. However, the δ^{66}Zn values for macaques are likely not solely controlled by fruit consumption, as values similar to the

predominantly frugivorous orangutans would be expected (>60% fruits; Fox et al., 2004; Wich et al., 2006; Hardus et al., 2012). As such, a comparatively higher proportion of animal matter in their diet (primarily insects in this case, which exhibit low δ^{66}Zn values [Evans et al., 2016] similar to other animal matter) is a reasonable inference for the position of macaques and other omnivorous taxa at the lower end on the omnivore spectrum of δ^{66}Zn values. Notwithstanding the evidence for animal matter, the incorporation of plant matter into the diet of TPL1 is still supported, as almost all carnivore specimens (92%, $n = 12$) exhibit lower δ^{66}Zn values, typically associated with a higher trophic level (Jaouen et al., 2016a, b).

Furthermore, the δ^{66}Zn values from TPL1 are similar to those of the M^2 from the early Holocene tropical hunter-gatherer population of Lapa do Santo from Brazil (δ^{66}Zn = +0.26 ± 0.14‰ 1σ, $n = 13$; Jaouen et al., 2020), whose diet was described as a mixture of plants and small-to-mid-sized animals (Strauss et al., 2016, Strauss, 2017). However, this comparison with the TPL1 individual remains tentative, as trophic levels based on δ^{66}Zn could not be assigned to Lapa do Santo's individuals because no sympatric fauna from the site was analyzed. Nevertheless, δ^{15}N values also obtained from the Lapa do Santo individuals (Strauss, 2017) lend support to an omnivorous diet and thus further validate the interpretation of δ^{66}Zn values for TPL1 as indicative of a mixture of animal and plant material. Additional δ^{66}Zn data from human populations are available (Jaouen et al., 2017, 2018), but comparisons between TPL1 *H. sapiens* individuals and these human populations are ill-advised because they concern French individuals of the 13th to 20th century from various locations for which cultural, social, religious, and economic factors contributed to dietary choices (Quellier, 2013). While the absolute range of δ^{66}Zn values of previous studies (Jaouen et al., 2017, 2018) is similar to what is seen for the Laotian Late Pleistocene sites presented in the current study, data presented from Koobi Fora (where a defined food web was studied; Jaouen et al., 2016a) further highlight that baselines vary from one setting to another, just as they do for δ^{15}N values. Failure to properly

account for baseline variation would lead to incorrect assessment of consumer trophic positions (Post, 2002; Woodcock et al., 2012).

The omnivorous diet determined for TPL1 strongly contrasts with most trophic level assessments obtained from nitrogen isotope data of humans in other regions of the world for that period, where a meat-rich diet is almost consistently observed (Richards et al., 2003; Bocherens et al., 2005; Richards and Trinkaus, 2009; Naito et al., 2016; Jaouen et al., 2019). Even when data attest to plant consumption, their overall dietary contribution remains unknown (Henry et al., 2014; Power et al., 2018) or relatively low (Naito et al., 2016), while the δ^{15}N values remain high and within the range of other carnivores. Moreover, δ^{15}N values are biased from the relative importance of plants in the diet being overprinted by meat consumption, owing to the fact that plants usually contain much less protein than meat (Phillips and Koch, 2002). This bias consequently hampers accurate reconstruction of overall dietary reliance made through nitrogen stable isotope analysis by favoring animal-based diets, which is observed in most cases for other regions of the world for that period.

Even when considering only Southeast Asian sites from Late Pleistocene, the omnivorous diet assigned for TPL1 offers some much-needed insights into our species' overall dietary reliance and behaviors for this region of the world for that period. Indeed, current data are scarce and rely mostly on archaeological material evidence (Barker et al., 2007; Wedage et al., 2019; Langley et al., 2020) and, to a lesser extent, on carbon stable isotope measurements (Roberts et al., 2017). Even when archeaological material such as organic remains (i.e., animal bones or plant remains) and stone tools are found, they mostly prove inadequate for reconstructing overall dietary reliance, as they offer indirect evidence of past human diets and may be unrepresentative of daily subsistence practices. In contrast, enamel δ^{66}Zn analysis provides new insights into human diets (i.e., degree of herbivory vs. faunivory) during the period of tooth crown formation. While differences between sites could also suggest different

adaptations of humans to tropical forest environments, methods such as zinc stable isotope analysis offer additional insights and nuances by providing direct evidence of overall dietary reliance. The omnivorous diet determined for TPL1 could also be in line with behaviors associated with specialized adaptations to tropical rainforest environments seen from archaeological records from other Southeast Asian sites, perhaps even such as exploitation and possible processing of plants (Barton, 2005; Barker et al., 2007; Summerhayes et al., 2010; Wedage et al., 2019) and diverse specialized hunting strategies (Barton et al., 2009; Bacon et al., 2015; Roberts et al., 2017; Wedage et al., 2019; Langley et al., 2020).

Alongside trophic level assessment using δ^{66}Zn measurements, the stable carbon isotope analysis of the M[1] of TPL1 provides additional dietary information (Fig. 3). The $\delta^{13}C_{diet}$ value of TPL1 is relatively low ($\delta^{13}C_{diet} = -26.4‰$) and falls well within the range of food obtained from a forested C_3 environment (Cerling and Harris, 1999; Cerling et al., 2015). This range of values is also in line with a humid climate and forested environments highlighted for northeast Laos before the onset of the Last Glacial Maximum (Milano et al., 2018). Within the studied Late Pleistocene food web of tropical forest, this value is most similar to that of taxa such as *Macaca* sp., *Sus* sp., and *Muntiacus* sp. The $\delta^{13}C_{diet}$ value of TPL1 is also similar to that of human specimens from terminal Pleistocene and Holocene deposits at Fa Hien-Lena and Balangoda Kuragala (~12–3 ka cal BP) and slightly lower than that from Late Pleistocene Batadomba-Lena fauna (dated to 36–12 ka; Roberts et al., 2015, 2017). While studies from other Late Pleistocene Southeast Asian sites suggested that semi-open rainforest and rainforest edges were preferred by humans (Roberts et al., 2015, 2017), the $\delta^{13}C_{diet}$ value from the TPL1 individual clearly highlights a strict reliance on dietary resources from forest environments. This value is also very close to the threshold ($\delta^{13}C_{diet} = -27.2‰$) established for a diet entirely consisting of resources from closed-canopy forests (Roberts et al., 2017, 2018; Tejada et al.,

2020), further contrasting with an initial assessment that tropical forests could not support human foraging in the absence of agriculture (Bailey and Headland, 1991).

5. Conclusions

Collectively, δ^{66}Zn values from TPL1 and comparison with fauna strongly suggest that the diet of this *H. sapiens* individual contained both plant and animal material, while the δ^{13}C value indicates that the resources consumed came strictly from forested environments. This omnivorous diet contrasts with most trophic level assessments obtained from nitrogen isotope data of humans in other regions of the world for that period, where a meat-rich diet is almost consistently supported. The current results are also the oldest direct evidence of overall dietary reliance and subsistence strategies for Late Pleistocene humans in rainforests. These results clearly reinforce a growing body of evidence for early human foragers' exploitation of a broad resource base and long-term occupation of tropical rainforest environments. Moreover, this study demonstrates the potential of δ^{66}Zn analysis on teeth where archeological material evidence is absent, and other forms of trophic level assessment are not possible. Finally, distinctions observed between other sympatric primates suggest that δ^{66}Zn analysis proves especially valuable for studying the diet and ecology of fossil primates but, most importantly, to explore adaptations and dietary reliance of different fossil hominins.

Author contributions

N.B., K.J., and T.T. designed the research; A.-M.B., T.E.D., F.D., L.L.S., A.Z., P.D., J.-L.P., Q.B., E.S., S.F., C.Z., F.C., F.A., E.P.-E., K.E.W., R.J.-B, T.L., V.S., S.B., T.S., P.S., D.S., and J.-J.H. contributed new reagents/analytic tools; N.B., E.D., D.F., J.M., M.T., and N.-H.T.

performed research; N.B. analyzed data; N.B. wrote the paper with input from all authors; N.B., A.-M.B., T.E.D., F.D., L.L.S., A.Z., P.D., J.-L.P., Q.B., E.S., S.F., C.Z., F.C., F.A., E.P.-E., K.E.W., R.J.-B, T.L., V.S., S.B., T.S., P.S., and D.S. contributed to excavation and recovery of the materials as part of the Laos Project.

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Declaration of competing interest

The authors declare that they have no competing interests.

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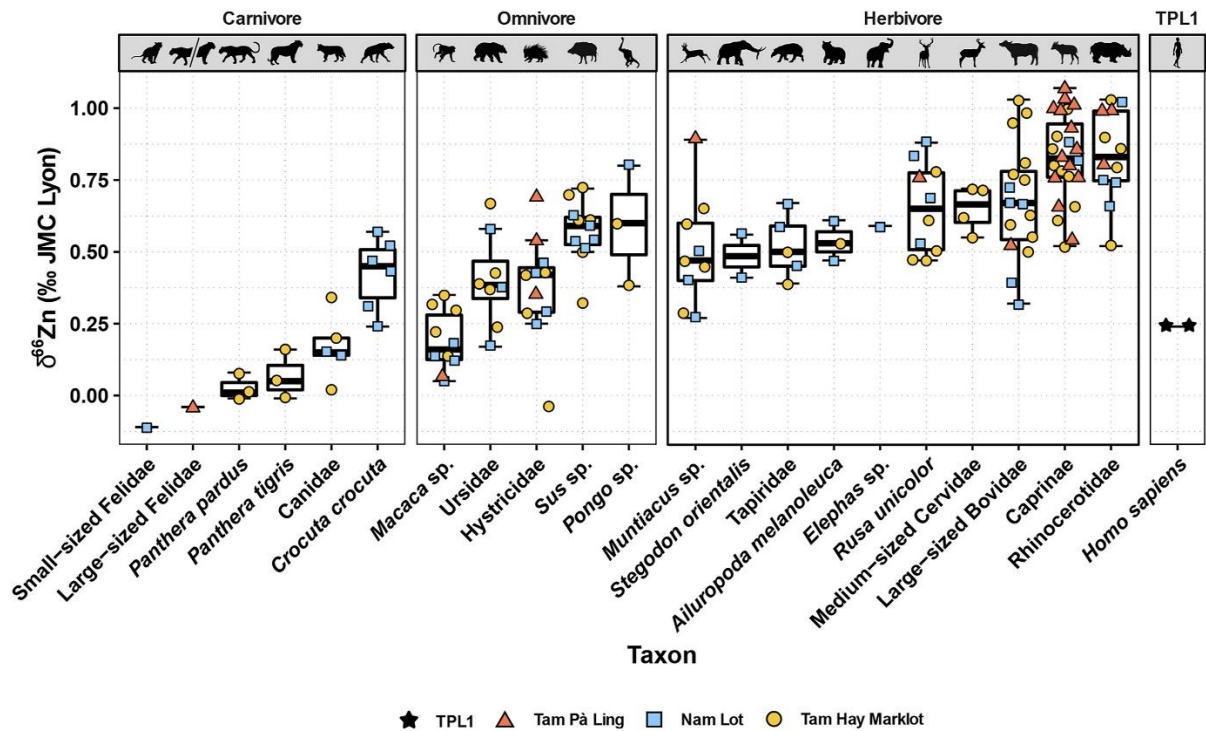
Figure

Figure 1. Box and whisker plots of the range of δ⁶⁶Zn values (relative to the JMC-Lyon Zn isotope standard) in tooth enamel for each taxon and the TPL1 *H. sapiens* individual (63–46 ka; ★ black star). Each of the other colors and symbols represents specimens from different sites: Tam Pà Ling (70–1.1 ka; orange triangles), Nam Lot (86–72 ka; blue squares), and previously published data from Tam Hay Marklot (Bourgon et al., 2020; 38.4–13.5 ka; yellow circles). Note the inclusion of the bone-eating carnivore *C. crocuta* with other carnivores. The boxes represent the 25th–75th percentiles, with the median represented by a bold horizontal line. The average analytical repeatability of samples was 0.01‰. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
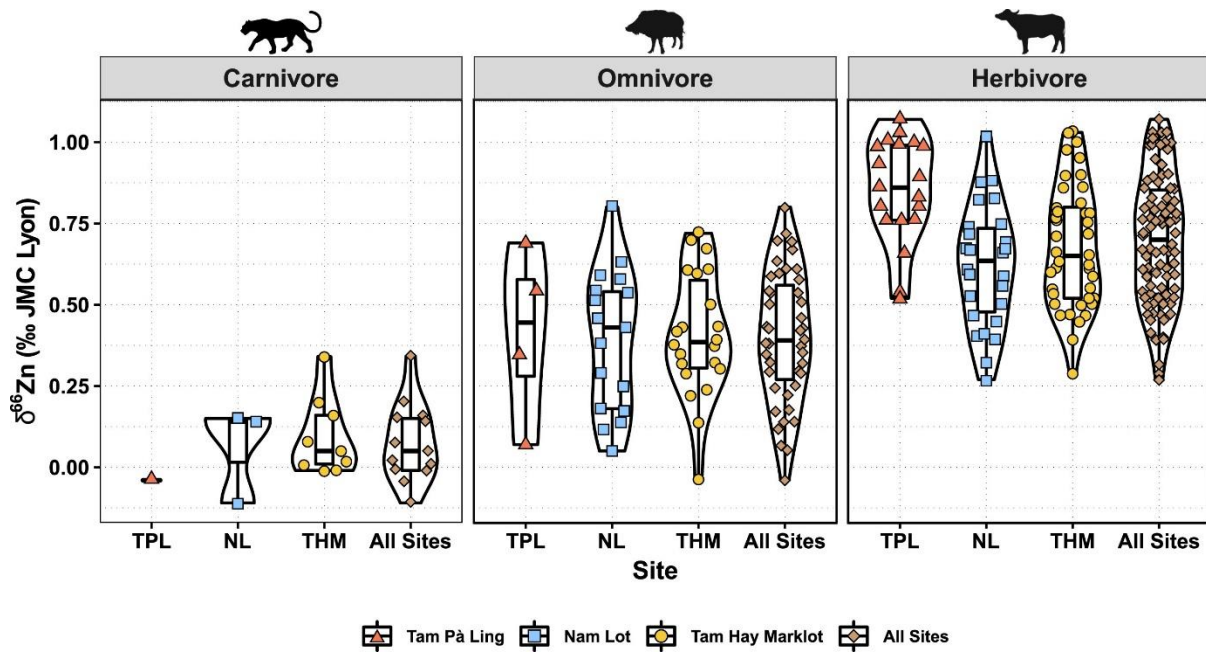
Figure 2. Violin plots presenting the range of δ^{66}Zn values (relative to the JMC-Lyon Zn isotope standard) of tooth enamel from each site for carnivores (excluding the bone-eating carnivore *C. crocuta*), omnivores, and herbivores. The color of each plot represents specimens coming from different sites: Tam Pà Ling (70–1.1 ka; orange triangles), Nam Lot (86–72 ka; blue squares), previously published data from Tam Hay Marklot (Bourgon et al., 2020; 38.4–13.5 ka; yellow circles), and all sites combined (brown diamonds). The outline of the plots illustrates kernel probability density, where the width represents the proportion of the data found therein. The boxes from the box and whisker plots inside the violin plots represent the 25th–75th percentiles, with the median represented by a bold horizontal line. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
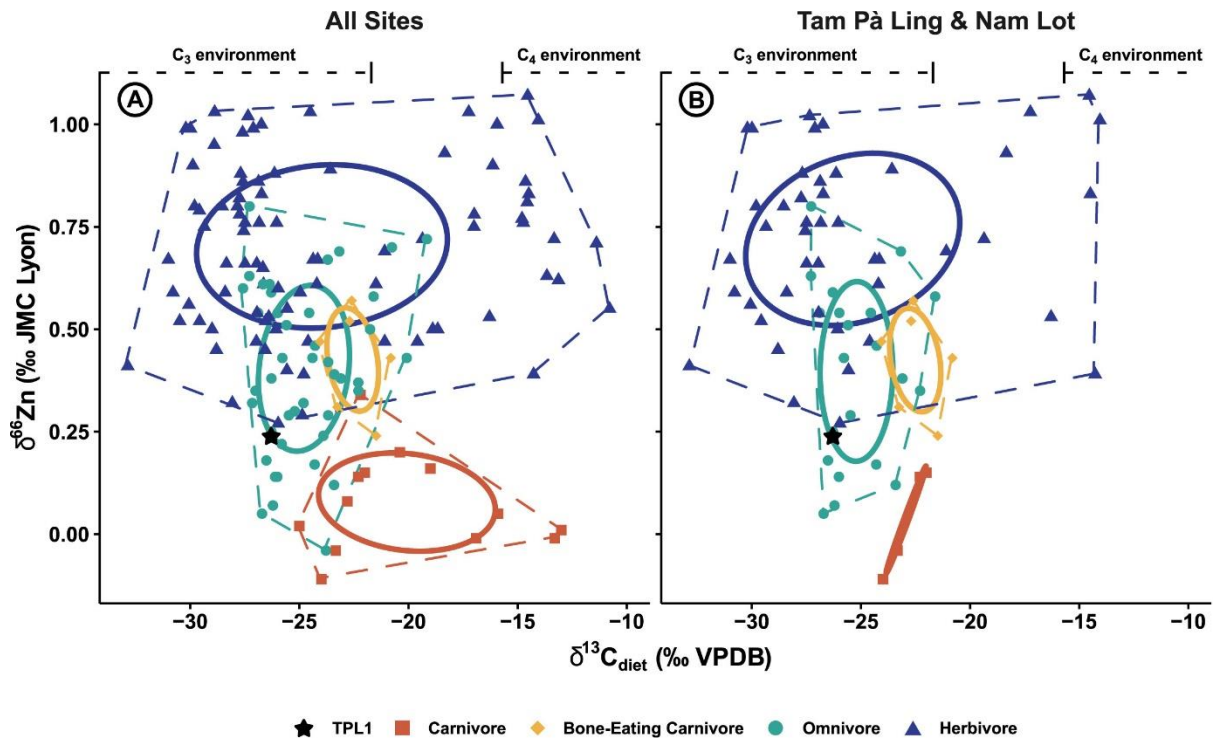
Fig. 3. Distribution of enamel δ^{66}Zn versus δ^{13}C$_{diet}$ values for TPL1 individual (black star), carnivores (red square), omnivores (turquoise circle), bone-eating carnivore *C. crocuta* (yellow diamond), herbivores (blue triangle) for A) all three Late Pleistocene sites of Tam Pà Ling (70–1.1 ka), Nam Lot (86–72 ka) and previously published data from Tam Hay Marklot (Bourgon et al., 2020; 38.4–13.5 ka) and B) only Tam Pà Ling and Nam Lot (this study and Bacon et al., 2018). 'C$_3$ environment' and 'C$_4$ environment' are respectively defined by δ^{13}C$_{diet}$ <−21.7‰ and >−15.7‰. Dashed lines for convex hulls represent the full range of variation and full lines for ellipses represent 40% predictive ellipses. VPDB = Vienna Pee Dee Belemnite.