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Earliest molecular evidence of Pu-erh tea uncovered in northwest China

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Dear Editor,

Since its origins in the Himalayan foothills of Yunnan province, southwest China, the salubrious tea produced from *Camellia sinensis* has been disseminated to every corner of the globe and has been embraced by countless people captivated by its aroma.

There are ample historical records to demonstrate that tea had become firmly entrenched as China's favored beverage. For instance, Lu Yu's *Classic of Tea* (茶经) during the Tang Dynasty (618-907 CE) is a huge milestone. However, the direct chemical evidence of tea remains scarce. Up to now, the earliest tea remains were discovered in a tomb in Zouchen, Shandong province, east China, during the early Warring States period (475-221 BCE).[1] Then, the tea is believed to have spread along the Silk Road to Tibet around 200 CE.[2] These studies used caffeine and theanine as biomarkers to identify tea, but they do not specify the exact type of tea. While tea originates from the species *Camellia sinensis*, diverse processing methods result in a wide array of flavor profiles. Based on the degree of fermentation, tea is generally categorized as green, white/yellow, Oolong, black, and Pu-erh. In particular, Pu-erh tea, derived from the variety *Camellia sinensis* var. *assamica*, is a unique fermented tea indigenous to the Pu-erh, Xishuangbanna, Linchang regions, Yunnan province, southwest China, since antiquity. As a historical icon of the Ancient Tea Horse Road, Pu-erh tea has maintained significant economic and cultural importance in East Asia for centuries. However, the early exploitation history of Pu-erh tea remains obscure owing to the lack of precise botanical records in ancient times and definitive archaeological evidence. Namely, ancient texts are inadequate to explicitly determine whether tea from the Pu-erh region should be classified as Pu-erh tea or categorized as common tea. This study uses Py-GC/MS to characterize nine ancient soil samples (coded as XP1-9) uncovered in the Xipang Jingjiao (Eastern Syriac Christianity) Monastery Relics Site, Turpan basin, Xinjiang, northwest China (Figures 1A-E).

Screening of nine samples from the Xipang site revealed distinct chemical profiles for samples XP6 and XP8. The results of Py-GC/MS analysis of nine samples mainly contain fatty acids (e.g., palmitic acid, stearic acid), sterols, nitrogen-containing heterocycles, and/or some unique biomarkers (Figures 1I~L). In particular, caffeine (Figure 1G), theacrine (Figure 1H) and the (tri)methoxybenzene family were consistently identified in both XP6 and XP8 samples, but were absent in the other seven samples, which suggests that these biomarkers are not the result of external contamination but are instead endogenous. Sample XP6 from a pottery jar (Figure 1C) was unearthed in excavation unit T0605, and sample XP8 was found in a house remain (coded as F10) (Figure 1B). The archaeological layer of both samples belongs to Gaochang Uyghur period (9th-13th century CE). Under a microscope, sample XP6 contains many black pieces with vertical stripes arranged in a regular way (Figure 1D).

In archaeological context of China, caffeine is primarily regarded as a biomarker of tea prior to the introduction of coffee in the 19th century. More importantly, theacrine is just found in Pu-erh tea,[3] and in Kucha from *Camellia assamica* var. *kucha* due to its unique chemical production mechanism regulated by the 9-N-methyltransferases encoded by gene TEA024443.[4] Sometimes, Kucha is also considered a kind of Pu-erh tea on the modern tea market. In this case, both ancient samples could be identified as Pu-erh tea residues. Moreover, the proportion of caffeine and theacrine would provide more species information. The ratio of caffeine and theacrine (C/T) is less than 0.5 in Kucha[5] and more than 2 in Pu-erh tea, and unavailable in common tea due to the absence of theacrine.[4] When brewing for 30 minutes, the leaching of theacrine is slow, about 0.67-fold of caffeine in Pu-erh tea; and when this principle is applied to Kucha and common tea, C/T in tea will decrease after brewing, especially the ratio of Kucha is still less than 1 and that of common tea will still be very high. Based on the peak integration method (namely peak area) of GC/MS data analysis, the C/T ratios in samples XP6 and XP8 are 4.8 and 2.0, respectively, so Kucha and common tea would be excluded, indicating that both samples contain Pu-erh tea residues.

Furthermore, the (tri)methoxybenzene family also provides some important information. A suite of methoxybenzenes, specifically 1,2,3-trimethoxybenzene, 1,2,4-trimethoxybenzene, 1,2-dimethoxy-3-methylbenzene, and 1,2-dimethoxybenzene, was detected in XP6, while 1,2,3-trimethoxybenzene and 1,2-dimethoxybenzene were identified in XP8. These compounds are characteristic volatile markers found in ripe Pu-erh tea, contributing to its distinct aromatic profile.[6] The trimethoxybenzene family was neither found in fresh Kucha nor in fermented black tea made of fresh Kucha.[7] In other types of tea, such as green tea, black tea, and oolong tea (semi-fermented), it is hard to detect the (tri)methoxybenzene family.[8] Although 1,3,5-trimethoxybenzene was found in the Warring States tea remains,[1] it is distinct from the methoxybenzene compounds in this study. Thus, the (tri)methoxybenzene profile further supports that tea residues in samples XP6 and XP8 originated from Pu-erh tea, instead of Kucha or common tea.

Pu-erh tea tree (Figure 1F) in China is solely distributed in the Pu-erh and its surrounding regions in Yunnan Province, southwest China;[9] thus, the source of the Pu-erh tea residues identified at the Xipang site can be traced back to these regions. The tea production in the Pu-erh and its surrounding regions could be traced back to the Tang Dynasty, when *the History of Barbarians* (蛮书) written by Fan Chuo (樊绰) during the late 9th century CE documents that "tea originates from mountains in Yinsheng region (including today's Pu-erh region). (茶出银生城界诸山)". In the Southern Song Dynasty (1127-1279 CE), *the Bowuzhi* (续博物志), written by Li Shi (李石), records that "tea originates from mountains in Yinsheng region. Get it at your leisure. (茶出银生诸山，采无时)". However, these historical records are insufficient to determine whether the tea from the Pu-erh, Xishuangbanna and Linchang regions was Pu-erh tea or common tea. Since wild-grown or cultivated Pu-erh tea is not found or recorded in other tea-producing regions of China (e.g., Zhejiang, Fujian, or Anhui province), then this study presents the first molecular evidence of ancient Pu-erh tea, indicating that it was at latest produced in the Pu-erh and its surrounding regions during the Gaochang Uyghur period.

Despite the vast geographical distances, tea has served as a bridge connecting diverse cultures and peoples. There is a famous wall-painting made in the Tang dynasty unearthed in Astana Necropolis, Turpan basin, Xinjiang. This painting, *a holding-cup maid trio* (托盏侍女三人图), is thought as the evidence of ancient tea consumption in Xinjiang.[10] Additionally, the tea trade is prosperous alongside the Silk Road. "The Hui Hu (Uyghurs) people in today's Mongolia plateau came to the court with many horses and traded them for tea", stated in *A Record of Things Seen and Heard by Mr. Feng* (封氏见闻记), compiled around the beginning of the 9th century. After the collapse of the Hui Hu Khaganate in the 9th century, some Uyghurs migrated from Mongolia plateau to Xinjiang, and established Gaochang Uyghur Kingdom, where they continued the practice of tea consumption. According to *XuZizhi Tongjian: Changbian* (续资治通鉴长编), "Our State (the Western Xia regime (1038-1227 CE) in northwest China) shares the boundary with northwestern

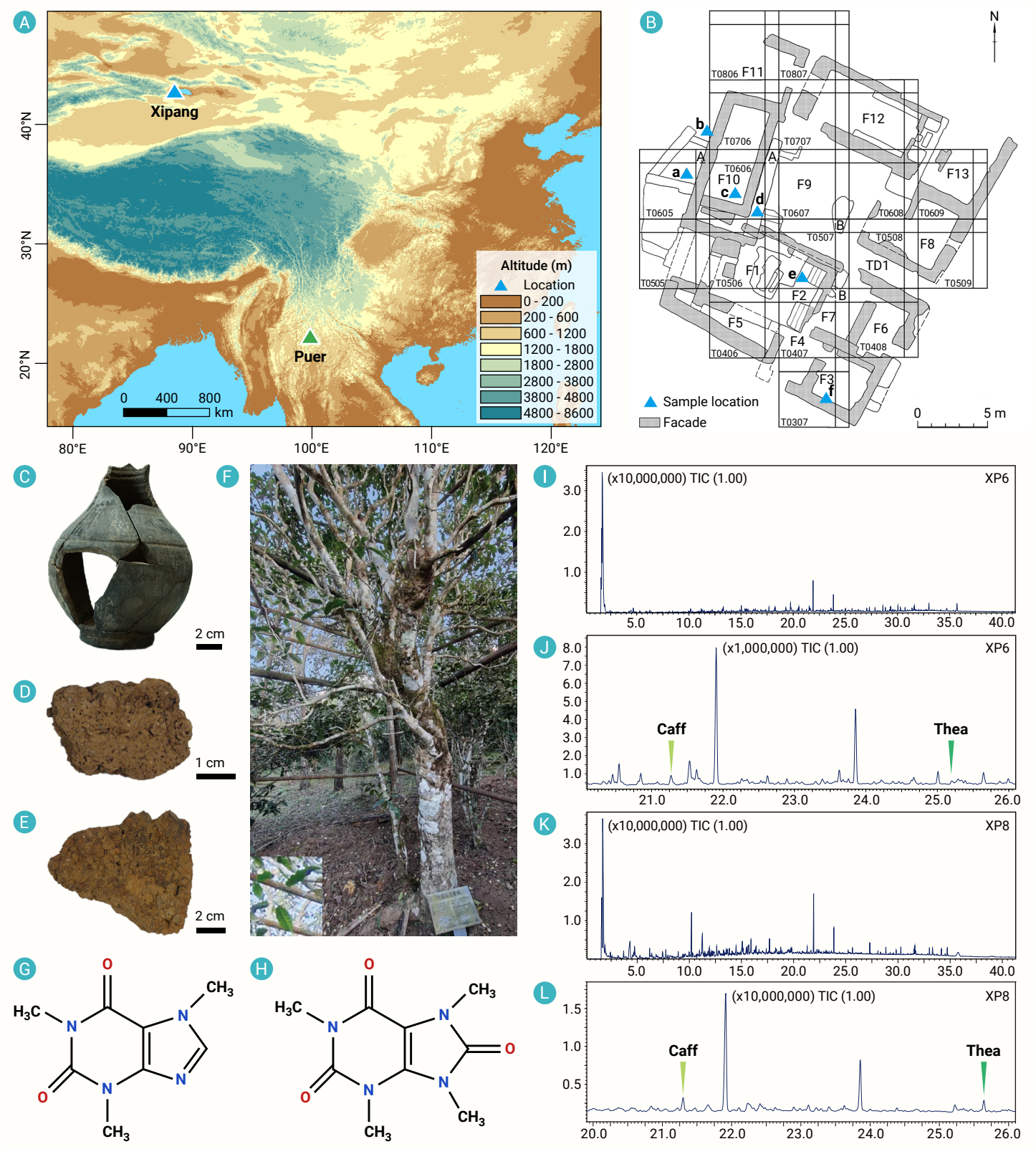

Figure 1. Ancient tea residue samples uncovered at the Xipang Site (A) the location of the Xipang site and Pu-erh region; (B) the layout of the Xipang Site; position a, where XP6 was taken; position b, where XP8 was taken; position c-F10,d-F10,e-F2,f-F3 where soil samples XP2/3/9, XP1, XP7, XP4/5 were takened respectively; (C) the pottery jar containing sample XP6; (D) part of sample XP6; (E) sample XP8; (F) an old Pu-erh tea tree grown in the Pu-erh region; (G) Caffeine; (H) Theacrine; (I) TIC (total iron chromatogram) of XP6; (J) part of TIC of sample XP6; (K) TIC of sample XP8; (L) part of TIC of sample XP8. (Caff, Caffeine; Thea, Theacrine)

states (including Gaochang Uyghur). May barter a few half-kilograms of tea for a sheep (本界西北连接诸蕃，以茶数斤，可以博羊一只)", which demonstrates the extensive tea trade conducted in Gaochang Uyghur Kingdom. Thus, the Pu-erh tea trade extending from the Pu-erh region in Yunnan to Turpan in Xinjiang over a long distance (Figure 1A) constitutes an integral component of this trade network.

Pu-erh tea has had a variety of positive effects on the local population in Xinjiang. It is commonly believed that Pu-erh tea can help refresh individuals leading a semi-nomadic lifestyle, particularly after consuming excessive amounts of greasy meat. Moreover, considering that regular drinking water

may have been contaminated during that period, the necessity of preparing tea also encouraged the local population to adopt the practice of drinking boiled water, which contributed to improving public health. As with many other distinctive economic and cultural products that traveled along the Silk Road from their place of origin, the word for tea spread alongside the commodity itself.

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AUTHOR CONTRIBUTIONS

Lyuting Wang, Conceptualization, Methodology, Software, Data curation, Writing- Original draft preparation, Visualization, Investigation.Wensuo Liu, Resources, Writing - Review & Editing. Long Wang, Resources, Writing - Review & Editing. Wenying Li, Resources, Writing - Review & Editing. Bin Han, Supervision, Software, Validation, Writing - Review & Editing. Yimin Yang, Supervision, Resources, Writing - Review & Editing, Project administration, Funding acquisition. All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

The raw data are available from the corresponding author on reasonable request.