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Uncovering the mysterious identity of Taisui—an old Chinese folk legend

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Dear Editor,

Taisui (太岁) has attracted great attention in China because it has been described in ancient Chinese texts as an entity possessing mysterious properties. In Chinese mythology and folklore, Jupiter is the God of the "Sui" (year) and is worshiped in Daoism. Jupiter appears at the same location in the sky approximately every 12 years (Jupiter's orbit is 11.86 years). However, since each cycle of Jupiter lasts for exactly 11.86 years and one Chinese year cycle comprises exactly 12 years, after approximately 84.7 years, Jupiter travels one cycle more than the number of cycles assumed by the ancient Chinese, which is called Taisui. According to the belief of the ancient Chinese, people are alerted when Taisui is digging in the ground, foretelling the destruction of houses or occurrence of natural disasters, such as floods and earthquakes (Zhao et al., 2018).

Taisui has also appeared under several different names in Chinese historical records, for example, as Feng, Shirou, Rouzhi, and Roulingzhi. The earliest historical records about Taisui date back to the earliest time between the 1st century BCE and the 1st century CE in old Chinese texts (Fang, 2009). The Daoist Ge Hong referred to Taisui as "Rouzhi" (meat). He described Taisui as one of the five types of excrescences, or "flesh excrescences" that will provide an individual with the invulnerability and longevity associated with Daoism. *The Compendium of Materia Medica* (Bencao Gangmu) also describes this medicine as "Feng" in Chapter 51 and cites "Shanhaijing", according to Guo's commentary, with "Zazhi" and "Xihu Zhi" as original sources. It refers to "Feng" as a class of peculiar organisms such as sea cucumbers or anemones endowed with supernatural qualities based on the supposition that they are spiritual beings (Li et al., 2013).

In modern China, Taisui was recorded for the first time in 1963 and reported again in 1992. Since then, the myth of Taisui has continued, and the Chinese media began to report a series of Taisui findings coupled with extraordinary pictures. In recent years, many unidentified entities were found and claimed to be Taisui (Dai, 2007; Wang et al, 2015). However, the properties of Taisui have thus far remained unclear, and there is no scientific evidence to support the medical properties of Taisui. Herein, we report that Taisui is more likely an abiotic entity because obvious characteristics were shared by seven Taisui samples from different regions in China (Figure 1A) as evidence by results of examinations of its physical appearance, DNA content, elemental and chemical compositions, microbial presence, and bioactivity.

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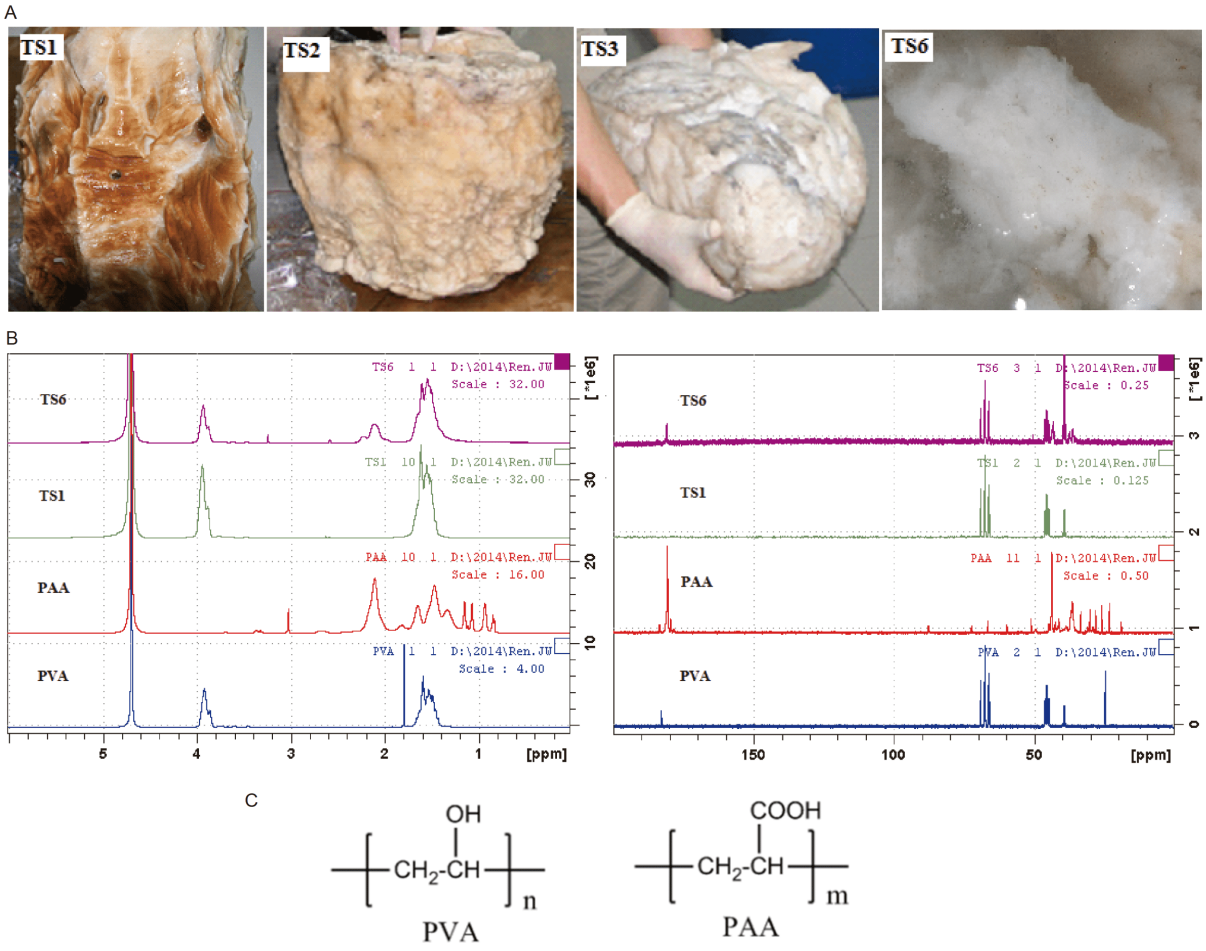


Figure 1 (Color online) Appearance and chemical identification of Taisui. A, Appearance and texture of various Taisui samples (TS1–3: meat-like Taisui; TS6: gel-like Taisui). B, ^{1}H and ^{13}C NMR spectra of TS1, TS6 with PAA and PVA standards measured with Bruker Avance III 500 ($^{1}H/^{13}C$ 500/125 MHz) spectrometer in D_2O. C, Chemical structures of PAA and PVA identified from Taisui.

In relation to the major non-metal elements in biomaterials, a majority of the examined Taisui tissues were composed of 42%–51% carbon with a very low phosphate and sulfur levels, indicating that the non-metal elements in biomaterials are quite different from the Taisui samples we examined (Tables S1 and S2 in Supporting Information). Compared to the level of trace metal and non-metal elements in regular biomaterials (Table S3 in Supporting Information), Taisui samples showed high levels of trace metal elements, including Cu, Ca, Mg, Fe, and Mn, and low levels of P and S elements (e.g., 0–0.017% P in Taisui; 0.2%–3.11% P in maize, bacterial, soil, and human body), indicating that several major trace metal elements may be trapped in the specific microstructure of Taisui tissues. A very low or non-detectable level of nitrogen was found in solid-like Taisui tissues (TS1–TS4), but a relatively high level of nitrogen, between 3% and 10%, was detected in soft gel-like Taisui tissues (TS5–TS7), which is higher than that in Zheng's report (0.012%) (Zheng et al., 2010). According to our observation of soft gel-like Taisui samples, a typical microstructure comprising a network of strings was observed under light microscope and SEM (Figures S1 and S2 in Supporting Information). Furthermore, liquid Taisui tissues much likely absorbed a lot of water during storage, resulting in the enrichment of inorganic nitrogen from our stored solution containing nitrate, which could also be confirmed by higher water content in liquid Taisui samples TS5–TS7 (>90%) than solid-like samples TS1–TS4 (48.3%–82.1%) (Table S1 in Supporting Information).

AMS-^{14}C measurements dated the Taisui samples back to 40,000 years (Table S4 in Supporting Information). With such long life span, it is impossible for biological entities to survive. Furthermore, NMR examination of Taisui samples confirmed that the chemical properties of Taisui were identical to those of polyvinyl alcohol (PVA) for meat-like types of Taisui samples, which showed two groups of proton signals at 1.21–1.83 and 3.77–4.10 ppm corresponding to two groups of carbon signals at 41.1–42.6 and 62.2–65.4 ppm and to those of a mixture of PVA and polyacrylic acid (PAA) for the soft gel-like types of Taisui samples, which showed

two groups of proton signals at 1.21–1.83 and 1.92–2.36 ppm corresponding to two groups of carbon signals at 36.1–37.3 and 42.9–44.1 ppm and a group of carbonyls at 180.7–181.3 ppm (Figure 1B and C). These results were confirmed by comparing the 1D NMR and 2D NMR data of a PAA standard. In addition to PVA, the two types of Taisui samples possessed different types of PAA as the other primary ingredient. The higher relative molecular weight of PAA in the TS5–TS7 samples is likely influenced by the existence of PAA, leading to differential formation of intramolecular and intermolecular ether bonds of PVA molecules. This resulted in two types of Taisui samples—solid Taisui with higher polymerization degree of PAA polymers or soft gel-like Taisui with a lower polymerization degree of PAA polymers.

All Taisui samples showed lower DNA content than soil samples from their original location, which was most likely due to microbes found in the environment (Table S5 in Supporting Information). Compared to protein concentrations, nitrogen content was low in the Taisui samples based on the measurements of peptides, proteins, and amino acids (Tables S6 and S7 in Supporting Information). Furthermore, no Taisui sample directly showed detectable antimicrobial activities, and their cytotoxicity to cultured tumor cell lines at 10 mg mL^{-1} after bioactive screenings included antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus fumigatus* as well as cytotoxicity against A549, MCF-7, HCT116, and HepG2 cell lines using the MTS assay. Based on analyses using light microscopy and SEM, DNA content, element composition, AMS-^{14}C measurement, NMR data, and bioactive screenings, the examined Taisui samples were scientifically suggested to more likely belong to naturally formed organic materials with special microstructures, which may allow the trapping of metal elements and organic materials for the potential health benefits recorded in old Chinese texts.

Previous studies also speculated that Taisui is a huge microorganism complex composed of slime mold and myxcobacteria, or certain fungi alone, or myxcobacteria alone (Dai, 2007; Zhu et al., 2011). Two *Myxomycetes* spp., 18 filamentous fungal genera, two yeasts, three gammaproteobacterial species, and one *Bacillus* sp. have been reportedly found in Taisui (Dai, 2007; Lin, et al., 2013; Li et al., 2015). However, these previous studies on Taisui reported doubtful results because the suspected reports were mostly derived from the partial examination of only one or two Taisui samples. These samples might have been contaminated due to long-term storage, or the microbial examinations were most likely performed on samples collected only from the surface of the Taisui, leading to a false positive detection of fungal isolates inside the Taisui. In this study, all the collected samples were subjected to strict precautions to avoid microbial contamination, and the samples were held for minimal storage periods. In terms of Taisui sample handling, all samples were first sterilized to make sure that there were no traces of accidental microbial contamination, and fungal and bacterial examinations were performed on the specimens collected from within the Taisui samples. Among the seven Taisui samples (Table S8 in Supporting Information), fungal isolates were only obtained from TS3 and TS4, and only *Fusarium solani*, *Acremonium* sp., and *Engyodontium album* were identified. These fungal isolates likely came from contamination due to air, soil, water, microbes, and plants. In addition to the culture-dependent approach, we used high-throughput sequencing to detect microbes in the Taisui samples (Figures S3–S6 in Supporting Information) and discovered that one OTU belonged to *Paecilomyces* sp. and three *Trichoderma* spp. made up 62% to 99% of the total fungal relative abundance (Table S9 in Supporting Information). *Paecilomyces* spp. are commonly found in soil, food products, and cosmetics. *Trichoderma* spp. are often opportunistic, avirulent plant symbionts that commonly occur in soil, wood, and vegetables. *P. chrysogenum*, the dominant filamentous fungus of *Eurotiomycetes*, is common in temperate and subtropical regions, particularly in indoor environments.

Evidently, the OTUs detected from Taisui likely came from environmental contamination since the chemical composition of Taisui samples was verified as consisting of high-molecular weight polymers detected in our NMR and molecular weight distribution analyses (Table S10, Figure S7 in Supporting Information). Based on multiple aspects of our structural and biological analyses of Taisui samples, we conclude that Taisui is not biotic in nature; rather, it has an abiotic origin and has gone through a long geological evolution.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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SUPPORTING INFORMATION

Supplementary Methods

Figure S1 Microstructure of the sections of solid-like and soft gel-like Taisui under light microscope.

Figure S2 Scanning electron microscopy images of the inner structure of solid-like (TS1) and soft gel-like (TS6) Taisui using a Hitachi (Tokyo, Japan) SU8010 cold-emission scanning electron microscope at 2 or 3 kV.

Figure S3 Relative abundance of the dominant fungal phyla across Taisui samples collected from different places in China. Others were designated as minor classes detected at lower concentrations.

Figure S4 Ordination diagram of principal component analysis (PCA) of the fungal community in seven Taisui samples.

Figure S5 Relative abundance of the dominant bacterial phyla across Taisui samples collected from different places in China. Others were designated as minor classes detected at lower concentrations. Molecular formula of Taisui samples.

Figure S6 Ordination diagram of principal component analysis (PCA) of bacterial community in seven Taisui samples.

Figure S7 GPC graphic of Taisui samples as determined by GPC with RI detector (Agilent).

Table S1 Percentage of non-metal and metal elements in the total elements from Taisui tissues by element analyzer and ICP-OES spectrometer

Table S2 Percentage of O and Si in total elements of Taisui tissues was measured by EM-EDS methods (Hitachi 8020 with Oxford EDS)

Table S3 Summary of non-metal elements in typical organisms and soil

Table S4 AMS-14C results of Taisui samples

Table S5 DNA contents of Taisui and soil around Taisui (S) samples collected from different places in China

Table S6 Protein quantitation was performed using Bradford assay, and peptide quantitation was performed by measuring absorbance with DeNovix DS-11

Table S7 Amino acid composition of Taisui

Table S8 Fungal isolates from Taisui samples

Table S9 Diversity and evenness indices of fungal and bacterial communities in Taisui samples collected from different places in China

Table S10 Weighted-average molecular weight of Taisui samples

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