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ORIGINAL ARTICLE

Sequencing and quantifying plastid DNA fragments stored in sapwood and heartwood of *Torreya nucifera*

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Abstract The selection of wood species and the styles of sculpture play key roles in the characterization of Buddhist statues. After Jianzhen, a Chinese Buddhist monk, visited Japan in the mid-eighth century, wood of the genus *Torreya* had been frequently used to produce single-bole statues. Establishing measures for the accurate identification of wood in the genus *Torreya* is effective for investigating the drastic change in the production of statues during this period. Analyzing the plastid deoxyribonucleic acid (DNA) fragments extracted from wood is considered helpful in the identification of species in the same genus. This study analyzed the sequences and residual amounts of plastid DNA fragments in the wood of *Torreya nucifera*. Nucleotide substitutions in the plastid DNA were clearly identified between *T. nucifera* and the species distributed in China, indicating that the wood of *Torreya* sp. can be discriminated based on the plastid DNA sequences. DNA polymorphism analyses revealed sequence diversity for the intergenic spacers on the *T. nucifera* plastid DNA. A series of polymerase chain reaction (PCR) analyses demonstrated that the plastid DNA fragments with a length of approximately 100 bp could be amplified from the residual DNA extracted from the *T. nucifera* sapwood with longer elapsed years after cutting. Therefore, an identification of wood species in the genus *Torreya* based on their plastid DNA is considered to be one of the most effective measures taken in the study regarding the historical changes of Buddhist statues.

Keywords DNA · Plastid · *Torreya* · Wood identification

Introduction

The genus *Torreya* belongs to the family Taxaceae, and consists of six species worldwide. In Asia, *Torreya nucifera* Siebold & Zucc. is distributed on the Honshu, Shikoku, and Kyushu islands of Japan, and in Jeju and on the Wando islands of South Korea [1]. *T. nucifera* is a big tree up to 25 m tall, with a trunk measuring 2 m d.b.h, and grows scattered in mixed conifer and hardwood forests [2]. In China, three species are distributed: *T. fargesii* Franch. mainly in Chongqing, Hubei, Hunan, Jiangxi, Shaanxi, and Sichuan; *T. grandis* Fortune *ex* Lindl. mainly in Anhui, Fujian, Guizhou, Hunan, Jiangsu, Jiangxi, and Zhejiang; and *T. jackii* Chun. in Fujian Jiangxi and Zhejiang [2]. The wood of this genus is moderately hard and durable, and silky in texture. The grain is fine and fairly even.

In Japan, the wood of *Torreya* sp. played an important part in the history of Buddhism; woods of this genus were frequently used for Buddhist statues made in the eighth and ninth centuries [3, 4]. Many statues in this period were carved from the single stem of a tree of *Torreya* sp., a peculiar characteristic of them. In the seventh century, the wood of *Cinnamomum* sp. was used for many Buddhist statues, that is, the wood species used for statues suddenly changed from hardwood to softwood in the eighth century. This major change in wood material might be needed to produce the single-bole statues using sophisticated techniques

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during that period. However, a question still remains about the process of transition to the production of Buddhist statues in the eighth century, that is, how the wood of *Torreya* sp. was introduced into the production of single-bole statues. Researchers who study Buddhist statues infer that Jianzhen, a Chinese Buddhist monk, influenced the major change in the styles of wooden statues after the eighth century [3]. Therefore, to clearly answer the question about the Buddhist statues produced in the eighth century, it is essential to identify the wood species used for the statues in the genus *Torreya* distributed in Japan and China.

As the tracheids of *Torreya* sp. demonstrate their characteristic helical thickening on the inner surfaces of the walls [5], wood of this species can be easily distinguished from other coniferous woods using a microscope. However, an anatomical investigation cannot identify the wood species in the genus *Torreya*. Generally, it is difficult to precisely identify wood species at the species level by microscopic observation [6]. For the identification of wood at the species level, a multiple approach using a number of analyzing methods must be adopted. The genomic deoxyribonucleic acid (DNA) stored in wood includes the nucleotide sequences inherent in its species. Several studies have proved the effectiveness of DNA identification of wood materials that are difficult to distinguish based on anatomical features [7–13]. This method is also expected to function in identifying the woods used as the materials of historically valuable products and buildings. The characteristics of Buddhist statues and their changes strongly relate to the history of Buddhist beliefs. Especially, the production of single-bole statues in the eighth century is speculated to be a major turning point in the history of Buddhist statues. Accurate identification of wood species in the genus *Torreya* based on DNA barcoding can contribute to clarify the transition process of Buddhist statues during this period.

More copies of plastid DNA exist in wood than nuclear DNA since plant cells generally possess a number of plastids. On the other hand, the residual DNA in old wood is considered to be fragmented or degraded for long elapsed years after cutting. Therefore, estimating the properties of plastid DNA fragments stored in the wood of *Torreya* sp., such as the nucleotide substitutions and residual amounts, is of particular importance in establishing a measure for identifying the species used for the Buddhist statues produced in the eighth and ninth centuries. In this study, the sequences and residual copy numbers of plastid DNA stored in the wood of *T. nucifera* were analyzed to consider the accurate identification of wood species in the genus *Torreya* distributed in Japan and China.

Materials and methods

Wood materials

Sapwood and heartwood of *T. nucifera* were selected from the collections of xylariums of the Forestry and Forest Products Research Institute. Table 1 lists the material names, parts, elapsed years after cutting, the Wood ID Nos., and sampling location for all materials.

Preparation of wood powder and extraction of residual DNA

Small pieces of 1 mm × 1 mm × 5 mm were prepared from each material listed in Table 1. After being pre-cooled in liquid nitrogen, the materials were powdered with a disruptor (Multi-Beads Shocker, Yasui Kikai). The revolution speed was 2500 rpm for a milling time of 30 s. All wood powder samples were prepared in 2014, and then stored at temperatures below −80 °C just prior to the extraction of residual DNA.

The residual DNA contained in 500 mg of each wood powder was extracted using the DNeasy Plant Maxi Kit and Mini Kit (Qiagen), and then finally eluted in 50 µl of ultrapure water. The concentrations of DNA extracted were measured using a fluorometer (Qubit 2.0, Life Technologies). The extracting operation was conducted twice for each wood powder sample.

Table 1 Wood materials for extraction of residual deoxyribonucleic acid (DNA)

Material name	Part	Elapsed years after cutting	Wood ID no.	Sampling location
12-S	Sapwood	12	TWTw19683	Kamitsusima, Nagasaki Pref
12-H	Heartwood	12	TWTw19683	Kamitsusima, Nagasaki Pref
50-S	Sapwood	50	TWTw14506	Hachioji, Tokyo
50-H	Heartwood	50	TWTw14506	Hachioji, Tokyo
63-S	Sapwood	63	TWTw26856	Tokyo
63-H	Heartwood	63	TWTw26856	Tokyo
85-S	Sapwood	85	TWTw3321	Kamogawa, Chiba Pref
85-H	Heartwood	85	TWTw3321	Kamogawa, Chiba Pref

Detection and sequencing of plastid DNA fragments

The partial sequences of *rbcL* (accession No. JQ512623, nucleotide positions 190 to 559), *trnL-trnF* (EF660644, 568 to 939), and *psbA-trnH* (EF660697, 269 to 647) were amplified from the residual DNA extracted by polymerase chain reaction (PCR). Table 2 lists the sense and anti-sense primer sequences used for the amplification. The PCR enzyme and buffer solution used were the TaKaRa ExTaq™ HotStart version (Takara Bio Inc.) and Ampdirect® Plus (Shimadzu Corporation), respectively. The reaction mixtures contained 5% extracted DNA (volume), 0.025 unit/μl PCR enzyme, and 0.5 μM sense and anti-sense primers. Amplifications were performed with an initial denaturation at 98.0 °C for 10 min, followed by 40 cycles of denaturation at 98.0 °C for 10 s, annealing at 55.5 °C (*rbcL*), 53.7 °C (*trnL-trnF*), and 60.0 °C (*psbA-trnH*) for 30 s, and then extension at 72.0 °C for 25 s. The amplicons were detected by 3% agarose gel electrophoresis with ethidium bromide. The nucleotide sequences of amplicons were analyzed to investigate the nucleotide substitutions. For the sequences of *trnL-trnF* and *psbA-trnH* obtained, DNA polymorphism analyses were conducted to estimate the nucleotide diversity with DnaSP Ver. 5.10.01 [14]. The sequences analyzed were also compared with the corresponding sequences of *Torreya* spp. in China as contained in the National Center for Biotechnology Information (NCBI): NC_029398:54797–56248 (*rbcL*), NC_029398:47089–48078 (*trnL-trnF*), and NC_029398:136480–137075 (*psbA-trnH*) for *T. fargesii*, DQ478794 (*rbcL*); EF660623 (*trnL-trnF*) and EF660692 (*psbA-trnH*) for *T. grandis*; KJ589006 (*rbcL*), KJ589075 (*trnL-trnF*), and EF660693 (*psbA-trnH*) for *T. jackii*.

Quantitative PCR analysis of residual plastid DNA

The copy numbers of targeted DNA fragments of *rbcL, trnL-trnF*, and *psbA-trnH* in the extracted residual DNA were investigated using quantitative PCR (qPCR) analyses. The same equipment, amplification protocol, and estimating method as previously reported were adopted in this study [15]. Table 3 lists the amplicon lengths, sense and anti-sense primer sequences, and TaqMan® probe sequences (Dual-labeled probe and primers for QPCR, Sigma Aldrich Japan) for the three targeted regions. The DNA fragments amplified using the sense and anti-sense primers listed in Table 2 are purified (QIAquick Gel Extraction Kit, Qiagen) and then used to prepare 1:10 dilution series for a standard curve method. The initial copy numbers of the dilution series were as follows: with *rbcL*, 2.57×10^6 copies per μl, with *trnL-trnF*, 3.06×10^6 copies per μl, and with *psbA-trnH*, 3.81×10^6 copies per μl. The PCR efficiency of each measurement was determined from the amplification curves of the dilution series obtained. Experimental data with 85 to 95% of the efficiencies were used to estimate the copy numbers of targeted sequences

Table 2 Sequences of sense primers and anti-sense primers for the polymerase chain reaction (PCR)

Target	Amplicon length (bp)	Sense primer	Anti-sense primer
rbcL	370	GGTACATGGACCACTGTTTG (nucleotide positions 190-208)	TACCATAATTCTTGGCGGAT (nucleotide positions 559-540)
trnL-trnF	372	CTTTTCATAATTCTGTGAGCAA (nucleotide positions 568-589)	GTCCTCTGCTCTACCAACTG (nucleotide positions 939-920)
psbA-trnH	379	TTCAGCTATGGATGCTAAATAAAGC (nucleotide positions 269-293)	CGCATGGTGGATTCACAATCC (nucleotide positions 647-627)

Table 3 Sequences of sense primers, anti-sense primers, and probes for the quantitative polymerase chain reaction (PCR) analyses

Target	Amplicon length (bp)	Sense primer	Anti-sense primer	Probe
rbcL	99	TTACCAGTCTTGATCGTTACA AGG (nucleotide positions 221-244)	GATCTAAAGGGTAAGCTACAT AGGC (nucleotide positions 319-295)	CCAGGAACGGGCTCAATATCA TAGCATCG (nucleotide positions 275-247)
trnL-trnF	111	CGGAATTCTCACTTTATTTTA AATAGCGC (nucleotide positions 592-620)	CAATTGCTCCTACGATCAACT TGTC (nucleotide positions 702-678)	ACCCCACTATTTTTTGCTAAATAG CAAAATAGATC (nucleotide positions 639-673)
psbA-trnH	93	CTTGGAAGGAATGACCGT AGACA (nucleotide positions 555-577)	CGCATGGTGGATTCACAATCC (nucleotide positions 647-627)	CCTTTGAACCACTTGGCTACGTCC GC (nucleotide positions 622-597)

in the residual DNA extracted. The correlation coefficients of the standard curves in this experiment were very high ($r^2 = 0.99$), indicating that the primers and probes listed in Table 3 functioned precisely.

Results and discussion

Amount of total DNA extracted from the samples

Residual DNA was not detected from the samples using a fluorometer, except 12-S and 12-H. The amounts of total DNA extracted from 12-S were 645 ng/500 mg and 650 ng /500 mg. For 12-H, the amounts were 6.0 ng/500 mg and 8.5 ng/500 mg. The values for 12-S were larger than those obtained in the previous study for *Cryptomeria japonica* sapwood [15]. The values for 12-H were almost the same as those for *C. japonica* heartwood.

PCR amplifications and sequencing

Figure 1 shows the PCR amplifications of the 370 bp *rbcL* fragment, 372 bp *trnL-trnF* fragment, and 379 bp *psbA-trnH* fragment contained in the samples. The three fragments were amplified from 12-S, 12-H, 63-H, and 85-S. Additionally, the *trnL-trnF* and *psbA-trnH* fragments were amplified from 50-H. Slight or no amplification was observed for 50-S, 63-S, and 85-H, indicating that for these samples the plastid DNA was largely fragmented or that it was difficult to amplify the residual DNA.

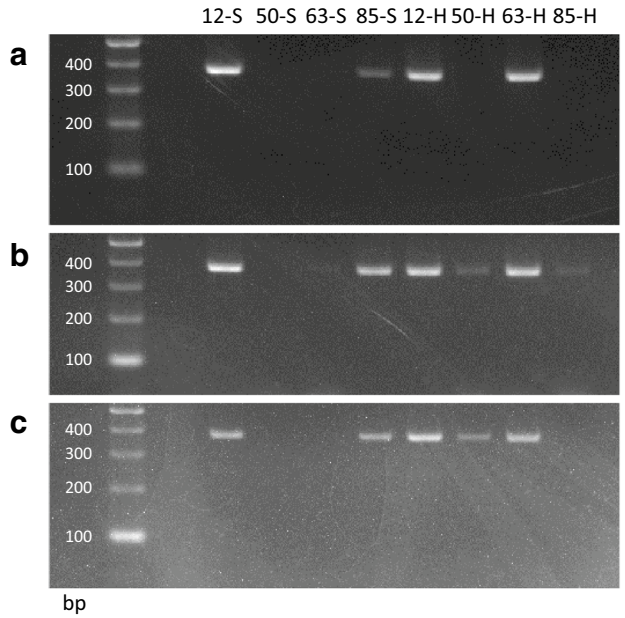

Fig. 1 Gel electrophoresis of amplified plastid DNA fragments of *T. nucifera*. **a***rbcL*, **b***trnL-trnF*, **c***psbA-trnH*

For the fragments amplified from 12-S, 12-H, 63-H, and 85-S, the nucleotide sequences were analyzed and compared to each other. These were also compared with the corresponding sequences of *Torreya* spp. distributed in China. Table 4 lists the nucleotide substitutions and deletions in the three fragments for *T. nucifera* and three Chinese *Torreya* spp. There was a nucleotide transition at position 247 in *T. nucifera rbcL*. *T. nucifera* is discriminated from *T. fargesii* and *T. grandis* with the difference in nucleotide at one position. Sequencing the amplified *T. nucifera trnL-trnF* fragments revealed that the length was one nucleotide longer than the corresponding sequence on EF660644. There were two nucleotide transitions at positions 600 and 681 in this sequence. The three Chinese *Torreya* spp. lack a nucleotide at position 752. The differences in nucleotides at four positions as well as the one deletion enable *T. nucifera* to be distinguished from the Chinese *Torreya* spp. No nucleotide substitution was observed within the four fragments of *T. nucifera psbA-trnH*. For this sequence, there are more differences and deletions in nucleotides than the other two sequences, thereby enabling a clearer distinction between *T. nucifera* and the Chinese *Torreya* spp.

This experiment analyzed the 1122 nucleotide sequences in *T. nucifera* plastid DNA. These are approximately 0.9% of whole plastid genome DNA sequences in reference to previous studies on conifers [16, 17]. As shown in Table 4, there are at least 21 nucleotide substitutions among the four *Torreya* spp. in Japan and China. Nucleotide substitution in the plastid genome provides the key base for wood identification at the species level. In *Cyclobalanopsis*, a subgenus of *Quercus*, nucleotide substitutions and insertion/deletion were detected at four positions in the 151 bp *trnT-trnL* fragment [7]. Between *Q. rubra* and *Q. robur*, there were five nucleotide insertions/deletions at the A/T-repeat region in the 290 bp *trnD-trnT* fragment [8]. The five nucleotide transitions and two transversions in the 441 bp *trnL-trnF* fragment were observed among four wood groups of *Shorea* spp [9]. Nucleotide substitutions of plastid DNA among the four *Torreya* spp. in Japan and China were detected at the almost same frequency with the species mentioned above, indicating that detecting appropriate regions in the plastid DNA was also effective for identifying wood in the genus *Torreya*.

Sequencing the amplified plastid DNA fragments revealed nucleotide substitutions among the three individuals of *T. nucifera*. The nucleotide diversity for the combined sequence of *trnL-trnF* and *psbA-trnH* was estimated to be 1.8×10^{-3} [Standard deviation (SD) 0.84×10^{-3}]. For the *trnL-trnF* fragment, the nucleotide diversity was estimated to be 3.6×10^{-3} (SD 1.7×10^{-3}). These values show the sequence diversity of the intergenic spacers on the *T. nucifera* plastid DNA. More sequence data for this plastid DNA should be collected from various areas in Japan,

Table 4 Nucleotide substitutions and deletions in the three fragments for *T. nucifera* and three Chinese *Torreya* spp.

Sample	*rbcL* (N.P)		*trnL-trnF* (N.P)						
	247	261	600	654	681	705	752	771	793
12-S	C	T	T	G	A	T	A	T	G
12-H	C	T	T	G	A	T	A	T	G
63-H	C	T	C	G	G	T	A	T	G
85-S	T	T	C	G	G	T	A	T	G
T. fargesii	C	C	T	G	A	C	–	T	G
T. grandis	C	C	T	T	A	C	–	T	A
T. jackii	C	T	T	G	A	T	–	G	G

Sample	*psbA-trnH* (N.P)															
	441	444	445	457	486	552	572	573	574	575	576	624	627	628	639	644
12-S	T	A	G	T	G	T	T	A	G	A	C	A	G	G	C	T
12-H	T	A	G	T	G	T	T	A	G	A	C	A	G	G	C	T
63-H	T	A	G	T	G	T	T	A	G	A	C	A	G	G	C	T
85-S	T	A	G	T	G	T	T	A	G	A	C	A	G	G	C	T
T. fargesii	T	A	G	T	A	C	T	A	G	A	C	A	G	G	C	C
T. grandis	T	G	T	C	A	T	T	A	G	A	C	A	G	G	C	T
T. jackii	C	A	G	T	A	T	–	–	–	–	–	T	T	–	–	T

N.P nucleotide position

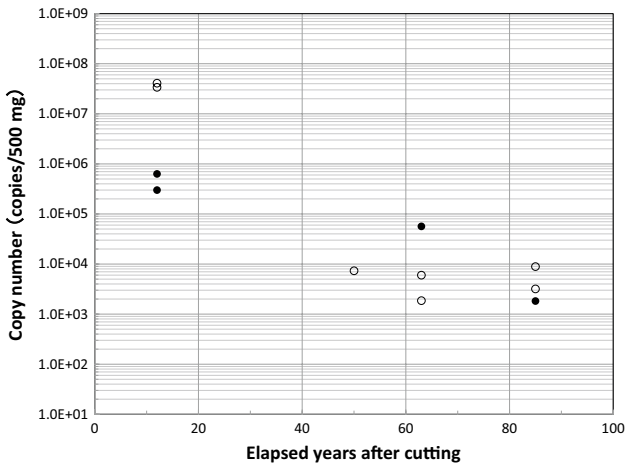

Fig. 2 Relationship between elapsed years after cutting and copy numbers of 99 bp *rbcL* fragments. *Open circle* sapwood, *closed circle* heartwood

and then analyzed in order to set the appropriate amplified regions for DNA identification among the wood of *Torreya* species.

Evaluation of copy numbers of plastid DNA fragments

Figure 2 shows the relationship between the elapsed years after cutting and the copy numbers of the 99 bp *rbcL* fragment. The copy numbers for 12-S were estimated to be 3.38×10^7 and 4.08×10^7 copies/500 mg, respectively. The copy numbers for 12-H were estimated to be 3.00×10^5 and 6.30×10^5 copies/500 mg, respectively, approximately one-hundredth of those for 12-S. The 99 bp *rbcL* fragment was not clearly detected for 50-S and 50-H, which corresponded to the results of PCR amplifications shown in Fig. 1a. This qPCR analysis detected the 99 bp fragment for 63-S, although the 370 bp fragment was not amplified. Their copy numbers were estimated to be approximately one ten-thousandth of those for 12-S. Conversely, the copy number was not clearly estimated for 63-H. Almost the same copy numbers were obtained for 85-S, but the copy number was not clearly estimated for 85-H.

Figure 3 shows the relationship between the elapsed years after cutting and the copy numbers of the 111 bp *trnL-trnF* fragment. The copy numbers for 12-S were estimated to be 9.44×10^7 and 8.40×10^7 copies/500 mg, respectively. The copy numbers for 12-H were approximately one-hundredth of those for 12-S. For these two samples, almost the same results were obtained between the *trnL-trnF* fragment and the *rbcL* one. The copy numbers were estimated for 63-S and 85-S as in the case of the *rbcL* fragments. The copy numbers were also estimated for 50-H. Figure 4 shows the relationship between the elapsed years after cutting and the copy numbers of the 93 bp *psbA-trnH* fragment. The copy numbers for 12-S were estimated to be 9.62×10^6 and 9.01×10^6 copies/500 mg, respectively, which were slightly smaller compared with the results for the other two fragments. Similar results were obtained for 12-H. The 93 bp fragment was not detected for 50-H

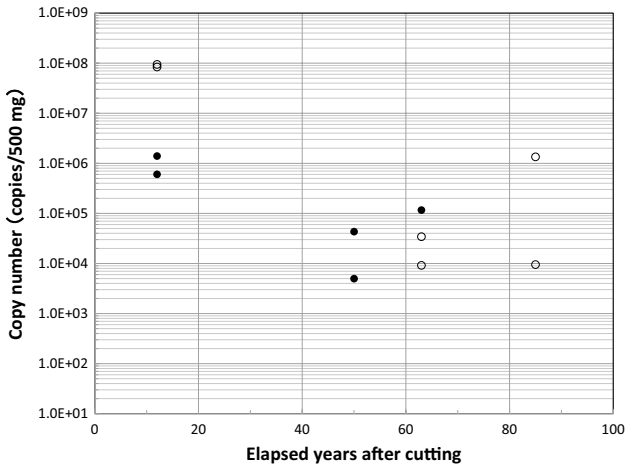

Fig. 3 Relationship between elapsed years after cutting and copy numbers of 111 bp *trnL-trnF* fragments. *Open circle* sapwood, *closed circle* heartwood

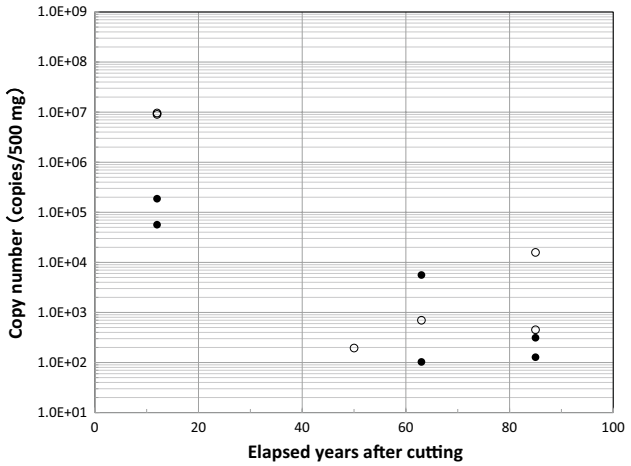

Fig. 4 Relationship between elapsed years after cutting and copy numbers of 93 bp *psbA-trnH* fragments. *Open circle* sapwood, *closed circle* heartwood

in contrast with PCR amplification of the 379 bp fragment shown in Fig. 1c. The copy numbers were also estimated for 63-H. The 93 bp fragment was detected for 85-H, although the 379 bp fragment was not amplified.

The qPCR analyses in this study generally estimated the amounts of plastid DNA fragments stored in the *T. nucifera* sapwood. The results shown in Figs. 1, 2, 3, and 4 suggest that the residual amounts of fragments in the sapwood scarcely depend on regions in the plastid genome. Copy numbers of the fragments decreased significantly in wood specimens cut over 50 years ago. It is considered that the plastid DNA in the *T. nucifera* sapwood has been fragmented with more elapsed years after cutting, but fragments with a length of approximately 100 bp have remained in the old sapwood materials. Conversely, it was difficult to detect the residual fragments of plastid DNA in the *T. nucifera* heartwood with longer elapsed years after cutting, which could be attributed to their unstable amplifications. Analyzing the *trnL-trnF* and *psbA-trnH* intergenic spacers on plastid DNA has functioned in the detailed discrimination of woody plants, such as their molecular evolutionary history [18], genetic relationship among cultivars [19], geographic distribution [20], climatic niche dynamics [21], and phylogeographic structure [22, 23]. In the conifer II clade that contains the five families except Pinaceae, plastid genomic organization relatively varies due to both the loss of inverted repeat and the shrinkage of intergenic spacers [24], which can be strongly related to the DNA identification of wood in the same genus. For *Torreya* sp. investigated in this study, the shrinkage of *psbA-trnH* intergenic spacer was observed, as shown in Table 4. Detecting a small number of nucleotide substitutions and deletions in the intergenic spacers on plastid DNA with a high degree of accuracy may enable the identification of wood species used in the single-bole statues.

Separation of wood of *T. nucifera* and *T. grandis* using DNA analysis

It has been clarified that the woods of *Torreya* spp. were used for many single-bole statues produced in the period from the 8th to ninth centuries, such as the standing statues of Shitenno (Daian-ji temple), Yakushinyorai (Jingo-ji temple), Den-yakushinyorai (Toshodai-ji temple), and Den-shuhououbosatu (Toshodai-ji temple) [3, 4]. Jianzhen visited Japan to teach the Chinese Buddhist precepts in the mid-eighth century. He also built Toshodai-ji temple in Nara as a place for Buddhist training, where the representative single-bole statues are enshrined as mentioned above. Actually, the number of single-bole statues made of the wood of *Torreya* sp. increased after his arrival. From these historical facts, it is inferred that Jianzhen and his party introduced and directed the production of statues with wood of this genus [3]. The following three processes are considered for the transition of production of statues: (1) the statues were carved in China and brought to Japan; (2) wood materials were brought to Japan from China, and the statues were carved in Japan; and (3) the statues were carved in Japan with the wood of Japanese *Torreya* sp. Jianzhen was also a Buddhist monk at Daming temple in Yangzhou, Jiangsu, China before his arrival in Japan. Jiangsu is one of the vegetation areas of *T. grandis*. This fact suggests a possibility that wood of this species had been introduced to Japan for making single-bole statues, at least in the mid-eighth century. Therefore, the wood identification between *T. nucifera* and *T. grandis* is a priority task in studying the major historical change of wooden Buddhist statues in Japan.

Plastid DNA sequences of *T. nucifera* and *T. grandis* exhibit relatively high homology to each other (Table 4), indicating that targeted regions should be carefully chosen from intergenic spacers to identify the wood of the genus *Torreya* based on the differences in plastid DNA sequences. For example, the results obtained by nucleotide sequencing in this study show that the difference in nucleotide at position 486 for *psbA-trnH* provides a distinction of wood between *T. nucifera* and *Torreya* sp. in China. Comparing the nucleotides at positions 654 and 793 in *trnL-trnF* and at positions 444, 445 and 457 in *psbA-trnH* enables the clear identification of wood between *T. nucifera* and *T. grandis*. This study demonstrates that plastid DNA fragments can be detected from the wood of *T. nucifera* placed in a room under stable conditions such as in a xylarium, although their PCR amplifications are somewhat unstable. The previous study indicated that residual plastid DNA in old wood materials was fragmented to a length of approximately 200 bp [15]. Wooden Buddhist statues that are registered as important cultural assets are being placed under constant conditions of temperature, humidity, and light to avoid the decay of the wood. Therefore, quite small amounts of plastid DNA fragments with a length of 100 to 200 bp are considered to remain in old wooden Buddhist statues. For practical application, a more efficient extraction of residual DNA and the detection of targeted sequences with higher accuracy and sensitivity are required for the identification of wood species used for statues, as only a small amount of wood pieces is collected. Sonication under an approximate condition may effectively extract the residual DNA from a small amount of thin wood sections or wood powder. Digital PCR system, a system for absolute quantification of DNA copy number, is considered to function in detecting the targeted DNA fragment stored in old wood [25]. Although there are remaining issues to be solved, a combined method of these two techniques will enable the DNA identification of wood used for the Buddhist statues in the eighth and ninth centuries.

Conclusion

This study conducted the extraction of residual DNA from the sapwood and heartwood of *T. nucifera* with different elapsed years after cutting, sequence analyses of their plastid DNA fragments, and an evaluation of their residual amounts. Nucleotide substitutions and deletions in plastid DNA sequences were clearly determined between *T. nucifera* and the species distributed in China, which could be the basis for discrimination among these woods. The results obtained by DNA polymorphism analyses exhibited sequence diversity of the intergenic spacers on the *T. nucifera* plastid DNA, indicating that more sequence data of the plastid DNA should be collected and analyzed to set the appropriate amplified regions for wood identification. The PCR amplification of plastid DNA fragments was unstable for the *T. nucifera* heartwood with longer elapsed years after cutting, whereas fragments with a length of approximately 100 bp were considered to be amplified for the sapwood. Multiple sequence analyses for intergenic spacers on plastid genome are considered to ensure the accurate discrimination of wood of *T. nucifera* from those of Chinese *Torreya* spp. Although there are remaining issues to be solved for practical application, DNA identification of the wood of *Torreya* spp. is considered to be a feasible measure to accurately identify the materials used for Buddhist statues after the mid-eighth century. The characteristics of statues strongly reflect an outlook on Buddhism during the period when the statues were produced. The choice of wood and the style of sculpture thus play key roles in the characterization of Buddhist statues. Therefore, DNA identification will contribute significantly to studies on the styles of wooden Buddhist statues.

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