Tree Genetics & Genomes (2025) 21:14
https://doi.org/10.1007/s11295-025-01696-8

GENOMIC RESOURCES

DNA markers targeting three cellular genomes for the discrimination between *Taxus baccata*, *T. cuspidata* and their hybrid

Daniel Bross[1] · **Hilke Schroeder**[1] · **Emilia Pers-Kamczyc**[2] · **Birgit Kersten**[1]

Received: 14 March 2024 / Revised: 25 February 2025 / Accepted: 1 March 2025

Abstract

The species identification in *Taxus* based on morphological differences has historically been difficult, and molecular markers are important tools to consistently identify *Taxus* species. The increasing availability of public DNA sequencing data supports efforts to develop such markers. In this study, a new molecular PCR-based marker for the differentiation of the three most abundantly occurring *Taxus* species in Europe, *T. baccata*, *T. cuspidata*, and their cultivated hybrid, *T.* × *media*, was identified in the ITS region in the nuclear genome, as well as markers for the identification of *T. baccata* and *T. cuspidata* that were identified in the *psbB_psaI* intergenic linker and *chlN* region of the chloroplast genome and in the *cox1* region of the mitochondrial genome. Markers identified in public data were validated with 106 *T. baccata*, 12 *T.* × *media* and 10 *T. cuspidata* individuals with pre-existing phenotypical species declarations. The marker test results matched the identity of all but seven samples, and all deviating samples were identified by the markers as *T.* × *media*. In combination, these markers can also identify the crossing direction of *T.* × *media* samples. The results of the tests on *T.* × *media* samples in this study support a paternal inheritance of the mitochondrial genome in *T.* × *media* and revealed the crossing direction of multiple cultivars for which it was previously unknown. A minimal marker set was defined for rapid and cost-efficient identification of the analysed *Taxus* species and the crossing direction in *T.* × *media*.

Keywords *Taxus* · Genomic data · Species delimitation · Hybridization

Introduction

The genus *Taxus* L. is a group of coniferous trees and shrubs whose native ranges mainly lie in the northern hemisphere (Li et al. 2001). In the past decades, public interest in this genus has risen as a result of the discovery of Paclitaxel (also known as Taxol®) in the 1960s (Wani and Horwitz 2014), which has since become one of the most used antitumor drugs (Gallego-Jara et al. 2020).

The differentiation of species in *Taxus* has historically been difficult due to an overall high extent of morphological similarity and phenotypic plasticity (Möller et al. 2007, 2020; Spjut 2007; Liu et al. 2011; Coughlan et al. 2020), with the number of reported species ranging from one (Pilger 1903) to 24 (and 55 varieties) (Spjut 2007) and more recent publications delimiting 15 (Liu et al. 2018), 16 (Fu et al. 2019) or nine (Coughlan et al. 2020) species, although the latter study did not include multiple known lineages. In the WFO Plant list (WFO 2024), the following 13 *Taxus* species names are currently listed as accepted names: *T. baccata* L., *T. brevifolia* Nutt., *T. calcicola* L.M. Gao & Mich.Möller, *T. canadensis* Marshall, *T. chinensis* (Pilg.) Rehder, *T. contorta* Griff., *T. cuspidata* Siebold & Zucc., *T. floridana* Nutt. ex Chapm., *T. florinii* Spjut, *T. globosa* Schltdl., *T. mairei* (Lemee & H.Lév.) S.Y. Hu, *T. sumatrana* (Miq.) de Laub. and *T. wallichiana* Zucc. Four potential cryptic species are

Communicated by G. G. Vendramin

✉ Birgit Kersten
birgit.kersten@thuenen.de

Daniel Bross
daniel.bross@thuenen.de

Hilke Schroeder
hilke.schroeder@thuenen.de

Emilia Pers-Kamczyc
epk@man.poznan.pl

[1] Thuenen Institute of Forest Genetics, Sieker Landstrasse 2, 22927 Grosshansdorf, Germany

[2] Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, Kórnik 62-035, Poland

Published online: 15 April 2025

also described (Liu et al. 2011), as well as two hybrid species, *T.* × *media* Rehder and *T. x hunnewelliana* Rehder, which resulted from crosses between *T. cuspidata* with *T. baccata* and *T. cuspidata* with *T. canadensis*, respectively (Chadwick and Keen 1976; Collins et al. 2003).

Seven of the *Taxus* species that are recognized by the IUCN Red List are considered "near threatened" (1), "vulnerable" (1), "endangered" (4), or "critically endangered" (1), with "decreasing" population trends (IUCN 2023), while the current population trend of European yew is categorized as "increasing".

T. baccata L. is the only naturally occurring *Taxus* species in Europe, and is also known as European or English yew. It is native to most of Europe, the Atlas Mountains, the Azores and Asia Minor (Schirone et al. 2010; Benham et al. 2016; Coughlan et al. 2020). Due to deforestation, selective felling and grazing (among other causes), natural populations of *T. baccata* have declined throughout Europe during the past 4000 years (Svenning and Magård 1999; and references therein). Consequently, forests harbouring yew are among the protected habitats designated by the European Community (Council Directive 92/43/EC 1992).

In addition to its natural populations, *T. baccata* has long been cultivated as an ornamental tree in Europe, along with the Japanese Yew *Taxus cuspidata* Siebold & Zucc. and its hybrid with *T. baccata*, *T.* × *media* (Chadwick and Keen 1976; Stöhr 2019). *T. cuspidata* naturally occurs in Japan and nearby continental regions on the Korean peninsula, China and Russia (Earle 2022), and was previously shown to be closely related to *T. baccata* (Liu et al. 2011; Coughlan et al. 2020; Möller et al. 2020). The divergence of the two species' ancestors was approximated to have occurred between 3.13 and 4.6 (Li et al. 2001) and 6.95 and 8.7 million years ago (Möller et al. 2020). Two varieties of *T. cuspidata* are currently accepted, var. *cuspidata* and var. *nana*, which differ in morphology and distribution area (Kondo 2016; Earle 2022; GBIF 2023; WFO 2024).

The *T.* × *media* hybrid cultivars originated from two initial crosses in early 1900s in the Hunnewell Pinetum, Boston, Massachusetts, and Hicks Nursery, Long Island, New York. Unfortunately, there is no sufficient documentation about the parents used in these crosses available, as noted by Collins et al. (2003). Therein, only the maternal parentage was determined as follows:" The original *T.* × *media* seedlings from the Boston area were grown from seed of *T. baccata* 'Fastigiata' (the Irish yew); and the original seedlings of *T.* × *media* from the Long Island area were grown from seed of *T. cuspidata*."

T. × *media* hybrids were also suspected– based on morphological assessment– in naturally regenerated yew populations in Europe. However, any influence of cultivated yews on the natural populations of *T. baccata* is unexplored in Europe (Stöhr 2019).

The difficulties in species recognition extend to *T.* × *media*, especially when only parts of a plant can be assessed. The main features used to distinguish between trees of *T. baccata* and *T. cuspidata* var. *cuspidata* are bark color and needle's shape. *T. baccata* bark is thin, smooth, and red-brown, it peels off in quite large patches with gently arched edges, revealing deeper, cherry-coloured layers. Moreover, on thinner shoots, patches of bark unroll at the edges. *T. cuspidata* has brown bark that peels off in narrow, thin, soft and jagged strands (Fig. 1). The falling pieces do not reveal cherry spots, showing no anthocyanin discoloration within the trunk. They have similar leaves, however, *T. baccata* can have longer leaves (2–3(4) cm) compared to T. *cuspidata* (up to 2.5 cm). In *T. baccata* they are flattened, slightly bent and gradually sharpened, with a thin, short green petiole at the base, whereas *T. cuspidata* leaves are quite stiff, sharp and short, arranged comb-like, but not very regularly, with leaves clearly yellowish underneath, and often yellow petioles. *T.* × *media* as a hybrid is characterized by intermediate characteristics of both species and it is very difficult to distinguish it from *T. baccata*. The most important morphological feature by which *T.* × *media* hybrids are identified is usually the intermediate colour of the trunk bark as well as the shape of the top of the needles, unfortunately in hybrids it is not always correlated with the quality of peeled-off bark. Some old *T.* × *media* varieties with a bushy shape, initially introduced into cultivation in the USA are *T.* × *media* 'Hicksii' (female clone) and *T.* × *media* 'Hatfieldii' (male clone), which are often mixed with the *T. baccata* 'Fastigiata' variety due to similar shape (Seneta et al. 2021).

Due to the difficulty, or in some cases impossibility, of clearly differentiating *T.* × *media* from *T. baccata* and *T. cuspidata* based on morphology, the development of DNA markers for genetic differentiation of these species and its hybrids as well as for the identification of the crossing directions in *T.* × *media* individuals is very important. The knowledge of the mode of organelle inheritance is important when interpreting results obtained from the application of chloroplast DNA (cpDNA) or mitochondrial DNA (mtDNA) markers for species identification. So far there is strong support for a predominantly paternal transmission of cpDNA (Chesnoy 1987; Pennell and Bell 1988; Collins et al. 2003) as well as mtDNA (Chybicki et al. 2016) in *T. baccata*. Thus, it is expected that cpDNA markers, such as the CAPS marker in the cpDNA region *trn*L-F (Collins et al. 2003), with primers that were published previously (Taberlet et al. 1991), allow to differentiate *T. baccata* from *T. cuspidata* only in the paternal line. DNA markers developed for differentiation of some *Taxus* species comprise randomly amplified polymorphic DNA (RAPD) markers

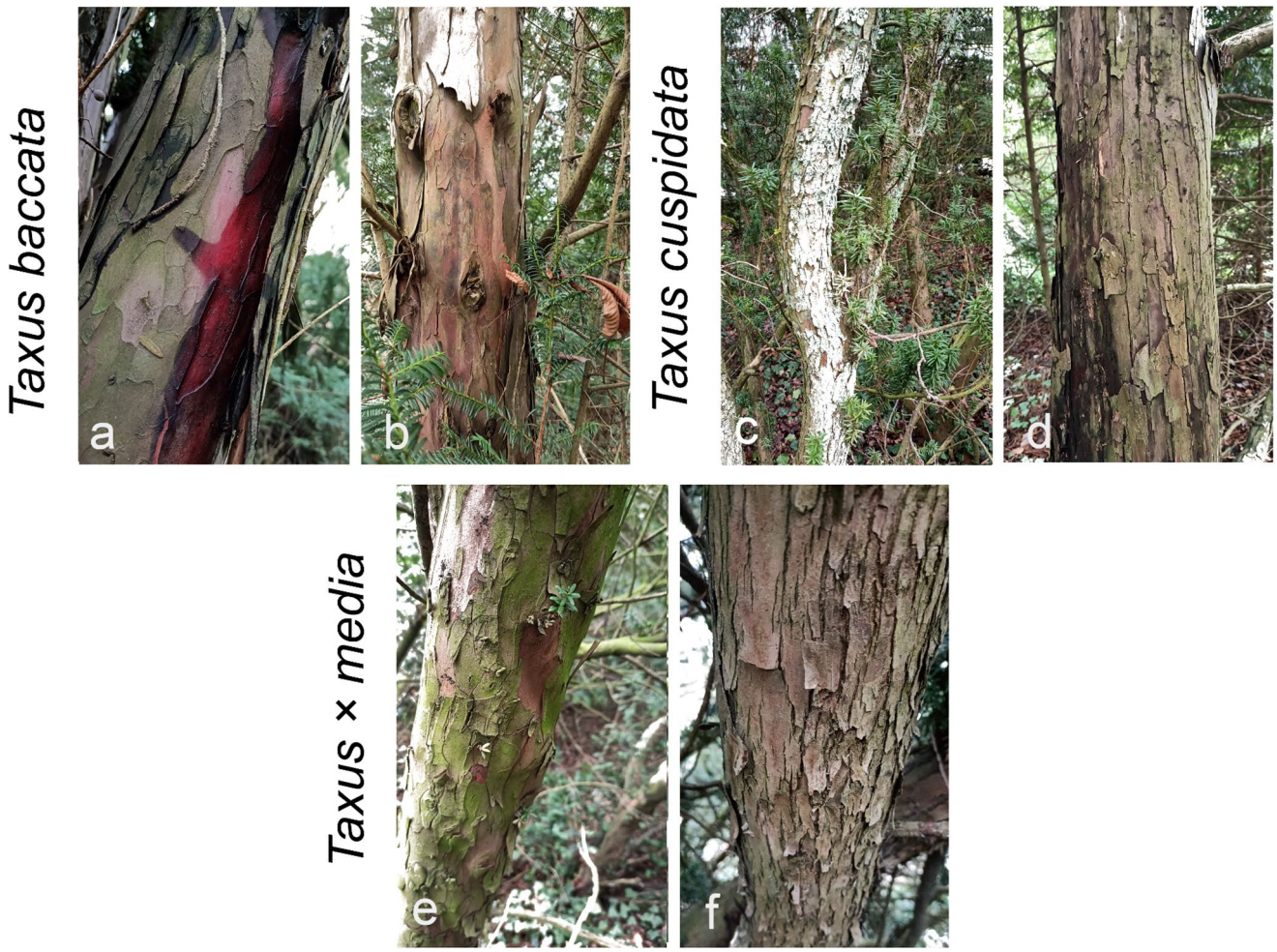

Fig. 1 Bark of *Taxus baccata* (**a**,**b**), *T. cuspidata* (**c**,**d**) and *T.* × *media* (**e**,**f**)

(Collins et al. 2003) and a sequence characterized amplified region (SCAR) marker (Hao et al. 2018). However, the reproducibility of results gained with RAPD markers is low and fragment patterns cannot be assigned to one of the three different genomes (cpDNA, mtDNA or nuclear DNA (nDNA) and related specific genomic regions. In the case of the SCAR marker, *T. cuspidata* and *T.* × *media* were successfully differentiated, but *T. baccata* was not included in the experiments (Hao et al. 2018). Another approach for species identification is DNA barcoding, i.e., the use of standardized genomic regions as taxon 'barcodes' (Hebert et al. 2003). This approach was used in a study focusing on *Taxus* previously (Liu et al. 2011, 2018), utilizing the ITS and *trn*L-F regions that were also examined in this study, and *trn*L-F was claimed as the ideal barcode for *Taxus* in general (Liu et al. 2018). Using this approach, the identification of interspecific hybrids relies on the ITS and *trn*L-F barcodes mismatching (see Liu et al. 2018), which is difficult to guarantee as the ITS genotype is inherited from both parent species. Hence, a PCR marker that allows differentiation between *T. baccata* and *T.* × *media* is, to our knowledge, still missing. However, the increasing abundance of publicly available sequencing data of *Taxus* species provides an opportunity to find DNA sequence variants capable of reliably differentiating the three *Taxus* species *T. baccata*, *T. cuspidata* and *T.* × *media*. In this study, we aimed to find and validate such variants and develop molecular PCR markers targeting cpDNA, mtDNA and nDNA in *Taxus* and to define a minimal easy-to-use and cost-efficient marker set (out of these markers) for the purpose of genetic differentiation between these species. Moreover, the marker set should be suitable to determine the crossing direction in *T.* × *media* hybrid individuals.

Materials and methods

Plant material

For the validation of in silico developed DNA markers, needles from yew trees with available (in some cases external) phenotypic species declaration (*T. baccata*, *T. cuspidata*, or *T.* × *media*) were collected at various geographic locations in Germany and Poland, including arboreta, botanical gardens, parks and two autochthonous stands of *T. baccata* in Poland (with permission of the Regional Directorate for Environmental Protection, Bydgoszcz, Poland: WOP.6400.37.2022, WOP.6205.72.2022.KLD, WOP.6205.73.2022.KLD, WOP.6400.36.2022.MKW). From these samples, some were excluded due to (1) mismatch between declared species of the related individual and cultivar name available for this species, or (2) mismatch between phenotypic plant sex and previously described exclusive cultivar sex allocation based on Chadwick and Keen (1976). A set of 128 samples remained (Online Resource 1, Table S1).

Whole-plastome alignment of *Taxus* species and identification of potential species-differentiating indels

Complete cpDNA sequences of four *T. baccata* and four *T. cuspidata* samples were downloaded from GenBank using CLC Genomics Workbench version 24.0.02. For each sequence, *psbA* was selected from the annotation track and set as the starting point in the circular sequence view. A whole-plastome alignment was performed using the "Create Alignment"-tool of CLC GWB (gap open cost = 10.0; gap extension cost = 1.0; end gap cost = as any other; alignment mode = very accurate; redo alignments = no; use fixpoints = no). The alignment was screened manually to identify potential *T. baccata*-specific or *T. cuspidata*-specific indels of at least 10 bp to enable simple genotyping based on agarose gel electrophoresis.

SNP identification in mitochondrial genes for the potential differentiation between *T. baccata* and *T. cuspidata* and alignment of *cox1* CDS of different *Taxus* species

All CDS sequences of the complete mtDNA sequence of *T. cuspidata* (accession number MN593023) (Kan et al. 2020) were downloaded from the GenBank entry and subjected to a BlastN analysis at NCBI (where "organism" was restricted to *Taxus*). Blast results were manually screened for genes with SNPs that potentially differentiate between *T. cuspidata* and *T. baccata* in the related CDS. The *cox1* gene was selected for an alignment of the related CDS of different *Taxus* species (CDS downloaded from Blast outputs), including *T. cuspidata* (MN593023), by the "Create Alignment"-tool of CLC GWB (default parameters). SNPs with different alleles in *T. baccata* compared to the *T. cuspidata* query sequence were identified manually based on the two *T. baccata* and two *T. cuspidata* accessions in the alignment.

SNP identification in nuclear genes for the potential differentiation between *T. baccata* and *T. cuspidata*

The *T. baccata* nuclear DNA sequence JX188555.1 (Liu et al. 2011), including internal transcribed spacer (ITS) 1 and 2, was used as a query in BlastN analysis at NCBI (https://blast.ncbi.nlm.nih.gov/) where "organism" was restricted to *T. baccata* and *T. cuspidata*. DNA sequences of the blast hits (including the sequence of JX188555.1) were downloaded from GenBank in FASTA format and filtered according to the following criteria: (1) Accessions from unpublished studies were removed; (2) Accessions of BLAST hits with less than 50% query cover or 98% identity were removed; (3) For accessions from samples for which *trn*L-F sequence data were available, samples were genotyped in silico based on the *trn*L-F marker (Collins et al. 2003), and samples with contradictory species name and *trn*L-F genotype were removed; (4) Accessions containing ambiguous base calls (meaning calls other than A, T, G or C) were removed. The remaining sequences were then subjected to an alignment using the "Create Alignment"-tool of CLC GWB (gap open cost = 10.0; gap extension cost = 1.0; end gap cost = as any other; alignment mode = very accurate; redo alignments = no; use fixpoints = no). The alignment was screened manually to identify SNPs potentially differentiating between *T. baccata* and *T. cuspidata*.

DNA extraction, PCR and genotyping

Fresh needles were cut into small pieces and afterwards frozen in liquid nitrogen in 2 mL tubes. Two stainless steel beads (diameter 4 mm) were added to the cut needles. The samples were then homogenized in the swing mill "MM300" (Retsch, Haan, Germany). The DNA was extracted using an inhouse protocol with an ATMAB based extraction buffer using the "standard operation protocol" (SOP) as described in detail in Bruegmann et al. (2022).

Primers flanking the DNA sequence variant of interest were designed using related *T. baccata* reference sequences (primers and references in Online Resource 1, Table S2). PCR reaction setups are given in Table 1 and temperature protocols used for PCR are listed in Table 2. For all markers, the used polymerase was DCS/Pol DNA Polymerase (DNA Cloning Service, Hamburg, Germany). Primers were

Table 1 PCR reaction set ups for the used markers. PCR buffers: [1]: BD PCR buffer (DNA cloning service, Hamburg, Germany), [2]: B PCR buffer (DNA cloning service, Hamburg, Germany). Indels means both indel-based markers TA_InDel1 and TA_InDel2 as they share their reaction setups

Ingredients	Amount [µl]			
	Indels	TA_cox1	TA_ITS	tmL-F
H_20	8.90	13.50	11.37	16.80
PCR buffer (10x)	1.50[1]	2.50[1]	2.50[1]	2.50[2]
$MgCl_2$ (25 mM)	1.20	2.00	2.50	2.50
dNTPs (10 mM)	0.30	0.50	0.50	0.50
DMSO (Enhancer)	0.30	0.30	1.50	-
Primer F (10 µM)	0.30	0.50	0.75	0.75
Primer R (10 µM)	0.30	0.50	0.75	0.75
DNA-Polymerase (5 U/µl)	0.20	0.20	0.13	0.20
Master mix	**13**	**20**	**20**	**24**
DNA (10 ng/µl)	2	5	5	1
Total	**15**	**25**	**25**	**25**

Table 2 Temperature protocols for PCR reactions for the used markers. For cells with multiple values, the order is: Indels/TA_cox1/TA_ITS/*trn*L-F. Indels means both indel-based markers TA_InDel1 and TA_InDel2 as they share their reaction setups

Step	Temperature [°C]	Duration	Cycles
Initialisation	94	4/4/3/1 min	1
Denaturation	94	45/45/60/45 sec	30/35/30/35
Annealing	57/55/57/55	45/45/90/45 sec	30/35/30/35
Extension	72	1/1/2/2 min	30/35/30/35
final Extension	72	10 min	1

acquired from Eurofins Genomics Europe Shared Services GmbH, Ebersberg, Germany. PCRs were carried out in SensoQuest Labcycler (SensoQuest GmbH, Göttingen, Germany).

Marker-specific genotyping procedures were carried out as follows:

TA_InDel1 + TA_InDel2

Individuals were genotyped using these simple PCR markers by amplicon size screening. The PCR products were separated by electrophoresis on a 2% agarose gel, using a 50 bp ladder (New England Biolabs GmbH (NEB), Frankfurt, Germany) for size comparison. For this, 5 µl of PCR product was mixed with 2 µl Orange G loading dye (Merck KGaA, Darmstadt, Germany). The gels were stained with Roti-Safe GelStain (Carl Roth GmbH & Co. KG, Karlsruhe, Germany).

TA_cox1

Genotyping of individuals using this cleaved amplified polymorphic site (CAPS) marker was performed by fragment size screening after amplicon restriction with BclI (recognition site: T/GATCA). The PCR products were digested in a reaction mix consisting of 7 µl H_20, 2.5 µl of 10x rCutSmart buffer (New England Biolabs GmbH (NEB), Frankfurt, Germany), 0.5 µl BclI and 15 µl PCR product in a final volume of 25 µl. Digestion was performed in a SensoQuest Labcycler (SensoQuest GmbH, Göttingen, Germany) with the following program: 1 h at 37 °C, followed by 20 s at 65 °C. The digested PCR products (25 µL and 2.5 µl Orange G loading dye (Merck KGaA, Darmstadt, Germany)) were then analysed by electrophoresis on a 2% agarose gel using a Smart Ladder (Eurogentec Deutschland GmbH, Köln, Deutschland) for size comparison. The gels were stained with Roti-Safe GelStain (Carl Roth GmbH & Co. KG, Karlsruhe, Germany).

TA_ITS

Genotyping was performed by Sanger sequencing of amplicons. The PCR products were checked by electrophoresis on a 1.2% agarose gel stained with Roti-Safe GelStain (Carl Roth GmbH & Co. KG, Karlsruhe, Germany), using a Smart Ladder (Eurogentec Deutschland GmbH, Cologne, Germany) for size comparison. Loading samples for the gel consisted of 5 µl H_20, 5 µl PCR product and 1 µl Orange G loading dye (Merck KGaA, Darmstadt, Germany). The samples were then sequenced by Sanger sequencing using forward- and reverse primer, respectively (StarSeq, Mainz, Germany). Sanger sequencing data were aligned to a suitable reference sequence by CLC GWB (version 22.0) using the "Assemble Sequences to Reference"-tool of the "Sanger Sequencing Analysis" tools to generate reference positions for SNPs. Base calls were identified at the reference positions (see results, Table 3).

*trn*L-F

Genotyping of individuals using this CAPS marker (Collins et al. 2003) was performed by fragment size screening after amplicon restriction with AseI. The PCR products were digested in a reaction mix consisting of 7 µl H_20, 2.5 µl of 10x NEBuffer r3.1 (New England Biolabs, Ipswich, MA), 0.5 µl of 10 U/µl of AseI (New England Biolabs, Ipswich, MA) and 15 µl PCR product in a final volume of 25 µl. Digestion was performed on a SensoQuest Labcycler (SensoQuest GmbH, Göttingen, Germany) with the following program: 1 h at 37 °C, followed by 20 s at 65 °C. The digested PCR products were then analysed on a 1.5% agarose gel stained with Roti-Safe GelStain (Carl Roth GmbH & Co. KG, Karlsruhe, Germany), using a Smart Ladder (Eurogentec Deutschland GmbH, Cologne, Germany) for size comparison. Loading samples consisted of 25 µl digested sample DNA and 2.5 µl Orange G loading dye (Merck KGaA, Darmstadt, Germany).

Table 3 Newly identified DNA sequence variants for a genetic differentiation between *T. baccata* and *T. cuspidata*

Genome	Type of variant	Reference	NCBI accession of reference	Reference position (in bp)	Allele in *T. baccata*	Allele in *T. cuspidata*	Derived marker
cpDNA	indel	*T. cuspidata* cpDNA sequence	NC_041498	64,069–64,090	-	AAATTAAATTAATGGAACAGTT	TA_InDel1
cpDNA	indel	*T. cuspidata* cpDNA sequence	NC_041498	125,773–125,799	-	ATGAAAAGCGAAGTAATTTTGCTTTTG	TA_InDel2
mtDNA	SNP	*T. cuspidata cox1*-CDS	MN593023.1_cds_QJS35647.1	842	T	C	TA_cox1
nDNA	SNP	*T. baccata* partial ITS sequence	JX188555.1	481	C	T	TA_ITS

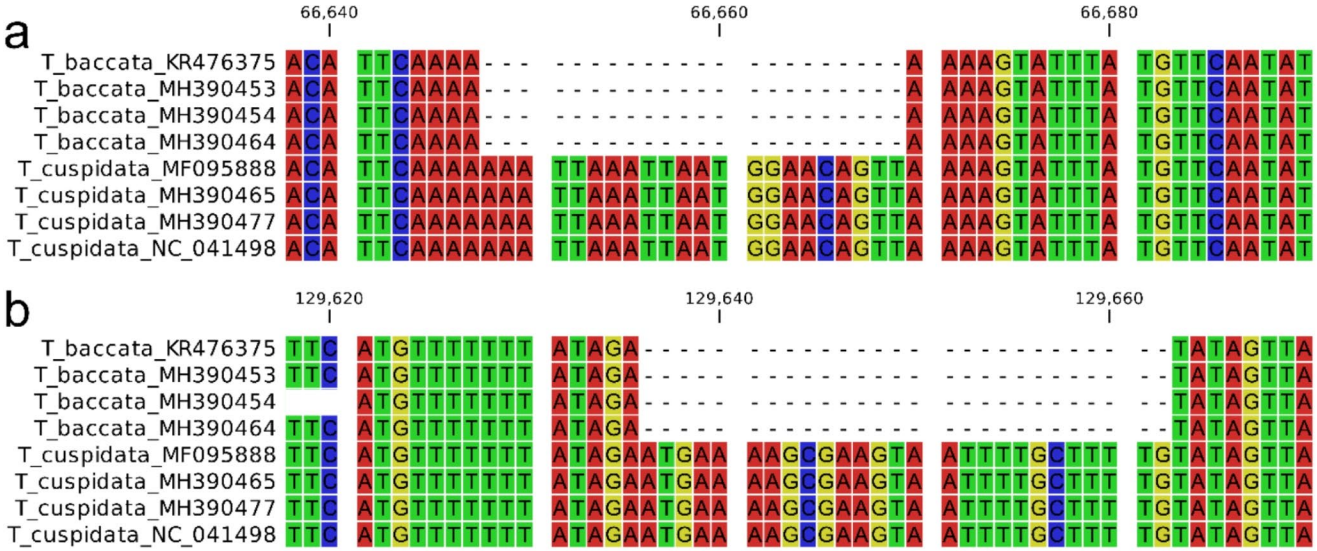

Fig. 2 Indels differentiating *T. baccata* and *T. cuspidata* in a whole-plastome alignment of four *T. baccata* and four *T. cuspidata* samples (complete alignment in Online Resource 2). NCBI accessions are given in the sequence name. (**a**) is related to the marker TA_InDel1 and (**b**) to marker TA_InDel2 (Table 3). Numbers on top of the alignment show the alignment position

Results

Identification of DNA sequence variants in public data

In case of the chloroplast genome, we focused on the identification of larger indels which can be implemented in simple PCR markers and made visible on agarose gels. Based on a whole-plastome alignment of eight accessions (four *T. baccata*, four *T. cuspidata*) (Online Resource 2), we identified two potential *T. baccata*-specific deletions of more than 20 bp when compared to the cpDNA sequence of *T. cuspidata* at alignment positions 66,648–66,669 (TA_InDel1) and 129,636–129,662 (TA_InDel2) (Table 3; Fig. 2). TA_InDel1 is located in the *psbB_psaI* intergenic spacer, and TA_InDel2 is located in the *chlN* gene of the chloroplast genome. Based on these indels, simple PCR markers were derived (TA_InDel1 and TA_InDel2; primer sequences in Online Resource 1, Table S2).

Aiming at the development of a mtDNA marker allowing for the potential differentiation between *T. baccata* and *T. cuspidata*, ten SNPs that show different alleles in *T. baccata* compared to *T. cuspidata* were identified in mtDNA CDS in silico based on two *T. baccata* and two *T. cuspidata* accessions (Online Resource 1, Table S3; Online Resource 3). Out of these, three SNPs were selected where the *T. baccata* allele is located in a recognition site of a restriction enzyme. One of these SNPs located in *cox1* (Online Resource 1, Table S3) at position 842 of the related reference sequence (MN593023.1_cds_QJS35647.1; see Table 3) was selected for CAPS marker development using the restriction enzyme BclI (marker TA_cox1 in Table 3; primers and fragment sizes in Online Resource 1, Table S2). In an alignment of *cox1* CDS of different *Taxus* individuals, the SNP differentiating *T. baccata* and *T. cuspidata* is located at alignment position 842 bp (Online Resource 3). Only the two *T. baccata* individuals in the alignment show the allele T at this position where the two *T. cuspidata* individuals as well as

individuals representing three other *Taxus* species show the allele C.

For potential species differentiation based on nDNA, a region of nuclear rRNA internal transcribed spacers (ITS) was analysed in silico in an alignment of 14 related publicly available sequences that were remaining after quality control (nine *T. baccata* and five *T. cuspidata*) (see Methods; Online Resource 4). Based on the alignment, a SNP potentially differentiating *T. baccata* and *T. cuspidata* was identified in the ITS region and a related marker was derived (TA_ITS in Table 3; alignment position 498 in Online Resource 4), allowing genotyping of individuals by PCR and Sanger sequencing (primer sequences in Online Resource 1, Table S2).

Validation of DNA markers in a set of species-declared *Taxus* individuals

To validate the species-specificity of the detected variants, all markers (Table 3) were tested with a set of 128 newly collected *Taxus* DNA samples ("validation set"; Online Resource 1, Table S1). In addition, the published cpDNA marker *trn*L-F (Collins et al. 2003) was applied to the validation set.

The validation set includes 106 *T. baccata*, 12 *T.* × *media* and 10 *T. cuspidata* individuals (Online Resource 1, Table S1). All samples were collected in Germany or Poland (Fig. 3). Since the provenance of most samples is undocumented, we cannot estimate the extent to which the natural distribution area of the species analysed is covered by our validation set.

The genotyping results for all 128 samples using the four markers developed in this study (Table 3) and the *trn*L-F marker (Collins et al. 2003) are presented in Online Resource 1, Table S1. The nDNA ITS markers TA_ITS (Table 3) enable the determination of both parental alleles at the given positions (without knowledge of the crossing direction). In case of these markers, nearly all 106 individuals phenotypically declared as *T. baccata* provided the *T. baccata*-specific allele in homozygote configuration at the ITS-SNP position (with one exception; Online Resource 1, Table S1; chromatograms available at https://osf.io/akd

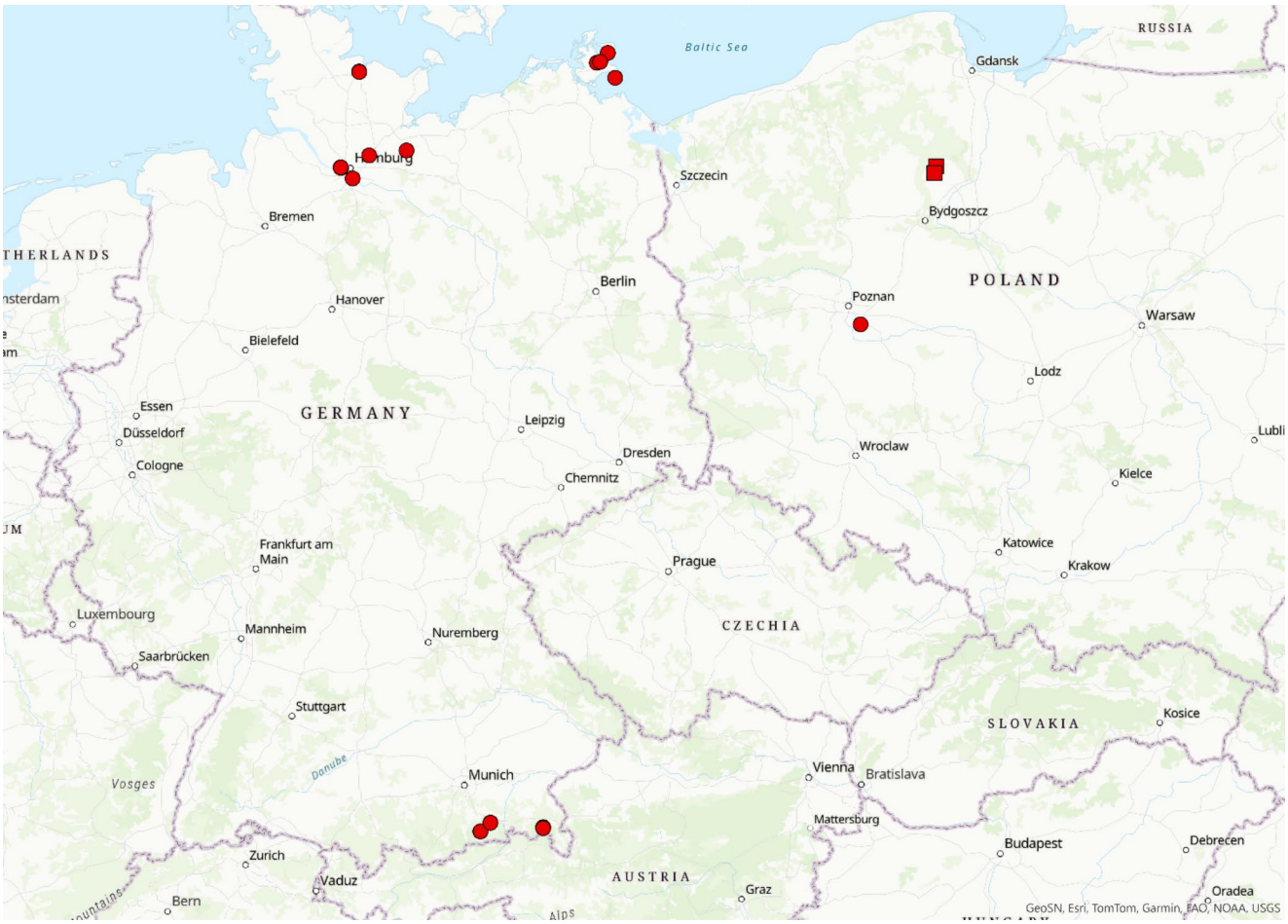

Fig. 3 Sampling locations of 128 *Taxus* individuals used for marker validation. Locations with autochthonous stands are marked with squares, all other locations are marked with circles. Note: Spatially close sampling locations (see Table S1) are represented by the same symbol

7r/?view_only=4244a737139942c18f70f862ccfa443b). In case of the 12 *T.* × *media* hybrid individuals, all genotyping results with the ITS markers showed heterozygous SNPs and thereby were in accordance with the phenotypic species declaration. In the phenotypically declared *T. cuspidata* individuals (10 samples), the *T. cuspidata*-specific allele was identified in four individuals in homozygous configuration as expected and in six individuals in heterozygous configuration, thus genetically classifying these six individuals as *T.* × *media* hybrids (Online Resource 1, Table S1; chromatograms available at https://osf.io/akd7r/?view_only=4244a737139942c18f70f862ccfa443b).

In all 128 samples, the cpDNA markers TA_InDel1 and TA_InDel2 as well as the previously published cpDNA marker *trn*L-F indicated the same species in the paternal line (*T. baccata* or *T. cuspidata*, respectively), confirming the species-specificity of the two newly developed indel markers. Considering the 116 individuals with phenotypic species declaration *T. baccata* or *T. cuspidata*, the genetic results obtained with the cpDNA markers were not following the phenotypic species declaration in only four cases (identified as hybrids by the ITS marker; Online Resource 1, Table S1). Genotyping of the 12 individuals declared as hybrids with the three cpDNA markers identified *T. baccata* in seven cases and *T. cuspidata* in five cases in the paternal line, underlining that both crossing directions are possible in *T.* × *media* hybrids. Genotyping of all 128 individuals with the newly developed mtDNA CAPS marker TA_cox1 (Table 3) provided the same genetic species identity like all three cpDNA markers applied, thus confirming that this marker allows to differentiate between *T. baccata* and *T. cuspidata* in the paternal line as well.

A comparison of the initial phenotypic species declaration and DNA marker-supported species identification for the validation set is presented in Fig. 4. The genetic species declaration is based on the four newly developed DNA markers (Table 3). Beyond genetic species identification, the applied marker set also allows the identification of crossing direction in *T.* × *media* hybrids, based on the application of cpDNA/mtDNA markers (paternal species identity of hybrids indicated in brackets at the right-hand side in Fig. 4). The crossing directions of seven of the 12 *T.* × *media* cultivars identified by these markers show a complete accordance with previously published data, as shown in Table 4. In case of the other five cultivars the crossing direction was previously unknown and was genetically identified in this study (Table 4; Fig. 5).

To define a minimal marker set, the species identification results were complemented with considerations of cost and ease-of-use. Given the predominantly paternal inheritance of cpDNA and mtDNA, the nDNA marker TA_ITS is the only marker capable of identifying *T.* × *media* hybrids in addition to *T. baccata* and *T. cuspidata*, and should therefore be included.

However, the ITS marker is not able to define the crossing direction in *T.* × *media* hybrids. To define the paternal line in *T.* × *media*, TA_InDel2 performs best, as it is not reliant on a digestion procedure like TA_cox1 or the

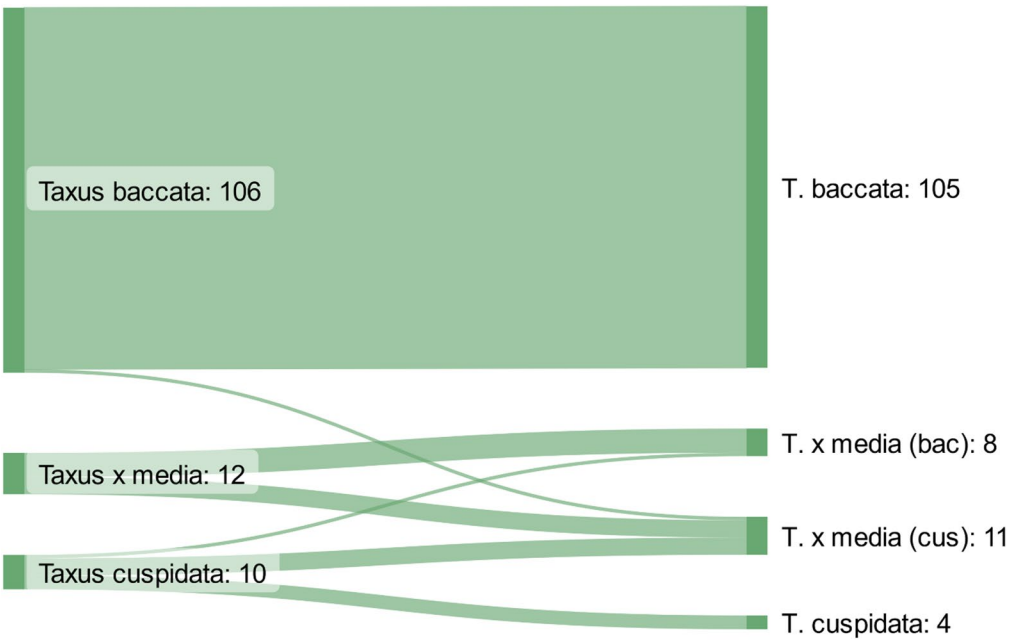

Fig. 4 Comparison between initial species declaration (left) of 128 *Taxus* samples of the validation set and marker-supported species identification (right) based on four newly developed DNA markers (Table 3). Bar heights are scaled to dataset size. For *T.* × *media*, the paternal genotype is given in brackets (bac = *T. baccata*, cus = *T. cuspidata*). Created using SankeyMATIC (https://sankeymatic.com/build/)

Table 4 Results of genetic identification of the chloroplast type, indicating crossing direction of different *T.* × *media* cultivars on the basis of the new cpDNA markers TA_InDel1 and TA_InDel2. Previously reported chloroplast types (using the *trn*L-F marker) taken from Collins et al. (2003). Genotyping of five cultivars with previously unknown crossing direction by using the cpDNA marker TA_InDel2 is presented in Fig. 5

Sample ID	Cultivar	This study	(Collins et al. 2003)
TXxBAC_1	Thayerae	*T. baccata*	*T. baccata*
TXxBAC_3	Hillii	*T. baccata*	*T. baccata*
TXxBAC_5	Hicksii	*T. baccata*	*T. baccata*
TXxBAC_7	Densiformis	*T. cuspidata*	*T. cuspidata*
TXxBAC_8	Hatfieldii	*T. cuspidata*	*T. cuspidata*
TXxBAC_9	Brownii	*T. cuspidata*	*T. cuspidata*
TXxBAC_10	Green Mountain	*T. baccata*	*T. baccata*
TXxBAC_11	Rising Star	*T. baccata*	-
TXxBAC_12	Farmen	*T. cuspidata*	-
TXxBAC_13	Groenland	*T. baccata*	-
TXxBAC_14	Straight Hedge	*T. baccata*	-
TXxBAC_15	Stricta Viridis	*T. cuspidata*	-

previously published marker *trn*L-F (Collins et al. 2003). It also produces larger DNA fragments during PCR than TA_InDel1, resulting in easier length polymorphism screening, while still being short enough for applications with heavily degraded DNA templates (e.g., wood samples). As such, the minimal marker set of TA_ITS and TA_InDel_2 allows for a complete and fast species differentiation between *T. baccata*, *T. cuspidata* and *T.* × *media* as well as for the identification of the crossing direction in *T.* × *media*.

Discussion

The markers developed in this study allow the differentiation of *T. baccata* and *T. cuspidata* in the nuclear, mitochondrial and chloroplast genome (Table 3). In the case of the nuclear marker, *T.* × *media* may also be identified. The cpDNA and mtDNA markers can provide the crossing direction in *T.* × *media* hybrids by defining the species in the paternal line (Table 4; Fig. 5).

Taxus is notorious for its difficult species identification, and yet, there have been successes in applying DNA-based techniques to support and supersede the traditional morphology-based approaches (Collins et al. 2003; Möller et al. 2007; Liu et al. 2011, 2018; Hao et al. 2018; Coughlan et al. 2020). Our study builds up on these works to achieve the identification of two *Taxus* species and their hybrid. As an alternative to barcoding approaches, which consider entire sequences in barcoding regions and require the construction

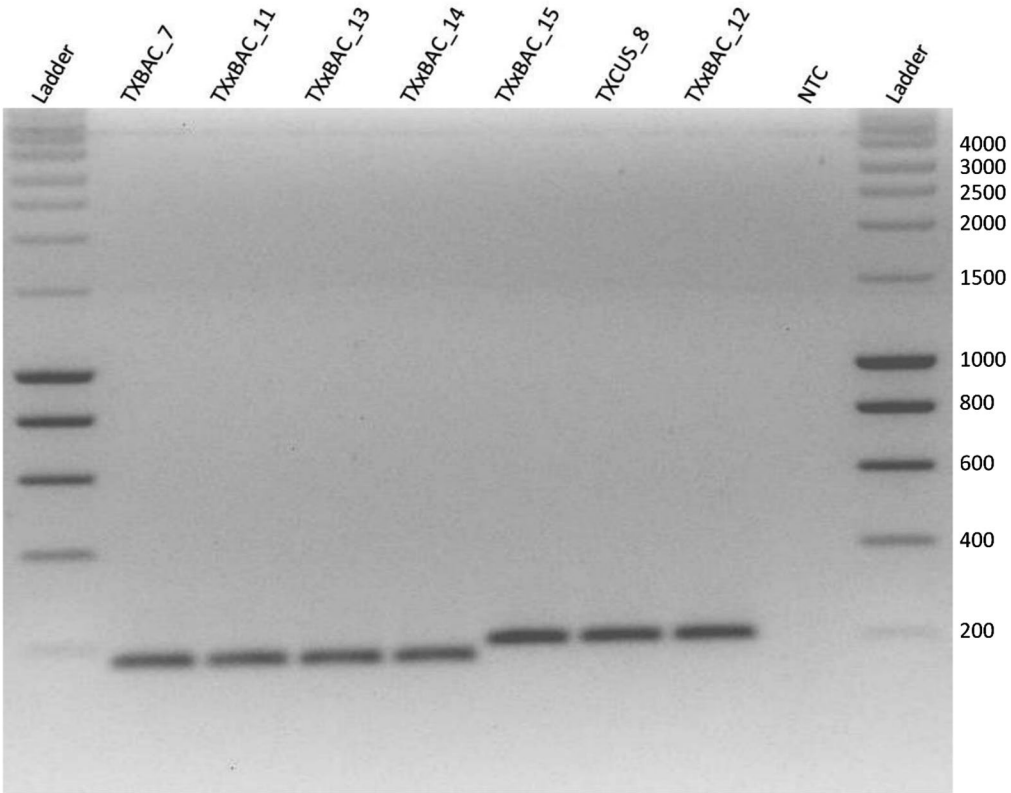

Fig. 5 Agarose gel showing genotyping of several *Taxus* × *media* individuals (Table 4) with the cpDNA marker TA_InDel2 to identify the species in the paternal line. Samples TXBAC_7 and TXCUS_8 function as positive controls for *T. baccata* and *T. cuspidata*, respectively. NTC = no template control. Ladder = Smart Ladder (Eurogentec Deutschland GmbH, Cologne, Germany). Ladder fragment size description in bp

of DNA barcode reference libraries based on large sample collections (Antil et al. 2023), the approach we followed focuses on the identification and validation of singular taxon/species-differentiating DNA sequence variants. The markers developed based on these variants are then used as a basis for genotyping of individual samples (e.g., Mader et al. 2020; Schroeder and Kersten 2023).

The markers of each cellular genome have different advantages when viewed in the context of previously published markers. Sequence comparison of the eukaryotic ITS regions is widely used in taxonomy and molecular phylogeny because of several favourable properties, despite some noticeable drawbacks for phylogenetic studies (Álvarez and Wendel 2003). For the nuclear marker in the ITS region developed in this study (Table 3), the causal SNP for the differentiation of *T. baccata* and *T. cuspidata* has likely been utilized before as part of a DNA barcoding approach (Liu et al. 2011), but the information on SNP positions with species-specific alleles, as well as a reference library for barcoding (Liu et al. 2018), was not accessible to us.

In this study, one SNP position was identified in the ITS region that shows *T. baccata* and *T. cuspidata*-specific alleles (Table 3; Online Resource 4), allowing species differentiation. In the case of interspecific *T.* × *media* hybrids, only the nuclear genome can be expected to contain species-specific DNA sequences from both parental lines and thereby indicate the hybrid status of a sample, as the inheritance of the mitochondrial and chloroplast genome in *T. baccata* were found to be (predominantly) paternal (Chesnoy 1987; Pennell and Bell 1988; Anderson and Owens 1999; Chybicki et al. 2016). Thus, the combination of the nuclear ITS marker with a cpDNA marker allows for the identification of the paternal line in *T.* × *media* hybrids, thereby identifying the crossing direction, which is not documented for many cultivars (Table 4; Figs. 4 and 5).

The chloroplast markers TA_InDel1 and TA_InDel2 amplify only small DNA segments (Table 3; Fig. 4; Online Resource 1, Table S1), which is preferable for heavily degraded DNA templates (e.g., wood samples (Mitchell 2015; Schroeder et al. 2016). Compared to the previously published marker in the chloroplast *trn*L-F region (Collins et al. 2003), the presented indel-based markers do not require an additional digestion step for the differentiation of *T. baccata* and *T. cuspidata*, thereby allowing a broader range of applications at a lower cost.

The inclusion of the mitochondrial TA_cox1 marker, which showed *T. baccata*-/*T. cuspidata*-specific alleles in this study (Table 3; Online Resource 1, Table S3), allows for the deduction of the mode of inheritance of the mitochondrial genome in *T.* × *media*. The results of this marker related to the identified species showed a complete accordance with the chloroplast markers in all *T.* × *media* samples (Online Resource 1, Table S1), indicating the same mode of inheritance. As mentioned above, the mode of inheritance of chloroplast DNA in *T. baccata* was found to be paternal (Chesnoy 1987; Pennell and Bell 1988; Anderson and Owens 1999), and a more recent study found the mode of inheritance of mitochondrial DNA to be predominantly paternal in *T. baccata* as well (Chybicki et al. 2016). Our results indicate that this is also true for the hybrid cultivar *T.* × *media*.

The validation process employed in this study showed few deviations from the expected marker results, correctly identifying all *T.* × *media* samples and >99% of *T. baccata* samples, but only 40% of *T. cuspidata* samples (Fig. 4; Online Resource 1, Table S1). Noticeably, all deviations involve samples that were considered to be either *T. baccata* or *T. cuspidata*, but identified by the TA_ITS marker as *T.* × *media*. The hybrid is described as intermediate between its parent species, showing mixed characteristics (Rehder 1923; Collins et al. 2003), which, combined with the general similarity between the parent species (Stöhr 2019), indicates that species declarations based on morphological attributes might be prone to errors. The inclusion of the previously published *trn*L-F marker (Collins et al. 2003) and its accordance with the newly presented cpDNA (and mtDNA) markers underline the possibility of a wrong initial species declaration in the cases of these deviations, as chloroplast types of *T. cuspidata* were found in a sample that was declared as *T. baccata* and vice versa (Fig. 3). To fully alleviate this issue, a broader range of samples is needed for a complementary marker validation, e.g., containing *T. cuspidata* samples from regions in which it is native.

Capturing within-species variation in the studied genetic regions was limited by the number of publicly available high-quality sequences. While we used all of the relevant sequence data that was available at GenBank at this time (respecting the QC filtering for ITS sequences, see Methods section), future studies will undoubtedly be able to benefit by a larger and more diverse dataset that better reflects the genetic diversity of the studied species.

Other aspects are the restricted number of samples in the validation set and the limited documentation of provenances of the included samples. A large part of *T. baccata* samples were received or collected from arboreta or botanical gardens in northern Germany, with their origin often being either regional or unknown. While the small set of more distant *T. baccata* samples from Bavaria and Poland (including samples from autochthonous stands) showed no divergent marker results (Online Resource 1, Table S1), a larger dataset of samples throughout the native range of *T. baccata* could provide an even better confirmation of the presented markers integrity.

Already the minimal marker set comprised of TA_ITS and TA_InDel2 (Table 3) allows for a fast taxon differentiation between *T. baccata*, *T. cuspidata* and *T.* × *media* as well as for the identification of the crossing direction in *T.* × *media* hybrids. However, the developed molecular markers share the drawback of inability to identify introgressions which might occur when *T.* × *media* hybrid individuals backcross and transfer genetic material between species potentially resulting in genomic admixture if the introgressed alleles are established (Mallet et al. 2016). Although, due to the multi-copy nature of the ITS region (Baldwin et al. 1995), the developed ITS marker might provide first indications on introgression (deviation from a 1:1 ratio of the two species-specific alleles in the base calls), such effects might still be confounded with the technical limitations of base calling during the sequencing process. These cases are better addressed in genome-wide studies with specialized tools such as NewHybrids (Anderson and Thompson 2002; Wringe et al. 2017). Genotyping using genome-wide distributed SNP-markers have been applied in several studies to detect introgression between closely related tree species (e.g., Fogelqvist et al. (2015), Reutimann et al. (2020)).

In Europe, *T. baccata* is the only naturally occurring species, while other *Taxus* species are usually restricted to cultivation. However, *T.* × *media* individuals have been observed outside of cultivation, bringing the identity of *Taxus* populations into question (Stöhr 2019). Given the difficulties of morphological species identification, the presented markers may assist future efforts to study the impact of *T.* × *media* on autochthonous populations of *T. baccata*.

Conclusions

In this study, we present novel DNA markers targeting all three cellular genomes to aid in the identification of *Taxus* species occurring in Europe, aiming at specific advantages over previously published markers. A minimal marker set comprised of the nDNA marker TA_ITS and the cpDNA marker TA_InDel2 developed in this study allows for a fast and cost-efficient species differentiation between *T. baccata*, *T. cuspidata* and *T.* × *media* as well as for the identification of the crossing direction in *T.* × *media* hybrids. The future application of the mtDNA marker TA_cox1 in combination with cpDNA markers in more *T.* × *media* hybrids will show if the mode of mitogenome inheritance is exclusively paternal in hybrids or if exceptions occur.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11295-025-01696-8.

Acknowledgements We thank the technicians Katrin Groppe, Imke Burau and Anke Schellhorn for technical assistance and the gardeners of the Thuenen Institute of Forest Genetics in Grosshandorf for the care of the plants. We are grateful to Muhidin Seho (Bavarian Office for Forest Genetics, Teisendorf, Germany) and Matthias Fladung (Thuenen Institute of Forest Genetics, Grosshansdorf, Germany) who provided us with material of some *T. baccata* individuals. Stefan Rust, Jürgen Schöttler (both: Loki Schmidt Botanical Garden Hamburg, Germany) and Susanne Petersen (Botanical Garden Kiel, Germany) are acknowledged for providing access to the related botanical gardens and supporting sample collections. We thank Malte Mader for support with the Linux cluster and Niels Müller for helpful discussions (both: Thuenen Institute of Forest Genetics).

Author contributions Conceptualization: Birgit Kersten, Daniel Bross; Plant material acquisition: Daniel Bross, Birgit Kersten, Emilia Pers-Kamczyc, Hilke Schroeder; Formal analysis and investigation: Daniel Bross, Birgit Kersten; Writing - original draft preparation: Daniel Bross, Birgit Kersten, Hilke Schroeder; Writing - review and editing: Daniel Bross, Birgit Kersten, Hilke Schroeder, Emilia Pers-Kamczyc; Funding acquisition: Birgit Kersten, Hilke Schroeder, Emilia Pers-Kamczyc; Supervision: Birgit Kersten.

Funding Open Access funding enabled and organized by Projekt DEAL.
This study was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)– project number 497528752– in the scope of the TaxGen project (funding of DFG to B.K.; DFG grant no. KE 916/10−1), the Deutsche Bundesstiftung Umwelt (DBU, German Federal Environmental Foundation; grant number 33949/01–31) and by the Institute of Dendrology, Polish Academy of Sciences (DEC-2022/03/ZB/FBW/00008).

Data availability Sanger sequencing data for ITS-based marker genotyping are available at https://osf.io/akd7r/?view_only=4244a737139942c18f70f862ccfa443b

Declarations

Ethical approval and consent to participate Not applicable.

Human and animal ethics Not applicable.

Consent for publication All authors have read and agree with the publication of the manuscript.

Competing interests The authors declare no competing interests.

References

Álvarez I, Wendel JF (2003) Ribosomal ITS sequences and plant phylogenetic inference. Mol Phylogenet Evol 29:417–434. https://doi.org/10.1016/s1055-7903(03)00208-2

Anderson ED, Owens JN (1999) Megagametophyte development, fertilization, and cytoplasmic inheritance in taxus brevifolia. Int J Plant Sci 160:459–469. https://doi.org/10.1086/314143

Anderson EC, Thompson EA (2002) A Model-Based method for identifying species hybrids using multilocus genetic data. Genetics 160:1217–1229. https://doi.org/10.1093/genetics/160.3.1217

Antil S, Abraham JS, Sripoorna S, Maurya S, Dagar J, Makhija S, Bhagat P, Gupta R, Sood U, Lal R, Toteja R (2023) DNA barcoding, an effective tool for species identification: a review. Mol Biol Rep 50:761–775. https://doi.org/10.1007/s11033-022-08015-7

Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CS, Donoghue MJ (1995) The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Ann Missouri Bot Gard 82:247–277

Benham SE, Houston Durrant T, Caudullo G, de Rigo D (2016) Taxus baccata in Europe: distribution, habitat, usage and threats. In: San-Miguel-Ayanz J, de Rigo D, Caudullo G, Houston Durrant T, Mauri A (eds) European atlas of forest tree species. Publ. Off. EU. Luxembourg. e015921+

Bruegmann T, Fladung M, Schroeder H (2022) Flexible DNA isolation procedure for different tree species as a convenient lab routine. Silvae Genet 20–30. https://doi.org/10.2478/sg-2022-0003

Chadwick LC, Keen RA (1976) A study of the genus taxus. Ohio Agricultural Research and Development Center Research Bulletin 1086

Chesnoy L (1987) L'origine des organites du cytoplasme embryonnaire Chez les gymnospermes. Bulletin de La Société botanique de France. Actualités Botaniques 134:51–56. https://doi.org/10.1080/01811789.1987.10826862

Chybicki IJ, Dering M, Iszkuło G, Meyza K, Suszka J (2016) Relative strength of fine-scale spatial genetic structure in paternally vs biparentally inherited DNA in a dioecious plant depends on both sex proportions and pollen-to-seed dispersal ratio. Heredity 117:449–459. https://doi.org/10.1038/hdy.2016.65

Collins D, Mill RR, Möller M (2003) Species separation of taxus baccata, T. canadensis, and T. cuspidata (Taxaceae) and origins of their reputed hybrids inferred from RAPD and CpDNA data. Am J Bot 90:175–182. https://doi.org/10.3732/ajb.90.2.175

Coughlan P, Carolan JC, Hook ILI, Kilmartin L, Hodkinson TR (2020) Phylogenetics of taxus using the internal transcribed spacers of nuclear ribosomal DNA and plastid trnL-F regions. Horticulturae 6:19. https://doi.org/10.3390/horticulturae6010019

Council Directive 92/43/EEC of 21 (1992) May 1992 on the conservation of natural habitats and of wild fauna and flora. Official Journal of the European Communities

Earle CJE (2022) Gymnosperm database. The Gymnosperm Database. https://www.conifers.org/ta/Taxus.php. Accessed 23 Nov 2022

Fogelqvist J, Verkhozina AV, Katyshev AI, Pucholt P, Dixelius C, Rönnberg-Wästljung AC, Lascoux M, Berlin S (2015) Genetic and morphological evidence for introgression between three species of willows. BMC Evol Biol 15:1–10. https://doi.org/10.1186/s12862-015-0461-7

Fu C-N, Wu C-S, Ye L-J, Mo Z-Q, Liu J, Chang Y-W, Li D-Z, Chaw S-M, Gao L-M (2019) Prevalence of isomeric plastomes and effectiveness of plastome super-barcodes in yews (Taxus) worldwide. Sci Rep 9:1–11. https://doi.org/10.1038/s41598-019-39161-x

Gallego-Jara J, Lozano-Terol G, Sola-Martínez RA, Cánovas-Díaz M, de Diego Puente T (2020) A compressive review about Taxol®: history and future challenges. Molecules 25:5986. https://doi.org/10.3390/molecules25245986

GBIF Secretariat (2023) GBIF Backbone Taxonomy. https://www.gbif.org/species/5284524. Accessed 22 Jul 2024

Hao J, Jiao K, Yu C, Guo H, Zhu Y, Yang X, Zhang S, Zhang L, Feng S, Song Y, Dong M, Wang H, Shen C (2018) Development of SCoT-based SCAR marker for rapid authentication of taxus media. Biochem Genet 56:255–266. https://doi.org/10.1007/s10528-018-9842-0

Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003) Biological identifications through DNA barcodes. Proc R Soc Lond B Biol Sci 270: 313–321. https://doi.org/10.1098/rspb.2002.2218

IUCN (2023) The IUCN Red List of threatened species. https://www.iucnredlist.org/. Accessed 9 Oct 2023

Kan S-L, Shen T-T, Gong P, Ran J-H, Wang X-Q (2020) The complete mitochondrial genome of taxus cuspidata (Taxaceae): eight protein-coding genes have transferred to the nuclear genome. BMC Evol Biol 20:1–17. https://doi.org/10.1186/s12862-020-1582-1

Kondo T (2016) Development of polymorphic microsatellite markers for Japanese Yew, taxus cuspidata, and T. cuspidata Var. Nana (Taxaceae). Appl Plant Sci 4:1600020. https://doi.org/10.3732/apps.1600020

Li J, Davis CC, Tredici PD, Donoghue MJ (2001) Phylogeny and biogeography of taxus (Taxaceae) inferred from sequences of the internal transcribed spacer region of nuclear ribosomal DNA. Harvard Papers in Botany

Liu J, Möller M, Gao L-M, Zhang D-Q, Li D-Z (2011) DNA barcoding for the discrimination of Eurasian yews (Taxus L., Taxaceae) and the discovery of cryptic species. Mol Ecol Resour 11:89–100. https://doi.org/10.1111/j.1755-0998.2010.02907.x

Liu J, Milne RI, Möller M, Zhu G-F, Ye L-J, Luo Y-H, Yang J-B, Wambulwa MC, Wang C-N, Li D-Z, Gao L-M (2018) Integrating a comprehensive DNA barcode reference library with a global map of yews (Taxus L.) for forensic identification. Mol Ecol Resour 18:1115–1131. https://doi.org/10.1111/1755-0998.12903

Mader M, Schroeder H, Schott T, Schöning-Stierand K, Leite Montalvão AP, Liesebach H, Liesebach M, Fussi B, Kersten B (2020) Mitochondrial genome of fagus sylvatica L. as a source for taxonomic marker development in the Fagales. Plants 9:1274. https://doi.org/10.3390/plants9101274

Mallet J, Besansky N, Hahn MW (2016) How reticulated are species? BioEssays 38:140–149. https://doi.org/10.1002/bies.201500149

Mitchell A (2015) Collecting in collections: a PCR strategy and primer set for DNA barcoding of decades-old dried museum specimens. Mol Ecol Resour 15:1102–1111. https://doi.org/10.1111/1755-0998.12380

Möller M, Gao L-M, Mill RR, Li D-Z, Hollingsworth ML, Gibby M (2007) Morphometric analysis of the taxus Wallichiana complex (Taxaceae) based on herbarium material. Bot J Linn Soc 155:307–335. https://doi.org/10.1111/j.1095-8339.2007.00697.x

Möller M, Liu J, Li Y, Li J-H, Ye L-J, Mill R, Thomas P, Li D-Z, Gao L-M (2020) Repeated intercontinental migrations and recurring hybridizations characterise the evolutionary history of Yew (Taxus L.). Mol Phylogenet Evol 153:106952. https://doi.org/10.1016/j.ympev.2020.106952

Pennell RI, Bell PR (1988) Insemination of the archegonium and fertilization in taxus baccata L. J Cell Sci 89:551–560. https://doi.org/10.1242/jcs.89.4.551

Pilger R (1903) Taxaceae. In: Engler A (ed) Das Pflanzenreich, 5 edn. Wilhelm Engelmann, Leipzig, pp 99–105

Rehder A (1923) New species, varieties and combinations from the herbarium and the collections of the Arnold arboretum. J Arnold Arboretum 4:107–109

Reutimann O, Gugerli F, Rellstab C (2020) A species-discriminatory single-nucleotide polymorphism set reveals maintenance of species integrity in hybridizing European white Oaks (Quercus spp.)

despite high levels of admixture. Ann Bot 125:663–676. https://doi.org/10.1093/aob/mcaa001
Schirone B, Ferreira RC, Vessella F, Schirone A, Piredda R, Simeone MC (2010) Taxus baccata in the Azores: a relict form at risk of imminent extinction. Biodivers Conserv 19:1547–1565. https://doi.org/10.1007/s10531-010-9786-0
Schroeder H, Kersten B (2023) A small set of nuclear markers for reliable differentiation of the two closely related oak species Quercus Robur and Q. Petraea Plants 12:566. https://doi.org/10.3390/plants12030566
Schroeder H, Cronn R, Yanbaev Y, Jennings T, Mader M, Degen B, Kersten B (2016) Development of molecular markers for determining continental origin of wood from white Oaks (Quercus L. Sect. Quercus). PLoS ONE 11:e0158221. https://doi.org/10.1371/journal.pone.0158221
Seneta W, Dolatowski J, Zieliński J (2021) Dendrologia. Wydawnictwo Naukowe PWN, Warszawa
Spjut RW (2007) Taxonomy and nomenclature of taxus (Taxaceae). J Bot Res Inst Tex 1:203–289
Stöhr O (2019) Zur Frage der identität Junger eibenverwilderungen (Taxus sp.) Im siedlungsraum von Osttirol (Österreich). Braunschweiger Geobotanische Arbeiten 13:93–117. https://doi.org/10.24355/dbbs.084-201902151100-0
Svenning J-C, Magård E (1999) Population ecology and conservation status of the last natural population of english Yew taxus baccata in Denmark. Biol Conserv 88:173–182. https://doi.org/10.1016/s0006-3207(98)00106-2
Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of Chloroplast DNA. Plant Mol Biol 17:1105–1109. https://doi.org/10.1007/bf00037152
Wani MC, Horwitz SB (2014) Nature as a remarkable chemist: a personal story of the discovery and development of taxol. Anticancer Drugs 25:482. https://doi.org/10.1097/cad.0000000000000063
WFO (2024) World Flora Online. Version 2023.03. Published on the Internet.https://wfoplantlist.org/. Accessed 22 Jul 2024
Wringe BF, Stanley RRE, Jeffery NW, Anderson EC, Bradbury IR (2017) parallelnewhybrid: an R package for the parallelization of hybrid detection using newhybrids. Mol Ecol Resour 17: 91–95. https://doi.org/10.1111/1755-0998.12597