A multidisciplinary approach reveals hidden taxonomic diversity in the morphologically challenging *Taxus wallichiana* complex

Michael Möller,[1,4] Lian-Ming Gao,[1] Robert R. Mill,[4] Jie Liu,[1,2] De-Quan Zhang,[1,2] Ram C. Poudel[1,2,3] & De-Zhu Li[1,2]

1 *Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China*
2 *Plant Germplasm and Genomics Center, Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China*
3 *Kunming College of Life Sciences, University of the Chinese Academy of Sciences, Kunming, Yunnan 650201, China*
4 *Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, Scotland, U.K.*
Authors for correspondence: *M. Möller, m.moeller@rbge.ac.uk; L.-M. Gao, gaolm@mail.kib.ac.cn; D.-Z. Li, dzl@mail.kib.ac.cn*

Abstract Addressing the taxonomy of morphologically complex groups can be challenging. Correlating results from multivariate morphological analyses and molecular data and reconciling these with geographic distributions allowed us to address the classification of the taxonomically difficult *Taxus wallichiana* complex in China and surrounding areas. We used 27 morphological characters (phenotype) and sequences of the chloroplast *trnL-F* intron/spacer region (genotype) on 1240 samples of 79 populations and 43 type and paratype specimens and demonstrated a strong correlation between phenotype and genotype and geographic distribution. We were able to define the number of taxonomically discrete units in the complex and delineate the geographic distribution of five species, *T. chinensis*, *T. florinii*, *T. mairei*, *T. wallichiana* and the here described new species *T. calcicola* in China and adjacent regions. These are much fewer than the number of recently proposed species and these exhibit greatly non-overlapping and continuous distributions. Based on descriptions of species-"typical" specimens, we prepared species-specific morphometric profiles, that allowed the generation of a species differentiation matrix, allowing the quick selection of the most suitable characters for the separation of any species pair. This work represents a step forward towards the completion of a workable taxonomy of Asian *Taxus*.

Keywords differentiation matrix; multivariate analysis; phytogeography; population-level sampling; taxonomy; *Taxus calcicola*

Supplementary Material The Electronic Supplement (Figs. S1–S8; Tables S1–S7) is available in the Supplementary Data section of the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

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■ INTRODUCTION

Morphologically complex species groups where few consistent characters exist can pose considerable problems and result in widely divergent taxonomic treatments and an excess of synonymy, as for example in *Camellia* L. (Sealy, 1958; Chang & Ren, 1998; Ming, 2000; Jiang & al., 2010) or *Taxus* L. (Pilger, 1903; Fu & al., 1999; Spjut, 2007; Farjon, 2010).

The genus *Taxus* has been difficult taxonomically from early on, and different views have resulted in drastically different systematic treatments, with the extremes being Pilger (1903) treating the genus to include globally one species with seven subspecies, while more recently Spjut (2007) distinguished 24 species with 55 varieties. De Laubenfels (1988) regarded all Asian *Taxus*, except *T. cuspidata* Siebold & Zucc., as belonging to one variable species, *T. sumatrana* (Miq.) de Laub., while Spjut (2007) distinguished 29 taxa in Asia alone. In the *Flora of China* (Fu & al., 1999), three species (*T. fuana* Nan Li & R.R. Mill, now *T. contorta* Griff., *T. cuspidata*, *T. wallichiana* Zucc.) and two varieties (*T. wallichiana* var. *chinensis* (Pilger) Florin, *T. wallichiana* var. *mairei* (Lemée & H. Lév.) L.K. Fu & Nan Li) were recognised, while Spjut (2007) distinguished 16 species including five new species, with seven varieties, in the same area. Farjon (2010) recently accepted the varieties in *T. wallichiana* at species rank, *T. chinensis* (Pilg.) Rehd., *T. mairei* (Lemée & Lév.) S.Y. Hu ex T.S. Liu, and *T. wallichiana*.

None of these past taxonomic treatments utilised molecular data in support of their proposed classifications. Several recent publications on *Taxus* have used chloroplast and low-copy and multicopy nuclear gene sequences, though these addressed species relationships, rather than species delineation and often included only a single sample representing a taxon (Li & al., 2001; Hao & al., 2008a, b). Multiple samples were included in a recent paper using the barcoding concept to distinguish molecular entities among the Eurasian yews and 11 distinct lineages were found (Liu & al., 2011).

The potential of utilising morphological data and molecular data in combination in resolving difficult taxonomic groups has not been fully exploited for *Taxus*, but has been used in different plant groups, though often in a phylogenetic framework (e.g., MacMaster & al., 2005; Wortley & Scotland, 2006), and with varying results (reviewed by Duminil & Di Michele, 2009). Shah & al. (2008) used a multivariate morphometric approach in parallel with DNA sequence data to unambiguously distinguish *T. baccata* L., *T. contorta* (as *T. fuana*) and *T. wallichiana*, and clarified the species status of the latter two in the west Himalayas where they were considered previously to constitute a single species (i.e., *T. baccata*, Aitchison, 1880).

In a recent multivariate morphometric study conducted on the *Taxus wallichiana* complex, using 27 vegetative characters originating from 128 herbarium specimens (Möller & al., 2007), four discrete clusters were identified corresponding to the taxa of *T. wallichiana* as defined in the *Flora of China* (Fu & al., 1999). These represented the three varieties comprising the complex, *T. wallichiana* var. *chinensis*, *T. wallichiana* var. *mairei* and *T. wallichiana* var. *wallichiana*, plus a fourth, named "Hengduan Mountain type" (Möller & al., 2007). These morphological clusters also showed a strong geographic pattern. The varieties of *T. wallichiana* have recently been raised to species status but with a cautionary note: "This genus is perhaps a good example of a group of species for which it could be illuminating to apply some more explicit methodology than intuitive taxonomy based on the careful observation of specimens, as has been done so far with the widely divergent results cited above [i.e., Pilger, 1903; Spjut, 2007]. Morphometric analysis of leaf characters could test these in theory, but may not find many gaps between what are mostly quantitative characters. Another (and currently popular) approach is of course genetics (DNA), but here the comprehensive sampling of all the dispersed areas in which the genus is found to grow naturally becomes a major obstacle to obtain results that we can have any confidence in" (Farjon, 2010: 969).

Indeed, our multivariate morphological approach on leaf characters alone showed a great potential in *Taxus* with the differentiation of the *T. wallichiana* varieties and the indication of a further morphological entity, the "Hengduan Mountain type" (Möller & al., 2007). In contrast to Farjon's scepticism quoted above regarding the usefulness of DNA data, our molecular phylogeographic study of 50 *T. wallichiana* populations collected across China and north Vietnam (Gao & al., 2007), revealed 19 different chloroplast PCR-RFLP haplotypes for the cpDNA *trnL-F* region, with a strikingly similar geographic distribution compared to the vegetative phenotype clusters found in Möller & al. (2007). Recent work, including a barcoding approach on the *T. wallichiana* complex revealed several taxon-specific haplotype lineages in China and north Vietnam, representing the three *T. wallichiana* varieties, and the Hengduan type, plus three additional lineages, all showing strongly discrete geographic distributions (Liu & al., 2011, 2013). However, no morphological data were analysed alongside to test the congruence between the molecular lineages and proposed taxonomic units.

Because only a small number of herbarium specimens was included in our previous morphometric study, with each specimen representing a population or locality (Möller & al., 2007), and because consecutive molecular work indicated the presence of more genetic entities among the *T. wallichiana* complex (Liu & al., 2011), we acquired morphological data for 1240 newly collected specimens of 79 populations comprehensively covering the distribution range of the complex (Fu & al., 1999), in the east Himalayas, mainland China and north Vietnam. In parallel, their cpDNA lineages were determined based on our PCR-RFLP and sequencing approach (Gao & al., 2007; Liu & al., 2011). Since Spjut (2007) recognised 16 species and seven varieties in the distribution range of *T. wallichiana*, with often parallel and disjunct distributions, we included 36 of his designated type specimens in our analysis.

In the present study, we used a multidisciplinary approach, combining molecular chloroplast haplotype lineage data, morphological multivariate analyses and geographic mapping, at the population-level for the *T. wallichiana* complex in China and adjacent areas to (1) test the correlation of morphologically definable clusters (i.e., taxa) with the cpDNA haplotype lineages, and link these to geographic distribution patterns; (2) test the coherence and independent status of the clusters found, and compare the results with recent taxonomic treatments (e.g., Spjut, 2007; Farjon, 2010), and (3) to propose taxonomic consequences, and describe a new species in *Taxus*. We have deliberately not set any a priori importance on any of our characters, because of the subjectivity that would be introduced. For poorly performing ones, this may be linked to a perceived subjectivity in the scoring of their states. Thus, our approach will identify characters that are reliable to score and perform strongly in the differentiation and identification of *Taxus* taxa. The study will show that morphologically complex groups can be tackled effectively taxonomically when combining information from fundamentally different sources, such as morphological and molecular data and analyse these in a geographic context.

■ MATERIALS AND METHODS

Plant material. — For the present paper we collected 79 populations of *Taxus wallichiana* as defined by Fu & al. (1999) from the east Himalayas, across China and north Vietnam, to comprehensively cover the entire distribution range of the species (Fig. 1). We excluded population samples from Taiwan at this point, as the link between Taiwan and mainland China will be dealt with in a forthcoming paper focussing on that area. The core dataset comprised a total of 1240 specimens, with 2 to 32 individuals per population (Electr. Suppl.: Table S1). Leaves for DNA extraction from each individual were dried in silica gel until used. Herbarium vouchers for each plant were prepared and are deposited at KUN and/or E.

Morphological data for 12 herbarium type specimens (including types of seven of Spjut's taxa) were already available from Möller & al. (2007), and data for an additional 29 specimens representing designated types and paratypes of taxa named by Spjut (2007) from China, east Himalayas and north Vietnam were acquired here. Two additional type specimens representing *T. wallichiana* and *T. wallichiana* var. *yunnanensis*

(W.C. Cheng & L.K. Fu) C.T. Kuan were further added (Electr. Suppl.: Table S2). Farjon (2010) regarded *T. wallichiana* var. *yunnanensis* as a synonym of *T. wallichiana*.

cpDNA haplotype lineage determination. — DNA extraction, *trnL-F* PCR, sequencing and cpDNA-RFLP genotyping followed Gao & al. (2007) and Liu & al. (2011). Definition of the seven genotype lineages for the *T. wallichiana* complex (cf. Fu & al., 1999) was based on Gao & al. (2007) and Liu & al. (2011) (Electr. Suppl.: Table S3). The haplotype lineages of the populations were plotted on maps using ArcGIS v.9.3 (ESRI, Redlands, California, U.S.A.). Locality data came from GPS data obtained during field collections of the populations.

Morphological characters. — The same 27 characters (26 leaf characters and one bud scale character) and character states as defined in Möller & al. (2007) were used (Electr. Suppl.: Table S4). Seven characters were continuous (characters 2–7 and 25) and twenty were discrete (1, 8–24, 26, 27). Coding was performed as described in Poudel & al. (2012), ensuring consistency. In Möller & al. (2007) we defined character 11 based on the insertion of the leaf on the stem. Here, as in Poudel & al. (2012), we used the leaf arrangement on branches, which is more reliable to score.

Character correlations with extrinsic variables. — Prior to the PCA analyses, the correlation of the discrete intrinsic leaf variables with extrinsic variables measured for each tree, such as height and stem diameter at breast height (DBH) and altitude, was carried out to investigate the datasets for effect of plant age (i.e., height and DBH) and elevation on the leaf characters used in the PCA. Pearson correlation coefficients were calculated to measure the strength of the linear relationship between pairs of variables, and statistically tested in JMP 6 (SAS Institute Inc., Cary, North Carolina, U.S.A.).

Principal component analysis (PCA) and cluster analyses (CA). — The PCA and CA procedures followed Möller & al. (2007) using R-pack Le Progiciel R.4.0d10 (Casgrain & al., 2005) and JMP 6 respectively. The seven continuous characters showed a normal distribution. The PCA results were displayed as three-dimensional scatter plots using JMP 6.

For the CA we used *K*-means clustering to test the integrity of the PCA clustering. We allowed JMP 6 to allocate group identifications based on cluster means (*K*-means). We used a range of numbers of clusters (*K*, see below) for the disjoint cluster analyses. Beginning with a set of temporary, random clusters, each sample is assigned to the nearest cluster mean. Repeated replacing of the random mean with the actual mean occurs until no further change in sample assignment occurs.

Dataset 1 – *K*-means clustering and correlation of genotype and phenotype. — In Möller & al. (2007) for 128 herbarium specimens, four morphologically distinct groups (phenotypes) were found among the *T. wallichiana* samples from China: *T. chinensis*, *T. mairei*, *T. wallichiana* (s.str.) and the "Hengduan Mountain type". In Liu & al. (2011), seven chloroplast haplotype lineages were detected, *T. chinensis*, *T. mairei*, and *T. wallichiana*, the Hengduan type, the Emei type, the Qinling type and the Tonkin type. To determine how many haplotype lineages could be defined morphologically, we correlated the genotype (chloroplast haplotype lineages) with the phenotype (*K*-means clusters). To this end we performed a PCA on the 27 morphological characters on the population dataset that comprised 1240 samples from 79 populations, and assigned phenotype identities by *K*-means clustering, using 2 to 10 clusters. Each of the 1240 samples was assigned to one of the seven genotypes and associated with its phenotype identity from the *K*-means clustering. This led to the optimal number of clusters to be used to best define the structure among the 1240 samples.

Dataset 1 – Phenotype mapping. — The phenotypes with the optimal number of *K*-means clusters were also plotted onto a map using ArcGIS v.9.3, to illustrate their spatial distribution.

Dataset 1 – Population coalescence. — To investigate the morphological diversity of individual populations, we highlighted samples of individual populations across the PCA space, to investigate their level of coherence or indicate their levels of morphological diversity by the degree of spread across (i.e., morphologically variable) or between (i.e., potential hybrid origin) individual phenotype clusters.

Dataset 2 – Coherence of datasets and types. — To test the coherence of the newly acquired dataset with our previous morphological data and Spjut's taxonomy, we combined the newly assembled morphological dataset here with data from seven type specimens of the three taxa, *T. chinensis*, *T. mairei*, and *T. wallichiana* from our previous work (Möller & al., 2007) and data for 36 additional samples representing six types and thirty paratypes designated by Spjut (2007) for his taxa with distribution ranges in China and the east Himalayas, *T. florinii* Spjut, *T. kingstonii* Spjut, *T. mairei* var. *speciosa* (Florin) Spjut, *T. obscura* Spjut, *T. phytonii* Spjut, and *T. suffnessii* Spjut. Together with these 43 specimens the total number of samples in this analysis was thus 1283. This would test the coherence of the two different morphometric datasets, and support the validity of conclusions drawn from their analysis. We performed a PCA with the 27 morphological characters as described above, focussing here on the position of the types among the newly collected population samples.

Dataset 3 – "Typical" specimens. — In order to obtain a more defined clustering of unique lineages, a reduced set of samples was analysed by PCA, omitting individuals from populations of mixed genetic lineages and morphological clusters. This resulted in the exclusion of ten entire populations (BK, DB, EB, EM, GG, HL, LB, MEK, SL, TB), all but one sample of QJ, and 81 individuals that fell outside their phenotype clusters and that were possibly the result of introgression. The resulting reduced dataset included 958 individuals from 69 populations. A PCA using the 27 morphological characters was performed as described above. This analysis also provided information on the most-discriminating characters between the clusters, and allowed a morphological description and discrimination of the taxa without interference from "atypical" specimens.

■ RESULTS

cpDNA haplotype lineage distribution. — Seventeen haplotypes detected already in Gao & al. (2007) were found among the 1240 samples analysed. These were assigned to seven lineages, representing *T. chinensis* (122 individuals), *T. mairei* (409), *T. wallichiana* (274), the Emei type (84), the Hengduan type (148), the Qinling type (123) and the Tonkin type (80) (Electr. Suppl.: Table S1). Plotting the seven lineages onto a map, a discrete geographic pattern emerged with little overlap (Fig. 1). The *T. mairei* lineage occupied the southeast and southwest of China to the southern fringes of the Sichuan basin. The *T. chinensis* lineage occupied the Daba Mountains around the northern edge of the Sichuan basin. The Qinling lineage formed a ring of populations to the North in parallel to the *T. chinensis* populations from the western Sichuan basin across the northern Qinling Mountains to the southern Taihang Mountains. The Emei type populations clustered around the southwestern fringe of the Sichuan basin, centred around the Emei Mountains and Qiaojia (QJ) in the northeast of Yunnan province. Here, they meet the populations of the *T. wallichiana* lineage. The *T. wallichiana* lineage extends from northeast Yunnan westwards to the Yunnan plateau and into the east Himalayas. The most southerly population of *T. wallichiana* included here was from Jinping (MA) near the border between China and Vietnam. The eight populations of the Hengduan lineage formed a tight cluster in the Hengduan Mountains region, to the North of the *T. wallichiana* populations in Yunnan. The majority of populations comprising the Tonkin type lineage occurred in southeast Yunnan, along the border region between Yunnan and Vietnam, with one population sampled further south in Vietnam, and two from Guizhou, one from Anlong (AL) in the southwest of that province and the other from the Northeast (Jiangkou, FJS). Nine populations were heterogeneous possessing haplotypes from different combinations of two lineages (i.e., MN, TQ, HSH, EM, QJ, JZG, JFS, BK, FJS) (Electr. Suppl.: Table S1).

Correlation of morphological characters with intrinsic and extrinsic characters. — As expected, plant height was strongly positively correlated with DBH ($r = 0.7749$, 943 pairs, $P < 0.0001$). However, the relationship appears non-linear, with an excess of taller plants possessing lower stem diameters, suggesting that the plants grow tall quickly, then increase in DBH over time (linear fit: $r^2 = 0.6$; polygonal fit 2nd degree: $r^2 = 0.628$) (Fig. 2). Neither plant height ($r = -0.05$, 1034 pairs, $P = 0.1081$) nor DBH ($r = 0.0304$, 968 pairs, $P = 0.3446$) was correlated to altitude (Table 1).

No strong correlations ($P < 0.001$) were found between the continuous characters char2 to char7 and char25, and height and DBH (Table 1). The only exception is the correlation between char3 (minimum leaf length) and height, but this character did not correlate with DBH ($r = 0.0549$, 968 pairs, $P = 0.0877$), and the slope of the positive correlation was very

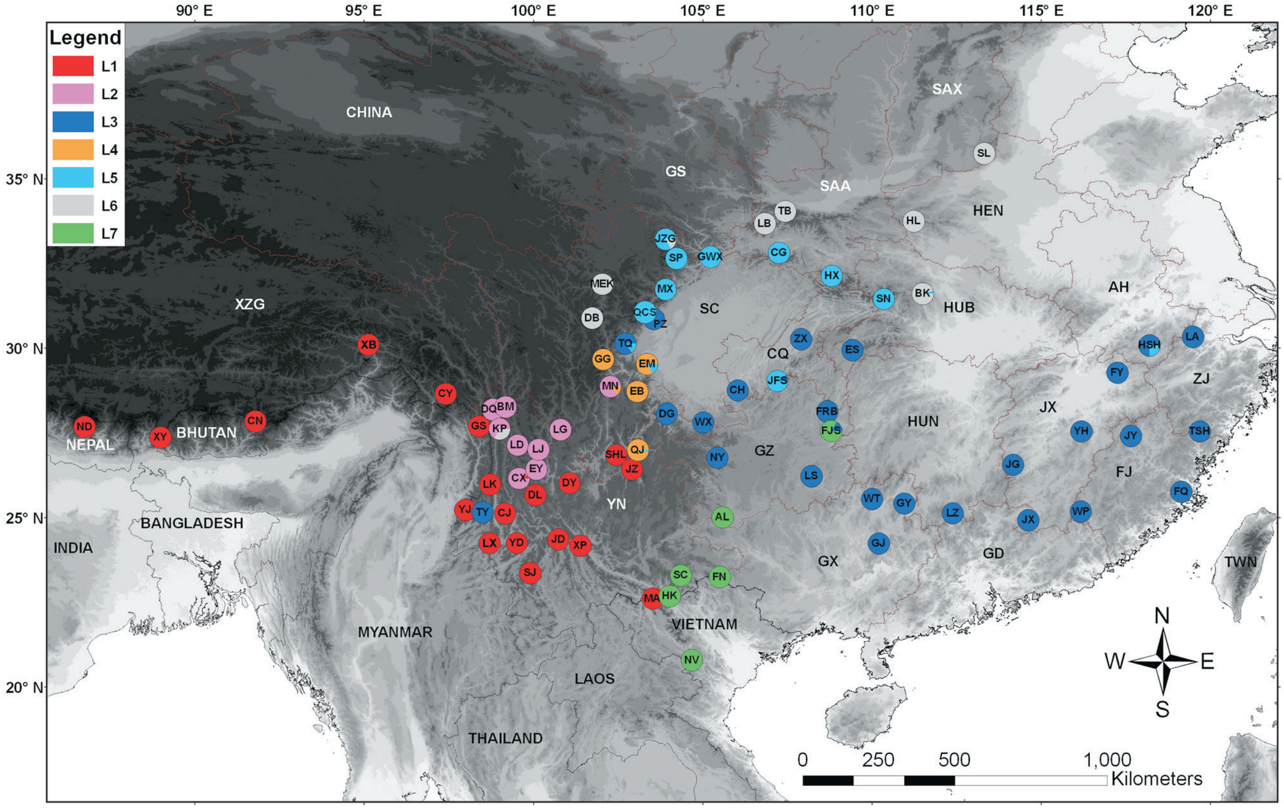


Fig. 1. Localities of the 79 populations of the *Taxus wallichiana* complex included in the present study from China and adjacent areas, and the distribution of 7 haplotype lineages (L1–L7) defined on chloroplast *trnL-F* data from Gao & al. (2007) and Liu & al. (2011). AH, Anhui; CQ, Chongqing; FJ, Fujian; GD, Guangdong; GS, Gansu; GX, Guangxi; GZ, Guizhou; HEN, Henan; HUB, Hubei; HUN, Hunan; JX, Jiangxi; SAA, Shaanxi; SAX, Shanxi; SC, Sichuan; TWN, Taiwan; XZG, Xizang; YN, Yunnan; ZJ, Zhejiang. Population codes inside circles as in Electr. Suppl. Table S1.

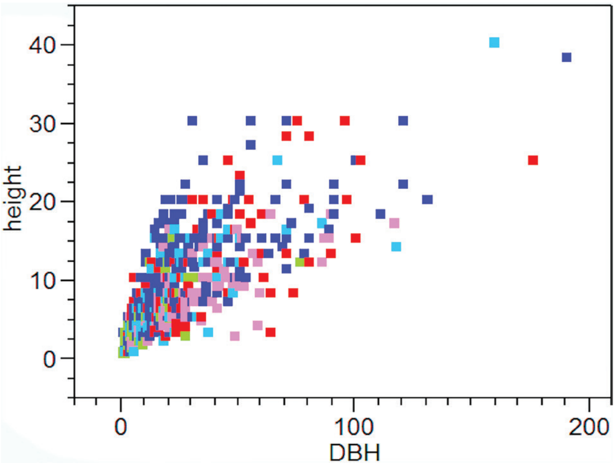


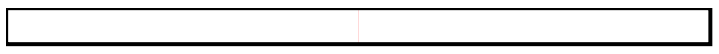
Fig. 2. Scatter plot of tree height [m] and stem diameter at breast height (DBH) [cm] for 943 comparisons of samples from 79 populations of the *Taxus wallichiana* complex with both tree height and DBH values, coded by five *K*-means clusters after PCA on 27 morphological characters. Colour code as in Fig. 4.

shallow ($r = 0.1135$). Overall, there was no consistent effect of plant age on the leaf and bud scale characteristics observed. Altitude, on the other hand, consistently showed very strong correlations with most continuous characters ($P < 0.0001$), except char4 (longest leaf length, $P = 0.0104$) and char25 (number of stomatal lines, $P = 0.1417$), with a tendency of higher leaf density (char2, $r = 0.3474$), and shorter (char3, $r = -0.1105$) and narrower (char5, $r = -0.5730$; char6, $r = -0.5334$) leaves, and a higher leaf length/width ratio (char7, $r = 0.4184$) with increasing altitude.

Dataset 1

Principal components analysis. — The distribution of variance in the PCA on 1240 samples was 28.5%, 14.8% and 6.7% for the first three axes, respectively, resulting in a cumulative value of 50%. The scree test (Cattell, 1966) suggested that three axes included significant information and needed to be explored. The PCA plot showed a strong structure (Electr. Suppl.: Fig. S1).

Table 1. Pairwise correlations of altitude [m], stem diameter at breast height (DBH) [cm], tree height [m] and the continuous morphological characters used in the PCA on samples of the *Taxus wallichiana* complex, providing slope of Correlation, number of comparisons (Count), levels of statistical significances (Signif Prob), and a correlation plot (Corr Plot).

Variable	by Variable	Correlation	Count	Signif Prob	Corr Plot
height	alt	−0.0500	1034	0.1081	
DBH	alt	0.0304	968	0.3446	
DBH	height	0.7749	943	<0.0001	
char2	alt	0.3474	1264	<0.0001	
char2	height	0.0060	1037	0.8474	
char2	DBH	0.0947	968	0.0032	
char3	alt	−0.1105	1264	<0.0001	
char3	height	0.1135	1037	0.0002	
char3	DBH	0.0549	968	0.0877	
char4	alt	−0.0721	1264	0.0104	
char4	height	0.0782	1037	0.0118	
char4	DBH	−0.0288	968	0.3715	
char5	alt	−0.5730	1264	<0.0001	
char5	height	0.0538	1037	0.0834	
char5	DBH	−0.0220	968	0.4933	
char6	alt	−0.5334	1264	<0.0001	
char6	height	0.0257	1037	0.4084	
char6	DBH	−0.0859	968	0.0075	
char7	alt	0.4184	1264	<0.0001	
char7	height	0.0573	1037	0.0653	
char7	DBH	0.0538	968	0.0943	
char25	alt	−0.0414	1264	0.1417	
char25	height	0.0596	1037	0.0551	
char25	DBH	−0.0241	968	0.4537	

***K*-means clustering and correlation of genotype and phenotype. —** The plot of phenotype lineage/haplotype cluster correlations over number of clusters displayed the proportion of the largest unit within the respective lineage as a measure of robustness of a cluster (Table 2; Electr. Suppl.: Fig. S2). It illustrated that the Tonkin type lineage was highly defined across all numbers of clusters (>87.5%), and the *T. wallichiana* lineage up to eight clusters (>91.6%). The Hengduan type and *T. mairei* lineages were highly defined up to five clusters (>90.5%), but the values declined at higher cluster numbers. The *T. chinensis* and Emei lineages had lower values across the clusters tested, and reached their highest proportions with five clusters (68.5% and 90%, respectively) (apart from two clusters, but this clustering is not very descriptive) (Electr. Suppl. Fig. S2B). The Qinling type was morphologically the least definable lineage, with at most barely half the samples falling into the highest category. Summing up the percentages, overall, the results suggest that five clusters best represented

Table 2. Correlation between the 7 cpDNA haplotype lineages and 4 **(A),** 5 **(B)** or 7 **(C)** morphological clusters of 1240 samples of the *Taxus wallichiana* complex after PCA and *K*-means clustering of the 27 morphological characters.

A, 4 *K*-means clusters:

		morphological clusters					
		1	2	3	4		
lineage	cpDNA type	*wall*	Hd	*mai*	*chin*	sum	largest block (%)
1 *T. wallichiana*	4, 5, 6, 7, 8	**270**	1	–	3	274	98.5
2 Hengduan type	9,	2	**145**	1	–	148	98.0
3 *T. mairei*	13, 14, 16	1	1	**398**	9	409	97.3
4 Emei type	10	24	17	–	42	84	(50.0) *T. chinensis*
5 *T. chinensis*	11, 12, (15)	11	29	5	**76**	122	62.3
6 Qinling type	17	10	12	62	39	123	(50.4) *T. mairei*
7 Tonkin type	1, 2, 3,	–	–	3	77	80	(96.3) *T. chinensis*
sum		318	207	469	246	**1240**	

B, 5 *K*-means clusters:

		morphological clusters						
		1	2	3	4	5		
lineage	cpDNA types	*wall*	Hd	*mai*	*chin*	Ton	sum	largest block (%)
1 *T. wallichiana*	4, 5, 6, 7, 8	**270**	1	–	–	3	274	98.5
2 Hengduan type	9,	2	**134**	1	11	–	148	90.5
3 *T. mairei*	13, 14, 16	1	1	**398**	3	6	409	97.3
4 Emei type	10	23	1	–	58	3	84	(69.1) *T. chinensis*
5 *T. chinensis*	11, 12, (15)	9	16	6	**84**	7	122	68.9
6 Qinling type	17	10	6	61	39	7	123	(49.6) *T. mairei*
7 Tonkin type	1, 2, 3,	–	–	3	3	**74**	80	92.5
	sum	315	159	469	198	99	**1240**	

C, 7 *K*-means clusters:

		morphological clusters								
		1	2	3	4	5	6	7		
lineage	cpDNA types	*wall*	Hd	*mai*	*chin*	Ton	Emei	Qinl	sum	largest block (%)
1 *T. wallichiana*	4, 5, 6, 7, 8	**269**	–	–	–	3	2	–	274	98.2
2 Hengduan type	9,	0	**102**	–	7	0	39	–	148	68.9
3 *T. mairei*	13, 14, 16	1	–	**220**	1	4	12	171	409	53.8
4 Emei type	10	17	–	–	46	2	19	–	84	(54.8) *T. chinensis*
5 *T. chinensis*	11, 12, (15)	7	8	1	77	6	17	6	122	63.1
6 Qinling type	17	2	2	22	31	5	21	**40**	123	(32.5)
7 Tonkin type	1, 2, 3,	–	–	–	3	**73**	–	4	80	91.3
	sum	296	112	243	165	93	110	221	**1240**	

Column headers are as follows: *wall*, *Taxus wallichiana*; Hd, Hengduan type; *mai*, *T. mairei*; *chin*, *T. chinensis*; Ton, Tonkin type; Emei, Emei type; Qinl, Qinling type
Numbers in bold, where molecular lineages correspond to morphological clusters.

the seven cpDNA lineages, since at higher numbers of clusters the proportion of the largest category in a lineage dropped for most lineages (Fig. 3).

When matching the cluster number with lineage number, i.e., seven, only two lineages, *T. wallichiana* and the Tonkin type, were well defined. All other lineages had lower values, suggesting that they included a significant proportion of samples from another morphological lineage, i.e., there was little correlation between the morphology and the haplotypes in five of the lineages. On the contrary, with four and five morphological clusters, four haplotype lineages were well defined (>90.5%). However, with four morphological groups, samples of the Tonkin-type haplotype fell in the *T. chinensis* morphological group, with five they formed an independent group (Table 2).

Phenotype clustering. — The PCA plot of the 1240 samples identified on the basis of five *K*-means clusters showed the strong spatial separation between the five morphological units (Electr. Suppl.: Fig. S3). The first axis separated *T. mairei* from the remaining clusters. These were separated in the second axis with the *T. wallichiana* cluster at the top, the *T. chinensis* cluster in the middle and the Tonkin type at the bottom. The Hengduan-type cluster overlapped with the *T. wallichiana* cluster, but was effectively separated from this in the third axis (Electr. Suppl.: Fig. S3B). Re-orienting the axes in three dimensions allowed a relatively overlap-free spatial view of the five clusters (Fig. 4A).

Genotype clustering. — Highlighting the individual cpDNA haplotype lineages on the 1240 sample PCA plot shows that the samples of *T. mairei*, *T. wallichiana*, the Hengduan and the Tonkin type occupied discrete spaces (Fig. 4B–E) (the few outliers will be dealt with below). The cpDNA lineages of *T. chinensis* and the Emei and Qinling types clustered rather widely (Fig. 4F–H). The *T. chinensis* haplotype lineage covered the entire space of the *T. chinensis* morphology cluster, but included many samples of the Hengduan type and a few of the Tonkin type, and the *T. mairei* and *T. wallichiana* samples. A

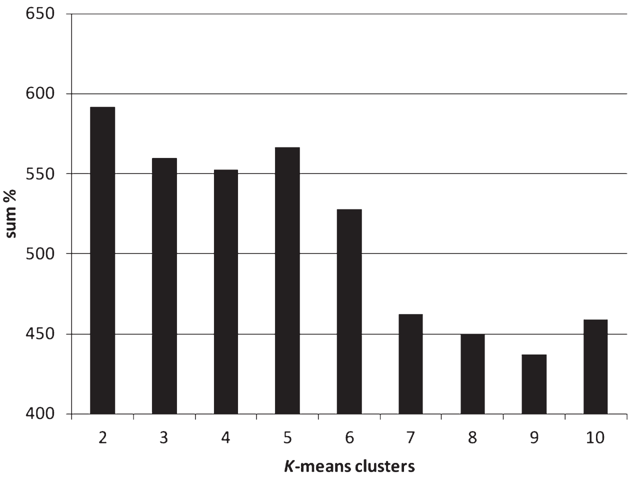


Fig. 3. Sum of the percentages of the largest clusters within the seven cpDNA lineages over 2 to 10 *K*-means clusters after PCA based on 27 morphological characters of 1240 samples of the *Taxus wallichiana* complex from 79 populations.

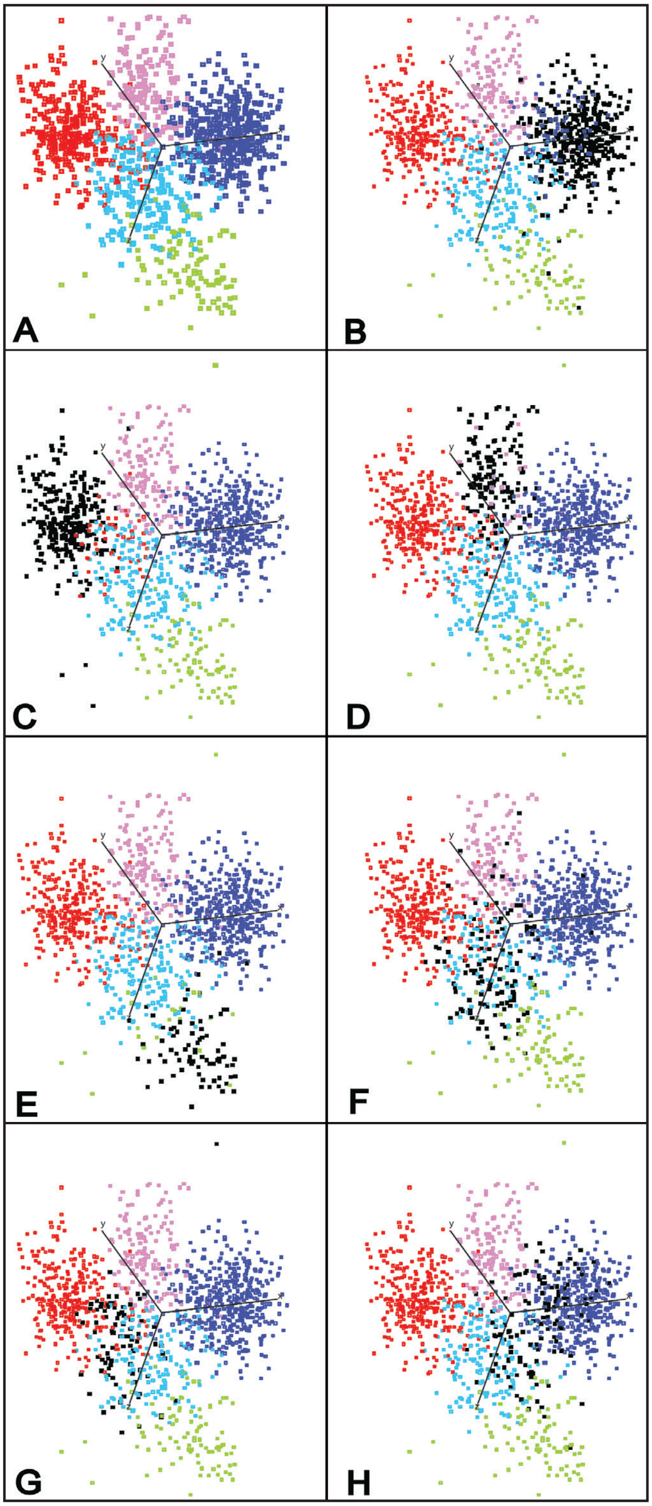


Fig. 4. Rotated 3D PCA scatter plots based on 27 morphological characters of 1240 samples of the *Taxus wallichiana* complex from 79 populations, coloured by five clusters in the *K*-means analysis **(A),** and highlighting (black) the seven different cpDNA lineages **(B–H): B,** *T. mairei*; **C,** *T. wallichiana*; **D,** the Hengduan type (*T. florinii*); **E,** the Tonkin type (*T. calcicola*); **F,** *T. chinensis*; **G,** the Emei type; **H,** the Qinling type. PCA phenotype cluster colours represent: red, *T. wallichiana*; pink, Hengduan type (*T. florinii*); dark blue, *T. mairei*; light blue, *T. chinensis*; green, Tonkin type (*T. calcicola*).

few samples of the Emei type spread into the *T. chinensis* morphological cluster and also clustered among *T. wallichiana* and Tonkin-type samples. The Qinling-type lineage spread mostly across the *T. mairei* cluster, but also covered some samples of the Hengduan and Tonkin types and of the *T. chinensis* cluster.

Phenotype mapping. — Plotting the geographic distribution of the sample identities using 5 *K-means* clustering onto a map showed strong geographic patterns (Fig. 5). These matched very tightly to the patterns of the seven cpDNA lineages (Fig. 1). Most populations were homogenous for their morphological assignation. Mixed populations occurred predominantly around the Sichuan basin. The distribution of the *T. mairei* and *T. wallichiana* lineages and Hengduan and Tonkin types were virtually identical with the cpDNA lineage patterns. Of the eight populations with a *T. chinensis* cpDNA lineage, only two populations, Maoxian (MX) and Shennongjia (SN), included only *T. chinensis* phenotypes (Fig. 5; Electr. Suppl.: Table S1). All other populations of *T. chinensis*, as well as the Emei and the Qinling types, were highly polymorphic, comprising various mixtures of phenotypes of all of the five morphological clusters.

Population coalescence. — Highlighting individual populations in the 1240 five *K*-means cluster PCA showed that populations had different clustering patterns. Some, like the *T. wallichiana* population SHL (Huili) clustered very tightly within the species cluster (Electr. Suppl.: Fig. S4A), while others, like the *T. mairei* population FRB (Yinjiang) or WX (Weixin) clustered widely, but within the species cluster boundary (Electr. Suppl.: Fig. S4B). However, some populations clustered across species boundaries, such as populations TB (Taibai), SP (Pingwu), KP (Weixi), or EM (Emei), BK (Baokang) (Electr. Suppl.: Fig. S4C–G). In others, only a few individuals (sometimes just one) fell in a neighbouring cluster, e.g., populations FJS (Jiangkou), GS (Gongshan) and HSH (Huangshan) (Electr. Suppl.: Fig. S4H–J, Table S1). This analysis allowed the selection of cluster-specific populations and individuals and indicates candidates for removal for dataset 3, the PCA on cluster representative samples and populations.

Dataset 2

Principal components analysis. — The axis loading in the PCA on 1283 samples was 27.91%, 14.59% and 6.69% respectively (cumulative 49.19%), and the scree test (Cattell, 1966) suggested three axes to be shown. The five clusters formed a very similar spatial constellation compared to the analysis on 1240 samples (Electr. Suppl.: Fig. S5).

Coherence of datasets and types. — The type and paratype specimens of *T. chinensis*, *T. mairei*, and *T. wallichiana* fell in their respective taxon clusters (Electr. Suppl.: Fig. S6B–D). Spjut's *T. mairei* var. *speciosa* fell in the *T. chinensis* cluster at the boundary with the Tonkin type cluster (Electr. Suppl.: Fig. S6E). Where more than one type specimen was included, none, except for the holotype and paratypes of *T. florinii* (see below), fell into one cluster entirely (Table 3). Most fell across

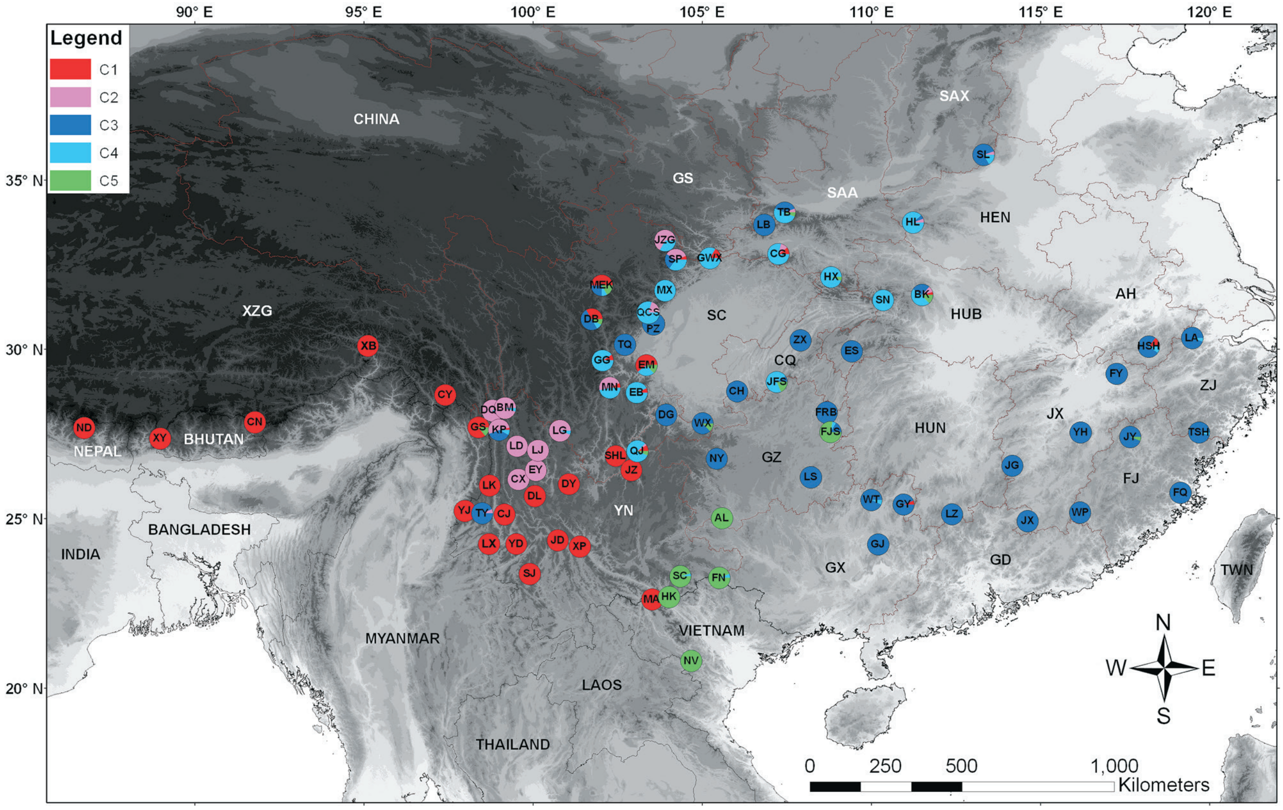


Fig. 5. Distribution of the 5 *K*-means phenotype clusters (C1–C5) after PCA based on 27 morphological characters across 79 populations of the *Taxus wallichiana* complex included in the present study from China and adjacent areas. Province and population codes as in Fig. 1.

Table 3. Allocation of type and paratype specimens assigned by Spjut to six *Taxus* species in China and adjacent areas in a PCA analysis of 27 morphological characters of 1283 samples and *K*-means clustering using 5 clusters, and their treatment by Farjon (2010).

		falling in cluster with						
Spjut type	no.	*T. wallichiana*	Tonkin	Hengduan	*T. chinensis*	*T. mairei*	fit	Farjon (2010)
		1	2	3	4	5		
T. florinii	1 (+10 p)	–	–	10	1	–	Hengduan type	*T. wallichiana*
T. kingstonii	1 (+ 6 p)	3	2	–	1	1	poor	*T. mairei*
T. mairei var. *speciosa*	1	–	–	–	1	–	*T. mairei*	*T. mairei*
T. obscura	1 (+ 7 p)	2	–	–	6	–	poor	*T. wallichiana*
T. phytonii	1 (+ 4 p)	2	–	–	2	1	poor	*T. wallichiana*
T. suffnessii	1 (+ 3 p)	1	2	1	–	–	poor	*T. wallichiana*
sum	6 (+30 p)	8	4	11	11	2		

p = paratypes

three or four species clusters, indicating their morphological incoherence (Electr. Suppl.: Fig. S6F–I). The holotype of *T. kingstonii* (Tk-2) fell into the *T. chinensis* cluster while two paratype specimens of that taxon (Tk-1, Tk-4) were assigned Tonkin-type identities in the *K*-means cluster analysis. However, these paratypes fell in the interphase with *T. mairei* and were not typical of the Tonkin type (Electr. Suppl.: Fig. S6F). The holotype (Tsf-1) and one paratype (Tsf-2) of *T. suffnessii* approached the Tonkin type morphologically, but fell outside of the *T. wallichiana* cluster in the third axis (Electr. Suppl.: Fig. S6I). Together with three high-altitude samples from population GS (GS13, GS14, GS15) they formed a discrete cluster (Electr. Suppl.: Fig. S7, circle). The holotype of *T. florinii*, as well as nine of the ten paratypes cited in its protologue, fell in the Hengduan-type morphological cluster; the remaining paratype (Tfl-1) fell into *T. chinensis* (Table 3).

Dataset 3

Principal components analysis. — The PCA of the 958 typical specimens showed higher axis loading than the previous analyses with 30.84%, 15.18% and 7.35% (cumulative 53.37%). The scree test (Cattell, 1966) suggested three axes to be displayed. Removing the mixed lineages and mixed cluster samples resulted in a discrete spatial distribution of the five clusters with little overlap (Fig. 6A; Electr. Suppl.: Fig. S8A, C). The spatial arrangement of the clusters and their separation over the three dimensions was similar to the analyses on 1240 and 1283 samples (Fig. 4; Electr. Suppl.: Fig. S5).

"Typical" specimen descriptors. — The descriptor loadings for the 1st and 2nd (Electr. Suppl.: Fig. S8B) and 1st and 3rd axes (Electr. Suppl.: Fig. S8D) indicated several variables of importance to separate the clusters. In the first axis, 13

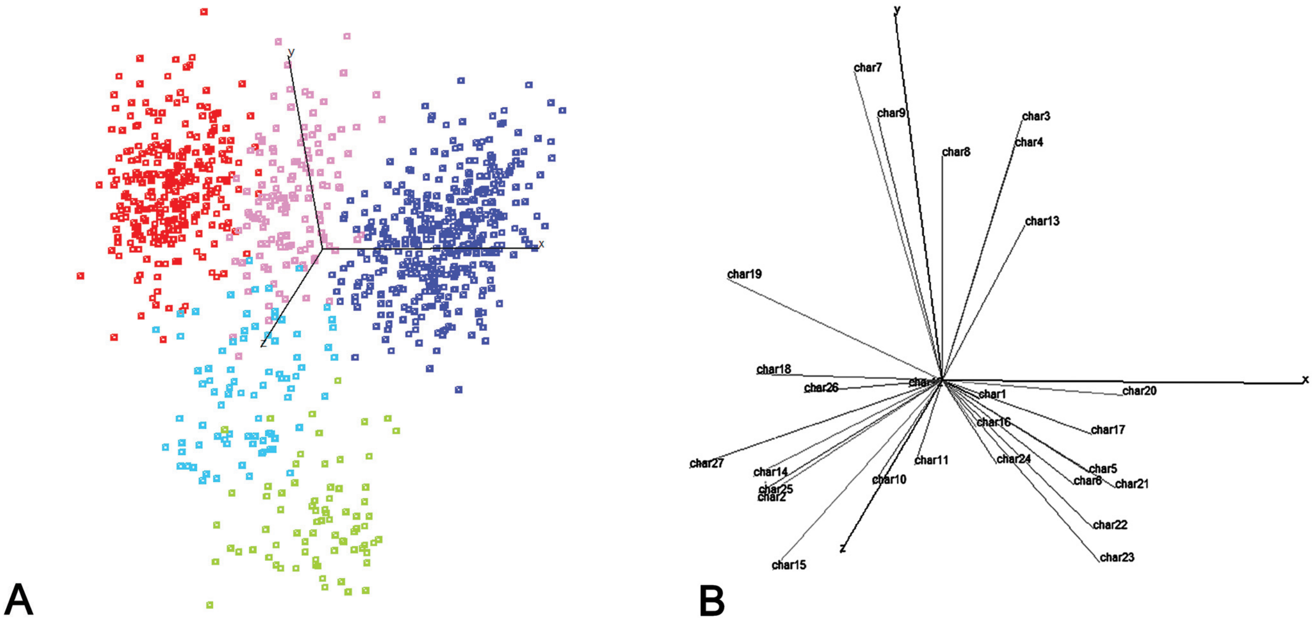


Fig. 6. Rotated 3D PCA scatter plot based on 27 morphological characters of 958 "typical specimens" of the *Taxus wallichiana* complex from 69 populations, coloured by five clusters in the *K*-means analysis **(A),** and corresponding descriptor plot **(B).** Colour code as in Fig. 4.

characters (char21, midrib shininess; char19, midrib papillation; char23, margin shininess; char5, narrowest width; char6, widest width; char17, midrib elevation; char14, mucro; char22, margin colour; char27, bud scale persistence; char2, leaf density; char20, midrib colour; char15, leaf thickness; and char13, apex shape) were responsible for separating *T. mairei* from the rest; these are predominantly characters concerning the midrib papillation, leaf colour and shininess and leaf width. The 2nd axis separated *T. chinensis* and the Tonkin type from *T. wallichiana*, with the six most important characters (char9, margin taper; char7, leaf length/width ratio; char8, leaf curvature; char3, shortest length; char4, longest length; and char13, apex shape) mainly based on leaf length, leaf length/width ratio and leaf curvature and margin characteristics. The Hengduan type was best separated from all other clusters in the third axis, strongest with char25 (number of stomatal lines) (Electr. Suppl.: Fig. S8D, Table S5).

The summary of the results for the continuous characters shows that despite relatively high rankings in the PCA, in most cases greatly overlapping ranges existed between the clusters, except for char7 (leaf length/width ratio) and char25 (number of stomatal lines) (Electr. Suppl.: Table S6). Among the discrete characters, some ranked consistently low, such as char10 (base symmetry), char11 (leaf arrangement) and char12 (apex symmetry). The reason was found in their invariability across the samples and species included. Others, such as char16 (leaf edges), char24 (margin width), or char26 (stomata density), were heterogeneous within the five clusters and greatly overlapping with other clusters (Electr. Suppl.: Table S7).

Morphological differentiation of the five clusters. — Focussing on the main characters separating the clusters in the first three axes, char21 (midrib shininess), char19 (midrib papillation), and char23 (margin shininess) in axis 1, char9 (margin taper), char7 (leaf length/width ratio) and char8 (leaf curvature) in the second and char25 (number of stomatal lines) in the third and char13 (apex shape), fourth in the overall ranking of the characters (Electr. Suppl.: Table S5), are sufficient to morphologically differentiate the five clusters. The clusters can be differentiated against each other using different characters (Table 4). For example, char21 (midrib shininess), char19 (midrib papillation) and char23 (margin shininess) all very effectively separated *T. mairei* and the Tonkin type from the remaining three clusters. The Tonkin type can be effectively separated from *T. mairei* by char9 (margin taper) and char8 (leaf curvature), though there is some overlap (4.8%) in character state 1 between the two clusters. They also differ in char13 (apex shape), with virtually no overlap. *Taxus chinensis* can be distinguished from *T. wallichiana* and the Hengduan type by char7 (leaf length/width ratio) which is much lower in the former (6.72) compared to the latter two (9.37 and 9.26 respectively). Finally, *T. wallichiana* differs from the Hengduan type in char25 (number of stomatal lines) that shows no overlap (*T. wallichiana* = 12–18, Hengduan type = 8–11). Another distinguishing feature between the two clusters concerns the apex shape (char13), though with some overlap (5.2%) in state 2 (Table 4).

■ DISCUSSION

In our previous study of *Taxus* in China and adjacent areas using solely morphological data (Möller & al., 2007), we found a strong geographic structure among the morphotypes (i.e., species) identified in the PCA, which were strongly confirmed by molecular data (Gao & al., 2007; Liu & al., 2011). We also identified additional morphological (the Hengduan type) and molecular entities (the Tonkin type, the Qinling type) in previous studies (Gao & al., 2007; Möller & al., 2007; Liu & al., 2011, 2013). Here, we expanded on the sampling, since only 128 individual herbarium specimens were included in the morphological study (Möller & al., 2007), and combined morphological and molecular data to delineate an existing species anew, *T. florinii* (the Hengduan type), and describe a new species, *T. calcicola* (the Tonkin type). The extended sampling (from 50 to 79 populations) and geographic genotype mapping here did not change the distribution of the chloroplast haplotype lineages compared to previous studies (Gao & al., 2007), but allowed a more precise depiction of the distribution of the individual genotype lineages (Fig. 1).

Correlation of morphological characteristics. — We found interesting correlations between morphological characteristics that could be traced to age-related and species-related aspects. For example, DBH and height were closely correlated but the scatter plot showed a wide cone-shaped distribution of data points, indicating that plants with similar heights showed a wide range of stem diameters. This indicates that *Taxus* plants reach their maximum height early and later mainly the stem diameter increases (Fig. 2), a phenomenon also observed for other understory trees (e.g., *Nyssa* L., Wang & al., 2012). The different phenotype groups did not show a tendency for a specific height/DBH limit and appeared mixed together, suggesting that there is no difference in plant size between the *Taxus* species here. Furthermore, there was no statistical link between plant height or DBH with altitude (Table 1), indicating that (1) there is no preference or ecological or species effect of altitude on plant size, and (2) our samples came from a similar mixed size pool for each group.

The absence of strong correlations between the continuous characters and plant size suggests that there are no juvenile forms of *Taxus*, as in other conifers such as *Juniperus* L., or in *Eucalyptus* L'Hér. On the other hand, the strong correlations of some characters with altitude does not necessarily mean that the latter has an effect on plant form, but is more likely a reflection of species-specific characteristics (and character stability), since the groups or lineages (or species) here have distinct but often overlapping altitudinal preferences (Fig. 7). Whether altitude per se has an effect on the leaf characters of *Taxus* can only be addressed in transplanting experiments.

Discrepancies in character states between studies. — When comparing the character states per species in our previous analysis (Möller & al., 2007) with the current work, some differences become apparent, for example for char11, leaf arrangement. In Möller & al. (2007) it was defined based on the insertion of the leaf on the stem, which is not always easily determined, while here we used an easier to score leaf

Table 4. Differentiation matrix summarizing the differences of important characters determined in a PCA on 27 morphological characters (Electr. Suppl.: Tables S6, S7) in pairwise species comparisons of 958 typical specimens of the *Taxus wallichiana* complex. The first column (shaded grey) gives the character states for each 'taxon'. The remaining columns give the differences in the proportion of character states in pairwise comparisons. Comparisons with highly diverging proportion of states, or little (l.olap) or no overlap (no olap) are in bold.

Taxon		*T. wallichiana*	Hengduan (*T. florinii*)	*T. mairei*	Tonkin (*T. calcicola*)	*T. chinensis*
T. wallichiana	char21	0(100%)	86.7%(0)	**0.5%(0)**	12.2%(0)	100%(0)
	char19	2(100%)	100%(2)	**0.5%(2)**	**no olap**	93.8%(2)
	char23	0(100%)	96.3%(0)	**no olap**	**no olap**	50.6%(0)
	char9	3(87%)	49.6%(3)	19.8%(3)	**1.4%(2&3)**	**9.9%(3)**
	char7	9.37	−0.11	−1.62	**−3.55**	**−2.65**
	char8	3(68.1%)	45.2%(3)	68.1%(3)	**2.7%(2)**	**3.7%(2)**
	char25	12–18	**8–11 no olap**	10–17 olap	10–16 olap	9–15 olap
	char13	2(92.6%)	**5.2%(2)**	**4.1%(3)**	**2.7%(1)**	**4.1%(3)**
Hengduan type (*T. florinii*)		char21	0(86.7%)	13.3.%(1)	13.3%(1)	86.7%(0)
		char19	2(100%)	**0.5%(2)**	**no olap**	93.8%(2)
		char23	0(96.3%)	**3.7%(1)**	**3.7%(1)**	50.6%(0)
		char9	3(49.6%)	19.8(3)	**8.1%(1)**	34.1%(1)
		char7	9.26	−1.51	**−3.44**	**−2.54**
		char8	3(45.2%)	45.2%(3)	16.2%(1)	33.3%(1)
		char25	8–11	**10–17 l.olap**	**10–16 l.olap**	**9–15 l.olap**
		char13	1(91.9%)	**3%(3)**	**2.7(1)**	18.5%(1)
T. mairei			char21	1(99.5%)	87.8%(1)	**0.5%(0)**
			char19	0(60.3%)	60.3%(0)	**4.9%(1)**
			char23	1(100%)	100%(1)	49.4%(1)
			char9	2(78.1%)	**1.8%(1)**	**9.9%(3)**
			char7	7.75	−1.93	−1.03
			char8	3(81.7%)	**4.8%(1)**	**4.8%(1)**
			char25	10–17	10–16 olap	9–15 olap
			char13	3(97%)	**0.3%(0)**	12.3%(3)
Tonkin type (*T. calcicola*)				char21	1(87.8)	12.2%(0)
				char19	0(77%)	**4.9%(1)**
				char23	1(100%)	49.4%(1)
				char9	0(89.2%)	34.6%(0)
				char7	5.82	+0.9
				char8	0(81.1%)	54.3%(0)
				char25	10–16	9–15 olap
				char13	0(97.3%)	65.4%(0)
T. chinensis					char21	0(100%)
					char19	2(93.8%)
					char23	0/1(50.6/49.4%)
					char9	1/0(53.1/34.6%)
					char7	6.72
					char8	0/1(54.3/39.5%)
					char25	9–15
					char13	0(65.4%)

arrangement on the branch. This caused the character state to differ between Möller & al. (2007) and here. Other character state differences (e.g., char8, leaf curvature; char9, margin taper or char16, leaf edges) were due to the fewer individuals included in Möller & al. (2007) (e.g., for the Hengduan type three individuals in the earlier paper, here 135; *T. wallichiana* 50, here 270), and the fact that the species characterisation here was based on "typical" samples, excluding samples with mixed character states.

Congruence of clustering between studies. — Despite the differences in the characteristics between Möller & al. (2007) and here, the clustering results were highly congruent as demonstrated by the correct positioning of type specimens in their respective clusters (Electr. Suppl.: Fig. S6B–D). The same was true for the geographic mapping of the clusters, though the increased sampling here allowed a much finer definition of the distribution of the clusters.

Congruence between morphological groups, molecular lineages and geographic distribution. — It was complex to integrate the morphological (phenotypic) and molecular (genotypic) data without prejudgment of the identity of the samples. We allowed the *K*-means clustering to determine the most appropriate number of clusters, by calculating the highest sum of linkage percentages between phenotype and genotype (Fig. 3). Excluding two, five clusters were found to represent most optimally the distribution of the seven genotype lineages, representing the species *T. wallichiana*, *T. mairei*, *T. chinensis* and the Hengduan and Tonkin types. The correlations between the genotype and phenotype lineages of these five groups was very high for most (>90%), except for *T. chinensis* (68.9%) (Table 2B). The Emei and Qinling type lineages could not be morphologically characterised and consisted predominantly of *T. chinensis* (69.1%) and *T. wallichiana* (27.4%), or *T. chinensis* (31.7%) and *T. mairei* (49.6%) phenotypes respectively.

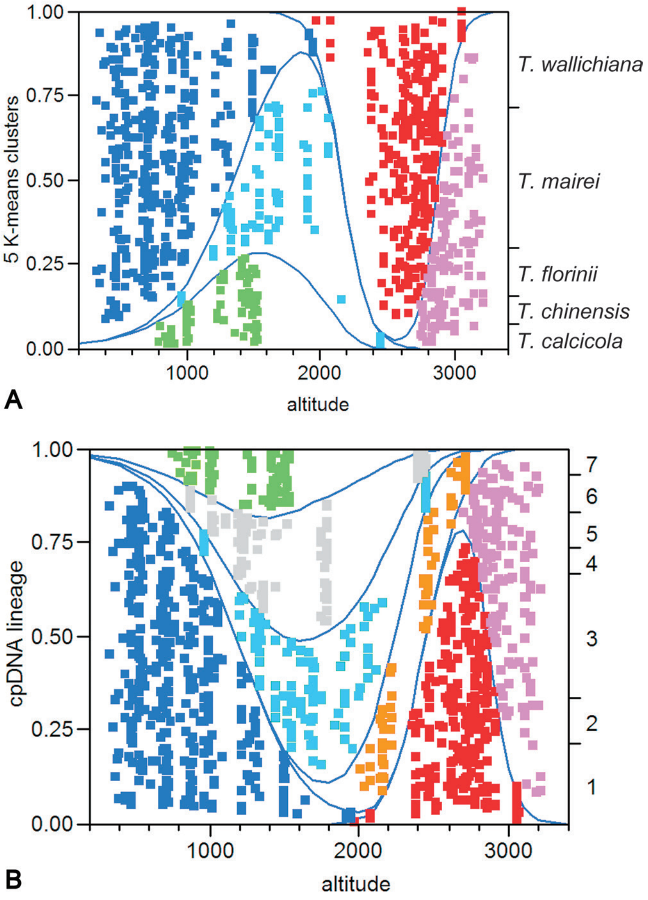


Fig. 7. Relationships (logistic fits) between altitude [m] and **A,** 5 *K*-means clusters of 958 typical samples of 69 populations after PCA on 27 morphological characters (colour code as in Fig. 5), and **B,** seven cpDNA lineages of 1240 samples of 79 populations (colour code as in Fig. 1) of the *Taxus wallichiana* complex.

This morphological heterogeneity was reflected in the geographic distribution of the phenotypes, that clearly showed the mixed populations of the Emei and Qinling type phenotypes (Fig. 5), while those for the three species showed very strongly defined distribution ranges, with only few, more mixed populations (e.g., GS for *T. wallichiana*, HSH for *T. mairei*, CG, JFS, JZG, SP for *T. chinensis*) (Fig. 5; Electr. Suppl.: Table S1). The two newly defined lineages of the Hengduan and Tonkin types (Gao & al., 2007; Möller & al., 2007; Liu & al., 2011) occupied discrete geographic ranges in the Hengduan Mountains region and Tonkin Bay respectively, with almost no overlap with the other three species except for the occasional mixed population (KP for the Hengduan type, and FJS for the Tonkin type).

Identity of "atypical" specimens and mixed populations. — The majority of "atypical" specimens occurred in mixed phenotype populations in the Emei and Qinling lineages. These are restricted to areas around the Sichuan basin. Preliminary molecular evidence indicates that these two lineages are the result of past hybridisation events (Zhang 2010; Gao & al. in prep.) between *T. chinensis*, *T. mairei* and *T. wallichiana*.

The remainder of "atypical" specimens concern a few isolated mixed populations and mostly represent either ecological altitudinal zonation (e.g., Ohsawa & al., 1985) or recent hybridisation events. For instance, the plants of the different lineages at the KP locality occurred at two different altitudinal ranges, nine plants between 1739 m and 1761 m and 18 plants between 2685 m and 2850 m respectively. The second group conformed in altitude, genotype and phenotype predominantly to the Hengduan type, while the nine at lower altitude belonged genotypically to the morphologically variable Qinling type. Other such cases of ecological niche-partitioning are apparent for the EM population, with the *T. chinensis* lineage occupying altitude ranges of 1700–1740 m while the *T. wallichiana* lineage grew at much higher altitudes of 2110–2200 m. Also at the HSH locality, plants with the *T. chinensis* haplotype lineage occur at high altitudes of the mountain at 1362–1632 m, while those growing at 380–472 m possessed the *T. mairei* haplotypes. In these cases the genotype differences are mostly matched by morphological differences (e.g., Electr. Suppl.: Fig. S4E, F) and thus represent cases of allopatric species separation by ecological adaptation as observed for other *Taxus* species (Poudel & al., 2012) or *Rhododendron* sect. *Vireya* H.F. Copel. (Argent, 2008).

At the FJS locality, lineages of *T. mairei* (600–736 m) and the Tonkin type (810–850 m) analysed here were collected at slightly different altitudes, but intervening populations may exist, and the species occur in sympatry. Here, the parallel morphological and molecular discontinuities break down and plants with Tonkin type morphology and *T. mairei* genotype exist, and vice versa. This suggests that secondary contact, hybridization and introgression likely occurred here. A similar hybridisation scenario is present at population locality BK, where one *T. chinensis* haplotype with *T. chinensis* phenotype (and Qinling type ITS sequence, Zhang 2010) is found among 22 plants of the Qinling type lineage. The above cases suggest that, while the boundaries between the lineages are very clear, in some cases ecological differences separate species locally, and rare secondary contacts, leading to introgression in *Taxus*, occur.

Taxonomic considerations. — There are two recent, but widely disparate, publications on the taxonomy of *Taxus*. Spjut (2007) has a very narrow species concept, based on a subjective evaluation of two or more correlated characters with the primary objective "to classify specimens in the fewest number of species and varieties that can be reasonably distinguished from one another". With this in mind he proposed the existence of 21 *Taxus* species (29 taxa) in Asia, 13 (15 taxa) of them occurring in China, in addition to the three species recognised by Farjon (2010), *T. chinensis*, *T. mairei* and *T. wallichiana*.

Spjut's treatment of *Taxus* species in China is in stark contrast to our previous findings (Möller & al., 2007; Gao & al., 2007; Liu & al., 2011) and our findings here, which differed slightly from that of Farjon (2010). We can recognise *T. chinensis*, *T. mairei*, *T. wallichiana*, *T. florinii* (the Hengduan type), and *T. calcicola* (the Tonkin type). The first three are also recognised by both Spjut (2007) and Farjon (2010). However, Farjon (2010), with a broader species concept, included *T. florinii* in *T. wallichiana* while his delineation of *T. chinensis* covered plants of the Tonkin type here described as *T. calcicola*. He also included *T. kingstonii* in *T. mairei*, and *T. obscura*, *T. phytonii* and *T. suffnessii* in *T. wallichiana*.

Taxus florinii is the only species described by Spjut (2007) whose type and paratype specimens fell in our analyses consistently in one morphometric cluster, i.e., the Hengduan type, indicating that it does form a coherent species. For this species, Spjut (2007) gave the distribution Xinjiang Uygur, Sichuan, Yunnan in China. The locality information for the specimen from "Xinjiang Uygur ('SoC.W. Sikiang'): 'NW Yunnan,' Tamichung, tree 20–30 ft, in fr., open pasture, *R.C. Ching 21505*" must be treated with caution since Tamichung lies near Lijiang in northwest Yunnan. It is clear that the localization of Tamichung in Xinjiang Uygur by Spjut (2007) was a mistake and that *T. florinii* is restricted to southwest Sichuan and northwest Yunnan. Plants found in intervening areas between Xinjiang Uygur and the Hengduan Mountains, in southeast Xizang, were here identified as *T. wallichiana* (Fig. 5). Excluding the incorrect locality for *T. florinii* in Xinjiang Uygur, the distributions of this species overlaps greatly with the distribution of the Hengduan type (Gao & al., 2007; Möller & al., 2007), and they must be regarded as the same (Figs. 1, 5).

The Tonkin type is morphologically and genetically a distinct entity occurring in the north of Vietnam, southeast Yunnan, southwest Guizhou with a relatively isolated population in east Guizhou (FJS) (Figs. 1, 5). In Möller & al. (2007) it came under *T. chinensis*, though only a few herbarium specimen individuals had been included. Spjut (2007) and Farjon (2010) also regarded material from this area as belonging to *T. chinensis*. In Gao & al. (2007) and Liu & al. (2011) the distinctness of samples from the Tonkin Bay area from *T. chinensis* samples from around the Sichuan Basin was clearly demonstrated. With the enlarged sampling here the Tonkin type could also be morphologically separated from *T. chinensis* samples (see below). The holotype of *T. suffnessii* of Spjut (2007) fell on the edge of the Tonkin type cluster in our morphological analysis, and did not conform to a typical *T. calcicola*. This holotype came from non-limestone habitats above Ahkail in the North Triangle of Myanmar, and is thus geographically disjunct from the limestone habitats of *T. calcicola* in southeast Yunnan/Guizhou. There is also a strong altitudinal difference, the holotype of *T. suffnessii* having been collected at 9000–10000 ft (2740–3050 m), whereas the Tonkin type occurs at 810–1525 m. The three high-altitude specimens (GS13, GS14, GS15 from 2700–2800 m) of the *T. wallichiana* population GS from Gongshan, which is near the type locality of *T. suffnessii*, differed morphologically from typical *T. wallichiana* specimens occurring at lower altitudes (1960–2650 m), by their short, straight and thick textured leaves, and clustered separately from the more typical *T. wallichiana* GS specimens. These high-altitude GS individuals clustered closely to the holotype and paratypes of *T. suffnessii* in the morphological PCA analyses (Electr. Suppl.: Fig. S7). Furthermore, the GS population belonged to a very different haplotype lineage compared to the Tonkin type, and molecular sequence data showed that the high-altitude GS specimens differ consistently by four mutational steps from the lower-altitude GS specimens (Gao & al., 2007). Thus, the *T. suffnessii* samples from Myanmar and the high-altitude GS specimens may belong to the same taxonomic unit. Further molecular data is necessary to ascertain whether this hypothesis is correct. However, the morphological, geographical, geological and ecological (altitude) discontinuities between the *T. suffnessii* holotype (and paratypes) and the Tonkin type specimens are strong evidence for their independent taxonomic identities. We therefore describe a new species here, *Taxus calcicola* L.M. Gao & Mich. Möller, in reference to its exclusive occurrence on limestone karst formations.

Since the holotype and paratype specimens of four of Spjut's additional taxa in China, *T. kingstonii*, *T. obscura*, *T. phytonii* and *T. suffnessii*, did not form coherent morphometric clusters (Electr.: Suppl.: Fig. S6F–I), it can be concluded that (1) they do not form morphologically coherent groups, and (2) they do not represent morphologically unique entities since they did not fall outside the clusters here. Additionally, the geographic distributions of Spjut's recognised species often have a disjunct pattern (*T. kingstonii*: India, Myanmar, China [including Taiwan]; *T. obscura*: Myanmar, China [including Taiwan], Philippines [Luzon], Indonesia; *T. phytonii*: Nepal, northeast India, Thailand, China [including Taiwan], Philippines [Luzon]). *Taxus obscura* and *T. phytonii* have conspicuous parallel disjunct occurrences from the eastern Himalayas across China and

Taiwan into SE Asia (Spjut, 2007). These disjunctions appear to trace the distributions of individual character states rather than biological species (see below). Farjon's (2010) broader species concept inevitably results in a more continuous distribution of species, and is perhaps more guided by geography, since at least based on Spjut's included types here, Farjon's species form morphologically rather heterogeneous groups (Table 3).

Morphologically, Spjut's (2007) types can readily be assigned to *T. chinensis*, *T. mairei*, *T. wallichiana*, *T. florinii* and *T. calcicola*, at least those from China and adjacent areas (Table 3; Electr. Suppl.: Fig. S6F–I; Table S2). Too little material is at present available from the Philippines and Indonesia to make far-reaching taxonomic decisions. Indian material of *Taxus* from the Khasia hills has been morphologically and molecularly analysed by Poudel & al. (2012) and found to belong to *T. mairei*. The specimen of *T. kingstonii* included here from the same area in India has also been identified as belonging to *T. mairei*. *Taxus mairei* has also been found in southeast Nepal and south Vietnam (Poudel & al., 2012). Together with the at first sight isolated population in Tengchong (TY), this extends the range of *T. mairei* considerably to the West, from southeast Nepal to east China, and southward to south Vietnam. This differs significantly from the range of this species defined by both Spjut (2007) and Farjon (2010), although for a complete geographic definition more population material needs to be studied.

The single sample of *T. mairei* var. *speciosa* fell morphologically on the boundaries between *T. chinensis*, *T. calcicola* and *T. mairei* in our analysis. It is recognized in Spjut (2007) by "branchlets arising anisodichotomously, and by leaves appearing greenish and unequally spaced along branchlets in dried specimens". These characters have not been used by us, because none of them is strongly discrete. The distribution of *T. mairei* by province is virtually identical with that of *T. mairei* var. *speciosa* (in 10 out of 13 provinces) and it is more likely that the characteristics used to distinguish the two varieties occur as mixed states across the distribution range of *T. mairei*. Thus, there is no geographic reason for recognising *T. mairei* var. *speciosa*.

Taxus wallichiana var. *yunnanensis* is given a distribution in India (Sikkim, Nagaland), Myanmar and China (Xizang, Yunnan, Sichuan) by Spjut (2007), and overlaps greatly with *T. wallichiana* and *T. florinii* in China. We found no genetic or morphometric evidence for the existence of a further taxonomic entity in our analyses in this area apart from *T. wallichiana* and *T. florinii*. In addition, the holotype specimen of this variety was collected from Chayu, southeast Xizang. The cpDNA haplotype of the population from the same locality belonged to *T. wallichiana* (Fig. 1). Since some characters used by Spjut (2007), such as epidermal cell shape, have not been used in the present study, it is difficult to ascertain what taxonomic value (and geographic relevance) these characters would exhibit when added to our data matrix.

Morphological characteristics of species in the *Taxus wallichiana* complex. — With the rejection of Spjut's species, except *T. florinii*, and the recognition of *T. calcicola*, five species can be morphologically and genetically defined in China with minimal geographical overlap, namely *T. calcicola*, *T. chinensis*, *T. florinii*, *T. mairei*, and *T. wallichiana*. The two remaining genotype lineages with mixed morphological populations, the Emei type and Qinling type, may be the result of past hybridisation and chloroplast capture events (Zhang, 2010). More detailed studies are required to ascertain their status. To describe the five morphologically recognisable species, we only included morphologically typical specimens and generated their morphometric profile (Electr. Suppl.: Tables S6, S7). This allowed us to generate a differentiation matrix between the species (Table 4). This matrix allowed the selection of the most suitable characters to differentiate any species pair.

Using morphological and molecular data and correlating these with geographic distributions allowed us to address, without prejudice on species identities, the number of taxonomically definable units in the genus *Taxus* and their distribution in China and adjacent areas. This enabled us to correct previous taxonomic treatments based on subjective species concepts. Unlike Spjut (2007) who used few selected characters that resulted in over-splitting and disjunct distributions that are difficult to reconcile with biological explanations, our multivariate morphological/molecular approach resulted in much fewer but morphologically and molecularly definable units with mostly clear geographic delineations. A few cases of recent migrants and present (and past) hybridisation events may blur the boundaries, but do not distort the final taxonomic consequences drawn here. Five taxa, one, *T. florinii* (the "Hengduan Mountain type"), split off from *T. wallichiana* var. *wallichiana* by Spjut (2007), three previously accepted varieties raised to species level, *T. chinensis*, *T. mairei* and *T. wallichiana* (Farjon, 2010), and a newly described one here, *T. calcicola* (the Tonkin type), exist in China and adjacent areas.

Here we made great progress in reconciling results from data from different sources and different analytical methods, to address the taxonomically difficult genus *Taxus* primarily in China and surrounding areas. *Taxus* occurs as far south as Sulawesi and Sumatra in Indonesia and the Philippines. In these areas, *Taxus* has not been studied sufficiently at the population level. Such studies are necessary to understand the genus there. Individual sporadically collected samples from these areas will not be sufficient to draw meaningful conclusions, and *Taxus* in Indonesia and the Philippines should be the focus of future research at the population level.

■ SPECIES DESCRIPTION

Taxus calcicola L.M. Gao & Mich. Möller, **sp. nov.** – Holotype: CHINA. Yunnan, Hekou County, Nanxi town, Longyincong, 1000 m, 22°41′N 104°01′E, 20 Sep. 2006, *L.M. Gao & M. Moeller GLM-06239* (KUN; isotype: E).

Affinis *T. chinensi* Pilger a qua costa papillas nullas vel sparsas habenti (character 19, costae papillatio) et costa haud nitenti (character 21, costae nitor) differt.

For drawing of holotype see Fig. 8.

Description. – Trees to 10 m tall. Trunk of trees monopodial, to 20 cm DBH. Branchlets green, dried green or pale green or pale brown. Winter buds ovoid; scales at base of shoots keeled abaxially, bud scales persistent. Foliage branchlets irregularly alternate, slender, terete, with fine grooves alongside decurrent leaf bases, green turning yellowish green to ochre or bronze-coloured. Leaves spirally inserted, base twisted to become distichously arranged, spreading at nearly right angles, dense arrangement on leaf branchlets, linear, (0.9–)1.4–2.3(–3.1) cm long, slender, straight, upper parts almost as broad as the lower parts, twisted at the short petiolate or nearly sessile base, straight or curved near the base, (1.9–)2.7–3.5(–4.5) mm wide, thick and coriaceous, margin ± revolute, apex abruptly pointed, ending in a mucronate apex. Midrib on the adaxial (upper) side raised in fresh leaves, 0.2 mm wide, continuous to the apex, on the abaxial (lower) side elevated, without papillae or sparsely papillate, 0.4–0.6 mm wide and continuous to the apex, equal or thinner than the margin with 0.5–0.7 mm wide, leaf colour lustrous dark green above, yellowish green with two pale yellowish green bands below. Stomata in two bands on the abaxial side, 10–16 lines per band, randomly and densely dispersed, 0.7–1.2 mm wide. Pollen cones axillary, solitary, forming rows on either side along fertile shoots, ovoid, yellowish green to pale brown, increasingly larger bracts at base. Seed-bearing structures axillary, solitary, sessile, with minute triangular scales covering a very small dwarf shoot and the single terminal ovule except the micropyle. Seeds ovoid, ca. 5.2–6.5 × 4.0–5.0 mm, somewhat compressed, with slightly obtuse lateral ridges in upper parts, apex obtuse and with a mucro, dark brown, enclosed in a cup-shaped, red succulent

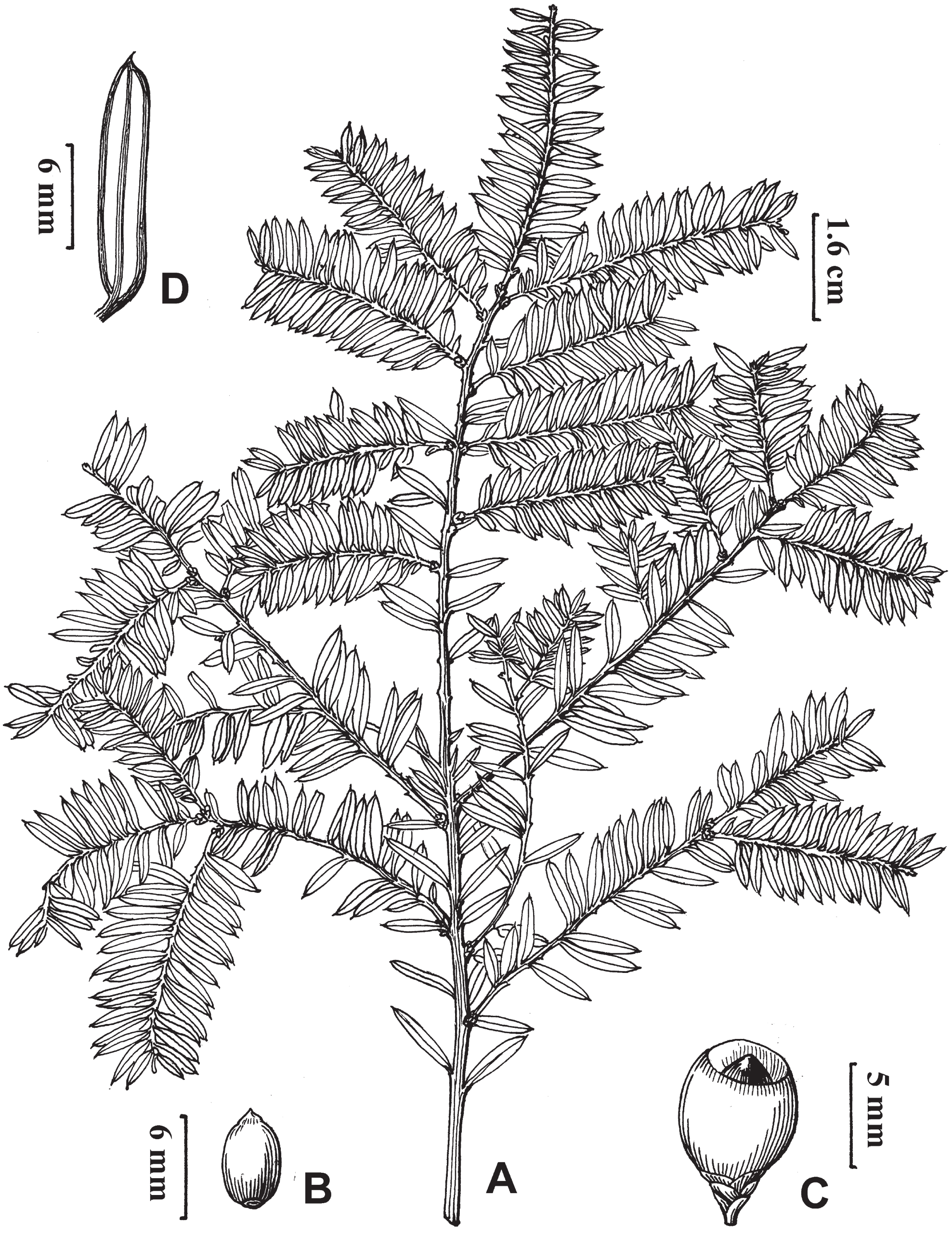


Fig. 8. *Taxus calcicola*. **A,** branch with leaves and bud scales; **B,** seed; **C,** fleshy aril and seed; **D,** leaf, showing midrib and margin on the abaxial surface. — From holotype specimen *L.M. Gao & M. Moeller GLM-06239*, drawn by Ling Wang, KUN.

aril, with the apical mucro subequalling or slightly protruding from the aril; hilum ellipsoid.

Specimens studied. – CHINA. Yunnan prov.: Hekou county, Nanxi town, Longyincong, evergreen mixed angiosperm/conifer forest, 990–1020 m, 20 Sep. 2006, *L.M. Gao & M. Moeller GLM-06224–06238 & 06240–06244* (KUN); Funing county, Tianpeng town, Heping village, mixed angiosperm/conifer forest on limestone hill, 1393–1420 m, 18 Sep. 2006, *L.M. Gao & M. Moeller GLM-06141–06168* (KUN); Xichou county, Fadou town, Gagou village, mixed angiosperm/conifer forest on limestone hill, 1465–1525 m, 9 Oct. 2003, *L.M. Gao GLM-2363–2383* (KUN); Xichou county, Fadou town, Yanming Shan, mixed angiosperm/conifer forest on limestone hill, 1440–1460 m, 8 Oct. 2003, *L.M. Gao GLM-2357–2358* (KUN); Dongma town, Daping village, mixed angiosperm/conifer forest on limestone hill, 1240 m, 22 Oct. 2012, *L.M. Gao & M. Moeller GLM-123950–123951* (KUN). — Guizhou prov.: Anlong county, Xinglong town, Paijian village, mixed angiosperm/conifer forest on limestone hill, 1350–1450 m, 20 Jul. 2006, *L.M. Gao & J. Wu GLM-06001–06003* (KUN); Jiangkou county, Taiping town, mixed angiosperm/conifer forest, 810–900 m, 16 Sep. 2003, *L.M. Gao & M. Moeller*, *KIBE 03-319, 03-320, 03-324, 03-325,03-328–03-338, 03-343, 03-344 & 03-350* (KUN, E). — VIETNAM. Son La, Môc Châu, from Tân Lập to Mường Lum, in evergreen forest on limestone hill, 1224–1246 m, 27 Feb. 2004, *NVSDE 82, 87 & 88* (E), 1251–1277 m, 28 Feb. 2004, *NVSDE 94, 95 & 98–101* (E); Hoa Binh, Mai Chau, Pa Co, in mixed forest, 1000 m, 20 Oct. 2002, *NVSDE 12* (E); Ha Giang, Yen Minh, Lao Ba Chai Commune, in mixed forest, 1500 m, 13 Nov. 2002, *NVSDE 167* (E).

Distribution. – China: southeast Yunnan, northeast and southwest Guizhou, and north Vietnam (Laocai, Son La, Hoa Binh and Ha Giang).

Habitat. – On karst limestone hills in mixed angiosperm/conifer forests or evergreen forest; alt. 810–1525 m.

Associate taxa. – *Taxus calcicola* grows alongside angiosperm tree taxa such as *Clausena excavata* Burm. f., *Cleidion bracteosum* Gagnep., *Eurya groffii* Merr., *Litsea cubeba* (Lour.) Pers., *Pavieasia anamensis* Pierre, *Sinosideroxylon pedunculatum* (Hemsl.) H. Chuang, *Xantolis boniana* (Dubard) P. Royen, *Yunnanopilia longistaminea* (W.Z. Li) C.Y. Wu & D.Z. Li,, and the conifers *Amentotaxus yunnanensis* H.L. Li, *Cephalotaxus mannii* Hook. f., *Podocarpus neriifolius* D. Don, and *Podocarpus wangii* C.C. Chang.

Etymology. – The species epithet, *calcicola*, refers to the preference of the species to grow on limestone hills. Farjon (2010) gives limestone karst for *T. chinensis* and *T. mairei*, though his distribution ranges (and his species circumscriptions) for these species include *T. calcicola*.

Proposed IUCN conservation category. – Endangered (EN A2acd; C1). The entire extent of occurrence (EOO) of this species is around 35,000 km² but the plants grow only in small and isolated patches of forests in very low densities, with a total number of individuals of less than 2000. The known localities are all outside of protected areas and are subject to degradation from surrounding agriculture and forest clearance, thereby changing the microclimate of the limestone karst habitat.

Key to the species of *Taxus* in China and adjacent areas

1 Midrib without papillae or with few papillae and midrib and leaf margin shiny on abaxial leaf surface 2
1 Midrib with dense papillae and midrib and leaf margin not shiny on abaxial leaf surface 3
2 Leaves sigmoid, leaf margins parallel to 2/3–3/4 of the leaf from the base, apex acuminate, with relatively high leaf length/width ratio (5.21–11.55, mean 7.75) ...... ***T. mairei***
2 Leaves mostly straight, leaf margins parallel, apex acute, with low leaf length/width ratio (4.11–8.12, mean 5.82) .... ***T. calcicola*** sp. nov.
3 Leaves mostly sigmoid, sometimes straight (*T. florinii*), leaf margins tapered from the base or near middle of the leaf, leaf length/width ratio relatively high (mean >9) 4
3 Leaves mostly straight, sometime falcate, leaf margins parallel, with relatively low leaf length/width ratio (5.37–8.88, mean 6.72) .... ***T. chinensis***
4 Stomata bands on the abaxial surface each with 12–18 lines, most bud scales persistent, apex acuminate .... ***T. wallichiana***
4 Stomata bands on the abaxial surface each with 8–11 lines, few bud scales persistent, apex acute-obtuse .. ***T. florinii***

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