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Species/geographic boundaries and evolutionary interrelationships of cultivated linden-trees (*Tilia* L.) based on morphological and nrDNA ITS characteristics

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Abstract

Nuclear ribosomal transcribed spacers (ITS1-5.8S rRNA-ITS2) is a popular marker that has superior taxa resolution in some groups of organisms. A taxonomic reassessment of 27 *Tilia* taxa combining a molecular marker (ITS1-5.8S rRNA-ITS2) and morphological characters (40 characteristics of 1,307 leaves and 1,146 fruits) was performed to: (1) evaluate nrDNA ITS as a potential barcode for *Tilia* species-level identification, (2) detect geographic differentiation pattern of *Tilia* trees originated from Europe, Asia, and North America and cultivated in common garden conditions, (3) compare *Tilia* hybrids and their putative parental species. We demonstrate that: (1) intra-individual and intra- morphospecies site polymorphism (2ISP) in ITS sequences occurs; (2) ITS variants in vegetatively propagated hybrids differ from variants in putative parental species; (3) geographical patterns of genetic and morphological differentiation were detected; (4) the majority of hybrids clustered around one of the parental species. The resulting poorly resolved relationship in the phylogenetic analyses (Maximum Parsimony and Maximum Likelihood) can be explained in terms of data quality (low number of parsimony informative sites, high level of homoplasy), the influence of hybridization on the phylogeny, or other issues. The ITS spacers should be excluded as a potential single barcode due to the existence of 2ISPs. We concluded that our ITS survey is not exhaustive because ITS variants in vegetatively propagated hybrids differ from variants in their parental species. A dichotomous key based mainly on qualitative morphological traits is constructed for the cultivated *Tilia* taxa.

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Introduction

Linden- (lime-) trees (*Tilia* L, Tiliaceae) are a widespread and taxonomically complex genus with a complicated evolutionary history. *Tilia* is native to three parts of the northern hemisphere: Europe and western Asia, eastern Asia, and eastern North America. Four species are native to Europe and western Asia: *T. cordata* Mill., *T. platyphyllos* Scop., *T. dasystyla* Steven, and *T. tomentosa* Moench. These species are further divided into several subspecies and varieties (Pigott, 2012). Moreover, several cultivars are described, e.g., *T. platyphyllos* Laciniata or *T. p.* Vitifolia, and *T. tomentosa* Varsaviensis (Borowski and Solecka, 1980; Boratyńska and Dolatowski, 1991; Seneta and Dolatowski, 2008). European species naturally hybridize, e.g., *T. cordata* and *T. platyphyllos* produce hybrid swarms *T. x europea* L. (Wicksell and Christensen, 1999; Fromm and Hattemer, 2003), whereas *T. cordata* and *T. dasystyla* are parental species to a hybrid *Tilia x euchlora* K. Koch. There are two eastern North American species: *T. americana* L. and *T. caroliniana* Mill., which are further divided into subspecies or varietas, e.g., *T. a.* var. *americana, T. caroliniana* ssp. *heterophylla* (Vent.) Pigott., or the controversial *T. a.* var. *neglecta* (Spach) Fosberg. *Tilia americana* and *T. c.* ssp. *heterophylla* are parental species to a hybrid *T. x stellata* Hartig. (=*T. neglecta* sensu Braun) located in the southern part of the distribution range of *T. americana*. *Tilia. a.* var. *neglecta* Spach is presumably a hybrid between *T. americana* and *T. caroliniana* ssp. *caroliniana*. (Pigott, 2012). There are several hybrids between American and European species that have arisen in cultivation. For example, *Tilia a. x moltkei* Spaeth ex C.K. Schneid. is presumably derivative of *T. tomentosa* and *T. americana*, whereas *T. a. x moltkei* Zamoyskiana is a hybrid between *T. tomentosa* Moench Petiolaris and *T. americana* (Boratyńska and Dolatowski, 1991). *Tilia x flaccida* Host ex Bayer is a derivative of *T. americana* and *T. platyphyllos* (Pigott, 2012). According to the recent treatment, 17 species and five subspecies were recognized in eastern Asia (Pigott, 2012). Besides the widespread species that have an inevitable impact on forests, e.g., *T. amurensis* Rupr., *T. mandshurica* Rupr. et Maxim., *T. japonica* (Miq.) Bayer., several other species of local importance were described, which include, among others, two Japanese endemic species *T. kiusiana* Shiras., *T. maximowicziana* Shiras, and the Chinese species *T. chinensis* Maxim, *T. miqueliana* Maxim., *T. henryana* Szyszyl., *T. paucicostata* Maxim., and *T. olivieri* Szyszyl. The latter could be one of the parental species of the more widely distributed and variable *T. tuan* Szyszyl. (Pigott, 2012). The status of some taxa or their origin are uncertain (e.g., *T. hupehensis* Cheng ex Chang is morphologically similar to *Tilia tuan* or *T. insularis* Nakai, see Szymanowski, 1970; Pigott, 2012). The recurrent formation of polyploids makes the situation even more complicated. In *Tilia*, two major classes of polyploids can be found. The large variation in chromosome numbers may indicate autopolyploidization in *Tilia maximowicziana* (2n=164), whereas hybridization coupled with allopolyploidyzation is postulated, e.g., for *T. x euchlora* K. Koch. (Pigott, 2012, 2002).

There have been a growing number of studies that aim to improve and clarify the systematics of the genus (e.g. Fromm and Hattemer, 2003; Fineschi *et al.*, 2003; Liesebach and Sinkó, 2008; Yousefzadeh *et al.*, 2012). However, in some studies, taxon sampling has had a strong influence on the results. A molecular study has recently been undertaken on microsatellite loci in the *Tilia* species to investigate further the phylogeography and hybridization in the genus (Phuekvilai and Wolff, 2013). Several studies have also provided estimates of *Tilia* morphology (Banerjee, 1976; Białobok, 1991; Pigott, 2012, 2008, 1997, and references therein) using traits such as the size and shape (asymmetry) of a leaf blade, shape of marginal teeth, types of hairs and veins, structure of bracts and cymes, and size of fruits and their wall structures.

Here, nrDNA variation [ITS1-5.8S rRNA-ITS2 region (ITS)], the most frequently used marker for DNA barcoding and phylogenetics (Álvarez and Wendel,

2003; China Plant BOL Group, 2011, Schoch *et al*., 2012, Stern *et al*., 2012), was used to check the utility of this region as a DNA barcode for *Tilia* species-level identification and molecular phylogenetics. The *Tilia* phylogeny was inferred with maximum parsimony (MP) and maximum likelihood (ML) analyses. The MP analysis has been already successfully applied by Yousefzadeh *et al*. (2012) for inferring phylogeny and molecular identification of the *Tilia* species from the Hyrcanian Forest. Moreover, here a phylogenetic network method has been used, as a valuable alternative to the regular phylogenetic analyses (Bryant and Moulton, 2004, see also Chen *et al*., 2013), for the first time in *Tilia*. This method is based on a criterion similar to that used in the neighbor-joining algorithm for tree construction.

To reveal possible differences in morphological traits (qualitative and quantitative) between *Tilia* taxa, and to check the accuracy of their identification, morphometric analyses of morphological traits of leaves and fruits were performed. Specifically, we tested the hypothesis, that *Tilia* hybrids are intermediate between their parents with respect to these traits. If true, this may offer an explanation for the blurring of species boundaries in the genus *Tilia*. Furthermore, in a biological monograph of the genus *Tilia* (Pigott, 2012), taxa are sorted out by their broad-scale geographic distribution. Thus, we have tested, whether the cultivated trees analyzed here, differ by geography in terms of leaf and fruit morphology.

Methods

Taxon sampling

Analyzed materials were obtained from Linden-trees growing in the Adam Mickiewicz University Botanical Garden in Poznań (BG) (52° 25' N 16° 53' E) and the Kórnik Arboretum (KA) (52°14' N 17° 5' E), which is a part of the Institute of Dendrology of the Polish Academy of Science (Poland). The materials have been collected from the trees under cultivation, but in the majority of cases of known wild provenance. Details of the plant material are shown in Table 1. The full documentation of analyzed *Tilia* trees (their origin and way of propagation) is available upon request in the investigated botanical gardens. Herbarium samples of analyzed taxa, available upon request, are preserved in the Department of Genetics, Adam Mickiewicz University in Poznań, Poland. Numbers of herbarium accessions are provided in Table 1. The taxa representation used in this investigation covers almost the entire geographical range of the genus, as well as the different ploidy levels (diploids and tetraploids, 2n=82 and 2n=164, respectively), varied taxonomic ranks (species, subsp, var.), and origin (hybrids/cultivated variety).

Table 1. *Tilia* taxa (n=27) used for morphological and molecular analyses (ITS), tree number in the field, GenBank accession number, and places of cultivation of *Tilia* trees.

	***Tilia* species names**				
no.	**BG[a]/KA[b]/ GenBank**	**Pigott *et al*. 2012 (♦ Seneta and Dolatowski 2008)**	**Section**	**Tree number/ *herbarium number (IM-XXXX)***	**Accession number (GenBank database)**
		Europe and western Asia			
1	*dasystyla* Steven	*dasystyla* Steven	*Anastraea*	[a]8006_7738, *IM-7161*	KF897531-33
2	*dasystyla* Steven	*dasystyla* Steven	*Anastraea*	–	HQ439433.1*
3	*platyphyllos* Scop. Vitifolia	*platyphyllos* Scop. Vitifolia♦	*Anastraea*	[a]8963_1294, *IM-7159*	KF445425
4	*platyphyllos* Scop. Laciniata	*platyphyllos* Scop. Laciniata♦	*Anastraea*	[b]204, *IM-7155*	KF445421
5	*platyphyllos* Scop.	*platyphyllos* Scop.	*Anastraea*	[a]8XXX_0089,	KF897516-18

	Tilia species names				
no.	**BG[a]/KA[b]/ GenBank**	**Pigott *et al.* 2012 (♦Seneta and Dolatowski 2008)**	**Section**	**Tree number/ *herbarium number (IM-XXXX)***	**Accession number (GenBank database)**
				IM-7163	
6	*platyphyllos* Scop.	*platyphyllos* Scop.	*Anastraea*	–	AF250292.1*
7	*tomentosa* Moench	*tomentosa* Moench	*Astrophilyra*	[b]2724, *IM-7174*	KF694727
8	*tomentosa* Moench	*tomentosa* Moench	*Astrophilyra*	[b]201, *IM-7133*	KF445417
9	*tomentosa* Moench	*tomentosa* Moench	*Astrophilyra*	–	AF250023.1*
10	*cordata* Mill.	*cordata* Mill.	*Anastraea*	[a]8925_0192, *IM-7158*	KF897519-21
11	*cordata* Mill.	*cordata* Mill.	*Anastraea*	[a]8008_7914, *IM-7168*	KF445434
12	*cordata* Mill.	*cordata* Mill.	*Anastraea*	[b]200, *IM-7150*	KF445416
13	*hyrcana* Tabari & Colagar	–	?	–	JX051606.1*
			Eastern Asia		
14	*amurensis* Rupr.	*amurensis* Rupr.	*Anastraea*	[a]8969_1850, *IM-7160*	KF445426
15	*henryana* Szyszyl.	*henryana* Szyszyl.	*Henryana*	[b]6953, *IM-7153*	KF445419
16	*henryana* Szyszyll.	*henryana* Szyszyl.	*Henryana*	[a]8964_0559, *IM-7157*	KF445423
17	*japonica* (Miq.) Simonk.	*japonica* (Miq.) Bayer.	*Anastraea*	[b]1094, *IM-7171*	KF694724
18	*insularis* Nakai	*amurensis* Rupr.	*Anastraea*	[a]8989_3356, *IM-7166*	KF445432
19	*kiusiana* Makino et Shiras.	*kiusiana* Shiras.	*Anastraea*	[b]13505, *IM-7149*	KF445415
20	*miqueliana* Maxim.	*miqueliana* Maxim.	*Astrophilyra*	[a]8XXX_6565, *IM-7165*	KF445431
21	*miqueliana* Maxim.	*miqueliana* Maxim.	*Astrophilyra*	–	DQ120724.1*
22	*mongolica* Maxim.	*mongolica* Maxim.	*Anastraea*	[a]8006_7811, *IM-7164*	KF445430
23	*olivieri* Szyszyl.	*olivieri* Szyszyl.	*Astrophilyra*	[a]8937_0605, *IM-7178*	KF897522-24
24	*olivieri* Szyszyl.	*olivieri* Szyszyl.	*Astrophilyra*	[b]3006, *IM-7179*	KF897525-27
25	*tuan* Szyszyl.	*tuan* Szyszyl.	*Astrophilyra*	[b]3009, *IM-7175*	KF694728
26	*hupehensis* Cheng ex H. T. Chang	*tuan* Szyszyl.	*Astrophilyra*	–	AF46019_7.1*
27	*paucicostata* Maxim.	*paucicostata* Maxim	*Anastraea*	–	AF460198.1*
			North America		
28	*americana* L.	*americana* L	*Astrophilyra*	[a]8937_1052, *IM-7156*	KF445422
29	*americana v. heterophylla* (Vent.) Loud	*caroliniana* Miller ssp. *heterophylla* (Vent.) Pigott	*Astrophilyra*	[b]198, *IM-7170*	KF694723
30	*americana v. heterophylla* (Vent.) Loud.	*caroliniana* Miller ssp. *heterophylla* (Vent.) Pigott	*Astrophilyra*	–	AF174639.1*
			Hybrids		
31	*tomentosa* Moench Varsaviensis	*tomentosa* Varsaviensis (x *varsaviensis* Kobendza)♦	*T. tomentosa* x *T. platyphyllos*	[a]8966_1404, *IM-7172*	KF694725
32	x *europaea* L. Euchlora Dolatowski	x *euchlora* K. Koch	*T. cordata* x *T. dasystyla*	[a]8XXX_3825, *IM-7180*	KF897528-30

no.	*Tilia* species names: BG[a]/KA[b]/ GenBank	Pigott *et al.* 2012 (♦ Seneta and Dolatowski 2008)	Section	Tree number/ *herbarium number (IM-XXXX)*	Accession number (GenBank database)
33	x *europea* L. Euchlora Dolatowski	x *euchlora* K. Koch	*T. cordata* x *T. dasystyla*	[a]8XXX_6564, *IM_7181*	KF897534
34	x *zamoyskiana* Wróbl.	*americana* x *moltkei* Zamoyskiana	*T. americana* x *T. tomentosa* Moench Petiolaris	[b]2723; *IM-7173*	KF694726
35	x *flaccida* Host	x *flaccida* Host ex Bayer	*T. americana* x *T. platyphyllos*	[a]8990_3476, *IM-7167*	KF445433
36	x *spaetchi* (?)	–	?	[b]7082, *IM-7152*	KF445418
37	x *neglecta* Spach♦	*americana* L. *v. neglecta* (Spach) Fosberg	*T. americana* x *T. caroliniana* ssp. *heterophylla* (?)	[a]8000_7497, *IM-7176*	KF694729
38	*americana* L. Moltkei	*T. americana* x. *moltkei* Späth *ex* C.K. Schneid.	?	[a]8XXX_6383, *IM-7162*	KF445428
			Rootstock		
39	*olivieri* rootstock	*platyphyllos* Scop.	?	[a]8937_0605, *IM-7148*	KF445414
			Outgroup		
40	*Craigia yunnanensis* W.W. Sm. & W.E. Evans	–	–	–	AF460199.1

[a] Botanical Garden of Adam Mickiewicz University, Poznań, Poland, [b] Kórnik Arboretum near Poznań, Poland

DNA extraction, ITS amplification, cloning, and sequencing

The total DNA was extracted from freeze tissue of leaves following the CTAB (Hexadecyl trimethyl-ammonium bromide) method (Doyle and Doyle, 1987). A homogenization of the frozen material was performed in 2 ml tubes with steel beads using a mixer mill. The CTAB extraction buffer was modified by adding 2% (w/v) polyvinylpyrrolidone and 0.2% (v/v) beta-mercaptoethanol. The ITS1-5.8S-ITS2 rDNA region was amplified using nested PCR with primer pairs published by Shaw *et al.* (2003). PCR fragments were purified using Exonuclease I-Shrimp Alkaline Phosphatase (Thermo Scientific). These fragments were directly sequenced in both directions using the BigDye Terminator Mix and ABI 3130xl automated sequencer (Applied Biosystems, California, USA) at the Faculty of Biology, Adam Mickiewicz University, Poznań, Poland.

Several individuals, including parental species and hybrids, were cloned to detect any (and presumably *all*) base pair variants in the ITS region. Amplified DNA was ligated in pGEM ®-TEasy vector (Promega) overnight at room temperature. The ligated DNA was subsequently transformed in DH5α competent cells. The Blue-White screening method was used for the detection of recombinant bacterial clones. The presence of inserted DNA in the recombinant clones was confirmed by the colony PCR. The inserted DNA was sequenced.

The DNA sequence edition was performed using DNA sequence chromatogram trace viewer FinchTV v.1.3.1 (Geospiza, Inc., www.Geospiza.com/Products/ finchtv.shtml). Contigs were assembled using Lasergene-DNAstar (www.dnastar.com). The molecular genetic analysis and sequence alignment were conducted using MEGA 5.2.2. for Windows (Tamura *et al.*, 2007). The initial automated

alignment was further adjusted manually. Sequences were submitted to GenBank through Bankit Submission Tool (accession numbers, Table 1).

Phylogenetic analysis

The ITS sequences were successfully collected from 39 accessions representing 27 taxa (nine sequences were gained from GenBank, www.ncbi.nlm.nh.gov). Molecular analyses were performed in two stages. For a small subset of samples, i.e., *T. euchlora* (BG, 3825), *T. platyphyllos* (BG, 0089), *T. cordata* (BG, 0192), *T. dasystyla* (BG, 7738), and *T. olivieri* (BG 0605, KA 3006), intra- and inter-array heterogeneity at a site and variation at the species-level were evaluated by molecular cloning. This analysis was followed by phylogenetic analyses, which were performed on the whole dataset.

The partition homogeneity/incongruence-length difference test (ILD) implemented in the TNT (Tree analysis using new Technologies) program (Goloboff *et al.*, 2003) was used to investigate whether different partitions of the ITS data have significantly different signals. Sequence identity was calculated in the program Geneious v. 7.0.4 created by Biomatters (www.geneious.com). The average number of nucleotide differences per site between two sequences (Nei, 1987) was calculated in a program called DnaSP v. 5.10.1 (Rozas *et al.*, 2003). The Maximum Likelihood (ML) framework implemented in the RAxML (Randomized Axelerated Maximum Likelihood) program 7.2.6 (Stamatakis, 2006) was employed, as recommended by Potts *et al.* (2014). This particular option (-m MULTIGAMMA –k GTR) allows us to treat each IUPAC nucleotide code as a unique character. Moreover, the ML analysis was performed using the program PHYML v.3.0. (Guindon *et al.*, 2010) under different substitution models. The model choice was based on the Akaike information criterion (AIC) implemented in the programs jModelTest 2.1.4 (Darriba *et al.*, 2012) and PartitionFinder v. 1.1.1 (Lanfear *et al.*, 2012). The latter program was used for selecting the best model of molecular evolution for different sets of sites. These two datasets were also analyzed using maximum parsimony (MP) criterion implemented in the TNT program (heuristic searches with 1000 replicates, using TBR branch swapping and equally weighted characters). The exclusion or inclusion of hybrids allows the researcher to assess their influence on a phylogeny.

As an alternative to these regular phylogenetic analyses, the phylogenetic network was created using SplitsTree v. 4.8 (Huson and Bryant, 2006) for the entire set of sequences and for ITS variants detected in a subset of individuals by molecular cloning. A general structure of molecular data was visualized employing a distance-based Neighbor-Net method (Bryant and Moulton, 2004). The network was constructed employing the "Average" option for calculated uncorrectedP distances and the equal angle splits transformation. Gaps were coded as informative sites. Moreover, a network from the NJ tree (in the bootstrap network) was constructed based on 1000 replicates. In all analyses, the nearest neighbor, *Craigia yunnanensis*, was used as an out-group.

Morphological analysis

Accessible taxa of *Tilia* were studied in terms of 40 characteristics of leaves and fruits. A total of 1,307 leaves and 1,146 fruits from 44 trees belonging to 27 taxa were studied. The measured traits and their codes are presented in Table 2, Fig. 1. Of these features, eight were continuous, 15 described percentages, and 17 were coded as binary or multistate. Some quantitative variables were a Lag10 transformed to approximate normality better (Howell, 2007; Tabachnick and Fidell, 2007). To test the normality and homogeneity of variances in the morphological data, the Lilliefors (Kolomogorov-Smirnov) and Levene`s tests were employed. A canonical discriminant analysis (DA) was applied to the additive, transformed data (eight traits) to determine which variable allows for the best discrimination between the geographical groups of taxa and the group of hybrids. Coefficients of determination were calculated as the square of the

multiple correlation coefficient multiplied by the percentage of variation for discriminant function DF1 and DF2, respectively. Squared Mahalanobis distances were used as a measure of the separation of these groups. Moreover, percentages of well-classified samples were presented (Stanisz, 2007).

Table 2. Continuous and discrete characters used in morphometric analyses of *Tilia* leaves and fruits.

No	Trait description		Character abbreviation	Type of characters: discrete (D) continuous (C)	Units of measurements / coding
1	Leaf –blade (LB)	length	L-LB	C	mm
2		maximum width	MW-LB	C	mm
3		length of LB apex	AL-LB	C	mm
4		no of pairs of transverse veins	VN-LB	C	4-35
5		length of petiole	L-LP	C	mm
6		shape of LB base	BS-LB	D	1-5
7		presence/absence of additional extensions (lobule)	E-LB	D	0-1
8	Marginal teeth on the middle part of LB	number of teeth per 2cm	TN-LB	C	2-14
9		shape	TS-LB	D	1-2
10	Presence/absence of hairs on LB	upper side	H-LBU	D	0-1
11		on veins (upper side)	HV-LBU	D	0-1
12		on lower side	H-LBL	D	0-1
13		on main veins on lower side	HV-LBL	D	0-1
14		in the axils of main veins and at the base on lower side	HF-LBL	D	1-3
15		on petiole	HP	D	0-1
16	Color of hairs	on lower side	HC-LBL	D	0-3
17		on main veins on lower side	HCV-LBL	D	0-1
18		in the axils of main veins on lower side	HFC-LBL	D	0-3
19		on petiole	HC-P	D	0-3
20	Type of hairs on lower side of LB	simple	SH-LBL	P	%
21		stellate with 2 arms	STH-LBL	P	%
22		star-shaped (4 -armed)	SRH4-LBL	P	%
23		star-shaped (6 -armed)	SRH6-LBL	P	%
24		star-shaped (8 -armed)	SRH8-LBL	P	%
25	on main veins on lower side of LB	simple	SH-LBV	P	%
26		stellate with 2 arms	STH-LBV	P	%
27		star-shaped (4 -armed)	SRH4-LBV	P	%
28		star-shaped (6 -armed)	SRH6-LBV	P	%
29		star-shaped (8 -armed)	SRH8-LBV	P	%
30	on petiole	simple	SH-P	P	%
31		stellate with 2 arms	STH-P	P	%
32		star-shaped (4 armed)	SRH4-P	P	%
33		star-shap (6 -armed)	SRH6-P	P	%
34		star-shap (8 -armed)	SRH8-P	P	%
35	Fruits	length	FL	C	mm
36		width	FW	C	mm
37		surface ornamentation	FSO	D	0-2
38		type of hairs	FH	D	0-1
39		longitudinal lines/ribs	FR	D	0-3
40		apical cavity	FAC	D	0-2

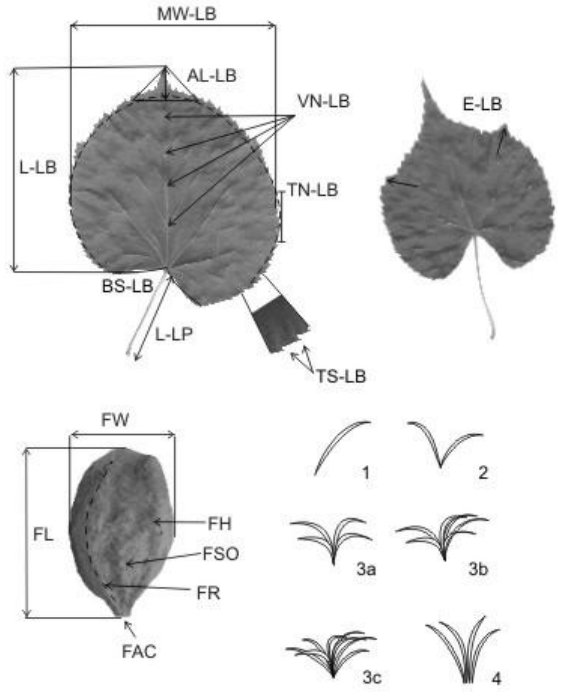

Fig. 1. Graphical descriptions of measured traits of leaves and fruits in *Tilia* (for detailed description see Table 2)

Leaf traits: L-LB – length of blade [mm], MW-LB – maximum width of blade [mm], AL-LB – length of leaf-blade apex [mm], L-LP – length of petiole [mm], VN-LB – number of pairs of transverse veins, BS-LB – shape of leaf blade base, E-LB – presence/absence of additional extensions (lobule)

Marginal teeth: TN-LB – number of teeth per 2 cm, TS-LB – shape of teeth

Fruit traits: FL – length [mm], FW – width [mm], FSO – surface ornamentation, FR – longitudinal lines/ribs, FAC – apical cavity, FH – type of hairs: 1 – simple, 2 – double, 3 – stellate (a – four-armed, b – six-armed, c – eight-armed), 4 –fasciculate.

The group of hybrids was composed of the following taxa: *T. a. x moltkei, T. euchlora, T. tomentosa* Varsaviensis*, T. a. x moltkei* Zamoyskiana*, T. x flaccida*, *T. x spaechi*, and *T. a.* var. *neglecta*. Two species — *T. insularis,* and *T. tuan* — were not considered as hybrids because their origins are uncertain (Pigott, 2012). To reveal possible differences in morphological traits among hybrids and putative parental species, a one-way MANOVA followed by Turkey's post-hoc tests for unequal sample sizes were performed. The hybrids and parental species were included as a fixed factor, and continuous traits as random factors. However, *Tilia americana* Moltkei, a hybrid of unknown paternity, and *Tilia a. x moltkei* Zamoyskiana were not analyzed by MANOVA. For the latter, only one parental species was available – *T. americana,* whereas we did not have access to *T. tomentosa* Petiolaris, the second parental species.

For nominal discrete (qualitative) data, contingency tables were used to compute Pearson's chi-square test for independence. This test assessed the association between qualitative traits and native geographical distribution of species. Descriptive statistics were calculated for the quantitative variables of each species based on the entire dataset. For qualitative variables, mode values were computed. The percentage variables derived from count data were also included. All of these data served as a basis for the construction of the dichotomous key for *Tilia* taxa. Data management and analyses were performed using the program STATISTICA 10 package, (StatSoft Inc., Tulusa, OK, USA).

In our work, we treated the original *Tilia* species identifications as valid and reliable. However, to ensure the accuracy all of the examined samples, these identifications were additionally checked by the second author (MC) using the morphological characters of leaves and fruits. The species nomenclature follows that of Pigott (2012), and original identification derived from the BG and AK was also specified (Table 1). In the case of GenBank sequence records the original nomenclature was maintained.

Results

Morphological analysis

The means of the samples (1-40) for which the full datasets were obtained are presented in the system of two discriminant variables (U1-U2), Fig. 2. As was shown in Fig. 2, analyzed *Tilia* trees formed three areas, relatively well isolated, which correspond to the

native geographical distribution of species, i.e., groups of trees from: (1) eastern Asia; (2) Europe and western Asia; and (3) North America. Based on the squared Mahalanobis distances, the centroids of these groups are significantly different (Table 3). Values of well-classified samples ranged from 85.71 to 100%. An insignificant Mahalanobis distance was detected between the group of hybrids and the group of trees that originated from Europe and western Asia ("European"). This seems to be justified since at least one parental species originated from this European group. The remaining Mahalonobis distances are significant, indicating separation between the hybrids and the eastern Asian and American taxa, the values of these distances are larger.

Table 3. Values of squared Mahalanobis distances between centroids of *Tilia* geographical groups and hybrids.

	E Asia	Europe and W Asia	N. America	Hybrids
E Asia	-			
Europe & W Asia	7.09[a]	-		
N. America	20.81[a]	9.51[c]	-	
Hybrids	6.46[c]	2.02	13.57[b]	-

[a] $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.05$

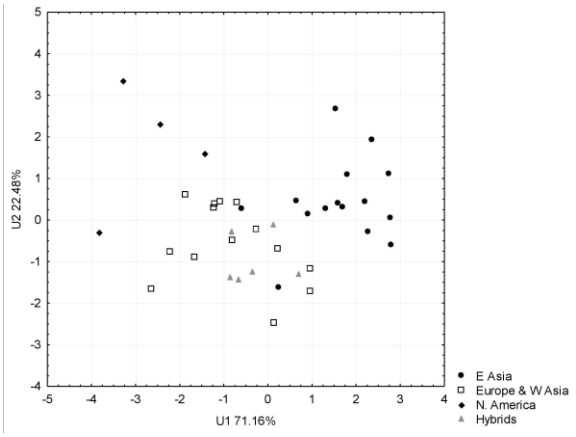

Fig. 2. Scatter plot of the first two discriminant functions (U1, U2) discriminated among *Tilia* geographical groups and groups of hybrids, accounting for 93.64% variance of eight morphological continuous traits of leaves and fruits. Each dot on the scatter plot represents a single tree.

Based on the coefficients of the determination of the canonical variables, four traits affect the most in the geographical group classification: the number of pairs of transverse veins, the maximum width of the leaf blade, fruits' width, and the number of teeth per 2 cm on the middle part of the leaf blade (Tables 4 and 5).

Table 4. Coefficients of determination for analyzed variables of leaves and fruits in cultivated *Tilia* trees.

Variable (transformation)	DF 1	DF2
L-LB (Log10)	3.99	6.06
L-LP	0.21	2.73
MW-LB	12.05	8.44
AL-LB (Log10)	2.43	6.06
VN-LB (Log10)	16.72	1.86
TN-LB	1.12	14.42
FL	3.94	1.45
FW	11.85	2.12
% of variation	71.16	22.48

The third function obtained in this analysis accounted for 6.36% between-groups variance.

The MANOVA has revealed a significant main effect involving species factor — a hybrid and parental taxa (Table 6). Based on the Turkey's post-hoc tests for unequal sample sizes, *Tilia x europea* (=T. x *europea* Euchlora) shows intermediate morphological characteristics between the parental species (*T. cordata* and *T. dasystyla*), ($P<0.01$).

However, in the majority of cases, hybrids resemble one of their parental species, e.g. *T. tomentosa* var. *Varsoviensis* (hybrid variety) does not significantly differ in quantitative traits from one of its parent (*T. tomentosa)*. There is also no significant difference between *T. x flaccida* and its parental species *T. platyphyllos*. Moreover, *T. x neglecta* does not differ significantly from *T. americana* (but also *T. platyphyllos*). On the contrary, significant differences in these traits are detected between these hybrids and the second parental species ($P<0.05$).

There is no such clear trend in qualitative traits based on analyses of contingency tables. Depending on considered traits, external resemblance between a hybrid and either the one or the second parental species is visible. For example, the mode values of BS-

LB, H-LBL, HC-LBL are equal for *T. tomentosa* var. *Varsoviensis* and *T. tomentosa,* and E-LB, TS-LB for *T. tomentosa* var. *Varsoviensis* and *T. platyphyllos*. Some of qualitative traits are unique for a particular species (e.g. traits: H-LBU, HF-LBL).

Below, a key for *Tilia* species identification based on the morphological traits of leaves and fruits is presented. A summary of the statistics of the analyzed traits is shown in Table 1 in the Online Resources section.

Table 5. Mean (St. dev.) of variables with the highest coefficients of determination for geographical groups and hybrids of cultivated *Tilia* trees.

	Mean ±St. dev. (n)			
Group Variable	**E Asia**	**Europe and W Asia**	**America**	**Hybrids**
MW-LB	62.06 ±19.98 (557)	67.83±22.39 (390)	95.27±38.19 (150)	62.81±13.00 (210)
VN-LB	6.95±1.17 (557)	8.17±3.03 (390)	10.36±1.96 (150)	7.98±1.38 (210)
TN-LB	7.02±2.35 (557)	8.98±2.09 (390)	6.21±1.63 (150)	9.08±1.74 (210)
FW	5.19±1.35 (437)	5.95±1.40 (390)	7.86±0.83 (126)	5.77±1.23 (180)

Table 6. Effects of species (hybrids and parental taxa) as grouping variable on continuous traits (dependent variables), assessed by one-way MANOVA.

***Tilia* hybrids and parental species**	**Wilks` Lambda**	**F value and degrees of freedom**	**Sig. of F (p-value)**
tomentosa Varsaviensis *tomentosa platyphyllos*	0.03	$F_{(16,\ 412)}$=129.26	0.001
x euchlora *cordata* *dasystyla*	0.18	$F_{(16,\ 460)}$=38.14	0.001
x zamoyskiana *americana* *tomentosa* Petiolaris	0.04	$F_{(16,\ 280)}$=68.80	0.001
x flaccida *americana* *platyphyllos*	0.20	$F_{(12,\ 284)}$=28.64	0.001
x neglecta *americana* *caroliniana* ssp. *heterophylla* *platyphyllos*	0.06	$F_{(24,\ 421)}$=29.21	0.001

Phylogenetic analysis of samples showing intra-individual variation in the ITS region

Generally, a small number of parsimony-informative sites were detected in the analyzed dataset representing five taxa. The total alignment matrix had 579 characters with 36 potentially parsimony-informative characters. The ITS1 was the most variable and had the highest number of parsimony-informative sites (21) in comparison to 5.8S (1) and ITS2 (14). Three ITS sequence variants were detected based on the three clones within each analyzed individual, except *T. olivieri*, for which two individuals were cloned and six different ITS variants were recovered. Variable sites, restricted to parsimony-informative sites within each analyzed taxon, are shown in Table 7. Our study has revealed varying patterns of nucleotide diversity per base pair (π) within a particular species - from 0.93 ± 0.27 in diploid species to 3.19-3.60 ± 1.01 in polyploids *T. x euchlora* and *T. dasystyla*, Table 8.

Maximum likelihood analyses of the ITS region for the small subset of cloned samples (all variants) were conducted using two different models (TrN+G and HKY+G) for datasets without and with hybrids, respectively. Likelihood scores were estimated using

PHYML. The same sample set was also analyzed under the GTR model of nucleotide substitution using the -m MULTIGAMMA –k GTR option implemented in RAxML, either including or excluding hybrids. Due to the general similarity in the topology of the strict consensus parsimonious tree and ML trees, only the MP phylograms, having higher resolutions, are presented in Figs. 3-4.

Table 7. Variable sites, restricted to parsimony-informative sites in ITS sequences of *Tilia*, recovered by molecular cloning.

		ITS 1																								5.8S	ITS 2																	
	position in alignment	1	3	4	13	17	22	24	27	36	53	85	99	102	126	146	150	152	154	157	178	181	217	359	366	370	372	378	389	401	426	434	437	473	476	550	576
	RefSeq.[a]	G	C	C	A	T	C	T	C	A	T	T	G	T	T	T	T	T	T	T	T	A	T	T	A	T	T	G	A	C	G	A	T	T	C	T	C
species name	clone no.																																										
T. x euchlora	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3825	2	T	T	.	G	C	T	.	G	G	C	.	A	.	.	.	.	.	.	G	.	.	.	C	.	C	.	A	.	.	.	.	C	.	.	C	
	3	T	T	.	G	C	T	.	G	G	C	.	.	.	.	.	C	C	G	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
T. platyphyllos	1	A	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	G	.	C	.	.	.	.	.	A	T	.	.	.	T	C	.
0089	2	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	.	.	.	.	.	C	.
	3	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	G	.	C	.	.	.	.	.	A	T	.	.	.	T	C	.
T. cordata	1	T	T	.	G	C	T	.	G	G	C		A	.	.	.	C	C	G	C	C	.	.	C	.	C	.	A		.	.	.	.	.	.	C	.
0192	2	T	T	.	G	C	T	.	G	G	C	.		.	.	.	C	C	G	C	.	.	.	C	.	C	.	A	T	.	.	.	.	.	.	C	.
	3	T	T	.	G	C	T	.	G	G	C	.	A	.	.	.	C	C	G	C	C	.	.	C	.	C	.	A		.	.	.	.	.	.	C	.
T. dasystyla	1	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	C	.	C	.	A	.	.	.	.	.	.	.	C	.
7738	2	T	T	.	G	C	T	.	G	G	C	.	A	.	.	.	C	C	G	C	C	.	.	C	.	.	.	.	.	A	.	.	.	.	.	.	.
	5	.	T	.	.	.	.	.	.	G	C	.		.	.	.	C	C	G	C	C	.	.		.	.	.	.	.	A	.	.	.	.	.	C	.
T. olivieri	1	.	.	.	G		T	C	G	.	C	C	A	C	A	C	C	C	G	C	C	.	.	C	G	.	C	A	G	.	.	C	C	C	.	C	G
0605	2	.	.	.	G	C	T	C	G	.	C	C	A		A		C	C	G	C	C	.	.	C	G	.	C	A	G	.	.	G		C	.	C	.
	3	.	.	.	G	C	T	C	G	.	C	C	A	C	A	C	C	C	G	C	C	.	C	C	G	.	C	A	G	.	.	C	C	C	.	C	G
T. olivieri	1	.	.	.	G	C	T	C	G	.	C	C	A	C	A	C	C	C	G	C	C	.	.	C	.	.	C	A	G	.	.	C	C	C	.	C	G
3006	2	.	.	.	G	C	T	C	G	.	C	C	A	C	A	C	C	C	G	C	C	.	.	C	G	.	C	A	G	.	.	G		C	.	C	.
	5	.	.	.	G	C	T	C	G	.	C	C	A		A	C	C		G	C	C	.	.	C	.	.	C	A	G	.	.	C	C	C	.	C	.

[a] The ITS sequence obtained for the first clone of *T. x euchlora* was treated as the reference sequence.

Table 8. Estimates of nucleotide diversity (π) ($x10^{-2}$) at ITS loci in *Tilia* based on: A. cloned individuals; B. results of bootstrap network analysis; C. native distribution of species. Gaps treated as missing data.

	Number of sequences	Number of chromosomes (2n)	Nucleotide diversity (π) [$x10^{-2}$] ± SD
A			
T. x euchlora	3	164	3.19 ±0.95
T. platyphyllos	3	82	1.16 ± 0.50
T. cordata	3	82	1.16 ± 0.50
T. dasystyla	3	164	3.60 ± 1.01
T. olivieri (total)	6	82	1.29 ± 0.19
T. olivieri (0605)	3	-/-	1.74 ± 0.50
T. olivieri (3006)	3	-/-	0.93 ± 0.27
Total	18		3.42 ± 0.23
B			
1	14		1.96 ± 0.32
2	5		0.42 ± 0.20
3	4		2.30 ± 0.52
4	4		0.47 ± 0.11
5	3		2.00 ± 0.62
6	7		1.875 ± 0.75
Total	37		2.87 ± 0.25
C			
Europe and W Asia	17		2.545 ± 0.32
E Asia	14		2.72 ± 0.45
North America	5		1.98 ± 0.75
Hybrids	3		2.68 ± 0.85
Total	39		2.83 ± 0.24

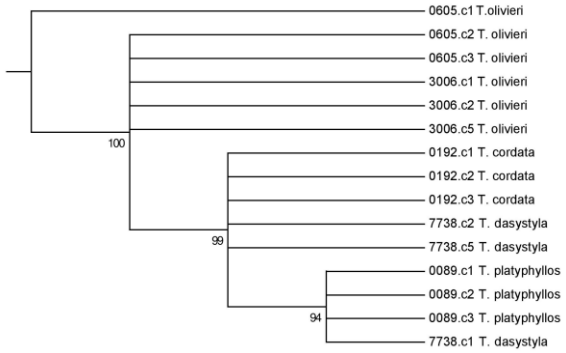

Fig. 3. Strict consensus tree of the 2 most parsimonious trees of length 94 (CI = 0.83, RI = 0.86) for a small set (n = 15) of cloned samples of *Tilia* (excluding *T. x euchlora*) derived from the analysis of ITS1-5.8S-ITS2. Standard bootstrap values > 70 are shown above branches.

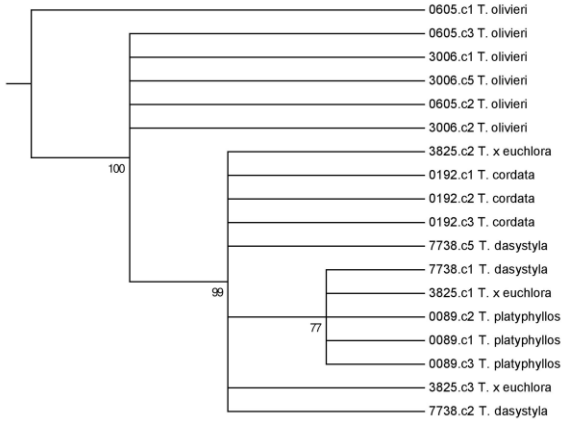

Fig 4. Strict consensus tree of the 2 most parsimonious trees of length 120 (CI = 0.73, RI = 0.79) for a small set (n = 18) of cloned samples of *Tilia* (including *T. x euchlora*) derived from the analysis of ITS1-5.8S-ITS2. Standard bootstrap values > 70 are shown above branches.

Generally, in both types of analyses, tree topologies were largely unresolved; in fact, only one monophyletic clade consisting of ITS variants of *T. olivieri* was observed. *Tilia x euchlora*, depending on the ITS variant, tended to cluster together with *T. cordata* and *T. dasystyla*, considered as its parental species, or *T. platyphyllos* and *T. dasystyla*. The inclusion of the hybrid taxon (*T. x euchlora*) negatively affected the support values and the level of homoplasy. Most nodes had higher bootstrap support with a set of consensus sequences (not shown) than with the matrix of the original variants (Figs. 3-4).

The neighbor-net and bootstrap network were constructed to gain better understanding of how the conflicting signals were contained in the dataset and to observe the reciprocal relationships between samples. These two data evaluations are largely congruent. Here, the bootstrap network is presented in Fig. 5. Generally, the composition of the clusters identified in the graph split (Fig. 5) was similar to the clades in the MP tree (Fig. 4). The split shows a strong support for a set of ITS sequence variants detected in *T. olivieri*, whereas the remaining clusters are characterized by many conflicting signals.

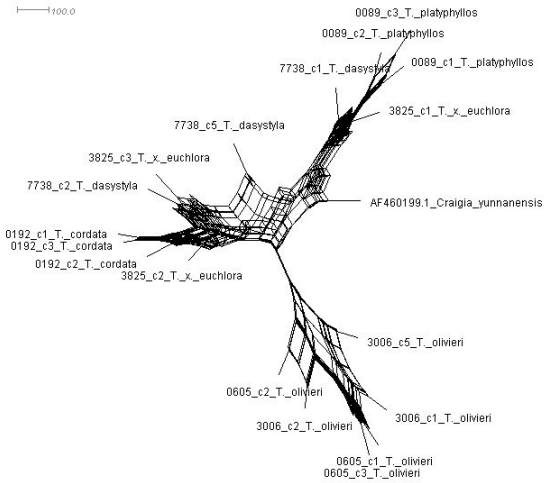

Fig. 5. Bootstrap network based on 1000 bootstrap replicates for a small data set n = 18 of *Tilia* ITS sequences obtained by molecular cloning. The scale bar indicates the scale of the network.

Neighbor-net pattern of the total data set

A summary of characters used in the phylogenetic analyses is shown in Table 2, Online Resources. The total alignment matrix had 580 characters with 36 potentially parsimony-informative characters. The ITS1 was the most variable and had the highest number of parsimony-informative sites (24) in comparison to 5.8S (0) and ITS2 (12). However, no significant conflicts between the ITS partitions were detected based on the ILD test (P=0.90 and P=0.96

for alignments without and with hybrids, respectively). The average nucleotide diversity per base pair, was 2.83 ± 0.24 for the whole set of ITS sequences, Table 8. Different congeneric species share identical variants of ITS2, i.e., *T. cordata* (7914) and *T. hyrcana* (JX051606.1) and three other taxa: (*T. dasystyla* HQ 439433.1), *T. platyphyllos* (AF 250292.1), and *T. x flaccida* (3476).

The full set of accessions was analyzed using both the neighbor-net method and the network from the NJ tree (bootstrap network). These two data evaluations are fully congruent. Fig. 6 presents the rooted phylogenetic network from the NJ tree based on the bootstrap values. The clusters identified in the split reflect a largely native geographical distribution of taxa. One cluster containing eastern Asian taxa (*T. miqueliana, T. amurensis, T. henryana, T. insularis, T. japonica*, and *T. hupehensis*) corresponds largely to a clade with high support (90) identified in the MP tree (Fig. 7). The remaining clusters visible in the neighbor-net method are unresolved in the phylogenetic regular analyses MP (Figs. 7-8) and ML (not shown). These clusters are composed of taxa from eastern Asia (*T. tuan, T. kiusiana, T. paucicostata)* and the more separated *T. mongolica*, North America (*T. americana*, *T. caroliniana* ssp. *heterophylla, Tilia a. x moltkei* Zamoyskiana), one geographically heterogeneous cluster (*T. olivieri, T. tomentosa,* and *T. a. x moltkei*), and two other "European" clusters. In the centum of the network, short central edges forming extensive cycles imply that the data support conflicting splits. The bootstrap network (Fig. 6), has revealed clusters that are characterized by varying patterns of nucleotide diversity - pi values ranged from 0.42 ± 0.20 (cluster 2) to 2.30 ± 0.52 (cluster 3), Table 8. Generally, the Linden-trees originated from eastern Asia are characterized by the highest nucleotide diversity at ITS loci.

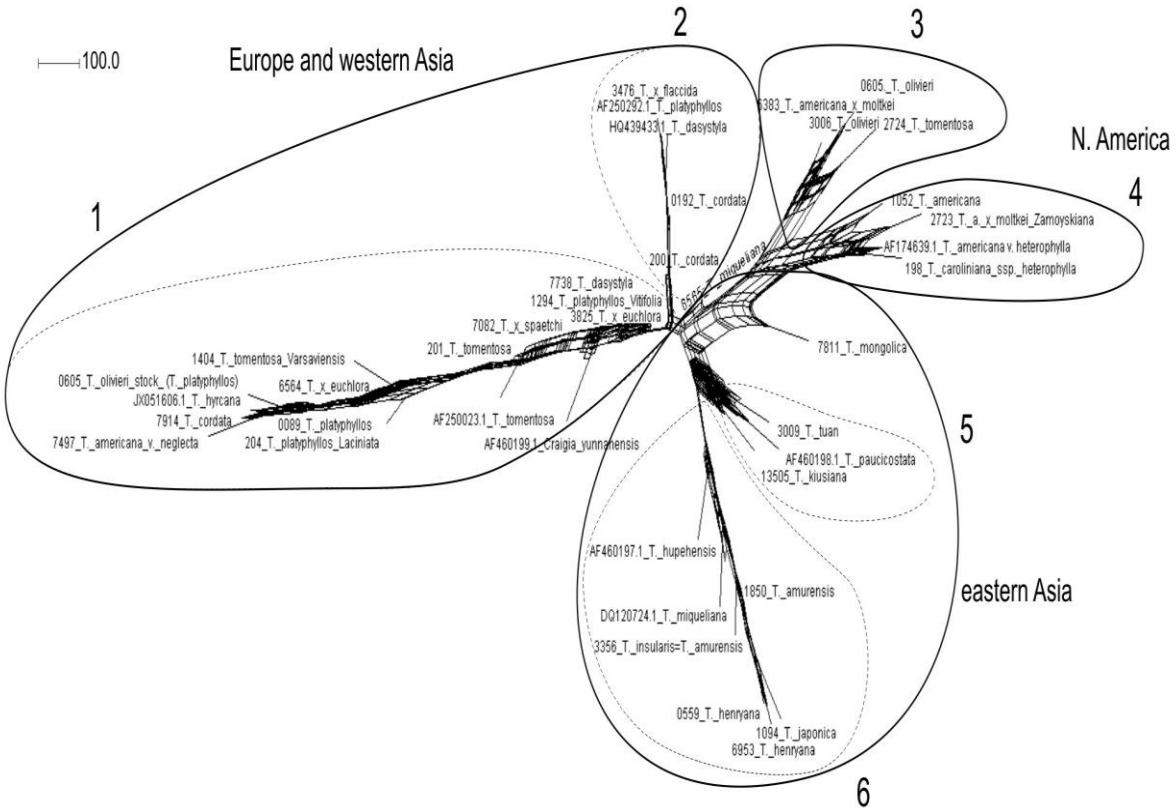

Fig. 6. Bootstrap network based on 1000 bootstrap replicates for a total data set n = 39 of *Tilia* ITS sequences. Native geographical distribution of analyzed taxa is specified. The scale bar indicates the scale of the network.

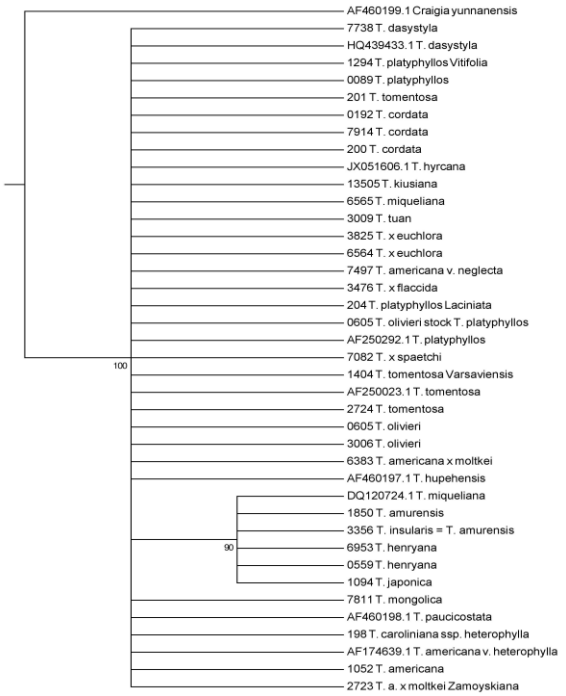

Fig. 7. Strict consensus tree of 100 most parsimonious trees of length 272 (CI = 0.52, RI = 0.45) for a total data set (n = 39) of *Tilia*, including hybrids, derived from the analysis of ITS1-5.8S-ITS2. Standard bootstrap values > 70 are shown above branches.

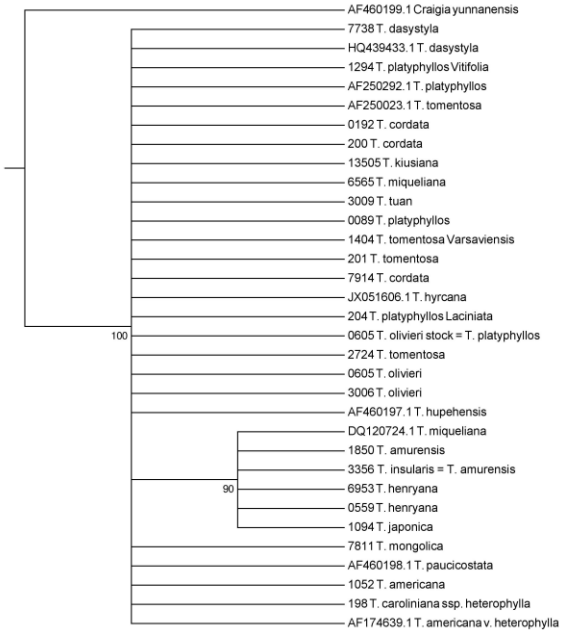

Fig. 8. Strict consensus tree of 530 most parsimonious trees of length 199 (CI = 0.68, RI = 0.68) for a data set (n = 32) of *Tilia*, excluding hybrids, derived from the analysis of ITS1-5.8S-ITS2. Standard bootstrap values > 70 are shown above branches.

Only the local incongruences between the native distribution of taxa and split clustering have been detected. The cluster composed of *T. olivieri, T. tomentosa*, and *T. a. moltkei* is heterogeneous in terms of its geographical affiliation with trees and their origins. The network favors two trees of *T. olivieri* (from BG and KA close to *T. tomentosa* (KA, 2724). One of these trees named *T. olivieri* (BG, 0605), presumably grafted, is composed of two parts: *T. platyphyllos* (rootstock) and one big branch of *T. olivieri*. This branch has leaves and fruits typical for *T. olivieri*, i.e., a leaf blade with very asymmetric, triangular teeth; an underside of leaves densely covered with white (8)-16 stellate hairs; and fruits prominently mammillate. The characteristics of the tree named *T. olivieri* (KA, 3006) vary in degree, resembling in several aspects *T. tomentosa*. Thus, based on the collected leaves and fruits, we are not able to confirm unambiguously the original identification.

A tree named *Tilia. a.* var. *neglecta* Spach (BG, 7497), being presumably a hybrid between *T. americana* and *T. caroliniana* ssp. *heterophylla*, is located between European and west Asian taxa in the graph split. The tree (grown from seeds in BG) shows typical characteristics of *T. platyphyllos*. It does not significantly differ in eight continuous traits from *T. platyphyllos* (and *T. americana*). It has ellipsoidal fruits 5-7 mm in diameter with prominent ribs, as well as leaves with small patches of simple and fasciculare hairs in axils of the main veins on the lower surface. Besides this, occasionally star-shaped hairs with six arms were detected on the lower side of the leaf blade. In *T. americana* fruits are larger, without ribs, and considerable variation in hairiness of leaves is observed. The set of these traits makes the original identification (*T. a.* var. *neglecta* Spach) deeply ambiguous and implies that the tree represents *T. platyphyllos*. *Tilia neglecta* sensu Braun non Spach is considered by Piggott (2012) as the hybrid *T. americana* x *T. caroliniana* ssp. *caroliniana* with four-armed stellate hairs, not observed in the tree no 7497.

Tilia tomentosa Varsaviensis and *T. x flaccida*, are grouped close to one of their parental species. *Tilia x spaetchi*, for which the parental species are unknown, and *T. x euchlora* are located among the European and west Asian taxa in the graph split, although the latter taxon, represented by two trees, does not cluster together. The ITS sequence identity between the hybrids and parental taxa never reached 100%.

Discussion

Concerted evolution is a process of DNA sequence homogenization among different loci within tandemly repeated gene families via unequal crossing-over combined with gene conversion (Dover, 1982, reviewed in Nei and Rooney, 2005). This process may result in the fixation of one sequence or, if relaxed, it may lead to intra-individual site polymorphism (2ISP), defined as any polymorphic site (Potts *et al*., 2014, see also Amheim *et al*., 1980; Wendel *et al*., 1995; Koch *et al*., 2003; Volkov *et al*., 2007). Two co-dominant variants can represent intra-array paralogs, allelic variants (between NORs), homoeologous variants (between orthologous NORs/5S loci), or paralogs between NORs/5S loci originated by duplication and translocation (Potts *et al*., 2014). The 2ISP phenomenon has been detected in different taxa (e.g., Wissemann, 1999; Koch and Al-Shehbaz, 2000; Harris and Crandall, 2000; Thornhill *et al*., 2007; Sani *et al*., 2008; Lindner and Banik, 2011; Hřibová *et al*., 2011), in which it can create a problem for species phylogeny when intra-individual variability exceeds intraspecific variability (i.e., when variants did not cluster together) (see also Buckler IV *et al*., 1997; Potts *et al*., 2014). Different processes are considered to be responsible for the 2ISP, among others, autopolyploidyzation or introgression, and hybridization, which is often coupled with allopolyploidy (see also King and Roalson, 2008; Potts *et al*., 2014).

One result to emerge from our study is that variations in the ITS region are detected across individuals within a particular *Tilia* species and within one individual (intragenomic variation). Because a fixed variation in different clones and individuals among products of independent PCR was detected, we excluded random PCR artifacts (Baldwin *et al*., 1995). The ITS sequence polymorphism apparently persisted because of polyploidy, which is a common phenomenon in the genus (Pigott, 2012, 2002).

Nevertheless, our ITS survey, which we did via molecular cloning, is not exhaustive. Hybrids vegetatively propagated should have combinations of traits derived from both parental types (e.g., two ITS variants). However, ITS sequence identity between hybrids and parental taxa never reached 100%.

The presence of ITS variants does influence substantially the species-level phylogeny. These variants not always clustered together into separate clades in the phylogenetic analysis, e.g., variants detected within *T. dasystyla* or *T. x euchlora*. The intragenomic variation of the ITS region discovered in *Tilia* could be further complicated by the fact that different congeneric species share identical variants of ITS2 (see also Song *et al*., 2012). The resulting poorly resolved relationship in the regular phylogenetic analyses can also be explained in terms of data quality, i.e., by demonstrating a small number of parsimony-informative sites, high level of homoplasy (CI=0.52-0.68 for the whole dataset), or other issues (e.g., heuristic nature of the tree search algorithms or not satisfactory fitting models) (Morrison, 2010). Bifurcating tree methods (MP, ML) may also appear inadequate when hybridization and polyploidyzation are fairly common phenomena, as in the genus *Tilia*. In some cases, improper delineation of species based on morphological data may also result in an improper interpretation of phylogeny. This could be demonstrated in the case of *T. olivieri* (KA) or *T. americana v. neglecta* (BG). However, some overlap between morphological data, lack of inflorescences and flowers, and a limited number of specimens within each analyzed taxon make well-documented identification difficult.

Nevertheless, the results of morphological quantitative analyses of hybrids *Tilia tomentosa* Varsaviensis*, T. x flaccida* and *T. x euchlora*, and their parental species revealed a high congruence with the molecular analyses (Fig. 6).

Most recently, literature has emerged that offers contradictory findings for the *Tilia* species based on ITS regular phylogenetic analysis. Yousefzadeh *et al.* (2012) found that this region is highly conserved among individuals of each of the studied *Tilia* species. However, the authors make no attempt to differentiate the ITS sequences by molecular cloning. Another major drawback of this approach is the limited number of species that were investigated.

Conclusion

Significant differences in the continuous morphological traits of leaves and fruits were found for groups of *Tilia* trees that reflect their native geographical distribution. In the majority of cases, both qualitative and continuous traits allow for species identification. A dichotomous key for *Tilia* taxa cultivated in Poland was presented. Geographical grouping based on morphology was largely confirmed by the molecular analyses of ITS, with some exceptions. However, the utility of this nuclear region is limited in *Tilia* phylogenetic reconstruction and in species diagnosis due to a small number of phylogenetically informative sites and the presence of intra-individual site polymorphism. The presence of more than one type of ITS sequence within one individual and within one species of *Tilia* was reported for the first time.

A dichotomous key to the genus *Tilia*

A dichotomous key to the genus *Tilia* growing in the Adam Mickiewicz University Botanical Garden in Poznań and the Kórnik Arboretum (Institute of Dendrology of the Polish Academy of Sciences). Measurements of continuous traits: mean [±2SD] in mm, qualitative traits mode [min.-max.].

Leaf blade 106 [24-241] - 90 [16-164], with large marginal teeth ca 7 [2-12]. Pairs of pinnate veins from 5 to 14. Fruits almost spherical or ellipsoidal, large 9.12 [6.19-12.04] – 7.86 [6.20-9.52]

American taxa

Leaf blade 74 [20-128] – 66 [24-107] with small marginal teeth ca 9 [5-13]. Pairs of pinnate veins from 3 to 14. Fruits ovoid or spherical, small 7.83 [4.99-10.67] – 5.90 [3.32-8.48].

European and western Asian taxa

Leaf blade 73 [33-114] – 62 [23-102] with large marginal teeth ca 7 [2-12]. Pairs of pinnate veins from 5 to 9. Fruits ovoid or spherical, small 7.43 [3.63-11.23] – 5.24 [2.52-7.95].

eastern Asian taxa

European and western Asian taxa

1 Lower (abaxial) surface of leaf covered with a dense tomentum of white, stellate hairs. Lack of small patches of fasciculate hairs in the axis of main veins.

2

2 Fruits obovoid.

T. tomentosa

2* Fruits almost spherical.

***T. tomentosa* Varsaviensis**

1* Lower surface of leaf glabrous or with sparse simple or stellate hairs.

3

3 Lower (occasionally also upper) surface of leaf covered with simple hairs. Fruits spherical and covered with a dense tomentum of stellate hairs. Longitudinal ribs prominent.

4

4 Leaf blades ovate with cordate base, leaf without lobes on the leaf margin. Leaf blades with white hairs on both sides.

T. platyphyllos

4* Other shape of leaf blades.

5

5 Leaf blades ovate with cordate base and additional lobes on the leaf margin. Veins on the lower surface

covered with reddish-brown hairs. The most often occurring eight pairs of lateral veins.

***T. platyphyllos* Vitifolia**

5* Varied shape of leaf blades, leaf often with additional lobes on the margin. Number of pairs of lateral veins>10.

***T. platyphyllos* Laciniata**

3* Lower surface of leaf glabrous or rarely and irregularly covered with stellate hairs (4- to 8-armed). Fruits ellipsoidal or obovoid, smooth or with weak ribs.

6

6 In the axils of main veins patches of fasciculate, reddish-brown hairs.

7

7 Peduncle glabrous or rarely with simple hairs. Fruits ovoid, smooth, only with weak ribs visible at the base when dry; with asymmetric apiculus.

T. cordata

7* Peduncle covered with stellate hairs (4-, 6-, and 8-armed). Fruits spherical, symmetric with weak ribs.

T. spaethii

6* In the axils of main veins patches of fasciculate white (sometimes straw-colored) hairs.

8

8 Lower surface of leaf glabrous. Fruits with prominent ribs.

T. dasystyla

8* Lower surface of leaf with simple hairs sparsely distributed along the small veins. Fruits with weak ribs.

T. x euchlora

3** Lower surface of leaf covered with simple and stellate (8-armed) hairs. Main veins with stellate hairs (8-armed).

***Tilia** americana x moltkei* **Zamoyskiana**

(T. americana x T. tomentosa* Pendula*)

American taxa

1 Lower surface glabrous.

T. americana

1* Lower surface covered with stellate hairs (8-armed).

2

2 Lower surface of leaf without patches of fasciculate hairs in the axils of main veins.

***T. americana* x Moltkei**

2* Patches of fasciculate hairs at the leaf base and/or in the axils of main veins.

3

3 Lower surface of leaf with patches of fasciculate hairs only in the axils of main veins.

T. heterophylla

3* Patches of reddish-brown fasciculate hairs at the leaf base and in the axils of main veins.

T.* x *flaccida

Eastern Asian taxa

1 Leaf margin serrate, with wide teeth with long apiculus; both sides of leaf with hairs, upper side with simple hairs, lower side with stellate hairs. Patches of white fasciculate hairs in the axils of the main veins on the lower surface. The blossom season is in September. Fruits rare.

T. henryana

1* Leaf margin dentate with marginal teeth with short apiculus. The blossom season is from June to July. Fruits mature before the end of vegetation season.

2

2 Fruits small, 7x5 mm, with smooth, thin-walled surface, occasionally with longitudinal lines or weak ribs.

3

3 Leaves small, 50 x 30 mm. Leaf ovoid with shallow, cordate base. Peduncles very short (ca 10 mm).

T. kiusiana

3* Leaves large, <60 x 50 mm. Leaf orbicular or sub-orbicular with deep, cordate base. Peduncles longer < 30 mm.

4

4 Leaf with large, deep teeth; leaf often with additional lobes on the margin. Fruits very small, spherical (ca 4 mm), with smooth surfaces.

T. mandshurica

4* Leaf with small teeth; leaf margin without lobes. Fruits ovoid with longitudinal lines on the surface.

5

5 Leaves orbicular, tapering gradually to an apex.

6

6 Lower surface of leaf with sparsely stellate hairs. Simple hairs on veins only.

T. insularis

6* Lower surface of leaf glabrous, simple or double hairs on veins only.

T. mongolica

5* Leaves orbicular, tapering to the long and narrow apex.

7 Lower surface of leaf glabrous, with stellate hairs on the base only. Simple hairs on veins.

T. japonica

2* Fruits larger, 10 x 7 mm, elongated, with mammillate and/or tomentose walls. Fruit wall thick, difficult to break.

8

8 Fruits covered with dense tomentum, only with sparse mammilla.

9

9 Peduncle covered with stellate hairs.

T. maximoviciana

9* Peduncle glabrous.

T. migueliana

8* Fruits with numerous mammilla.

10

10 Leaf orbicular with small teeth—ca 7-8 teeth/2 cm of leaf-margin. Lower surface of leaf with silver tomentum, without patches of fasciculate hairs in the axils of main veins.

T. oliveri

10* Leaf ovate, shallowly serrate, teeth large—ca 4-5 teeth/2 cm of leaf margin. Lower surface of leaf green, sparsely covered with stellate hairs. Patches of fasciculate hairs in the axils of main veins.

Supplementary materials

Species/geographic boundaries and evolutionary interrelationships of cultivated Linden-trees (*Tilia* L.) based on morphological and nrDNA ITS characteristics

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Table 1. Summary statistics of morphological traits of leaves and fruits: mean/mode values, range, and standard deviation in cultivated *Tilia* species.

Type of traits	Continuous (C)				Percentage (P)					Discrete (D)			
Trait.	Mean	Min	Max	SD	Trait	Mean	Min	Max	SD	Trait.	Mode	Numerousness of mode '	Min	Max
Tilia americana L., n=30														
VN-LB	29.60	8	70	13.66	SH-LBL	0	0	0	0.00	BS-LB	1	29	1	2
L-LB	9.93	8	12	1.01	STH-LBL	0	0	0	0.00	E-LB	0	30	0	0
L-LP	87.17	55	152	21.12	SRH4-LBL	0	0	0	0.00	TS-LB	2	30	2	2
MW-LB	85.37	47	178	27.11	SRH6-LBL	0	0	0	0.00	H-LBU	0	30	0	0
AL-LB	42.13	30	67	8.93	SRH8-LBL	0	0	0	0.00	HV-LBU	1	23	0	1
TN-LB	5.50	3	9	1.36	SH-LBV	100	100	100	0.00	H-LBL	0	30	0	0
FL	10.03	7.52	11.69	0.87	STH-LBV	0	0	0	0.00	HC-LBL	0	30	0	0
FW	8.24	6.70	10.16	0.70	SRH4-LBV	0	0	0	0.00	HV-LBL	1	30	1	1
					SRH6-LBV	0	0	0	0.00	HCV-LBL	1	30	1	1
					SRH8-LBV	0	0	0	0.00	HF-LBL	2	23	2	3
					SH-P	50	0	100	50.85	HFC-LBL	1	30	1	1
					STH-P	0	0	0	0.00	HP	0	15	0	1
					SRH4-P	0	0	0	0.00	HC-P	0	15	0	1
					SRH6-P	0	0	0	0.00	FSO	1	30	1	1
					SRH8-P	0	0	0	0.00	FH	2	30	2	2
										FR	0	30	0	0
										FAC	2	30	2	2
T. caroliniana ssp. *heterophylla*, n=60														
VN-LB	11.45	9	16	1.61	SH-LBL	1	0	30	5.55	BS-LB	1	31	1	4
L-LB	175.43	62	336	74.82	STH-LBL	0	0	0	0.00	E-LB	0	60	0	0
L-LP	51.12	30	75	11.27	SRH4-LBL	6	0	60	13.80	TS-LB	2	60	2	2
MW-LB	123.72	61	231	42.59	SRH6-LBL	10	0	50	15.57	H-LBU	0	39	0	1

Type of traits	Continuous (C)				Percentage (P)						Discrete (D)			
Trait.	Mean	Min	Max	SD	Trait	Mean	Min	Max	SD	Trait.	Mode	Numerousness of mode '	Min	Max
AL-LB	67.45	26	186	25.86	SRH8-LBL	83	20	100	25.33	HV-LBU	0	59	0	1
TN-LB	5.25	2	7	1.07	SH-LBV	24	0	100	36.67	H-LBL	1	60	1	1
FL	9.49	6.45	12.40	1.45	STH-LBV	2	0	50	8.98	HC-LBL	1	60	1	1
FW	7.90	6.28	10.04	0.90	SRH4-LBV	0	0	0	0.00	HV-LBL	1	46	0	1
					SRH6-LBV	2	0	40	7.63	HCV-LBL	1	45	0	1
					SRH8-LBV	51	0	100	46.06	HF-LBL	2	60	2	2
					SH-P	0	0	0	0.00	HFC-LBL	1	60	1	1
					STH-P	0	0	0	0.00	HP	0	60	0	0
					SRH4-P	0	0	0	0.00	HC-P	0	60	0	0
					SRH6-P	0	0	0	0.00	FSO	2	60	2	2
					SRH8-P	0	0	0	0.00	FH	2	60	2	2
										FR	0	60	0	0
										FAC	1	60	1	1
Tilia amurensis, n=60														
VN-LB	6.70	5	8	0.72	SH-LBL	32	0	100	38.99	BS-LB	1	44	1	5
L-LB	80.68	47	106	14.46	STH-LBL	0	0	0	0.00	E-LB	0	58	0	1
L-LP	38.88	23	61	8.47	SRH4-LBL	10	0	60	20.71	TS-LB	1	37	1	2
MW-LB	66.35	48	86	8.99	SRH6-LBL	11	0	100	25.18	H-LBU	0	60	0	0
AL-LB	31.28	19	58	7.17	SRH8-LBL	0	0	0	0.00	HV-LBU	1	47	0	1
TN-LB	7.22	5	10	1.34	SH-LBV	93	0	100	21.48	H-LBL	1	32	0	1
FL	7.20	5.30	9.09	1.15	STH-LBV	1	0	10	2.20	HC-LBL	1	32	0	1
FW	4.47	3.02	6.61	1.03	SRH4-LBV	0	0	0	0.00	HV-LBL	1	58	0	1
					SRH6-LBV	1	0	60	7.83	HCV-LBL	1	58	0	1
					SRH8-LBV	2	0	70	10.10	HF-LBL	3	60	3	3
					SH-P	46	0	100	42.84	HFC-LBL	3	31	1	3
					STH-P	1	0	20	4.15	HP	1	60	1	1
					SRH4-P	2	0	30	5.96	HC-P	1	60	1	1
					SRH6-P	15	0	80	22.05	FSO	1	30	0	1
					SRH8-P	26	0	90	31.10	FH	2	60	2	2
										FR	2	60	2	2
										FAC	2	60	2	2
Tilia cordata, n-150														
VN-LB	6.57	4	11	1.47	SH-LBL	29	0	100	39.70	BS-LB	1	89	1	5
L-LB	64.85	25	125	24.58	STH-LBL	8	0	100	20.21	E-LB	0	120	0	1
L-LP	30.25	13	59	10.31	SRH4-LBL	2	0	20	4.69	TS-LB	1	105	1	2
MW-LB	58.16	26	112	20.93	SRH6-LBL	5	0	100	13.35	H-LBU	0	102	0	1
AL-LB	21.81	5	54	10.60	SRH8-LBL	11	0	100	21.47	HV-LBU	0	86	0	1
TN-LB	10.07	7	14	1.66	SH-LBV	61	0	100	41.63	H-LBL	1	83	0	1
FL	6.57	4.28	9.19	1.18	STH-LBV	19	0	100	30.48	HC-LBL	1	81	0	3
FW	4.81	3.48	7.39	0.60	SRH4-LBV	0	0	40	3.72	HV-LBL	1	126	0	1
					SRH6-LBV	3	0	80	9.39	HCV-LBL	1	123	0	1
					SRH8-LBV	1	0	40	4.21	HF-LBL	3	150	3	3
					SH-P	20	0	100	40.13	HFC-LBL	3	150	3	3
					STH-P	0	0	0	0.00	HP	0	120	0	1
					SRH4-P	0	0	0	0.00	HC-P	0	120	0	1
					SRH6-P	0	0	0	0.00	FSO	0	150	0	0
					SRH8-P	0	0	0	0.00	FH	2	150	2	2
										FR	2	60	0	2
										FAC	2	150	2	2
T. dasystyla, n=60														
VN-LB	9.27	7	11	1.02	SH-LBL	1	0	50	6.56	BS-LB	1	34	1	5
L-LB	101.23	61	135	18.37	STH-LBL	26	0	100	43.39	E-LB	1	35	0	1
L-LP	45.08	24	68	11.21	SRH4-LBL	0	0	0	0.00	TS-LB	2	48	1	2
MW-LB	91.88	55	128	16.65	SRH6-LBL	0	0	0	0.00	H-LBU	0	60	0	0
AL-LB	32.22	18	48	7.43	SRH8-LBL	0	0	0	0.00	HV-LBU	0	51	0	1
TN-LB	7.95	5	10	1.20	SH-LBV	23	0	100	27.40	H-LBL	0	44	0	1
FL	9.16	7.24	11.41	1.13	STH-LBV	59	0	100	37.54	HC-LBL	0	45	0	1
FW	6.12	4.97	8.13	0.62	SRH4-LBV	0	0	0	0.00	HV-LBL	1	48	0	1
					SRH6-LBV	0	0	0	0.00	HCV-LBL	1	49	0	1
					SRH8-LBV	0	0	0	0.00	HF-LBL	3	60	3	3
					SH-P	4	0	100	19.25	HFC-LBL	1	30	1	3
					STH-P	1	0	30	4.06	HP	0	57	0	1
					SRH4-P	0	0	0	0.00	HC-P	0	57	0	1
					SRH6-P	0	0	0	0.00	FSO	2	30	0	2
					SRH8-P	0	0	0	0.00	FH	2	60	2	2
										FR	2	60	2	2

Type of traits	Continuous (C)				Percentage (P)					Discrete (D)			
Trait.	Mean	Min	Max	SD	Trait	Mean	Min	Max	SD	Trait.	Mode	Numerousness of mode '	Min	Max
										FAC	2	60	2	2
T. x euchlora, n=60														
VN-LB	7.45	4	11	1.57	SH-LBL	6	0	100	18.17	BS-LB	1	42	1	5
L-LB	54.77	36	94	10.64	STH-LBL	30	0	100	44.55	E-LB	0	55	0	1
L-LP	31.83	20	51	6.67	SRH4-LBL	8	0	100	24.92	TS-LB	1	59	1	2
MW-LB	51.78	33	73	9.08	SRH6-LBL	25	0	50	25.21	H-LBU	0	30	0	1
AL-LB	18.43	7	37	6.40	SRH8-LBL	25	0	50	25.21	HV-LBU	1	40	0	1
TN-LB	9.58	7	12	1.24	SH-LBV	20	0	100	33.16	H-LBL	1	56	0	1
FL	8.28	6.82	9.87	0.60	STH-LBV	29	0	100	39.88	HC-LBL	1	56	0	1
FW	5.06	4.21	5.84	0.35	SRH4-LBV	0	0	0	0.00	HV-LBL	1	59	0	1
					SRH6-LBV	25	0	50	25.21	HCV-LBL	1	59	0	1
					SRH8-LBV	25	0	50	25.21	HF-LBL	3	30	0	3
					SH-P	1	0	40	5.16	HFC-LBL	0	30	0	3
					STH-P	1	0	60	7.75	HP	1	31	0	1
					SRH4-P	0	0	0	0.00	HC-P	1	31	0	1
					SRH6-P	25	0	50	25.21	FSO	0	60	0	0
					SRH8-P	25	0	50	25.21	FH	2	60	2	2
										FR	2	60	2	2
										FAC	1	60	1	1
T. x *flaccida*, n=30														
VN-LB	6.80	5	9	0.92	SH-LBL	0	0	0	0.00	BS-LB	1	25	1	5
L-LB	60.57	46	77	8.42	STH-LBL	1	0	20	3.65	E-LB	1	18	0	1
L-LP	34.23	23	58	8.22	SRH4-LBL	0	0	0	0.00	TS-LB	1	30	1	1
MW-LB	63.90	49	80	8.02	SRH6-LBL	0	0	0	0.00	H-LBU	1	16	0	1
AL-LB	21.53	13	32	4.22	SRH8-LBL	99	80	100	3.65	HV-LBU	1	28	0	1
TN-LB	10.57	7	14	1.65	SH-LBV	44	10	100	28.24	H-LBL	1	30	1	1
FL	-	-	-	-	STH-LBV	46	0	80	22.51	HC-LBL	1	17	1	3
FW	-	-	-	-	SRH4-LBV	0	0	0	0.00	HV-LBL	1	30	1	1
					SRH6-LBV	0	0	0	0.00	HCV-LBL	1	17	1	3
					SRH8-LBV	10	0	40	11.89	HF-LBL	3	30	3	3
					SH-P	74	0	100	37.83	HFC-LBL	3	30	3	3
					STH-P	5	0	10	5.09	HP	1	24	0	1
					SRH4-P	0	0	0	0.00	HC-P	1	24	0	1
					SRH6-P	0	0	0	0.00	FSO	-	-	-	-
					SRH8-P	1	0	10	3.46	FH	-	-	-	-
										FR	-	-	-	-
										FAC	-	-	-	-
T. henryana, n=60														
VN-LB	7.22	4	10	1.38	SH-LBL	0	0	10	1.81	BS-LB	1	40	1	2
L-LB	93.90	60	145	17.56	STH-LBL	0	0	0	0.00	E-LB	0	60	0	0
L-LP	44.92	32	67	6.40	SRH4-LBL	6	0	50	12.23	TS-LB	2	30	1	2
MW-LB	94.12	65	122	12.66	SRH6-LBL	11	0	40	15.12	H-LBU	1	60	1	1
AL-LB	29.88	10	58	10.80	SRH8-LBL	83	40	100	21.10	HV-LBU	1	60	1	1
TN-LB	3.53	2	5	0.60	SH-LBV	32	0	60	13.08	H-LBL	1	60	1	1
FL	-	-	-	-	STH-LBV	0	0	0	0.00	HC-LBL	1	60	1	1
FW	-	-	-	-	SRH4-LBV	0	0	0	0.00	HV-LBL	1	60	1	1
					SRH6-LBV	6	0	30	9.74	HCV-LBL	1	60	1	1
					SRH8-LBV	62	40	100	11.12	HF-LBL	2	59	2	3
					SH-P	29	0	90	21.11	HFC-LBL	1	59	1	3
					STH-P	0	0	0	0.00	HP	1	60	1	1
					SRH4-P	0	0	20	2.58	HC-P	1	60	1	1
					SRH6-P	10	0	90	17.94	FSO	-	-	-	-
					SRH8-P	61	0	100	18.85	FH	-	-	-	-
										FR	-	-	-	-
										FAC	-	-	-	-
T. insularis, n=60														
VN-LB	7.42	6	10	0.93	SH-LBL	40	0	100	49.02	BS-LB	1	34	1	5
L-LB	77.45	46	127	20.89	STH-LBL	0	0	0	0.00	E-LB	0	59	0	1
L-LP	39.08	21	68	13.28	SRH4-LBL	0	0	0	0.00	TS-LB	1	38	1	2
MW-LB	66.82	40	103	14.41	SRH6-LBL	43	0	100	49.97	H-LBU	0	43	0	1
AL-LB	29.23	10	58	14.90	SRH8-LBL	0	0	10	1.81	HV-LBU	1	48	0	1
TN-LB	8.58	5	14	2.29	SH-LBV	49	0	100	47.79	H-LBL	1	50	0	1
FL	5.91	4.69	7.16	0.64	STH-LBV	30	0	100	37.57	HC-LBL	1	48	0	1
FW	3.68	2.73	4.61	0.50	SRH4-LBV	0	0	0	0.00	HV-LBL	1	60	1	1
					SRH6-LBV	20	0	90	30.75	HCV-LBL	1	60	1	1
					SRH8-LBV	2	0	20	4.43	HF-LBL	3	60	3	3
					SH-P	11	0	50	13.37	HFC-LBL	3	60	3	3
					STH-P	2	0	10	4.27	HP	1	56	0	1

Type of traits	Continuous (C)				Percentage (P)					Discrete (D)			
Trait.	Mean	Min	Max	SD	Trait	Mean	Min	Max	SD	Trait.	Mode	Numerousness of mode '	Min	Max
					SRH4-P	0	0	0	0.00	HC-P	1	28	0	3
					SRH6-P	1	0	30	5.03	FSO	2	30	1	2
					SRH8-P	79	0	100	24.87	FH	2	60	2	2
										FR	2	30	0	2
										FAC	2	60	2	2
T. japonica, n=60														
VN-LB	5.82	4	8	0.75	SH-LBL	0	0	0	0.00	BS-LB	1	40	1	5
L-LB	75.97	44	112	15.96	STH-LBL	0	0	0	0.00	E-LB	0	59	0	1
L-LP	30.10	20	48	5.54	SRH4-LBL	49	0	100	49.57	TS-LB	1	59	1	2
MW-LB	52.87	37	74	8.57	SRH6-LBL	2	0	50	8.20	H-LBU	0	60	0	0
AL-LB	23.40	11	39	5.79	SRH8-LBL	0	0	0	0.00	HV-LBU	1	38	0	1
TN-LB	7.22	5	10	1.53	SH-LBV	61	0	100	39.58	H-LBL	1	30	0	1
FL	6.43	4.50	8.45	1.17	STH-LBV	20	0	80	31.89	HC-LBL	1	30	0	1
FW	4.43	3.35	6.65	0.77	SRH4-LBV	5	0	50	12.00	HV-LBL	1	55	0	1
					SRH6-LBV	6	0	80	16.91	HCV-LBL	1	55	0	1
					SRH8-LBV	0	0	0	0.00	HF-LBL	3	57	0	3
					SH-P	37	0	100	43.52	HFC-LBL	3	58	0	3
					STH-P	1	0	30	4.54	HP	1	49	0	1
					SRH4-P	26	0	100	35.48	HC-P	1	49	0	1
					SRH6-P	17	0	100	27.20	FSO	0	30	0	1
					SRH8-P	1	0	50	6.66	FH	2	60	2	2
										FR	2	30	0	2
										FAC	2	60	2	2
T. kiusiana, n=60														
VN-LB	6.53	4	9	1.14	SH-LBL	20	0	100	38.58	BS-LB	1	48	1	5
L-LB	49.23	30	66	7.20	STH-LBL	0	0	0	0.00	E-LB	0	59	0	1
L-LP	9.15	5	13	1.84	SRH4-LBL	3	0	100	17.24	TS-LB	1	60	1	1
MW-LB	28.27	15	36	3.91	SRH6-LBL	0	0	0	0.00	H-LBU	1	30	0	1
AL-LB	21.05	13	38	4.90	SRH8-LBL	7	0	100	22.82	HV-LBU	1	59	0	1
TN-LB	10.83	7	14	1.43	SH-LBV	100	90	100	1.29	H-LBL	0	42	0	1
FL	-	-	-	-	STH-LBV	0	0	0	0.00	HC-LBL	0	42	0	1
FW	-	-	-	-	SRH4-LBV	0	0	0	0.00	HV-LBL	1	60	1	1
					SRH6-LBV	0	0	0	0.00	HCV-LBL	1	60	1	1
					SRH8-LBV	0	0	10	1.29	HF-LBL	1	34	1	3
					SH-P	99	90	100	2.52	HFC-LBL	3	54	1	3
					STH-P	0	0	0	0.00	HP	1	60	1	1
					SRH4-P	0	0	0	0.00	HC-P	1	60	1	1
					SRH6-P	0	0	0	0.00	FSO	-	-	-	-
					SRH8-P	1	0	10	2.52	FH	-	-	-	-
										FR	-	-	-	-
										FAC	-	-	-	-
T. mandshurica, n=30														
VN-LB	6.93	5	8	0.78	SH-LBL	43	0	90	17.65	BS-LB	1	17	1	5
L-LB	59.00	36	82	10.83	STH-LBL	0	0	10	1.83	E-LB	0	16	0	1
L-LP	30.37	2	44	7.95	SRH4-LBL	50	10	80	14.26	TS-LB	2	30	2	2
MW-LB	62.23	40	90	10.75	SRH6-LBL	7	0	40	8.77	H-LBU	1	16	0	1
AL-LB	22.17	11	53	7.67	SRH8-LBL	0	0	0	0.00	HV-LBU	1	22	0	1
TN-LB	6.13	4	9	1.01	SH-LBV	65	0	100	28.97	H-LBL	1	30	1	1
FL	4.44	3.78	5.57	0.43	STH-LBV	15	0	80	20.80	HC-LBL	1	30	1	1
FW	4.04	3.45	5.29	0.41	SRH4-LBV	15	0	60	17.76	HV-LBL	1	29	0	1
					SRH6-LBV	2	0	40	8.05	HCV-LBL	1	30	1	1
					SRH8-LBV	0	0	0	0.00	HF-LBL	3	30	3	3
					SH-P	76	0	100	23.13	HFC-LBL	3	30	3	3
					STH-P	11	0	80	16.80	HP	1	30	1	1
					SRH4-P	12	0	60	14.00	HC-P	1	30	1	1
					SRH6-P	1	0	40	7.30	FSO	0	30	0	0
					SRH8-P	0	0	0	0.00	FH	2	30	2	2
										FR	2	30	2	2
										FAC	0	30	0	0
T. maximowicziana, n=60														
VN-LB	6.95	5	9	0.85	SH-LBL	0	0	0	0.00	BS-LB	1	60	1	1
L-LB	69.03	46	102	12.23	STH-LBL	0	0	0	0.00	E-LB	0	60	0	0
L-LP	41.02	23	57	7.29	SRH4-LBL	0	0	0	0.00	TS-LB	1	54	1	2
MW-LB	66.00	49	86	8.86	SRH6-LBL	0	0	0	0.00	H-LBU	0	32	0	1
AL-LB	21.93	10	38	6.19	SRH8-LBL	100	100	100	0.00	HV-LBU	1	60	1	1
TN-LB	6.68	5	10	0.97	SH-LBV	12	0	30	8.92	H-LBL	1	60	1	1
FL	8.66	6.36	10.33	0.88	STH-LBV	0	0	0	0.00	HC-LBL	1	60	1	1
FW	6.84	5.61	8.23	0.54	SRH4-LBV	0	0	0	0.00	HV-LBL	1	60	1	1

Type of traits	Continuous (C)				Percentage (P)					Discrete (D)			
Trait.	Mean	Min	Max	SD	Trait	Mean	Min	Max	SD	Trait.	Mode	Numerousness of mode '	Min	Max
					SRH6-LBV	0	0	0	0.00	HCV-LBL	1	60	1	1
					SRH8-LBV	88	70	100	8.92	HF-LBL	3	60	3	3
					SH-P	1	0	10	2.79	HFC-LBL	3	60	3	3
					STH-P	1	0	30	4.90	HP	1	60	1	1
					SRH4-P	0	0	0	0.00	HC-P	1	60	1	1
					SRH6-P	0	0	0	0.00	FSO	2	30	0	2
					SRH8-P	98	70	100	5.46	FH	2	30	2	3
										FR	0	60	0	0
										FAC	2	60	2	2
T. americana x. *moltkei*, n=30														
VN-LB	18.53	5	36	7.93	SH-LBL	0	0	0	0.00	BS-LB	1	29	1	5
L-LB	7.90	6	9	0.84	STH-LBL	0	0	0	0.00	E-LB	0	30	0	0
L-LP	65.23	43	92	14.31	SRH4-LBL	0	0	0	0.00	TS-LB	2	18	1	2
MW-LB	60.07	24	85	14.21	SRH6-LBL	0	0	0	0.00	H-LBU	0	24	0	1
AL-LB	38.67	22	54	9.11	SRH8-LBL	100	100	100	0.00	HV-LBU	0	29	0	1
TN-LB	6.77	4	10	1.19	SH-LBV	79	0	100	34.01	H-LBL	1	30	1	1
FL	7.54	6.19	8.91	0.59	STH-LBV	0	0	0	0.00	HC-LBL	1	30	1	1
FW	7.32	6.26	8.49	0.48	SRH4-LBV	0	0	0	0.00	HV-LBL	1	27	0	1
					SRH6-LBV	0	0	0	0.00	HCV-LBL	1	27	0	1
					SRH8-LBV	11	0	100	21.45	HF-LBL	0	24	0	2
					SH-P	30	0	100	46.61	HFC-LBL	0	24	0	1
					STH-P	0	0	0	0.00	HP	0	21	0	1
					SRH4-P	0	0	0	0.00	HC-P	0	22	0	1
					SRH6-P	0	0	0	0.00	FSO	2	30	2	2
					SRH8-P	0	0	0	0.00	FH	2	30	2	2
										FR	1	30	1	1
										FAC	2	30	2	2
T. mongolica, n=60														
VN-LB	6.50	5	9	0.93	SH-LBL	5	0	100	17.02	BS-LB	1	46	1	5
L-LB	67.00	34	106	20.36	STH-LBL	2	0	100	12.91	E-LB	0	51	0	1
L-LP	29.68	21	45	5.18	SRH4-LBL	14	0	100	30.24	TS-LB	1	42	1	2
MW-LB	47.47	32	71	8.72	SRH6-LBL	8	0	100	22.31	H-LBU	0	60	0	0
AL-LB	21.22	14	29	3.69	SRH8-LBL	10	0	100	29.34	HV-LBU	0	52	0	1
TN-LB	7.05	6	9	0.79	SH-LBV	65	0	100	24.67	H-LBL	0	37	0	1
FL	6.18	5.21	7.08	0.39	STH-LBV	28	0	70	18.94	HC-LBL	0	36	0	1
FW	4.72	4.24	5.37	0.27	SRH4-LBV	0	0	0	0.00	HV-LBL	1	56	0	1
					SRH6-LBV	0	0	0	0.00	HCV-LBL	1	56	0	1
					SRH8-LBV	0	0	10	1.29	HF-LBL	3	30	1	3
					SH-P	0	0	0	0.00	HFC-LBL	3	60	3	3
					STH-P	0	0	0	0.00	HP	0	48	0	1
					SRH4-P	3	0	100	15.13	HC-P	0	48	0	1
					SRH6-P	15	0	100	33.62	FSO	1	30	0	1
					SRH8-P	2	0	30	6.59	FH	2	60	2	2
										FR	2	60	2	2
										FAC	2	30	1	2
T. miqueliana, n=30														
VN-LB	7.93	6	11	1.20	SH-LBL	0	0	0	0.00	BS-LB	1	25	1	5
L-LB	75.17	53	106	14.40	STH-LBL	0	0	0	0.00	E-LB	0	30	0	0
L-LP	33.13	20	53	9.18	SRH4-LBL	0	0	0	0.00	TS-LB	1	19	1	2
MW-LB	68.60	43	102	14.00	SRH6-LBL	0	0	0	0.00	H-LBU	0	30	0	0
AL-LB	22.10	10	42	7.88	SRH8-LBL	100	100	100	0.00	HV-LBU	0	16	0	1
TN-LB	8.10	6	10	1.09	SH-LBV	31	0	100	15.61	H-LBL	1	30	1	1
FL	9.44	7.89	10.51	0.59	STH-LBV	28	0	40	18.64	HC-LBL	1	30	1	1
FW	6.45	5.60	8.10	0.51	SRH4-LBV	0	0	0	0.00	HV-LBL	1	29	0	1
					SRH6-LBV	0	0	0	0.00	HCV-LBL	1	29	0	1
					SRH8-LBV	38	0	100	21.44	HF-LBL	3	30	3	3
					SH-P	10	0	90	25.66	HFC-LBL	3	30	3	3
					STH-P	3	0	40	9.52	HP	0	26	0	1
					SRH4-P	0	0	0	0.00	HC-P	0	26	0	1
					SRH6-P	0	0	0	0.00	FSO	0	30	0	0
					SRH8-P	1	0	10	2.54	FH	3	30	3	3
										FR	0	30	0	0
										FAC	2	30	2	2
T. oliveri, n=47														
VN-LB	7.66	6	10	1.03	SH-LBL	0	0	0	0.00	BS-LB	1	23	1	5
L-LB	70.96	48	105	15.30	STH-LBL	0	0	0	0.00	E-LB	0	47	0	0
L-LP	52.02	15	78	12.92	SRH4-LBL	0	0	0	0.00	TS-LB	1	31	1	2
MW-LB	68.43	47	95	12.53	SRH6-LBL	36	0	100	48.57	H-LBU	0	25	0	1

Type of traits	Continuous (C)				Percentage (P)						Discrete (D)			
Trait.	Mean	Min	Max	SD	Trait	Mean	Min	Max	SD	Trait.	Mode	Numerousness of mode '	Min	Max
AL-LB	26.30	17	40	5.57	SRH8-LBL	64	0	100	48.57	HV-LBU	0	24	0	1
TN-LB	6.47	5	9	1.00	SH-LBV	6	0	10	4.86	H-LBL	1	47	1	1
FL	9.17	7.25	12.85	1.43	STH-LBV	0	0	0	0.00	HC-LBL	1	47	1	1
FW	6.24	5.02	7.88	0.61	SRH4-LBV	0	0	0	0.00	HV-LBL	1	47	1	1
					SRH6-LBV	36	0	100	48.57	HCV-LBL	1	47	1	1
					SRH8-LBV	57	0	90	43.71	HF-LBL	0	47	0	0
					SH-P	0	0	0	0.00	HFC-LBL	0	47	0	0
					STH-P	0	0	0	0.00	HP	1	30	0	1
					SRH4-P	0	0	0	0.00	HC-P	1	30	0	1
					SRH6-P	0	0	0	0.00	FSO	2	47	2	2
					SRH8-P	64	0	100	48.57	FH	2	47	2	2
										FR	2	30	0	2
										FAC	2	47	2	2
T. platyphyllos, n=90														
VN-LB	25.47	11	47	6.33	SH-LBL	75	0	100	27.48	BS-LB	1	54	1	5
L-LB	8.86	5	16	2.06	STH-LBL	3	0	40	9.63	E-LB	0	82	0	1
L-LP	79.51	39	119	16.24	SRH4-LBL	8	0	90	19.67	TS-LB	2	61	1	2
MW-LB	92.47	58	165	23.05	SRH6-LBL	12	0	100	21.37	H-LBU	1	77	0	1
AL-LB	37.72	19	79	11.47	SRH8-LBL	0	0	10	1.05	HV-LBU	1	82	0	1
TN-LB	8.19	5	12	1.19	SH-LBV	91	0	100	18.07	H-LBL	1	89	0	1
FL	9.00	6.56	10.70	0.68	STH-LBV	7	0	100	16.33	HC-LBL	1	89	0	1
FW	8.57	6.03	10.22	0.81	SRH4-LBV	0	0	10	1.05	HV-LBL	1	90	1	1
					SRH6-LBV	2	0	50	8.32	HCV-LBL	1	90	1	1
					SRH8-LBV	0	0	0	0.00	HF-LBL	3	90	3	3
					SH-P	88	0	100	21.68	HFC-LBL	1	90	1	1
					STH-P	6	0	60	9.91	HP	1	86	0	1
					SRH4-P	0	0	0	0.00	HC-P	1	86	0	1
					SRH6-P	1	0	20	3.81	FSO	2	60	0	2
					SRH8-P	0	0	0	0.00	FH	2	36	2	3
										FR	3	36	1	3
										FAC	2	36	0	2
T. platyphyllos Laciniata, n=30														
VN-LB	14.40	4	35	6.88	SH-LBL	97	80	100	5.83	BS-LB	2	13	1	4
L-LB	101.53	35	164	35.41	STH-LBL	0	0	0	0.00	E-LB	1	26	0	1
L-LP	45.90	29	65	8.63	SRH4-LBL	0	0	10	1.83	TS-LB	2	30	2	2
MW-LB	54.67	32	78	11.92	SRH6-LBL	1	0	10	2.54	H-LBU	1	30	1	1
AL-LB	70.97	11	142	31.96	SRH8-LBL	2	0	10	3.79	HV-LBU	1	30	1	1
TN-LB	4.97	3	7	1.25	SH-LBV	99	90	100	2.54	H-LBL	1	30	1	1
FL	6.49	4.00	7.42	0.77	STH-LBV	0	0	10	1.83	HC-LBL	1	30	1	1
FW	5.81	4.09	7.14	0.62	SRH4-LBV	0	0	0	0.00	HV-LBL	1	30	1	1
					SRH6-LBV	0	0	0	0.00	HCV-LBL	1	30	1	1
					SRH8-LBV	0	0	10	1.83	HF-LBL	3	16	1	3
					SH-P	75	10	100	31.92	HFC-LBL	1	30	1	1
					STH-P	0	0	0	0.00	HP	1	30	1	1
					SRH4-P	11	0	80	18.18	HC-P	1	30	1	1
					SRH6-P	13	0	70	19.36	FSO	0	30	0	0
					SRH8-P	1	0	20	5.07	FH	2	30	2	2
										FR	2	30	2	2
										FAC	2	30	2	2
T. platyphyllos Vitifolia, n=30														
VN-LB	8.23	6	11	1.10	SH-LBL	12	0	100	27.05	BS-LB	1	30	1	1
L-LB	69.00	49	103	14.14	STH-LBL	45	0	100	47.18	E-LB	1	15	0	1
L-LP	27.40	18	43	6.43	SRH4-LBL	0	0	0	0.00	TS-LB	1	30	1	1
MW-LB	60.90	47	82	9.68	SRH6-LBL	0	0	0	0.00	H-LBU	0	30	0	0
AL-LB	25.87	18	37	4.94	SRH8-LBL	0	0	0	0.00	HV-LBU	1	25	0	1
TN-LB	10.77	9	14	1.41	SH-LBV	18	0	100	28.70	H-LBL	1	17	0	1
FL	7.33	6.54	8.19	0.48	STH-LBV	82	0	100	28.70	HC-LBL	1	16	0	1
FW	6.22	5.33	8.24	0.62	SRH4-LBV	0	0	0	0.00	HV-LBL	1	30	1	1
					SRH6-LBV	0	0	0	0.00	HCV-LBL	1	30	1	1
					SRH8-LBV	0	0	0	0.00	HF-LBL	3	30	3	3
					SH-P	80	0	100	40.68	HFC-LBL	3	30	3	3
					STH-P	0	0	0	0.00	HP	1	24	0	1
					SRH4-P	0	0	0	0.00	HC-P	1	24	0	1
					SRH6-P	0	0	0	0.00	FSO	1	30	1	1
					SRH8-P	0	0	0	0.00	FH	3	30	3	3
										FR	3	30	3	3
										FAC	1	30	1	1

Type of traits	Continuous (C)				Percentage (P)					Discrete (D)			
Trait.	Mean	Min	Max	SD	Trait	Mean	Min	Max	SD	Trait.	Mode	Numerousness of mode '	Min	Max
T. x *spaethi*, n=30														
VN-LB	8.13	5	9	1.01	SH-LBL	33	0	100	21.96	BS-LB	1	28	1	5
L-LB	96.40	49	131	18.90	STH-LBL	0	0	0	0.00	E-LB	0	30	0	0
L-LP	31.50	17	45	6.31	SRH4-LBL	33	0	70	16.01	TS-LB	1	30	1	1
MW-LB	69.93	35	94	11.75	SRH6-LBL	26	0	50	12.51	H-LBU	0	29	0	1
AL-LB	22.57	13	32	4.78	SRH8-LBL	9	0	30	11.06	HV-LBU	1	20	0	1
TN-LB	10.20	9	12	0.89	SH-LBV	19	0	60	20.90	H-LBL	1	30	1	1
FL	4.55	3.46	8.31	1.27	STH-LBV	9	0	40	12.52	HC-LBL	1	30	1	1
FW	4.19	3.02	5.65	0.72	SRH4-LBV	29	0	50	14.70	HV-LBL	1	30	1	1
					SRH6-LBV	38	10	100	20.01	HCV-LBL	1	30	1	1
					SRH8-LBV	5	0	40	10.42	HF-LBL	3	30	3	3
					SH-P	3	0	20	6.91	HFC-LBL	3	30	3	3
					STH-P	0	0	0	0.00	HP	1	22	0	1
					SRH4-P	32	0	100	32.70	HC-P	1	22	0	1
					SRH6-P	28	0	100	29.87	FSO	0	30	0	0
					SRH8-P	11	0	100	21.39	FH	2	30	2	2
										FR	2	30	2	2
										FAC	2	30	2	2
T. tomentosa, n=90														
VN-LB	19.81	1	41	6.14	SH-LBL	0	0	0	0.00	BS-LB	1	61	1	5
L-LB	8.39	6	12	1.13	STH-LBL	0	0	0	0.00	E-LB	0	59	0	1
L-LP	62.09	32	147	16.18	SRH4-LBL	0	0	0	0.00	TS-LB	1	61	1	2
MW-LB	61.72	14	137	15.32	SRH6-LBL	17	0	50	23.70	H-LBU	1	88	0	1
AL-LB	30.36	15	51	7.36	SRH8-LBL	83	50	100	23.70	HV-LBU	1	87	0	1
TN-LB	9.26	6	14	1.43	SH-LBV	3	0	10	4.50	H-LBL	1	90	1	1
FL	8.98	7.37	10.73	0.68	STH-LBV	3	0	10	4.74	HC-LBL	1	90	1	1
FW	5.68	4.28	6.86	0.54	SRH4-LBV	0	0	0	0.00	HV-LBL	1	90	1	1
					SRH6-LBV	17	0	50	23.70	HCV-LBL	1	90	1	1
					SRH8-LBV	77	50	100	19.49	HF-LBL	0	90	0	0
					SH-P	4	0	10	4.85	HFC-LBL	0	90	0	0
					STH-P	0	0	0	0.00	HP	1	90	1	1
					SRH4-P	0	0	0	0.00	HC-P	1	90	1	1
					SRH6-P	17	0	50	23.70	FSO	2	30	0	2
					SRH8-P	80	50	100	21.49	FH	3	60	2	3
										FR	2	90	2	2
										FAC	2	60	1	2
T. tomentosa Varsaviensis, n=60														
VN-LB	8.62	5	10	1.08	SH-LBL	0	0	10	1.81	BS-LB	1	45	1	5
L-LB	66.87	29	89	10.79	STH-LBL	0	0	0	0.00	E-LB	0	50	0	1
L-LP	39.92	12	65	10.38	SRH4-LBL	0	0	0	0.00	TS-LB	1	44	1	2
MW-LB	65.05	29	87	11.13	SRH6-LBL	0	0	0	0.00	H-LBU	1	44	0	1
AL-LB	28.52	14	47	6.38	SRH8-LBL	100	90	100	1.81	HV-LBU	0	34	0	1
TN-LB	8.27	5	11	1.55	SH-LBV	5	0	10	5.03	H-LBL	1	60	1	1
FL	7.47	6.27	8.92	0.63	STH-LBV	0	0	0	0.00	HC-LBL	1	60	1	1
FW	6.41	5.33	7.46	0.45	SRH4-LBV	0	0	0	0.00	HV-LBL	1	55	0	1
					SRH6-LBV	0	0	0	0.00	HCV-LBL	1	55	0	1
					SRH8-LBV	86	0	100	26.68	HF-LBL	0	60	0	0
					SH-P	0	0	0	0.00	HFC-LBL	0	60	0	0
					STH-P	0	0	0	0.00	HP	1	56	0	1
					SRH4-P	0	0	0	0.00	HC-P	1	56	0	1
					SRH6-P	0	0	0	0.00	FSO	1	30	0	2
					SRH8-P	93	0	100	25.15	FH	3	60	3	3
										FR	2	30	1	2
										FAC	2	60	2	2
T. tuan, n=30														
VN-LB	7.83	5	10	1.39	SH-LBL	0	0	0	0.00	BS-LB	1	30	1	1
L-LB	88.10	43	135	27.17	STH-LBL	0	0	0	0.00	E-LB	0	30	0	0
L-LP	41.50	26	56	7.07	SRH4-LBL	0	0	0	0.00	TS-LB	1	30	1	1
MW-LB	70.47	36	105	16.70	SRH6-LBL	48	0	100	36.11	H-LBU	1	29	0	1
AL-LB	40.50	21	64	12.39	SRH8-LBL	52	0	100	36.11	HV-LBU	1	29	0	1
TN-LB	3.70	2	6	0.95	SH-LBV	4	0	20	5.68	H-LBL	1	30	1	1
FL	9.55	8.90	10.61	0.46	STH-LBV	0	0	10	1.83	HC-LBL	1	30	1	1
FW	7.10	6.62	8.31	0.39	SRH4-LBV	0	0	0	0.00	HV-LBL	1	30	1	1
					SRH6-LBV	51	0	100	37.82	HCV-LBL	1	30	1	1
					SRH8-LBV	44	0	100	35.40	HF-LBL	2	30	2	2
					SH-P	1	0	10	3.05	HFC-LBL	1	30	1	1
					STH-P	0	0	0	0.00	HP	1	30	1	1
					SRH4-P	0	0	0	0.00	HC-P	1	30	1	1

Type of traits	Continuous (C)				Percentage (P)					Discrete (D)			
Trait.	Mean	Min	Max	SD	Trait	Mean	Min	Max	SD	Trait.	Mode	Numerousness of mode '	Min	Max
					SRH6-P	52	0	100	33.88	FSO	2	30	2	2
					SRH8-P	47	0	100	33.65	FH	3	30	3	3
										FR	0	30	0	0
										FAC	2	30	2	2
T. americana x *moltkei* Zamoyskiana, n=30														
VN-LB	8.83	7	11	0.95	SH-LBL	77	60	90	7.11	BS-LB	1	30	1	1
L-LB	72.53	50	114	16.98	STH-LBL	0	0	0	0.00	E-LB	0	30	0	0
L-LP	42.43	28	72	11.66	SRH4-LBL	1	0	10	2.54	TS-LB	1	21	1	2
MW-LB	72.17	51	109	13.79	SRH6-LBL	3	0	20	5.21	H-LBU	0	30	0	0
AL-LB	26.63	20	40	4.87	SRH8-LBL	20	10	40	7.43	HV-LBU	0	18	0	1
TN-LB	7.10	5	9	0.92	SH-LBV	21	10	70	15.96	H-LBL	1	30	1	1
FL	7.78	3.88	9.59	1.18	STH-LBV	0	0	0	0.00	HC-LBL	1	30	1	1
FW	7.48	5.30	8.83	0.79	SRH4-LBV	0	0	10	1.83	HV-LBL	1	30	1	1
					SRH6-LBV	10	0	50	12.73	HCV-LBL	1	30	1	1
					SRH8-LBV	69	10	90	22.09	HF-LBL	0	30	0	0
					SH-P	2	0	40	8.17	HFC-LBL	0	30	0	0
					STH-P	0	0	0	0.00	HP	0	18	0	1
					SRH4-P	0	0	0	0.00	HC-P	0	18	0	1
					SRH6-P	3	0	30	7.50	FSO	1	30	1	1
					SRH8-P	35	0	100	44.08	FH	3	30	3	3
										FR	1	30	1	1
										FAC	2	30	2	2

Supplementary materials

Species/geographic boundaries and evolutionary interrelationships of cultivated Linden-trees (*Tilia* L.) based on morphological and nrDNA ITS characteristics

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Table 2. Characteristics of the aligned ITS data matrix used for phylogenetic analysis

A: for the whole data set (n = 39 with hybrids (upper values); n = 32 without hybrids (lower values) and B: for a small subset of ITS data obtained by molecular cloning (n = 18 with hybrids (upper values); n = 15 without hybrids (lower values); [a] Tamura-Nei model, complete deletion gaps treatment; alignment-ambiguous region positions: 372–375 was removed.

A

Nucleotide composition (%)	ITS1 1-192 bp	5.8S 193- 351 bp	ITS2 352-580 bp	ITS1-5.8S-ITS2
T	19.3 19.3	20.7 20.7	19.5 19.5	19.8 19.8
C	30.6 30.7	27.7 27.7	35.1 35.1	31.6 31.6
A	20.5 20.5	23.3 23.3	16.2 16.1	19.6 19.6
G	29.5 29.5	28.3 28.2	29.2 29.2	29.0 29.0
total	186.8 187.4	158.6 158.7	221.8 221.8	567.2 568.1
Total length	192	159	229	580
Conserved sites	148 150	146 146	198 200	492 496
Variable sites	42 40	13 13	29 27	84 80
Parsimony informative sites	24 24	0 0	12 11	36 35
Singleton	18	13	17	48

	16	13	16	45
G+C content range	60.1 60.2	56 55.9	64.3 64.3	60.6 60.6
Identical pairs	176.00 177.00	158.00 158.00	216.00 216.00	550.00 551.00
R=si/sv	2.34 2.26	2.25 2.25	3.22 3.42	2.56 2.53
Overall transition/transversion bias [a]	1.391 1.352	29.851 29.657	12.861 10.815	2.432 2.210

B

Nucleotide composition (%)	**ITS1 1-190 bp**	**5.8S 191- 349 bp**	**ITS2 350-579 bp**	**ITS1-5.8S-ITS2**
T	19.2 18.8	20.5 20.5	20.2 20.1	19.9 19.8
C	31.1 31.4	28.0 28.0	35.1 35.2	31.8 31.9
A	19.9 20.0	23.2 23.3	15.9 15.8	19.2 19.3
G	29.7 29.8	28.4 28.3	28.8 28.9	29.0 29.0
total	190.0 190.0	158.9 158.9	226.3 226.4	575.2 575.3
Total length	190	159	230	579
Conserved sites	156 159	147 151	198 200	501 510
Variable sites	34 31	12 8	31 29	77 68
Parsimony informative sites	21 21	1 1	14 12	36 34
Singleton	13 10	11 7	17 17	41 34
G+C content range	60.9 61.2	56.3 56.3	63.9 64.1	60.8 61.0
Identical pairs	179.00 179.00	157.00 158.00	218.00 218.00	555.00 555.00
R=si/sv	3.87 4.38	nc nc	3.51 3.29	4.09 4.18
Overall transition/transversion bias [a]	4.2 4.781	378.948 299.067	3.637 3.413	4.256 4.361

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Author Contributions

Conceived and designed research: IM, MC, KW; performed the molecular analyses: KW, GK, IM; performed the morphological research: MC; analyzed the data: IM, KW, MC; wrote the paper (text): IM; provided the tables and drawings: KW, MC, IM; provided the key: MC, IM.

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