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**Title: Urban tree management: diversity of *Tilia* genus in streets and parks of Paris
based on morphological and genetic characteristics**

Iry Andrianjara ^{a,b}, Marianne Bordenave-Jacquemin^a, Virginie Roy ^a, Cécile Cabassa ^a, Pierre Federici ^a, David Carmignac ^a, Yoan Marcangeli ^a, Germinal Rouhan ^c, Mathilde Renard ^b, François Nold ^b, Jean-Christophe Lata ^{a,d}, Patricia Genet ^{a,*}, Séverine Planchais ^{a,*}.

^a Sorbonne Université, IRD, CNRS, INRAE, Université de Paris, UPEC, UMR 7618, Institute of Ecology and Environmental Sciences – Paris, Tour 44-45, 4 place Jussieu, 75005 Paris, France

^b Soil and Plant Expertise Division (DESV), Plant Science and Technology - Urban Agriculture Service (SSTV-AU), Paris Green Space and Environmental Department (DEVE), Ville de Paris, Parc Floral - Pavillon 5 - Rond-Point de la Pyramide, 75012 Paris, France

^c Institut de Systématique, Evolution, Biodiversité (ISYEB), Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, École Pratique des Hautes Études, Université des Antilles, 57 rue Cuvier CP39, 75005 Paris, France

^d Department of Geocology and Geochemistry, Institute of Natural Resources, Tomsk Polytechnic University, 30, Lenin Street, Tomsk 634050, Russia

(*) Authors equally supervised this work

Corresponding author: Iry Andrianjara. E-mail: iry.andrianjara@paris.fr; Phone: +331 44 27

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Author's contribution

I.A. led the writing of the manuscript, acquired, analysed and interpreted data; M.B-J. analysed, determined morphological identification, wrote and revised manuscript; V.R. analysed and interpreted genetic data; G.R. contributed to morphological identification and revised manuscript; C.C. and J-C.L. designed sampling and revised manuscript; P.F. contributed to the acquisition and analysis of genetic data; D.C. contributed to harvest and prepare samples; Y.M. contributed to harvest and prepare samples and conceived graphical abstract; M.R. and F.N. revised manuscript; P.G. and S.P. conceived the ideas and designed methodology, harvested samples, analysed and interpreted data, contributed to the writing and supervised the research group. All authors gave final approval for publication.

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1 **Article type: Research Article**

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3 **based on morphological and genetic characteristics**

4

5

6 Abstract

- 7 1. The development of tree inventories and open data bases provides green space managers a
8 better understanding of tree morphological diversity in urban areas. However, studies on
9 tree genetic diversity in cities remain rare.
- 10 2. The aim of our study is to provide better knowledge on tree intra- and interspecific
11 diversity to improve tree management in urban areas. We characterized 83 individuals of
12 *Tilia* species and clones in streets and parks in Paris (France) based on both
13 morphological characters (leaves, fruits and twigs) and genetic profiling by using nuclear
14 microsatellites markers.
- 15 3. We found five species through morphological identification: *Tilia cordata*, *Tilia dasystyla*,
16 *Tilia ×euchlora*, *Tilia ×europaea* and *Tilia platyphyllos*. Analysis of genetic profiles
17 allowed to distinguish three genetic groups correlated to *T. cordata*, *T. ×euchlora* and *T.*
18 *platyphyllos*. *T. ×euchlora* had the largest number of the same clonal profile (20
19 individuals out of 36) in Paris while no clone was found in *T. platyphyllos* group.
- 20 4. In some cases, clones were planted in the same street for aesthetic purposes. This can
21 increase disease risk and diminish resilience to climate change. Knowledge on genetic
22 variability between and within species is essential to hedge those risks and for evaluating
23 individual responses to environmental conditions.
- 24 5. Our study shows that production and purchasing practices by green space managers need
25 new insights and that tree nurseries can play a crucial role in supplying trees with diverse
26 genetics. Green space managers can reinforce diversity where possible in some areas
27 (development of urban forests, greening of secondary streets or sidewalks, etc.) to
28 improve ecosystem services in urban areas.

29 **Keywords**

30 green space management, microsatellite markers, morphological identification, urban ecology

31

32 **Introduction**

33 In a world of ever-expanding urbanization, urban trees contribute to ecosystem service
34 bundles including the mitigation of urban heat islands or the improvement of air quality (Livesley et
35 al., 2016). These services depend on morphological and physiological behaviors of urban trees and
36 their adaptation to urban pressures such as space constraints, soil compaction, limitation in the access
37 to water and air pollution (Roman and Scatena, 2011). Tree species or genotypes do not respond in the
38 same manner to these constraints and this response may change with age. This leads to potential and
39 effective services (Sieghardt et al., 2005) or even disservices, e.g. spreading of allergenic pollens
40 (Zong et al., 2020). Moreover, for citizens and policymakers, historical or cultural/patrimonial aspects
41 for some trees also matter (Blicharska and Mikusiński, 2014). All these aspects must be taken into
42 consideration by managers when selecting the right tree species or genotype in order to successfully
43 achieve, and monitor, city development plans.

44 Urban trees are often considered by citizens and managers as a “green patrimony”. Green
45 space managers would like to integrate criteria such as the resilience of species to future climate
46 change and city conditions, and promote tree diversity in cities together with the use of indigenous
47 species. Beyond media aspects, this issue is scientifically interesting. In Paris city (France), there are
48 currently 205.311 trees (2020), originating from German and Dutch tree nurseries or from Paris
49 municipal nurseries (DEVE - City of Paris, 2020; Laurian, 2019). During the 1980’s and 1990’s,
50 concerns about the origin of trees were less prevalent and a large proportion came from the East of
51 France, Italy and Germany (Personal communication, L. Chadirac, DEVE, 06/09/2020). A better
52 control of supplies provided by tree nurseries, in particular the knowledge of tree genetics, and a clear
53 traceability of the production methods are needed (Wolff et al., 2019). There are both advantages and
54 disadvantages of having genetically diverse trees. Diverse urban trees can facilitate the connectivity
55 between natural or naturalized populations (birds, insects, trees) by providing diverse resources and
56 habitats (Dallimer et al., 2012; Rivkin et al., 2019). However, this requires the planting and managing
57 of heterogeneous batches of trees that may not meet all of the desired aesthetic standards. Planting
58 clones also has some advantages. For example, retaining homogeneous tree alleys contributes to the

59 prestige of famous avenues. Moreover, managers usually plant clones because, in addition to
60 aesthetics, they are genetically tested and selected to resist urban pressures (pollution, soil
61 compaction, etc). It also facilitates tree management, e.g. managers ensure how clones develop and
62 when pruning is required (Alphand, 1867; Roloff, 2016). They also have more control on the losses
63 and financial costs of replacing clone trees. The question of the genetic diversity of trees in urban
64 areas is therefore complex.

65 Among the most planted tree species in Europe, Linden are often present as ornamental trees
66 in streets and parks (Hansen et al., 2014). In Paris city, after Plane trees and Horse-Chestnut trees they
67 represent the third most planted species, representing more than 10% of planted trees in streets
68 (APUR, 2010; DEVE - City of Paris, 2020). They are considered as resistant to urban stresses such as
69 water scarcity and drought, pollution and pruning (Sjöman and Busse Nielsen, 2010). All over
70 Europe, different linden species are planted in cities, such as *Tilia platyphyllos* Scop. (large-leaved
71 lime), *Tilia cordata* Mill. (small-leaved lime) and *Tilia dasystyla* Steven (Caucasian lime) (Pigott,
72 2012). *Tilia cordata* is also a parent in two different inter-specific hybrids named *Tilia ×euchlora*
73 K.Koch (Crimean lime - a hybrid between *T. cordata* and *T. dasystyla*) and *Tilia ×europaea* L.,
74 known as common lime (a hybrid between *T. cordata* and *T. platyphyllos*), both described in Pigott
75 (2012). Morphological identification of the different linden species is complex as leaves and fruits are
76 not always visible when young trees are in nursery. Furthermore, when looking at adult street trees, it
77 is often difficult to unequivocally identify species, especially for hybrids combining characteristics of
78 both parents. This *Tilia* genus has even been viewed by some as ‘taxonomic torture’ (Santamour and
79 McArdle, 1985).

80 Knowledge of tree genetics for reliable affiliation to a particular linden species is therefore a
81 crucial point. Different molecular biology tools are available to study *Tilia* species. Species
82 boundaries amongst North American *Tilia* have been studied by phylogenetic analysis of plastid DNA
83 sequences (McCarthy and Mason-Gamer, 2020). Nuclear markers such as microsatellite repeated
84 sequences were characterized in *Tilia platyphyllos* and then used to test for cross-amplification in 23
85 species in the *Tilia* genus (Phuekvilai and Wolff, 2013). These microsatellite loci were used to
86 characterize the natural clonal propagation of *Tilia americana* L. in North America (Evans and

87 Morris, 2016) and many other studies used microsatellite loci on planted taxa and clones of *Tilia spp.*
88 in cities over Europe. For example, in Denmark (Hansen et al., 2014; Wolff et al., 2019), UK (Pigott,
89 1992; Pigott, 2012; Wolff et al., 2019), Belgium and the Netherlands (Vanden Broeck et al., 2018),
90 researchers studied the historical plantations of *T. cordata* and *T. ×europaea*. They found that, during
91 the 17th & 18th centuries, a majority of *T. ×europaea* planted in avenues in England and Sweden
92 were clones exported from Netherlands and Belgium nurseries. Their results revealed that most of *T.*
93 *×europaea* planted in Northwest Europe were highly uniform and belonged to few clones (Wolff et
94 al., 2019). However, there is a paucity of information on the genetic diversity of *Tilia* species in Paris
95 as compared to other European cities. Moreover, for city managers, this implies complex and costly
96 laboratory work.

97 Inexpensive identification according to morphological criteria is still helpful for green space
98 managers to determine the identity of planted *Tilia sp.* and for maintaining traceability in tree
99 inventories. Organs that are most used for species determination are: leaves (shape, margin dentation,
100 and color), hairs (presence/absence, and type), flower bracts, fruits (form, hairiness and ribs) and
101 twigs (color and hairiness) (Pigott, 2012). These criteria have led to two morphological keys for *Tilia*
102 species. The first morphological key took all organs into account (Pigott, 2012) and the second
103 morphological key for *Tilia* species cultivated in Western Europe was based on vegetative
104 characteristics visible during a large part of the year (De Langhe, 2005). Although morphological
105 methods are more easily applicable in the field and population genetics methods are more used in the
106 laboratory, both provide information that will allow managers to better understand the history and
107 origin of their linden trees in parks and streets and to adapt their management to the local context.
108 Moreover, morphology gives information on the plasticity due to environmental effects. The choice
109 between morphological and/or molecular methods depends on the objectives of the study and access
110 to laboratory techniques.

111 Here, we wished to increase the knowledge base on tree intra-interspecific diversity in urban
112 areas and, as a result, improve management of these trees by taking into account their potential
113 resilience. We also wanted to establish a clear correspondence between morphological and genetic
114 analysis. First, the identification of different linden species was done by morphological criteria on

115 leaves, twigs and fruits (both on fresh and dry materials). Then, by microsatellite loci analysis,
116 different genetic groups were assigned to different species to check for intraspecific variations in each
117 group.

118

119 **1. Materials and methods**

120 *1.1 Study sites and sampling methods*

121 In total, 80 linden trees from streets (named T01 to T40) and parks (named TP01 to TP40),
122 plus 3 *Tilia cordata* reference trees (SU1, SU2, SU3) from Sorbonne University campus, were
123 sampled in Paris (France) (48.8534°N; 2.3488°E) in September 2018 when leaves were fully mature.
124 These trees were selected with city managers, across different districts, urban landscapes (streets and
125 parks), and varying tree age.

126 To prepare herbarium specimens, two sun-exposed twigs, when possible with fruits, were
127 collected from the lower part of the crown (2-5m above the ground). Three trees (“Léon Bollée”
128 avenue) were higher and not accessible with a ladder, and were sampled at 6-7m above ground with a
129 gondola. Best-preserved twigs with leaves and - when available - fruits were selected and dried by
130 pressing samples within folded newspapers for several days. They were then mounted on white paper
131 labelled with their inventory number, localization description and collection date.

132 For genetic analysis, leaves were sampled and pooled in a plastic bag labelled accordingly.
133 Leaves were washed, towel-dried and then frozen in liquid nitrogen and stored immediately at -20°C.
134 Age of trees was determined using dendrochronology by estimating the number and width of growth
135 rings and abnormal wood rings for each tree. Tree wood cores were collected in 80 linden trees with a
136 sterilized Pressler increment borer (length: 400 mm; diameter: 4.3 mm). Due to the tree health policy
137 of Paris municipality, only one core was sampled per tree. The dating was conducted by Xylotree
138 (www.xylotree.fr, Saintes, France).

139 *1.2 Morphological analysis of collected specimens*

140 Several studies have shown that if pruning has an effect on leaves, the effect is visible on leaf
141 area rather than on leaf shape (Pinkard and Beadle, 2000; Rajaona et al., 2011; Albarracín et al.,
142 2019). No information was found on the impact of pruning on leaf margins. Parisian green space
143 managers prune trees generally every seven years (Personal communication, B. Rizzo, DEVE,
144 22/01/2021). The pruning history is therefore not considered as a major impact in our morphological
145 determination. As Bengtsson (2005) said “[...] it is of great importance to study the samples when
146 they are fresh. Otherwise some important characters like the sheen or lustre and the micromorphology
147 of the upper lamina may soon be lost when the leaves are dried”. Therefore, photographs of fresh
148 samples were used to compare characters that can be lost when leaves are dried. Some of the collected
149 specimens had no reproductive organs, and therefore lacked floral and fruit characters.

150 Each collected specimen (fresh and dry) was assigned to one species by using both De Langhe
151 (2005) identification key that relies solely on vegetative character together with the authoritative
152 reference, and Pigott’s world monograph (2012), which describes the characters of all vegetative and
153 reproductive organs of the twigs. The samples were also compared to the following references: i) type
154 specimens of *Tilia* species (Supp. Table 1) using high definition images accessed through JStor
155 (<https://plants.jstor.org>), ii) *T. cordata* well-identified specimens collected from the Sorbonne
156 university campus (Paris) (named SU1, SU2 and SU3), and iii) herbarium specimens kept in the
157 Herbarium of the National Museum of Natural History (MNHN) in Paris, France. Finally, each
158 character and their description were added to a database in Xper3 biodiversity collaborative
159 management platform which helped us discriminate available traits in our specimens (Vignes-Lebbe
160 et al., 2016). An interactive key was then easily generated using the Xper3 platform tools (Supp.
161 Table 2). The main distinction between species is based on the characteristics of the lower laminar
162 surfaces. Morphological identification of a tree was based on the combination of several criteria.

163

164 *1.3 Microsatellite marker analyses*

165 Genetic diversity of our 80 trees was assessed by using 9 microsatellites (Tc915, Tc7, Tc6,
166 Tc5, Tc11, Tc927, Tc31, Tc943, and Tc937) previously characterized in *T. platyphyllos* (Phuekvilai

167 and Wolff, 2013) and successfully used on trees of the *Tilia* genus (Hansen et al., 2014; Erichsen et
168 al., 2019). Sequence and labelling of primers are shown in Supp. Table 3. Our references *T. cordata*
169 (SU1, SU2 and SU3) collected and determined through morphological analysis were included in the
170 same run. One *T. tomentosa* tree (CLT01) from the arboretum of Versailles-Chèvreloup (Yvelines,
171 France; Latitude: 48°50'1.02"N, Longitude: 2° 6'7.88"E) was added as a reference, and its allele sizes
172 are shown in Supp. Table 4.

173

174 *1.3.1 DNA extraction*

175 Frozen leaves were ground in liquid nitrogen. Fifty mg of leaf powder was dissolved in 800 µl
176 of CTAB (CetylTrimethylAmmonium Bromide) buffer and DNA extraction was done as described by
177 Phuekvilai and Wolff (2013). Since our leaf samples contained mucilages, CTAB buffer was heated
178 to 60°C at the first step of DNA extraction. After DNA precipitation, pellets were resuspended in 100
179 µl of water. DNA concentration was estimated at 260 nm and its purity at 280 nm (NanoPhotometer,
180 Implen®).

181

182 *1.3.2 PCR*

183 Forward primers were 5' labeled with NED, PET, VIC or 6-FAM dyes (Life technologies;
184 Supp. Table 3). PCR amplifications were performed in 10 µL reactions containing 10 ng of DNA, 1×
185 reaction buffer, 0.2 mM of each dNTP, 0.4 µM of each primer, and 0.5 U of *GoTaq* G2 DNA
186 polymerase (Promega). PCR program was 95°C for 5 min, followed by 15 cycles of 95°C for 15 s,
187 54°C for 15 s, and 72°C for 15 s, and 20 cycles of 89°C for 20 s, 52°C for 20 s, 72°C for 20 s, and a
188 final extension step of 72°C for 30 min. DNA amplification was made in three multiplexes combining
189 different primers and fluorochromes (M1: Tc915, Tc7, Tc6 and Tc5; M2: Tc11 and Tc927; M3: Tc31,
190 Tc 943, and Tc937). Samples were diluted 1:10 in Hi-Di Formamide (Life Technologies SAS), a size
191 standard was added (Genescan 600 LIZ size standard v2.0, Life Technologies SAS) and runs were
192 made in a Genetic Analyzer from Applied Biosystems (AB3500). Length of alleles at each locus was

193 determined by using GeneMapper® software (Applied Biosystems). Raw data were analysed with
194 AutoBin v 0.9 to homogenize allele sizes (Salin, 2010).

195

196 *1.3.3 Ploidy estimation*

197 Because some *Tilia* species are known to be polyploid, determining allele copy number in
198 partially heterozygous genotypes is challenging (Dufresne et al., 2014). We used the POLYSAT
199 version 1.7 package in R to determine the maximum number of microsatellite alleles and to estimate
200 ploidy for each individual (Clark and Jasieniuk, 2011). The ploidy results were used for exporting the
201 data (Supp. Table 4) for subsequent genetic analyses.

202

203 *1.3.4 Cluster analysis*

204 In an exploratory phase, microsatellite data were used for delimiting putative species, i.e.
205 partitioning sampled individuals into putative species without *a priori* knowledge about the number of
206 species (Hausdorf and Hennig, 2010). For this, we used a Bayesian clustering approach implemented
207 in the software STRUCTURE 2.2 (Pritchard et al., 2000). As STRUCTURE is searching for groups of
208 individuals that are in approximate Hardy–Weinberg equilibrium, a condition that is not realized in
209 planted trees, it was used in a first approach to evaluate the number of genetic groups and each
210 individual probability of belonging to a genetic group. Genetic clusters proposed by STRUCTURE
211 were seen as species hypotheses (i.e. groups whose members share similar patterns of variation) that
212 were re-evaluated with morphological characters. Simulations were run with an admixture model,
213 allele frequencies correlated, a burn-in of 50,000 and 100,000 Markov chain Monte Carlo (MCMC)
214 repetitions. In order to discard all dosage information in the input file and perform the analysis
215 correcting for the unknown dosage, the RECESSIVEALLELES flag was set to 1 (Meirmans et al.,
216 2018). Ten iterations were run for each assumed cluster ($K= 1$ to $(n+2)$, with n the number of species
217 found during morphological identification). Structure Harvester software version 0.6.94 (Earl and
218 vonHoldt, 2012) was used to determine the optimal K -value following Delta K method (Evanno et al.,

219 2005). Once the number of clusters was defined, all iterations were aligned using the Greedy
220 algorithms implemented in cluMPP (Jakobsson and Rosenberg, 2007) and the final plot was edited
221 using Structure Plot V2.0 (Ramasamy et al., 2014).

222

223 *1.3.5 Clone assignment*

224 Clones were identified by sorting allele sizes for each locus in an Excel table. We considered
225 that our individuals were clones if they did not differ by more than two alleles at one or two loci. For
226 each genetic cluster, we evaluated clones and their proximities to other genotypes through trees based
227 on a matrix of genetic dissimilarities (Lynch.distance function) obtained using the POLYSAT
228 package. The trees were constructed by Unweighted Pair Group Method with Arithmetic mean
229 (UPGMA) with the hclust function (plot in R version 3.4.2).

230

231 **2. Results**

232 *2.1 Morphological determination of species*

233 Tree ages determined by dendrochronology varied between 20 and 146 years old and are
234 distributed across all districts and local environments (parks, streets) (Supp. Table 4.). The analysis of
235 seven major characters on our specimens compared to our references (collected specimen, MNHN
236 herbarium, jstor database, Pigott (2012) and De Langhe (2005)) shows that, regardless of age, our
237 specimens could be divided into 5 morphogroups that correspond to 5 species, including two
238 described hybrids (Table 1; Fig.1).

239 Tree identification, based on several morphological characters, was therefore possible even if
240 certain characters were sometimes absent (e.g. fruits). Uncertainty persists for only 3 trees: T05, T35
241 and T36. The T05 and T36 individuals were assigned respectively to *T. dasystyla* and *T. platyphyllos*.
242 The individual T35 could not be determined because only a small amount of leaf sample was
243 available and this was used for genetic analysis.

244 Initial identification was based mainly on the shape of leaf margins. Indeed, *T. cordata*, *T.*
245 *platyphyllos* and *T. dasystyla* exhibit distinct leaf margin teeth shapes, very triangular with a very
246 acute apex in *T. dasystyla*, convexly triangular with an acute apex in *T. platyphyllos*, and convexly
247 triangular with a blunt apex in *T. cordata*. Interestingly, hybrids between *T. cordata* and the two other
248 species showed intermediate leaf margin shapes as compared to their two parent species (Fig. 1, D). In
249 order to further confirm the identification, a closer examination was performed on the leaf abaxial
250 and/or adaxial side hairs, which persist when dry (Figs 1, E and F). It is important to note that the
251 observed leaves were at the same stage of maturity because hair color changes according to leaf stage
252 in this species. Both *T. xeuclora* and *T. dasystyla* exhibited numerous club-shaped brownish hairs
253 along veins, often on both sides of the leaves, a feature generally absent in the other species, where
254 club-shaped hairs are less numerous, restricted to the abaxial side of the leaf, smaller, and lighter
255 colored (Fig. 1, G). Distinction between *T. xeuclora* and *T. dasystyla* could then be based on fruit
256 shape (Fig. 1, A). The color of twig of the year turned out to be useful (Fig. 1, C), being usually green
257 in *T. xeuclora*, and brownish in *T. dasystyla*. Among the studied taxa, *T. platyphyllos* was the easiest
258 species to determine, with leaf veins harbouring distinctive, numerous, perpendicular simple hairs.
259 The shape, hardness and size of its ribbed fruits were also unique among studied species. In contrast,
260 *T. xeuropaea* was more difficult to identify, as its leaves closely look like those of *T. cordata* (Fig. 1,
261 B). However its fruits are distinctive, similar to both *T. cordata* regarding their size (although slightly
262 larger), and to *T. platyphyllos* regarding their shape and the presence of marked ribs (Fig. 1, A).
263 Moreover, its leaf abaxial fascicular hairs exhibited a duller color than *T. cordata* ones. Finally, *T.*
264 *cordata* could easily be identified by its reddish tufts of fascicular hairs in the vein axils on the leaf
265 abaxial side, and by its small, easily breakable mature fruits, with slightly asymmetric apiculus, and
266 very faint ribs.

267 In our tree population, there were 36 *T. xeuclora*, 19 *T. cordata*, 18 *T. platyphyllos*, 6 *T.*
268 *dasystyla* and 3 *T. xeuropaea* (Table 2). According to these identifications, a map of the location of
269 our samples was drawn and showed the distribution of *Tilia spp.* trees across different Parisian
270 districts (Fig. 2).

271 2.2 Genetic profiling by using microsatellite markers

272 2.2.1 Genetic group repartition of urban *Tilia* spp.

273 Structure Harvester analysis found the highest Delta K value at K=2 ($\Delta K=208.4$), followed by
274 K=3 ($\Delta K=175.6$), which is also interesting for analysing substructuring of the data. References for *T.*
275 *cordata* (SU1, SU2 and SU3) and *T. tomentosa* (CLT01) were first integrated in this analysis (Supp.
276 Table 4). Since none of the individuals matched to *T. tomentosa* genetic profile, CLT01 is not shown
277 in these results. For K=2, the first cluster included individuals affiliated to *T. cordata*, *T. dasystyla*, *T.*
278 *×europaea* and *T. platyphyllos*, and the second cluster included individuals affiliated to *T. ×euchlora*
279 (Supp. Fig. 1). For K=3, genetic group 1 showed a clear correspondence with *T. cordata*, genetic
280 group 2 with *T. ×euchlora* and genetic group 3 with *T. platyphyllos* (Fig. 3). Four individuals
281 morphologically identified as *T. dasystyla* (TP11, TP31, TP32 and TP37) showed an admixed profile,
282 with a posterior probability of assignment to genetic group 2 of 0.56 (TP11), genetic group 1 of 0.55
283 (TP31), genetic group 3 of 0.61 (TP32), genetic group 3 of 0.72 (TP37). The TP04 and TP07
284 individuals, identified as *T. ×europaea* showed a mixture of group 1 (*T. cordata*) and group 3 (*T.*
285 *platyphyllos*) corresponding to *T. ×europaea* parents. Seven individuals had their genetic group not
286 corresponding to their morphological assignation (T05, T06, T12, T33, TP03, TP19 and TP33). For
287 example, we observed that the T06 and T12 individuals were assigned to genetic group 3, while their
288 morphological assignments were respectively *T. cordata* and *T. ×europaea*. For K=4, a cluster may
289 correspond to *T. dasystyla*, but the ΔK was 0.563 which is too low for adequate interpretation of the
290 results (Supp. Fig. 2).

291 2.2.2 Genetic similarity

292 Distance trees were constructed (Fig. 4) for each genetic group/species identified by
293 STRUCTURE analysis (K=3). Conflicting genetic data were related specifically to 4 lindens (TP11,
294 TP16, TP32, and TP37). Morphological identification of TP16 led to *T. ×euchlora*; TP11, TP32, and
295 TP37 led to *T. dasystyla*, but they were indexed to genetic group 3 (*T. platyphyllos*). Indeed, these
296 individuals had a low genetic distance with each other in genetic group 3 (Fig. 4, C), although TP16,
297 TP32 and TP37 were a mixture of genotypes between the three groups (Fig. 3).

298 Concerning genetic groups, different clonal subgroups were found in group 1 (*T. cordata*) as
299 in group 2 (*T. ×euchlora*), no clonal subgroups were found for *T. platyphyllos* (Table 3, Supp. Tables
300 5, 6 and 7). These clonal groups come from tree nurseries that probably use propagation by cuttings or
301 layering to produce new trees instead of planting seeds (Bengtsson, 2005; Pigott, 2012). In group 1, 6
302 trees (T01, T05, T07, T16, TP10, TP13) corresponded to the same clonal subgroup, TP04 and TP07 to
303 another clonal subgroup (Fig. 4, A, Supp. Table 5). SU3 and TP06 belong to a third clonal subgroup
304 but this is a hypothesis because of missing data (Fig. 4, Supp. Table 5). In total, group 2 showed 20
305 individuals belonging to the same clonal subgroup (T03, T08, T17, T23, T25, T26, T27, T29, T31,
306 T39, T40, TP08, TP09, TP19, TP24, TP25, TP28, TP29, TP35, TP36), 2 individuals to another
307 subgroup (T20 and TP27) and 3 individuals to a third subgroup (T24, TP40, TP30) (Fig. 4, B,
308 Supp. Table 6).

309 Geographical location of the genetic profiles of the 80 trees is random inside Paris city,
310 without distinction between streets or parks. Moreover, the same clonal group could have been
311 indifferently planted in different locations or in the same street for several decades (Supp. Tables 5, 6
312 and 7). For example, a clone was planted in “Louis Vicat” street (15th district, T08, T17, T26, T27,
313 T29, T39, T40), in “Léon Bollée” avenue (13rd district, T03) and in a park named “Square des
314 Epinettes” (17th district, TP28 and TP36). Dendrochronology revealed different ages among clonal
315 individuals, indicating possible use of the same clone for more almost fifty years (Table 4).

316 **3. Discussion**

317 *3.1 Morphological vs genetic identification*

318 Genetic variability (genotypes) results in morphological variations (phenotypes). Local
319 environment also plays a role and can have significant impact on urban tree growth (Kjelgren and
320 Clark, 1993). Genetic variability between and within a species is important because each individual
321 responds differently to environmental conditions. For example, twig color is one of the criteria used to
322 differentiate *T. ×euchlora* and *T. dasystyla* but one should keep in mind that twigs well exposed to
323 light often turn from green to red (Pigott, 2012).

324 Our integrative study shows that there is a good correspondence (73 out of 80 trees) between
325 morphological criteria and genetic analysis by microsatellites. Some individuals (TP04, TP07, T12)
326 were difficult to differentiate from their *T. cordata* parent but were finally assigned to *T. ×europaea*.
327 For these hybrids, one parental genome could be expressed more than the other on its progeny
328 genome, thus exacerbating some associated characters (Rapp and Wendel, 2005). During our
329 morphological analysis, concerns were raised that we could have mistakenly considered the cultivar
330 ‘Pallida’ or ‘Zwarte linde’ of *T. ×europaea* as *T. ×euchlora* individuals. However, our *T. ×euchlora*
331 individuals had all the characters described by Pigott (2012) and did not correspond to the description
332 of these cultivars. Tree identification therefore involves several criteria, some discriminating more
333 than others, such as fruits, leaf margins and the position and nature of hairs on the abaxial side of
334 leaves.

335 We defined 5 species based on morphological characters, microsatellite analysis allowed us to
336 show that we have only 3 genetic groups among our trees (genetic groups 1, 2 and 3 showed a clear
337 correspondence respectively with *T. cordata*, *T. ×euchlora* and *T. platyphyllos*). When morphology
338 was difficult, genetic analysis (assignment of an individual to a genetic group) although complex,
339 costly and time-consuming, is necessary to confirm species determination (Wolff et al., 2019).
340 However, some individuals identified as *T. dasystyla* and *T. ×europaea* and whose morphology was
341 not obvious also proved difficult to identify through genetic analysis because of their mixed profile. In
342 our study, the number of individuals identified as *T. dasystyla* and *T. ×europaea* was low compared to
343 the other species, making it difficult to differentiate between species with a Bayesian clustering
344 approach.

345

346 3.2 Genetic diversity within the genus *Tilia* in Parisian streets and parks

347 Twenty trees of *T. ×euchlora* belonged to the same clonal group and other individuals in
348 genetic group 2 were closely related to this clone. Often they were located within the same street,
349 illustrating the practice of selecting a homogeneous batch to create a landscape alignment, with a

350 particular aesthetic goal. In contrast, when clones were present in several places, scattered across
351 Paris, they probably correspond to a clone produced in nurseries over several decades. These clones
352 are likely easy to reproduce and appreciated by the services of the city for their aesthetic characters
353 (Personal communication, L. Chadirac, DEVE, 06/09/2020). In our study the most planted clones was
354 *T. ×euchlora*, while other studies described *T. ×europaea* clones in different European cities (Hansen
355 et al., 2014; Vanden Broeck et al., 2018; Wolff et al., 2019). As our sampling includes only a small
356 number of *T. ×europaea*, it is difficult to compare the clone planting policy between Paris and
357 European cities.

358 The fact that we have a significant proportion of clonal individuals in urban areas is not very
359 surprising when we consider that a small number of nurseries supply the municipality with linden
360 trees (Personal communication, L. Chadirac, DEVE, 06/09/2020). Moreover, vegetative
361 multiplication is favoured over sowing seeds in tree nurseries because germination is often a difficult
362 and time consuming task (Tylkowski, 2006). Nevertheless, tree production from seeds is possible for
363 species with easier seed germination, leading to more genetic diversity such as for *T. platyphyllos*
364 whose seeds show higher germination rates than *T. cordata* (Magherini and Nin, 1994). The high
365 degree of genetic similarity within urban tree species is not unique to Paris, nor to the urban
366 environment. For example, in Fredensborg Slotshave (Denmark), old *T. ×europaea* were removed
367 and replaced by trees of the same genetic profile found in commercial tree nurseries in order to restore
368 an historical avenue (Hansen et al., 2014).

369 Nurseries when possible should therefore develop programs dedicated to seed storage,
370 viability and testing for clones. Indeed, promoting planting of different clonal groups of the same
371 species would provide genetic diversity while maintaining positive aspects of clonality. Aesthetic
372 aspects would remain the responsibility of managers.

373 *3.3 Scientific and management perspectives on the genetic diversity of urban trees*

374 In a context of global change, which tree species should be planted in our cities to conserve all
375 the ecological services they provide? How can we ensure sufficient diversity? The low genetic
376 diversity between trees of the same species probably reflects management practices that have been in

377 place for decades. The aim was to plant, as much as possible, trees that are as similar to each other as
378 possible, mainly for aesthetic purposes, but that are also resistant to urban challenges (Laurian, 2019).
379 However, poor genetic diversity could be a threat to urban trees in the case of emerging diseases, pest
380 proliferation or an abrupt change in the environmental situation (e.g., air pollution, extreme
381 temperatures, increased dust formation) (Raupp et al., 2006; Roloff, 2016).

382 The existence of this clonality could lead to fruitful interactions between managers and
383 scientists interested in comparisons of growth and resistance of trees isolated from different
384 environments. For instance, Lobo et al. (2018) studied *T. cordata* scions of twelve populations
385 originated from different geographical areas in Denmark (including Northern and Southern areas), and
386 grown for eight years in the same orchard located in the South of Denmark. Scions from the Northern
387 part of Denmark kept their original phenology and showed a delayed bud burst compared to those
388 from the South. Phenotypic changes which were monitored in this study could be due to adaptation of
389 trees to local conditions.

390 In addition to the observance of Santamour's guidelines (1990), cities have many challenges
391 to face in order to increase tree diversity in their streets. They need to identify new insights and
392 progress in terms of production and purchasing practices. According to the location in the city,
393 different possibilities of tree management can be considered. When the location is classified as an
394 historical or cultural landscape, managers are required to maintain homogeneous alignments, taking
395 great care of phytosanitary status of trees. In other places, plantations of diversified batches of trees
396 (intra- and interspecific diversity) should be prioritized. By increasing tree diversity with a wise
397 selection of species in one location, they will also support the diversity of tree-associated species
398 (insects, herbaceous plants etc.) (Omar et al., 2019). Other advantages, such as having a varied
399 architecture for cooling and air circulation (Vos et al., 2013) and reducing allergic risks (Zong et al.,
400 2020) can also be anticipated.

401 **Conclusion**

402 This study showed that there is a strong correspondence between morphological identification, which
403 is an important tool for green space managers to identify *Tilia* species, and genetic based
404 determinations. Cities have developed tree inventories and open data to improve understanding of tree
405 diversity in urban areas. Adding genetic profiles of trees to inventories could be an interesting tool for
406 identifying where clones are planted in the same street and for evaluating potential disease risks. Only
407 *Tilia* species were mentioned in this study but it is clear that similar investigations on other species is
408 needed if we are to maintain and enhance tree diversity and improve ecosystem services in urban
409 areas.

410 **Data Availability Statement**

411 <http://www.xper3.fr/xper3GeneratedFiles/publish/identification/-3498028717715930076/>

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549 **Figures Legends**

550 **Figure 1.** Comparison of morphological characters of the five *Tilia* species, identified in the samples
551 collected in streets and parks of Paris. (A): fruit; (B): general view of the second leaf from the apex of
552 the twig of the year; (C): twig of the year; (D): leaf margin; (E): base of main veins on the abaxial leaf
553 side; (F): minor veins on the abaxial leaf side; (G): club-shaped hairs along minor veins on the abaxial
554 leaf side. Inset bars represent 2 mm in (A), 1 cm in (B), 5 mm in (C) and (D), 1 mm in (E), 500 μm in
555 (F), or 200 μm in (G). In (G) plain arrows indicate club-shaped hairs, and 4-time magnified views of
556 these hairs are included inside inset circles, corresponding to the 50 μm scale bars

557

558 **Figure 2.** Map of *Tilia spp.* sampled in Paris. Smaller maps numbered 1 to 10 show magnified areas
559 corresponding to pentagons 1 to 10 on the general map of Paris. Gray numbers are district numbers

560

561 **Figure 3.** Analyses of sample structures by Structure Harvester with $K=3$. Each color represents a
562 genetic group, and species noted under the graph correspond to morphological identification. Genetic
563 group 1 (light gray), group 2 (medium gray), group 3 (dark gray) correspond respectively to *T.*
564 *cordata*, *T. xeuclora* and *T. platyphyllos*. Name of trees and parks are respectively T01 to T40 and
565 TP01 to TP40. The proportion of each color bar represents the probability of belonging to a genetic
566 group. ui = unidentified individual

567

568 **Figure 4.** Genetic distance trees (UPGMA method) of 83 linden in Paris, separated in three genetic
569 groups 1 (A), 2 (B) and 3 (C), determined by STRUCTURE with $K=3$. Individual trees in red showed
570 an admixed profile. Name of trees are T01 to T40 (in streets) and TP01 to TP40 (in parks)

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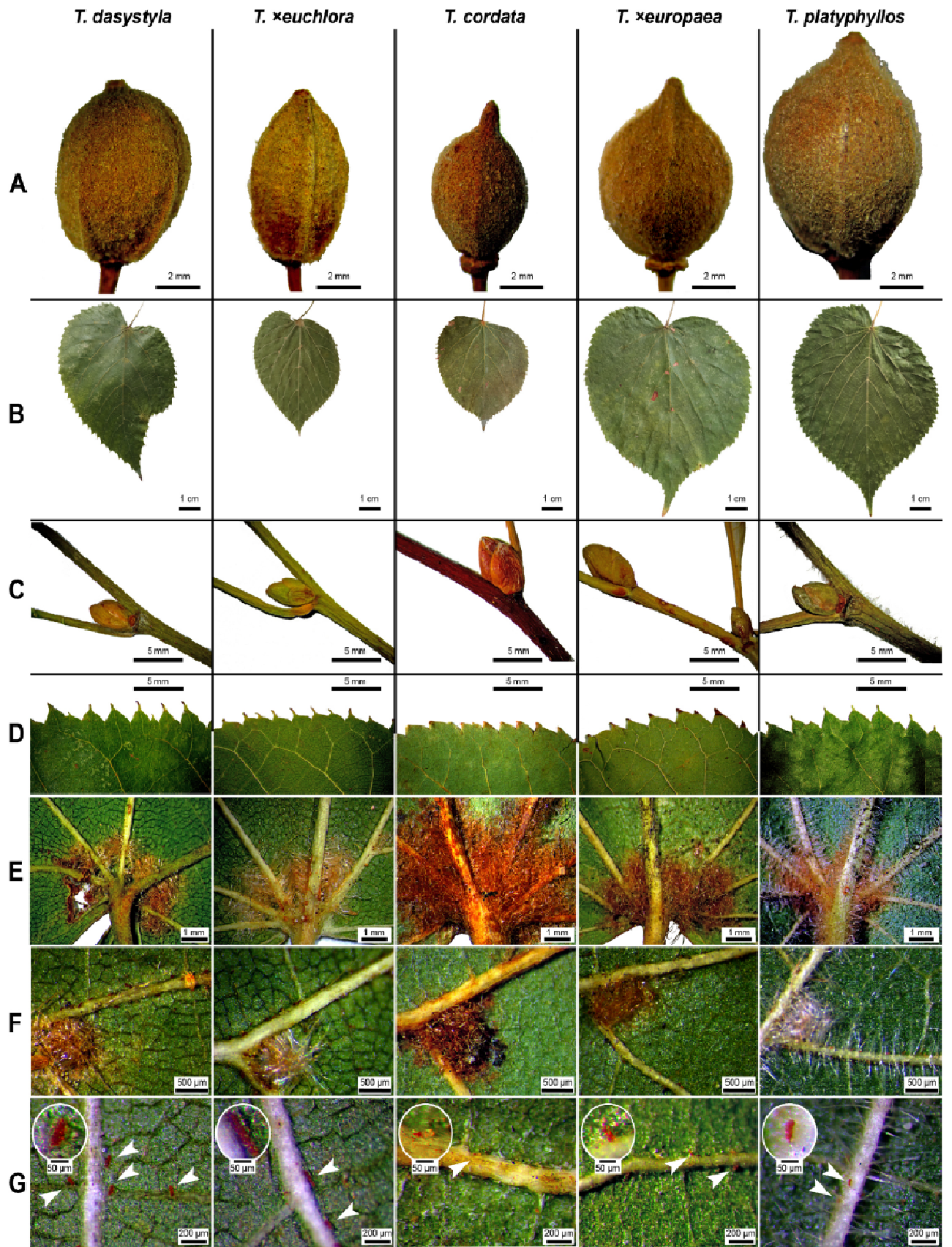
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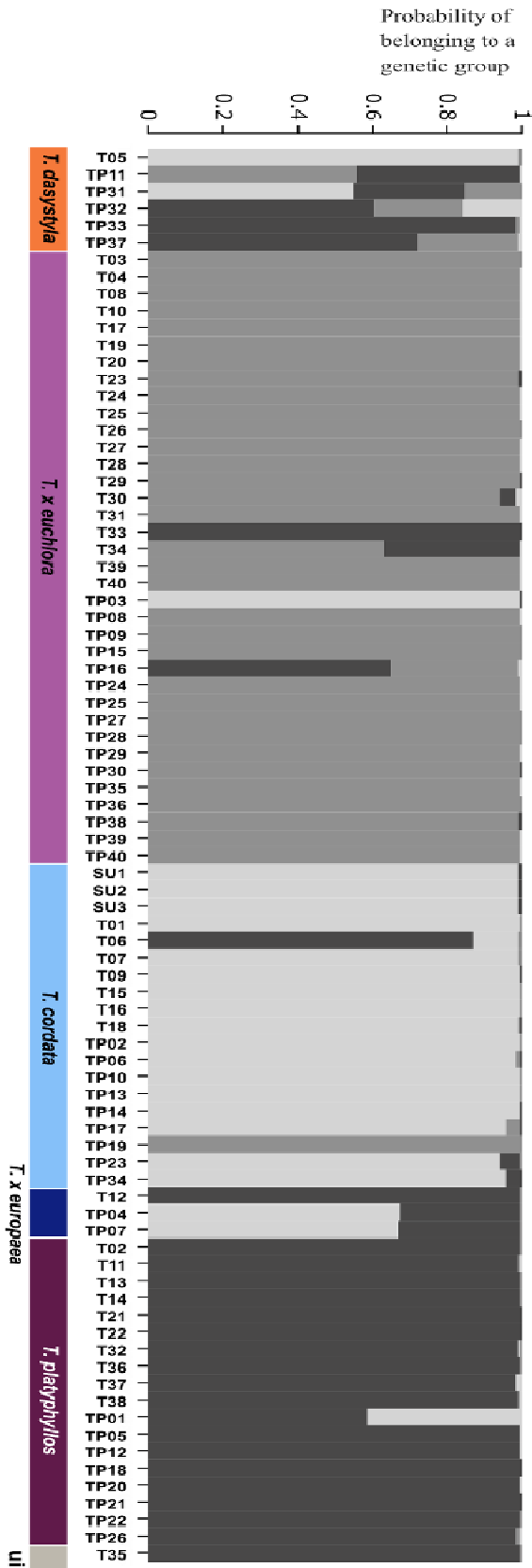
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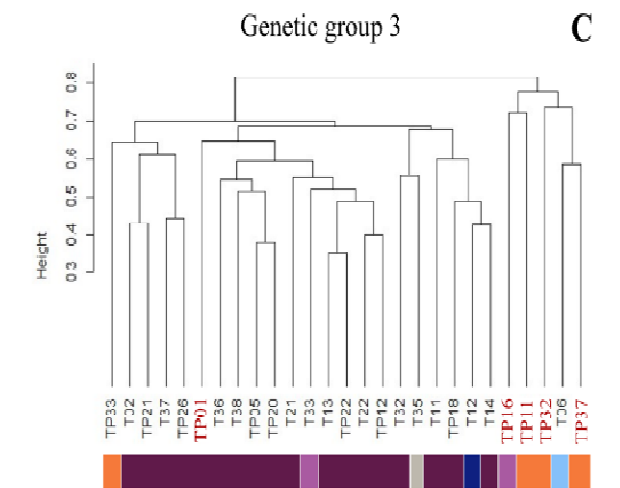
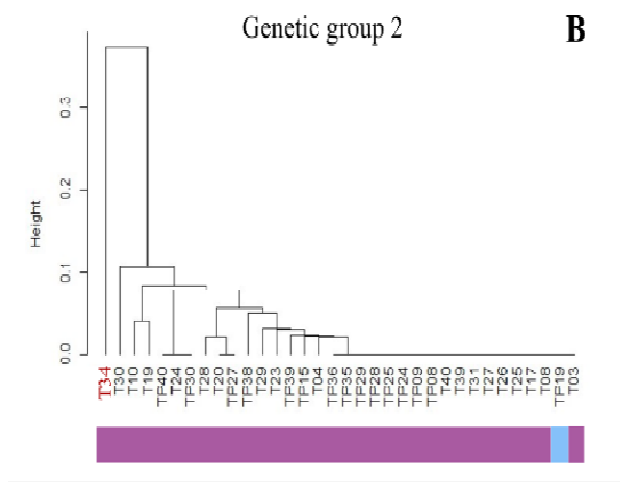
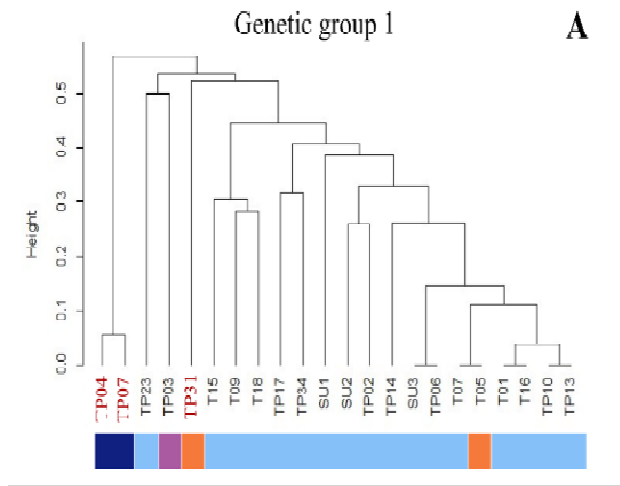
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Morphological identification

- T. dasystyla*
- T. ×euchlora*
- T. cordata*
- T. ×europaea*
- T. platyphyllos*
- Unidentified

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589 **Tables legends**

590 **Table 1.** Character states of seven major morphological characters assigned the 83 linden trees to five
 591 morphogroups subsequently identified as the five mentioned species based on Pigott's monograph
 592 (2012) and De Langhe determination keys (2005)

| | <i>Morphogroup 1 identified as T. cordata</i> | <i>Morphogroup 2 identified as T. dasystyla</i> | <i>Morphogroup 3 identified as T. x euchlora</i> | <i>Morphogroup 4 identified as T. x europaea</i> | <i>Morphogroup 5 Identified as T. platyphyllos</i> |
|---------------------------------|--|---|---|--|--|
| <i>Adaxial side of leaves</i> | Glabrous | Glabrous | Glabrous | Glabrous | Hairy |
| <i>Abaxial side of leaves</i> | Brownish orange fasciculate hairs in the axils of main veins | Straw colored to light brown fasciculate hairs in the axils of main veins | Brush-shape fasciculate hairs at the junction of the main and secondary veins; single, short, brown hairs along veins | Pink to pale brown fasciculate hair in the axils of the main veins. Exceptionally, stellate hair in all surfaces | Fasciculate hairs along the main veins, sometimes also along secondary veins |
| <i>Twigs</i> | Glabrous | Glabrous | Glabrous for long shoot. Hairs for short shoot | Glabrous | Fine hairs falling during winter |
| <i>Buds</i> | With two scales visible | With three scales | With three scales | Unknown | With three scales |
| <i>Bracts</i> | Glabrous | Glabrous | Glabrous | Hairs | Hairs |
| <i>Fruits form</i> | Fragile and easily broken. Spherical when sterile, ellipsoid to obovoid 6-7 mm long, 3.5-4.2 mm wide | Not easily broken. Spherical 6.6-11.4 mm long, 5.7-8.2 mm wide | Not easily broken. Ovoid, sterile 8-10 mm long, 5.0-6.5 mm wide | Easily broken. Spherical | Not easily broken. Spherical 9.1-11.9 mm long, 7.9-9.7 mm wide |
| <i>Hairs and ribs on fruits</i> | When fertile, covered with fasciculate hairs, without ribs | Longitudinal ridges covered with dense white silky tomentum | Weakly ribbed (5 ribs) covered with brown fasciculate hairs | Ribbed, light brown tomentum | 5 ribs covered with white hairs |

596 **Table 2.** Number of individuals by species identified in our study based on morphological characters

| Species | Number of individuals |
|--------------------------------|-----------------------|
| <i>Tilia dasystyla</i> | 6 |
| <i>Tilia</i> × <i>euchlora</i> | 36 |
| <i>Tilia cordata</i> | 19 |
| <i>Tilia</i> × <i>europaea</i> | 3 |
| <i>Tilia platyphyllos</i> | 18 |
| Unidentified individual | 1 |

597
598

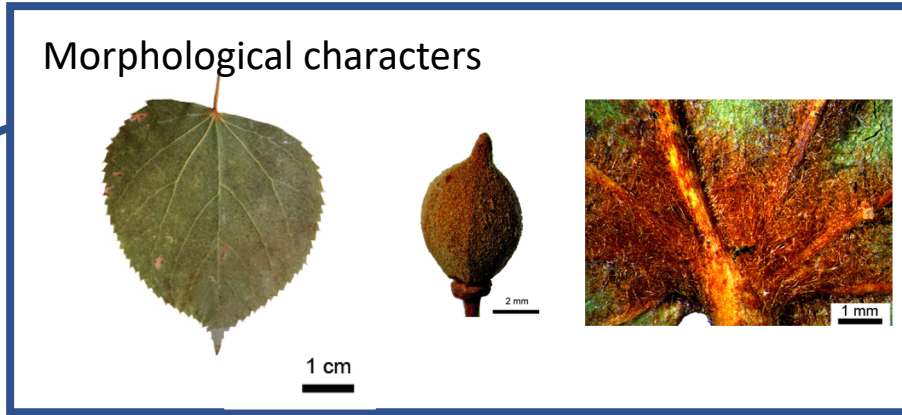
599 **Table 3.** Detailed analysis of genetic group 1, group 2 and group 3 diversity corresponding,
600 respectively, to morphological identification of *T. cordata*, *T. ×euchlora* and *T. platyphyllos*

| <i>Genetic group</i> | Species | Number of individuals | Number of genetic profiles | Number of clonal groups |
|----------------------|---------------------------|--------------------------|----------------------------|-------------------------|
| Group 1 | <i>Tilia cordata</i> | 22 | 15 | 3 |
| Group 2 | <i>Tilia ×euchlora</i> | 34 | 14 | 3 |
| Group 3 | <i>Tilia platyphyllos</i> | 26+1 unaffiliated | 27 | 0 |

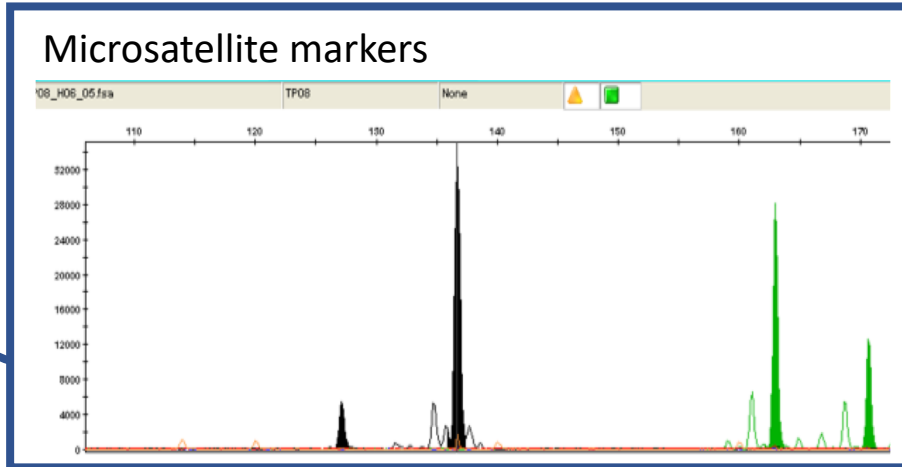
602 **Table 4.** List of some trees planted in streets and parks that belong to the same clonal group of *T.*
 603 *xeuchlora*

| Species | Tree name | | Localisation | Estimated age (years) |
|---------------------|-----------|--------|----------------------|-----------------------|
| <i>T. xeuchlora</i> | T08 | street | rue Louis Vicat | 20 |
| | T17 | street | rue Louis Vicat | 58 |
| | T26 | street | rue Louis Vicat | 61 |
| | T27 | street | rue Louis Vicat | 61 |
| | T29 | street | rue Louis Vicat | 64 |
| | T39 | street | rue Louis Vicat | 67 |
| | T40 | street | rue Louis Vicat | 64 |
| | T03 | street | avenue Léon Bollée | 33 |
| | TP28 | park | square des Epinettes | 44 |
| | TP36 | park | square des Epinettes | 48 |

How genetically diverse is *Tilia* sp. in a city?



Linden species identification



→ Many clones
» Need to increase
diversity in
tree nurseries

Scientists

Urban tree managers