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Article

Verification of names for certain *Populus* L. clones (Salicaceae) commonly grown in the United Kingdom

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Abstract

For eleven *Populus* clones, *P.* 'Beaupre', *P.* 'Boelare' *P.* 'Columbia River', *P.* 'Fritzi Pauley', *P.* 'Gaver', *P.* 'Ghoy', *P.* 'Gibecq', *P.* 'Primo', *P.* 'Robusta', *P.* 'Scott Pauley', *P.* 'Trichobel' widely cultivated across Europe and grown in the UK, their taxonomy and clonal names were verified using morphological and molecular markers. Correct scientific names were applied to all clones. For nine of them, the correct clonal names were found. One new combination was made, and seven scientific names were typified.

Keywords: correct names, cultivation, herbarium vouchers, microsatellite genotyping, morphology, nomenclature, taxonomy, typification, 'UNAL' poplar clones

Introduction

The inventory of worldwide and regional floras is an important target for modern botanical research (Paton *et al*., 2008; Christenhusz and Byng, 2016; Nic Lughandha *et al*., 2016; Paton *et al*., 2016; Borsch *et al*., 2020; Garnett *et al*., 2020). The study of the biodiversity in different regions results in establishing the number of taxa growing naturally and taxa that have been introduced either accidentally or through cultivation. In the United Kingdom (UK)

there are three main aspects to plant cultivation through which plants are distributed across the country: agriculture, forestry and horticulture. During the multiple stages of plant movement, mistakes in identification and labelling can occur and as a result the same clones could be cultivated in different places under different names (Kuzovkina *et al*., 2016a, b; Belyaeva *et al*., 2018; Belyaeva, 2020; Belyaeva *et al*., 2020).

The challenges in identification and naming in Salicaceae *sensu stricto* were described recently (Belyaeva, 2020). All plant research should start with answering two main questions: (1) To which taxon does a plant belong? and (2) What is the correct name of this taxon? These questions cannot be resolved without involving taxonomy and nomenclature. Taxonomic opinions can change over time when using advanced approaches in plant research (Humphreys and Linder, 2009; Culley, 2013; Belyaeva, 2020). However, nomenclature is determined by the concept of nomenclatural type which is required for the scientific names of plants to be validly published (Art. 7.2 of the International *Code of Nomenclature for Algae, Fungi, and Plants*, ICN, (Turland et al., 2018). As the nomenclatural type is defined as an element to which the name of the taxon is permanently attached, there is the possibility to compare the plant in question to the type, which could be, in the case of plants, the herbarium specimen.

For cultivated plants, such an element is not required by the *International Code of Nomenclature for Cultivated Plants*, ICNCP (Brickell *et al*., 2016) for validly published cultivar names. Thus, most cultivars cannot be compared to an herbarium specimen. In this connection, it is becoming very important to use verified herbarium voucher specimens to document plants used in research. It has been emphasised by many taxonomists that herbarium specimens deposited by researchers as vouchers are essential in providing credibility and concrete and verifiable evidence about the taxa that have been used in published research (Goldblatt et al., 1992; Funk, 2004; Funk *et al*., 2005; Culley, 2013; Funk *et al*., 2014; Goodwin et al., 2015; Carranza-Rojas *et al*., 2017).

When comparative morphology cannot help in the identification of cultivated plants, the application of genetic markers is a very important tool to support botanical research. It allows the identification of clones, which is very helpful especially in plants such as poplars that have a long breeding history, including complex hybridisation and clones, as members of full-sib families. Molecular markers can be evaluated independently from environmental conditions, ontogenetic stages or seasonal changes that may influence morphological traits. The system of nuclear microsatellite markers applied here is very similar to that used in forensic medicine, where the identity of individuals (e.g. unknown samples compared with buccal cells

from known candidates) and the direct ancestry between individuals (e.g. paternity testing) can be detected without doubts.

As a model we chose to investigate the nomenclature and taxonomy of certain *Populus* clones that have been widely distributed and grown throughout Europe, combining a traditional morphological approach with molecular fingerprinting and supporting this study by typification of scientific names and deposited voucher specimens for the cultivar names.

Material and methods

The *Populus* L. clones studied in this research are listed in Table 1. All are commonly grown in the UK for forestry and horticulture (Jobling, 1990).

Primary information about the names of the clones, possible parentage, taxonomy and nomenclature, etymology and usage were obtained from the cited references and the following webpages: *The International Commission on Poplars and Other Fast-Growing Trees Sustaining and Environment* (IPC, 2020), *International Plant Names Inde*x (IPNI, 2020), *The World Checklist of Vascular Plants* (WCVP, Govaerts, 2020) and *Plants of the World Online* (POWO, 2020).

Table 1. List of *Populus* clones analysed in this research.

No	Name of cultivar	Scientific name
1.	*Populus* 'Beaupre'	***Populus* × *generosa*** A.Henry (= ***P. deltoides*** W.Bartram ex Marshall × ***P. tristis*** Fisch.)
2.	*Populus* 'Boelare'	***Populus* × *generosa*** A.Henry
3.	*Populus* 'Columbia River'	***Populus tristis*** Fisch.
4.	*Populus* 'Fritzi Pauley'	***Populus tristis*** Fisch.
5.	*Populus* 'Gaver'	***Populus* × *canadensis*** Moench (= ***P. deltoides*** W.Bartram ex Marshall × ***P. nigra*** L.).
6.	*Populus* 'Ghoy'	***Populus* × *canadensis*** Moench
7.	*Populus* 'Gibecq'	***Populus* × *canadensis*** Moench
8.	*Populus* 'Primo'	***Populus* × *canadensis*** Moench
9.	*Populus* 'Robusta'	***Populus* × *canadensis*** Moench
10.	*Populus* 'Scott Pauley'	***Populus tristis*** Fisch.
11.	*Populus* 'Trichobel'	***Populus tristis*** Fisch.

Herbarium specimens consulted, herbarium vouchers and the typification process

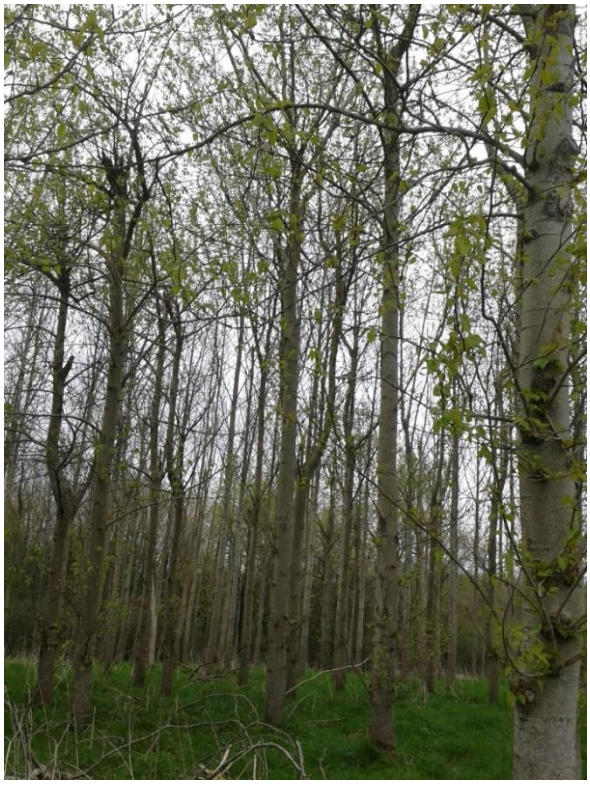


Figure 1. Poplar plantation, Little Burton Farm, Leweston, near Sherborne, UK. 9 April 2019. Photograph by Irina Belyaeva.

For the correct identification of studied plants herbarium specimens of listed taxa and their cultivars were studied in A, B, BM, BR, GH, K, LE, MHA, NBGW, NMW, NY, P, SLBI, SVER and WSY **(**herbarium codes are cited according to Thiers, 2020) and their morphological characters were compared to the original descriptions of taxa. Accepted scientific names are given in bold and follow Belyaeva and Govaerts (2020). Typifications were made according to the International Code of Nomenclature for Algae, Fungi and Plants (ICN; Turland *et al*., 2018) and recommendations provided by McNeill (2014; 2015).

Herbarium voucher specimens were produced from the 11 *Populus* clones listed in Table 1 growing at the plantation near Sherborne, UK (Fig. 1) and placed in the following herbaria: NMW (National Museum of Wales), WSY (Royal Horticultural Society Garden, Wisley) and NBGW (National Botanic Garden of Wales).

Morphological characters

Morphological characteristics used for the taxonomic division and identification of *Populus* taxa include leaves on the long and short shoots, petioles, buds, and male and female flowers (Loudon, 1838; Komarov, 1934; 1936; Cansdale,1938, Sokolov *et al*., 1951; Nagaraj, 1952; Critchfield, 1960; Van Broekhuizen, 1964; Bogdanov, 1965; Radu, 1966; Müller and Sauer, 1972; Curtis and Lersten, 1974; Bugała, 1976; Tsaryov, 1979, 1985; Starova, 1980; Meikle, 1984; Eckenwalder, 1977, 1984a, 1984b; 1996; 2010; Jobling, 1990; Koltzenburg, 1999; Kostina and Schanzer, 2014; Kostina and Nasimovich, 2014, Kostina *et al*., 2017; Skvortsov, 2006, 2007a, 2007b; 2008, 2010, 2011; Dickmann and Kuzovkina, 2014; Feodorova and Alexandrov, 2020). However, these characteristics are very variable and make identification of *Populus* taxa extremely difficult. The identification of hybrids is especially difficult as they can exhibit features of one or both parent taxa in all combinations and with great variation. It is thereby practically impossible to differentiate clones of the same taxon

unless there are some extraordinary morphological or other noticeable characteristics. For this purpose, there should be living reference collections in different countries in which every *Populus* clone has been verified by molecular methods, with corresponding herbarium vouchers and a molecular identifier or a recorded DNA fingerprint.

DNA Fingerprinting

Leaf material for DNA fingerprinting was collected from the trees at the poplar plantation, Little Burton Farm, Leweston near Sherborne, UK (Fig. 1) and from verified clones at the INBO (Instituut voor Natuur en Bosonderzoek) tree nursery, Geraardsbergen, Belgium (Fig. 2), separately as two sets of nominally the same eleven poplar clones. For DNA fingerprinting of all samples, a set of 18 highly variable nuclear microsatellite markers was applied, established and standardised in the laboratory of Thünen Institute of Forest Genetics, Waldsieversdorf, Germany (Table 2). The standardised laboratory protocols and marker characteristics were described previously (Liesebach *et al*. 2010, 2011, 2015). The genotypes

Figure 2. Poplar plantation Geraardsbergen, Belgium. 26 May 2019. Photograph by Irina Belyaeva.

were compared with all recorded genotypes previously analysed. This unpublished list contains about 700 different genotypes from the sections *Aigeiros* and *Tacamahaca* and their intersectional hybrids, collected over about 13 years from breeding material at the Thünen Institute and cooperating institutes, reference samples from several clone collections (including samples from Belgium) and some collections in natural habitats.

Table 2. Overview of 18 nuclear microsatellite markers applied to standardised poplar genotyping.

ID	Locus abbreviation	Range (bp)	Primers sequence (5' → 3')	Motif	Number of alleles *	Number of alleles**	Reference ***
1	PMGC456	76-136	TGTAGGAGATATCCACGTGG AACAATATGCTTCATAGCACAG	GA	>30	6	Poplar Molecular Genetics Cooperative (PMGC, 2010)
2	PMGC2852	91-172	ATAATCTCCCTAGCTTAATTCC GAATAACATGGATAATGTGTTTG	GA	>30	7	Poplar Molecular Genetics Cooperative (PMGC, 2010)
3	ORPM30_1	187-193	ATGTCCACACCCAGATGACA CCGGCTTCATTAAGAGTTGG	TC	4	3	Tuskan *et al*. 2004; Lexer *et al*. 2005
4	PMGC14	179-228	TTCAGAATGTGCATGATGG GTGATGATCTCACCGTTTG	CTT	28	6	Poplar Molecular Genetics Cooperative (PMGC, 2010)
5	PMGC2163	188-269	CAATCGAAGGTAAGGTTAGTG CGTTGGACATAGATCACACG	GA	>30	6	Poplar Molecular Genetics Cooperative (PMGC, 2010)
6	ORPM30_2	209-260	ATGTCCACACCCAGATGACA CCGGCTTCATTAAGAGTTGG	TC	>30	8	Tuskan *et al*. 2004; Lexer *et al*. 2005
7	WPMS5	263-320	TTCTTTTTCAACTGCCTAACTT TGATCCAATAACAGACAGAACA	GT	>30	8	Van der Schoot *et al*. 2000
8	PMGC2550	112-167	AGGTTACAAACTTTGTTGTAGC GAACAAACTCTCACTGTGGTC	GA	25	7	Poplar Molecular Genetics Cooperative (PMGC, 2010)
9	PMGC510	132-202	AGTCCTGGTCCTGGATTGG CTACATTAATTTCCCTGTCATC	GA	24	7	Poplar Molecular Genetics Cooperative (PMGC, 2010)
10	PTR2	201-234	AAGAAGAACTCGAAGATGAAGAACT ACTGACAAAACCCCTAATCTAACAA	–	12	4	Dayanandan *et al*. (1998)
11	WPMS20	204-310	GTGCGCACATCTATGACTATCG ATCTTGTAATTCTCCGGGCATCT	TTCTGG	14	5	Smulders *et al*. (2001)
12	WPMS18	210-264	CTTCACATAGGACATAGCAGCATC CACCAGAGTCATCACCAGTTATTG	GTG	14	6	Smulders *et al*. (2001)
13	PMGC2679	98-138	GGAATCCGTTTAGGGATCTG CGTCTGGAGAACGTGATTAG	GA	15	7	Poplar Molecular Genetics Cooperative (PMGC, 2010)
14	WPMS16	125-185	CTCGTACTATTTCCGATGATGACC AGATTATTAGGTGGGCCAAGGACT	GTC	16	7	Smulders *et al*. (2001)
15	WPMS15	188-218	CAACAAACCATCAATGAAGAAGAC AGAGGGTGTTGGGGGTGACTA	CCT	10	5	Smulders *et al*. (2001)
16	PMGC433	179-228	GCAGCATTGTAGAATAATAAAAG AAGGGGTCTATTATCCACG	GA	27	9	Poplar Molecular Genetics Cooperative (PMGC, 2010)
17	WPMS14	209-304	CAGCCGCAGCCACTGAGAAATC GCCTGCTGAGAAGACTGCCTTGAC	CGT	23	9	Smulders *et al*. (2001)
18	WPMS9	235-332	CTGCTTGCTACCGTGGAACA AAGCAATTTGGGTCTGAGTATCTG	GT	>30	6	Van der Schoot *et al*. (2000)

* Number of alleles in all reference samples from sections *Tacamahaca* and *Aigeiros*

** Number of alleles in the samples analysed in this study (Table 1)

******* PMGC markers were obtained from the International *Populus* Genome Consortium. They are available from ORNL (Oak Ridge National Laboratory), website: https://fair.ornl.gov/poplar/ssr_resource.htm

To evaluate the reliability of a set of markers to identify clones it is usual to estimate the probability that two samples are identical by chance at all analyzed markers, although they are not ramets of a clone. CERVUS is a freely available software programme (http://www.fieldgenetics.com/pages/aboutCervus_Overview.jsp) and is one of the packages commonly in use for marker-based parentage analyses in populations (CERVUS package, version 3.0.7, Marshall *et al*. 1998, Kalinowski *et al*. 2007). It needs codominant diploid data (Mendelian loci) of any marker type and considers the input data as a population in Hardy-Weinberg equilibrium. Allele frequencies and then non-exclusion probabilities for identity and sibling identity were calculated from the input dataset for each locus separately and then combined for all marker loci under the precondition of no linkage. The calculated combined non-exclusion probabilities are one part of the standard output that can be used independently from parentage analyses.

In addition to the power to identify clones, the applied set of nuclear microsatellites can provide taxonomical information. Besides a number of species-specific alleles, a multivariate data evaluation can visualize more or less exactly the affiliation of samples to sections, species or hybrids. For this kind of analysis, pairwise genetic distances were calculated, followed by a multidimensional scaling to produce scatter plots (Software: SAS 9.4 TS Level 1M5, Copyright (c) 2016 by SAS Institute Inc., Cary, NC, USA.).

Results and discussion

All research that involves plants start with investigation of their distribution and the history of where, when and by whom they were originally described. The clones listed in Table 1 are now distributed worldwide as valuable trees for forestry and horticulture, but the species involved in their selection are native to different continents. There are three species involved in the hybrid combinations of the clones studied, two from the New World, *Populus deltoides* and *P. tristis*, and one from the Old World, *P. nigra*

Historical background

According to Henry (1914: 2–3) the American Black Poplar, *Populus deltoides*, was introduced to Europe (Holland, France and Great Britain) under this name in 1700, before it was described by Marshall in 1785, and started hybridising with the indigenous European Black Poplar (*P. nigra*) so that nurseries were filled with seedlings from this spontaneous hybridisation. The hybrid plants grew more quickly and, because of this, were more widely cultivated than their parent species. There were also two infraspecific taxa of the European Black Poplar with remarkable features, one with a fastigiate crown (***Populus nigra*** L. f. ***italica***

(Münchh.) A.Andersen Bot. Tidsskr. 30: 405. 1910 ≡ *P. nigra* L. var. *italica* Münchh., Hausvater 5: 229. 1770) and the other with birch-like leaves and pubescent shoots (***Populus nigra*** L. f. ***betulifolia*** (Pursh) I.V.Belyaeva, **comb. & stat. nov.** [urn:lsid:ipni.org:names: 77213518-1] ≡ *P. betulifolia* Pursh, Fl. Amer. Sept. 2: 619. 1813 ≡ *P. nigra* var. *betulifolia* Pursh, Fl. New York 2: 216. 1843 ≡ *P. nigra* subsp. *betulifolia* (Pursh) Wettst. ex Buttler & Hand, Kochia 2: 46. 2007). The spontaneous hybrids between *P. deltoides* and *P. nigra* called *P.* × *canadensis* embrace all possible combinations of characters from the parent taxa that were involved in the crossing. In addition, the fastigiate form of *P. nigra* reached the United States of America (USA) in 1784 and there it was hybridised with the local *P. deltoides* (Henry, 1914). According to Houtzagers (1937) the variety *P. deltoides* var. *missouriensis* Henry was the first to be introduced to Europe and was cultivated also under the name *P. angulata* Aiton. Henry (1914: 258) wrote that an artificial cross was made at Kew Gardens, UK between an old tree of *P. angulata* and the English Black Poplar (*P. nigra* var. *betulifolia*) from which two seedlings were chosen for their uniformity and vigorous growth. Later these Euro-American spontaneous and artificial hybrids became very popular in many countries including the UK.

As reported by Jobling (1990) eleven new poplar clones, some of them bred as so called 'UNAL' clones at the Government Poplar Research Station at Geraardsbergen, Belgium (now INBO), were introduced in 1985 into the UK (see Table 1). These clones were first established in the populetum at Alice Holt Forest, Hampshire, and then at sites with a wide range of environmental conditions throughout the UK. A map of the locations of poplar experiments with 'UNAL' clones in UK is given by Tabbush and Beaton (1998: 358) with the comment "Not all clones were represented at all sites, although *P.* 'Robusta', *P.* 'Ghoy', *P.* 'Scott Pauley', *P.* 'Trichobel' and *P.* 'Beaupre' were included everywhere as 'core' clones." They were provisionally approved by the Forestry Commission Research Division in 1989 and were added to the list of clones approved for commercial production under Forest Reproductive Material Regulations. As most of these clones were bred for conditions in Belgium, in the UK they grow best in the south of England.

Application of scientific names to the cultivated *Populus* clones

The clones studied belong to three taxonomic groups:

(1) hybrids between poplars from the section *Aigeiros* Duby or black poplars (*Populus* 'Gaver', *P.* 'Ghoy', *P.* 'Gibecq', *P.* 'Primo' and *P.* 'Robusta'), the parent species of which are *P. deltoides* and *P. nigra*;

(2) hybrids between poplars that belong to different sections, *Aigeiros* and *Tacamahaca* Spach (*P.* 'Beaupre' and *P.* 'Boelare') the parent species of which are *P. deltoides* and *P. tristis*;

(3) poplars from the section *Tacamahaca* or balsam poplars (*P*. 'Columbia River', *P*. 'Fritzi Pauley', *P*. 'Scott Pauley' and *P*. 'Trichobel') which resulted from the selection of clones of the same species, *P*. *tristis* (= *P*. *trichocarpa* Torr. & A.Gray ex Hook.).

Morphological characters and their use in identification and verification of *Populus* clones

As the hybrid *Populus* clones in Groups 1 and 2 combine the morphological features of *P*. *deltoides, P*. *nigra* and *P*. *tristis* respectively, their distinct characters are given in Table 3.

Cansdale (1938) underlined six morphological features that are important for the identification of black poplars:

(1) translucent (cartilaginous) leaf margins – the character that is present only in *Populus* of the section *Aigeiros*

(2) ciliation on leaf margins – the character that is present in the American Black Poplars and their hybrids but not in European Black Poplars

(3) glands at the junction of the petiole and leaf blade (basilaminar glands) – two or more glands are present in the American species but not in the European one

(4) shape of the petioles in cross-section – this character is similar in American and European Black Poplars

(5) shape of the branchlets in cross-section – the presence of ridges or angles in the American species and their absence in the European species

(6) variation in leaf type within the species depending on their position on the branchlet, the type of branchlet (long or short).

Recent work by Russian scientists (Kostina and Schanzer, 2014; Kostina and Nasimovich, 2014) emphasised that European Black Poplars have a 2-carpellate capsule and American Black poplars a 3–4-carpellate capsule which had also been noticed earlier by Cansdale (1938), Starova (1980) and (Eckenwalder, 2010).

Each of the clones discussed in this paper has a unique combination of variable characters which is reflected in their genetics, morphology and economically important features such as rate of growth and resistance to different pests and diseases (Van Broekhuizen, 1964, 1970, 1972; Ceulemans and Impens, 1980; Van Slycken and Stevens, 1987; Jobling, 1990; Smith, 2016).

The names of all the clones were established during the evaluation of their propagation, growth and resistance (Van Broekhuizen, 1970; Steenackers and Van Slycken, 1982; Van Slycken, 1984a; 1984b; Van Slycken and Stevens, 1987) according to the rules of the International Code of Nomenclature for Cultivated Plants (ICNCP, Brickell *et al*., 2016: Article 27).

Table 3. Morphological characters of *Populus nigra* L., *P. deltoides* W.Bartram ex Marshall and *P. tristis* Fisch.

Character	*Populus nigra*	*Populus deltoides*	*Populus tristis*
Long shoots	glabrous to sparsely hairy, always round and without corky ribs, green or brown in summer, grey-yellowish in winter, shiny*	glabrous or thinly long-hairy, 5-angular with more or less marked corky ribs or round**	usually densely hairy, coarse, round, reddish brown becoming grey
Leaves on long shoots	broadly triangular or deltoid, 5–7 cm long and 4–6 cm wide, at the base straight, broadly cuneate or rounded, seldom narrowly cuneate with short, evenly tapering tips, margin translucent, bluntly, finely crenate, not ciliate; basilaminar glands absent	broadly triangular ovate, with truncate to cordate or broadly cuneate base, 3–9 cm long and 3–9 cm wide; with round or tubular basilaminar glands (2–6); margin translucent, ciliate, crenate-serrate; apex abruptly short or long-acuminate	usually triangular or narrowly ovate, 5–9 cm long and 5–6 cm wide, base rounded to cordate, with 2 round basilaminar glands, margin not translucent, not ciliate, finely evenly crenate-serrate apex obtuse to acute, abaxial surface white to greyish white or greenish white with red resin stains, sparsely pubescent, adaxial dark green and abaxially pale grey-green, glabrous
Petioles on long shoots	glabrous, distally side-flattened, slightly reddish	glabrous, distally side-flattened	glabrous or sparsely pubescent, cylindrical or distally slightly flattened, often markedly swollen distally, half of blade length
Petioles on short shoots	glabrous, 3–5 cm long, distally side-flattened	glabrous, 3–8 cm long, equal leaf blade length, distally side-flattened	
Buds	brown, glabrous, coated with aromatic balsamic substance, flower buds bigger than leaf buds, recurvate, set on short shoots or at the base of long shoots	greenish yellow, glabrous or stiffly hairy, resinous, slightly fragrant	red, sparsely hairy or glabrous, resinous, sticky, very fragrant***
Male generative shoots	catkins 5–6 cm long, rachis green, glabrous, with densely set flowers	catkins to 10 cm long, with densely set flowers	catkins densely flowered, 7–10 cm
Female generative shoots	catkins 6–8 cm long in flowers, light green, 10–16 cm in fruits	catkins loosely flowered with 15–40 flowers, 5–8 cm long in flowers, to 18–24 cm in fruits	catkins densely flowered, 7–10 cm, 25–50 flowers per catkin, to 17 cm long in fruit, pedicel to 3 cm in fruit
Male flowers	15–30 stamens with dark red anthers	40–60 stamens with truncate anthers	30–50 stamens with truncate anthers
Female flowers	ovaries 2 carpellate, spherical, shiny green, on distinct peduncles, half enveloped with lighter perianth; two sessile stigmas are lighter in colour than ovary and cucullate; bracts yellowish green laciniate, dark brown on the edge	ovaries 3–4 carpellate, ovoid, glabrous; 3–4 stigmas, platelike, spreading, bracteoles of both sexes laciniate, not ciliate	ovary 3–4 carpelled, spherical, (hairy), stigmas 2–4 platelike, spreading;
Fruits	capsules roundish or oval, smooth with a short tip	capsules ovoid, glabrous sulcate or wrinkled	capsules spherical, densely hairy to glabrate

* petioles of young leaves on long and short shoots with indumentum (Feodorova and Alexandrov, 2020);
** petioles of young leaves on long and short shoots with sparse indumentum (Feodorova and Alexandrov, 2020);
*** only ***P. deltoides*** subsp. ***wislizenii*** (S.Watson) Eckenw. has hairy buds. (Eckenwalder, 1977)

Group 1. Clones based on hybrids between species of the section *Aigeiros* are known under the clonal names *Populus* 'Robusta', *P.* 'Gaver', *P.* Gibecq', *P.* 'Ghoy' and *P.* 'Primo'. They all belong to a hybrid taxon, ***Populus* × *canadensis***,

Populus* × *canadensis Moench, Verz. Ausländ. Bäume: 81. 1785 ≡ *P.* × *euramericana* Guinier, Rapp. Comiss. Int. Peuplier: 6. 1950, nom. illeg. superfl.

Type: United Kingdom, Royal Botanic Gardens Kew, Arboretum, 29.III.1965, *sine col. 406/65k*, ♂ (K! – neotype, **designated here** by I.V.Belyaeva); United Kingdom, Royal Botanic Gardens Kew, Arboretum, 1964, *A. Neumann 124/84g* (K! – epitype, **designated here** by I.V.Belyaeva).

Note: This name has 24 synonyms in the *World Checklist of Vascular Plants* (WCVP, Govaerts, 2020), one of which, *P.* × *euramericana* Guinier, was erroneously used widely in cultivation, mostly by breeders and foresters, as this name has been accepted incorrectly by the International Poplar Commission of FAO despite it not complying with the rules of the ICN (Turland *et al*., 2018). The full and comprehensive nomenclatural and taxonomical analysis for *Populus* × *canadensis* and *P.* × *euramericana* was given by Boom (1957) and the latter was recognized as a superfluous name, thus out of use.

P.* × *canadensis Moench **f. *robusta*** Simon-Louis ex Schelle, Handb. Laubholzben. (L.Beissner, E.Schelle & H.Zabel): 16. 1903 ≡ *P.* × *robusta* (Simon-Louis ex Schelle) C.K.Schneid., Ill. Handb. Laubholzk. 1: 11. 1904 ≡ *P.* × *canadensis* Moench var. *robusta* (Simon-Louis ex Schelle) Hyl., Nordisk Karlvaxtfl. 2: 387. 1966.

(= *P. deltoides* var. missouriensis (A.Henry) A.Henry × *P. nigra* f. *italica*)

Type: United Kingdom, Royal Botanic Gardens Kew, Arboretum, 31.VIII.1964, *sine col. 401* (K! – neotype, **designated here** by I.V.Belyaeva); United Kingdom, Royal Botanic Gardens Kew, Arboretum, 29.III.1965, *sine col. 191/92m*, ♂ (K! – epitype, **designated here** by I.V.Belyaeva).

= *P. vernirubens* A.Henry, Gard. Chron. 87: 24. 1930

Type: United Kingdom, between Effingham junction and Ockham Surrey, heathy pasture near May's Green, 7.VI.1914, *C.E. Britton 1180* (K! – neotype, **designated here** by I.V.Belyaeva).

Note: According to Henry (1914) *P.* × *robusta* was found in 1895 in Simon-Louis' nursery near Metz and he saw an old female tree of *P. angulata* Aiton (= ***P. deltoides***) near to where the seedlings of this hybrid were growing. This female individual was most likely pollinated by pollen of *P.* × *plantierensis* A.Henry (= *P. nigra* f. *betulifolia* × *P. nigra* f. *italica*). The microsatellite genotypes of the clones *P.* × *plantierensis*, *P. nigra* f. *betulifolia* and *P. nigra* f. *italica* are consistent with this pedigree. Henry (1914) wrote that from *P.* × *plantierensis*, hairy

twigs and a fastigiate crown with ascending (not vertical) branches were derived, which characterise *P.* × *robusta*. The name of this male *Populus* clone refers to its "robust" growth. There were two similar seedlings originally found and one of them was described by Henry in 1930 as *P. vernirubens* from its leaf colour. *P.* × *canadensis* f. *robusta* was established later as the cultivar *Populus* 'Robusta'.

From our molecular research the offspring *P.* 'Robusta' is consistent with the mother *P. deltoides* var. *missouriensis* (= *P. angulata*), in the Thünen Institute database under the clonal name *P.* 'Angulata de Chautagne,' and the father *Populus nigra* L. f. *italica*, in the same database as *P.* 'Italica,' but not with the father *P.* × *plantierensis*. It is therefore likely that *P.* 'Robusta' inherited its upright habit from *P. nigra* f. *italica* rather than from *P.* × *plantierensis*.

A further four cultivars of *Populus* × *canadensis*, *P.* 'Gaver', *P.* 'Ghoy', *P.* 'Gibecq' (named after places in Belgium) and *P.* 'Primo' (translated into English as 'top quality'), originated and were established in Belgium (Steenackers and Van Slycken, 1982, Smith *et al*., 2016). These clones have very similar morphological features but differ in their growth and disease resistance. Below are short descriptions according to Van Broekhuizen (1970), Steenackers and Van Slycken (1982), Jobling (1990), Tabbush and Beaton (1998) and Smith *et al*. (2016).

Populus 'Gaver' is a male clone having a straight trunk with a tendency to form heavy branches, low in the crown. Leaves emerge early, growth is rapid and it suffers from breaking branches at an early age and therefore is not used as an avenue tree. It is moderately resistant to rust, *Melampsora larici-populina* Kleb., and the fungal leaf spot pathogen *Marssonina brunnea* (Ellis & Everh.) Magnus and has good resistance against bacterial cancer, *Xanthomonas populi* (Ridé) Ridé.

Populus 'Ghoy' is a female clone and is the fastest growing of the four UNAL clones of *Populus* × *canadensis* mentioned above. It has a straight trunk and fine branches, having the least problems with branch breakage and is therefore in an experimental scale trial as an avenue tree (Bomen boek, 2019). This clone is resistant to rust, leaf spot disease and bacterial canker.

Populus 'Gibecq' is a male clone. It has a very good trunk shape, but also forms heavy branches and so is not suitable for avenue plantings. Like *P.* 'Gaver' this clone is very good for forest planting in pure stands. It is still little known in cultivation.

Populus 'Primo' is a male clone with a good straight trunk and a few heavy branches but is quite wind resistant. It is a suitable tree for avenue plantings because of its habit. This clone is not too sensitive to rust and quite resistant to *Marssonina brunnea* and bacterial canker.

All five clones from this group are listed for sale in the tree nursery Udenhout (Bomen boek, 2019).

Group 2. Clones based on hybrids between species of the section *Aigeiros* and species of the section *Tacamahaca*. Known under the clonal names *Populus* 'Beaupré' and *P.* 'Boelare,' they belong to the hybrid taxon ***Populus* × *generosa***.

Figure 3. *Populus* × *generosa* A.Henry at Royal Botanic Gardens, Kew, 2017. Photo by Kevin McGinn.

Populus* × *generosa A.Henry, Gard. Chron., ser. 3, 56: 258. 1914.

(***P. deltoides*** × ***P. tristis***) [Fig. 3].

Type: United Kingdom, Royal Botanic Gardens Kew, Arboretum, location 242, 22.VII.2016, *Kevin McGinn* Accession number: *1969-17235* (K! – neotype, **designated here** by I.V.Belyaeva); United Kingdom, Royal Botanic Gardens Kew, Arboretum, on Seven Sisters Lawn, 4.V.1933, *W. Dallimore s.n.*, ♀, fruits (K! – epitype, **designated here** by I.V.Belyaeva).

– *P. wettsteinii* Janch., Phyton (Horn) 8: 232. 1959, **nom. inval.** (without Latin description).

Note: the story of this hybrid's origin at the Royal Botanic Gardens, Kew was written by Henry (1914) as follows: "In March 1912, a pistillate Carolina Poplar (*P. angulata*) at Kew was crossed with the pollen of *P. trichocarpa*, of which there is a fine specimen, then sixteen years old in that garden. From the few seeds, which ripened towards the end of June and were sown immediately there were raised, at Cambridge, four seedlings. These attained about 2 inches in height by the end of October, 1912. Starting next season as tiny plants in good garden soil at Glasnevin, they grew remarkably in 1913." He also gives a general description of this hybrid poplar underlining its intermediacy between parents in width and colour of leaves, their lower surface being pale grey and their rounded petioles resembling *P. trichocarpa* leaves but their leaves having a coarsely serrate translucent margin and cordate base liken it to *P. angulata* (= ***P. deltoides***). Jobling (1990) reported that *P.* × *generosa* was widely planted in the UK for amenity purposes, often at the roadside and in parks and gardens, in preference to other poplars. He also concluded that "the

number of clones in cultivation, and the extent of their variation in appearance and behaviour, is unknown. Both male and female of this historically important hybrid were planted at one time." A collective name for this group of hybrids was suggested, *P.* × *interamericana* (Van Broekhuizen, 1972) and was invalidly published without Latin description and type citation. Although rare, *P.* × *generosa* has also since been recorded in the wild in the USA where the native distribution of *P. deltoides* and *P. tristis* narrowly overlap (Idaho, Montana, Washington and Wyoming) (Eckenwalder, 1984a).

Below are short descriptions of two cultivars, *Populus* 'Beaupré' and *P.* 'Boelare', compiled according to Steenackers and Van Slycken (1982), Jobling (1990) and Smith *et al.* (2016). Both cultivars were selected in the 1970s because of their extremely fast growth rate, their straight trunk, crown shape and their resistance to bacterial canker, the rust *Melampsora larici-populina* Kleb., and *Marssonina brunnea*. Their names were also established in 1982 (Steenackers and Van Slycken, 1982). The name of *P.* 'Beaupré is derived from the name of the Beaupré Abbey, located in Geraardsbergen and close to the INBO tree nursery and *P.* 'Boelare' is named after the Boelare Castle, located in the municipality Nederboelare, which is now part of the town of Geraardsbergen. These poplars are rightly regarded as the fastest growing broad-leaved trees in Western Europe (Jobling, 1990; Tabbush and Beaton, 1998; Bomen boek, 2019). *Populus* 'Beaupré' and *P.* 'Boelare' being female clones produce fluffy wind-dispersed seeds which are seen as a nuisance by some. Because of this and their susceptibility to rust, they are no longer in use in cultivation as ornamental trees although they have been used in breeding programmes for dense wood and for mixed forest plantings. According to the *FAO International Register of Cultivars of Populus* (2020), *Populus* 'Beaupré' is an artificial hybrid between *P. trichocarpa* 'Fritzi Pauley' and *P.* 'S.1-173' (= *P. deltoides* from Iowa ×*P. deltoides* from Missouri). Microsatellite genotypes of *P.* 'Beaupré' and *P.* 'Fritzi Pauley' are consistent with this pedigree. *P.* 'Fritzi Pauley' is the mother of *P.* 'Boelare' too. These clones are listed for sale in the tree nursery Udenhout (Bomen boek, 2019).

Group 3. Clones based on selection from different individuals of the same species of the section *Tacamahaca* – ***Populus tristis*** Fisch. They are known under the clonal names *P.* 'Columbia River', *P.* 'Fritzi Pauley', *P.* 'Scott Pauley' and *P.* 'Trichobel'.

Populus tristis Fisch., Allg. Gartenzeitung 9: 402. 1841.

Type: Russia, Botanical Gardens, St. Petersburg, 1933, in cultivation, *sine coll.* as *Populus candicans* Aiton (LE01064206! – lectotype, designated by Skvortsov, 2008: 66 [Fig. 4]).

= *P. trichocarpa* Torr. & A.Gray ex Hook., Hooker's Icon. Pl. 9: t. 878. 1852.

≡ *P. balsamifera* subsp. *trichocarpa* (Torr. & A.Gray ex Hook.) Brayshaw, Canad. Field-Naturalist 79: 95. 1965.

Type: United States, California, Santa Clara River near Beneventano, *sine date*, *Mss. R.M. Austin ex Herb. A.Gray*, ♀ (K000592057! – lectotype, **designated here**); United States, California, Plumas Co., *Mss. R.M. Austin ex Herb. A.Gray 10/78*, ♂ (K000592056! – epitype, **designated here** by I.V.Belyaeva).

Note: This *Populus* was introduced to the UK in 1892 under the name *P. trichocarpa* (Jobling, 1990) but had been known in Russia and Europe under the name *P. tristis*, the earlier name that has priority (Skvortsov, 2008, 2010). It was recognised as the fastest growing poplar and a very ornamental tree and so it was very quickly distributed widely in cultivation.

According to Jobling (1990) "the first few clones of *P. trichocarpa* introduced to the UK had a northern, probably Canadian origin" and "In the 1970s, two of the most disease resistant and vigorous clones imported from Washington State were released to the nursery trade in this country and approved by the Forestry Commission for planting for timber production. They were named *Populus* 'Fritzi Pauley' and *P.* 'Scott Pauley'. At about the same time the former was released in Belgium, France, Germany and the Netherlands, where a full account was published, and the latter was released in Germany, where a botanical description was prepared". *P. trichocarpa* has been much used in artificial breeding programmes in several European countries as well as in the USA.

***Populus* 'Columbia River'**

According to Smith (2016: 114) this clone was selected in the early 1960s in Canada. In *FAO International Register of Cultivars of Populus* (2020) it is recorded as a male clone. *Populus* 'Columbia River' was established in 1982 (Steenackers and Van Slycken, 1982) and was named after the river on the Pacific Northwest USA. An artificial hybrid between selected clones of *P. trichocarpa*. It is a vigorously growing tree with upright long slender branches up to approx. 20 m high, densely branched, more or less columnar and forms straight trunks. It is a male cultivar that is fairly resistant against rust, *Marssonina brunnea* and bacterial cancer. It could be used as a park tree but is not suitable for avenue planting. A strong wind can cause breakage of branches and trunks. This is one of the cultivars of ***P. tristis*** (= *P. trichocarpa*) available at nurseries. It is listed for sale in the tree nursery Udenhout (Bomen boek, 2019).

***Populus* 'Fritzi Pauley'**

Named after the wife of Prof. Scott Pauley, Harvard University, USA, this female, rapidly growing cultivar was established in 1970 (Van Broekhuizen, 1970).

Figure 4. Lectotype of *Populus tristis* Fisch. LE01064206

As reported by Van Broekhuizen (1970) the original tree was selected by Prof. Scott Pauley together with Dr. H. Johnson near Skagit River, Mount Baker National Park, Washington State, USA, in 1947. Cuttings of this poplar under the number V235 were sent to the Geraardsbergen, Belgium in 1948. Later this cultivar was introduced to the UK in 1950 and, at about the same time, to other countries of Europe (Jobling, 1990). It was used in the breeding programme in Belgium as a parent for crossing with *P. deltoides*. It was characterised by Smith (2016: 114) as a worthless tree that blows down in the Netherlands. It is fairly resistant to rust and very resistant against *Marssonina brunnea* and bacterial cancer, and because of this may be suitable for short rotation plantation as wood for green energy. It is hardly in cultivation anymore although this cultivar is still growing at RHS Garden, Wisley, UK, and is listed on the RHS website.

***Populus* 'Scott Pauley'**

Named after Prof. Scott Pauley, Harvard University, *Populus* breeder, this clone was established in 1977 (Hoffmann *et al*., 1977).

According to Jobling (1990), it is a vigorous cultivar from Washington State that has a very fine form and is not prone to grow epicormics unless very heavily pruned. It is not damaged by wind and is approved by the Forestry Commission for timber production.

***Populus* 'Trichobel'**

An artificial hybrid between selected clones of *P. trichocarpa*, it was established in 1982 (Steenackers and Van Slycken, 1982). The clone's name refers to the species epithet

Figure 5. Lateral adventitious roots developed on a twig of *Populus* 'Trichobel' collected from Little Burton Farm, Leweston, UK on 19 July 2019. Photograph by Irina Belyaeva.

'*trichocarpa*' (tricho-) and Belgium where it originated (-bel). It is a male clone and a very strong growing tree with a straight trunk. Little or no sensitivity to *Marssonina brunnea*, resistant to poplar canker and moderately sensitive to rust. A very ornamental tree. *P.* 'Trichobel' is different from other clones of this group in the quick rooting of its cuttings which take just 3-5 days in moist condition to start growing roots (Fig. 5). This clone is listed for sale in the tree nursery Udenhout (Bomen boek, 2019).

DNA Fingerprinting in identification and verification of *Populus* clones

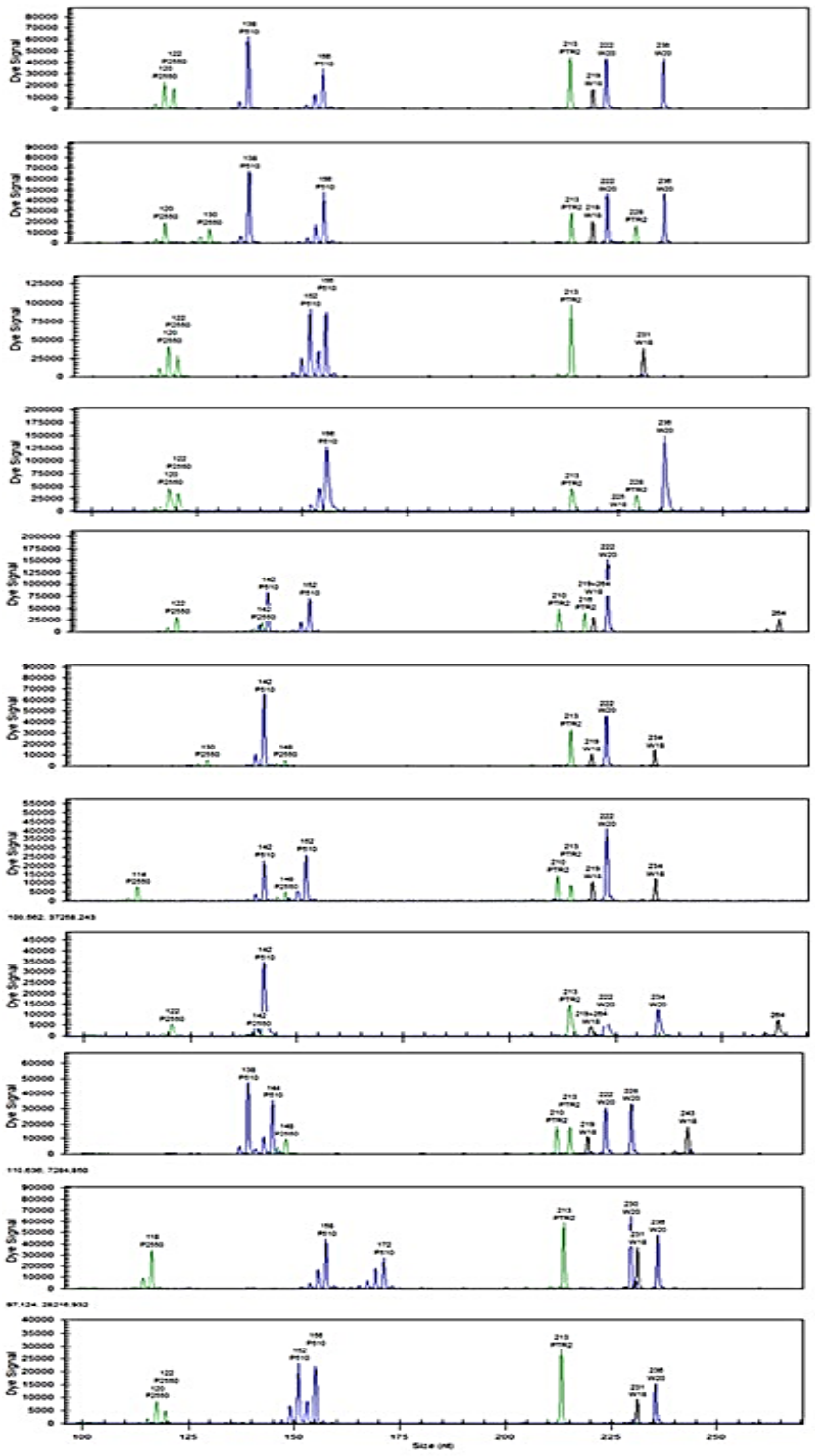
A reliable clone identification with the applied marker set is demonstrated by extremely low probabilities for sample identity by chance. Estimated non-exclusion probabilities for sibling identities amount to 7×10^{-6} for *P. tristis*, to 5×10^{-6} for *P. nigra*, and to 2×10^{-4} for *P. deltoides*. The estimates for unrelated individuals are 8×10^{-15}, 5×10^{-15}, and 6×10^{-11} respectively (see Table 4).

Table 4: Calculated combined non-exclusion probabilities for identity respective sibling identity with help of the software CERVUS for three poplar species

Species	Number of included samples	Combined non-exclusion probability (identity)	Combined non-exclusion probability (sibling identity)
P. trichocarpa	100	7.792E-0015	0.00000676
P. nigra	123	5.083E-0015	0.00000504
P. deltoides	85	5.648E-0011	0.00022241

According to our results the 11 genotyped poplar accessions (Fig. 6) collected at the poplar plantation, Little Burton Farm, Leweston near Sherborne,UK, represent 10 different and clearly distinguishable multilocus genotypes. These 10 genotypes fit to the respective reference material derived from the Belgian collection (Table 5). The Sherborne sample of *P.* 'Primo' is identical with the sample of *P.* 'Ghoy' from Sherborne as well as from Belgium (No. 8 in Table 5). Obviously, this sample was mislabelled, which might happen at any time during or after introduction from Belgium in 1985, the vegetative propagation and transfers within the UK or leaf collection and during genotyping in the laboratory.

All genotypes from this study were compared to the existing entries in the poplar genotype list of the Thünen Institute, Germany. The clones *P*. 'Beaupre', *P*. 'Boelare', *P*. 'Fritzi Pauley', *P*. 'Gaver', *P*. 'Robusta' and *P*. 'Scott Pauley' (Table 4) fit exactly to several other



P. 'Beaupre'

P. 'Boelare'

P. 'Columbia River'

P. 'Fritzi Pauley'

P. 'Gaver'

P. 'Ghoy'

P. 'Gibecq'

P. 'Primo'

P. 'Robusta'

P. 'Scott Pauley'

P. 'Trichobel'

Figure 6: Example of the fingerprint patterns for 5 of the markers, 11 clones listed as in Table 1 (material from Little Burton Farm, Leweston, UK, with the exception of clone 'Primo' from INBO, Belgium)

independent samples. For the clones *P*. 'Ghoy', *P*. 'Gibeqc', *P*. 'Primo' and *P*. 'Trichobel' (Table 4) no former entries were available, but the reference samples originating from the breeding institute (INBO) were available and, therefore, their identities are without any doubt.

The samples of *P*. 'Columbia River' (Sherborne, UK and INBO, Belgium) are both identical to a genotype in the database of the Thünen Institute, Germany from a certain clone which was used as a female parent in crossings. This sample is also identical to an accession with the name 'Blom' from the German Bundessortenamt. Its genotype clearly fits to *P. tristis* (= *P. trichocarpa*) and has no hint of any other species contributions.

Another genotype in the database named *P*. 'Columbia River' has *Populus tristis* (= *P. trichocarpa*) and an Asian balsam poplar (*P. suaveolens* Fisch. ex Poit. & A.Vilm.) in its pedigree. However, this is a single sample without repetition from other sources.

A visualisation of taxonomic information derived from microsatellite genotyping is presented in Figure 7. 396 samples belonging to taxonomic groups relevant in this study (the three species *P. tristis, P. nigra, P. deltoides* or hybrids between them) were selected from the entire available data pool. Based on multidimensional scaling, a two-dimensional plot was created for graphical display that clearly confirms the status of the 11 clones genotyped in this study.

Table 5. Result of assignment of analysed genotypes with the reference list.

No.	Name of cultivar	Result of genotyping and comparison with previously analysed samples
1.	*Populus* 'Beaupre'	Identical genotype with the reference sample from Belgium and with other independent samples of *Populus* 'Beaupre'
2.	*Populus* 'Boelare'	Identical genotype with the reference sample from Belgium and with other independent samples of *Populus* 'Boelare'
3.	*Populus* 'Columbia River'*	Identical genotype with the reference sample from Belgium. See comment below
4.	*Populus* 'Fritzi Pauley'	Identical genotype with the reference sample from Belgium and with other independent samples of *Populus* 'Fritzi Pauley'
5.	*Populus* 'Gaver'	Identical genotype with the reference sample from Belgium and with other independent samples of *Populus* 'Gaver'
6.	*Populus* 'Ghoy'	Identical genotype with the reference sample from Belgium
7.	*Populus* 'Gibecq'	Identical genotype with the reference sample from Belgium
8.	*Populus* 'Primo'	Identical with the genotype of *Populus* 'Ghoy'
9.	*Populus* 'Robusta'	Identical genotype with the reference sample from Belgium and with other independent samples of *Populus* 'Robusta'
10.	*Populus* 'Scott Pauley'	Identical genotype with the reference sample from Belgium and with other independent samples of *Populus* 'Scott Pauley'
11.	*Populus* 'Trichobel'	Identical genotype with the reference sample from Belgium

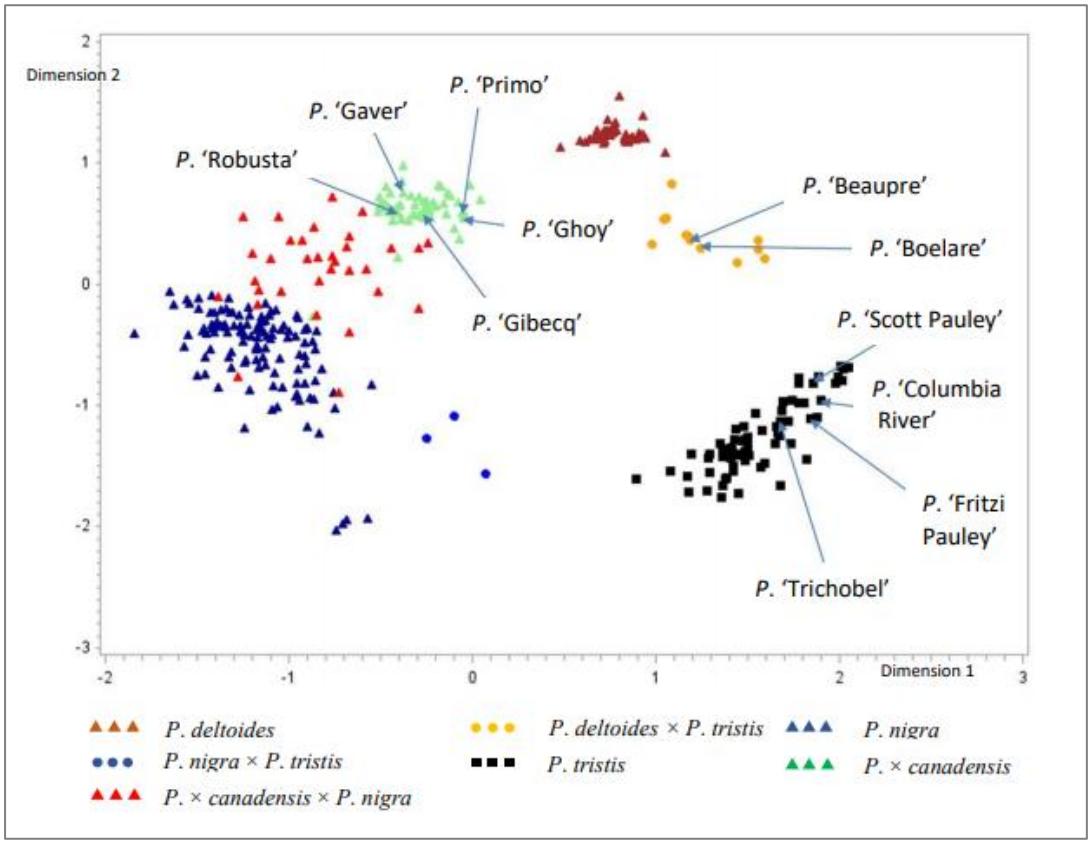


Figure 7. Scatter plot derived from genotypes of samples belonging to three species (*Populus deltoides, P. nigra, P. tristis*) and their hybrids (section *Aigeiros*: triangles, section *Tacamahaca*: squares, intersectional hybrid: dots), 11 clones analysed in this study are highlighted by arrows.

Conclusions

Nuclear microsatellites have long been used for the identification of individual genotypes and the analysis of pedigrees, being independent of morphology and environmental influences. Regarding the assessment of reliability of clone identification for a given marker set, the combined non-exclusion probabilities were usually considered as raw estimates, because the strong precondition of Hardy-Weinberg equilibrium can hardly be fulfilled in experimental data from natural or breeding populations. Therefore, the calculated probabilities may be underestimated and the real probabilities may be higher, but even 10 fold higher probabilities could be sufficient to expect reliable results. However, uncertainty might come from mislabelled material that has entered the genotype database, especially, if there is only one sample of a certain clone. Such mislabelling could be a result of confusion in the long history of poplar breeding and exchange of material among institutes worldwide.

Nine clones of *Populus* included in this research were identified as such by using morphological and molecular markers. Their herbarium vouchers can be seen in the herbaria at NBGW, NMW and WSY. Although two clones, *P.* 'Primo' and *P.* 'Columbia River' are

confirmed with their taxonomy, further investigation of their genetic identity is needed, and their herbarium vouchers could not be used in other research as proof of identity.

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Authors' contributions

Irina V. Belyaeva (IB) initiated the project together with JP, CD and GT; studied live and herbarium material, collected and prepared samples for DNA-analysis from trees growing in UK, wrote the first version of the manuscript, made necessary nomenclatural and taxonomic statements together with RG and KM, made a new combination and typifications, participated in discussion of the results and text of the manuscript and coordinated the project.

Charles Dutton (CD) initiated the project together with JP, IB and GT, organised and curated collection of poplars at Sherborne (UK), organised collection and primary identification of the clones, analysed and discussed results and the text of the manuscript.

Rafaël H.A. Govaerts (RG) together with IB and KM discussed nomenclatural and taxonomic decisions, participated in preparation and discussion of the manuscript.

Heike Liesebach (HL) analysed DNA-samples prepared from leaves collected by IB and MS, carried out molecular research, the data analysis and statistics, participated in preparation and discussion of the manuscript.

Kevin McGinn (KM) collected, prepared and identified herbarium samples together with IB at K, analysed and hosted voucher herbarium specimens at NBGW, participated in preparation and discussion of the manuscript.

Marijke Steenackers (MS) organised and collected together with IB herbarium specimens of 'UNAL' clones at the poplar plantation of INBO (Belgium) for taxonomic research and

identification, collected and prepared plant material from poplar clones for DNA-analysis, participated in preparation and discussion of the manuscript.

Graham Taylor (GT) together with CD, JP and IB initiated the project, planned and discussed details of organisation of research and results, participated in preparation and discussion of the manuscript.

John Pickett (JP) together with CD, GT and IB initiated the project, lead and advised in organisation of scientific collaboration and planned stages of the research, lead scientific meetings and discussions on the necessary research and scientific results, participated in preparation and discussion of the manuscript.

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