

# Identification of weeping crabapple cultivars by microsatellite DNA markers and morphological traits



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## ABSTRACT

Ornamental crabapples are small landscape trees with charming flowers, colourful fruits and many growth forms. The first weeping crabapple cultivars, *Malus prunifolia* 'Pendula' and 'Pendula Nova', were described in Sweden around 150 years ago. Our study was aimed at identification and characterization of weeping crabapple clones by microsatellite markers and morphological traits. We analysed 13 Swedish and Finnish trees and 8 reference accessions including *M. prunifolia* 'Pendula' and three international cultivars belonging to its progeny. The 21 trees represented 13 distinct genotypes. Five local trees were identified as the historical 'Pendula', assumed to be extinct from the nursery trade. On the basis of morphological traits and historical records, two old Swedish trees were concluded to represent 'Pendula Nova'. The authenticity of the trees could not be confirmed by DNA markers because no known plant of the old cultivar was found in botanical collections. The Finnish clone 'Hyvingiensis' proved unique among the crabapple accessions studied. 'Hyvingiensis' was probably raised from seed at the Finnish State Railways Nurseries about 110 years ago. Several mislabellings were revealed among both the local and the reference samples. A novel identification key was created to aid discrimination between the clones by their morphological traits. A combination of DNA fingerprints, comparison of morphological traits and tracing information in relevant archives and old garden literature proved useful for solving the origin and identity of weeping crabapples. The results contribute to conservation of garden plants and stabilization of horticultural nomenclature.

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## 1. Introduction

Ornamental crabapples (*Malus* spp.) are small trees and shrubs valued for their charming flowers, handsome summer and autumn foliage, and colourful fruits that often remain on the tree long after leaf fall. Crabapples provide a wide range of growth forms as well as flower and fruiting characteristics. Many crabapples are more winter hardy than the common apple (*Malus domestica*) and the fruits of several cultivars are suitable for economic uses, facts that have certainly raised the interest in growing crabapples at high latitudes.

Crabapples are distinguished from apple trees grown for fruit production by fruit size: members of the genus *Malus* that have fruits 2 in. (ca. 5 cm) or less in diameter are considered as crabapples (Wyman, 1955). Some wild *Malus* species and varieties are grown as ornamental crabapples, but most of the cultivated forms

are hybrids between different *Malus* taxa. In the twentieth century, breeding of ornamental crabapples was carried out mainly in Canada and in the USA, where approximately 400–600 different forms and cultivars are grown (Dirr, 2009).

A special group of ornamental crabapples is those with pendulous branches, the weeping or hanging cultivars. The first two weeping *Malus* varieties described in horticultural literature arose in 1860 and 1873 at the experimental station of the Royal Swedish Academy of Agriculture in Stockholm (Lindgren, 1878a, 1878b). The clones were named as *Pyrus prunifolia pendula* and *P. prunifolia pendula nova*. The earlier of them, *Malus prunifolia* 'Pendula' according to current nomenclature, proved to be very winter hardy (up to 66°N) and was soon introduced to tree nurseries in Europe and North America (Lindgren, 1878a, 1878b).

The classic weeping crabapple cultivars, 'Excellenz Thiel' (introduced by the German nursery L. Späth in 1909), 'Oekonomierat Echtermeyer' (introduced by Späth in 1914) and 'Red Jade' (introduced by the Brooklyn Botanic Garden in 1953), belong to the first- and second-generation progeny of *M. prunifolia* 'Pendula'. 'Excellenz Thiel' is a seedling of *M. p.* 'Pendula', with *M. floribunda* as the putative pollen parent (Späth, 1909),

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while 'Red Jade' is an open-pollinated seedling of 'Excellenz Thiel' (Jefferson, 1970). The pink-flowering 'Oekonomierat Echtermeyer' is described as a hybrid between 'Excellenz Thiel' and *Malus pumila* var. *niedzwetzkyana* (Jefferson, 1970). *Malus* 'Elise Rathke' (first recorded in 1884 and in our opinion correctly named as *M. domestica* 'Pendula') is another old weeper that is not a crab, but rather a common apple on grounds of fruit diameter, 5.9 cm in average, according to the database of UK National Fruit Collections (<http://www.nationalfruitcollection.org.uk>).

In the second half of the twentieth century, some 50 new weeping crabapple cultivars were released (Fiala, 1994). Nearly all the new weepers were bred in the USA and only a few of them are marketed in Europe, to judge from the Plant Finder of the Royal Horticultural Society (<http://www.rhs.org.uk/plants/>) and the name list of the European Nurserystock Association (<http://www.internationalplantnames.com/>).

The two oldest weepers, *M. prunifolia* 'Pendula' and 'Pendula Nova', have not been available in Sweden for a long time. Andréasson and Wedelsbäck Bladh (2011) noted both cultivars in the catalogues of two Swedish nurseries between 1864 and 1900, but not thereafter. From an historical inventory list (Anon., 1879), we know that both were imported to Finland before the end of the nineteenth century. *M. prunifolia* 'Pendula' was cited in Finnish garden literature until the 1940s (e.g. Sorma, 1932; Schalin, 1935) and marketed by local nurseries until the early 1960s (e.g. Harviala Plantskolor Och Växthus, 1939; Olsson, 1962). Most Finnish and a few Swedish tree nurseries currently offer a white-flowering crabapple with strongly weeping branches, named as *Malus* 'Hyvingiensis'. The cultivar was first mentioned in garden literature in 1953 (Lehtonen and Jokela, 1953) and it

was originally spread by the Finnish State Railways Nurseries (active 1873–1990 at Hyvinkää), but the origin and uniqueness of 'Hyvingiensis' remain uncertain.

The aim of our research was to identify and characterize weeping crabapple clones cultivated in old parks in Sweden and Finland. Our specific objectives were (1) to find out if extant plants of the Swedish cultivars, 'Pendula' and 'Pendula Nova', can still be found, (2) to determine the cultivar identity of the Finnish clone 'Hyvingiensis' and (3) to confirm cultivar names of a few weeping crabapples grown in Swedish and Finnish botanical collections. For cultivar identification, we used microsatellite (SSR)-based DNA markers and morphological traits.

## 2. Materials and methods

### 2.1. Plant material

The 13 local accessions studied (Table 1) involved un-named crabapple trees recorded in connection with park inventories or through observations of plant experts as well as a few doubtfully named trees in botanical gardens or arboreta. For 'Hyvingiensis', an about 65 years old tree of documented origin at the Finnish State Railways Nurseries was used as the authentic accession and a tree propagated by Ahonen's Nursery represented plant material currently sold as 'Hyvingiensis'. Reference samples of the weeping cultivars 'Elise Rathke', 'Excellenz Thiel', 'Oekonomierat Echtermeyer', *M. prunifolia* 'Pendula' and 'Red Jade' were obtained from botanical gardens in Europe and North America (Table 1). *M. prunifolia* 'Pendula Nova' could not be traced in any of the 25 plant collections

**Table 1**  
The list of weeping crabapple accessions genotyped.

Accession name	Location	Age and origin
1 <i>Malus</i> sp.	Sunnersta Manor, Uppsala, Sweden	Ca. 100-year-old tree in the park of the Manor House, origin unknown
2 <i>Malus</i> sp.	Ulleråker Hospital, Uppsala, Sweden	Ca. 130-year-old tree in the hospital park, origin unknown
3 <i>Malus</i> sp.	Mattias Iwarsson, Uppsala, Sweden	A young graft on A2 from a ca. 50-year-old tree of unknown origin in the Botanical Garden, Uppsala University, Sweden
4 <i>Malus</i> sp.	Mattias Iwarsson, Uppsala, Sweden	A young graft on A2 from a ca. 130-year-old tree of unknown origin at the Park Maria Kronbergs Minne in Falun, Sweden
5 <i>Malus</i> sp.	Lindesberg Church Square, Sweden	Ca. 100-year-old tree at the square close to the church, origin unknown
6 <i>Malus</i> 'Hyvingiensis'	Hyvinkää Railway Park, Finland	Planted in 1953, originating from the Finnish State Railways Nurseries
7 <i>Malus</i> "Hyvingiensis" <sup>a</sup>	Ahonen's Nursery, Karttula, Finland	A young tree grafted on seedling rootstock, scion originating from a mother plant of Ahonen's own
8 <i>Malus</i> sp.	Kauppilanaukio Park, Hyvinkää, Finland	Planted in 1997, purchased from an unknown Finnish nursery
9 <i>Malus</i> sp.	Esplanadi Park, Helsinki, Finland	Planted in 1998, purchased from Sundberg Nurseries, Lohja, Finland
10 <i>Malus</i> sp.	Annanpuisto Park, Tuusula, Finland	Planted in 1998, purchased from an unknown Finnish nursery.
11 <i>Malus</i> hybr.1952–3893	Gothenburg Botanical Garden, Sweden	Received in 1952 from Magnus Johnson's Nursery, Södertälje, Sweden, a putative hybrid between <i>M. × zumi</i> 'Calocarpa' and <i>M. 'Oekonomierat Echtermeyer'</i>
12 <i>Malus</i> "Elise Rathke" <sup>a</sup> 1990-0214	Botanic Garden of the University of Turku, Finland	Planted in 1990, purchased from Viksten's Nursery, Tammela, Finland
13 <i>Malus</i> "Red Jade" <sup>a</sup>	Kellokoski-Ohkola Arboretum, Tuusula, Finland	Planted in 1998, purchased from Lalla's Nursery, Järvenpää, Finland
Reference cultivars with accession numbers		
14 <i>M. prunifolia</i> 'Pendula' 1982.0273*A	Sir Harold Hillier Gardens, UK	Age and origin unknown
15 <i>M. prunifolia</i> 'Pendula' <sup>b</sup>	Dubrava Arboretum, Lithuania	Planted in 2001 or 2002, origin unknown
16 <i>Malus</i> 'Excellenz Thiel' H901501	RHS Garden, Hyde Hall, UK	Planted between 1955 and 1993, origin unknown
17 <i>Malus</i> 'Oekonomierat Echtermeyer' 9069-1937A	Montreal Botanical Garden, Canada	Received from Herm. A. Hesse Nursery, Weener-Ems, Germany
18 <i>Malus</i> 'Red Jade' 532-75	Arnold Arboretum, USA	Received from Ruth Birkhoff, Cambridge, USA
19 <i>Malus</i> 'Elise Rathke' 56-99	Arnold Arboretum, USA	Received from Jac. Jurissen & Sons Nursery, Naarden, the Netherlands
20 <i>Malus</i> 'Elise Rathke' 1977.3941*Z	Sir Harold Hillier Gardens, UK	Age and origin unknown
21 <i>Malus baccata</i> 'Pendula' <sup>b</sup>	Botanical Garden of Tartu University, Estonia	Age and origin unknown

<sup>a</sup> Cultivar names to be confirmed are enclosed in double quotation marks to set them apart from the accessions of the reference panel.

<sup>b</sup> Accession number not available.

that were asked. Instead, we received a sixth reference sample of *M. baccata* 'Pendula' from the Botanical Garden of the Tartu University.

Young leaf samples of the Swedish and Finnish trees were collected in 2009 and 2012. Reference samples were acquired in 2009, 2010 and 2012. All samples were shipped to the Department of Agricultural Sciences, University of Helsinki, sealed in plastic bags and stored at  $-20^{\circ}\text{C}$  until DNA analysis, performed in 2012.

## 2.2. DNA isolation and SSR analysis

For DNA extraction, 1  $\text{cm}^2$  of leaf tissue per genotype was ground to a fine powder with a Retsch Mixer Mill MM400 (Retsch GmbH, Germany). Genomic DNA was extracted using E.Z.N.A.<sup>®</sup> plant DNA kit (Omega Bio-Tek Inc., USA) following the supplier's instructions. The DNA isolates were kept at  $-20^{\circ}\text{C}$  before polymerase chain reactions (PCR).

Seven SSR primer pairs, originally developed for the domestic apple (Gianfranceschi et al., 1998; Liebhard et al., 2002), were used: CH01d03, CH01h02, CH02c06, CH02c09, CH02c11, CH02d08 and COL. All chosen primers have been utilized for varietal identification of apple genotypes (e.g. Laurens et al., 2004; Guarino et al., 2006; Garkava-Gustavsson et al., 2013). The forward primers were fluorescently labelled with HEX<sup>®</sup>, FAM<sup>®</sup> or TET<sup>®</sup> dyes.

For PCR amplifications, 2  $\mu\text{l}$  of genomic DNA was used in an 8  $\mu\text{l}$  reaction containing 10 $\times$  reaction buffer (KCl), 1.5 mM  $\text{MgCl}_2$ , 2.0 mM dNTP mixture, 0.3/0.6 U Taq DNA polymerase (Finnzymes, Thermo Fisher Scientific, USA) and 0.3  $\mu\text{M}$  of both primers. The PCR reactions were performed using a Mastercycler gradient (Eppendorf) with the following thermal program: (1)  $95^{\circ}\text{C}$  for 2 min, (2) 28 cycles of  $94^{\circ}\text{C}$  for 20 s, 55 or  $60^{\circ}\text{C}$  (depending on the primer) for 40 s and  $65^{\circ}\text{C}$  for 45 s and (3)  $65^{\circ}\text{C}$  for 5 min.

The PCR products were separated in the Sequencing Laboratory of the Institute of Biotechnology, University of Helsinki, by a capillary electrophoresis system (3730 DNA Analyzer; Applied Biosystems Inc., USA) and fragment sizes were determined by Peak Scanner Software 1.0 (Applied Biosystems, Inc., USA). In cases where only one peak was visible, its size was recorded twice since the locus was considered homozygous.

## 2.3. Morphological traits

The local accessions were examined for their morphological characteristics, adopted from the descriptor list for ornamental crabapples published by the International Union for the Protection of New Varieties of Plants (UPOV, 2003). Measurements were taken on 3–10 well developed leaves, flowers and fruits. To separate between the accessions in a key, observations on a set of additional characters (not UPOV) were used. No morphological traits were recorded for tree no. 7.

The plants were documented by taking digital images of both the general appearance and morphological details. Dried voucher specimens of the Swedish trees are preserved at the Museum of Evolution, Uppsala University, and those of the Finnish trees are kept at the Herbarium of the Finnish Museum of Natural History, University of Helsinki.

## 2.4. Data analyses

The number of alleles per locus, the effective number of alleles and the observed and expected heterozygosity were computed from the allelic profiles. For calculation of genetic distances between the accessions, the allele sizes were transformed into binary scores because two of the accessions appeared to be triploid. The measures of genetic variation and the genetic distances were calculated with GenAlEx 6.5 (Peakall and Smouse, 2012). For visualization of the similarity relationships, a dendrogram was

constructed by the unweighted pair-group method with arithmetic average (UPGMA) in the MEGA software version 5.10 (Tamura et al., 2011).

The cultivar identity of the 13 local accessions was investigated by comparing their DNA profiles with those of the 8 reference samples. The assumed parent–offspring relationships were checked by comparing the multilocus allelic profiles of the putative relatives. The morphological traits were used to confirm the cultivar identifications indicated by allelic data. The phenotypic differences between the local accessions were summarized in an identification key.

## 3. Results and discussion

### 3.1. SSR polymorphism

The primer pairs CH01h02 and CH02c11, both of the two primer pairs amplified two different loci, one of which was monomorphic in all accessions. The two monomorphic loci were discarded from data analyses. The seven polymorphic loci amplified 62 alleles in the entire set of accessions studied. The two triploid accessions displayed four unique alleles not found in the diploid genotypes. In the 19 diploid genotypes, the average number of alleles per locus was 8.3 (Table 2), which is somewhat less than the average allele number (9.3) in the 85 traditional Swedish and Finnish domestic apple cultivars studied by Garkava-Gustavsson et al. (2013). Other studies on domestic apple germplasm collections revealed an average of 9.2 alleles (Guarino et al., 2006) and 9.7 alleles (Gasi et al., 2010) per locus, respectively. The numbers of both accessions and SSR loci were smallest in our study, which may explain the differences.

In the present set of accessions, the expected heterozygosity varied from 0.72 to 0.85 per locus (Table 2) reflecting the high frequency of cross-pollination and self-incompatibility in the genus *Malus*. The mean expected heterozygosity values reported for traditional domestic apple cultivars range from 0.74 (Garkava-Gustavsson et al., 2013) to 0.81 (Guarino et al., 2006).

### 3.2. Morphological traits

All 13 local accessions had single, white flowers often with a shade of pink on unopened flower buds and on the outer side of petals. 'Hyvingiensis' (no. 6) and "Red Jade" (no. 13) had large flowers with an average diameter more than 6 cm, while the flowers of the other accessions were of medium size, approx. 5 cm in diameter.

The leaves of the local accessions were green and unlobed. The leaf margin was crenate to serrate in all accessions except for 'Hyvingiensis' that had doubly serrate leaf margins. Accessions no. 11 and 13 had small fruits with a deciduous calyx. The other trees had medium-sized fruits with a persistent calyx, apart from tree no. 8, some fruits of which shed their calyx at an early stage. For all local accessions, the predominant colour of the mature fruit skin was yellow with a varying amount of orange or red blush on the exposed side.

The morphological characteristics distinguishing the local accessions were summarized as an identification key (Fig. 1). Four accessions were relatively easy to discriminate: in accession no. 2, the fruit stalk was remarkably short, no. 6 was distinguished by its large, pure white flowers and thick leaves with doubly serrate margins, and nos. 11 and 13 had small berry-like fruit. The remaining accessions were distinguishable only by the minor differences in their vegetative parts (Fig. 1).

### 3.3. Cultivar identification

The 13 local accessions represented six distinct genotypes. One Swedish (no. 5) and four Finnish trees (nos. 8, 9, 10 and 12)

1. Fruit with deciduous calyx.....*Malus* hybrid, no. 11 and *Malus* “Red Jade”, no. 13  
Fruit with persistent calyx.....2
2. Fruit stalk short, 2–16 mm.....*Malus* sp., no. 2  
Fruit stalk long 16–50 mm, often with two glands present at the base.....3
3. Leaf margin doubly serrate, flower buds white ..... *Malus* ‘Hyvingiensis’, no. 6  
Leaf margin crenate to serrate, flower buds pinkish.....4
4. Young shoots and buds grey or brown....*Malus prunifolia* ‘Pendula’, nos. 5, 8, 9, 10 and 12  
Young shoots and buds brownish-red to red.....5
5. Blade length of the 4<sup>th</sup> to 6<sup>th</sup> leaf <6 cm.....*Malus* sp., no. 1  
Blade length of the 4<sup>th</sup> to 6<sup>th</sup> leaf > 6 cm.....*Malus* sp., no. 3

**Fig. 1.** Identification key to the weeping ornamental crabapples studied for their morphological traits.

**Table 2**  
Observed variation at 7 microsatellite loci tested in 19 diploid weeping crabapple accessions.

SSR locus	Size range (in bp)	Number of alleles	Effective number of alleles	Observed heterozygosity	Expected heterozygosity
CH01d03	127–189	10	5.65	1.00	0.82
CH01h02	227–273	10	6.86	1.00	0.85
CH02c06	216–252	7	6.00	0.92	0.83
CH02c09	231–259	7	4.97	0.75	0.80
CH02c11	201–243	9	5.24	1.00	0.81
CH02d08	204–257	9	5.88	0.92	0.83
COL	215–241	6	3.51	0.50	0.72
Mean		8.3	5.44	0.87	0.81

Accessions showing an identical or triploid allelic profile were excluded from calculation of the diversity parameters.

displayed an SSR profile identical with the reference cultivar no. 14, *M. prunifolia* ‘Pendula’ from the Sir Harold Hillier Gardens. The morphological traits of the five local accessions were similar, with fruit size and colour matching those of *M. prunifolia* ‘Pendula’ as described by Lindgren (1878a, 1878b). However, our reference panel included another accession (no. 15) named as *M. prunifolia* ‘Pendula’, but its allele profile differed from that of the previous accessions (Table 3). Because fruits of accession no. 15 from the Dubrava Arboretum proved to be dissimilar (small and always without calyx) from Lindgren’s description of *M. prunifolia* ‘Pendula’, the Dubrava tree was regarded as mislabelled.

Unfortunately, the origin of *M. prunifolia* ‘Pendula’ from the Sir Harold Hillier Gardens is unknown. Nevertheless, the authenticity of the six ‘Pendula’ trees is supported by an additional Swedish *M. prunifolia* ‘Pendula’ accession that has grown at the Bergius Botanic Garden, Stockholm since 1925 at the latest, and was discovered late in our study. The morphological characteristics of this accession were consistent with those of the five previous ‘Pendula’ trees. The Bergius Botanic Garden is located next to the grounds once

occupied by the experimental station of the Royal Swedish Academy of Agriculture (Lange, 2000), where *M. prunifolia* ‘Pendula’ originated.

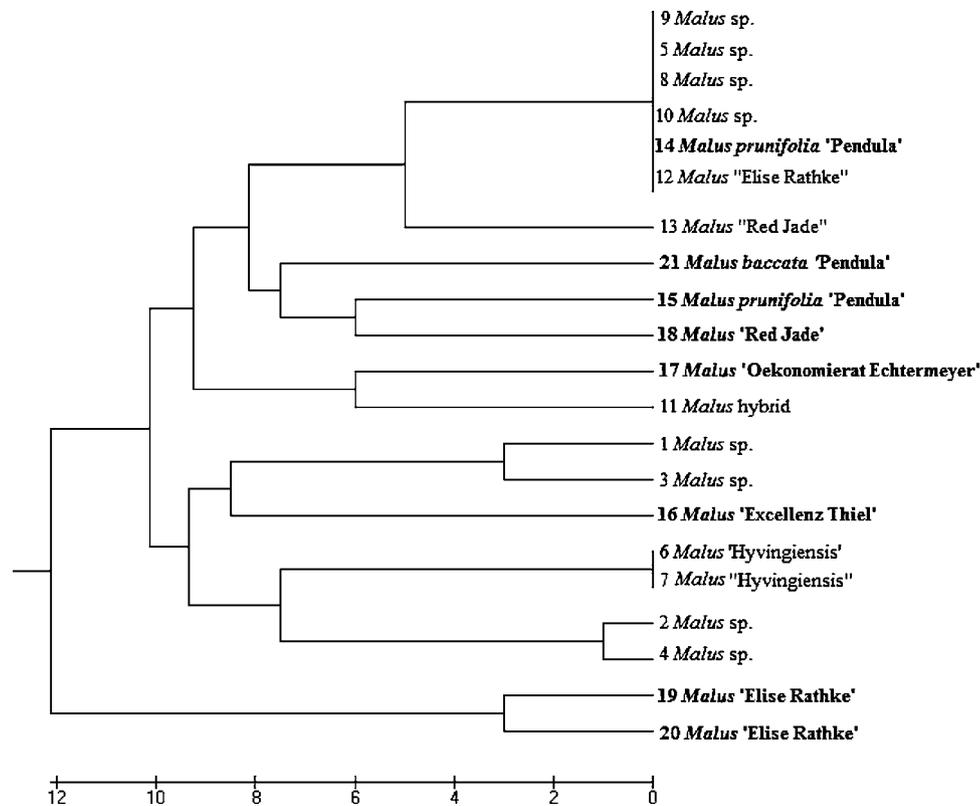
While looking for *M. prunifolia* ‘Pendula’ in local plantings, we quite unexpectedly found four relatively young Finnish trees representing this 150-year-old cultivar, which has not been listed in domestic nursery catalogues for 50 years. Practically all apple trees planted in Finland are produced by local nurseries, as the cold climate restricts cultivation of most international cultivars. It seems probable that some Finnish nurseries hold mother stock of *M. prunifolia* ‘Pendula’, mislabelled and sold most likely as *Malus* ‘Hyvingiensis’, the only white-flowering weeper common in present-day Finnish nursery trade.

The two ‘Hyvingiensis’ accessions (nos. 6 and 7) had identical DNA profiles, unique from the rest of the accessions analysed (Table 3). Three distinguishable alleles were determined at four loci in both ‘Hyvingiensis’ samples, indicating triploidy of the clone. The field observations also were consistent with polyploidy: ‘Hyvingiensis’ has thick, large leaves, large flowers and big fruits with poor seed set.

**Table 3**  
Allele sizes (in bp) at 7 loci for 14 weeping crabapple accessions analysed in this study.

Accession name	SSR primer pairs						
	CH01d03	CH01h02	CH02c06	CH02c09	CH02c11	CH02d08	COL
Local accessions							
1 <i>Malus</i> sp.	141:150	252:254	250:252	244:244	217:229	212:250	215:231
3 <i>Malus</i> sp.	142:150	248:250	250:252	244:244	217:229	212:250	231:231
4 <i>Malus</i> sp.	142:158	237:250	242:252	233:250	205:229	212:218	231:231
6 <i>Malus</i> ‘Hyvingiensis’	137:141:150	235:237:239	242:246	233:250	225:229:233	212:218	229:231:233
11 <i>Malus</i> hybr.	129:150	250:273	216:250	231:246	201:215	212:226	215:241
13 <i>Malus</i> “Red Jade”	139:150	227:239	238:246	231:250	215:229	214:214	231:231
Reference accessions							
14 <i>M. prunifolia</i> ‘Pendula’	141:150	239:254	238:246	233:250	215:233	204:214	231:241
15 <i>M. prunifolia</i> ‘Pendula’	127:150	250:252	216:238	231:233	215:229	214:216	241:241
16 <i>Malus</i> × <i>scheideckeri</i> ‘Excellenz Thiel’	137:141	237:254	216:250	231:254	205:229	216:226	231:231
17 <i>M.</i> × <i>gloriosa</i> ‘Oekonomierat Echtermeyer’	150:189	237:254	216:216	231:246	215:243	212:214	241:241
18 <i>Malus</i> ‘Red Jade’	127:160	231:254	238:248	231:231	223:229	214:216	237:241
19 <i>Malus</i> ‘Elise Rathke’	137:141	237:242	248:252	246:259	207:217	227:257	221:237
20 <i>Malus</i> ‘Elise Rathke’	135:139	237:242	–	246:259	207:217	227:257	221:237
21 <i>Malus baccata</i> ‘Pendula’	127:150	227:254	238:246	231:246	207:229	204:214	215:235

Allelic profiles are not shown for the remaining seven accessions that were identical to those of other local or reference trees.



**Fig. 2.** UPGMA dendrogram of 21 weeping crabapple accessions based on a genetic distance matrix calculated for codominant data, using seven SSR loci. The reference accessions from botanical collections are printed in bold and cultivar names to be confirmed are enclosed in double quotation marks.

The results of this study proved the authenticity of 'Hyvingiensis' plants currently grown by one local nursery. In an earlier SSR analysis, four 'Hyvingiensis' plants from four different nurseries were shown to be identical with the 65-year-old tree no. 6 of known origin (Vuorinen, 2012). The name *P. prunifolia pendula* 'Hyvingiensis' seems to have been recorded for the first time in an old inventory list, preserved in the archive of the Finnish State Railways Nurseries, that was written between 1893 and 1903 by the then head gardener. The cultivar name is quoted in the same form in a contemporary article on the Finnish State Railways Nurseries (Kornman, 1904). Another report of the same year refers to "a weeping crabapple grown from local seed at the State Railways Nurseries" (Karsten, 1904). 'Hyvingiensis' seems thus to have arisen around the turn of the previous century, though it was not described until the early 1950s (Lehtonen and Jokela, 1953).

Two of the un-named Swedish accessions (nos. 2 and 4) gave the same band profile for all but one marker (CH01h02), where no fragment was amplified from accession no. 2. The two accessions were assumed to be identical. Another two of the Swedish accessions (nos. 1 and 3) had similar allele sizes at four loci, but slightly different bands at the remaining three loci (Table 3). The accessions could not be properly compared by their morphological traits because tree no. 3 was a young graft with few flowers and fruits. Judging from the vegetative characteristics and the origin of the trees, accessions no. 1 and 3 seem to represent different genotypes.

One of our original targets was to determine whether *M. prunifolia* 'Pendula Nova' can still be found in old plantings. The fruit size, colour and taste of accession no. 2 seemed to match Lindgren's short description of 'Pendula Nova' (Lindgren, 1878a, 1878b). The original growing sites of accessions no. 2 and no. 4 are both parks constructed in the 1880s. The Ulleråker hospital tree no. 2 was described 60 years ago in a dendrological publication as "an

old, unidentified weeping crabapple" (Hylander, 1955). In its early days, the hospital ran a plant nursery that sold seed and living plants to the public. In 1888, the nursery's catalogue offered both old Swedish weepers ("*P. prunifolia pendula* and *P. prunifolia pendula nova*") for sale (Anon., 1888). From these morphological and historical data, we suggest that accessions no. 2 and 4 may very likely represent the historical 'Pendula Nova'.

The hybrid accession no. 11 from Gothenburg Botanical Garden shared at least one allele with its presumed parent cultivar 'Oekonomierat Echtermeyer' at all but one locus (Table 3). The small difference (four base pairs) might have originated from a mutation in either the parental or our reference accession of *Malus* 'Oekonomierat Echtermeyer' or have arisen from an experimental error. Further parent-offspring relationships were sought for reference sample no. 16, supposed to represent the cultivar 'Excellenz Thiel' that is reported to be both a seedling of *M. prunifolia* 'Pendula' and a parent of both 'Red Jade' and 'Oekonomierat Echtermeyer'. Nevertheless, the microsatellite allele profile of the reference accession no. 16 did not lend support to any of these relationships (Table 3). The small discrepancies between the allele profiles of the two 'Elise Rathke' accessions (Table 3) were probably due to an error in fragment sizing (for marker CH01d03) and a failure in PCR amplification (for marker CH02c06).

In our data, three local trees (nos. 1, 3 and 13) did not match any of the reference cultivars, and two reference accessions (nos. 15 and 16) seemed not to be true to their names. These five samples represented five unique genotypes. The 100-year-old tree no. 1 could be a local seedling, and the same may hold true for tree no. 3 that was originally discovered as a graft in an apple hedge. The remaining three trees may belong to cultivars that were not included in the reference panel, or may be seedlings. As pointed out by Jefferson (1970) and Fiala (1994), misnamed crabapples are

common in the nursery trade, and one reason for mistaken naming lies in the propagation of cultivars by seed.

In the dendrogram (Fig. 2), the two accessions representing the domestic apple cultivar 'Elise Rathke', form a group of their own, and the proper ornamental crabapples are arranged in two clusters. *M. prunifolia* 'Pendula', its synonyms and descendants comprise one group that also includes *M. baccata* 'Pendula' and the two small-fruited, mislabelled accessions no. 13 and 15 (Fig. 2). The second crabapple group consists of the four old Swedish accessions, the two 'Hyvingiensis' trees and the presumably misnamed 'Excellenz Thiel' from the RHS Garden Hyde Hall (Fig. 2).

The genetic relationships displayed by the dendrogram (Fig. 2) match the known parentage relations of the cultivars. The morphological traits of fruits are also in agreement with the clustering in Fig. 2: the large-fruited domestic apple accessions form a distinct group, while the first crabapple group is composed of accessions with relatively small fruit and either deciduous or persistent calyx and the second group includes those accessions with larger fruit and persistent calyx. However, as the dendrogram is based on only seven SSR loci per genotype, it could be unstable, and a small change in fingerprint data could alter the topology of the tree.

#### 4. Conclusions

In this study, we were able to identify several old weeping crabapple clones by a combination of microsatellite genotyping, morphological observations, old garden literature and archival research. Living plants of two valuable historical weepers, one of which is the ancestor of three classic crabapple cultivars, were discovered, the origin and authenticity of one domestic cultivar was solved, and the three clones will be preserved in the national gene banks. As indicated by our results, parks and plant collections may hold historically valuable, though unidentified or misnamed garden plant genotypes. The methods presented here could be applied to other ornamental plant groups for stabilization of horticultural nomenclature and for conservation of garden plant genetic resources.

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