



# Genetic assessment of the pomological classification of plum *Prunus domestica* L. accessions sampled across Europe

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**Abstract** The genotyping of European fruit tree collections has helped to identify synonyms, determine parentage, reveal key specimens in the collections and provide information on the development of modern cultivars from one or several progenitors. However, studies on European plum *Prunus domestica* L. accessions have been lagging behind, mainly because of the hexaploid chromosome number. In this co-operative study, 104 accessions conserved by 14 partners across Europe were phenotyped for 20

descriptors, and genotyped for 8 SSR loci together with 8 reference cultivars. Based on the descriptors and additional information supplied by the partners, as well as the scientific and horticultural literature, each accession was assigned to one of six pomological groups; (1) egg plums sensu lato (E), (2) prunes of the French d’Agen type (P/A), (3) prunes of the Central-Southeast European Zwetschen type (P/Z), (4) green-gages (G), (5) mirabelles (M) and (6) bullaces, damsons and var. *pomariorum* (D/B). A MANOVA conducted on descriptor data revealed significant differentiation among the pomological groups as well as a geographic impact on the differentiation of local plum accessions in Europe. SSR data showed that two

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trios and seven pairs of genotypes had very similar allele profiles and possibly are genetically identical in spite of different accession names. An AMOVA indicated sparse genetic differentiation when accessions were grouped according to geographic origin whereas significant differences were obtained among pomological groups. A Bayesian analysis of genetic structure, as well as a discriminant analysis of principal components (DAPC), further revealed levels of similarity among and within the different pomological groups, suggesting that egg plums *sensu lato* (E) and greengages (G) can be referred to subsp. *domestica* while damsons and bullaces (D/B) but also Central-Southeast European prunes (P/Z) show more affinity to subsp. *insititia*. The small and possibly heterogeneous groups with mirabelles (M) and prunes of the d'Agen type (P/A) take an intermediate position suggesting a hybridogenic origin.

**Keywords** DNA · Genebank · Microsatellite markers · Plant conservation · SSR

## Introduction

Recently, genotypic and phenotypic characterisation of fruit tree germplasm has been undertaken in several

large international projects, e.g., FruitBreedomics targeting apple and peach (Laurens et al. 2018). Genotyping efforts have identified numerous synonyms and mislabelling in European fruit tree collections (e.g. apple: Urrestarazu et al. 2016) thus enabling cost-effective management of the conserved material. Parentage has been certified, sometimes for several generations, and key individuals that hold a central position in the germplasm have been identified (e.g. apple: Ordidge et al. 2018; Muranty et al. 2020). Combining genotypic and phenotypic data for the same material can help to ensure that suitable germplasm is incorporated in modern plant breeding programs, and facilitate the identification of particular genes of interest, e.g., apple (Urrestarazu et al. 2017), peach (Micheletti et al. 2015; Aranzana et al. 2017), apricot (Bourguiba et al. 2012) and sweet cherry (Mariette et al. 2010). Additionally, association between genetic structure and geographic origin has been revealed in several fruit crops, providing information on the development of modern cultivars from one or several progenitor species and the movement of plant material along trade routes to final establishment in present-day production areas (Micheletti et al. 2015; Urrestarazu et al. 2016).

All of the previously mentioned fruit crops are primarily diploid and thus amenable to genotyping

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with SSR (simple sequence repeat) markers, and each crop represents a single, relatively homogenous species. By contrast, the plums grown in Europe mostly belong to the hexaploid *P. domestica* L. s.l., making genotyping with microsatellite markers a somewhat more complicated and laborious task. *Prunus domestica* probably originated in southeastern Europe or western Asia around the Caucasus Mountains and the Caspian Sea. Results of a recent sequence-based genotyping study agree with the generally accepted view that *P. domestica* derives from the diploid cherry plum or myrobalan (*P. cerasifera* Ehrh.) and possibly also the tetraploid wild species sloe or blackthorn (*P. spinosa* L.) (Reales et al. 2010; Zhebentyayeva et al. 2019). However, the aforementioned studies indicate that a third, so far unknown Eurasian plum species could be involved. A closely related and also hexaploid species, *P. insititia* L., differs from common *P. domestica* by smaller and more compact trees, smaller and ovate leaves, and smaller fruits. This taxon is better treated as *P. domestica* subsp. *insititia* (L.) C.K. Schneider due, e.g. to the great similarities in chloroplast DNA between this taxon and *P. domestica* (Reales et al. 2010).

Plum cultivars are commonly divided into various pomological groups, for example (1) prunes with small to medium-sized, elliptic to oblong, usually blue to purplish fruits and relatively firm, spicy, free-stone flesh that is suitable for drying (e.g. the French ‘d’Ente’/‘Prune d’Agen’ and the central-southeast European ‘Hauszwetsche’/‘Bistrica’/‘Požegača’/‘Besztercei’), (2) egg plums with medium to large, ovate or elliptic fruits with rounded ends and tender, sweet flesh that often clings to the stone (e.g. the British ‘Victoria’), (3) greengages with medium-sized, rounded and usually greenish fruits with tender and very sweet cling-stone flesh (e.g. the French ‘Reine-Claude Verte’), (4) damsons and bullaces with small, rounded to oval fruits with bluish or yellow skin (many local cultivars as well as the commonly used rootstock ‘St. Julien’), and (5) mirabelle plums with small rounded fruits with yellow to orange skin and very sweet, free-stone flesh (e.g. the French ‘Mirabelle de Nancy’).

The pomological groups are reflected by a sub-specific taxonomical classification; prunes are usually referred to as *P. domestica* subsp. *domestica*, and egg plums to either the same subspecies or sometimes to *P. domestica* subsp. *intermedia* Röder, while damsons,

bullaces and ‘St. Julien’ plums are classified as *P. domestica* subsp. *insititia*. The mirabelles are treated as a subspecies of their own, subsp. *syrriaca* (Borkh.) Janchen ex Mansfeld, or as the variety *syrriaca* within subsp. *insititia* (Halapija Kazija et al. 2014). Similarly, the greengages have been described as a subspecies, *P. domestica* subsp. *italica* (Borkh.) Gams, or as the variety *italica* within subsp. *insititia*. Greengages have, however, also been regarded as hybrids between subsp. *domestica* and the mirabelles due to similarities with mirabelles in fruit morphology and taste (Hedrick 1911). Classification has varied over time for other groups with small and rounded fruits, like the French perdrigons (e.g. ‘Perdrigon Violette’) and the yellow-fruited landraces in var. *pomariorum* (Boutigny) Dostál (e.g. the German ‘Spilling’).

European plum, a term that commonly encompasses most if not all of the previously described groups, is a rather heterogeneous crop. However, there are relatively few molecular marker-aided studies on the genetic diversity of this crop, probably because of difficulties in scoring SSR loci in hexaploids. Genotyping projects carried out in Croatia and other European countries (Halapija Kazija et al. 2014), France (Horvath et al. 2011), Greece (Athanasiadis et al. 2013; Merkouropoulos et al. 2016), Hungary (Makovics-Zsohár et al. 2017), Scandinavia (Sehic et al. 2015) and Spain (Urrestarazu et al. 2018) have shown that local cultivars often differ considerably from widespread international cultivars. However, the question of whether there is a true geographic component to the genetic variation, or whether the differentiation between widespread and local cultivars is mainly an effect of biased sampling of the subspecies/pomological groups, has not been fully resolved.

The European Cooperative Programme for Plant Genetic Resources (ECPGR, <http://www.ecpgr.cgiar.org/>) is aimed at ensuring long-term conservation of important germplasm in Europe as well as facilitating an increased utilization of this germplasm, e.g. in plant breeding. Special attention is given to the selection of unique accessions with valuable traits, of European origin or importance to Europe, in order to establish decentralized European Collections under the rules of AEGIS (A European Genebank Integrated System <http://www.ecpgr.cgiar.org/aegis/>). The ECPGR *Prunus* workgroup carried out two projects, ‘PRUNDOC’ and ‘Prunus Alignment’, in 2015 and 2018

respectively, both funded by Bioversity International. During PRUNDOC, a number of descriptors for describing and discriminating plum (*P. domestica* L. s.l.) accessions, were selected from lists available in the literature in order to harmonize characterization and evaluation of accessions across collections (Hjeltnes et al. 2017). Within the two projects, morphological and evaluation data on local plum accessions, conserved in 14 different European countries, was collected based on the PRUNDOC descriptor list.

This study aims to quantify the genetic variation and determine the impact of pomological/taxonomical classification and geographic origin on the genetic differentiation among a representative set of plum accessions maintained in Europe. For this purpose, 104 accessions conserved and phenotyped by partners in the ‘PRUNDOC’ and ‘Prunus Alignment’ projects, were classified into pomological groups and genotyped using eight SSR primer pairs.

## Materials and methods

### Plant material

A total of 104 local plum accessions were analysed in this study (Table 1). Each of the 14 partners, from Great Britain in the West to Estonia and Latvia in the East and from Norway and Sweden in the North to Italy and Greece in the South, contributed plant material and phenotyping data for 4–18 accessions that are presently conserved by the project partners. Eight previously used reference cultivars, ‘Bistrica’, ‘Čačanska Rodna’, ‘Hanita’, ‘Mirabelle de Nancy’, ‘Reine-Claude Violette’, ‘Stanley’, ‘Topfirst’ and ‘Valor’, were included to enable comparison with the data set of Sehic et al. (2015).

### Phenotyping and classification

All accessions were scored for 20 descriptors by each partner using trees and fruit in their own collections (Table 2). Based on the descriptors as well as additional information supplied by the partners and available scientific and horticultural literature, each accession was assigned to one of six pomological groups; (1) egg plums sensu lato (E), (2) prunes of the French d’Agen type (P/A), (3) prunes of the Central-Southeast European Zwetschen type (P/Z), (4)

greengages (G), (5) mirabelles (M) and (6) bullaces, damsons and var. *pomarium* (D/B). Differentiation between the two prune types is not clear-cut but P/A are described as having more pointed ends on both fruits and stones. In addition, egg plums were divided into two groups based on average fruit size; above 40 g (Eb) or below (Es). The subdivision of two pomological groups (prunes and egg plums) into four new groups (P/A, P/Z, Eb and Es) was done in order to investigate if observed pomological differences had a genetic basis. In several previous papers, the term ‘European plum’ was applied to most cultivars that could not be referred to as greengages, mirabelles or damsons/bullaces (Horvath et al. 2011; Halapija Kazija et al. 2014; Sehic et al. 2015). ‘European plum’ is, however, also used to distinguish *P. domestica* as opposed to e.g. Asian plum *P. salicina* Lindl., and should therefore be avoided for assignment of pomological groups within the species.

### SSR analyses

Young branches were collected in early spring from a single tree for each of the investigated accessions, and sent to Balsgård, SLU in Sweden, where they were forced indoors until leaves could be harvested. DNA was extracted from frozen leaf material using the Qiagen DNeasy™ Plant Mini Kit (Qiagen AB) according to manufacturer’s instructions. A set of 8 previously published SSR primer pairs, which have already been used for a genetic study on plum (Sehic et al. 2015) (Table 3) was employed for the analyses. For DNA sequences, references, amplification procedures and annealing temperatures, see Sehic et al. (2015) with the minor change that Taq DNA polymerase (recombinant) (Thermo Fischer Scientific) was used. Diluted PCR products were mixed with Hi-Di formamide (Applied Biosystems) and an in-house prepared size standard, after which the amplified fragments were separated on an ABI 3130xl Genetic Analyser (Applied Biosystems). Software package Gene-Marker v. 1.85 (SoftGenetics LLC) was used for scoring of DNA fragments. In case of any uncertainty regarding the scoring process, PCR amplification was repeated. Multilocus SSR profiles were scored as ‘allelic phenotypes’ based on the presence of alleles but not their frequencies.

**Table 1** All accessions studied, with accession code including country of sampling (BEL Belgium, DEU Germany, DNK Denmark, EST Estonia, FRA France, GBR Great Britain, GRC Greece, HUN Hungary, ITA Italy, LVA Latvia, NOR Norway, SRB Serbia, SVO Slovakia, SWE Sweden, REF Reference), cultivar name and country of origin if different from sampling (CAN Canada, CZE Czech Republic, USA United States of America), fruit weight, fruit shape, fruit basic colour, fruit over colour (amount of covering and colour), pomological group (E = egg plum sensu lato, G = greengage, P/A = prune of French d’Agen type, P/Z = Central–Southeast European prune/Zwetsche, M = mirabelle, D/B = damson/bullace, C = *P. cerasifera*, ? = possibly diploid). Entries ordered according to a Bayesian genetic structure analysis (Fig. 6), and assignment to a reconstructed panmictic population (RPP) for  $K = 2$ ,  $K = 4$  and  $K = 8$ , with probability of membership  $q_i > 80\%$  (values within parentheses  $80\% > q_i > 50\%$ ), as well as assignment to discriminant analysis of principal components (DAPC) clusters, selected according to the Bayesian information criterion (BIC)

Sample	Accession	Cultivar	Weight (g)	Shape	Basic colour	Over colour	Classification	No. in RPP, RPP, DAPC		
								Fig. 5		
								k = 2	k = 6	k = 8
BEL4	CRA PRU 370	Reine-Claude Souffriau	41–55	Round	Violet/blue	None	G	1	1	1
GRC6	PD0004	Mpardaki Circular (BEL)	26–40	Round	Yellow/green	None	G	2	1	1
EST4	EST2186	Tartu Punane	41–55	Ovate	Green	Violet	E	3	1	1
NOR3	1429	Helgøypomme (FRA)	41–55	Oval	Yellow/green	Medium, pink	G	4	1	1
GBR7	1976-046	Victoria	41–55	Ovate	Yellow/green	Medium, red	E	5	1	(1)
FRA15	P3692	Bonjour	41–55	Oblong	Violet/blue	Slight, red	E	6	1	1
DEU6	PFL0012	Gräfin Cosel	41–55	Elliptic	Purple/red	Wide, black	E	7	1	(2)
EST2	EST2170	Ave	41–55	Ovate	Purple/red	Violet	P/Z x E	8	1	1
REF2		Valor (CAN)	41–55	Ovate	Purple/red	Wide, black	E	9	1	1
LVA9	LVADPrU2	Julius (EST)	26–40	Elliptic	Violet/blue	Wide, violet	E	10	1	(1)
GBR3	2003-002	Grand Duke	56–70	Ovate	Purple/red	None	E	11	1	1
REF8		Topfirst (DEU)	26–40	Ovate	Violet/blue	Violet	E	12	1	(2)
EST1	EST2169	Amitar	41–55	Oblong	Yellow/green	Violet	E	13	1	1
GBR5	2000-127	Late Orange	> 70	Elliptic	Yellow/green	Medium, orange	E	14	1	(1)
LVA8	LVADPrU1	Suhkruploom (EST)	11–25	Elliptic	Dark blue	Slight, violet	P/Z x E	15	1	(1)
GBR6	1949-255	Utility	56–70	Elliptic	Yellow/green	Wide, red	E	16	1	1
SWE2	BF0229	Hackman	56–70	Ovate	Yellow/green	Slight, orange	G	17	1	1
BEL1	CRA PRU 137	Belle de Louvain	56–70	Elliptic	Purple/red	Wide, violet	E	18	1	1
FRA2	P3727	D’Ente Double	41–55	Elliptic	Purple/red	Violet	P/A	19	1	2
SWE6	BF0337	Opal	26–40	Heart-shaped	Orange	Medium, violet	G x E	20	1	1
REF4		Reine-Claude Violette (FRA)	26–40	Round-ovate	Violet/blue	Wide, violet	G	21	1	2
FRA8	P0389	Reine-Claude Davion	26–40	Elliptic	Yellow/green	Slight, red	G	22	1	(1)
FRA5	P0062	Abricotée Jaune	26–40	Elliptic	Yellow/green	Slight, orange	G	23	1	1
EST5	EST2250	Kihelkonna	41–55	Ovate	Purple/red	Red	E	24	1	(2)
HUN6	prudo530	Tarka perdrigon (FRA?)	26–40	Round	Yellow/green	Wide, red/violet	G	25	1	2
GBR1	1977-186	Czar	26–40	Elliptic	Purple/red	Wide, black	E	26	1	1
SWE3	BF0237	Herman	26–40	Ovate	Purple/red	Wide, violet	E	27	1	2
DNK4	POM B70	Kongehomme	41–55	Round	Orange	Medium, violet	G	28	1	2

Table 1 continued

Sample	Accession	Cultivar	Weight (g)	Shape	Basic colour	Over colour	Classification	No. in Fig. 5	RPP, k = 2	RPP, k = 6	RPP, k = 8	DAPC
FRA10	P0449	Impériale Epineuse	41–55	Oblong	Purple/red	Medium, violet	E	29	1	2	7	1
SWE5	BF0249	Jubileum	> 70	Elliptic	Purple/red	Wide, violet	E	30	1	(2)		1
DEU4	PFL0030	Ruth Gerstetter	26–40	Elliptic	Violet/blue	Wide, black	E	31	1	2		1
ITA9	214	Prunella	26–40	Round	Green	Wide, violet	E	32	1	2	7	1
DEU3	PFL0011	Reine-Claude d'Althann (CZE)	41–55	Round	Yellow/green	Medium, violet	G	33	1	2	(7)	1
BEL3	CRA PRU 114	Belle de Thuin	56–70	Elliptic	Yellow/green	None	E	34	1			1
SRB3	PD 0103	Čaćanska Lepotica	26–40	Elliptic	Dark blue	Violet	P/A x E	35	1	(2)		1
EST3	EST2176	Liisu	41–55	Ovate	Purple/red	Violet	E	36	1	(3)		1
ITA1	264	Susino Secondo	56–70	Oblong	Purple/red	Wide, violet	E	37	1			1
FRA11	P0812	Double Robe	26–40	Ovate	Violet/blue	Medium, red	P/A	38	1	2	7	1
LVA6	LVA01009	Lāse	41–55	Oblong	Yellow/green	None	E	39	1	(3)		1
FRA7	P0328	Madame Guttin	26–40	Ovate	Violet/blue	Medium, red	E	40	1	(1)		1
REF7		Stanley (USA)	c. 55–60	Oblong	Violet/blue		P/A	41	1			1
LVA1	LVA01016	Zemgale	26–40	Round	Yellow/green	Wide, red	E	42	1	2	(7)	1
NOR1	255	Edda	41–55	Heart-shaped	Dark blue	Wide, violet	E	43	1			1
BEL6	CRA PRU 57	Wignon	26–40	Ovate	Yellow/green	Wide, violet	E	44	1			1
FRA3	P0302	Impériale Murat	> 70	Oblong	Purple/red	Medium, violet	E	45	1			1
GBR2	1977-187	Marjorie's Seedling	41–55	Elliptic	Green	Medium, violet	E	46	1	(1)		1
ITA6	147	Lazzaninu	11–25	Elliptic	Purple/red	Wide, blackish	I	47	1	(2)		1
NOR5	1398	Tråneplomme	26–40	Drop-shaped	Yellow/green	Slight, orange	E	48	1			1
SWE4	BF0243	Ive	41–55	Round	Yellow/green	Wide, violet	G x E	49	1	2		1
REF5		Mirabelle de Nancy (FRA)	c. 7–8	Round	Yellow	Slight, red	M	50	(1)			1
LVA3	LVA01013	Latvijas Sarkanā Oplūme	26–40	Elliptic	Purple/red	Wide, violet	E	51	(1)	(3)		1
GRC2	PD0005	Glyka Skopelou	26–40	Elliptic	Yellow/green	Wide, violet	P/A	52	(1)			1
LVA7	LVA02549	Zilā Lāse	11–25	Oblong	Violet/blue	Wide, violet	E	53	(1)			1
ITA4	438	Agostana	26–40	Elliptic	Purple/red	Wide, violet	P/A	54	(1)			1
DNK2	POM B141	Gul Rosinblomme	< 10	Round	Yellow/green	None	M	55	(1)		2	
LVA2	LVA01006	Aizputes	11–25	Ovate	Violet/blue	Wide, black	E	56	(1)			1
LVA4	LVA01010	Latvijas Dzeltenā Oplūme	26–40	Drop-shaped	Yellow	None	E	57	(1)	3		1
SWE1	Elite Pl. Stat.	Experimentalfältets Sviskon	11–25	Oblong	Purple/red	Wide, black	P/Z	58	(1)			1
FRA1	P3691	Reine-Claude Diaphane	41–55	Rounded flat	Orange	Red	G	59	(2)			1
SVK2	svk-pd-002	Trenčianska Okružička	11–25	Round	Violet/blue	None	D/B	60	(2)			1
GRC3	PD0006	Ksina Skopelou	26–40	Elliptic	Yellow/green	Slight, red	D/B	61	(2)			1

Table 1 continued

Sample	Accession	Cultivar	Weight (g)	Shape	Basic colour	Over colour	Classification	No. in Fig. 5	RPP, k = 2	RPP, k = 6	RPP, k = 8	DAPC
FRA6	P0072	Mirabelle Parfumée de Septembre	11–25	Round	Orange	Medium, red	M	62	(2)			2
REF6		Čačanska Rodna (SRB)	26–40	Ovate-oblong	Violet/blue	Violet	P/A x P/Z	63	(2)	(4)	(3)	2
DNK3	POM B25	Gul Sveskeblomme	11–25	Elliptic	Yellow/green	Slight, pink	P/Z	64	(2)			2
BEL5	CRA PRU 38	Prune de Prince	11–25	Round	Dark blue	Slight, black	M	65	(2)			1
ITA5	I28	Gaiotti	26–40	Round	Purple/red	Wide, black	E	66	(2)	(2)		1
DEU5	PFL0022	Mirabelle de Flotow (FRA)	11–25	Round	Orange	Slight, pink	M	67	(2)			1
REF3		Hanita (DEU)	26–40	Oblong	Violet/blue		E	68	(2)			2
FRA4	P3720	Oustenque Bleue	11–25	Elliptic	Violet/blue	Slight, pink	D/B	69	(2)			2
FRA18	P3764	Briquetch	< 10	Elliptic	Green	Slight, orange	D/B	70	2			2
GRC1	PD0003	Mpardaki Elliptic	26–40	Elliptic	Yellow/green	None	D/B	71	2			2
HUN1	prudo20	Duránci	26–40	Ovate	Violet/blue	Wide, blackish	E	72	2			2
FRA16	P3705	Saint-Léonard	< 10	Drop-shaped	Violet/blue	Wide, violet	D/B	73	2	(4)		2
DEU2	PFL0010	Ersinger Frühzwetsche	11–25	Elliptic	Violet/blue	Wide, violet	P/Z	74	2			2
ITA11	240	Sanacore	11–25	Obovate	Yellow/green	None	D/B	75	2			1
ITA13	59	Caleca	11–25	Elliptic	Green	Medium, red	D/B	76	2			2
FRA12	P1671	Verdanne	26–40	Elliptic	Yellow/green	Slight, red	E	77	2			2
ITA8	196	Paradisus	26–40	Oblong	Green	Slight, red	E	78	2			2
BEL7	CRA PRU 319	Altesse Dorée	26–40	Ovate	Orange	Slight, pink	P/Z	79	2	(4)	(3)	2
SVK1	svk-pd-001	Kozienka	26–40	Elliptic	Violet/blue	None	P/Z	80	2			2
NOR4	1422	Blåplomme fra Lier	11–25	Ovate	Violet/blue	Wide, black	D/B	81	2	(3)		2
GRC4	PD0007	Avgata Skopelou	26–40	Drop-shaped	Yellow/green	None	E	82	2			2
DEU1	PFL0002	Bühler Frühzwetsche	11–25	Elliptic	Violet/blue	None	P/Z	83	2	(4)		2
GBR4	1949-225	Winesour	11–25	Elliptic	Purple/red	Wide, violet	D/B	84	2			2
FRA9	P0410	Quetsche de Wagenstadt	11–25	Elliptic	Yellow/green	Slight, orange	P/Z	85	2			2
ITA12	249	Sighera	11–25	Elliptic	Green	Medium, violet	D/B	86	2			2
ITA3	216	Ramassin Ramassin	< 10	Oblong	purple/red	Wide, black	D/B	87	2			2
HUN3	prudo436	Potyó fehér	< 10	Elliptic	Yellow/green	Slight, red	D/B	88	2			2
FRA13	P2737	Prune de Vars	11–25	Rounded flat	Yellow/green	Wide, red	G	89	2			2
HUN5	prudo522	Potyó szilva	5–15	Elliptic	Dark blue	Wide, black	D/B	90	2			2
ITA10	219	Ramassin di Pagnò	< 10	Oblong	Purple/red	Wide, black	D/B	91	2			2
FRA14	P3344	Prune de Chien	11–25	Round	Dark blue	None	D/B	92	2			2
FRA17	P3726	Prune de Chien	11–25	Round	Dark blue	None	D/B	93	2			2
NOR2	1389	Eikerplomme	< 10	Elliptic	Orange	Medium, red	D/B	94	2	3		2



Table 1 continued

Sample	Accession	Cultivar	Weight (g)	Shape	Basic colour	Over colour	Classification	No. in Fig. 5	RPP, k = 2	RPP, k = 6	RPP, k = 8	DAPC
SRB2	PD 0202	Moravka	11–25	Elliptic	Dark blue	Violet	D/B	95	2			2
SVK4	svk-pd-004	63 MY BO 1	11–25	Round	Purple/red	Medium, red	C	96	2			2
DNK5	POM B205	Spilling (DEU)	< 10	Round	Orange	Medium, red	D/B	97	2	3		2
DEU7	PFL0014	Haferpfauwe	11–25	Elliptic	Yellow/green	None	D/B	98	2	(4)	(3)	2
BEL2	CRA PRU 88	Sainte-Catherine	26–40	Oblong	Yellow/green	Slight, red	P/Z	99	2	(4)	(3)	2
ITA14	62	Cariadoggia	11–25	Oblong	Yellow	None	D/B	100	2			2
ITA2	220	Ramassin Giallo	11–25	Elliptic	Yellow	None	D/B	101	2			2
SVK3	svk-pd-003	55 S BO 1	11–25	Round	Violet/blue	None	D/B	102	2			2
SRB1	PD 0203	Rosička Zrtka	11–25	Elliptic	Violet/blue	Violet	D/B	103	2			2
SRB4	PD 0101	Požegača (Balkan?)	11–25	Elliptic	Dark blue	Violet	P/Z	104	2	4	3	2
REF1		Bistrica (Balkan?)	c. 11–25	Elliptic	Dark blue	Violet	P/Z	105	2	4	3	2
DNK1	POM B197	Gul Havreblomme	26–40	Round	Yellow/green	Slight, pink	?	106	2			2
GRC5	PD0001	Asvestochoriou	41–55	Round	Yellow/green	Slight, red	E	107	2	(6)		2
HUN2	pnudo229	Tölcser koronájú	11–25	Oblong	Dark blue	Wide, black	P/Z	108	2	4	3	2
ITA7	189	Muninca	11–25	Oblong	Yellow	None	D/B	109	2			2
LVA5	LVA01008	Kārsavas	26–40	Round	Violet/blue	Wide, violet	E	110	2			2
GRC7	PD0002	Prausti	26–40	Obovate	Orange	None	M	111	2			2
HUN4	pnudo484	Besztercei 5/a (Balkan?)	11–25	Oblong	Dark blue	Wide, black	P/Z	112	2	4	3	2



**Table 2** Phenotypic traits scored in situ for 104 local plum accessions according to the List of Plum Descriptors prioritized during PRUNDOC. Additional categories (italicized) were

added to most of the observed traits in order to accurately classify a wide range of distinct, as well as transitional phenotypic forms found among the examined accessions

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Trait

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Measured and derived traits

1. Fruit size: 1 = very small (< 10 g), 2 = *very small/small (10–11 g)*, 3 = small (11–25 g), 4 = *small/medium (25–26 g)*, 5 = medium (26–40 g), 7 = large (41–55 g), 8 = very large (56–70 g), 9 = extremely large (> 70 g)
2. Stone: ratio length–width (= thickness)

Observed traits

3. Season of flowering: 1 = extremely early, 2 = very early, 3 = early, 4 = *early/intermediate*, 5 = intermediate, 6 = *intermediate/late*, 7 = late, 8 = very late, 9 = extremely late
  4. Harvest maturity: 1 = extremely early, 2 = very early, 3 = early, 4 = *early/mid-season*; 5 = mid-season, 6 = mid-season/late, 7 = late, 8 = very late, 9 = extremely late
  5. Fruit shape: 1 = rounded flat, 2 = round, 3 = elliptic, 4 = elongated elliptic, 5 = ovate, 6 = heart shaped, 7 = drop shaped, 8 = inverted ovate
  6. Fruit skin colour: 1 = whitish, 2 = green, 3 = yellow/green, 4 = *yellow/green/orange*, 5 = orange, 7 = purple/red, 8 = violet/blue, 9 = dark blue
  7. Over colour of the skin: 0 = none, 1 = orange, 2 = pink, 5 = red, 6 = *red/violet*, 7 = violet, 8 = *violet/black*, 9 = black
  8. Stone adherence to flesh: 1 = freestone, 2 = semi-freestone, 3 = clingstone
  9. Stone shape: 1 = rounded flat, 2 = *rounded flat/rounded*, 3 = rounded, 4 = *rounded/ovate*, 5 = ovate, 7 = elliptic, 9 = elongated
  10. Colour of flesh: 1 = whitish, 2 = green, 3 = yellowish green, 4 = yellow, 5 = orange, 6 = red
  11. Sensory analysis of sugar acid ratio: 1 = very acidic, 3 = acidic, 4 = *acidic/good balance*, 5 = good balance, 6 = *good balance/sweet*, 7 = sweet, 9 = very sweet
  12. Flesh firmness: 1 = extremely soft, 3 = soft, 4 = *soft/medium*, 5 = medium, 6 = *medium/firm*, 7 = firm, 9 = extremely firm
  13. Fruit: depth of suture towards stalk end: 1 = absent, 2 = *absent/shallow*, 3 = shallow, 4 = *shallow/medium*, 5 = medium, 6 = *medium/deep*, 7 = deep
  14. Fruit: depth of stalk cavity: 1 = absent, 2 = *absent/shallow*, 3 = shallow, 4 = *shallow/medium*, 5 = medium, 7 = deep
  15. Extent of over colour: 1 = none, 2 = *none/slight*, 3 = slight, 5 = medium, 6 = *medium/widespread*, 7 = widespread
  16. Fruit: skin bloom: 1 = *none*, 3 = poor, 4 = *poor/medium*, 5 = medium, 6 = *medium/high*, 7 = high
  17. Fruit: flesh juiciness: 2 = *none/low*, 3 = low, 4 = *low/medium*, 5 = medium, 6 = *medium/high*, 7 = high
  18. Stone: size: 3 = small, 4 = *small/medium*, 5 = medium, 6 = *medium/large*, 7 = large
  19. Leaf blade shape: 1 = ovate, 2 = elliptic, 3 = obovate
  20. Tree habit: 1 = upright, 2 = upright/semi-upright, 3 = semi-upright, 4 = semi-upright/spreading, 5 = spreading, 7 = drooping, 9 = weeping
- 

## Evaluation of phenotypic data

Based on all 20 descriptors, relationships among the 104 local plum accessions were examined using a Factorial Analysis of Mixed Data (FAMD) (Pagès 2014), which enabled the analysis of data sets containing both quantitative and qualitative variables. The FAMD analysis was carried out in R package “FactoMineR” v. 2.41, function “FAMD” (Le et al. 2008). The distances matrix obtained through FAMD was used in order to construct a dendrogram using R package “factoextra” v. 1.0.5, function “fviz\_dend”

(Kassambara and Mundt 2017). A dendrogram was used instead of the default options because it provided a simpler overview of the relationships among analysed accessions.

Geographic as well as pomological differentiation of the phenotyped plum accessions was investigated with a multivariate analysis of variance (MANOVA), performed in R v. 3.5.1, function “manova”. Two presumably diploid accessions were removed from the data set, resulting in 102 accessions. The accessions were a priori divided into groups depending on (a) presumed geographic origin within one of five

**Table 3** Number of alleles for eight SSR loci, allele size range and Nei's gene diversity, calculated for all plum genotypes, reference cultivars, local accessions, as well as for K = 2 reconstructed panmictic populations (RPPs) and admixed accessions. For origin and DNA sequences of these loci, see Sehic et al. (2015)

Locus code	Size range (bp)	All (n = 112)		Reference (n = 8)		Local (n = 104)		RPP1 (n = 49)		RPP2 (n = 43)		Admixed (n = 20)	
		No alleles	Gene diversity	No alleles	Gene diversity	No alleles	Gene diversity	No alleles	Gene diversity	No alleles	Gene diversity	No alleles	Gene diversity
UDP 98-407	157/231	29	0.886	10	0.912	29	0.885	14	0.8651	25	0.8956	10	0.8628
Pac A 33	169/254	38	0.932	15	0.909	38	0.932	28	0.8938	21	0.9002	25	0.9489
CPSCT 026	165/216	23	0.916	13	0.904	23	0.917	17	0.8973	21	0.9196	18	0.9282
BPPCT 040	118/154	17	0.878	8	0.883	17	0.878	10	0.8477	17	0.8983	14	0.8865
BPPCT 007	121/159	19	0.910	10	0.898	19	0.911	13	0.8992	19	0.9106	14	0.8992
BPPCT 014	186/289	47	0.934	16	0.939	47	0.934	22	0.9171	40	0.9397	27	0.9263
BPPCT 034	213/277	27	0.915	12	0.914	27	0.915	17	0.8979	24	0.9215	18	0.9181
UDP 96-005	092/169	34	0.917	11	0.858	34	0.920	15	0.8928	28	0.9137	20	0.9315
Mean		29.25	0.911	11.88	0.902	29.25	0.912	17.00	0.8889	24.38	0.9124	18.25	0.9127

areas in Europe: Central, Eastern, Northeastern, Northern and Western, or (b) assigned to one of seven pomological groups (Eb, Es, G, M, P/A, P/Z and D/B).

#### Evaluation of molecular data

Genetic variation in the plant material was estimated with SSR allele phenotypes, and gene diversity (average expected heterozygosity, Nei 1978) was calculated across all 112 accessions using population genetics software SPAGeDI 1.3 (Hardy and Veke-mans 2002). Similarity among putatively identical genotypes was quantified as the fraction of shared bands  $S$ , i.e. number of common bands divided by the number of bands exhibited by genotypes  $x$  and  $y$ :  $S_{xy} = 2n_{xy}/(n_x + n_y)$ .

Similarity among all 112 accessions was estimated with an Unweighted Pair Group Method with Arithmetic mean (UPGMA) cluster analysis based on a matrix with pairwise comparisons using Jaccard's similarity coefficient. Calculations were carried out with NTSYSpc v. 2.1 (Rohlf 2000) and the dendrogram was constructed in MEGA 6 software (Molecular Evolutionary Genetics Analysis) (Tamura et al. 2013).

Differentiation of accessions depending on (a) geographic origin (defined as for the phenotypic data analysis described above) and (b) assignment to pomological groups (as above), was investigated with Analyses of Molecular Variance (AMOVA) (Ex-coffier et al. 1992), based on the stepwise mutation model (Ohta and Kimura 1973) and performed using GenoType software [GenoType/GenoDive package (Meirmans and Van Tienderen 2004)] with 1000 permutations.

In order to further analyse the differentiation and relationships among the pomological and geographical groups, the multivariate factorial correspondence analysis (FCA), based on a matrix of binary microsatellite allele presence/absence data, was performed using the "dudi.coa" routine in R 3.5.1 (R Core Team 2018) as suggested by Muller and McCusker (2009). The graphical display of the FCA results was done using the scatterplot3d (2D option is presented for simplicity of viewing) package v. 0.3-41 (Ligges and Mächler 2003) in the same statistical software. Four apparently synonymous accessions were removed from the data set, as well as two diploids (Danish 'Gul Havreblomme' and Slovakian

*P. cerasifera* selection 63 MY BO 1), resulting in 106 accessions for AMOVA and FCA.

The Bayesian model-based cluster procedure within Structure v. 2.2.3 (Pritchard et al. 2000) was used to determine genetic structure within the set of 112 accessions. For individuals with less than six allelic variants per locus, absent allele(s) was assigned as missing data (-9). *K* (unknown) RPPs (reconstructed panmictic populations) were computed on individuals, testing *K* (log-likelihood) = 1–10 for all accessions assuming that sampled cultivars were from unknown origin. Ten independent runs were conducted for each *K*. A burn-in period of 200,000 and 500,000 iterations was applied. Structure Harvester v. 0.6.1 (Earl and von Holdt 2011), which implements the Evanno method (Evanno et al. 2005), was used to estimate *K* values for the analysed data. After determination of the most probable *K* values, runs with maximum likelihood were used to assign individuals to specific clusters (Vigouroux et al. 2008). The assignment of a cultivar to an RPP was provided by the probability of membership *qi* chosen at 80% according to corresponding studies on plums (Urrestarazu et al. 2018). All input files were compiled using MADC v. 1.2 (Grahic and Grahic 2017). Additionally, we conducted a discriminant analysis of principal components (DAPC) using the package adegenet 2.0.0 (Jombart et al. 2010) in R software (R Core Team 2018). Two clusters were selected according to the Bayesian information criterion (BIC).

#### Comparison of phenotypic and molecular data

A positive correlation between phenotypic and molecular data was investigated with a Mantel test (Mantel 1967) with 10,000 permutations, using a Gower distance matrix (phenotyping data) (Gower 1971) calculated in R package “Cluster” v. 2.0.7-1, function “daisy” (Maechler et al. 2018) and a Jaccard’s distance matrix (molecular data). The test was conducted in R package “ade4” v. 1.7.13, function “mantel.rtest” (Dray and Dufour 2007).

## Results

### Classification of accessions

Out of the 112 accessions (Table 1), a total of 44 were classified as egg plums *sensu lato* (E) including three accessions reported to derive from crosses between egg plums and prunes; the Estonian ‘Ave’ (‘Wilhelmine Späth’ × ‘Tartu Kaunitar’) and ‘Suhkruploom’ (‘Wilhelmine Späth’ o.p.) and the Serbian ‘Čačanska Lepotica’ (‘Stanley’ × ‘Ruth Gerstetter’, according to Decroocq et al. (2004)). Additionally, the 44 egg plums were subdivided into two groups based on their average fruit weight (above or below 40 g), with each group consisting of 22 accessions.

Fifteen accessions were classified as greengages (G), including five with ‘Reine-Claude’ in the name, two that were previously described as synonymous with well-known greengages, Danish ‘Kongebloome’ (‘Reine-Claude van Mons’ but fruit more similar to ‘Prune Pêche’, Henk Woldring pers. comm.) and Norwegian ‘Helgøypomme’ (‘Reine-Claude d’Oullins’) and the documented Swedish greengage offspring ‘Ive’ and ‘Opal’. Six accessions, only occasionally referred to as greengages, were also included: French ‘Abricotée Jaune’ and ‘Prune de Vars’, Italian ‘Prunella’, Swedish ‘Hackman’, Greek ‘Mpardaki Circular’ and finally ‘Tarka Perdrigon’, which is conserved by the Hungarian partner but probably derives from France (synonyms ‘Bunter Perdrigon’ and ‘Perdrigon Bariolé’).

The mirabelle group (M) contained three French accessions although ‘Mirabelle de Flotow’ deviates by having stones that are more similar to subsp. *insittia* (Henk Woldring pers. comm.). Three additional accessions were included: Belgian ‘Prune de Prince’, Danish ‘Gul Rosinblomme’ (which may be synonymous with ‘Herrenhauser Mirabelle’, Henk Woldring pers. comm.), and ‘Praousti’, defined as a mirabelle in the Greek collection but with larger fruit (26–40 g) than other mirabelle accessions.

Prunes were split into two groups; the French d’Agen prunes (P/A) and the Central-Southeast European Zwetschen (P/Z). In this study, French accessions ‘d’Ente Double’ and ‘Double Robe’, Italian ‘Agostana’, Greek ‘Glyka Skopelou’ (reported to be a sport of ‘d’Ente’ but does not have the characteristic pointed ends), and American reference cultivar ‘Stanley’ (cross between ‘d’Ente’ and British egg plum ‘Grand

Duke') were referred to P/A. Thirteen accessions were classified as P/Z including Serbian reference cultivar 'Čačanska Rodna', which originated in a cross between 'Stanley' and 'Požegača' (P/Z). All P/A accessions were dark blue to purple and had medium-sized to large fruit (26–60 g) whereas P/Z accessions were blue, black or reddish and most of them had smaller fruit. Four P/Z accessions had intermediate sized fruit (26–40 g): 'Čačanska Rodna', Belgian 'Altesse Dorée' and 'Sainte-Catherine' (which has stones more similar to P/A, Henk Woldring pers. comm.), and Slovakian 'Kozlienka'. Danish 'Gul Sveskeblomme' deviates by its yellow fruit colour and may be synonymous with 'Hartwiss Gelbe Zwetsche' (Henk Woldring pers. comm.).

Twenty-eight accessions were classified as damsons, bullaces or var. *pomariorum* (D/B), all of which are usually treated as subsp. *insititia*. Three of these, Danish 'Gul Havreblomme' (which to some extent also resembles a greengage but had a maximum of only two alleles/locus, see below) and Greek 'Ksina Skopelou' and 'Mpardaki Elliptic' had fruit that weighed over 25 g while the other accessions had smaller fruit.

Finally, the data set also contained the Slovakian *P. cerasifera* selection 63 MY BO 1.

### Phenotype-based relationships

Relationships among accessions were assessed using a factorial analysis (FAMD) with data from the 20 descriptors. Based on the matrix obtained, all 104 local plum accessions were placed into a dendrogram (Fig. 1). The accessions were first split into two main clusters, A ('Abricotée Jaune'–'d'Ente Double') and B ('Briquetch'–'Paradis'). These were each split into two subclusters, A1 ('Abricotée Jaune'–'Zemgale') and A2 ('Gräfin Cosel'–'d'Ente Double'), and B1 ('Briquetch'–'Haferpflaume') and B2 ('Tarka Perdrigon'–'Paradis'). Subcluster A1 contained 28 accessions representing all sampled plant collections except those in Italy and Slovakia. Representation of pomological groups was, however, heavily skewed towards egg plums (11) and greengages (9). In subcluster A2, all collections except those in Italy and Slovakia were again represented among the 37 accessions, with 21 egg plums and 3 greengages. Clusters B1 and B2 instead had a substantial representation of damsons/bullaces (D/B): 11 out of 25 (B1

and 7 out of 14 (B2). In B1, one small subcluster contained all the 4 Slovakian accessions (two D/B, one P/Z and one *P. cerasifera*) while another small subcluster contained 5 French D/B accessions together with the German 'Bühler Frühzwetsche'. The geographic influence was even larger in B2, with 13 out of 14 accessions from Italy (mostly D/B but also two egg plums, one P/A and one greengage).

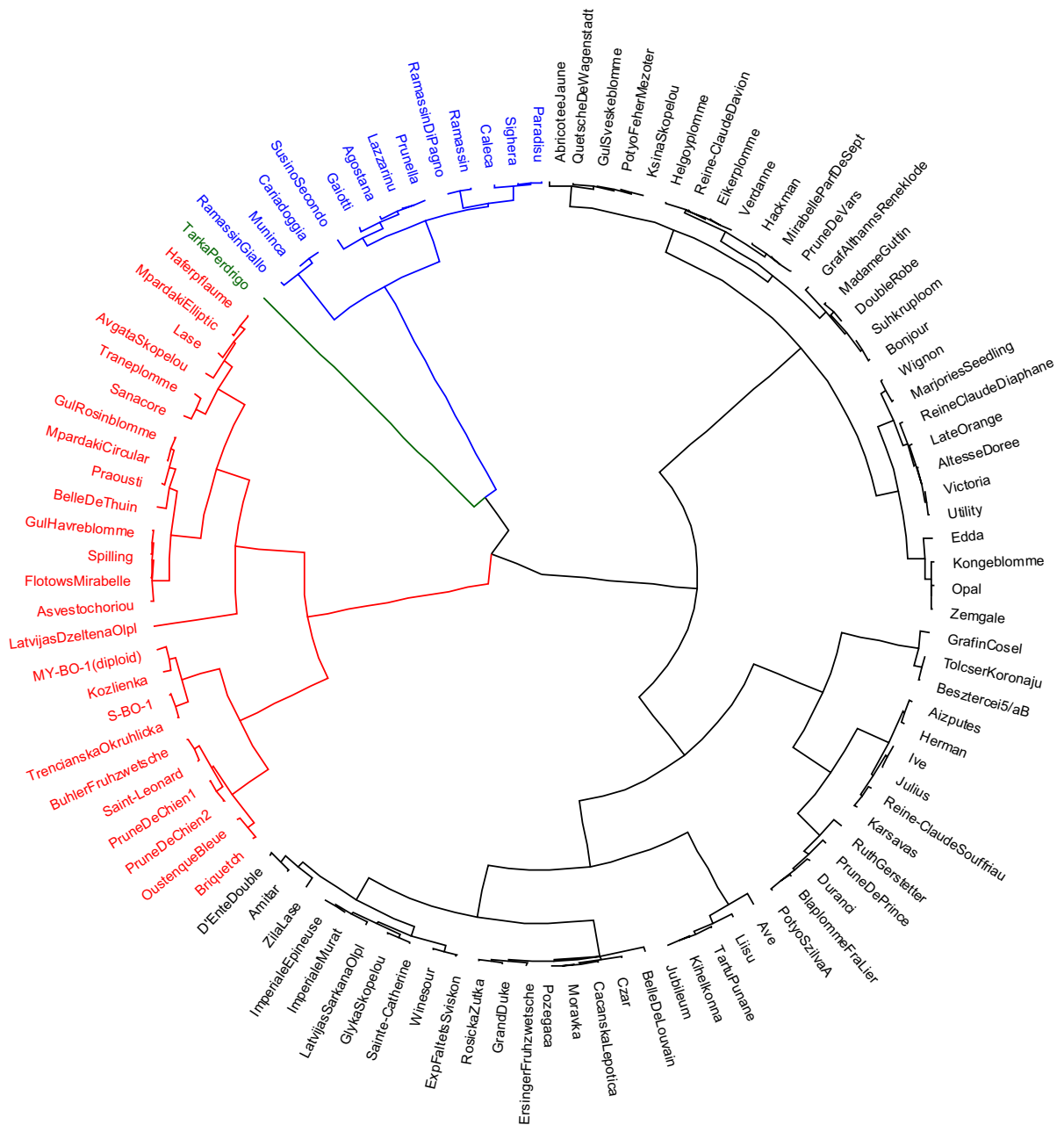
### Phenotype-based differentiation

Two MANOVAs were used to assess the differentiation (1) among accessions with different geographic origins, and (2) among accessions assigned to different pomological groups. The first analysis revealed a highly significant,  $P < 0.001$ , effect of geographic origin (when different, known or presumed origin of the cultivar was used instead of origin of the sample) when the 102 local accessions (excluding two diploids) were divided into 5 groups of countries; North (11 accessions from Denmark, Norway and Sweden), North-East (14 accessions from Estonia and Latvia), West (38 accessions from Belgium, France and Great Britain, Central (22 accessions from Germany and Italy) and East (17 accessions from the Czech Republic, Greece, Hungary, Serbia and Slovakia).

Differentiation among pomological groups was similarly investigated, with the 102 accessions grouped as large-fruited egg plums (21), small-fruited egg plums (20), greengages (14), mirabelles (5), French prunes (P/A; 4), Central–Southeast European prunes (P/Z; 11), and damsons/bullaces (27). Again, a highly significant differentiation was revealed ( $P < 0.001$ ).

### SSR polymorphism and gene diversity

Eight primer pairs amplified 234 distinct alleles in this study, or on average 29.3 alleles per locus (Table 3). Number of alleles differed considerably between loci, with only 17 detected for locus BPPCT 40 while 47 were detected for locus BPPCT 014. By contrast, gene diversity as calculated according to Nei (1978) was more similar, ranging from 0.878 to 0.934 for the same two loci, and an overall average of 0.911. SSR polymorphism and gene diversity were determined for  $K = 2$  reconstructed panmictic populations (RPPs), as well as a group with admixed accessions



**Fig. 1** Factorial analysis of mixed data (FAMD)-based dendrogram using descriptor data for 104 local plum accessions. Different colours highlight the main clusters. (Color figure online)

(Table 3) assigned through Bayesian structure analysis. Number of distinct alleles was the lowest within the largest group (RPP1, 49 samples) with 17 alleles per locus on average and a gene diversity of 0.889. Highest value for alleles per locus, 24.38, was noted for RRP2 (43 samples) while both RPP2 and the

admixed accessions (20 samples) possessed high gene diversity (0.912 and 0.913, respectively).

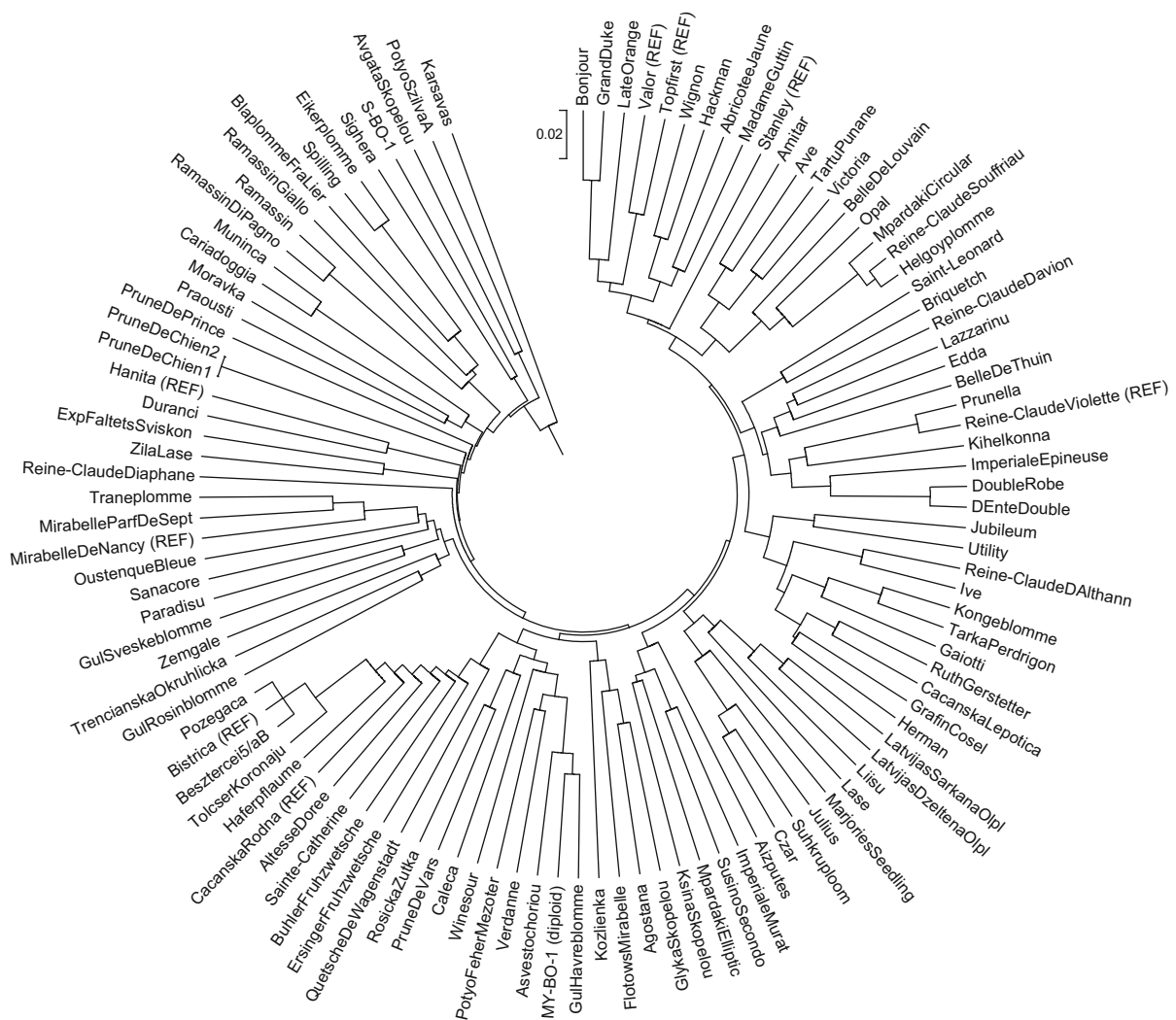
DNA-based evaluation of hexaploid plum accessions is considerably more difficult than for diploid genotypes since up to six alleles may occur in each locus, but the number scored is usually somewhat

lower. Overlooking a present but faint band is thus possible as well as mistakenly scoring an artefactual band as an allele. All but four accessions produced up to five (23 accessions) or six (85 accessions) alleles per locus in keeping with their presumed hexaploidy. Two accessions produced a maximum of four alleles per locus, Greek ‘Asvestochoriou’ and Latvian ‘Latvijas Sarkanā Olplūme’, but are most likely also hexaploid. In addition, the diploid *P. cerasifera* had, as expected, only one or two alleles in all loci except for UDP 96 where a third, most likely artefactual band was scored. More surprising, ‘Gul Havreblomme’ exhibited only

one or two alleles in every locus. Whether this cultivar is truly a diploid, needs to be investigated further e.g. using flow cytometry.

#### Molecular marker-based similarities

Results of a DNA-based UPGMA cluster analysis including all 104 local plum accessions and 8 reference cultivars, are shown in a dendrogram (Fig. 2). Axes differentiating the major clusters were very short and discrimination therefore unclear. The analysis did, however, reveal several cases of highly similar or even



**Fig. 2** Unweighted pair group method with arithmetic mean (UPGMA)-based dendrogram using molecular data for 104 local plum accessions and 8 reference cultivars



identical accessions. The two slightly morphologically divergent accessions of French damson cultivar ‘Prune de Chien’ had 33 completely identical alleles and are treated as genetically identical in this study.

Three greengages also appear to be close to identical although sampled in different collections under different names; the Greek ‘Mpardaki Circular’, the Norwegian ‘Helgøypomme’ and the Belgian ‘Reine-Claude Souffriau’ which is reported to originate from an orchard with ‘Reine-Claude Verte’. They shared 28, 31 and 32 alleles, respectively, and the minor differences (either 2-bp differences in allele size, or the occurrence of additional alleles) are most likely caused by mutations or experimental artefacts. ‘Helgøypomme’ is usually regarded as a sport of ‘Reine-Claude d’Oullins’, which was substantiated by comparison with a previously analysed sample of ‘Reine-Claude d’Oullins’ in our data base (from the study of Sehic et al. 2015). The fraction of shared bands,  $S$ , varied between 0.89 and 0.94 for all pairwise comparisons among ‘Mpardaki Circular’, ‘Helgøypomme’, ‘Reine-Claude Souffriau’ and ‘Reine-Claude d’Oullins’. Whether the two Reine-Claude cultivars are truly clonal or if one of the sampled trees may have been mislabeled, is presently not known. It should also be noted that none of the analysed greengages appears to be synonymous with ‘Reine-Claude Verte’ since they differed clearly ( $S < 0.80$ ) when compared to a sample of this cultivar in our database.

Another case involved three P/Z accessions representing an old and well-known type of prune, which has been widely grown in Central and Southeastern Europe for use in desserts and liquors: Hungarian ‘Besztercei 5/a’, Serbian ‘Požegača’ and Croatian ‘Bistrica’ (the latter used as a reference cultivar). ‘Besztercei 5/a’ and ‘Bistrica’ were identical in 28 out of 30 alleles ( $S = 0.97$ ), while both of them shared 27 out of 31 alleles ( $S = 0.93$ ) with ‘Požegača’. Very restricted variation in SSR profiles among ‘Požegača’/‘Bistrica’ accessions has been reported previously by Halapija Kazija et al. (2013), who speculated that this could be due to its potential status as a landrace cultivar. A fourth accession, ‘Tölcsér Koronájú’ from Hungary, was similar but differed at several loci (25 alleles out of 34 were identical with ‘Besztercei’,  $S = 0.85$ ) and may instead be a close relative, e.g. a seedling offspring.

Several additional cases of very similar and potentially identical genotypes were encountered. P/A prunes ‘d’Ente Double’ and ‘Double Robe’ shared 28 out of 35 alleles ( $S = 0.89$ ). By contrast, Greek ‘Glyka Skopelou’ which has been described as a sport of ‘d’Ente’, shared only 19 out of 50 alleles ( $S = 0.55$ ) with ‘d’Ente Double’ and 15 out of 44 ( $S = 0.51$ ) with ‘Double Robe’ thus being clearly different. Italian greengage ‘Prunella’ shared 28 out of 36 alleles ( $S = 0.88$ ) with the reference cultivar ‘Reine-Claude Violette’, which it also resembles in its rounded green fruits with a violet over colour. Relatively similar but genetically different genotypes were noted also for two other greengages: Danish ‘Kongebomme’ and Hungarian ‘Tarka Perdrigon’,  $S = 0.78$ . Finally, several cases of relatively high similarity involved D/B accessions; ‘Ramassin Ramassin’ and ‘Ramassin di Pagno’ from Italy with very small purple fruit,  $S = 0.86$ , ‘Cariadoggia’ and ‘Muninca’ also from Italy but with slightly larger and yellow fruit,  $S = 0.84$ , and ‘Spilling’ from Germany and ‘Eikerplomme’ from Norway, both with small yellow–orange fruit of the var. *pomariorum* type,  $S = 0.80$ .

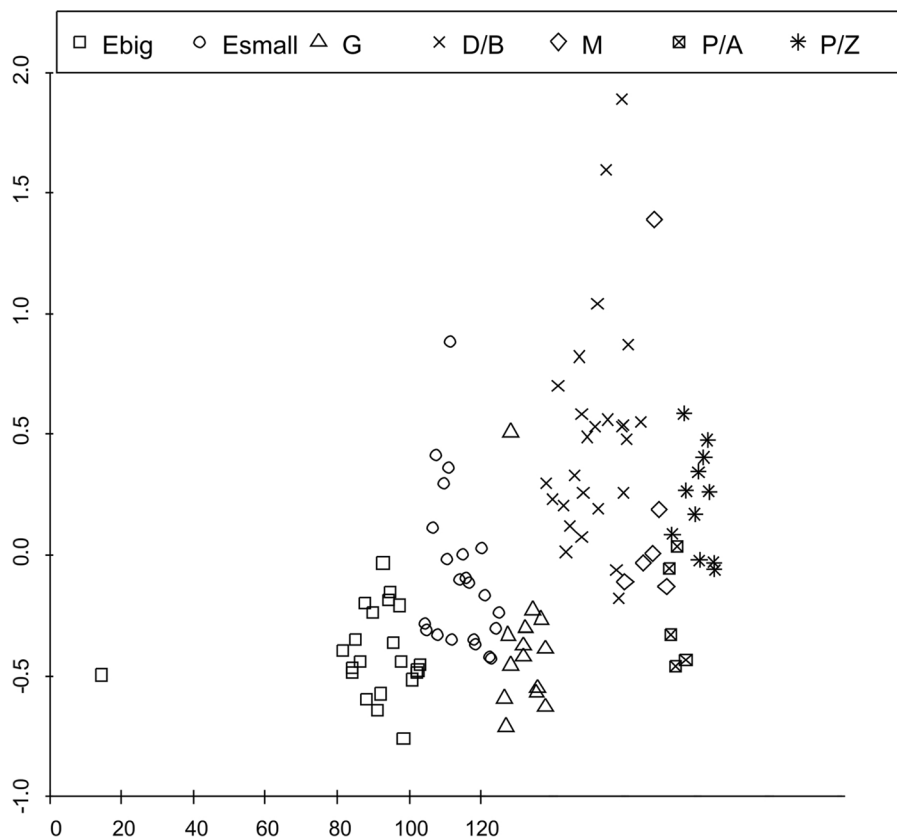
#### Molecular marker-based differentiation

A possible differentiation linked to geographic origin was investigated for 106 accessions (including reference cultivars but excluding 4 synonymous accessions and two diploids) applying AMOVA among 5 groups; North (11 accessions), North-East (14), West (40, including the American reference ‘Stanley’ and the Canadian reference ‘Valor’ with parents from Great Britain and France), Central (24) and East (17). The AMOVA results showed that only 0.7% of the total variation occurred between geographically defined groups, suggesting that very little of the genetic differentiation among local plum cultivars in Europe is associated with their immediate geographic origin.

Differentiation between pomological groups, when the 106 accessions were divided into large-fruited egg plums (22), small-fruited egg plums (22), greengages (14), mirabelles (6), French prunes (P/A, 5), Central-Southeast European prunes (P/Z, 11) and damsons/bullaces (26), explained a small (1.6%) but statistically significant ( $P < 0.001$ ) portion of the total variation.

Relationships between the 7 pomological groups were further investigated with an FCA (Fig. 3). In a





**Fig. 3** Two-dimensional plot of a factorial correspondence analysis (FCA) of the molecular data for 106 plum accessions divided into 7 pomological groups: egg plums large (Ebig, fruit over 40 g), egg plums small (Esmall, fruit below 40 g),

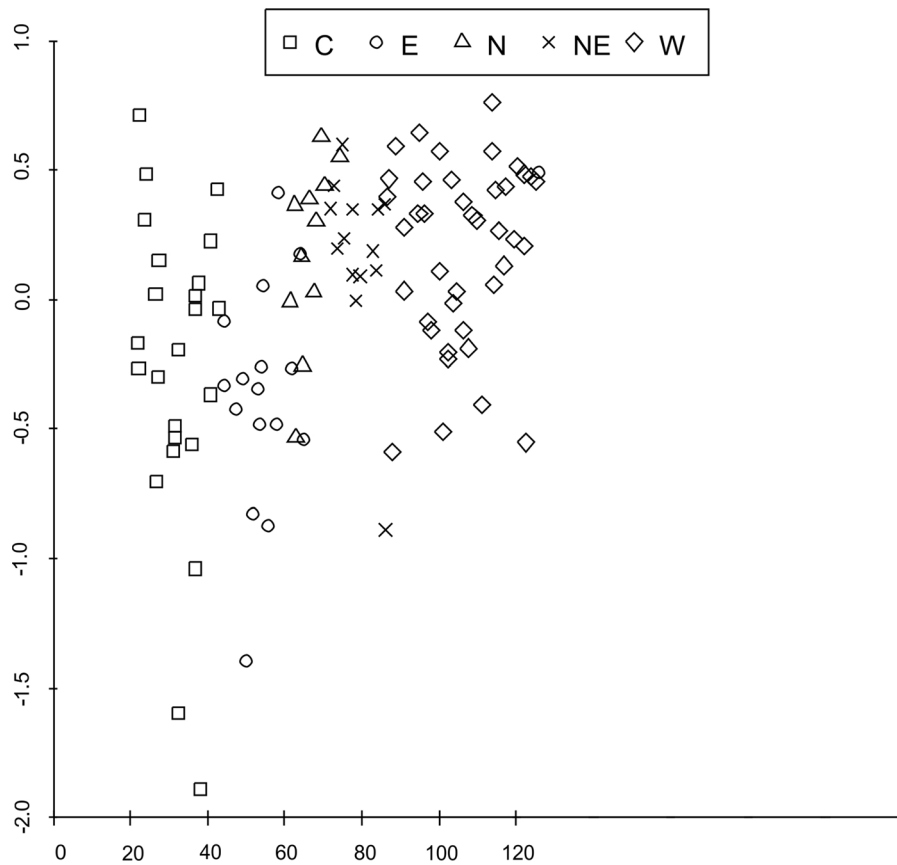
greengages (G), mirabelles (M), French type prunes (P/A), Central–Southeast European type prunes (P/Z) and damsons and bullaces (D/B)

two-dimensional plot, small-fruited egg plums and greengages were placed side-by-side with the large-fruited egg plums found in the lower left-hand corner (Fig. 3). On the right-hand side, P/A and P/Z prunes appeared close together but still with a degree of separation. Mirabelles and damsons/bullaces have a more central position but are closer to the prunes than to the egg plums and greengages. In an FCA of the 5 geographic groups, there was considerable overlap although the two largest groups, Central and Western, appeared on opposite sides in the 2-dimensional plot (Fig. 4).

#### Genetic structuring and DAPC

$\Delta K$  analyses (Evanno et al. 2005) revealed a maximum value for  $K = 2$  and two smaller peaks for  $K = 6$  and  $K = 8$  (Fig. 5a). Bayesian structure analyses were

carried out on 104 local cultivars and 8 reference cultivars (Fig. 6). The two RPPs inferred for  $K = 2$  contained 49 and 43 accessions respectively, that were assigned with a probability of membership  $qI > 80\%$  (Table 1). RPP2:1 was dominated by egg plums (32 accessions) and greengages (13), but 3 P/A prunes were also included, ‘d’Ente Double’, ‘Double Robe’ and ‘Stanley’, as well as Italian ‘Lazzarinu’ classified as a damson (D/B). By contrast, RPP2:2 contained 24 accessions classified as D/B and 10 P/Z prunes. In addition, one mirabelle (‘Praousti’), one greengage (‘Prune de Vars’), and 6 egg plums (French ‘Verdanne’, Hungarian ‘Duránci’, Italian ‘Paradis’, Latvian ‘Kārsavas’, and Greek ‘Avgata Skopelou’ and ‘Asvestochoriou’) were allocated to RPP2:2. All but the last of these egg plums, do, however, have fruit below 40 grams (belonging to the Es pomological group). Finally, the *P. cerasifera* accession as well as

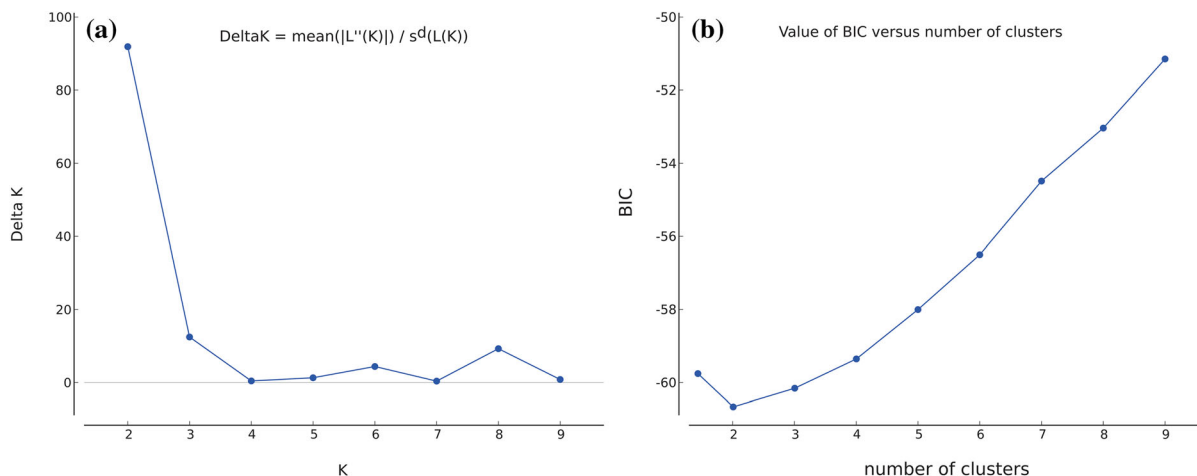


**Fig. 4** Two-dimensional plot of a factorial correspondence analysis (FCA) of the molecular data for 106 plum accessions divided into 5 groups based on geographic origin in Europe: central, eastern, northern, north-eastern and western

another putative diploid, ‘Gul Havreblomme’, were also included in this RPP. Provided that egg plums, greengages and possibly also some of the P/A prunes belong to subsp. *domestica*, while damsons/bullaces and P/Z prunes have a stronger affinity to subsp. *insititia*, differentiation between the two RPPs appears to be associated with interspecific taxonomy. The 20 admixed cultivars (assigned to RPP1 or RPP2 with  $80\% > qI > 50\%$ ) included 5 mirabelles, 6 egg plums (all with fruits below 40 grams), one greengage, 2 P/A prunes, 3 P/Z prunes and 3 D/B.

RPP2:1 (from  $K = 2$ ) was divided into two groups when  $K = 6$ ; 13 accessions in RPP6:1 (7 egg plums and 6 greengages) and 12 in RPP6:2 (4 egg plums, 6 greengages and 2 P/A prunes) (Table 1). In addition, there were two smaller groups; RPP6:3 with three accessions: egg plum ‘Latvijas Dzeltēnā Olplūme’ and the very similar D/B accessions ‘Eikerplomme’ and ‘Spilling’, and RPP6:4 with the three almost identical

P/Z accessions ‘Besztercei’, ‘Bistrica’ and ‘Požegača’ together with very similar ‘Tölscéř Koronájú’. Evaluation of additional accessions that displayed probability of membership above 50% with one of the RPP6 groups, supported the conclusion that RPP6:1 and RPP6:2 were made up mainly of subsp. *domestica* accessions but with no further differentiation associated to pomological group, whereas RPP6:4 contained a group of synonymous or closely related P/Z accessions. Interestingly several other P/Z accessions (the ‘Požegača’-offspring ‘Čačanska Rodna’ together with ‘Altesse Dorée’, ‘Bühler Frühzwetsche’, ‘Ersinger Frühzwetsche’ and ‘Sainte-Catherine’) showed more than 50% affiliation with RPP6:4, as did also German ‘Haferpflaume’ (D/B). Accessions with more than 50% affiliation to RPP6:3 include both egg plums and damsons/bullaces, mostly from northern or north-eastern Europe. Numerous accessions contained a substantial influence of two additional genomic groups



**Fig. 5** Plot of delta K values from the Structure analyses (a) and Bayesian information criterion (BIC) values (b), based on SSR data on 104 local plum accessions and 8 reference cultivars

(RPP6:5 and RPP6:6), but none of the accessions could be assigned with 50% probability to either one of them.

For  $K = 8$ , most of the accessions appeared to be admixed and only three groups had any accessions assigned with at least 80% probability; RPP8:1 contained 12 of the 13 accessions from RPP6:1, RPP8:7 contained 5 of the 12 accessions in RPP6:2, while RPP8:3 contained the same four accessions as RPP6:4.

Considering the weak  $\Delta K$  signals for  $K = 6$  and  $K = 8$  (Fig. 5a), as well as a high proportion of admixed genotypes (Fig. 6a, b and c), results obtained for these  $K$  values must be treated with great caution since the suggested population structure cannot be resolved properly until more data becomes available. Instead, the results of the  $K = 2$  as well as the FCA, formed the basis for the discussion and conclusions in this study.

Using the Bayesian information criterion (BIC) (Fig. 5b), a population structure with two groups was indicated for the discriminant analysis of principal components (DAPC) (Fig. 6d). Assignment of the 112 genotypes into two clusters (DAPC lacks the category *admixed*) was very similar to the results of the Bayesian Structure analysis for  $K = 2$ . The only substantial difference between the results of the Bayesian Structure analysis ( $K = 2$ ) and DAPC, was the Italian accession ‘Sanacore’ which belonged to RPP2:1 in the first analysis and to the second cluster in the latter analysis (Table 1). All other, somewhat

divergent results concerned accessions that were admixed in the Structure analysis (probability of membership lower than 80%, to either RPP for  $K = 2$ ).

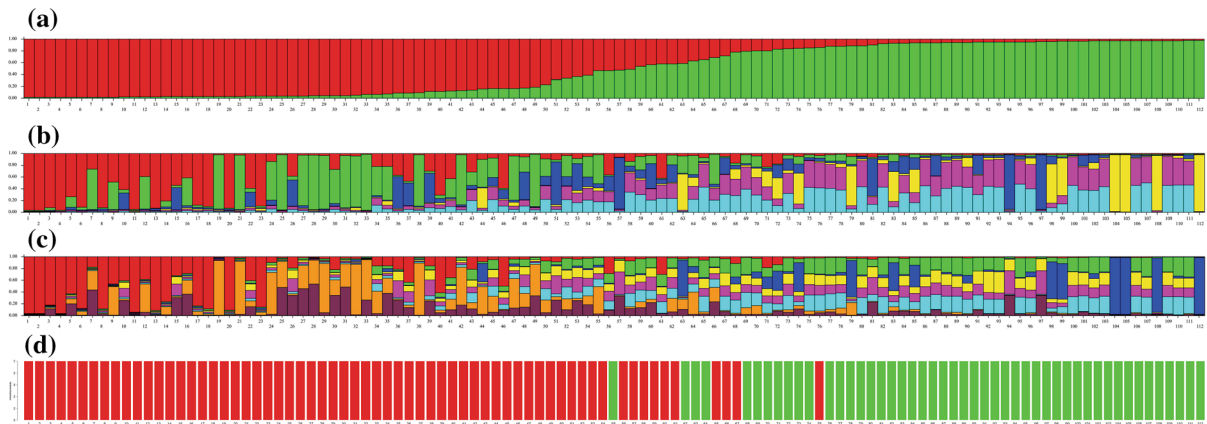
#### Correlation between genetic and phenotypic data sets

A Mantel test, performed to determine the correlation between the descriptor-based data and the SSR marker data, showed a relatively low correlation ( $R = 0.1693$ ) which was nonetheless statistically significant ( $P < 0.0001$ ).

## Discussion

### Phenotyping

A total of 20 standardized descriptors were used, and methods for measuring and scoring were carefully defined to avoid unnecessary bias. Nevertheless, one must take into consideration that the collected data might have been influenced by environmental factors (the accessions being grown in different countries), and through subjectivity of persons undertaking the scoring. We noted substantial phenotypic differences for some accessions that had identical or nearly identical SSR profiles. Some of this variation may, however, be attributable to mutations and the selection of ‘sports’. For example, even when grown and scored in one place, morphological variation was previously



**Fig. 6** Bar plot of the results from three Bayesian genetic structure analyses of 112 plum accessions with  $K = 2, 6$  and  $8$ , respectively (**a, b** and **c**), as well as from discriminant analysis of

principal components (DAPC) based on two clusters (**d**). For accession names, see Table 1

noted among clones of ‘Reine-Claude Verte’ despite their identical SSR profiles (Gharbi et al. 2014). Many ‘colour sports’ are well known to exist in other fruit crops.

The MANOVA used for differentiating between 7 pomological groups was highly significant, and the phenotype-based FAMD (Fig. 1) revealed a crude differentiation between, on the one hand, egg plums, greengages and P/A prunes and, on the other hand, P/Z prunes and damsons/bullaces. A similar level of differentiation was obtained when the material was grouped into 5 geographic areas of origin. The FAMD-based dendrogram indicated particularly strong grouping among accessions from Italy, and from Slovakia.

Since representation of pomological groups varied in the samples from different countries, it is difficult to disentangle the influence of pomological grouping from that of geographic origin. Northern (Denmark, Norway and Sweden) and Western (Belgium, France and Great Britain) material had a mixed representation among the different pomological groups, whereas the 14 accessions sampled in collections of Estonia and Latvia (group North-East) were all egg plums. Accessions originating in Germany or Italy (group Central) instead included 10 accessions classified as D/B, two each of P/Z and greengages, one P/A, and only six egg plums. Similarly, a considerable number of D/B (8) and P/Z (5) but only 4 egg plums originated from countries in the Eastern group (Greece, Hungary, Serbia and Slovakia). To what extent these differences reflect the genetic structure of plum germplasm in Europe is difficult to say. Policies for collecting and

maintaining plant material in national fruit tree collections differ considerably among countries (Nybom and Garkava-Gustavsson 2009).

#### Molecular data

The mean number of alleles per locus was 29.3, which is higher than in most previous studies: 18.7 alleles in 62 traditional Croatian, regional and international accessions (Halapija Kazija et al. 2014), 19.3 alleles in 55 mostly Hungarian accessions (Makovics-Zsohár et al. 2017), 20.0 alleles in 45 European plum accessions preserved in Germany (Xuan et al. 2011), 22.7 alleles in 76 traditional Nordic and international accessions (Sehic et al. 2015), 23.4 alleles in 166 Spanish and international accessions (Urrestarazu et al. 2018) and 29.0 in 80 accessions from the French National Collection (Horvath et al. 2011). It is possible that the reason for this, and the reason for the closest similarity to the study by Horvath et al. (2011), is the broad representation of different pomological groups in our samples.

In our study, the highest number of different alleles was detected for BPPCT 014, and the lowest for BPPCT 040, which is similar to the study by Sehic et al. (2015). Gene diversity calculated for all analysed samples (0.911) is almost identical to the values reported by Halapija Kazija et al. (2014) and Sehic et al. (2015).

The most interesting results regarding number of different alleles and gene diversity, were noted when we compared accessions with a probability of

membership  $qI > 80\%$  to RPP2:1 and RPP2:2 in Bayesian structure analysis for  $K = 2$ . Contrary to expectations, the largest RPP (RPP2:1), consisting of 49 accessions, had the lowest values for these parameters. Higher values for both mean number of alleles and gene diversity were detected for accessions assigned to RPP2:2 ( $n = 43$ ). Even the admixed group of accessions ( $n = 20$ ) possessed a higher number of distinct alleles and gene diversity comparable to RPP2:1. The homogeneity found within RPP1:2 is a likely consequence of the fact that this group includes numerous cultivars derived from modern breeding programs involving mainly subsp. *domestica*. By contrast, RPP2:2, which consists mainly of old local accessions belonging to the more primitive subsp. *insititia*, is notably more diverse. Similar logic can be applied for the admixed accessions, which presumably represent the results of hybridization between the various plum groups or subgroups and thus in spite of their low number ( $n = 20$ ) possess significant diversity.

Neither Horvath et al. (2011) nor Halapija Kazija et al. (2014) found any correlation between the majority of scored morphological traits and the molecular data. However, in this study a low but significant correlation was found between the data sets. This is probably due to inclusion of a number of phenotypic descriptors which are effective in classifying plum accessions into various pomological groups, among which different levels of genetic differentiation were detected using molecular data. Also, similar patterns of separation of e.g. egg plums and greengages on one side and damsons/bullaces and P/Z prunes on the other side, were noticeable in both the FAMD-based dendrogram (using phenotypic data) and in the FCA (using molecular data).

#### Differentiation of plum cultivars

Although accessions of subsp. *insititia* show more primitive features (smaller trees, smaller and often more sour fruits) than accessions of subsp. *domestica* generally do, validated wild forms have never been located for either taxon (Reales et al. 2010; Zhebentyayeva et al. 2019). Moreover, the very similar chloroplast haplotypes reported in studies of a wide variety of accessions from both subspecies (Reales et al. 2010; Horvath et al. 2011; Urrestarazu et al. 2018) suggest that they originate from the same

ancestral line but have become increasingly dissimilar due to differences in exposure to domestication, including active selection for genotypes with traits that could be perpetuated through centuries by vegetative propagation (Woldring 2000; Zhebentyayeva et al. 2019).

Division of plum germplasm between the two taxa is handled very differently in different studies. In some previous multivariate and/or SSR-based studies on plum diversity, almost all accessions were classified as *P. domestica* and only a few to *P. insititia* or subsp. *insititia* (Milošević and Milošević 2012). In another study, mirabelles as well as damsons were treated as *P. insititia* (Halapija Kazija et al. 2014). In yet another, mirabelles, greengages and Quetche/Zwetschen (termed ‘damsons’), were all treated as subsp. *insititia* (Horvath et al. 2011) whereas several small-fruited cultivars were treated as ‘European plum’ including some accessions classified as damsons/bullaces (D/B) in our study.

Several SSR-based studies have focused on the comparison of local plum germplasm to sets of international reference cultivars (Horvath et al. 2011; Halapija Kazija et al. 2014; Sehic et al. 2015; Merkouropoulos et al. 2016; Makovics-Zsohár et al. 2017; Urrestarazu et al. 2018). Not surprisingly, a major division was found between local germplasm belonging mainly to subsp. *insititia*, and international cultivars belonging mainly to subsp. *domestica* (egg plums *sensu lato*) (Halapija Kazija et al. 2014; Sehic et al. 2015). Two groupings of Hungarian germplasm, Zwetsche and subsp. *insititia*, were distinguished from a third group with international reference cultivars (egg plums *sensu lato*, greengages and P/A prunes) in a Bayesian structure analysis by Makovics-Zsohár et al. (2017). In a study comparing Spanish germplasm with international reference cultivars, a grouping with greengages was the first to split off at  $K = 2$ , while the remaining accessions divided into two subgroups at higher  $K$  values; one containing local Spanish cultivars together with old Spanish and French reference cultivars, and another more heterogeneous subgroup containing the majority of reference cultivars including several P/A and P/Z accessions (Urrestarazu et al. 2018).

In the study most similar to ours, in terms of sample range, Horvath et al. (2011) analysed a total of 80 accessions in France. Eighteen of these were included in our study although we found virtually no overlap in

the structuring of accessions in the two studies. Both RPP2:1 and RPP2:2 for  $K = 2$  by Horvath et al. (2011), contained accessions that were found to differ widely in our study where they were classified as egg plums and D/B, with the addition of P/A and P/Z prunes in RPP2:1, and one mirabelle and one greengage in RPP2:2. Five additional greengages were admixed. For  $K = 4$  similar groups were obtained by Horvath et al. (2011), except that RPP4:3 now contained the mirabelle, two greengages and one D/B.

A recent study, again based mainly on accessions conserved in France, describes the outcome of sequence-based genotyping of 405 plum accessions (Zhebentyayeva et al. 2019), 34 of which were represented also in our study. Greengages (mainly ‘Reine-Claude Verte’ and its offspring) and a group called “DAP” (mainly ‘d’Ente’ and its offspring) constituted the two best defined pomological groups. Also, mirabelles and ‘European plums’ formed clusters, with P/Z prunes in a subcluster within the ‘European plums’. It should, however, be noted that most of the pomological groups were very narrowly defined whereas the definition used for ‘European plum’ was relatively wide and included several accessions treated as D/B in our study.

#### Pomological plum groups

In our study, several different pomological groups were defined, and to a considerable extent verified by SSR data.

1. Egg plums sensu lato: Proper egg plums are mostly oval and large-fruited (above 40 grams) and belong to subsp. *domestica* (or subsp. *intermedia* in some treatises). We chose to also classify several small-fruited cultivars as egg plums when they could not be referred to any of the other pomological groups. In the FCA (Fig. 3), large-fruited egg plums occurred at one end of the 2-dimensional plot, with small-fruited egg plums and greengages as closest neighbours. In the Bayesian structure analysis, almost all large-fruited egg plums clustered together with greengages, and were very different from groups with affinity to subsp. *insititia*. By contrast, some of the small-fruited egg plums were intermingled with damsons and bullaces, and probably represent hybridisation products.
2. Greengages: A widely grown genotype named ‘Reine-Claude Verte’ but with many synonyms as well, has been identified using SSR loci (Gharbi et al. 2014) as well as DNA sequencing (Zhebentyayeva et al. 2019). This particular greengage is reported to have been introduced to Europe from Armenia through Greece and Italy, and cultivated in France since the end of the 15th century. Depending on author, a variable number of other genotypes, some of which appear to be direct offspring of ‘Reine-Claude Verte’, are included in the pomological group ‘greengages’. This group was very homogenous in a study of mainly Spanish germplasm and some reference cultivars (Urrestarazu et al. 2018). In a sequence-based study, greengages also formed a well-defined group (Zhebentyayeva et al. 2019). By contrast, greengages were less well circumscribed in our study. Several of our greengages were, however, classified as ‘European plum’ by Zhebentyayeva et al. (2019), as well as in the study of Horvath et al. (2011) in which many also showed admixture. The affinity to large-fruited egg plums is, however, much larger than the affinity to subsp. *insititia*, and we suggest that greengages should be treated as var. *italica* under subsp. *domestica*.
3. Mirabelles: Although 5 of the 6 mirabelles grouped close together in our Bayesian structure analysis, they all demonstrated an admixed genome and could not be allocated to either subsp. *domestica* or subsp. *insititia*. In the FCA, they were closer to subsp. *insititia* and showed some affinity also to the two groups of prunes. An origin involving crosses with different ancestral groups was suggested by Horvath et al. (2011) who also found admixed genomes. By contrast, Halapija Kazija et al. (2014) analysed 25 mostly Croatian mirabelle accessions which were genetically homogenous and appeared to have a very similar ancestry. It is, however, not possible to ascertain how well these Croatian accessions represented the diversity of mirabelles since no other mirabelle samples were included in their study.
4. P/A prunes: Two separate types of prunes are sometimes mentioned in the horticultural literature and can be treated as separate entities (Horvath et al. 2011). The French type is represented primarily by the ‘Prune d’Agen’ also known as ‘d’Ente’, which dates back to the times



of the crusades when Benedictine monks brought the ‘Date Plum’ from Turkey or Persia to Europe (Hedrick 1911). These cultivars, in Table 1 designated as ‘P/A’, are described as having medium-sized, long to egg-shaped fruits and flat stones with pointed ends. In a DNA sequence-based study, DAP (mainly ‘d’Ente’ and its offspring, roughly equivalent to P/A in the present study) formed a well-defined group but almost all of the studied accessions belonged to the same clone (Zhebentyayeva et al. 2019). Since they also had a unique cpDNA haplotype, DAP are probably derived from a unique hybridisation event that did not involve any plums from the other pomological groups. Our study included only 5 P/A accessions; the two French accessions and their offspring clustered together with other subsp. *domestica* accessions in the Structure analysis while the remaining two P/A accessions were admixed and probably had a different origin. A similar situation was reported by Horvath et al. (2011) and this was attributed to crosses with local germplasm.

5. P/Z prunes: The second type of plums for drying is the Central–Southeast European ‘Quetsche’ (in French) or ‘Zwetsche’ (in German), sometimes also known as German/Austrian prunes ‘Hauszwetschen’ or Hungarian prunes ‘Musquée de Besztercei’. These cultivars, defined as ‘P/Z’ in Table 1, have smaller, oval fruits and thicker stones with rounded ends. The largest group of identical genotypes in our study contained P/Z accessions ‘Besztercei’, ‘Bistrica’, ‘Požegača’ and the somewhat less similar (i.e. fewer shared alleles) ‘Tölcser Koronájú’. In a previous study, 30 out of 33 accessions from Bosnia and Herzegovina, Croatia and Serbia labelled as ‘Požegača’ or ‘Bistrica’, were either identical or similar in all but one or two SSR loci (Halapija Kazija et al. 2011); in addition, ‘Hauszwetche’ from Austria shared the most common genotype. In yet another study, 6 clones of ‘Besztercei’ proved to have unique but still very similar SSR marker profiles, with ‘Besztercei Szilva’ regarded as a synonym for ‘Požegača’ (Makovics-Zsohár et al. 2017). According to our study, other cultivars in this group are e.g. the German ‘Bühler Frühzwetsche’ and ‘Ersinger Frühzwetsche’ as well as the Belgian ‘Altesse dorée’ and ‘Sainte-Catherine’.
6. Damsons/bullaces: The English damsons and bullaces are well-known but rather primitive plum types that have been selected especially for culinary purposes, and are usually treated as subsp. *insititia* although e.g. the German ‘Spilling’ has been classified as *P. domestica* subsp. *pomariorum*. Corresponding plum types have been selected and grown also in many other countries, and are assigned to ‘D/B’ in Table 1. The D/B accessions are strongly differentiated from commercially cultivated plums (egg plums and greengages). Thus, most of the analysed Norwegian local plum germplasm as well as some Swedish landrace accessions of D/B (e.g. ‘Krikon’) were quite distinctive from international reference cultivars (Sehic et al. 2015).

## Conclusions

Geographic origin and pomological assignment were equally important factors in explaining a descriptor-based grouping structure in European plum germplasm. By contrast, pomological assignment was more important than geographic origin according to SSR



marker data. Egg plums *sensu lato* and greengages can be classified as subsp. *domestica* while the genetically more diverse damsons and bullaces along with the prunes of the Central-Southeast European type (Zwetschen) show more affinity to subsp. *insititia*. The small and possibly heterogeneous (in our study) groups with mirabelles and prunes of the French d' Agen type, take an intermediate position suggesting a hybridogenic origin.

In this study, several different approaches were used in order to infer the underlying genetic structure of the examined plum germplasm. The importance of geographic origin in explaining a descriptor-based grouping structure in European plum germplasm, could at least in part be attributed to differences in climate and orchard management as well as differences in character scorings among the collection sites throughout Europe. In this aspect, characterization based on SSR markers has a clear advantage. Furthermore, the obtained SSR marker data was evaluated using several different approaches (FCA, Bayesian Structure analysis and DAPC) in order to verify the classification of the examined accessions into different pomological groups. Since it is highly probable that a number of the examined accessions originate from hybridisation between members of different pomological groups, the ability of the Bayesian Structure analysis to identify admixed genotypes is very useful, giving a certain advantage to this approach over DAPC. The factorial correspondence analysis (FCA) efficiently illustrates the relationships among individual genotypes as well as between pomological groups, and thus complements the Structure analysis.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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