



SZENT ISTVÁN UNIVERSITY
FACULTY OF HORTICULTURAL SCIENCE
DEPARTMENT OF BOTANY

**SPECIATION BY TRIPARENTAL
HYBRIDIZATION IN *SORBUS* IN THE EASTERN
AND SOUTH-EASTERN EUROPEAN REGIONS
(Nothosubgenus *Triparens*)**

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1. INTRODUCTION AND OBJECTIVES

Because of frequent hybridization, polyploidization and apomixis, *Sorbus* s.l. is one of the most diverse and fast evolving genera of the *Rosaceae*, comprising more than 250 species. From Europe so far 201 taxa have been reported. predominantly stenoendemics and apomictic species with hybridogenous origin and very narrow distribution area (Kurtto et al. 2018).

Sorbus subgen. *Aria* includes the diploid *S. aria* s.str. and numerous polyploids (*S. aria* agg.). They often hybridize with the diploid *S. torminalis* (Subg. *Torminaria*), *S. aucuparia* (Subg. *Sorbus*) or *S. chamaemespilus* (Subg. *Chamaemespilus*) resulting in a broad scale of either homoploid or allopolyploid combinations which can be grouped in three biparental and two triparental nothosubgenera: subgenus *Tormaria* (*Torminaria* × *Aria*), subgenus *Soraria* (*Sorbus* × *Aria*), subgenus *Chamaespilaria* (*Chamaemespilus* × *Aria*), subgenus *Chamsoraria* (*Chamaemespilus* × *Sorbus* × *Aria*) and subgenus *Triparens* (*Sorbus* × *Aria* × *Torminaria*). Triparental taxa, belonging to the subgenus *Chamsoraria* (*Chamaemespilus* × *Sorbus* × *Aria*), are predominantly known from the subalpine regions of the Northern Carpathians and the Alps (nine taxa) (Düll 1961, Bernátová & Májovský 2003, Meyer et al. 2005). The lower regions are represented by two taxa of subgenus *Triparens*: *S. intermedia* and

S. × liljeforsii, the latter is a backcross hybrid of *S. intermedia* to *S. aucuparia* (Kurtto et al. 2018).

In the course of studying both herbarium and live material of the European *Sorbus*, beside the known *S. intermedia* and *S. × liljeforsii*, three further species, *S. dacica*, *S. paxiana* and *S. tauricola* reported formerly to be of biparental origin with either *S. aria* agg. × *S. aucuparia* (Borbás 1883, Jávorka 1915, Kárpáti 1940, 1960, Somlyay & Sennikov 2016, Sennikov & Kurtto 2017) or *S. aria* agg. × *S. torminalis* (Popov 1959, Kárpáti 1960, Zaikonnikova 1985, Sennikov és Phipps 2013, Sennikov & Kurtto 2017) lineage, are hypothesized to have evolved by triparental hybridization. *S. dacica* is a stenoendemic species of Transylvania, Romania, *S. paxiana* is a local endemic of the Cerna Mountains, Southeastern region of Banat, Romania. *S. tauricola* is an indigenous species of the Crimean Peninsula. They more or less combine morphological features of the three supposed ancestors: (i) the densely tomentose abaxial leaf side of *S. aria* agg., (ii) the greenish-yellowish colour of the abaxial leaf surface (iii) and the brownish tinge of their fruits during ripening special trait of *S. torminalis*, (iv) the more or less deep and rounded lobes and the more or less separated lower leaflet pair at least in some leaves of the long sterile shoots that indicate genetic involvement of *S. aucuparia*.

This combination of morphological features suggested taxonomic re-evaluation of *S. dacica*, *S. paxiana* and *S. tauricola*.

The overall objectives of this research were as follows:

- to collect plant materials of our 3 new trigenomic candidates, *S. dacica*, *S. paxiana* and *S. tauricola* as well as their putative parents (*S. aria* s.l., *S. aucuparia* and *S. torminalis*) and to embed our results in a wider taxonomic context where further 30 European related *Sorbus* taxa were also involved.
- to investigate evidence for triparental origin of *S. dacica*, *S. paxiana* and *S. tauricola* by means of biochemical (HPLC-ESI-qTOFMS) and molecular (cpSSR) methods.
- to provide evidence for taxonomic heterogeneity of *S. dacica* by means of morphometric and biochemical (HPLC-ESI-qTOFMS) methods as well as by flow cytometry.
- based on flow cytometry to assess the ploidy level (FCM) and reproduction mode (FCSS) of *S. dacica* and *S. paxiana*.
- to delineate putative scenarios of *Triparens* speciation.

2. MATERIALS AND METHODS

2.1. Plant materials

37 *Sorbus* taxa (including the two morphotypes of *S. dacica* s.l.) collected at different sites across Europe were involved in the surveys (**Tab. 1, Fig. 1**).

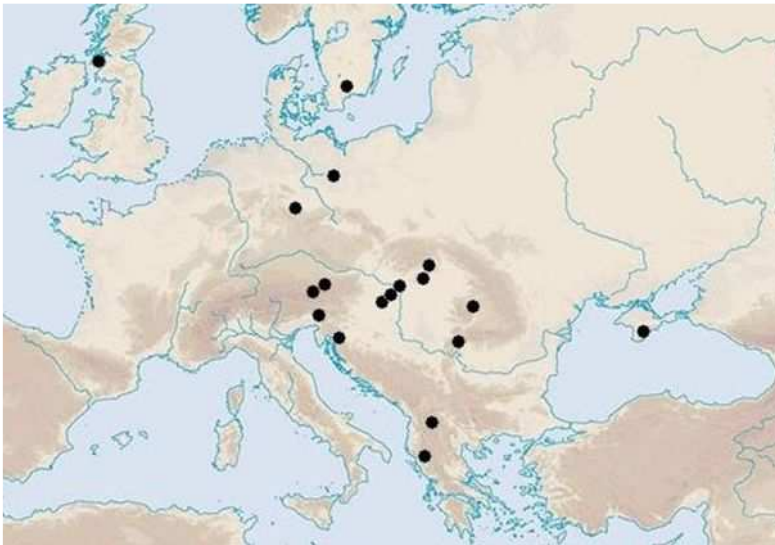


Figure 1. Localities of *Sorbus* taxa sampled for this study.

Table 1. Number of samples involved in biochemical, molecular, flow cytometric and morphometric studies.

Subgenus/Nothosubgenus	Taxon	Biochemical studies (HPLC)	Molecular studies (cpSSR)	Flow cytometry (FCM)	Morphometric studies (PCA) (individual/leaf)
Torminaria	<i>S. torminalis</i>	3	1		
Chamaemespilus	<i>S. chamaemespilus</i>	3	1		
Sorbus	<i>S. aucuparia</i>	3	1		
Aria	<i>S. aria</i> s.str.	3	1		
	<i>S. danubialis</i>	4	2		
	<i>S. domugledica</i>	2	2		
	<i>S. graeca</i>	3	2		
	<i>S. pannonica</i>	3	2		
	<i>S. ulmifolia</i>	3	2		
	<i>S. umbellata</i> agg.	13	1		
	<i>S. vajdae</i>	3	2		
Chamaespilaria	<i>S. x ambigua</i>	3	2		
Soraria	<i>S. arranensis</i>	1	1		
	<i>S. austriaca</i>	3	2		
	<i>S. borbasii</i>	3	2		
	<i>S. dacica</i> KL morphotype	4	2	9	12/39
	<i>S. hazslinszkyana</i>	3	2		
	<i>S. hohenesteri</i>	1	1		
	<i>S. macedonica</i> n.p.	3	2		
	<i>S. mougeotii</i>	2	2		
	<i>S. pseudofennica</i>	1	1		
	<i>S. pseudothuringiaca</i>	1	1		
	<i>S. pulchra</i>	1	1		
	<i>S. x thuringiaca</i>	3	2		
	<i>S. tuzsoniana</i>	3	2		
Tormaria	<i>S. badensis</i>	2	2		
	<i>S. bakonyensis</i>	3	2		
	<i>S. balatonica</i>	2	2		
	<i>S. bohemica</i>	2	2		
	<i>S. gayeriana</i>	3	2		
	<i>S. pelsoensis</i>	3	2		
	<i>S. pseudolatifolia</i>	3	2		
	<i>S. semincisa</i>	4	2		
Triparens	<i>S. dacica</i> SZL morphotype	7	2	10	9/40
	<i>S. intermedia</i>	3	1	1	
	<i>S. paxiana</i>	6	2	12	
	<i>S. tauricola</i>	1	2		
Összesen:	37	114	63	32	21/79

2.2. Morphological studies (PCA)

Complete revision of the *Sorbus* herbarium preserved in the Hungarian Natural History Museum Budapest (BP) was carried out by the author. Several type specimens were designated including the lectotype of *S. paxiana*, a species emphatically discussed in the dissertation.

To reveal morphological variability of *S. dacica* s.l. a multivariate morphometric analysis was carried out using 14 morphological characters of the leaves. Data evaluation was done with R package version 3.4.1.

2.3. Biochemical studies (HPLC-ESI-qTOFMS)

Chromatographic separation was carried out on a Phenomenex Kinetex C18, 4.6×150 mm, 2.6 µm column (Phenomenex, Macclesfield, UK) using an Agilent 1200 series high performance liquid chromatography (HPLC) system (Waldbronn, Germany). For the chromatography, 0.5 % (v/v) formic acid in water (mobile phase A) and 0.5 % (v/v) formic acid in acetonitrile (mobile phase B) were used as solvents at a flow rate of 500 µl/min.

The HPLC system including a diode array detector (DAD) was coupled to an Agilent (Santa Clara, CA USA) 6530 quadrupole – time-of-flight mass spectrometer (q-TOFMS), which was equipped with a dual spray ESI source.

The quantification of vitexin (apigenin-8-C-glucoside) was carried out using a reference standard. In absence of the other apigenin-glycoside (apigenin O-glucuronide) reference standard only the relative peak areas were compared.

2.4. Molecular studies (cpSSR)

DNA extractions were made from silica-dried leaves using a modified CTAB protocol (Doyle and Doyle 1987, Xu et al. 2004). Amplification of two variable chloroplast DNA regions was carried out by using *rpl16 pm1* and *trnT-L pm1* primer pairs targeting *rpl16* gene intron as well as intergenic spacer between *trnT* (UGU) exon and *trnL* (UAA) 5' exon following Chester et al. (2007). For allele size evaluation, the chromatograms were analyzed with the Peak Scanner v1.0 (Thermo Fisher Scientific, MA, Waltham, USA).

2.5. Flow cytometry (FCM, FCSS)

2.5.1. Estimation of somatic DNA ploidy level (FCM)

DNA ploidy level of *S. paxiana*, *S. dacica* and *S. intermedia* was assessed by flow cytometry with DAPI (4',6-diamidino-2-phenylindole) as a fluorescent stain. *Bellis perennis* L. ($2C=3.38$ pg; Schönswetter et al. 2007) or *Carex acutiformis*-t ($2C=0,82$ pg; Lipnerová et al. 2013) were used as an internal standard. Preparation of samples generally followed a two-step procedure described by Doležel et al. (2007).

Following sample preparation fluorescence intensity was measured on a CyFlow ML (Partec GmbH, Münster, Germany) equipped with a UV LED 365 nm. Fluorescence intensity of 3000 particles was recorded and fluorescence histograms were further analysed using FloMax 2.0 software (Partec GmbH, Germany). Ploidy levels were deduced based on the previous karyological studies of the genus *Sorbus* (Lepší et al. 2008, Lepší et al. 2015).

2.5.2. Determination of reproduction modes (FCSS)

The modes of reproduction were determined based on the flow cytometric seed screen (Matzk et al. 2000). The procedure generally copied the one used for the leaf analysis however, some small modifications were made. Modes of reproduction were inferred based on DNA ploidy levels of the embryo and the endosperm following Matzk et al. (2000) and Talent & Dickinson (2007).

3. RESULTS

3.1. Biochemical studies (HPLC-ESI-qTOFMS)

3.1.1. Flavone-O-glycoside (Apigenin O-glucuronide)

Apigenin O-glucuronide was found to be a characteristic flavonoid component occurring exclusively in the leaves of *S. torminalis* and its progenies. The highest concentrations of apigenin O-glucuronide was detected in *S. torminalis*, whilst its putative descendants (taxa grouped in subgenus *Tormaria* and subgenus *Triparens*) also contained it in different and considerably smaller amounts. We found apigenin O-glucuronide in *S. intermedia*, also detected by Challice & Kovanda (1978), reflecting its close relation to *S. torminalis*. We detected apigenin O-glucuronide in the leaves of *S. dacica*, *S. paxiana* and *S. tauricola* confirming their *S. torminalis* origin. The compound was not observed in the leaves of *S. aria* s.l., *S. aucuparia* and *S. chamaespilus* as well as their putative descendants (Nothosubg. *Soraria*, Nothosubg. *Chamaespilaria*) (**Table 2**).

Table 2. Results of the biochemical studies (HPLC-ESI-qTOFMS) (nd: not detected).

Subgenus/Nothosubgenus	Taxon	average content of vitexin in dried leaves (mg/g)	average content of spiglerin O-glucuronide in dried leaves (millions cps)	Type of flavonoids
Terminalia	<i>S. terminalis</i>	0.368	10.922	TERMINALIS
Chamaemespilus	<i>S. chamaemespilus</i>	3.841	nd	
Sorbus	<i>S. aucuparia</i>	nd	nd	
Aria	<i>S. aria</i> s.str.	nd	nd	
	<i>S. danubialis</i>	nd	nd	
	<i>S. domugledica</i>	0.068	nd	
	<i>S. greeca</i>	nd	nd	
	<i>S. pannonica</i>	0.189	nd	
	<i>S. ulmifolia</i>	nd	nd	
	<i>S. umbellata</i> agg.	0.579	nd	
	<i>S. veldae</i>	0.030	nd	
Chamaespilaria	<i>S. x ambigua</i>	1.667	nd	
Soraria	<i>S. arvensis</i>	nd	nd	
	<i>S. austriaca</i>	nd	nd	
	<i>S. borbasii</i>	nd	nd	
	<i>S. dacica</i> KL morphotype	nd	nd	
	<i>S. hazslinszkyana</i>	nd	nd	
	<i>S. hohenesteri</i>	nd	nd	
	<i>S. macedonica</i> n.p.	nd	nd	
	<i>S. mougeotii</i>	nd	nd	
	<i>S. pseudofennica</i>	nd	nd	
	<i>S. pseudothuringiaca</i>	nd	nd	
	<i>S. pulchra</i>	nd	nd	
	<i>S. x thuringiaca</i>	nd	nd	
	<i>S. tuzsoniana</i>	nd	nd	
Tormentum	<i>S. badensis</i>	0.089	2.027	TERMINALIS
	<i>S. bakonyensis</i>	0.047	5.951	TERMINALIS
	<i>S. balatonica</i>	0.093	3.167	TERMINALIS
	<i>S. bohemia</i>	0.119	1.972	TERMINALIS
	<i>S. gayeriana</i>	0.199	8.801	TERMINALIS
	<i>S. pilsensis</i>	nd	4.523	TERMINALIS
	<i>S. pseudolatifolia</i>	0.057	5.427	TERMINALIS
	<i>S. semilancea</i>	0.235	5.346	TERMINALIS
Tripartena	<i>S. dacica</i> SZL morphotype	0.029	2.530	TERMINALIS
	<i>S. intermedia</i>	0.130	5.315	TERMINALIS
	<i>S. paziana</i>	0.205	0.787	TERMINALIS
	<i>S. tauricola</i>	0.551	3.718	TERMINALIS

3.1.2. Flavone-C-glycoside (apigenin-8-C-glucoside, vitexin)

In agreement with the earlier observations (Challice & Kovanda 1978, 1986), of the European 'basal' *Sorbus* species examined, vitexin occurred exclusively in the leaves of *S. torminalis* and *S. chamaemespilus* as well as their descendants (each taxon of the biparental subgenus *Tormaria* and subgenus *Chamaespilaria* in addition to the trigenomic subgenus *Triparens*). Furthermore the compound was found in *S. umbellata* agg. belonging to subgenus *Aria* as well (Challice & Kovanda 1985). *S. domugledica* contained also this compound confirming its *S. umbellata* origin, presumed formerly by Kárpáti (1960). Unexpectedly *S. pannonica* and *S. vajdae* also contained vitexin suggesting their possible *S. umbellata* origin. As we expected, subgenus *Triparens* taxa also contained vitexin suggesting their *S. torminalis* origin. We confirmed the earlier observation of vitexin content in the leaves of *S. intermedia* (Challice & Kovanda 1978). *S. tauricola* had vitexin in the highest concentration, whereas it was present in *S. paxiana* and *S. intermedia* in smaller amount. In *S. dacica* it was found only in a trace amount. We did not detect vitexin in *S. aucuparia* and any representatives of subgenus *Soraria* (**Table 2.**).

3.2. Molecular studies (cpSSR)

Fragment length of the simple-leaved 'basal' taxa *S. torminalis*, *S. chamaemespilus* and each taxon of subgenus *Aria* in *rpl16* and *trnT-L* loci proved to be quite similar. *S. aucuparia*, the fourth member of the hybridization matrix, however, shows a very different pattern of fragment length in the two studied loci ('*aucuparia*-type' chloroplast). Taxa of Nothosubg. *Chamaespilaria* and Nothosubg. *Tormaria* are characterized by low levels of polymorphism regarding the studied loci alike their parents (*S. torminalis*, *S. chamaemespilus*, Subg. *Aria*). The conspicuously characteristic '*aucuparia*-type' chloroplast of all studied *Soraria* taxa provides evidence for the key role of *S. aucuparia* in the speciation. Nevertheless, since plastid DNA is known to be of maternal inheritance in angiosperms, *S. aucuparia* tends to take part in the hybridization processes as pollen acceptor, receiving pollen from a member of the *Aria* group. The '*aucuparia*-type' plastid of proved (*S. intermedia*) and putative (*S. dacica*, *S. paxiana* and *S. tauricola*) subgenus *Triparens* taxa also indicates their *S. aucuparia* origin and the participation of *S. aucuparia* in the hybridogenous speciation as maternal line (**Table 3**). Our results are in agreement with former results by Chester et al. 2007 and Uhrinová et al. 2017.

Table 3. Results of the molecular studies (cpSSR).

Subgenus/Nothosubgenus	Taxon	rpl16 locus (bp)	trnT-L locus (bp)	Type of chloroplast
Tomnaria	<i>S. torminalis</i>	146	272	
Chamaemespilus	<i>S. chamaemespilus</i>	141	271	
Sorbus	<i>S. aucuparia</i>	110	238	AUCUPARIA
Aria	<i>S. aria</i> s.str.	142	272	
	<i>S. danubialis</i>	142	272	
	<i>S. damugledica</i>	142	272	
	<i>S. graeca</i>	143	272	
	<i>S. pannonica</i>	143	272	
	<i>S. ulmifolia</i>	142	272	
	<i>S. umbellata</i> agg.	142	272	
	<i>S. vajdae</i>	142	272	
Chamaespilaria	<i>S. × ambigua</i>	141	272	
Soraria	<i>S. arranensis</i>	109	238	AUCUPARIA
	<i>S. austriaca</i>	109	238	AUCUPARIA
	<i>S. barbasii</i>	111	238	AUCUPARIA
	<i>S. dacica</i> KL morphotype	109	238	AUCUPARIA
	<i>S. hazslinszkyana</i>	109	238	AUCUPARIA
	<i>S. hohensteri</i>	108	238	AUCUPARIA
	<i>S. macedonica</i> n.p.	109	238	AUCUPARIA
	<i>S. mougeotii</i>	109	238	AUCUPARIA
	<i>S. pseudofennica</i>	109	238	AUCUPARIA
	<i>S. pseudothuringiaca</i>	109	238	AUCUPARIA
	<i>S. pulchra</i>	110	238	AUCUPARIA
	<i>S. × thuringiaca</i>	109	238	AUCUPARIA
	<i>S. tuzsoniana</i>	109	238	AUCUPARIA
Tomaria	<i>S. badensis</i>	143	273	
	<i>S. bakonyensis</i>	144	273	
	<i>S. balatonica</i>	144	273	
	<i>S. bohemia</i>	144	273	
	<i>S. gayeriana</i>	144	273	
	<i>S. pelsoensis</i>	143	273	
	<i>S. pseudolatifolia</i>	146	272	
	<i>S. semiincisa</i>	141	272	
Triparens	<i>S. dacica</i> SZL morphotype	111	238	AUCUPARIA
	<i>S. intermedia</i>	111	238	AUCUPARIA
	<i>S. paxiana</i>	112	238	AUCUPARIA
	<i>S. tauricola</i>	111	238	AUCUPARIA

3.3. Flow cytometry (FCM, FCSS)

3.3.1. Estimation of somatic DNA ploidy level (FCM)

Among species of subgenus *Triparens* the ploidy level of *S. intermedia* was previously published (Pellicer et al. 2012) and the chromosome number of *S. tauricola* is also known (Zaikonnikova & Kipiani 1980). Both of them are tetraploids, ($2n=68$). Our measurement confirmed this, strengthening that *S. intermedia* is indeed tetraploid. *S. dacica* and *S. paxiana* proved also to be tetraploid.

3.3.2. Determination of reproduction modes (FCSS)

Seed analysis revealed only tetraploid embryos in all three studied taxa (*S. dacica*, *S. paxiana* and *S. intermedia*). The endosperm/embryo ratios indicate pseudogamous reproduction mode of all three species.

The pollen contribution to endosperm development was exclusively tetraploid in the case of *S. intermedia*, while mostly tetraploid and rarely diploid in *S. dacica* and *S. paxiana*. Triploid pollen contribution was observed in one *S. paxiana* seed.

3.4. Taxonomic revision of *Sorbus dacica* s.l.

3.4.1. Morphological variability of *S. dacica* s.l.

Morphometric analyses revealed that *S. dacica* s.l. can be separated into two different morphotypes, a broad-leaved (SZL) and a narrow-leaved (KL) one. The first and the second ordination axes account for 48.09 % and 16.71 % of the variation, respectively (**Figure 2**).

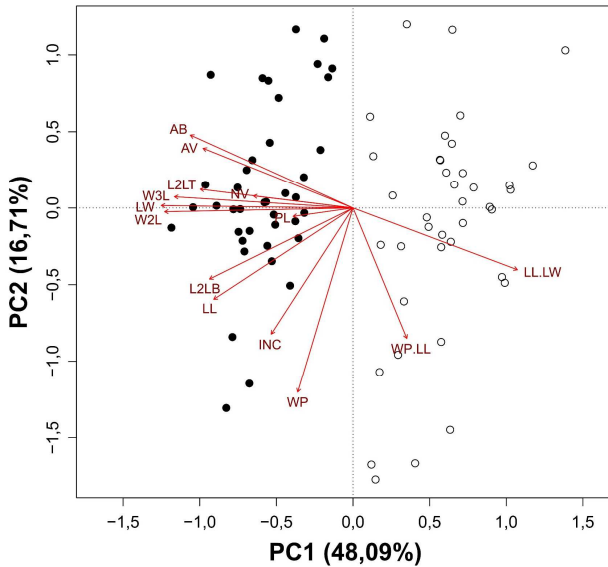


Figure 2. Principal component analysis based on 14 morphological characters of the leaves of *Sorbus dacica* s.l. ● broad-leaved morphotype; ○ narrow-leaved morphotype.

The two morphotypes were well separated by two characters, LW (leaf width) and LL/LW (leaf length/width ratio) with only a slight overlap in these values (**Figure 3**).

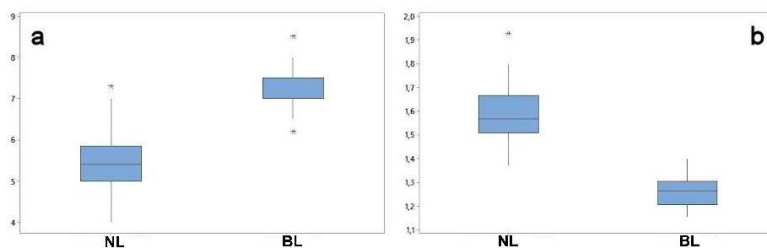


Figure 3. Box-plots of LW (leaf width) (a) and LL/LW (leaf length/width ratio) (b) characters of *Sorbus dacica* s.l. NL: narrow-leaved morphotype; BL: broad-leaved morphotype.

3.4.2. Chemotaxonomical variability of *Sorbus dacica* s.l.

The two morphotypes can be characterized by their difference in the flavonoid content. Leaves of the broad-leaved morphotype contain vitexin, whilst we did not detect this compound in the leaves of the narrow-leaved one. Furthermore, the broad-leaved morphotype contained also apigenin O-glucuronide confirming its *S. torminalis* origin, contrary to the narrow-leaved one (**Table 2**).

3.4.3. Chloroplast variability of *Sorbus dacica* s.l.

Both morphotypes are characterised by 'aucuparia-type' plastid having 111 bp (broad-leaved) and 109 bp (narrow-leaved) in *rpl16* locus, as well as 272 bp in *trnT-L* locus (**Table 3**).

3.4.4. Cytotype variability of *Sorbus dacica* s.l.

3.4.4.1. Ploidy level

The two morphotypes are characterized by different ploidy levels. The broad-leaved one proved to be tetraploid, whilst the narrow-leaved one was triploid.

3.4.4.2. Reproduction mode

The endosperm/embryo ratios indicate pseudogamous reproduction mode of both morphotypes. The pollen contribution to endosperm development was tetraploid, triploid and diploid in the case of the broad-leaved morphotype, whereas tetraploid, triploid and haploid pollen contributions were observed in the narrow-leaved one.

4. CONCLUSIONS AND SUGGESTIONS

4.1. Putative scenarios of *Triparens* speciation

Subg. *Triparens* comprises apomictic *Sorbus* taxa that combine the genomes of *S. aria* agg., *S. aucuparia* and *S. torminalis*. They have evolved at least by two independent cross events. Since the chloroplast genome is maternally inherited in angiosperms including *Sorbus*, and each *Triparens* species shows 'aucuparia-type' plastid, *S. aucuparia* must be the ovule donor in at least one hybridization event.

The most probable evolutionary pathway is that as a first step, a diploid sexual *S. aucuparia* as pollen acceptor (contributing with a reduced haploid egg) hybridized with a tetraploid apomictic taxon from the *S. aria* agg. (contributing with a reduced diploid pollen) producing a triploid apomictic *Soraria* taxon with 'aucuparia-type' plastid inherited maternally. Subsequently during a second crossing event this *Soraria* hybrid as maternal progenitor (providing an unreduced triploid egg and 'aucuparia-type' plastid) hybridized with the sexual diploid *S. torminalis* (providing a reduced haploid pollen containing gene(s) of apigenin O-glucuronide and vitexin synthesis) leading to the formation of a tetraploid *Triparens* hybrid with *aucuparia*-type' plastid and apigenin O-glucuronide content. Unreduced diploid pollen coming from diploid *S. aria* s.str. is unlikely. This was supported also by

the results of Lepší et al. (2016) who did not observe unreduced pollen in any studied individuals of diploid sexual *S. aria* s.str. Additional support for this scenario is that the diploid *S. aria* s.str. as a potential pollen donor is not native in the Baltic-Scandinavian region where *S. intermedia* has evolved (Sennikov & Kurtto 2017). The tetraploid *S. rupicola*, the only *Aria* species occurring sympatrically is supposed to be one of its parents (Rich et al. 2010). *S. aria* s.str. was not reported in the Crimean Peninsula either (Sennikov & Kurtto 2017) and only tetraploid members of *Aria* have been found to co-exist with *S. tauricola* (Zaikonnikova & Kipiani 1980, Zaikonnikova 2001, Sennikov & Kurtto 2017). We have not detected any diploid *S. aria* s.str. growing together with *S. dacica* and *S. paxiana* either, but several tri- and tetraploid representatives of subgenus *Aria* were revealed by flow cytometric cytotype mapping (Nosková et al. unpublished data). This concept is supported also by the fact that at least one *Soraria* taxon always occurs sympatrically with all known *Triparens* species (Jávorka 1915, 1927, Liljefors 1953, Kárpáti 1960, Zaikonnikova 2001, Németh et al. unpublished data) (**Figure 4**).

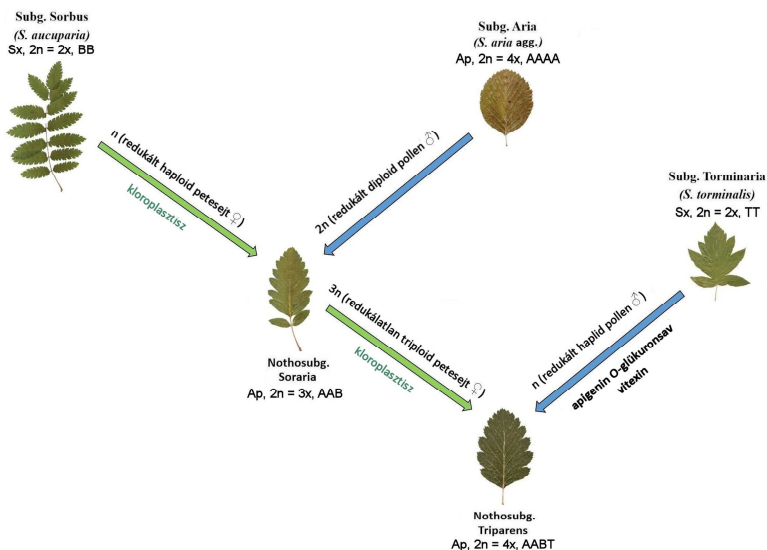


Figure 4. The most probable hypothetical scenario of *Sorbus* subg. *Triparens* speciation. Abbreviations: A: subgenus *Aria* genome, B: *S. aucuparia* genome, T: *S. torminalis* genome, Sx: sexual, Ap: apomictic (original).

4.2. Chorological and phytogeographical considerations

With re-classification of *S. dacica*, *S. paxiana* and *S. tauricola* the earlier known distribution of nothosubgenus *Triparens* becomes larger, and along with the Scandinavian-Baltic area (*S. intermedia* and *S. × liljeforsii*) includes also the Transylvanian (*S. dacica*) and Banatian (*S. paxiana*) regions in Romania, as well as the Crimean Peninsula (*S. tauricola*) (**Figure 5**).

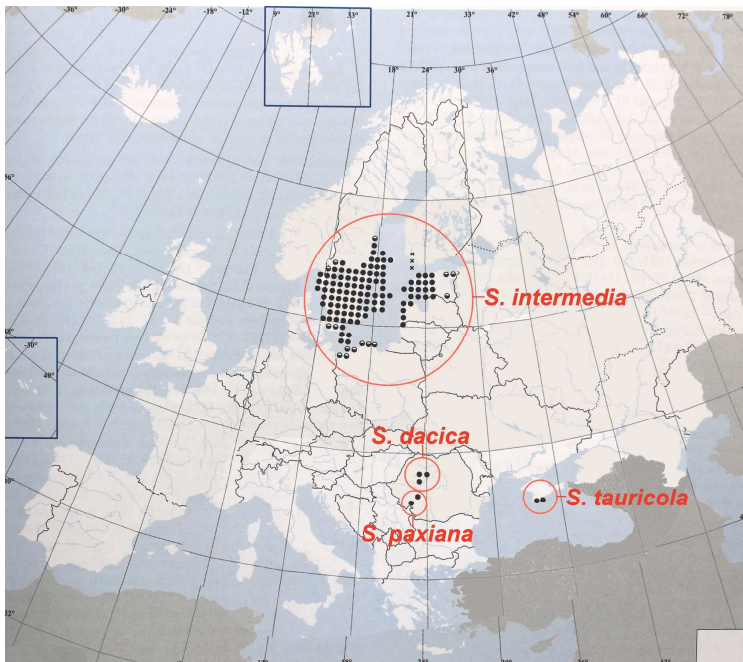


Figure 5. Map showing natural distribution of *Sorbus* subg. *Triparens*. ●: native; ◐: status unknown or uncertain; x: probably extinct native. (based on Kurtto et al. 2018, modified and completed).

4.3. Taxonomical and nomenclatural consequences

4.3.1. Taxonomical and nomenclatural considerations of Nothosubg. *Triparens*

Molecular studies provided evidence that *Sorbus* s.l. is a polyphyletic genus including five main evolutionary lineages (traditionally classified as subgenera *Sorbus*, *Cormus*, *Aria*, *Chamaemespilus*, *Torminaria*) (Campbell et al. 2007). The

taxonomic status of the numerous intermediate taxa of hybrid origin has also been long disputed. Sennikov and Kurtto (2017) have proposed that hybridogenous subgenera should be recognized as genera of their own and subgenus *Triparens*, composed of *S. intermedia*, the only species of hybrid origins between *S. aria* agg., *S. aucuparia* and *S. torminalis* should be classified in genus *Borkhausenia* (later the name *Borkhausenia* was replaced for *Scandosorbus* for nomenclatural reasons; Sennikov 2018).

Since *S. dacica* had formerly been classified in the biparental subgenus *Soraria*, in their taxonomic approach it was placed into the genus *Hedlundia*, a newly introduced name for taxa of hybrid origin with *S. aria* agg. × *S. aucuparia* lineage. *S. paxiana* and *S. tauricola* treated previously as species of *S. aria* agg. × *S. torminalis* origin were included in subgenus *Karpatiosorbus*. However, under *Sorbus* concept by Sennikov (2018), all the three-way hybrid taxa among *S. aria* agg., *S. aucuparia* and *S. torminalis* including *S. dacica*, *S. paxiana* and *S. tauricola* should be accommodated in the genus *Scandosorbus*.

4.3.2. Taxonomical and nomenclatural considerations of *Sorbus dacica* s.l.

According to the results, our morphological, biochemical, molecular and flow cytometry studies revealed taxonomic heterogeneity and heterogeneous evolutionary past of *S. dacica* s.l.

Morphometric analyses revealed that *S. dacica* s.l. can be separated into two different morphotypes: 1. a broad-leaved one having greenish-yellowish colour of leaf underside and larger fruits (14–17 mm in diameter) with brownish tinge during ripening; 2. a narrow-leaved one having whitish-grayish colour of leaf underside and smaller fruits with 13–14 mm in diameter. Leaves of the broad-leaved morphotype contain apigenin O-glucuronide and vitexin confirming its *S. torminalis* origin, whereas we did not detect either of them in the narrow-leaved morphotype suggesting the lack of *S. torminalis* among the ancestors. The 'aucuparia-type' plastid indicates the participation of *S. aucuparia* as maternal parent resulting in the formation of both morphotypes. The broad-leaved morphotype proved to be tetraploid, whilst the narrow-leaved can be characterized with triploid cytotype. One member of *S. aria* agg. as the third parent of both morphotypes is obvious from morphological features such as dense hairiness on the abaxial side of the leaves. Consequently, the broad-leaved morphotype has evolved by triparental hybridization of *Sorbus aria* agg., *Sorbus aucuparia* and *Sorbus torminalis*. It is unambiguously identifiable

with the specimens of *S. dacica* s.str. growing in the *locus classicus* Torda Gorge. The narrow-leaved morphotype has originated via a biparental cross event between *S. aria* agg. and *S. aucuparia*. Taxonomic position of this morphotype is still unclear and its distinction on the species-level needs further investigations.

5. NEW SCIENTIFIC ACHIEVEMENTS

1. Using biochemical (flavonoid), molecular (chloroplast microsatellites) and morphological markers we proved that *S. dacica* (Transylvania, Romania), *S. paxiana* (Banat, Romania) and *S. tauricola* (Crimean Peninsula) have evolved by triparental hybridisation combining the genomes of *S. aria* agg., *S. aucuparia* and *S. torminalis*.
2. Using flow cytometry we determined the ploidy level and reproduction mode of *S. dacica* and *S. paxiana*. Both species proved to be tetraploid ($2n=4x=68$) with pseudogamous reproduction mode.
3. Using morphological and biochemical methods as well as flow cytometry we proved that *S. dacica* includes two morphologically different pseudogamous taxa which differ from each other in flavonoid content, ploidy level and evolutionary past as well. One of them is tetraploid and has evolved by triparental hybridization, whilst the other one is triploid and has originated via a biparental cross event.
4. Using flavonoid markers and high performance liquid chromatography we detected vitexin in the leaves of *S. domugledica*, a Banatian endemism, confirming its *S.*

umbellata subsp. *banatica* origin. This was formerly presumed based on morphological features. We detected vitexin in the leaves of *S. pannonica* and *S. vajdae* suggesting also *S. umbellata* among the ancestors.

5. Using chloroplast markers we proved that each taxon of the hybridogenous subgenus *Soraria* and *Triparens* studied is characterized by 'aucuparia-type' chloroplast, indicating their *S. aucuparia* origin and the participation of *S. aucuparia* in the hybridogenous speciation as maternal line.

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7. PUBLICATIONS CONNECTED TO THE DISSERTATION

Paper in impact factored journals:

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