

NORDIC JOURNAL OF BOTANY

Research

Hybrids between *Rubus idaeus* and *Rubus* sect. *Corylifolii* and their relation to *R. pruinus* and *R. rosanthus*

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Nordic Journal of Botany

2021: e03111

doi: 10.1111/njb.03111

Subject Editor and
Editor-in-Chief: Torbjörn Tyler
Accepted 2 June 2021
Published 31 August 2021



We have studied hybrids between *Rubus idaeus* and various members of *R.* sect. *Corylifolii*, primarily in Sweden. With the help of DNA-ploidy level determinations using flow cytometry and microsatellite DNA analysis of over 500 samples, we show that the material can be divided into four stable apomictic species (belonging to subsect. *Subidaei*) and a large number of primary hybrids. Stable species can be recognised by a distribution that is distinct from the *Corylifolii* parent, a uniform morphology and an almost invariant genetic pattern. We confirm that *R. cordatiformis* has arisen from the hybrid *R. eluxatus* × *idaeus*. Moreover, our study shows that *R. lagerbergii* has arisen from the hybrid *R. dissimulans* × *idaeus*. For *R. onsalaënsis*, we unexpectedly identify the *Corylifolii* ancestor as a previously unidentified species with a very restricted distribution in the northern part of the province of Halland, which is here described as *R. antecedens*. Furthermore, we show that *R. pruinus* s. str. has arisen from the hybrid *R. aureolus* × *idaeus* and that it is known only from a rather restricted area in the provinces of Småland, Östergötland and Södermanland in eastern Sweden. On the other hand, the great majority of what has been called *R. pruinus* is a diverse collection of primary hybrids between *R. idaeus* and various *R.* sect. *Corylifolii* species, which show a large variation both genetically and morphologically. The same applies to *R. rosanthus*, which represents *R. norvegicus* × *idaeus* hybrids: The microsatellite analysis shows that they have arisen independently and therefore should be considered as primary hybrids. We also identified primary hybrids between *R. idaeus* and *R. gothicus* (by far the most common hybrid in Sweden), *R. aureolus*, *R. camptostachys*, *R. decurrentispinus*, *R. eluxatus*, *R. friesianus*, *R. hallandicus*, *R. lidforsii* and *R. norvegicus*.

Keywords: apomixis, *Rubus antecedens*, *Rubus idaeus* hybrids, *Rubus lagerbergii*, *Rubus pruinus*, *Rubus rosanthus*, Weberian species concept

Introduction

Rubus sect. *Corylifolii* Lindl. is a large and difficult group of blackberries, characterised by a creeping stem, broad stipules, overlapping leaflets with short petiolules and broad, overlapping and somewhat crispate petals (Weber 1981). Like most of the other blackberries (*Rubus* sect. *Rubus* L.), the species are apomictic (aposporous and pseudogamous) (Gustafsson 1939) and a large number of similar species have been described.



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They have probably arisen from crossings between members of *R. sect. Rubus* and *R. caesius* L., dewberry. However, apomixis is only facultative. Therefore, hybrids are rather common and hybridisation is an important source of new species (Lidforss 1914, Gustafsson 1939, Sochor et al. 2015).

Some of the *Corylifolii* taxa share a set of common characteristics: conspicuously red prickles, even on the shadow-side of the stem, densely hairy ovaries, occurrence of seven-foliate leaves, straight narrow prickles, rounded stems and leaves that are densely hairy on the lower surface (although each taxon seldom exhibits all of them). Several of these characters indicate some influence from raspberry (*R. idaeus* L.) and consequently, they are assigned to a separate group, either as subsection *Subidaei* (Focke) Hayek (Weber 1981) within *Corylifolii* or section *Subidaei* (Focke) A.Beek, at the same level as *Corylifolii*, to emphasise that they have a partly differing origin (Van De Beek 2014).

In Sweden, five species of *Subidaei* have been reported: *R. pruinus* Arrh., *R. rosanthus* Lindeb., *R. lagerbergii* Lindeb., *R. cordatiformis* (Neuman) Ryde and *R. onsalaënsis* Ryde. The latter was recently described and has a local distribution in the northern part of the province of Halland on the Swedish west-coast (Ryde 2009, 2011). *Rubus cordatiformis* was first described as a variety of *R. rosanthus* (Neuman 1901) and it also has a quite local distribution in the central part of the province of Halland (Ryde 2009, 2011). *Rubus rosanthus* was described by Lindeberg (1885) and it has a rather wide distribution along the northern and eastern coast of Skagerrak from Arendal in Norway to the Göteborg archipelago in Sweden (Weber 1981). *Rubus lagerbergii* was also described by Lindeberg (1885) and has a disjunct distribution in three areas along the Swedish west coast, but it also occurs on the Danish island Anholt and on northern Jutland (Pedersen and Schou 1989, Tyler et al. 2007, Blomgren and Falk 2011). *Rubus pruinus* was described by Arrhenius from the island Öro in the north-eastern part of the province of Småland on the Swedish east coast (Arrhenius 1839). However, Lidforss reported it from most of the southern provinces in Sweden (Lidforss 1901) and it is now considered to have a wide distribution in northern, western and central Europe (Weber 1981).

Unlike the other four *Subidaei* species, *R. pruinus* is morphologically quite diverse. Already in 1854, Lindeberg pointed out that it is not uniform and shows transitions to other *Corylifolii* species (Lindeberg 1854) and many taxa of lower rank have been suggested (Areschoug 1885, Neuman 1901, Gustafsson 1938, Weber 1981). However, Weber rejected all of them as shadow forms or insignificant modifications, except for *f. warmingii* (Jens. ex Neuman) H.E. Weber (Weber 1981).

In fact, already Areschoug (1885) pointed out early on that *R. pruinus* gives the impression of being a hybrid between *R. idaeus* and either *R. aureolus* Allander or *R. wahlbergii* Arrh. This idea was reiterated by subsequent authors (Lindman 1918), but Lidforss suggested that one of the parents might instead be *R. gothicus* (Lidforss 1899, 1901) and he also reported a fourth potential parent, *R. fasciculatus*

P.J.Müll. (Lidforss 1901). He showed by cultivation experiments that *R. pruinus* gives rise to variable progeny (Lidforss 1905), indicating that it is not a constant agamosperous species, but a sexual hybrid. Hylander brought this one step further by suggesting that *R. pruinus* is a collective species, consisting of primary hybrids of *R. idaeus* with various *Corylifolii* species (Hylander 1958a). However, he proposed that the hybrids should nevertheless be described as varieties of *R. pruinus* with the name of the parent species preceded by the prefix 'sub', e.g. *Rubus pruinus* v. *subgothicus* for the *R. gothicus* × *idaeus* hybrid. Several such taxa, but without any formal descriptions, were presented in a provincial flora (Holmgren 1942). In all, he reported *R. idaeus* hybrids with 15 *Corylifolii* species (Hylander 1958b).

On the other hand, Kanér suggested that *R. pruinus* is a collection of triple hybrids between the hybrid *R. caesius* × *idaeus* and a *Corylifolii* species (Kanér 1953). He reported five such hybrids, viz. with *R. aureolus*, *R. tiliaster* H.E. Weber, *R. fasciculatus*, *R. camptostachys* G. Braun and *R. wahlbergii*. The latter two hybrids were also experimentally produced by crossing experiments. Weber seemed to have similar ideas, although he also suggested that the species of *Subidaei* could have arisen from back-crossings between *R. caesius* × *idaeus* and *R. caesius* (Weber 1981). Still, he considered them as proper apomictic species and not as primary hybrids.

Recently, one of us renewed and promoted the idea that *R. pruinus*, at least in Sweden, is a collection of unrelated primary hybrids between *R. idaeus* and various members of *R. sect. Corylifolii*, based on an inventory in Halland (Ryde 2009, 2011). Thus, it was suggested that they should be reported as hybrids and that such a treatment may reduce some of the confusion that has plagued the treatment of the *Corylifolii* in Sweden. However, there also undoubtedly exist proper apomictic species that have originated from *R. idaeus* hybrids. Therefore, it is not fully clear how these two categories should be discerned. In this article, we clarify this issue with the help of flow cytometry to establish ploidy levels and microsatellite DNA analysis to describe genetic differentiation patterns.

The taxonomical treatment of the apomictic blackberries has varied (Gustafsson 1943, van de Beek 1974, Weber 1996). Attempts to give names to every biotype and various artificial systems have plagued the taxonomy and led to a stagnation during the mid 20th century. This situation was solved by the Weberian reform (Holub 1997), which was based on mapping projects over large areas, evaluation of type material, identification of the species from their classical localities and a restriction of what is considered as a species based on the size of the distribution (Weber 1972, 1977, 1981, van de Beek 1974). The latter rules, specifying that only taxa with a distribution larger than 20–50 km should be given a scientific name, strongly reduced the number of species recognised and opened for a general understanding of the blackberries in Europe.

However, recently this species concept has been criticised (Hörandl 1998, Loos 2008, Ryde 2011, Haveman and Ronde 2013). Several arguments against it have been provided:

- A species concept based on the size of the distribution is practical, but artificial and without any scientific ground.
- It underestimates biodiversity.
- It does not reflect the process of speciation.
- It leads to practical problems both for the field botanist (locally abundant taxa may lack a scientific name and may therefore be assigned to incorrect species, which gives rise to chaos in the local floras or botanical collections).
- It gives practical problems in red listing (only the moderately uncommon taxa can be red listed, not the most rare ones, because the latter are not accepted as species; furthermore, if the distribution of accepted species decreases, they may be removed from the list, because they are no longer recognised as species).
- It gives rise to strange emphases on some taxa (for example, why is a species occupying two localities 50 km apart more interesting than another species with hundreds of localities within 10 km?).
- If local species are not described, it becomes hard to find out whether they actually occur in several areas.
- It is scientifically unsatisfactory that different and arbitrary species concepts are employed for different groups.
- The Weberian species concept has been useful for gaining a continent-wide overview of the genus, but as the *Rubus* flora is now reasonably well-known in most parts of Europe, it has played out its role. The availability of GPS, cytometry and genetic methods have made it possible to take the next step and try to describe all stable species.

An important argument against the Weberian species concept is also that it is not based on any deeper analysis of the actual taxonomical problems in *Rubus*. Haveman and Ronde (2013) report four problems (also partly discussed by Loos 2008 and Ryde 2011):

1. Primary hybrids, mainly between sexual and apomictic species.
2. Locally distributed stabilised biotypes.
3. Variation within the widely distributed species.
4. Unstabilised swarms of putative hybridogenic biotypes.

They argued that species belonging to group 2 should be described as species, irrespective of the size of the distribution, whereas unstabilised biotypes and primary hybrids should not. The variation in group 3 should be described

as separate species only if they form independently evolving lineages with consistent distributions, independent from related taxa.

We fully agree with this view and have previously discussed these problems from a Swedish viewpoint, where hybrids with *R. caesius* and *R. idaeus* are the main problem (Ryde 2011). However, it then becomes important to decide whether a local biotype is a stabilised independently evolving lineage or an occasional biotype or a hybrid. In this article, we discuss this problem for putative hybrids between *Corylifolii* and *R. idaeus* and suggest how the problem can be solved.

Methods

A total of 546 samples (young leaves from the tip of the annual shoot) of *R. idaeus*, several *Corylifolii* species and putative hybrids were collected from Sweden. The localities are specified in the Supporting information and a number of vouchers have been deposited at herbarium LD. The samples were stored in a freezer at -80°C until they were used.

DNA was extracted with the 2× CTAB method (Doyle and Doyle 1987). The concentration of the DNA was determined by a fluorometer and the samples were diluted with ddH₂O to $\sim 14\text{ ng }\mu\text{l}^{-1}$. Four pairs of microsatellite primers, described in Table 1, were used to analyse variable microsatellite loci. The three loci Ru105b, Ru117b and Ru275a were described for *R. idaeus* (Graham et al. 2004), whereas locus mRaCIRRIV2A8 was described from *Rubus alceifolius* (Ansellem et al. 2001). They will be called 105b, 117b, 275a and 2A8, respectively, in the following. They have also been successfully used also for other *Rubus* species (Stafne et al. 2005, Zhou 2014, Lewin 2016, Hedrén et al. 2019). One primer in each primer pair was tagged with a fluorescing molecule (Cy5-) to enable the identification of the polymerase chain reaction (PCR) products in the automatic sequencing machine (ALF Express II).

The PCR reactions consisted of 35 cycles of denaturation at 94°C for 30 s, hybridisation at varying temperatures (Table 1) for 30 s and polymerisation at 72°C for 1 min (10 min in the last cycle). Each PCR reaction was performed in a solution consisting of (in a total volume of ca 6.4 μl): 4.4 μl ddH₂O, 0.66 μl 10× reaction buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl and 15 mM MgCl₂), 0.56 μl dNTPs (2.5 mM for each nucleotide), 0.25 μl Cy5-tagged primer

Table 1. Details for the primer pairs (F=forward, R=reverse, T_a =annealing temperature in $^{\circ}\text{C}$).

Primer name	SSR	Primer sequence (5'→3')	T_a	Product size (bp)	Size marker (bp)
Rubus 105b	(AG) ₈	F GAAATGCAAGGCGAATTGT R TCCATCACCAACACCACTA	52	140–190	224
Rubus 117b	(CATA) ₆ (GA) ₈	F CCAACTGAAACCTCATGCAC R ACTTGGTCCTGTTGGTCTGG	59	100–180	181
Rubus 275a	(AG) ₂₇	F CACAACCAGTCCCGAGAAAT R CATTTCATCCAAATGCAACC	52	90–200	224
mRaCIRRIV2A8	(CA) ₁₂ (CT) ₁₁	F TAAAAAGGCGCAACAGTCG R AGACACAGAAACAGGCATCG	52	150–260	291

(10 µg ml⁻¹), 0.1 µl untagged complementary primer (25 µmol ml⁻¹), 0.03 µl Taq polymerase (5 u µl⁻¹; Applied Biosystems) and 0.6 µl template DNA (14 ng µl⁻¹).

The PCR products were mixed with formamide and proper size markers, and were then heated to 94°C for 25 min before they were separated with electrophoresis in a polyacrylamide gel. The DNA fragments were detected and analysed with the help of an ALF Express II analysis system. The relative height of the bands was estimated on a five-graded scale. Samples that resulted in poor PCR reactions were purified with a Qiagen purification kit, after which the PCR and electrophoresis were redone.

DNA-ploidy levels were estimated by flow cytometry for 68 samples. This analysis was performed by Plant Cytometry Services (<www.plantcytometry.nl>). Fresh leaf material was homogenised and mixed with ice-cold buffer with DAPI (Arumuganathan and Earle 1991). The amount of DNA per cell nucleus was determined in a CyFlow ML with iceberg lettuce *Lactuca sativa* as internal standard. The estimates were calibrated by using *Rubus* samples with known chromosome numbers. The DNA-ploidy levels are presented as fractional chromosome numbers from this calibration line between the ratio in fluorescence between the samples of known chromosome numbers and the internal standard.

Results and discussion

DNA-ploidy levels

Chromosome numbers and DNA-ploidy levels of *Rubus* species have been studied many times before (Gustafsson 1933, Meng and Finn 2002, Ryde 2011, Sochor et al. 2019). It is thus known that *R. idaeus* is a sexual diploid species with $2n=2x=14$. *Rubus caesius* act as a strictly apomictic – (It has been shown that *R. caesius* (and several other tetraploid *Rubus* species) actually forms embryo sacks by regular meiosis (Christen 1950, Dowrick 1961), but it still produces embryos without fusion with a sperm cell, either by restitutional meiosis or by fusion of nuclei in the embryo sac (Dowrick 1961, 1966). Neither Lidforss nor Dowrick were able to construct any hybrids with *R. caesius* as the mother (Lidforss 1914, Dowrick 1966), indicating that it effectively functions as a strict apomict. On the other hand, Sochor et al. 2015 have found that many *Corylifolii* species carry a chloroplast haplotype common with *R. caesius* (Sochor et al. 2015), indicating a common origin on the maternal side.) – tetraploid with $2n=4x=28$ (Gustafsson 1943), whereas most species of *R. sect. Corylifolii* are facultatively apomictic tetraploids with $2n=4x=28$, although a few species are apomictic pentaploids with $2n=5x=35$ (e.g. all *R. subsect. Subidaei*, as well as *R. wahlbergii* and *R. vikensis* A. Pedersen ex G. Wendt) or hexaploids with $2n=6x=42$ (e.g. *R. slesvicensis* Lange).

We estimated the DNA-ploidy levels of 68 samples with flow cytometry (results given in the Supporting information). All 44 samples of putative hybrids between *R. idaeus* and members of *R. sect. Corylifolii* turned out to

be pentaploids ($2n=35$). This is the expected ploidy level if the hybrids arise from the union of a haploid gamete of normally reduced pollen of *R. idaeus* (with $n=7$) with an unreduced egg cell of the *Corylifolii* species (which normally has $2n=28$; $28+7=35$) (Gustafsson 1939). The same DNA-ploidy levels were also found in *R. lagerbergii*, *R. cordatiformis* and *R. onsalaënsis*, as has been reported previously (Ryde 2009, 2011), supporting the suggestion that they have arisen from hybrids with *R. idaeus*. All samples of putative *R. rosanthus* and *R. pruinusos* also gave values corresponding to 35 chromosomes.

As also noted before (Ryde 2011), the estimated chromosome number (from the calibration line between the ratio in fluorescence between samples with known chromosome numbers) for the *R. idaeus* hybrids is somewhat below the calibration line obtained from other *Corylifolii* samples, 34.2 ± 0.1 , with a variation from 31.2 to 36.3. This indicates that *R. idaeus* has a lower amount of DNA than the *Corylifolii* and it has been argued that this is a good indication of such an origin, in contrast to other pentaploid *Corylifolii* species with different origins, e.g. *R. wahlbergii* and *R. vikensis*, which have a higher amount of DNA (Ryde 2011).

In a few cases, the flow cytometry indicated that the interpretation of a sample as a *R. idaeus* hybrid was not correct. This is in particular true for a sample from Snårestad in Skåne. This sample had characters of both a *R. idaeus* hybrid, with brightly red and narrow prickles (but neither hairy ovaries nor seven-foliolate leaves), and a *R. caesius* hybrid with sepals that embrace the fruit. However, the cytometry showed that it is a hexaploid ($2n=42$), which indicates that it actually is a *R. caesius* hybrid (14 chromosomes from reduced pollen from *R. caesius* and 28 chromosomes from the unreduced egg cell of the *Corylifolii* species (Gustafsson 1939)). In this respect, it is notable that Lidforss in his cultivation studies of a putative *R. wahlbergii* $\times$ *caesius* hybrid sometimes obtained specimens that were similar to *R. pruinusos* (Lidforss 1901, 1905).

Conversely, two samples from Gödelöv in Skåne, collected from two stands that are only 20 m apart, were distinctly different in terms of morphology – one gave the impression of being a *R. idaeus* hybrid, whereas the other was more similar to a *R. caesius* hybrid. However, both samples gave $2n=35$, indicative of *R. idaeus* hybrids, although *R. caesius* hybrids may occasionally also have this number (Gustafsson 1933). Yet, the cytometry estimate for one of the samples was quite high, 35.8 (the other was more normal, 34.6). Still, microsatellite DNA analysis (below) indicates that both samples are *R. gothicus* $\times$ *idaeus*, but with independent genetic origins. Thus, we conclude that *R. idaeus* hybrids occasionally can have characters reminiscent of *R. caesius* hybrids, in particular sepals that embrace the fruits.

Microsatellite DNA analysis of *R. idaeus*

The main genetic method employed in this article is microsatellite DNA analysis, based on four loci, 105b, 117b, 275a and 2A8, as was described in the Methods section. We have analysed 126 samples of *R. idaeus* from Sweden and

Germany, collected from many scattered localities, but sometimes also in groups of ~10 samples from the same locality (but not within the same vegetative clone). The results show a high degree of variability, as was expected from a sexual species. Even for samples from the same locality, the variation is extensive. Within individuals, most loci showed one or two alleles, as would be expected for a diploid species.

Moreover, alleles of many different sizes were observed in *R. idaeus* (Supporting information), although some alleles were more common than others, especially at the 105b and 117b loci. Therefore, it is hard to assign certain alleles to *R. idaeus*. Instead, in the following we will assume that alleles that cannot be assigned to a *Corylifolii* parent come from *R. idaeus* in putative hybrids.

***Rubus cordatiformis* and the difference between an apomictic species and primary hybrids**

Next, we examined 25 samples of *R. cordatiformis*. This species is known only from a restricted area in the province of Halland (Ryde 2009, 2011). It is very characteristic, with large flowers with brightly pink filaments. However, it also has clear characters of a *R. idaeus* hybrid with conspicuously red prickles and slightly hairy ovaries. It is clear from the morphology that the *Corylifolii* parent is *R. eluxatus* Neuman, which is rather common in the same area. Interestingly, the primary hybrid *R. eluxatus* × *idaeus* is also known from three

localities in the province of Halland (Ryde 2009). This allows us to discuss the difference between an apomictically reproducing independent species and primary hybrids and how they can be distinguished in practise.

First, the distribution pattern is different. Primary hybrids occur at isolated localities within the distribution of the *Corylifolii* parent (in most parts of southern Sweden, *R. idaeus* occurs essentially everywhere). The hybrids often occur together with one or both of the parent species and spread only vegetatively. An independent species, on the other hand, has a distribution of its own: It is often common in a local area and its distribution is partly distinct from that of the *Corylifolii* parent. This situation is illustrated in Fig. 1, showing that *R. eluxatus* has a much larger distribution than *R. cordatiformis*. It is most common around the city of Varberg and in the parishes of Steninge and Eftra. The distributions of the two species overlap partly in the latter area, but the distribution of *R. cordatiformis* extends farther to the north. On the other hand, the hybrid (red stars in Fig. 1) occurs only at three isolated localities, scattered within the distribution of *R. eluxatus*, two around Varberg and one in Steninge. Thus, the first thing to check when finding a putative hybrid is whether it occurs in more localities in the same neighbourhood or not.

Second, an independent species is morphologically uniform, i.e. all plants share essentially the same characters, whereas primary hybrids are morphologically variable and

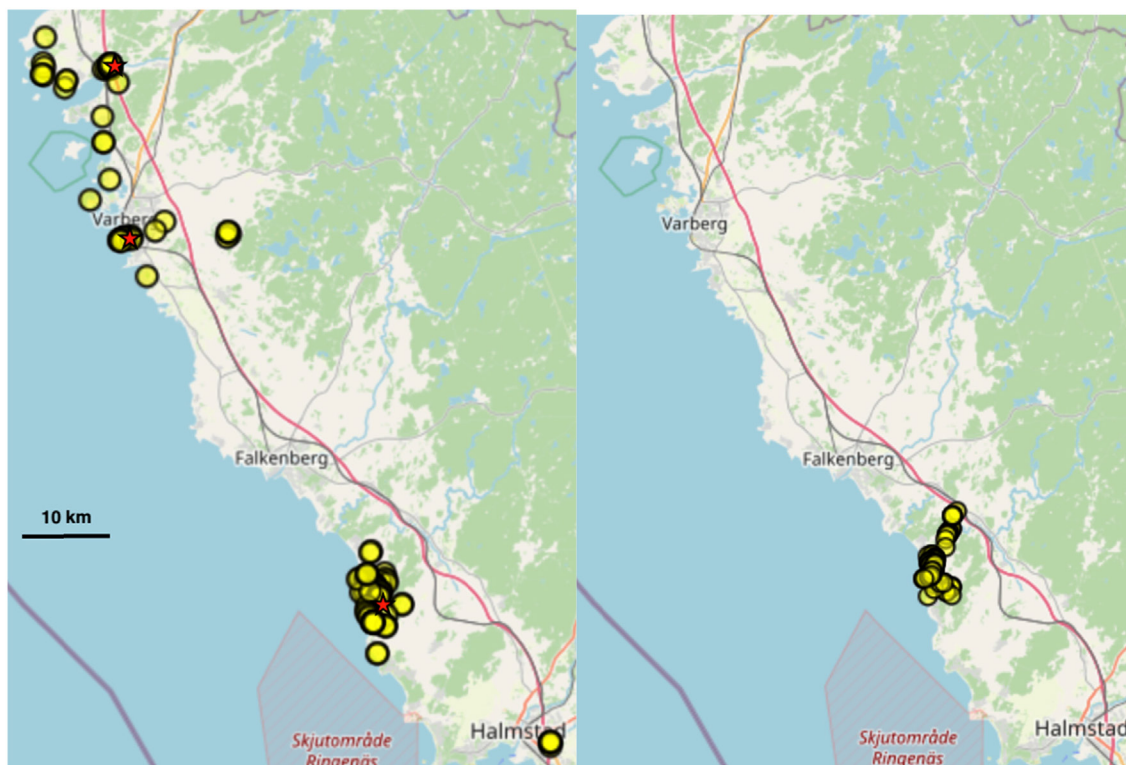


Figure 1. Distributions of *R. eluxatus* (left) and *R. cordatiformis* (right) in the province Halland, western Sweden (Ryde 2009, 2011), showing reports to Artportalen 2000–2020 (Leidenberger et al. 2016). The three localities of the *R. eluxatus* × *idaeus* hybrid are shown by red stars in the distribution of *R. eluxatus*.



Figure 2. Comparison of *R. cordatiformis* (to the left) and the *R. eluxatus* × *idaeus* hybrids from three localities (Värö, Apelviken and Steninge, from left to right). The upper row shows the leaves on the stem, the middle row the inflorescence and the bottom row the flower.

exhibit different combinations of characters from the two parent species. The apomictic species *R. cordatiformis* always has large flowers with pink filaments (it is the only *Corylifolii* species in Sweden with deeply pink filaments, cf. Fig. 2). The prickles are always red, but the ovaries are only slightly hairy (the mature fruits are glabrous). The leaves are mostly 3–5-foliate. On the other hand, the three primary hybrids are distinctly different. The one from Apelviken (middle red star in Fig. 1) has seven-foliate leaves, but no red prickles (Fig. 2). The one from Värö (upper star) has five-foliate leaves and brightly red prickles. The one from Steninge (lower star) has five-foliate leaves and prickles that are only slightly red at the base. All three have pink flowers and hairy ovaries, but the hairs are longer and denser in the hybrid from Apelviken. None of the hybrids has the pink filaments,

the small, dense, dark red prickles on the stem or the long sharp prickles on the pedicels of *R. cordatiformis*. The hybrid from Steninge has led to some confusion, because it appears in the same area as *R. cordatiformis*. Therefore, it was previously taken as a deviant morphotype of *R. cordatiformis* (Ryde 2009, 2011).

Third, a stable species is genetically uniform. This is illustrated by the microsatellite DNA results in Fig. 3. It can be seen that for locus 105b all 21 samples of *R. eluxatus* gave identical results, showing four alleles with lengths of 146, 148, 152 and 164 bp, reflecting that the species is tetraploid. The results for *R. cordatiformis* are similar: all 15 samples show the same four alleles as *R. eluxatus*. However, there is also one extra allele for all samples with a length of 156 bp, originating from *R. idaeus*. The three primary hybrids are also similar:

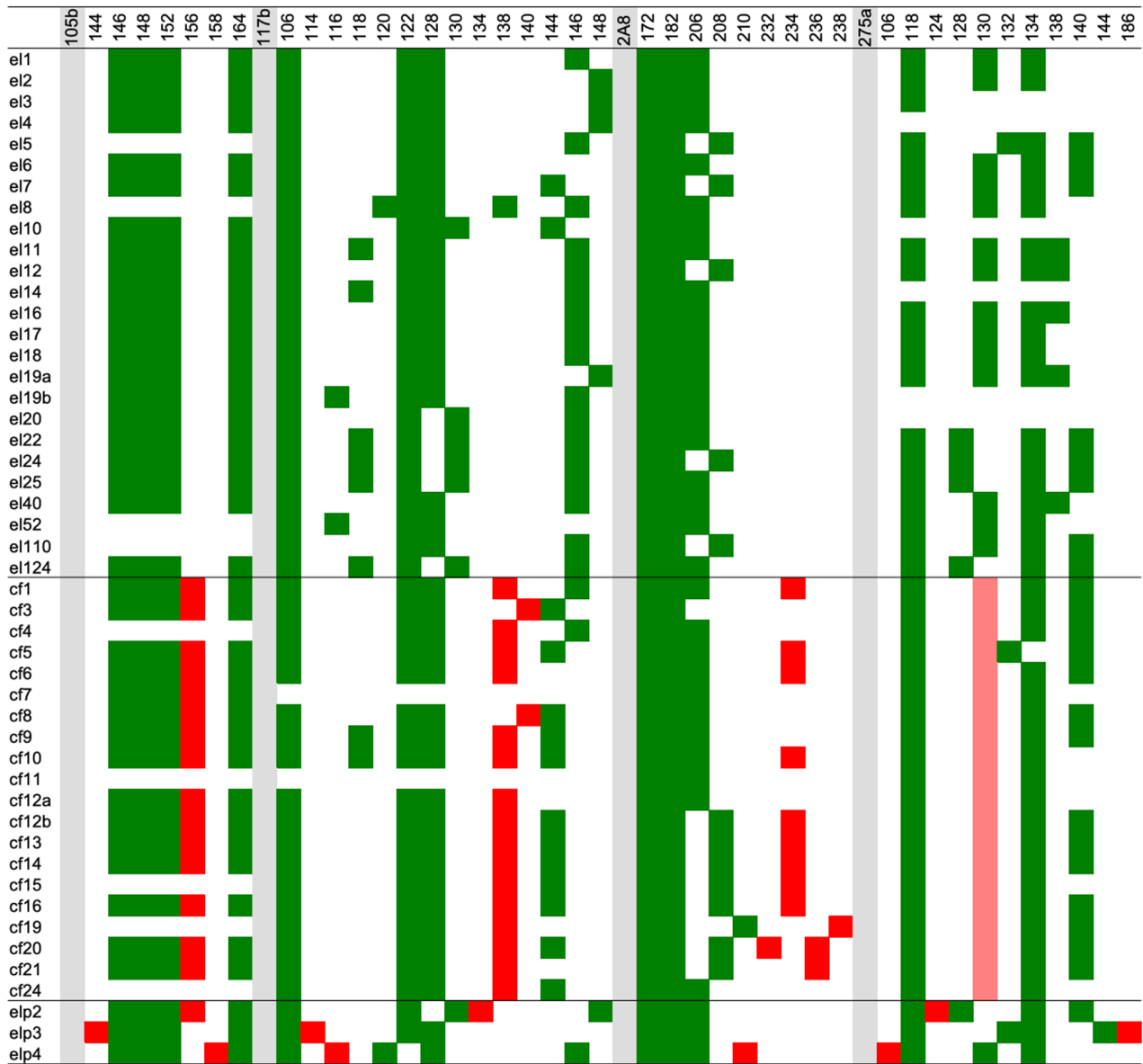


Figure 3. Microsatellite DNA results for 25 samples of *R. eluxatus* (sample names starting with 'el'), 20 samples of *R. cordatiformis* (cf) and three samples of *R. eluxatus* $\times$ *idaeus* (elp: Värö, Apelviken and Steninge in this order) at the four loci. The coloured areas reflect the presence of an allele of this length (in bp). The green alleles are common for most samples, whereas the red alleles occur only in *R. cordatiformis* and the hybrids, and most likely originate from *R. idaeus*. The light-red allele (for locus 275a) has an increased intensity, because it is a combination of alleles both from *R. eluxatus* and *R. idaeus*.

They share four of the alleles with *R. eluxatus*, and they also have an additional, fifth, allele from *R. idaeus*. However, the fifth allele in the three hybrids has different length, illustrating that they originate from different *R. idaeus* parents: In the sample from Apelviken, it has a length of 144 bp, whereas in the sample from Steninge, it has a length of 158 bp. For the sample from Värö, it has a length of 156 bp, i.e. the same as for *R. cordatiformis*.

At locus 117b, the results are similar: *R. cordatiformis* and the hybrids share the same alleles as *R. eluxatus* (although the results are slightly blurred by some variations in the length of

the alleles), but they also have an extra allele. In *R. cordatiformis*, it has a length of 138 bp, whereas in the three hybrids, the length is 114, 116 and 132 bp, respectively. This variation clearly shows that they have separate origins.

The results at locus 2A8 are also similar, but the extra allele from *R. idaeus* is quite long and weak, so it shows a larger variation and is sometimes not detected. An extra allele is observed in only one of the three hybrids (the one from Steninge).

At locus 275a, *R. cordatiformis* and the hybrids also share the four alleles from *R. eluxatus*. In the hybrids, there is an

extra allele for each of them, but with different lengths (106, 124 and 186 bp). However, in *R. cordatiformis*, there is no fifth allele, because the allele contributed by *R. idaeus* is of the same size as one of the alleles from *R. eluxatus*. This pattern is indicated by a shift of the relative intensities of the PCR products; in *R. eluxatus*, the fragment of 118 bp length is the most intense, whereas in *R. cordatiformis*, the most intense fragment is instead of 130 bp length.

Based on these results and principles, we are prepared to interpret results for the other samples.

***Rubus pruinus* s. str.**

Arrhenius described *R. pruinus* in 1839 from the island Öro close to Oskarshamn on the Swedish east coast (Arrhenius 1839). Both on this island and on the adjacent mainland, there is a taxon that is morphologically constant and gives the impression of having arisen from the hybrid *R. aureolus* × *idaeus*. It has pink flowers and often pink styles (cf. Fig. 4). The terminal leaflets are triangular, with a cordate base, a broad tip and some tendency towards becoming

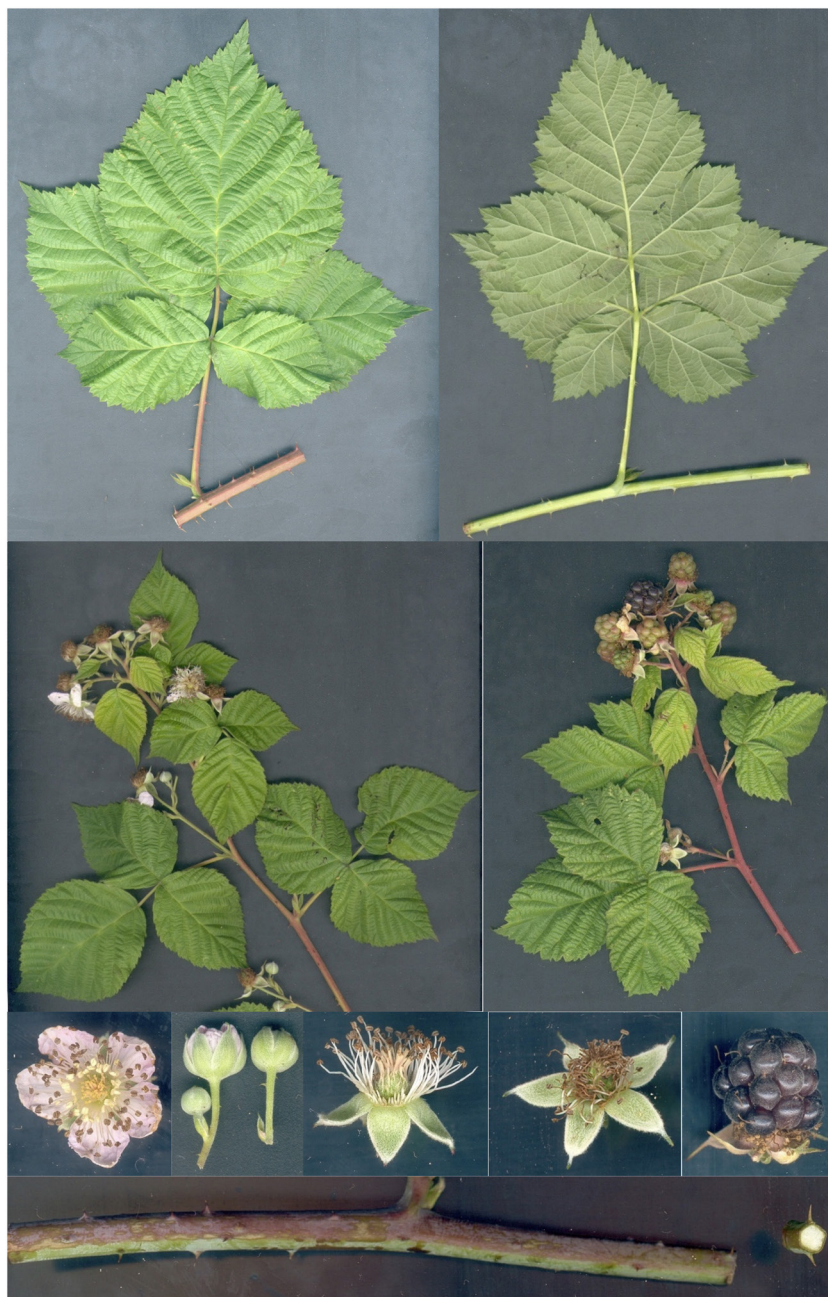


Figure 4. Pictures of *R. pruinus* s. str.: upper and lower side of leaves, inflorescence, flower bud, flower from above and dissected, ripe fruit and stem.

seven-foliolate. The leaf stalk is rather short (< 7 cm) and with few prickles. The ovaries and even the ripe fruits are densely hairy. The fruits ripen early and are large and well-developed. The prickles are small and not conspicuously red. The stems are prominently pruinose. It agrees well with Arrhenius' description and the type material. It is particularly significant, as Weber (1981) also noted, that Arrhenius does not mention any red prickles, which otherwise has been one of the hallmarks of *R. pruinus* s. lat. Therefore, we identify this taxon with Arrhenius' *R. pruinus* and we recognise it as *R. pruinus* s. str. in the following. It has $2n=35$, as expected for a species that has arisen from a hybrid between *R. idaeus* and a member of *R. sect. Corylifolii* (four samples tested).

In the microsatellite DNA analysis, patterns observed at locus 105b strongly support that *R. pruinus* s. str. has arisen from the hybrid *R. aureolus* $\times$ *idaeus*: All the 14 samples of *R. pruinus* s. str. have the same pattern of five alleles of the same length, as can be seen in Fig. 5. Four of the alleles are the same as the most common alleles for *R. aureolus*, whereas the fifth most likely originates from *R. idaeus*. However, three of the samples of *R. aureolus* have one of the alleles replaced by another, shorter allele. This does not correlate to any differences in morphology or to any differences in the other studied loci, so the difference does not have any apparent taxonomic value and might represent a somatic mutation in *R. aureolus* after its origin. Interestingly, one sample has a similar

pattern, together with another extra allele, from *R. idaeus*, found at a different position compared to *R. pruinus* s. str. Thus, this sample most likely represents a primary *R. aureolus* $\times$ *idaeus* hybrid, genetically unrelated to *R. pruinus* s. str.

Loci 2A8 and 275a give similar results, although there is a larger variation (up to 6 bp) in the length of especially the longer alleles, including the alleles from *R. idaeus*. The results for locus 117b also support the conclusion, although the extra allele from *R. idaeus* in *R. pruinus* s. str. has the same length as an allele from *R. aureolus*, so that it is only observed by a change in the intensities of the corresponding PCR fragments. All three loci, also support the interpretation of the putative *R. aureolus* $\times$ *idaeus* hybrid, showing an extra allele with lengths distinct from those found for *R. pruinus* s. str.

We have observed *R. pruinus* s. str. in a restricted area in the north-eastern part of the province of Småland and in the eastern part of Östergötland, up to Norrköping (yellow symbols in the map in Fig. 6). However, we have not performed any systematic inventory so it can most likely be found at more localities in this area. Indeed, studies of herbarium material at LD gave several additional putative localities (orange symbols in Fig. 6), including a sample from Trosa in Södermanland, further to the north. There is 155 km between the most distant localities (130 km excluding the one from Trosa). However, we have not found any *R. pruinus* s. str. among reports of *R. pruinus* s. lat. outside

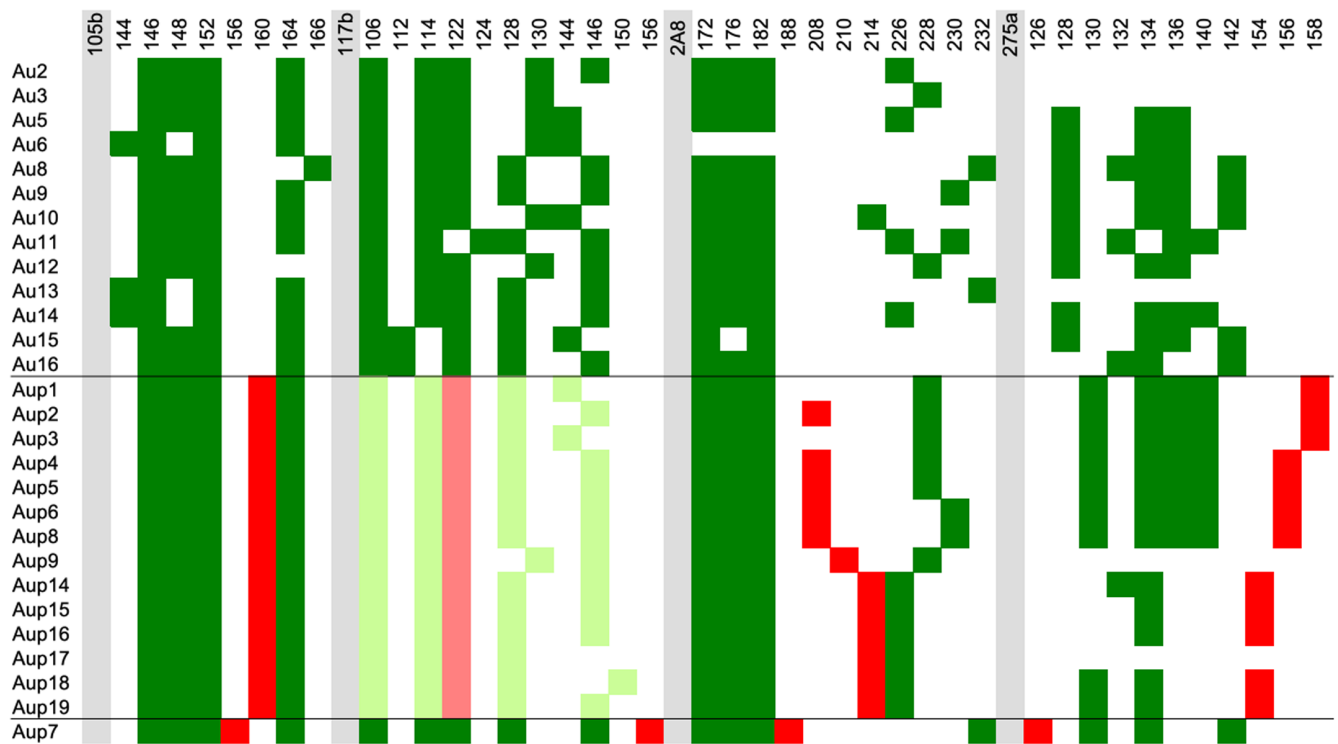


Figure 5. Microsatellite DNA results for 13 samples of *R. aureolus* (sample names starting with 'Au'), 14 samples of *R. pruinus* s. str. (Aup) and one sample of *R. aureolus* $\times$ *idaeus* (Aup7) at the four loci. The coloured areas reflect the presence of an allele of this length (in bp). Green alleles are common for most samples and probably come from *R. aureolus*, whereas the additional red allele most likely originates from *R. idaeus*. Light-green alleles for locus 117b have decreased intensity, whereas the light-red allele has increased intensity, because it is a combination of alleles both from *R. aureolus* and *R. idaeus*.

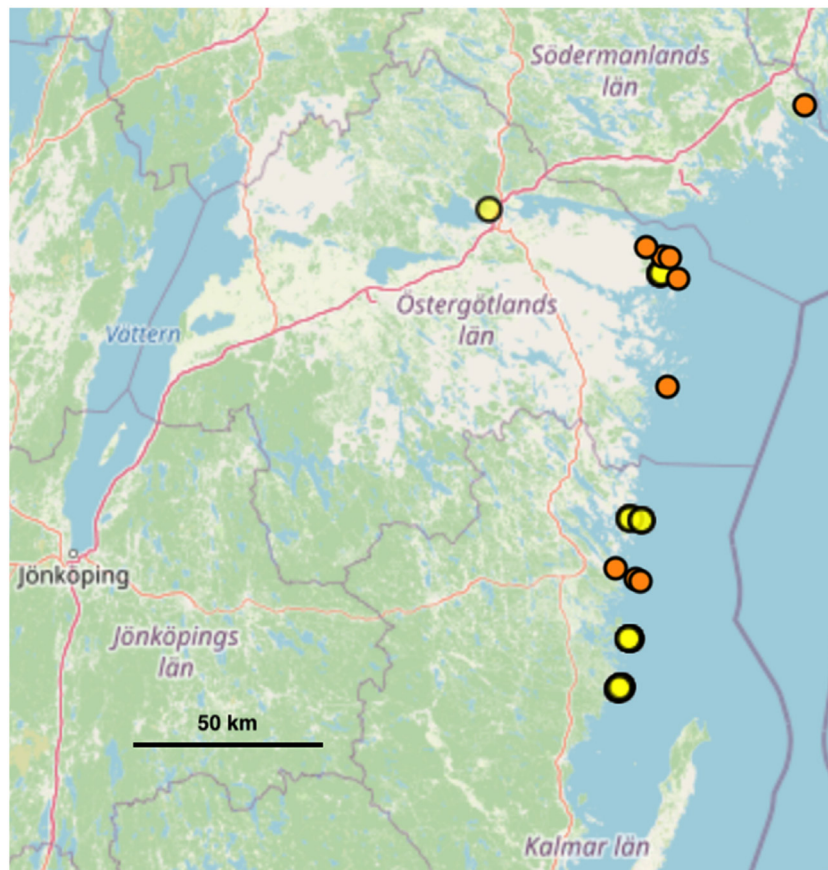


Figure 6. Known distribution of *R. pruinosus* s. str. based on visited localities (yellow symbols) and older localities identified from dried material in LD (orange symbols).

this area, although *R. aureolus* occurs up to northern Uppland and we have observed other putative *R. aureolus* $\times$ *idaeus* hybrids around Stockholm.

In conclusion, our results show that there is an asexually reproducing species in the north-eastern part of the province of Småland, eastern Östergötland and possibly in eastern Södermanland, which most likely represents Arrhenius' *R. pruinosus*. The microsatellite-DNA analysis shows that it has arisen from the combination *R. aureolus* $\times$ *idaeus*, but that it is clearly an independent apomictic species (it has a distribution of its own, it is uniform in morphology and it gives identical microsatellite-DNA analysis patterns). Therefore, the name *R. pruinosus* should be retained for this taxon and should not be used for other hybrids or apomictic species in other regions. One sample interpreted as the sexual primary hybrid *R. aureolus* $\times$ *idaeus* is also found in the same area as *R. pruinosus* s. str. Such hybrids have also been observed in other areas on the Swedish east coast, where *R. aureolus* is rather common.

Rubus pruinosus s. str. is quite easily separated from other hybrids between *R. idaeus* and members of *Corylifolii* by the pink flowers with pink styles, the triangular leaves, the pruinose stem and the large, hairy and early ripening fruits (Fig. 4). In the latter respect, it might be confused with the cultivar *R. 'Bedford giant'*, which is sometimes found as an

old garden escape (Oredsson 2005). However, *R. 'Bedford giant'* differs by other characters and has numerous prominently red, dense prickles on the stem and densely tomentose leaves on the lower side (and $2n=42$).

Rubus onsalaënsis

Rubus onsalaënsis is known from six localities in a restricted area in the northern part of the province of Halland in western Sweden (Ryde 2009, 2011). It is characterised by small white flowers, heart-shaped terminal leaflets, a sparsely hairy rachis and brightly red prickles on the stem (illustrated in Ryde 2009, 2011). The latter condition indicates that it has arisen from a hybrid with *R. idaeus*, but the *Corylifolii* parent has been unknown, because it is not similar to any species in the vicinity. *Rubus onsalaënsis* has $2n=35$, as expected for an apomictically reproducing species.

We examined three samples of *R. onsalaënsis* with microsatellite DNA analysis. They gave nearly identical microsatellite patterns, but they were not similar to any known species from Sweden. However, two samples of an unknown taxon that is found in six closely situated localities in Onsala, about 6 km from the localities of *R. onsalaënsis*, gave similar results. The two taxa shared the same alleles at loci 105b, 117b and 275a, but *R. onsalaënsis* had one extra allele at each locus.

For locus 2A8, the two taxa gave identical results with only three alleles.

Morphologically, the unknown taxon is quite similar to *R. onsalaënsis*, but it does not have the conspicuously red prickles, i.e. the *idaeus*-like character of *R. onsalaënsis*. Moreover, it had $2n=28$. All this strongly indicates that this taxon actually is the *Corylifolii* parent of *R. onsalaënsis*. This species has a very restricted distribution, but owing to its relation to *R. onsalaënsis*, which indicates that it might once have had a wider distribution (and therefore may be found in more localities in this area), we here describe this taxon with the name *Rubus antecedens*. In fact, the microsatellite DNA analysis indicates a putative *R. antecedens* $\times$ *caesius* from a locality 4 km west of *R. antecedens* and 4 km south of *R. onsalaënsis*. A wider comparison of the microsatellite data shows that both *R. antecedens* and *R. onsalaënsis* share many alleles with *R. arrhenii*, belonging to *R. sect. Rubus*, not known from Sweden, but occurring in Denmark.

Owing to its small distribution, *R. antecedens* does not merit recognition according to the Weberian species concept (Weber 1981). However, we and others have objected to this concept, as discussed in the Introduction (Hörandl 1998, Loos 2008, Ryde 2011, Haveman and Ronde 2013). In addition to the discussed problems with the Weberian species concept, *R. antecedens* illustrates yet another problem. If this species is not described, it becomes hard to describe and trace the origin of *R. onsalaënsis*. If the ancestor does not have any name, it is not possible to specify the *Corylifolii* parent and the present findings become meaningless. Thus, the Weberian species concept makes it harder to trace the phylogeny and evolution in *Subidaei* and in *Rubus* sect. *Rubus* in general.

Rubus lagerbergii

The fourth *Subidaei* species in Sweden is *R. lagerbergii*. It is the species within this subsection that shows the least prominent *idaeus* characters: It does not have any conspicuously red prickles, nor does it have seven-foliolate leaves. However, it has densely hairy ovaries and these hairs remain even on the ripe fruits. An evident question is whether the microsatellite DNA analysis may give any indication about the origin of this species, which is not obvious based on the morphology or on its distribution.

The microsatellite DNA analysis directly identified the *Corylifolii* parent of *R. lagerbergii* as a part of the species hitherto recognised as *R. dissimulans* Lindeb. (our microsatellite DNA analysis show that *R. dissimulans* s. lat. in Sweden should be divided into two species; *R. dissimulans* s. str. occurs only in restricted areas in the middle and northern parts of the province of Bohuslän, in the parishes of Bro, Tanum and Kville, whereas the segregate recognised here occurs along the coast of Skagerrak from northern Halland to southern Norway (Ryde et al. unpubl.)). At each of the four loci studied, *R. lagerbergii* and this undescribed taxon share the same four alleles (occasionally with some small variations for a few samples), but *R. lagerbergii* has one extra allele (at locus 2A8

sometimes two extra; cf. Fig. 7). The same pattern is shown by a standard clustering analysis which places the two species next to each other.

This is a quite attractive suggestion. The two species share several prominent characters (cf. Fig. 8). First, both species have numerous and dense prickles. As expected, the prickles are smaller and shorter on *R. lagerbergii*, reflecting the small prickles of *R. idaeus*. Second, both species have white flowers. Third, both species have elongated and narrow tips on their terminal leaflets. Fourth, both have a tendency towards forming 3–4-foliolate leaves. Fifth, both have rather many glands both on the stem and in the inflorescence. Sixth, both have narrow stipules (~1 mm). Finally, the leaves of the *R. dissimulans* segregate have only scattered hairs on the veins on the lower side of the leaves, whereas the leaves are somewhat more densely hairy on *R. lagerbergii* (as expected for an *idaeus* hybrid), but they are still not especially hairy.

Of course, there are also conspicuous differences. In particular, the leaves of *R. lagerbergii* are purely green with broad heart-shaped terminal leaflets, whereas those of the *R. dissimulans* segregate are yellowish green with much more narrow terminal leaflets. Moreover, the inflorescence of *R. lagerbergii* has only scattered hairs, whereas that of the other species is densely hairy.

The two species have distributions that are close to each other, but with a minimal overlap only in northern Halland (Fig. 8). *Rubus dissimulans* s. lat. is rare with scattered localities throughout its distribution, whereas *R. lagerbergii* is common in several areas.

Rubus rosanthus

Rubus rosanthus is a species known from the Swedish west coast in Västergötland and Bohuslän, as well as the south coast of Norway to Arendal (Weber 1981). It is characterised by small, straight, purple prickles, broadly heart-like terminal leaflets, pink flowers, hairy ovaries and fruits, and pinkish styles (but almost never seven-foliolate leaves). These characters indicate that it is related to *R. norvegicus* H.E. Weber & A. Pedersen, which is supported by the fact that the distribution of *R. rosanthus* mirrors that of *R. norvegicus*, as can be seen in Fig. 9.

The microsatellite DNA analysis shows that the material is genetically highly heterogeneous, cf. Fig. 10. For example, for locus 2A8, *R. rosanthus* shares four alleles with *R. norvegicus*, but it also has one additional allele from *R. idaeus*. However, the latter can have almost any size and only very few samples share alleles of the same length. The results for the other three loci are similar, but show somewhat less variation in the length of alleles from *R. idaeus* (five, six and four different alleles from *R. idaeus* are found at loci 105b, 117b and 275a, respectively). Still, there is no covariation of the samples among the four loci.

Moreover, *R. rosanthus* shows a rather extensive variation in morphology, e.g. regarding the number and size of the prickles on the stem, the amount of glands and hairs on the stem and in the inflorescence, the width of the stipules, the

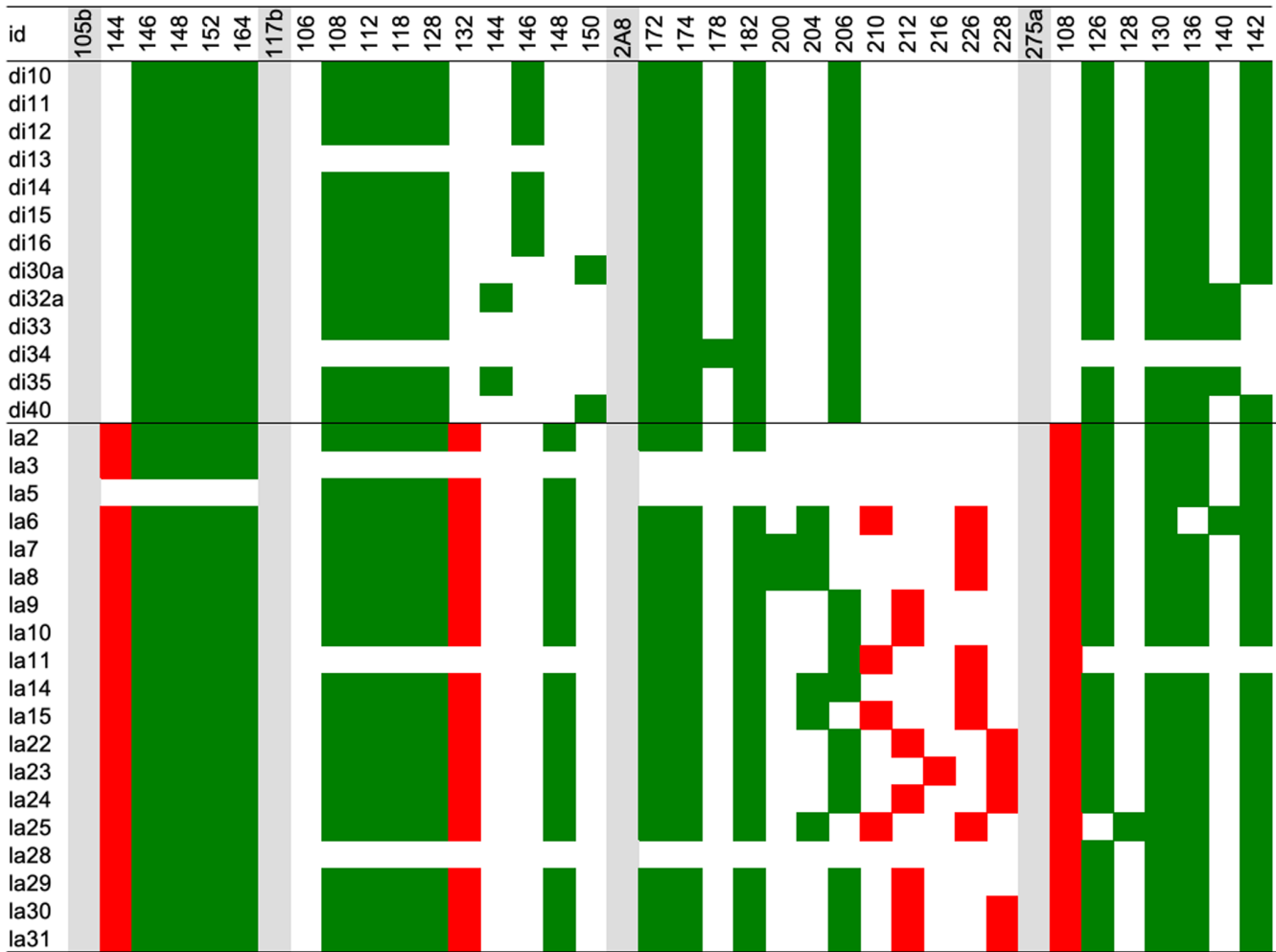


Figure 7. Microsatellite DNA results for 13 samples of *R. dissimulans* pro parte (di) and 19 samples of *R. lagerbergii* (sample names starting with 'la') at the four loci. The coloured areas reflect the presence of an allele of this length (in bp). Green alleles are common for all samples and come from *R. dissimulans*, whereas the additional red allele most likely originates from *R. idaeus*.

amount of hairs on the leaves, the colour of the petals (from light to dark pink), the amount and length of the hairs of the fruits, as well as how much the lower and middle leaflets are joined.

Therefore, we conclude that *R. rosanthus* is a collective name of a set of *R. norvegicus* $\times$ *idaeus* hybrids that have arisen independently many times. They do not seem to disperse by seeds (i.e. they have not inherited the capacity for asexual seed formation, in agreement with the conclusion by Gustafsson that primary *Rubus* hybrids are sexual (Gustafsson 1943)) and therefore do not form a distribution distinct from that of *R. norvegicus*. Consequently, the name *R. rosanthus* should be discarded and they should instead be called *R. norvegicus* $\times$ *idaeus*.

Other *R. idaeus* hybrids

The same seems to apply to all the other samples in this investigation, i.e. representing what has traditionally been called *R. pruinosis* in Sweden. They show an extensive variation in

morphology, distribution and microsatellite allele composition. In many cases, the *Corylifolii* parent is quite apparent, because it grows in the neighbourhood and is morphologically similar.

For example, *R. hallandicus* $\times$ *idaeus* is found at one locality in the province of Halland (Ryde 2009, 2011), where it grows together with the parents. It is characterised by very dense, red prickles, leaves that are almost glabrous underneath and that are often seven-foliolate. This was confirmed by the microsatellite DNA analysis, showing the same alleles as *R. hallandicus* (Gabr. ex Aresch.) Neuman, but with one extra allele or with a modified intensity pattern indicating a difference in allele copy number.

Likewise, *R. camptostachys* $\times$ *idaeus* (collected from four localities in Skåne) is characterised by hairy anthers (*R. camptostachys* G. Braun is the only *Corylifolii* species in Sweden with hairy anthers), combined with red prickles, seven-foliolate leaves and hairy ovaries. Again, this hybrid origin is supported by the microsatellite DNA analysis, showing the same alleles as in *R. camptostachys*, supplied with an extra allele

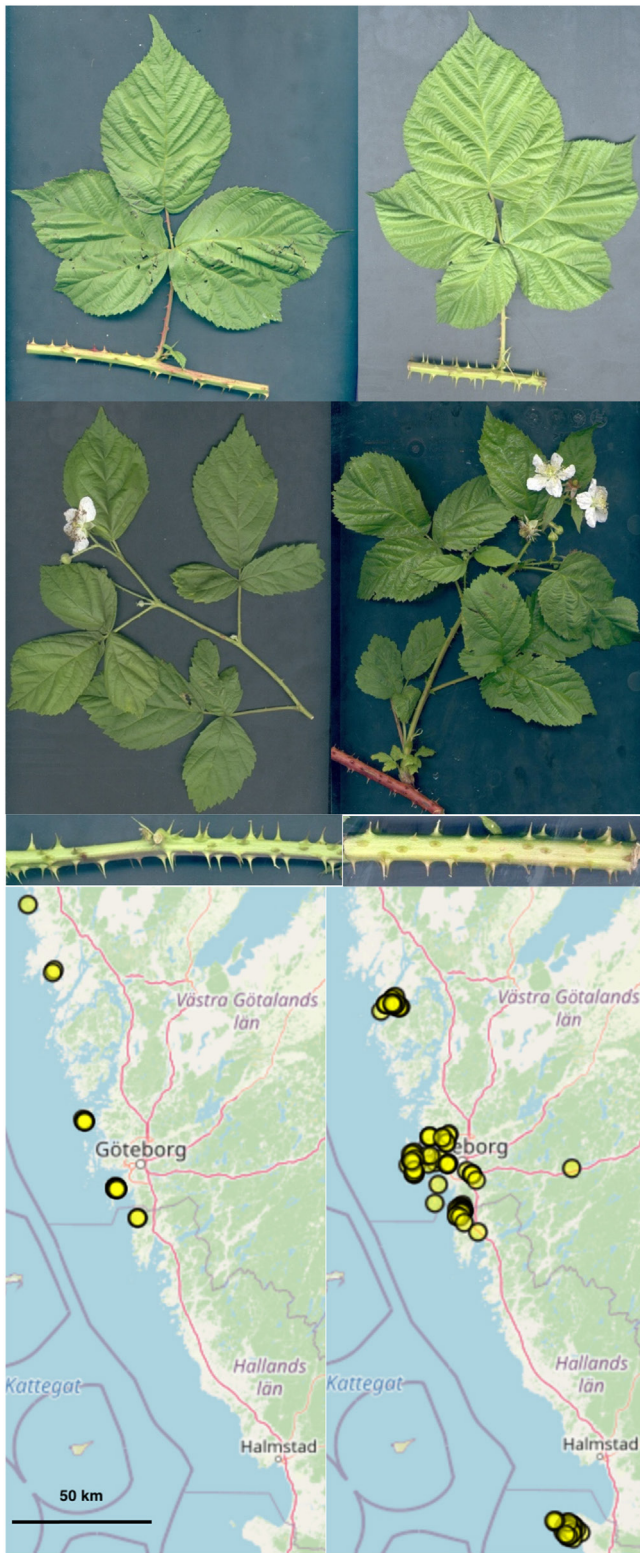


Figure 8. Comparison between *R. dissimulans* pro parte (left) and *R. lagerbergii* (right; stem leaf, upper side, inflorescence and stem), together with distribution maps in Sweden (reports 2000–2020 to Artportalen (Leidenberger et al. 2016)).

from *R. idaeus*, although not of the same lengths for the four samples (indicating independent origins).

Rubus lidforsii × *idaeus* has been confirmed for two samples from Löderup (which is the only place in Skåne where *R. lidforsii* (Gelert) Lange is abundant). They were sampled at a distance of 500 m from each other, but they are clearly genetically distinct. Two samples from Arrie in Skåne probably also belong to this hybrid. They were sampled from two localities at a distance of 2 km, but their microsatellite patterns are nearly identical. Two samples from Rörum in Skåne seem to be the hybrid *R. decurrentispinus* H.E. Weber × *idaeus*. In fact, the lectotype of *R. pruinus* v. *silvaticus* C. E. Gust. (in LD) probably represents the latter hybrid.

The most common *Corylifolii* × *idaeus* hybrid in Sweden is *R. gothicus* × *idaeus*, reflecting that *R. gothicus* (Frid. & Gelert) ex E.H.L.Krause is one of the most common *Corylifolii* species in most parts of southern Sweden. This hybrid is often easy to recognize by small, narrow leaves that are shiny above and sparsely hairy below. We have collected 31 samples of this hybrid from six provinces. They were confirmed by the microsatellite DNA analysis, but they do not share the same alleles at all four loci, illustrating that they have arisen independently. The only exception is two samples from two different provinces, which are not morphologically similar. Therefore, their agreeing microsatellite allele composition is most likely coincidental. The microsatellite DNA analysis also clearly shows that what has previously been interpreted as *R. mortensenii* × *idaeus* from Tranås in Skåne (because it grows together with *R. mortensenii* (Frid. & Gelert) ex E.H.L.Krause), actually is *R. gothicus* × *idaeus*.

The investigation also included 15 samples of putative *R. pruinus* from Sleswig–Holstein in Germany. The microsatellite DNA analysis clearly shows that none of the samples represents *R. pruinus* s. str. One of them is undoubtedly *R. gothicus* × *idaeus*. Furthermore, our results show that only two of the samples share the same alleles at all four loci. These two samples were collected at nearby localities, so they may represent primary *R. idaeus* hybrids that are spreading vegetatively. Our results also indicate that five samples probably have a common *Corylifolii* ancestor and another six samples have another *Corylifolii* parent (but neither of the two groups is genetically uniform, i.e. they represent primary hybrids). The other three samples seem to have three different parents. Unfortunately, it is not possible to identify any of the *Corylifolii* parents based on the microsatellite DNA analysis, indicating that none of the parents are found among the Swedish *Corylifolii*. Furthermore, our results do not support the presence of any uniform and apomictically reproducing *R. idaeus* hybrid in this area and show that what has previously been included in *R. pruinus* does not form any genetically coherent taxon.

Finally, there are two samples from Sweden (from Väsby in Skåne and Kalmar in Småland) that morphologically show characters of a *R. idaeus* hybrid, but for which the microsatellite DNA analysis could not establish any likely parent among the investigated Swedish (and a few additional Danish, German and Norwegian) *Corylifolii* and sect. *Rubus*

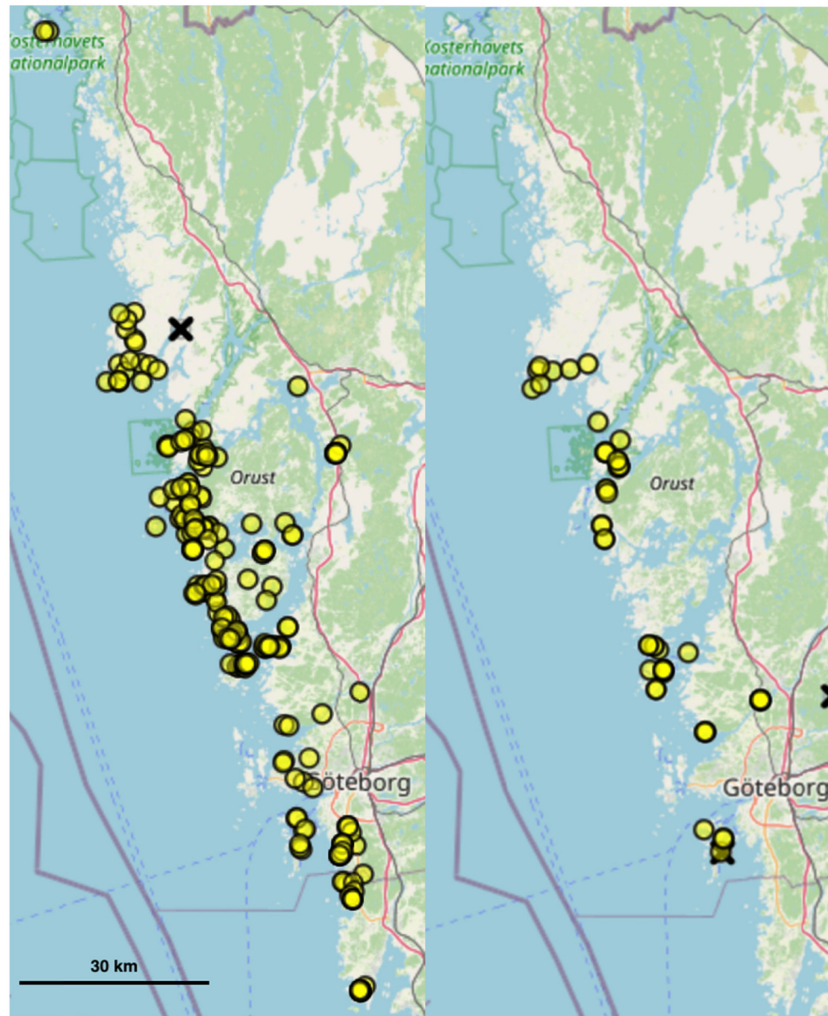


Figure 9. Distributions of *R. norvegicus* (left) and '*R. rosanthus*' = *R. norvegicus* × *idaeus* (right) in Sweden, according to Artportalen (reports 2000–2020) (Leidenberger et al. 2016).

species (about 100 in total). It is possible that the two samples have arisen from *R. idaeus* hybrids with species that are no longer present in Sweden. Alternatively, they may represent *R. caesius* hybrids that have got some morphological characters of *R. idaeus* hybrids, as was reported by Lidforss (1905). In our investigations, *R. caesius* hybrids are much harder to identify by microsatellite DNA analysis than *R. idaeus* hybrids (results to be published). However, both samples gave $2n=35$ by flow cytometry, indicating that the samples represent *R. idaeus* hybrids. Still another possibility is that they have arisen from crossings between a *Corylifolii* species and the *R. caesius* × *idaeus* hybrid, as was suggested by Kanér (1953). To obtain the observed chromosome number of $2n=35$, they should then have arisen by fusion of an unreduced egg cell with $2n=21$ from the primary hybrid with a reduced sperm cell with $n=14$ in pollen from the *Corylifolii* parent. Such a pattern would be rather hard to verify in the microsatellite analysis, as the allelic variation in *R. caesius* is quite large. However, besides this possibility, we find no support in our analysis to Kanér's suggestion that *R. pruinus*

s. lat. has arisen from crossings between *Corylifolii* species and *R. caesius* × *idaeus*. It is also very rarely observed that *R. pruinus* s. lat. is found together with *R. caesius* × *idaeus* (although the latter is rather common in some areas of southern Sweden), whereas it is often found together with both *R. idaeus* and the putative *Corylifolii* parents.

We have observed putative *R. idaeus* hybrids of a number of additional species, including *R. friesianus* H.Hyl. ex T.Mattsson & Oredsson and *R. glauciformis* (C.E.Gustafsson) H.Hyl. A few samples in nature were interpreted as *R. wahlbergii* × *idaeus*, which is interesting since such hybrids would be expected to have $2n=42$, because *R. wahlbergii* itself is pentaploid. However, the three investigated samples were found to be *R. gothicus* × *idaeus* and to have $2n=35$. Accordingly, we have so far not confirmed any hybrid between any pentaploid *Corylifolii* species and *R. idaeus*.

Hybrids between *R. idaeus* and members of *R. sect. Rubus* are much less common, but at least *R. plicatus* × *idaeus*, *R. insularis* F.Aesch. × *idaeus* and *R. radula* Weihe × *idaeus* have been reported (Krause 1891, Lidforss 1899, Ryde 2009). In

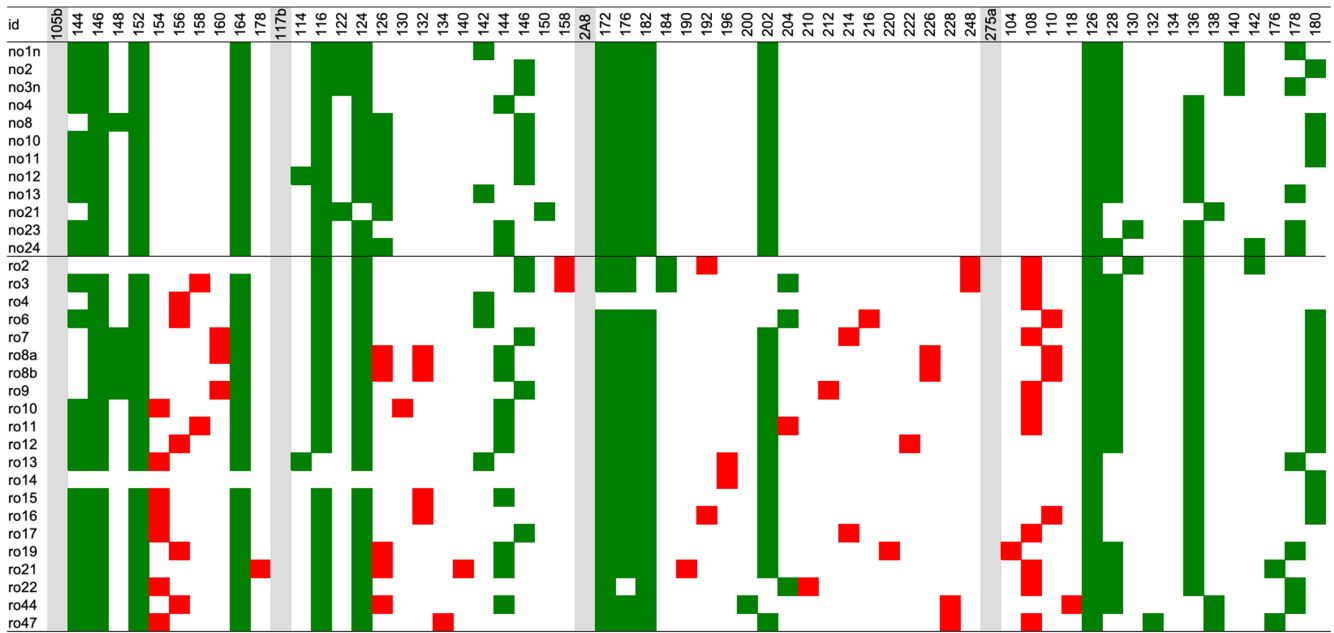


Figure 10. Microsatellite DNA results for 12 samples of *R. norvegicus* (upper part with sample names starting with 'no') and 21 samples of *R. 'rosanthus'* (ro) = *R. norvegicus* × *R. idaeus* at the four loci. The coloured areas reflect the presence of an allele of this length (in bp). The green alleles are common for both species, whereas the red ones are extra for *R. 'rosanthus'*, most likely originating from *R. idaeus*.

fact, we interpret the lectotype of *R. pruinosis* v. *norvegicus* C.E. Gustafsson (in LD) as *R. plicatus* × *R. idaeus*.

It should also be noted that several species of *R. subsect. Rubus* (formerly *Suberecti* e.g. *R. nessensis* Hall, *scissus* W.C.R. Watson and *scissoides* H.E. Weber) show features of *R. idaeus*, viz. conspicuously red prickles and seven-foliolate leaves. It has been shown that *R. idaeus* has been involved in the development of these species (Sochor et al. 2015). In fact, *R. nessensis* has sometimes been considered as *R. plicatus* × *R. idaeus*, although Areschoug argued against this hypothesis (Areschoug 1872, 1885). Our microsatellite analysis does not give any indication that *R. nessensis*, *scissus* or *scissoides* should have arisen from hybridisation of any of the investigated Swedish *Rubus* species and *R. idaeus*.

Finally, it should also be noted that standard clustering analysis works poorly for the *R. idaeus* hybrids: In such analyses, the hybrids often cluster together with their parent species, because they share the same alleles (in addition to an extra allele from *R. idaeus*) and small variations among the pure species result in a pattern where they appear close to the hybrids. Special similarity measures need to be developed to discern the hybrids from their parents, based on the occurrence of extra alleles and also the relative intensities of the corresponding PCR fragments, if such analysis should be successful for the hybrids.

Subidaei, a section or a subsection

As mentioned in the introduction, it has recently been suggested that the *Subidaei* should be treated as a section of its own, separate from *R. sect. Corylifolii* (Van De Beek 2014).

The argument was that *R. sect. Corylifolii* represents species that have arisen from hybrids between *R. caesius* and species from *R. sect. Rubus*, whereas *Subidaei* represents hybrids with *R. idaeus* from *R. subgen. Ideobatus*. However, our microsatellite results clearly show that at least all the Swedish species of *Subidaei* have arisen as hybrids of species of *R. sect. Corylifolii* with *R. idaeus*. Therefore, they have a common origin within *R. sect. Corylifolii* and share 4/5 of their genome with members of this section. Consequently, it is hard to argue that the *Subidaei* should be treated as a sister group to *R. sect. Corylifolii*. They also share most of their characters with *R. sect. Corylifolii* and cluster with them in a cluster analysis of the blackberries, much closer to their *Corylifolii* parent species than to each other or to *R. idaeus*. Thus, our results support Weber's view that the *Subidaei* are best regarded as a subsection within *R. sect. Corylifolii*.

Conclusions

We have studied the relationship between Swedish species of *Rubus* sect. *Corylifolii* subsect. *Subidaei* and primary hybrids between *R. idaeus* and various *Corylifolii* species using microsatellite DNA analysis and flow cytometry. We argue that uniform, separately evolving apomictic species are characterised by:

- a uniform morphology
- a distinct distribution, which is separate from the *Corylifolii* parent
- a (nearly) identical microsatellite DNA pattern.

With these criteria, we show that four *Subidaei* species are apomictic and that they all have arisen from *R. idaeus* hybrids:

- *R. pruinus* s. str. has arisen from *R. aureolus* × *idaeus*
- *R. lagerbergii* has arisen from *R. dissimulans* p.p. × *idaeus*
- *R. cordatiformis* has arisen from *R. eluxatus* × *idaeus*
- *R. onsalaënsis* has arisen from *R. antecedens* × *idaeus*

On the other hand, *R. rosanthus*, which has arisen from *R. norvegicus* × *idaeus*, should not be accepted as a proper species, because it does not fulfil any of the three criteria. Instead, it is apparently a collection of primary *R. norvegicus* × *idaeus* hybrids.

Likewise, most of what has been called *R. pruinus* in Sweden is a diverse group of *R. sect. Corylifolii* × *idaeus* hybrids that are not genetically related. We strongly argue

that they should be reported as primary hybrids and the name *R. pruinus* should be restricted to *R. pruinus* s. str. of Arrhenius, which has a limited distribution in eastern Sweden. Primary *R. idaeus* hybrids can probably arise from all *Corylifolii* species, but we have so far not found any such hybrids with any of the pentaploid species.

It has been argued that without the Weberian species concept, the number of *Rubus* species would become prohibitively large (tens of thousands) (Kurtto et al. 2010). The present study suggests another solution. In Sweden, there is also a large number of local biotypes. However, the great majority of these are easily recognised as hybrid products between *Corylifolii* and either *R. idaeus* or *R. caesius* (the latter will be dealt with in a forthcoming article). If these are treated as hybrids, as suggested in this article, only a handful of



Figure 11. Pictures of *Rubus antecedens* sp. nov. Upper and lower side of leaves, inflorescence, flower bud, flower from above and dissected, young fruit and stem.

unknown taxa remain in Sweden. We argue that these should be described, regardless of their distribution. It remains to be shown if the same approach works in other countries or if additional restrictions are still needed. However, it is clear that for Sweden, the Weberian species concept is no longer needed and will only give rise to practical problems for the field botanist and in conservation (Hörandl 1998, Loos 2008, Ryde 2011, Haveman and Ronde 2013).

***Rubus antecedens* Ryde sp. nov.**

Type: Sweden, Halland, Onsala: the cross between Stora Enens väg and Tranas väg, RT90 6370770 1272789 (WGS84 57.405561, 12.024136), leg. U. Ryde. 20 Jun 2020 (holotype: LD, isotypes: GB, S, UPS).

Stem procumbent, slightly sulcate, 5–6 mm in diameter, light red, glabrous and with occasional stipitate glands. Internodes 5–15 cm. Prickles recurved to slightly bent, with the same colour as the stem, 5–15 per 5 cm, 4.5–6.0 mm, with a base of 4–5 mm. Stipules 1.5–2.5 mm wide, typically with conspicuously red veins. Leaves 14–19 cm long, with 5 leaflets, slightly darkish green. Petiole 5–7 cm (somewhat longer than the basal leaflets), with 3–7 prickles, 2–3 mm long with a curved tip. Terminal leaflets cordate, 7–9 × 6–9 cm, plicate, widest around the middle, with occasional hairs on the upper side and sparsely hairy beneath, with the hairs perpendicular to the veins. Petiolule of terminal leaflet 2.5–3.5 cm, with 0–5 prickles. Leaf margin typically biserrate and periodic with teeth of unequal size, 1–3 mm. Tip of the terminal leaflet elongated, ~1.5 cm. Lateral leaflets 6–9 cm long with a petiolule of 11–15 mm. Basal leaflets 4–7 cm with a petiolule of 2–5 mm. Inflorescence typically short, with a slightly hairy rachis and quite many stalked glands in the upper part. Prickles in the inflorescence recurved and 2–5 mm long, 1–3 per 5 cm. Pedicels 10–15 mm with 1–4 prickles of ~1.4 mm, almost straight and erect. Upper leaves mostly three-lobed, rather finely serrate and grey beneath owing to rather dense short hairs. Flowers fairly small, ~2 cm. Petals purely white, also in bud. Filaments and styles white. Sepals green, tomentose, with white borders, rather many stipitate glands and no prickles, with short tips and patent in fruit. Hypanthium with no or occasional hairs. Carpels glabrous. Stamens exceed the styles in length; anthers glabrous. Fruits usually aborted. The species is illustrated in Fig. 11 and Supporting information.

The scientific epithet is selected to point out that this species was identified as the ancestor of *R. onsalaënsis*. So far, it is known only from six very nearby (within 250 m, but well separated) localities in a residential area in Onsala, Halland, in total covering ~170 m². It grows in an abandoned pastureland, a green area, a parking lot, roadsides and in the outskirts of a garden. The DNA-ploidy level is tetraploid ($2n=28$).

Acknowledgements – We thank Werner Jansen for collecting samples of *R. idaeus* and putative *R. pruinus* from Sleswig–Holstein and Per

Wahlén for notifying us about the first locality of *R. antecedens*. We also thank Gerard Geenen and Krit Raemakers at Plant Cytometry Services for excellent service.

Funding – We thank the Botanical Societies in Lund and Göteborg, as well as Magnus Bergvalls stiftelse for economic support.

Author contributions

Ulf Ryde: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Project administration (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Melanie S. Montes:** Investigation (supporting); Writing – review and editing (supporting). **Mingyue Zhou:** Investigation (lead); Writing – review and editing (supporting). **Tore Mattsson:** Formal analysis (equal); Writing – review and editing (equal). **Tomas Burén:** Formal analysis (equal); Investigation (supporting); Writing – review and editing (supporting). **Mikael Hedrén:** Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Resources (equal); Software (equal); Supervision (equal); Validation (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.3j9kd51j3>> (Ryde et al. 2021).

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