NORDIC JOURNAL OF
BOTANY

Research article

Rubus wahlbergii and related species in Sweden

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Nordic Journal of Botany
2024: e04373
doi: 10.1111/njb.04373
Subject Editor: Torbjörn Tyler
Editor-in-Chief: Torbjörn Tyler
Accepted 22 May 2024
Published 25 June 2024

We studied *Rubus wahlbergii* and related species in *R.* sect. *Corylifolii*, primarily in Sweden, using DNA microsatellites. The results show that the material can be divided into three species: *R. wahlbergii* s.s. which occurs along the Swedish coast from Bohuslän to Uppland, *R. nordicus* which occurs on the western coast all the way south to north-western Skåne, and a previously unrecognized taxon which we here describe as *R. wahlbergioides*. The latter has scattered localities in Halland, Skåne, Blekinge, Öland and Småland, but is common around Kivik in Skåne and Kalmar in Småland. It is characterised by leaves that are without felt underneath, terminal leaflets with a somewhat more triangular shape and coarser serration than those of *R. wahlbergii* and also has more glands on the stem and the inflorescence. In the northern part of the distribution, *R. nordicus* is easily distinguished morphologically from *R. wahlbergii*, e.g. by a strong tendency to form 7-foliolate leaves, but in Skåne and Halland, the two species are harder to distinguish. Still, genetically *R. nordicus* is more similar to *R. sordirosanthus* than to *R. wahlbergii* s.s.

Keywords: apomixis, *R. nordicus*, *R. wahlbergii*, *R. wahlbergioides*, *Rubus*

Introduction

Rubus sect. *Corylifolii* Lindl. is a group of blackberries that most likely have arisen from crosses between dewberry (*R. caesius*) and various species of *Rubus* sect. *Rubus* (and sometimes also species from other sections) (Weber 1981, Pedersen and Schou 1989). Species of this section are characterised by creeping stems, broad stipules, overlapping leaflets with short petiolules, as well as overlapping and (at least in Scandinavia) somewhat crispate petals. Like most blackberries and dewberry, they are predominantly apomictic.

One of the most common species of this group in many parts of southern Sweden is *R. wahlbergii* Arrh. It is typically a vigorous plant with broad heart-shaped leaves that are prominently grey tomentose beneath, thick and five-foliolate, and with a rather even serration. The stem has quite strong prickles (for a *Corylifolii*), which

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are straight to slightly bent with a broad base, but few or no glands or hairs. The flowers are pink and relatively small, and the fruits are well-developed. The inflorescence has many flowers and broad, curved, yellowish prickles with prominent purple base. Outside Sweden, it occurs in Denmark, Germany, southern Norway, Poland, Czech Republic and North Macedonia (Weber 1981, Hegi and Weber 1995, Mirek 1995, Kurtto et al. 2010, Teofilovski 2011).

Most species of *R.* sect. *Corylifolii* are tetraploids, but *R. wahlbergii* is a pentaploid (Gustafsson 1933). This ploidy level is mainly found in hybrids between raspberry *R. idaeus* and species of *R.* sect. *Corylifolii*. However, *R. wahlbergii* shows no sign of such an origin, which has also been confirmed by genetic studies (Ryde et al. 2021). Instead, Lidforss (1905, 1907), based on artificial hybrids, suggested that *R. wahlbergii* may have arisen from the hybrid between *R. grabowskii* Weihe ex Günther et al. (*R.* sect. *Rubus*) and *R. caesius*. This is consistent with the chromosome number: *R. caesius* is a tetraploid and provides two sets of chromosomes in reduced pollen, whereas *R. grabowskii* is a triploid, and can contribute all three sets in an unreduced egg cell. *Rubus grabowskii* may also have contributed the pink flowers and the tomentose underside of the leaves.

The only other pentaploid Swedish species of *R.* sect. *Corylifolii* that does not seem to have arisen from a *R. idaeus* hybrid is *R. vikensis* A. Pedersen ex. G. Wendt (Ryde et al. 2021). Before its formal description as a species in 2008, it was often considered as a infraspecific taxon of *R. wahlbergii* or *R. tiliaster* H. E. Weber (Wendt 2008). However, genetic studies confirmed that it is distinct from these two species (Ryde 2010, Lewin et al. 2022) and should be treated as a species of its own.

Rubus wahlbergii is known to be notoriously variable, with distinct geographically restricted morphotypes (Hylander 1958b, Weber 1981, Pedersen and Schou 1989). This problem has been reduced by the recognition of five morphologically related species, viz. *R. glauciformis*, *R. sordirosanthus*, *R. friesianus*, *R. nordicus* and *R. wahlbergii* s.s. *Rubus glauciformis* Gustaf. Ex H. Hyl., which was originally suggested to represent the hybrid *R. wahlbergii* × *caesius* (Gustafsson 1938). However, Hylander argued that it is a separate species (Holmgren 1942, Hylander 1958b) and this has been confirmed by later field work (Burén 2009) and molecular studies (Lewin et al. 2022). It is a tetraploid (Gustafsson 1933, Lewin et al. 2022).

Rubus sordirosanthus H. Hyl. ex Ryde (Hylander 1958a, Ryde and Werlemark 2010) has a restricted distribution in central Halland and is also a tetraploid. The leaves are less tomentose on the lower side and are typically more elongated than those of *R. wahlbergii* s.s. The styles are normally weakly pink, especially at the base. Genetic studies have shown that it is distinct from *R. wahlbergii* (Ryde and Werlemark 2010).

Rubus friesianus H. Hyl. ex T. Mattsson and Oredsson has a restricted distribution in southern Bohuslän (Hylander 1958a, Mattsson and Oredsson 2010). It differs from *R. wahlbergii* by having smaller, but more numerous and falcate prickles, leaves that are green beneath and flowers that are paler. It is a tetraploid (Mattsson and Oredsson 2010).

The fourth species was first described as *R. trominsularis* E. H. L.Krause (Krause 1891), but Gustafsson preferred to treat it as a variety, first as *R. wahlbergii* var. *trominsularis* (Gustafsson 1936) and later as *R. wahlbergii* var. *partitus* Gust. (Gustafsson 1938). However, Weber pointed out that *R. trominsularis* is a synonym to *R. pruinosus* (i.e. a *R. idaeus* hybrid (Ryde et al. 2021)) and that the description of *R. wahlbergii* var. *partitus* is improper. Therefore, he instead named it *R. wahlbergii* subsp. *nordicus* H. E. Weber and A. Pedersen (Weber 1998) and later gave it species rank (Weber 2008). *Rubus nordicus* (H. E. Weber and A. Pedersen) H. E. Weber has a strong tendency to have seven-foliolate leaves or at least lobed terminal leaflets, a more coarse and uneven serration, a less dense indumentum on the lower side of the leaves compared to *R. wahlbergii* s.s., and a broad and divaricate inflorescence. The species almost fully replaces *R. wahlbergii* in Norway from Vest-Agder to Østfold and in the northern part of the Swedish province of Bohuslän, but it becomes less characteristic and hard to distinguish from *R. wahlbergii* further to the south.

Moreover, during field inventories in the southern and eastern provinces of Sweden, we observed another characteristic taxon (Ryde 2009). It has terminal leaflets that are green underneath without any felt. The terminal leaflets also have a more triangular shape with a somewhat coarser serration. It has more numerous stalked glands. It does not seem to have been noticed before.

Apparently, important taxonomical problems still remain within this group. In this study, we use molecular methods to study *R. wahlbergii* and related taxa in Sweden and adjacent countries. We show that genetically there are three distinct and widespread taxa and discuss how they can be distinguished based on their morphology.

Methods

About 560 samples (young leaves from the tip of the annual shoot) of *R. wahlbergii* and several related *Rubus* species were collected, mainly from Sweden, but also from Denmark, Norway and Germany. The localities are specified in the Supporting information. Vouchers are deposited in LD. The samples were stored in a freezer at –80°C until DNA was extracted.

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 ddH_2O to ~14 ng μL^{-1}. Four pairs of microsatellite primers, described in the Supporting information, were used to analyse variable microsatellite loci. The three loci Ru105b, Ru117b and Ru275a were described in *R. idaeus* (Graham et al. 2004), whereas the locus mRaCIRRIV2A8 was described in *R. alceifolius* Poir. (Ansellem et al. 2001). They will here be referred to as 105b, 117b, 275a and 2A8, respectively. They have been successfully used also for other *Rubus* species (Stafne et al. 2005, Zhou 2014, Hedrén et al. 2020, Ryde et al. 2021, Lewin et al. 2022). One primer in each pair was tagged with a fluorescing molecule (Cy5-) to enable the identification of the polymerase chain reaction (PCR) products in an automated sequencer (ALF Express II).

The PCR amplification consisted of 35 cycles of denaturation at 94 °C for 30 s, annealing at varying temperatures (Supporting information) for 30 s and polymerisation at 72 °C for 1 min (10 min in the last cycle). Each PCR was performed in a solution consisting of (in a total volume of ca 6.4 µL): 4.4 µL ddH_2O, 0.66 µL 10× reaction buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl and 15 mM $MgCl_2$), 0.56 µL dNTPs (2.5 mM for each nucleotide), 0.25 µL Cy5-tagged primer (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 appropriate size markers and were then heated to 94 °C for 25 min before they were separated by electrophoresis in a polyacrylamide gel. The DNA fragments were detected and analysed with the help of an ALF Express II analysis system. Samples that resulted in poor PCR reactions were purified with a Qiagen purification kit, after which the PCR and electrophoresis were redone. Samples that still resulted in poor amplification (unspecific amplification, dominance of short fragments etc.) were excluded.

The relative height of the amplified fragments on the resulting electropherogram was recorded on a five-graded scale for each sample to obtain an estimate of band intensity. Differences in relative band intensity between plants with overlapping banding patterns were matched with information on ploidy levels and interpreted as possibly due to variation in allele copy numbers. Similarly, differences in relative band intensity between plants of the same ploidy expressing different numbers of bands were also interpreted as due to variation in allele copy numbers. Several samples were re-amplified by PCR and rerun to verify the patterns of relative band intensity. Such analyses gave rise to closely similar differences in band intensity as the original runs, with only minor variation in peak height (typically, at most by a single unit along the five-graded scale used by us).

DNA-ploidy levels were estimated by flow cytometry for 22 samples. This analysis was performed by Plant Cytometry Services (http://www.plantcytometry.nl). Fresh leaf material was homogenised and mixed with an 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 an 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.

The search for putative genetic relationships based on the microsatellite results was facilitated by the use of computer programs taking into consideration both the length of the various microsatellite alleles and the relative intensity of the PCR products (Lewin et al. 2022). Principal-coordinate (PCO) analyses were performed with the NTSYSpc ver. 2.20 software (Rohlf 1994), using a pair-wise comparison of Jaccard coefficients (Jaccard 1908) based on the occurrence of alleles for all four loci.

Morphological characters follow descriptions in the relevant literature (Oredsson 1969, Weber 1981, Pedersen and Schou 1989). Characters of leaves and the stem refer to the middle part of the primocane. Morphological characters were primarily studied during the flowering period. Pictures of the various species were obtained with an Agfa Snapscan e50 digital flatbed scanner on fresh material at resolutions of 150–1200 dpi.

Result and Discussion

Microsatellite DNA analysis results: three distinct species

We analysed ~ 560 samples of *R. wahlbergii* and related species with microsatellite DNA analysis at four different loci. In

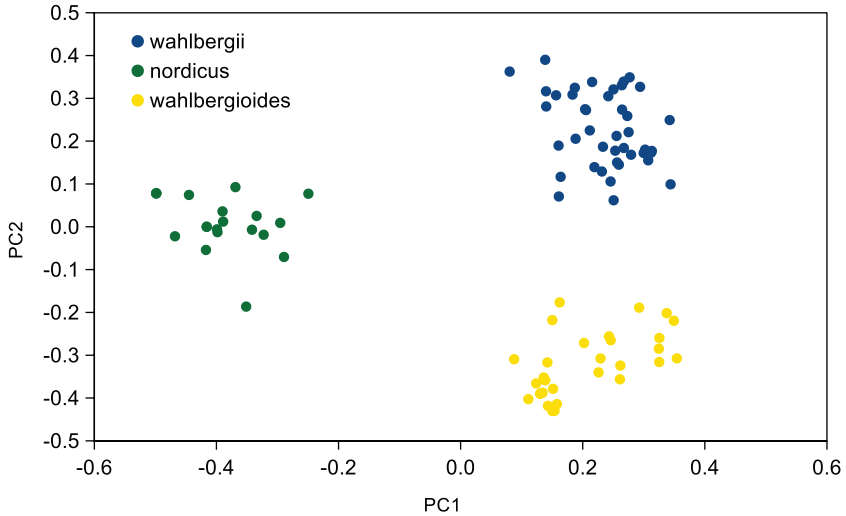

Figure 1. Two-dimensional representation of the PCO results based on the microsatellite DNA analysis, showing the three species within the *R. wahlbergii s.l.* complex. The two components explain 41% of the variation.

Fig. 1, the results of *R. wahlbergii*, *R. nordicus* and the third taxon mentioned at the end of the Introduction is presented in a PCO plot (in the following, we will refer to these three taxa collectively as *R. wahlbergii* s.l.). It can be seen that the material falls into three distinct groups.

The first group (blue in Fig. 1) comprises 23 samples from seven Swedish provinces, along the coast from Bohuslän, Västergötland, Halland, Skåne and Blekinge to Småland and Öland (i.e. from all provinces where we have collected samples; cf. Fig. 2a). These samples represent mainly material that has been interpreted as *R. wahlbergii* s.s. based on morphological characters (cf. Fig. 3), e.g. with a thick white felt on the lower side of the leaves and few glands on the stem and in the inflorescence. The lectotype of *R. wahlbergii*

Figure 2. Distribution of the tested localities of a) *R. wahlbergii*, b) *R. nordicus* and c) *R. wahlbergioides* in southern Sweden (in the latter case also with localities identified from field studies).

is from southeast Sweden (Arrhenius without date in UPS) and has all these characters. We therefore identify this group as *R. wahlbergii* sensu stricto.

The second group (green in Fig. 1) comprises 44 samples collected from the Swedish west coast, in the provinces of Bohuslän, Västergötland, Halland and north-western Skåne (Fig. 2b). The southernmost locality is from Helsingborg and the other four samples from Skåne are from Brunnby parish. Some representative pictures are shown in Fig. 4. It includes all specimens judged on morphological grounds as typical *R. nordicus* from Bohuslän, with seven-foliolate leaves that are less felty on the lower side like the holotype from Aust Agder in Norway (Pedersen 1990, in C). We therefore identify this group as *R. nordicus*. However, many samples that were originally judged as *R. wahlbergii* s.s. based on the morphology also belong to this group. Some examples are shown in the lower part of Fig. 4. Furthermore, some samples that morphologically were judged to be intermediates between *R. wahlbergii* s.s. and *R. nordicus*, with a tendency towards 7-foliolate leaves belong to the first group. Some examples are shown in the lower part of Fig. 3.

Consequently, somewhat modified characters are needed to discern the two species. Table 1 collects useful differences. It should be noted, however, that no character always applies, so that several need to be examined. Still, the two species can often be distinguished in nature already at a distance by the general appearance: *R. nordicus* is a vigorous plant with a general somewhat disorganised appearance, a greyish-green tint, larger leaves that are bullate and somewhat elongated with a rather coarse serrature and less felt on the lower surface and more brownish turions, often hairy towards the tip, whereas *R. wahlbergii s.s.* is somewhat smaller, with a very orderly appearance, a pure green colour, smaller leaves that are flat, smooth and heart-shaped with a regular serrature and thick felt on the lower side, as well as sulcate and purple turions, typically with no hairs. In the northern part of its distribution (northern Bohuslän and Norway), *R. nordicus* is easily recognised by the frequent occurrence of 7-foliolate leaves. However, in the southern part of the distribution, such leaves are not seen and both species may have lobed terminal leaflets. It is often a challenge to distinguish between the two species on dried material from this area; the more hairy carpels of *R. nordicus* seems to be the most reliable character.

The third group (yellow in Fig. 1) comprises 37 samples with a more uneven distribution. One comes from Halland, representing what was described as a deviating morphotype from Spannarp in a previous inventory of this province (Ryde 2009). Sixteen come from Skåne, four from Blekinge, one from Öland and ten samples are from Småland, mainly around the city of Kalmar (cf. Fig. 2c). One sample from Germany, Wentorf in Holstein, also belongs to this group (not in the map). Morphologically, this group is quite distinct and easily separated from the other two species (cf. Fig. 5 and Table 1). The terminal leaflet has somewhat more triangular shape with a coarser serration and is grey-green underneath, hairy, but without any tomentose felt. The upper side is often somewhat shiny. It typically also has more glands on the stem and in the inflorescence and the serration of the leaves are slightly

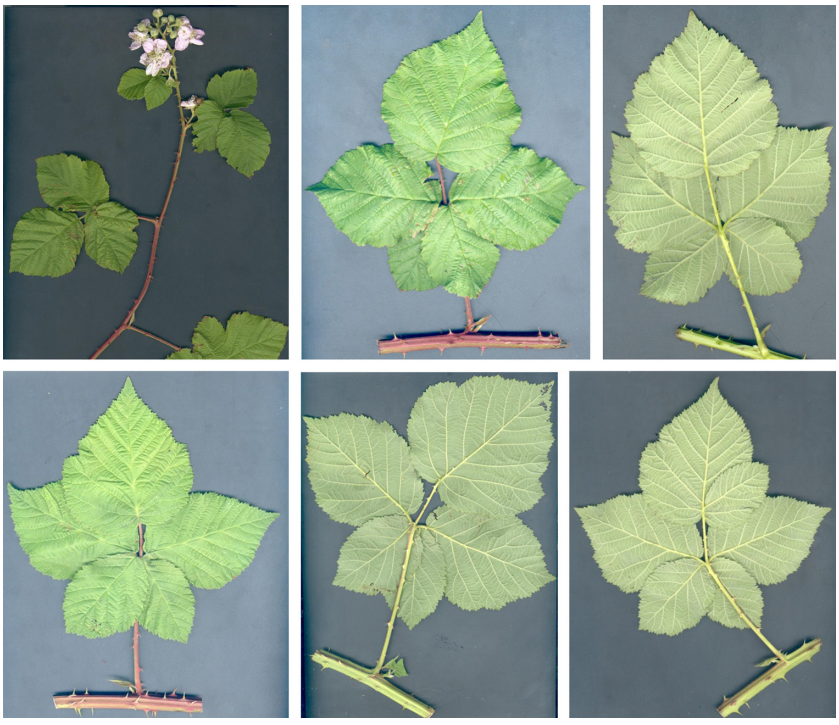

Figure 3. Pictures of *R. wahlbergii* s.s. (inflorescence, as well as upper and lower side of leaves). The upper figures show typical material, whereas the lower figures show forms with lobated leaves. The pictures are from localities A (6 July 2004, first row), B (18 Aug 2004) and C (4 July 2005, last two pictures) in the Supporting information.

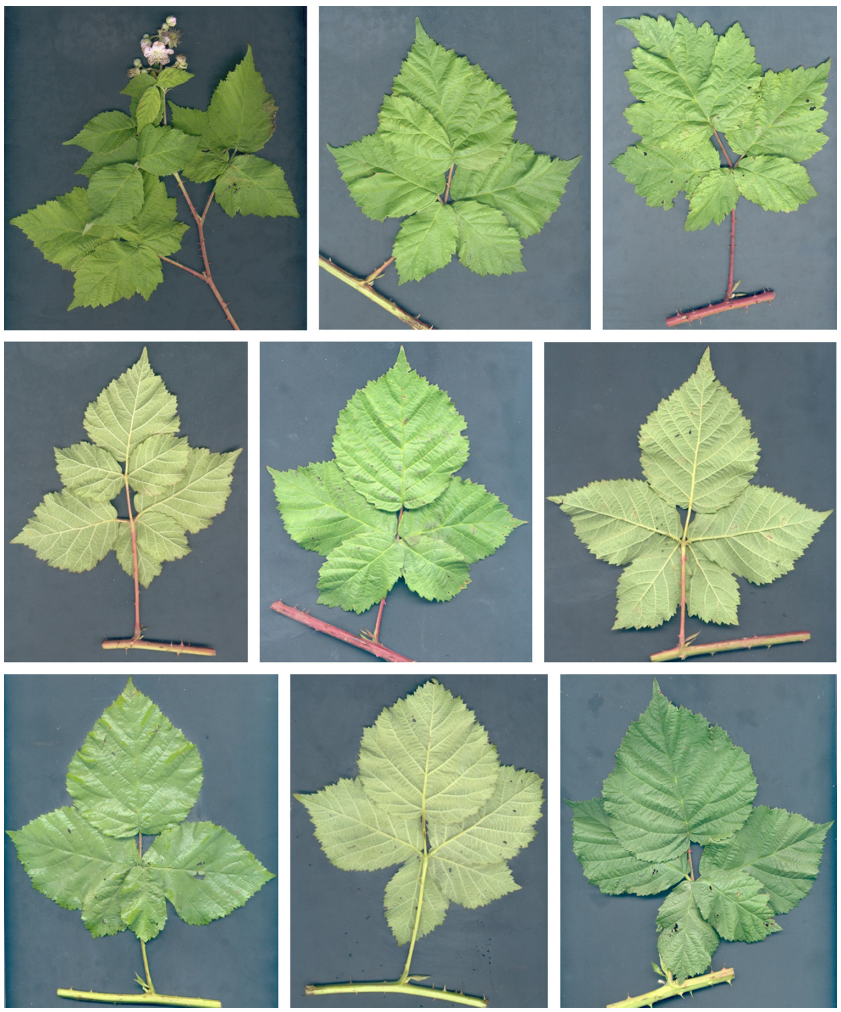

Figure 4. Pictures of *R. nordicus* (inflorescence, as well as upper and lower side of leaves) emphasizing the variation in the leaf shape. The pictures are from localities D (29 July 2012, four first from top left), E (7 Aug 2004, next two), F (8 July 2004, next two) and G (31 July 2001, lower right picture) in the Supporting information.

coarser than in typical *R. wahlbergii* s.s., but less coarse than in *R. nordicus*. The stem is normally sulcate and the prickles in the inflorescence are slightly larger than in the other two species. Since this group is distinct morphologically and it has an independent distribution over large parts of southern Sweden, we describe it below as *Rubus wahlbergioides*.

Flow cytometry results

Twenty-one samples of *R. wahlbergii* s.l. were studied by cytometry and all were found to be pentaploid (2n = 35) as expected. There is no significant difference between the three species: the samples of *R. wahlbergii* s.s., *R. nordicus* and *R. walbergoides* give average estimated chromosome numbers of 35.5, 35.4 and 35.2, respectively. As mentioned earlier, *R. wahlbergii* s.l. has a larger amount of DNA (genome size estimated from the correlation line between the cytometry results and samples of known ploidy levels) than hybrids between *R. idaeus* and various *R.* sect. *Corylifolii* species (Ryde 2009 2011, Ryde et al. 2021). The estimated chromosome number of the latter typically is around 34, whereas the average of our *R. wahlbergii* s.l. samples is 35.4. This indicates that *R. wahlbergii* s.l. has not arisen from *R. idaeus* hybrids.

Relationship between *R. wahlbergii* and other R. *sect.* Corylifolii *species*

Another question is how *R. wahlbergii* s.l. relates to other *R.* sect. *Corylifolii* species. A clustering analysis of a number of *R.* sect. *Corylifolii* species related to *R. wahlbergii* s.l. (no species of *Rubus sect. Rubus* come close) based on the studied four microsatellite loci is shown in Fig. 6. It can be seen that *R. vikensis*, which is the only other Swedish pentaploid species that most likely has not arisen from hybridisation with *R. idaeus*, is only distantly related to *R. wahlbergii* s.l. Instead, it clusters with *R. dissimulans* Lindeb. (p.p.) and *R. lagerbergii* Lindeb. Ex F. Aresch. (the latter is a pentaploid that has most likely arisen from the *R. dissimulans* × *idaeus* hybrid (Ryde et al. 2021)). *R. gothicus* Frid. and Gelert ex E. H. L. Krause and *R. friesianus* are genetically somewhat more similar to *R. wahlbergii* s.l., but they are even closer to *R. allanderi* H.Hyl. ex Burén and H. E. Weber and *R. rugulosus* Ryde,

Table 1. Variation in morphological characters of *R. wahlbergii, R. nordicus and R. wahlbergioides*.

Character	*R. wahlbergii*	*R. nordicus*	*R. wahlbergioides*
Stem	often sulcate	angled but typically not sulcate	often sulcate
Stem colour	purple in sun	brownish in sun	purple in sun
Hairs on turion	usually no	often hairy at the tip	occasional
Stipitate glands on the turion	no–occasional	no–occasional	occasional–scattered
Leaf colour	pure green	mixed (mosaic) and greyish green	pure green, often shiny
Shape of terminal leaflet	heart-shaped	elongated heart-shaped	heart-shaped, triangular
Serration	fine and regular	coarse and doubly serrated	intermediate, doubly serrated
Surface of terminal leaflet	smooth	more bubbly (caused by the tertiary nerves that are more distinct and impressed	smooth
Terminal leaflet	flat	tip bent downwards	flat
Number of prickles on the petiolule of terminal leaflet	1–5	6–9	5–8
Lower leaf surface	felty, whiteish	less felted and grey-green	no felt and grey-green
Hairs on lower surface	upright	more lying	more lying, rather sparse
Inflorescence hairiness	densely hairy with patent hairs	less hairy with more lying hairs	densely hairy with patent hairs
Inflorescence glands	no–occasional	no–occasional	many stalked glands
Inflorescence prickles length (mm)	2.4–4.6	2.7–4.2	2.5–4.0
Upper leaves	often several entire leaves at the top	mostly 3-foliolate (except the uppermost)	often several entire leaves at the top
Serration of uppermost leave	typically fine	coarse, with teeth that are directed forwardly	coarse
Flowers size (cm)	1.6–2.2	1.8–2.3	2.0–2.8
Ovaries	glabrous or occasionally with one hair that falls off early	more hairy and hairs remain longer. Occasionally with a crown of hairs	glabrous or occasionally with one hair that falls off early

respectively. Likewise, *R. glauciformis* Gust. ex H. Hyl. is more similar to *R. mortensenii* Frid. and Gelert ex E. H. L. Krause, while *R. walsemannii* H. E. Weber is a closer neighbour, and even more so is a group including *R. decurrentispinus* H. E. Weber, *R. lidforssii* (Gelert) Lange and *R. soendrumensis* Ryde. However, the species with the highest genetic similarity to *R. wahlbergii* s.l. are *R. polybracteatus* Ryde, *R. wendtii* Ryde, and especially *R. sordirosanthus*. It can also be concluded that *R. wahlbergii* s.s. and *R. wahlbergioides* are more similar to each other than to *R. nordicus*. Moreover, *R. sordirosanthus* is actually closer to *R. nordicus* than are either *R. wahlbergii* s.s. or *R. wahlbergioides*. Still, it should be remembered that evolution in *Rubus* is reticulate, so this clustering analysis is rather approximate and only reflects the genetic similarity in the studied four microsatellite loci.

In two previous studies (Ryde 2009, Ryde and Werlemark 2010), a taxon from three localities close to the city of Varberg in the province of Halland was discussed. A study based on random amplified polymorphic DNA (RAPD) analysis of one sample of this taxon indicated that it deviated from *R. vikensis*, *R. wahlbergii* and *R. sordirosanthus* (but closest to the latter). However, the present more detailed study clearly shows that the microsatellite alleles from two samples of the Varberg taxon and three samples of *R. sordirosanthus* are essentially identical. This is also confirmed by the morphology (pictures are shown in Ryde and Werlemark 2010)) and the chromosome number (tetraploid).

Origin of *R. wahlbergii s.l.*

An often discussed question is how *R. wahlbergii s.l.* has arisen. As discussed in the Introduction, it has been suggested that it has arisen from the *R. grabowskii* × *caesius* hybrid (Lidforss 1905, 1907). The microsatellite DNA analysis fully supports this suggestion as can be seen in Fig. 7. At locus 105b, *R. grabowskii* has three alleles (reflecting that it is triploid), all overlapping with alleles of *R. wahlbergii* s.l. The remaining two alleles of *R. wahlbergii* s.l. are the most common alleles of *R. caesius*.

At 2A8, *R. grabowskii* has two or three alleles and all three are encountered also in *R. wahlbergii* s.l. The other one or two alleles are again the two most common alleles in *R. caesius.*

At 117b, *R. grabowskii* has three alleles. All are also encountered in *R. wahlbergii* s.l., although with some variability. The other alleles of *R. wahlbergii* s.l. are also found in *R. caesius*. The only exception is the shortest allele of *R. nordicus* (108 bp), which was not encountered in our investigation of *R. caesius* (but alleles 2 bp shorter were found).

At 275a, there are some variation in the three alleles of *R. grabowskii*, but all alleles are encountered also in *R. wahlbergii* s.l. The additional two or three alleles of the latter overlap with alleles encountered in *R. caesius* (but alleles of most lengths are encountered in that species for this locus). The fact that *R. wahlbergii* s.s., *R. nordicus* and *R. wahlbergioides* show distincly different and reproducible patterns of alleles at

Figure 5. Pictures of *R. wahlbergioides,* upper and lower side of leaves, inflorescence, flower, flower bud, dissected flower, young fruit, ripe fruit, upper and lower side of upper leaves, younger leaf, primocane with detail and transect. The pictures are from localities H (13 Aug 2004, top row) and J (17 Aug 2003, lower two rows) in the Supporting information.

these loci indicates that they have arisen from independent hybridisations, rather than from a single hybridisation event that has later diverged by mutations. Corresponding patterns are seen at the 2A8 and 117b loci, but are less obvious.

Similar analyses show that other possible origins of *R. wahlbergii* s.l., are less likely (discussed in the Supporting information), e.g. *R. montanus* × *caesius* (*R. montanus* Lib. Ex Lej. is also triploid and belongs to the same series as *R. grabowskii*, viz. *Discolores*), *R. polybrachteatus* × *idaeus, R. wendtii* × *idaeus*, *R. walsemanii* × *idaeus, R. decurrentispinus* × *idaeus, R. gothicus* × *caesius* or *R. sordirosanthus* × *idaeus*. Of course, we have not compared with all possible *Rubus* species, only all occurring naturally in Sweden, but this analysis shows that *R. grabowskii* × *caesius* is a possible origin of *R. wahlbergii* s.l.

Rubus wahlbergioides Ryde and Burén sp. nov. (Fig. 5)

Type: Sweden, Småland, Kalmar: W end of Lybecksvägen, 56°41'26.2"N, 16°19'12.3"E, leg. T. Burén 2 July 2021 (holotype: LD, isotypes: GB, S, UPS). Illustrated in the Supporting information.

Etymology

The scientific epithet is selected to point out the similarity to *R. wahlbergii*.

Description

Stem low-arched to procumbent, sulcate to angled, 4–7 mm in diameter, purple, with occasional to scattered hairs and stipitate glands. Internodes 7–13 cm. Prickles recurved to slightly curved, with the same colour as the stem, 8–16 per 5 cm, 3.5–6.5 mm long and 4.5–7.5 mm wide at the base. Stipules 1.0–2.5 mm wide. Leaves 15–19(–24) cm long, with 5 leaflets, pure green on the upper side, thick. Petiole 4.5–6.5 cm (approximately as long as the basal leaflets), with 5–12 prickles, 1.5–3.0 mm long, falcate. Terminal leaflet cordate, 8–11 × 6.5–11.5 cm, widest at base, with scattered hairs on the upper side and hairy, but without felt, and grey beneath. Petiolule of terminal leaflet 2.4–4.1 cm, with 5–8 prickles, directed both downwards and to the sides. Leaf margin typically biserrate with teeth of unequal size, 2–3 mm. Tip of the terminal leaflet 1.0–1.6 cm. Lateral leaflets 7.5–9.5 cm long with a petiolule of 1–15 mm. Basal leaflets 5–7 cm with a petiolule of 0–2 mm. Inflorescence dense, with densely hairy rachis and many stalked glands. Prickles in the inflorescence

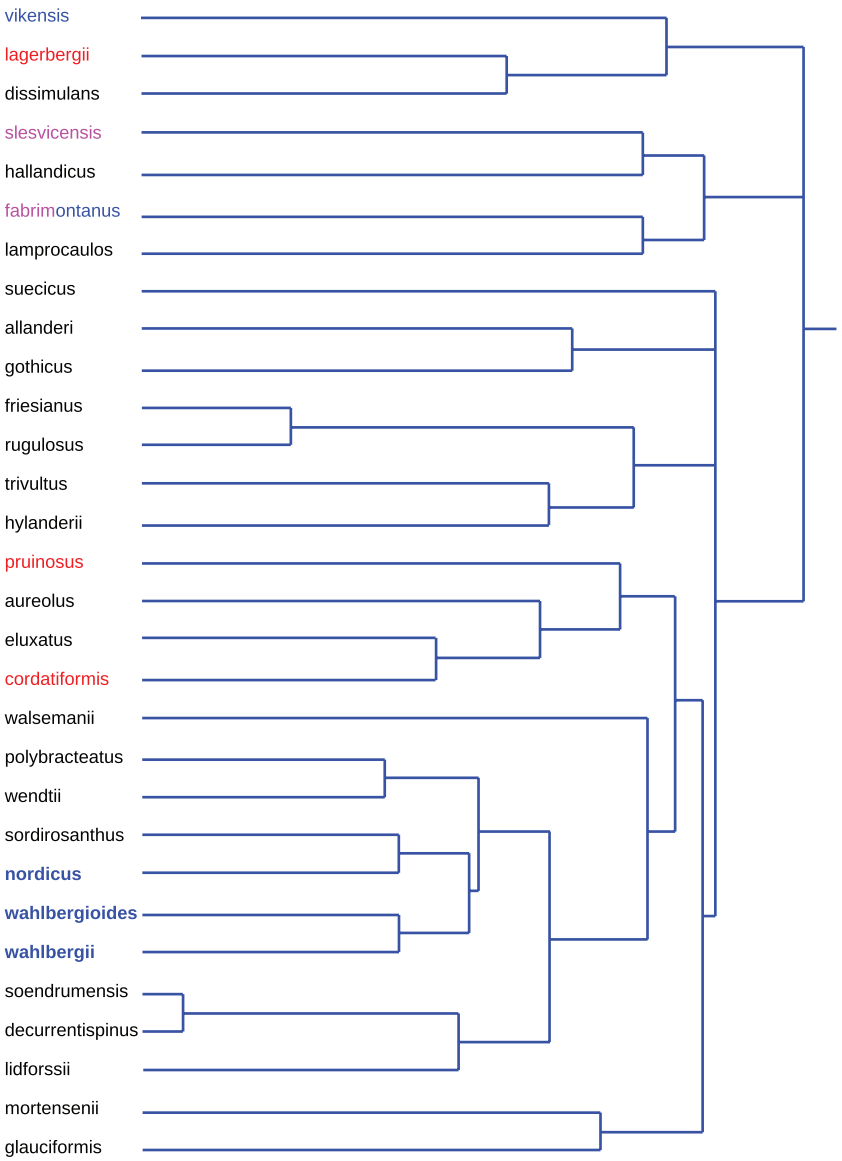

Figure 6. Cluster analysis (unweighted pair group method with arithmetic mean) of *R. wahlbergii* s.l. and their closest relatives based on DNA microsatellite data. The ploidy level is indicated with the colour of the species name: black – tetraploid, red – pentaploid, arisen from a *R. idaeus* hybrid, blue – pentaploid, not arisen from a *R. idaeus* hybrid, or magenta – hexaploid. *R. fabrimontanus* can be either pentaploid or hexaploid.

bent and 2.5–4.0 mm long, 5–12 per 5 cm. Pedicels 4–10 mm with 2–6 prickles of 1.0–1.6 mm, bent–falcate. Upper leaves trilobed to entire, coarsely serrate and whitish felted. Flowers 2.0–2.8 cm with pink petals. Filaments and styles white. Sepals grey, tomentose, often with several stipitate glands and occasional prickles, with short tips, deflexed in fruit. Hypanthium densely hairy. Carpels glabrous or with occasional hairs. Stamens of the same length as the styles or slightly shorter; anthers glabrous. Fruits rather well developed with >10 drupelets. DNA-ploidy level pentaploid (2n = 35).

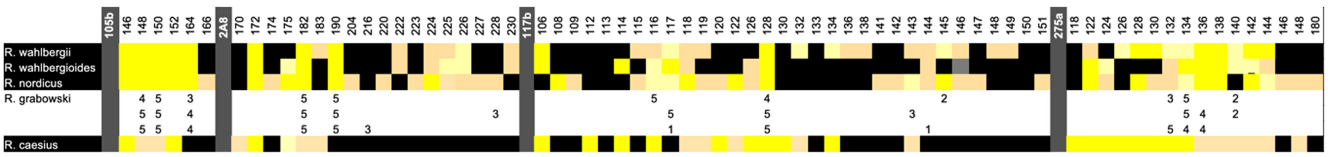

Figure 7. Microsatellite DNA analysis results for all samples of *R. wahlbergii* s.l., *R. caesius* and *R. grabowskii*. The former two are summarised by colour coding: Dark yellow for alleles found in almost all samples, light yellow for alleles found in ~10% of the samples and light orange for alleles found only a few samples. For *R. grabowskii*, the three samples are shown as the relative intensities (1–5) of the PCR products. The lengths in bp are shown in the first row.

Distribution and habitat

A distribution map based on the investigated material and field studies is shown in Fig. 2c (one sample from Wenstof in Holstein also belongs to *R. wahlbergioides*, showing that it occurs also in Germany). It grows at similar places as *R. wahlbergii*, on road sides, forest edges, pasturelands, shrubberies, outskirts of gardens, etc.

Conclusions

We studied the genetic relation within *R. wahlbergii s.l.* in Sweden using microsatellite DNA analysis and flow cytometry. We show that the material can be divided into three species. Two are *R. wahlbergii* s.s. and *R. nordicus*, although with a somewhat modified circumscription of the latter species: *R. nordicus* actually has a distribution that reaches in the south to north-western Skåne and it does not necessarily have seven-foliolate leaves (and *R. wahlbergii s.s.* sometimes have lobed terminal leaflets). Instead, the two species need to be distinguished by more subtle characters (listed in Table 1). The third species has not been recognized before, except in a local inventory of the province of Halland (Ryde 2009) and we describe it here as *R. wahlbergioides*. It has more triangular leaves that are somewhat shiny above and without any felt below. It also has more glands in the inflorescence. It has one locality in Halland, scattered localities in Skåne but also a distribution centre around Kivik, many localities in Blekinge, Småland and Öland, especially around Kalmar. It is surprising that *R. wahlbergioides* has not been noticed in the literature before. We have checked most Swedish floras but do not find any description that fits to *R. wahlbergioides.* Not even Hylander's lengthy discussion of *R. wahlbergii* (Hylander 1958b) includes anything that can be identified with *R. wahlbergioides.*

Acknowledgements – We thank Leszek Mackiewicz and the late Kåre A. Lye for collecting samples and showing localities. We are also indebted to 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

Mingyue Zhou: Investigation (lead). **Melanie S. Montes**: Investigation (supporting); Writing – review and editing (supporting). **Tore Mattsson**: Formal analysis (supporting); Writing – review and editing (equal). **Tomas Burén**: Formal analysis (supporting); Investigation (supporting); Writing – review and editing (supporting). **Mikael Hedrén**: Data curation (lead); Formal analysis (supporting); Supervision (lead); Validation (supporting); Writing – review and editing (supporting). **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).

Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.3bk3j9ktf (Zhou et al. 2024).

Supporting information

The Supporting information associated with this article is available with the online version.

References

Ansellem, L., Dutech, C. and Billotte, N. 2001. Isolation and characterization of polymorphic microsatellite loci in *Rubus alceifolius* Poir. (*Rosaceae*), an invasive weed in la Réunion Island. – Mol. Ecol. Notes 1: 33–35.
Arumuganathan, K. and Earle, E. D. 1991. Estimation of nuclear DNA content of plants by flow cytometry. – Plant Mol. Biol. Rep. 9: 229–241.
Burén, T. 2009. Tre förbisedda och missförstådda krypbjörnbär från sydöstra Sverige. – Sven. Bot. Tidskr. 103: 196–204.
Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. – Phytochem. Bull. Bot. Soc. Am. 19: 11–15.
Graham, J., Smith, K., MacKenzie, K., Jorgenson, L., Hackett, C. and Powell, W. 2004. The construction of a genetic linkage map of red raspberry (*Rubus idaeus subsp. idaeus*) based on AFLPs, genomic-SSR and EST-SSR markers. – Theor. Appl. Genet. 109: 740–749.
Gustafsson, Å. 1933. Chromosomenzahlen in der Gattung *Rubus*. – Hereditas 18: 77–80.
Gustafsson, C. E. 1936. Vorbereitende *Rubus*-Studien in Norwegen. – Nyt Mag. Naturvidensk 76: 151–153.
Gustafsson, C. E. 1938. Skandinaviens Rubusflora. – Bot. Not. 91: 378–420.
Hedrén, M., Zhou, M., Montes, M. and Ryde, U. 2020. Bjärebjörnbär, ett nytt krypbjörnbär i Skåne. – Sven. Bot. Tidskr. 114: 22–31.
Hegi, G. and Weber, H. E. 1995. Illustrierte Flora um Mitteleuropa, Band IV, Teil 2A. – Rubus.
Holmgren, B. 1942. Blekinges fanerogamer och kärlkryptogamer. – J. A. KrooksBokhandel, Karlskrona, pp.179–180.
Hylander, H. 1958a. Några nya eller kritiska *Rubi Corylifolii*. – Bot. Not. 111: 517–534.
Hylander, H. 1958b. *Rubus*-släktet i Sverige. – Manuscript kept at the Botanical Museum Library at Lund University. pp. 1–462.
Jaccard, P. 1908. Nouvelles rechèrches sur la distribution florale. – Bull. Soc. Sci. Nat 44: 223–270.
Krause, E. H. L. 1891. Bastarde des *Rubus idaeus* L. – Abh. Natl. Ver. Bremen 7: 156–159.
Kurtto, A., Weber, H. E., Lampinen, R. and Sennikov, A. N. 2010. Atlas florae Europaeae. Distribution of vascular plants in Europe 15. *Rosaceae* (*Rubus*). – The Committee for Mapping the Flora of Europe & Societas Biologica Fennica Vanamo.
Lewin, M., Zhou, M., Montes, M. S., Mattsson, T., Burén, T., Hedrén, M. and Ryde, U. 2022. Hybrids between *Rubus caesius* and *Rubus* sect. Corylifolii (*Rosaceae*) and their relation to *R. cyclomorphus*, *R. tiliaster*, *R. glauciformis*, *R. slesvicensis* and *R. firmus*. – Nord. J. Bot. 2022: e033759.
Lidforss, B. 1905. Studier öfver artbildningen inom släktet *Rubus*. – Ark. Bot. 4: 1–41.

Lidforss, B. 1907. Studier öfver artbilningen inom släktet *Rubus* II. – Ark. Bot. 6: 1–43.
Mattsson, T. and Oredsson, A. 2010. Falkbjörnbär Rubus friesianus - ännu ett återuppstånded krypbjörnbär. – Sven. Bot. Tidskr. 104: 85–93.
Mirek, Z. 1995. Vascular plants of Poland. A checklist. – Polish Botanical Studies Guidebook Series 15 (Cracow). p. 1.
Oredsson, A. 1969. Drawings of Scandinavian Plants 17–20, Rubus L. Subgen. Rubus. – Bot. Not. 122: 1–1.
Pedersen, A. and Schou, J. C. 1989. Nordiske brombær, AAU Report 21. – Univ. of Aarhus, Denmark.
Rohlf, F. J. 2005. NTSYS-pc, Numerical Taxonomy System, ver. 2.20. – Exeter Publishing, Setauket, NY, USA.
Ryde, U. 2009. Krypbjörnbär I Halland. – Sven. Bot. Tidskr. 103: 279–299.
Ryde, U. 2010. Genetic analysis shows that *Rubus vikensis* is a distinct species with a disjunct distribution. – Nord. J. Bot. 28: 246–250.
Ryde, U. 2011. Arguments for a narrow species concept in *Rubus* sect. Corylifolii. – Nord. J. Bot. 29: 708–721.
Ryde, U. and Werlemark, G. 2010. Steningebjörnbär, ett nygammalt krypbjörnbär från Halland. – Sven. Bot. Tidskr. 104: 405–413.
Ryde, U., Montes, M. S., Zhou, M., Mattsson, T., Burén, T. and Hedrén, M. 2021. Hybrids between Rubus idaeus and Rubus sect. Corylifolii and their relation to R. pruinosus and R. Rosanthus. – Nord. J. Bot. 39: e03111.
Stafne, E. T., Clark, J. R., Weber, C. A., Graham, J. and Lewers, K. S. 2005. Simple sequence repeat (SSR) markers for genetic mapping of raspberry and blackberry. – J. Am. Soc. Hortic. Sci. 130: 722–728.
Teofilovski, A. 2011. Prilozi za florata na republika Makedonija, Skopje.
Weber, H. E. 1981. Revision der Sektion *Corylifolii* (Gattung *Rubus*, *Rosaceae*) in Skandinavien und im nördlichen Mitteleuropa. – Sonderb. Naturwiss. Ver. Hamburg 4: 1–229.
Weber, H. E. 1998. Batological notes on Flora Nordica. – Nord. J. Bot. 18: 39–40.
Weber, H. E. 2008. Notizen zur brombeer-Flora in Dänemark und Skandinavien (Rubus L. sect. Corylifolii Lindley). – Osnabr. Naturwiss. Mitt. 33/34: 59–61.
Wendt, G. 2008. Vikenbjörnbär, ett nytt krypbjörnbär. – Sven. Bot. Tidskr. 102: 147–152.
Zhou, M. 2014. Population genetics and phylogeny of European blackberries. – MSc. thesis, Lund Univ., Sweden, http://lup.lub.lu.se/student-papers/record/4675509.
Zhou, M., Montes, M. S., Mattsson, T., Burén, T., Hedrén, M. and Ryde, U. 2024. Data from: *Rubus wahlbergii* and related species in Sweden. – Dryad Digital Repository, https://doi.org/10.5061/dryad.3bk3j9ktf.