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Evolutionary patterns in South African brambles (*Rubus* L.) – new insights from molecular markers

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Background: South African brambles (*Rubus* L., Rosaceae) represent a complex group of six native species and at least 12 introduced taxa with different ploidy levels and varying tendencies to hybridisation. The role of hybridisation, introgression and apomixis in the ongoing evolution has been hypothesised based on morphological observations, but it has not been rigorously studied to date, and nor has the phylogeny of the group.

Objectives and methods: This paper aims to reveal the evolutionary patterns and mechanisms in South African brambles by employing three types of molecular markers: plastid and nuclear ribosomal DNA sequences, and nuclear microsatellites.

Results: The data confirmed the tetraploid *R. thaumasius* A.Beek and diploid *R. ludwigii* Eckl. & Zeyh. as distinct native species, while the other four native species are shown to be closely related and likely derived from three ancestors.

Conclusion: Ancient hybridisation and limited gene flow between regions (particularly between winter- and summer-rainfall zones) appear to be the main drivers of current patterns in the tetraploid *R. pinnatus* Willd. and hexaploid *R. rigidus* Sm. Current hybridisation is also likely, although rare. The mechanism of 'octoploid bridge' is proposed, which overcomes the ploidy reproduction barrier between *R. pinnatus* (or other tetraploids) and *R. rigidus*. No gene flow was detected between native and alien taxa, but clonal duplications were discovered in the *R. bergii* × *pinnatus* hybrid, which implies the possibility of apomictic spread of homoploid hybrids formed between native and introduced brambles and the potential for a new invasion. On the other hand, heteroploid hybrids (*R. bergii* × *rigidus*) are formed recurrently and spread only vegetatively.

Keywords: apomixis, clonal spread, hybridisation, introgression, reticulate evolution

Introduction

Rubus L. (Rosaceae: Rosoideae), commonly known as brambles, blackberries, raspberries, dewberries etc., is a complex genus due to its thousands of species and diverse evolutionary mechanisms, of which hybridisation, polyploidisation and apomixis are among the most important (Sochor et al. 2015), and often exhibiting strong phylogeographic patterns (Sochor & Trávníček 2016; Sochor et al. 2017). The genus has been relatively intensively studied in some parts of the world, e.g., in central and northwestern Europe, where an elaborate morphology-based system of a few sexual species and > 750 recognised apomictic microspecies (i.e., asexual genotypes of certain distribution areas and distinct morphology) organised in series, subsections and sections is in use

(Weber 1996; Kurtto et al. 2010). On the other hand, the genus has been relatively neglected in other regions (e.g., North America or the Caucasus: Alice et al. 2015; Sochor &Trávníček 2016). This uneven distribution of knowledge is reflected not only in the taxonomy but also in the phylogenetics, phylogeography and evolutionary biology of the group (biosystematics in a wide sense).

The African continent belongs among the understudied regions of the world. None of the native African *Rubus* taxa (disregarding North Africa, which is home to a few species of predominantly European distribution) were included in the two worldwide phylogenetic studies (Alice & Campbell 1999; Carter et al. 2019), and just a single DNA sequence from a native sub-Saharan African *Rubus* species is present in the NCBI GenBank nucleotide database (*R. rigidus* Sm., accession number U95229). Genetic diversity in African accessions has been analysed only among selected Kenyan specimens (unfortunately mostly undetermined and thus of unknown primary origin) using morphological and microsatellite markers with the aim of characterising potential breeding material (Ochieng et al. 2018, 2019). The evolution and phylogeny of African brambles are therefore unexplored.

Within the African continent, the taxonomy and fundamental biological properties (reproduction mode, polyploidy) are best explored in the South African *Rubus* taxa owing to recent advances in our understanding of the species in the region (Sochor et al. 2018, 2022; Van de Beek 2021). Applying a very narrow morphology-based (micro-)species concept, inspired by the one used for European *Rubus* apomicts, Van de Beek (2021) distinguished 16 native species in six series in the Cape Floristic Region alone and commented on the existence of a number of other 'species', so far insufficiently studied. On the other hand, Sochor et al. (2022) incorporated ploidy level and reproductive mode data, and identified six native South African *Rubus* species in total (see Table 1 for overview), all of them sexual di-, tetra- or hexaploids (2n = 14, 28, 42, respectively), and some of them phenotypically highly variable. In addition, 12 introduced taxa and 12 hybrids were identified, which indicated potential ongoing evolution in South African brambles via hybridisation, introgression and apomixis. However, the real effect of these processes on natural populations could not be evaluated properly based on phenotypic and cytometric data only.

In this work, we used the *Rubus* material that was studied in recent biosystematic/taxonomic investigations (Van de Beek 2021; Sochor et al. 2022) and analysed the sampled individuals by employing three types of DNA markers to address South African *Rubus* evolution from two perspectives. First, plastid and ribosomal nuclear sequence data were used for phylogenetic and phylogeographic reconstructions, and for identifying/confirming the identity of hybrids and potential introgressants. Specifically, we aimed at revealing not only phylogenetic relationships among taxa, but also at detecting any signatures of potential ancient or ongoing gene flow between native species or between native and introduced taxa. Second, simple sequence repeats (SSR, microsatellites) were used for primary evaluation of genotypic diversity and microevolutionary processes in a model group of *R. bergii* (Cham. & Schltdl.) Eckl. & Zeyh., *R. rigidus*, *R. pinnatus* Willd. and their hybrids. In particular, we aimed at detecting clonal duplications and quantifying the degree of apomixis at a regional scale, and thus evaluating the evolutionary and invasive potential of the hybrids. The new DNA data helped us understand the evolutionary history and phenotypic patterns in this relatively young and species-poor (in the context of the Cape flora) but evolutionary complex plant group.

Table 1. Overview of *Rubus* taxa occuring in South Africa with their distribution, ploidy level and reproductive mode (all from Sochor et al. 2022) and plastid haplotypes (detected in this study); LP = Limpopo; MP = Mpumalanga; G = Gauteng; FS = Free State; KZN = KwaZulu-Natal; EC = Eastern Cape; WC = Western Cape

Taxon/hybrid	Distribution in South Africa (provinces)¹	DNA ploidy level	Reproduction	Plastid haplotype
Native taxa				
R. apetalus Poir.	LP, MP, KZN, EC	4x	sexual	*Ape1*, *Ape2*
R. ludwigii Eckl. & Zeyh.	MP, FS, KZN, EC, WC	2x	sexual	*Lud1*
R. pinnatus subsp. *pinnatus* Willd.	WC	4x	sexual	*Pin1*
R. pinnatus subsp. *pappei* (Eckl. & Zeyh.) Sochor	MP, KZN, EC	4x	sexual	*Pin3*, *Rig6*
R. rigidus Sm.	All except Northern Cape	6x	sexual	*Rig1–11*
R. thaumasius A.Beek	EC	4x	sexual	*Tha1*
R. transvaalensis Gust.	MP, KZN	6x	sexual	*Rig5*

Table 1. Overview of *Rubus* taxa occuring in South Africa with their distribution, ploidy level and reproductive mode (all from Sochor et al. 2022) and plastid haplotypes (detected in this study); LP = Limpopo; MP = Mpumalanga; G = Gauteng; FS = Free State; KZN = KwaZulu-Natal; EC = Eastern Cape; WC = Western Cape (continued)

Taxon/hybrid	**Distribution in South Africa (provinces)¹**	**DNA ploidy level**	**Reproduction**	**Plastid haplotype**
North American taxa				
R. sect. *Arguti* (Rydb.) L.H.Bailey	LP, MP, G, FS, KZN, EC	4x	apomictic	*Arg1*
R. sect. *Cuneifolii* (L.H.Bailey) L.H.Bailey	KZN, EC	4x	apomictic	*Cun2*
R. titanus L.H.Bailey	WC	6x	sexual	*Urs1*
R. trichogynus A.Beek	LP, MP, FS, KZN, EC, WC	7x	sexual	*Urs1*
European/Caucasian taxa				
R. armeniacus Focke	EC, WC	4x	apomictic	*Dol1*
R. bergii (Cham. & Schltdl.) Eckl. & Zeyh.	EC, WC	4x	apomictic	*Ulm1*
R. aff. *bergii*	KZN	4x	apomictic	*Ulm2*
R. ulmifolius Schott	MP, KZN	2x	sexual	*Ulm1*, *Ulm2*
Asian taxa				
R. ellipticus Sm.	KZN	2x	sexual	*Eli1*
R. niveus Thunb.	LP, MP, KZN, EC	2x	sexual	*Niv1*
R. phoenicolasius Maxim.	KZN, EC	2x	sexual	*Phe1*
R. rosifolius Sm.	KZN, (WC)	2x	sexual	*Ros1*
Hybrids				
R. apetalus × *R. ludwigii*	MP	3x	not analysed	*Lud1*
R. apetalus × *R. pinnatus*	MP	4x	sexual	*Ape2*
R. sect. Arguti × *R. pinnatus*	MP	4x	apomictic	*Rig6*
R. sect. Arguti × *R. rigidus*	M, KZN, EC	5x, 8x	sexual	*Rig1*, *Rig4*, *Rig5*, *Rig9*
R. bergii × *R. pinnatus*	WC	4x		*Pin1*, *Rig6*
R. bergii × *R. rigidus*	EC, WC	5x	sexual	*Rig2*, *Rig3*, *Rig4*, *Rig6*, *Rig9*
R. bergii × *R. thaumasius*	EC	4x	apomictic	*Tha1*
R. ludwigii × *R. pinnatus*	KZN	3x	not analysed	*Lud1*
R. niveus × *R. transvaalensis*	MP	4x	not analysed	*Niv1*
R. pinnatus × *R. rigidus*	WC, EC	5x, 8x	sexual	*Rig3*
R. rigidus × *R. ulmifolius*	MP	4x	not analysed	*Rig5*
R. pinnatus × *R. thaumasius*	EC	not analysed	not analysed	*Tha1*

Materials and methods

Sampling and DNA extraction

DNA samples (see Supplementary Table S1) were collected during fieldwork for a biosystematic treatment of South African *Rubus* (see Sochor et al. 2022 for details). The specimens were simultaneously thoroughly studied morphologically and mostly also analysed for ploidy level and reproduction mode. Of the available collections, 224 specimens (particularly from non-apomictic taxa and hybrids) were used for sequencing, whereas only a selection of 45 specimens of *R. bergii*, *R. rigidus*, *R. pinnatus* and their hybrids, mostly from Western Cape (see Supplementary Table S1), was used for SSR analysis for primary evaluation of genotypic and allelic diversity, confirmation of phenotypic determinations, as well as assessment of the suitability of the markers for further studies. Six specimens of *R. bergii* from its native range in Western Europe were included as well. DNA was extracted from silica gel-dried leaves using the CTAB method (Doyle & Doyle 1987). Eight specimens, four of them being the type specimens, were provided by A. van de Beek, which represented his new species or his conception of old species (Supplementary Table S1; Van de Beek 2021; see also Sochor et al. 2022 for further discussion and revised taxonomic concepts); their DNA was extracted from two seeds per specimen using GenElute™ Plant Genomic DNA Miniprep kit (Sigma-Aldrich, USA).

Sequencing

Two plastid regions were analysed: the *matK* intron was amplified and sequenced with primers XFA and AST_R (Dunning & Savolainen 2010) and the *trnL-trnF* intergenic spacer with primers c and f (Taberlet et al. 1991). The ribosomal nuclear locus ITS (internal transcribed spacer) was amplified and sequenced with primers ITS1 and ITS4 (White et al. 1990). Polymerase chain reactions (PCRs) were performed using EliZyme FAST Taq mix (Elisabeth Pharmacon, Czechia) according to the manufacturer's protocol in reaction volume of 15 μL. PCR products were checked on agarose gel electrophoresis, purified by precipitation with polyethylene glycol (10% PEG 6000 and 1.25 M NaCl in the precipitation mixture) and sequenced using the Sanger method at Macrogen Europe (the Netherlands). In selected specimens, the ITS amplicon was cloned into a bacterial vector to obtain sequences of different ITS alleles (ribotypes) within one individual. In these cases, PCR was performed using EliZyme HIFI polymerase (Elisabeth Pharmacon) with proofreading activity. The PCR product was purified, its concentration estimated by Nanodrop 2000, and 18 ng of the PCR product was ligated in the total volume of 10 μL ligation mixture into pJET1.2/blunt cloning vector using CloneJET PCR Cloning Kit (Thermo Scientific, USA). The plasmid was further used for transformation of *Escherichia coli* strain DH5α using TransformAid Bacterial Transformation Kit (Thermo Scientific) following the overnight bacterial culture protocol, with a modification that the initial cultivation in C-medium was not longer than six hours and the colony used for its inoculation was not older than one day. Transformed bacterial colonies were used as a template in a colony PCR with primers pJET1.2 forward and reverse (supplied with the cloning kit). PCR products were checked, purified and sequenced with the amplification primers similarly to direct sequencing as described above.

SSR analysis

Ten microsatellite loci (Graham et al. 2004, 2006; Woodhead et al. 2008) were selected based on amplification efficiency and variability in a selection of samples, and amplified using the EliZyme FAST Taq (Elisabeth Pharmacon) in 10μL reaction volume with 7.5 ng template DNA following the standard manufacturer's protocol (see Supplementary Table S2 for further details). Fluorescent labelling was performed using a nested PCR containing three primers: a template-complementary forward primer with M13 tail at its 5′ end (final concentration 0.1 μM), a template-complementary reverse primer (concentration 0.4 μM), and a fluorescently 5′-modified M13 primer (5′-TGTAAAACGACGGCCAGT; NED™, PET®, VIC™ or FAM™ modification; concentration 0.4 μM). To facilitate annealing of the universal M13 primer the annealing temperature was lowered to 53°C in the last nine PCR cycles. Such labelled PCR products were separated together with the GeneScan 600LIZ® size standard on an ABI 3730XL capillary sequencer at Macrogen Europe.

Data analysis

DNA sequence editing, alignments and haplotype/ribotype identification were performed in Geneious 8 (Biomatters, New Zealand). Plastid haplotypes were compared with the sequences of Sochor et al. (2015) and Sochor and Trávníček (2016), and their codes assigned accordingly. A median-joining algorithm was used to create a phylogenetic haplotype network in Network 10.1.0.0 (Bandelt et al. 1999). All sequences were deposited in NCBI GenBank (accession numbers OL899048–OL899299 [ITS], OL954095–OL954503 [*matK* and *trnL-trnF*]). ITS data were checked for the presence of contaminations by microorganisms, pseudogenes and PCR recombinants as in Sochor et al. (2015). The filtered alignment was analysed in Network using star contraction (number of mutations set to three) and median-joining algorithms, and in SplitsTree 4 (Huson & Bryant 2006) using NeighbourNet algorithm with uncorrected P character transformation.

SSR chromatograms were analysed and scored manually in Peak Scanner 1.0 (Applied Biosystems). Alleles were coded according to their length in bp and saved as both codominant and binary data. Shannon information index was computed and principal coordinate analysis (PCoA) using the distance method with standardisation was performed in GenAlEx 6.5 (Peakall & Smouse 2012) based on the binary data matrix. Histogram of genetic distances and genotype identification were performed in Genotype 2.0b23 (Meirmans & Van Tienderen 2004).

Results

Patterns in plastid DNA variation

Plastid DNA data were obtained for 219 specimens. Among native South African *Rubus* taxa, 18 plastid haplotypes were distinguished when both single nucleotide polymorphisms (SNPs) and indels were considered, and 14 haplotypes when indels were rejected (Figure 1). Two haplotypes characterised *R. ludwigii* and *R. thaumasius*, respectively, and grouped separately from other native species. The other haplotypes formed two mutually related groups shared mainly by *R. pinnatus* and *R. rigidus*. The haplotype of *R. transvaalensis* (haplotype *Rig5*) was shared with *R. rigidus*, and two haplotypes (*Ape1, Ape2*) were found only in *R. apetalus* and its hybrid. Each of the Asian species bore a single unique haplotype. Tetraploid Euro-Caucasian taxa were also characterised by their haplotypes, but two haplotypes were detected in the diploid *R. ulmifolius* – one shared with *R. bergii* and one with *R.* aff. *bergii*. Four haplotypes were distinguished among North American taxa, one borne by at least three morphotypes of *R.* sect. *Arguti*, one by *R.* sect. *Cuneifolii*, one by two undetermined morphotypes (one of them possibly belonging to *R.* sect. *Alleghenienses*), and one haplotype was shared by *R. titanus* and *R. trichogynus* (*Urs1*; presumably derived from the western North American *R.* sect. *Ursini*).

The haplotypes of *Rubus rigidus* and *R. pinnatus* exhibited clear patterns in geographic distribution. *Rubus rigidus* bore only three haplotypes in the westernmost part of the range, all from the A group (Figure 1D), whereas only haplotypes of the C group (with one exception of the *Rig6* haplotype detected once near Alexandria, EC) were detected in eastern regions outside of the Cape Floristic Region, and the highest diversity was discovered in KwaZulu-Natal (KZN). A roughly similar pattern (Figure 1C) was detected in *R. pinnatus* and corresponded to its subspecific classification, in which western *R. pinnatus* subsp. *pinnatus* bore only the *Pin1* haplotype or its derivative *Pin4*, whereas *R. pinnatus* subsp. *pappei* had mostly the *Rig6* haplotype shared with western *R. rigidus*, or one haplotype from the C group differing from the eastern *R. rigidus* haplotypes only in one indel (*Pin3*).

All of the studied hybrids of *R. rigidus* [*R. bergii* × *rigidus* – 22 individuals (ind.); *R.* sect. *Arguti* × *rigidus* – 11 ind.; *R. rigidus* × *ulmifolius* – 3 ind.; *R. rigidus* × *pinnatus* – 1 ind.] exhibited haplotypes derived from that species. Similarly, *R. thaumasius* served as the pistillate parent of all of its studied hybrids (with *R. bergii* – 3 ind.; and with *R. pinnatus* – 1 ind.), as did *R. ludwigii* (with *R. apetalus* – 1 ind.; and *R. pinnatus* – 1 ind.). *Rubus pinnatus* served as pistillate parent in all of the studied hybrids with *R. bergii* (6 ind. representing 4 genotypes) but not in the hybrids with *R. apetalus*, whose pistillate parent was the latter species (1 ind.). The hybrid *R. niveus* × *transvaalensis* shared the haplotype with the first species.

Variation in ITS

ITS data were generated from 118 individuals in total, of which 90 were sequenced directly (individuals without length variation in the amplicon) and 28 were cloned (Supplementary Table S1). One to eight cloned sequences (185 in total, 6.6 on average) were obtained per individual after the exclusion of contaminants (10 sequences in total) and recombinants (14 sequences). 275 sequences were included in the final analyses. ITS exhibited more variation than plastid DNA, but part of it was not shared among individuals and was thus uninformative. The cloned sequences from hybrids always confirmed their hybrid origin. Similarly, two or more divergent orthologous ITS alleles were detected in alien apomictic polyploids. Except for the (putatively primary) hybrids, no gene flow/introgression was detected between native and introduced taxa.

Among native taxa, *R. thaumasius* and *R. ludwigii* formed distinct phylogenetic lineages, while the other species formed three groups (A, B, C, corresponding to the plastid haplotype groups according to their presumed origin), two of which could be subdivided into three subgroups each (Figure 2A). *Rubus apetalus* formed a separate distinct branch diverging from the base of the A group. *Rubus transvaalensis* was restricted to the C1 subgroup, which was not shared by any other species, but was placed at the split of C2 and C3 subgroups belonging to *R. rigidus*. Specimens of *R. pinnatus* subsp. *pinnatus* had only B2 ribotypes, but specimens from the transitional zone in the eastern parts of WC, as well as *R. pinnatus* subsp. *pappei* from MP bore mostly B1 ribotypes, and the remaining eastern populations had B3 ribotypes (Figure 2B). The B1 subgroup was the only one shared with *R. rigidus*, although only rarely in KZN and MP. *Rubus rigidus* was otherwise represented in A and C groups (Figure 2C): A was detected almost throughout the studied area, C3 dominated the lowlands of KZN and C2 was detected in MP always as an ortholog together with B1.

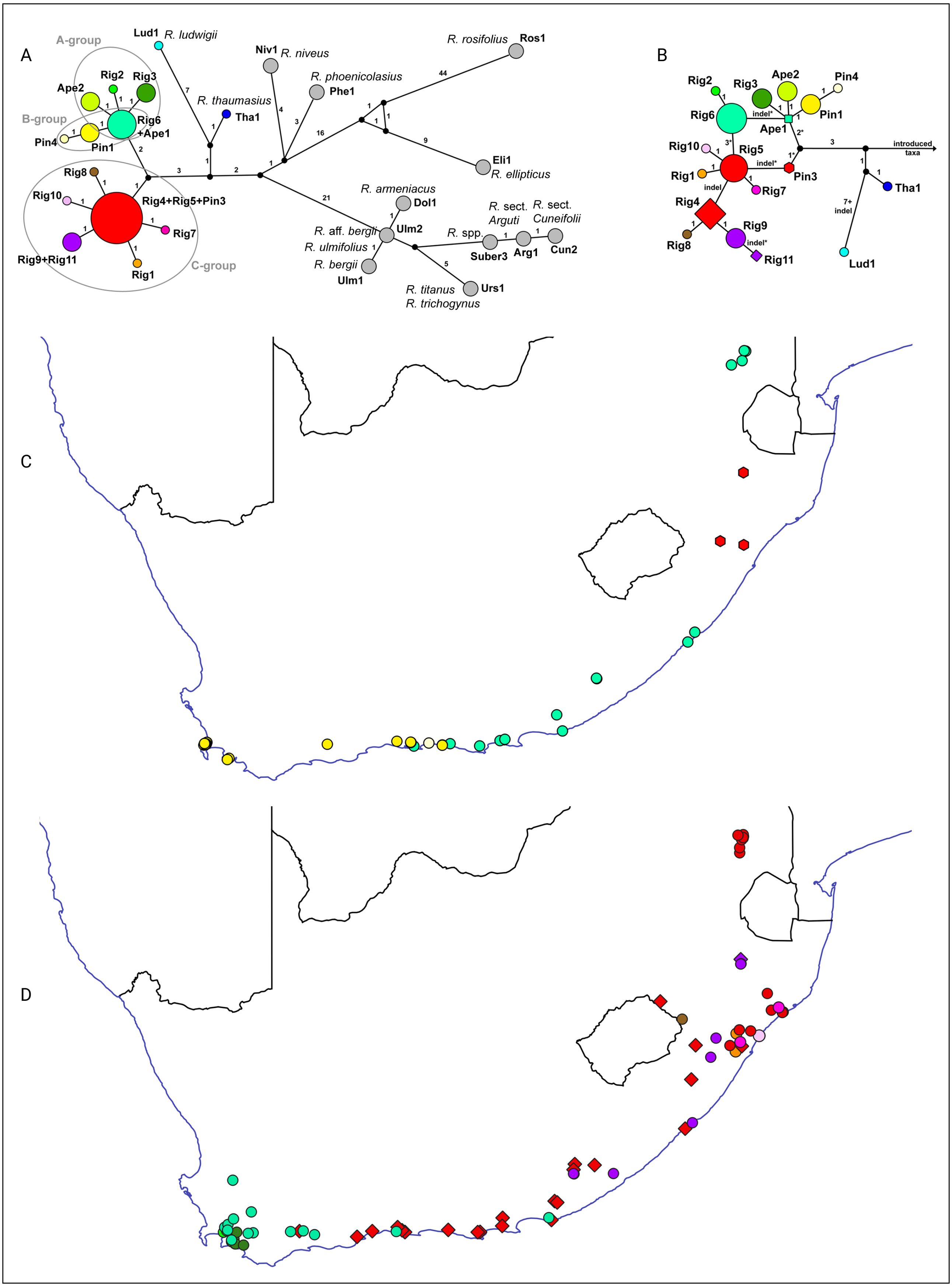

Figure 1. Plastid haplotype diversity patterns in South African *Rubus* taxa. A, haplotype network based on SNPs; B, haplotype network based on SNPs and indels (number of SNP mutations or indels shown above branches, forward and reverse mutations at the same position indicated by asterisks; symbol size corresponds with the frequency of the haplotype in the dataset); C and D, geographic distribution of haplotypes in *R. pinnatus* and *R. rigidus* (including their hybrids), respectively, in South Africa (symbol shapes and colours correspond with A and B). *Rubus apetalus* (haplotypes *Ape1*, *Ape2*) and *R. transvaalensis* (*Rig5*) are not included in the maps.

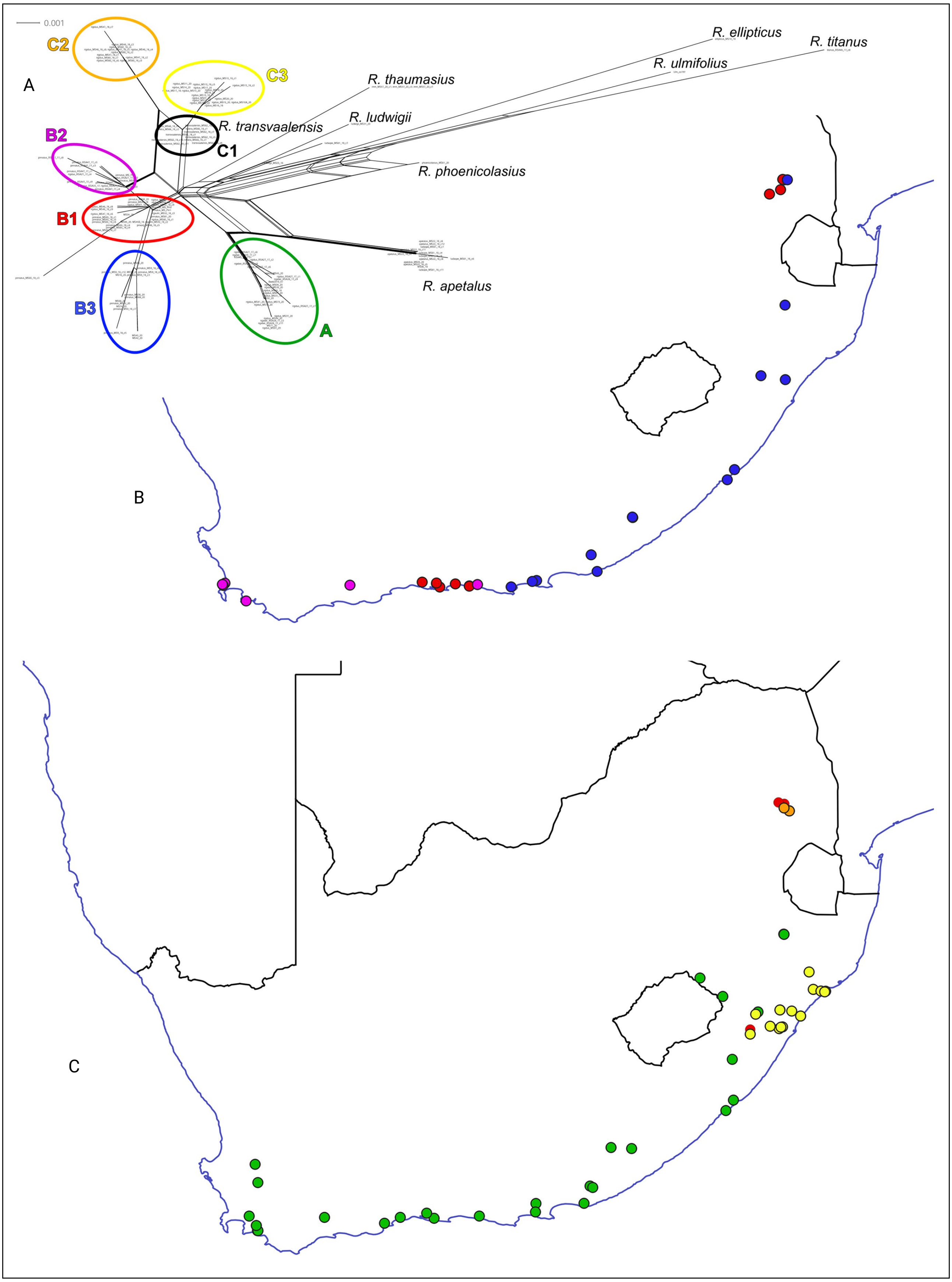

Figure 2. ITS ribotype diversity patterns in South African *Rubus* taxa. A, SplitsTree phylogenetic network based on cloned and directly sequenced ITS amplicons. B and C, geographic distribution of ribotypes in *R. pinnatus* and *R. rigidus* (including their hybrids), respectively, in South Africa (symbol colours correspond with A). Note that the C1 group is exclusive for *R. transvaalensis* and is not included in the maps.

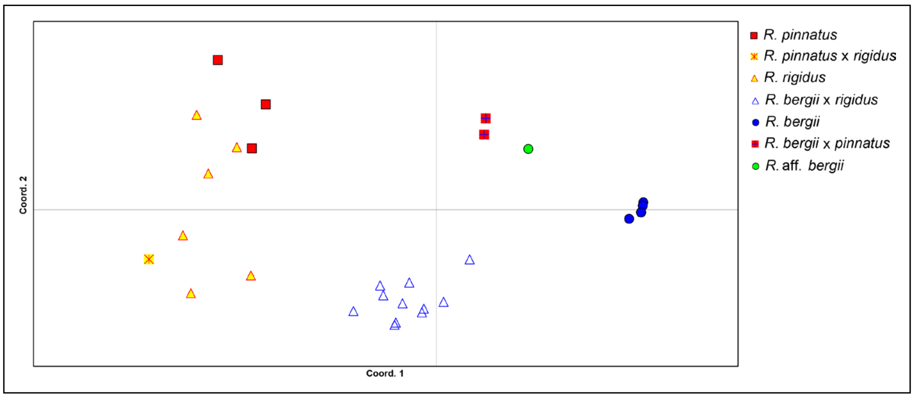

Figure 3. PCoA analysis of SSR data; the two axes explain 43% and 18% of the whole variation, respectively.

Variation in microsatellites

Ten SSR loci were selected following our previous work (e.g., Király et al. 2017), but Rubus123a was excluded due to poor amplification efficiency. In total, 99 alleles were detected in the studied sample set of 45 individuals across the nine loci (3–21 alleles per locus, mean ± standard deviation 11.0 ± 5.8; Supplementary Table S3). However, only null alleles (no PCR products) were detected at two loci (Rubus26a and ERubLR_SQ01_G16) in *R. bergii* and relatives. Distribution of genetic distances among individuals indicated the threshold between within-genotype and among-genotype variation to be set at three mutations (not shown). Applying this threshold, genotype assignment was almost identical to analysis with the threshold of zero (i.e., no mutation within a genotype allowed; Table 2); only *R. bergii* exhibited three different mutations in three individuals (one per individual; Supplementary Table S3). Despite that, this species was clearly monoclonal in both its native and secondary range (Table 2). Besides *R. bergii*, clonality was detected in the hybrid *R. bergii* × *pinnatus*. In contrast, *R. rigidus*, *R. pinnatus* and *R. bergii* × *rigidus* exhibited no clonal duplication. PCoA analysis supported identification of the parents in all of the presumed hybrids (Figure 3).

Discussion

Evolutionary history is complex in native species

Both the ITS and cpDNA confirm that *R. ludwigii* and *R. thaumasius* are distinct native species that diverged from the common ancestor of all South African *Rubus* taxa. This finding is contrary to previous interpretations of the origin of *R. thaumasius*, which was originally presumed to be a hybrid of *R. rigidus* and some other taxon (Gustafsson 1934) or even of purely European origin (Stirton 1981; Henderson 2011). However, its presumed relationship with tropical African species, such as *R. runssorensis* Engl. and *R. friesiorum* Gust. (Van de Beek 2021; Sochor et al. 2022), needs to be confirmed, as no material from tropical Africa was available for this study.

A different pattern was observed in the other four native species. *Rubus apetalus* is well differentiated for both ITS and cpDNA data and is not participating in the current evolution of the other species. It is undoubtedly closely related to both *R. pinnatus* and *R. rigidus*. *Rubus transvaalensis* is even more closely related to

Table 2. Summary statistics of SSR data; N = number of individuals; G = number of genotypes identified at different mutation thresholds (th = 0 or 3 allowing no or up to three mutations within a genotype, respectively); Alleles = average number of observed alleles per individual across all nine loci (± standard deviation); Shannon index = Shannon information index computed in GenAlEx from a binary matrix (± standard error)

Taxon	N	G (th = 0)	G (th = 3)	Alleles (± S.D.)	Shannon index (± S.E.)
R. pinnatus	3	3	3	15 (± 3.454)	0.092 (± 0.022)
R. rigidus	6	6	6	19.5 (± 2.950)	0.285 (± 0.026)
R. bergii	17	4	1	17 (0)	0.012 (± 0.005)
R. bergii × *rigidus*	11	11	11	22.8 (± 1.940)	0.195 (± 0.025)
R. bergii × *pinnatus*	5	2	2	23.8 (± 0.448)	0.082 (± 0.018)
R. pinnatus × *rigidus*	1	1	1	20 (NA)	NA
R. aff. bergii	1	1	1	18 (NA)	NA

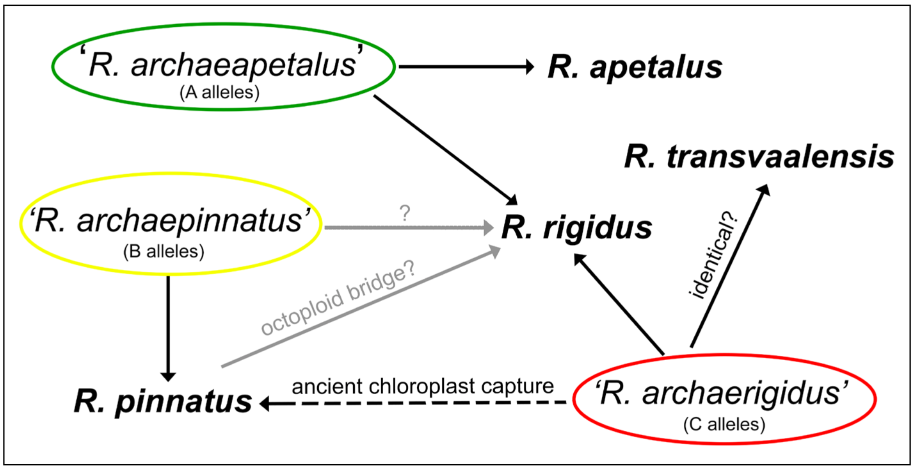

Figure 4. Scheme of proposed evolutionary relationships between modern species of the *R. rigidus–pinnatus–apetalus* group and their hypothetical ancestors; see text for explanation.

R. rigidus as inferred from phenotype (see Sochor et al. 2022) and DNA sequences (Figures 1, 2). Relationships between *R. pinnatus* and *R. rigidus* appear to be complex due to shared haplotypes and ribotypes, but in relation to the geographic distributions, this pattern cannot be explained simply by free recurrent gene flow. Taking into account the phylogenetic relationships among haplotypes and ribotypes and taxonomic and geographic distribution patterns, the following scenario can be hypothesised (Figure 4).

Three ancestral species, possibly already tetraploid or even hexaploid, migrated through the coastal regions from northeast to southwest, occasionally hybridised and further evolved into the species as currently recognised, although the ancestral species themselves disappeared. The first ancestor, '*R. archaeapetalus*', is represented in our data as the basal ribotypes and haplotypes of the A group (Figures 1A & 2A). This ancestor evolved directly into *R. apetalus* but must have contributed to the formation of *R. rigidus*, as implied from the A ribotypes throughout its range and the A haplotypes in the west (which, however, may have been derived also from the second ancestor despite the fact that the current geographic patterns rather contradict this possibility; Figures 1C & 1D). A second ancestor, '*R. archaepinnatus*' (B alleles) gave rise to *R. pinnatus* with considerable geographic genetic variation between the winter-rainfall and summer-rainfall zones but also contributed to the genome of *R. rigidus* to some extent, at least in the east (see ITS; Figure 2). A third ancestor, '*R. archaerigidus*' (C groups), must have had an identical ribotype (C1) and haplotype (*Rig5*) as *R. transvaalensis* and may have therefore also been very similar to this modern species in other respects (e.g., in hexaploidy?). This ancestor probably did not spread to westernmost South Africa as no traces of it have been detected in any modern taxon there. It must, however, have contributed to the formation of *R. rigidus* (mainly in eastern regions), of *R. transvaalensis*, and to a lesser degree also *R. pinnatus*. However, as far as we know, *R. pinnatus* only bears one haplotype derived from '*R. archaerigidus*' (*Pin3*). This haplotype differs from *Rig5* in the absence of one 6-bp repetition, which makes *Pin3* the basal-most haplotype within the C group. Therefore, the *Pin3* haplotype can only be a result of an ancient chloroplast capture, rather than a continuous gene flow from '*R. archaerigidus*' to *R. pinnatus*.

Similar reticulate evolution pathways are often observed in polyploid complexes. For example, Fehrer et al. (2009) revealed very complex evolutionary patterns in both diploid and polyploid accessions of European *Hieracium* s.str. (Asteraceae). Highly reticulate evolution associated with late Quaternary phylogeography of sexual ancestors was reconstructed in European blackberries, among which more than 750 species are recognised, but these originate in just around six ancestral diploids, some of them extinct (Sochor et al. 2015, 2017). However, hybridisation has long been recognised as an important process in plant evolution and speciation in general, not only in apomictic genera (Rieseberg 1995; Nolte & Tautz 2010).

Current gene flow among taxa seems to be limited

In our previous paper (Sochor et al. 2022), we reported on the occurrence of 12 hybrid combinations in South African brambles, some of which are locally even more frequent than their parents (e.g. *R. bergii* × *R. rigidus*). The hybrid origins of all of these taxa were supported by the molecular data presented here (Figure 3; see also Supplementary Table S1 for plastid haplotypes). The frequent occurrence of hybrids and the successful production of seeds and even the occurrence of facultative apomixis in some of them made us consider the evolutionary potential of hybridisation in South African brambles. Furthermore, two octoploid fertile sexual hybrids derived from *R. rigidus* (with *R. pinnatus* or *R.* sect. *Arguti*) were also discovered, which implies that such hybrids are not rare (the two specimens represented 4.5% of the 44 hybrid individuals with known ploidy in our dataset). Hypothetically, these octoploids could backcross with the tetraploid parent (2x gamete) due to the formation of regular reduced 4x gametes (see Sochor et al. 2022). The offspring (6x) would then share ploidy level and ± 50% of the genome with the first parent. Therefore, only two generations can be sufficient to overcome the ploidy reproduction barrier between tetra- and hexaploids.

Although potentially very effective and explanatory for the extraordinary phenotypic variability of *R. rigidus* (see Sochor et al. 2022), this 'octoploid bridge' (parallelism of triploid bridge sensu Ramsey & Schemske 1998) does not appear to be a common evolutionary mechanism, because no shared alleles have so far been detected between native and introduced taxa (except for the apparent hybrids), and only a few shared alleles were detected among native species. An example is the *Rig6* haplotype in *R. rigidus* near Alexandria, EC, where this haplotype is shared with *R. pinnatus*, but *R. rigidus* bears it in regions much further west (because of shared ancestry) and a transition zone was only documented in the eastern parts of WC (Figure 1D). Another possible example are the B1 ribotypes, which seem to originate from the *R. pinnatus*/'*archaepinnatus*' lineage but were found also in *R. rigidus* in KZN and MP, in all cases together with the C ribotypes in each individual. This last fact could imply that the five *R. rigidus* individuals (all confirmed hexaploids) can actually be early-generation introgressants, because the ribosomal cistron has not yet been homogenised. However, due to the rather limited sample set, we cannot rule out the possibility of ancient gene flow between the two species and the local preservation of genes of *R. pinnatus*/'*archaepinnatus*' in *R. rigidus*.

Genetic diversity is geographically structured in *R. pinnatus* and *R. rigidus*

Genotypic and allelic diversity and its structuring are crucial information for the management of both introduced and native taxa (especially those of conservation concern), and for understanding their evolutionary behaviour. Our data provide two perspectives. While DNA sequences from the conservative markers provide a wide and superficial overview, the population-genetic data from microsatellites enable much finer and deeper insights, but were restricted in this study to a single model system of *R. bergii*, *R. rigidus*, *R. pinnatus* and their hybrids.

From the wider, phylogeographic perspective, our sequence data imply relatively low genetic diversity in *R. thaumasius*, *R. apetalus*, *R. transvaalensis,* and also *R. ludwigii*. The latter was, however, included only marginally in this study and its geographic variation may not have been sampled. In contrast, *R. pinnatus* and particularly *R. rigidus* exhibit high diversity in plastid DNA and ITS, which is clearly geographically structured. This structuring appears to reflect not only the reticulate evolution discussed above, but also a long-term isolation of populations and limited gene flow among regions. The most conspicuous genetic differences are between the summer-rainfall and winter-rainfall zones (Figures 1 & 2), implying that the haplotypic/ribotypic geographical differentiation may have been accompanied by niche shift, which in turn may be associated with a slight phenotypic shift in *R. pinnatus*. These geographically linked differences justify its subdivision into two subspecies (Sochor et al. 2022).

In *R. rigidus*, on the other hand, major phenotypic traits (e.g. structure of leaves, fruit colour, leaf indumentum) do not correspond with haplotypes or ribotypes. Consequently, putatively distinct morphotypes (or species sensu Van de Beek 2021) are widespread across South Africa, but are obviously composed of diverse genotypes of different phylogenetic/genealogical history. In other words, taxonomic treatment of such morphotypes on the species level is contradicted not only by their obligate sexuality (Sochor et al. 2022), but also their diverse polytopic origin. A narrow species concept, such as that used in Europe for apomictic genotypes, is, therefore, clearly inapplicable in South African native taxa.

Clonality implies apomictic spread in *R. bergii* and *R. bergii* × *pinnatus*

Originally, we suspected the *R. bergii* × *R. rigidus* hybrids to be partly apomictic and able to persist and spread without recurrent formation of new genotypes via hybridisation (Sochor et al. 2018). This would result in the presence of the same genotype at different locations, and later in the dominance of one or a few successful hybrid genotypes within each region. However, no clonal (i.e., apomictic) duplication was detected among the 11 hybrid individuals in our dataset, despite the fact that the sampling was focused on a small area in westernmost WC (see Supplementary Table S3). This fact supports our later conclusion (Sochor et al. 2022) that these pentaploid hybrids are possibly exclusively sterile and can persist and spread only via vegetative means. On the other hand, the high frequency of occurrence of the hybrid in some regions implies its easy and common recurrent formation.

Surprisingly, clonal duplications were identified in the hybrid *R. bergii* × *pinnatus*, although this was not in our primary focus and was therefore represented by only five individuals in our SSR data set. Four of the individuals turned out to belong to a single genotype (Table 2; Supplementary Table S3). The sampled area was very small with distances between the individuals of the clone being 0.33–1.36 km. Such distances, however, seem to be too long to be explained by the spontaneous vegetative spread. As human-mediated propagation can be most likely excluded, the most probable explanation for our finding is asexual dispersal via seeds – apomixis. We have reported on apomictic seeds in two other homoploid hybrids between native and introduced *Rubus* taxa (*R. bergii* × *thaumasius* and *R. pinnatus* × sect. *Arguti*; Sochor et al. 2022) but it was not clear whether these seeds were viable and able to secure dispersal.

Similarly, Clark and Jasieniuk (2012) detected (rare) hybridisation among native and introduced *Rubus* taxa in western United States, as well as apomixis at the level of the embryo. However, seedlings derived from the hybrids exhibited higher allelic variation than would be expected for apomictic offspring, and apomixis, therefore, was not confirmed on the level of seedlings. In contrast, the frequent occurrence of hybrids between *Taraxacum officinale* (alien) and *T. japonicum* (native) (Asteraceae) was reported in western Japan despite a very low hybridisation rate (Matsuyama et al. 2018). The number of hybrid genotypes detected in that study in natural populations was surprisingly high but still indicated their apomictic spread. A combination of apomixis, high genotypic diversity, and hybrid origin from a native species seemed to promote effective natural selection and propagation of well-adapted genotypes, and thus enhanced invasiveness.

Rubus bergii × *pinnatus*, as well as the other two hybrids with apomictic seeds, is only locally common and of rather low importance as an invader at this moment. However, these hybrids may potentially pose an initial phase of new invasion that can take advantage of local adaptations of the native parent (Pfennig et al. 2016), clonal multiplication of a superior genotype (Parepa et al. 2014), potential hybrid vigour (Ayres 2004) or simply of being an evolutionary novelty (Ellstrand & Schierenbeck 2006). Targeted sampling of the tetraploid hybrids with subsequent assessment of genotypic diversity and invasive potential is required to evaluate this hypothesis.

High genotypic and allelic diversity were detected in *R. rigidus* (Table 2), three or four alleles per locus and individual being no exception, which is consistent with its sexual mode of reproduction and allopolyploid origin. In contrast, *R. bergii* was confirmed to be monoclonal with no signs of recombination or introgression from other taxa, yet with relatively high allelic diversity (reflecting its allopolyploid origin; Table 2). Monoclonality in our dataset also confirmed the identity of South African *R. bergii* and European plants usually treated under the name *R. vigorosus* P.J.Müll. & Wirtg. (Kurtto et al. 2010; Van de Beek 2014). Such extremely low genotypic diversity is consistent with data from other apomictic *Rubus* microspecies (Király et al. 2017; Šarhanová et al. 2017). Although the monoclonality is contradictory to the relatively high proportion of sexually derived embryos as detected by flow cytometric seed screen in the demonstrably monoclonal microspecies (cf. Šarhanová et al. 2012; Sochor et al. 2022), this paradox appears to be a common phenomenon in *Rubus*, so far without explanation (see also Šarhanová et al. 2017). Similar patterns are therefore presumed to occur in other South African alien apomictic blackberries such as *R. armeniacus* and *R.* sect. *Cuneifolii* (both likely monoclonal in South Africa), and *R.* sect. *Arguti* with two widespread clones and several genotypes of local occurrence (Sochor et al., 2022).

Conclusion

South Africa is not a hotspot for *Rubus* diversity, but the genus is taxonomically challenging and has been rather overlooked in this region (Van de Beek 2021; Sochor et al. 2022). A combination of traditional phenotype-based, molecular, and cytometric methods have improved our understanding of its diversity and evolutionary behaviour.

Contrary to previous concerns and notions that the group (or at least some of the taxa) is a hardly intelligible tangle (Sochor et al. 2018; Van de Beek 2021), the biosystematics of South African *Rubus* is not intractable. Despite frequent hybridisation, gene flow among modern species appears to be weak, as the hybrids mostly do not contribute to further evolution via hybridogenesis or introgression. However, clonal duplications and asexually derived seeds detected in tetraploid hybrids of native and introduced taxa may indicate incipient new plant invasions, and this process deserves further attention.

High phenotypic variability in some species, which has caused much confusion, can readily be explained by their allopolyploid origin and phylogeographic patterns. For example, the extreme variability in *R. rigidus* seems to be caused by: 1) its hexaploidy; 2) its origin in (at least) three ancestral species (Figure 4); 3) among-population isolation and subsequent differentiation particularly between winter-rainfall and summer-rainfall zones but also within the zones to some extent; and 4) probably weak but possibly continuous gene flow from other species, such as *R. pinnatus* and *R. transvaalensis*.

The data presented here and in our previous papers are not exhaustive and should be regarded rather as a foundation for further studies. Besides the invasive potential of the tetraploid hybrids, the most challenging task for the future is to unearth evolutionary links between the South African and tropical African *Rubus* flora, as well as better characterise the diversity of alien, particularly North American taxa, which seem to be quite rich, yet underexplored in the eastern regions of South Africa. However, our experiences show that new and often surprising discoveries can be expected around every corner of (not only) South African batology.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

MS performed the sampling, laboratory work, data analyses and wrote the first draft of the manuscript, JCM contributed to the fieldwork and data interpretations, and edited the manuscript.

Ethical considerations

This article followed all ethical standards for research without direct contact with human or animal subjects.

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Data availability statement

All sequences were deposited in NCBI GenBank (accession numbers OL899048–OL899299 [ITS], OL954095–OL954503 [*matK* and *trnL-trnF*]). SSR data matrix is available in Supplementary table S3. Herbarium vouchers are deposited in public herbaria OL, NBG, PRE, NU and L.

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Supplementary Material

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
RSA01/17	*R. bergii*	SA, WC, Table Mt., Cape Town, Valley of Isolation, ca. 800 m NW of Waterworks Museum, at S oriented rock wall at a tourist path	890	-33.970	18.402	3/11/2017	4×*	OL, NBG	Ulm1			dir. seq.	
RSA02/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Table Mt., Cape Town, Skeleton Gorge, 300 m SE of the beginning of Hely-Hutchinson dam	660	-33.980	18.417	3/11/2017	4×	OL, NBG	Pin1	B2		dir. seq.	
RSA04/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Table Mt., Cape Town, Woodhead Dam, 60 m W of Waterworks Museum	740	-33.975	18.407	3/12/2017	4×	OL	Pin1		1		
RSA05/17	*R. bergii* × *pinnatus*	SA, WC, Cape Town, Newlands, Riverside Road	38	-33.985	18.445	3/15/2017	4×	OL, NBG	Pin1		1		
RSA06/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Cape Town, Rondebosch, 300 m NW of the Rhodes Memorial, at the road	209	-33.951	18.456	3/16/2017		OL, NBG	Pin1	B2	1	dir. seq.	
RSA07/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Cape Town, Rondebosch, 750 m W of the Rhodes Memorial	351	-33.953	18.451	3/16/2017			Pin1				
RSA08/17	*R. bergii*	SA, WC, Cape Town, Rondebosch, 750 m W of the Rhodes Memorial	351	-33.953	18.451	3/16/2017		NBG	Ulm1		1	dir. seq.	
RSA09/17	*R. bergii* × *pinnatus*	SA, WC, Cape Town, Kirstenbosch arboretum, SW margin at the stream	94	-33.989	18.437	3/16/2017	4×	NBG	Pin1		1		
RSA10/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Cape Town, Kirstenbosch arboretum, S margin at the stream	84	-33.989	18.439	3/16/2017	4×*	OL	Pin1				

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
RSA11/17	*R. bergii* × *pinnatus*	SA, WC, Cape Town, Kirstenbosch arboretum, central part	90	-33.988	18.440	3/16/2017		n/a			1		
RSA12/17	*R. titanus*	SA, WC, Cape Town, at the road Kirstenbosch - Hout Bay, behind the crossroads to Alphen	215	-34.008	18.413	3/18/2017	6×	OL, NBG	Urs1			dir. seq.	
RSA13/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Hout Bay, NE end of the town, at the road to Constantia	48	-34.015	18.384	3/18/2017		NBG	Pin1		1		
RSA14/17	*R. armeniacus*	SA, WC, Hout Bay, at the road Kirstenbosch - Hout Bay, 0.5 km W of Constantia Neck	176	-34.012	18.400	3/18/2017	4×	OL, NBG	Dol1				
RSA15/17	*R. bergii* × *pinnatus*	SA, WC, Cape Town, at the road Kirstenbosch - Hout Bay, at the crossroads to Alphen	182	-34.007	18.418	3/18/2017	4×	OL, NBG	Pin1		1		
RSA16/17	*R. bergii*	SA, WC, Stellenbosch, Devonvallei, at the main road to Cape Town	87	-33.948	18.819	3/21/2017		NBG	Ulm1		1	dir. seq.	
RSA17/17	*R. bergii*	SA, WC, Stellenbosch, base of Pappegaaiberg, S of the top	98	-33.940	18.845	3/21/2017	4×	OL, NBG	Ulm1		1	cloned	
RSA18/17	*R. bergii* × *rigidus*	SA, WC, Stellenbosch, Idasvallei, 0.6 km SW of Idasvallei Dam	186	-33.923	18.896	3/21/2017	5×	OL, NBG	Rig6		1		
RSA19/17	*R. bergii*	SA, WC, Kylemore, at the road Stellenbosch - Pniel	264	-33.912	18.944	3/21/2017	4×	NBG	Ulm1		1		
RSA20/17	*R. bergii* × *rigidus*	SA, WC, Kylemore, at the road Stellenbosch - Pniel	264	-33.912	18.944	3/21/2017	5×	OL, NBG	Rig2		1	dir. seq.	
RSA21/17	*R. bergii* × *rigidus*	SA, WC, at the road R45 between Simondium and Drakenstein	164	-33.858	18.972	3/21/2017		OL, NBG	Rig6		1	dir. seq.	
RSA22/17	*R. bergii*	SA, WC, at the road R45 between Simondium and Cillie	127	-33.816	18.951	3/21/2017		NBG	Ulm1		1		

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
RSA23/17	*R. bergii*	SA, WC, Paarlsberg, at the picnic place 0.9 km NE of the top	379	-33.735	18.947	3/21/2017	4×*	OL	Ulm1		1		
RSA24/17	*R. bergii*	SA, WC, Paarlsberg, 2 km S of the top, under Victoria Dam	241	-33.759	18.948	3/21/2017		NBG	Ulm1		1		
RSA25/17	*R.* sp. *(North American)*	SA, WC, Kogelberg Nature Reserve, 300 m W of Visitors Centre	41	-34.323	18.964	3/22/2017	7×	OL, NBG	Urs1			cloned	
RSA26/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Kogelberg Nature Reserve, 500 m NW of Visitors Centre	24	-34.319	18.964	3/22/2017	4×	OL, NBG	Pin1				
RSA27/17	*R. pinnatus* × *rigidus*	SA, WC, at the road R44 (Kleinmond - Bot River), N of Arabella Country Estate	31	-34.304	19.135	3/22/2017	5×	OL, NBG	Rig3	A+B2	1	cloned	
RSA28/17	*R. bergii* × *rigidus*	SA, WC, at the road R44 (Kleinmond - Bot River), N of Arabella Country Estate	31	-34.304	19.135	3/22/2017	5×	OL, NBG	Rig3	A	1	cloned	
RSA29/17	*R. bergii*	SA, WC, ca. 8.7 km NE of Kleinmond, Elgin Valley, among vineyards	411	-34.280	19.086	3/22/2017		OL	Ulm1		1	dir. seq.	
RSA30/17	*R. bergii* × *rigidus*	SA, WC, Elgin Valley,10 km NNE of Kleinmond, among orchards at the stream	138	-34.254	19.054	3/22/2017	5×	OL, NBG	Rig3		1	dir. seq.	
RSA31/17	*R. bergii* × *rigidus*	SA, WC, Grabouw, Highlands Road	235	-34.216	19.055	3/22/2017		n/a	Rig6		1		
RSA32/17	*R. bergii* × *pinnatus*	SA, WC, Kirstenbosch Botanical Garden, 200 m NW of the N entrance, in forest	170	-33.985	18.430	3/23/2017	4×	OL, NBG	Pin1		1	dir. seq.	
RSA33/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Constantia, between Spilhaus Ravine and Constantia Nek, at Contour Path	325	-34.002	18.416	3/26/2017		OL	Pin1	B2		dir. seq.	
RSA34/17	*R. armeniacus*	SA, WC, 450 m NNE of Constantia Nek	254	-34.008	18.408	3/26/2017	4×	OL, NBG	Dol1				

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
RSA35/17	*R. bergii*	SA, WC, Table Mt., Disa Gorge	668	-33.978	18.399	3/26/2017		n/a	Ulm1		1		
RSA36/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Table Mt., Disa Gorge	668	-33.978	18.399	3/26/2017		n/a	Pin1	B2		dir. seq.	
RSA37/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Table Mt., Disa Gorge	690	-33.978	18.401	3/26/2017		n/a	Pin1				
RSA38/17	*R. bergii*	SA, WC, Table Mt., Cape Town, Woodhead Dam, 60 m W of Waterworks Museum	740	-33.975	18.407	3/26/2017	4×	NBG	Ulm1		1		
RSA39/17	*R. bergii* × *rigidus*	SA, WC, at the road N2 between Patryslaagte and Houwhoek (E of Grabouw)	237	-34.192	19.110	3/29/2017	5×	OL, NBG	Rig3	A	1	cloned	
RSA40/17	*R. rigidus*	SA, WC, at the road R43, 6.2 km NNE of Fisherhaven	11	-34.306	19.146	3/29/2017	6×	OL, NBG	Rig3	A	1	dir. seq.	
RSA41/17	*R. bergii* × *rigidus*	SA, WC, at the road between Sandbaai and Caledon, Creation	244	-34.333	19.331	3/29/2017	5×	OL, NBG	Rig3		1	dir. seq.	
RSA42/17	*R. bergii* × *rigidus*	SA, WC, Genadendal, crossroads on SW margin of the town	231	-34.049	19.553	3/29/2017		OL, NBG	Rig6		1		
RSA43/17	*R. bergii*	SA, WC, Genadendal, bushes 240 m SW of the Moravian Church	257	-34.036	19.556	3/29/2017		OL, NBG	Ulm1		1		
RSA44/17	*R. bergii* × *rigidus*	SA, WC, at the road between Genadendal and Helderstroom (at the river in the middle of the distance)	237	-34.062	19.438	3/29/2017	5×	OL, NBG	Rig6		1	dir. seq.	
RSA45/17	*R. bergii* × *rigidus*	SA, WC, Helderstroom	253	-34.066	19.370	3/29/2017		n/a			1		
RSA47/17	*R. pinnatus* subsp. *pinnatus*	SA, Betty's Bay Botanical Garden, 500 m NW of the entrance	66	-34.348	18.925	4/4/2017	4×	NBG	Pin1	B2		cloned	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
RSA48/17	*R. titanus*	SA, Betty's Bay Botanical Garden, 170 m NW of the entrance	34	-34.351	18.926	4/4/2017	6×	NBG	Urs1			cloned	
MS02/18	*R.* sp. *(North American)*	SA, KZN, Royal Natal, Tugela Valley, 0.4 km SSE of Thendele Upper Camp, secondary/disturbed woodland	1490	-28.713	28.935	2/20/2018	6×	OL	Fla1			cloned	
MS03/18	*R. ludwigii*	SA, KZN, Royal Natal, Tugela Valley, 0.4 km SSE of Thendele Upper Camp, secondary/disturbed woodland	1490	-28.713	28.935	2/20/2018	2×	OL	Lud1			dir. seq.	
MS04/18	*R. rigidus*	SA, KZN, Royal Natal, Tugela Valley, 0.5 km S of Thendele Upper Camp, primary montane grassland/woodland	1530	-28.715	28.935	2/20/2018	6×	OL	Rig4	A	1	dir. seq.	
MS05/18	*R.* sect. *Arguti*	SA, KZN, Howick, 1.5 km W of the city centre, ruderal shrubland at the river	1021	-29.486	30.218	2/22/2018	4×	OL	Arg1				*R. originalis* sensu Beek
MS06/18	*R.* sect. *Arguti*	SA, KZN, top of Beacon Hill, edge of Eucalyptus plantation	1175	-29.471	30.212	2/22/2018	4×	OL	Arg1			cloned	*R. originalis* sensu Beek
MS07/18	*R.* sect. *Cuneifolii*	SA, KZN, top of Beacon Hill, edge of Eucalyptus plantation	1168	-29.471	30.211	2/22/2018	4×	OL	Cun2				
MS08/18	*R. apetalus*	SA, KZN, Karkloof, 0.4 km N of Canopy Tours, primary forest	1224	-29.319	30.261	2/22/2018	4×	OL	Ape2	D		dir. seq.	
MS09/18	*R. pinnatus* subsp. *pappei*	SA, KZN, Karkloof, 0.2 km NW of Canopy Tours, primary forest	1197	-29.321	30.262	2/22/2018	4×	OL	Pin3	B3		cloned	
MS10/18	*R.* sect. *Cuneifolii*	SA, KZN, Karkloof, 0.2 km S of Canopy Tours, edge of dirt road	1155	-29.324	30.263	2/22/2018	4×	OL	Cun2				

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	**Species**	**Locality**	**Elevation**	**GPS**	**GPS**	**Date**	**Ploidy**	**Voucher in**	**Haplotype**	**Ribotype (pinnatus–rigidus group)**	**Used for SSR**	**ITS analysis**	**Note**
MS11/18	*R.* sect. *Cuneifolii*	SA, KZN, 1.25 km NNW of Karkloof Waterfall, edge of Eucalyptus plantation	1073	-29.390	30.273	2/22/2018	4×	OL	Cun2			cloned	
MS12/18	*R.* cf. sect. *Procumbentes* × *rigidus*	SA, KZN, Assagay, Assagay Crescent street, at a road	675	-29.784	30.740	2/23/2018	7×	OL	Rig1				
MS13/18	*R. rigidus*	SA, KZN, Assagay, Assagay Crescent street, at a road	675	-29.784	30.739	2/23/2018	6×	OL, PRE	Rig1	C3	1	cloned	
MS14/18	*R. ellipticus*	SA, KZN, Assagay, Assagay Crescent street, at a road	675	-29.785	30.738	2/23/2018	2×	OL	Eli1			dir. seq.	
MS15/18	*R. ellipticus*	SA, KZN, Assagay, N of Assagay Road, secondary shrubby vegetation near an ornamental garden	672	-29.769	30.750	2/23/2018	2×	OL	Eli1				
MS16/18	*R. rigidus*	SA, KZN, Waterfall town, Brackenhill Rd., roadside/riverbank	544	-29.746	30.814	2/23/2018	6×	OL	Rig4	C3		dir. seq.	
MS17/18	*R. rigidus*	SA, KZN, N of Hillcrest, Ngwele Rd., roadside	643	-29.752	30.778	2/23/2018	6×	OL, PRE	Rig7	C3	1	dir. seq.	
MS18/18	*R. rigidus*	SA, KZN, E of Camperdown, side of highway	736	-29.731	30.540	2/23/2018	6×	OL, PRE	Rig5	C3		dir. seq.	
MS19/18	*R. ludwigii*	SA, KZN, at road R617, N of Ncwadi	1376	-29.717	29.957	2/24/2018	2×	OL, PRE	Lud1				
MS20/18	*R.* cf. sect. *Allegheniensis*	SA, KZN, at road R617, N of Ncwadi	1376	-29.717	29.957	2/24/2018	6×*	OL	Fla1			cloned	
MS21/18	*R. transvaalensis*	SA, KZN, at road R617, N of Ncwadi	1376	-29.717	29.957	2/24/2018	6×	OL, PRE	Rig5	C1		dir. seq.	
MS22/18	*R. apetalus*	SA, KZN, N part of Marutswa Nature Reserve E of Bulwer, at primary forest margin	1459	-29.808	29.787	2/24/2018	4×	OL	Ape2	D		cloned	
MS23/18	*R.* sect. *Cuneifolii*	SA, KZN, N part of Marutswa Nature Reserve E of Bulwer, at primary forest margin	1470	-29.808	29.786	2/24/2018		OL	Cun2				

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS24/18	*R.* aff. *bergii*	SA, KZN, Ingelabantwana Nature Reserve N of Bulwer, between forest and Eucalyptus plantation	1423	-29.725	29.744	2/24/2018	4×	OL	Ulm2		1		
MS25/18	*R. apetalus*	SA, KZN, Ingelabantwana Nature Reserve N of Bulwer, forest margin	1424	-29.726	29.745	2/24/2018	4×	OL, PRE	Ape2				
MS26/18	*R. rigidus* × sect. *Arguti*	SA, KZN, at the road NE of Ingelabantwana Nature Reserve, N of Bulwer	1409	-29.725	29.749	2/24/2018	5×	OL	Rig4				
MS27/18	*R. ludwigii* × *pinnatus*	SA, KZN, at the road E of Ingelabantwana Nature Reserve, N of Bulwer	1225	-29.734	29.760	2/24/2018	3×	OL	Lud1				
MS28/18	*R.* sect. *Arguti*	SA, KZN, Howick, below the waterfall, in forest gap	907	-29.485	30.239	2/25/2018	4×	OL	Arg1				*R. originalis* sensu Beek
MS29/18	*R. niveus*	SA, KZN, Howick West, junction of Main Rd. and Ogilvie Rd.	1046	-29.509	30.232	2/25/2018	2×	OL	Niv1				
MS30/18	*R. niveus*	SA, KZN, at former railway Merrivale - Howick	1043	-29.502	30.236	2/25/2018	2×	OL	Niv1				
MS31/18	*R.* sect. *Cuneifolii*	SA, KZN, Howick, pasture/meadow W of the town	1038	-29.485	30.204	2/26/2018		OL	Cun2				
MS32/18	*R.* sect. *Arguti*	SA, KZN, Howick, roadside/marsh W of the town	1024	-29.484	30.205	2/26/2018	4×	OL	Arg1				*R. originalis* sensu Beek
MS33/18	*R. trichogynus*	SA, KZN, Howick, N end of Park Rd., garden margin	1037	-29.483	30.226	2/26/2018	7×	OL	Urs1			cloned	
MS34/18	*R. ludwigii*	SA, KZN, Giant's Castle, Langalibalele Ridge, montane grassland	2162	-29.286	29.478	2/28/2018	2×	OL	Lud1				
MS35/18	*R.* sect. *Arguti*	SA, KZN, W of Estcourt, at White Mountain Resort, in secondary Acacia bushveld	1550	-29.107	29.614	3/1/2018	4×	OL	Arg1				*R. revealii* sensu Beek
MS36/18	*R.* sect. *Cuneifolii*	SA, KZN, W of Estcourt, at White Mountain Resort, in secondary Acacia bushveld	1550	-29.107	29.613	3/1/2018	4×	OL	Cun2				

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS37/18	*R. rigidus*	SA, KZN, Drakensberg, 0.5 km S of Injasuti Camp, roadside in montane grassland	1449	-29.123	29.440	3/4/2018	6×	OL	Rig8	A	1	dir. seq.	
MS38/18	*R. ulmifolius*	SA, KZN, in pass 5 km S of Charlestown (near Volksrust), in a small ravine at margin of pine plantation	1680	-27.461	29.873	3/4/2018	2×	OL, PRE	Ulm2			dir. seq.	
MS39/18	*R. ulmifolius*	SA, MP, Volksrust, behind houses in the 1st Ave, at a wall	1707	-27.373	29.867	3/4/2018	2×	OL	Ulm2				
MS40/18	*R. rigidus* × sect. *Arguti*	SA, MP, at road R37 near its junction with R539, pine plantation margin	1082	-25.288	30.766	3/5/2018	5×	OL, PRE	Rig5				
MS41/18	*R. rigidus* × sect. *Arguti*	SA, MP, forest fragment 2.5 km NNW of Hendriksdal	1448	-25.169	30.764	3/5/2018	5×	OL	Rig5				
MS42/18	*R. pinnatus* subsp. *pappei*	SA, MP, forest fragment 2.5 km NNW of Hendriksdal	1448	-25.169	30.764	3/5/2018	4×	OL	Rig6	B1		dir. seq.	
MS43/18	*R. rigidus* × sect. *Arguti*	SA, MP Graskop, forest margin S of Panorama Camp	1438	-24.949	30.846	3/6/2018	8×	OL, PRE	Rig5				
MS44/18	*R. niveus*	SA, MP, Graskop, forest margin S of Panorama Camp	1438	-24.949	30.846	3/6/2018	2×	OL	Niv1				
MS45/18	*R. apetalus* × *pinnatus*	SA, MP, Graskop, forest margin S of Panorama Camp	1435	-24.949	30.846	3/6/2018	4×	OL, PRE	Ape2	B3+D		dir. seq.	
MS45B/18	*R. pinnatus* subsp. *pappei*	SA, MP, Graskop, forest margin S of Panorama Camp	1435	-24.949	30.846	3/6/2018	4×	OL	Rig6	B1		dir. seq.	
MS46/18	*R. rigidus*	SA, MP, Graskop, forest margin on edge of the kloof W of the waterfall	1400	-24.944	30.841	3/6/2018	6×	OL, PRE	Rig5	B1+C2	1	cloned	
MS47/18	*R. rigidus*	SA, MP, Graskop, at road R533 in S part of the town	1421	-24.947	30.843	3/6/2018	6×	OL	Rig5	B1+C2		cloned	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS47B/18	*R. rigidus*	SA, MP, Graskop, at road R533 in S part of the town	1421	-24.947	30.843	3/6/2018	6×	OL	Rig5	B1+C2		dir. seq.	
MS48/18	*R.* sect. *Arguti* × *pinnatus*	SA, MP, Graskop, 300 m SE of Panorama Camp, overgrown pasture/grassland	1428	-24.949	30.846	3/6/2018	4×	OL, PRE	Rig6	B1		cloned	
MS49/18	*R. rigidus* × sect. *Arguti*	SA, MP, Graskop, 250 m SSW of the waterfall, in secondary shruby vegetation	1414	-24.945	30.839	3/6/2018	5×	OL, PRE	Rig5				
MS50/18	*R. rigidus* × *ulmifolius*	SA, MP, Graskop, 250 m SSW of the waterfall, in secondary shruby vegetation	1414	-24.945	30.839	3/6/2018	4×	OL, PRE	Rig5				
MS51/18	*R. rigidus* × sect. *Arguti*	SA, MP, Graskop, edge of pine plantation at the Pinnacle rock	1443	-24.913	30.852	3/7/2018	5×	OL	Rig5				
MS52/18	*R. rigidus* × *ulmifolius*	SA, MP, Graskop, at the Pinnacle rock, grassland	1439	-24.912	30.853	3/7/2018	4×	OL	Rig5	B1		cloned	
MS53/18	*R. rigidus* × sect. *Arguti*	SA, MP, 7 km N of Graskop, junction of R532 and R534, roadside in pine plantations	1429	-24.868	30.847	3/7/2018	5×	OL	Rig5				
MS54/18	*R. pinnatus* subsp. *pappei*	SA, MP, 3.4 km W of Graskop, 0.9 km S of junction of R532 and R533, roadside in (secondary?) forest	1480	-24.939	30.811	3/7/2018	4×	OL	Rig6	B1		dir. seq.	
MS55/18	*R. rigidus* × sect. *Arguti*	SA, MP, 130 m NE of Mary Shire Falls SW of Graskop, roadside between forest and pine plantation	1261	-24.984	30.812	3/7/2018	5×	OL	Rig5				
MS58/18	*R. ulmifolius*	SA, MP, Graskop, pastures at electric transformer station	1431	-24.935	30.843	3/8/2018	2×	OL	Ulm1				

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS59/18	*R. rigidus* × *ulmifolius*	SA, MP, Graskop, pastures at electric transformer station	1431	-24.935	30.843	3/8/2018	4×	OL	Rig5				
MS60/18	*R. rigidus*	SA, MP, view point 3.4 WNW of Pilgrimsrest, montane grassland	1508	-24.881	30.725	3/8/2018	6×	OL	Rig5	B1+C2		cloned	
MS61/18	*R. apetalus* × *ludwigii*	SA, MP, roadside 14 km SSE of Lydenburg	1740	-25.222	30.478	3/8/2018	3×	OL	Lud1	D		cloned	
MS62/18	*R. transvaalensis*	SA, MP, Buffelskloof Nature Reserve, secondary shrubland 1.6 km S of headquarters	1692	-25.303	30.522	3/8/2018	6×	OL	Rig5	C1		cloned	
MS63/18	*R. apetalus*	SA, MP, Buffelskloof Nature Reserve, secondary shrubland 1.6 km S of headquarters	1692	-25.303	30.522	3/8/2018	4×	OL	Ape2	D		dir. seq.	
MS64/18	*R. pinnatus* subsp. *pappei*	SA, MP, Buffelskloof Nature Reserve, 2.5 km NNW of headquarters, forest	1861	-25.267	30.518	3/9/2018	4×	OL	Rig6				
MS65/18	*R. pinnatus* subsp. *pappei*	SA, MP, Buffelskloof Nature Reserve, 2.5 km NNW of headquarters, forest	1861	-25.267	30.518	3/9/2018	4×	OL	Rig6	B1		cloned	
MS66/18	*R. niveus* × *transvaalensis*	SA, MP, Buffelskloof Nature Reserve, 300 m W of headquarters, riverside	1752	-25.289	30.521	3/9/2018	4×	OL	Niv1	C1		cloned	
MS67/18	*R. apetalus*	SA, MP, Buffelskloof Nature Reserve, N margin of headquarters, grassland	1768	-25.288	30.524	3/9/2018	4×	OL	Ape2				
MS68/18	*R.* sect. *Arguti*	SA, MP, Buffelskloof Nature Reserve, 0.5 km N of headquarters, roadside/pine plantation margin	1782	-25.284	30.524	3/10/2018	4×	OL	Arg1				*R. originalis* sensu Beek
MS01/20	*R. rigidus*	SA, KZN, Vryheid Hill, 500 m S of North Gun Point, margin between grassland and woodland	1403	-27.743	30.790	3/6/2020	6×	NBG	Rig11	A		dir. seq.	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS02/20	*R.* sect. *Cuneifolii*	SA, KZN, Vryheid Hill, 400 m S of North Gun Point, margin between grassland and woodland	1428	-27.742	30.789	3/6/2020		NBG	Cun2				
MS03/20	*R. pinnatus* subsp. *pappei*	SA, KZN, Vryheid Hill, 400 m S of transmitting tower, grassland/woodland	1443	-27.745	30.794	3/6/2020		OL	Pin3	B3		dir. seq.	
MS04/20	*R. rigidus*	SA, KZN, Vryheid Hill, 900 m SSE of transmitting tower, roadside in forest	1276	-27.749	30.796	3/6/2020	6×	OL	Rig9	A		dir. seq.	
MS05/20	*R. apetalus*	SA, KZN, Vryheid Hill, 900 m SSE of transmitting tower, roadside in forest	1366	-27.749	30.796	3/6/2020		NBG	Ape1	D		dir. seq.	
MS06/20	*R. rigidus* × sect. *Arguti*	SA, KZN, Vryheid, W end of Noord Street, abandoned garden/orchard	1215	-27.757	30.789	3/6/2020	5×	OL	Rig9				
MS07/20	*R. rigidus*	SA, KZN, 6 km N of Melmoth, roadside	743	-28.530	31.402	3/6/2020	6×	OL	Rig5	C3		dir. seq.	
MS09/20	*R.* sect. *Arguti*	SA, KZN, Eshowe, at Fort Nonquai, park margin, potentially originally planted	496	-28.904	31.446	3/7/2020		NBG	Arg1				*R. originalis* sensu Beek
MS100/20	*R. bergii* × *rigidus*	SA, WC, Heidelberg, VanRiebeeck Street, E margin og the town, roadside among pastures	77	-34.092	20.967	3/24/2020	5×	OL	Rig6				
MS101/20	*R. bergii* × *rigidus*	SA, WC, Suurbraak, Tradouw Pass road, bank of Gootvaderbosch River, roadside	129	-34.005	20.707	3/24/2020	5×	OL	Rig6				
MS102/20	*R. bergii* × *rigidus*	SA, WC, Swellendam, at R60, 3 km W of the city centre	119	-34.022	20.415	3/25/2020	5×	OL	Rig6				
MS103/20	*R. bergii* × *rigidus*	SA, WC, Worcester, N2 roadside 10 km NE of the city centre	318	33.567	19.511	3/25/2020	5×	OL	Rig6				

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS104/20	*R. rigidus*	SA, WC, Paarl, at R101, 4.4 km SSE of Du Toitskloof Pass, roadside in fynbos	551	-33.729	19.100	3/25/2020	6×	OL	Rig6			dir. seq.	
MS106/20	*R. thaumasius*	SA, EC, at a road R345, 20 km NNE of Hogsback, roadside/Eucalyptus plantation	1390	-32.438	27.036	3/13/2020	4×	OL	Tha1			dir. seq.	
MS10A/20	*R. rigidus*	SA, KZN, 4 km SE of Eshowe, roadside in rural ladscape	339	-28.916	31.494	3/7/2020	6×	OL		C3		dir. seq.	
MS10B/20	*R. rigidus*	SA, KZN, 4 km SE of Eshowe, roadside in rural ladscape	339	-28.916	31.494	3/7/2020		OL	Rig5	C3		dir. seq.	
MS11/20	*R. rigidus*	SA, KZN, near R102, at a dirt (W) road to Obanjeni, woodland	54	-28.952	31.664	3/7/2020		NBG	Rig7	C3		dir. seq.	
MS12/20	*R. rigidus*	SA, KZN, Mtunzini, uMlalazi, T-junction N of camping place, roadside/reed bed	4	-28.956	31.768	3/7/2020	6×	OL	Rig5	C3		dir. seq.	
MS13/20	*R. rigidus*	SA, KZN, Mtunzini, Valley Drive, 1.5 km SW of former railway station, roadside between railway and Eucalyptus plantations	17	-28.971	31.747	3/8/2020	6×	OL	Rig5	C3		dir. seq.	
MS14/20	*R. rigidus*	SA, KZN, Shaka's Rock, roadside 600 m SE of N2 e×it 212	78	-29.506	31.215	3/8/2020	6×	OL	Rig10	C3		dir. seq.	
MS15/20	*R. rigidus*	SA, KZN, Shaka's Rock, roadside 500 m SE of N2 e×it 212	81	-29.505	31.214	3/8/2020	6×	OL	Rig10	C3		dir. seq.	
MS16/20	*R. pinnatus* subsp. *pappei*	SA, KZN, 21 km NNW of Tongaat, R614 roadside among fields	632	-29.409	30.801	3/8/2020		OL	Pin3	B3		dir. seq.	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS17/20	*R. rigidus*	SA, KZN, 21 km NNW of Tongaat, R614 roadside among fields	632	-29.395	31.018	3/8/2020	6×*	OL	Rig5	C3		dir. seq.	
MS18/20	*R. rigidus* × sect. *Arguti*	SA, KZN, 19 km ENE of Wartburg, R614 roadside among fields	1001	-29.371	30.757	3/8/2020		OL	Rig1				
MS19/20	*R. rigidus*	SA, KZN, 19 km ENE of Wartburg, R614 roadside among fields	1001	-29.371	30.759	3/8/2020	6×	NBG	Rig5	C3		dir. seq.	
MS20/20	*R. rigidus*	SA, KZN, 19 km ENE of Wartburg, R614 roadside among fields	1008	-29.371	30.756	3/8/2020	6×	OL	Rig5	C3		dir. seq.	
MS21/20	*R. rosifolius*	SA, KZN, Queen Elizabeth Park NW of Pietermaritzburg, N part of the reserve, bushes in abandoned plantations in a small valley	914	-29.562	30.317	3/9/2020	2×	OL	Ros1			dir. seq.	
MS23/20	*R.* aff. *bergii*	SA, KZN, Hilton, N end of Hayfields Road, heavily invaded anthropogenic habitats (R. aff. bergii, R. sect. Cuneifolii, R. sect. Arguti)	1119	-29.543	30.296	3/9/2020	4×*	NBG	Ulm2				
MS28/20	*R.* sect. *Arguti*	SA, KZN, 8 km WNW of Richmond, at a dirt road in Eucalyptus plantations	970	-29.854	30.198	3/10/2020		OL	Arg1				*R. originalis* sensu Beek
MS29/20	*R. rigidus*	SA, KZN, Mkomazi River Valley, 300 m N of Hela Hela camp	555	-29.906	30.098	3/10/2020	6×	OL	Rig9	B1+C3		dir. seq.	
MS30/20	*R. niveus*	SA, KZN, right bank of Mkomazi River, 400 m WNW of Hela Hela camp	553	-29.908	30.094	3/10/2020		NBG	Niv1			dir. seq.	
MS31/20	*R. ludwigii*	SA, KZN, E of Kokstad, at the road P570, 1 km from junction with N2, bushes among plantations	1286	-30.514	29.650	3/10/2020		OL	Lud1			dir. seq.	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS32/20	*R. rigidus*	SA, KZN, E of Kokstad, at the road P570, 1 km from junction with N2, bushes among plantations	1286	-30.514	29.650	3/10/2020	6×	NBG	Rig4	A		dir. seq.	
MS35/20	*R.* sect. *Cuneifolii*	SA, EC, Wild Coast, Mbizana, 1.2 km NNW of R394 and R61 junction	1008	-30.857	29.604	3/11/2020	4×*	OL	Cun2				
MS36/20	*R. pinnatus* subsp. *pappei*	SA, EC, Wild Coast, Lusikisiki area, 4.8 km NE of Magwa Falls, woodland	505	-31.416	29.676	3/11/2020		NBG	Rig6	B3		dir. seq.	
MS37/20	*R. rigidus*	SA, EC, Wild Coast, Lusikisiki area, 4.8 km NE of Magwa Falls, woodland	505	-31.416	29.676	3/11/2020	6×	NBG	Rig9	A		dir. seq.	
MS38/20	*R. pinnatus* subsp. *pappei*	SA, EC, Wild Coast, Port St John's area, right bank of the Bulolo River, 950 m N of its mouth	-10	-31.643	29.515	3/12/2020		NBG	Rig6	B3		dir. seq.	
MS39/20	*R. rigidus*	SA, EC, Wild Coast, Port St John's area, at a dirt road to Sileka Nature Reserve, 1 km NW of the Bulolo River Mouth	94	-31.648	29.508	3/12/2020	6×*	NBG	Rig4	A		dir. seq.	
MS40/20	*R. rigidus*	SA, EC, 3.5 km W of Komgha, R63 roadside	680	-32.587	27.856	3/13/2020	6×	OL	Rig9				
MS41/20	*R. rigidus*	SA, EC, Fort Cunynghame Forestry Station, 9 km N of Stutterheim, grasslands among tree plantations	973	-32.488	27.421	3/13/2020	6×	OL	Rig4	A		dir. seq.	
MS42/20	*R. pinnatus* subsp. *pappei*	SA, EC, Fort Cunynghame Forestry Station, 9 km N of Stutterheim, grasslands among tree plantations	971	-32.487	27.421	3/13/2020		NBG	Rig6	B3		dir. seq.	
MS43/20	*R. pinnatus* subsp. *pappei*	SA, EC, Fort Cunynghame, 10.5 km N of Stutterheim, roadside among tree plantations	1056	-32.475	27.413	3/13/2020		OL	Rig6	B3		dir. seq.	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS44/20	*R. bergii*	SA, EC, at a road R345, 15 km N of Hogsback, roadside/small ravine	1374	-32.466	26.965	3/13/2020	4×*	OL	Ulm1				
MS45/20	*R. rigidus*	SA, EC, at a road R345, 15 km N of Hogsback, roadside/small ravine	1371	-32.465	26.965	3/13/2020	6×*	OL	Rig4	A		dir. seq.	
MS46/20	*R. apetalus*	SA, EC, 1 km SE of Hogsback, forest gap	973	-32.602	26.947	3/14/2020	4×*	NBG	Ape2				
MS47/20	*R.* sect. *Arguti*	SA, EC, 900 m E of Hogsback, in tree plantations	1144	-32.598	26.948	3/14/2020	4×	OL	Arg1				*R. revealii* sensu Beek
MS48/20	*R. armeniacus*	SA, EC, 900 m E of Hogsback, stream bank in tree plantations	1137	-32.597	26.948	3/14/2020		NBG	Dol1			cloned	
MS49/20	*R. bergii* × *rigidus*	SA, EC, 900 m E of Hogsback, stream bank in tree plantations	1144	-32.595	26.947	3/14/2020	5×	OL	Rig9				
MS50/20	*R. thaumasius*	SA, EC, Hogsback, arboretum, stream bank	1230	-32.591	26.936	3/14/2020	4×	OL	Tha1			dir. seq.	
MS51/20	*R. trichogynus*	SA, EC, Hogsback, NW margin of the town, park/ garden	1275	32.592	26.930	3/14/2020	7×	OL	Urs1				
MS52/20	*R. bergii* × *thaumasius*	SA, EC, Hogsback, Hydrangea Lane, roadside	1178	-32.598	26.940	3/14/2020	4×	OL	Tha1				
MS53/20	*R. bergii* × *thaumasius*	SA, EC, Hogsback, Hydrangea Lane, roadside	1201	-32.599	26.941	3/14/2020	4×	OL	Tha1			cloned	
MS55/20	*R. armeniacus*	SA, EC, Hogsback, S margin of the town, Away With The Fairies camp garden	1163	-32.602	26.939	3/15/2020	4×*	NBG	Dol1				
MS57/20	*R. thaumasius*	SA, EC, Hogsback, arboretum, among bushes in tree plantations	1232	-32.590	26.937	3/15/2020	4×*	OL	Tha1			cloned	
MS58/20	*R. pinnatus* × *thaumasius*	SA, EC, Hogsback, arboretum, among bushes in tree plantations	1232	-32.590	26.937	3/15/2020		OL	Tha1				

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS59/20	*R. bergii* × *thaumasius*	SA, EC, Hogsback, arboretum, among bushes in tree plantations	1200	-32.590	26.933	3/15/2020	4×	OL	Tha1			cloned	
MS60/20	*R. rigidus* × sect. *Arguti*	SA, EC, Hogsback, arboretum, among bushes in a park	1208	-32.590	26.935	3/15/2020		OL	Rig9				
MS61/20	*R. phoenicolasius*	SA, EC, Hogsback, 200 m NW of the entrance to arboretum, roadside/margin of plantation	1221	-32.592	26.934	3/15/2020	2×	OL	Phe1			dir. seq.	
MS62/20	*R. bergii* × *rigidus*	SA, EC, Hogsback, 130 m SE of the entrance to arboretum, roadside/margin of plantation	1213	-32.594	26.937	3/15/2020		OL	Rig4				
MS63/20	*R.* sect. *Arguti* *(different sp.!)*	SA, EC, Hogsback, 350 m SE of the entrance to arboretum, garden hedge	1229	-32.595	26.938	3/15/2020		OL	Arg1				
MS64/20	*R. thaumasius*	SA, EC, Hogsback, S margin of the town, Away With The Fairies camp garden	1200	-32.603	26.939	3/16/2020	4×	OL	Tha1				
MS65/20	*R. rigidus*	SA, EC, Makhanda (Grahamstown), 2.1 km WSW of Albany Museum, grassland	654	-33.318	26.500	3/16/2020	6×*	OL	Rig4	A		dir. seq.	
MS66/20	*R. pinnatus* subsp. *pappei*	SA, EC, Makhanda (Grahamstown), 2.1 km WSW of Albany Museum, grassland	644	-33.318	26.500	3/16/2020		OL	Rig6	B3		dir. seq.	
MS67/20	*R. rigidus*	SA, EC, Makhanda (Grahamstown), 5.3 km SE of Albany Museum, roadside in thickets	346	-33.350	26.560	3/17/2020		OL	Rig4	A		dir. seq.	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS68/20	*R. pinnatus* subsp. *pappei*	SA, EC, Boesmanriviermond (Kenton on Sea area), 2.1 km WSW of the town cente, among bushes in pastures	27	-33.689	26.632	3/18/2020	4×	OL	Rig6	B3		dir. seq.	
MS69/20	*R. rigidus*	SA, EC, Cannon Rocks (Alexandria area), 15 km WSW of the Boknes River mouth, roadside in grassland/pastures	176	-33.754	26.428	3/18/2020	6×	NBG	Rig4				
MS70/20	*R. rigidus*	SA, EC, Alexandria, at the main facilities of Addo Woody Cape Section of Addo Elephant NP, grassland	176	-33.700	26.367	3/18/2020		OL	Rig6	A		dir. seq.	
MS71/20	*R. rigidus*	SA, EC, Groendal, Lower Blindekloof hiking trail, riverbank in forest	115	-33.704	25.302	3/19/2020	6×*	OL	Rig4	A		dir. seq.	
MS72/20	*R. pinnatus* subsp. *pappei*	SA, EC, Uitenhage area, 3.4 km NE of Witteklip Railway Station, R334 roadside	266	-33.892	25.292	3/20/2020		OL	Rig6	B3		dir. seq.	
MS73/20	*R. rigidus*	SA, EC, Uitenhage area, 3.4 km NE of Witteklip Railway Station, R334 roadside	266	-33.892	25.292	3/20/2020	6×*	OL	Rig4	A		dir. seq.	
MS74/20	*R. pinnatus* subsp. *pappei*	SA, EC, left bank of Van Stadens River at R102, park/forest	94	-33.912	25.196	3/20/2020		OL	Rig6	B3		dir. seq.	
MS75/20	*R. bergii* × *rigidus*	SA, EC, Humansdorp, Voortrekker Road, NE of hospital, roadside/park/pasture	127	-34.028	24.783	3/20/2020	5×	OL	Rig4				
MS76/20	*R. pinnatus* subsp. *pappei*	SA, EC, Humansdorp, at R102, 4 km WSW of the town centre	107	-34.034	24.728	3/20/2020		OL	Rig6	B3		dir. seq.	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS77/20	*R. bergii* × *rigidus*	SA, EC, Humansdorp, at R102, 4 km WSW of the town centre	107	-34.034	24.728	3/20/2020		OL	Rig4				
MS78/20	*R. bergii*	SA, EC, left bank of Elands River, at R102, roadside	229	-33.982	24.051	3/20/2020		OL	Ulm1				
MS79/20	*R. bergii* × *rigidus*	SA, EC, left bank of Elands River, at R102, roadside	229	-33.982	24.051	3/20/2020		NBG	Rig4				
MS80/20	*R. pinnatus* *(transitional)*	SA, EC, right bank of Elands River, at R102, roadside/plantation	230	-33.981	24.050	3/20/2020	4×*	OL		B2		dir. seq.	
MS81/20	*R. pinnatus* *(transitional)*	SA, EC, right bank of Elands River, at R102, 150 S of entrance to Wolf Sanctuary, roadside/pasture	237	-33.982	24.048	3/20/2020	4×*	OL	Rig6	B1		dir. seq.	
MS82/20	*R. rigidus*	SA, EC, right bank of Elands River, at R102, 150 S of entrance to Wolf Sanctuary, roadside/pasture	237	-33.982	24.048	3/20/2020		NBG	Rig4	A		dir. seq.	
MS83/20	*R. pinnatus* *(transitional)*	SA, EC, Storms River Mouth, at the main gate, forest/park	201	-34.011	23.869	3/21/2020	4×*	OL	Pin1	B1		dir. seq.	
MS84/20	*R. pinnatus* *(transitional)*	SA, WC, Nature's Valley, left bank of Groot River, at R102, roadside among forests	17	-33.967	23.560	3/21/2020		OL	Pin4	B1		dir. seq.	
MS85/20	*R. trichogynus*	SA, WC, rest place at N2 in the middle between Plettenberg Bay and Knysna, forest margin	265	-34.038	23.215	3/21/2020	7×	OL	Urs1				
MS86/20	*R. bergii* × *pinnatus*	SA, WC, Knysna Forest, 400 m W of King Edward VII Big Tree, roadside in forest	403	-33.957	23.148	3/22/2020		NBG	Rig6				
MS87/20	*R. pinnatus* *(transitional)*	SA, WC, Knysna Forest, 1.2 km W of King Edward VII Big Tree, roadside in forest	446	-33.958	23.140	3/22/2020	4×*	OL	Pin1	B1		dir. seq.	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS89/20	*R. bergii* × *pinnatus*	SA, WC, Knysna Forest, 800 m NNW of King Edward VII Big Tree, roadside in forest/old forest clearing	506	-33.950	23.148	3/22/2020		OL	Pin1				
MS90/20	*R. rigidus*	SA, WC, Knysna, S end of Gardiners Road, edge of plantation, stream bank	79	-34.031	23.047	3/22/2020	6×*	OL	Rig4	A		dir. seq.	
MS92/20	*R. bergii* × *rigidus*	SA, WC, Knysna, continuation of Lakeview Street, former plantation/garden	50	-34.033	23.053	3/22/2020	5×	OL	Rig4				
MS93/20	*R. bergii* × *rigidus*	SA, WC, Knysna area, Rheenendal Road, 5 km NNE of junction with N2, roadside among plantations	186	-33.992	22.985	3/23/2020		OL	Rig4				
MS94/20	*R. rigidus*	SA, WC, Sedgefield area, Seven Passes Road, 4 km E of Karatara, roadside among pastures	235	-33.918	22.882	3/23/2020	6×*	OL	Rig4	A		dir. seq.	
MS95/20	*R. pinnatus* subsp. *pinnatus*	SA, WC, Sedgefield area, Seven Passes Road, T-junction 1.5 km SW of Karatara, roadside	213	-33.928	22.825	3/23/2020		OL	Pin1	B1		dir. seq.	
MS96/20	*R. bergii* × *rigidus*	SA, WC, Sedgefield area, Barrington Link Road, 500 m NNW of junction with N2	6	-34.020	22.852	3/23/2020		OL	Rig6				
MS97/20	*R. rigidus*	SA, WC, Mosselbay area, at R327, 7.7 km NW of its junction with N2, roadside among grasslands	224	-34.141	21.948	3/24/2020	6×	OL	Rig4	A		dir. seq.	
MS98/20	*R. pinnatus* subsp. *pinnatus*	SA, WC, Riversdale area, at R323, 11 km NWN of Riversdale, roadside among plantations	416	-33.995	21.225	3/24/2020	4×	OL	Pin1	B2		dir. seq.	
MS-Pin1	*R. pinnatus* *(transitional)*	SA, WC, E of Knysna, Bitou Municipality	264	-34.038	23.215	3/21/2020		n/a	Rig6	B1		dir. seq.	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS-Pin2	*R. pinnatus (transitional)*	SA, WC, Storms River Mouth	106	-34.018	23.868	3/21/2020		n/a	Pin1	B1		dir. seq.	
MS-Pin3	*R. pinnatus (transitional)*	SA, WC, Knysna Forest	364	-33.947	23.142	3/22/2020		n/a	Pin1	B1		dir. seq.	
Young 2620	*R. rosifolius*	SA, KZN, road from Kranskop to Tugela River, 11.5 km from Kranskop				5/9/2017		NU	Ros1				
Beek2019.97	*R. rigidus*	SA, WC, Tulbagh, Winterhoek road	220	-33.241	19.144	11/21/2019	6×*	L		A		dir. seq.	*R. ecklonii* sensu Beek, epitype
Beek 2020.07	*R. rigidus*	SA, WC, along the R102 between Groot Brakrivier and George	160	-34.011	22.298	1/13/2020	6×*	L	Rig4	A		dir. seq.	*R. fertilis* A. Beek, holotype
Beek 2019.4	*R. rigidus*	SA, WC, Stellenbosch, Jonkershoek reserve, along the circular drive ± 1.5 or 2 km after the bridge at the right side	330	-33.987	18.964	1/23/2019	6×*	L	Rig6				*R.* cf. *chrysocarpus* "anthraco-carpus"
Beek 2019.39	*R. rigidus*	SA, WC, Stellenbosch, Jonkershoek Reserve, along the left turn off along the river from the circular drive, beginning short after the dam	290	-33.983	18.951	2/24/2019	6×*	L	Rig6	A		dir. seq.	*R. chrysocarpus* sensu Beek
Beek 2020.11	*R. pinnatus* subsp. *pappei*	SA, EC, Uitenhage, Van Stadenspas	275	-33.881	25.308	1/15/2020	4×*	L	Rig6				*R. anas* A.Beek, holotype
Beek 2019.100	*R. rigidus*	SA, WC, Grabouw, Vyeboom, along the R321	320	-34.036	19.174	11/28/2019	6×*	L	Rig3				*R. rigidus* sensu Beek, epitype
Beek 2019.94	*R. rigidus*	SA, WC, Citrusdal, south of Theerivier	240	-32.837	19.083	11/20/2019	6×*	L	Rig6	A		dir. seq.	*R. leptodytus* A.Beek
Beek 2020.05	*R. rigidus*	SA, WC, along the road from Swellendam	130	-34.012	20.620	1/13/2020	6×*	L	Rig4	A		dir. seq.	*R. mundtii* sensu Beek
Manning 3601	*R.* sect. *Arguti*	SA, KZN, Howick						NBG	Arg1				*R. originalis* sensu Beek

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
Manning 3602	*R. rigidus*	SA, KZN, Howick						NBG	Rig9	A+C3		dir. seq.	
Beek2018-15	*R. bergii* × *rigidus*	SA, WC, Stellenbosch, Helshoogte, entrance of Hillcrest	280	-33.912	18.942	2/24/2018		L	Rig2		1		
R151/11	*R. bergii*	Germany, Lower Sa×ony, Voltlage	50	52.430	7.753			OL	Ulm1		1		
VŽ-Vig1	*R. bergii*	Germany, Hessen, NE Jügesheim, Rodfeld umweit des Reitplatzes	135	50.035	8.903	8/27/2009		Žíla	Ulm1		1		
VŽ-Vig2	*R. bergii*	Germany, Hessen, SW Urberach, Thomashüttenschneise	195	49.956	8.773	8/26/2009		Žíla	Ulm1		1		
VŽ-Vig3	*R. bergii*	Germany, Hessen, S Mernes, grosses Feldkreuz und Scheune	290	50.225	9.488	10/1/2007		Žíla	Ulm1		1		
VŽ-Vig4	*R. bergii*	Germany, Hessen, Babenhausen, Staatstr.	125	49.980	8.929	9/3/2006		Žíla			1		
VŽ-Vig5	*R. bergii*	Germany, Lower Sa×ony, oppidum Recke, pagus Voltlage	40	52.430	7.753	8/16/2011		Žíla	Ulm1		1		

* analysed via FCSS from seed(s)

Supplementary Table S2. Primer sequences, PCR conditions and number of observed alleles for the used SSR loci

Working name	Original name	Sequence of F primer (5′-3′)	Sequence of R primer (5′-3′)	Ta [°C]	Cycles	Reference	No. of alleles
RUB4	Rubusr47a	(M13)-AAGCAGGACACCTCAGATGC	CAGCCAACCATCATCAGCTA	59	25+9	Graham et al. (2004)	12
RUB5	Rubusr76b	(M13)-CTCACCCGAAATGTTCAACC	GGCTAGGCCGAATGACTACA	63	24+9	Graham et al. (2004)	4
RUB6	Rubus105b	(M13)-GAAAATGCAAGGCGAATTGT	TCCATCACCAACACCACCTA	59	24+9	Graham et al. (2004)	16
SSR05	Rubus275a	(M13)-CACAACCAGTCCCGAGAAAT	CATTTCATCCAAATGCAACC	51	33	Graham et al. (2004)	18
SSR27	Rubus26a	(M13)-AACACCGGCTTCTAAGGTCT	GATCCTGGAAAGCGATGAAA	53	32	Graham et al. (2004)	11
SSR17	Rub236b	(M13)-TGTGCCAAGAACTCATCGTC	CCGAACGCCTCCCTACTT	62	25+9	Graham et al. (2006)	20
SSR13	ERubLR_SQ01_G16	(M13)-GCACCCTAATCTCCATGACC	CCGCTGTAGTTCCTGTAGGC	59	22+9	Woodhead et al. (2008)	6
SSR14	RubPara_SQ005_K23	(M13)-AGGTGAGGTGGGAGATGATG	ATCCTCGGTTCCTCCAAAAT	63	25+9	Woodhead et al. (2008)	3
SSR18	Rub238h	(M13)-GTGACCTCCGAGAGCTTGAG	CTTCACCGCCACTAGTACCC	63	25+9	Woodhead et al. (2008)	9
RUB7*	Rubus123a	(M13)-CAGCAGCTAGCATTTTACTGGA	GCACTCTCCACCCATTTCAT	62	26+10	Graham et al. (2004)	1

* Excluded from final analysis

Supplementary Table S3. Samples used for SSR analyses, allelic data and genotype assignment as determined by GENOTYPE

					Genotype assignment at different thresholds						Detected alleles								
Taxon/hybrid	**Collection code**	**Locality**	**Latitude**	**Longitude**	**Th=0**	**Th=1**	**Th=2**	**Th=3**	**Th=4**	**Th=5**	**Rub5 = Rubusr76b**	**Rub6 = Rubus105b**	**SSR5 = Rubus275a**	**SSR17 = Rub236b**	**SSR27 = Rubus26a**	**SSR18 = Rub238h**	**SSR14 = RubPara_SQ005_K23**	**Rub4 = Rubusr47a**	**SSR13 = ERubLR_SQ01_G16**
R. bergii × pinnatus	RSA05/17	SA, WC, Newlands, Riverside Road	-33.985	18.445	1	1	1	1	1	1	169+209+221	163+167+181+193	140+160+248	165+199	176	145+154+156+163	215+244	233+237+244+250	222
R. bergii × pinnatus	RSA09/17	SA, WC, Kirstenbosch arboretum, SW margin	-33.989	18.437	1	1	1	1	1	1	169+209+221	163+167+181+193	140+160+248	165+199	176	145+154+156+163	215+244	233+237+244+250	222
R. bergii × pinnatus	RSA11/17	SA, WC, Kirstenbosch arboretum, central part	-33.988	18.440	1	1	1	1	1	1	169+209+221	163+167+181+193	140+160+248	165+199	176	145+154+156+163	215+244	233+237+244+250	222
R. bergii × pinnatus	RSA15/17	SA, WC, at the road Kirstenbosch - Hout Bay	-34.007	18.418	2	2	2	2	2	2	209	163+169+181+193	144+160+178+248	161+180+197	162	145+154+156+163	215+244	223+233+244	222
R. bergii × pinnatus	RSA32/17	SA, WC, Kirstenbosch Bot. Garden, 200 m NW of the N entrance	-33.985	18.430	1	1	1	1	1	1	169+209+221	163+167+181+193	140+160+248	165+199	176	145+154+156+163	215+244	233+237+244+250	222
R. pinnatus	RSA04/17	SA, WC, Table Mt., Woodhead Dam	-33.975	18.407	3	3	3	3	3	3	209	163+181	140+164	161+199	160	145+154	215	244	222
R. pinnatus	RSA06/17	SA, WC, Rondebosch, 300 m NW of the Rhodes Memorial	-33.951	18.456	4	4	4	4	4	4	209	163+181	140+144+168	161+165+197+201	160+176	145+151+154	215	233+244	222
R. pinnatus	RSA13/17	SA, WC, Hout Bay, NE end of the town	-34.015	18.384	5	5	5	5	5	5	209	163+181	144+162+164	201	158	145+154	215	244	222
R. rigidus × pinnatus	RSA27/17	SA, WC, at the road R44 (Kleinmond - Bot River)	-34.304	19.135	6	6	6	6	6	6	209	163+173+181+200	134+152+162	167+188	158+182	145+151	215+242	233+244	222+231
R. rigidus	RSA40/17	SA, WC, at the road R43, 6.2 km NNE of Fisherhaven	-34.306	19.146	7	7	7	7	7	7	209	163+173+202	134+162	167	158	145	215+242	233+244	231
R. rigidus	MS04/18	SA, KZN, Royal Natal, Tugela Valley	-28.715	28.935	8	8	8	8	8	8	209	163+173+196	138+148+156	165+174	148	145+151+156	215+242	221+235+244	231
R. rigidus	MS37/18	SA, KZN, Drakensberg, 0.5 km S of Injasuti Camp	-29.123	29.440	9	9	9	9	9	9	209	163+173+198	134+148+158	167+178+180	148	145+151+156	215+242	221+235+244	231
R. rigidus	MS46/18	SA, M, Graskop, edge of the kloof	-24.944	30.841	10	10	10	10	10	10	209+215	163+177+198	146+152+158	163+184+188	156+178	145+149+156	244	235+241+246	233+235
R. rigidus	MS13/18	SA, KZN, Assagay	-29.784	30.739	11	11	11	11	11	11	209+215	163+177+200+204	128+238+144+152	187+189	158	145+156	215	233+235+244	233
R. rigidus	MS17/18	SA, KZN, N of Hillcrest, Ngwele Rd.	-29.752	30.778	12	12	12	12	12	12	209	163+171+177+179+193	128+140+152	165+191+203	154+158	145+148+156	215	235+239+244	233
R. bergii × rigidus	RSA20/17	SA, WC, Kylemore	-33.912	18.944	13	13	13	13	13	13	209+221	163+167+185+198	148+160	167+176+180	158	145+156+163	215+242+244	231+235+237+244	231
R. bergii × rigidus	RSA21/17	SA, WC, between Simondium and Drakenstein	-33.858	18.972	14	14	14	14	14	14	209	163+173+185+198	148+162+178+248	167+172+180	158	145+156	215+242+244	223+233+235+237+244	231
R. bergii × rigidus	RSA28/17	SA, WC, at the road R44 (Kleinmond - Bot River)	-34.304	19.135	15	15	15	15	15	15	209+221	163+167+173+181+185	134+160	167+180+186	158	145+163	215+242+244	231+233+244	231
R. bergii × rigidus	RSA30/17	SA, WC, Elgin Valley,10 km NNE of Kleinmond	-34.254	19.054	16	16	16	16	16	16	209+221	163+173+193	136+160+178	167+186	158	145+156	242+244	231+233+244	231

Supplementary Table S3. Samples used for SSR analyses, allelic data and genotype assignment as determined by GENOTYPE (continued)

					Genotype assignment at different thresholds						Detected alleles								
Taxon/hybrid	**Collection code**	**Locality**	**Latitude**	**Longitude**	**Th=0**	**Th=1**	**Th=2**	**Th=3**	**Th=4**	**Th=5**	**Rub5 = Rubusr76b**	**Rub6 = Rubus105b**	**SSR5 = Rubus275a**	**SSR17 = Rub236b**	**SSR27 = Rubus26a**	**SSR18 = Rub238h**	**SSR14 = RubPara_SQ005_K23**	**Rub4 = Rubusr47a**	**SSR13 = ERubLR_SQ01_G16**
R. bergii × *rigidus*	RSA31/17	SA, WC, Grabouw, Highlands Road	-34.216	19.055	17	17	17	17	15	15	209+221	163+167+173+181+185	134+160+178+248	167+186	158	145+156+163	215+242+244	231+233+244	231
R. bergii × *rigidus*	RSA39/17	SA, WC, between Patryslaagte and Houhoek	-34.192	19.110	18	18	18	18	17	17	209	163+173+185+200	134+162+178	167+184	158	145+163	215+242+244	233+237+244	231
R. bergii × *rigidus*	RSA41/17	SA, WC, between Sandbaai and Caledon, Creation	-34.333	19.331	19	19	19	19	18	18	209+221	163+167+173+185+204	134+162+248	167+188	158	145+156+163	215+242+244	231+233+235+237+244	231
R. bergii × *rigidus*	RSA42/17	SA, WC, SW margin of Genadendal	-34.049	19.553	20	20	20	20	19	19	209+221	163+173+185+200	134+160+178	167+174	158	145+156+163	215+242+244	233+237+244	231
R. bergii × *rigidus*	RSA44/17	SA, WC, between Genadendal and Helderstroom	-34.062	19.438	21	21	21	21	20	20	209+221	163+167+171+193+200	148+160	167+174+180	162	135+145+156+163	215+242+244	223+231++233+244	225
R. bergii × *rigidus*	RSA45/17	SA, WC, Helderstroom	-34.066	19.370	22	22	22	22	21	21	209+221	163+173+193+200	152+160	167+174+180	138	135+145+156+163	215+242+244	231+235+237+244	231
R. bergii × *rigidus*	Beek2018.15	SA, WC, Stellenbosch	-33.912	18.942	23	23	23	23	22	22	209	163+167+173+185+198	158+162+178	165+176	158	145+156+163	215+242+244	233+235+237+244	231
R. bergii	RSA29/17	SA, WC, ca 8.7 km NE of Kleinmond, Elgin Valley	-34.280	19.086	24	24	24	24	23	23	209+221	167+185+193	160+178+248	180	null	156+163	244	223+233+237	null
R. bergii	RSA35/17	SA, WC, Table Mt., Disa Gorge	-33.978	18.399	24	24	24	24	23	23	209+221	167+185+193	160+178+248	180	null	156+163	244	223+233+237	null
R. bergii	RSA38/17	SA, WC, Table Mt., Woodhead Dam	-33.975	18.407	25	25	24	24	23	23	209+221	167+185+193	160+178+248	182	null	156+163	244	223+233+237	null
R. bergii	RSA43/17	SA, WC, Genadendal	-34.036	19.556	26	26	24	24	23	23	209+221	167+185+193	162+178+248	180	null	156+163	244	223+233+237	null
R. bergii	RSA08/17	SA, WC, Rondebosch, 750 m W of the Rhodes Memorial	-33.953	18.451	24	24	24	24	23	23	209+221	167+185+193	160+178+248	180	null	156+163	244	223+233+237	null
R. bergii	RSA16/17	SA, WC, Stellenbosch, Devonvallei	-33.948	18.819	24	24	24	24	23	23	209+221	167+185+193	160+178+248	180	null	156+163	244	223+233+237	null
R. bergii	RSA17/17	SA, WC, Stellenbosch, base of Pappegaaiberg	-33.940	18.845	24	24	24	24	23	23	209+221	167+185+193	160+178+248	180	null	156+163	244	223+233+237	null
R. bergii	RSA19/17	SA, WC, Kylemore, at the road Stellenbosch - Pniel	-33.912	18.944	24	24	24	24	23	23	209+221	167+185+193	160+178+248	180	null	156+163	244	223+233+237	null
R. bergii	RSA22/17	SA, WC, between Simondium and Cillie	-33.816	18.951	24	24	24	24	23	23	209+221	167+185+193	160+178+248	180	null	156+163	244	223+233+237	null
R. bergii	RSA23/17	SA, WC, Paarlsberg	-33.735	18.947	24	24	24	24	23	23	209+221	167+185+193	160+178+248	180	null	156+163	244	223+233+237	null
R. bergii	RSA24/17	SA, WC, Paarlsberg, under Victoria Dam	-33.759	18.948	24	24	24	24	23	23	209+221	167+185+193	160+178+248	180	null	156+163	244	223+233+237	null
R. bergii	R151/11	Germany, Lower Saxony, Voltlage	52.430	7.753	24	24	24	24	23	23	209+221	167+185+193	160+178+248	0	null	156+163	244	223+233+237	null
R. bergii	VŽ-Vig1	Germany, Hessen, Rotgau	50.035	8.903	24	24	24	24	23	23	209+221	167+185+193	160+178+248	180	null	156+163	244	223+233+237	null
R. bergii	VŽ-Vig2	Germany, Hessen, Urberach	49.956	8.773	24	24	24	24	23	23	209+221	167+185+193	160+178+248	180	null	156+163	244	223+233+237	null

Supplementary Table S3. Samples used for SSR analyses, allelic data and genotype assignment as determined by GENOTYPE (continued)

					Genotype assignment at different thresholds						Detected alleles								
Taxon/hybrid	**Collection code**	**Locality**	**Latitude**	**Longitude**	**Th=0**	**Th=1**	**Th=2**	**Th=3**	**Th=4**	**Th=5**	**Rub5 = Rubusr76b**	**Rub6 = Rubus105b**	**SSR5 = Rubus275a**	**SSR17 = Rub236b**	**SSR27 = Rubus26a**	**SSR18 = Rub238h**	**SSR14 = RubPara_SQ005_K23**	**Rub4 = Rubusr47a**	**SSR13 = ERubLR_SQ01_G16**
R. bergii	VŽ-Vig4	Germany, Hessen, Babenhausen	49.980	8.929	24	24	24	24	23	23	209+221	167+185+193	160+178+248	180	null	156+163	244	223+233+237	null
R. bergii	VŽ-Vig5	Germany, Lower Saxony, Voltlage	52.430	7.753	27	27	24	24	23	23	209+221	167+185+193	160+178+248	176	null	156+163	244	223+233+237	null
R. aff. bergii	MS24/18	SA, KZN, Ingelabantwana Forest, N of Bulwer	-29.725	29.744	28	28	25	25	24	24	209	165+169+193	138+148+160	165+172	null	156+163+165	244	215+233+244	null