

Article

Assessment of Genetic Diversity and Interspecific Relationships of the Genus *Viburnum* Inferred from Start Codon Targeted (SCoT) Polymorphism Markers

I-Jin Choi *, Hey-Min Park and Hyun-Kyung Kim

Plant Management & Research Division, Seoul Botanic Park, Seoul 07789, Republic of Korea; plannerhm3@seoul.go.kr (H.-M.P.); hnknng0315@seoul.go.kr (H.-K.K.)

* Corresponding author. E-mail: ijin2080@seoul.go.kr (I.-J.C.); Tel.: +82-2-2104-9760 (I.-J.C.)

Received: 21 December 2025; Revised: 28 January 2026; Accepted: 6 March 2026; Available online: 16 March 2026

ABSTRACT: The genus *Viburnum* (Adoxaceae) comprises deciduous broad-leaved shrubs with a thicket-like growth habit, and globally about 150–200 species are recognized. In Korea, several native *Viburnum* taxa have recently been listed as threatened, emphasizing the need for robust genetic information to support conservation and management. This study aimed to evaluate genetic diversity and interspecific relationships among 33 *Viburnum* taxa and to establish a practical framework for their identification and management using start codon targeted (SCoT) markers. SCoT markers were chosen because they are easier to apply than simple sequence repeat (SSR) and generally provide richer nuclear variation than chloroplast DNA (cpDNA), offering a simple yet informative tool for distinguishing closely related members of this shrub genus. Seventeen SCoT primers produced 489 polymorphic bands, revealing substantial nuclear variation among the 33 *Viburnum* taxa. An unweighted pair-group method with arithmetic mean (UPGMA), we grouped the 33 accessions into four major genetic clusters, and this clustering pattern was in good agreement with the structure inferred from principal component analysis. These clusters highlighted the genetic isolation of the *V. plicatum* group and the close affinity of the *V. carlesii* complex, while also indicating complex relationships among East Asian species. In contrast, *V. plicatum* formed Cluster IV, highlighting the taxonomic positions of these lineages and their potential priority for conservation and breeding. Overall, the results demonstrate that SCoT markers provide an efficient, operationally simple system for discriminating between closely related accessions and major genetic lineages within *Viburnum*. The SCoT-based approach developed here provides baseline information for species and cultivar identification. It also supports germplasm conservation and the selection of genetically divergent parents for future breeding programs.

Keywords: SCoT markers; Genetic diversity; Phylogenetic relationships; *Viburnum*; Germplasm conservation; Taxonomy



1. Introduction

The genus *Viburnum* (Adoxaceae) comprises about 150–200 shrub species in the temperate and subtropical regions of the Northern Hemisphere. These are plants of ecological and ornamental importance [1]. In South Korea, 10 native *Viburnum* taxa have been reported, and several native *Viburnum* taxa are considered vulnerable [2–4]. Among them, *V. burejaeticum* has been designated and protected as an Endangered Wild Species Class II in South Korea since 2017 [5]. This situation highlights the need for robust genetic information to support conservation and management at the national and institutional levels.

Morphology-based taxonomy alone has often been insufficient to resolve relationships among closely related *Viburnum* taxa due to phenotypic plasticity and hybridization [6]. Therefore, a molecular approach is required to achieve more reliable identification and to clarify taxonomic boundaries [1,7,8]. Chloroplast DNA (cpDNA) and nuclear markers have been used to examine the phylogenetic and evolutionary characteristics of *Viburnum*, including East Asian and Korean taxa. However, detailed interspecies relationships are complex to specify, and a method for definitive discrimination of species characteristics for collection is needed [3,9–12].

Marker selection, which reduces high polymorphism and analysis time in institutions that collect and manage various plant genetic sources, is an attractive tool for identification, redundancy testing, and representative conservation fields [13,14]. Start codon targeting (SCoT) markers are key PCR-based markers that use primers designed around conserved ATG start codons to generate highly polymorphic, reproducible banding profiles without prior sequence information [15–17]. These markers have been applied to many crops and wild plants to evaluate genetic diversity and structure. They are simpler and more efficient than SSR markers, which require time-consuming development of species-specific primers [12,13,18]. Markers such as SCoT are handy for resolving relationships in *Viburnum*, as cpDNA often shows limited sequence divergence between closely related taxa [10].

This study aims to provide a SCoT-based genetic information for the *Viburnum* Collection at Seoul Botanic Park. This study investigates two main groups: the *V. carlesii* complex (*V. carlesii*, *V. × burkwoodii*, *V. × carcalcephalum*, *V. carlesii* var. *botchiuense*, and *V. botchiuense*). This complex enables detailed analysis within closely related lineages. It includes a variety of *V. plicatum* forms and varieties (e.g., ‘Rosacea’, ‘Lanarth’, and ‘Summer Snowflake’) that differ significantly in branch structure and inflorescence morphology. Using SCoT primers, 33 *Viburnum* species were analyzed with *Lonicera japonica* as an outgroup, providing basic data for identification, systematic management, and off-site conservation of collection plants.

2. Materials and Methods

2.1. Plant Materials and Genomic DNA Extraction

A total of 33 *Viburnum* taxa [19–21] and *L. japonica* (Caprifoliaceae) as the outgroup were analysed (Table 1). These 33 taxa were selected to represent the major infrageneric sections of *Viburnum* occurring in Korea and to span the diversity from wild native species (e.g., *V. furcatum*, *V. wrightii*) to two focal taxa of high conservation and horticultural importance, *V. burejaeticum* and *V. setigerum*, which are priority targets for ex situ conservation in Korea [16,18]. *L. japonica* was used as the outgroup because it belongs to the order Dipsacales, as does *Viburnum*, but is placed outside Adoxaceae, providing a closely related yet distinct lineage for rooting the dendrogram [16].

Table 1. The thirty-three *Viburnum* Taxa and outgroup (*L. japonica*) used in the SCoT primer analysis.

No.	Species/Cultivar	Origin	Breeding	History	Source Code *
1	<i>Viburnum dilatatum</i> ‘Daruma’	Horticultural selection	unknown	unknown	RHS
2	<i>V. dilatatum</i> ‘Michael Dodge’	Horticultural selection	unknown	unknown	RHS
3	<i>V. erosum</i> ‘Mongoloid’	Horticultural selection	unknown	unknown	HORT
4	<i>V. opulus</i> var. <i>sargentii</i>	Northeast Asia	unknown	unknown	IPNI
5	<i>V. opulus</i> ‘Aureum’	Horticultural selection	unknown	yellow-leaved cultivar	RHS
6	<i>V. opulus</i> ‘Xanthocarpum’	Horticultural selection	unknown	yellow-fruited cultivar	RHS
7	<i>V. opulus</i> ‘Hallasan’	Korea	unknown	Korean selection	K-HORT
8	<i>V. × burkwoodii</i>	UK	1920s	<i>V. carlesii</i> × <i>V. utile</i>	TSO/RHS
9	<i>V. × burkwoodii</i> ‘Mohawk’	Horticultural selection	1960s	selection from <i>V. × burkwoodii</i>	RHS
10	<i>V. × burkwoodii</i> ‘Conoy’	Horticultural selection	unknown	compact selection	RHS
11	<i>V. × burkwoodii</i> ‘Park Farm Hybrid’	Horticultural selection	unknown	unknown	HORT
12	<i>V. carlesii</i> Hemsl.	Korea	1888	wild species	IPNI
13	<i>V. erubescens</i> ‘Eskimo’	unknown	unknown	unknown	UNK
14	<i>V. opulus</i> f. <i>hydrangeoides</i>	Japan	unknown	morphological form	IPNI
15	<i>V. lantana</i> ‘Variegatum’	Europe	unknown	variegated cultivar	RHS
16	<i>V. lentago</i>	Canada	1753	wild species	IPNI
17	<i>V. lobophyllum</i> Gräbn	China	1901	wild species	IPNI
18	<i>V. bitchiuense</i> Makino	Japan	1902	wild species	IPNI
19	<i>V. cotinifolium</i> D. Don	Himalayan region	1825	wild species	IPNI
20	<i>V. burejaeticum</i>	China	1862	wild species	IPNI
21	<i>V. plicatum</i> ‘Rosacea’	Horticultural selection	unknown	unknown	HORT
22	<i>V. plicatum</i> f. <i>plicatum</i> ‘Pink Sensation’	Horticultural selection	unknown	unknown	HORT
23	<i>V. carlesii</i> var. <i>bitchiuense</i>	Korea/Japan	unknown	wild form	IPNI
24	<i>V. × bodnantense</i>	UK	1930s	<i>V. farreri</i> × <i>V. grandiflorum</i>	TSO/RHS
25	<i>V. × bodnantense</i> ‘Dawn’	UK	1935	selection: ‘Dawn’	RHS
26	<i>V. rhytidophyllum</i>	China	1888	wild species	IPNI
27	<i>V. rhytidophyllum</i> ‘Variegatum’	Horticultural selection	unknown	variegated cultivar	RHS
28	<i>V. macrocephalum</i>	China	1847	wild species	IPNI
29	<i>V. setigerum</i> Hance	China	1882	wild species	IPNI
30	<i>V. plicatum</i> f. <i>tomentosum</i> ‘Lanarth’	UK	unknown	selection	HORT
31	<i>V. plicatum</i> f. <i>tomentosum</i> ‘Summer Snowflake’	Horticultural selection	unknown	unknown	RHS
32	<i>V. japonicum</i>	Japan/Korea	1824	wild species	IPNI
33	<i>V. × carlcephalum</i>	USA	1950s	<i>V. carlesii</i> × <i>V. macrocephalum</i>	TSO/RHS
Out group	<i>L. japonica</i>	East Asia	1784	wild species	IPNI

* Source codes: IPNI = International Plant Names Index; RHS = Royal Horticultural Society Plant Finder; TSO = Trees and Shrubs Online; HORT = general horticultural sources; K-HORT = Korean horticultural sources; UNK = unknown.

Plant materials were sampled from accessions preserved at Seoul Botanic Park. Genomic DNA was extracted from 0.2 g of fresh leaf material using the i-genomic Plant DNA Extraction Kit (iNtRon Biotechnology, Seongnam, Republic of Korea). DNA was diluted to 20 ng/μL for subsequent PCR reactions. The sampling design emphasizes two groups: the *V. carlesii* complex (including *V. carlesii*, *V. × burkwoodii*, *V. × carlcephalum*, *V. carlesii* var. *bitchiuense*, and *V. bitchiuense*), which allows fine-scale resolution of relationships within a closely related lineage, and a diverse set of *V. plicatum* (e.g., ‘Rosacea’, ‘Lanarth’, ‘Summer Snowflake’) that differ markedly in branching architecture and inflorescence morphology.

2.2. SCoT Marker Analysis

In this study, we initially screened 60 SCoT primers and selected 17 highly polymorphic and reproducible primers for genetic analysis (Table S1), following previously described SCoT protocols with minor modifications [15,16]. PCR amplification was performed in a total volume of 20 μ L containing 20 ng genomic DNA, 1 \times PCR buffer, 2.5 mM MgCl₂, 0.2 mM of each dNTP, 0.5 μ M primer, and 1 U Taq DNA polymerase. PCR conditions were as follows: an initial denaturation at 95 °C for 5 min; followed by 35 cycles of 95 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min; and a final extension at 72 °C for 10 min.

2.3. Gel Electrophoresis and Data Analysis

The amplified products were separated on 1.5% agarose gels at 130 V for 120 min. Bands were scored in a binary format (1 = present, 0 = absent). Specifically, PCR products were separated on 1.5% agarose gels, stained with Dyne Loading Star (Dyne Bio, Seongnam, Republic of Korea), and visualized under UV light using a Gel Doc XR+ imaging system (Bio-Rad, Hercules, CA, USA). A 100 bp DNA ladder (NanoHelix, Daejeon, Republic of Korea) was used as a molecular size standard. All PCR amplifications were performed in triplicate independently to confirm the reproducibility of the banding patterns.

2.4. Statistical Analysis

Pairwise Jaccard distance coefficients were calculated from the binary matrix (Table S2) using NTSYSpc version 2.2 (Exeter Software, Setauket, NY, USA) and used to construct a UPGMA dendrogram. [22]. Principal component analysis (PCA) was performed on the Jaccard distance matrix to visualize multilocus patterns of variation.

3. Results

3.1. SCoT Marker Polymorphism

A total of 60 SCoT primers were initially screened, of which 17 highly polymorphic and reproducible primers were selected for genetic analysis, generating 489 polymorphic bands. All screened primers are listed in Table S1, and the key characteristics of the 17 selected primers are summarized in Table 2. The number of polymorphic bands per selected primer ranged from 19 (SCoT 45) to 35 (SCoT 18), with an average of 28.76 bands per primer. Among these, SCoT34 and SCoT48 showed relatively high polymorphism information content (PIC) and clear, reproducible banding patterns. They were therefore chosen as representative examples for the gel image in Figure 1.

Table 2. Sequences and polymorphism statistics of the 17 SCoT primers used for genetic analysis of *Viburnum* taxa.

No.	Primer	Sequence (5'-3')	Melting Tem. (°C)	GC Content (%)	Polymorphic Bands	PIC
1	SCoT 12	ACGACATGGCGACCACCG	59.8	61.1	33	0.38
2	SCoT 13	ACGACATGGCGACCATCG	59.3	61.1	31	0.41
3	SCoT 14	CAACAATGGCTACCACCT	62.5	66.7	33	0.36
4	SCoT 16	CAACAATGGCTACCACGC	58.1	61.1	33	0.35
5	SCoT 18	CAACAATGGCTACCACGT	61.1	66.7	35	0.35
6	SCoT 34	ACCATGGCTACCACCGCA	60.3	61.1	31	0.44
7	SCoT 45	ACAATGGCTACCACTGAC	53.2	50.0	19	0.38
8	SCoT 47	ACAATGGCTACCACTGCC	56.3	55.6	22	0.38
9	SCoT 48	ACAATGGCTACCACTGGC	56.3	55.6	21	0.41
10	SCoT 50	ACAATGGCTACCACTGGG	55.5	55.6	34	0.33
11	SCoT 51	ACAATGGCTACCACTGTC	53.2	50.0	33	0.33
12	SCoT 52	ACAATGGCTACCACTGCA	55.5	50.0	28	0.36
13	SCoT 53	ACAATGGCTACCACCGAC	56.0	55.6	29	0.39

14	SCoT 54	ACAATGGCTACCACCAGC	56.3	55.6	29	0.33
15	SCoT 55	ACAATGGCTACCACTACC	52.5	50.0	26	0.37
16	SCoT 59	ACAATGGCTACCACCATC	53.2	50.0	23	0.26
17	SCoT 60	ACAATGGCTACCACCACA	55.0	50.0	27	0.38
Total					489	
Mean					28.76	0.36

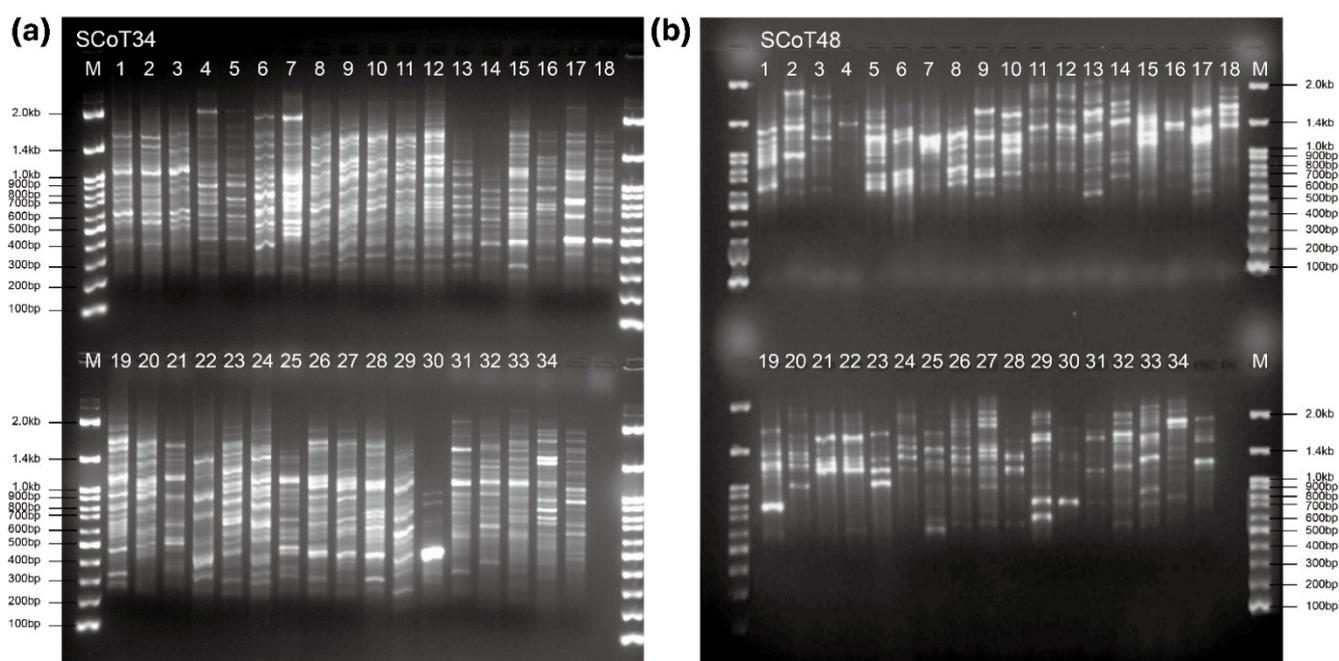


Figure 1. SCoT banding patterns of 33 *Viburnum* accessions and the outgroup were generated with primers SCoT 34 (a) and SCoT 48 (b). M, 100 bp DNA ladder with size markers from 100 to 2000 bp indicated on the left (a) and right (b); lanes 1–33 correspond to the *Viburnum* accessions listed in Table 1, lane 34 represents the outgroup, and lane 35 was not used in the analyses.

The PIC values ranged from 0.26 to 0.44, with a mean of 0.36. According to commonly used criteria, markers with PIC values between 0.25 and 0.50 are considered moderately informative, whereas values below 0.25 indicate only slightly informative markers [23]. Thus, the SCoT primers used in this study are moderately informative and provide sufficient discriminatory power for diversity and relationship analyses in this collection. The high number of polymorphic bands per primer indicates that a compact SCoT primer set can effectively capture genetic variation across the 33 *Viburnum* taxa.

3.2. Genetic Similarity and Phylogenetic Analysis (UPGMA)

The UPGMA dendrogram separated the *Viburnum* taxa from the outgroup, *L. japonica* (Figure 2). Cluster analysis indicated substantial genetic differentiation, with four distinct clusters being recognized at a similarity coefficient of 0.46: Cluster I contained 23 accessions, Cluster II 3, Cluster III 2, and Cluster IV 5 accessions. The largest group (Cluster I) encompassed the majority of species, whereas Clusters II–IV represented more restricted assemblages. The clustering pattern broadly corresponded to traditional infrageneric sections (Tomentosa, Odontotinus, Lentago, Euviburnum, and Opulus), although several taxa showed placements inconsistent with morphology-based classification. Pairwise Jaccard distances among the 33 *Viburnum* taxa (and *L. japonica* as outgroup) are given in Table S2.

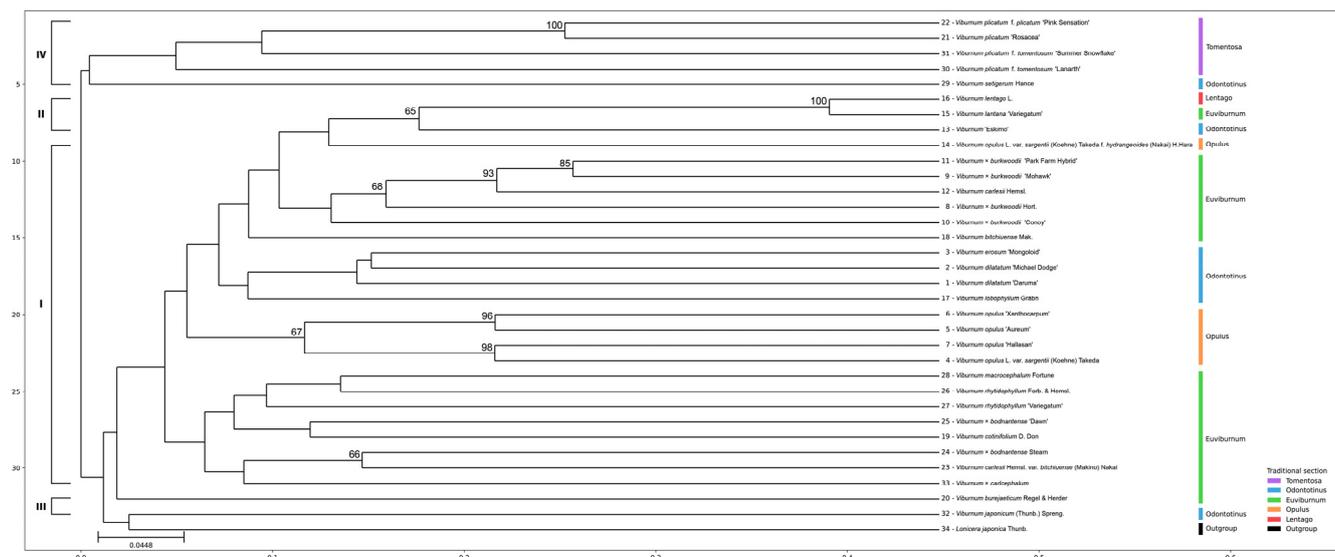


Figure 2. UPGMA phylogram inferred from SCoT primer data for 33 *Viburnum* taxa, with *L. japonica* as the outgroup. Branch lengths are proportional to Jaccard distance among taxa (scale bar). Taxon numbers correspond to those listed in Table 1, and taxa are color-coded according to traditional section classification. Numbers above branches indicate bootstrap support values (%) based on 1000 replicates (only values $\geq 50\%$ are shown).

Within this structure, the *V. carlesii* complex (including *V. carlesii*, *V. × burkwoodii*, and *V. bitchiuense*) consistently formed a subgroup in Cluster I. In contrast, *V. plicatum* and its forms and cultivars were placed together in Cluster IV as a clearly separated group, emphasizing how divergent they are from the remaining taxa.

Several species, such as *V. dilatatum*, *V. erosum*, and *V. wrightii*, showed intermediate positions within Cluster I, suggesting partial admixture within this cluster. *V. japonicum* grouped most closely with *V. burejaeticum* in Cluster III.

3.3. Principal Component Analysis (PCA)

The first two principal components explained a substantial proportion of the total variance (PC1: 26.5%, PC2: 15.8%), allowing a clear visualization of genetic relationships among the accessions (Figure 3). Most *Viburnum* taxa were widely scattered along the two PCA axes, indicating substantial genetic diversity within the genus. In contrast, members of the *V. carlesii* complex were clustered closely together, whereas *V. plicatum* occupied a more distant position. This distribution pattern is consistent with the relationships inferred from the UPGMA dendrogram.

Within the *V. carlesii* complex, putative hybrids such as *V. × burkwoodii* and *V. × carlcephalum* occupied intermediate positions between their parental taxa, consistent with their hybrid origin. In contrast, *V. plicatum* accessions were more widely distributed along the PC1 axis, indicating clear separation from the *V. carlesii* complex but relatively weak structure within the *V. plicatum* group itself. This distribution pattern was similar to the clustering pattern observed in the UPGMA dendrogram.

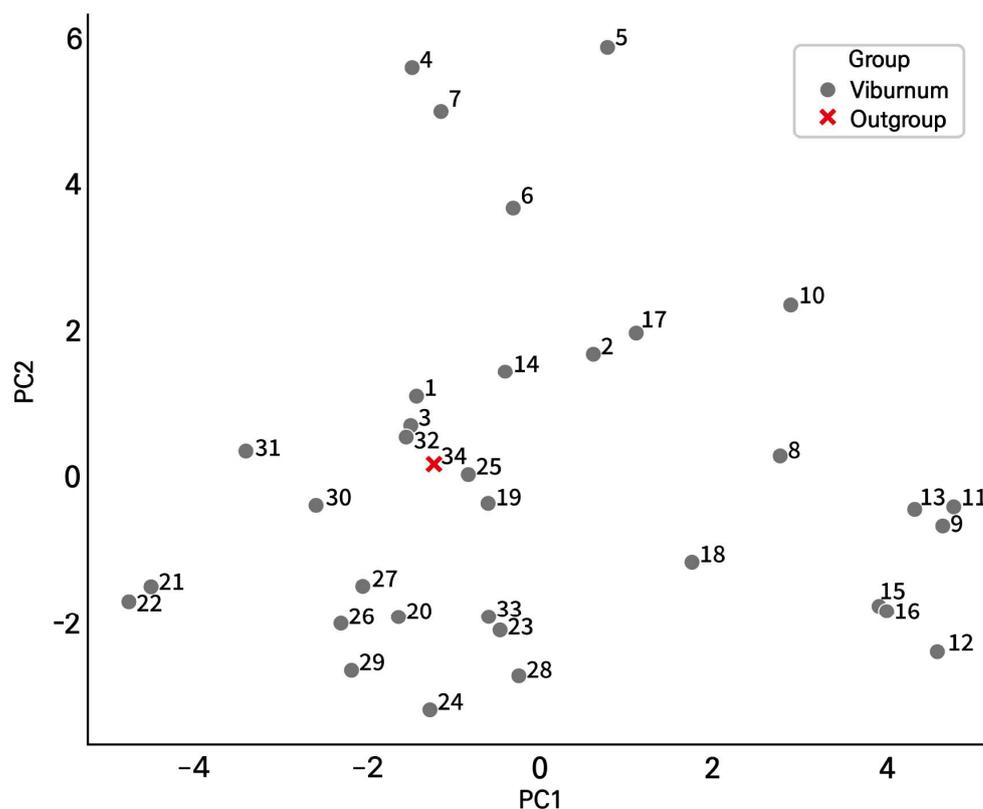


Figure 3. Principal component analysis of *Viburnum* taxa with *L. japonica* as the outgroup (red cross). Sample numbers correspond to those in Table 1.

4. Discussion

4.1. Effectiveness of SCoT Markers

The high level of polymorphism observed in this study (489 polymorphic bands; mean PIC of 0.36) indicates that the selected SCoT primers are highly informative and reliable for assessing genetic diversity within *Viburnum*. These values lie at the upper end of the ranges reported for SCoT markers in crop and woody species, supporting the strong discriminating power of this marker system for germplasm characterization [15,16].

4.2. Interspecific Relationships and Taxonomic Implications

The UPGMA dendrograms and PCA arrays revealed well-structured interspecific relationship patterns that are mainly consistent with the proposed morphology and DNA-based classification of *Viburnum* species [1,7,9,10]. The four genetic clusters (I–IV) showed broad concordance with traditional infrageneric sections, as Cluster IV (*V. plicatum* group) corresponds to section Tomentosa, and Cluster II (*V. lentago*, *V. lantana*, *V. erabescens*) to section Lentago [10]. The tight clustering of the *V. carlesii* complex confirms its close taxonomic affinity. Indicates that relatively few, well-defined lineages can capture much of the genetic and morphological variation represented by these taxa, which is advantageous for targeted breeding and ex situ conservation planning [1,9,11,12,18]. By contrast, East Asian species such as *V. dilatatum*, *V. erosum*, and *V. wrightii* showed partially intermixed positions within the main cluster, whereas *V. japonicum* grouped with *V. burejaeticum* in Cluster III. These relationships are not entirely consistent with current morphological

classifications. Together, suggesting a complex evolutionary history and possible historical hybridization among East Asian *Viburnum* lineages. These patterns indicate that multiple, partially diverged lineages coexist within East Asian *Viburnum*, and that conservation and taxonomic work in this group should explicitly account for this hidden evolutionary complexity [3,4,8–10].

The genetic isolation of *V. plicatum* (Cluster IV) is particularly noteworthy. Although morphologically classified within section Tomentosa, *V. plicatum* and its cultivars formed a strongly supported monophyletic group, markedly distant from all other clusters. These patterns support a unique systematic position and are consistent with the characteristic stratified horizontal branch described in taxonomic and horticultural treatments, as well as with striking sterile marginal flowers [7,10,24]. The strong genetic isolation of the *V. plicatum* group, together with its distinctive branching architecture and showy sterile marginal flowers, suggests a long-term divergence from other *Viburnum* lineages and supports its recognition as a distinct conservation unit and a valuable source of genetically divergent parents for breeding [1,10].

The close association of *V. burejaeticum* with *V. japonicum* in Cluster III likewise highlights their genetic differentiation from other East Asian taxa. It suggests that this lineage represents an evolutionarily and conservationally important component of *Viburnum* diversity that has so far received relatively little detailed genetic study [1,3,4,9].

4.3. Implications for Conservation and Breeding

The genetic marker information selected here may provide basic information for the discrimination, conservation, and management of plants of the genus *Viburnum* maintained at the Seoul Botanic Park [9,10,24]. In particular, identifying genetically distant lineages such as *V. plicatum* and *V. burejaeticum* can help minimize redundancy within the collection [1,3,4,10]. These patterns indicate that multiple, partially diverged lineages coexist within East Asian *Viburnum*. They also suggest that conservation and taxonomic work in this group should explicitly account for this hidden evolutionary complexity [9,25].

The substantial genetic distances observed between clusters (Table S2) provide a framework for selecting genetically diverse parental combinations in future breeding programs. For example, some of the highest pairwise Jaccard distances in Table S2 occur between members of the *V. plicatum* group (Cluster IV; e.g., *V. plicatum* f. tomentosum ‘Lanarth’, no. 30) and taxa in the *V. carlesii* complex (Cluster I; e.g., *V. carlesii*, no. 12, and *V. burkwoodii*, nos. 8–11), indicating that such inter-cluster combinations would maximize nuclear genetic divergence in breeding crosses. Inter-cluster crosses, particularly those involving the genetically isolated *V. plicatum* lineage (Cluster IV), may maximize genetic diversity in hybrid offspring [1,7,10,12]. In particular, genetically isolated lineages such as the *V. plicatum* group and the *V. burejaeticum*-*V. japonicum* lineage should be given high priority in conservation programmes. They can serve as strategically important parents for generating hybrids with novel combinations of ornamental and adaptation-related traits [11,12,26].

Moreover, *V. burejaeticum* (no. 20) shows consistently high genetic differentiation from the main East Asian cluster, reinforcing its status as a genetically unique lineage and a priority candidate for conservation-oriented breeding together with *V. japonicum* (no. 32) in Cluster III [3,4,9]. Distinct genetic differences between major clusters may indicate new allelic combinations that could be beneficial for resource conservation. Furthermore, crossbreeding between genetically distant taxa may facilitate the development of ornamental cultivars with novel or improved traits [12,26–28].

4.4. Future Perspectives

Future research should integrate these SCoT-based results with additional molecular approaches, such as ISSR, SSR, and SNP markers, which have already been developed or applied in *Viburnum* and provide complementary, codominant information on allelic and lineage variation [12,18,26]. Combining such

multilocus molecular data with detailed morphological, anatomical, and ecological trait analyses is expected to yield a more highly resolved phylogenetic framework and help clarify remaining taxonomic uncertainties within the genus [1,7,9,10]. In addition, plastid phylogenomic work on Adoxaceae suggests that including additional outgroups from closely related genera would improve root placement and allow more rigorous tests of *Viburnum* monophyly in future phylogenetic analyses. In this context, *Sambucus* (Adoxaceae) could serve as an appropriate outgroup candidate [3].

Integrating these SCoT data with chloroplast sequences and newly developed SSR markers from the *Viburnum* collection at Seoul Botanic Park will enable finer-scale inference of demographic history, gene flow, and population structure, thereby strengthening both taxonomic and conservation applications in *Viburnum* [3,12,18,29].

5. Conclusions

This study provides the first systematic application of SCoT markers to assess genetic diversity and interspecific relationships in the genus *Viburnum*. Using 17 SCoT primers, we detected 489 polymorphic bands across 33 *Viburnum* taxa, including several endangered and vulnerable native species. We resolved four distinct genetic clusters that clearly separated *Viburnum* from the outgroup *Lonicera japonica*.

The cluster pattern well reflected the independent systematic location of *V. plicatum* and the close relationships in the *V. carlesii* complex. While revealing partially mixed groups of East Asian taxa and distinct *V. japonicum* and *V. burejaeticum* taxa in cluster III, consistent with complex evolutionary histories in these taxa. These results provide practical genetic markers that can be utilized for taxonomic identification and help refine ex-situ strategies by selectively obtaining genetically unique lineages and contributing to reducing intra-cluster duplication.

The pronounced genetic differentiation between the major clusters indicates that significant genetic variation exists that has not yet been fully utilized in ornamental breeding. Crossbreeding between genetically distant taxa may promote the development of varieties with new combinations of adaptation-related traits and horticultural/corrosive traits. Overall, the SCoT-based framework developed here will support long-term conservation planning, germplasm management, and the strategic selection of breeding parents in *Viburnum*. Furthermore, this study highlights the value of SCoT markers as an efficient tool for managing woody ornamental plant germplasm. It provides basic data to support long-term conservation plans for some *Viburnum* resources, including endangered taxa.

Supplementary Materials

The following supporting information can be found at: <https://www.sciepublish.com/article/pii/910>, Table S1: List of all 60 SCoT primers screened in this study and their basic characteristics; Table S2: Jaccard distance matrix calculated from the binary SCoT dataset for the 33 *Viburnum* accessions.

Statement of the Use of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used Grammarly and Perplexity (AI-assisted tools) to improve readability and language. After using these tools, the authors reviewed and edited the content as needed and took full responsibility for the publication's content.

Acknowledgments

The authors thank Wan-Hee Lee, Head of the Plant Management & Research Division, and Su-Mi Park, Director of Seoul Botanic Park, for administrative support and facilitation of this project. The authors also thank the staff of Seoul Botanic Park for their assistance with field sampling and cultivation of *Viburnum* accessions.

Author Contributions

I.-J.C.: conceptualization, data curation, formal analysis, supervision, writing—original draft, writing—review & editing. H.-M.P.: investigation, long-term collection, and *ex situ* conservation of *Viburnum* germplasm, establishment and management of the *Viburnum* display garden at Seoul Botanic Park. H.-K.K.: investigation, monitoring of growth performance, and ongoing propagation and maintenance of *Viburnum* accessions.

Ethics Statement

Not applicable. This study did not involve humans or animals. The plant materials used in this study are not listed in the IUCN Red List, and no endangered or protected species were harmed during sample collection. All plant sampling and subsequent experiments were conducted in accordance with the guidelines and regulations of Seoul Botanic Park.

Informed Consent Statement

Not applicable. This study did not involve humans.

Data Availability Statement

Processed data supporting the findings of this study are provided in the Supplementary Materials. The raw data (including gel images and the binary SCoT marker matrix) are available from the corresponding author upon reasonable request.

Funding

This research was funded by the Seoul Metropolitan Government.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

References

1. Spriggs EL, Clement WL, Sweeney PW, Madrinán S, Edwards EJ, Donoghue MJ. Temperate radiations and dying embers of a tropical past: The diversification of *Viburnum*. *New Phytol.* **2015**, *207*, 340–354. DOI:10.1111/nph.13305.
2. Choi YG, Oh SH. A comparative morphological study of *Viburnum* (Adoxaceae) in Korea. *Korean J. Plant Taxon.* **2019**, *49*, 107–117. DOI:10.11110/kjpt.2019.49.2.107.
3. Ran H, Liu Y, Wu C, Cao Y. Phylogenetic and comparative analyses of complete chloroplast genomes of Chinese *Viburnum* and *Sambucus* (Adoxaceae). *Plants* **2020**, *9*, 1143. DOI:10.3390/plants9091143.
4. Zhu H, Liu J, Li H, Yue C, Gao M. Complete chloroplast genome structural characterization and comparative analysis of *Viburnum japonicum* (Adoxaceae). *Forests* **2023**, *14*, 1819. DOI:10.3390/f14091819.
5. National Institute of Biological Resources. *V. burejaeticum* Species Information. Available online: <https://species.nibr.go.kr> (accessed on 18 December 2025).
6. Moeglein MK. The Roles of Polyploidy, Climate, and Genetic Architecture in the Evolution of Leaf Form in *Viburnum* (Adoxaceae). Ph.D. Thesis, Yale University, New Haven, CT, USA, 2021.
7. Ghimire B, Son DC, Park BK, Oh SH. Comparative wood anatomy of Korean *Viburnum* L. (Adoxaceae) and its taxonomic implication. *PhytoKeys* **2020**, *156*, 27–46. DOI:10.3897/phytokeys.156.52031.
8. Zhao L, Wang Y, Lyu W, Tang Z, Qiu L, Tang M, et al. A new synonym for *Viburnum erosum* (Viburnaceae) in East China, based on morphological and molecular evidence. *PLoS ONE* **2025**, *20*, e0312920. DOI:10.1371/journal.pone.0312920.
9. Choi YG, Youm JW, Lim CE, Oh SH. Phylogenetic analysis of *Viburnum* (Adoxaceae) in Korea using DNA sequences. *Korean J. Plant Taxon.* **2018**, *48*, 206–217. DOI:10.11110/kjpt.2018.48.3.206.

10. Clement WL, Arakaki M, Sweeney PW, Edwards EJ, Donoghue MJ. A chloroplast tree for *Viburnum* (Adoxaceae) and its implications for phylogenetic classification and character evolution. *Am. J. Bot.* **2014**, *101*, 1029–1049. DOI:10.3732/ajb.1400015.
11. Dean D. Assessing the Genetic Diversity of the Genus *Viburnum* Using Simple Sequence Repeats. Ph.D. Thesis, University of Tennessee, Knoxville, TN, USA, 2014.
12. Hamm TP, Nowicki M, Boggess SL, Ranney TG, Trigiano RN. A set of SSR markers to characterize genetic diversity in all *Viburnum* species. *Sci. Rep.* **2023**, *13*, 5343. DOI:10.1038/s41598-023-31878-0.
13. Hamm TP, Nowicki M, Boggess SL, Klingeman WE, Hadziabdic D, Huff ML, et al. Development and characterization of 15 novel genomic SSRs for *Viburnum farreri*. *Plants* **2021**, *10*, 487. DOI:10.3390/plants10030487.
14. Yun YE, Yu JN, Lee BY, Kwak M. An introduction to microsatellite development and analysis. *Korean J. Plant Taxon.* **2011**, *41*, 299–314. DOI:10.11110/kjpt.2011.41.4.299.
15. Ahmad FK, Noori IM. Phytochemical analysis and SCoT molecular marker used to determine genetic diversity of many fig accessions distributed in various locations of Sulaymaniyah province. *Kufa J. Agric. Sci.* **2024**, *16*, 86–118. DOI:10.36077/kjas/2024/v16i4.12074.
16. Altaf MT, Nadeem MA, Ali A, Liaqat W, Bedir M, Baran N, et al. Applicability of start codon targeted (SCoT) markers for the assessment of genetic diversity in bread wheat germplasm. *Genet. Resour. Crop. Evol.* **2025**, *72*, 1205–1218. DOI:10.1007/s10722-024-02016-0.
17. Collard BCY, Mackill DJ. Start codon targeted (SCoT) polymorphism: A simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Mol. Biol. Rep.* **2009**, *27*, 86–93. DOI:10.1007/s11105-008-0060-5.
18. Barish S, Arakaki M, Edwards EJ, Donoghue MJ, Clement WL. Characterization of 16 microsatellite markers for the Oreinotinus clade of *Viburnum* (Adoxaceae). *Appl. Plant Sci.* **2016**, *4*, 1600103. DOI:10.3732/apps.1600103.
19. International Plant Names Index. Available online: <https://www.ipni.org> (accessed on 15 February 2025).
20. Korea National Arboretum Plant List. Available online: <http://www.nature.go.kr> (accessed on 15 February 2025).
21. Trees and Shrubs Online. Available online: <https://treesandshrubsonline.org> (accessed on 15 February 2025).
22. Rohlf FJ. *NTSYSpc: Numerical Taxonomy and Multivariate Analysis System*, version 2.2; Exeter Software: Setauket, NY, USA, 2009.
23. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* **1980**, *32*, 314–331.
24. Royal Horticultural Society Plant Finder. Available online: <https://www.rhs.org.uk/plants> (accessed on 15 February 2025).
25. Winkworth RC, Donoghue MJ. *Viburnum* phylogeny based on combined molecular data: Implications for taxonomy and biogeography. *Am. J. Bot.* **2025**, *92*, 653–666. DOI:10.3732/ajb.92.4.653.
26. Hasan N, Choudhary S, Naaz N, Sharma N, Laskar RA. Recent advancements in molecular marker-assisted selection and applications in plant breeding programs. *J. Genet. Eng. Biotechnol.* **2021**, *19*, 128. DOI:10.1186/s43141-021-00231-1.
27. Hong JH, Shim EJ, Park WH, Soh EH. Construction of SSR profile database for variety identification of plum collected in Korea. *Korean J. Breed. Sci.* **2015**, *47*, 97–104. DOI:10.9787/KJBS.2015.47.2.97.
28. Ran H, Liu Y, Wu C, Cao Y. Phylogenetic and comparative analyses of complete chloroplast genomes of Chinese *Viburnum* and *Sambucus* (Adoxaceae). *Plants* **2020**, *9*, 1143. DOI:10.3390/plants9091143.
29. Le TY, Park J. The complete chloroplast genome sequence of *Viburnum odoratissimum* and phylogenetic relationship with other close species in the Adoxaceae family. *Plant* **2021**, *9*, 28–35. DOI:10.11648/j.plant.20210902.12.