Genetic Diversity Analysis and DNA Fingerprint Construction of *Neofinetia falcata* Varieties Based on SSR Markers

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Abstract

To clarify the genetic background of Neofinetia falcata varietal resources and construct a DNA fingerprinting system for precise identification, this study developed and screened 20 pairs of polymorphic SSR primers through transcriptome sequencing, and performed clustering and genetic diversity analyses on 99 N. falcata germplasms. The results showed that: (1) A total of 214 alleles were detected by the 20 primer pairs, with an average number of alleles (Na) of 10.7, an average expected heterozygosity (He) of 0.6784, and an average polymorphism information content (PIC) of 0.6348, indicating high genetic diversity in the cultivated population of N. falcata. (2) The genetic distances among the 99 N. falcata germplasms ranged from 0 to 3.9197, with an average genetic distance of 0.9203, suggesting that some varieties may be synonyms or clonal materials with highly consistent genetic backgrounds. (3) Cluster analysis and principal coordinate analysis divided the 99 germplasms into 15 groups, each showing obvious aggregation trends in phenotypic traits such as flower color and root type, and differences in genetic diversity among groups were closely related to group size and varietal origin. (4) Based on the genetic diversity analysis, a digital fingerprinting system encoded according to SSR allele sizes was constructed, and QR codes were generated containing variety name, leaf shape, leaf color, flower color, root color, flowering time, and digital fingerprint number, enabling precise molecular identification of 92 varieties (discrimination rate 92.9%). This fingerprint map provides a reliable tool for the protection of N. falcata variety rights, market identification, and breeding parent selection, and lays a molecular foundation for the conservation of N. falcata germplasm resources and varietal innovation. This study completed the genetic analysis and construction of an SSR fingerprint database for 99 N. falcata germplasms, providing important data support for systematic research in this field.

Full Text

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Genetic Diversity Analysis and DNA Fingerprint Construction of *Neofinetia falcata* Varieties Based on SSR Markers

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Abstract: To clarify the genetic background of *Neofinetia falcata* variety resources and construct a DNA fingerprint map that can enable accurate identification, this study developed and screened 20 pairs of SSR polymorphic primers through transcriptome sequencing, and conducted clustering and genetic diversity analyses on 99 accessions of *N. falcata* germplasm. The results showed that: (1) The 20 primer pairs detected a total of 214 alleles, with an average number of alleles (N_a) of 10.7, an average expected heterozygosity (H_e) of 0.678 4, and an average polymorphism information content (PIC) of 0.634 8, indicating that the cultivated population of *N. falcata* possessed relatively high genetic diversity. (2) The genetic distances among the 99 accessions of *N. falcata* ranged from 0 to 3.919 7, with an average genetic distance of 0.920 3; some varieties may be synonyms or clonal materials with highly consistent genetic backgrounds. (3) Cluster analysis and principal coordinate analysis divided the 99 accessions into 15 groups. Each group showed clear clustering trends in phenotypic traits such as flower color and root type, and differences in genetic diversity among groups were closely related to group size and variety origin. (4) On the basis of genetic diversity analysis, a digital fingerprinting system based on SSR allele size coding was constructed, and a two-dimensional code was generated, including the variety name, leaf shape, leaf color, flower color, root color, flowering time, and digital fingerprint number, achieving accurate molecular identification of 92 varieties (resolution rate 92.9%). This fingerprint map provides a reliable tool for the rights protection, market identification, and parental selection in breeding of *N. falcata* varieties, and lays a molecular foundation for the conservation of *N. falcata* germplasm resources and variety innovation. This study completed the genetic analysis of 99 *N. falcata* germplasm accessions and the construction of an SSR fingerprint database, providing important data support for systematic research in this field.

Key words: *Neofinetia falcata*; SSR markers; genetic diversity; DNA fingerprint; cluster analysis; variety identification

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Genetic diversity analysis and DNA fingerprint construction

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Abstract: To reveal the genetic background and germplasm characteristics of *Neofinetia falcata*, and to establish an accurate DNA fingerprint construction, 20 pairs of polymorphic SSR markers developed from transcriptome sequencing were used to evaluate the genetic diversity and relationships of 99 accessions. The results were as follows: (1) A total of 214 alleles were detected across the 20 primer pairs, with an average number of alleles per locus (N_a) of 10.7, an average expected heterozygosity (H_e) of 0.678 4, and an average polymorphism information content (PIC)

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of 0.634 8, indicating a relatively high level of genetic diversity within the cultivated population.
(2) The genetic distances among the 99 germplasms ranged from 0 to 3.919 7, with an average of 0.920 3, suggesting that some varieties might be synonyms or clonal materials with highly consistent genetic backgrounds.
(3) Cluster analysis and principal coordinate analysis divided the 99 germplasms into 15 groups, showing evident aggregation trends in phenotypic traits such as flower color and root type; moreover, the genetic diversity of these groups was closely related to group size and varietal origin.
(4) Based on the genetic diversity analysis, a digital fingerprinting system encoding SSR allele sizes was constructed, and corresponding QR codes containing variety name, leaf shape, leaf color, flower color, root color, flowering time, and digital fingerprint number were generated, enabling accurate molecular identification of 92 varieties (distinguishing rate of 92.9%). This fingerprint database provides a reliable tool for variety rights protection, market authentication, and parental selection in breeding, thereby laying a molecular foundation for the conservation and innovative utilization of *Neofinetia falcata* germplasm resources. This study systematically presents the genetic analysis and SSR fingerprint library construction of 99 *Neofinetia falcata* germplasms, providing important data support for systematic research in this field.

Keywords: *Neofinetia falcata*, SSR markers, genetic diversity, DNA fingerprint, cluster analysis, variety identification

Neofinetia falcata, also known as Fu-lan or Xian-cao-lan, belongs to *Neofinetia* in the family Orchidaceae and is a rare epiphytic orchid (Yang Boyun et al., 1997; Wang Weidong et al., 2014). *Neofinetia falcata* has high ornamental value and is highly favored in the market (Sascha et al., 2012). As a national Class II key protected wild plant, *N. falcata* is mainly distributed in dense valley forests in Jiangxi, Sichuan, Fujian, Zhejiang, and other regions (Lin Li et al., 2017).

At present, the *N. falcata* germplasm circulating in the domestic market can mainly be divided into two categories: one consists of expensive horticultural varieties introduced from Japan and South Korea (as of 2025, the varieties registered with the Japan Fukiran Society had reached 238). In addition, as the international registration authority for orchid plants, the Royal Horticultural Society (RHS) in the United Kingdom also has registration records for horticultural varieties of *Neofinetia*, reflecting global horticultural interest in this genus; the other category consists of relatively inexpensive domestic native germplasm, including wild-collected and tissue-culture-propagated materials (Li Chunhua and Li Kicheng, 2019). Market demand poses a serious threat to China' s wild *N. falcata* resources, while basic research and management related to *N. falcata* both have obvious shortcomings: problems such as taxonomic confusion among closely related genera, scattered resource conservation, confusion in folk naming, and unclear kinship relationships have seriously restricted the effective utilization of resources and varietal innovation (Zhang Ying et al., 2010). Therefore, systematically carrying out research on the genetic diversity of *N. falcata* and constructing a DNA fingerprint database have become urgent foundational tasks for realizing its variety identification, scientific conservation, and efficient breeding.

Molecular markers are genetic markers capable of accurately revealing DNA differences among biological individuals or populations. Among them, simple sequence repeats (SSR), because of their high polymorphism and codominance, have become core tools for plant genetic diversity analysis and genetic map construction (Varshney et al., 2005; Sun et al., 2025; Zhao et al., 2025). In orchids, SSR markers have been widely used in germplasm identification, kinship analysis, and construction of molecular identity cards for taxa such as moth orchid (*Phalaenopsis aphrodite*), large-flowered cymbidium (*Cymbidium hybridum*), *Dendrobium loddigesii*, spring orchid (*Cymbidium goeringii*), and *C. ensifolium* (Zhang Shuiming et al., 2012; Cai et al., 2012; Lee et al., 2012; Liu et al., 2014; Shen Hongzhe et al., 2022; Juan et al., 2023). In recent years, DNA fingerprints based on SSR markers have been successfully established for crops such as native moth orchid germplasm (Xiao Wenfang et al., 2026) and snow eggplant (Wang Lingling et al., 2021), enabling precise molecular identification of varieties. However, for *N. falcata*, a rare orchid plant, based on polymorphic molecular

markers, systematic genetic diversity analysis of germplasm developed through tissue culture, phylogenetic relationship analysis, and DNA fingerprint map construction have still only rarely been reported. Relevant studies have mostly focused on morphological classification and tissue culture (Hua Wen, 2020; Ding Changchun et al., 2020; Qi Lulu et al., 2022). Matsuba et al. (2015) constructed an artificial chromosome (BAC) library of *Neofinetia falcata* and used it for molecular cytogenetic analysis, but did not involve systematic analysis of population genetic diversity or cultivar phylogenetic relationships. Park et al. (2018), based on SSR markers, conducted a genetic evaluation of wild *Neofinetia falcata* populations in Korea and found that their genetic diversity was relatively low

and that they had a genetic bottleneck; however, the study subjects were limited to wild populations and did not cover the numerous horticultural cultivars with complex genetic backgrounds. Therefore, assessing genetic diversity and constructing DNA fingerprint maps for *Neofinetia falcata* cultivated germplasm have become urgent tasks for supporting the conservation and sustainable utilization of its resources; at present, related studies remain relatively scattered and lack systematic data support.

In summary, although existing studies have provided preliminary clues for the conservation of *Neofinetia falcata*, for the large number of cultivated cultivars with confused nomenclature, the following three scientific questions have not yet been systematically answered: what the level of genetic diversity is, how phylogenetic relationships among cultivars should be delimited, and whether a reliable molecular fingerprinting system can be established to achieve accurate identification. Therefore, using 99 *Neofinetia falcata* cultivated germplasm accessions as the research objects and relying on SSR molecular marker technology, this study carried out genetic diversity analysis, cluster analysis, and fingerprint map construction, aiming to answer the above questions and provide key technical support for *Neofinetia falcata* cultivar-right protection, market authenticity identification, and marker-assisted molecular breeding.

1 Materials and Methods

1.1 Experimental Materials

All experimental materials were obtained from Ningbo Xingyuan Agricultural Technology Co., Ltd., comprising a total of 99 *Neofinetia falcata* germplasm materials (Table 1). In mid-August 2025, disease- and pest-free young and mature leaves were collected and temporarily stored in liquid nitrogen before being placed in a −80 °C freezer for later use.

Table 1 Names of 99 *Neofinetia falcata* germplasm resources

No.	Cultivar Name	No.	Cultivar Name	No.	Cultivar Name
1	'Taijixian' *N. falcata* 'Taegeuksun'	34	'Honghuadian' *N. falcata* 'Benikaden'	67	'Cuihuadian' *N. falcata* 'Suikaden'

No.	Cultivar Name	No.	Cultivar Name	No.	Cultivar Name
2	‘Fuguidian’ *N. falcata* ‘Fukiden’	35	‘Zhu Tianwanghu’ *N. falcata* ‘Shutenno Tora’	68	‘Qiuhongjin’ *N. falcata* ‘Shuukou Nishiki’
3	‘Xichudu’ *N. falcata* ‘Nishidemiyako’	36	‘Zizhi’ *N. falcata* ‘Shiori’	69	‘Jinlouge’ *N. falcata* ‘Kinkakurou’
4	‘Xihe’ *N. falcata* ‘Saikaku’	37	‘Hongtiangou’ *N. falcata* ‘Benitengu’	70	‘Bainiao’ *N. falcata* ‘Shiratori’
5	‘Yinshijie’ *N. falcata* ‘Ginsekai’	38	‘Beimihu’ *N. falcata* ‘Himiko’	71	‘Qingzhou Fugui’ *N. falcata* ‘Seijiku Fugaku’
6	‘Yujin’ *N. falcata* ‘Tamanishiki’	39	‘Qinghai’ *N. falcata* ‘Seikai’	72	‘Dajiang Wanjin’ *N. falcata* ‘Oemarushima’
7	‘Zhenhe’	40	‘Jianguo’	73	‘Xiansan’

No.	Cultivar	No.	Cultivar	No.	Cultivar
	N. falcata ‘Manazuru’		*N. falcata* ‘Kenkoku’		*N. falcata* ‘Konsan’

No.	Cultivar	No.	Cultivar	No.	Cultivar
8	‘古都’ *N. falcata* ‘Koto’	41	‘贯雪’ *N. falcata* ‘Kansetsu’	74	‘朝鲜铁’ *N. falcata* ‘Chousen Tetsu’
9	‘古都之雪’ *N. falcata* ‘Ko-toyuki’	42	‘桃山锦’ *N. falcata* ‘Momoya-man-ishiki’	75	‘红水晶’ *N. falcata* ‘Kousu-ishou’
10	‘日向猛虎’ *N. falcata* ‘Hyug-amoko’	43	‘桃山香’ *N. falcata* ‘Seijyu’	76	‘憧’ *N. falcata* ‘Dou’
11	‘天明’ *N. falcata* ‘Cheon-myung’	44	‘圣寿’ *N. falcata* ‘Seijyu’	77	‘宝生殿’ *N. falcata* ‘Houshouden’
12	‘奄美冠白’ *N. falcata* ‘AmamiKan-paku’	45	‘豆叶缟’ *N. falcata* ‘Mameba Gashima’	78	‘神曲’ *N. falcata* ‘Shinkyoku’
13	‘高千穗之缟’ *N. falcata* ‘Takachiho no Shima’	46	‘天宫殿’ *N. falcata* ‘Tenkyu-den’	79	‘允庆丸’ *N. falcata* ‘Inkei Maru’
14	‘湖东之剑’ *N. falcata* ‘Ko-tokonot-surugi’	47	‘海王丸’ *N. falcata* ‘Kaioumaru’	80	‘信玄’ *N. falcata* ‘Shingen’
15	‘金广锦’ *N. falcata* ‘Koga-hon-ishiki’	48	‘黑豹’ *N. falcata* ‘Kurohyo’	81	‘红玄’ *N. falcata* ‘Kougen’

No.	Cultivar	No.	Cultivar	No.	Cultivar
16	‘宝锦’ *N. falcata* ‘Takaran-ishiki’	49	‘翠扇’ *N. Falcata* ‘Suisen’	82	‘黑龙宝’ *N. falcata* ‘Kokuryu-uhou’
17	‘织姬’ *N. falcata* ‘Orihime’	50	‘金福’ *N. falcata* ‘Kim-puku’	83	‘红牡丹’ *N. falcata* ‘Benib-otan’
18	‘幽谷锦’ *N. falcata* ‘Yugan-ishiki’	51	‘晃贵殿’ *N. falcata* ‘Kouki-den’	84	‘紫宸殿’ *N. falcata* ‘Shishin-den’
19	‘樱锦’ *N. falcata* ‘Sakura Nishiki’	52	‘绿云’ *N. falcata* ‘Ryokuun’	85	‘源照寺’ *N. falcata* ‘Gen-shouji’
20	‘秋之舞姬’ *N. falcata* ‘Akino-maihime’	53	‘吟风白缟’ *N. falcata* ‘Ginfuu Shi-rogashima’	86	‘都羽二重’ *N. falcata* ‘Miyako-habutae’
21	‘贵妇人’ *N. falcata* ‘Kifujin’	54	‘寿光’ *N. falcata* ‘Jukou’	87	‘伯令’ *N. falcata* ‘Hakurei’
22	‘翠宝’ *N. falcata* ‘Suihou’	55	‘绀波流’ *N. falcata* ‘Kon-haryuu’	88	‘红岗’ *N. falcata* ‘Koukou’
23	‘满天’ *N. falcata* ‘Manten’	56	‘凤玉’ *N. falcata* ‘Hougyoku’	89	‘公主’ *N. falcata* ‘Hime’
24	‘黎明’ *N. falcata* ‘Reimei’	57	‘花衣’ *N. falcata* ‘Hanagoromo’	90	‘花簪’ *N. falcata* ‘Hana kanzashi’

No.	Cultivar	No.	Cultivar	No.	Cultivar
25	‘红扇’ *N. falcata* ‘Benniougi’	58	‘浚河牡丹’ *N. falcata* ‘Shungawa Botan’	91	‘真月’ *N. falcata* ‘Shingetsu’

No.	Germplasm	No.	Germplasm	No.	Germplasm
26	‘万华镜’ *N. falcata* ‘Mangekyou’	59	‘雪中松’ *N. falcata* ‘Setsuchuu Matsu’	92	‘龙泉锦’ *N. falcata* ‘Ryuzen nishiki’
27	‘火影’ *N. falcata* ‘Hwayeong’	60	‘八千代’ *N. falcata* ‘Yachiyo’	93	‘杨贵姬’ *N. falcata* ‘Yokihime’
28	‘旭升’ *N. falcata* ‘Asahizakari’	61	‘剑王’ *N. falcata* ‘Kenou’	94	‘翠月湖’ *N. falcata* ‘Cuiyuehu’
29	‘曼珠沙华’ *N. falcata* ‘Manjushage’	62	‘秋宝’ *N. falcata* ‘Shuuhou’	95	‘童话’ *N. falcata* ‘Douwa’
30	‘丰明殿’ *N. falcata* ‘Houmeiden’	63	‘黄云龙’ *N. falcata* ‘Kiunryuu’	96	‘银雪岭’ *N. falcata* ‘Ginsetsurei’
31	‘紫金天’ *N. falcata* ‘Shikinten’	64	‘日月光’ *N. falcata* ‘Nichigetsukou’	97	‘武藏’ *N. falcata* ‘Musashi’

DNA agarose gel electrophoresis results of partial Neofinetia falcata germplasms

Figure 1: DNA agarose gel electrophoresis results of partial Neofinetia falcata germplasms

No.	Germplasm	No.	Germplasm	No.	Germplasm
32	‘朱天童子’ *N. falcata* ‘Shuten-doji’	65	‘金银罗莎’ *N. falcata* ‘Kingin-rasha’	98	‘梦幻之缟’ *N. falcata* ‘Mugenoshima’
33	‘豆天红梅’ *N. falcata* ‘Mame-teng Koubai’	66	‘雷山’ *N. falcata* ‘Raizan’	99	风兰 *N. falcata*

1.2 Experimental Methods

1.2.1 DNA Extraction and Detection

A magnetic-bead reagent kit from Beijing Boyoushun Biotechnology Co., Ltd. was used to extract genomic DNA from 99 samples. Three μL of DNA sample was taken and detected by 1.5% agarose gel electrophoresis; finally, the DNA was diluted to 50 ng $\cdot$ μL$\hat{}$-1 and stored at -20 °C (Fig. 1).

Lanes from left to right correspond to varieties 1–24 (upper panel) and 25–48 (lower panel); **M.** DNA marker (DL2 000).

Fig. 1 DNA agarose gel electrophoresis results of partial *Neofinetia falcata* germplasms

1.2.2 Transcriptome Sequencing and SSR Primer Design

Using wild plants as the test material, mature leaves were collected, stored in dry ice, and sent to Shanghai Majorbio Bio-pharm Technology Co., Ltd. for transcriptome sequencing. The sequencing platform was Ilumina Noveseq 6000, with three repeated sequencing runs. After the raw data were filtered and quality-checked,

Clean reads were obtained; Trinity v2.8.5 was used to perform de novo assembly of the clean reads, yielding unigene sequences. MISA v1.0 was used to search all unigenes for SSR loci, and unigene sequences containing SSR loci were randomly selected. Primer 3.0 was then used to design 100 pairs of SSR candidate primers.

Fig. 2 Preliminary screening of candidate primer pairs by agarose gel electrophoresis

Figure 2: Fig. 2 Preliminary screening of candidate primer pairs by agarose gel electrophoresis

1.2.3 Primer screening and capillary electrophoresis

Using the DNA of six preselected cultivars– 'Hikari Mouko' , 'Benitengu' , 'Qinghai' , 'Beniougi' , 'Koto no Tsurugi' , and 'Fugui Dian' –as templates, PCR amplification and preliminary screening were performed for the 100 pairs of candidate primers (Appendix Table 1). During primer screening, the annealing temperature differed among primers. All primer PCR amplification products were preliminarily screened using 1.5% agarose gel electrophoresis (Fig. 2), and samples with clear bands and consistent brightness were selected. These screened samples were then subjected to 6% denaturing polyacrylamide gel electrophoresis to further verify primer polymorphism (Fig. 3). Finally, based on the clarity of amplified bands, polymorphism richness, and stability, 20 pairs of core SSR primers were selected for subsequent population genetic analysis (Table 2). The PCR reaction system (total 20 μL) was as follows: ddH_2O 7 μL, 2× Easy Taq PCR Super Mix 10 μL, 10 μmol $\cdot$ L^{-1} forward and reverse primers 0.5 μL each, and DNA template 2 μL. The reaction program was: predenaturation at 94 °C for 5 min; denaturation at 94 °C for 30 s, annealing for 35 s (the annealing temperatures of each primer are shown in Table 2), and extension at 72 °C for 40 s, for a total of 35 cycles; final extension at 72 °C for 3 min. PCR products were subjected to capillary electrophoresis using a 3730 XL sequencer (ABI, USA), and the original peak profiles were analyzed with Genemarker V2.2.0 software to determine the allele sizes of each sample at different loci.

P1–P16 and P85–P100 are primers; lanes from left to right correspond to *Neofinetia falcata* cultivars 10, 37, 39, 25, 14, and 2; **M**. DNA marker (DL2 000).

Primers used: P1–P16, P85–P100; Lanes from left to right correspond to *Neofinetia falcata* varieties 10, 37, 39, 25, 14, and 2; **M**. DNA marker (DL2 000).

Fig. 2 Preliminary screening of candidate primer pairs by agarose gel electrophoresis.

Primers are denoted as P; lanes from left to right correspond to *Neofinetia falcata* varieties 10, 37, 39, 25, 14, and 2; **M.** DNA marker (DL 500).

Fig. 3 PAGE analysis results

1.2.4 Genetic diversity analysis

Based on the genotype data of alleles amplified in 99 *Neofinetia falcata* germplasms using 20 pairs of core SSR primers, the following population genetic diversity parameters were calculated with Popgene v1.32 software: the

number of alleles per locus (N_a), effective number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), Nei' s gene diversity index (H), and Shannon' s information index (I). Meanwhile, PowerMarker software was used to calculate the polymorphism information content (PIC) of each locus, classify the polymorphism level of the primers, and evaluate the level of genetic variation within the population.

1.2.5 Analysis of population genetic relationships

Based on Nei' s (1972) standard genetic distance, Mega 7.0 software was used to construct a phylogenetic tree by the unweighted pair-group method with arithmetic mean, so as to visually display the genetic relationships and grouping patterns among the 99 *Neofinetia falcata* germplasms. The core genetic diversity parameters of each group and the genetic distances among core groups were calculated. At the same time, based on the genetic distance matrix, principal coordinate analysis (PCoA) was performed using GenAlEx software.

1.2.6 Construction of SSR fingerprint profiles

To achieve accurate molecular identification of *Neofinetia falcata* germplasms, the method of Wang Qiongqiong et al. (2021) was referenced to construct a unique digital fingerprint code for each germplasm. The specific method was as follows: the sizes of allele fragments in each sample were encoded in a fixed order as numeric strings to form core fingerprint data. SSR primers were sequentially coded as A, B, C, ……. The amplified band data corresponding to each SSR primer for each material were recorded; when the product was a single band, only the band size was recorded (omitting the unit bp). If the amplification product contained multiple bands, the numbers were connected with "~" ; if there was no amplified band, it was indicated by 0. In this way, different varieties were encoded as band-type codes composed of letters and numbers. Thereafter, online software was submitted to ···

(http://cli.im/) to generate QR codes, and the information such as species name, botanical classification, and fingerprint-band type number was entered together. On this basis, an SSR fingerprint map for *Cymbidium* germplasm was constructed.

2 Results and Analysis

2.1 Screening of polymorphic SSR primers

Using six cultivars, 'Rixiang Menghu' , 'Hong Tiangou' , 'Qinghai' , 'Hongshan' , 'Hudong Zhijian' , and 'Fugui Dian' , 20 SSR primer pairs with good polymorphism and clear bands were screened from 100 pairs of preselected primers (Table 2). Among the amplified SSR sequences, dinucleotide repeats were the predominant type (75%), especially the `(AG)n` repeat, which had the highest frequency; hexanucleotide repeats also accounted for a certain proportion (20%),

whereas trinucleotide repeats were relatively few (5%). The expected amplification lengths ranged from 140 to 300 bp.

Table 2 Sequence information of 20 SSR primers

Table 2 Sequence information of 20 SSR primers

Number	Primer	Repeat motifs	Forward sequence (5′–3′)	Reverse sequence (5′–3′)	Average GC content (%)	Annealing temperature (°C)
1	P4	(CT)	CACACA[illegible]GAAA TGTC	[illegible] GCGA	50.0	60
2	P11	(AG)	CCTTATA[illegible]TCCC AGCC	[illegible] ATCC	52.5	60
3	P16	(AG)	TCGACA[illegible]CTCTC CTTCT	[illegible] GGC	52.6	60
4	P17	(CA)	TCAATC[illegible]AATGG CCACGA	[illegible] GCA	43.0	60
5	P22	(TC)	AGCTGA[illegible]GGGA AG-CAC	[illegible] GAGA	57.5	60
6	P23	(AG)	GTTATG[illegible]TACA AGCTG	[illegible] AGCG	52.5	62
7	P24	(GA)	GAAAAT[illegible]ATCTC CCTG	[illegible] AAT	47.5	62
8	P27	(TA)	GGTGCT[illegible]CCAAA CAAT	[illegible] ACA	45.0	60
9	P43	(GA)	TTGCTC[illegible]TTATT CTTTCA	[illegible] TGA	43.0	60
10	P51	(CT)	CTGGGA[illegible]CTAAC CG-GTG	[illegible] AA-GAAA	44.9	60
11	P52	(AG)	TCTCTC[illegible]TCGGG CTC	[illegible] TAT	55.0	60
12	P53	$(AG)_{16}$	TCAGCG[illegible]CTCGT CCAC	[illegible] CCC	52.5	62
13	P58	$(CA)_{15}$	CAGTCTA[illegible]CTCCA CC-CAA	[illegible] GCC	55.0	60
14	P59	$(AG)_{31}$	CCTTCTC[illegible]CAGAA TCGC	[illegible] GTG	52.5	60
15	P63	$(TC)_{21}$	ACTGGG[illegible]CAGA TGAC	[illegible] CCGA	55.0	62

16	P71	$(CTT)_{10}$	GGCTATT[illegible] AGAA	[illegible]ATGGC TGG	47.5	60
17	P96	$(CACCTC)_5$	CCTCTCC[illegible] TCCT	[illegible]ATTCT TGG	52.5	58
18	P97	$(GGGGAA)_5$	ACATCCT[illegible] ACCG	[illegible]AGCA ACTA	47.5	60
19	P99	$(AGAGGA)_5$	ATCAAGT[illegible] GAAAC	[illegible]TCGAA ACA	45.0	58
20	P100	$(CCCTAA)_5$	CTTTCGT[illegible] GAGG	[illegible]CTGGT AGT	55.0	60

2.2 Genetic Diversity of 99 *Neofinetia falcata* Germplasm Accessions

Twenty pairs of primers were used to amplify the 99 *Neofinetia falcata* germplasm accessions, and a total of 214 allelic loci were detected. The number of alleles (N_a) per primer pair ranged from 4 to 26, with an average of 10.7 (Table 3). Among all primers, P58 and P59 produced the largest number of alleles, with 26 each; P43 followed with 21; and P63 produced the fewest alleles, with only 4. The effective number of alleles (N_e) ranged from 1.668 3 (primer P27) to 11.887 2 (primer P59), with an average of 4.461 4. The polymorphism information content (PIC) of the primers ranged from 0.366 6 to 0.910 6, with a mean of 0.634 8. Among the 20 selected primer pairs, 16 had PIC values >0.5 and were highly polymorphic primers; the PIC values of primers P22, P11, P27, and P63 were slightly lower than 0.5, indicating moderate polymorphism. This shows that the selected primers generally exhibited relatively high polymorphism. The mean Shannon' s information index (I) of the *Neofinetia falcata* population was 1.533 1, with the highest value of 2.816 5 (primer P58) and the lowest value of 0.750 6 (primer P63). The observed heterozygosity (H_o) ranged from 0.060 6 to 0.626 3, with a mean of 0.340 9; the expected heterozygosity (H_e) ranged from 0.402 6 to 0.920 5, with a mean of 0.678 4. Nei' s gene diversity index (H) ranged from 0.400 6 to 0.915 9, with an average of 0.675 0, indicating that the genetic diversity level of the *Neofinetia falcata* germplasm was relatively high.

Table 3 Polymorphism of 20 SSR primers in the 99 *Neofinetia falcata* germplasm samples

Primer	Number of alleles (N_a)	Effective number of alleles (N_e)	Shannon' s information Index (I)	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)	Nei' s gene diversity index (H)	Polymorphism information content (PIC)

P4	15.0	5.548 3	1.990 4	0.505 1	0.823 9	0.819 8	0.797 0
P11	9.0	2.072 3	1.163 2	0.404 0	0.520 1	0.517 4	0.494 6
P16	7.0	3.087 9	1.270 8	0.363 6	0.679 6	0.676 2	0.617 4
P17	6.0	2.720 2	1.163 9	0.060 6	0.635 6	0.632 4	0.573 3
P22	10.0	2.895 0	1.340 1	0.333 3	0.657 9	0.654 6	0.593 8
P23	10.0	2.505 4	1.296 0	0.383 8	0.603 9	0.600 9	0.564 9
P24	9.0	3.692 2	1.518 2	0.222 2	0.732 9	0.729 2	0.686 4
P27	6.0	1.668 3	0.860 0	0.252 5	0.402 6	0.400 6	0.379 7
P43	21.0	11.765 9	2.705 1	0.444 4	0.919 7	0.915 0	0.909 2
P51	16.0	7.720 4	2.351 1	0.424 2	0.874 9	0.870 5	0.859 8
P52	7.0	3.460 8	1.433 0	0.434 3	0.714 7	0.711 0	0.661 4
P53	10.0	4.080 3	1.735 7	0.252 5	0.758 8	0.754 9	0.731 3
P58	26.0	11.880 0	2.816 5	0.232 3	0.920 5	0.915 8	0.910 6
P59	26.0	11.887 2	2.798 6	0.626 3	0.920 5	0.915 9	0.910 4
P63	4.0	1.922 3	0.750 6	0.282 8	0.482 2	0.479 8	0.382 8
P71	7.0	2.535 5	1.127 3	0.404 0	0.608 7	0.605 6	0.546 9
P96	9.0	2.778 1	1.333 1	0.393 9	0.643 3	0.640 0	0.598 0
P97	5.0	1.715 1	0.759 8	0.262 6	0.419 1	0.416 9	0.366 6
P99	6.0	2.560 3	1.057 8	0.131 3	0.612 5	0.609 4	0.529 8
P100	5.0	2.731 6	1.190 8	0.404 0	0.637 1	0.633 9	0.583 0
Mean value	10.7	4.461 4	1.533 1	0.340 9	0.678 4	0.675 0	0.634 8
Mean value							

2.3 Genetic distances among 99 cultivars of *Cymbidium* spp.

Based on SSR molecular markers, Nei' s genetic distances among cultivars were calculated. The genetic distances of the 99 *Cymbidium* cultivars ranged from 0 to 3.919 7, with an average of 0.920 3. Among them, the genetic distance between 'Heilongbao' (No.82) and the original *Cymbidium* species (No.99) was the greatest (3.919 7), indicating the most distant genetic relationship. The genetic distance between many groups of cultivars was 0, including 'Xichudu' (No.3), 'Gudu' (No.8), and 'Gudu Zhi Xue' (No.9); among 'Xihe' (No.4), 'Yinshijie' (No.5), 'Yujin' (No.6), and 'Zhenhe' (No.7), all four of which are evolved cultivars of 'Xichudu' ; between 'Tianming' (No.11) and 'Bowstream' (No.55); among 'Baojin' (No.16), 'Yuan Zhaosi' (No.85), and 'Longquanjin' (No.92); between 'Zhu Tian Tong Zi' (No.32) and 'Leishan' (No.66); and between the original *Cymbidium* species (No.99) and 'Shengshou' (No.44), 'Bainiao' (No.70),

This indicates that these varieties may be clonal materials with synonyms or highly consistent genetic backgrounds.

2.4 Cluster Analysis among 99 *Neofinetia falcata* Germplasm Accessions

The clustering results based on Nei' s genetic distance (Fig. 4) showed that the 99 *Neofinetia falcata* germplasm accessions could be divided into 15 groups at a similarity threshold of 0.62. Among them, Group I contained the original *Neofinetia falcata* species (No. 99), "White Bird" (No. 70), and four other classic varieties, and was the basic representative group among the *Neofinetia falcata* resources. Groups II and III were single-variety groups ["Manzhushahua" (No. 29) and "Fengmingdian" (No. 30)], belonging to classic superior varieties of jewel-root type, with independent and unique genetic backgrounds. Groups V (19 accessions), VI (14 accessions), and XV (19 accessions) were the groups containing the largest numbers of varieties. Group V was dominated by jewel-root white-flowered and mud-root white-flowered types, and included a large number of key hybrid varieties, with relatively high genetic diversity. Group VI consisted entirely of white-flowered types, encompassing multiple evolved varieties of "Xichudu," and the genetic associations among varieties were close. Group XV was dominated by mud-root types and had rich flower colors (including colored flowers, red flowers, pink flowers, and white flowers), with relatively concentrated variation characteristics.

The genetic diversity parameters of the 15 groups and the genetic distances among groups are shown in Table 4. The genetic differentiation characteristics of the clustered groups were highly consistent with the clustering results. Differences in genetic diversity among the 15 clustered groups were closely related to group size and variety origin. The H_e and PIC values of the highly polymorphic groups (V and XV) were higher than those of the other groups, and their genetic distances from the basic Group I were 0.541 7 and 0.512 1, respectively, indicating that although they originated from the basic group, genetic variation had increased significantly after long-term hybrid improvement. The H_e (0.534 8) and PIC (0.511 1) of Group VI were relatively low, and its genetic distance from Group V was only 0.430 4, indicating that the genetic associations among varieties in this group were close and the range of gene exchange was relatively narrow. The H_e and PIC values of the single-variety groups (II and III) were both close to 0, and their genetic distances from the other groups were all greater than 0.7, reflecting the independence of their genetic backgrounds. The remaining medium-sized groups (IV, VIII, X, etc.) had H_e and PIC values at intermediate levels, and their genetic distances from the core groups (V and XV) were concentrated between 0.423 3 and 0.591 3, indicating that they had certain genetic associations with the core groups and were important supplements to the core groups.

Fig. 4 Cluster analysis of 99 *Neofinetia falcata* germplasm resources based on SSR markers

Fig. 4 Cluster analysis of 99 *Neofinetia falcata* germplasm resources based on SSR markers

Figure 3: Fig. 4 Cluster analysis of 99 *Neofinetia falcata* germplasm resources based on SSR markers

Table 4 Core genetic diversity parameters and inter-group genetic distances of 15 clustered groups of *Neofinetia falcata*

Group	Number of germplasms	Number of alleles (N_a)	Effective number of alleles (N_e)	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)	Polymorphism information content (PIC)	Genetic distance I	Genetic distance V	Genetic distance XV
I	4	3.9500	3.2156	0.3125	0.6609	0.6335	0.0000	0.5417	0.5121
II	1	1.1500	1.1500	0.1500	0.0750	0.0656	1.0614	0.9147	0.7321
III	1	1.2500	1.2500	0.2500	0.1250	0.1094	0.9228	0.8431	0.8338
IV	4	3.5000	2.7075	0.5000	0.5812	0.5554	0.5974	0.4602	0.4909
V	19	6.3000	3.7893	0.4038	0.6303	0.6118	0.5417	0.0000	0.3257
VI	14	4.3500	2.9001	0.2857	0.5348	0.5111	0.5183	0.4304	0.3552
VII	2	1.8000	1.6300	0.2250	0.3062	0.2779	0.7355	0.5585	0.4908
VIII	6	2.5500	2.0126	0.2500	0.4194	0.3911	0.6575	0.4925	0.3989
IX	3	2.3500	2.0413	0.2833	0.4111	0.3831	0.5532	0.5830	0.3388
X	7	3.2000	2.5418	0.3643	0.4612	0.4373	0.6004	0.5913	0.3625
XI	6	3.9500	2.8489	0.3917	0.5271	0.5073	0.6183	0.4233	0.3027
XII	2	1.7000	1.6100	0.1500	0.2812	0.2537	0.6041	0.6583	0.3509
XIII	5	3.0000	2.1598	0.2400	0.4310	0.4096	0.6660	0.5081	0.3110
XIV	6	3.0500	2.4846	0.2333	0.4569	0.4301	0.5623	0.4154	0.2620

Group	Number of germplasms	Number of alleles (N_a)	Effective number of alleles (N_e)	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)	Polymorphism information content (PIC)	Genetic distance I	Genetic distance V	Genetic distance XV
XV	19	4.8500	2.8133	0.4132	0.5474	0.5255	0.5121	0.3257	0.0000

2.5 Principal Coordinate Analysis

The principal coordinate analysis (PCoA) based on Nei' s genetic distance matrix showed that the cumulative variance explained by the first two principal coordinates (PC1 and PC2) reached 63.9% (PC1 contribution rate 35.2%, PC2 contribution rate 28.7%), effectively reflecting the pattern of genetic differentiation among germplasms and showing a high degree of agreement with the clustering analysis results (Figure 5). In the two-dimensional PCoA scatter plot, the 15 clustered groups displayed clear spatial distribution characteristics: the groups with the largest numbers of accessions, V, VI, and XV, each formed independent aggregation regions—Group V

the inter-germplasm spatial distance was relatively close (genetic diversity was relatively high but genetic association was tight), and the overlap of scatter points in group VI was high (the genetic distance between varieties was small). The dispersion of scatter points in group XV was slightly higher than that in groups V and VI (variation characteristics were concentrated). The single-variety groups II ("Manzhu Shahua" No. 29) and III ("Fengmingdian" No. 30) appeared as independent scatter points, located at the far ends of the positive and negative axes of PC2, respectively, with spatial distances from other groups being significant, verifying the independence of their genetic backgrounds. The basal group I (four classical varieties) was distributed in the central-axis region, with a moderate degree of scatter aggregation, consistent with its position as a basal representative group. Groups with closer spatial distances had smaller genetic distances; for example, the scatter regions of group V and group VI were adjacent, with an average genetic distance of only 0.28. In summary, the PCoA results not only intuitively revealed the genetic differentiation pattern of the 99 *Neofinetia falcata* germplasms, but also verified the rationality of the group classification by cluster analysis from the spatial dimension, providing dual support at the molecular level for genetic diversity evaluation and group delimitation of *N. falcata* germplasm.

Fig. 5 Principal coordinate analysis (PCoA) of 99 *Neofinetia falcata* germplasm samples

Fig. 5 Principal coordinate analysis (PCoA) of 99 Neofinetia falcata germplasm samples

Figure 4: Fig. 5 Principal coordinate analysis (PCoA) of 99 Neofinetia falcata germplasm samples

2.6 DNA Fingerprinting

Using 20 pairs of SSR primers, 92 of the 99 *Neofinetia falcata* varieties could be completely distinguished. Three evolved varieties of "Xichudu" – "Xihe" (No. 4), "Yujin" (No. 6), and "Zhenhe" (No. 7)—as well as "Gudu" (No. 8) and "Gudu Zhixue"(No. 9), and "Baojin"(No. 16) and "Longquanjin"(No. 92), which are identical or extremely similar varieties, require further screening and identification (temporarily, digits from small to large were sequentially appended after their identical fingerprint codes). Through the online website (http://cli.im/), based on the unique digital fingerprint code of each germplasm, DNA fingerprints of the 99 *Neofinetia falcata* varieties were constructed (Appendix 2). Scanning the QR code provides access to the variety name, leaf shape, leaf color, flower color, root color, flowering time, and digital fingerprint code of *N. falcata* (Fig. 6).

'Taigeuksun'
N. falcata 'Taegeuksun'

'Fukiden'
N. falcata 'Fukiden'

'Nishidemiyako'
N. falcata 'Nishidemiyako'

'Saikaku'
N. falcata 'Saikaku'

'Ginsekai'
N. falcata 'Ginsekai'

'Kotoyuki'
N. falcata 'Kotoyuki'

'Hyugamoko'
N. falcata 'Hyugamoko'

'Cheonmyung'
N. falcata 'Cheonmyung'

'AmamiKanpaku'
N. falcata 'AmamiKanpaku'

'Taegeuksun'
N. falcata 'Taegeuksun'

Fig. 6 QR codes of some *Neofinetia falcata* varieties

Fig.6 QR codes of part *Neofinetia falcata* varieties

3 Discussion and Conclusion

3.1 Analysis of genetic diversity in *Neofinetia falcata* germplasm resources

In this study, 20 pairs of SSR primers were used to evaluate 99 accessions of *Neofinetia falcata* germplasm. The results showed that the level of population genetic diversity was relatively high (mean N_a = 10.7, He = 0.678 4, H = 0.675 0, PIC = 0.634 8); among them, primers such as P58, P59, and P43 exhibited extremely high polymorphism (PIC > 0.9) and are core markers for variety identification. The genetic diversity indices of the materials in this study were significantly higher than those reported by Park et al. (2018) for wild Korean *Neofinetia falcata* populations (Ne = 2.15, H = 0.42, I = 0.83). The intrinsic reasons for this difference are, on the one hand, that artificial selection and germplasm exchange have enriched variation—the 99 materials cover Japanese and Korean horticultural cultivars, Chinese native species, and artificial hybrid progenies. Long-term artificial selection for ornamental traits such as flower color, root type, and leaf variegation has not only retained superior genotypes but also introduced new genetic combinations through hybridization, accumulating abundant allelic variation. On the other hand, the difference is also related to the genetic background provided by the distinctive low-elevation wild populations native to China (such as Ningbo) (Sun Jijing, 2008; Lin Li et al., 2017; Hua Wen, 2020). Meanwhile, the 20 pairs of SSR primers developed through transcriptome sequencing in this study detected a total of 214 alleles among the 99 *Neofinetia falcata* germplasm accessions, with an average of 10.7 alleles per locus and including 16 pairs of highly polymorphic primers. They have strong discriminatory power and can effectively support genetic-diversity analysis and fingerprint construction for *Neofinetia falcata* germplasm. Therefore, although wild *Neofinetia falcata* faces threats from habitat fragmentation and overcollection, cultivated populations have effectively preserved and enriched its genetic variation through cross-regional introduction and hybridization, laying a key foundation for varietal innovation and conservation of genetic resources in the genus *Neofinetia*.

3.2 Genetic basis of inter-varietal relationships and cluster division

Cluster analysis based on Nei' s genetic distance divided the 99 germplasm accessions into 15 clusters, and the cluster division was highly associated with phenotypic traits such as flower color, root type, and leaf type. Cluster I comprised the original *Neofinetia falcata* species and classic cultivars such as 'Bainiao' ; its genetic background was relatively conservative and highly consistent with the original morphological characteristics of the genus *Neofinetia* (Editorial Committee of *Flora of China*, Chinese Academy of Sciences, 1999), indicating that this cluster has been subject to relatively little artificial intervention. Cluster VII was dominated by white-flowered cultivars, whereas Cluster

XV covered multiple flower-color types such as yellow flowers and red flowers, and its root type was mainly mud-rooted, reflecting directional selection for ornamental traits during breeding. Principal coordinate analysis (PCoA) showed that the first two principal coordinates cumulatively explained 63.9% of the genetic variation, further verifying the rationality of the cluster division. Young et al. (2023), based on chloroplast *matK* and nuclear ITS sequences, conducted systematic analysis of the genus *Neofinetia* and its close relatives.

Phylogenetic analysis also indicated that the genetic clustering results of this species and its varieties were significantly associated with key morphological traits (such as leaf shape and root type), corroborating the conclusion in this study that "cluster division is associated with phenotypic trait aggregation."

In addition, the genetic distance between some varieties was 0 (for example, between 'Xichudu' and its evolved varieties), suggesting that these varieties may be synonyms, sister lines, or clone materials with extremely similar genetic backgrounds. This naming confusion is widespread among orchid plants (Hinsley et al., 2018), and its fundamental causes lie in the prevalence of asexual reproduction and the lack of unified standards for folk nomenclature. By combining SSR markers with clustering and PCoA analyses, this study more accurately revealed the kinship relationships among varieties, providing direct molecular evidence for variety sorting and standardized nomenclature; at the same time, it also compensates for the previous shortcomings of scattered and unsystematic studies on orchid kinship relationships (Zhang Ying et al., 2010). Clear cluster division not only helps elucidate the evolutionary process of orchids from wild species to horticultural varieties, but also provides genetic grouping references for the subsequent construction of core germplasm banks and the selection of hybrid parents, thereby avoiding inbreeding depression in offspring caused by close hybridization.

3.3 Construction and Application Prospects of SSR Fingerprint Profiles

Based on 20 pairs of core SSR primers, this study successfully constructed a digital fingerprint coding system for 99 orchid germplasms and generated a corresponding QR-code information database, with a discrimination rate of 92.9%. This fingerprint profile can completely distinguish 92 varieties; the remaining 7 varieties have highly similar genetic backgrounds and require further detailed identification through high-resolution markers or phenotypes. In view of the bottleneck that long-term identification of orchid varieties relies heavily on morphological traits that are easily affected by growth stage and environment, the SSR fingerprint profile constructed in this study provides an objective and stable solution. Compared with traditional methods, molecular fingerprint identification has the advantages of high accuracy and good reproducibility (Zhu Xudong et al., 2014). Compared with dominant markers such as RAPD, the codominant nature of SSR markers can more accurately distinguish homozygous/heterozygous states (Zhao et al., 2025), such as identifying subtle genetic

differences between 'Xichudu' and its evolved varieties, thereby providing more reliable evidence for variety-right protection. Meanwhile, this study further integrated QR-code information technology, linking phenotypic information such as variety name, leaf shape, flower color, and root color with molecular fingerprints, making queries more intuitive and convenient and better suited to the practical application scenarios of orchids as ornamental plants.

This fingerprint profile has prominent application value, mainly reflected in the following three aspects: market regulation—rapid identification of the authenticity of high-priced varieties, curbing market chaos such as passing off inferior products as superior ones; resource management—connecting with phenotypic databases to digitize variety information and resolve the dilemma in which traditional records do not match cultivation reality; breeding practice —guiding parental selection and authenticity identification of hybrid offspring, thereby improving breeding efficiency. In addition, by comparing fingerprint characteristics between cultivated varieties and wild populations, it can also trace germplasm origins, providing molecular evidence for combating illegal collection and strengthening the protection of wild resources. In summary, the fingerprint profile and application system constructed in this study directly respond to practical problems in the current orchid market, such as difficulty in distinguishing authentic varieties and confusion in germplasm management, and provide a reliable technical solution for precise molecular identification of cultivated varieties.

3.4 Research Limitations and Prospects

This study systematically completed the genetic analysis of orchid cultivated germplasm and the construction of an SSR fingerprint database, providing important data support for systematic research in this field and demonstrating outstanding application value. However, the samples were still mainly horticultural varieties; in the future, wild germplasm should be supplemented to comprehensively evaluate the genetic background. At the same time, SSR markers have limited ability to distinguish extremely closely related varieties. Future research can be deepened in three directions: first, combining high-throughput markers such as SNPs for fine-scale analysis to improve identification accuracy; second, based on the genetic framework of this study, carrying out association analysis of important traits; and third, promoting molecular marker-assisted breeding to systematically support the conservation and innovative utilization of orchid resources.

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A. 'Houmeiden'(Group III, medium-sized plant, pre-illuminated porcelain-white variegation, white flower, jewel root); **B.** 'Nishidemiyako' (Group XV, fukurin (edge) variegation, white flower, mud root); **C.** 'Cuiyuehu' (Group V, mameba (bean-leaf), striped variegation, green root); **D.** 'Mangekyou' (Group XV, green leaf, pink flower, mud root).

Supplementary Fig. 1 Phenotypic characteristics of *Neofinetia falcata*

Supplementary Table 1 Information of 100 primers

Primer No.	Forward sequence (5′-3′)	Reverse sequence (5′-3′)	Product size (bp)	Repeat motifs
P1	[illegible]	[illegible]	280	$(TC)_{16}$
P2	[illegible]	[illegible]	227	$(TG)_{15}$
P3	[illegible]	[illegible]	247	$(AG)_{21}$

Primer No.	Forward sequence (5′-3′)	Reverse sequence (5′-3′)	Product size (bp)	Repeat motifs
P4	CACACACCGT[illegible]CAGAAAG[illegible]A	[illegible]	226	$(CT)_{15}$
P5	TGTTCAAATA[illegible]GATTCG[illegible]	[illegible]	262	$(CT)_{18}$
P6	CCATCTCCTT[illegible]CCACAAA[illegible]G	[illegible]	142	$(GA)_{23}$

Primer	Sequence 1	Sequence 2	Size	Repeat motif
P7	AACGCTCGCC[illegible]GTTTCTT[illegible]GG	[illegible]	276	$(GA)_{15}$
P8	CATGGTGAAT[illegible]TCATACC[illegible]AA[illegible]	[illegible]	253	$(CA)_{20}$
P9	CCCAAAAGTG[illegible]CAGCAGC[illegible]C	[illegible]	161	$(TC)_{16}$
P10	ATTGTTCGGA[illegible]TTGGCTC[illegible]G	[illegible]	244	$(TC)_{23}$
P11	CCTTATAGGC[illegible]AATCCCA[illegible]C	[illegible]	191	$(AG)_{15}$
P12	ACTGGACAGA[illegible]CCTCCTCC[illegible]T	[illegible]	193	$(TC)_{30}$
P13	CCTCCATCTC[illegible]TCGACTCA[illegible]TT	[illegible]	137	$(TC)_{16}$
P14	CCTTGCTCTG[illegible]TCGTCTG[illegible]G	[illegible]	273	$(CT)_{15}$
P15	ATTTCTCCTC[illegible]GAGGTCA[illegible]C	[illegible]	165	$(AG)_{24}$
P16	TCGACACCGG[illegible]GCTCTCG[illegible]C	[illegible]	205	$(AG)_{21}$
P17	TCAATCTATA[illegible]AATGGC[illegible]A	[illegible]	268	$(CA)_{25}$
P18	CAAGCAATAA[illegible]TTAAAGG[illegible]G	[illegible]	274	$(AG)_{18}$
P19	TGCTCTCCTT[illegible]GGGAGAC[illegible]T	[illegible]	180	$(GA)_{20}$
P20	CTGTGCGTTA[illegible]TAGTCGC[illegible]A	[illegible]	172	$(TC)_{21}$
P21	TTAGAGAAGG[illegible]AGGGCG[illegible]G	[illegible]	241	$(CT)_{22}$
P22	AGCTGAGGGA[illegible]CGGGAGA[illegible]A	[illegible]	156	$(TC)_{26}$
P23	GTTATGCCAC[illegible]GTACAAC[illegible]G	[illegible]	275	$(AG)_{20}$
P24	GAAAATCCAC[illegible]CATCTCA[illegible]T	[illegible]	170	$(GA)_{15}$
P25	GAAGACGAAG[illegible]AGAAGGA[illegible]T	[illegible]	164	$(GA)_{22}$
P26	AATGCCTGTG[illegible]TTATTTG[illegible]A	[illegible]	278	$(TG)_{15}$
P27	GGTGCTTGAT[illegible]AACCAAAA[illegible]A	[illegible]	261	$(TA)_{15}$
P28	CAACCTCAGC[illegible]CTCTCTTC[illegible]T	[illegible]	274	$(CT)_{26}$
P29	AGAAGAAGCC[illegible]GCGTGCC[illegible]A	[illegible]	223	$(AG)_{15}$
P30	TTCCTCTTCC[illegible]TGGTGA[illegible]A	[illegible]	278	$(TC)_{21}$
P31	TCTATGTTTC[illegible]G	[illegible]	162	$(TC)_{20}$
P32	CCCAATGGAT[illegible]GTAACGTC[illegible]C	[illegible]	165	$(TC)_{15}$
P33	GTTGCTTGCC[illegible]GTCAACC[illegible]T	[illegible]	206	$(TC)_{15}$
P34	GTACGGAGGT[illegible]AACCGTC[illegible]G	[illegible]	214	$(CT)_{21}$
P35	TGAAAGAATC[illegible]GCCA[illegible]T	[illegible]	173	$(TC)_{24}$
P36	AAACCCTTCA[illegible]CATCTCAC[illegible]G	[illegible]	165	$(TC)_{15}$
P37	ACGTGTAAAT[illegible]GGCGACA[illegible]A	[illegible]	229	$(GA)_{22}$
P38	CGAAGAAGCT[illegible]CTTCTTC[illegible]T	[illegible]	277	$(TC)_{17}$
P39	CCGAACACGG[illegible]CGTCTGC[illegible]C	[illegible]	277	$(CT)_{19}$
P40	CCTCTCTTCT[illegible]CTCCAAA[illegible]A	[illegible]	239	$(AG)_{19}$

Primer	Sequence 1	Sequence 2	Size	Repeat motif
P41	GTGGAAACTA[illegible]	[illegible]	280	$(TC)_{23}$
P42	TGGTGACTTT[illegible]	[illegible]	229	$(GA)_{15}$
P43	TTGCTCAACC[illegible]	[illegible]	157	$(GA)_{23}$
P44	TGCCTCTGGG[illegible]	[illegible]	279	$(AT)_{15}$
P45	AAATTTGACA[illegible]	[illegible]	280	$(GA)_{18}$
P46	TGCCCTACTC[illegible]	[illegible]	150	$(CT)_{22}$
P47	TTCCAACCCC[illegible]	[illegible]	182	$(AG)_{20}$
P48	CGAGGTCGGG[illegible]	[illegible]	121	$(GA)_{17}$
P49	TAAGCCAGCC[illegible]	[illegible]	209	$(TC)_{34}$

Primer	Forward primer sequence	Reverse primer sequence	Product size	Repeat motif
P50	CAAGTCAACT[illegible]	[illegible]	260	$(AG)_{26}$
P51	CTGGGAGAAC[illegible]	[illegible]	174	$(AT)_{16}$
P52	TCTCTCTTTC[illegible]	[illegible]	203	$(AG)_{23}$
P53	TCAGCGGCTA[illegible]	[illegible]	225	$(AG)_{16}$
P54	CTGCTCGAGA[illegible]	[illegible]	256	$(CT)_{15}$
P55	TTTTCTTTTC[illegible]	[illegible]	129	$(CT)_{24}$
P56	GGAATAGAAG[illegible]	[illegible]	248	$(AG)_{21}$
P57	CACACAAAGG[illegible]	[illegible]	177	$(CA)_{15}$
P58	CAGTCTACCC[illegible]	[illegible]	219	$(AG)_{31}$
P59	CCTTCTCTAT[illegible]	[illegible]	256	$(CT)_{20}$
P60	CCACCAACTT[illegible]	[illegible]	162	$(CT)_{19}$
P61	CCCTGACCTG[illegible]	[illegible]	241	$(CA)_{15}$
P62	CCCTTTGCTT[illegible]	[illegible]	205	$(CT)_{19}$
P63	ACTGGGTTCA[illegible]	[illegible]	223	$(TC)_{21}$
P64	ACCTCTCTGC[illegible]	[illegible]	177	$(AG)_{30}$
P65	ATGTGATTCA[illegible]	[illegible]	217	$(CT)_{25}$
P66	CTGGATTCAT[illegible]	[illegible]	273	$(TCT)_{10}$
P67	GAGAAGACTG[illegible]	[illegible]	216	$(CTG)_{10}$
P68	GAAAAGAAAC[illegible]	[illegible]	268	$(TAA)_{11}$
P69	AAAATGGACT[illegible]	[illegible]	233	$(TGC)_{10}$
P70	AACGCCGATC[illegible]	[illegible]	178	$(TTA)_{13}$
P71	GGCTATTTGG[illegible]	[illegible]	226	$(CTT)_{10}$
P72	TAAAGCAGCC[illegible]	[illegible]	258	$(GAA)_{11}$
P73	GGAACAGTTA[illegible]	[illegible]	184	$(TTA)_{10}$
P74	ATGGAGCAGG[illegible]	[illegible]	157	$(AGG)_{11}$
P75	ATCTCTACGC[illegible]	[illegible]	251	$(TCC)_{11}$
P76	GAAGGGGGAC[illegible]	[illegible]	224	$(CGG)_{10}$
P77	ACCACCGTGA[illegible]	[illegible]	265	$(GCC)_{11}$

Primer	Forward primer sequence	Reverse primer sequence	Product size	Repeat motif
P78	TCACCTGTCA[illegible]	[illegible]	220	$(TCT)_{13}$
P79	TGTTGCCTCA[illegible]	[illegible]	165	$(TCT)_{12}$
P80	AGCGATGGAT[illegible]	[illegible]	163	$(AGG)_{11}$
P81	TCACACCAGG[illegible]	[illegible]	177	$(ATC)_{10}$
P82	ACAGCCATAT[illegible]	[illegible]	251	$(TAT)_{10}$
P83	AGCACCATCC[illegible]	[illegible]	261	$(CCA)_{12}$
P84	TGAATTTCTT[illegible]	[illegible]	258	$(TCA)_{10}$
P85	TTGCTATAAT[illegible]	[illegible]	166	$(CAA)_{19}$
P86	AAAGCTCATA[illegible]	[illegible]	160	$(AAT)_{10}$
P87	GAATCTTTCA[illegible]	[illegible]	169	$(GGA)_{10}$
P88	AAAGGATGTT[illegible]	[illegible]	262	$(CAA)_{15}$
P89	TGGAAAAGGC[illegible]	[illegible]	180	$(ACA)_{10}$
P90	AAACTTCCCA[illegible]	[illegible]	244	$(AAG)_{10}$
P91	CTCGTCTTTC[illegible]	[illegible]	241	$(TCT)_{14}$
P92	GGTTCTTCGC[illegible]	[illegible]	251	$(CCACCT)_{7}$

P93	CTCTCATGGC[illegible]	[illegible]	176	$(CAACCA)_{5}$
P94	AAGGTGTTGC[illegible]	[illegible]	175	$(CCACAT)_{5}$
P95	TCATGAAAAT[illegible]	[illegible]	263	$(CATCAC)_{5}$
P96	CCTCTCCCTC[illegible]	[illegible]	210	$(CACCTC)_{5}$
P97	ACATCCTTCA[illegible]	[illegible]	242	$(AGGGGAA)_{5}$
P98	CCCAAATTAC[illegible]	[illegible]	218	$(TAAAA)_{6}$
P99	ATCAAGTGAT[illegible]	[illegible]	197	$(AGAGGA)_{5}$
P100	CTTTCGTCCT[illegible]	[illegible]	170	$(CCCTAA)_{5}$

Supplementary Table 2 SSR fingerprinting of 99 *Neofinetia falcata* germplasm resources

Germplasm No.	SSR fingerprinting
1	$A232_{235B177C175}185D269E99_{115F259}261G171H217_{220I127J179}183K$
2	$A235_{237B201}203C181D269E145F259_{267G169}175H220I147J185_{193K}$
3	$A237_{248B181}193C175_{181D269E145F259}267G167H220I145_{152J185K171}$
4	$A241B193_{197C185D286E145F259G169H220I152}170J169_{191K167}171L323$
5	$A241B193_{197C185D286E145F259G169H220I152}170J169_{191K167}171L323$
6	$A241B193_{197C185D286E145F259G169H220I152}170J169_{191K167}171L323$
7	$A241B193_{197C185D286E145F259G169H220I152}170J169_{191K167}171L323$
8	$A237_{248B181}193C175_{181D269E145F259}267G167H220I145_{152J185K171}$
9	$A237_{248B181}193C175_{181D269E145F259}267G167H220I145_{152J185K171}$
10	$A235B193C175_{185D286E141}145F259G167H220I154J193K173L323$

Germplasm No.	SSR fingerprinting
11	A235B193C185D269E139$_{145F259G167H220I160J185K163}$171L325M19
12	A224$_{235B193C175}$185D269$_{271E145F261G167H220}$224I127$_{150J169}$179K1
13	A235B193C181D269E145F259G167H220I164J179K173L327M19
14	A237B193$_{201C175D269E139F259G167}$175H217I150$_{152J185K171L334M19}$
15	A246B193$_{201C185D288E145F267G169H220I156J169K167L325M200N264O20}$
16	A237B193C181$_{185D288E139}$145F249$_{259G169H220I145}$176J189K163$_{1}$
17	A235$_{250B181}$193C181D269E139F259G169H220I152J177$_{185K163L3}$
18	A237B193C185D271E139F267G175H220I166$_{172J191K163}$171L323
19	A235$_{237B193C181D288E145F259G167H220I145}$156J185$_{189K163L327M212}$2
20	A235B193C175D288E141$_{145F259}$267G171H220I150$_{154J185K163}$167
21	A219$_{232B193}$197C175$_{185D269E99}$115F259$_{261G169H220I127J167}$169K1
22	A226$_{250B181}$201C175$_{181D286E145F259}$261G167$_{175H217}$220I154J175$_{}$
23	A239B193C181$_{185D288E139F259G169H220I152J193K171L325M227N252}$27
24	A232$_{246B193C185D286E145F259G157}$169H217$_{220I170J193K163L334M210N}$
25	A241B181$_{193C175}$181D288E125$_{141F259G169}$175H220I147J175$_{189K}$
26	A230$_{232B177}$193C175$_{181D286E139}$145F249$_{259G167}$171H215$_{220I154}$16
27	A232B193$_{197C175D269E139F261}$267G169H220$_{222I127}$150J179$_{183K163}$
28	A232B193$_{197C175}$187D258$_{269E139F261G169H220I133J173}$179K167L32
29	A235B177C181D286E141F259$_{267G167H220I172J185K171L329M202}$240
30	A219$_{237B193C181D288E139F259G171H217}$220I154J179$_{185K163L334}$336M

No.	Sequence
31	A232$_{246B193C187D286E125F267G167}$171H220$_{222I156J165K155}$171L325I
32	A230$_{237B193}$201C181$_{185D269E139}$145F249$_{259G167}$171H215I154$_{164J}$
33	A232B193C175$_{187D269E139F263G157}$167H220$_{222I133J165}$183K163$_{16}$
34	A230$_{232B193}$201C175$_{185D269E139}$145F259$_{269G167H215I145}$164J177$_{18}$
35	A232B193$_{201C185D269E139}$145F249$_{259G167}$171H220I145$_{164J177K163}$
36	A235B193C181D269E125F259G169H220I145$_{168J189K163}$171L336
37	A232B201C185D269E139F267G167H217I156J193K163$_{171L336M2}$
38	A230B193C185D286E139F259$_{267G169H220I145J187K163}$167L323M2
39	A235$_{246B193C175D269E145F259G167}$171H220I152$_{184J177}$193K171L33
40	A237B177C181D286E139$_{145F259G167H220I145}$172J189K171L325M
41	A235$_{243B193C175}$185D286E145F267G169H217$_{220I158}$166J185K163
42	A232$_{235B197C175D269E139F249}$259G157$_{169H220}$222I123J177$_{185K155}$1
43	A235$_{246B201C175}$181D269E139$_{145F249}$267G169H220I152$_{156J191K16}$
44	A232$_{248B201C181}$185D286E139F269G157H220I166$_{174J187K171L334}$
45	A232$_{235B193C185D286E139F259G175H215}$220I152J187K171L325M240
46	A232B193C185D286E139F259G175H215$_{220I152}$166J187K171L32
47	A232B193C175$_{181D269E145F249}$259G159$_{169H220I152J179}$193K163$_{16}$
48	A232$_{243B201C185D288E139F249}$267G167H220I150J185K163L325M1
49	A235$_{239B201C175}$185D269E141$_{145F259}$267G171H217$_{220I143}$152J18
50	A235$_{241B193C185D269E139F259}$267G169H220I145J193K171L336M2

No.	Sequence
51	A232$_{239B193C175}$185D286E139F259G157$_{169H220I164J167K171L325M}$
52	A237B193C175$_{185D269E145F259G175H220I168J189K167}$171L323M196
53	A232$_{235B177}$193C175D269E139F261$_{269G167H220}$224I127J175K163
54	A235B193C185D269E139$_{145F267G167H215I154J185K171L334}$338M19
55	A235B193C185D269E139$_{145F259G167H220I160}$168J185K163$_{171L325}$
56	A237$_{252B193C185D269E139}$145F259G167H220I168$_{172J189K171L332}$33
57	A232B193C185D269E141F259G167H220I164J185K163L332M20
58	A237$_{246B181}$193C181$_{185D269E143F267G163H217}$220I147J169$_{177K163}$1
59	A232$_{237B177}$201C175$_{187D269E139F249}$259G167H217$_{220I150J173}$185K
60	A232$_{246B193}$201C185D269E139$_{145F259}$267G169H220I152$_{174J185K}$
61	A232$_{237B191}$193C183$_{185D250}$271E141F259$_{261G169H220}$222I168J165
62	A232B193C175D269$_{271E125}$139F249$_{259G167H222}$224I127$_{158J167}$179
63	A235$_{246B193C181D288E141F259G171H220I154J189K163}$167L323M198N2
64	A232B193$_{197C183}$185D269E139$_{145F259G169}$177H220I158J179$_{185K}$
65	A241B193C185D288E139$_{145F259G167H217I147}$158J185K163$_{171L325}$
66	A237B193$_{201C181}$185D269E139$_{145F249}$259G157$_{167H215}$220I154$_{164}$
67	A232B193$_{197C185D288E139F267G169}$175H220I170J185K163L325$_{327}$
68	A235B175$_{201C185D286E145F246}$267G167H220I154J165$_{169K167L325M}$
69	A232B193C181$_{185D269E139}$145F259G169$_{175H217I152J185K171L336M}$
70	A235B193C185D288E145F259G175H217I174J193$_{197K171L323}$336
71	A235$_{246B193C185D269E145F259G171H220I152J169K163}$167L325M204N2
72	A232$_{246B193C181}$185D269E141$_{145F249}$259G167H220I166J187K163
73	A246B193C185D269E145F259$_{267G171H220I152}$156J169K167L325

74	A246B193C181D269E145F259G167H217$_{220I152J185K171L332}$334M
75	A232$_{235B177}$193C175D269$_{271E99}$115F263G169H220I127J165$_{179K}$
76	A237B193C181D269E145F259G167H220I160J185K163L323M19
77	A235B193C181D288E141F259G167H220I154~170J189K163L323
78	A235B181$_{193C185D286E145F259G169H220I156}$170J169$_{189K163}$167L325
79	A235$_{246B193C175D269E145F259G169H220I152J185K163L332M238N250O199}$
80	A235B193C181D269E139F267G175H220I170J189K163L327M20
81	A230$_{237B193}$201C175$_{181D286E145F249}$269G167$_{175H215}$220I145$_{164J1}$
82	A232$_{246B181}$193C185D288E139F259$_{267G169H220I168}$172J189$_{193K1}$
83	A232$_{235B193C181D288E145F249G171}$175H220I154$_{172J191K167}$171L325
84	A232$_{246B193C181}$185D269E125$_{139F259G167H220I147}$154J177$_{185K175}$
85	A237B193C181$_{185D288E139}$145F249$_{259G169H220I145}$176J189K171L
86	A235$_{237B193C181D286}$288E139$_{145F259G167H220I156J185}$191K163L327
87	A232$_{235B191}$193C175$_{185D286E99}$115F259$_{263G167H217}$222I170J165$_{1}$
88	A230$_{246B193C181D286E145F259G169}$175H220I154J185K163L325M19
89	A232B193$_{197C175D286E139F259}$265G167H220I133J165$_{183K163}$167L
90	A230$_{232B193C181D269E145F259G167H215}$220I164J199K163L332M202
91	A237$_{246B193}$201C181D286E139F259G173H220I152J181K171L32

92	A237B193C181$_{185D288E139}$145F249$_{259G169H220I145}$176J189K163$_{1}$
93	A237$_{246B193}$201C185D288E145F249G167H220I154J169$_{187K163}$16
94	A235B193C185D269E139F259G171H220I154J185K167L332M22
95	A232$_{250B193C175D269E139}$145F259G157H217$_{220I145}$154J187$_{193K171}$
96	A235$_{246B177}$191C187D269E115$_{129F267G169H220I158J169}$191K163L3
97	A235B193C185D269E145F267G169H220I152J189$_{193K163}$171L32
98	A232$_{235B193C185D286E139F259G175H215I152}$166J187K171L325M204
99	A217B175$_{189C195}$203D269E154$_{161F257}$276G167$_{171H243I125}$154J173

Note: Figure translations are in progress. See original paper for figures.