A New Set of Microsatellite Primers for Coelogyne fimbriata (Orchidaceae) and Cross-Amplification in C. ovalis

Authors: Huang, Wei-Chang, Jiang, Kai, Hu, Chao, Xiao, Yue-E, Seyler, Barnabas C., et al.

Source: Applications in Plant Sciences, 5(5)

Published By: Botanical Society of America

URL: https://doi.org/10.3732/apps.1700025
A NEW SET OF MICROSATELLITE PRIMERS FOR *Coelogyne fimbriata* (Orchidaceae) AND CROSS-AMPLIFICATION IN *C. ovalis*

Wei-Chang Huang2,3,4, Kai Jiang2,3,5,7, Chao Hu2,3, Yue-E Xiao2,3, Barnabas C. Seyler6, and Yuan-Yuan Li5

2Shanghai Chenshan Plant Science Research Center, Chinese Academy of Sciences, Chenshan Botanical Garden, Shanghai 201602, People’s Republic of China; 3Shanghai Key Laboratory of Plant Functional Genomics and Resources, Shanghai Chenshan Botanical Garden, Shanghai 201602, People’s Republic of China; 4College of Landscape Architecture, Fujian Agriculture and Forestry University, Fuzhou 350002, People’s Republic of China; 5School of Ecological and Environmental Sciences, Shanghai Key Laboratory of Urban Ecological Processes and Eco-Restoration, East China Normal University, Shanghai 200241, People’s Republic of China; and 6Department of Botany, University of Hawai‘i at Mānoa, Honolulu, Hawai‘i, USA

**Premise of the study:** Declining orchid populations have made it necessary to prioritize the study of population structure and genetic diversity for species including *Coelogyne fimbriata* (Orchidaceae).

**Methods and Results:** A biotin-streptavidin capture method was used to construct a microsatellite library for *C. fimbriata*. A total of 15 polymorphic nuclear microsatellite loci were isolated and characterized using 47 *C. fimbriata* individuals from two natural populations in China. The number of alleles per locus for the two populations ranged from two to 17. The observed and expected heterozygosities ranged from 0.000 to 1.000 and from 0.000 to 0.867, respectively. Among these polymorphic primers, 11 loci were also successfully amplified in *C. ovalis*, and 10 loci showed moderate to high-level polymorphism. Cross-amplification of the 15 polymorphic loci was tested in five related species: *C. cumingii*, *C. eberhardtii*, *C. mayeriana*, *C. peltastes*, and *C. velutina*.

**Conclusions:** Fifteen microsatellites in *C. fimbriata* and 10 in *C. ovalis* have moderate to high-level genetic variation, indicating their utility in population genetic studies, thus contributing to orchid conservation.

**Key words:** *Coelogyne fimbriata; Coelogyne ovalis*; medicinal orchid; microsatellites; Orchidaceae; polymorphic markers.

*Coelogyne fimbriata* Lindl. (Orchidaceae), a medicinal orchid, is mainly distributed in southern China, Cambodia, northeastern Indonesia, Laos, Malaysia, Thailand, and Vietnam (Clayton and Beaman, 2002). Because southern China is the northernmost edge of its distribution region, Chinese *C. fimbriata* populations are of particular concern because populations on distribution margins are most vulnerable to disturbance (Channell and Lomolino, 2000). Furthermore, in consideration of global climate change and habitat fragmentation, it is urgent to design effective conservation strategies for endangered natural orchid populations (Swarts and Dixon, 2009). *Coelogyne fimbriata* is an epiphytic or lithophytic orchid, which requires a dormant period in winter. This species grows on its substrate with creeping and slender rhizomes. It can reproduce both sexually via seed and vegetatively by rhizomatic growth. Usually blooming in late summer, it produces one or two flowers on a scape. The flowers exhibit a type of pollinator deception in which the flower odor mimics food for foraging female wasps (Cheng et al., 2009).

Many studies have focused on the pollination syndromes of orchids (Tang et al., 2014); however, there is a lack of genetic information documented for this species. Because genetic information is important for the conservation and sustainable utilization of orchids (Gijbels et al., 2015), we developed microsatellite markers to allow studies of the genetic diversity, genetic structure, and mating system of *C. fimbriata*. In total, 15 polymorphic microsatellite loci were isolated and characterized to study genetic variation within this species clade. These highly polymorphic loci displayed high genetic variation and extensive usability in congeneric species, and may serve as a universal tool for orchid genetic studies.

**METHODS AND RESULTS**

A biotin-streptavidin capture method was employed to construct a microsatellite-enriched DNA library (Jiang et al., 2011). First, we extracted genomic DNA from silica gel-dried leaves of one *C. fimbriata* individual using a Plant Genomic DNA Extraction Kit (Tiangen, Beijing, China). The enzyme *MseI* (New England Biolabs, Beverly, Massachusetts, USA) was used to...
digest approximately 300 ng of genomic DNA in a 25-μL reaction volume for 2 h at 37°C. Fragments 200–1000 bp in length were then ligated to an Msel-adaptor pair (F: 5′-TACTCGAGACTCAT-3′ and R: 5′-GAGTAGTGTCCCT-
GAG-3′). The ligation-digestion mixture was diluted with ultrapure water (1:4), and the diluted fragments were amplified using Msel-N primer (5′-GATGTCCTGTAGTAAN-3′) in a 25-μL PCR reaction volume at 95°C for 5 min, followed by 23 cycles of 94°C for 30 s, 53°C for 1 min, and 72°C for 1 min. Next, to obtain microsatellite-enriched DNA fragments, the PCR products were hybridized with 5′-biotinylated (AC)₅ probes. We used streptavidin-coated magnetic beads (Promega Corporation, Madison, Wisconsin, USA) to capture single-stranded DNA fragments containing microsatellites. The enriched products were amplified using Msel-N primers for 28 cycles. After the PCR products were purified using a multifunctional DNA Extraction Kit (Bioteke Corporation, Beijing, China), they were ligated into Excherichia coli strain DH5α with the pMD19-T vector (TaKaRa Biotechnology Co., Dalian, Liaoning, China).

We randomly selected and sequenced 249 positive clones using M13+/M13- primers on an ABI 3730 DNA Sequence Analyzer (Applied Biosystems, Foster City, California, USA). Of the 249 sequenced clones, 136 contained microsatellites. Twenty-four sequences were discarded because of short flanking regions for primer design. Finally, we designed 112 primer pairs using Premier 5.0 (PREMIER Biosoft International, Palo Alto, California, USA). We selected 28 individuals from Dawei Mountain, Yunnan Province, and 19 from Diabloo Mountain, Hainan Province, China (Appendix 1), for PCR using these 112 primers. Of the 112 primers, 47 produced an expected band on 1% agarose gel, 40 failed to obtain amplification products, and 25 others produced multiple bands. These primer pairs were selected for further testing.

Table 1. Characteristics of 15 polymorphic microsatellite loci developed for Coelogyne fimbriata.

| Locus   | Primer sequences (5′-3′)* | Tₘ (°C) | Repeat motif | A | Allele size range (bp) | GenBank accession no. |
|---------|--------------------------|---------|--------------|---|-----------------------|----------------------|
| CFI-11  | F: <6-FAM>CGACCATCTCCCGCATAT | 60      | (GT)₉        | 7 | 429–443               | KP676048             |
|         | R: GACACACACTACCTACAC     |         |              |   |                       |                      |
| CFI-26  | F: <6-FAM>ATATACACAGCGGATTC | 60      | (CT)₃        | 3 | 210–218               | KP676049             |
|         | R: CTGTGTGTCCTGTGT        |         |              |   |                       |                      |
| CFI-30  | F: <6-FAM>CACTCTTCTACATTCA | 58      | (ATC)₉       | 2 | 103–109               | KP676050             |
|         | R: AGTGGGCGTTAGGCTATAG    |         |              |   |                       |                      |
| CFI-51  | F: <6-FAM>TGAGAAGTGCTCGAGTT | 58      | (AG)₁₄      | 7 | 336–362               | KP676052             |
|         | R: GGGATGGAGTAAAGGTT      |         |              |   |                       |                      |
| CFI-60  | F: <6-FAM>AAACCTCTGTCCGCTTT | 60      | (TC)₃,…(CT)₃,…(TC)₃ | 2 | 344–346               | KP676053             |
|         | R: GTGCTGAGTGGTTCACA      |         |              |   |                       |                      |
| CFI-120 | F: <6-FAM>GGRATCACTCCATTCACAC | 60      | (GT)₆       | 5 | 362–372               | KP676054             |
|         | R: ATCATAGGATTGACTCTG     |         |              |   |                       |                      |
| CFI-167 | F: <6-FAM>CAAGAACGCAAGCAGGAA | 58      | (AG)₆       | 8 | 259–293               | KP676055             |
|         | R: GAGCACTAACCAGCTAGTTA   |         |              |   |                       |                      |
| CFI-229 | F: <6-FAM>AGGCTTACCTGGCATACTCT | 52      | (CT)₇,…(TC)₇ | 2 | 181–187               | KP676056             |
|         | R: ATTCCTGGCTGGCTCACA     |         |              |   |                       |                      |
| CFI-231 | F: <6-FAM>GCGGTGAGTGTGAA | 52      | (AG)₃,…(GA)₈ | 2 | 285–289               | KP676057             |
|         | R: CAGAGCCATACAGGACGATA   |         |              |   |                       |                      |
| CFI-26  | F: <6-FAM>CTCCCATACCACTAATTT | 55      | (AG)₁₆,…(AG)ₖ | 4 | 153–171               | KX237659             |
|         | R: ATAGCTCATCCCAAGAGCA    |         |              |   |                       |                      |
| CFI-29  | F: <6-FAM>TTGAGTGTGTGTCTTCTT | 52      | (TG)₅       | 5 | 270–308               | KX237660             |
|         | R: TCTAGTCTACACTATCTT     |         |              |   |                       |                      |
| CFI-57  | F: <6-FAM>GGAGAAGAGAAGGAG | 55      | (CT)₇       | 17 | 173–213              | KX237661             |
|         | R: GAGCAAGGAGGAGGAGAGA    |         |              |   |                       |                      |
| CFI-126 | F: <6-FAM>CTCCGGTTGCTGTTTTC | 52      | (CT)₁₀      | 4 | 253–259               | KX237663             |
|         | R: ATTCCTGGCTTGTGCAGTA    |         |              |   |                       |                      |
| CFI-147 | F: <6-FAM>GGAGGTGGTGGATG | 52      | (CT)₁₀,CA,CT₁₀ | 2 | 254–260               | KX237664             |
|         | R: ATGAGGATATATGCAGTA     |         |              |   |                       |                      |
| CFI-172 | F: <6-FAM>CTGGTTTTTTCTCTGTT | 52      | (CT)₁₀,CA,k | 4 | 257–271               | KX237666             |
|         | R: TCTGAGAATACAAACAGA     |         |              |   |                       |                      |

Note: A = number of alleles; Tₘ = annealing temperature.

*Fluorescent dyes (i.e., HEX, ROX, and 6-FAM) are presented with the forward primers.
Table 2. Characteristics of 15 polymorphic microsatellite loci in Coelogyne fimbriata and C. ovalis populations, respectively.*

| Locus | Coelogyne fimbriata | Coelogyne ovalis |
|-------|----------------------|------------------|
|       | DWS population (n = 28) | DLS population (n = 19) | MHX population (n = 21) | JGX population (n = 16) |
| CF1-11 | 7 | 0.259* | 0.740 | 4 | 0.500 | 0.475 | — | — | — | — |
| CF1-26 | 1 | 0.000 | 0.000 | 3 | 0.053* | 0.101 | 9 | 0.333* | 0.859 | 5 | 0.188* | 0.756 |
| CF1-30 | 2 | 1.000* | 0.500 | 2 | 0.158 | 0.229 | 1 | 0.000 | 0.000 | 2 | 0.000* | 0.469 |
| CF1-51 | 7 | 0.286* | 0.665 | 4 | 0.250 | 0.736 | 4 | 0.095 | 0.255 | 5 | 0.500* | 0.585 |
| CF1-60 | 2 | 0.643 | 0.436 | 2 | 0.688 | 0.451 | 1 | 0.000 | 0.000 | 4 | 0.000 | 0.516 |
| CF1-120 | 4 | 0.889* | 0.658 | 3 | 0.316 | 0.277 | — | — | — | — | — | — |
| CF1-167 | 7 | 0.964* | 0.766 | 5 | 0.579* | 0.672 | 3 | 0.929 | 0.554 | 6 | 0.875* | 0.809 |
| CF1-229 | 2 | 0.179 | 0.316 | 2 | 0.063 | 0.061 | 1 | 0.000 | 0.000 | 5 | 0.200* | 0.391 |
| CF1-231 | 2 | 0.680 | 0.449 | 1 | 0.000 | 0.000 | 1 | 0.000 | 0.000 | 5 | 0.200* | 0.391 |
| CF2-26 | 2 | 0.750 | 0.469 | 3 | 0.368 | 0.597 | 6 | 0.667* | 0.604 | 10 | 0.625* | 0.813 |
| CF2-29 | 3 | 0.769 | 0.500 | 4 | 0.333 | 0.474 | 4 | 0.900 | 0.546 | 3 | 0.929 | 0.554 |
| CF2-57 | 5 | 0.926* | 0.598 | 14 | 0.789* | 0.867 | — | — | — | — | — | — |
| CF2-126 | 2 | 0.111* | 0.500 | 4 | 0.474* | 0.669 | 6 | 0.952* | 0.585 | 6 | 0.875* | 0.809 |
| CF2-147 | 1 | 0.000 | 0.000 | 3 | 0.842 | 0.554 | 2 | 0.000 | 0.000 | 4 | 0.000 | 0.000 |
| CF2-172 | 2 | 0.036 | 0.035 | 3 | 0.421 | 0.639 | 10 | 0.529 | 0.877 | 7 | 0.063* | 0.510 |

Note: — = failed to amplify; A = number of alleles; H_e = expected heterozygosity based on Hardy–Weinberg equilibrium; H_o = observed heterozygosity; n = number of individuals genotyped.

*Voucher and locality information for the populations are shown in Appendix 1.

* Indicates significant deviation from Hardy–Weinberg equilibrium (P < 0.001).

CONCLUSIONS

In the current study, although a majority of the developed loci showed monomorphism (68.1%), 15 polymorphic loci were identified in C. fimbriata. These polymorphic loci are valuable for orchid population genetic studies. For example, these markers can be used to characterize the clonal structure of C. fimbriata to estimate seed and pollen flow at a fine scale. Furthermore, these polymorphic loci can provide more information, such as genetic diversity indices, which are important for the conservation and management of the species.

LITERATURE CITED

Channell, R., and M. V. Lomolino. 2000. Dynamic biogeography and conservation of endangered species. Nature 403: 84–86.

Cheng, J., J. Shi, F. Z. Shangguan, A. Dafni, Z. H. Deng, and Y. B. Luo. 2009. The pollination of a self-incompatible, food-mimic orchid, Coelogyne fimbriata (orchidaceae), by female vespula wasps. Annals of Botany 104: 565–571.

Clayton, D., and R. S. Beaman. 2002. The genus Coelogyne: A synopsis. Natural History Publications, Kota Kinabalu, Borneo.

George, É., and J. C. George. 2011. Les Coelogynes. Belin, Paris, France.

Gibbs, P., K. De Hert, H. Jacquemyn, and O. Honnay. 2015. Reduced fecundity and genetic diversity in small populations of rewarding versus deceptive orchid species: A meta-analysis. Plant Ecology and Evolution 148: 153–159.

Goudet, J. 1995. FSTAT: A computer program to calculate F-statistics, version 1.2. Journal of Heredity 86: 485–486.

Govarts, R. 1999. World checklist of seed plants 3 (1, 2a & 2b). MIM, Deurne, Belgium.

Jiang, K., H. Gao, N. N. Xu, E. P. K. Tsang, and X. Y. Chen. 2011. A set of microsatellite primers for Zostera japonica (Zosteraceae). American Journal of Botany 98: e236–e238.

Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research–An update. Bioinformatics (Oxford, England) 28: 2537–2539.

Pelser, P. B., B. Gravendeel, and E. F. De Vogel. 2000. Revision of Coelogyne section Fuliginosae (Orchidaceae). Blumea 45: 253–273.

Table 3. Amplification of 15 microsatellite loci developed for Coelogyne fimbriata in five other Coelogyne species.

| Locus | C. cumingii (n = 5) | C. mayeriana (n = 5) | C. eberhardtii (n = 5) | C. peltastes (n = 5) | C. velutina (n = 5) |
|-------|---------------------|---------------------|-----------------------|---------------------|---------------------|
| CF1-11 | +                   | —                   | —                     | —                   | +                   |
| CF1-26 | +                   | +                   | +                     | +                   | +                   |
| CF1-30 | —                   | —                   | —                     | —                   | +                   |
| CF1-51 | +                   | —                   | —                     | —                   | —                   |
| CF1-60 | —                   | —                   | —                     | —                   | —                   |
| CF1-120 | —                   | —                   | +                     | —                   | —                   |
| CF1-167 | +                   | +                   | +                     | +                   | +                   |
| CF1-229 | —                   | —                   | —                     | —                   | —                   |
| CF1-231 | —                   | —                   | —                     | —                   | —                   |
| CF2-26 | —                   | —                   | +                     | —                   | —                   |
| CF2-29 | —                   | —                   | +                     | —                   | —                   |
| CF2-57 | —                   | —                   | +                     | —                   | —                   |
| CF2-126 | —                   | —                   | —                     | —                   | —                   |
| CF2-147 | +                   | +                   | +                     | +                   | +                   |
| CF2-172 | +                   | +                   | +                     | +                   | +                   |

Note: + = primer successfully amplified; — = primer failed to amplify; n = number of individuals.
1. Locality information for the Coelogyne fimbriata and *C. ovalis* samples used in this study.\(^a\)

| Species                  | Locality ID | Collection locality | Geographic coordinates | Collector       | Collection no. | \(n\) |
|--------------------------|-------------|---------------------|------------------------|-----------------|----------------|------|
| *Coelogyne fimbriata* Lindl. | DWS         | Yunnan, China       | 22.931°N, 103.685°E    | Wei-Chang Huang | CS-HWC201606-2 | 28   |
| *Coelogyne fimbriata*     | DLS         | Hainan, China       | 18.659°N, 109.916°E    | Ming-Zhong Huang| CS-HMZ201610-6 | 19   |
| *Coelogyne ovalis* Lindl. | MHX         | Yunnan, China       | 23.051°N, 103.356°E    | Wei-Chang Huang | CS-HWC201606-5 | 21   |
| *Coelogyne ovalis*        | JGX         | Yunnan, China       | 23.523°N, 100.646°E    | Wei-Chang Huang | CS-HWC201509-8 | 16   |
| *Coelogyne cumingii* Lindl.\(^b\) | —           | Taiwan              | —                      | Wei-Chang Huang | —              | 5    |
| *Coelogyne eberhardtii* Gagnep.\(^b\) | —           | Thailand             | —                      | Wei-Chang Huang | —              | 5    |
| *Coelogyne mayeriana* Rchb. \(^b\) | —           | Taiwan              | —                      | Wei-Chang Huang | —              | 5    |
| *Coelogyne peltastes* Rchb. \(^b\) | —           | Taiwan              | —                      | Wei-Chang Huang | —              | 5    |
| *Coelogyne velutina* de Vogel\(^b\) | —           | Taiwan              | —                      | Wei-Chang Huang | —              | 5    |

*Note:* — = no detailed information available; \(n\) = number of individuals sampled.

\(^a\) All voucher specimens were deposited in Shanghai Chenshan Herbarium (CSH), Shanghai, China.

\(^b\) Samples of *Coelogyne cumingii*, *C. eberhardtii*, *C. mayeriana*, *C. peltastes*, and *C. velutina* were collected from living plants at Shanghai Chenshan Botanical Garden (introduced from Taiwan and Thailand according to the record).
## Appendix 2. Characteristics of 32 monomorphic microsatellite markers developed for *Coelogyne fimbriata*.

| Locus | Primer sequences (5′–3′) | $T_a$ (°C) | Repeat motif | Allele size (bp) | GenBank accession no. |
|-------|-------------------------|-----------|--------------|-----------------|----------------------|
| CF1-33 | F: TAATGATATTCCAGCCTCCCC | 55 | (CT)$_8$ | 197 | KP676050 |
|        | R: CAGGAGATTTCCAGGCTATTAA |           |              |                 |                      |
| CF1-2  | F: CAAATCCAAAATCAGGGAGAAGG | 56 | (AG)$_{15}$ | 152 | KY744706 |
|        | R: TCCAGAAATATATGAGGCGAGGC |           |              |                 |                      |
| CF1-3  | F: GAGAAATTTCAAGGACCAATG | 56 | (TCT)$_3$ | 198 | KX237656 |
|        | R: TCTGAGACAGAAGGAGGGC |           |              |                 |                      |
| CF1-8  | F: TTAGGGTTGGAGGAGGAA | 56 | (AG)$_{13}$ | 402 | KX237657 |
|        | R: CCAAGATGCAAGAGAAAACAA |           |              |                 |                      |
| CF1-15 | F: CGACTTCACTTCCAGTATCTC | 56 | (AG)$_{14}$ | 248 | KY744707 |
|        | R: CACTCACTGAGCCACTTC |           |              |                 |                      |
| CF1-20 | F: GGAATATTGATAAAAAGCACT | 56 | (TC)$_{18}$ | 181 | KY744708 |
|        | R: TCCCAACTCTTCAACACCC |           |              |                 |                      |
| CF1-23 | F: TTCCCGCTTGGTATATCCAATCAT | 64 | (CT)$_{12}$ | 178 | KY744709 |
|        | R: GTTCCCTCTTGGCCTAGTTTAG |           |              |                 |                      |
| CF1-24 | F: ACCCTTCTATGCTGTATT | 62 | (CT)$_{7}$ | 186 | KX237658 |
|        | R: CTTTCTCAACCAAGCTTTTT |           |              |                 |                      |
| CF1-27 | F: GAGGAGGCTGAGTGAGGAA | 62 | (AG)$_{6}$ | 111 | KY766112 |
|        | R: GGAGGAGGCTCAGGAGGAG |           |              |                 |                      |
| CF1-59 | F: GAAGCAGAAAATACATA | 56 | (TC)$_{22}$ | 90 | KY744710 |
|        | R: TCTCACTCCACTCTATCT |           |              |                 |                      |
| CF1-101 | F: TGTCAGCTCCGAGGAGG | 64 | (TC)$_{27}$ | 355 | KY744711 |
|        | R: ATGGAGGGTGGTAGTGTGUG |           |              |                 |                      |
| CF1-112 | F: GGGATTCGGACTGAGATT | 64 | (GA)$_{39}$ | 228 | KX237663 |
|        | R: TTAGTAGGGATGCGAGGAG |           |              |                 |                      |
| CF1-127 | F: TCAAAGTCCTACATC | 53 | (CT)$_{23}$ | 153 | KY744712 |
|        | R: TTTTAGGTCCACACATT |           |              |                 |                      |
| CF1-129 | F: TTGGCATTTTCGCTTCT | 59 | (CT)$_{12}$–(TC)$_{16}$ | 235 | KY744713 |
|        | R: CCTGCTTTTTGTGGTTT |           |              |                 |                      |
| CF1-136 | F: TCGACCGCTGATAGCGCAACA | 64 | (TC)$_{6}$ | 285 | KY744714 |
|        | R: ATGGACCACTCGGCCAGGAC |           |              |                 |                      |
| CF1-137 | F: GGAAGGCTTACGAGAAT | 64 | (TC)$_{10}$–(CT)$_{3}$ | 126 | KY744715 |
|        | R: ATGGGATGACAGGAGGAG |           |              |                 |                      |
| CF1-140 | F: GAGATGCGGAGAGAAGAAAG | 62 | (GA)$_{10}$ | 108 | KY744716 |
|        | R: TGAAGGAGGAGTGGAGGAG |           |              |                 |                      |
| CF1-146 | F: TATCCAATGATATGATGA | 53 | (TC)$_{15}$–(AC)$_{10}$ | 347 | KY744717 |
|        | R: GGAATGCGGAGAAAAGGT |           |              |                 |                      |
| CF1-148 | F: TGAAATAGATATCCGATGATTA | 56 | (AG)$_{20}$ | 227 | KY744718 |
|        | R: AAATCGGTGTATAGGGAC |           |              |                 |                      |
| CF1-149 | F: GTCAACAGAAAGACCGAAG | 56 | (CT)$_{18}$ | 350 | KY738665 |
|        | R: AAAGATCCCTCCTCATTAT |           |              |                 |                      |
| CF1-155 | F: TCTGTGCTTTTCTCTCTTACC | 56 | (CT)$_{20}$ | 104 | KY744719 |
|        | R: CCAATGCTCCCTGAGATAC |           |              |                 |                      |
| CF1-160 | F: GAATCCCTCTGCTCCATT | 53 | (TG)$_{12}$–(GA)$_{15}$ | 109 | KY744720 |
|        | R: GTGTTGTTAAGTGTGTTCGAGTA |           |              |                 |                      |
| CF1-171 | F: TCCCTGTCCGGTGAAAC | 59 | (GA)$_{31}$ | 232 | KY744721 |
|        | R: GAGATCCCTCCGACACATAC |           |              |                 |                      |
| CF1-177 | F: AAGGTTAGAATGGTGAGGGG | 59 | (AG)$_{14}$–AA(AG)$_{13}$ | 405 | KY744722 |
|        | R: GGGAGATGAGCTTTATGAT |           |              |                 |                      |
| CF1-192 | F: ACCTGTTGATATCTCCGAGTC | 64 | (CA)$_{16}$ | 274 | KY744723 |
|        | R: GAGGGTGTGAGAATCTCCATTTA |           |              |                 |                      |
| CF1-213 | F: ACCAAATAGGAAGATGAGGAGAAGGAA | 64 | (CT)$_{16}$ | 151 | KY744724 |
|        | R: ATGGCGGAGCAAGAAAGG |           |              |                 |                      |
| CF1-217 | F: CTTGTTGTCTATATTAGG | 64 | (CT)$_{12}$–(CA)$_{13}$ | 181 | KX237667 |
|        | R: CTTTTTCTACAGCCTTCATT |           |              |                 |                      |
| CF1-222 | F: TACGGAAATCAGGGAGAACAA | 63 | (CT)$_{46}$ | 181 | KY744725 |
|        | R: CGATTTAGGATTAGGAGGTT |           |              |                 |                      |
| CF1-232 | F: AATAAGATAATGGGAGGAGA | 62 | (GA)$_{9}$ | 108 | KY744726 |
|        | R: ACGGAGAGCTGCCTTTTA |           |              |                 |                      |
| CF1-234 | F: ATCAAAACTCTATTATCC | 64 | (TC)$_{8}$ | 292 | KY744727 |
|        | R: AGATTTACGCTGGCAGC |           |              |                 |                      |
| CF1-238 | F: AACCCCGACGACACAATA | 64 | (GA)$_{11}$ | 379 | KY744728 |
|        | R: CGCCGCTATTCCCTACACA |           |              |                 |                      |
| CF1-240 | F: TACAGGCCCTCTAATACCCA | 60 | (TC)$_{13}$ | 488 | KY744729 |
|        | R: CGGAGGGAGGTGTGAGAT |           |              |                 |                      |

*Note: $T_a$ = annealing temperature.*