



Figure 5 Amplification and detection of primers 4, 18, SF3, SF14, SF4, SF12 and SF13

Note: 1~8 The number of strains; M: 50bp DNA ladder

Table 6 Information of microsatellite primers used in this study

Locus name	Dye	Primer sequence (5'-3')	Tm (°C)	Allele size (bp)	Repeated motif
SF12	5'-FAM	F:AAACGAATGAGGCTGAATGG R:GGATGCACGCAATTTTCTTT	58	150-200	(AT) <sub>6</sub>
SF3	5'-FAM	F:GAGAGCTCTGCTGCCATCTT R:ATAACGTTCCCTGGCATCTG	58	150	(TC) <sub>6</sub>
SF4	5'-FAM	F:ATAAAAAAGTCCCCGGAGCAT R:GCCAGTGAAATTGAGGTTGC	58	100-150	(AG) <sub>9</sub>
18	5'-FAM	F:ACATTGATTTGCATTGGGGT R:AGAGCACATTCCGGTACCAC	58	200-250	(CA) <sub>6</sub>
SF13	5'-HEX	F:ACGGCCTTTGTTTTCTCTCA R:AAACCGCCAACACAGGTAAT	58	250-300	(GT) <sub>7</sub>
SF14	5'-HEX	F:CTTCGTCCCCGATACAAGAG R:CATCATGCCCCGATATCATCA	58	200-300	(CAG) <sub>6</sub>
4	5'-HEX	F:AGTGAGAGCACCTGCTGGAT R:AGCAGTGGGCTTTACCCTTT	58	300	(TTC) <sub>5</sub> /(GGGTAAA) <sub>3</sub>

Table 7 The PCR reaction system of the microsatellite markers

Component	Volume (μL)
(Vazyme)2×Taq Master mix	12.5
Forward primer	1.5
Reverse primer	1.5
ddH <sub>2</sub> O	6
DNA template	1.5
Total	20

The PCR reaction program was as follows: 94 °C for 3 min; 30 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min; followed by a final extension at 72 °C for 5 min, and then held at 4 °C. After completion of the PCR reactions, the products were examined by electrophoresis on 3% agarose gels. Qualified PCR amplification products were sent to an automated sequencer (Applied Biosystems) for allele genotyping. GeneMarker software was used to read allele sizes, and genotyping results were obtained for 100 *Platygladus orientalis* individuals.