DEVELOPMENT OF EST-SSR MARKERS FOR *TAXILLUS NIGRANS* (LORANTHACEAE) IN SOUTHWESTERN CHINA USING NEXT-GENERATION SEQUENCING

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**METHODS AND RESULTS**

*Taxillus nigrans* (Hance) Danser (Loranthaceae) is a mistletoe species that is found attached to many canopy tree species in low mountains, hills, and river basins in subtropical areas of southwestern China at elevations of 300–1300 m. Flowering can occur throughout the year, and the fruiting period is mainly in November. The entire plant of this species can be used as raw material for Chinese traditional medicine (Jiang, 1998). However, because the range of the species has undergone rapid expansion mediated by birds in the urban area of Chengdu (Sichuan Province, China), it forms large groves on garden tree species and is sometimes harmful to its host trees, so that individuals of this species are often removed by gardeners. To date, apart from some basic taxonomic data on the species (Gong et al., 2004) and genome studies on other species of *Taxillus* Tiegh. (Rist et al., 2011; Wei et al., 2017), nearly all published research has focused on aspects relating to its medicinal value, for example, the extraction and identification of medicinal components and the optimization of extraction methods (Li et al., 2006, 2009; Zhang et al., 2016; Zhao et al., 2016). There is little information on the genetic diversity and population structure of the species. We are also interested in developing genetic approaches for identification of individuals and assignment testing, which will help in understanding how this species expands its distribution and jumps from host to host in urban areas as well as in the field.

Simple sequence repeat (SSR) markers, also known as microsatellites or short tandem repeats, are highly polymorphic and are therefore useful as molecular markers in population genetic studies (Zhang et al., 2012; Jiang et al., 2015). Transcriptome sequencing has proven to be a powerful and cost-effective tool that has greatly accelerated the process of discovering molecular markers, including single nucleotide polymorphisms (SNPs) and SSRs (Ashrafi et al., 2012; Qi et al., 2016). In this study, we sequenced and assembled the transcriptome of *T. nigrans* and developed a set of expressed sequence tag (EST)–SSR markers for population genetic studies of *T. nigrans*. We also tested the transferability of these markers in herbarium samples of *T. delavayi* (Tiegh.) Danser and five individuals of *Scutella parasitica* L. (collected from the field), another Loranthaceae parasite that co-occurs with *T. nigrans*.

Approximately 10 μg (400 ng/μL) of total RNA was extracted from fresh leaf material of one individual of *T. nigrans* using TRIzol Reagent (Invitrogen, Carlsbad, California, USA). Subsequently, mRNA was isolated using magnetic oligo (dT) beads (Illumina, San Diego, California, USA); it was then fragmented into short fragments using the Ambion RNA Fragmentation Kit (Ambion, Austin, Texas, USA) according to the manufacturer’s protocols. First-strand cDNA synthesis was performed using reverse transcriptase (Invitrogen) with random primers, and second-strand cDNA was synthesized by RNase H and DNA Polymerase I (Invitrogen). Finally, the transcriptome was sequenced on an Illumina HiSeq 2000 system at Novogene (Beijing, China). Prior to the assembly, a stringent filtering process of raw sequencing reads was conducted. The number of

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