Chapter 3
The Evolution of Genetic Studies on *Baccharis*

Patrícia de Abreu Moreira, Heloiza Navarro de Novaes, and G. Wilson Fernandes

Abstract The genetic studies on the genus *Baccharis* started in 1945 and accompanied the development of tools for genetics investigation. First of all, the karyotype of some *Baccharis* species was determined, followed by reports on the chromosome number for some species. The majority of the information on the molecular biology of the *Baccharis* genus was generated to clarify the taxonomic identity of the taxon. In the 2000s, an intraspecific genetic study with the rare and endemic *Baccharis concinna* using randomly amplified polymorphic DNA markers was performed in an altitudinal gradient in Southeastern Brazil. Despite the high genetic variability within populations of *B. concinna*, the populations studied were very similar, and genetic variability was not related to variation in altitude. It was an important study that marked the population genetic investigation on the genus *Baccharis*. Then, next-generation sequencing technology was used to develop microsatellite markers for *B. dracunculifolia*. This set of microsatellite markers was efficient in kinship and gene flow analyses, and a low combined probability of genetic identity was attained when the six loci were included in the analysis. Two other species, *B. concinn*a and *B. aphylla*, were evaluated for the transferability of microsatellite markers developed for *B. dracunculifolia*. Five microsatellite markers that successfully amplified fragments were obtained both in *B. concinna* and *B. aphylla.* Otherwise, more genetic studies on *Baccharis* genus are called for as the importance of its species in community assembly and ecosystem services is increasing.

P. de Abreu Moreira (✉)
Laboratório de Genética Evolutiva e de Populações, Universidade Federal de Ouro Preto, Ouro Preto, Brasil

H. N. de Novaes · G. W. Fernandes
Ecologia Evolutiva & Biodiversidade, Departamento de Genética, Ecologia & Evolução / ICB, Universidade Federal de Minas Gerais, Belo Horizonte, Brasil

G. W. Fernandes et al. (eds.), *Baccharis*,
https://doi.org/10.1007/978-3-030-83511-8_3

Keywords Genetic variability · Heterozygosity · Molecular biology · Microsatellite markers · Phylogeny

Until the mid-1960s, the genetic diversity of populations was accessed by morphological traits as sizes, shapes, or color patterns. These kinds of markers contributed to broaden our knowledge about population genetics. However, there are innumerous limitations to morphological traits; for instance, genetic variation could be overestimated because of phenotypic plasticity (Freeland 2005). Later, the genetic diversity was accessed by the sizes, shapes, and numbers of chromosomes, which was used to reconstruct the evolutionary history of *Drosophila pseudoobscura* by Sturtevant and Dobzhansky (1936). Chromosomal variation was studied between species and populations, but there was no consistent relationship between morphological and chromosomal variation (Rowe et al 2004). Hence, the development of molecular markers revolutionized this scenario, and genetic variation could be accessed from polymorphic proteins or DNA sequences.

A very large amount of information about different species could be performed, which allowed quantifying genetic diversity, population subdivision, gene flow, effective population size, breeding structure, inbreeding depression, natural selection, and genetic drift. All these studies are predominantly intraspecific (within a particular species). Nevertheless, molecular markers as DNA sequences are used in both systematics and phylogenetic studies which focus on the species level of classification (Rowe et al 2004). All this genetic knowledge has become extremely important for the effective conservation of many species.

The studies on the genus *Baccharis* accompanied the development of tools for genetics investigation. Genetic studies of *Baccharis* started in 1945 when chromosome numbers of 28 families of angiosperms were described by Bowden (1945) and included *Baccharis pingraea*, *B. genistelloides*, *B. genistifolia*, *B. halimifolia*, and *B. phyteumoides*. This report contained polar views of meiotic stages and mitotic metaphases of different species. The same number of chromosomes was registered for all these species ($n = 9$ or $2n = 18$). On the other hand, reports on the chromosome number for species in the genus have increased dramatically (see Table 3.1), mainly to better understand the systematics and phylogeny of the group. The chromosome number of *B. dracunculifolia* ($n = 9$) was first described in 1970 using botanical materials collected in the state of Minas Gerais, Brazil (Coleman 1970). It was suggested that the ancestral chromosome number of the family Asteraceae is $n = 9$ (Solbrig et al. 1969; Solbrig 1977; Nesom 2000; Mota et al. 2016). While the majority of *Baccharis* species has a basic chromosome number of $x = 9$, some exceptions were detected as in *B. latifolia* (Turner et al. 1967; Powell and King 1969; Spooner et al. 1995), *B. glutinosa* (Ariza-Espinar 1974), and *B. salicifolia* (Solbrig et al. 1969), all of them with $n = 18$ or in *B. nitida* where $n = 25$ (Powell and King 1969). There is a description of $n = 10$ for *B. tricuneata* (Turner et al.

Table 3.1 Gametic (*n*) and somatic (2*n*) chromosome numbers in *Baccharis*

Species	*n*	2*n*	References
B. acaulis (Wedd. ex R.E.Fr.) Cabrera	–	18 + 1B	Hellwig (1990)
B. aliena (Spreng.) Joch.Müll. (as *Heterothalamus alienus* (Spreng.) O. Kuntze)	9	–	Bernardello (1986)
B. anomala DC.	–	18	Ruas et al. (1989)
B. articulata (Lam.) Pers.	9	–	Rozenblum et al. (1985)
B. boliviensis (Wedd.) Cabrera (as *Heterothalamus boliviensis* Wedd.)	9	–	Rozenblum et al. (1985)
B. brachyphylla A.Gray	9	–	Keil and Pinkava (1976)
	9	–	Spellenberg and Ward (1988)
B. braunii (Polak.) Standl.	9	–	Anderson et al. (1974)
B. brevifolia DC.	9	–	Coleman (1970)
	9	–	Solbrig et al. (1969)
B. breviseta DC. (as *Baccharidastrum argutum* (Less.) Cabrera	9	–	Coleman (1968)
B. burchellii Baker	9	–	Coleman (1970)
B. buxifolia (Lam.) Pers. (as *B. revoluta* Kunth)	9	–	Hunziker et al. (1989)
B. chachapoyasensis Cuatrec.	ca. 9	–	Turner et al. (1967)
B. chilco Kunth	9	–	Turner et al. (1967)
B. coridifolia DC.	9	–	Ariza-Espinar (1974)
	9	18	Hunziker et al. (1990)
B. cutervensis Hieron. (as *B. spathulata* Klatt)	9	–	Turner et al. (1967)
B. darwinii Hook. & Arn. (as *B. heterothalamoides Britton*)	9	–	Turner et al. (1979)
B. decussata (Klatt) Hieron.	9 + 3–5 fragments	–	Powell and King (1969)
	9 + 1B	–	Turner et al. (1967)
B. dracunculifolia DC.	9	–	Casas (1981)
	9	–	Coleman (1970)
B. effusa Griseb.	9 II	–	Wulff et al. (1996)
B. elaeoides Remy	–	18	Hellwig (1990)
B. flabellata Hook. & Arn.	9II	–	Wulff et al. (1996)
B. flabellata Hook. & Arn. var. *argentina* (Heering) Ariza	9II	–	Wulff et al. (1996)
B. genistelloides (Lam.) Pers.	9	–	Coleman (1968)
	9	–	Bowden (1945)
B. genistifolia DC.	9	–	Bowden (1945)

(continued)

Table 3.1 (continued)

Species	*n*	2*n*	References
B. glutinosa Pers.	ca. 9	–	Anderson et al. (1974)
B. glutinosa Pers. (as *B. douglasii* DC.)	9	–	Keil and Pinkava (1976)
	9	–	Solbrig et al. (1964)
B. glutinosa Pers. (as *B. pingraea* DC.)	9	–	Bowden (1945)
	9	–	Covas and Schnack (1946)
	18	–	Ariza-Espinar (1974)
	9	–	Hunziker et al. (1989)
	9	–	Turner et al. (1979)
B. gnidiifolia Kunth (as *B. sternbergiana* Steud.)	9 II	–	Sundberg et al. (1986)
B. grandicapitulata Hieron.	9	–	Turner et al. (1967)
	9II	–	Sundberg et al. (1986)
B. halimifolia L.	9	–	Bowden (1945)
	9	–	Westman et al. (1975)
B. helichrysoides DC.	9	–	Coleman (1968)
B. heterophylla Kunth	9	–	Keil and Stuessy (1977)
B. latifolia (Ruiz & Pav.) Pers.	ca. 18	–	Powell and King (1969)
	18	–	Spooner et al. (1995)
	ca. 18	–	Turner et al. (1967)
	–	18	Müller (2006)
B. linearifolia (Lam.) Pers. (as *B. leptophylla* DC.)	9	–	Hunziker et al. (1989)
B. linearifolia (Lam.) Pers. (as *B. rufescens* Spreng.)	9	–	Ariza-Espinar (1974)
B. linearifolia (Lam.) Pers. (as *B. subcapitata* Gardner)	ca. 9		Turner et al. (1967)
B. ligustrina DC.	9	–	Coleman (1970)
B. linearis (Ruiz & Pav.) Pers.	9	–	Jansen and Stuessy (1980)
	9	–	Hunziker et al. (1990)
	–	18	Hellwig (1990)
B. mexicana Cuatrec.	9	–	Jackson (1970)

(continued)

Table 3.1 (continued)

Species	*n*	2*n*	References
B. mesoneura DC.	9	–	Coleman (1970)
B. microdonta DC.	–	18	Müller (2006)
B. montana DC. (as *B. elaegnoides* Steud. ex Baker)	9	–	Coleman (1970)
B. multiflora Kunth	9	–	Jackson (1970)
B. nitida (Ruiz & Pav.) Pers.	25	–	Powell and King (1969)
B. nivalis (Wedd.) Sch. Bip. ex Phil.	9	–	Solbrig et al. (1964)
B. notosergila Griseb.	9	–	Hunziker et al. (1989)
B. oblongifolia (Ruiz & Pav.) Pers.	9	–	Turner et al. (1967)
B. obovata (Ruiz & Pav.) DC.	–	18	Hellwig (1990)
B. oxyodonta DC. (as *B. melastomifolia* Hook. & Arn.)	9	–	Turner and Irwin (1960)
B. oxyodonta DC.	9	–	Coleman (1968)
B. patagonica Hook. & Arn.	–	18	Dollenz (1976)
B. petiolata DC.	9	–	Hunziker et al. (1989)
B. phylicoides Kunth	9 II	–	Sundberg et al. (1986)
	ca. 9	–	Turner et al. (1967)
B. phyteumoides (Less.) DC.	9	–	Bowden (1945)
B. pilularis DC. subsp. *consanguinea* (DC.) C.B.Wolf	9	–	Raven et al. (1960)
B. pilularis DC. subsp. *pilularis*	9	–	DeJong and Montgomery (1963)
B. plummerae A.Gray	9	–	Raven et al. (1960)
B. poeppigiana DC. subsp. *ocellata* F.H. Hellwig	–	18 + 1B	Hellwig (1990)
B. polifolia Griseb.	9	–	Turner et al. (1979)
B. prunifolia Kunth	9 + 2B	–	Powell and Cuatrecasas (1970)
B. punctulata DC.	9	–	Carr et al. (1999)
	9	–	Rozenblum et al. (1985)
	9 + 4B	–	Rozenblum et al. (1985)
	9	–	Turner et al. (1979)
B. racemosa (Ruiz & Pav.) DC.	9	–	Jansen and Stuessy (1980)
B. rhomboidalis Remy var. *rhomboidalis*	–	18	Hellwig (1990)
B. rhomboidalis Remy subsp. *truncata* (Phil.) F.H. Hellwig	–	18	Hellwig (1990)

(continued)

Table 3.1 (continued)

Species	*n*	2*n*	References
B. riograndensis Teodoro & J.E.Vidal		18	Heiden et al. (2006)
B. rupicola Kunth	9	–	Jansen et al. (1984)
B. salicifolia (Rui & Pav.) Pers.	9	–	DeJong and Montgomery (1963)
	9	–	Jackson (1970)
	9	–	Keil and Stuessy (1975)
	9	–	Keil and Stuessy (1977)
	9	–	Pinkava and Keil (1977)
	9	–	Rozenblum et al. (1985)
	9	–	Solbrig et al. (1964)
	18	–	Solbrig et al. (1969)
	9	–	Turner and Irwin (1960
	ca. 9	–	Turner et al. (1979)
	–	18	Müller (2006)
B. salicifolia Pers. (as *B. lanceolata* Kunth)	9	–	Covas and Schnak (1946)
B. sarothroides A.Gray	9	–	DeJong and Montgomery (1963)
	9	–	Keil and Pinkava (1976)
B. scandens (Ruiz & Pav.) Pers. (as *B. alnifolia* Meyen & Walp.)	–	18	Dillon and Turner (1982)
B. sergiloides A.Gray	9	–	DeJong and Montgomery (1963)
B. serranoi H.Rob.	–	18	H. Rob.
B. serrifolia DC.	9 + 1 fragment + 2B	–	Anderson et al. (1974)
	–	18	Sundberg et al. (1986)
B. sordescens DC.	9 + 1B	–	Keil and Stuessy (1977)
	9	–	Powell and Turner (1963)
B. tarchonanthoides Baker	9	–	Coleman (1970)

(continued)

Table 3.1 (continued)

Species	*n*	2*n*	References
B. thesioides Kunth	9 + 3–4 s	–	Spellenberg and Ward (1988)
Baccharis tola Phil. subsp. *sanctelicis* (Phil.) Joch.Müll. (published as *B. santelicis* Phil.)	–	18	Hellwig (1990)
B. tricuneata (L. f.) Pers. (as *B. magellanica* (Lam.) Pers.)	9	–	Wulff (1984)
	–	18 + 1B	Hellwig (1990)
B. tricuneata Pers. (as *B. tricuneata* (L. f.) Pers. var. *callaenis* Cuatrec.	10	–	Turner et al. (1967)
B. tricuneata Pers. (as *B. tricuneata* (L. f.) Pers. var. *paramorum* Cuatrec.)	9	–	Powell and King (1969)
B. trinervis (lam.) Pers.	Ca. 9 + fragments	–	Anderson et al. (1974)
	9	–	Jackson (1970)
	9	–	Solbrig et al. (1969)
	9	–	Turner and Irwin (1960)
B. trinervis (Lam.) Pers. (as *B. trinervis* (Lam.) Pers. var. *rhexioides* (Kunth) Baker)	9	–	Powell and Cuatrecasas (1970)
	9 II	–	Wulff et al. (1996)
B. vulneraria Baker (as *Baccharidastrum triplinervium* (Less.) Cabrera))	9	–	Coleman (1970)
B. tucumanensis Hook. & Arn.	9 II	–	Wulff et al. (1996)
B. ulicina Hook. & Arn.	9	–	Ariza-Espinar (1974)
	9	–	Turner et al. (1979)
B. vanessae R.M.Beauch.	9	–	Beauchamp (1980)
Baccharis ventanicola (Cabrera) Soria & Zardini (published as *B. rufescens* var. *ventanicola* Cabrera)	9	–	Hunziker et al. (1989)
B. vernalis F.H.Hellw.	–	18	Hellwig (1990)
B. wrightii A.Gray	9 II + 1I		Powell and Powell (1977)
	9 II + 2B		Weedin and Powell (1978)

Adapted from Heiden et al. (2006)

1967), but it is a questionable count since authors pointed that it is possible that a supernumerary or "B" chromosome was mistaken as a bivalent. In fact, B chromosome and some fragments were described in species of the genus as in *B. decussata* (Turner et al. 1967; Powell and King 1969), *B. flabellata* (Wulff et al. 1996), *B. prunifolia* (Powell and Cuatrecasas 1970, 1975), *B. punctulata* (Rozenblum et al. 1985), *B. serrifolia* (Anderson et al. 1974), and *B. thesioides* (Spellenberg and Ward 1988). However, as the genus *Baccharis* appears to exhibit chromosomal stability

(Solbrig et al. 1969; Solbrig 1977), molecular markers are needed to provide information about genetic diversity within and between populations.

The majority of the information on the molecular biology of the *Baccharis* genus was generated to clarify the taxonomic identity of this taxon. Despite being the fourth largest genus of the family Asteraceae and the most specious within the tribe Astereae (Heiden 2014), the evolutionary relationship of the genus is still in discussion. Zanowiak (1991) studied the systematic and phyletic relationships within the subtribe Baccharidinae. This chloroplast DNA study suggests that South American *Conyza* spp. should be included in the subtribe Baccharidinae, that the Baccharidinae consists of some species of *Baccharis* (published as *Heterothalamus*) and *Archibaccharis* clades, while another clade includes South American *Exostigma notebellidiastrum* (published as *Conyza notebellidiastrum*), *Baccharis*, and *Baccharidastrum*. In this same study, Zanowiak (1991) verified that *B. neglecta* and *B. halimifolia* hybridize, with *B. neglecta* being the maternal parent. Some novelties towards a phylogenetic infrageneric classification of *Baccharis* were published by Heiden and Pirani (2016) which includes names of new taxa, new combinations, and names at new rank for subgenera and sections of the genus. Later, Heiden et al. (2019), based mostly on phylogenetic grounds, proposed that *Baccharis* should comprise 440 species classified into 7 subgenera and 47 sections.

In the 2000s, Gomes et al. (2004), for the first time, conducted an intraspecific genetic study in this genus. These authors investigated the genetic variability in *Baccharis concinna* using randomly amplified polymorphic DNA (RAPD) markers. This species is a rare, dioecious, and threatened shrub, endemic to Serra do Espinhaço, Southeastern Brazil. The authors studied 335 individuals belonging to 6 populations along an altitudinal gradient. Despite the high genetic variability within populations of *B. concinna*, the populations studied were very similar, and genetic variability was not related to the altitudinal gradient. The authors argued that their findings could be explained by the *B. concinna* mating system. This shrub is pollinated and dispersed by wind, which may promote an intense gene flow among the studied species patches, independent of elevation. The authors also emphasized the absence of a physical barrier to gene flow by pollen and seed dispersal among the studied patches of individuals in the landscape.

The RAPD technique was developed in the 1990s (Welsh and Mcclelland 1990; Williams et al. 1990); it is quick and easily generated by PCR and requires no prior DNA sequence information. Although RAPD markers were commonly used for genetic diversity in plants (e.g., Wachira et al. 1995; Iqbal et al. 1997; Ram et al. 2008), they are subject to some limitations. Due to the dominance of the RAPD markers, it is not possible to distinguish between homozygotes (one copy of allele) and heterozygotes (two copies of allele) individuals. Furthermore, the RAPD markers do not allow the investigation of direct gene flow using paternity analysis. In addition, RAPD markers are of limited reproducibility because the segments of DNA are amplified by PCR using arbitrary primers that copy genome regions according to the annealing temperature of user selection. Nowadays, the use of other molecular markers is needed to investigate the genetic diversity of plant species with confidence.

Alternatively, the microsatellite markers, also known as simple sequence repeats (SSRs) and short tandem repeats (STR) (Jacob et al. 1991; Edwards et al. 1991), have been used in population and conservation genetics studies (Guichoux et al. 2011). The microsatellite markers are repeating motifs in tandem that are found at high frequency in most taxa genomes and exhibit high levels of polymorphism due to the high mutation rate that make them more informative than other molecular markers (i.e., single nucleotide polymorphism – SNP) (Bhargava and Fuentes 2010). The microsatellite markers are relatively uniformly distributed in the genomes of species, and due to their co-dominance, the distinction between homozygote and heterozygote individuals is possible. Traditionally, microsatellite development was slow, costly, and labor-intensive and required the construction of genomic libraries using recombinant DNA enriched for a few targeted SSR motifs. The repeating motifs can be mono-, di-, tri-, tetra-, penta-, and hexanucleotide repeats (Litt and Luty 1989; Zane et al. 2002).

Dinucleotide microsatellite repeats are commonly produced by the genomic library technique. However, dinucleotide repeats are prone to polymerase slippage during the PCR amplification (slipped-strand mispairing) and suffer from stutter bands (PCR products from the same fragment that are shorter by one or a few repeats) (Chambers and MacAvoy 2000). Unfortunately, this feature of dinucleotide repeats may lead to genotype scoring errors (Clarke et al. 2001; McDowell et al. 2002) making allele definition difficult (Levinson and Gutman 1987; Meldgaard and Morling 1997), especially for heterozygotes with adjacent alleles (Guichoux et al. 2011). Besides that, tri-, tetra-, and pentanucleotide loci perform better than dinucleotides because they are less prone to enzyme slippage (Edwards et al. 1991; Acharige et al. 2012).

The conservation of the sequence in the primer sites flanking the microsatellite loci and the stability of those sequences during evolution (Dayanandan et al. 1997; Ciampi et al. 2008; Feres et al. 2009) allows the use of SSR markers developed from one species to another. The transfer of polymorphic markers in plants is mainly successful within genera, and it has been successfully applied to the genetic analysis of tropical species (Zucchi et al. 2002; Cota et al. 2012; Moreira et al. 2012). Thus, in the last two decades, microsatellite markers have been used to assess gene flow at the population level and recent demographic events and aided in phylogenetic inferences (Braga et al. 2007; Ciampi et al. 2008; Moreira et al. 2008; Cruz et al. 2012; Muñoz-Pajares et al. 2017; Larranaga et al. 2017; Morris and Shaw 2018).

Despite the great number of species in the genus *Baccharis*, there are microsatellite markers developed for *B. dracunculifolia* only (Belini et al. 2016). A set of 17 markers was developed for *B. dracunculifolia* using a genomic microsatellite library (Belini et al. 2016), but out of 17, 12 are dinucleotide microsatellite that implies genotype scoring errors detailed above. Besides, six of them were monomorphic for three *B. dracunculifolia* populations (N = 315 individuals), which reinforced the need to advance in the development of new microsatellite markers for this species.

1 Development of Microsatellite Markers for *Baccharis dracunculifolia* Using NGS Technology

A good strategy to develop microsatellite markers with better performance is using next-generation sequencing (NGS) technology (Zalapa et al. 2012; Ambreen et al. 2015; Bonatelli et al. 2015; Hodel et al. 2016). The NGS allows the rapid and efficient development of microsatellite markers for non-model organisms for ecological and evolutionary studies. Moreover, the advent of NGS provided a cheaper and faster microsatellite development (Guichoux et al. 2011). We followed this approach to develop microsatellite markers for *B. dracunculifolia*, as described next.

A genomic library was built from 100 μg of one individual of *B. dracunculifolia*, in which DNA was extracted from leaves, through paired-end strategy that was sequenced using MiSeq® platform (Illumina©, San Diego, CA) to produce paired-end 250 base reads. A total of 21.4 million reads were obtained, and we used the Perl script PAL_FINDER_v0.02.04 (see Castoe et al. 2012) to identify 11,296 potentially amplifiable locus (PAL) (Table 3.2). We extracted reads that contained perfect dinucleotide, trinucleotide, tetranucleotide, pentanucleotide, and hexanucleotide tandem SSRs, totaling 7,277 PALs.

The SSR repeat motifs consisted of 24.08% dinucleotide, 58.55% trinucleotide, 6.95% tetranucleotide, 5.35% pentanucleotide, and 5.04% hexanucleotide repeat units (Fig. 3.1a). The AT/TA motif repeats were the most abundant dinucleotide SSR, accounting for 76.32% of all dinucleotide repeats (Fig. 3.1b). The trinucleotide AAT/TAA motif repeats were the most abundant type, accounting for 27.20% of all trinucleotide repeat motifs, while ATA/TAT and ATT/TAA repeats accounted for 17.10% and 14.90%, respectively (Fig. 3.1c).

Then, a manual filtering step was performed to select exclusively SSR loci with long and perfect repeats motifs since they tended to be more polymorphic (Zalapa et al. 2012). Thus, we chose tri-, tetra-, penta-, or hexanucleotide repeats present in long reads, larger than 274 bp, and obtained 1356 microsatellite loci candidates for microsatellite markers. To ensure that SSR loci chosen for *B. dracunculifolia* could

Table 3.2 Simple sequence repeat types in *Baccharis dracunculifolia* contigs sequences

Motif length	Number of SSR	Frequency (%)
Mononucleotide	523	4.6
Dinucleotide	1753	15.5
Trinucleotide	4261	37.7
Tetranucleotide	506	4.5
Pentanucleotide	390	3.5
Hexanucleotide	367	3.2
Compound	3240	28.7
Broken	256	2.3
Total	11,296	

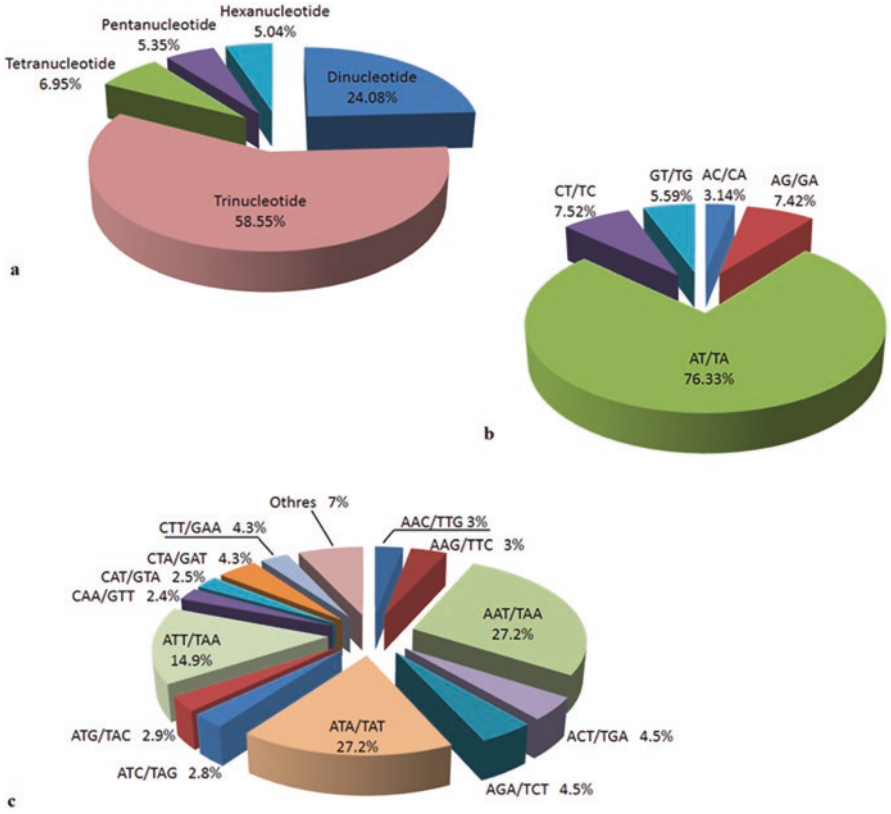

Fig. 3.1 Characteristics of SSR motifs identified in *Baccharis dracunculifolia* using NGS sequencing. (**a**) Frequency of di-, tri-, penta-, tetra-, and hexanucleotide SSR motif repeats. (**b**) Frequency of different dinucleotide SSR motifs. (**c**) Frequency of different trinucleotide SSR motifs

follow as much as possible the stepwise mutation model used in coalescent-based methods to infer demographic events as proposed by Estoup et al. (2001), we selected just perfect motifs. Thus, 36 primer pairs flanking the SSR loci were designed using Primer3 software (Untergasser et al. 2012). Following, a set of 17 perfect microsatellite markers were chosen for amplification screening using 15 *B. dracunculifolia* individuals from the rupestrian grassland vegetation in Serra do Cipó, Brazil (Fig. 3.2). The PCR products were viewed on a polyacrylamide gel electrophoresis (PAGE) 6% and stained with silver nitrate (Sanguinetti et al. 1994).

A total of 12 microsatellite loci (Bdr6, Bdr7, Bdr9, Bdr11, Bdr13, Bdr20, Bdr21, Bdr22, Bdr25, Bdr26, Bdr31, Bdr34) produced clear amplicons with expected size in the acrylamide gel (Fig. 3.3). Then, we designed all these 12 primer pairs, and the forward primers were marked with 4 dyes: VIC®, 6-FAM™, PET®, and NED™.

To assess the polymorphism and population genetic parameters with these microsatellite markers, we genotyped 60 individuals of *Baccharis dracunculifolia* from the Serra do Cipó region: 20 individuals between 760 and 839 m, 20 between

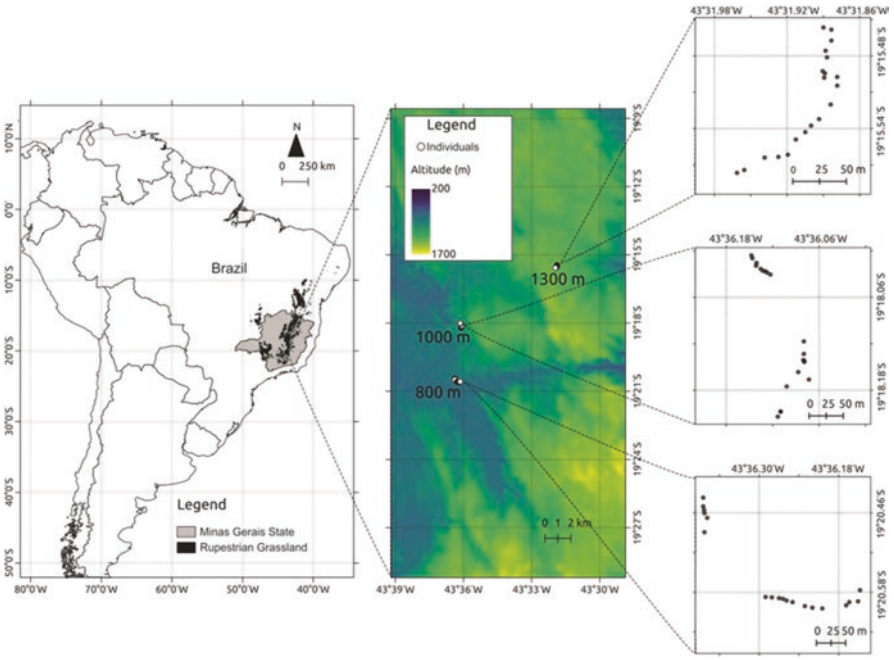

Fig. 3.2 Sites where individuals of *Baccharis dracunculifolia* were sampled in the rupestrian grassland vegetation in Serra do Cipó, Brazil

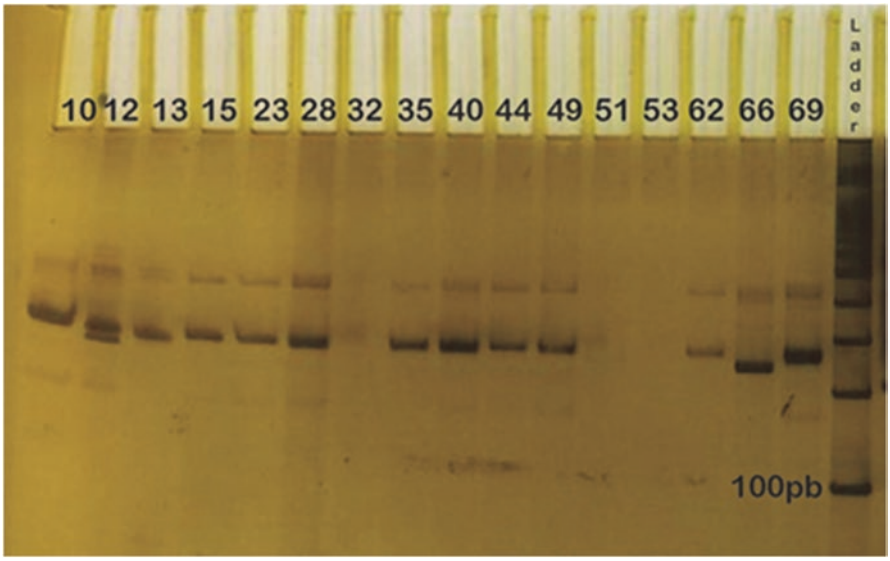

Fig. 3.3 An acrylamide gel stained with silver nitrate to verify polymorphism in Bdr6 microsatellite marker developed for *Baccharis dracunculifolia* before labeling them with fluorophores. The numbers above correspond to *B. dracunculifolia* individuals

Table 3.3 Characterization of 12 polymorphic microsatellite markers developed in *Baccharis dracunculifolia*

Locus	Primer sequence (5′–3′)	Repeat motif	Expected allele size (bp)	T_a (°C)	[$MgCl_2$]
Bdr6[a]	F: ACAGGCGGGAATACTTACCA R: CCCTAATGAAACCAGGACCA	$(AATT)_6$	231	60	2.15 mM
Bdr7[a]	F: GAGAAGGGGGAGAGGCTTTA R: CCCATTTTAAGGCTGTTTGA	$(AGAA)_6$	245	52	2.15 mM
Bdr9	F:GGAGCCGAAAGTGAAAAACA R: TGTTCAGCGGGTGTTGTAAA	(TGA)7	272	52	2.15 mM
Bdr11	F: TCCTTCATCTTGTTGCTCCA R: TGTCCGCCATTTTCTTCTCT	(GGAT)6	213	60	2.15 mM
Bdr13	F: GATGGTGGTTCGGGTAAGAA R: CGCCATTGAAATTGTTGTTG	(TATC)6	200	62	0.43 mM
Bdr20	F: CCCAAAGAAATGGATGAAGC R: TGGAATGGAGTTGTGTGTTGA	(TCTT)6	195	60	2.15 mM
Bdr21	F: TGCCACCATCTCTCTCTCTCT R: AATTAGCACCCACGCCATT	(TTTA)6	197	56	0.86 mM
Bdr22[a]	F: CCAATTTGAAACGACATGACTC R: CGGCTACGTCAACGACTATG	$(ATTT)_6$	157	58	0.43 mM
Bdr25[a]	F: GGAGCCGAAAGTGAAAAACA R: TGTTCAGCGGGTGTTGTAAA	$(TGA)_7$	272	52	2.15 mM
Bdr26[a]	F: AGCTGTTGTTGTGCCTGAGA R: GGATCGTCATCTCGTGTCCT	$(ATG)_8$	171	60	0.215 mM
Bdr31[a]	F: CCTGCATATTGAAAGCTCGTC R: GCTTGAATGACCCACGAAC	$(GCTCG)_5$	246	60	2.15 mM
Bdr34	F: CCGAGGCCAAATGAAATCT R: CTTGTCGAATGCCGAAAAAT	(TATTT)7	221	52	2.15 mM

[a]Microsatellite markers used to genotype a *Baccharis dracunculifolia* population

1026 and 1040 m, and 20 from 1348 to 1356 m altitudes (Fig. 3.2). The DNA was extracted from leaves using CTAB 2% protocol (Doyle and Doyle 1990). DNA purity and concentration were checked using NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

All *B. dracunculifolia* individuals were genotyped with six microsatellite loci (Bdr6, Bdr7, Bdr22, Bdr25, Bdr26, Bdr31). PCR amplifications were performed in a 13 μL volume containing 10.0 μM of each primer, 1.5 μL of 5X special IVB PCR buffer, 1 unit of Taq DNA polymerase (Phoneutria, BR), 0.25 mM of each dNTP, 0.215–2.15 mM of $MgCl_2$ (according to each primer – Table 3.3), and 10.0 ng of template DNA. DNA amplification was accomplished in a PCR system (Veriti™ 96-Well Thermal Cycler; Applied Biosystems, California, USA) under the following conditions: 94 °C for 5 min (one cycle); 94 °C for 1 min, 52–62 °C for 1 min (according to each primer – Table 3.3); 72 °C for 1 min (35 cycles); and 72 °C for 50 min. The PCR products were electrophoresed on an ABI Prism 3730 automated DNA sequencer (Fig. 3.4) (Applied Biosystems, California, USA) and were sized by comparison to a *GeneScan* 500 LIZ dye Size Standard (Applied Biosystems,

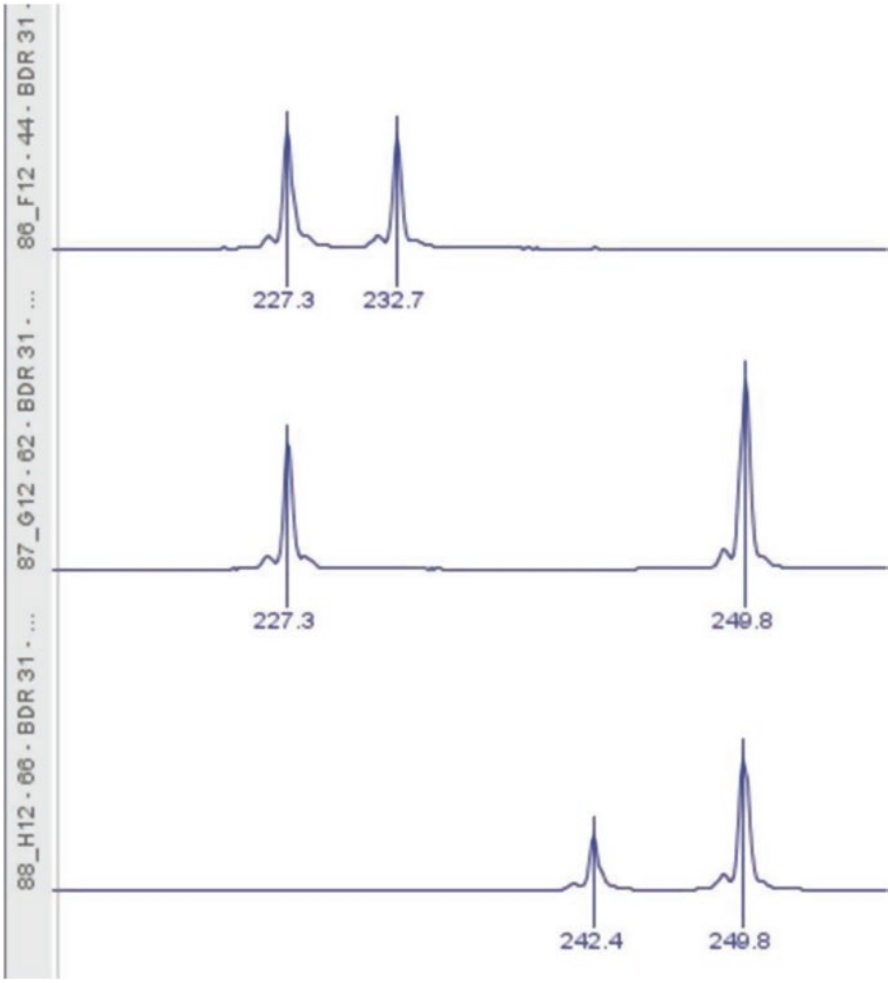

Fig. 3.4 Microsatellite profile of Bdr31 marker developed for *Baccharis dracunculifolia* detecting different heterozygote peaks

California, USA). Fluorescent PCR products were automatically sized using Geneious 10.2.3 (Kearse et al. 2012).

The number of alleles (*A*) ranged from 2 to 8 per locus, and the average number of alleles in this population was 5.33 (Table 3.4). Despite the use of just 6 microsatellite markers, we found higher allelic richness than Belini et al. (2016) using 11 microsatellite markers based on 315 samples from 3 populations. Besides, Belini et al. (2016) developed six other microsatellite markers which were monomorphic in all these individuals. All these monomorphic markers were dinucleotide repeats, and the polymorphic markers were composed of six dinucleotide and five compound markers. Also, these authors used the traditional genomic microsatellite

Table 3.4 Characterization of 6 microsatellite loci based on a sample of 60 adult individuals of *Baccharis dracunculifolia* from Serra do Cipó, Brazil

Locus	Alleles range (bp)	A	Ho	He	Fis	Q	I
Bdr6	131–234	8	0.231	0.808	0.714^{***}	0.743	0.061
Bdr7	225–253	8	0.545	0.594	0.082^{***}	0.743	0.187
Bdr22	152–168	5	0.333	0.605	0.449^{***}	0.595	0.210
Bdr25	270–273	2	0.286	0.408	0.300^{ns}	0.187	0.433
Bdr26	164–170	3	0.789	0.547	-0.443^{*}	0.370	0.270
Bdr31	225–249	6	0.583	0.659	0.115^{**}	0.659	0.178
Over all loci	194–224	5.33	0.461	0.603	0.202	QC = 0.995	IC = 5.1×10^{-05}

A number of alleles, *He* expected heterozygosity, *Ho* observed heterozygosity, *Fis* fixation index, *Q* probability of paternity exclusion, *QC* combined probability of paternity exclusion, *I* probability of genetic identity, *IC* combined probability of genetic identity

*Loci deviating from HWE equilibrium after Bonferroni corrections (values followed by ns did not statistically differ from zero, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$)

library to develop microsatellite markers. This result reinforces the better outcomes when using NGS to identify microsatellites that could enable the selection of tri-, tetra-, or pentanucleotide motif repeats to avoid "stutter" bands and genotype scoring errors because of dinucleotide repeat. All microsatellite markers developed by us have tri- or more motif repeats, and all of them are perfect microsatellite markers, which are considered more polymorphic. These characteristics highlight the potential of our markers.

The observed heterozygosity (*Ho*) ranged from 0.231 to 0.789 per locus, and the average was 0.461. The expected heterozygosity (*He*) ranged from 0.408 to 0.807 per locus, and the average was 0.603. For most loci (except Bdr25), the observed heterozygosity was lower than expected under the Hardy-Weinberg equilibrium (HWE), with fixation indexes (*Fis*) significantly different from zero (Table 3.4). These HWE deviations may be the presence of null alleles or due to the low number of analyzed individuals, which must be insufficient to reveal all possible genotypic combinations (López-Márquez et al. 2016), hence leading to a possible underestimation of allele frequencies and heterozygosity (McInerney et al. 2011). In addition, the observed heterozygosity was lower than expected under HWE which may be due to excess homozygotes since all evaluated individuals belong to only one population and inbreeding can occur in this population.

The probability of excluding two individuals as related when they are not was 74.3% with the Bdr6 locus. However, the combined probability of paternity exclusion increased to 99.5% when the six loci were included in the analysis (QC = 0.995 – Table 3.4), indicating that this set of microsatellite markers is efficient in kinship and gene flow analyses. The probability of genetic identity (I) ranged from 0.187 to 0.934 per locus (Table 3.3), and a low combined probability of genetic identity (IC) was attained when the six loci were included in the analysis (IC = 5.1×10^{-05} – Table 3.3). Although some loci presented a significant excess of homozygotes, the higher combined probability of paternity exclusion and lower combined probability of genetic identity show that this battery of microsatellite markers is suitable for population genetic analyses.

2 Cross-Amplification in *Baccharis*

We sampled other two species, *B. concinna* and *B. aphylla*, to evaluate the transferability of microsatellite markers developed for *B. dracunculifolia*. All of these three species occur in sympatry in the rupestrian grasslands of Serra do Cipó. We collected leaves from eight individuals of *B. concinna* and nine individuals of *B. aphylla* and extracted their DNA using the CTAB 2% protocol (Doyle and Doyle 1990). DNA purity and concentration were checked using NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

The cross-amplification analysis was realized with the six microsatellite markers characterized in *B. dracunculifolia* (see Table 3.3) under the same PCR conditions used to amplify *B. dracunculifolia*. The PCR fragments were viewed on a 6% polyacrylamide gel electrophoresis (PAGE) stained with silver nitrate (Sanguinetti et al. 1994). Five microsatellite markers that successfully amplified fragments were obtained both in *B. concinna* (Bdr6, Bdr7, Bdr25, Bdr26, Bdr31) (Fig. 3.5a) and in *B. aphylla* (Bdr6, Bdr7, Bdr22, Bdr25, Bdr31) (Fig. 3.5b).

Modifications of the tested PCR conditions (mainly annealing temperature, DNA and $MgCl_2$ concentration) may have increased this preliminary success of cross-species amplification in *Baccharis*. In addition, other microsatellites developed for *B. dracunculifolia*, but not yet characterized for this species, can be used in future cross-amplification in this genus. These microsatellite markers must provide new information about the population genetic structure of *B. dracunculifolia* and related species and may help elucidate more details on the evolutionary relationships in this genus. Besides, this new molecular tool may help in the management and conservation of *B. dracunculifolia* as well as other species in the genus. We argue that urgent genetic studies on the genus *Baccharis* are called for as the importance of its species in community assembly, ecosystem services, and potential invisibility of disturbed communities is increasing in the recent decade.

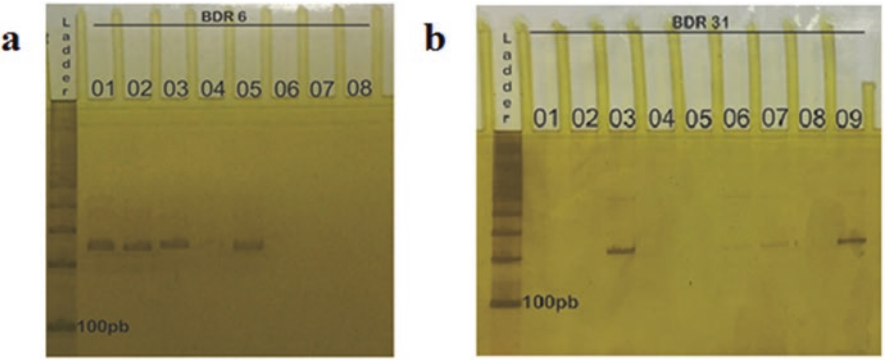

Fig. 3.5 Microsatellite profile of microsatellite markers developed for *Baccharis dracunculifolia* amplified in congeneric species, (**a**) Bdr6 profile in *Baccharis concinna*, PCR fragments were detected in individuals 1–5, (**b**) Bdr31 profile in *Baccharis aphylla*, PCR fragments were detected in individuals 3, 6, 7, and 9

Acknowledgements We thank the Universidade Federal de Ouro Preto (UFOP) for logistical support, Coordenação de Aperfeiçoamento de Pessoalde Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Conselho Nacional deDesenvolvimento Científico e Tecnológico (CNPq) for financial support.

References

Acharige SD, Chand V, Mather P (2012) Development and characterization of tri- and tetranucleotide polymorphic microsatellite markers for skipjack tuna (*Katsuwonus pelamis*). Ceylon J Sci Biol Sci 41:11–17

Ambreen H et al (2015) Development of genomic microsatellite markers in *Carthamus tinctorius* L. (Safflower) using next generation sequencing and assessment of their cross-species transferability and utility for diversity analysis. PLoS One 10:e0135443

Anderson LC, Kyhos DW, Mosquin T, Powell AM, Raven PH (1974) Chromosome numbers in Compositae. IX. Haplopappus and other Astereae. Am J Bot 61:665–671

Ariza Espinar L (1974) Las especies de *Baccharis* (Compositae) de Argentina Central. Museo Botánico, Universidad Nacional de Córdoba, Córdoba

Beauchamp RM (1980) *Baccharis vanessae*, a new species from San Diego County, California. Phytologia 46:216–222

Belini CM et al (2016) Characterization of microsatellite markers for *Baccharis dracunculifolia* (Asteraceae). Appl Plant Sci 4:apps.1500093

Bernardello LM (1986) Números cromosómicos en Asteraceae de Córdoba. Darwin 27:169–178

Bhargava A, Fuentes FF (2010) Mutational dynamics of microsatellites. Mol Biotechnol 44:250–266

Bonatelli IA, Carstens BC, Moraes EM (2015) Using next generation RAD sequencing to isolate multispecies microsatellites for *Pilosocereus* (Cactaceae). PLoS One 10:e0142602

Bowden WM (1945) A list of chromosome numbers in higher plants. I. Acanthaceae to Myrtaceae. Am J Bot 32:81–92

Braga AC, Reis AMM, Leoi LT, Pereira RW, Collevatti RG (2007) Development and characterization of microsatellite markers for the tropical tree species *Tabebuia aurea* (Bignoniaceae). Mol Ecol Notes 7:53–56

Carr GD, King RM, Powell AM, Robinson H (1999) Chromosome numbers in Compositae. XVIII. Am J Bot 86:1003–1013

Castoe TA et al (2012) Rapid microsatellite identification from Illumina paired-end genomic sequencing in two birds and a snake. PLoS One 7:e30953

Chambers GK, MacAvoy ES (2000) Microsatellites: consensus and controversy. Comp Biochem Physiol B Biochem Mol Biol 126:455–476

Ciampi AY, Azevedo VCR, Gaiotto FA, Ramos ACS, Lovato MB (2008) Isolation and characterization of microsatellite loci for *Hymenaea courbaril* and transferability to *Hymenaea stigonocarpa*, two tropical timber species. Mol Ecol Res 8:1074–1077

Clarke LA, Rebelo CS, Goncalves J, Boavida MG, Jordan P (2001) PCR amplification introduces errors into mononucleotide and dinucleotide repeat sequences. Mol Pathol 54:351–353

Coleman JR (1968) Chromosome numbers in some Brazilian Compositae. Rhodora 70:228–240

Coleman JR (1970) Additional chromosome numbers in Brazilian Compositae. Rhodora 72:94–99

Cota LG, Moreira PA, Menezes EV, Gomes AS, Ericsson AR, Oliveira DA, Melo AF Jr (2012) Transferability and characterization of simple sequence repeat markers from *Anacardium occidentale* to *A. humile* (Anacardiaceae). Genet Mol Res 11:4609–4616

Covas G, Schnak B (1946) Número de chromosomas enantofites de la region de Cuyo (Republica Argentina). Rev Argent Agron 13:153–166

Cruz MV, Rodrigues JG, Souza HA, Lovato MB (2012) Isolation and characterization of microsatellite markers for *Plathymenia reticulata* (Fabaceae). Am J Bot 99:e210–e212

Dayanandan S, Bawa K, Kesseli R (1997) Conservation of microsatellites among tropical trees (Leguminosae). Am J Bot 84:1658

Dejong D, Montgomery F (1963) Chromosome numbers in some Californian Compositae: Astereae. Aliso 5:255–256

Dillon M, Turner BL (1982) Chromosome numbers of Peruvian Compositae. Rhodora 84:131–137

Dollenz AO (1976) Números cromósomicos de *Verbena tridens* Lag., *Baccharis patagonica* Hook. & Arn. Y *Adesmia boronoides* Hook. f. Anal Inst Patagonia 7:163–167

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Edwards A, Civitello A, Hammond HA, Caskey CT (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am J Hum Genet 49:746–756

Estoup A, Wilson IJ, Sullivan C, Cornuet JM, Moritz C (2001) Inferring population history from microsatellite and enzyme data in serially introduced cane toads, *Bufo marinus*. Genetics 159:1671–1687

Feres JM, Martinez ML, Martinez CA, Mestriner MA, Alzate-Marin AL (2009) Transferability and characterization of nine microsatellite markers for the tropical tree *species Tabebuia roseo-alba*. Mol Ecol Res 9:434–437

Fernandez Casas J (1981) Recuentos cromosomaticos de algunas angiospermas de Bolivia y Peru. Saussurea 12:157–164

Freeland JR (2005) Molecular ecology. Wiley-Blackwell, Hoboken

Gomes V, Collevatti R, Silveira F, Fernandes G (2004) The distribution of genetic variability in *Baccharis concinna* (Asteraceae), an endemic, dioecious and threatened shrub of rupestrian fields of Brazil. Conserv Genet 5:157–165

Guichoux E, Lagache L, Wagner S, Chaumeil P, Léger P, Lepais O, Lepoittevin C, Malausa T, Revardel E, Salin F, Petit RJ (2011) Current trends in microsatellite genotyping. Mol Ecol Res 11:591–611

Heiden G (2014) Systematics of *Baccharis* (Asteraceae: Astereae). Thesis, Universidade de São Paulo

Heiden G, Pirani JR (2016) Novelties towards a phylogenetic infrageneric classification of *Baccharis* (Asteraceae, Astereae). Phytotaxa 289:285–290

Heiden G, Iganci J, Stein V, Bobrowski V (2006) Número cromossômico de *Baccharis riograndensis* Malag. & J.E.Vidal (Asteraceae). Pesq Bot 57:121–136

Heiden G, Antonelli A, Pirani JR (2019) A novel phylogenetic infrageneric classification of *Baccharis* (Asteraceae: Astereae), a highly diversified American genus. Taxon 68:1048-1081

Hellwig FH (1990) Die Gattung *Baccharis* L. (Compositae – Asteraceae) in Chile. Mitt Bot Staatssamml München 29:1–456

Hodel RGJS-SMC, Landis JB, Crowl AA, Sun M, Liu X, Gitzendanner MA, Douglas NA, Germain-Aubrey CC, Chen S, Soltis DE, Soltis PS (2016) The report of my death was an exaggeration: a review for researchers using microsatellites in the 21st century. Appl Plant Sci 4:1–13

Hunziker J, Escobar A, Xifreda CC, Gamerro J (1990) Estudios cariológicos en Compositae VI. Darwin 30:115–121

Hunziker JH, Wulff AF, Wulf AF, Xifreda CC, Escobar A (1989) Estudios cariologicos en Compositae V. Darwiniana 29:25–39

Iqbal MJ, Aziz N, Saeed NA, Zafar Y, Malik KA (1997) Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. Theor Appl Genet 94:139–144

Jackson J (1970) IOPB chromosome numbers reports XXV. Taxon 19:102–113

Jacob HJ, Lindpaintner K, Lincoln SE, Kusumi K, Bunker RK, Mao YP, Ganten D, Dzau VJ, Lander ES (1991) Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. Cell 67:213–224

Jansen RK, Stuessy TF (1980) Chromosome counts of Compositae from Latin America. Am J Bot 67:585–594

Jansen RK, Stuessy TF, Díaz-Piedrahíta S, Funk VA (1984) Recuentos cromosómicos en Compositae de Colombia. Caldasia 14:7–20

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649

Keil DJ, Pinkava DJ (1976) Chromosome counts and taxonomic notes for Compositae from the United States and Mexico. Am J Bot 63:1393–1403

Keil DJ, Stuessy TF (1975) Chromosome counts of Compositae from the United States, Mexico and Guatemala. Rhodora 77:171–195

Keil DJ, Stuessy TF (1977) Chromosome counts of Compositae from Mexico and the United States. Am J Bot 64:791–798

Larranaga N, Albertazzi FJ, Fontecha G, Palmieri M, Rainer H, van Zonneveld M, Hormaza JI (2017) A Mesoamerican origin of cherimoya (*Annona cherimola* Mill.): implications for the conservation of plant genetic resources. Mol Ecol 26:4116–4130

Levinson G, Gutman GA (1987) Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Mol Biol Evol 4:203–221

Litt M, Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44:397–401

López-Márquez V, García-Jiménez R, Templado J, Machordom A (2016) Development and characterization of 26 novel microsatellite loci for the trochid gastropod *Gibbula divaricata* (Linnaeus, 1758), using Illumina MiSeq next generation sequencing technology. PeerJ 4:e1789

McDowell JR, Diaz-Jaimes P, Graves JE (2002) Isolation and characterization of seven tetranucleotide microsatellite loci from Atlantic northern bluefin tuna *Thunnus thynnus thynnus*. Mol Ecol Notes 2:214–216

McInerney CE, Allcock AL, Johnson MP, Bailie DA, Prodohl PA (2011) Comparative genomic analysis reveals species-dependent complexities that explain difficulties with microsatellite marker development in molluscs. Heredity 106:78–87

Meldgaard M, Morling N (1997) Detection and quantitative characterization of artificial extra peaks following polymerase chain reaction amplification of 14 short tandem repeat systems used in forensic investigations. Electrophoresis 18:1928–1935

Moreira RG, McCauley RA, Cortés-Palomec AC, Lovato MB, Fernandes GW, Oyama K (2008) Isolation and characterization of microsatellite loci in *Coccoloba cereifera* (Polygonaceae), an endangered species endemic to the Serra do Cipó, Brazil. Mol Ecol Resour 8:854–856

Moreira PA, Sousa SA, Oliveira FA, Araujo NH, Fernandes GW, Oliveira DA (2012) Characterization of nine transferred SSR markers in the tropical tree species *Enterolobium contortisiliquum* (Fabaceae). Genet Mol Res 11:3729–3734

Morris AB, Shaw J (2018) Markers in time and space: a review of the last decade of plant phylogeographic approaches. Mol Ecol 27:2317–2333

Mota L, Torices R, Loureiro J (2016) The evolution of haploid chromosome numbers in the sunflower family. Genome Biol Evol 8:3516–3528

Müller J (2006) Systematics of *Baccharis* (Compositae-Astereae) in Bolivia, including an overview of the genus. Syst Bot Monogr 76:1–341

Muñoz-Pajares AJ, García C, Abdelaziz M, Bosch J, Perfectti F, Gómez JM (2017) Drivers of genetic differentiation in a generalist insect-pollinated herb across spatial scales. Mol Ecol 26:1576–1585

Nesom GL (2000) Generic conspectus of the tribe Astereae (Asteraceae) in North America and Central America, the Antilles, and Hawai. Botanical Research Institute of Texas, Texas

Pinkava D, Keil D (1977) Chromosome counts of Compositae from the United States and Mexico. Am J Bot 64:680–686

Powell AM, Cuatrecasas J (1970) Chromosome numbers in Compositae: Colombian and Venezuelan Species. Ann Mo Bot Gard 57:374–379

Powell AM, Cuatrecasas J (1975) In IOPB chromosome number reports L. Taxon 24:671–678

Powell AM, King RM (1969) Chromosome numbers in the Compositae: Colombian species. Am J Bot 56:116–121
Powell AM, Powell SA (1977) Chromosome numbers of gypsophilic plant species of the Chihuahuan desert. Sida, Contrib Bot (Online) 7:80–90
Powell A, Turner B (1963) Chromosome numbers in the Compositae VII. Additional species from the southwestern United States and Mexico. Madrono 17:128–140
Ram SG, Parthiban K, Kumar RS, Thiruvengadam V, Paramathma M (2008) Genetic diversity among *Jatropha* species as revealed by RAPD markers. Genet Resour Crop Evol 55:803–809
Raven PH, Solbrig OT, Kyhos DW, Snow R (1960) Chromosome numbers in Compositae. I. Astereae. Am J Bot 47:124–132
Rowe G, Sweet M, Beebee TJC (2004) An introduction to molecular ecology. Oxford University Press, New York
Rozenblum E, Waisman CE, Hunziker JH (1985) Estudios cariológicos en Compositae. II Darwiniana 26:15–25
Ruas PM, Bertillacchi SRA, Vieira AOS, Matzenbacher NI (1989) Citogenética do gênero *Baccharis* (Compositae). Ciên Cult 41:702
Sanguinetti CJ, Dias Neto E, Simpson AJ (1994) Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. BioTechniques 17:914–921
Solbrig OT (1977) Chromosomal cytology and evolution in the family Compositae. In: Heywood VH, Harborne JB, Turner BL (eds) The biology and chemistry of the Compositae. Academic Press, London, pp 267–281
Solbrig OT, Anderson LC, Kyhos DW, Raven PH, Rudenberg L (1964) Chromosome numbers in Compositae V. Astereae II. Am J Bot 51:513–519
Solbrig OT, Anderson LC, Kyhos DW, Raven PH (1969) Chromosome numbers in Compositae VII: Astereae III. Am J Bot 56:348–353
Spellenberg R, Ward DE (1988) Chromosome counts of angiosperms from New Mexico and adjacent areas. Phytologia 64:390–398
Spooner DM, De Jong DCD, Byung-Yun S, Stuessy TF, Gengler KM, Nesom GL, Berry PE (1995) Chromosome counts of Compositae from Ecuador and Venezuela. Ann Mo Bot Gard 82:596–602
Sturtevant AH, Dobzhansky T (1936) Inversions in the third chromosome of wild races of *Drosophila pseudoobscura*, and their use in the study of the history of the species. Proc Natl Acad Sci U S A 22:448–450
Sundberg S, Cowan CP, Turner BL (1986) Chromosome counts of Latin American Compositae. Am J Bot 73:33–38
Turner BL, Irwin HS (1960) Chromosome numbers in the Compositae II. Meiotic counts for 14 species of Brazilian Compositae. Rhodora 62:122–126
Turner BL, Powell AM, Cuatrecasas J (1967) Chromosome numbers in Compositae. XI. Peruvian Species. Ann Mo Bot Gard 54:172–177
Turner BL, Bacon J, Urbatsch L, Simpson B (1979) Chromosome numbers in South American Compositae. Am J Bot 66:173–178
Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG (2012) Primer3 – new capabilities and interfaces. Nucleic Acids Res 40:e115–e115
Wachira FN, Waugh R, Powell W, Hackett CA (1995) Detection of genetic diversity in tea (*Camellia sinensis*) using RAPD markers. Genome 38:201–210
Weedin J, Powell A (1978) IOPB chromosome number reports LX. Taxon 27:223–231
Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218
Westman WE, Panetta FD, Stanley TD (1975) Ecological studies on reproduction and establishment of the woody weed, groundsel bush (*Baccharis halimifolia* L.: Asteraceae). Aust J Agric Res 26:855–870
Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Wulff AF (1984) Estudios cromosómicos en Compuestas de las floras Patagonica y Bonaerense. Darwin 25:17–26

Wulff A, Hunziker JH, Escobar A (1996) Estudios cariológicos en Compositae. Darwin 34:213–231

Zalapa JE, Cuevas H, Zhu H, Steffan S, Senalik D, Zeldin E, McCown B, Harbut R, Simon P (2012) Using next-generation sequencing approaches to isolate simple sequence repeat (SSR) loci in the plant sciences Am J Bot 99:193–208

Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: a review. Mol Ecol 11:1–16

Zanowiak DJ (1991) An analysis of systematic and phyletic relationships within Baccharidinae (Asteraceae: Astereae). PhD Thesis, Texas A&M University.

Zucchi MI, Brondani RPV, Pinheiro JB, Brondani C, Vencovsky R (2002) Transferability of microsatellite markers from *Eucalyptus* spp. a *Eugenia dysenterica* (família Myrtaceae). Mol Ecol Notes 2:512–513