Review Article

Studies on diversity and evolution of Iridaceae species in southern Brazil

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Abstract

Plants of the family Iridaceae are well represented in the grassland vegetation of southern Brazil, occurring in the Pampa and Atlantic Forest biomes. Nevertheless, little is known about the taxonomy and evolution of Iridaceae species in southern Brazil. The main goal of this review is to compile published information about South American Iridaceae, and to discuss the evolution and genetic diversity of the family presenting our own research data in the light of the published literature. The main focus is on the genera *Calydorea*, *Cypella*, *Herbertia*, and *Sisyrinchium*. Aspects of reproductive system and of pollinator attraction are also discussed.

Keywords: cytotaxonomy, molecular phylogenetics, Iridoideae, population genetics, diversity.

Iridaceae

Iridaceae (Asparagales, Monocots) encompasses approximately 2,000 species distributed among 65 to 75 genera (Goldblatt *et al*., 2008). Among these species, approximately 160 occur in Brazil, representing 18 genera, and 12 of these genera are found in southern Brazil (Eggers *et al*., 2010). In terms of diversification and abundance, the most important Iridaceae genera in Brazil are *Sisyrinchium* L. (58 species), *Neomarica* Sprague, *Pseudotrimezia* R.C. Foster (21 species both), *Trimezia* Salisb. ex Herb. and *Cypella* Herb. (14 species both).

Iridaceae is one of the largest families of Monocots. It comprises plants with wide diversity of flowers and can be recognized by their petaloid perianth, or corolla, with three tepals of the inner whorl and the three of the outer whorl usually alike in texture, shape and often in color (but not in *Sisyrinchium*). Iridaceae is distinguished of other Asparagalean families by the three stamens in the androecium (Goldblatt and Manning, 2008). The family is quite interesting by multiple strategies and intimate associations with pollinating insects or birds due to the diversity of the flowers. Furthermore, some genera of Iridaceae are focus of research concerning the evolution of oil-producing struc-

tures related to specialized oil-bee pollination (Chauveau *et al.*, 2011).

The tribes Tigrideae, Sisyrinchieae and Trimezieae from the subfamily Iridoideae have representatives in southern Brazil. Notwithstanding, species richness in Trimezieae is poor for this southern region, contrasting with the high diversity in southeastern Brazil. Important genera found in southern Brazil include *Calydorea* (nine species), *Cypella* (11 species) and *Herbertia* (six species) which belong to tribe Tigrideae and *Sisyrinchium* (45 species, tribe Sisyrinchieae) (Eggers *et al*., 2010). Tigrideae are characterized by the presence of bulbs and plicate leaves, whereas the Sisyrinchieae have rhizome or fibrous root system and plane or terete leaves. Also, very particular of *Sisyrinchium* are the partially or entirely united filaments (Goldblatt *et al*., 2008).

According to Rodriguez and Sytsma (2006), the clade Tigridieae groups several genera with very similar vegetative characteristics, making the differentiation of Tigridieae genera impracticable except during the flowering period. Also, broad morphological variability in the flowers is found within species, which is a source of confusion for the circumscription of many species. New World species of Iridaceae are found in Trimezieae, Tigridieae and Sisyrinchieae, the latter a well-supported clade that includes six genera (*Libertia* Spreng., *Orthrosanthus* Sweet, *Olsynium* Raf., *Sisyrinchium*, *Solenomelus* Miers, and

Tapeinia Comm. ex Juss.) (Goldblatt *et al.*, 2008). Among these genera, *Sisyrinchium* is the most diverse.

Sisyrinchium flowers have filaments that are partly connected or completely fused in a staminal column that may bear trichomes of different types. Elaiophores of various densities and extensions may be present in the staminal column, usually at its base, and function to attract specialized pollinator bees that use the oil produced by these trichomes to feed their larvae (Chauveau *et al.*, 2011).

Iridaceae species exhibit a broad variety of flower colors and shapes that may serve as signal to pollinators (Goldblatt and Manning, 2008). Three major types of pollinator rewards are known. For example, the non-volatile oils produced in the elaiophores of many species (particularly in *Sisyrinchium*) attract specialized oil-collecting bees belonging to two Hymenoptera families, Mellitidae and Apidae (Michener, 2007). In an analysis of the evolutionary aspects of oil-bee pollination in almost 200 species, Renner and Schaefer (2010) showed that oil-bee pollination has evolved independently at least 28 times in different angiosperm families. A recent investigation of the evolution of oil-producing structures related to specialized oil-bee pollination in *Sisyrinchium* and the phylogeny of the genus indicated that glandular trichomes have evolved independently three times (Chauveau *et al.*, 2011). The occurrence of this type of plant-pollinator interaction in flowers providing oil as a reward is found in only 11 angiosperm families (Renner and Schaefer, 2010).

Great morphological variation can be observed in certain groups of *Sisyrinchium* species. *S. micranthum* Cav. is the most variable species, with morphotypes that can be characterized by a combination of different plant size and habit, flower size and color, and tepal organization. This variation has been discussed elsewhere (Chukr and Capellari Jr, 2003) and is not directly associated with ploidy levels (Tacuatiá *et al.*, 2012b). When Chauveau *et al.* (2011) examined nine accessions of *S. micranthum* related to different morphotypes, they were unable to separate the taxa even though the phylogenetic tree displayed groups within the species.

Other taxa with morphological variation and taxonomic problems are related to *Sisyrinchium palmifolium* L. and *S. vaginatum* Spreng. Each of these species have many synonyms, representing species considered difficult to distinguish from the main cited ones (Johnston, 1938; Chukr and Capellari Jr, 2003). Notwithstanding, our preliminary investigations suggest that many of these synonyms must be again evaluated and that studies on a better characterization of these species are needed.

Besides the three taxa cited above, other *Sisyrinchium* species from southern Brazil are focused by our research group. These species are primarily members of the sections *Scirpeocharis*, *Sisyrinchium*, and *Lenitium* (*sensu* Ravenna). Examples of such species are *S. luzula* Klotzsch

ex Klatt, *S. pachyrhizum* Baker, and *S. sellowianum* Klatt, respectively.

All taxa mentioned previously and which are under investigation by our research group do not have detailed information about the diversity and evolution of the species. We now address such questions in this review, keeping in focus this taxonomic group as a whole.

Cytogenetic Data for Iridaceae

Cytological data are now available for approximately 65% of Iridaceae species, primarily for those from the Old World taxa (Goldblatt and Takei, 1997; Alves *et al.*, 2011). Although most of these data are limited to chromosome counts, the data available from cytogenetic studies suggest an interesting scenario for chromosome evolution. The great variation in aspects of Iridaceae karyology, such as karyotypic architecture, basic (monoploid) number (x), and ploidy level, makes cytological analyses important for clarifying the systematics and evolution of this family (Goldblatt and Takei, 1997).

The basic chromosome number for Iridaceae remains uncertain, although ancestral chromosome numbers have been suggested for almost all genera (Goldblatt, 1990; Goldblatt and Takei, 1997). The most likely basic number is 10, with many derived numbers such as 9, 8, 7, and 5. Goldblatt and Takei (1997) suggested that much of the variation in chromosome number results from descending disploidy. Heteromorphisms of the chromosome arms in some species also indicate the occurrence of unequal reciprocal translocations.

Chromosome numbers ($2n$) in the Iridaceae vary from 6 to approximately 230, suggesting that polyploidy has been important in the evolution of Iridaceae species (Alves *et al.*, 2011). Such variability is probably related to cycles of polyploidy and downward disploidy. Neopolyploidy is also common in the family, since intrageneric and intraspecific polyploidy are common, especially in the Northern Hemisphere.

Chromosome morphology and size vary greatly among Iridaceae species. Karyotype bimodality and asymmetry are frequent in the family, particularly in Iridoideae. Phylogenetic data indicate that bimodality has arisen several times in different lineages (Goldblatt *et al.*, 2008). Although the Neotropics are considered the second most important center of diversity in Iridaceae (Goldblatt and Manning, 2008), studies of Neotropical Iridaceae have been few in number; cytogenetic studies have focused primarily on North American species, and karyological data from Brazilian species are scarce. Recently, Alves *et al.* (2011) reported chromosomal characterizations of 15 species of Iridaceae from northeastern Brazil, increasing to 24 the number of species in that region with cytological data.

There are few data for most of the Brazilian taxa, even for chromosome numbers counts, and for some of them, the only cytological data available are from species collected in

other South American countries (Goldblatt and Takei, 1997; De Tullio *et al.*, 2008; Moreno *et al.*, 2009). Chromosome numbers were determined for the *Calydorea* species *C. amabilis* (Ravenna) Goldblatt & Henrich, *C. azurea* Klatt, *C. nuda* Baker, *C. pallens* Griseb., and *C. xiphioides* (Poepp.) Espinosa, all of them with $x = 7$, although three different diploid chromosome numbers were also found ($2n = 14$, 28, or 42) (Goldblatt, 1982; Goldblatt and Takei, 1997). In a more recently published cytological report for *Calydorea* (De Tullio *et al.*, 2008), the karyotype of Argentinean material of *C. undulata* Ravenna ($2n = 14$) was described as bimodal, with two pairs of large chromosomes and five pairs of small chromosomes. Asymmetry was moderate, with meta- and submetacentric chromosomes, two of them satellited. Our preliminary cytological analyses for *Calydorea alba* Roitman & A. Castillo*, C. approximata* R.C. Foster*, C. campestris* (Klatt) Baker, and *C. crocoides* Ravenna confirm the monoploid number $x = 7$. Previous counts are not available for these species.

Chromosome numbers were described for seven species of *Cypella* (Ravenna, 1981; Goldblatt, 1982; Kenton *et al.*, 1990; Goldblatt and Takei, 1997); for all but one, $2n = 14$ and $x = 7$. Interestingly, *C. coelestis* (Lehm.) Diels, for which $2n = 10$, has a different generic position by some authors [as *Phalocallis coelestis* (Lehm.) Ravenna]. Chromosome sizes and total DNA content vary considerably among the species, although the relative chromosome sizes and bimodality are maintained within the karyotypes of each species. Our studies on *Cypella* species from southern Brazil (*C. fucata* Ravenna*, C. hauthalii* R.C. Foster*, C. herbertii* (Lindl.) Herb. and *C. osteniana* Beauverd ssp. *aurantiaca* Roitman & A. Castillo) have confirmed $x = 7$ for these species.

Like *Cypella* and *Calydorea* species, *Herbertia* species have a basic number of $x = 7$. Four ploidy levels ($2x$, $4x$, $6x$, and $8x$) have been found in South American *Herbertia* species (Winge, 1959; Goldblatt and Takei, 1997; Roitman and Castillo, 2004; Moreno *et al.*, 2009). Intraspecific polyploid series were also reported for *Herbertia lahue* (Molina) Goldblatt ($2n = 14$, 28, 42, or 56) and *H. pulchella* Sweet ($2n = 14$, 28, or 42) (Kenton and Heywood, 1984; Goldblatt and Takei, 1997; Moreno *et al.*, 2009). Moreno *et al.* (2009) carried out a detailed karyotype analysis of three *Herbertia* species from Argentina and confirmed the four ploidy levels already described for the genus. *H. darwinii*, whose chromosome number is reported for the first time in this paper is diploid ($2n = 14$), whereas *H. quareimana* is a tetraploid confirming previous data (Kenton and Heywood, 1984; Goldblatt and Takei, 1997). Two cytotypes were observed for *H. lahue* ssp. *amoena*, one hexaploid with $2n = 42$ and one octoploid with $2n = 56$. The mean chromosome length ranges between 2.6 and 2.9 μm. According to the authors, the polyploid cytotype has a symmetric karyotype, while the diploid *H. darwinii* has a bimodal one. Chromosome banding and fluorescence *in situ* hybridiza-

tion were also performed. One ongoing study of our group concerning six *Herbertia* species endemic from southern Brazil shows that all of them have $x = 7$ and four ploidy levels can be found, confirming previous studies.

Sisyrinchium is the Iridaceae genus with the largest amount cytological data, although data for the South American *Sisyrinchium* species are still scarce (Goldblatt, 1982; Kenton *et al.*, 1986; Goldblatt and Takei, 1997). Although $x = 5$, 8, 9, and 17 have been found, $x = 8$ and $x = 9$ are the more frequent ones, with 9 being considered the ancestral chromosome number (Chauveau *et al.*, 2011). The remaining secondary basic numbers apparently have evolved through decreasing disploidy and/or ancestral hybridization between $x = 8$ and $x = 9$ species, followed by polyploidization (Rudall *et al.*, 1986; Goldblatt and Takei, 1997). According to Kenton and Heywood (1984), the most frequent x value for *Sisyrinchium* from the Southern Hemisphere is 9, whereas for those in the Northern Hemisphere $x = 8$ is more common.

Our research group has obtained chromosome numbers for 14 *Sisyrinchium* species, 10 of them for the first time; all but one have an $x = 9$ (Corrêa, unpublished data), the exception is *S. micranthum* with $x = 8$ (Tacuatiá *et al.* 2012b), consistent with literature data (Goldblatt and Takei, 1997).

Intraspecific cytotypes were observed in *S. micranthum*, and although three ploidy levels were found, diploidy was most common. Polyploidy seems to be an important factor in *Sisyrinchium* evolution, as more than 70% of the studied species are polyploids (Goldblatt and Takei, 1997). Whereas the Southern Hemisphere has more diploid than polyploid *Sisyrinchium* species, higher ploidy levels, such as octo- and dodecaploidy, have been found in *Sisyrinchium* species of the Northern Hemisphere. Cytological data of Goldblatt (1982), Kenton and Heywood (1984), Kenton *et al.* (1986) and Rudall *et al.* (1986) suggested that ploidy level increases with latitude, also indicating a higher level of heterozygosity for the colonization of peripheral habitats. An allopolyploid origin of many cytotypes was suggested by Kenton *et al.* (1986); however, consistent data are not available. *Sisyrinchium* chromosomes are very small, especially in $x = 8$ species, making it difficult to perform karyotypical analyses (Kenton *et al.*, 1986). Karyotype symmetry tends to correlate with large genomes in *Sisyrinchium* species (Kenton and Heywood, 1984; Kenton *et al.*, 1986).

Goldblatt *et al.* (1984) suggested that karyotype evolution in Iridaceae has been accompanied by increases and decreases in genome size. The DNA content of several Iridaceae species has been determined, primarily for the genus *Sisyrinchium*, (Goldblatt *et al.*, 1984; Kenton *et al.*, 1986; Kenton *et al.*, 1990). The haploid genomes (*C*-value) of *Sisyrinchium* species range from 0.25 to 3.26 pg DNA, with the largest variation in genome size found in $x = 9$ species of the *Echthronema* section. Kenton *et al.* (1986) re-

ported that diploid species in the Southern Hemisphere exhibit a positive and significant relationship between genome size and latitude. Variation in genome size seems to be also related to life cycle, with annual plants tending to have smaller genomes than perennials. DNA measurements in *Sisyrinchium* species from southern Brazil show little variation in monoploid genome size (Cx), although hexaploids have less DNA (Souza-Chies and Kaltchuk dos Santos, unpublished data). Such reduction may be a result of the "downsizing" phenomenon. We have observed that genome size is considerably larger in *Calydorea*, *Cypella*, and *Herbertia* than in *Sisyrinchium*, as expected considering the respective chromosome sizes. In *Cypella* species, which have larger chromosomes than *Sisyrinchium* species, genome size ranges from 2.03 to 2.39 pg (Kenton *et al.*, 1990). Species of the genera with $2n = 14$ have C values of approximately 4 pg. *Herbertia* polyploids have proportionally higher DNA contents, but all have similar Cx values.

Evolution and General Features

Calydorea

Calydorea comprises approximately 16 species native to South America (Goldblatt and Manning, 2008; Goldblatt *et al.*, 2008). *Calydorea* species have equal or subequal tepals that range in color from white to dark purple. In southern Brazil, the genus presents an interesting distribution, with typical species belonging to different biomes: *C. basaltica* Ravenna, *C. crocoides* (Figure 1A)*,* and *C. longipes* Ravenna occur mainly in the Atlantic Forest biome, whereas *C. alba* (Figura 1B) and its allies are endemic of the Pampa biome (Ravenna, 1965, 2005a,b; Roitman and Castillo, 2005).

The taxonomy of *Calydorea* is bewildering because of the placement of many different genera that expand its circumscription. Based on a cladistic analysis using morphological data, Goldblatt and Henrich (1991) concluded that *Cardiostigma* Baker, *Salpingostylis* Small, *Catila* Ravenna, and *Itysa* Ravenna are synonyms of *Calydorea*. Goldblatt and Manning (2008) proposed that *Calydorea* includes provisionally *Catila*, *Itysa*, *Lethia* Ravenna, and *Tamia* Ravenna. The most recent study of Iridaceae phylogeny included only two *Calydorea* species, which did not group together in the phylogenetic trees (Goldblatt *et al*., 2008). Considering the number of species involved in the analysis, phylogenetic relationships still need to be clarified.

De Tullio *et al.* (2008), through morphological and cytological approaches, evaluated the validity of *Tamia pallens* (Griseb.) Ravenna and *C. undulata* and proposed that *Tamia pallens* should be regarded as a synonym of *C. pallens* because the floral divergences are insufficient for segregation to the genus *Tamia*. This conclusion was corroborated by their very similar karyotypes. On the other hand, the authors argued for the validity of *C. undulata*.
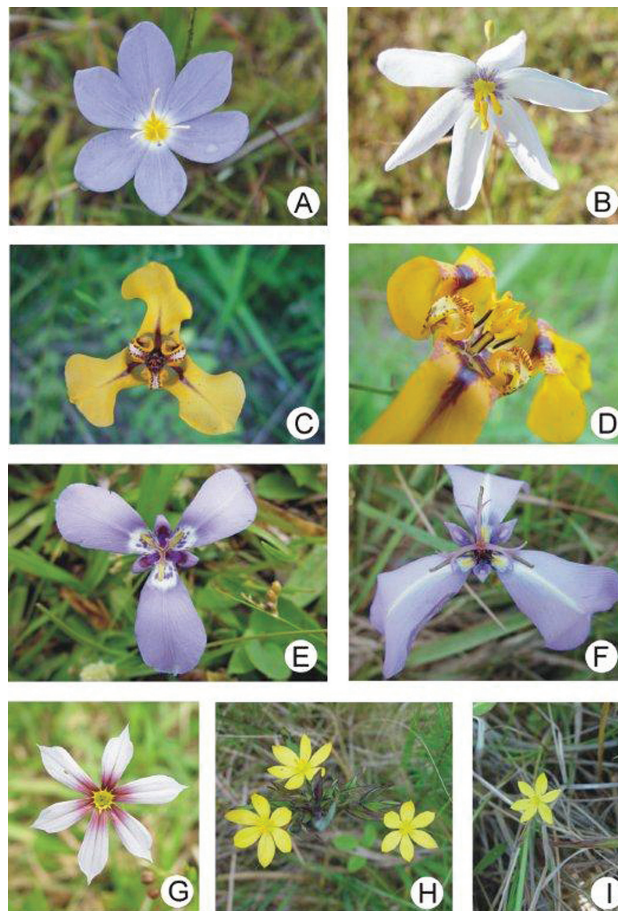

Figure 1 - Flowers of species cited in this study: (A) *Calydorea crocoides* Ravenna; (B) *Calydorea alba* Roitman & A. Castillo; (C) *Cypella herbertii* (Lindl.) Herb.; (D) *Cypella fucata* Ravenna; (E) *Herbertia lahue* (Molina) Goldblatt; (F) *Herbertia pulchella* Sweet; (G) *Sisyrinchium micranthum* Cav.; (H) *Sisyrinchium palmifolium* L.; (I) *Sisyrinchium vaginatum* Spreng. Photographs: L. Eggers.

This panoramic survey clearly shows that a detailed phylogenetic study of *Calydorea* is necessary to clarify these relationships and our team is now working toward this goal. Data about the genetic divergence or evolutionary aspects of *Calydorea* species are lacking, even though the plants are representative of the south Brazilian vegetation. We are now focusing on the genetic variability of *C. crocoides*, an endemic species from the Campos de Cima da Serra region of Rio Grande do Sul, based on inter-sequence simple repeat (ISSR) markers. Preliminary data suggest that the analyzed populations are structured ($\phi_{ST} = 0.240$), indicating little gene flow among populations. These data are still preliminary and few populations were analyzed until this moment. However, it is interesting to mention that the species *C. crocoides* presents a very limited geographical distribution and therefore the populations are geographically close. Thus, it seems that other factors besides geographical separation between populations are influencing the geographical structure of the populations

analyzed, such as breeding system and dispersion of pollen and seeds.

Cypella

Most *Cypella* species are native to South America (Goldblatt *et al.*, 2008), with some species occurring in Central America and Mexico. Their flowers are characterized by two different whorls of tepals and thickened style branches with terminal crests that provide complex flower morphology for preservation and analysis. The flowers are usually orange or yellow, but creamy, white and blue ones also occur. Most of the *Cypella* species in southern Brazil were described by Ravenna (1981), but a comprehensive review of the genus is needed. The most common known species of *Cypella* in southern Brazil are *C. herbertii* and *C. fucata* (Figure 1C, 1D, respectively).

Cypella provisionally includes the genera *Kelissa* Ravenna, *Onira* Ravenna, and *Phalocallis* Herb. (Goldblatt and Manning, 2008). The genus consists of approximately 30 species (Goldblatt *et al.*, 2008), but this number varies according to the accepted circumscription. Taxonomy of *Cypella* remains controversial, and recent phylogenetic research has indicated that it is paraphyletic (Celis *et al.*, 2008; Goldblatt *et al.*, 2008). In phylogenetic studies of Iridaceae (Souza-Chies *et al.*, 1997; Reeves *et al.*, 2001; Rodriguez and Sytsma, 2006; Celis *et al.*, 2008; Goldblatt *et al.*, 2008), *Cypella* is usually underrepresented. In the most recent and most complete phylogenetic overview of Iridaceae, Goldblatt *et al.* (2008) analyzed three species of *Cypella* and the genus appeared as polyphyletic, including *Cipura* Aubl., *Nemastylis* Nutt., *Herbertia*, *Onira*, and *Calydorea.* The relationships among *Cypella*, *Phalocallis*, and the monotypic genera *Kelissa* and *Onira* remain to be determined using molecular data (Goldblatt and Manning, 2008).

The morphologically related *Cypella* species *C. fucata*, *C. herbertii*, and *C. osteniana* remain unanalyzed in the published phylogenies. *Cypella fucata* and *C. herbertii* have been the focus of some evolutionary studies, including studies of the breeding systems of *C. herbertii* (Devoto and Medan, 2003) and of the molecular diversity among populations of *C. fucata* (De Marco *et al.*, 2009). Like other Iridaceae, some *Cypella* species, such as *C. herbertii*, are known to produce lipidic oils in elaiophores as a reward for their highly specific pollinator bees, a feature that is very important in the evolution of the genus (Goldblatt and Manning, 2008).

After testing the effects of grazing disturbance on *C. herbertii* reproduction, Devoto and Medan (2003) concluded that grazing disturbance might greatly reduce the quantity and quality of pollinators for one population of the species. In three years of observations, visits by the specialist oil-bee pollinator *Chalepogenus* Holmberg (1903) were recorded only for flowers inside cattle-free exclosures and never for those outside the exclosures. Although little im-

pact on the mating system was noted, this finding raises the possibility of a risk of extinction of this species highly dependent of specialized pollinators (Devoto and Medan, 2003).

Cypella fucata is the only *Cypella* species for which a population analysis has been done (De Marco *et al.*, 2009). It is a wild plant with populations that are often formed by few individuals only. Two populations of *C. fucata* separated by 22 km were studied using dominant ISSR markers and the data indicated gene flow between the populations ($\phi_{ST} = 0.0851$ with an intermediate structure) and high identity between the two collection sites (98%). These results suggest that *C. fucata* cross-pollinates and that vegetative propagation does not play an important role in the maintenance of the populations (De Marco *et al.*, 2009).

Herbertia

Herbertia comprises approximately seven species distributed in temperate regions of South America and the southern United States (Goldblatt and Manning, 2008). *Herbertia* flowers are predominantly violet, with tepals of different sizes, and anthers appressed to style branches. The two most common species of the genus in southern Brazil are *H. lahue* (Figure 1E) and *H. pulchella* (Figure 1F). These two plants can be readily distinguished by the larger size and the presence of a conspicuous white middle streak on the outer tepals of *H. pulchella*.

Reconstruction of Iridaceae phylogeny using DNA sequences has demonstrated a close relationship between *Cypella* and *Herbertia*, which together form a clade with *Calydorea* and *Cipura* (Reeves *et al.*, 2001; Goldblatt and Manning, 2008, Goldblatt *et al.*, 2008). To date, the major taxonomic problem concerning *Herbertia* is the delimitation of species which exhibit both a great affinity in vegetative features and a high variability in some aspects of floral morphology. Moreover, the flowers are ephemeral and poorly preserved as herbarium material, making the identification of herbarium specimens difficult (Rodriguez and Sytsma, 2006; Goldblatt *et al.*, 2008). Thus, morphotype differentiation and characterization of species based exclusively on morphological characters are not recommended and a combination of data sets on morphological and molecular traits is advised for a reasonable analysis.

There are no reported studies of *Herbertia* using molecular markers for the comprehension of intra- and interspecific diversity. Our research group is currently conducting a study based on dominant markers, such as ISSR-PCR and preliminary results already permit some differentiation between morphotypes of *H. lahue*, consistent with other studies on *Crocus* L. and *Iris* L. (Caiola *et al.*, 2004; Sik *et al.*, 2008; Saad and Mahy, 2009). A preliminary analysis of *H. lahue* has indicated that the largest source of variation occurs among populations ($\phi_{ST} = 0.82$, $p < 0.001$). These differences may be related to self-fertilization, which was indicated already in earlier results on

the *H. lahue* reproductive system. Additionally, variation in floral morphology verified among populations is possibly related to differentiation associated with selfing. Similar results were found in the *Iris* section *Oncocyclus* (Saad and Mahy, 2009) and in *Iris bismarckiana* Dammann & Sprenger (Sadder, 2007), both of which were analyzed with Random Amplified Polymorphic DNA (RAPD) markers.

In three populations of *H. quareimana*, our initial ISSR study points to a situation different from that in *H. lahue*, as a similar degree of differentiation was observed between ($\phi_{ST} = 0.475$) and within ($\phi_{IS} = 0.52$) *H. quareimana* populations (p < 0.001). So far, these results differ from those obtained by RAPD analyses of *Iris aphylla* L. and *Crocus sativus* L., in which the greatest source of diversity was observed within populations (Wroblewska *et al.*, 2003; Beiki *et al.*, 2010). Factors like number of markers used in the analysis, the distribution across the genome and the number of sampled individuals can affect distance estimates. The estimates obtained so far are preliminary and more loci need to be added to the analysis. Nevertheless, some inference is already possible. Initial experiments have indicated that *H. quareimana* is mainly cross-pollinated, while *H. lahue* produces fruit also by self-fertilization. Observations of floral visitors in *H. quareimana* and *H. lahue* sympatric populations have shown that the main visitors are honey bees, *Apis mellifera* L., which prefer flowers of *H. quareimana* and visite *H. lahue* only secondarily. The geographical distance between populations is also considered an influential factor in estimates of genetic differentiation, highlighting the need for sampling more populations, especially in the case of *H. lahue*, which has a wider distribution, occurring in virtually all regions of the State of Rio Grande do Sul, where it is also in sympatry with other *Herbertia* species such as *H. darwinii* and *H. pulchella*. In the case of *H. quareimana*, its geographical distribution is concentrated in the western region of the state and some populations occur in sympatry with *H. lahue*. In the case of *H. quareimana,* none of its populations was observed to occur in sympatry with other *Herbertia* species.

Sisyrinchium

Sisyrinchium extends throughout South, Central and North America, with one species in Greenland. The species are associated primarily with two major centers of distribution, Mexico and South America, with a conservative estimate of approximately 140 species in the Americas (Goldblatt and Manning 2008). *Sisyrinchium* has been suggested to be of South American origin (Goldblatt *et al.*, 2008; Chauveau *et al.*, 2011). For the Southern Hemisphere, its biogeographical pattern indicates expansions from Central America and the northern Andes to the sub-Andean ranges between Chile and Argentina and to the area of the Paraná river basin (Chauveau *et al.*, 2011).

Sisyrinchium has radially symmetric yellow, pale, dark blue or purple flowers. Its species can be distinguished by leaf traits (*e.g.*, cylindrical *vs.* linear, presence of caulinate leaves) and flower characters, particularly aspects of androecium organization. Filaments can be partly connected or completely fused in a staminal column that may bear trichomes of different types, including elaiophores (Goldblatt and Manning, 2008).

Sisyrinchium plants are usually found in open areas, grasslands, rocky formations, or wet environments and are sometimes found in ruderal habitats. Some species, such as *S. micranthum* (Figure 1G) are widespread in southern Brazil, whereas others, such as *S. rambonis* R.C. Foster, occur only in environments with specific ranges of humidity and altitude (Foster, 1950; personal observation).

Phylogenetic evidence from Iridoideae based on five plastid DNA regions strongly suggests that *Sisyrinchium* is closely related to the four other genera of tribe *Sisyrinchieae* (*Libertia*, *Orthrosanthus*, *Olsynium*, and *Solenomelus*) (Goldblatt *et al.*, 2008). The infrageneric taxonomy of *Sisyrinchium* has been studied over many years, based primarily on morphological traits and, more recently, on molecular characteristics. However, the systematics of the genus remain poorly resolved because of the weedy, self-fertile nature of its species and the high morphological similarity between closely related taxa, which is attributable to a recent adaptive radiation process resulting in the emergence of species complexes (Goldblatt, 1982; Chauveau *et al.*, 2011).

A recent phylogenetic study of *Sisyrinchium* combining eight molecular markers of the plastidial, mitochondrial, and nuclear genomes (Chauveau *et al.*, 2011) confirmed its monophyly and revealed nine major clades weakly connected to the unfinished subdivision established by Ravenna, with some exceptions. Chauveau *et al.* (2011) suggested that changes in the classification of the sections are needed, especially in the clades including species from southern Brazil. The section *Hydastylus* appears to be polyphyletic, and a new taxonomic treatment of the species allocated to this section is required. Tree topology showed a clade formed by the *S. palmifolium* complex (section *Hydastylus*) and the *S. vaginatum* complex (section *Viperella*), which is a well-supported monophyletic group. A similar result was observed for the section *Sisyrinchium*, whose circumscription needs to be reviewed because species of the section were present in at least three well-supported clades, the main one including specimens of *S. micranthum*, the related *S. laxum* Otto ex Sims and *S. rosulatum* E.P. Bicknell. Species belonging to section *Lenitium* and to section *Scirpeocharis* are grouped in a single clade in the phylogeny, suggesting that these sections correspond to a unique natural group. *Sisyrinchium sellowianum*, placed by Ravenna in the section *Lenitium*, is related to species belonging to section *Scirpeocharis*, including *S. luzula,* both found in southern Brazil.

We have examined the genetic structure within and among populations belonging to four complexes of *Sisyrinchium* species (*S. micranthum, S. palmifolium* - Figure 1H, *S. sellowianum*, and *S. vaginatum* - Figure 1I) based on ISSR markers (Table 1). Genetic differentiation among populations of *S. micranthum*, *S. palmifolium*, *S. sellowianum,* and *S. vaginatum* was considerable (ϕ_{ST} = 0.3528, 0.4700, 0.4370, and 0.4537, respectively) and a higher intrapopulation genetic variability was observed (ϕ_{IS} = 0.6472, 0.5300, 0.5630 and 0.5463, respectively), the genetic differentiation among populations indicates a high structure. The Nei (h) genetic identity index (Nei, 1973) and the Shannon (I) index of phenotypic diversity (Shannon and Weaver, 1949) were tested for these four groups of species with the following results: *S. micranthum* (h = 0.1819, I = 0.2750), *S. palmifolium* (h = 0.1563, I = 0.2626), *S. sellowianum* (h = 0.2945, I = 0.4535) and *S. vaginatum* (h = 0.2445, I = 0.3839). The lowest values of diversity were observed for *S. micranthum* (Tacuatiá *et al.*, 2012a) and *S. palmifolium*, whereas *S. sellowianum* showed the highest values, which is consistent with the identity of the Nei index calculated for the analyzed complexes.

The genetic variability and genetic differentiation observed for the populations of these complexes may indicate reduced gene flow, probably due to geographical distance and fragmentation of populations, resulting in high genetic structure. High levels of genetic differentiation might be explained by several factors, such as the species breeding system, genetic drift, demographic fluctuations, or the genetic and geographic isolation of populations (Hogbin and Peakall, 1999).

At the present time, the analysis indicated no significant correlation between geographic and genetic distances for the four complexes of species (Table 1). The lack of correlation may be due to the fact that we analyzed complexes of species, encompassing more than one taxonomic entity (or species) and with distinct genomes. Furthermore, several evolutionary processes can affect the lack of correlation, for example, a founder effect and habitat fragmentation. The sites of collection of these plants are areas of agricultural use and grasslands, factors which normally reduce population size and restrict the possibility of gene flow between populations.

According to Hamrick and Godt (1989), reproductive biology is the most important determinative factor in the genetic structure of plant populations. They showed that outcrossing plant species tend to have 10% to 20% of genetic variation among populations, whereas selfing species have, on average, 50% of variation among populations. Thus, our data suggest that the analyzed species preferentially reproduce by selfing. Genetic diversity within populations is considered to be of high importance for the adaptation to fluctuating environments and, as a consequence, for the long-term survival of a species.

Conclusions

The present work reviews what is known about the genetic diversity and evolution of species belonging to the genera *Calydorea*, *Cypella*, *Herbertia*, and *Sisyrinchium* from southern Brazil. Diversity indexes obtained illustrate the genetic differentiation of populations that occur in grasslands of southern Brazil. With the exception of *C. fucata*, these populations are generally well structured. The available cytogenetic evidence indicates that polyploidy events have been important in Iridaceae speciation processes. Phylogenetic studies have clearly demonstrated the need for a review of the taxonomical circumscription of these genera, an exception being the genus *Sisyrinchium*. Phylogenetic results for *Sisyrinchium* allow to reconstruct the evolution of glandular trichomes, which occurred independently three times in this genus. Evolutionary studies of *Sisyrinchium* suggest that oil-producing trichomes may have played a key role in its diversification. The other genera should also be tested for glandular trichomes that attract specialized pollinators.

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Table 1 - Indices of genetic variability for *Sisyrinchium* species.

Species	Φ		h	I	r
	ST	IS			
S. micranthum	0.3528*	0.6472*	0.1819	0.2750	0.266
S. palmifolium	0.4700*	0.5300*	0.1563	0.2626	0.16
S. sellowianum	0.4370*	0.5630*	0.2945	0.4535	0.101
S. vaginatum	0.4537*	0.5463*	0.2445	0.3839	0.240

Φ_{ST} = interpopulation genetic differentiation coefficient; Φ_{IS} = intrapopulation genetic differentiation coefficient; h = Nei's genetic identity (Nei, 1973); I = Shannon index of phenotypic diversity (Shannon and Weaver, 1949); r = Mantel test.
*p > 0.01.

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