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► **To cite this version:**

Olivier Chauveau, Lilian Eggers, Tatiana T Souza-Chies, Sophie Nadot. Oil-producing flowers within the Iridoideae (Iridaceae): evolutionary trends in the flowers of the New World genera. *Annals of Botany*, 2012, 110 (3), pp.713-729. 10.1093/aob/mcs134. hal-04267983

HAL Id: hal-04267983

<https://universite-paris-saclay.hal.science/hal-04267983>

Submitted on 2 Nov 2023

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Oil-producing flowers within the Iridoideae (Iridaceae): evolutionary trends in the flowers of the New World genera

Olivier Chauveau^{*1}, Lilian Eggers², Tatiana T. Souza-Chies² and Sophie Nadot¹

¹Université Paris-Sud, Laboratoire Ecologie Systématique et Evolution, UMR 8079, Orsay, F-91405, France and

²Instituto de Biociências, Departamento de Botânica, Universidade Federal do Rio Grande do Sul, Porto Alegre, Av. Bento Gonçalves, 9500 – Prédio 43433, Brazil

*For correspondence. E-mail oli.chauveau@laposte.net

Received: 10 January 2012 Returned for revision: 6 March 2012 Accepted: 16 April 2012 Published electronically: 10 July 2012

• **Background and Aims** Oil-producing flowers related to oil-bee pollination are a major innovation in Neotropical and Mexican Iridaceae. In this study, phylogenetic relationships were investigated among a wide array of New World genera of the tribes Sisyrinchieae, Trimezieae and Tigridieae (Iridaceae: Iridoideae) and the evolution of floral glandular structures, which are predominantly trichomal elaiophores, was examined in relation to the diversification of New World Iridaceae.

• **Methods** Phylogenetic analyses based on seven molecular markers obtained from 97 species were conducted to produce the first extensive phylogeny of the New World tribes of subfamily Iridoideae. The resulting phylogenetic hypothesis was used to trace the evolutionary history of glandular structures present in the flowers of numerous species in each tribe. Hypotheses of differential diversification rates among lineages were also investigated using both topological and Binary-State Speciation and Extinction methods.

• **Key Results and Conclusions** Floral glandular structures and especially trichomal elaiophores evolved multiple times independently in the American tribes of Iridoideae. The distribution pattern of species displaying glandular trichomes across the phylogeny reveals lability in the pollination system and suggests that these structures may have played a significant role in the diversification of the Iridoideae on the American continent.

Key words: Elaiophores, glandular trichomes, Iridoideae, nectaries, pollination systems, Sisyrinchieae, Tigridieae, Trimezieae.

INTRODUCTION

Bees are the world's dominant pollinating agents. Their role in pollination and in turn in sexual reproduction ranges from simply beneficial to essential for the pollination of much of the natural vegetation of the world (Michener, 2007). In addition to or instead of pollen and nectar, which are the two most common resources collected by bees, flowers of 11 unrelated families distributed throughout the angiosperms produce non-volatile oils (Buchmann, 1987; Rasmussen and Olesen, 2000; Steiner and Whitehead, 2002; Neff and Simpson, 2005; Renner and Schaefer, 2010). These oils are collected and mainly used as food stores for larvae by specialized bees of the Melittidae and Apidae families of Hymenoptera (Michener, 2007). Floral epithelial or trichomal oil-producing organs, so-called elaiophores, are present in more than 1500 species of flowering plants. Twenty-eight transitions towards oil-bee pollination were identified among angiosperms, and Iridaceae is one of the two families where elaiophores have evolved more than once (Renner and Schaefer, 2010). The actual number of transitions in Iridaceae is unknown due to phylogenetic uncertainties, but the latest comprehensive phylogeny of Iridaceae (Goldblatt *et al.*, 2008) allowed the identification of at least three independent origins of oil-offering flowers within the family (Renner and Schaefer, 2010), although numerous New World genera were missing or poorly sampled regarding their morphological diversity and species richness.

Floral oils are the third type of resource collected by pollinators in Iridaceae (Goldblatt and Manning, 2008), coming after pollen and nectar. All transitions but one towards oil-offering flowers concern strictly American taxa of the Iridoideae (Renner and Schaefer, 2010), where oils are produced by conspicuous glandular trichomes located on the tepals or the staminal column. *Tritoniopsis parviflora* (Jacq.) G.J.Lewis (Crocoideae), from South Africa, is the only oil-producing species outside Iridoideae and outside America. It stands out from the rest of Iridaceae by secreting floral oils from epithelial elaiophores (Manning and Goldblatt, 2002).

The subfamily Iridoideae is a major evolutionary branch of the family, comprising at least 900 species and making up about 40% of the species richness within the Iridaceae (Goldblatt and Manning, 2008). The Iridoideae are subdivided into four large tribes plus the Australian genus *Diplarrena* Labill., the single member of the tribe Diplarreneae, sister to all other tribes (Goldblatt and Manning, 2008). The predominantly Old World Irideae (with some species of *Iris* Tourn. ex L. in North America), and the largely New World Sisyrinchieae (*Libertia* Spreng. and *Orthrosanthus* Sweet include Australasian species), sister to the two strictly New World tribes, Trimezieae and Tigridieae, form a monophyletic lineage defined by molecular and morphological synapomorphies (Goldblatt, 1990; Reeves *et al.*, 2001; Rodríguez and Sytsma, 2006; Goldblatt *et al.*, 2008; Goldblatt and Manning, 2008). The major dichotomy in the Iridoideae is the separation

of the Old World genera, and the New World genera including *Libertia* and *Orthrosanthus* (Goldblatt *et al.*, 2008).

Trichomal elaiophores are particularly widespread in *Sisyrinchium* L., the largest genus in the New World Iridaceae (approx. 140 species). They are located either on the staminal column or on the adaxial side of the tepals, sometimes on both parts, a distribution pattern that has only been observed on flowers of this genus (Goldblatt and Manning, 2008; Chauveau *et al.*, 2011). A recent comprehensive study of *Sisyrinchium* suggested that floral glandular trichomes evolved three times independently in the genus, including two transitions towards oil-secreting trichomes (Chauveau *et al.*, 2011). Trichomal elaiophores evolved also probably once in the common ancestor of Trimezieae and Tigridieae, with at least six reversions (Renner and Schaefer, 2010).

However, these estimates rely on a phylogeny in which, due to incomplete sampling of genera and lack of representativeness in

the most species-rich genera, phylogenetic and taxonomic relationships inside the American tribes Trimezieae and Tigridieae remain poorly understood, precluding the identification of all the transitions toward oil-bee pollination within these tribes (Reeves *et al.*, 2001; Rodríguez and Sytsma, 2006; Goldblatt *et al.*, 2008).

It has been suggested that elaiophores may have evolved from perigonal nectaries (Rudall *et al.*, 2003), as both trichomal nectaries, composed of nectar-producing trichomes, and trichomal elaiophores are widespread among the three mostly American tribes of the Iridoideae with high topological and anatomical similarities, although this hypothesis remains untested.

The goal of this study was to elucidate phylogenetic relationships among and within the tribes Sisyrinchieae, Trimezieae and Tigridieae (Fig. 1) using a large sampling of species, representing the taxonomic diversity of these tribes.

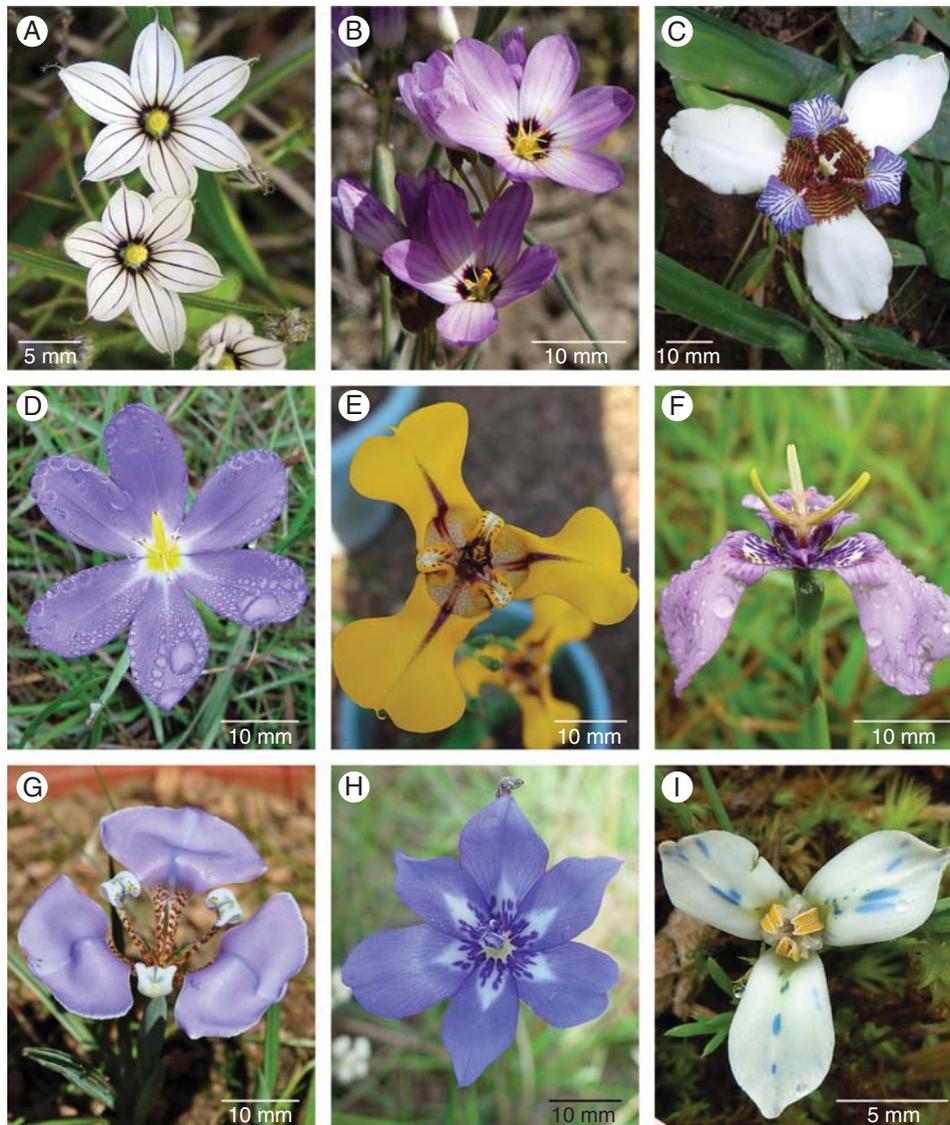


FIG. 1. Floral morphology in tribes Sisyrinchieae, Trimezieae and Tigridieae: (A, B) Sisyrinchieae: (A) *Sisyrinchium sellowianum*, (B) *Olsynium junceum*, (C) Trimezieae: *Neomarica candida*, (D–I) Tigridieae: (D) *Calydorea crocoides*, (E) *Cypella herbertii*, (F) *Herbertia quareimana*, (G) *Onira unguiculata*, (H) *Gelasine elongata* and (I) *Cardenanthus vargasii*.

A total-evidence approach was carried out to produce molecular phylogenetic trees and to test the monophyly of the generic divisions defined in the published classifications. The resulting historical framework was used to infer the evolutionary history of floral trichomes, mainly nectaries and elaiophores, towards a better understanding of the evolution of pollination strategies in relation to floral secretions. Differential diversification rate analyses were used to estimate whether floral trichomes, and more specifically trichomal elaiophores, may have contributed to the diversification of the Iridaceae on the American continent.

MATERIALS AND METHODS

Taxonomic sampling

The list of taxa sampled, voucher information and GenBank accession numbers is given in the Appendix. We sampled a total of 97 species covering most of the genera proposed in the literature for the tribes Sisyrinchieae, Trimezieae and Tigridaeae. Based on the systematic study published by Goldblatt and Manning (2008), only three monospecific genera are missing: *Tapeinia* Juss. (Sisyrinchieae), *Pseudiris* Chukr & A.Gil (Trimezieae) and *Salpingostylis* Small (Tigridaeae). Within the tribe Tigridaeae, our sampling included species which formerly belonged to genera reduced or provisionally retained in *Tigridia* Juss. (*Ainea* Ravenna, *Cardiostigma* Baker, *Colima* (Ravenna) Aarón Rodr. & Ortiz-Cat., *Fosteria* Molseed, *Rigidella* Lindl. and *Sessilanthera* Molseed & Cruden), *Cypella* Herb. (*Kelissa* Ravenna, *Onira* Ravenna and *Phalocallis* Herb.), *Calydorea* Herb. (*Catila* Ravenna and *Tamia* Ravenna) and *Mastigostyla* I.M.Johnst. (*Cardenanthus* R.C.Foster). The monophyly of these recent taxonomic circumscriptions remains to be tested, and variation in the presence/absence of trichomal nectaries and elaiophores is widespread in these genera (Ravenna, 1979, 1981, 1983, 2009; Goldblatt and Henrich, 1991; Goldblatt and Manning, 2008), accounting for our extensive sampling. Outgroups were selected from the genera *Iris*, *Diets* Salisb. ex Klatt and *Diplarrena* (one species per genus), and represent the two other tribes of Iridoideae (Goldblatt et al., 2008). Plant material was sampled mostly from the wild, or from cultivated specimens obtained from seeds collected in the wild and grown in botanical gardens or national collections.

DNA extraction, amplification and sequencing

Total DNA was extracted from fresh or silica gel-dried leaves using the NucleoSpin Plant II (Macherey-Nagel, Düren, Germany) extraction kit, following the manufacturer's instructions. A combination of coding and non-coding cpDNA regions was used to infer phylogenetic relationships among the taxa sampled. We selected the five DNA regions already used by Goldblatt et al. (2008): three coding regions (*rps4*, *matK* and *rbcL*), the *trnL-F* region (*trnL* intron, *trnL* exon 2 and the intergenic spacer *trnL-trnF*) and the *rps16* intron (Taberlet et al., 1991; Johnson and Soltis, 1995; Oxelman et al., 1997; Souza-Chies et al., 1997; Reeves et al., 2001). These loci have shown their potential to complement each

other in improving the phylogenetic signal at the family and subfamily taxonomic levels within Iridaceae (Souza-Chies et al., 1997; Reeves et al., 2001; Goldblatt et al., 2008). Our study includes molecular characters from two additional non-coding cpDNA regions (spacer *rps4-trnS* and *matK-5'trnK* intron) in order to improve the current resolution of the phylogeny among Sisyrinchieae, Trimezieae and Tigridaeae (Shaw et al., 2005, 2007). Primers used to amplify each DNA region and additional primers used for sequencing are given in Supplementary Data Table S1. PCR amplifications were performed using a PTC-100 MJ-Research thermal cycler in a 30- μ L total volume reaction with the following reaction components: 1.5 μ L of genomic DNA (approx. 15–50 ng), 0.5 μ M of each primer, 100 μ M dNTP, 1 \times *rTaq* buffer, 1.5 mM MgCl₂ and 0.2 U *Taq* polymerase (*Taq* CORE Kit 10, MP Biomedicals, Illkirch, France). The *rps4* + spacer *rps4-trnS*, *matK* + *matK-5'trnK* intron, and *trnL-F* loci were successfully amplified with the addition of DMSO (1.2 μ L) to the PCR mix. Detailed PCR conditions for each DNA locus used in this study are given in Supplementary Data Table S2. Each DNA region was amplified as a single fragment except in a few cases where internal primers originally designed for sequencing were used to amplify the targeted region in smaller fragments. PCR products were purified and automatically sequenced by Cogenics (Meylan, France) using an ABI PRISM automatic sequencer (Perkin Elmer, Courtaboeuf, France) with forward and reverse primers. Raw forward and reverse sequences for each sample were assembled with CodonCode Aligner 3.5.3 (CodonCode Corp., Dedham, MA, USA); ambiguous bases were corrected after examination of chromatograms, and consensus sequences were edited.

Phylogenetic analyses

Alignments were first produced using Clustal x (Thompson et al., 1997) and MUSCLE (Edgar, 2004) and were further improved manually using MEGA5 (Tamura et al., 2011). All detected polymorphisms [single nucleotide polymorphisms (SNPs) and insertion-deletions (indels)] were visually checked and further validated using a base quality threshold above 20. The indels of the non-coding regions (spacer *rps4-trnS*, *rps16* intron, *matK-5'trnK* intron and *trnL-F* region) shared by two or more taxa were coded as binary characters using GapCoder (Young and Healy, 2003), a program based on simple indel coding *sensu* Simmons and Ochoterena (2000). All seven cpDNA regions were first analysed independently (results not shown). As the comparison of the resulting topologies of separate analyses revealed no instances of well-supported topological differences and, because a better resolution was obtained when these regions were analysed together than separately, they were combined into a single supermatrix for subsequent analysis.

Maximum parsimony (MP) analysis

Data sets including indels coded with GapCoder were analysed using the parsimony criterion in PAUP* version 4.0b10 (Swofford, 2002). Parsimony ratchet (Nixon, 1999) searches consisting of 20 independent replicates of 200 iterations with 15 % of characters reweighted per iteration were

scripted using the program PAUPRat (Sikes and Lewis, 2001) and run in PAUP*. Strict and majority-rule consensus trees were calculated from all most-parsimonious trees and the robustness of nodes was evaluated using MP with 1000 bootstrap replicates of new heuristic searches [100 random addition replicates, tree-bisection-reconnection (TBR) branch swapping and multrees on].

Maximum-likelihood and Bayesian analysis

Maximum-likelihood (ML) analyses (Felsenstein, 1981) were performed using RAxML 7.2.6 (Stamatakis, 2006) with the data partitioned as indicated in Supplementary Data Table S3 and the most appropriate evolutionary model implemented for each type of partition. The GTRGAMMA model (GTR model + optimization of substitution rates + Γ model of rate heterogeneity) was used for the DNA partitions and the BINGAMMA model (model for binary substitution using the Γ model of rate heterogeneity) for the coded indel partitions; the alpha parameter was estimated separately for each DNA and binary partition. To assess the stability of the tree topology and branch length values, 200 independent ML searches were conducted to find the best-scoring ML tree; RAxML used different randomized stepwise addition parsimony starting trees for each ML search. The reliability of ML topologies was evaluated by non-parametric standard bootstrap tests using 1000 pseudo-replicates. Bootstrap values were then summarized and reported on the best-scoring ML tree.

Bayesian Monte Carlo Markov chain (MCMC) (Yang and Rannala, 1997) analyses were run using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) with the data partitioned and the most appropriate evolutionary model implemented for each partition as indicated in Supplementary Data Table S3. Model parameters were set to those calculated by MrModeltest 2.3 (Nylander, 2004). The Akaike information criterion was chosen to select the most appropriate model of DNA substitution for each data set or data partition used in the analyses. Coded indels were included in a separate data matrix and treated using a simple model with variable rates. Two independent runs, each comprising four Markov chains (one cold and three heated) and starting with a random tree, were performed simultaneously for 10^7 generations, sampling trees at every 100th generation. The convergence diagnostic was calculated every 10^4 generations and its critical value was set in order to automatically stop the analysis when the standard deviation of the split frequencies had reached the value defined by the stopval command (stoprule = yes stopval = 0.01). In all analyses, the first 25 % trees from each run were discarded as burnin. Resulting trees from the two independent runs were then pooled to produce one 50 % majority-rule consensus tree and Bayesian posterior probabilities were generated for the resulting tree.

Combined dataset phylogenies

Node supports resulting from all three analyses (MP, ML and Bayesian) were reported on the best-scoring ML tree. For each analysis, a given support value was kept if the bootstrap support for MP or ML was higher than 50 %, or if the

posterior probability (PP) was higher than 0.90. Nodes were considered fully supported if they had a parsimony bootstrap value (PBS) and an ML bootstrap value (LBS) of 100 %, as well as a Bayesian PP of 1.00. Strongly supported nodes were defined with a threshold of at least 80 % for the PBS and LBS values, and a PP value ≥ 0.95 . Nodes for which only two of the three support values reach these minimum values and the third support value was at least 50 % (PBS), 60 % (LBS) and 0.95 (PP) were considered moderately supported, and nodes for which only one of the three support values reaches the minimum values and the two other support values were at least 50 % (PBS), 50 % (LBS) and 0.90 (PP) were considered weakly supported.

Characterization of glandular structures and trichomes

Glandular structures secreting nectar or oils are present in the flowers of numerous species within tribes Sisyrinchieae, Trimezieae and Tigridaeae. Among them, glandular trichomes are predominant. They are located mainly on the stamens but also on the adaxial side of tepals in *Sisyrinchium* species (Sisyrinchieae), and the nature of their secretion has been identified in many species (Chauveau et al., 2011). Among Trimezieae and Tigridaeae, glandular trichomes are found only on the adaxial side of inner tepals, except for the genus *Herbertia* Sweet, where they are located on both series of tepals, although much more scattered on the outer tepals (Ravenna, 1989; Goldblatt and Manning, 2008). These structures are sometimes called nuptial glandular structures or nuptial trichomes to emphasize their involvement in the relationship with pollinators (Delpino, 1874; Fahn, 2000; Chauveau et al., 2011). They are referred to hereafter simply as glandular structures. Morphological data were obtained from the literature and from observations on herbarium specimens (BAA, CTES, ICN, K and P) or specimens held in living collections in the botanical gardens of the Université Paris-Sud (France) and Porto Alegre (Brazil) as indicated in Supplementary Data Table S4. When the literature indicated that the presence of secretory structures was not constant within a genus, this trait was systematically verified for each species included in the study, using herbarium specimens or living plants. Because we were unable to identify the type of secretion for the trichomes of *Larentia mexicana* (Tigridaeae), this character was coded as unknown. Similarly, uncertainties concerning the presence of glandular structures remain for species of the genus *Olsynium* Raf. (Sisyrinchieae). *Olsynium douglasii* produces nectar from an extensive vascular tissue located just beneath the outer epidermis of the swollen part of the staminal column whereas *O. junceum* produces nectar from anticlinally elongated epidermal cells on both surfaces of the staminal swollen tube (Forcone et al., 1997; Rudall et al., 2003). It has been suggested that several other species of *Olsynium*, which have a similar swollen base of the staminal column, could produce nectar in the same manner (Rudall et al., 2003). Although this kind of staminal column was observed in the flowers of *O. filifolium* and *O. scirpoideum*, the presence of nectaries was not confirmed. The absence/presence of glandular structures and the type of secretion were then coded as unknown.

For character optimization, glandular structures were coded using three discrete characters, respectively the anatomical type (A), the type of secretion (B) and the localization (C) of glandular structures. Five states were defined for the first character, namely (0) absent, (1) unicellular trichomes, (2) pluricellular scales, (3) carpel margins cells and (4) epithelial cells. The type of secretion was coded with six states: (0) absence of glandular structure, (1) trichomal nectaries, (2) trichomal elaiophores, (3) pluricellular scales nectaries, (4) septal nectaries and (5) epithelial nectaries. Glandular structure localization was coded as follows: (0) absent, and present (1) on the staminal column, (2) on the inner tepals, (3) on both whorls of tepals and (4) on the ovary. The ML best scoring tree was used for character optimization with the MP and ML methods implemented in MESQUITE 2.75 (Maddison and Maddison, 2010). With MP, character states were treated as unordered, allowing any transition among states. ML optimization was conducted using the MK1 model of evolution (Schluter *et al.*, 1997; Pagel, 1999), which gives equal probability for changes between all character states.

Detection of differential diversification rates

Two methodological strategies were used to estimate differential diversification rates across the phylogeny inferred. The topological strategy allows the identification of differential diversification rates along a phylogeny independently of character states, whereas the likelihood-based BISSE method (Maddison *et al.*, 2007) estimates whether speciation (λ) and extinction (μ) rates depend on the state of a particular character, and allows the assessment of a state-dependent effect of this character on diversification rates.

The program SymmeTREE (Chan and Moore, 2005) compares the topological species distribution observed with a random species distribution. This method was employed to determine if diversification rate shifts exist across lineages. Being a topological method, it relies on the order of branching events and does not require information on branch lengths. Six whole-whole tree tests of differential diversification rates, each sensitive to different scenarios and nodal depths, were used. I_c tests for diversification at deeper nodes by determining if the observed trees are more or less asymmetric than expected (Page, 1993). M_π and M_Σ both consider the asymmetry of internal nodes, with M_π sensitive to deeper nodes through multiplication of individual node probabilities and M_Σ sensitive to nodes closer to the tips of the tree through addition of node probabilities. M_π^* and M_Σ^* differ from the two previous statistical tests by having each node probability weighted by its size (Chan and Moore, 2002). B_1 measures tree balance as opposed to imbalance; this latter statistical test excludes the root and is sensitive to changes at the tips of the tree (Shao and Sokal, 1990). The MP strict consensus tree, ML best-scoring tree and Bayesian majority-rule consensus tree were all used for the SymmeTREE analysis.

The BISSE speciation/extinction likelihood analysis implemented in MESQUITE 2.75 was used to calculate the likelihood and estimate the parameters of a six-parameter model of speciation, extinction and character change. This analysis required a fully resolved phylogram and binary characters.

Branch lengths were needed for likelihood calculation and for estimating each character state along branches. Therefore, a range of constrained BISSE models were explored to test the hypotheses regarding differential diversification of lineages in the absence (state 0) or presence (state 1) of glandular trichomes (character D) on the one hand, and of trichomal elaiophores (character E) on the other hand. The different states were coded for each character according to the observations made for characters A and B. The fully relaxed six-parameter model (FR model) using the ML best-scoring tree was first estimated. More constrained models were then compared in a hierarchical manner [models having one parameter constrained (1C models) to be equal versus FR model, models having two parameters constrained (2C models) versus 1C models, and fully constrained model versus 2C model] to test whether the fully relaxed model was a significantly better fit than models in which speciation, extinction or transition rates were equal. Because the BISSE constrained models are nested within the fully relaxed one, the significance of differences in model was estimated with likelihood ratio tests. Conversely, statistical comparisons were started with the most constrained model (each parameter equal in states 0 and 1) and compared with models relaxing one constraint at a time (1C models). The significance of differences in models was estimated in the same way and when a significant improvement in likelihood was observed, the corresponding model was compared with models relaxing two constraints at a time (2C models) and, finally, the best 2C model was compared with the FR model.

RESULTS

Phylogenetic analyses

No major incongruences were detected among topologies based on the separate analysis of each cpDNA region, allowing us to combine the datasets. All cpDNA markers were combined into a single supermatrix totalling 6787 characters (including the 310 coded indels), of which 1267 (18.6%) were phylogenetically informative. The parsimony-informative characters were distributed mainly among non-coding regions (21.6%), but the percentage of informative characters was also high in coding regions (16.2%), the *matK* region totalling more than 61.9% of the parsimony-informative characters of the coding regions.

Parsimony ratchet analysis of all seven cpDNA regions yielded 3969 equally most-parsimonious trees of 3449 steps, consistency index (CI, excluding uninformative characters) = 0.66 and retention index (RI) = 0.91. ML searches produced a best-scoring ML tree with $-\ln L = 30\,001$ (Supplementary Data Fig. S1). The 9000 trees retained from the Bayesian analysis were summarized into a 50% majority-rule consensus tree. Tree topologies resulting from the total-evidence analyses with parsimony ratchet, likelihood and Bayesian approaches were largely congruent, as shown by the number of shared nodes reported on the ML best-scoring tree (Fig. 2). MP, ML and Bayesian analyses fully support the monophyly of Sisyrrinchieae, Trimezieae and Tigridieae (PBS and LBS = 100% and PP = 1 for each tribe), confirming the results of Goldblatt *et al.* (2008).

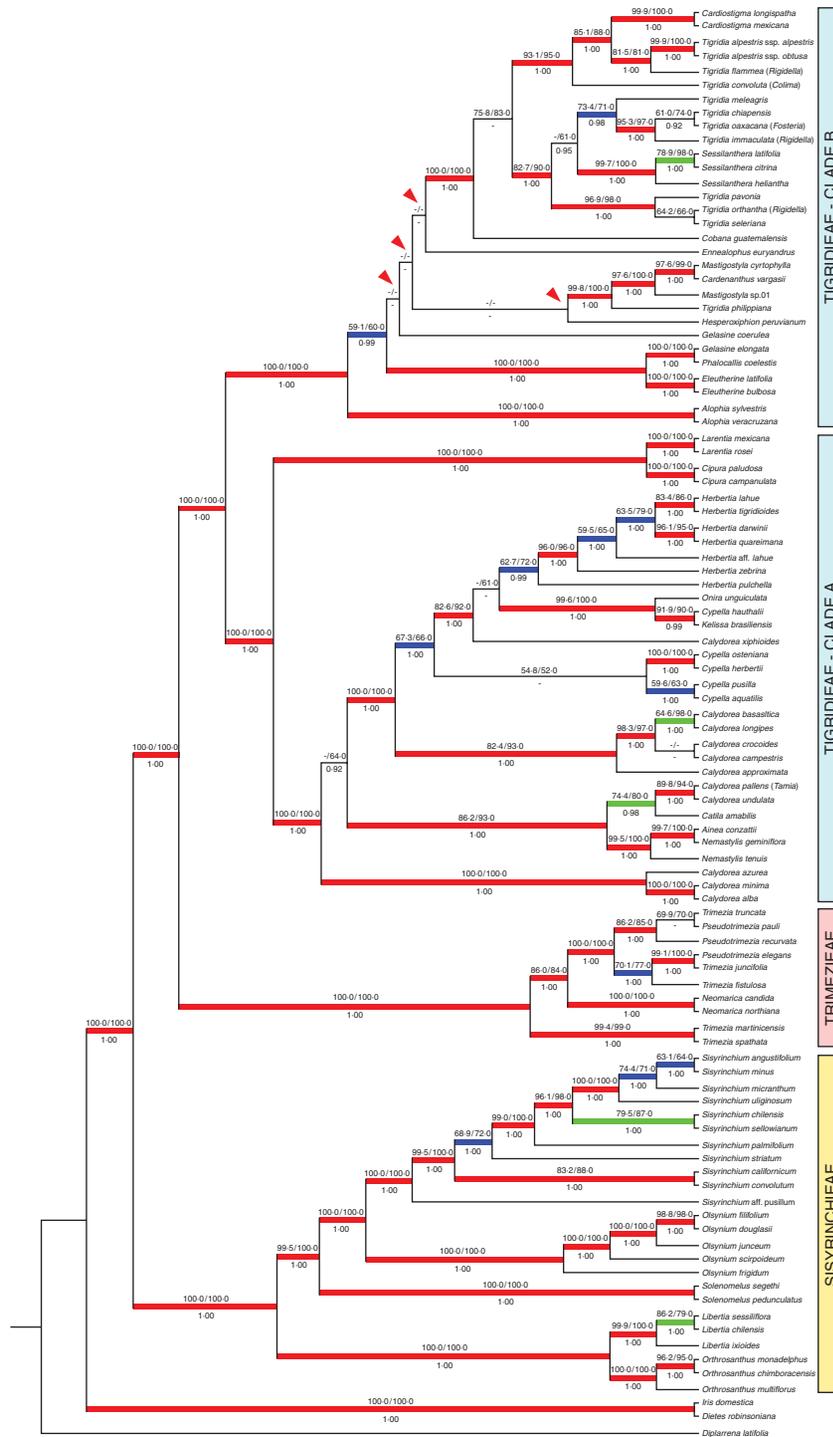


FIG. 2. Maximum-likelihood best-scoring topology obtained from the combined dataset. Support values at nodes are parsimony bootstrap (PBS, on the left, above branches), likelihood bootstrap (LBS, on the right, above branches) and Bayesian posterior probability (PP, below branches). Thick red branches indicate support of at least 80 % (PBS), 80 % (LBS) and 0.95 (PP). Thick green lines indicate branches for which only two of the three support values reach these minimum values and the third support value is at least 50 % (PBS), 60 % (LBS) and 0.95 (PP). Thick blue lines indicate branches for which only one of the three support values reaches the minimum values set above while the two other support values are at least 50 % (PBS), 50 % (LBS) and 0.90 (PP). Red arrowheads indicate the nodes that are supported in none of the analyses.

Sisyrrinchieae. The monophyly of all genera included in the present study is fully or strongly supported. A dichotomy is observed between *Libertia* + *Orthrosanthus* on the one hand,

which are distributed on the American and Australasian continents, and the three exclusively American genera on the other hand.

Trimezieae. The genus *Trimezia* is clearly non-monophyletic, including all representatives of *Pseudotrimezia* (non-monophyletic) and *Neomarica*. All of these phylogenetic relationships are fully supported.

Tigridieae. The tribe is divided into two fully supported clades hereafter named clade A and clade B. Clade A consists of genera exclusively included in the subtribe Cipurinae Benth. & Hook.f. (Goldblatt, 1990). *Cipura* and *Larentia* are sister to the rest of clade A with full support. The results reveal that two major genera within clade A, *Calydorea* and *Cypella*, are not monophyletic. *Calydorea* is split into three unrelated strongly supported lineages within clade A, none of which is related to *C. xiphioides*, the type species of the genus. In the genus *Cypella*, *C. hauthalii* stands apart from the rest of the genus and forms a strongly supported clade together with *Onira unguiculata* and *Kelissa brasiliensis*. The four other representatives of the genus are retrieved in another unrelated clade. *Herbertia* is a moderately supported genus, but *H. pulchella* is sister to the rest of the genus with strong support. *Herbertia* is nested within the *Calydorea* and *Cypella* lineages.

Clade B comprises the remaining genera of Cipurinae (*Cardenanthus*, *Eleutherine*, *Ennealophus*, *Gelasine* and *Mastigostyla*) and all of the subtribe Tigridiinae Pax (Goldblatt, 1990). The phylogeny confirms that neither Tigridiinae nor Cipurinae are monophyletic, as already suggested by Rodríguez and Sytsma (2006). *Tigridia philippiana*, the only South American member of the genus included in this study, is sister to *Mastigostyla* and *Cardenanthus* with strong support.

As suggested by earlier molecular and morphological studies (Rodríguez, 1999; Rodríguez and Sytsma, 2006; Goldblatt et al., 2008), most of the genera grouped by Goldblatt et al. (2008) in *Tigridia* (namely *Cardiostigma*, *Colima*, *Fosteria*, *Rigidella*, *Sessilanthera* and *Tigridia*) are gathered in a monophyletic lineage, which forms with its sister genus *Cobana* a fully supported clade. Within this lineage *Cardiostigma* and *Sessilanthera* form strongly supported clades. The species formerly included in the genus *Rigidella* are nested within different *Tigridia* lineages with strong support, confirming that this genus is not monophyletic. Likewise, the representatives of the monospecific genera *Colima* (*T. convoluta*) and *Fosteria* (*T. oaxacana*) are retrieved within two different *Tigridia* lineages. The monophyly of the two subgenera (*Tigridia* and *Hydrotaenia*) recognized by Molseed (1970) and accepted by Goldblatt et al. (2008) for the genus *Tigridia sensu stricto* is not supported by our study and the species included in each of the subgenus are split into several unrelated clades among the *Tigridia* complex.

Evolution of glandular structures and trichomes

The MP optimizations of three aspects of glandular structures (Characters A, anatomical type; B, secretion type; C, distribution within flowers) are shown on Figs 3 and 4. Values obtained from the ML optimizations of characters A–C are detailed in Supplementary Data Figs S2–S4. The numbers of transitions and reversions among character states found

with the MP and ML optimizations of characters A–C within each tribe are summarized in Table 1.

Character A: anatomical type of glandular structures. The MP optimization shows that the state for the common ancestor of the three study tribes is the absence of glandular structures, as for the tribes Sisyrinchieae and Tigridieae, for the common ancestor of Trimezieae and Tigridieae, and for clades A and B within Tigridieae. These ancestral nodes appear mostly ambiguous with the ML optimisation (Fig. S2A, B) with two more likely states (absence of glandular structures and presence of unicellular trichomes), except for Sisyrinchieae, where the absence of glandular structures is the ancestral state with both methods (ML optimization: $P_0 = 0.88$). For Trimezieae, the ancestral state is the presence of unicellular trichomes, with both MP and ML optimizations ($P_1 = 0.99$).

Both MP and ML optimizations suggest that unicellular trichomes evolved three times independently within Sisyrinchieae and that this character state is derived in *Sisyrinchium* and *Solenomelus*. A single transition from the absence of glandular structures towards epithelial glandular cells is reported in *Olsynium*, and pluricellular scales evolved once from an ambiguous ancestral state in *Solenomelus*.

Within Trimezieae, the ML optimization favours unequivocally that glandular structures were lost three times independently in *Pseudotrimezia*, although the MP optimization results in a more ambiguous scenario with one to three reversions.

Within Tigridieae, the MP and ML optimizations lead to two different scenarios: with MP, seven to 12 transitions towards unicellular trichomes are recorded among clades A and B with few reversions, while with ML only four to seven transitions are observed, with numerous reversions. Whatever method is used, at least four transitions from the absence of glandular structures towards unicellular trichomes occurred within this tribe.

Character B: secretory type of glandular structures. The MP optimization (Fig. 3) shows that the state for the common ancestor of the three study tribes, as well as for the tribes Sisyrinchieae and Tigridieae, for the common ancestor of Trimezieae and Tigridieae, and for clades A and B within the latter tribe, is the absence of secretory structures. All these results are congruent with the MP optimization for character A. Similarly, the ML optimization for character B (Fig. S3A, B) favours the absence of secretory structures for most of these ancestral nodes, except for the common ancestor of the three study tribes and of Trimezieae and Tigridieae, in which two states are more likely (absence of secretory structures and presence of trichomal elaiophores). For Trimezieae, the ancestral state is the presence of trichomal elaiophores with the ML optimization ($P_1 = 0.92$), in agreement with the MP and ML optimisations for character A.

Both MP and ML optimizations suggest that trichomal elaiophores are derived within *Sisyrinchium* and evolved probably twice independently, with one subsequent reversion. A single transition from the absence of secretory structures towards epithelial nectaries was recorded in *Olsynium*, and pluricellular scale nectaries evolved once from the absence of glandular secretory structures in *Solenomelus*.

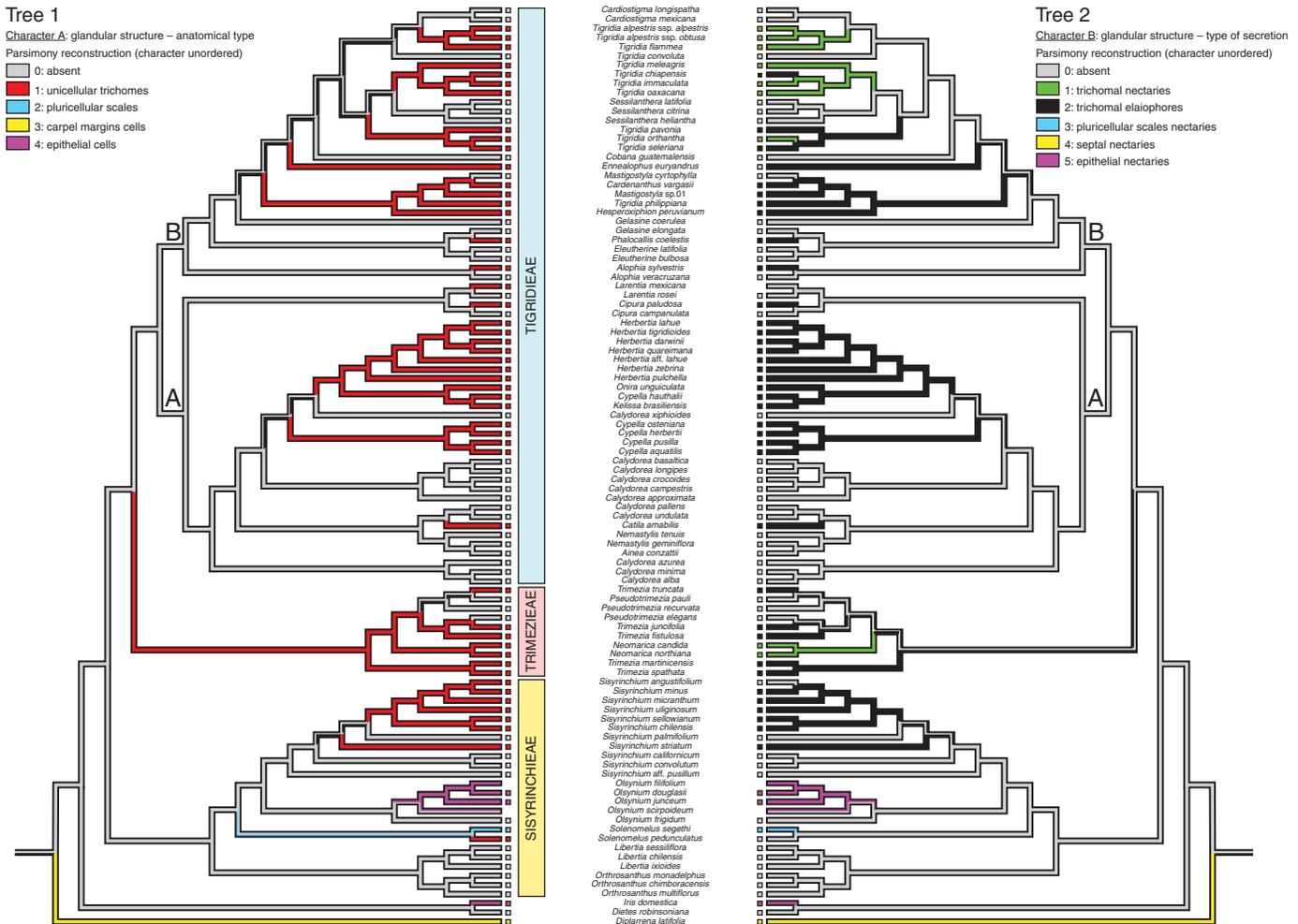


FIG. 3. Mirror trees showing the optimization of the anatomical type, character A (Tree 1), and the secretion type, character B (Tree 2), of glandular structures on the ML best-scoring tree, with maximum parsimony. External nodes are coloured according to the character state observed, and internal nodes are coloured according to the ancestral state inferred. Multi-coloured branches show unresolved ancestral character states under parsimony analysis. The absence of a square in front of the external nodes corresponds to missing data. Major clades within Tigridaeae are labelled A and B above the corresponding branches.

Within Trimezieae, both ML and MP optimizations favour an ancestral state with the presence of trichomal elaiophores, which were lost once or three times independently within *Pseudotrimezia*. Trichomal nectaries evolved once in the ancestor of *Neomarica*.

Both MP and ML optimizations suggest that trichomal elaiophores and trichomal nectaries are derived within clades A and B of Tigridaeae. At least seven independent transitions from flowers devoid of secretory structures towards flowers with trichomal elaiophores were recorded. In addition, trichomal elaiophores evolved once from trichomal nectaries in *Tigridia chiapensis*. Trichomal nectaries evolved only in clade B, twice from ancestors devoid of secretory structures, but also once from trichomal elaiophores in *Tigridia orthantha*.

Character C: distribution of glandular structures. Similarly to the results obtained for characters A and B, the MP optimization (Fig. 4) shows that the absence of glandular structures is the ancestral state for the common ancestor of the three study tribes as well as for the tribes Sisyrinchieae and

Tigridaeae, for the common ancestor of Trimezieae and Tigridaeae, and for clades A and B within Tigridaeae. The results obtained from the ML optimization are mostly ambiguous (absence of glandular structures and presence of unicellular trichomes), except for Sisyrinchieae, where the absence of glandular structures is the ancestral state with both methods (ML optimization: $P_0 = 0.88$). In Trimezieae, the ancestral state is the presence of glandular structures on the inner tepals, with both MP and ML optimizations ($P_2 = 0.99$).

Both MP and ML optimizations suggest that glandular structures located on the staminal column evolved three times independently among Sisyrinchieae (respectively, epithelial cells within *Olsynium*, glandular trichomes within *Sisyrinchium*, and both pluricellular scales and glandular trichomes within *Solenomelus*). Furthermore, glandular trichomes located on tepals evolved once independently within *Sisyrinchium*.

In Trimezieae, the ML optimization favours unequivocally that unicellular trichomes located on the inner tepals are ancestral and were lost three times independently in *Pseudotrimezia*.

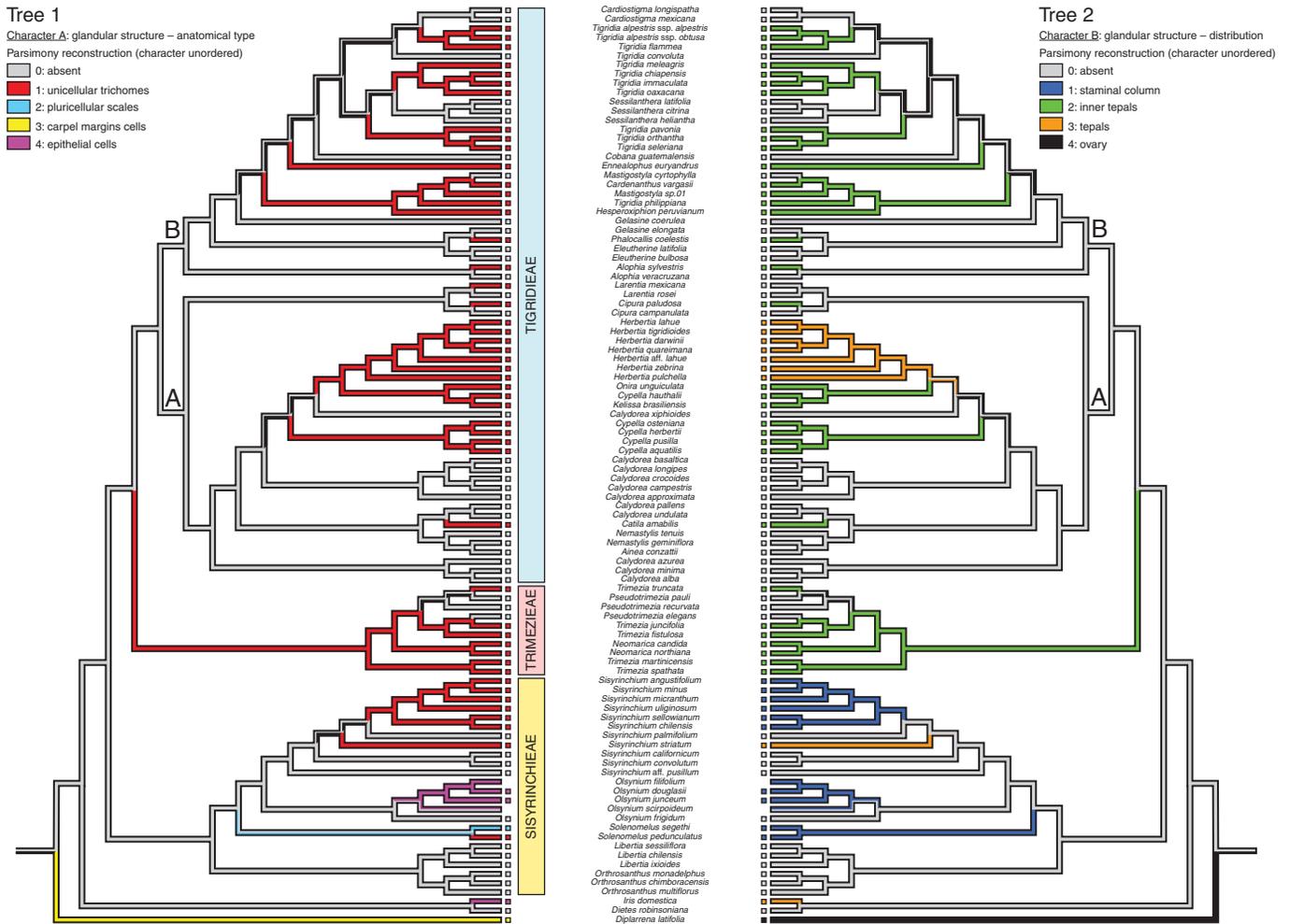


FIG. 4. Mirror trees showing the optimization of the anatomical type, character A (Tree 1), and the distribution within the flower, character C (Tree 2), of glandular structures on the ML best-scoring tree, with maximum parsimony. External nodes are coloured according to the character state observed, and internal nodes are coloured according to the ancestral state inferred. Multi-coloured branches show unresolved ancestral character states under parsimony analysis. The absence of a square in front of the external nodes corresponds to missing data. Major clades within Tigridieae are labelled A and B above the corresponding branches.

The MP optimization results in an ambiguous scenario with one to three reversions.

Because unicellular secretory trichomes are distributed mostly on the inner tepals among Tigridieae, except within *Herbertia* where they occur on both whorls of the perianth, the MP and ML optimizations for character C lead respectively to the same results as obtained for character A. The same pattern of transitions and reversions is observed and the only difference concerns the unusual distribution of trichomal elaiophores within *Herbertia*. The ML optimization suggests that the ancestor possessed trichomal elaiophores only on the inner tepals. Even though two ancestral states (absence or presence of glandular structures on the inner tepals) are equally parsimonious for character C with the MP optimization, the ancestral state for *Herbertia* and its sister clade is probably the presence of trichomal elaiophores because the results obtained for characters A and B with the MP and ML optimizations and for character C with the ML optimization favour this scenario (Figs 4 and 5 and S2–S4).

Detection of differential diversification rates

The values of the different parameters, including the rates of net diversification and the speciation/extinction ratios obtained from the best ML BISSE model for lineages in the absence (state 0) or presence (state 1) of glandular trichomes (character D) on the one hand, and of trichomal elaiophores (character E) on the other hand, are given in Table 2. Detailed comparisons of BISSE models for characters D and E are reported in Supplementary Data Tables S5 and S6, respectively, while results obtained with SymmeTREE are presented in Supplementary Data Table S7.

The results of the six whole-tree statistics computed in SymmeTREE using the ML best-scoring tree topology reveal the presence of at least one significant shift in diversification rates within our study group. Because the *P*-values tend to decrease from I_c to M_{Σ} and then increase from M_{Σ} to B_1 (*P*-values are not significant only for the I_c and B_1 statistical tests), shifts in diversification rate probably occur at an intermediate phylogenetic scale. This is supported by the statistical

TABLE 1. MP and ML optimization results (ancestral states and number of transitions and reversions observed within each tribe) for character A (anatomical type of glandular structures), B (secretory type of glandular structures) and C (distribution of glandular structures).

Taxon / Character A	Ancestral state		Transitions						Reversions	
	MP	ML	0 > 1		0 > 2		0 > 4		1 > 0	
			MP	ML	MP	ML	MP	ML	MP	ML
Sisyrrinchieae	0	0	2 to 3	3	1	1	1	1	0 to 1	0
Trimezieae	1	1	0 to 1	0	0	0	0	0	1 to 3	3
Tigridieae (clade A)	0	0 or 1	4 to 5	2 to 3	0	0	0	0	0 to 1	3 to 4
Tigridieae (clade B)	0	0 or 1	3 to 7	2 to 4	0	0	0	0	1 to 5	5 to 8

Character states: (0) absent, (1) unicellular trichomes, (2) pluricellular scales, (3) carpel margin cells, (4) epithelial cells.

Taxon / Character B	Ancestral state		Transitions								Reversions					
	MP	ML	0 > 1		0 > 2		0 > 3		0 > 5		1 > 2		2 > 0		2 > 1	
			MP	ML	MP	ML	MP	ML	MP	ML	MP	ML	MP	ML	MP	ML
Sisyrrinchieae	0	0	0	0	1 to 2	2	1	1	1	1	0	0	1 to 2	1	0	0
Trimezieae	0 or 2	2	0 to 1	0	0 to 4	0	0	0	0	0	0	0	0 to 3	3	0 to 1	1
Tigridieae (clade A)	0	0	0	0	3 to 4	3	0	0	0	0	0	0	0 to 1	2	0	0
Tigridieae (clade B)	0	0	2	2	4 to 5	4 to 5	0	0	0	0	1	1	1	1 to 2	1	1

Character states: (0) absent, (1) trichomal nectaries, (2) trichomal elaiophores, (3) pluricellular scales nectaries, (4) septal nectaries, (5) epithelial nectaries.

Taxon / Character C	Ancestral state		Transitions								Reversions	
	MP	ML	0 > 1		0 > 2		0 > 3		2 > 3		2 > 0	
			MP	ML	MP	ML	MP	ML	MP	ML	MP	ML
Sisyrrinchieae	0	0	3	3	0	0	1	1	0	0	0	0
Trimezieae	2	2	0	0	0 to 1	0	0	0	0	0	1 to 3	3
Tigridieae (clade A)	0	0 or 2	0	0	4 to 5	2 to 3	0 to 1	0	0 to 1	1	0 to 1	3 to 4
Tigridieae (clade B)	0	0 or 2	0	0	3 to 7	2 to 4	0	0	0	0	1 to 5	5 to 8

Character states: (0) absent, (1) staminal column, (2) inner tepals, (3) tepals, (4) ovary.

TABLE 2. Speciation rates (λ_0 and λ_1) in the absence (state 0) vs. presence (state 1) of glandular trichomes (character D) and trichomal elaiophores (character E) in the lineages, extinction rates (μ_0 and μ_1), and forward and reverse transition rates (q_{01} and q_{10}) obtained from the best ML BISSE model for each character; the rates of net diversification ($r = \lambda - \mu$) and the speciation/extinction ratios ($a = \lambda/\mu$) are reported on the right

Best model	Constraints	λ_0	λ_1	μ_0	μ_1	q_{01}	q_{10}	r_0	r_1	a_0	a_1
Character D											
D-3	$\mu_0 = \mu_1$	129.36	259.83	80.33	80.33	70.62	149.50	49.03	179.50	1.61	3.23
Character E											
E-5	$\lambda_0 = \lambda_1, \mu_0 = \mu_1$	215.25	215.25	123.28	123.28	126.40	208.21	92.0	92.0	1.75	1.75

tests performed on the MP strict consensus tree and the Bayesian majority rule consensus tree, of which the P -values, although being only marginally significant, vary following the same pattern. The Δ statistics implemented in SymmeTREE give statistical support ($P = 0.05$ for each tree) for a single shift of diversification rate located at the base of the clade including *Cobana guatemalensis* and the *Tigridia* complex (*Cardiostigma*, *Colima*, *Fosteria*, *Rigidella*, *Sessilanthera* and *Tigridia*).

Comparisons of BISSE models, from the fully relaxed model (model D-1) to models with one parameter equal for lineages where each character examined is present or absent (models D-2 to D-4), were not significant. Because constraining extinction rates to be equal (model D-3, $\mu_0 = \mu_1$) resulted in the smallest change in ln-likelihood, model D-3 was accepted as better than model D-1. Next, model D-3 was compared with nested models with two parameters equal (models D-5 and D-7), testing for equal speciation rates and transitions

rates, respectively. Both tests resulted in significant differences in ln-likelihood values, indicating that D-3 is the best model. When comparisons were started from the most constrained model towards models with two parameters equal for both types of lineage, only the comparison with model D-7 ($\mu_0 = \mu_1$ and $q_{01} = q_{10}$) was marginally significant. This model was selected as a better model than the fully constrained one and compared with nested models D-3 and D-4 with only one parameter equal. Only model D-3 with equal extinction rates ($\mu_0 = \mu_1$) resulted in a significant improvement in likelihood, and was selected as the best model to evaluate the six parameters among lineages in the absence or presence of glandular trichomes. We found a diversification rate 3.66 times higher within lineages with glandular trichomes than within the other lineages.

Similarly, a range of constrained BISE models were explored to test for differential diversification of lineages in the absence (state 0) or presence (state 1) of trichomal elaiophores. The best model was the one in which speciation and extinction rates were constrained to be equal among the different lineages.

DISCUSSION

The results presented in this study are fully concordant with the current classification of the tribes Sisyrinchieae, Trimezieae and Tigridaeae proposed by Goldblatt *et al.* (2008). The total-evidence approach supports Sisyrinchieae as a monophyletic group and its sister-group relationship with Trimezieae and Tigridaeae. These two tribes are also clearly monophyletic, but within Tigridaeae, the circumscription of the subtribes Cipurinae and Tigridiinae as monophyletic groups is not supported.

Phylogenetic relationships within the American tribes of Iridoideae

Sisyrinchieae. The phylogenetic relationships among genera of the tribe Sisyrinchieae resulting from the latest comprehensive phylogeny published (Chauveau *et al.*, 2011) are fully confirmed by the present study, using different cpDNA regions. Our results also confirm, with a larger sampling, the sister-group relationship suggested by Goldblatt *et al.* (2008) between the *Libertia*–*Orthrosanthus* lineage and the clade including the three exclusively American genera. According to the divergence times estimation proposed by Goldblatt *et al.* (2008), the present phylogeny suggests that the two Australasian and American genera *Libertia* and *Orthrosanthus* diverged from one to another before the separation of Antarctica from South America and Australasia.

Trimezieae. Within this tribe, our results question the monophyly of *Pseudotrimezia* (endemic to Minas Gerais in Central Brazil) and *Trimezia*. These two genera differ by their leaf shape, number of bracts on the floral stem, tepal shape and number of style branches, and *Trimezia* has been divided into two sections on the basis of leaf shape (Chukr, 2001; Chukr and Giuliotti, 2008): section *Trimezia* includes all species with unifacial leaves whereas section *Juncella* includes all species with terete leaves. The centre of diversity

of *Trimezia* is located in Central Brazil, but several species of section *Trimezia* occur from Central America to Argentina. Based on leaf anatomical traits, Rudall (1993) suggested a close evolutionary relationship between the genus *Pseudotrimezia* and the section *Juncella* of *Trimezia*.

The phylogeny (Fig. 2) shows that the *Trimezia* species with unifacial leaves (*T. martinicensis* and *T. spathata*) are sister group to a clade including *Neomarica*, *Pseudotrimezia* and all *Trimezia* species with terete leaves (*T. fistulosa*, *T. juncifolia* and *T. truncata*). These results suggest that the generic delimitations of *Trimezia* and *Pseudotrimezia* should be revised. The clade encompassing *Pseudotrimezia*, *Trimezia fistulosa*, *T. juncifolia* and *T. truncata* could be elevated to the generic level, as all these species share highly similar vegetative features. The minor differences observed in floral traits, including glandular structures, are probably related to shifts in pollination strategies. Further phylogenetic and morphological investigations are clearly needed here.

Tigridaeae. The generic delimitations in this tribe have already been widely debated, especially concerning the genera that were included in the *Tigridia* complex (Rodríguez and Sytsma, 2006; Goldblatt and Manning, 2008; Goldblatt *et al.*, 2008), but also concerning several monospecific genera closely related to *Calydorea* (Goldblatt and Henrich, 1991; Ravenna, 2001) and *Cypella* (Goldblatt and Manning, 2008; Ravenna, 2009). The taxonomic treatment of *Tigridia* proposed by Goldblatt and Manning (2008) expanded the circumscription of the genus and encompassed a range of species displaying adaptations to various pollination strategies. Wide variation in floral morphology in relation to pollination systems is not uncommon in the African genera of Iridaceae. In the subfamily Crocoideae, *Babiana* Ker Gawl., *Gladiolus* Tourn. ex L. and *Tritoniopsis* L. Bolus are striking examples of genera of Iridaceae that have undergone explosive adaptive radiation, leading to profound floral diversification (Goldblatt and Manning, 2006, 2008). Within Iridoideae, *Moraea* Mill. is another example of a genus encompassing small species clusters with derived floral morphology (Goldblatt and Bernhardt, 1999). Each of these genera from Crocoideae and Iridoideae includes species showing floral morphological variations associated with various pollination systems (Goldblatt and De Vos, 1989; Goldblatt, 1990; Goldblatt *et al.*, 2008).

The phylogenetic relationships observed among genera belonging to clade A, which includes a significant sample of species, suggest that several genera should be examined critically for alternative generic delimitations. The close relationship of *Ainea konzattii* with species of *Nemastylis* is quite unexpected, as it was nested in the *Tigridia* complex in the phylogeny of Goldblatt *et al.* (2008). The inclusion of *Ainea* within *Tigridia* was supported by their identical basic chromosome number ($x = 14$) and bisulcate pollen grains (Goldblatt and Manning, 2008). However, *Ainea konzattii* also shares morphological features with *Cardiostigma* and *Nemastylis*, supporting the phylogenetic relationships observed in the present work (Ravenna, 1979). Note that *Cardiostigma longispatha*, which appears nested with strong support within the *Tigridia* complex in our phylogeny, has monosulcate pollen grains, suggesting that pollen aperture type has weak

systematic significance in Tigridaeae (Goldblatt and Manning, 2008). Further investigations will be necessary to clarify the phylogenetic relationships between *Ainea konzattii* and the rest of Tigridaeae. The clade formed by *Nemastylis* and *Ainea* is sister to a monophyletic group including *Calydorea undulata*, *Calydorea pallens* and *Catila amabilis*. Following a narrow generic concept, Ravenna (1983, 2001) referred *Calydorea pallens* and *Catila amabilis* to the monotypic genera *Tamia* and *Catila*, respectively, while other systematic studies reduced them to *Calydorea* (Goldblatt and Henrich, 1991; Goldblatt and Manning, 2008). The present phylogeny shows that *Calydorea* is clearly not monophyletic, and that the type species *C. xiphioides* is totally unrelated to the rest of *Calydorea*. Similarly, *Cypella* is split into two clades, with *C. hauthalii* related to the monospecific genera *Kelissa* and *Onira* and to *Herbertia*. The genera *Herbertia*, *Cypella*, *Kelissa* and *Onira* share similar features in vegetative habit, and the limits among these genera are mainly based on trivial perigonal, staminal or stylar differences. Morphological observations showed that there is a trend in the genus *Cypella* towards reduction of the inner tepals, and in few species towards partial or complete fusion of the filaments, two morphological features that are characteristic of *Herbertia* (Goldblatt and Manning, 2008). The absence of clear morphological differences among *Calydorea*, *Cypella*, *Herbertia* and *Nemastylis* suggests that the generic circumscriptions of these genera should be revised to maintain the principle of monophyly at the genus level. Further investigations are required, especially concerning the pollination biology of these species, to assess whether the floral variations observed are related to pollination adaptations.

Within clade B of Tigridaeae, the close relationship between *Phalocallis coelestis*, long included in *Cypella*, and *Gelasine elongata* suggests that *Phalocallis* should be regarded as a separate genus from *Cypella*. *Gelasine coerulea* appears unrelated to *G. elongata*, suggesting that the generic circumscription of this poorly understood genus should be revised. The phylogenetic relationships of *Cardenanthus* and *Mastigostyla* are evidenced for the first time here and support the reduction of *Cardenanthus* within *Mastigostyla* as it was proposed by Goldblatt and Manning (2008). Both are closely related to the South American *Tigridia philippiana*. In spite of floral similarities between this latter species and the rest of *Tigridia*, our results suggest that *T. philippiana* should not be longer retained in *Tigridia*.

The tree topology presented here is consistent with the phylogenetic analyses of Rodríguez and Sytsma (2006) and Goldblatt et al. (2008) concerning the *Tigridia* lineages, the only exception being *Ainea konzattii*, which falls into clade A in the present phylogeny. Based on a larger sampling and several additional cpDNA regions, our results confirm that the genus *Tigridia sensu stricto* is not monophyletic. The reduction of *Cardiostigma*, *Colima*, *Fosteria*, *Rigidella* and *Sessilanthera* within *Tigridia* proposed by Goldblatt and Manning (2008) is fully supported.

Evolution of glandular structures and trichomes

More than 90 % of the glandular structures identified among the three tribes studied are unicellular glandular trichomes,

mostly of the elaiophores type, which represent more than 77 % of the glandular trichomal areas identified. These results illustrate the overwhelming development of oil secretion in the New World Iridaceae, compared with the Old World where only one species of Crocoideae is known to secrete floral oils. Oil-secreting structures in numerous species of Sisyrinchieae, Trimezieae and Tigridaeae are borne on either the perianth or the androecium. In Sisyrinchieae, trichomal elaiophores are present only within *Sisyrinchium* and they are mostly distributed on the staminal column, a unique distribution pattern in the family, although they can also be located on the basal part of the adaxial side of tepals in a few species (Chauveau et al., 2011). Trichomal elaiophores distributed on the staminal column are usually fully exposed, except in *S. micranthum* and its closely allied species where they are concealed by the base of the perianth (Cocucci and Vogel, 2001; Chauveau et al., 2011). Among Trimezieae and Tigridaeae, trichomal elaiophores are exclusively located on the mid or lower part of the adaxial side of the inner tepals, and are usually concealed by folds or pockets of the tepal surface. The only exception concerns *Herbertia* (Tigridaeae) where small areas of oil-producing trichomes occur also on the adaxial side of the outer tepals. Within both tribes, the glandular areas located on the inner tepals may consist also of trichomal nectaries, found within *Neomarica* (Trimezieae) and the *Tigridia* complex (Tigridaeae). Character optimizations suggest that, in several cases, trichomal nectaries and trichomal elaiophores might share a common evolutionary history. This would be expected, as it seems unlikely that nectar and oil secretions should be produced within similar glandular structures, unless they share a common developmental and evolutionary origin.

Our results show that glandular structures evolved repeatedly among the three tribes studied and that trichomal elaiophores evolved independently within each tribe. Within Sisyrinchieae, trichomal elaiophores evolved twice independently within the genus *Sisyrinchium*, followed by one reversion, as already suggested by Chauveau et al. (2011). Trichomal elaiophores probably evolved only once at the base of the tribe Trimezieae, followed by one transition towards trichomal nectaries (in *Neomarica*) and several reversions (in *Pseudotrimezia*). Most transitions from the absence of glandular structures towards trichomal elaiophores and trichomal nectaries occur within Tigridaeae, with respectively at least seven and two transitions. Furthermore, trichomal nectaries and elaiophores evolved twice independently from one to another in this tribe. These figures reveal a high degree of lability in the evolutionary pattern of glandular trichomes among the New World tribes of Iridoideae, and more specifically of trichomal elaiophores. Among the 11 families of Angiosperms where elaiophores evolved, this pattern of evolution is highly unusual, except perhaps for Orchidaceae, in which multiple transitions towards the presence of elaiophores probably occurred (Renner and Schaefer, 2010). Repeated losses of trichomal elaiophores were recorded in *Calceolaria*, although after a single transition towards the presence of elaiophores. The frequent loss of oil-secreting structures in this genus could be related to the dispersion of the genus in areas where oil-collecting bees are scarce (Cosacov et al., 2009). The unusual lability in presence/absence of

glandular trichomes (either trichomal elaiophores or trichomal nectaries) might reflect frequent pollinator shifts among the three American tribes of Iridoideae. It also suggests that the most frequent pollination systems are oil-bee pollination and a pollination system where pollen is the only reward available for pollinators. Among the Iridaceae of sub-Saharan Africa, pollination shifts are assumed to be one of the driving forces for speciation by promoting reproductive isolation in peripheral and ecologically distinct populations (Goldblatt and Manning, 2006). In the same way, the repeated transitions observed towards the presence of glandular trichomes may indicate that specialized interactions promoted speciation through local evolutions and favoured the evolutionary success of the Iridoideae on the American continent, a hypothesis that requires future testing.

Detection of differential diversification rates

The difference in speciation rates found between lineages with and without glandular trichomes (3.66) does not necessarily indicate that the occurrence of these structures of secretion directly affects the speciation rates. It is possible that other floral characters having evolved in a coordinated manner with these glandular structures are causally responsible. The absence of variation in speciation and extinction rates between lineages with and without trichomal elaiophores suggests that oil-flowers alone cannot account for lineage diversification at the taxonomic level of the subfamily Iridoideae on the American continent. However, the results obtained by the topological method indicate that shifts in diversification rates occurred probably at an intermediate phylogenetic level among the three tribes studied and the only shift localized was at the base of a clade that holds numerous shifts of pollination systems, which is the reason why numerous small genera now reduced to synonymies in *Tigridia* were described. This body of observations leads us to assume that repeated shifts between pollination systems may have played a role in the diversification of the Iridoideae on the American continent and that glandular trichomes are among the characters involved in these processes of adaptation, a hypothesis that requires further investigations to be tested.

Conclusions

In this study, molecular and morphological evidence was used to clarify phylogenetic relationships among the American tribes of Iridoideae and provide insight into the evolutionary history of glandular structures, and more specifically of oil-producing trichomes in these tribes. Our results confirm the monophyly of each of the three New World tribes recognized in Iridoideae and allow us to identify for the first time two main clades within the tribe Tigridieae. We suggest that further taxonomic studies should be conducted to reflect the phylogenetic relationships observed in Tigridieae and Trimezieae, especially concerning the genera *Trimezia* and *Pseudotrimezia* within the tribe Trimezieae, and *Calydorea*, *Cypella* and *Herbertia* within clade A of the tribe Tigridieae. Glandular structures evolved multiple times in the lineages studied, indicating that repeated shifts in pollination systems probably occurred among the three tribes. Two

main categories of reward to pollinators were identified, namely pollen and oils secreted from trichomal elaiophores in the subfamily, and the optimization of various floral characters showed that transitions and reversions occurred mainly between these two categories. Floral nectaries were found in less than 15 % of the species studied. Trichomal elaiophores and trichomal nectaries evolved three times from one to another, suggesting that these two glandular structures might have a common developmental and evolutionary history. Analyses of differential diversification rates suggest that glandular trichomes may have played a role in the diversification of the clade including the tribes Sisyrinchieae, Trimezieae and Tigridieae, increasing significantly the speciation rates within the lineages concerned. However, the analyses show that trichomal elaiophores, which are the most common secretory type of trichomes, do not affect the diversification rates. Further investigations are therefore needed to assess whether the repeated evolutionary shifts observed for this character are related to shifts in pollination systems and to understand how far the occurrence of glandular trichomes was involved in these adaptive processes.

This work provides a global framework for future studies on the evolution of relationships between species of the American Iridoideae and their pollinators. Further phylogenetic studies including nuclear molecular markers and floral morphological traits are needed to improve the resolution at the tribal level, especially in the Trimezieae and the two clades identified within the Tigridieae, and to further clarify the circumscription of genera within these clades. Information concerning the pollination biology of most of the species included in the American tribes of Iridoideae is scarce and often nothing is known about the range of pollinators involved in the reproduction process of these species. The secretion of floral oils related to specialized oil-bee pollination, prevalent in Neotropical and Mexican Iridoideae, has opened pollination niches for Iridaceae outside of Africa. This study suggests that oil flowers may be one of the driving forces involved in the diversification of Iridaceae on the American continent, especially in the Neotropical area. The lability observed in the presence/absence of glandular trichomes may indicate that frequent evolutionary transformations from one pollination system to another have occurred during the course of evolution, reinforcing the capacity for adaptation to different environmental conditions.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxford-journals.org and consist of the following. Table S1: primers used for amplifying and sequencing. Table S2: PCR profiles for DNA amplification. Table S3: data and models used in analyses. Table S4: type and distribution of glandular structures among the genera and species studied. Table S5: comparison of full and constrained ML BISSE analyses in absence vs. presence of glandular trichomes (character D) in the lineages. Table S6: comparison of full and constrained ML BISSE analyses in absence vs. presence of trichomal elaiophores (character E) in the lineages. Table S7: detection of diversification rate variations among the tribes Sisyrinchieae, Trimezieae and Tigridieae using the SymmeTREE software. Figure S1:

maximum-likelihood best-scoring topology obtained from the combined molecular dataset showing branch lengths. Figure S2: trees showing the ML optimization of the anatomical type (character A) of glandular structures on the ML best-scoring tree. Figure S3: trees showing the ML optimization of the secretory type (character B) of glandular structures on the ML best-scoring tree. Figure S4: trees showing the ML optimization of the distribution (character C) of glandular structures within the flower on the ML best-scoring tree.

ACKNOWLEDGEMENTS

We are grateful to J. Lovo (University of São Paulo, Brazil) who provided the plant material for the genus *Pseudotrimezia* and most of the *Trimezia* species. We are also indebted to R. and E. Heaton (NCCPG Sisyrrinchium National Collection, Newton Abbot, UK), S. Aubert and R. Douzet (Jardin Botanique de la Station Alpine du Lautaret, France) and F. Pautz (Jardin Botanique de Lyon, France) for generously providing seeds, exsiccates or leaf material for DNA extractions, and J. F. Bertrand for his investment in maintaining the living collection of Iridoideae developed in our University. This work received funding from the French/Brazilian CAPES/COFECUB cooperation project Sv550/07 and from the IFR87 'La plante et son environnement'.

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- vouchers [new voucher specimens are deposited in the following herbaria: Universidade Federal do Rio Grande do Sul (ICN), Université Paris-Sud (UPS)]. Two samples are only cultivated in the R. & E. Heaton (NCCPG) *Sisyrinchium* collection without voucher deposited), and GenBank accession number (*rps4* + spacer *rps4-trnS*, *rps16* intron, *matK* + *matK-5'trnK* intron, *rbcL*, *trnL-F* region). Names in parentheses are names that are not accepted by the world Checklist of Monocots (Barker, 2004). na, no sequence available for the accession.
- Data are presented in the following format:
Species; geographical origin; **Voucher** (Herbarium); GenBank accession numbers: (*rps4* + spacer *rps4-trnS*, *rps16* intron, *matK* + *matK-5'trnK* intron, *rbcL*, *trnL-F* region).
- (*Ainea conzattii* (R.C.Foster) Ravenna) – SP572; Mexico: Oaxaca (RBGK 21846); Rodríguez & Ortiz-Catedral 2948 (IBUG); JQ670269, AM940171, JQ670431, JQ670511, JQ670588. *Alophia silvestris* (Loes.) Goldblatt – SP587; Mexico: State of Mexico (RBGK 21847); Rodríguez & Ortiz-Catedral 3179 (IBUG); JQ670270, na, JQ670432, JQ670512-, JQ670589. *Alophia veracruzana* Goldblatt & T.M.Howard – SP571; Mexico: Veracruz (RBGK: I-199); Goldblatt & Howard 9070 (MO); JQ670271, JQ670357, JQ670433, AJ309678, AJ409592. *Iris domestica* (L.) Goldblatt and Mabb. – G (2008); Z68235, AJ578794, AJ579961, AJ309694, GQ351358. *Calydorea alba* Roitman & A.Castillo – SP522; Brazil: Rio Grande do Sul (PABG); Eggers & Souza-Chies 519 (ICN); JQ670272, JQ670358, JQ670434, JQ670513, JQ670590. *Calydorea approximata* R.C.Foster – SP516; Brazil: Paraná (PABG); Eggers & Souza-Chies 249 (ICN); JQ670273, JQ670359, JQ670435, JQ670514, JQ670591. *Calydorea azurea* Klatt – SP577; Argentina: Entre Ríos (RBGK 30880); Roitman T-14 (no data); JQ670274, JQ670360, JQ670436, JQ670515, JQ670592. *Calydorea basaltica* Ravenna – SP890; Brazil: Paraná; Eggers & Souza-Chies 606 (ICN); JQ670275, JQ670361, JQ670437, JQ670516, JQ670593. *Calydorea campestris* (Klatt) Baker – SP526; Brazil: Paraná (PABG); Eggers & Souza-Chies 330 (ICN); JQ670276, JQ670362, JQ670438, JQ670517, JQ670594. *Calydorea crocoides* Ravenna – SP515; Brazil: Rio Grande do Sul (PABG); Eggers & Souza-Chies 218 (ICN); JQ670277, JQ670363, JQ670439, JQ670518, JQ670595. *Calydorea longipes* Ravenna – SP588; Brazil: Paraná; Eggers & Souza-Chies 390 (ICN); JQ670278, JQ670364, JQ670440, JQ670519, JQ670596. *Calydorea minima* Roitman & J.A.Castillo – SP578; Argentina: Corrientes (RBGK 30875); Roitman T-8 (no data); na, JQ670365, JQ670441, JQ670520, JQ670597. *Calydorea pallens* Griseb. – SP547; Argentina: Córdoba (RBGK I-200); Goldblatt 9579 (MO); JQ670279-, JQ670366, JQ670442, AJ309682, AJ409596. *Calydorea undulata* Ravenna – SP576; Argentina (RBGK 30878); Roitman T-12 (no data); na, JQ670367, JQ670443, JQ670521, JQ670598. *Calydorea xiphioides* (Poepp.) Espinosa – SP875; Chile: Region VII (UPSBG); Chauveau H11001 (ICN); JQ670280, JQ670368, JQ670444, JQ670522, JQ670599-. (*Cardenanthus vargasii* R.C.Foster) – 025-2011; Peru: Cuzco; Eggers et al. 025-2011 (ICN, CUZ); JQ670281, JQ670369, JQ670445, JQ670523, JQ670600. (*Cardiostigma longispatum* (Herb.) Baker) –

APPENDIX

Taxa. Identification number or literature information for all species of Iridaceae included in this study. G (2008): Goldblatt et al. (2008), geographical origin [specimens cultivated in the botanical garden of the Université Paris-Sud (UPSBG), in the botanical garden of Porto Alegre (PABG), or elsewhere are noted after the geographical origin information; the reference numbers for the specimens obtained from the RBG Kew DNA Bank (RBGK) are also indicated],

SP548; Mexico: Michoacán (RBGK 22039); *Rodríguez & Ortiz-Catedral 3050* (IBUG); JQ670282, JQ670370, JQ670446-, JQ670524, JQ670601. (*Cardiostigma mexicanum* (R.C.Foster) Ravenna) – SP546; Mexico (RBGK 21848); *Rodríguez & Ortiz-Catedral 2993* (IBUG); JQ670283, AM940172, AM940207, AM940184, JQ670602. (*Catila amabilis* Ravenna) – SP503; Brazil: Rio Grande do Sul (PABG); *Eggers 697* (ICN); JQ670284, JQ670371, JQ670447, JQ670525, JQ670603. *Cipura campanulata* Ravenna – SP569; Nicaragua: Estelí (RBGK I-201); *Henrich & Stevens 143* (MO); JQ670285-, AJ578772, AJ579939, AJ309681, AJ409595. *Cipura paludosa* Aubl. – SP570; Bolivia: Santa Cruz (RBGK 37119); Huaylla 3231 (LPB); na, na, JQ670448, JQ670526, JQ670604. *Cobana guatemalensis* (Standl.) Ravenna – SP568; Guatemala: Guatemala (RBGK 21849); *Rodríguez et al. 2831* (IBUG, WIS); JQ670286, JQ670372, AM940208, JQ670527, JQ670605. *Cypella aquatilis* Ravenna – SP565; Argentina (RBGK I-202); *Castillo s.n.* (MO); JQ670287, AJ578775, JQ670449, AJ309683, AJ409597. *Cypella hauthalii* (Kuntze) R.C.Foster – SP524; Brazil: Rio Grande do Sul (PABG); *Eggers & Souza-Chies 541* (ICN); na, JQ670373, JQ670450, JQ670528, JQ670606. *Cypella herbertii* (Lindl.) Herb. – SP504; Brazil: Rio Grande do Sul (PABG); *Eggers 698* (ICN); JQ670288, JQ670374, JQ670451, JQ670529, JQ670607-. *Cypella osteniana* Beauverd – SP585; Uruguay: Lavalleja (RBGK 30882); RBGK living collection 1987–209); JQ670289, JQ670375, JQ670452, JQ670530, JQ670608. *Cypella pusilla* (Link & Otto) Benth. & Hook.f. – SP506; Brazil: Rio Grande do Sul (PABG); *Eggers 700* (ICN); JQ670290, JQ670376, JQ670453, JQ670531, JQ670609. *Dietes robinsoniana* (F.Muell.) Klatt – SP564; Lord Howe Island (RBGK I-8); *Pickard 3377* (MO); JQ670291, JQ670377-, JQ670454, AJ307080, AJ307237. *Diplarrena latifolia* Benth. – G (2008); Australia: Tasmania; *Chase I-220* (K); AJ409036, AJ578779, AJ579946, AJ309686, AJ409600. *Eleutherine bulbosa* (Mill.) Urb. – SP575; Guatemala: Alta Verapaz (RBGK 22041); *Rodríguez 2830* (IBUG, WIS); JQ670292, JQ670378, JQ670455, JQ670532, JQ670610. *Eleutherine latifolia* (Standl. & L.O.Williams) Ravenna – G (2008); Mexico: San Luis Potosí; *Goldblatt 9072* (MO); AJ409031, AJ578782, AJ579949, Z77283, AJ409591. *Ennealophus euryandrus* (Griseb.) Ravenna – SP563; Bolivia: Santa Cruz (RBGK 30885); *Wood 20309* (no data); JQ670293, AJ578783, AJ579950, JQ670533, AJ409598. *Gelasine coerulea* (Vell.) Ravenna -SP519; Brazil: Paraná (PABG); *Eggers & Souza-Chies 335* (ICN); JQ670294, JQ670379, JQ670456, JQ670534, JQ670611. *Gelasine elongata* (Graham) Ravenna – SP560; Brazil (RBGK I-48); *Goldblatt 5925* (MO); JQ670295, AJ578787, JQ670457, AJ309674, AJ409587. *Herbertia darwinii* Roitman & J.A.Castillo – SP520; Brazil: Rio Grande do Sul (PABG); *Eggers & Souza-Chies 502* (ICN); JQ670296, JQ670380, JQ670458, JQ670535, JQ670612. *Herbertia lahue* (Molina) Goldblatt – SP001; Brazil: Rio Grande do Sul; *Eggers & Souza-Chies 211* (ICN); JQ670297, JQ670381, JQ670459, JQ670536, JQ670613. *Herbertia* aff. *lahue* (Molina) Goldblatt – SP518; Brazil: Rio Grande do Sul (PABG); *Eggers 704* (ICN); JQ670298, JQ670382, JQ670460, JQ670537, JQ670614. *Herbertia pulchella* Sweet – SP002; Brazil: Rio Grande do Sul; *Eggers & Souza-Chies 260* (ICN); JQ670299, JQ670383, JQ670461, JQ670538-, JQ670615. *Herbertia quareimana* Ravenna – SP505; Brazil: Rio Grande do Sul (PABG); *Eggers 699* (ICN); JQ670300, JQ670384, JQ670462, JQ670539, JQ670616. *Herbertia tigridioides* (Hicken) Goldblatt – SP586; Argentina (RBGK 30879); *Roitman T-13* (no data); na, JQ670385, JQ670463, JQ670540, JQ670617. *Herbertia zebrina* Deble – SP003; Brazil: Rio Grande do Sul; *Eggers & Souza-Chies 179* (ICN); JQ670301, JQ670386, JQ670464, JQ670541, JQ670618. *Hesperoxiphion peruvianum* (Baker) Baker – SP527; Peru (UPSBG); *Chauveau H11002* (ICN); JQ670302, JQ670387, JQ670465, JQ670542, JQ670619. *Kelissa brasiliensis* (Baker) Ravenna – SP507; Brazil: Rio Grande do Sul (PABG); *Eggers 701* (ICN); JQ670303, JQ670388, JQ670466, JQ670543, JQ670620. (*Larentia mexicana* (C.V.Morton & R.C.Foster) Goldblatt) – SP566; Mexico: Jalisco (RBGK 21851); *Ortiz-Catedral 212* (IBUG); JQ670304, AM940175, AM94021, AM940187, JQ670621. *Larentia rosei* (R.C.Foster) Ravenna – SP567; Mexico: Jalisco (RBGK 22040); *Rodríguez & Martinelli 2855* (IBUG, WIS); JQ670305, AM940174, AM940209, AM940186, JQ670622. *Libertia chilensis* (Molina) Guncel – SP876; Chile: Region VII (UPSBG); *Chauveau H11003* (ICN); JQ670306, JQ670389, JQ670467, JQ670544, JQ670623. *Libertia ixioides* (G.Forst.) Spreng. – SP535; New Zealand: Stewart Island (RBGK I-218); *Chase I-218* (K); JQ670307, AJ578801, AJ579968, AJ309687, AJ409601. *Libertia sessiliflora* (Poepp.) Skotts. – SP877; Chile: Region V (UPSBG); *Chauveau H11004* (ICN); JQ670308, JQ670390, JQ670468, JQ670545, JQ670624. *Mastigostyla cyrtophylla* I.M.Johnst. – SP880; Chile: Region I (UPSBG); *Chauveau H11005* (ICN); JQ670309, JQ670391, JQ670469, JQ670546, JQ670625. *Mastigostyla* I.M.Johnst. sp.01 – 046-2011; Peru: Cuzco; *Eggers et al. 046-2011* (ICN, CUZ); JQ670310, JQ670392, JQ670470, JQ670547, JQ670626. *Nemastylis geminiflora* Nutt. – SP557; USA (RBGK 22043); *Went s.n.* (Source: A. Rodríguez); JQ670311, JQ670393, JQ670471, JQ670548, JQ670627. *Nemastylis tenuis* (Herb.) S.Watson – SP559; Mexico: Jalisco (RBGK 22042); *Rodríguez 2636* (IBUG, WIS); JQ670312, AM94017, AM940213, AM940190, AM940165. *Neomarica candida* (Hassl.) Sprague – SP500; Brazil (PABG); *Eggers 694* (ICN); JQ670313, JQ670394, JQ670472, JQ670549, JQ670628. *Neomarica northiana* (Schneev.) Sprague – G (2008); Paraguay: Amambay; *Solomon 6950* (MO); na, AJ578805, AJ579972, AJ309679, AJ409593. *Olsynium douglasii* (A.Dietr.) E.P.Bicknell – SP007; Canada: British Columbia (NCCPG); *R. & E. Heaton OLS100-02* (living collection); JQ670314, JQ670395, JQ670473, JQ670550, JQ670629. *Olsynium filifolium* (Gaudich.) Goldblatt – SP549; Falkland Islands; *Chase I-243* (K); JQ670315, AJ578807, AJ579974, AJ309688, AJ409602. (*Olsynium frigidum* (Poepp.) Goldblatt) – SP546; Chile: Region VII (UPSBG); *Chauveau H11006* (ICN); JQ670316, JQ670396, JQ670474, JQ670551, JQ670630. *Olsynium junceum* (E.Mey. ex C.Presl) Goldblatt – SP837; Argentina: Mendoza; *Chauveau & Aubert H09050* (ICN); JQ670317, JQ670397, JQ670475, JQ670552, JQ670631. *Olsynium scirpoideum* (Poepp.) Goldblatt – SP009; Chile: Region Metropolitana (NCCPG); *R. & E. Heaton OLS104-17* (living collection); JQ670318, JQ670398, JQ670476, JQ670553, JQ670632. *Onira unguiculata* (Baker) Ravenna – SP517;

- Brazil: Rio Grande do Sul (PABG); *Eggers & Souza-Chies* 273 (ICN); JQ670319, JQ670399, JQ670477, JQ670554, JQ670633. *Orthrosanthus chimboracensis* (Kunth) Baker – SP532; Mexico: Oaxaca; *Chase I-231* (K); AJ409039, AJ578809, AJ579976, JQ670555, AJ409605. *Orthrosanthus monadelphus* Ravenna (SP174); Mexico: Oaxaca (UPSBG); *Chauveau & Pautz H09049* (UPS); JQ670320, JQ670400, JQ670478, JQ670556, JQ670634. *Orthrosanthus multiflorus* Sweet – SP531; Australia (UPSBG); *Chauveau H11007* (ICN); JQ670321, JQ670401, JQ670479, JQ670557, JQ670635. *Phalocallis coelestis* (Lehm.) Ravenna – SP510; Brazil: Rio Grande do Sul (PABG); *Eggers* 703 (ICN); JQ670322, JQ670402, JQ670480, JQ670558, JQ670636. *Pseudotrimezia elegans* Ravenna – SP538; Brazil: Minas Gerais; *Lovo 144* (SPF); JQ670323, JQ670403, JQ670481, JQ670559, JQ670637. (*Pseudotrimezia pauli* Chukr) – SP539; Brazil: Minas Gerais; *Lovo 152* (SPF); JQ670324, JQ670404, JQ670482, JQ670560, JQ670638. *Pseudotrimezia recurvata* Ravenna – SP540; Brazil: Minas Gerais; *Lovo 175* (SPF); JQ670325, JQ670405, JQ670483, JQ670561, JQ670639. *Sessilanthera citrina* Cruden – SP550; Mexico: Guerrero (RBGK 22044); *Rodríguez* 2885 (IBUG, WIS); JQ670326, JQ670406, JQ670484, JQ670562, JQ670640. *Sessilanthera heliantha* (Ravenna) Cruden – SP579; Mexico: Guerrero (RBGK 22045); *Rodríguez* 2892 (IBUG); JQ670327, JQ670407, JQ670485, JQ670563, JQ670641. *Sessilanthera latifolia* (Weath.) Molseed & Cruden – SP582; Mexico: Guerrero (RBGK 22046); *Rodríguez* 2884 (IBUG); na, JQ670408, JQ670486, JQ670564, JQ670642. *Sisyrinchium angustifolium* Mill. – SP014; USA (UPSBG); *Chauveau H09002* (ICN); JQ670328, JQ670409, JQ670487, JQ670565, JQ670643. *Sisyrinchium californicum* (Ker Gawl.) Dryand.-SP022; USA (UPSBG); *Chauveau H09006* (ICN); JQ670330, JQ670411, JQ670489, JQ670567, JQ670645. *Sisyrinchium chilense* Hook. – SP238; Peru: Apurimac (UPSBG); *Chauveau et al. H09054* (ICN); JQ670331, JQ670412, JQ670490, JQ670568, JQ670646. *Sisyrinchium convolutum* Nocca – SP108; Mexico: Mexico (UPSBG); *Chauveau et al. H09019* (ICN); JQ670332, JQ670413, JQ670491, JQ670569-, JQ670647. *Sisyrinchium micranthum* Cav. – SP839; Uruguay: Colonia; *Chauveau & Aubert H09052* (ICN); JQ670333, JQ670414, JQ670492, JQ670570, JQ670648. *Sisyrinchium minus* Engelm. & A.Gray – SP502; Brazil (PABG); *Eggers* 696 (ICN); JQ670334, JQ670415, JQ670493, JQ670571, JQ670649. *Sisyrinchium palmifolium* L. subsp. *palmifolium* – SP175; unknown origin, cultivated in UPS Bot. Gard.; *Chauveau H09020* (ICN); JQ670335, JQ670416, JQ670494, JQ670572, JQ670650. *Sisyrinchium* aff. *pusillum* Kunth – SP237; Peru: Apurimac (UPSBG); *Chauveau & Heaton H09052* (UPS); JQ670329, JQ670410, JQ670488, JQ670566, JQ670644. *Sisyrinchium sellowianum* Klatt – SP091; Brazil: Santa Catarina; *Eggers & Souza-Chies* 253 (ICN); JQ670336, JQ670417, JQ670495, JQ670573, JQ670651. *Sisyrinchium striatum* Sm. – SP844; Chile: Región Metropolitana (UPSBG); *Chauveau H11008* (ICN); JQ670337, JQ670418, JQ670496, JQ670574, JQ670652. *Sisyrinchium uliginosum* Ravenna – SP154; Brazil: Paraná; *Eggers & Souza-Chies* 393 (ICN); JQ670338, JQ670419, JQ670497, JQ670575, JQ670653. *Solenomelus pedunculatus* (Gillies ex Hook.) Hoch. – SP221; Chile: Region V (UPSBG); *Chauveau H09044* (ICN); JQ670339-, JQ670420, JQ670498, JQ670576, JQ670654. *Solenomelus segethi* (Phil.) Kuntze (SP005); Argentina: Neuquen; *Chauveau & Aubert H09001* (ICN); JQ670340, JQ670421, JQ670499, JQ670577, JQ670655. *Tigridia alpestris* Molseed subsp. *alpestris* – SP553; Mexico: Hidalgo (RBGK 22047); *Rodríguez* 2768 (IBUG, WIS); JQ670341, AM940179, AM940214, AM940191, JQ670656. *Tigridia alpestris* subsp. *obtusa* Molseed – SP554; Mexico: Michoacán (RBGK 22048); *Rodríguez & Ortiz-Catedral*, 3069 (IBUG); JQ670342, AM940180, JQ670500, AM940192, AM940167. *Tigridia chiapensis* Molseed – SP529; Mexico: Chiapas (UPSBG); *Chauveau H11009* (ICN); JQ670343, JQ670422, JQ670501, JQ670578, JQ670657. *Tigridia convoluta* (Ravenna) Goldblatt – SP558; Mexico: Jalisco (RBGK 21854); *Ramírez* 3390 (IBUG); JQ670344, AM940177, AM940212, AM940189, JQ670658. *Tigridia flammea* (Lindl.) Ravenna – SP552; Mexico: Michoacán (RBGK 22062); *Rodríguez et al.* 2813 (IBUG, WIS); JQ670345, AM940181, JQ670502-, JQ670579, AM940168. *Tigridia immaculata* (Herb.) Ravenna – SP581; Guatemala: Sacatepequez (RBGK 22067); *Rodríguez* 2832 (IBUG, WIS); JQ670346, JQ670423, JQ670503, JQ670580, JQ670659. *Tigridia meleagris* (Lindl.) G.Nicholson – SP580; Mexico: state of Mexico (RBGK 22070); *Rodríguez & Ramirez* 2903 (IBUG); JQ670347, JQ670424, JQ670504, JQ670581, JQ670660. *Tigridia oaxacana* (Molseed) Goldblatt – SP561; Mexico: Oaxaca (RBGK 21853); *Rodríguez* 2747 (IBUG, WIS); JQ670348-, AM940176, AM940211, AM940188, JQ670661. *Tigridia orthantha* (Lem.) Ravenna – SP556; Mexico: Oaxaca (RBGK 22235); *Rodríguez* 2836 (IBUG); JQ670349, AM940182, AM940217, AM940194, JQ670662. *Tigridia pavonia* (L.f.) DC. – SP555; Mexico (RBGK I-242); *Chase I-242* (K); JQ670350, AJ57882, AJ579987, AJ309680, AJ409594. *Tigridia philippiana* I.M.Johnst. – SP530; Chile: Region II (UPSBG); *Chauveau H11010* (ICN); JQ670351, JQ670425, JQ670505, JQ670582, JQ670663. *Tigridia seleriana* (Loes.) Ravenna – SP551; Mexico: Oaxaca (RBGK 22238); *Rodríguez* 2835 (IBUG, WIS); JQ670352, JQ670426, JQ670506, JQ670583, JQ670664. *Trimezia fistulosa* R.C.Foster – SP542; Brazil: Minas Gerais; *Lovo 128* (SPF); JQ670353, JQ670427, JQ670507, JQ670584, JQ670665. *Trimezia juncifolia* (Klatt) Benth. & Hook.f. – SP573; Brazil: Minas Gerais; *Lovo 36* (SPF); JQ670354, JQ670428, JQ670508, JQ670585, JQ670666. *Trimezia martinicensis* (Jacq.) Herb. – G (2008); Venezuela; *Berry* 3802 (MO); na, AJ578821, AJ579988, AJ30967, AJ409583. *Trimezia spathata* (Klatt) Baker – SP501; Brazil: Rio grande do Sul (PABG); *Eggers* 695 (ICN); JQ670355, JQ670429, JQ670509, JQ670586, JQ670667. *Trimezia truncata* Ravenna – SP574; Brazil: Minas Gerais; *Lovo 47* (SPF); JQ670356, JQ670430, JQ670510, JQ670587, JQ670668.