UNIVERSIDAD NACIONAL DE COLOMBIA

RELACIONES FILOGENÉTICAS EN LA TRIBU TIGRIDIEAE (IRIDACEAE)

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FACULTAD DE CIENCIAS-ÁREA CURRICULAR DE BIOLOGÍA

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GENERAL ABSTRACT

The florally diverse tribe Tigrideae (ca. 170 species) is restricted to the Americas. While vegetatively uniform, variation in floral morphology includes tepal orientation, color patterning, type of nectar produced, and structure of the stamens and style branches, such that over 45 genera have been described.

Our research, aimed largely at providing an improved understanding of relationships and then a revised generic delimitation consistent with phylogenetic principles, expands coverage of South American genera not previously included in molecular studies (*Cardenathus*, *Kelissa, Mastigostyla* and South American *Tigridia*) and widens sampling within *Cardiostigma*, *Cypella* and *Herbertia*, making it the largest sampling of the tribe to date. The results of molecular analyses based on plastid DNA (5 genes) and nuclear ITS, emphasize the presence of two well supported lineages, which do not correspond with the current circumscription of Cipurinae and Tigridiinae subtribes proposed by Goldblatt (1982).

Pollen studies show differences in surface sculpturing and aperture number. Optimization of pollen characters on the molecular phylogeny, and statistical analyses suggest that differences in number of sulci, lumina size, muri size, and patterns of ornamentation are taxonomically and phylogenetically informative and can be used to distinguish some taxa and clades in Tigridieae. Although the number of pollen apertures (i.e., two sulci) do not support the recognition of subtribe Tigridiinae as traditionally circumscribed, phylogenetic results and pollen studies support the expansion of the Mexican-Guatemalan clade to include seven other genera (*Ainea* Ravenna, *Cardiostigma* Baker*, Cobana* Ravenna, *Colima* (Ravenna) AarónRodr. & Ortiz-Catedral, *Fosteria*

Molseed, *Rigidella* Lindl., *Sessilanthera* Molseed & Cruden), as was proposed in previous studies (Goldblatt & Manning 2008).

Even though there is limited resolution within the South American genera, the analysis suggests that *Cypella* is not a polyphyletic group and recommends at least the inclusion of the monotypic *Kelissa* in *Cypella* and the inclusion of *Onira* in *Herbertia*. Results also show that the South American species of *Tigridia* should be referred to *Mastigostyla*. Molecular synapomorphies were identified for the different clades. Finally, assuming the circumscription of subtribes, proposed here, and based on several indirect calibration points, we proposed possible divergence times for the different groups. Cipurinae and Tigridiinae, shared a common ancestor 38.91 million years ago (MA). Tigridiinae diverged 25.11 MA where as Cipurinae diverged 26.41 MA.

Key words: Cipurinae, character evolution, cpDNA, Internal Transcribed Spacer (ITS), Iridaceae, distribution, divergence times, Tigridieae, Tigridiinae, phylogenetics, floral morphology, pollen morphology, sulci.

GENERAL INTRODUCTION

The family Iridaceae includes 65-70 genera and approximately 1900 species (Goldblatt, 2001; Reeves *et al*., 2001). This group represents one of the largest families of the order Asparagales (APG III, 2009) although it has a world wide distribution, its radiation center is Africa where at least 1000 species are reported, many of them restricted to Southern Africa (Goldblatt *et al*., 1998). Although the family is considered a monophyletic group, some doubt remains about the inclusion of *Isophysis* and *Geosiris* which have been considered as belonging to different families: Isophysidaceae and Geosiridaceae respectively (Goldblatt, 1990). About half of the species belong to one of seven genera *Iris* (280), *Gladiolus* (260), *Moraea* (195), *Romulea* (92), *Gleissorhiza* (85), *Crocus* (80) y *Sisyrinchium* (80).

The first classification of the family based on cladistic methods was proposed by Goldblatt (1990) and later by Rudall (1994). These authors each recognized four subfamilies (Isophysioideae, Nivenioideae, Iridoideae and Ixioideae) each one with one or more tribes for a total of seven tribes. Four tribes (Trimezieae, Tigridieae, Irideae, Sisyrinchieae) within subfamily Iridoideae with 30 genera and approximately 765 species are recognized. The tribe Tigridieae (15 genera, 140 species) is restricted to the New Word and its classification has been interpreted under different criteria. First, it was circumscribed by Kittel in 1840 by having bulbs, plicate leaves and style branches forming a complex and specialized structure which contrasted with other members of the subfamily (Rodríguez and Ortiz, 2001). Later, Bentham and Hooker (1883) divided the genera in two tribes: Moraeeae and Sisyrinchieae. Other authors proposed the inclusion and exclusion of genera within tribes (Engler, 1964; Hutchinson, 1973). The division of Tigridieae into two subtribes Tigridiinae and Cipurinae, based on the pollen form, the branches of the style, filaments and chromosome number was proposed by Goldblatt (1982), who included 18 genera in the tribe (Goldblatt, 2001).

Currently, Tigridieae includes about 140 species in 15 genera, 13 of which are referred to Cipurinae and the remaining two, to Tigridiinae. Some genera such as *Ainea*, *Gelasine*, *Kelissa* and *Onira* are monotypic or have few species, representing probably divergent members of other larger genera. However evolutionary relationships among members of the tribe have not been extensively explored.

The exclusively New World Tigridieae has radiated extensively in the Andes (*Cardenanthus*, *Mastigostyla*) and in southern South America, to where many endemic genera with few species occur (*Gelasine*, *Kelissa*, *Onira*). A second center of radiation is in northern Central America and Mexico where *Ainea*, *Cobana*, *Fosteria* and *Sessilanthera* (now in *Tigridia*) are endemic. *Tigridia* is also present in the Andes, and *Nemastylis* is shared between the southern United States and Mexico and Guatemala. *Cypella* has a wide range across South and Central America including the Caribbean Islands. *Herbetia* has an interesting disjunct distribution, with five species in Argentina, Brazil, Uruguay, and southern Chile, and one subspecies in the southern United States (Goldblatt *et al.,* 1998).

Recently, a phylogenetic study was used to explain the origin and the geographic distribution of Tigridiinae (Rodríguez and Systma, 2002). This molecular analysis suggested that Tigridiinae had its origin in South America with a subsequent migration and radiation in Mexico. The origin of Cipurinae is not clear, but the low sequence divergence in the ITS region suggests that Tigridiinae has undergone a recent burst of radiation, driven in part by shifts in pollination strategy.

Studies about the genera of Tigridieae include reviews and taxonomic notes for *Alophia* (Goldblatt and Howard, 1992), *Calydorea* (Goldblatt and Henrich, 1991) *Cipura* (Ravenna, 1988; Goldblatt and Henrich, 1987; Celis *et al.,* 2003) and *Catila and Onira* (Ravenna, 1983). Others works include a cytogenetic suprageneric analysis, in which the difference among the subtribes is established (Goldblatt 1982).

The phylogeny of Tigridieae is poorly understood and a complete systematic study of the tribe has not been produced so far. Some genera have been taxonomically treated such as *Eleutherine* (Goldblatt and Snow, 1991) and *Tigridia* (Molseed, 1970). Additionally, there is a study of the pollen morphology of the tribe (Rudall and Wheeler, 1988), where Goldblatt's (1982) division into two subtribes is supported. Rodríguez (1999), using ITS sequences showed that Trimezieae and Tigridieae are monophyletic, a result confirmed by Reeves *et al.,* (2001) using plastid DNA. Rodríguez (1999) concluded that Cipurinae was not monophyletic, since some taxa cluster with a basal clade in Tigridieae, whereas others cluster with Tigridiinae. This study did not include South American species, but only Mexican ones, which limits some of the inferences.

This doctoral thesis explored the phylogenetic relationships of subtribe Tigridieae, and assessed the monophyletic status of Tigridiinae and Cipurinae when more species of their distribution are included, particularly South American ones. I also explored the implications of the phylogeny for the classification of Tigridieae and the relevance of palinologycal characters for the generic delimitation of Tigridiinae and Cipurinae. In order to acomplish these objetives, I used plastid DNA genes (the non-coding *trnL-trnF*, the *trnL* intron, the non-coding trnH-psbA intergenic spacer, the *mat*K gene and the trnKK3 gene) and the nuclear ITS region. Both genomes have proved to be useful at different levels of resolution to infer the relationships among different groups of plants. Particularly the phylogenetic resolution of ITS at lower taxonomic levels is well known (Baldwin *et al.,* 1995; Sang *et al.,* 1995, Kelly, 1998) for some genera, but also limited in others because of low sequence diversity (Bain and Jansen, 1995; Leclerc *et al.*, 1998).

I performed phylogenetic analysis using three different methods of reconstruction maximum parsimony, maximum likelihood and Bayesian inference. Different combinations of genes as well as single gene analyses were designed in order to have a complete view of the phylogenetic history of Tigridieae. Once the phylogeny of the group was reconstructed and the pollen variation of the group determined, I evaluated distribution ancestral areas of

Tigridieae, to understand the geographical scenario for this succesfully diversified group of plants, assuming that the group originally had a more restricted distribution than it has today.

The introduction includes a general view of the research questions based on the available literature. The first chapter includes a detalied study of the phylogeny of the group based on both plastid and nuclear genes, with inferences on taxonomy, ancestral distribution and divergence times for the different clades. A second chapter is a description of the pollen morphology based on scanning electron microscopy, and the inferences obtained from mapping pollen characters on the resolved phylogeny. A third chapter treats *Zygella* S. Moore, as a synonym of *Larentia* Klatt (Iridaceae). Finally the conclusions section synthesizes the main inferences reached from this study.

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CHAPTER 1.

PHYLOGENETIC RELATIONSHIPS IN TIGRIDIEAE (IRIDACEAE) BASED ON PLASTID AND NUCLEAR ITS DNA SEQUENCES

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ABSTRACT

The florally diverse tribe Tigrideae (ca. 170 species) is restricted to the Americas. While vegetatively uniform, variation in floral morphology includes tepal orientation, color patterning, type of nectar produced, and structure of the stamens and style branches, such that over 45 genera have been described. Our research, aimed largely at providing an improved understanding of relationships and then a revised generic delimitation consistent with phylogenetic principles, expands coverage of South American genera not previously included in molecular studies (*Cardenathus*, *Kelissa* and *Mastigostyla*) and widens sampling within *Cardiostigma*, *Cypella* and *Herbertia* making it the largest sampling of the tribe to date. The results of molecular analyses based on plastid DNA (5 genes) and nuclear ITS sequences support the paraphyly of several genera including *Tigridia* and *Cypella.* Phylogenetic results indicate that the circumscription of *Tigridia* should be expanded to include *Ainea*, *Cardiostigma*, *Cobana*, *Colima*, *Fosteria*, *Rigidella* and *Sessilanthera*. Even though there is limited resolution within the South American genera, the analysis suggests that *Cypella* is not polyphyletic and suggests at least the reduction of the monotypic *Kelissa* within *Cypella*. Results also show that the South American species of *Tigridia* should be referred to *Mastigostyla*. Molecular synapomorphies are identified for the different clades. Finally based on different calibration points and different approaches, we propose possible divergence times for the different groups. The subtribes Tigridiinae and Cipurinae shared a common ancestor 38.91 million years ago (MA). Tigridiinae diverged 25.11 MA where as Cipurinae diverged 26.41 MA. We did not find large differences between the estimates from standard

Bayesian analysis under a relaxed clock and nonparametric rate-smoothing methods.

Key words: Cipurinae, cpDNA, internal transcribed spacer (ITS), Iridaceae, distribution, divergence times, Tigridieae, Tigridiinae, phylogenetics

RESUMEN

La Tribu Tigrideae, floralmente diversa (ca. 170 especies) se restringe a las Américas. Aunque son vegetativamente uniformes, la variación en morfología de tépalos incluye orientación de tépalos, patrón de coloración, tipo de néctar producido, y la estructura de estambres y ramas del estilo, al punto que más de 45 géneros han sido descritos. Nuestra investigación, destinada principalmente a proporcionar un mayor entendimiento de las relaciones evolutivas y luego una delimitación genérica consistente con principios filogenéticos, aumenta el muestreo taxonómico a géneros Sur Americanos que previamente no habían sido incluidos en estudios moleculares (*Cardenathus*, *Kelissa* y *Mastigostyla*), e incrementa el muestreo dentro de *Cardiostigma*, *Cypella* y *Herbertia* haciéndolo el muestreo más grande de la tribu a la fecha. Los resultados de los análisis moleculares basados en DNA plastídico (5 genes) y secuencias de ITS nucleares enfatizan en la parafilia de varios géneros incluyendo *Tigridia* y *Cypella*. Resultados filogenéticos indican que la circunscripción de *Tigridia* debe expandirse para incluir a *Ainea*, *Cardiostigma*, *Cobana*, *Colima*, *Fosteria*, *Rigidella* y *Sessilanthera*. Aun cuando hay resolución limitada dentro de los géneros Sur Americanos, los análisis sugieren que *Cypella* no es polifilético y recomienda por lo menos la reducción del monotípico *Kelissa* dentro de *Cypella*. Los resultados también muestran que las especies Sur Americanas de *Tigridia* deben ser referidos a *Mastigostyla*. Se identificaron sinapomorfías moleculares para los diferentes clados. Finalmente, y basados en diferentes enfoques en la calibración del reloj molecular, se estimaron los puntos de divergencia para los diferentes clados. Las subtribus Tigridinae y Cipurinae compartieron un ancestro común aproximadamente hace 38,91 millones de años (MA). Tigridinae divergió hace

25,11 MA, mientras que Cipurinae divergió hace 26,41 MA. No se encontraron grandes diferencias en los estimativos de métodos estándar bayesianos bajo un reloj relajado y métodos no paramétricos de suavizamiento de tasas evolutivas.

Palabras clave: Cipurinae, DNA plastídico, secuencias nucleares de ITS, Iridaceae, distribución, tiempos de divergencia, Tigridieae, Tigridiinae, filogenética.

1. INTRODUCTION

The family Iridaceae, one of the most diverse among the Asparagales (Goldblatt 2001, Chase *et al.,* 2006), includes about 2028 species in 66 genera (Goldblatt and Manning 2008, Goldblatt *et al.,* 2008). These species are distributed world-wide although the family is particularly abundant in the eastern Mediterranean, Mexico, South America and specially Africa where there are some 1000 species, most of which are restricted to southern Africa (Goldblatt *et al.,* 1998, Rodriguez and Sytsma 2006, Goldblatt and Manning 2008). They occupy a broad range of habitats that include open scrubs, deserts and grasslands. Iridaceae is defined by having isobilateral, equitant leaves, calcium oxalate crystals in the form of styloid crystals, and flowers with an inferior ovary flowers and three stamens (Goldblatt 1990).

Except for the circumscription of the genera *Isophysis* and *Geosiris*, sometimes treated as different families in Isophysidaceae and Geosiridaceae respectively (Dahlgren *et al.,* 1985; Goldblatt 1990; Rudall 1994; Chase *et al.,* 2006), the family is considered monophyletic. Goldblatt (1990) proposed the first classification of the Iridaceae using cladistic methods and it is the base of the current classification, with four subfamilies and seven tribes recognized. Rudall's (1994) anatomical work provided additional support for Goldblatt's hypothesis. DNA data has been important to understand generic relationships within the family and to provide new information regarding its classification. Goldblatt and Manning (2008) produced the most recent and complete work on the family to date, including data from previous molecular analysis (Souza-Chies *et al.,* 1997, Reeves *et al.*, 2001, Goldblatt *et al.,* 2006, and Rodríguez and Systma 2006). They proposed seven subfamilies for Iridaceae: Isophysidoideae (1: 1), Patersonioideae (1: 21), Geosiridoideae (1: 1), Aristeoideae (1: ca. 55), Nivenioideae (3: 15), Crocoideae (29: ca. 1032), Iridoideae (29: ca. 890) (Goldblatt *et al.*, 2008, Goldblatt and Manning 2008).

The subfamily Iridoideae is the second largest of the family and includes 5 tribes: Diplarreneae (1:2) distributed in Australia and Tasmania, Sisyrinchieae (6: ca. 177) from the New World and the Pacific Region, Trimezieae (3: 42) and

Tigridieae (15: ca. 172), both restricted to the New World, and Irideae (5: ca. 510) which occurs in the Old World, although a good number of species of the genus *Iris* are present in North America (Goldblatt *et al.,* 1998).

Currently, Tigridieae and Trimezieae are considered sister groups based on ITS (Rodríguez, 1999) and plastid DNA sequences (Reeves *et al.,* 2001). The ancestral conditon for both tribes includes free filaments, well developed style branches opposed to the stamens and anthers joined at the apical portion of the style branches. The tribes differ by the presence of the bulb and the plicate leaves in Tigridieae both absent in Trimezieae (Goldblatt and Manning 2008).

Tigridieae as described by Kittel (1840) is vegetatively uniform, by contrast it exhibits sizeable variation in floral morphology, including tepal orientation, color patterning, type of nectar produced, and structure of the stamens and style branches (Celis *et al.,* 2008). Goldblatt (1982) divided the Tigridieae into two subtribes based on pollen morphology, the branches of the style, the union of the filaments and basic chromosome number: 1) Tigridiinae, with disulcate pollen grains, a chromosome number of n=14, and a geographic distribution centered in Mexico and Guatemala, and 2) Cipurinae, with monosulcate pollen grains, n=7, and a center of diversification in South America with some species extending into the Southern US. Goldblatt (1990) included 18 genera in the tribe Tigridieae. Rodríguez (1997) and Rodríguez and Systma (2006) proposed a reduction in the number of Tigridiinae genera, but those taxonomic changes were never formally published. Subsequently, Goldblatt proposed several generic reductions in a revised classification of the tribe (Goldblatt, 2001). Currently, Tigridieae includes about 172 species in 15 genera, 13 of which are referred to Cipurinae and the remaining two, to Tigridiinae (Goldblatt and Manning 2008, Goldblatt *et al.,* 2008).

The first phylogenetic study in Tigridieae was done by Rodríguez and Systma (2006), based on a combined analysis of morphological characters and DNA sequences. Their result confirmed (1) the monophyly of the subtribe Tigridiinae (including *Cardiostigma longisphatha* and *Nemastylis convoluta* both previously included in Cipurinae), and (2) that Cipurinae was not monophyletic: some taxa

comprise a basal clade in Tigridieae, whereas others were associated with Tigridiinae. This study included only Mexican and Central American species from five of the 13 genera of Cipurinae, and only two South American species: *Ennealophus foliosus* and *Tigridia lutea* (Rodriguez and Systma, 2006).

Recently, Goldblatt and Manning (2008) and Goldblatt *et al.,* (2008) based on DNA sequences and morphological features concluded that several genera of the tribe Tigridieae should not retain generic status. They placed *Ainea*, *Cardiostigma*, *Colima*, *Fosteria*, *Rigidella* and *Sessilanthera* within *Tigridia*; the Bolivian *Cardenanthus* was included within *Mastigostyla*; *Kelissa* and *Onira* within *Cypella*; *Tamia* within *Calydorea*, and *Tucma* within *Ennealophus*. Despite these substantial advances in the taxonomy and phylogenetics of the tribe from different sources of data, the phylogeny of Tigridieae is still poorly understood, particularly because South American species have not been included.

In order to study the phylogenetic relationships of Tigridieae, to evaluate the monophyly of Tigridiinae and Cipurinae, and to contribute to the understanding of generic delimitation of the tribe, we grouped species used in previous studies (Souza-Chies *et al.,* 1997, Reves *et al.,* 2001, Rodríguez and Sytsma 2006, Goldblatt *et al.,* 2008), expanded the molecular analysis of South American Tigridieae by including the genera *Cardenathus* and *Mastigostyla* and increased broadly the sampling of *Calydorea*, *Cardiostigma*, *Cypella*, *Herbertia* and *Tigridia*; making it the largest sampling of the tribe to date. Our analysis included five plastid DNA regions (*trnL-trnF*, *trnL* intron, *trnH-psbA*, *matK*, *trnKK3*) and the nuclear ribosomal internal transcribed spacer (nrITS). These have been shown by Reeves *et al.* (2001), Rodriguez and Systma 2006 and Goldblatt *et al.,* 2008 to provide sufficient sequence divergence when combined with other DNA sequences to resolve relationships of species or species groups within Tigridieae. In order to provide a broad phylogenetic perspective, we reconstructed the phylogenetic relationships by Maximum Parsimony as well as probabilistic methods (Maximum Likelihood and Bayesian Inference).

2. MATERIAL AND METHODS

2.1. Taxa and genetic material

A total of 85 accessions from 59 taxa were included in the different analyses (Table 1): the ingroup represented sixty-two accessions, including three genera of Tigridinae provisionally accepted by Goldblatt *et al.,* (2008) and all the genera of Cipurinae except *Larentia*. In this study we expanded the molecular analysis of South American Tigridieae by including for the first time the genera *Cardenathus*, *Mastigostyla* and South American *Tigridia*; also we increased the sampling of *Calydorea*, *Cardiostigma*, *Cypella*, *Herbertia* and *Tigridia.* For outgroup comparisons we included 10 species from tribes of the Iridoideae subfamily i.e. Diplarrheneae (1), Irideae (2), Sisyrinchieae (3), and Trimezieae (4). Thus, this is the largest sampling of the tribe to date. Additionally, this work includes sequences from GenBank (Souza-Chies *et al.,* 1997, Reeves *et al.,* 2001, Rodríguez and Sytsma 2006 and Goldblatt *et al.,* 2008) and the sequences generated by the authors at the Molecular Systematics Section of the Royal Botanical Garden (Kew) and at the Molecular Systematics Lab of Guadalajara University (Table 1).

The DNA analyzed in this study was extracted from plant material obtained in Herbarium specimens of Universidad Mayor San Francisco Xavier de Chuquisaca (HSB), the Royal Botanic Gardens KEW living plant collection (K), the personal collection of Dr Germán Roitman at Buenos Aires University (Argentina) and leaf tissue collected by the authors in Peru and Bolivia. The specimens were dried and preserved in silica gel following Chase and Hills (1991). In addition, our DNA datasets were complemented with samples from the Kew DNA Bank and from the collection of Rodríguez and Sytsma 2006 stored at Guadalajara University.

In order to provide a better perspective of the evolution of Tigridiedae (Iridaceae), we included data from a total of six genes from both plastid (cpDNA) and nuclear (nDNA) genomes. The cpDNA regions included the non-coding *trnL-trnF* intergenic spacer, the *trnL* intron, the non-coding *trnH-psbA* intergenic spacer, the *mat*K gene and the *trnKK3* gene. The

nuclear gene used in this study was the internal transcribed spacer (nrITS).

2. 2. DNA extraction and amplification

Total genomic DNA was extracted following the method of Doyle and Doyle (1987, 1990) modified in Smith *et al.,* (1991), and subsequently purified with QIAquick™ silica columns (Qiagen, Crawley, West Sussex, UK) according to the manufacturer's protocol for PCR products.

Primers for DNA amplification of the non-coding *trnL-trnF*, and the *trnL* intron cpDNA regions were as published by Taberlet *et al.,* (1991). The primer sequences used for the amplification or the *matK* gene are found in Hilu *et al.,* (2003). In several cases we used alternatively *matK* X and matK5 primers according with the Barcode of Life Project (http://www.kew.org/barcoding/protocols.html). Likewise, primers for *trnH-psbA* intergenic spacer are in Kress and Erickson 2007. PCR reactions had a total volume of 50 µl containing 10-30 ng of genomic DNA, 200 µl of each dNTP, 0,4 µM of each primer, 5 µl of 10X reaction buffer, 2.5 µl BSA (40mg/ml), 1-1.5 units of Taq DNA polymerase (Promega Co.) The reaction profile included an initial denaturation step at 94° C for 3 min, followed by 35 cycles with denaturation at 94° C for 1 min, annealing at 48-52° C for 1 min and extension at 72° C for 2 min and a final elongation at 72° C for 10 min.

The nuclear ITS region, was amplified using the primers ITS5, ITSLeu.1, and ITS4 (White *et al.,* 1990; Baldwin 1995). In some cases, we got a first PCR product with the primers ITS5 and ITS2, for a second PCR with ITS3B and ITS4 (Baum *et al.,* 1994) with the first PCR as a template. PCR reactions of 20µl contained 10-30ng of total genomic DNA, 1µl of dNTPs (5mM), 0,5 µM of each primer, 2 µl of 10X reaction buffer, 1 µl BSA (2,5mg/ml), 2 µl $MgCl_2$ (25 mM), 0,05 units of Taq DNA polymerase (Promega Co.). The reaction profile included an initial denaturation step at 94° C for 3 min, followed by 25 cycles with denaturation at 94° C for 1.5 min, annealing at 54° C for 2 min and extension at 72° C for 3 min; a final

elongation was performed at 72° C for 15 min. PCR Products were cleaned with QIAquick™ (Quiagen, Crawley, West Sussex, UK), using the manufactur's instructions.

2.3. DNA sequencing, and DNA alignment

Both strands were sequenced by using the same amplification primers on an Applied Biosystems 303 capillary DNA automated sequencer using BigDye™ terminator v3.1 chemistry, following the manufacture's protocols (ABI). Sequences from both strands were assembled and edited with Sequencher version 4.5 (GeneCodes, Ann Arbor, Michigan, USA). All sequences were submitted to GenbanK (Appendix 1A-F). Alignments were obtained individually by gene using Clustal X version 2.0.10 (Larkin *et al.,* 2007) using the default parameters, and adjustments were made manually in BioEdit version 7.0.9.0 (Hall 1999). *MatK* gene and *trnKK3* genes required very little editing. In other genes, segments that could not be aligned unambiguously were excluded. In addition, unevenly sequenced ends were trimmed prior to phylogenetic analysis.

2.4. Phylogenetic analyses

Because the five regions offer different degrees of resolution at different phylogenetic levels, we built 16 different data sets, six of which corresponded to individual genes and ten to different combinations of genes. In order to have a detailed scenario of the performance of each combination on the phylogeny of the Tidridinae, we performed analyses of maximum parsimony with each data set (see Table 2). Because observed levels of sequence divergence were low, we did not expect signs of saturation, which could affect phylogenetic reconstruction as a result of multiple hits. In effect, saturation plots did not show any sign of saturation for any of the genes, when considering transitions and transversions separately (data not shown). Thus, we weighted equally all substitutions in phylogenetic analysis.

Genetic divergence at different levels and transition/transversion ratios were calculated with PAUP* 4.0b10 (Swofford, 2002) and Mega (Tamura *et al.,* 2007). Maximum parsimony heuristic searches were performed for each one of the 16 combinations and conducted with 1000 random taxon addition replicates, and tree-bisection-reconnection (TBR) branch swapping. Insertion or deletions characters (gaps) were considered as missing data. For the fundamental trees produced we calculated Consistency Index (CI), Retention Index (RI) and the rescaled consistency index (RC) obtained by multiplying the CI by the RI. When more than one most parsimonious tree was found, we calculated a Majority Rule Consensus Tree (MRCT). Clade supports were estimated with the bootstrap (Felsenstein, 1985) in PAUP on some of the combinations that provided better phylogenetic resolution: 1000 bootstrap replicates, simple taxon addition, TBR branch swapping and holding 10 trees at each step.

Due to computational time limitations, the Maximum likelihood analyses were performed only on the datasets that provided the best phylogenetic resolution in the MP analyses. For Maximum likelihood analysis, we first determined the substitution model that best fit the data with jModeltest (Posada, 2008). Since a given combination could have different genes, we performed the searches with Jmodeltest by considering each gene of a combination separately, or together as part of a unique “gene”. ML heuristic searches were performed in PAUP (Swofford *et al*., 2002) by stepwise addition, with the option as-is to add taxa, swapping was done on best trees only (TBR) and with the maximum number of trees to be saved=10. In the analysis, the PAUP block provided by jmodeltest was copied in the matrix file to specify the substitution model to be applied. Multiple search replicates (100) were performed to find the maximum-likelihood topology support with Garli (Zwickl, 2006) and specifying according to Garli options the most similar model obtained with jModeltest.

Bayesian analyses were conducted using MrBayes (Huelsenbeck and Ronquist 2001) version 3.1.2. on a personal computer or by means of Web Computing Resources: Computational Biology Service Unit, *Cornell*

University (http://cbsuapps.tc.cornell.edu/sfscode.aspx). We ran 20 million generations on two separate and independent runs, sampling every 1000th generation, for a total of 20,000 samples. Convergence was evaluated on both Tracer from the Beast package (Drummond *et al.,* 2007) and by checking for the standard deviation of split frequencies (< 0,01). The burn-in used corresponded to 25% of the samples (5000) and a majority consensus tree was computed with TreeAnotator (From the Beast Package). We visualized the trees to see the posterior probabilities on the different nodes of interests with FigTree from the Beast package.

Finally, molecular synapomorphies for the main clades were determined by using the MP tree. In order to do that, we first eliminated the non-informative characters in the matrix. Then, we mapped each remaining character in the phylogeny by using MacClade (Madison and Madison 2005) to establish whether or not particular characters could be assumed as derived characters for particular clades.

2.5. Ancestral area reconstruction

Species distributions were compiled from vouchers included in this study (Table 1), plus information from localities obtained in herbarium specimens. We consulted about 1300 specimens on loan from The Missouri Botanical Garden (MO) and the following South American herbaria: Herbario Nacional Colombiano (COL); Universidad Nacional San Antonio Abad del Cusco, Herbario Vargas (CUZ); Universidad de San Francisco Xavier de Chuquisaca, Herbario Chuquisaca (HSB); Universidad Nacional de San Austin de Arequipa, Herbario Arequipense (HUSA); Herbario Nacional de Bolivia (LPB); Universidad de San Marcos, and Herbario San Marcos (USM). Additional information was obtained from the List of Species of Flora of Brazil (Eggers *et al*., 2012) and from the World Checklist of Selected Plant Families (WCSP, 2012).

Georeferencing of both data sets was done by using the point-radius methodology according to the suggestions in the Guide to best practices for

georeferencing (Chapman and Wieczoreck, 2006). Maps were generated in DIVA-GIS 5.4 (Hijmans et al., 2005).

In order to have a geographical scenario and to infer implications of the radiation of the Tigridieae, we grouped species ranges into 17 regions, following biogeographic provinces proposed by Morrone for South America (2001, 2006 Urtubey *et al* 2010), and four regions were added to cover the whole area of tribe's distribution (Table 3, Fig 5A). Ancestral character state reconstructions for distribution were inferred using Parsimony approaches in Mesquite version 2.6 (Maddison and Maddison 2011). The distribution was considered as an unordered character

In order to estimate possible divergence times for the main clades of interest, we used two different approaches. The first one was a Bayesian search with a relaxed clock implemented in BEAST 1.7.2 (Drummond *et al* 2006; Drummond & Rambaut 2007), using the GTR+G substitution model, a Yule tree prior, and uncorrelated and log normally distributed rate variation. Two runs with 10,000,000 generations were performed with parameters sampled every 1,000 generation for a total of 10,000 samples; burn-in corresponded to 25% of the samples (2500). In this analysis we used a single calibration point based on fossil evidence for the divergence of *Libertia ixioides* and *Orthosanthus chimboracensis* of the Tribe Sisyrinchieae, estimated at 22 MA (Goldblatt *et al.,* 2008).

The second approach explored the nonparametric rate-smoothing (NPRS) algorithm developed by Sanderson (1997), which take into account rate variation among lineages and relaxes the stringency of the clock assumption using smoothing methods. For this approach, we included up to 10 calibration points (Table 4) or combination of those from indirect estimates for different divergence events within the Tribe (Goldblatt 2008).

In order to do this, we first used the ML tree obtained with Garli (Zwickl, 2006) as a starting point with branch lengths (numbers of substitutions along each branch and the MP tree with branch lengths) and used r8s (Sanderson 2003) and the set of calibration points to allow scaling of rates and times to real units. We performed analyses by combining single or

multiple calibration points in order to evaluate the effect of using single vs. multiple calibration points. Node ages were visualized in Fig Tree (from the Beast Package).

3. RESULTS

Some taxa do not have data for all genes. Levels of sequence divergence were, in general, low as expected. PsbA and mat K showed the lowest levels of divergence in most comparisons, including identical sequences when comparing different species of the same genus (Appendix 2). On the other hand *trnL-F* and *ITS* showed moderate levels of sequence divergence, for example species within the genus *Calydorea* had 5.48 % sequence divergence for ITS, where as for trnL-F species within *Eleutherine* had 3.19 % sequence divergence.

Considering individual regions ITS had the largest number of variable characters (43%) as well as the largest percentage of parsimonious informative characters out of the total: 24% (Table 2). *psbA-trnH* and *trnL* intron not only had the lowest percentage of variable characters out of the total (10 and 22%) but the lowest percentage of parsimonious informative characters (11 and 10%). For these individual data sets, CI parameter that explain levels of homoplasy were relatively high (between 0.67 and 0.89).

The combinations with more variable characters and parsimony informative characters were Combination 1 (that included all 6 genes), and Combination 7 (which included three of six). In both cases 28% of the characters were variable, and 15% of the total characters were informative under parsimony. For these combinations, we also detected good values of CI, RI, RC (Table 2).

Individual genes did not show the best resolution in the performed analysis, mainly for limited sampling. Figure 1 shows the phylogeny obtained with ITS sequences, and Figure 2 shows the phylogeny obtained with the plstid genes. In both analyses two lineages are clearly defined**:** Lineage A and Lineage B, although the resolution within these monophyletic groups is limited. It was

evident that combination of regions showed better resolution than topologies obtained with plastid or nuclear genes separately (see Appendix 3-1 A). Thus, we focused on the combinations that included more than one region. Most of them showed similar topological arrangements, mainly in the large clades, and sight variations appeared among for the terminal taxa in some of the larger clades. Different monophyletic groups product of different analyses were compared (Table 5.) and the different topologies obtained are showed in appendix 3A-H. The monophyletic status of the tribe Tigridieae, lineage A and lineage B (corresponding with two subtribes), as well as other small subclades within the large groups were consistent.

Because the combination (comb7) had the best taxonomic sampling as well as the best phylogenetic resolution, we chose it to perform additional analyses. This combination included all species that had sequences of at least 3 of the total of 6 genes studied (72 taxa: 10 for the outgroup, 62 for the ingroup).

For the MP analysis of this combination, the matrix included 3779 characters of which 1044 (28%) were variable and 551 (15%) were parsimoniously informative. The heuristic search produced 528,689 most parsimonious trees (L= 1715 CI=0,734, RI=0,797 and RC=0,585). We calculated a majority rule consensus tree (MRCT) from this analysis, which did not collapse too many branches since most of them swapped along terminal branches within the large clades.

The combination (Comb7) was submitted to additional analysis with Maximum Likelihood with bootstrap replicates (ML), Maximum parsimony with bootstrap replicates and Bayesian inference (BI). Support values obtained from these analyses were placed on the MRCT nodes from the previous analysis (Figure 3).

In this tree the molecular sampling shows that a monophyletic tribe Tigridieae includes both subtribes designated as Lineage A and Lineage B, all with the maximum support values for the three methods of reconstruction used. The tribe Trimezieae (2 genera and 4 species sampled) appears as the sister clade of the group formed by Clade A and Clade B. The tribes Sisyrinchieae, Irideae,

and Diplarreneae are basal in the tree with moderate to high levels of support in their relationships with other members of the subfamily.

In order to clarify the discussion, from this point of the manuscript, we will name the Lineage A as Cipurinae subtribe, and lineage B as Tigridiinae subtribe. The two clades formed by the subtribes Tigridiinae (clade B) and Cipurinae (Clade A) were evident in all other analysis. Tigridiinae consists of the Mesoamerican genera and the South American genera. In this topology, *Alophia* is the first divergent genus; five out of ten genera from which we had more than one species resulted as monophyletic groups (*Sessilanthera, Ennealophus, Eleutherine, Gelasine* and *Alophia*) with moderate to high support values; other genera with more than one sampled species resulted in an unresolved politomy (*Fosteria* and *Rigidella*), whereas two genera turned out to be not monophyletic in this analysis (*Tigridia* and *Mastigostyla*). In this clade, several subclades are also resolved. The first one is N, the closest relative of *Ennealophus*, as suggested by Goldblatt *et al.* (2008). However, in the BI tree (see Appendix 6-1) the N clade appears as a sister group of (*Cardenanthus*, *Mastigostyla*, *Tigridia*). L, K and J are three clades formed by different genera of the Subtribe.

The second largest clade includes the Cipurinae, in which two out of the five genera, for which we had more than one species turned out to be monophyletic (*Cypella* and *Cipura*). In this clade, two subclades are also present: Clade C formed by the *Cipura-Larentia* group and Clade D, formed by *Nemastylis tenuis, Calydorea pallens* 1, the subclade (Onira, Herbertia spp), Cypella spp., and the subclade Herbertia tigridoides and Calydorea spp (Fig.3-1.).

Additionally we were able to find 50 synapomorphies at different levels, which could be defined as specific positions for large groups (Table 6 and appendix 4). Twenty-three of those corresponded to transitions (12 T↔C, 11 A↔G), and twenty-seven to transversions (9 A↔T, 9 T↔G, 4 C↔G and 5 C↔A). All DNA regions included positions as synapomorphies, except *psbA-trnH* and *trnL* intron. The gene with the largest number of these synapomorphical positions corresponded to *matk* with 33 (66%), followed by *trnKK3* with 10 (20%), ITS

with 6 (12%) and finally *trnL-F* with 1(2%). A total of 13 synapomorphies defined the Subtribe Cipurinae whereas 6 defined the subtribe Tigridiinae. These positions defined well-supported clades with the three methods of reconstruction used, clades C and D within Cipurinae, and clades K, L, N within Tigridiinae.

We obtained estimates of divergence times for the different clades under two different approaches. The first approach included a single calibration point (22 MY) for the separation of *Liberthia ixioides - Orthrosanthus chimboracensis* under a relaxed clock with Beast (Drummond *et al.,* 2007). The second one used multiple calibration points and the nonparametric rate-smoothing method implemented in r8s (Sanderson 2003). For the starting tree in the r8s analyses, we used the MP tree and the ML tree from separate analysis. Divergence times for the different clades are shown in Figure 4-1 and a summary of the estimates are included in Table 7 (more detailed results from these analysis in Appendix 5). Although, as expected we did not get identical values for a given clade, in general standard Bayesian and NPRS estimates were similar. The topology shows the same arrangement of relationships for the different groups than the combination of different genes (Figure 3). For comparison purposes, we included values from Goldblatt *et al.,* 2008. The subfamily Iridoideae shared a common ancestor 52,07 MA (25,58-86,06), whereas the tribes Trimezieae-Tigridieae had a common ancestor 40,97 MA (21,65-70,27). The separation of the Cipurinae and Tigridiinae occurred ca. 38,5 MA. The first divergent event in subtribe Cipurinae (Lineage A) occurred ca. 26,41 MA whereas for the subtribe Tigridiinae it happened ca. 25,11 MA. Recent splitting events within the subtribes incude divergence times range between 5,26 MA (Clade X) and 20,69 MA (clade D).

4. DISCUSSION

The tribe Tigridieae within the Iridaceae is an example of successfull adaptive radiation, which has lead to great floral diversification patterns. That variation in floral morphology includes tepal orientation, color patterning, type of nectar, and the structure of the stamens and style branches. With such a broad variety of features, at least 45 genera have been described so far.

This research expands the sampling of South American genera not previously included in molecular studies (*Cardenathus*, *Mastigostyla* and South American species of *Tigridia*), and widens the sampling within *Cardiostigma* (now placed in *Tigridia*), *Cypella* and *Herbertia* making it the largest sampling of the tribe to date. The aims of this research are to better understand of the relationships of members of the subtribes Tigridinae and Cipurinae relative to other taxa of the Subfamily Iridoideae and to provide a phylogenetic framework for a revised generic classification. Although our sample still does not include species of *Kelissa, Itysa* and *Lethia,* we believe our results based on molecular data could be the basis for considerable generic restructuring of the Tribe and a framework for further morphological studies to define synapomorphies of the main groups.

4.1. The ITS region

The internal transcribed spacer has been used as a source of characthers for phylogenetic inference in many angiosperm families (Baldwin *et al.,* 1995). Among its chief advantages are universal primers for its amplification, it is a small region which can be sequenced relatively fast, and depending on the taxonomic level, it provides good phylogenetic signal. In this study, ITS showed the highest percentage of variable characters (46%) when all individual genes were compared. The next best gene in terms of variable characters was trnK3 with 34%. Also, ITS had the highest number of parsimony informative characters (24%) followed by

trnKK3 and *trnL_F* with 19%. Because we had data for only 46 taxa, it is not possible to compare the resulting phylogeny with the whole data set, however, the topology is congruent in defining the main large clades; Figure 1 (Tribe Tigridieae, subtribes Tigridiinae and Cipurinae).

Although ITS gene has been used to estimate the phylogeny of many organisms (Hillis and Dixon 1991), there exists evidence of thousands of copies, located in one or several arrays. Although the divergence of these copies is low, due mainly by concerted evolution (Arnheim 1983), there is concern about the rates of evolution of speciation events vs concerted evolution. When concerted evolution is slower than speciation, then the genome may contain divergent paralogues (descendants of a duplicated ancestral gene) (Baldwin *et al*., 1995). Unidentified paralogous relationships and recombination could result in erroneous species phylogenies (Sanderson and Doyle 1992). Although we can not rule out totally the presence of paralogous copies of ITS in our data set, it is possible to say, based on different lines of evidence, that the effect of paralogous genes is minimum. First, we did not find any evidence of double peaks in the electropherograms of the sequences; this could suggest more than one copy of the gene. Second, the A-T, G-C frequencies in comparisons among all taxa, did not show any statistical difference (data not shown). Third, levels of divergence among all comparisons, did not show evidence of paralogous copies under different evolutionary forces that could affect different mutation rates. For example, we did not find higher divergence values for comparisons among species than among genera. Fourth, all of the combinations that included ITS showed similar (and better resolved) phylogenies than when the ITS sequences were excluded (Figure 2).

4.2. The Chloroplast plus the ITS Regions

As described in the results section, most of analyses provided a similar evolutionary scenario for the diversification of these plants. Particularly, the combination that included at least three genes for most of the taxa turned out to be the most resolved tree with moderate to high values of support when using the three methods of reconstruction. Thus, the

following inferences are based on the topology of the MRCT of a MP analysis tree (Figure 3.).

As expected the Tribe Tigridieae is a monophyletic group and consists of two major groups both supported by high confidence values (Clade A and B, Figure 3). Our results are inconsistent with the currently followed taxonomic delimitation of the subtribes proposed by Goldblatt (1982). Althoug more recent studies (Goldblatt and Manning 2008, Rodríguez 1999) suggested that the 1982 classification had limitations, we now have conclusive evidence to justify a realignment of the subtribes.

Table A: Classification of the genera of Tigridieae within subtribes. C= Cipurinae, T = Tigridiinae.

GENERA	Goldblatt 1982	Celis & García (current study)
Alophia	C	T
Calydorea	C	C
Cipura	C	C
Cobana	T	T
Cypella	C	C
Eleutherine	C	T
Ennealophus	C	T
Gelasine	C	T
Herbertia	C	C
Hesperoxiphion	C	T
Larentia	C	C
Mastigostyla	C	T
Nemastylis	C	C
Salpingostylis	N/A	N/A
Tigridia	T	T

Considering our analysis, the first clade (Lineage A) comprised only genera of the subtribe Cipurinae (*Calydorea*, *Cipura, Cypella, Herbertia*, *Larentia*, *Onira* and *Nemastylis)* and the second (Clade B) corresponded to members of the subtribe Tigridiinae plus several genera previously recognized as belonging to Cipurinae (Figure 3). Additionally, the Clade B included a strongly supported monophyletic subclade N, previously called

Mexican-Guatemalan Tigridiinae by Rodriguez (1999), and an unresolved basal group formed by *Eleutherine-Gelasine* (Clade K), *Cardenanthus-Mastygostila*-Sout American *Tigridia* complex (Clade L)*, Alophia veracruzana* (Clade J)*,* and *Ennealophus* (Clade M).

This topology is consistent with previous studies (Goldblatt *et al.* 2008, Rodriguez and Systma 2006), however we found a different position for *Ennealophus* in our BI analysis. This genus is basal and sister to *Tigridia* (sensu Goldblatt and Manning 2008) in our MP tree, whereas it forms a clade with *Gelasine*, *Eleutherine* and the clade L in the BI tree (see Appendix 6).

Traditionally *Alophia*, *Cardenanthus*, *Ennealophus*, *Gelasine*, *Hesperoxiphion*, and *Mastigostyla* have been classified within the subtribe Cipurinae, as they all possess monosulcate pollen, and chromosome number n=7 (Goldblatt 1982). Our phylogenetic results complement those found by Goldblatt *et al.,* (2008), and Rodríguez and Systma (2006) and show that those genera belong to basal taxa of Tigridiinae and not in Cipurinae.

Although we have not found morphological synapomorphies that allow us to delimitate the two subtribes of Tigridieae, we propose a new circumscription based on molecular synapomorphies (see Table 6). According to this, Cipurinae includes only *Cipura*, *Cypella*, *Herbertia*, *Kelissa*, *Larentia* and *Onira*. Likewise, Tigridiinae would include *Alophia*, *Cardenanthus*, *Eleutherine*, *Ennealophus*, *Gelasine*, *Mastigostyla* (sensu lato, including *Cardenanthus* and Sout American *Tigridia*), and the genus *Tigridia* (sensu Goldblatt and Manning 2008).

The Cipurinae subtribe shows that *Cipura* and *Larentia* are related (Clade C). Although, *Cipura* is closely related to *Cypella*, the latter is considered a polyphyletic group (Rodriguez 1999, Rodriguez 2006) since some species appeared closely related to *Cipura* (*Cypella mexicana, Cypella rosei*) where as other species are closely related to *Herbertia* (*Cypella aquatilis* and others).

Including new South American taxa in phylogenetic analysis (*Cypella herbertii*, *Cypella osteniana*), and based on *Larentia* studies (Goldblatt and Celis, 2010), we can say that *Cypella* is a monophyletic group (Clade G) and related to *Herbertia*. Additionally, *Larentia* is sister to *Cipura*; currently, *Larentia* includes three species, two endemic to Mexico (*L. mexicana*, *L. rosei*) and *L. linearis*, which is distributed from Venezuela to Brazil and Bolivia. In subsequent studies, it will be necessary to include *Larentia linearis* and additional species of *Cypella* to confirm the monophyly of the genus (Goldblatt and Celis, 2010).

The genus *Onira* was placed at this rank by Ravenna (1983) who created this monotypic genus based on *Herbertia unguiculata* despite its obvious similarities to *Cypella*, *Catila*, *Calydorea* and *Kelissa*. Examples of this include the similarities with *Cypella hauthalli*, which shares morphological characters in the bulb, leaves and inflorescences. In particular the flowers of both species have spotted red-brown claws. Furthermore the filaments are filiform and free at the base, as in *Calydorea* and *Catila*; and the anthers clasping the style arms as in *Catila*. Ravenna (1983) concluded that *Onira* undoubtedly was related to *Kelissa* and *Cypella* but makes no reference to their differences with other *Herbertia* species. Later Roitman and Castillo (2007) proposed that *Kelissa* and *Onira* should be included in *Cypella* because the differences in the stamens and style branches are too trivial to define them as separate genera, and that the similarities between the flowers are sufficient to be placed in *Cypella* as *C. unguiculata.*

The inclusion of three *Herbertia* species in this study (Table 1), makes clear that *Cypella unguiculata* is nested within the *Herbertia* clade (Clade H) and contrary to the expectations of Goldtblatt and Manning (2008), *Herbertia* is not nested in *Cypella* (Clade G), but its sister group is supported with high values (Figure 3). In light of the molecular results, it is necessary to further expand the sampling within *Herbertia*, because most certainly the current *Cypella ungiculata* should be treated under *Herbertia* as *H. unguiculata*.

Despite differences in the inner tepals between the *Calydorea* species included in this study, and *Herbertia tigridioides,* they cluster in the same clade (Clade I). In *C. nuda*, *C. minima* and *C. azurea* inner tepals are subequal to the outer tepals, while in *H. tigridioides* the inner tepals are less than half long as the outer ones. Additionally, they are erect, just as they are in most *Herbertia* species (sensu stricto).

Further inclusion of species such as *Herbertia crosae* and *H. lahue*, considered close to *H. tigridioides* (Roitman and Castillo 2004), could help to better understand the relationships between *Herbertia* and *Calydorea*.

Furthermore, discovery of a clade (L) comprising South American species of *Tigridia* and numerous representatives of *Cardenanthus* plus and *Mastigostyla* supports the hypothesis that these species do not form part of the Mesoamerican *Tigridia* clade (Rodríguez and Rodríguez and Systma 2006a, 2006). Thus, Rodriguez and Systma (2006) did not misplace the South American *Tigridia*. Instead, our results confirm that they belong to the *Mastigostyla* clade, including *Cardenanthus*. In future studies it would be relevant to include additional species of *Cardenanthus* in order to know clearly whether *Cardenanthus* should be considered as a true genus and not as a synonym of *Mastigostyla* as Goldblatt and Manning (2008) concluded. For now, we propose the transfer of South American *Tigridia* to *Mastigostyla*.

4.3. Geographical distribution of Tigridieae and ancestral areas

Iridaceae has a worldwide distribution although it is especially well represented in Southern Africa as well as temperate and highland South and Central America. Evidence of this New World distribution is well exhibited by the tribe Trimezieae and its sister group Tigridieae. The latter extends from the southern United States and northern Central America to northern Chile, central Argentina and Uruguay (Rodriguez and Systma 2006). On the other hand, the Cipurinae subtribe includes endemic genera in Uruguay, southern Brazil and northern Argentina (*Gelasine*, *Herbertia*) and the Andes (*Hesperoxhiphion*, *Mastigostyla*). *Cypella* has a wide range

across temperate South America, including the West Indies. *Nemastylis* is shared between the southern United Sates, Mexico and northern Mesoamerica.

Our results indicate that diversification of Tigridieae occurred about 38,9 MA, and its ancestral region is inferred as corresponding to the north of Mexico and Mesoamerica (O area, see Table 3, Fig 5A). The Cipurinae and Tigridiinae subtribes began diversifying ca. 26,41 and 25,11 MA respectively, from their common ancestral area in Mesoamerica (area O, Fig 6) during the late Oligocene and early Miocene long before North and South America where finally joined via Central America. The ancestral lineage of the Cipurinae differentiated into two groups, the *Cipura-Larentia* (Clade C, Fig 3) and the Clade D.

The ancestor of *Cipura-Larentia* (Fig 3, node C) occured in Mesoamerica about 13 MA, later *Cipura* diverged in *C. campanulata* and *C. paludosa*) whose current distributions are in northern South America, particularly the high cordilleras of Venezuela, Colombia, Ecuador, and Peru; the Orinoco plains and Amazonia (Areas A, G, H; see Fig 5A). This migration or long distance dispersal happened long before the formation of the Isthmus of Panama around 3 MA ago.

One of our main interests in collecting South American species of *Tigridia* was to improve our knowledge on the relationship between the Mesoamerican and South American samples of the genus. Based on molecular evidence, current areas of distribution and group divergence times, we can infer there are two separate and well-differentiated lineages. The South American species of *Tigridia* diverged 15,7 MA when their ancestors inhabited the coastal Peruvian Desert and Puna provinces of Peru (see Table 3). Their current distribution is concentrated in the dry areas of Peru, Bolivia and the north of Argentina. Clade N (Fig 3, Fig 6) diverged almost simultaneously (15,3 MA), from its ancestral Mesoamerican region. Due to their similar floral characteristics, the South American species have been regarded as *Tigridia*, and a disjunt distribution has been hypothesized for the genus. However, our evidence shows that this corresponds to a morphological convergence rather than

divergence between groups, possibly correlated to their pollinators. This is remarkable given the great similarity between some *Tigridia* species from Mesoamerica (i.e. *T. multiflora*, see Fig 1, chapter.2) and South America (*Tigridia sp.*, see Fig 1, chapter 2) in the stigma and stamen arrangement, as well as tepal size and shape.

Current classification (Goldblatt 1982) recognizes a disjunct distribution of *Tigridia* (Tigridiinae) with two main areas of endemism, one in Mexico, Honduras and Guatemala, and one in southern Peru and northern Chile (Figure 5B). If we accept our molecular evidence (Figure 3) and place the South American species of the genus *Tigridia* in *Mastigostyla*, this would support that Tigridiinae (sensu Goldblatt and Manning 2008) has its center of diversification in the northern Central America and Mexico and does not include South American species. In this case *Mastigostyla* with its new circumscription would be distributed in the Bolivian Andes, the coast of Peru and their ecosystems of “Lomas” and northern Chile.

Finally, the estimation of divergence times was similar when using standard bayesian analysis and non-parametric smoothing methods. Other studies have tested the impact of different calibration scenarios (from the fossil record) under different methods (bayesian vs. NPRS). Although both methods provide similar estimates, this study suggests an important effect of using secondary calibration points, which yielded drastically younger age estimates (Sauquet *et al.* 2012). Our work used secondary calibration points, and obtained similar estimates for the different clades under different methods. Additionally these were similar to previous reports from the literature (Goldblatt 2008).

4. 4. Taxonomy and nomenclature

Based on our analysis, we propose to fix the placement of two South American species which have traditionally been placed among different genera in Cipurinae. Thus we recognize the following two species in their respective genera*, Cypella brasiliensis* and *Herbetia unguiculata.*

Cypella brasiliensis (Baker) Roitman & J.A.Castillo, Darwiniana 45: 238. 2007. Basionym: *Herbertia brasiliensis* Baker, Journal of the Linnean Society, Botany 16: 134. 1877. *Alophia brasiliensis* (Baker) Kuntze, Revisio Generum Plantarum 3(3): 305. 1898. *Trifurcia brasiliensis* (Baker) Goldblatt, Brittonia 27(4): 384. 1975. *Kelissa brasiliensis* (Baker) Ravenna, Bull. Mus. Natl. Hist. Nat., B, Adansonia 3: 106. 1981. TYPE: Brasil. "Brasilia meridionalis," s.d., *Sello 2863* (lectotype, designated by Ravenna 1981: 106, 108; B).

Herbertia unguiculata Baker, Hand. Irid.: 72. 1892. *Alophia unguiculata* (Baker) Kuntze, Revis. Gen. Pl. 3(3): 305. 1898. *Onira unguiculata* (Baker) Ravenna, Nordic J. Bot. 3(2): 204. 1983. *Cypella unguiculata* (Baker) Roitman & J.A. Castillo, Darwiniana 45: 238. 2007. TYPE: Brasil. South Brasil, *Sello* (holotype, K).

Recent treatments have placed this species in *Cypella* or *Onira* but our analysis (see Fig. 3, node H) clearly supports the placement of this species in *Herbertia*, as originally suggested by Baker (1892).

Finally, based on our molecular phylogenetic analysis and supported by the biogeographical inferences of this study, we propose the following taxonomic rearrangements and nomenclatural changes.

Calydorea tigridioides (Hicken) Celis, **comb. nov.** Basionym: *Alophia tigridioides* Hicken, Darwiniana 1: 116. 1924. *Trifurcia tigridioides* (Hicken) Goldblatt, Brittonia 27(4): 384. 1975. *Herbertia tigridioides* (Hicken) Goldblatt, Ann. Missouri Bot. Gard. 64(2): 379. 1977 [1978]. TYPE: Argentina. (Not found).

Mastigostyla albicans (Ravenna) Celis, **comb. nov.** Basionym: *Tigridia albicans* Ravenna, Revista Inst. Munic. Bot. 2: 59. 1962 [1964]. TYPE: Perú. "Cult. in Bonaria ex bulbis collectis in Andibus Peruviae australis, in declivis supra Tarartam (Tacna)", Dec 1960, *P.F. Ravenna 83* (holotype: herb. Ravenna).

Mastigostyla huyanae (J.F. Macbr.) Celis, **comb. nov.** Basionym: *Nemastylis huyanae* J.F. Macbr., Publ. Field Mus. Nat. Hist., Bot. Ser. 11(1): 13. 1931 (as '*Huyanae*'). *Tigridia huyanae* (J.F. Macbr.) Ravenna, Revista Inst. Munic. Bot. 2: 60. 1962 [1964]. TYPE: Perú. Matucna, Apr–May 1922, *Macbride & Featherstone 469* (holotype, F).

Mastigostyla minuta (Ravenna) Celis, **comb. nov.** Basionym: *Tigridia minuta* Ravenna, Revista Inst. Munic. Bot. 3(2): 29. 1969. TYPE: Perú. Ayacucho, *Ravenna 77** (holotype, herb. Ravenna).

Mastigostyla pearcei (Baker) Celis, **comb. nov.** Basionym: *Nemastylis pearcei* Baker, Handb. Irid.: 114. 1892. *Tigridia pearcei* (Baker) Ravenna, Revista Inst. Munic. Bot. 2: 60. 1962 [1964]. TYPE: Perú. Andes of Peru, Huanuco, 10,000 ft., *R. Pearce 85* (holotype, K)

Mastigostyla philippiana (I.M. Johnst.) Celis, **comb. nov.** Basionym: *Tigridia philippiana* I.M. Johnst., Contr. Gray Herb. 85: 26. 1929. TYPE: Chile. Vicinity of Aguada Grande ("Cachinal de la Costa") of Philippi, near Antofagasta–Atacama provincial boundary, *I. Johnston 5757* (holotype, GH; isotype, SGO).

Mastigostyla purruchucana (Herb.) Celis, **comb. nov.** Basionym: *Gelasine purruchucana* Herb. Bot. Mag. 66: t. 3779. 1840. *Gelasine purruchucana* var. *princeps* Herb., Bot. Mag. 66: t. 3779. 1840. *Nemastylis purruchucana* (Herb.) Benth. ex Baker, Handb. Irid.: 114. 1892. *Tigridia purruchucana* (Herb.) Ravenna, Revista Inst. Munic. Bot. 2: 60. 1962 [1964]. TYPE: Perú. Purruchuca [near Lima], *Mathews 784* (holotype, K).

Gelasine purruchucana var. *simplex* Herb., Bot. Mag. 66: t. 3779. 1840. *Tigridia purruchucana* subsp. *simplex* (Herb.) Ravenna, Revista Inst. Munic.

Bot. 2: 60. 1962 [1964]. TYPE: Perú. "*ex eodem loco*" [Purruchuca], *Mathews* (type, K).

Mastigostyla raimondii (Ravenna) Celis, **comb. nov.** Basionym: *Tigridia raimondii* Ravenna, Phytologia 64(4): 289. 1988. TYPE: Perú. Arequipa. Caravele, Altos de Atiquipa, *A. Raimondi 11652* (holotype, USM).

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6. TABLES

Table1-1: Voucher, molecular database accession and literature information for all species of Iridoideae included in this study.

Species	**Accession/ herbarium voucher or reference**	**Literature citation/or EMBL accession numbers for new sequences**						
		abr	***ITS***	**trnL intron**	***trn*L–F**	***mat*K**	***psbA***	***trnK3***
Alophia veracruzana Goldblatt & T.M. Howard	*Goldblatt 9070* (MO)	Alop_ver1	n/a	r/c	AJ409592	AJ579931	Celis	AJ579931
Alophia veracruzana Goldblatt & T.M. Howard	*Yucca Do Nursery*	Alop_ver3	R (2006)	n/a	R (2006)	n/a	R (2006)	n/a
Calydorea amabilis (Ravenna) Goldblatt & Henrich*	*Roitman 21P*	Caly_amab	n/a	Celis	n/a	n/a	n/a	n/a
Calydorea aproximata R.C. Foster*	*Roitman 35P*	Caly_appr	n/a	Celis	n/a	n/a	n/a	n/a
Calydorea azurea Klatt	*Roitman 14P*	Caly_azur	n/a	Celis	Celis	n/a	Celis	n/a
Calydorea minima Roitman & A. Castillo	*Roitman 8P*	Caly_mini	n/a	Celis	Celis	n/a	Celis	n/a
Calydorea nuda (Herb.) Baker	*Roitman 25P*	Caly_nud1	Celis	Celis	n/a	n/a	Celis	n/a
Calydorea nuda (Herb.) Baker	*Roitman 29P*	Caly_nud2	n/a	Celis	n/a	Celis	Celis	n/a
Calydorea pallens Griseb.	*San Antonio Botanical Gardens*	Caly_pal1	x	n/a	x	n/a	x	n/a
Calydorea pallens Griseb.*	*Goldblatt 9579* (MO)	Caly_pal2	n/a	r/c	AJ409596	AJ580606/7	n/a	n/a
Cardiostigma hintonii (R.C. Foster) Ravenna	*Rodríguez 4900* (IBUG)	Cardi_hint	Celis	Celis	Celis	Celis	Celis	n/a
Cardiostigma longispathum (Herb.) Baker	*Rodríguez 2794* (IBUG)	Cardi_lon1	DQ224198	Celis	Celis	Celis	R (2006)	n/a
Cardiostigma longispathum (Herb.) Baker	*Rodríguez 5213* (IBUG)	Cardi_lon2	Celis	Celis	Celis	Celis	Celis	n/a

Continued

Species	**Accession/ herbarium voucher or reference**	**Literature citation/or EMBL accession numbers for new sequences**						
		abr	***ITS***	**trnL intron**	***trn*L–F**	***mat*K**	***psbA***	***trnK3***
Cardiostigma mexicanum (R.C.Foster) Ravenna	*Rodríguez 5154* (IBUG)	Cardi_mexi	Celis	Celis	Celis	Celis	Celis	n/a
Cipura campanulata Ravenna	*Rodríguez 2710*	Cipu_cam1	x	n/a	x	x	x	n/a
Cipura campanulata Ravenna	*Rodriguez 2893*	Cipu_cam2	x	n/a	x	n/a	x	n/a
Cipura paludosa Aubl.	*Rodríguez 2644*	Cipu_pal1	n/a	x	n/a	x	x	n/a
Cipura paludosa Aubl.	*Hernrich 143*	Cipu_pal2	n/a	r/c	AJ409595	AJ579939	Celis	AJ579939
Cobana guatemalensis (Standl.) Ravenna	*Rodríguez 2831* (IBUG)	Coba_guat	DQ224202	Celis	AM940160	AM940208	R (2006)	AM940208
Cypella aquatilis Ravenna	*Castillo s.n.* (MO)	Cype_aqua	n/a	r/c	AJ409597	AJ580610/11	Celis	AJ580610/11
Cypella hautalii* subsp. *hautalii *	*Roitman 22P*	Cype_haut_haut	n/a	n/a	n/a	n/a	Celis	n/a
Cypella hautalii* subsp. *opalina Ravenna*	*Roitman 3P*	Cype_haut_opal	n/a	Celis	n/a	n/a	Celis	n/a
Cypella herberti (Lindl.) Herb. **subsp. *herbertii***	*Roitman 6P*	Cype_herb_herb	n/a	Celis	Celis	n/a	Celis	n/a
Cypella herberti (Lindl.) Herb. **subsp. *wolffhuegeli*** *	*Roitman 7P*	Cype_herb_wolf	n/a	n/a	n/a	n/a	Celis	n/a
Cypella laeta Ravenna*	*Roitman 11P*	Cype_laet	n/a	n/a	n/a	n/a	Celis	n/a
Cypella laxa Ravenna*	*Roitman 9P*	Cype_laxa	n/a	n/a	n/a	n/a	Celis	n/a
Cypella osteniana Beauv.	*Roitman 24*	Cype_oste	Celis	Celis	Celis	Celis	Celis	n/a
Cypella pabstiana Ravenna*	*Roitman 18*	Cype_pabs	n/a	Celis	Celis	n/a	n/a	n/a
Diplarrhena latifolia Benth.	*Chase 1220* (K)	Dipl_lati	n/a	Celis	AJ409600	AJ579946	Celis	x
Eleutherine latifolia (Standl.& Will.) Ravenna	*Goldblatt 9072* (MO)	Eleu_lat1	Celis	r/c	AJ409591	AJ579949	n/a	x
Eleutherinelatifolia (Standl.& Will.) Ravenna	*Rodriguez 2722*	Eleu_lat2	x	n/a	x	x	x	n/a
Ennealophus euryandrus (Griseb.) Ravenna	*Roitman 33*	Enne_eur1	n/a	Celis	n/a	Celis	Celis	n/a
Ennealophus euryandrus (Griseb.) Ravenna	*Solomon 9972* (MO)	Enne_eur2	Celis	r/c	AJ409598	AJ579950	Celis	AJ579950

Continued

Species	**Accession/ herbarium voucher or reference**	**Literature citation/or EMBL accession numbers for new sequences**						
		abr	***ITS***	**trnL intron**	***trn*L–F**	***mat*K**	***psbA***	***trnK3***
Ennealophus foliosus (Kunth) Ravenna	*Castillo s.n.*	Enne_foli	x	n/a	x	n/a	x	n/a
Ferraria crispa Burm.	*Goldblatt 9732 (MO)*	Ferr_cris	n/a	r/c	AJ409606	AJ579951	Celis	AJ579951
Gelasine elongata (Graham) Ravenna	*Roitman 23*	Gela_elo1	Celis	Celis	Celis	Celis	Celis	n/a
Gelasine elongata (Graham) Ravenna	*Goldblatt 5925* (MO)	Gela_elo2	x	r/c	AJ409587	AJ580618/9	n/a	X
Herbertia lahue (Molina) Goldblatt	*Roitman 27*	Herb_lahu	Celis	Celis	n/a	Celis	Celis	n/a
Herbertia lahue (Molina) Goldblatt subsp. ***Amoena*** (Griseb.) Goldblatt*	*Roitman 10P*	Herb_lahu_amoe	n/a	Celis	n/a	n/a	Celis	n/a
Herbertia lahue subsp. ***Intermedie*** (Ined.) *	*Roitman 5P*	Herb_lahu_inte	n/a	Celis	Celis	n/a	n/a	n/a
Herbertia pulchella Sweet	*Roitman 26*	Herb_pul1	n/a	Celis	Celis	Celis	Celis	n/a
Herbertia pulchella Sweet	*Goldblatt s.n.* (MO)	Herb_pul2	n/a	Celis	AJ409608	AJ580620/763	Celis	n/a
Herbertia tigridioides (Hicken) Goldblatt	*Roitman 14*	Herb_tigr	n/a	x	x	n/a	x	n/a
Hesperoxiphion peruvianum (Baker) Baker	*Goldblatt s.n.* (MO)	Hesp_peru	n/a	x	AJ409590	AJ579959	n/a	X
Iris versicolor L.	*Rodriguez s.n.*	Iris_vers	x	n/a	x	n/a	x	n/a
Kelissa brasiliensis (Baker) Ravenna*	*Roitman 15*	Keli_bras	n/a	Celis	n/a	n/a	n/a	n/a
Larentia mexicana (C.V.Morton & R.C.Foster) Goldblatt	*Ortiz-Catedral 212* (IBUG)	Cype_mexi	n/a	x	AM940162	AM940210	n/a	X
Larentia rosei (R.C.Foster) Ravenna	*Rodriguez 2855* (IBUG)	Cype_rose	R (2006)	Celis	AM940161	AM940209	R (2006)	AM940209

Continued

Species	**Accession/ herbarium voucher or reference**	**Literature citation/or EMBL accession numbers for new sequences**						
		abr	***ITS***	**trnL intron**	***trn*L–F**	***mat*K**	***psbA***	***trnK3***
Libertia ixioides (G. Forst.) Spreng.	*Chase I-218* (K)	Libe_ixio	n/a	r/c	AJ409601	AJ579968	Celis	AJ579968
Mastigostyla cyrtophylla I.M. Johnst.	*Sinca-Cansino 54*	Cype_cyrt	n/a	Celis	Celis	Celis	n/a	n/a
Mastigostyla hoppii R.C.Foster	*Sinca-Cansino 92*	Mast_hopp	Celis	Celis	Celis	Celis	Celis	n/a
Mastigostyla sp.	Sinca-Cansino 107	Mast_sp1	Celis	Celis	Celis	Celis	Celis	n/a
Mastigostyla sp.	Sinca-Cansino 69	Mast_sp2	Celis	Celis	Celis	Celis	Celis	n/a
Mastigostyla torotoroensis Huaylla & Wilkin	Wood 21952	Mast_toro	Celis	Celis	Celis	Celis	Celis	n/a
Mastigostyla tunariensis (R.C.Foster) Ravenna	*Roitman 30*	Carde_tuna	n/a	Celis	Celis	Celis	Celis	n/a
Nemastylis tenuis (Herb.) Baker*	*Rodríguez 2636* (IBUG)	Nema_tenu	x	x	AM940165	AM940213	x	X
Neomarica gracilis (Herb.) Sprague	*Rodríguez s.n.*	Neom_grac	x	n/a	x	n/a	x	n/a
Neomarica northiana Sprague	*Solomon 6950* (MO)	Neom_nort	x	AJ409593	x	AJ579972	x	AJ579972
Neomarica* sp.	Beck 3995	Neom_sp1	n/a	Celis	n/a	n/a	Celis	n/a
Olsynium filifolium (Gaudich.) Goldblatt	*Chase I-243* (K)	Olsy_fili	n/a	r/c	AJ409602	AJ579974	Celis	AJ579974
Onira unguiculata (Baker) Ravenna	*Chase 16592* (K)	Onir_ungi	n/a	x	AJ577606	AJ579975	n/a	X
Orthrosanthus chimboracensis Baker	*Chase I-231* (K)	Orth_chim	n/a	r/c	AJ409605	AJ579976	Celis	X
Sessilanthera citrina Cruden	*Rodriguez 2892*	Sess_citr	x	n/a	x	x	x	n/a
Sessilanthera heliantha (Ravenna) Cruden	*Rodriguez 2885*	Sess_heli	x	n/a	x	x	x	n/a
Sessilanthera latifolia (Weath.) Molseed&Cruden	*Rodriguez & Vargas 2791*	Sess_lati	DQ224197	n/a	x	x	x	n/a

Continued

Species	**Accession/ herbarium voucher or reference**	**Literature citation/or EMBL accession numbers for new sequences**						
		abr	***ITS***	**trnL intron**	***trn*L–F**	***mat*K**	***psbA***	***trnK3***
Tigridia alpestris Molseed **subsp. *alpestris***	*Rodríguez 2768* (IBUG)	Tigr_alpe_alpe	n/a	Celis	AM940166	AM940214	n/a	X
Tigridia alpestris* subsp. *obtusa Molseed	*Rodríguez & Ortiz-Catedral 3069* (IBUG)	Tigr_alpe_obtu	n/a	Celis	AM940167	AM940215	n/a	X
Tigridia convoluta (Ravenna) Goldblatt	*Ramírez 3390* (IBUG)	Nema_conv	x	n/a	AM940164	AM940212	x	X
Tigridia conzattii (R.C.Foster) Goldblatt	*Rodríguez 2948* (IBUG)	Aine_conz	n/a	Celis	AM940159	AM940206	n/a	n/a
Tigridia durangensis Molseed ex Cruden	*Rodríguez 2642*	Tigr_dura	DQ224193	n/a	x	n/a	x	n/a
Tigridia flammea (Lindl.) Ravenna	*Rodríguez 2813* (IBUG)	Tigr_flam	DQ224201	x	AM940168	AM940216	x	X
Tigridia huajuapanensis Molseed ex Cruden	*Rodriguez & Villegas* 2738	Tigr_huaj	DQ224194	n/a	x	n/a	x	n/a
Tigridia immaculata (Herb.) Ravenna	*Rodríguez* 2832	Rigi_imma	x	n/a	x	x	x	n/a
Tigridia immaculata (Herb.) Ravenna	*Rodríguez* 2890	Rigi_inus	x	n/a	x	x	x	n/a
Tigridia lutea Link, Klotzsch & Otto	*Calcinos s.n.*	Tigr_lute	x	n/a	x	n/a	x	n/a
Tigridia mexicana Molseed **subsp. *mexicana***	*Rodriguez 2805*	Tigr_mexi	DQ224200	n/a	x	n/a	x	n/a
Tigridia multiflora (Baker) Ravenna	*Rodriguez 2625*	Tigr_mult	DQ224192	n/a	x	n/a	x	n/a
Tigridia oaxacana (Molseed) Goldblatt	*Rodríguez 2747* (IBUG)	Fost_oax1	n/a	x	AM940163	AM940211	n/a	X
Tigridia oaxacana (Molseed) Goldblatt	*Rodríguez 2754* (IBUG)	Fost_oax2	DQ224196	n/a	x	x	x	n/a

Continued

Species	**Accession/ herbarium voucher or reference**	**Literature citation/or EMBL accession numbers for new sequences**						
		abr	***ITS***	**trnL intron**	***trn*L–F**	***mat*K**	***psbA***	***trnK3***
***Tigridia* sp**.	*Celis 454*	Tigr_sp1_39	Celis	Celis	Celis	Celis	Celis	n/a
***Tigridia* sp.**	*Celis 455*	Tigr_sp1_40	Celis	Celis	Celis	n/a	Celis	n/a
***Tigridia* sp.**	*Celis 456*	Tigr_sp2_43	Celis	Celis	Celis	Celis	n/a	n/a
Trimezia fosteriana Steyerm.	*Rodriguez s.n.*	Trim_fost	x	n/a	x	x	x	n/a
Trimezia martinicensis (Jacq.) Herb.	*Berry 3802* (MO)	Trim_mart	Celis	Celis	AJ409583	AJ579988	Celis	X

The species names are arranged alphabetically and include all species used as ingroup and outgroup.
C (1993) = Chase et al. (1993), R (2001) = Reeves et al. (2001a), S. (1997) = Souza-Chies et al. (1997), n/a = not available. R (2006)= Rodriguez &Systma (2006).

* Species excluded from the Comb7 analysis, for having less than 3 sequences available

Table 2-1: Individual Data sets (6) and Combined data sets (10)* analyzed under Maximum parsimony for taxa of the Subfamily Iridoideae.

*** Combination 1:** all six genes**; Combination 2:** includes all species which have at least trn L_Fsequences; **Combination 3:** includes all species which have at least ITS sequences; **Combination 4:** includes all species which have at least matK sequences; **Combination 5:** species with sequences of at least 4 of the 6 genes; **Combination 6:** the species with sequences at least of 5 to the 6 genes**; Combination 7**: the species with sequences at least of 3 to the 6 genes; **Combination 8:** include all the species with at least trnL intron sequences; **Combination 9**: the data set used is without trnkk3 genes, but at least have 3 of 5 sequences of genes, **Combination 10:** only plastid DNAS.

		Total Characters	Total Taxa	Variable Characters and percentage		P-inform Characters and percentage		MP trees	MP steps	CI	RI	RC
Individual Data Sets	*ITS*	582	46	249	43%	138	24%	8026	516	0.676	0.757	0.512
	psbA	488	68	96	20%	54	11%	100000	127	0.890	0.875	0.779
	matK	1639	55	427	26%	227	14%	100000	621	0.773	0.837	0.647
	trnK3	189	24	65	34%	36	19%	4	90	0.867	0.915	0.793
	trnLintron	503	60	110	22%	48	10%	100000	141	0.865	0.909	0.786
	trnL_F	378	70	114	30%	70	19%	8023	168	0.786	0.897	0.705
***Combined Data Sets**												
Comb 1	*ITS+psbA+matK+trnKK3+trnLintron+trnL_F*	3779	85	1061	28%	573	15%	1000000	1741	0.734	0.804	0.590
Comb 2	*All with trnL_F*	3779	70	1030	27%	542	14%	100000	1680	0.739	0.797	0.589
Comb 3	*All with ITS*	3779	46	733	19%	361	10%	5356	1160	0.747	0.759	0.567
Comb 4	*All with matK*	3779	55	979	26%	502	13%	2040	1517	0.758	0.798	0.605
Comb 5	*4 of 6*	3779	51	969	26%	494	13%	408	1505	0.763	0.796	0.607
Comb 6	*5 of 6*	3779	29	875	23%	383	10%	16	1270	0.791	0.741	0.586
Comb 7	*3 of 6*	3779	72	1044	28%	551	15%	528689	1715	0,734	0.797	0,585
Comb 8	*All with trnLintron*	3779	60	964	25%	478	13%	100000	1446	0.775	0.805	0.623
Comb 9	*3 of 5 w/o trnkk3*	3590	60	979	27%	515	14%	597126	1622	0.723	0.785	0.567
Comb 10 (chloroplasts)	*psbA+matK+trnKK3+trnLintron+trnL_F*	3197	60	780	24%	402	13%	1000000	1140	0.778	0.828	0.644

Tabla 3-1. Geographical areas used in the biogeographical analyses.

Areas following Morrone (2001a, 2001b, 2006)

Area code	Description	Circunscription
A	North Andean Paramo Province	High cordilleras fo Venezuela, Colombia, Ecuador, and Peru, above 3000 m altitude
B	Coastal Peruvian Desert province	Narrow strip along the Pacific Ocean coast, from northern Peru to northern Chile
C	Puna province	Bolivia, northern Argentina and Chile, and southern Peru
D	Atacama province	Northern Chile, between 18 and 28 south latitude
E	Prepuna province	Central and northwestern Argentina, from Jujuy to northern Mendoza
F	Monte province	Central Argentina, between 24 and 43 south latitude, from Salta to northeastern Chubut
G	Orinoco plains	Low areas in the north western side of Colombia an Venezuela.
H	Amazonian	Areas related to valley of the Amazon River that comprises the countries of Brazil, Bolivia, Venezuela, Colombia, Peru and Ecuador
I	Chacoan	This region comprises areas of Argentina Northern zone, Bolivia, Brazil Uruguay and Paraguay between the rivers Paraguay an Paraná and the Andean altiplan.
J	Parana	North of Paraguay and South of Brazil, valley around the Parana river.
K	Andean region Central Chilean	Andean region Central Chilean
L	Patagonian	Region located at the southern end of South America between Argentina and Chile, comprises the southern section of the Andes Cordillera.
M	Subantartic	South Western area of South America in Argentina
N	Septentrional Caribbean	Plains of the South America septentrional caribbean that includes Chocó biogeographic region, Panama and Costa Rica.
O	Central america	Comprises the South West area of United States, North of Mexico and Mesoamerica.
P	Gulf of Mexico	Areas from the South West coast of the United States, low basin of the Mississipi river, Florida and the Gulf of Mexico.
Q	Insular America (Antilles)	Insular America, Antilles.

Table 4-1. Calibration points used for estimation of divergent times in different clades of the Iridoideae.

All correspond to Indirect estimates from Goldblatt (2008).

(Most Common Recent Ancestor) Calibration Point	Age (Million Years: MA)
Libertia ixioides – Orthrosanthus chimboracensis	22
Cypella rosei – Trimezia martinicensis	35
A.*Calydorea azurea-Cypella rosei*	25.36
B.*Alophia veracruzana 1-Ainea conzati 1*	23.57
C.*Cypella rosei-Cipura campanulata* 1	12.86
F.*Cypella aquatilis-Onira*	15.71
H.*Herbertia lahue-Onira*	10.35
K.*Eleutherine latifolia 1-Gelasine elongate*	11.43
N.*Cobana guatemalesnis-Ainea conzatti*	13.93
X.*Tigridia alpestris obtusa-Tigridia flamea*	5

Table 5-1: Monophyletic status supported on different combinations of genes for different clusters within the Subfamily Iridoideae.

The reference topology corresponds to Combination 7, given the largest taxonomic sampling and number of genes sequenced*. (✓) means the combination supports a given monophyletic arrangement, (No) it does not support it. (Nd) means there is no data to support that given arrangement, e.g. only one taxon was present

*** It is possible that a given clade may have fewer taxa than the reference in Comb 7, or taxa not included in Comb 7**

Monophyletic Group	Combination									
	1	2	3	4	5	6	7	8	9	10
Tribe Tigridieae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Subtribe Cipurinae (Clade A)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Clade D	no	✓	✓	✓	✓	no	✓	✓	✓	✓
Clade C	no	✓	✓	✓	✓	no	✓	✓	✓	✓
Subtribe Tigridiinae (Clade B)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Clade N	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Clade M	✓	✓	✓	✓	nd	nd	✓	✓	✓	✓
Clade L	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Clade K	✓	✓	✓	✓	✓	✓	✓	✓	✓	nd
Clade J	✓	✓	nd	nd	nd	nd	✓	nd	✓	nd
Tribe Trimezieae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Tribe Sisyrinchieae	✓	✓	nd	✓	✓	✓	✓	✓	✓	✓
Tribe Irideae	✓	✓	nd	nd	nd	nd	✓	nd	✓	nd
Tribe Trimezieae sister group of Tigridieae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Alophia basal to Clade B (Tigridinae)?	Y	Y	Y	N	N	Y	Y	Y	Y	N

Table 6-1: Molecular synapomorphies supporting different clades within the Tribe Tigridieae.

Clade	No. Synapomorphies	TV	TI
A (Cipurinae)	13	10 (MatK: 1, trnKK3; 9)	3 (Matk:3)
B (Tigridinae)	6	3 (Matk:3)	3 (ITS: 1, Matk: 2)
C (Cipura-Larentia)	4	2 (MatK:1, ITS:1)	3 (MatK:3)
D (Calydorea-Cypella-Herbertia)	7	5 (ITS: 1, MatK 4)	2 (Matk:2)
E	1	-	1 (ITS:1)
K (Eleutherine-Gelasine)	1	1 (ITS:1)	-
L (Mastigostyla-Tigridia)	2	1 (trnL-F:1)	1 (Matk:1)
N (Tigridiinae sensu stricto)	14	4 (MatK: 3, trnKK3: 1)	10 (ITS: 1,MatK 9)
Tribe Tigridieae	1	1 (MatK: 1)	-

Table 7-1. **Estimates of Times of Divergence (MA) for different clades within the Subfamily Iridoideae based on Combination 7.**

Analysis includes standard Bayesian calculations with BEAST (Drummond *et al.,* 2007) and NPRS (non parametric rate-smoothing) using r8s (Sanderson 2003). For the NPRS analysis MP and ML starting trees were evaluated. Goldblatt *et al.,* 2008 estimates are included for comparison.This table summarizes the results with 4 calibration points *. Other detailed analysis with more calibration points are in appendix 5.

*Calibration points (Lib-Orth=22 MA Liberthiaixioides – Orthrosanthus chimboracensis), (A=25,32 MA Calydorea azurea-Cypella rosei), (B=23,57 MA Alophia veracruzana 1-Ainea conzati 1), (N=13,93 MA Cobana Guatemalesnis-Aineaconzatti**.**

Clade	Goldblatt et al., 2008	RELAXED CLOCK BEAST		NPRS WITH r8s STARTING TREE: ML				NPRS WITH r8s STARTING TREE: MP	
		Lib-Orth	95% credibility intervals	A	B	N	*Lib-Orth*A,B, N	A	B
Subfamily Iridoideae	62	52,07	25,58-86,06	63,98	62,31	59,55	63,27	67,07	59,01
Trimezieae-Tigridieae	35	40,97	21,65-70,27	41,58	40,5	38,7	41,04	43,57	38,33
Tribe Tigridieae	30,71	38,91	19,89-67,14	38,95	37,93	36,25	38,4	38,84	34,17
Subtribe Cipurinae CLADE A	25,36	26,41	12,72-46,72	25	24,35	23,27	24,63	25	21,99
SubtribeTigridinae CLADE B	23,57	25,11	13,03-43,16	23,62	23	21,98	23	26,14	23
Clade C	12,86	12,59	5,01-24,01	12,15	11,83	11,31	11,97	11,36	10
Clade D	20	20,69	9,54-35,58	20,3	19,77	18,9	20	19,42	17,08
Clade N	13	15,21	7,31-26,78	13,97	13,6	13	13	12,95	11,39
Clade O	12,86	12,75	6,06-22,01	11,54	11,24	10,74	11,01	10,55	9,29
Clade L	No	15,7	7,76-27,04	14,88	14,49	13,85	10,37	11,99	10,55
Clade K	11,43	12,12	4,48-21,99	12,84	12,5	11,95	12,55	9,79	8,61
Clade X	5	5,26	1,78-10,73	5,69	5,55	5,3	5,55	2,51	2,2

7. FIGURES

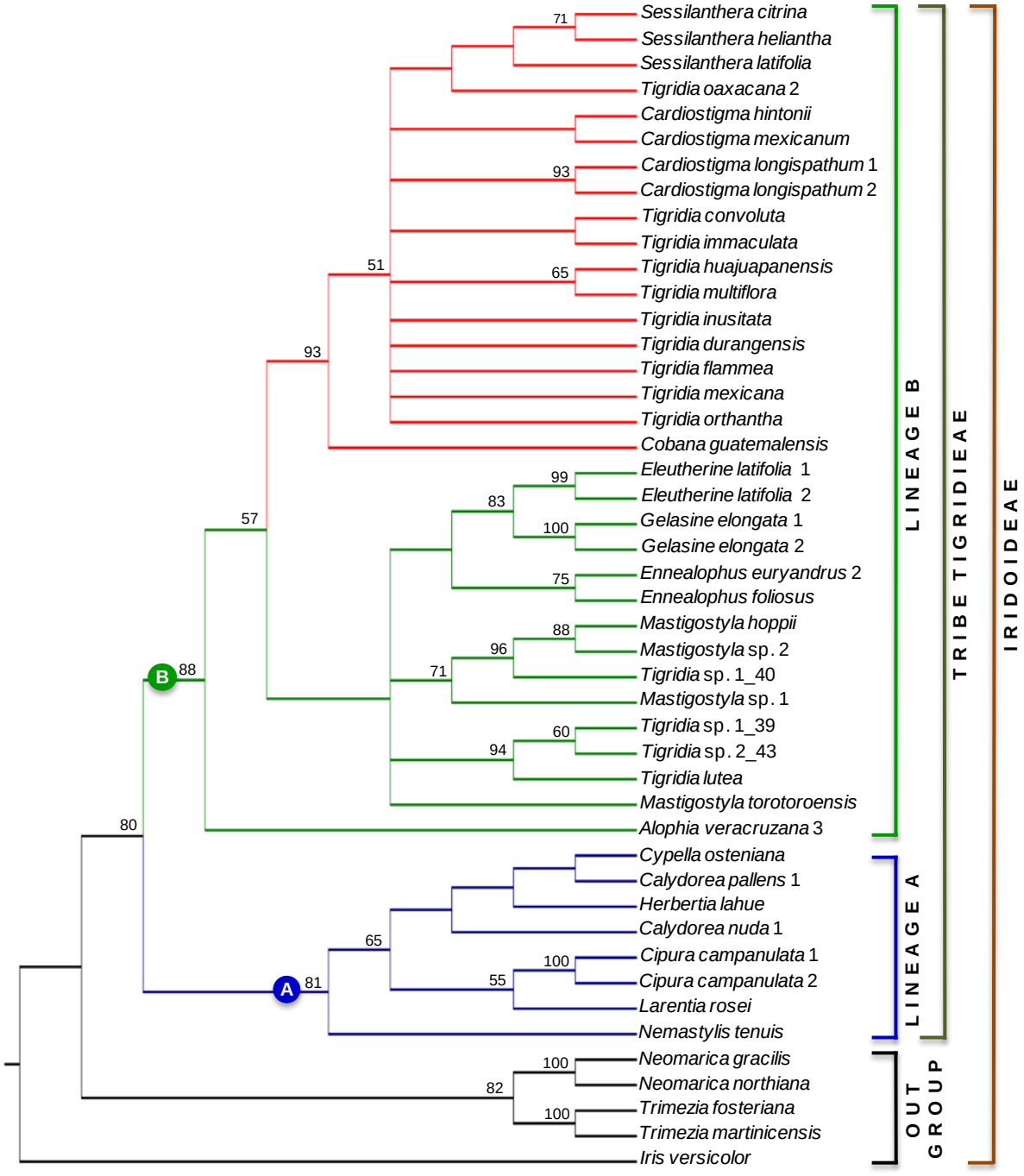

Figure 1.1: Phylogenetic relationships among members of the Tribe Tigridieae (Subfamily Iridoideae) based on nuclear sequences of the ITS gene.

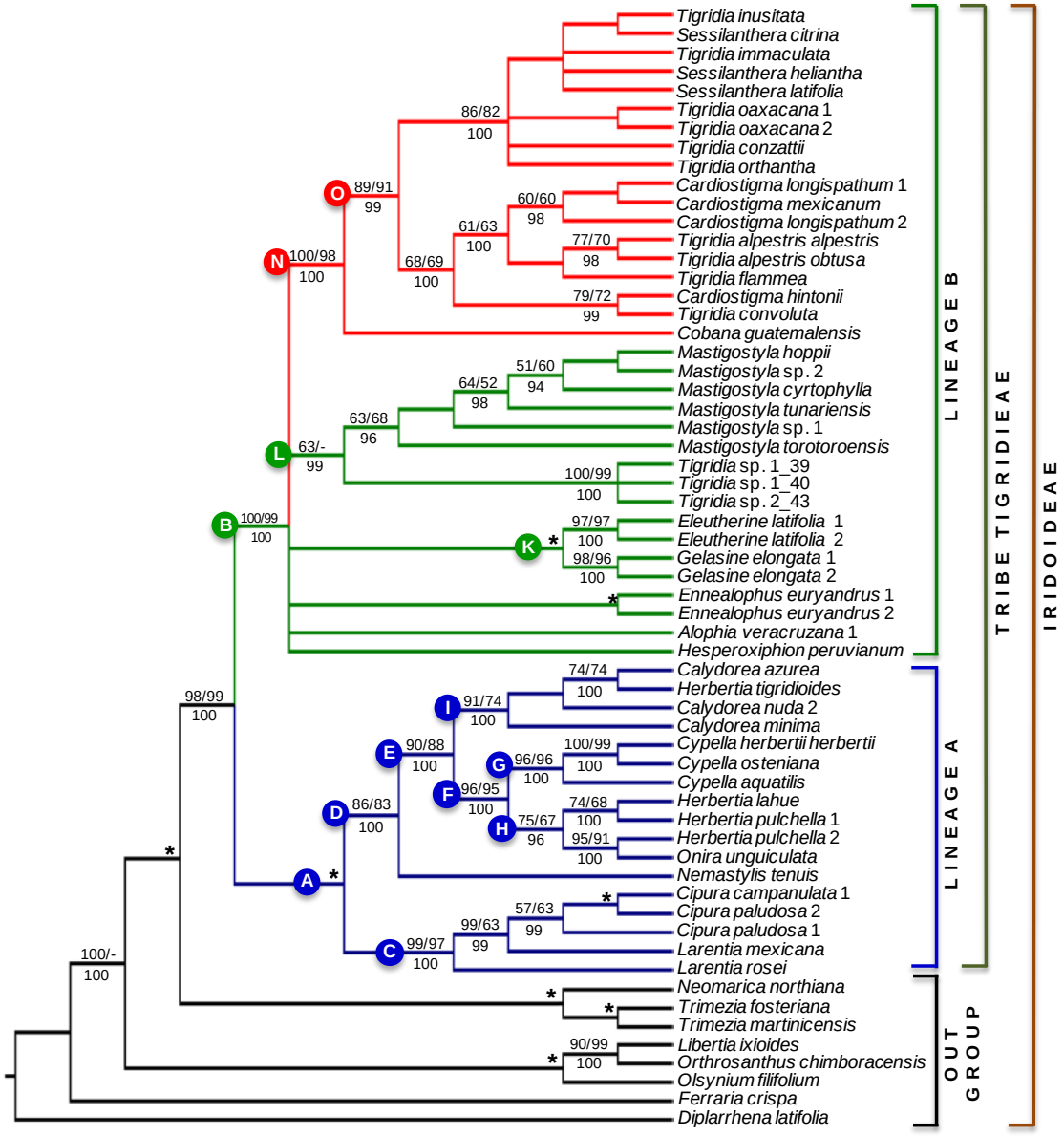

Figure 2.1: Phylogenetic relationships among members of the Tribe Tigridieae (Subfamily Iridoideae) based on Chloroplast sequences from Combination 10 (genes MatK, psbA, TrnKK3, trnL.F, and trnLintron).

Numbers on the nodes correspond to bootstrap replicates under under maximum likelihood (100) / under parsimony (100). On the bottom the values correspond to posterior probabilities under a Bayesian Inference analysis. An asterisk means that the support under the three methods were the maximum (100 % for the bootstrap analysis or posterior probability of 1 for the Bayesian analysis). The hyphen means that the obtained support was lower than 50 for the bootstrap or 0.5 por the bayesian Inference.

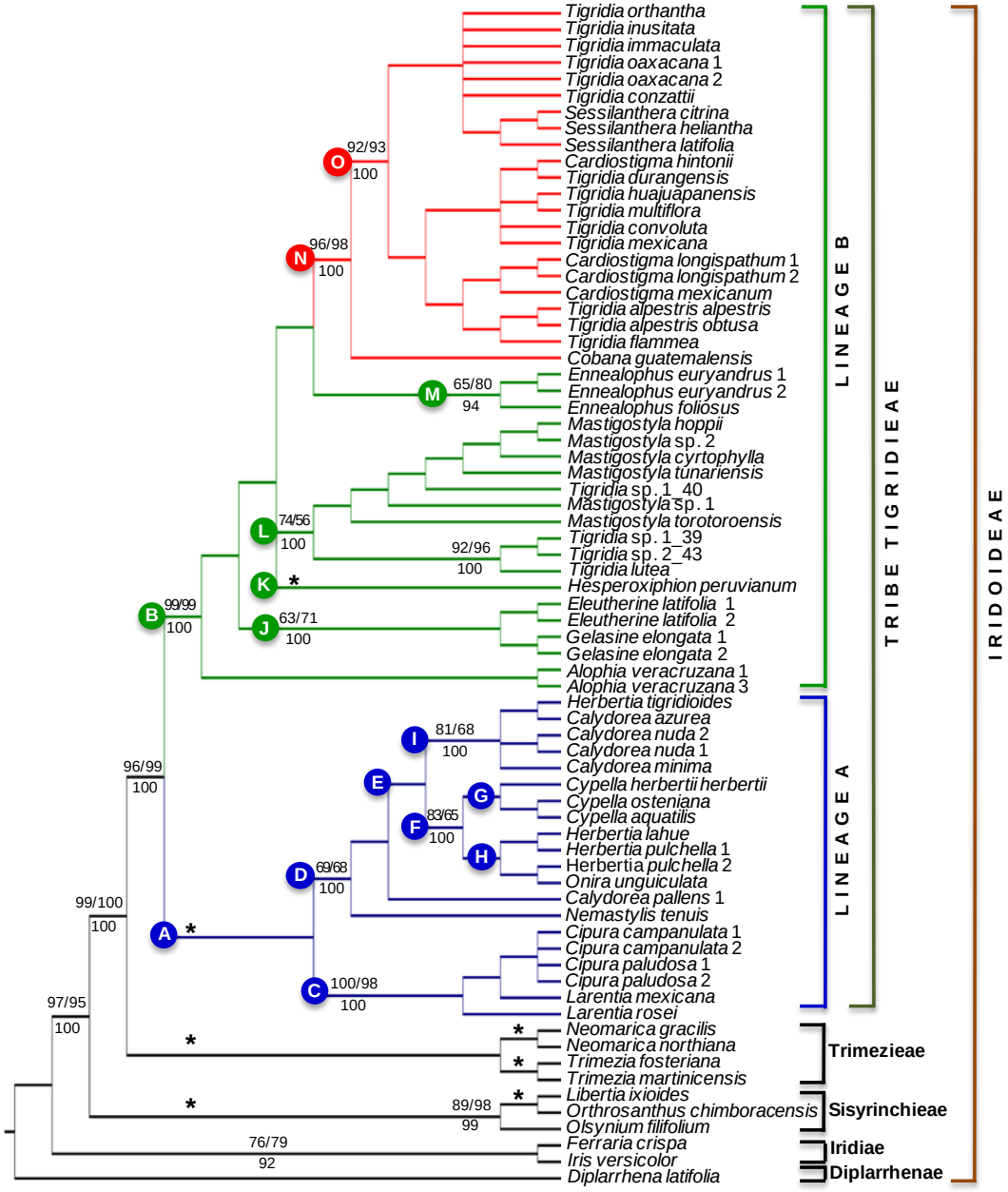

Figure 3.1: Phylogenetic relationships among members of the Tribe Tigridieae (Subfamily Iridoideae) based on sequences from Combination 7 (3 of 6 genes).

Support on the values correspond to bootstrap replicates under parsimony (100) / under maximum likelihood (100). On the bottom the values correspond to posterior probabilities under a Bayesian Inference analysis. An asterisk means that the support under the three methods were the maximum (100 % for the bootstrap analysis or posterior probability of 1 for the Bayesian analysis).

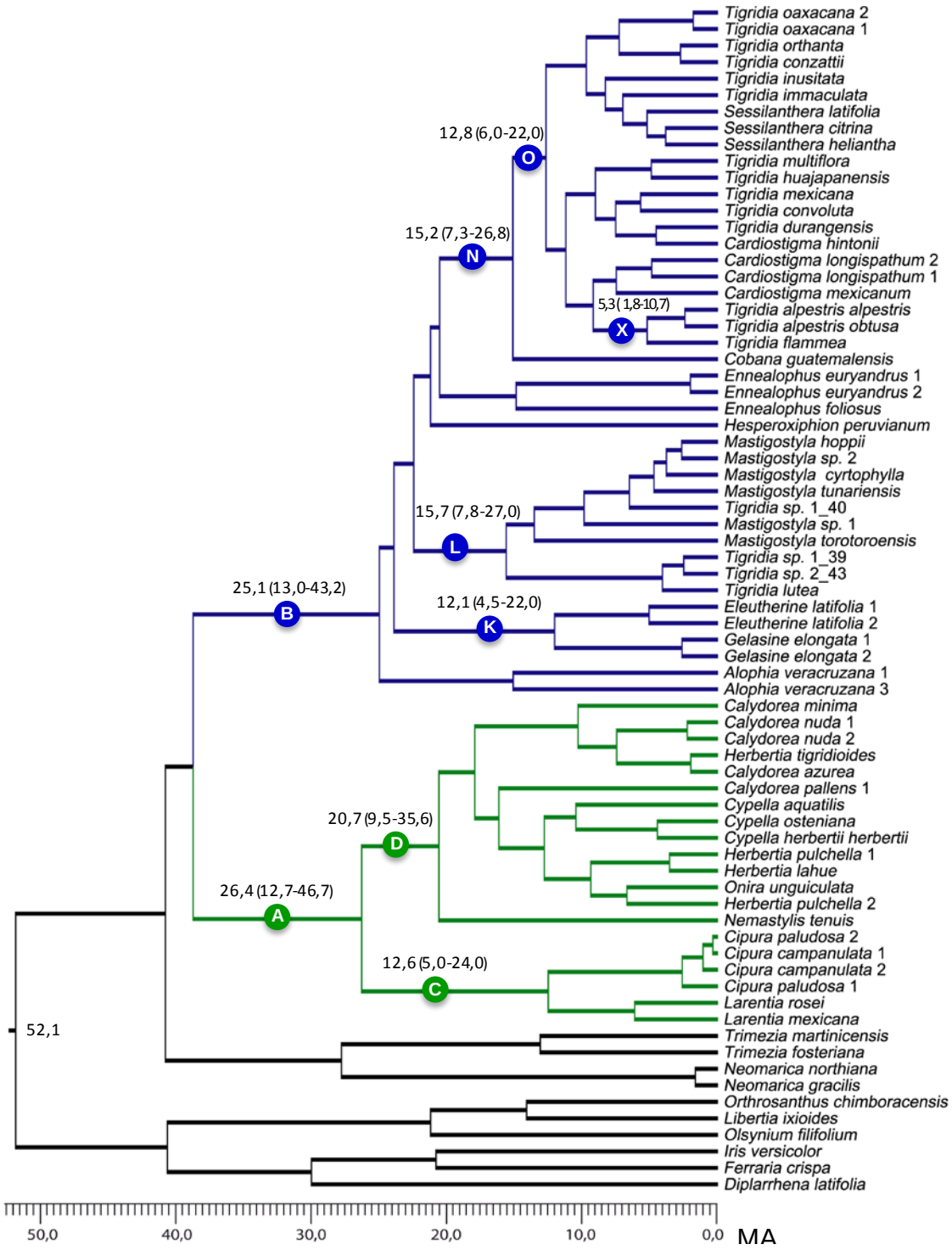

Figure 4.1: Times of Divergence for the main clades of Iridoideae based on Bayesian inference reconstruction. Scale bar in MA.

Figure 5-1 A: Geographical Areas distribution of Tigridieae.

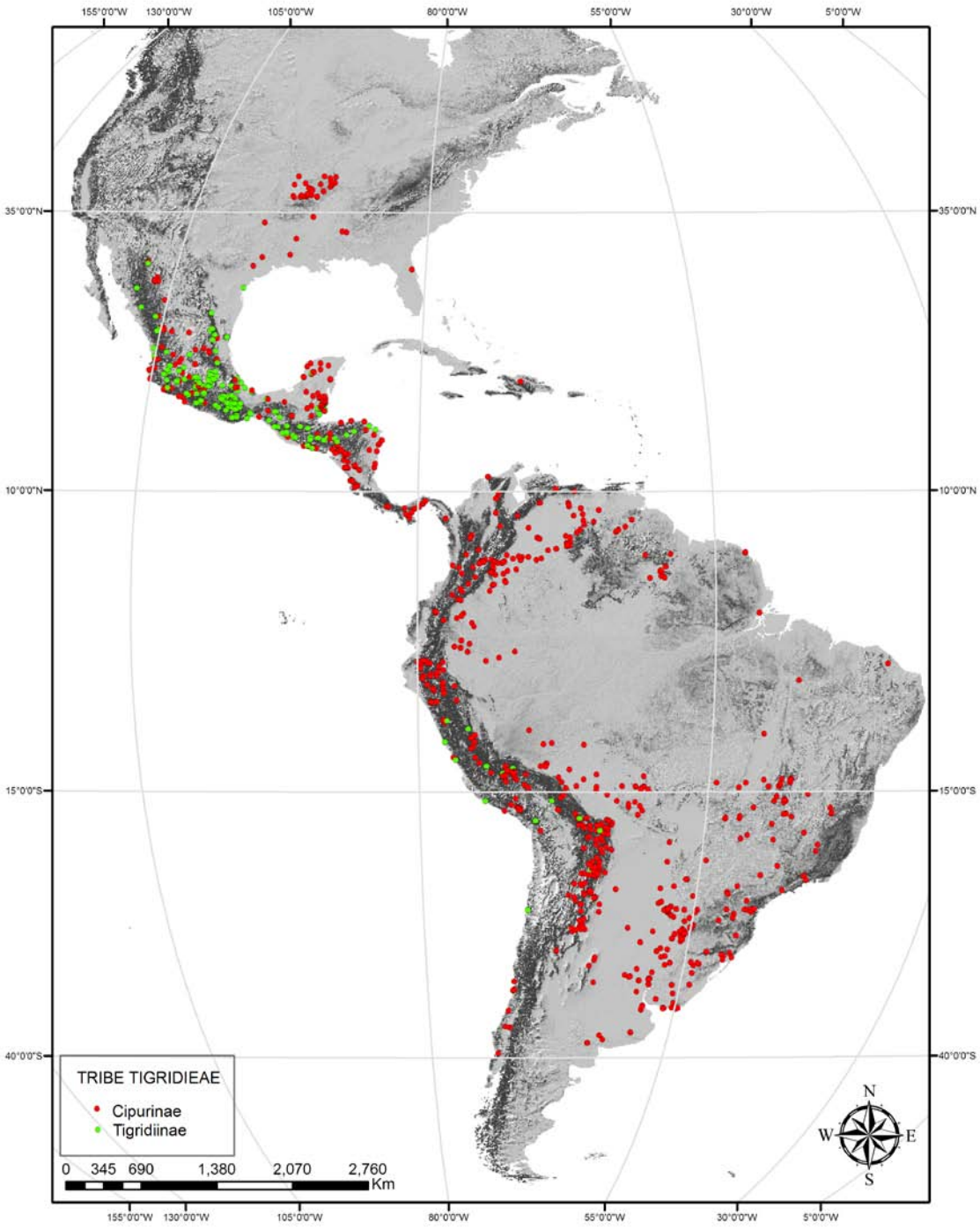

Figure 5-1 B: Geographical Distribution of Tigridieae based on current classification of the subtribes (GOLDBLATT 1982).

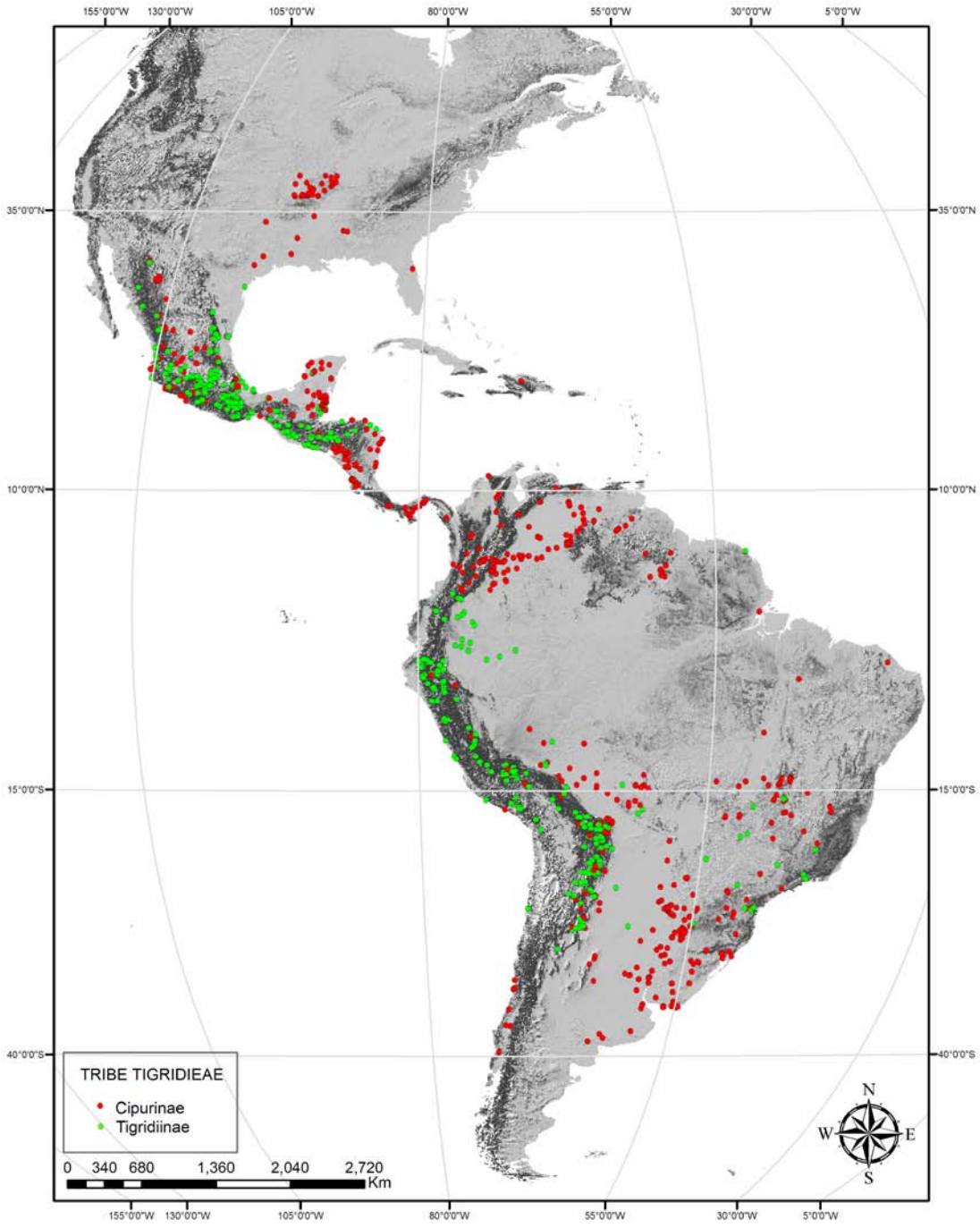

Figure 5-1 C: Geographical Distribution of Tigridieae based on new circumscription suggested in this study.

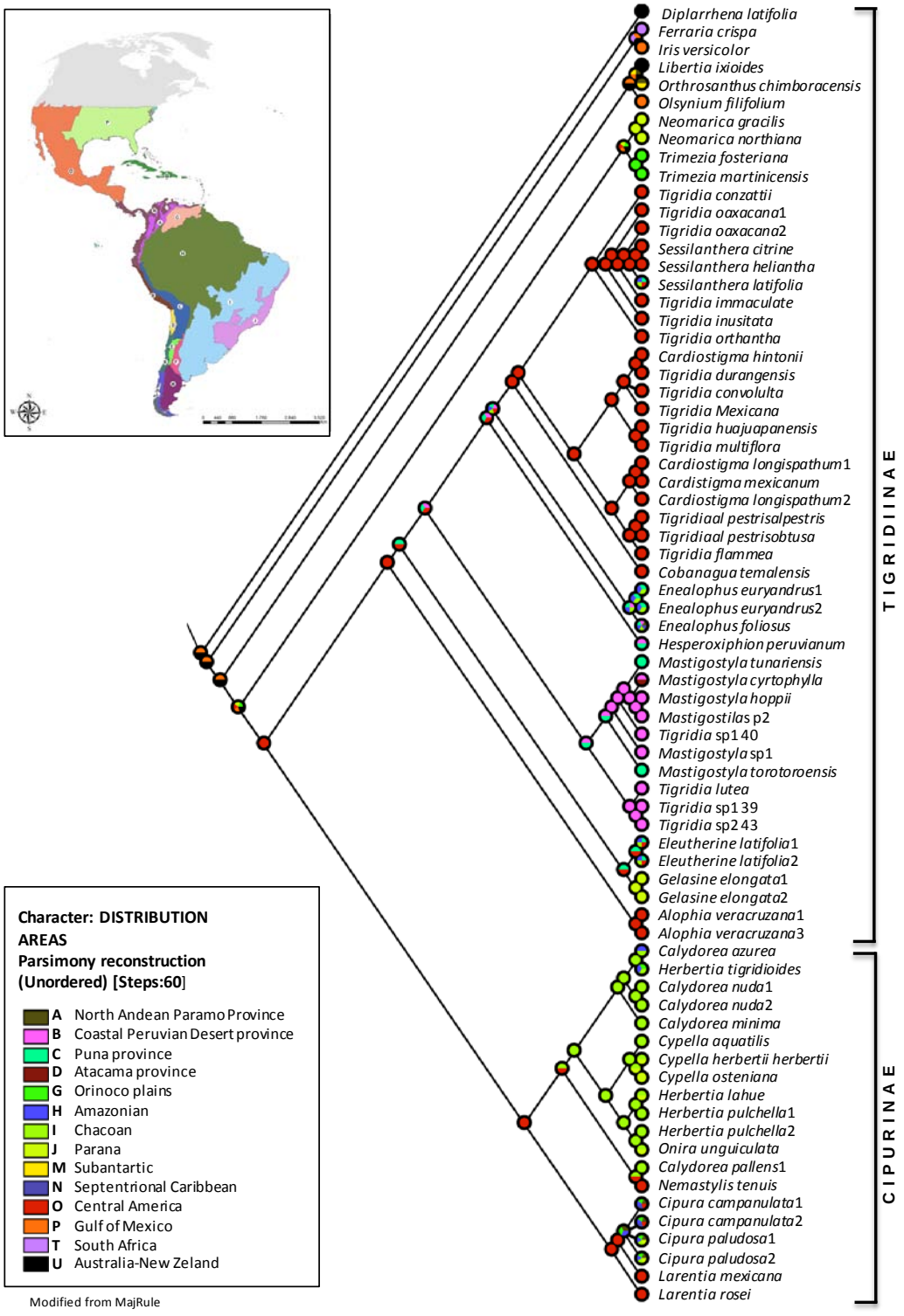

Figure 6-1: Optimization of Distribution in the Tigridieae.

Appendix 1-1 A: ITS sequence alignment for 46 taxa of the Iridoideae Subfamily. Only variable sites are included (249 out of 582 in the matrix) (Mega 5). Numbers on the top are the specific positions on the gene. A dot means same nucleotide relative to the first sequence used a reference. The hyphen means a gap.

```
[                                                                                      11111111111111111111111111111111111111111111111111111111]
[                   1111111222233333444444444555555555666666667777777888889990000000001122333333344455555666666667777777788888]
[           35678123457834783456901234568912345678912357890123478015676790134567890104013567128346890123567901234579034567]
Iris_vers   GGAACA-ACCATC-CCACGTCAGCACTT-TCGTCCCGC-CTCGGCGCACCCGTCGGGTCCGCGCGGCGGCACGCCGGTGGCCA---------TC-TACCGGC--------
Neom_grac   .AC...-..G...-A...AC..A.CT---A.......T-.C..A...C....G.C.T...A.......A.G-.....C.C...CGCCGGAGC..G.T..T..GACGGGCG
Neom_nort   .AC...-..G...-A...AC..A.CT---A.......T-.C..A...C....G.C.T...A.......A.G-.....C.C...CGCCGGAGC..G.T..T..GACGGGCG
Trim_fost   .AC...-..G...-A...T.T.ATTT---GA.C....T-.......TCATT...C......TA.T....T.G.T..A.CT...CGTTGAAGC.TG.TA.TT.GGCGGGTG
Trim_mart   .AC...-..G..ACAT..T.T.ATTT---AA.C..T.T-....AT.TCATGA..CT.....TAAT.T....G....A.CTT..CGTAAGAGC.TG.T..TT.GGCGGGTG
Alop_ver3   .AC..C-.AG.CA-A...CC....CTG.G...CG....-........C......T..C......CC....CA..GCC.....CCGTACGGGC..GCT...A.-ACGCGCG
Caly_nud1   .AC...-.AG...TA.......A..-.ACGGCC....-........C......C.TC.....T.C...N.-..........CATCGCGGC..N.C...A.AGCGGACG
Caly_pal1   .AC...-.AG...-A.G.....A.C-GACGGCC.....-.C.....AC......C.TC.....A.C.....-..........CATCACGGC..GCC...A.GGCGGGCA
Card_hint   .AC.T.-.AG.CA-AA..CC....CTG.CG..A.....-..........T....C..C.......A.....A.........T.CACAGGGGC..GNT...AGGGCGCGCG
Card_lon1   .AC.T.-.AG.CA-A...CC....CTGCCG........-...............C..C.......A.....A..........CACAGGGGC..GCT...A.GGCGCGCG
Card_lon2   .AC.T.-.AG.CA-A...CC....CTGCCG........-...............C..C.......A.....A..........CACAGGGGC..GCT...A.GGCGCGCG
Card_mexi   .AC.T.-.AG.CA-AT..CC....CTGCCG........-...............C.TC.......A.A...A..........CACAGGGGC..GCT...A.GGCGCGCG
Cipu_cam1   .AC...-.AG...-A.......A.C-GACGGCC.....-.C...-------...-.AC.............-..........CGACGCGGCGGCCC.A.A.GGCGGGCG
Cipu_cam2   .AC...-.AG...-A.......A.C-GACGGCC.....-.C...-------...-.AC.............-..........CGACGCGGCGGCCC.A.A.GGCGGGCG
Coba_guat   .AC.T--.AG..A-A...CC....CTGCCG........-......T...T....CT...............A..........GGCATGGGC..GCT..T..GGGACGCG
Cype_oste   .AC...-.AG...-A.......A.C-GACGGCC.....-........C......C.TC.......T.....-..........CA-ATCGCGCGACG..C-.AGCGGACG
Cype_rose   .AC...-.AG...-A.......A.C-GACGGCC.....C.....------------------------------------------------------------GGCGGGCG
Eleu_lat1   .AC..GA.AG.CA-A...TC....CTGCCG..C..T..-........T.T...TC.CC.GA..........A..........CGCAGGGGC..GCT....------CG
Eleu_lat2   .AC..GA.AG.CAAA...TC....CTGCCG..C..T..-..........T...TC.CC.GA..........A..........CACAGGGGC..GCT....------CG
Enne_eur2   .AC..G-.AG.CA-A...CC....CTGCCG........-...............CN.C.............A..........CACAGGGGC..GCT.....GGCGCGCG
Enne_foli   .AC..G-.AG.CA-A...CC....CTGCCG........-........G......C..C...T.........AA...A.....CGCAGGGGC..GCT.....GGCGTGCG
Fost_oax2   .AC.T--.AG.CA-A...CC....CTG.CG........-...............C..C.......A.....A..........CGCAGGGGC..GCT...A.GGCGCGCG
Gela_elo1   .AC..GATAG.CA-A...TC....CTGCCGT.C....T-..........T....-T.C...AC..A-....A..........CACAGGGGC.TGCC....------CG
Gela_elo2   .AC..GATAG.CA-A...TC....CTGCCGT.C....T-..........T....T.CC-..AC..A-....A..........CACAGGGGC.TGCC....------CG
Herb_lahu   -------------------------------------------------------------------------------------------------------------
Mast_hopp   .AC..G-.AG.CA-A...CC.....TGCA.....T..T-..T............C..CT............AN.........CACAGGGGC..GCT...A.GGCGCGCG
Mast_sp1    .NC..GT.AG.NA-N...CC....CTGCC..N...N.TA...............C..C.............A..........CACAGGGGC..GCT...A.GGCGCGCG
Mast_sp2    .AC..G-.AG.CA-A...CC.....TGCA.....T..T-..T............C..CT............A..........CACAGGGGC..GCT...A.GGCGCGCG
Rigi_inus   .AC.T--.AG.CA-A...CC....CTG.CG........-...............A..C.......A................CATAGGGGC..GCTA..A.GGCGCGCG
Sess_citr   .AC.T--.AG.CA-A...CC....CTCCCG........-...............C..C.......A....CA..........CACAGGGGC..GCT...A.GGCGCGCG
Sess_heli   .AC.T--.AG..A-A.G.CC.T..CTCCCG........-...............C..C.......A.....A..........CACAGGGGC..GCT...A.GGCGCGCA
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Sess_lati   .AC.T.-.GAC-A-A...CC....CTC.CG........-..............C..C.......A.....A...........CGCAGGGGC..GCT...A.GGCGCGCG
Tigr_dura   .AC.T.-.AG.-A-A...CC....CTGCCG........-...C..........C..C.......A.....A....A......CGCAGGGGC..GCT...A.GGCGCGCG
Tigr_flam   .AC....-.AG..G-A...CCA...TTGCCG........-T.............C..C.......A.....A...........CACAGCGGC..GCT...A.GGCGCGCG
Tigr_huaj   .AC.T.-.AG.CA-A...CC....CTGCCG........-T.............C..CT......A.....A...........CGCAGGGGC..GCT...A.GGCGCGCG
Tigr_lute   .AC..G-.AG..A-A...CC....CTGCCG........-..............C..C.....A.......A...........CACAGGGGC..GCT...A.TGCGCGCG
Tigr_mexi   .AC.T.-.AG.CA-A...CC....CTGCCG........-..............TC..C.......A.....A...........CGCAGGTGC..GCT...A.GGCGCGCG
Tigr_mult   .AC.T.-.AG.CA-A...CC....CTGCCG........-T.............C..C.......A.....A...........CGCATGGGT..GCT...A.GGCGTGCG
Tigr_orth   .AC.T.-.AG..A-A...CC....CTGCCG........-..............C..C.....T.A.....A...........CGCATGGAC..GCT...A.GGCGCGCG
Tigr_sp1_39 .-C..G-.AG..A-A...CC....CTGCCG........-..............C..C.....A.......A...........CACANGGGC..GCT...A.TGCGCGCG
Tigr_sp1_40 .AC..G-.AG.CA-A...CC.....TGCA.....T..T-..T............C..CT............A...........CACAGGGGC..GCT...A.GGCGCGCG
Tigr_sp2_43 .AC..G-.AG..A-A...CC....CTGCCG........-..............C..C.....A.......A...........CACATGGGC..GCT...A.TGCGCGCG

[           1111111111222222222222222222222222223333333333333333333444444444444444444444444444444444444455555555555555]
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[           8901245689012346780678902385670057851319534567848901458663892670149015723463567890161267890678901367804670139 0]
Iris_vers   ----------------------GTTCTTTCCAGGTGCTAGCGCCCGTCCTCCGCCGGTCCCATCCGCGCCCCA-CTCGTGCCCCGTGCGGTCGTCGCCGGCGCGTGA-
Neom_grac   ACGAGGAGCTCG-C-CGA-TACG-T.C.G.....------.....AT.C.......C.........T....-.CA.C........C........TC.......CG
Neom_nort   ACGAGGAGCTCG-C-CGA-TACG-T.C.G.....................AT.C.......C.........TN...-.CANC.....-...C........TC........G
Trim_fost   AGGAGGGTTTCG-C-CGA-TAAG-T...G.....................AT.C.........T.......T....-A.A.C.T..T..A.C.....T...C.......CG
Trim_mart   AGGAGGATTTCG-C-TGA-TAAG-T..TG.....................AT.C.T........T.G.T...T....-ACAAG.T..T..ATC.........C........G
Alop_ver3   AGCCGGAGCTCGCC-CGGCTACG-.C.....----------.....AT.C.......C...G.....AT.....-.CA.C....T....C.........C........G
Caly_nud1   AGGAGGGGCTCG-C-CGA-TACA-....A.....................AT.C.......C.........T.....-.CAAC.....A...C...T..N..NA...A...A
Caly_pal1   AGGAGGGGCTCA-C-CGA-CATA-....A.....................AT.C.......C.........AT.....-.CA.C.........C.......A.CA.......G
Card_hint   AGCCGAANCTCGTC-CGA-TACG-.........................AT.CT......C.........T.....-.A......T....C.....T...CA.A.....G
Card_lon1   AGCCGGAGCTCGTC-CGA-TACG-.........C...T.C........AT.CT......C.........T.....-.A......T....C.........CA.A.....G
Card_lon2   AGCCGGAGCTCGTC-CGA-TACG-.........C...T.C........AT.CT......C.........T.....-.A......T....C.........CA.A.....G
Card_mexi   AGACCGAGCTCGTC-CGA-TACG-T.........................AT.CT......C.........T.....-.A......T....C.....T...CA.A.....G
Cipu_cam1   AGGAGGGGCTCGCACCAAATACA-.........----------.....AT.C.......C-.....A.T.T.....-.CA.C.........C....C.C..CA.......G
Cipu_cam2   AGGAGGGGCTCCCACCAAATACA-..........T----------.....AT.C.......C.....A.T.T.....-.CA.C.........C....C.C..CA.......G
Coba_guat   ATCCGGA-CTCGCC-CGA-TACG-.........................AT.CT.....TC.........T.....-.CA......T....CA........CA.A.....G
Cype_oste   AGGAGGGGGCAGC--CGA-CATA-....A.....................AT.C.......C.........T.....-.CA.C....T....C.......T.CA.......G
Cype_rose   AGGAGCGGCGCGCCCCCAATGCCT..C...C.A.TTACA--........AT.C.......CG...C.....T.....-.CA.C.........C.........CA....C..G
Eleu_lat1   CGCCGGAGCTCGTC-CGA-TACG-.............C..........AT.C.......C.........T.....-.CA.C....T....C.........C.......CA
Eleu_lat2   CGCCGGAGCTCGTC-CGA-TACG-.............N..........AT.CT.N....C.......C..T....C.CATC.G.TT....C-------------------
Enne_eur2   AGCCGGGGCTCGTC-CGA-TACG-.........................AT.C..............-.ATN...-.CA.C....TN...C...-...-.C....-.T.T
Enne_foli   AGCCGGGGCTCGTC-CGA-TACG-.........................ATTC....T....T........T....-TCA.CA...TT...C..T......CA.......T
Fost_oax2   AGTCGGAGCTCGTC-CGA-TACG-T.........................AT.CT......C.........T.....-.A......T.A..C.........CA.A.....G
Gela_elo1   CGCCGGAGCTCGCC-CGA-TACG-.........................AT.C.......C.........T.....-.CA.C....T-...C........-.C....N..--
Gela_elo2   CGCCGGAGCTCGCC-CGA-TACG-.........................AT.C..N....C.........AT.....-.CA.C....TN...C.........C.--------
Herb_lahu   ---------------------CA-....A..TA.........N..N.AAT.C.N...T.C.N........T....G.CA.C.........C.......A.NA.N.....G
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Mast_hopp   AGCTGGAGCTCGTC-CGA-TACG-................AAAAAA.AT.C.......C........T.T...T-.CA.C....T...TC.........C........G
Mast_sp1    AGCNGGAGCTCGTC-CGA-TACG-.........................AT.N...T...C.....GAANATG....-.CAAG.N-------------------------
Mast_sp2    AGCTGGAGCTCGTC-CGA-TACG-.........................AT.C.......C........T.T...T-.CA.C....T...TC.........C........G
Mast_toro   AGCCGGAACTCGCC-CGA-TTCG-.........................AT.C..A....C.........NT.....-.CANC....TA...C-------------------
Nema_conv   AGCCGGAGCTCGTC-CGA-TACG-.........................AT.CT......C..........T.....-..A......TA...C.........CA.A.....G
Nema_tenu   AGGAGGGTCTCGCCGCCA-TACGA....AC...................AT.C.......C..........T.....-.CA.C....T....C.C..A..AACC.......G
Rigi_imma   AGCCGGAGCTCGTC-CGA-TACG-.........................AT.CT......C..........T.....-..A......TAA..C.........CA.A.....G
Rigi_inus   AGCCGGAGCTCGTC-CGA-TACG-.........-------G.......AT.CT......C..........T.....-..A......T.A.TC.........CA.A.....G
Sess_citr   AGCCGGAGCTCGTC-CGC-TACG-.........---------.......AT.CT......C.........AT.....-..A......TAA..C.........CA.A....CG
Sess_heli   AGCCGGAGCTCGTC-CGA-TACG-.........................AT.CT......C..........T.....-..A......T.A..C.........CA.A....CG
Sess_lati   AGCCGGAGCTCGTC-CGA-TACG-.........................AT.CT......C..........T.....-..A......T.A-.C.........CAAAA....G
Tigr_dura   AGCCGGAGCTCGTC-CGA-TACG-.........................AT.CT......C..........T.....-..A......T....C.........CA.A.....G
Tigr_flam   AGCCGGAGCTCGTC-CGA-TACG-.........................AT.CT......C..........T.....-..A......T....C.........CA.A.....G
Tigr_huaj   AGTCGGAGCTCGCC-CGA-TACG-.........................AT.CT......C........T.T.....-..A......T....C.........CA.A.....G
Tigr_lute   AGCCGGAGCTCGCC-CGA-TACG-.........................AT.C.......C..........T.TT.-.CA.C..T.T....C.........C........G
Tigr_mexi   AGTCGGAGCTCGTC-CGA-TACG-.........................AT.CT......C..........T.....-..A......T...AC.........CA.A.....G
Tigr_mult   AGTCTGAGCTCGCC-CGA-TACG-..............C........AT.CT......C..........T.....-..A......T....C.........CA.A.....G
Tigr_orth   AACCCGAGCTCGTC-CGA-TACG-.........................AT.CT......C..........T.....-.T......T.A..C.........CA.A.....G
Tigr_sp1_39 AGCCGGAGCTCGCC-CGA-TACG-.........................------.....C..........T.TT.-.CA.C..T.T...TC.........C........G
Tigr_sp1_40 AGCTGGAGCTCGTC-CGA-TACG-.........................AT.C.......C..........T.TT.-.CA.C..T.T...TC.........C........G
Tigr_sp2_43 AGCCGGAGCTCGCC-CGA-TACG-.........................AT.C.......C..........T.TT.-.CA.C..T.T...TC.........C........G

[           55555555555555555555555555555]
[           33333334444444555555566677788]
[           12345680123479123567918903412]
Iris_vers   CACGGCCTTCACCCCCGGCTGGTACGAGC
Neom_grac   A...A..G..G...GA..AC..-...GTT
Neom_nort   A.-------..GT.----------------
Trim_fost   G..........T..GA.AAC.A..T.GTG
Trim_mart   A............TGA.AA.......GTG
Alop_ver3   ..............GA.AGAA.....GTG
Caly_nud1   GN...GT..--------------------
Caly_pal1   ..........G...GA.AACA.-...G.G
Card_hint   ..........G...GACAGGA.....GTG
Card_lon1   ..........G...GACAGGA.....GTG
Card_lon2   ..........G...GACAGGA.....GTG
Card_mexi   .........TG...GACAGGA.....GTG
Cipu_cam1   .C........G...GA.AAC..-...GCG
Cipu_cam2   .C........G...GA.AAC..-...GCG
Coba_guat   ..........G...GACAGGA.....GTG
```

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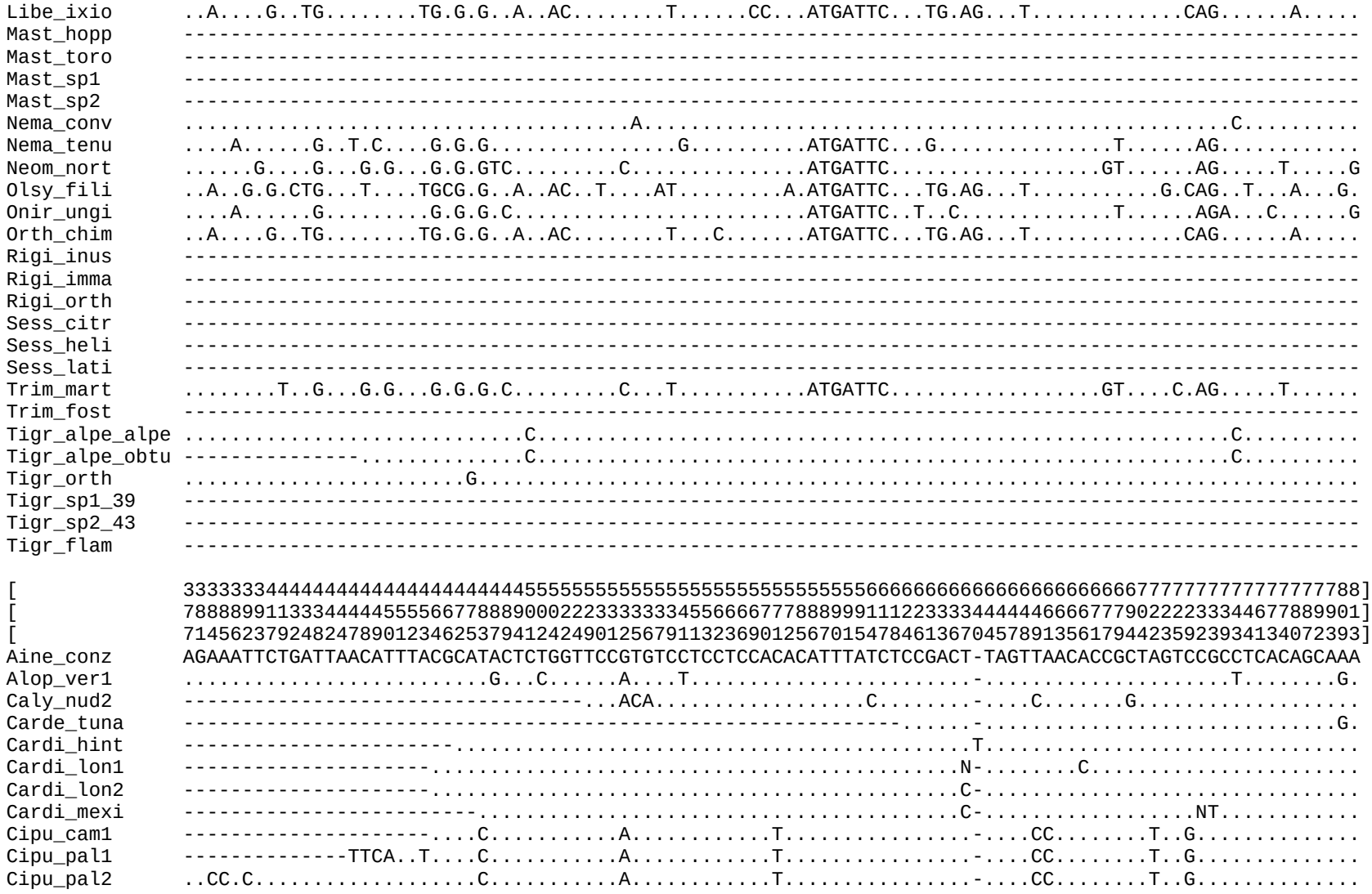
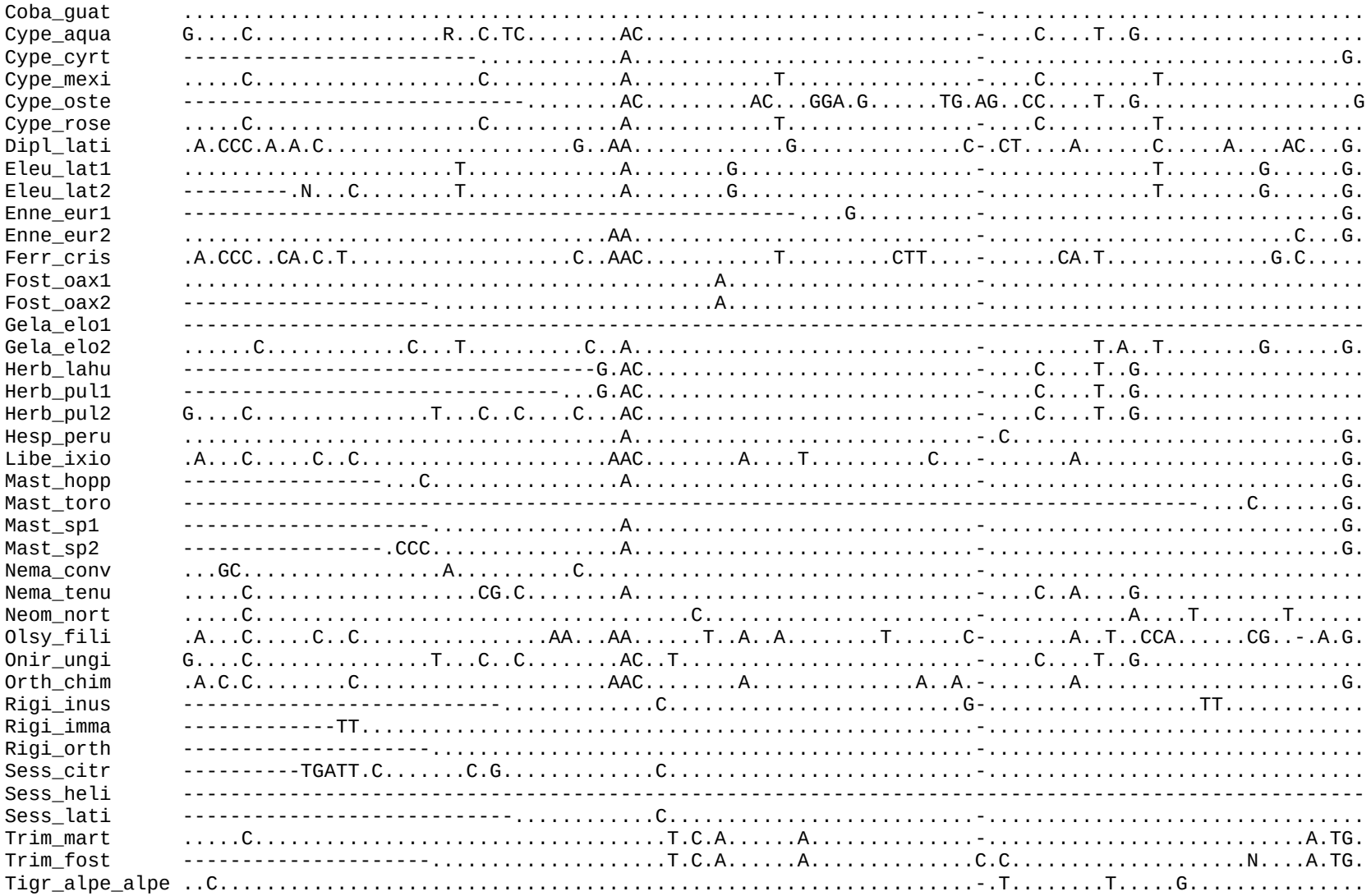
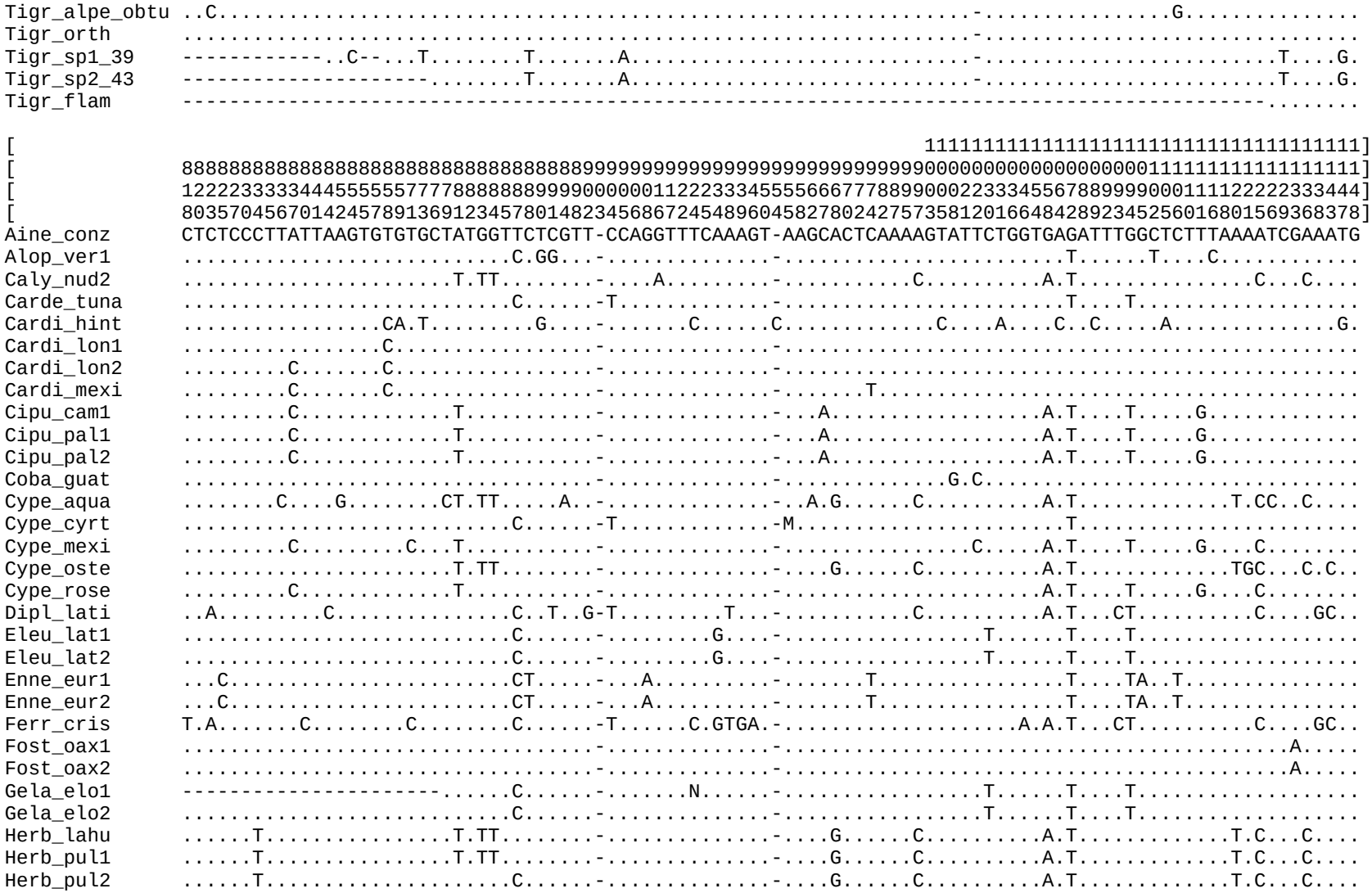
Cype_oste   ..........G...GA-AACA.-...GCG
Cype_rose   ..........G...GA.AACT.-...GCG
Eleu_lat1   ....A.........GA.AGCC.....GCG
Eleu_lat2   ----------------------------
Enne_eur2   ACACT.TCG.G.A---------------
Enne_foli   ..........GT..GACAGCAT....GTG
Fost_oax2   ..........GT..AACAGGA.....GTG
Gela_elo1   ----------------------------
Gela_elo2   ----------------------------
Herb_lahu   ..........G..GGA.AACA.-.NNGNG
Mast_hopp   ..........GT..GA.AGCA.-------
Mast_sp1    ----------------------------
Mast_sp2    ..........GT..GA.AGCA.-------
Mast_toro   ----------------------------
Nema_conv   ..........G...GACAGGA.....GTG
Nema_tenu   ..........G..TGA..ACA.-...G--
Rigi_imma   ..........G...GACAGGA.....GTG
Rigi_inus   ..........G...GACAG.A.....GTG
Sess_citr   ..........GT..GACAGGA.....GTG
Sess_heli   ..........GT..GACAGGA.....GTG
Sess_lati   ..........GT..GACAGGA.....GTG
Tigr_dura   ..........G...GACAGGA.....GTG
Tigr_flam   ..........G...GACAGGA.....GTG
Tigr_huaj   ..........G...GACAGGA.....GTG
Tigr_lute   ..........GT..GA.AGCA.AC.T---
Tigr_mexi   ..........GT..GACAGGA.....GTG
Tigr_mult   ..........G...GACAGGA.....GTG
Tigr_orth   ..........G...GACAGGA.....GTG
Tigr_sp1_39 ..........GT..GA.AGCA.-------
Tigr_sp1_40 ..........GT..GA.AGCA--------
Tigr_sp2_43 ..........GT..GA.AGCA.-------
;
end;
```

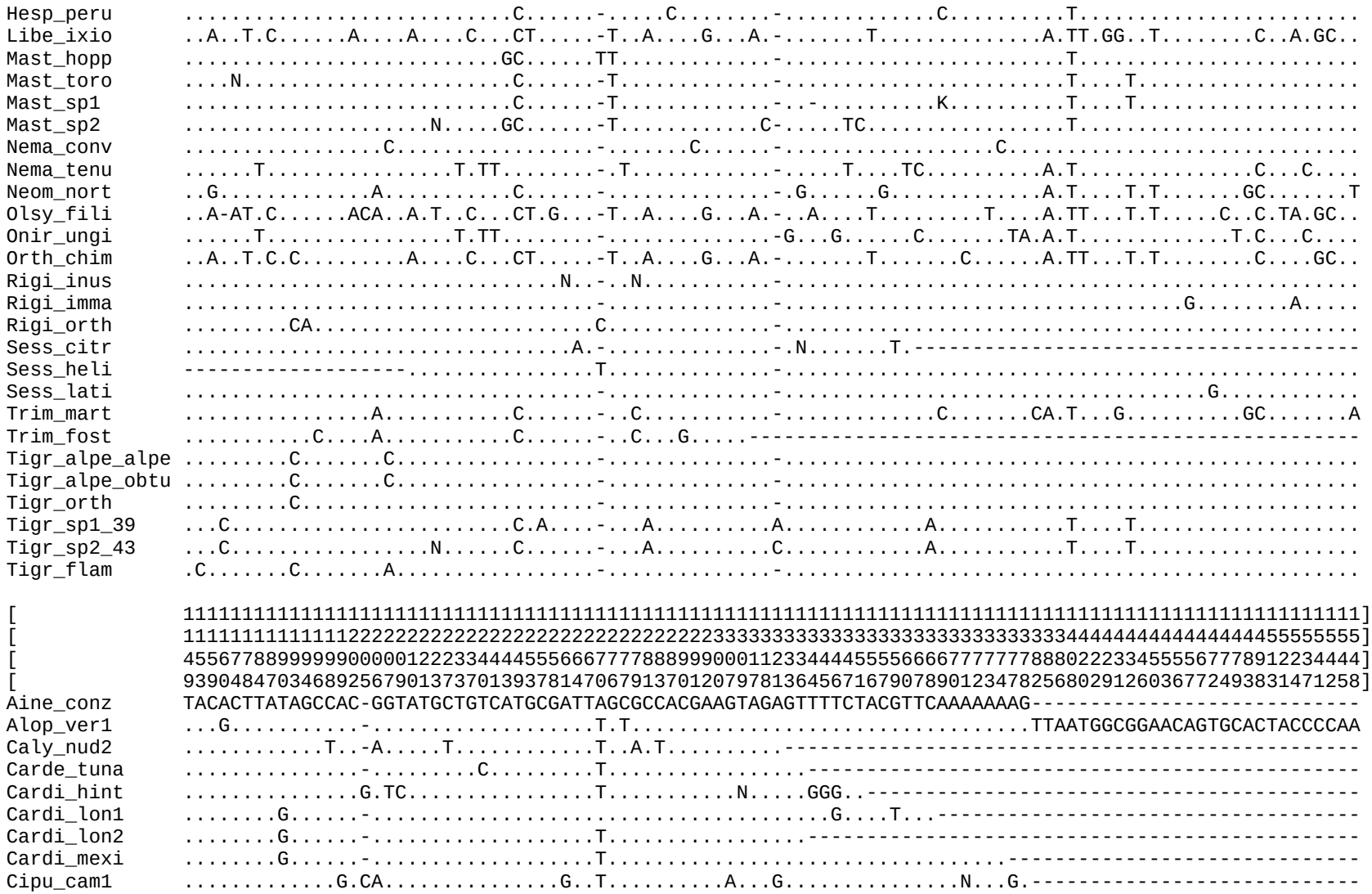
Appendix 1-1 B: MatK sequence alignment for 56 taxa of the Iridoideae Subfamily. Only variable sites are included (494 out of 1831 in the matrix)(Mega 5). Numbers on the top are the specific positions on the gene. A dot means same nucleotide relative to the first sequence used a reference. The hyphen means a gap.

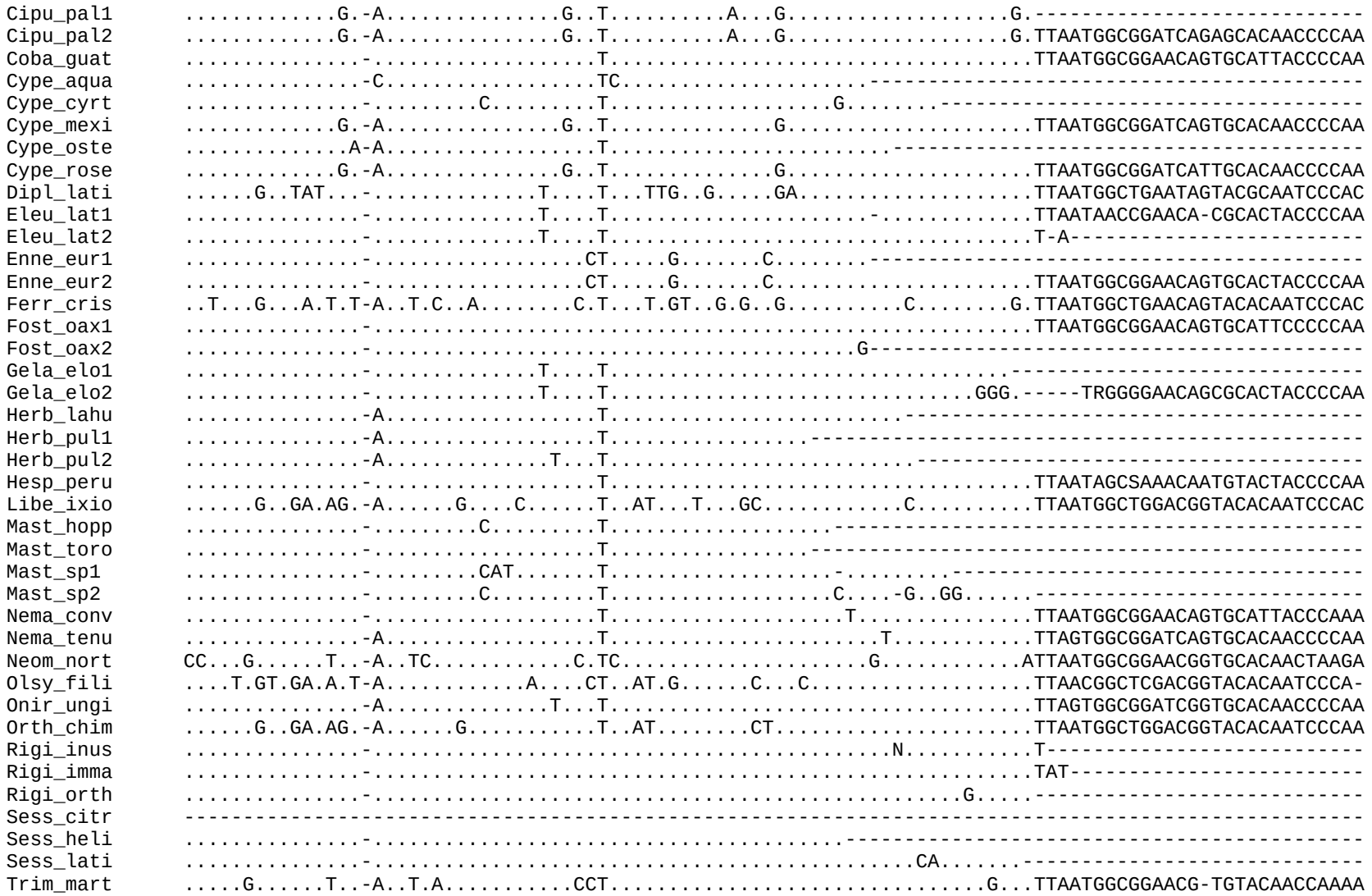
```
[                                11111111111111111111111222222222222222222222222222222222222222333333333333333333333]
[              1333334455566789990011122333334455667789900111112222333333345555566666777778899112234445555666677777]
[              1056896802539090685646707035678968452704716034568156734567894015680246802345780526233128178902590356]
Aine_conz      TGGACTCACTCTCTCATATGCATTTACTGTGGTGCCGTGTGCAATTATTTCGCGGATCATCCGCCTGCCACCACAACCAGAGGCATCCGAGTACTCGAAA
Alop_ver1      ----........T......A.G.G.G.C........C................ATGATCC.........GT.......GT.AT..............G..
Caly_nud2      ----------------------------------------------------------------------------------------------------
Carde_tuna     ----------------------------------------------------------------------------------------------------
Cardi_hint     ----------------------------------------------------------------------------------------------------
Cardi_lon1     ----------------------------------------------------------------------------------------------------
Cardi_lon2     ----------------------------------------------------------------------------------------------------
Cardi_mexi     ----------------------------------------------------------------------------------------------------
Cipu_cam1      ----------------------------------------------------------------------------------------------------
Cipu_pal1      ----------------------------------------------------------------------------------------------------
Cipu_pal2      C.......T..C.........G.G.G.C.........................ATTATTC................T.........A.....C.......
Coba_guat      ........................................................................G...........................
Cype_aqua      ....A......G.........G.G.G..C........................ATGATTC..T..C.............T......AG....C.......
Cype_cyrt      ----------------------------------------------------------------------------------------------------
Cype_mexi      ...........G.........G.G.G.C.........................ATGATTC................T..T......AG....C...A...
Cype_oste      ----------------------------------------------------------------------------------------------------
Cype_rose      ...........G.........G.G.G.C..................G......ATGATTC................T..T......AG....C...A...
Dipl_lati      ...T.......G........TG.G.G..A...C.TT...C...G..T....C.ACGATTC.G..G...T....ATT.T.T.....CAG......A..G..
Eleu_lat1      .....................G.G.G...........................ATGATCC............M...............A...........
Eleu_lat2      ----------------------------------------------------------------------------------------------------
Enne_eur1      ----------------------------------------------------------------------------------------------------
Enne_eur2      .A...................G.G.G.......A...................ATGATCC........................................
Ferr_cris      ...........G.C....C.AG.C.G..A.T.C..........GC.TA..T.TATGATTCA...G.............GTT....CAG...C..AA....
Fost_oax1      ......................................A.............................................................
Fost_oax2      ----------------------------------------------------------------------------------------------------
Gela_elo1      ----------------------------------------------------------------------------------------------------
Gela_elo2      .....................G...G...........................ATGATCC........................................
Herb_lahu      ----------------------------------------------------------------------------------------------------
Herb_pul1      ----------------------------------------------------------------------------------------------------
Herb_pul2      ....A......G.........G.G.G...........................ATGATTC..T..C.............T......AG....C......G
Hesp_peru      .....................G.G.G...........................ATGATCC........................................
```




Coba_guat
Cype_aqua
Cype_cyrt
Cype_mexi
Cype_oste
Cype_rose
Dipl_lati
Eleu_lat1
Eleu_lat2
Enne_eur1
Enne_eur2
Ferr_cris
Fost_oax1
Fost_oax2
Gela_elo1
Gela_elo2
Herb_lahu
Herb_pul1
Herb_pul2
Hesp_peru
Libe_ixio
Mast_hopp
Mast_toro
Mast_sp1
Mast_sp2
Nema_conv
Nema_tenu
Neom_nort
Olsy_fili
Onir_ungi
Orth_chim
Rigi_inus
Rigi_imma
Rigi_orth
Sess_citr
Sess_heli
Sess_lati
Trim_mart
Trim_fost
Tigr_alpe_alpe


Tigr_alpe_obtu ..C.....-.....G.....
Tigr_orth .....-.....
Tigr_sp1_39 ----------..C--...T.....T.....A.....-.....T....G.
Tigr_sp2_43 ----------.....T.....A.....-.....T....G.
Tigr_flam ----------.....
[11111111111111111111111111111111111]
[88888888888888888888888888888888999999999999999999999999999900000000000000000001111111111111111]
[12222333334445555557777888888899990000001122233345555666777889900022333455678899990001111222223334444]
[80357045670142457891369123457801482345686724548960458278024275735812016648428923452560168015693683 78]
Aine_conz CTCTCCCTTATTAAGTGTGTGCTATGGTTCTCGTT-CCAGGTTTCAAAGT-AAGCACTCAAAAGTATTCTGGTGAGATTTGGCTCTTTAAAATCGAAATG
Alop_ver1 .....C.GG...-.....-.....T.....T....C.....
Caly_nud2 .....T.TT.....-....A.....-.....C.....A.T.....C...C....
Carde_tuna .....C.....-T.....-.....T....T.....
Cardi_hint .....CA.T.....G....-.....C.....C.....C....A....C..C.....A.....G.
Cardi_lon1 .....C.....-.....-.....
Cardi_lon2 .....C.....C.....-.....-.....
Cardi_mexi .....C.....C.....-.....-.....T.....
Cipu_cam1 .....C.....T.....-.....-...A.....A.T....T.....G.....
Cipu_pal1 .....C.....T.....-.....-...A.....A.T....T.....G.....
Cipu_pal2 .....C.....T.....-.....-...A.....A.T....T.....G.....
Coba_guat .....-.....-.....G.C.....
Cype_aqua .....C....G.....CT.TT.....A..-.....-..A.G.....C.....A.T.....T.CC..C....
Cype_cyrt .....C.....-T.....-M.....T.....
Cype_mexi .....C.....C...T.....-.....-.....C.....A.T....T.....G....C.....
Cype_oste .....T.TT.....-.....-....G.....C.....A.T.....TGC...C.C..
Cype_rose .....C.....T.....-.....-.....A.T....T.....G....C.....
Dipl_lati ..A.....C.....C..T..G-T.....T...-.....C.....A.T...CT.....C....GC..
Eleu_lat1 .....C.....-.....G....-.....T.....T....T.....
Eleu_lat2 .....C.....-.....G....-.....T.....T....T.....
Enne_eur1 ...C.....CT.....-...A.....-.....T.....T....TA..T.....
Enne_eur2 ...C.....CT.....-...A.....-.....T.....T....TA..T.....
Ferr_cris T.A.....C.....C.....C.....-T.....C.GTGA.-.....A.A.T...CT.....C....GC..
Fost_oax1 .....-.....-.....A.....
Fost_oax2 .....-.....-.....A.....
Gela_elo1 ----------.....C.....-.....N.....-.....T.....T....T.....
Gela_elo2 .....C.....-.....-.....T.....T....T.....
Herb_lahu .....T.....T.TT.....-.....-....G.....C.....A.T.....T.C...C....
Herb_pul1 .....T.....T.TT.....-.....-....G.....C.....A.T.....T.C...C....
Herb_pul2 .....T.....C.....-.....-....G.....C.....A.T.....T.C...C....




Cipu_pal1 ..........G.-A..........G..T.........A...G..........G.----------
Cipu_pal2 ..........G.-A..........G..T.........A...G..........G.TTAATGGCGGATCAGAGCACAACCCCAA
Coba_guat ..........-..........T..........TTAATGGCGGAACAGTGCATTACCCCAA
Cype_aqua ..........-C..........TC..........----------
Cype_cyrt ..........-.....C.....T..........G.....----------
Cype_mexi ..........G.-A..........G..T.........G..........TTAATGGCGGATCAGTGCACAACCCCAA
Cype_oste ..........A-A..........T..........----------
Cype_rose ..........G.-A..........G..T.........G..........TTAATGGCGGATCATTGCACAACCCCAA
Dipl_lati ......G..TAT...-.........T....T...TTG..G.....GA..........TTAATGGCTGAATAGTACGCAATCCCAC
Eleu_lat1 ..........-.........T....T..........-.........TTAATAACCGAACA-CGCACTACCCCAA
Eleu_lat2 ..........-.........T....T..........T-A----------
Enne_eur1 ..........-.........CT.....G.....C.....----------
Enne_eur2 ..........-.........CT.....G.....C..........TTAATGGCGGAACAGTGCACTACCCCAA
Ferr_cris ..T...G...A.T.T-A..T.C..A.....C.T...T.GT..G.G..G.....C.....G.TTAATGGCTGAACAGTACACAATCCCAC
Fost_oax1 ....-..........TTAATGGCGGAACAGTGCATTCCCCCAA
Fost_oax2 ....-.....G----------
Gela_elo1 ..........-.........T....T..........----------
Gela_elo2 ..........-.........T....T..........GGG.-----TRGGGGAACAGCGCACTACCCCAA
Herb_lahu ..........-A.........T..........----------
Herb_pul1 ..........-A.........T..........----------
Herb_pul2 ..........-A.........T...T..........----------
Hesp_peru ..........-.........T..........TTAATAGCSAAACAATGTACTACCCCAA
Libe_ixio ......G..GA.AG.-A......G....C......T..AT...T...GC.....C.....TTAATGGCTGGACGGTACACAATCCCAC
Mast_hopp ..........-.....C.....T..........----------
Mast_toro ..........-.........T..........----------
Mast_sp1 ..........-.....CAT.....T..........-.....----------
Mast_sp2 ..........-.....C.....T..........C....-G..GG.....----------
Nema_conv ..........-.........T..........T.....TTAATGGCGGAACAGTGCATTACCCAAA
Nema_tenu ..........-A.........T..........T.....TTAGTGGCGGATCAGTGCACAACCCCAA
Neom_nort CC...G.....T..-A..TC.....C.TC..........G.....ATTAATGGCGGAACGGTGCACAACTAAGA
Olsy_fili ....T.GT.GA.A.T-A.....A....CT..AT.G.....C...C..........TTAACGGCTCGACGGTACACAATCCCA-
Onir_ungi ..........-A.....T...T..........TTAGTGGCGGATCGGTGCACAACCCCAA
Orth_chim ......G..GA.AG.-A......G.....T..AT.....CT..........TTAATGGCTGGACGGTACACAATCCCAA
Rigi_inus ..........-.....N.....T----------
Rigi_imma ....-.....TAT----------
Rigi_orth ..........-.....G.....----------
Sess_citr ----------
Sess_heli ....-.....----------
Sess_lati ..........-.....CA.....----------
Trim_mart .....G.....T..-A..T.A.....CCT.....G...TTAATGGCGGAACG-TGTACAACCAAAA

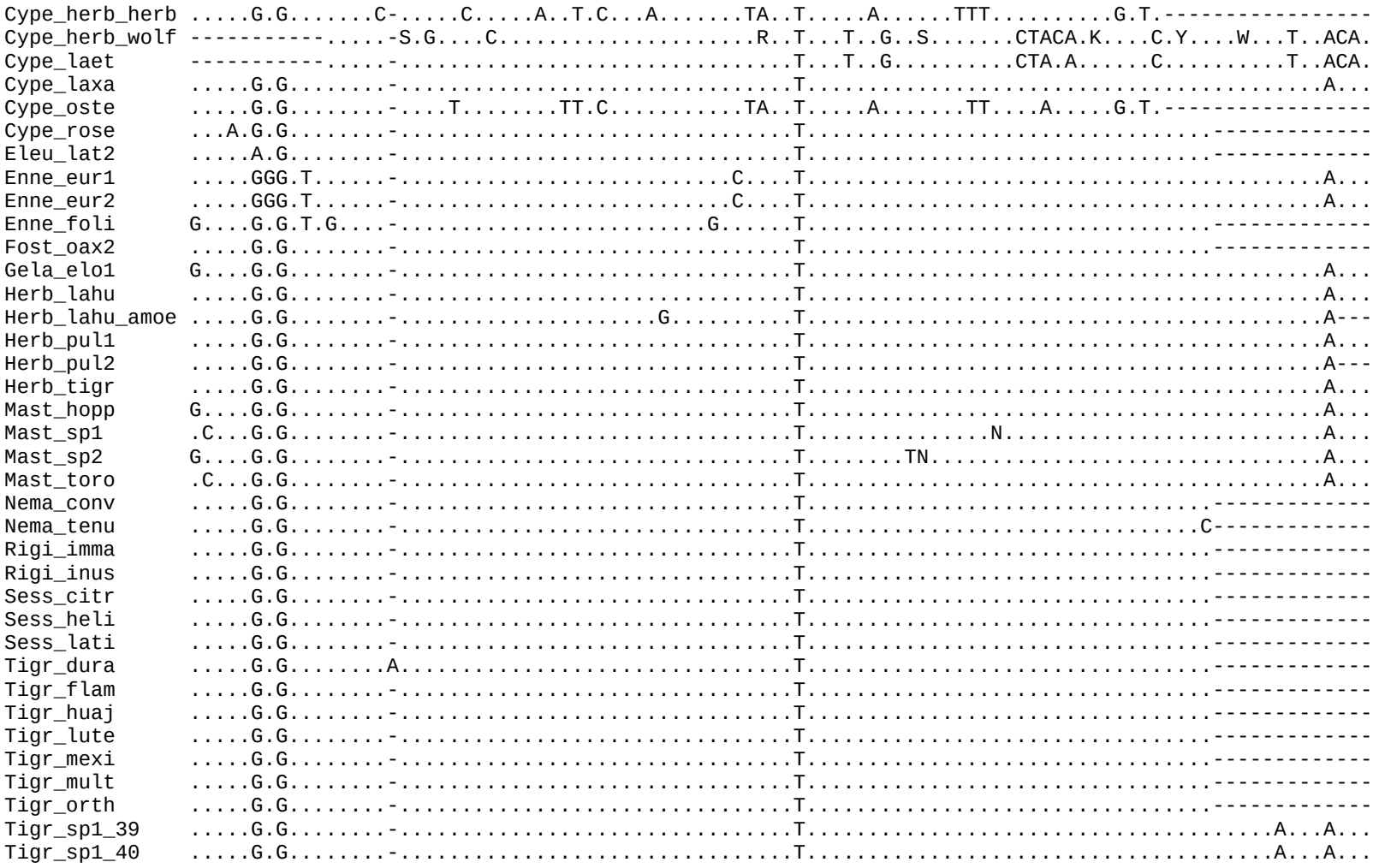
```
Trim_fost      ---------------------------------------------------------------------------------------------------
Tigr_alpe_alpe ........G....T.-..................CT...................................TTAATGGCGGAACAGTGCATTACCCCAA
Tigr_alpe_obtu ........G......-..................CT...................................GTAATGGCGGAACCGTGCATTACCCCAA
Tigr_orth      ...............-.......................................................TTAATGGCGGAACAGTGCATTACCCCAA
Tigr_sp1_39    ..............T-...................T..............G..............G.....----------------------------
Tigr_sp2_43    ..............T-...................T..............G.............-----------------------------------
Tigr_flam      ........G......-..................CT................................---------------------ATTACCCCAA

[              1111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111]
[              5555555555555555556666666666666666666666666666666666667777777777777777777777777777777777777888]
[              5555556666778888891112222333344556667778888888999999990111111122222334444455556666777778888023]
[              1245890256374567810252457678958095692583456789012345682345678914689151258927890134034580268481]
Aine_conz      ----------------------------------------------------------------------------------------------
Alop_ver1      TTCTTACTCAGAAACATTCTGCAGTATGAGCTCCGTCCGGG---------ACATG-CAGAGTGGATACCCTACTGTTTTCCGTAAGTTAAGTAG
Caly_nud2      ----------------------------------------------------------------------------------------------
Carde_tuna     ----------------------------------------------------------------------------------------------
Cardi_hint     ----------------------------------------------------------------------------------------------
Cardi_lon1     ----------------------------------------------------------------------------------------------
Cardi_lon2     ----------------------------------------------------------------------------------------------
Cardi_mexi     ----------------------------------------------------------------------------------------------
Cipu_cam1      ----------------------------------------------------------------------------------------------
Cipu_pal1      ----------------------------------------------------------------------------------------------
Cipu_pal2      TTTTTACTCGGCAACATTCCGCAGGATAAGCTTCGTCCGGATCAAGAGGGACATGGCGGAATGGATACTCAA---CTCGGATATTTATAAGTGG
Coba_guat      CTCTTATTCAGAAACATCCTGCAGGATGAGCTCCGTCCGGATCAAGAAGGA-GTGGCAGAGTGGATACCCAACTGTCTTCCGTAAGTTAGTTAG
Cype_aqua      ----------------------------------------------------------------------------------------------
Cype_cyrt      ----------------------------------------------------------------------------------------------
Cype_mexi      TTTTTACGCGGAAACATTCCGCAGGATAAGCTCCGTCCGGATCAAGAGGGACATGGCGGAATGGATACTCAAT--CTCGGATATTTAGAAGTAG
Cype_oste      ----------------------------------------------------------------------------------------------
Cype_rose      TTTTTACGCGGAAACATTCCGCAGGATAAGCTCCCTCCGGATCAAGAGGGACATGGCGGAATGGATACTCAAT--CTCGGATATTTAGAAGTAG
Dipl_lati      TTCTTTCTTGAAAACGTTCTTTAGGATGAGCTCAGTAAGGATCAAGAGGGACATGTAGTAGTAGCTACACAACTGTCTTCCGTAAGTTTAGTAG
Eleu_lat1      TTCTGACTCAGAAACATTCTGCAGGATGAGCTCCGTCCGCATCAAGAGGGACATGGCAGAGTGGATACCCAACTGTCTTCCGTAAGTTAAGTAG
Eleu_lat2      ----------------------------------------------------------------------------------------------
Enne_eur1      ----------------------------------------------------------------------------------------------
Enne_eur2      TTCTTACTCAGAAACATTCTGCAGGATGAGCTCCG----------AAAGGACATGGCAGAGTGGATACCCAATTG-CTTCCGTAAGTTAGGTAG
Ferr_cris      TTCTTTCTTAAAAACGTTCTTYAGGATAAGCCCAGTACGGATCAAGAGGGACATGCTGTAGGGGATCTACAACTGTCTTCCGTAAGTTTAGT--
Fost_oax1      TTCTTATTCAGAAACATCCTGCAGGATGAGCTCCGTCCGGATCAAGAAGGA-GTGGCAGAGTGGATACCCAACTGTCTTCCGTAAGTTAGTTAG
Fost_oax2      ----------------------------------------------------------------------------------------------
Gela_elo1      ----------------------------------------------------------------------------------------------
Gela_elo2      TTCTGACTCAGAAACATTCTGCAGGATGAGCTCCGTCCGGATCAAGAGGGACATGGCAGAGTGGATACCCAACTGTTTTCCGTAAGTTAAGTAG
Herb_lahu      ----------------------------------------------------------------------------------------------
```

```
Herb_pul1       ----------------------------------------------------------------------------------------------------
Herb_pul2       ----------------------------------------------------------------------------------------------------
Hesp_peru       TTCCTACTCAGAAACATTCTGCAGGATGAGCTCCGTCCGGATCAAGAAGGACATGGCAAAATGGATACCCAACTGTCTTCCGTAAGTTAAGTAG
Libe_ixio       TTCCTACTCAAAAATCCTCTTTAGGATGAGAT-AGTACTGATCAAGGGGGACATGTCGCAGTGGATACACAACTGTCTTTTGTAAGTTAAGTAG
Mast_hopp       ----------------------------------------------------------------------------------------------------
Mast_toro       ----------------------------------------------------------------------------------------------------
Mast_sp1        ----------------------------------------------------------------------------------------------------
Mast_sp2        ----------------------------------------------------------------------------------------------------
Nema_conv       TTCTTATTCAGAAACATCCTGCAGGATGAGCTCCGTCCGGAT--AGAAAGA-GTGGCAGGGTGGATACCCAACTGTCTTCCGTAAGTTAGTTAG
Nema_tenu       TTTTTCCTCAGAAGCATTCTGCACGATGAGCTCCGTCCGGATCAAGAGGGGCATGGCGGAATGGATACCCAAT--CTCGGATATTTAGAAGTAG
Neom_nort       TTCTTACTCAGAAACATTTTTCAGGTCTTGCTCCGTCCGGATTAAGAGGGACACGGCGGAGTGGATACTCCACTGTCTTCCCT----TAAGTAG
Olsy_fili       TGCTTACTCAAAAATCTTCTTCAGGATGACAG-AGTACTAAGGAGGAAAAATATGTCGTAGTGGATACAAAACGGTCTTTCGTAAGTTAAGT--
Onir_ungi       TGTTTACTCAGAAACATTCTGCACGATGAGCTCCGTCCGGATCAAGAGGGACATGGCGGAATGGAAACCCAAT--CTCGGATATTTAGAAGTAG
Orth_chim       TTCCTACTCAAAAATCCTCTTCAGGATGAGAT-AGTACTGATCGAGGGGGACATATCGTAGTGGATACACCACTTCTTTTGGTAAGTTAAGTAA
Rigi_inus       ----------------------------------------------------------------------------------------------------
Rigi_imma       ----------------------------------------------------------------------------------------------------
Rigi_orth       ----------------------------------------------------------------------------------------------------
Sess_citr       ----------------------------------------------------------------------------------------------------
Sess_heli       ----------------------------------------------------------------------------------------------------
Sess_lati       ----------------------------------------------------------------------------------------------------
Trim_mart       TTCTTACTCAGAAACATTCTTCCGGTCTTGCTTCGACCGGATCAAGAGGGACACGGTGGAGTGGATACTCAGCTGTCTTCCGTAAGTTAAGCAG
Trim_fost       ----------------------------------------------------------------------------------------------------
Tigr_alpe_alpe  TTCTTATTCAGAAGCATCCTGCAGGATGAGCTCCGTCCGGATCAAGAAGGA-GTGGCAGGGTGGATACCCAACTGTCTTCCGTAAGTTAGTTAG
Tigr_alpe_obtu  TTCTTATTCAGAAGCATCCTGCAGGATGAGCTCCGTCCGGATCAAGAAGGA-GTGGCAGGGTGTATACCCAACTGTCTTCCGTAAGTTAGTTAG
Tigr_orth       TTCTTATTCAGAAACATCCTGCAGGATGAGCTCCGTCCGGATCAAGAAGGA-GTGGCAGAGTGGATACCCAACTGTCTTCCGTAAGTTAGTTAG
Tigr_sp1_39     ----------------------------------------------------------------------------------------------------
Tigr_sp2_43     ----------------------------------------------------------------------------------------------------
Tigr_flam       TTCTTATTCAGAGGCATYCTGCAGGATGAGCTCCGTCCGGATCAAGAAGGA-GTGGCAGGGTGGATATCCAACTGTCTTCCGTAAGTTAGTTAG
```

Appendix 1-1 C: psbA sequence alignment for 68 taxa of the Iridoideae Subfamily. Only variable sites are included (96 out of 488 in the matix) (Mega 5). Numbers on the top are the specific positions on the gene. A dot means same nucleotide relative to the first sequence used a reference. A hyphen means a gap.

```
[                                                      11111111111111112222222222222222222223333333334444444444]
[                  11222222223344445555566667777888999900001222245689990122233444667778889990122678990112245778]
[              124658012567891201291238925670258459034515676346954161245145904135120243465785503507144396811465]
Dipl_lati      ATTGACC-TCAAAATA-CGTTATGTCCGCGCGAAGAGGCGTAATTCGATGCTTGTGACACTACCCCCACGTGGTATTATGTATAGTTGCGAGCAGT
Ferr_cris      .......G........-.........A......................T..........................C.........C.....A...
Iris_vers      .......G........-.......................C........T........................G......C.-------------
Libe_ixio      G....A.G........-.......................C.......CT.G........C.....T.........................A...
Neom_grac      .....G.G......G.-................................TT................................-------------
Neom_nort      .....G.G......G.-................................TT.........................................A..C
Neom_sp1       .....G.G...TTTG.-...G............................TT..........................-------------------
Olsy_fili      G..T.A.G........-.......................C.......C..........T.G....T......C.....A...TAAG.....A---
Orth_chim      G....A.G........-.......................C.......CT.G........C.....T.............G...........A---
Trim_fost      G....G.G...TTTG.-..................G.............TT.............................-..-------------
Trim_mart      G....G.G...TTTG.-..................G.............TT................................-------------
Alop_ver1      ------.G......-.-...N-..........G......A.C.A.....T................TC........................A.--
Alop_ver3      .....G.G........-......A............C.....G......T.................................-------------
Caly_azur      .....G.G........-................................T.......T.................................AA---
Caly_mini      -----------.....-................................T...T..G..........CTA.A......C..........T..ACA.
Caly_nud1      .....G.G........-................................T..............................G..........AA...
Caly_nud2      .....G.G........-................................T..............................G..........AA...
Caly_pall      .....G.G........-................................T.............................A..C-------------
Carde_tuna     G....G.G.......CA................................T..........................................A...
Cardi_hint     .....G.G........-................................T..........................................A...
Cardi_lon1     .....G.G........-................................T................................-------------
Cardi_lon2     T...GGAG......CT-......N.........................T.....................................C.N..T.C.
Cardi_mexi     ..C.CGGGA...GGCTGTA..............................T........................................T.A..-
Cipu_cam1      .....G.G........-..................T.............T................................-------------
Cipu_cam2      .....G.G........-..................T.............T................................-------------
Cipu_pal1      .....G.G........-..................T.............T..........................................A...
Cipu_pal2      .....G.G........-..................T.............T..........................................A...
Coba_guat      .....G.G..C.....-................................T..............................-..-------------
Cype_aqua      G....G.G........-..............T.............TA..T..C.G....................-................A---
Cype_haut_haut G....G.A.....-----.......TTT.AAT..A............C.T...AG..........A......T....T.............-----
Cype_haut_opal G....G.G.....-----.......TTT.AAT..A............C.T...A...........A......T....T..............A---
```


Cype_herb_herb .....G.G.......C-.....C.....A..T.C...A.......TA..T.....A......TTT.........G.T.----------
Cype_herb_wolf ----------.....-S.G....C.....................R..T...T..G..S.......CTACA.K....C.Y....W...T..ACA.
Cype_laet ----------.....-.....T...T..G.........CTA.A......C.........T..ACA.
Cype_laxa .....G.G.......-.....T.....A...
Cype_oste .....G.G.......-....T.......TT.C...........TA..T.....A.......TT....A.....G.T.----------
Cype_rose ...A.G.G.......-.....T.....----------
Eleu_lat2 .....A.G.......-.....T.....----------
Enne_eur1 .....GGG.T.....-.....C....T.....A...
Enne_eur2 .....GGG.T.....-.....C....T.....A...
Enne_foli G....G.G.T.G....-.....G.....T.....----------
Fost_oax2 .....G.G.......-.....T.....----------
Gela_elo1 G....G.G.......-.....T.....A...
Herb_lahu .....G.G.......-.....T.....A...
Herb_lahu_amoe .....G.G.......-.....G.....T.....A---
Herb_pul1 .....G.G.......-.....T.....A...
Herb_pul2 .....G.G.......-.....T.....A---
Herb_tigr .....G.G.......-.....T.....A...
Mast_hopp G....G.G.......-.....T.....A...
Mast_sp1 .C...G.G.......-.....T.....N.....A...
Mast_sp2 G....G.G.......-.....T.....TN.....A...
Mast_toro .C...G.G.......-.....T.....A...
Nema_conv .....G.G.......-.....T.....----------
Nema_tenu .....G.G.......-.....T.....C----------
Rigi_imma .....G.G.......-.....T.....----------
Rigi_inus .....G.G.......-.....T.....----------
Sess_citr .....G.G.......-.....T.....----------
Sess_heli .....G.G.......-.....T.....----------
Sess_lati .....G.G.......-.....T.....----------
Tigr_dura .....G.G.......A.....T.....----------
Tigr_flam .....G.G.......-.....T.....----------
Tigr_huaj .....G.G.......-.....T.....----------
Tigr_lute .....G.G.......-.....T.....----------
Tigr_mexi .....G.G.......-.....T.....----------
Tigr_mult .....G.G.......-.....T.....----------
Tigr_orth .....G.G.......-.....T.....----------
Tigr_sp1_39 .....G.G.......-.....T.....A...A...
Tigr_sp1_40 .....G.G.......-.....T.....A...A...

Apendix 1-1 D: TrnKK3 sequence alignment for 24 taxa of the Iridoideae Subfamily. Only variable sites are included (65 out of 189 in the matrix)(Mega 5). Numbers on the top are the specific positions on the gene. A dot means same nucleotide relative to the first sequence used a reference. A hyphen means a gap.

```
[                                                        111111111111111111111111]
[                 12223334444444445555556777777778888899000011111122233333444688]
[              36873470361234567890123460123456792467939036705678912812368046269]
Alop_ver1      GCTCCGTCCGGG---------ACATG-CAGAGTGGATACCCTACTGTTTTCCGTAAGTTAAGTAG
Cipu_pal2      ...T.......ATCAAGAGGG.....G.G..A.......T.A.---C.CGGATATTTA.....G.
Coba_guat      ...........ATCAAGAAGG.-G..G..............A.....C............GT...
Cype_mexi      ...........ATCAAGAGGG.....G.G..A.......T.A.T--C.CGGATATTTAG......
Cype_rose      .....C.....ATCAAGAGGG.....G.G..A.......T.A.T--C.CGGATATTTAG......
Dipl_lati      ....A..AA..ATCAAGAGGG.....TAGT...A.C...A.A.....C...........T.....
Eleu_lat1      ..........CATCAAGAGGG.....G..............A.....C.................
Enne_eur2      .....-----------AAAGG.....G..............A.T..-C............G....
Ferr_cris      ..C.A..A...ATCAAGAGGG.....CTGT..G....CTA.A.....C...........T...--
Fost_oax1      ...........ATCAAGAAGG.-G..G..............A.....C............GT...
Gela_elo2      ...........ATCAAGAGGG.....G..............A.......................
Hesp_peru      ...........ATCAAGAAGG.....G..A.A.........A.....C.................
Libe_ixio      .A.-A..A.T.ATCAAGGGGG.....T.GC.........A.A.....C..TT.............
Nema_conv      ...........AT--AGAAAG.-G..G...G..........A.....C............GT...
Nema_tenu      ...........ATCAAGAGGGG....G.G..A.........A.T--C.CGGATATTTAG......
Neom_nort      ...........ATTAAGAGGG...C.G.G..........T.C.....C....C.----.......
Olsy_fili      CAG-A..A.TAAGGAGGAAAA.T...T.GT.........AAA..G..C..T............--
Onir_ungi      ...........ATCAAGAGGG.....G.G..A....A....A.T--C.CGGATATTTAG......
Orth_chim      .A.-A..A.T.ATCGAGGGGG....AT.GT.........A.C...TC...TG............A
Trim_mart      ...T..A....ATCAAGAGGG...C.GTG..........T.AG....C..............C..
Tigr_alpe_alpe ...........ATCAAGAAGG.-G..G...G..........A.....C............GT...
Tigr_alpe_obtu ...........ATCAAGAAGG.-G..G...G...T......A.....C............GT...
Tigr_orth      ...........ATCAAGAAGG.-G..G..............A.....C............GT...
Tigr_flam      ...........ATCAAGAAGG.-G..G...G.......T..A.....C............GT...
```

Apendix 1-1 E: TrnL.F sequence alignment for 70 taxa of the Iridoideae Subfamily. Only variable sites are included (114 out of 378 in the matrix)(Mega 5). Numbers on the top are the specific positions on the gene. A dot means same nucleotide relative to the first sequence used a reference. A hyphen means a gap.

```
[                                            111111111111111111111111111111111111111112222222222222222222]
[               11111112223333344444556677889000001122333333344444445566677788888899990011222234555567788]
[              901346782592568901235050139018012486889034568901457892756806923458903454938012301123731804]
Dipl_lati      GCCATTTATTCTGT----GGTCTATTTACCGAAAACTCTTCCCATTTGTTGATAACCGAGCTGTAGGCGGATGCGCAGTAAGATCTTGAC
Ferr_cris      .T..........A.----...........A...........T...C.....G.................T...TC...CCC..A......
Iris_vers      --------...CAC-GGT--.....A.G....GT...........C....T.CGGT.AC..........T....C....CC..A......
Libe_ixio      ..TT......T...GGGT.....C..GTAA.................................A.....T..T.CT...TC----.....
Neom_grac      --------......GGGT.....C.---------............................A......T....C....TC..A......
Neom_nort      A......C......GGGT.....C.---------............................A......T....C....TC..A......
Olsy_fili      ...T..G.-----------------.GTAAAG.G.A..........C...A......-..A..A.....T..T.AT...TC----....-
Orth_chim      ..TT......T...GGGT.....C..GTAA.............G...T...............A.A...T..T.CT...TC----..A..
Trim_fost      --------.....CGGGT.....C.---------...................................T....C....TT..A...A..
Trim_mart      .T.....CC....CGAAAAA..AC.---------T.......................W..........T....C....TC..A......
Aine_conz      .T............GGGT.....C.---------........A.....C......T.............T....C....TC..A......
Alop_ver1      .T............GGGT...T.C.---------........A..........................T....C....TC..A......
Alop_ver3      --------.C.Y..GGGT...T.C.---------........A..........................T....C....TC..A......
Caly_azur      ..............GGGT.....C.---------.............T..................A..TG...C....TCT.A.C....
Caly_mini      ........-.....GGGN.....C.---------.............T..................A..TG...C....TCT.A......
Caly_pal1      --------.-.-..GGGT.....C.---------.............T..................A..T....C....TCT.A......
Caly_pal2      .T............GGGT.....C.---------........A.....C....................T....C....TC..A......
Carde_tuna     .T............GGGT.....C.---------........A..........................T....C....TC..A.....G
Cardi_hint     .T.G..........GGGT.....C.---------...AAG..A.....C....................T....C....TC..A......
Cardi_lon1     .T............GGGT.....C.---------........A.....C....................T....C....TC..A......
Cardi_lon2     .T............GGGT.....C.---------........A.....C....................T....C....TC..A......
Cardi_mexi     .T............GGGT.....C.---------........A.....C....................T....C....TC..A......
Cipu_cam1      --------......GGGT.....C.---------..........C.G............A......A.AT....C.C..TCT.AA.....
Cipu_cam2      --------......GGGT.....C.----------.........C.G............A......A.AT....C.C..TCT.AA.....
Cipu_pal2      ..............GGGT.....C.---------..........C.G............A......A.AT....C.C..TCT.AA.....
Coba_guat      .T..........A.----...........A...........T...C.....G.................T....C....CC.GA......
Cype_aqua      ........C.....GGGT.....C.---------......A......T..................A..T....C....TTT.A..A...
Cype_cyrt      .T............GGGT.....C.---------........A..........................T....C....TC..A.....G
Cype_herb_herb ..............GGGT.....C.---------......A......T..................A..T....C....TCT.A..A...
Cype_mexi      ........-.....GGGT.....C.---------..........C.....................A..T....C.C..TCT.A......
Cype_oste      ..............GGGT.....C.---------......A......T..................A..T....C....TCT.A..A...
Cype_pabs      .....GG.......GGGT.....C.---------......A......T..................A..T....C....TCT.A..A...
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Cype_rose      ------------------------------------------------------------------------------
Eleu_lat1      .T..A.........GGAT.....CG---------......AA.........................T....C....TC..A...A..
Eleu_lat2      --------.C.C..GGAT..G..CG---------G......AA......G..................T....C....TC..A...A..
Enne_eur2      .T............GGGT.....C.---------.......A.........................T....C....TC..A......
Enne_foli      --------.C....GGGT.....C.---------.......A.........................T....C....TC..A....G.
Fost_oax1      .T............GGGT.....C.---------.......A.....C......T.............T....C....TC..A......
Fost_oax2      ----------.....GGGT.....C.---------.......A.....C......T.............T....C....TC..A......
Gela_elo1      .T..A.........GGGT.....CG---------.......AA..................G.......T.G..C....TC..A...A..
Gela_elo2      .T..A.........GGGT.....CG---------.......AA..................G.......T.G..C....TC..A...A..
Herb_lahu_inte .T..........A.----............A...........T...C.....G.................T....C....CC.GA......
Herb_pul1      ..............GGGT.....C.----------.....A......T..................A..T.----------------
Herb_pul2      ..............GGGT.....C.----------..C...A......T........T.........A..T....C....NCT.A..A...
Herb_tigr      ..............GGGT.....C.---------.............T...................A..TG...C....TCT.A.C....
Hesp_peru      .T............GGGT.....C.---------.......A.........................T....C....TC..A......
Mast_hopp      .T............GGGT.....C.---------.......A.........................T....C....TC..A.....G
Mast_sp1       .T............GGGT.....C.---------.......A.........................T....C....TC..A.....G
Mast_sp2       .T............GGGT....NC.---------.......A.........................T....C....TC..A.....G
Mast_toro      .T............GGGT.....C.---------.......A.........................T....C..A--C..A.....G
Nema_conv      --------.C.C..GGGT.....C.---------.......A.....C...................T....C....TC..A......
Nema_tenu      .T............GGGT.....C.---------.......A.....C...................T....C....TC..A......
Onir_ungi      ............------.....C.----------......A......T........T.........A..T....C....TCT.A..A...
Rigi_imma      ----------.....GGGT.....C.---------.......A.....C......T.............T....C....TC..A......
Rigi_inus      ----------.....GGGT.....C.---------.......A.....C......T...........T.T....C....TC..A......
Sess_citr      ----------.....GGGT.....C.---------.......A.....C......T.............T....C....TC..A......
Sess_heli      ----------.....GGGT.....C.----------.............CC.....T........T....T....C....TC..A......
Sess_lati      ----------.....GGGT.....C.---------.......A.....C......T.............T....C....TC..A......
Tigr_alpe_alpe .T............GGGT.....C.---------.......A.....C...................T....C....TC..A......
Tigr_alpe_obtu .T............GGGT.....C.---------.......A.....C...................T....C....TC..A......
Tigr_flam      .T............AGGT.....C.---------.......A.....C...................T....C....TC..A......
Tigr_orth      .T............GGGT.....C.---------.......A.....C......T.............T....C....TC..A......
Tigr_dura      --------.C....GGGT.....C.----------...AAG..A.....C...................T....C....TC..A......
Tigr_huaj      --------.C....GGGT.....C.---------.......A....TC......T.............T....C....TC..A......
Tigr_lute      ----------.....CGGGT.....C.---------.......A.........................T....C....TC..A.....G
Tigr_mexi      ----------.....GGGT.....C.---------.......A.....C...................T....C....TC..A......
Tigr_mult      --------.C....GGGT.....C.---------.......A.....C...................T....C....TC..A......
Tigr_sp1_39    .T............CGGGT.....C.---------.......A.........................T....C....TC..A.....G
Tigr_sp1_40    .T............CGGGT.....C.---------.......A.........................T....C....TC..A.....G
Tigr_sp2_43    .T............CGGGT....NN.---------.......A.........................T....C....TC..A.....G
```

```
[              22233333333333333333333]
[              88901222223455555666677]
[              58007046899901345602363 6]
Dipl_lati      TGACAGAGCAAACTTGGTCCTAAG
Ferr_cris      .ACA....................
Iris_vers      .ACA.AG....G............
Libe_ixio      G.CA..................TC
Neom_grac      ..CA....T.C.......T...C.
Neom_nort      ..CA....T.C.......T...C.
Olsy_fili      ---A...........TT.TACC..
Orth_chim      G.CA..............---..C..
Trim_fost      ..CA.....CC.T.....A...C.
Trim_mart      ..CA....A.C.T.........C.
Aine_conz      ..CA......C..GC..C....C.
Alop_ver1      ..CA......G......C....C.
Alop_ver3      ..CA......G......C....CA
Caly_azur      ..CA...A..C.....A.....C.
Caly_mini      ..CA...A..C.....A.....C.
Caly_pal1      ..CA......C.....A.....CA
Caly_pal2      ..CA......C..G...C....C.
Carde_tuna     ..CA......C......C....C.
Cardi_hint     ..CA......C..G----T...C.
Cardi_lon1     ..CA......C..G...C....C.
Cardi_lon2     ..CA......C..G...C....C.
Cardi_mexi     ..CA......C..G...C....C.
Cipu_cam1      ..CA..G...C.....A.....C.
Cipu_cam2      ..CA..G...C.....A.....C.
Cipu_pal2      ..CA..G...C.....A.....C.
Coba_guat      .ACA....................
Cype_aqua      ..CA......C.....A.....C.
Cype_cyrt      ..CA......C......C....C.
Cype_herb_herb ..CA......C.....A.....C.
Cype_mexi      ..CA......C.....A.....C.
Cype_oste      ..CA......C.....A.....C.
Cype_pabs      ..CA......C.....A.....C.
Cype_rose      ------------------------
Eleu_lat1      ..CA..G...C......C....C.
Eleu_lat2      ..CGG.G...C......C....CA
Enne_eur2      ..CA......C......C....C-
Enne_foli      ..CAG.....C......C....CA
```

```
Fost_oax1      ..CA......C..GC..C....C.
Fost_oax2      ..CA......C..GC..C....CA
Gela_elo1      ..CA..G...C......C....C.
Gela_elo2      ..CA..G...C......C....C.
Herb_lahu_inte .ACA.....................
Herb_pul1      ------------------------
Herb_pul2      ..CA.............A.....C.
Herb_tigr      ..CA...A..C.....A.....C.
Hesp_peru      ..CA......C......C....C-
Mast_hopp      ..CA......C......C....C.
Mast_sp1       ..CA......C......C....C.
Mast_sp2       ..CA......C......C....N.
Mast_toro      ..CA......C......C....C.
Nema_conv      ..CAG.....C..G...CT...CA
Nema_tenu      ..CA......C..G...CT...C.
Onir_ungi      ..CA......C.....A.....-.
Rigi_imma      ..CA......C..GC..C....CA
Rigi_inus      ..CA......C..GC..C....C.
Sess_citr      ..CA......C..GC..C....C.
Sess_heli      ..CAG.....C..GC..C....CA
Sess_lati      ..CA......C..GC..C....CA
Tigr_alpe_alpe ..CA......C..G...C....C.
Tigr_alpe_obtu ..CA......C..G...C....C.
Tigr_flam      ..CA......C..G...CT...C.
Tigr_orth      ..CA......C..GC..C....C.
Tigr_dura      ..CA......C..G...CT...CA
Tigr_huaj      ..CA......C..GC..C....CA
Tigr_lute      ..CA......C......C....C.
Tigr_mexi      ..CA......C..G...CT...CA
Tigr_mult      ..CAG.....C..G...C....CA
Tigr_sp1_39    ..CA......C......C....C.
Tigr_sp1_40    ..CA......C......C....C.
Tigr_sp2_43    ..CA......C......C....C.
```

Apendix 1-1 F: TrnLIntron sequence alignment for 60 taxa of the Iridoideae Subfamily. Only variable sites are included (110 out of 503 in the matrix) (Mega 5). Numbers on the top are the specific positions on the gene. A dot means same nucleotide relative to the first sequence used a reference. A hyphen means a gap.

```
[                                11111111111111112222222222222222222222223333333333333333333333333333333334444444444]
[                   22357778899900134556778899990000113333444555669999990000112222333334455666667788899990022222344]
[              2567856161246745756995288130826784569380256129128074567890123685678456894702023596904801580802468027]
Dipl_lati      GAAGTAGAATTACGACAAGGTGTCGAGCCGGCTCCTTGGACTACCGAGAAACACGACCTGACAGAGTTGGTCCAGAGAGAGCTGACTTTAAAAAGATAAG
Ferr_cris      .................G.......T............TGTC.T......................C..........................C......
Libe_ixio      ...C.............GA......T.......T..C.TGT..TT........T.......A.AC.C..............A.C....----........
Neom_nort      ..............GGGG.......T......C.....-GTC.T...C.............A.T.AC......................GG.......G.
Neom_sp1       -------........GGG.......T......C.....-GTC.T...C...T.........A...AC.......................G.......G.
Olsy_fili      A...G.........---GT......TA.T.....T.CA.GT..TT........T...----A.AC.C..A........A....C.T..----....A...
Orth_chim      .................GA....T.T.....A....C.TGT..TT..A.....T.......AGAC.C................C....----........
Trim_mart      .T.............GGG.......T....A.C.....-GTC.T...C.....T.......A...AC.A.....A..........A....G.......G.
Aine_conz      ..........G....GGG....C..T............-GTC.T...C......A......A....C.............A.........G.........
Alop_ver1      ..........G....AGG....C..T............-GTCGT...C......A......A....C....TT.......A.........G.........
Caly_amab      ..-.......G....GGG....C.AT.......T.G..-GTC.T.A.C......M......A....C.......................G....T....
Caly_appr      ..-....C..G....GGG....C..T.T.T........-GTC.T...C......A......A....C.......................G..-------
Caly_azur      ..-......CG....GGG....C..T.T.T........-GTC.T...C......A...G..A....C...C...................G.........
Caly_mini      ..-.......G....GGG....C..T.T.T........-GTC.T...C....N.A...G..A....C.......................G......N..
Caly_nud1      ..-.......G....GGG..N.C..N.T.T........-GTC.T...C.N..C.AN..G..---------------------------------------
Caly_nud2      -------...G....GGG....C..T.T.T........-GTC.T...C......A...G..A....C.......................G.........
Caly_pal2      ..........G....GGG....C..T............-GTCGT...C......A....T.A....C.............A...G.....G.........
Carde_tuna     ..-.......G....GGG....C..T............-GTC.T...C......AC.....A....C.......................G.........
Cardi_hint     .......-..G....GGG....C..T......G.....-GTC.T...C......A......A....C.............A.........G.........
Cardi_lon1     .......-.CG....AGG....C..T............-GTC.T...C......A....T.A....C.............A.......GNG.........
Cardi_lon2     -----C.-T.GT...GGG....C..T............-GTC.T...C.G....A....T.A....C............GA.........G......-..
Cardi_mexi     .......-.CG....AGG....C..T............-GTC.T...C......A....T.A....C.............A.......G.G......T..
Cipu_pal1      ---....-..G.G..GGG....C..T.T.T........-GTC.T...C......A......A....C.......................G.........
Cipu_pal2      ..........G.G..GGG....C..T.T.T........-GTC.T...C......A......A....C.......................G.........
Coba_guat      .................G.......T......G.....TGT..T......................C.................................
Cype_aqua      ..........G.T..GGG.A..C..T.T.T........-GTC.T...C......A......A....C.......................G.........
Cype_cyrt      --------C.G....GGG....C..T............-GTC.T...C....N.NTNN...A....C.......................G.........
Cype_haut_opal ------------------------------------------------......A......A....C.......................G....T....
Cype_herb_herb ..-.......G....GGG....C..T.T.T........-GTC.T...C......A......A....C.......................G.........
Cype_mexi      NNNNN.....G.G..GGG....C..T.T.T........-GTC.T...C......A......A....C.......................G.........
Cype_oste      ..-.......G....GGG....C..T.T.T........-GTC.T...C......A......A....C.......................G.........
Cype_pabs      ..-.......G...GGGG....C..T.T.T........-GTC.T...C......A......A....C......................NG.........
Cype_rose      -----.....G....GGG....C..T........T...-GTCGT...C....N.A......A....C.......................G.........
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Eleu_lat1      ..G.......G....GGG....C.AT.......T.G..-GTC.T...C......A......A....C.......................G....T....
Enne_eur1      ..........G....GGG....C..T........T...-GTCGT...C......A......A....C.......................G.........
Enne_eur2      ..........G....GGG....C..T........T...-GTCGT...C......A......A....C.......................G.........
Fost_oax1      ..........G....GGG....C..T............-GTC.T...C......A......A....C.............A.........G.........
Gela_elo1      ..-........G....GGG....C.AT.......T.G..-GTC.T.A.C......A......A....C.......................G....T....
Gela_elo2      ..........G....GGG....C.AT.......T.G..-GTC.T.A.C......A......A....C.......................G....T....
Herb_lahu      ..........G..A.GGG....C..T.T.T........-GTC.T...C......A......A....C.......................G.........
Herb_lahu_amoe ..-........G....GGG....C..T.T.T........-GTC.T...C......A......A....C.......................G.........
Herb_lahu_inte ..................G.......T......G.....TGT..T.......................C.............................
Herb_pul1      ----------------G..G.C..T.T.T........-GTC.T...C....N.A......A.A..CG..............G.......G.........
Herb_pul2      ..........G....GGG...AC..T.T.T........-GTC.T..TCT...C.A......A....C......C.C.T.........C..GGC......A
Herb_tigr      ..-.......CG....GGG....C..T.T.T........-GTC.T...C......A...G..A....C...C...................G.........
Hesp_peru      ..........G....GGG....C..T............-GTC.T...C......A......A....C.......................G.........
Keli_bras      -.-........G....GGG....C..T.T.T........-GTC.T...C......A......A....C.......................G-........
Mast_hopp      .....N....G....GGG....C..T............-GTC.T...C......A......A....C.......................G.........
Mast_sp1       ..........GN...GGG....C..T............-GTC.T...C......A......A....C.......................G......N..
Mast_sp2       .....N....G....GGG....C..T............-GTC.T...C......A......A....C.......................G.......-.
Mast_toro      ..-........G....GGG....C..T............-GTC.T...C......A......A....C.......................G.........
Nema_tenu      ..........G....GGG....C..T............-GTC.T...C......A......A....C.............A.........G.........
Onir_ungi      ..........G....GGG....C..T.T.T........-GTC.T...C......A......A....C.......................G.........
Tigr_flam      ..........G....GGG....C..T............-GTC.T...C......A......A....C.............A.........G.........
Tigr_orth      ..........G....GGG....C..T............-GTC.T...C......A......A....C.............A.....C...G.........
Tigr_alpe_alpe ..........G....GGGT...C..T............-GTC.T...C......A......A....C.............A.........G.........
Tigr_alpe_obtu ..........G....GGGT...C..T............-GTC.T...C......A......A....C.............A.........G.........
Tigr_sp1_39    -....CC-..G....GGG....C..T............-GTC.T...C..G...A......A....C.........A.............G...A..-T.
Tigr_sp1_40    ..N.NNN-..G....GGG....C..T............-GTC.T...C..G...A......A....C.........A.............G...A.....
Tigr_sp2_43    ---------..G....GGG....C..T............-GTC.T...C..G.NNTTTT.NTA....C.........A.............G...A.....
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```
[              4444444445]
[              5556777990]
[              4781135672]
Dipl_lati      TCACAACTAG
Ferr_cris      ..G.......
Libe_ixio      ..G......A
Neom_nort      ........C.
Neom_sp1       ..........
Olsy_fili      ..........
Orth_chim      ..........
Trim_mart      ........T.
Aine_conz      C.........
Alop_ver1      C.........
Caly_amab      C.........-
Caly_appr      ----------
Caly_azur      ..........
Caly_mini      ..........
Caly_nud1      ----------
Caly_nud2      ..........
Caly_pal2      C.........
Carde_tuna     C.........
Cardi_hint     C.........
Cardi_lon1     C.........
Cardi_lon2     C...TCG...
Cardi_mexi     C.........
Cipu_pal1      ..........
Cipu_pal2      ..........
Coba_guat      .TG.......
Cype_aqua      ..........
Cype_cyrt      C.........
Cype_haut_opal C.........---
Cype_herb_herb ..........
Cype_mexi      ..........
Cype_oste      ..........
Cype_pabs      ..........
Cype_rose      C.........---
Eleu_lat1      C..T......
Enne_eur1      C......A..
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Enne_eur2      C.........
Fost_oax1      C.........
Gela_elo1      C.........
Gela_elo2      C.........
Herb_lahu      ..........
Herb_lahu_amoe ..........
Herb_lahu_inte .TG.......
Herb_pul1      ..........
Herb_pul2      ........T.
Herb_tigr      ..........
Hesp_peru      C.........
Keli_bras      .......---
Mast_hopp      C.........
Mast_sp1       C.........
Mast_sp2       C.........
Mast_toro      C.........
Nema_tenu      C.........
Onir_ungi      ..........
Tigr_flam      C.........
Tigr_orth      C.........
Tigr_alpe_alpe C.........
Tigr_alpe_obtu C.........
Tigr_sp1_39    C.-T-.....
Tigr_sp1_40    C.........
Tigr_sp2_43    C.........
```

Appendix 2-1: Sequence divergence within genera in six different genes for taxa of the SubtribeIridoideae.

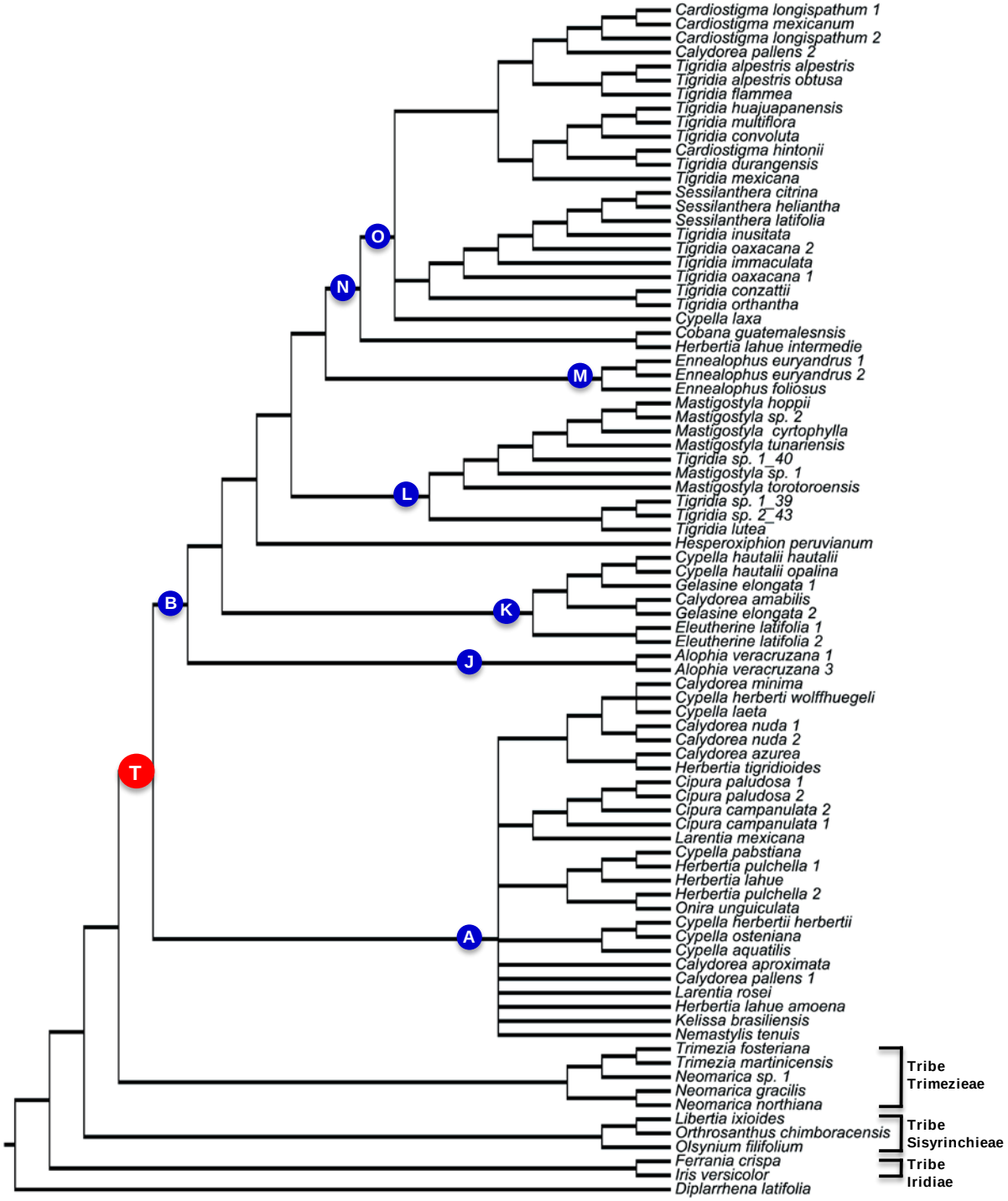
The divergences correspond to absolute differences without any nucleotide substitution model since divergences were very low and no saturations sign was found.

GEN	GENUS	Within genera	Between species
ITS	*Calydorea*	5,48	
	Cardiostigma	0	2,6
	Cipura	0,22	
	Cypella	6,6	
	Eleutherine	1,7	
	Ennealophus	5,6	
	Gelasine	0,7	
	Mastigostyla	1,2	5,8
	Nemastylis	8,5	
	Rigidella	2,7	
	Sessilanthera	2	2,9
	Tigridia	0,2	7
trnL_F	*Alophia*	0,77	
	Calydorea	0,4	4,5
	Cardiostigma	0	1,86
	Cipura	0	
	Cypella	0	8,9
	Eleutherine	3,19	
	Ennealophus	1,2	
	Herbertia	1,3	6,9
	Mastigostyla	0	0,36
	Nemastylis	1,57	
	Rigidella	0,78	
	Sessilanthera	0,4	2
	Tigridia	0	3,97
trnL-Intron	*Calydorea*	0	3,14
	Cardiostigma	0,22	2,5
	Cipura	0	
	Cypella	0	1,64
	Ennealophus	0,21	

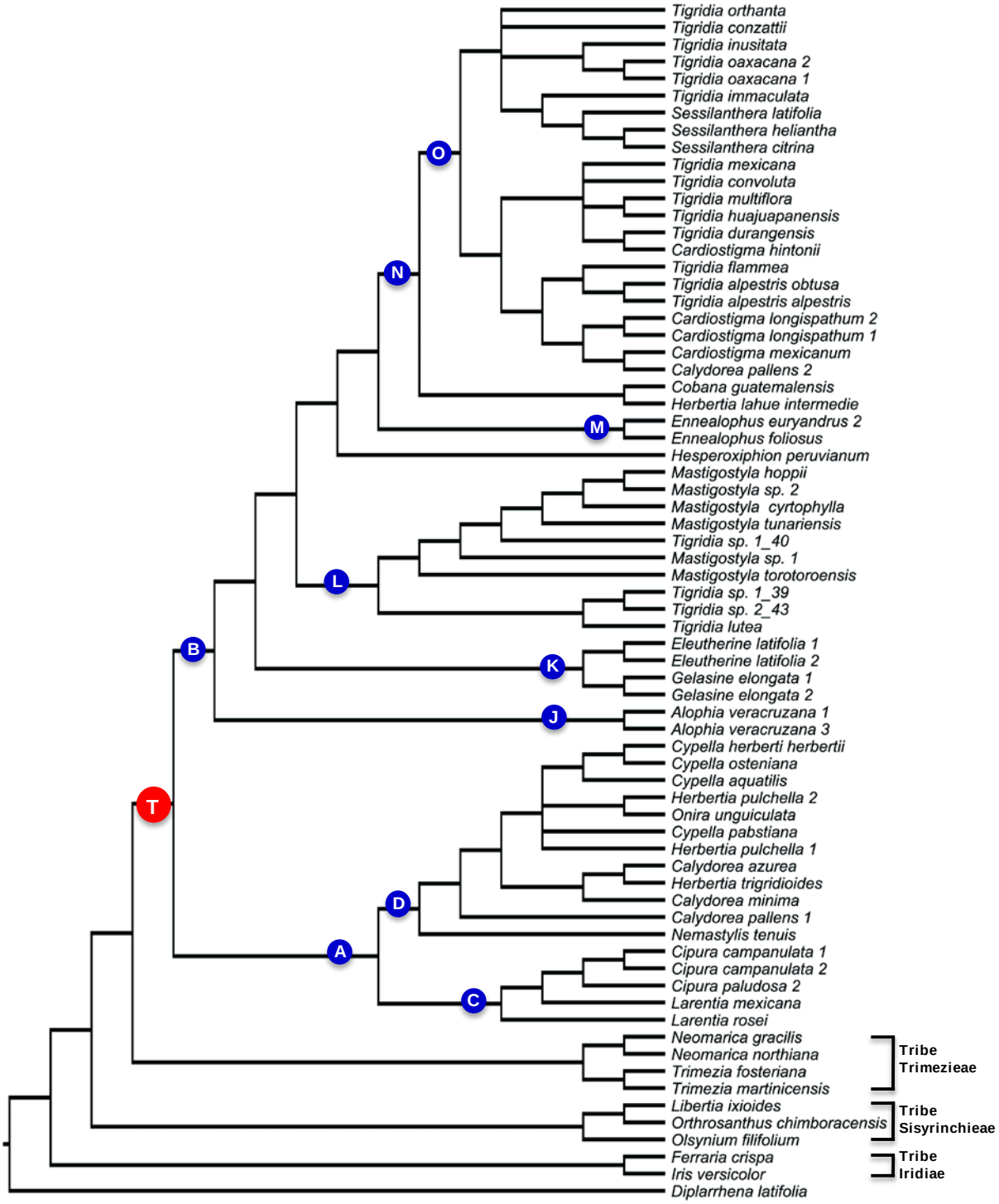
Continued

	Gelasine	0	
	Herbertia	0,22	5,91
	Mastigostyla	0	
	Tigridia	0,22	2,36
trnKK3	*Cypella*	0,55	
	Nemastylis	14,24	
	Tigridia	0,54	1,09
psbA	*Alophia*	3,09	
	Calydorea	0	3,03
	Cardiostigma	1,6	3,2
	Cipura	0	
	Cypella	0,3	8
	Ennealophus	0	1,7
	Herbertia	0	0,25
	Mastigostyla	0	0,7
	Nemastylis	0,3	
	Rigidella	0	
	Sessilanthera	0	
	Tigridia	0	
matK	*Cardiostigma*	0,25	2,5
	Cipura	0	0,58
	Cypella	0,26	4,28
	Eleutherine	0,16	
	Ennealophus	0,29	
	Fosteria	0,12	
	Gelasine	0,57	
	Herbertia	0	0,93
	Mastigostyla	0,48	1,08
	Nemastylis	4,1	
	Rigidella	0,72	
	Sessilanthera	0,44	1,96
	Tigridia	0,24	3,06

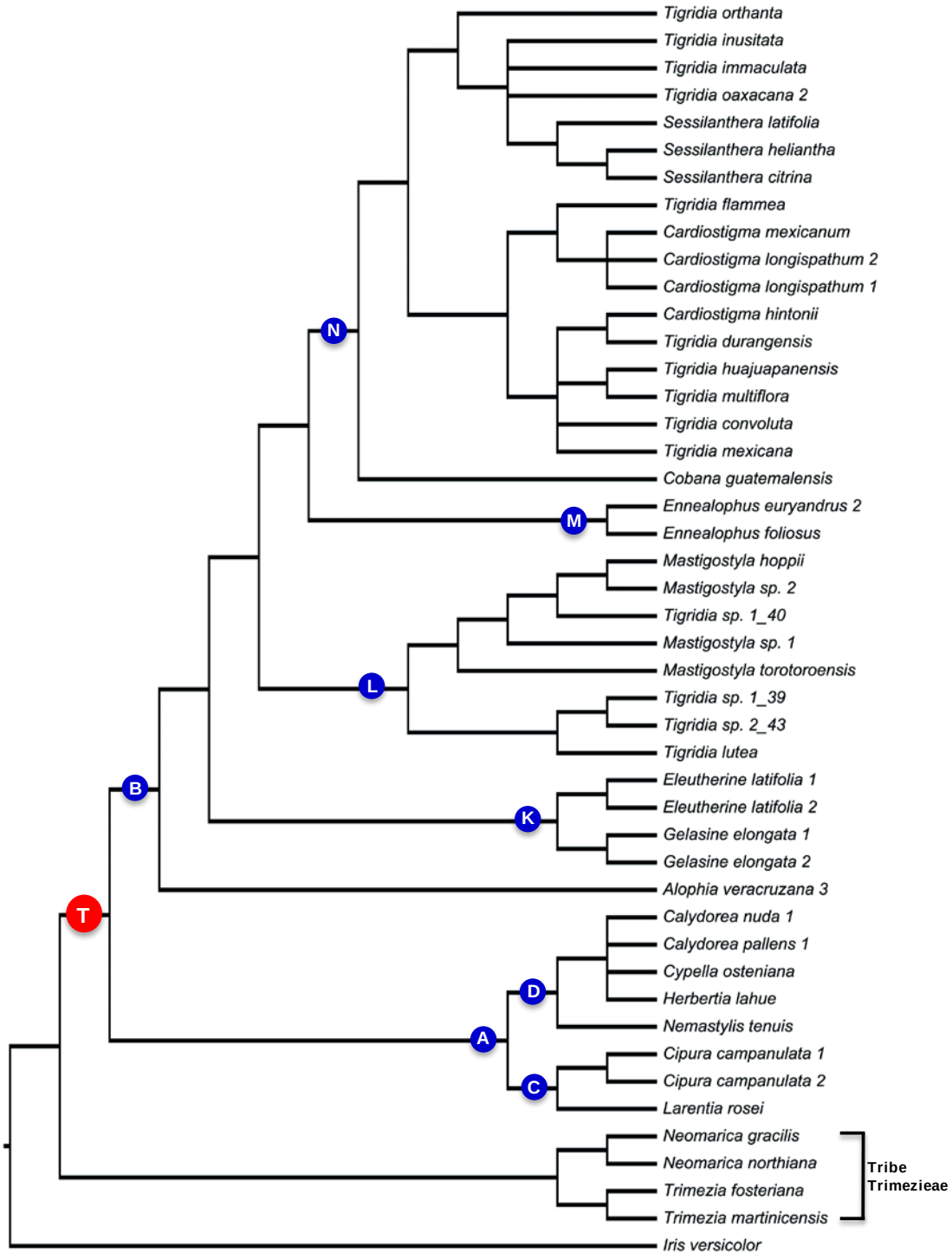
Appendix 3-1 A: Phylogenetic relationships among members of the Tribe Tigridieae (Subfamily Iridoideae) based on sequences from Combination 1 (ITS+psbA+matK+trnKK3+trnLintron+trnL_F).



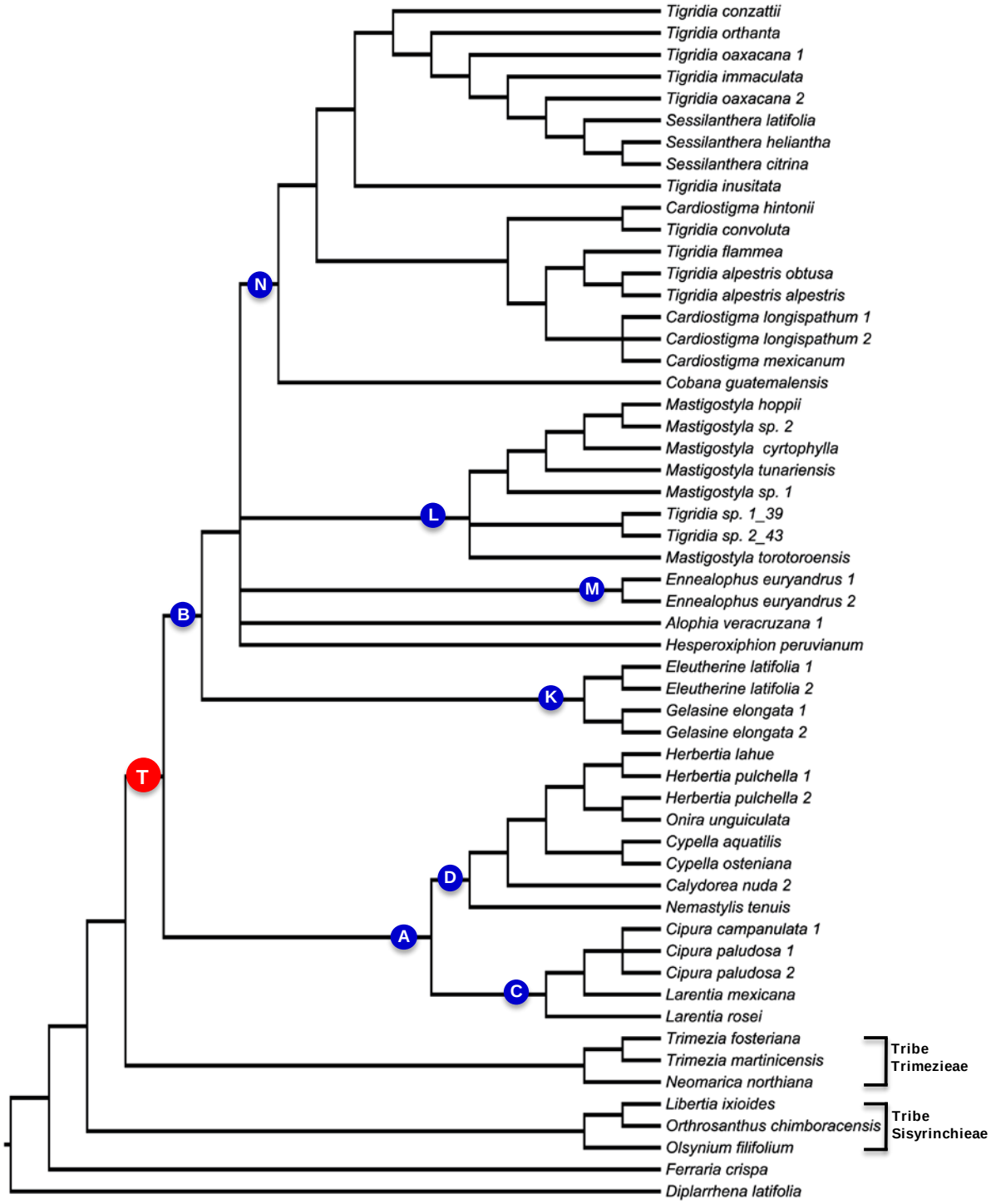
Appendix 3-1 B: Phylogenetic relationships among members of the Tribe Tigridieae (Subfamily Iridoideae) based on sequences from Combination 2 (all with trnL_F).



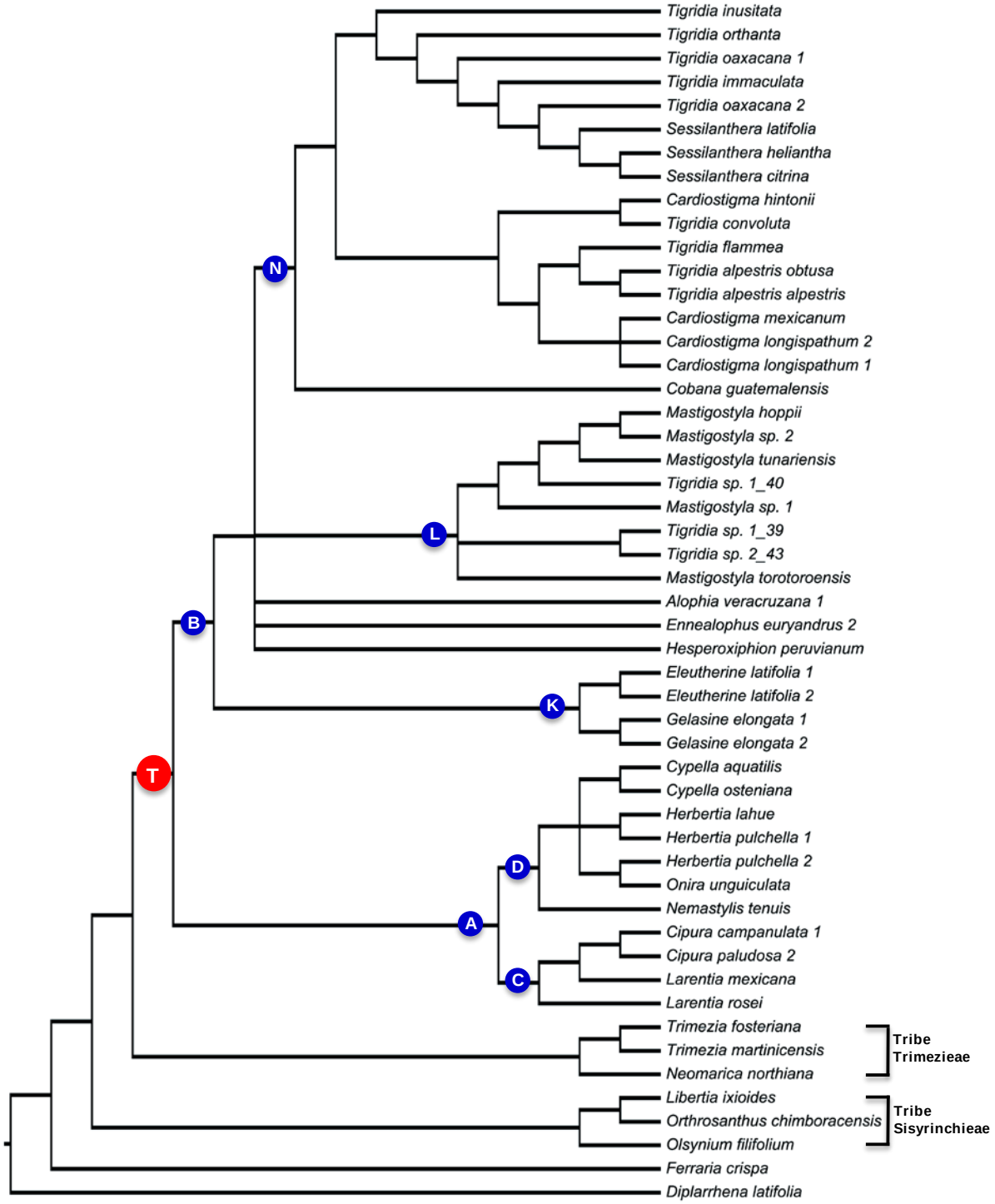
Appendix 3-1 C: Phylogenetic relationships among members of the Tribe Tigridieae (Subfamily Iridoideae) based on sequences from Combination 3 (all with ITS).



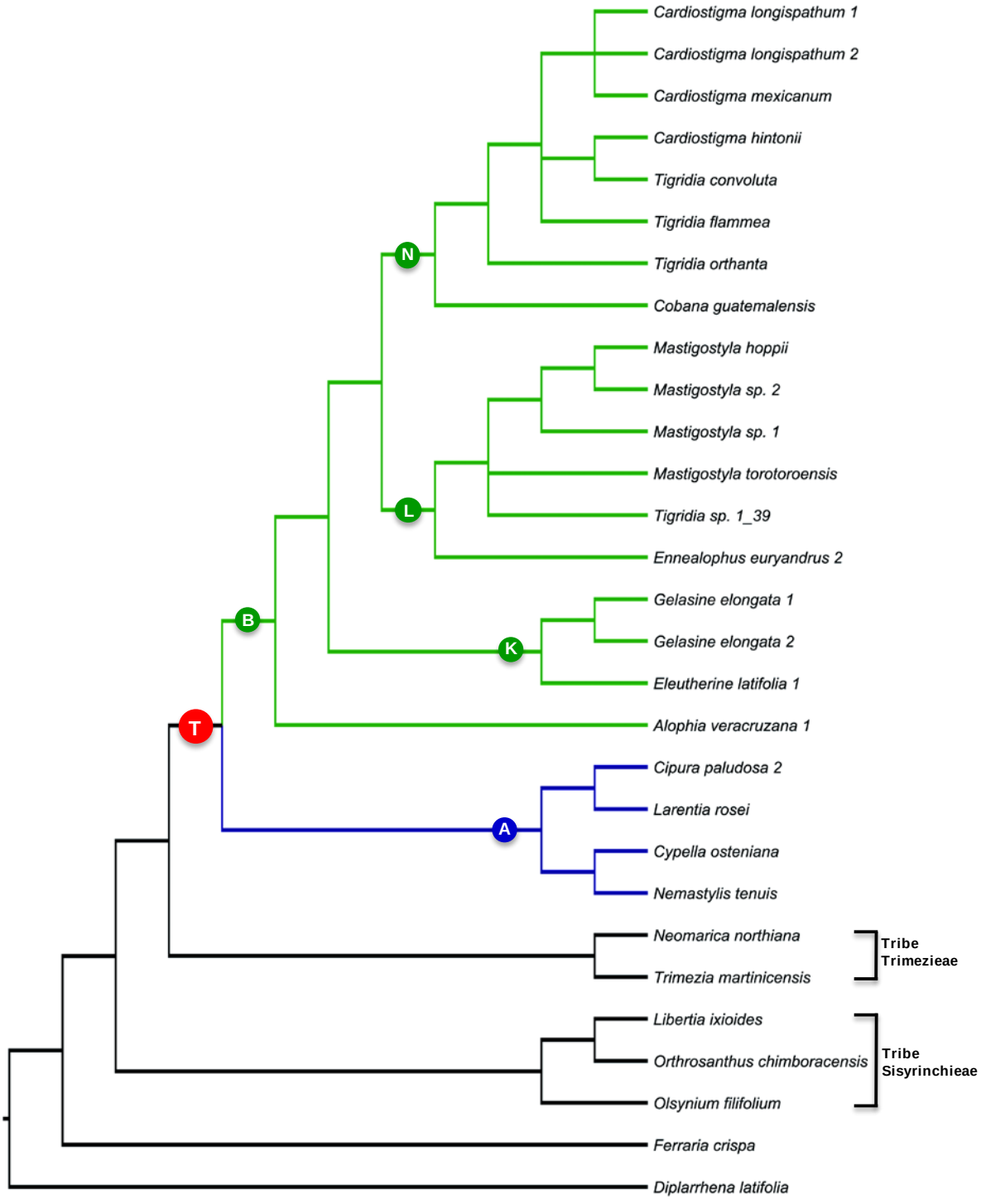
Appendix 3-1 D: Phylogenetic relationships among members of the Tribe Tigridieae (Subfamily Iridoideae) based on sequences from Combination 4 (all with matK).



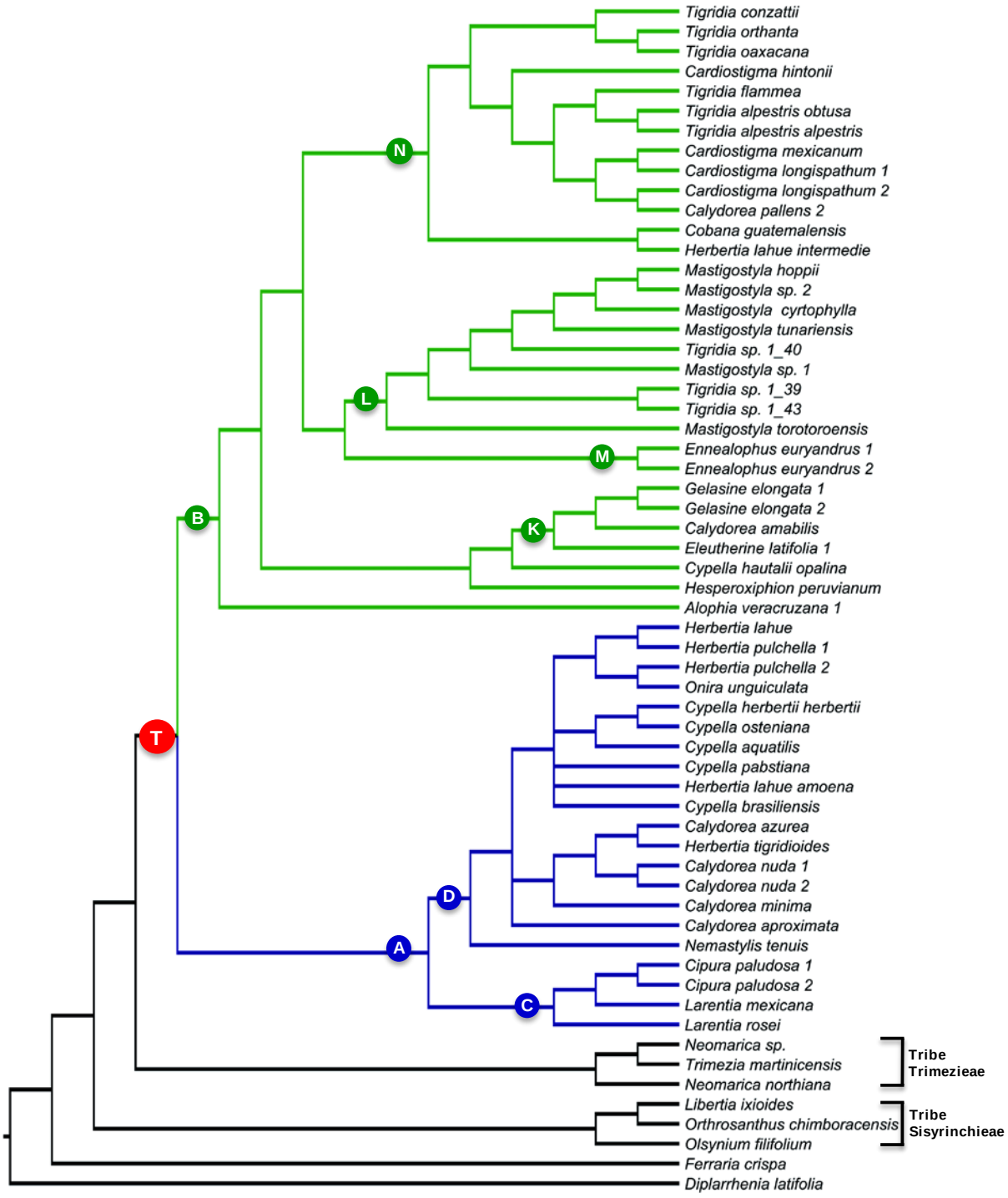
Appendix 3-1 E: Phylogenetic relationships among members of the Tribe Tigridieae (Subfamily Iridoideae) based on sequences from Combination 5 (4 of 6 genes).



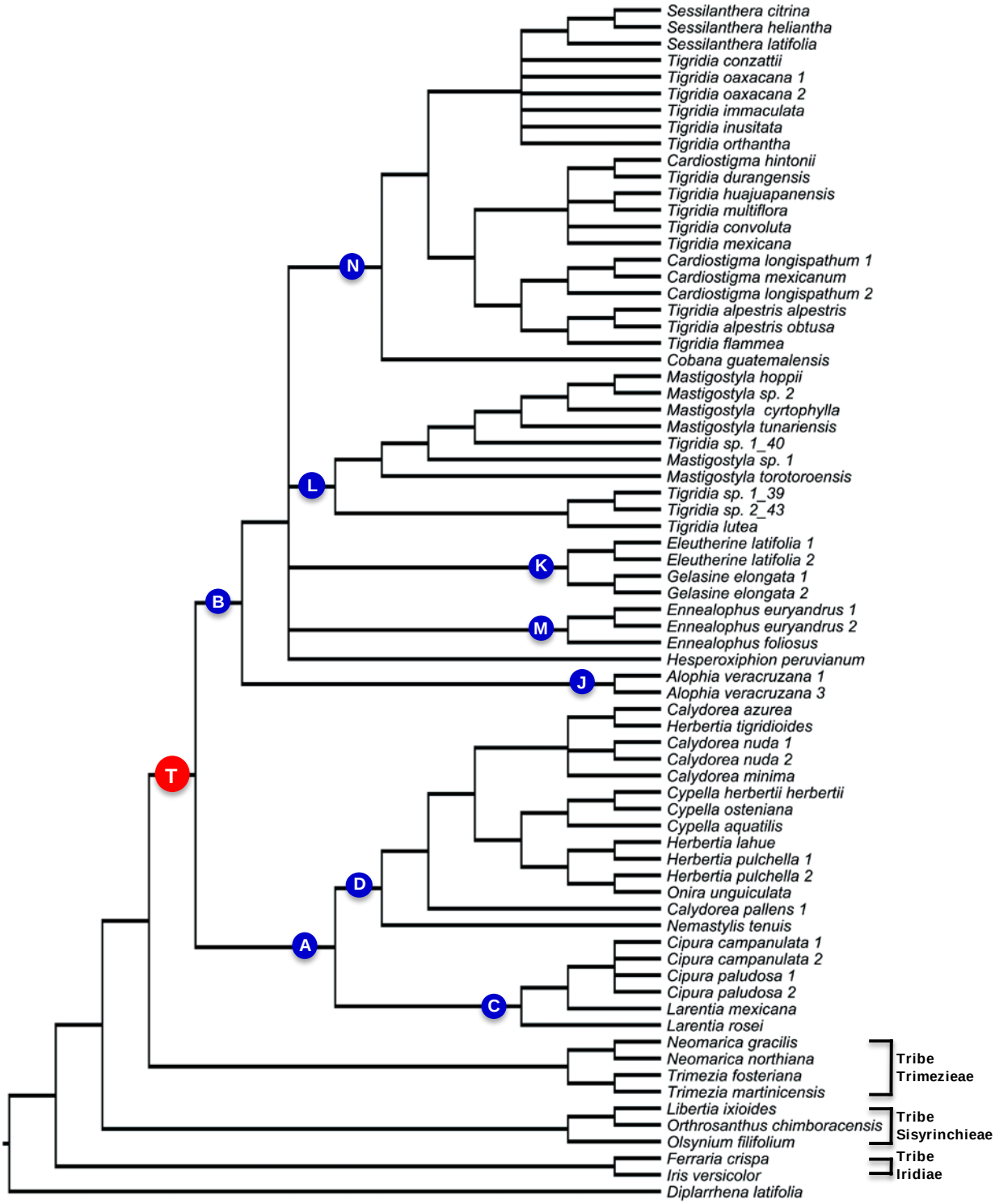
Appendix 3-1 F: Phylogenetic relationships among members of the Tribe Tigridieae (Subfamily Iridoideae) based on sequences from Combination 6 (5 of 6 genes).



Appendix 3-1 G: Phylogenetic relationships among members of the Tribe Tigridieae (Subfamily Iridoideae) based on sequences from Combination 8 (all with trnLintron).



Appendix 3-1 H: Phylogenetic relationships among members of the Tribe Tigridieae (Subfamily Iridoideae) based on sequences from Combination 9 (3 of 5 without trnkk3).



Appendix 4-1: Molecular synapomorphies for different clades of subfamily Iridoideae.

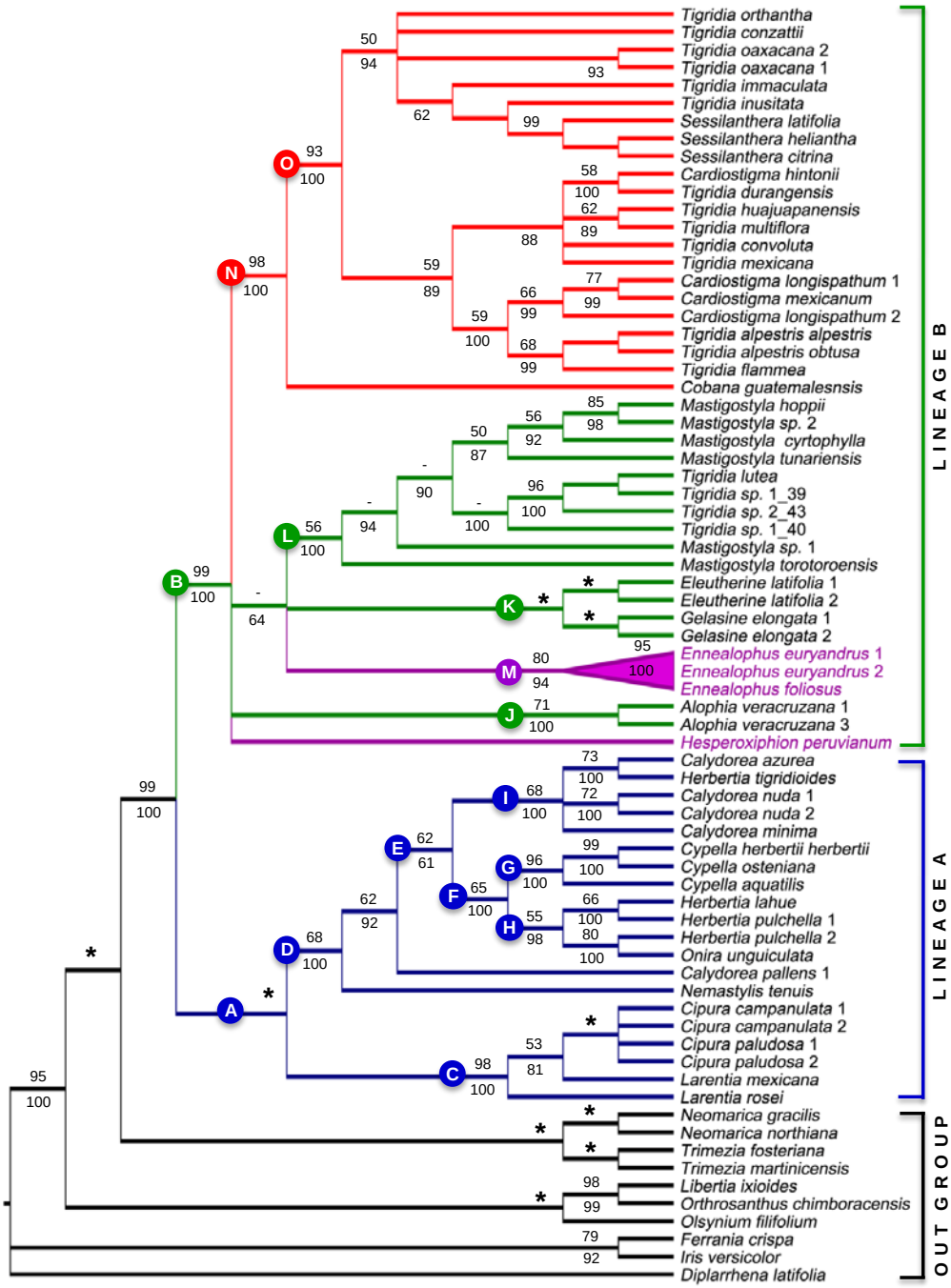
Clade	Change	Mutation	Character	GEN
A	T-C	TI	1553	Matk
A	T-C	TI	1719	Matk
A	C-T	TI	2624	Matk
A	A-T	TV	2523	Matk
A	T-G	TV	2827	trnKK3
A	C-G	TV	2828	trnKK3
A	C-A	TV	2830	trnKK3
A	G-T	TV	2831	trnKK3
A	T-A	TV	2837	trnKK3
A	A-T	TV	2840	trnKK3
A	A-T	TV	2841	trnKK3
A	G-T	TV	2842	trnKK3
A	T-A	TV	2845	trnKK3
B	A-G	TI	556	ITS
B	C-T	TI	1462	Matk
B	A-G	TI	2128	Matk
B	G-T	TV	1133	Matk
B	A-C	TV	1412	Matk
B	A-T	TV	2583	Matk
C	C-T	TI	1357	Matk
C	A-G	TI	2331	Matk
C	T-C	TI	2682	Matk
C	G-T	TV	268	ITS
C	T-G	TV	2181	Matk
D	T-C	TI	1564	Matk
D	A-G	TI	2478	Matk
D	T-A	TV	236	ITS
D	C-A	TV	1108	Matk
D	C-G	TV	1764	Matk
D	A-C	TV	2206	Matk
D	G-C	TV	2695	Matk
E	G-A	TI	185	ITS
K	A-C	TV	188	ITS
L	A-G	TI	2440	Matk
L	C-G	TV	3685	trnL-F
N	G-A	TI	516	ITS
N	G-A	TI	1186	Matk
N	G-A	TI	1200	Matk
N	A-G	TI	1303	Matk
N	G-A	TI	1305	Matk
N	T-C	TI	1307	Matk
N	C-T	TI	1309	Matk
N	C-T	TI	2568	Matk
N	C-T	TI	2630	Matk
N	T-C	TI	2661	Matk
N	T-G	TV	1304	Matk
N	A-T	TV	1306	Matk
N	T-A	TV	1308	Matk
N	G-T	TV	2855	trnKK3
Tribe	T-G	TV	2689	Matk

TV= Transversion; TI=Transition

Appendix 5-1: Estimates of the calibration points for the divergence of different clades of the subfamily Iridoideae.

Clade	Goldblatt et al. 2008	Lib-Orth relaxed clock (BEAST)	Non-Parametric Smoothing Rate													Range
			Lib-Orth	Cype-Trime	Lib-Orth, Cype-Trime	A	B	K	C	F	H	N	X	*Lib-Orth* and X	All	
Subfamily Iridoideae	62	52,07	71,21	53,85	53,85	64	62,3	54,8	63,2	66,9	61,3	59,6	56,17	67,77	58,6	52,07-71,21
Trimezieae-Tigridieae	35	40,97	46,28	35	35	41,6	40,5	35,7	41,1	43,5	39,8	38,7	36.51	44,03	35	35-46-28
Tribe Tigridieae	30,71	38,91	43,35	32,79	32,79	39	37,9	33,4	38,5	40,8	37,3	36,3	34,2	41,23	33,6	32,79-43,35
Subtribe Cipurinae CLADE A	25,36	26,41	27,82	21,04	21,04	25	24,4	21,4	24,7	26,2	23,9	23,3	21,95	26,45	23,3	21,04-27,82
Subtribe Tigridiinae CLADE B	23,57	25,11	26,29	19,88	19,88	23,6	23	20,2	23,3	24,7	22,6	22	20,74	24,97	23,9	19,88-26,29
Clade C	12,86	12,59	13,52	10,22	10,22	12,2	11,8	10,4	12	12,7	11,6	11,3	10,67	12,85	12	10,22-13,52
Clade D	20	20,69	22,6	17,09	17,09	20,3	19,8	17,4	20,1	21,2	19,4	18,9	17,83	21,48	19,5	17,09-22,06
Clade N	13	15,21	15,55	11,76	11,76	14	13,6	12	13,8	14,6	13,4	13	12,26	14,72	13	11,76-15,55
Clade O	12,86	12,75	12,84	9,71	9,71	11,5	11,2	9,89	11,4	12,1	11,1	10,7	10,13	12,12	10,8	9,71-12,84
Clade L	no	15,7	16,56	12,52	12,52	14,9	14,5	12,8	14,7	15,6	14,3	13,9	13,06	15,73	14,6	12,52-16,56
Clade K	11,43	12,12	14,28	10,8	10,8	12,8	12,5	11	12,7	13,4	12,3	12	11,27	13,57	11	10,8-14,28
Clade X	5	5,26	6,34	4,79	4,79	5,69	5,55	4,88	5,63	5,96	5,45	5,3	5	5	5	4,79-6,34

Appendix 6-1: MP analysis (on top, 1000 replicates) and posterior probabilities X 100 from Bayesian analysis (on the bottom). An asterisk mean the support on that node was the maximum in both analysis. A hyphen means that the given support is lower than 50% for bootstrap or 0,5 for posterior probabilities. The red lines denote the only difference in topology relative to the MP analysis of the same genes, the genus *Ennealophus* cluster in a different group.



CHAPTER 2.

POLLEN MORPHOLOGY OF THE TRIBE TIGRIDIEAE (IRIDOIDEAE, IRIDACEAE) AND ITS SYSTEMATIC IMPLICATIONS

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POLLEN MORPHOLOGY OF THE TRIBE TIGRIDIEAE (IRIDOIDEAE, IRIDACEAE) AND ITS SYSTEMATIC IMPLICATIONS

ABSTRACT

In this study we expanded the phylogenetic and palynological analyses of the South American *Tigridia* by studying for the first time the genus *Cardenanthus* R. C. Foster and increasing the sampling of *Calydorea* Herb., *Cypella* Herb., *Herbertia* Sweet and South American *Tigridia* Juss.; making it the largest sampling of the tribe to date. The palynological variation of pollen number of sulci (i.e., apertures) and other palynological characters (e.g., pollen size, pattern of pollen ornamentation, lumina and muri size) were carefully evaluated and described within the tribe. Pollen aperture number and pollen exine sculpture were studied in the contex of new molecular phylogenies based on the nuclear ribosomal internal transcribed spacer (nrITS) and five plastid DNA regions-- the non-coding *trnL-trnF*, the *trnL* intron, the non-coding *trnH-psb*A intergenic spacer, the *mat*K and the *trn*K3 genes. Evolution and ancestral characters states of aperture number and sculpture were inferred using parsimony and maximum likelihood approaches. Molecular phylogenetics, character optimizations, and statistical analyses suggested that differences in number of apertures, lumina size, muri size, and patterns of ornamentation are taxonomically and phylogenetically informative and can be used to distinguish some taxa and clades within Tigridieae. Although the number of pollen apertures (i.e., two sulci) did not support the recognition of subtribe Tigridiinae as traditionally circumscribed, the disulcate pollen supported the expansion of

Tigridia to include seven other genera: *Ainea* Ravenna, *Cardiostigma* Baker*, Cobana* Ravena, *Colima* (Ravenna) Aarón Rodr. and Ortiz-Catedral, *Fosteria* Molseed, *Rigidella* Lindl., *Sessilanthera* Molseed and Cruden.

Our preliminary results indicate that pollen morphology is potentially useful for understanding phylogenetic relationships within the tribe Tigrideae and should be further investigated.

Keywords: Pollen, Tigridieae, sulci, character evolution, South America, Iridaceae

RESUMEN

En este estudio se ampliaron los análisis filogenéticos y palinológicos de especies Suramericanas de la Tribu Tigridieae. Se incluye por primera vez el género *Cardenanthus* R. C. Foster y se incrementó el muestreo de especies de *Calydorea* Herb., *Cypella* Herb*., Herbertia* Sweet y *Tigridia* Juss. El muestreo aquí empleado es el más extenso de la tribu hasta la fecha. La variación del número de sulcos (i.e., aperturas) y otros caracteres palinológicos (por ejemplo, el tamaño del polen, el patrón de la ornamentación, y el tamaño del lumen y el muri) fueron cuidadosamente evaluados y descritos en la tribu. La evolución del número de aperturas y ornamentación de polen de la tribu se estudió a la luz de nuevas filogenias moleculares basadas en secuencias nucleares (nrITS) y cinco regiones de ADN cloroplástico: *trn*L-*trn*F, *trn*L intrón, *trn*H-*psb*A, *mat*K, y *trn*K3. La evolución de los caracteres y los estados ancestrales del número de aperturas y ornamentación de la exina fueron estimados usando métodos de parsimonia y máxima verosimilitud. Los análisis filogenéticos, optimizacion de caracteres y estudios estadísticos sugieren que las diferencias en el número de aperturas, el tamaño de lumen, el tamaño del muri, y los patrones de ornamentación son taxonómica y filogenéticamente informativos y pueden ser utilizados para distinguir taxones y clados en Tigridieae. Aunque el número de aperturas de polen (polen disulcado) no apoyó el reconocimiento de la subtribu Tigridiinae como tradicionalmente se circunscribe, el polen bisulcado apoyó la ampliación de *Tigridia* para incluir

otros siete géneros: *Ainea* Ravenna, *Cardiostigma* Baker, *Cobana* Ravena, *Colima* (Ravenna) Aarón Rodr. and Ortiz-Catedral, *Fosteria* Molseed, *Rigidella* Lindl., *Sessilanthera* Molseed and Cruden. El número de aperturas y la ornamentación del polen, apoyaron el reconocimiento de los linajes polifiléticos de *Cypella*. Nuestros resultados preliminares, indican que la morfología del polen es potencialmente útil para la comprensión de las relaciones filogenéticas dentro de la tribu Tigridieae. Estudios similares a los que aqui se presentan deben continuar desarrolladose en el futuro.

Palabras clave: Polen, Trigridieae, sulcos, evolución de caracteres, Suramérica, Iridaceae

1. INTRODUCTION

Tigridieae is an American tribe of the Iridoideae (Iridaceae) described by Kittel (1840). The tribe comprises bulb forming plants with plicate leaves and basic chromosome number of *n*= 7. Although vegetatively uniform, the group exhibits sizeable variation in floral morphology, including differences in tepal orientation, size and color patterning (Fig 1.). Septal nectaries are absent and replaced by elaiophores as floral secretion structures, which may be present on tepal claws in some species (Rudall *et al.,* 2003). The style branches frequently form a specialized and complex structure intimately associated with the stamens (Rodríguez and Systma, 2006; Fig. 2.). This florally diverse tribe consists of ca. 15 genera and 160 species (Goldblatt and Manning, 2008) subdivided into subtribes Cipurinae and Tigridiinae. Cipurinae is defined by its monosulcate pollen grains, basic chromosome number of *n*= 7, and a center of diversity in South American with some species of *Herbertia* Sweet (1 spp.) and *Nemastylis* Nutt. (5 spp.) extending into the Southern US. Tigridiinae is recognized by its disulcate pollen grains, basic chromosome number of *n*= 14, and its geographic distribution is centered in Mexico and Guatemala, with five species in Peru and North of Chile (Goldblatt, 1982; Rodríguez, 1999).

Rudall and Wheeler (1988) presented the first palynological study of the Tigridieae and tested the taxonomic value of pollen information. That study included 38 specimens representing 11 genera (15 spp.) of Cipurinae, and three genera (seven spp.) of Tigridiinae. Later Goldblatt and Thomas (1992) in their pollen study of the subfamily Iridoideae, expanded the sampling of the tribe and included about 9 genera (22 spp.) of Cipurinae and one genus (four spp.) of Tigridiinae. Goldblatt and Thomas (1992) sampled a total of seven species representing *Cipura* Aubl. and *Cypella* Herb. (including *Kelissa* Ravenna) and Rudall and Wheeler (1988) studied the palynological variation of *Catila* Ravenna, *Fosteria* Molseed and *Mastigostyla* I.M. Johnston, three genera not sampled before. Both studies suggested that at the subtribal (e.g.,Tigridiinae) and generic level (e.g., *Alophia* Herb., *Ennealophus* N.E. Br. and *Gelasine* Herb.), aperture number and exine sculpture provided valuable synapomorphies for the delimitation of taxa (Goldblatt and Thomas, 1992). These studies also concluded that the variability in aperture number and exine sculpturing could be significant in the taxonomy of the Tigridieae tribe and indicated the need for a phylogenetic framework to interpret the evolution of pollen within the tribe.

More recently, Rodriguez (1999) using an ITS based phylogeny of the tribe evaluated for the first time the evolution of pollen in Tigridieae. His study comprised six and five genera of Cipurinae and Tigridiinae, respectively, excluding South American species (except for *Ennealophus foliosus* (Kunth) Ravenna and *Tigridia lutea* Link, Klotzsch & Otto). His studies showed that Tigridieae includes monosulcate, zonasulcate, trichotomo sulcate, spiralsulcate and disulcate pollen grains and he discussed patterns of pollen aperture evolution. Pollen aperture evolution in the tribe, which appears to follow the trend in monocot pollen evolution described by Zavada (1983), where the monosulcate type is the ancestral condition and the bisulcate type is derived. The three goals of this study are to: 1) to evaluate and characterize pollen variation in the tribe, 2) to study pollen aperture number and pollen exine sculpture evolution in Tigridieae using new molecular-based phylogenies, and 3) to assess the taxonomic utility of several palynological characters.

2. MATERIALS AND METHODS

2.1. Palynological analyses

The pollen of 87 species was investigated (Table 2). Information for several species was taken from published palynological reports (e.g., Rudall and Wheeler, 1988; Goldblatt and Le Thomas, 1992). The terminology used in descriptions follows Erdtman (1952), Faegri and Iversen (1964), and Kremps (1965).

Pollen material was gathered from herbarium specimens (HSB, K, MO), spirit material, live plants in the field, and from Germán Roitman's living collection in Buenos Aires University, Buenos Aires, Argentina.

Living and spirit material was dehydrated through an alcohol series, critical point-dried using a Bal-Tec 030 critical point dryer, and mounted on aluminum stubs for observations. Herbarium material was mounted directly onto stubs. Samples were coated with platinum using an Emitech K550 sputter coater.

Observations using Scanning Electronic Microscopy (SEM) were made at Royal Botanic Gardens, Kew (K) and the Missouri Botanical Garden (MO), using a Hitachi Cold Fields Emission SEM S-4700 at 2 KV and a Jeol Neoscope JCM 5000 at 10 KV, respectively. Measurements and image analyses were obtained from 30 grains using the ImageJ 1.45 program (Image processing and Analysis in Java). Five pollen characters were studied: pollen size, number of apertures, membrane apertures, exine sculpture, lumina size, and muri size. Palynological variation of ten closely related species of *Cypella* (Cipurinae as defined by Celis *& García,* Chapter 1) was evaluated with univariate, bivariate, and multivariate statistical methods (Principal Component Analysis, PCA) using JMP software.

2.2 Molecular phylogenetic analyses

A Bayesian tree was estimated using a total of 72 accessions representing 69 taxa (including 10 spp. in the outgroup). The phylogeny was based on six markers: nuclear ITS, plastid *trnL-trnF, trnL* intron, *trnH-psbA, matK, trnKK3* markers (Celis *& García*, Chapter 1).

2.3 Character evolution

Patterns of evolution and ancestral character state reconstructions for pollen aperture number, and sculpture exine type were inferred using Parsimony and Maximum Likelihood approaches in Mesquite version 2.75 (Maddison and Maddison 2012). The two characters were weighted and considered unordered. Ambiguous resolutions were investigated using two different parsimony assumptions: the accelerated transformation (ACCTRAN) and delayed transformation (DELTRAN) schemes implemented in MacClade 4.08 (Maddison and Maddison 2005). To obtain a fully resolved tree topology a requirement imposed by ACCTRAN, DELTRAN and Maximum likelihood schemes, four taxa were removed from the newly proposed Tigrideae phylogeny (Celis *& García*, Chapter 1). Maximum likelihood optimizations were based in the Markov Chain (MK) evolutionary model with 1 parameter. Taxa removed from the tree neither affected main phylogenetic relationships or results and conclusion presented below.

3. RESULTS

3.1. General description of Tigridieae pollen

*Shape***.** Following Erdtman's (1952) pollen shape categories the pollen in Tigridieae is mostly subprolate (Figs. 3D-E,) to prolate spheroidal (Figs. 3A, 3B, 3C), with few representatives with prolate (Fig. 3I) or subprolate shapes (Table 2).

Size. Pollen size according to Kremp's (1962) classification is very large. Within the tribe size is variable across its genera (Table 1) and within species (Table 2). In Cipurinae the grains are in average 42.97 µm in length, with an average breadth of 36.78 µm. Tigridiinae grains have an average length of 43.62 µm and an average breadth of 36.38 µm.

The comparison of our and published data showed that known estimations of the pollen grain size were larger than the ones here observed (Table 1 and 2). *Calydorea approximata* (22.72 × 20.97 µm), and *Cypella osteniana* (29.17 × 27.30 µm) in average showed the smallest pollen grains within Tigridiinae and Cipurinae respectively (see Appendix 1). The biggest pollen grains recorded for Cipurinae and Tigridiinae respectively were found in *Cipura formosa* (68.1 × 56.8 µm) and *Gelasine corerulea* (58.80 × 43.80 µm).

Sulci number and orientation. Cipurinae have mainly monosulcate grains (Fig. 3), with the exception of few species of *Cipura*, which also exhibit disulcate grains as seen in some species of *Alophia* (Rudall and Wheeler, 1988). The sulci correspond to the the zonasulcate (Fig. 3G) or trichotomosulcate (Fig. 3H) apertures types.

In Tigridiinae the most common pollen is monosulcate and disulcate. In the last case the apertures are located in the longest axis of the grains.

Examples of monosulcate pollen in this subtribe are *Tigridia pavonia* (Rudall and Wheeler, 1988) and *Cardiostigma longispathum,* with zonasulcate grains.

The aperture membrane (Table 2) is commonly smooth (psilate) (Fig. 3B, E), verrucated as in *Alophia* (Fig. 3A) or granular as in *Hesperoxhipion* and *Ennealophus*.

Exine sculpturing. The exine sculpture in Tigridieae is complex and to understand its variation has been challenging because of the incomplete or often conflicting morphological interpretations published in previous studies (Table 4). Five characters of the exine (general sculpture, lumina size, continuity of the muri, muri walls facing the lumina, sculpture of the endexine) were identified as taxonomically useful and the variation in those characters was quantified and described (Table 3). This information was used to develop a new standardized exine classification and to construct descriptions of the exine for the tribe and each of its 12 genera (Table 5).

In general Tigridieae present four general types of exine sculpture: heterobrochate (e.g., heteroreticulate) (Fig. 4A), microheterobrochate (lumina < 1 µm) (Fig. 4D-4H), rugulate (Fig. 4B, 4C) and perforate (Fig. 11I). The microheterobrochate exine has in average a small lumina area of 0.003 to 0.017 μm^2 and is characteristic of pollen grains of *Alophia*, *Eleutherine* and *Herbertia* (Fig. 4D-H). Pollen grains of *Calydorea* and *Gelasine* have a rugulate exine (Fig. 4B, 4C). In the tribe the muri is continuous excepting in *Cypella herbertii* subsp. *wolffhuegelii* (Fig. 5H).

The individual lumen or meshes of the reticulum have several variations (Fig. 5). The shape of the lumina can be defined by straigtht (Fig. 4D, 4G) or curved and very large sinuous muri walls (Fig. 4A, 4J, 4K).

Additionally the heterogeneous lumina can show small lumina intermixed with larger lumina sizes (Fig. 4E, 4F, 4I) as in *Hesperoxhipion* where the largest lumina is surrounded by the smaller ones, sometimes the smallest forming a ring around the largest ones.

In general the tectated areas of the exine appear to be simplicolumellate (e.g., tectum sustained by a single row of columellas; Fig. 4F, 4G). Finally, the lumina endexine (intectated areas in the lumina) in the tribe can be granulate or psilate.

3.2. Pollen descriptions of genera of Tigridieae

The palynological descriptions below provide information about the shape and size of the grains, number of sulci, and sculpture of the aperture membrane and exine. The descriptions based on the sampling provided in Table 3, and supplemented with information from previous works (Rudall & Furnnes 1982, Goldblatt & Le Thomas 1992). The number studied of taxa and the total number of species per genera is annotated in parentheses after the genus. A summary of measurements and exine type is presented in Table 3.

Alophia (3/6). *Pollen shape* subprolate to prolate spheroidal*; pollen size* (31.9) 38 (40.05) µm length × (23.06) 30.25 (35.17) µm breadth. *Number of apertures* 1 (pollen monosulcate), excepting in *Alophia drummondii* (2 apertures -pollen disulcate; Rudall & Furness 1982); *membrane aperture* verrucate (Fig. 3A). *Exine* microheterobrochate to heterobrochate; *muri* (0.2) 0.34 (0.5) µm breadth, *muri walls* facing the lumina straight or not; *lumina endexine* granulate, *lumina area* of 0.017-0.55 μm^2, with an average of 0.14 μm^2. In *Alophia veracruzana* the lumina is larger than in *A. silvestris* (Table 3, Appendix 1).

Calydorea (4/16). *Pollen shape* subprolate, prolate-spheroidal to prolate; *pollen size* (21.1) 35.18 (48.6) µm length × (19.7) 27.59 (36.8) µm breadth. *Number of apertures* 1; *membrane aperture* psilate. *Exine*

microheterobrochate (as in *C. amabilis* and *C. approximata*) to heterobrochate as in *C. campestris*, *C pallens* and *C. undulata*; *muri* breadth in average 0.40 µm, *muri walls* facing lumina straight to sinuate curve; *lumina endexine* without granules (excepting *Calydorea undulata*), *lumina area* in the microheterobrochate grains 0.003-0.17 μm^2, lumina in the heterobrochate grains 1.06-2.44 μm^2.

Cipura (5/9)***.*** *Pollen shape* predominantly subprolate to prolate-spheroidal; *pollen size* the largest grains of the tribe (45.4) 54.89 (68.1) µm length × (39.39) 47.17 (59) µm breadth. *Number of apertures* 1 to 3, mono- or disulcate or zonasulcate; membrane aperture psilate. *Exine* heterobrochate to microheterobrochate; *muri walls* facing the lumina not straight to straight*, muri* 1 µm breadth; *lumina endexine* granulate, the *lumina area* is the largest in the tribe, 2.4-20.3 μm^2, with an average of 6.54 μm^2.

Cypella (12 spp. and 5 subspp./30). *Pollen shape* prolate-spheroidal, subrpolate to prolate; *pollen size* (26.05) 35.05 (56.40) µm length × (21.1) 30.97 (48.1) µm breadth, *Cypella herbertii* has the largest known grains of the genus and *C. osteniana* the smallest ones; *membrane aperture* psilate. *Exine* mostly heterobrochate, few microheterobrochate (*C. brasilensis*) or rugulate-porate (Table 3); *muri wall* facing the lumina straight or not, *muri* continuous or discontinuous, muri 0.53 µm breadth; *lumina endexine* with or without granules at the base (see Table 2). The variation of muri and lumina define four pollen types within the genus:

Type I. Sculpture micro- and heterobrocate-perforate (*C. laxa*, *C. herbertii* subsp *herbertii*), pori 0.002-0.5 μm^2*; muri* continuous, *muri walls* facing the lumina straight or not; *lumina endexine* psilate*, lumina area* 1-6 μm^2.

Type II. Sculpture microheterobrochate without pori; *muri* continuous, *muri walls* facing the lumina straight or not, *muri* 0.35 breadth; *lumina endexine* psilate, *lumina area* 0.008-0.951 μm^2.

Type III. Sculpture microheterobrocate-porate; *muri* continuous, *muri walls* facing the lumina straight or not, *muri* breadth 0.44 µm; *lumina endexine* psilate, *lumina area* 0.023-0.06 µm^2.

Type IV. Sculpture micro- and heterobrochate without pori; *muri dis*continuous, *muri walls* facing the lumina sinuate, *muri* 0.55-0.58 µm breadth; *lumina endexine* granulate or not, small lumina intermixed with larger lumina, the smallest lumina sometimes surrounding the largest one, *smallest lumina area* 0.016-1.7 µm^2, *largest lumina area* (0.5) 1.7-5.23 µm^2.

Eleutherine (1/2). *Pollen shape* subprolate; *pollen* length 27.87 µm, pollen breath unknown. *Number of apertures* 1; *membrane aperture* psilate. *Exine* microheterobrochate-porate; *muri walls* facing the lumina sinuate or curvy, muri 0.2 µm breadth in average; *lumina endexine* psilate, *lumina area* (0.01) 0.07 (0.21) µm^2.

Ennealophus (2/5). *Pollen size* (36) 41.6 (46.2) µm length × (29) 33 (37.8) breadth. *Number of apertures* 1; *membrane aperture* smooth or with exine islands and granules (as in *E. foliosus* and *E. euryandrus* according with Goldblatt & Le Thomas 1992). *Exine* reticulate; *muri* continuous, without perforations, 0.5 µm in average breadth; *lumina* polyhedral, small lumina sometimes round to polyhedral, without granules at the base of the lumen.

Gelasine (2/6). *Pollen shape* prolate-spheroidal, oblate-spheroidal, prolate; *pollen* size (30.19) 35.36 (58.8) µm length × (27.41) 32.23 (43.8) µm breadth. *Number of apertures* 1; *membrane aperture* psilate. *Exine* microheterobrochate; *muri walls* facing the lumina curvy or sinuate, muri 0.33 µm breadth in average; *lumina endexine* psilate, *lumina area* 0.18 µm^2.

Herbertia (4/7). *Pollen shape* mainly subpprolate to prolate-spheroidal; *pollen* size (25.97) 33.78 (49.8) µm length × (22.64) 19.14 (41.8) µm

breadth. *Number of apertures* 1; *membrane aperture* psilate. *Exine* microheterobrochate, rugulate, porate or not; *muri walls* facing the lumina curved or sinuate, muri 0.36 µm breadth in average; *lumina endexine* psilate, *lumina area* 0.10 μm^2.

Hesperoxiphion (2/4). *Pollen shape* prolate-spheroidal to prolate; *pollen size* (37.32) 42.79 (57.5) µm length × (34.10) 38.59 (49.9) µm breadth. *Number of apertures*; *membrane aperture* granulate. *Exine* heretobrochate; *muri walls* facing the lumina straight or not, muri 0.57 µm breadth in average; *lumina endexine* granulate, small lumina intermixed with larger lumina, the smallest lumina sometimes surrounding the largest one, *smallest lumina area* 0.074-0.761 μm^2, *largest lumina area* 2.046-4.89 μm^2.

Larentia (1/4). *Pollen shape*; *pollen size* (44.87) 47.18 (49.69) µm length pollen breadth unknown. *Number of apertures* 1; *membrane aperture* psilate. *Exine heterobrochate*; *muri walls* facing the lumina sinuate to curvy, muri 0.56 µm breadth in average; *lumina endexine* psilate, *lumina area* 1.33-7.88 μm^2.

Mastigostyla (4/20). *Pollen shape* prolate spheroidal; *pollen* size (30.51) 54.26 (69.31) µm length × (26) 28.44 (30.8) µm breadth. *Number of apertures* 1 to 2; *membrane aperture* psilate. *Exine* micro- and heterobrochate; *muri* walls facing the lumina sinuate or curvy, muri 0.33 µm breadth in average; *lumina endexine* psilate, *lumina area* 0.70 μm^2.

Tigridia (6/55). *Pollen shape* prolate to subprolate; *pollen* size (42) 48.02 (55.30) µm length × (29.7) 36.54 (43.9) µm breadth. *Number of apertures* 1 to 2; *membrane aperture* psilate. *Exine* microheterobrochate to rugulate; *muri walls* facing the lumina curvy or sinuate to straight, muri 38 µm breadth in average; *lumina endexine* psilate, *lumina area* 0.2-0.67 μm^2.

3.3 Pollen morphological variation: univariate, bivariate and multivariate analyses. A case study in *Cypella* species

To evaluate the taxonomic utility of continuous pollen variation within the Trigrideae we studied the sculpture exine variation as quantitative rather than qualitative data. In a pilot exploration we study *Cypella* species as a study case. The variation of exine sculpture was evaluated with univariate, bivariate, and multivariate statistical methods (Principal Component Analysis, PCA). Measurements and image analyses of exine sculpture images (Celis and Fuentes 2011, Fig. 2; see Appendix 2) were made for two pollen characters (lumina size and muri size).

Our SEM observations, image analyses, and statistical tests indicated that features of the exine ornamentation might be taxonomically useful and phylogenetically informative (Celis and Fuentes 2011, Fig. 5; see Appendix 2).

PCA analyses, showed that pollen length, muri width, and area lumen described 30% of the total pollen variation in species of *Cypella*, indicating the palynotaxonomic utility of these characters (Celis and Fuentes 2011, Fig. 6; see Appendix 2).

These results additionally indicated that the variation of pollen sculpture treated as a continuous variable might offer novel insights for understanding patterns of exine evolution. Differences in continuous variation of the lumen area and muri width, both defining ornamentation type, appear to be helpful to trace pollen evolution and to distinguish species with more certainty (Celis and Fuentes 2011, Fig. 5; see Appendix 2).

Analyses at the generic and subtribal level are in progress to explore the value of patterns of exine variation within Tigridieae (Celis and Fuentes in prep).

3.4 Character evolution

Pollen aperture number. Ancestral character reconstruction using parsimony and likelihood (99%) methods suggested the monosulcate pollen as the ancestral condition of the whole tribe (Fig. 6B, 13-14) and suggested that the disulcate pollen condition evolved exclusively within Tigridiinae (likelihood 99%; Fig. 8).

Within Tigridiinae parsimony reconstructions using the “all most parsimonious states” (MPR) trace option (Fig. 7A) and likelihood (Fig. 8) assumptions recovered at least two independent evolutions for the disulcate pollen condition (within clade L and N). Within this subtribe depending on the clade analyzed and/or scheme of optimization implemented it is inferred that the disulcate pollen type evolved from ancestor that presented a monosulcate pollen or reconstructions resulted ambiguous. Parsimony and likelihood reconstructions resolved unambiguosly that the ancestor of the N clade presented a monosulcate pollen. However, for the clade L, MPR and likelihood reconstructions were ambiguous (Fig. 7A, 8). In contrast DELTRAN and ACCTRAN (Fig. 7) estimated that the disulcate pollen in the clade L evolved from an ancestor with a monosulcate pollen.

The zonosulcate pollen condition evolved twice with the tribe, once in each of the two subtribes: in *Cardiostigma* within the clade N (Tigridiinae) and in *Cipura* within the clade C (Cipurinae). Interestingly, the derived zonosulcate condition appears to have evolved from different ancestral pollen aperture conditions. MPR and likelihood (98%) reconstructions suggest that the zonosulcate pollen in *Cardiostigma* clade evolved from an ancestral disulcate pollen type. On the other hand, for the *Cipura* clade MPR and likelihood optimizations are ambiguos (Fig. 7A, 8) and ACCTRAN and DELTRAN (Fig. 7A) optimizations resolved the monosulcate pollen type for the ancestor of the clade.

Pollen ornamentation. The heterobrochate (reticulate) pollen ornamentation is recovered unambiguously by both Parsimony and likelihood (>95%) methods for the ancestor of the whole Tigridieae and Cipurinae (Fig. 9-11). However, the reconstruction of the ancestral condition for Tigridiinae is ambiguous (MPR optimizations; Fig. 10A), heterobrochate (likelihood (93%) and DELTRAN optimizations; Fig. 10B), or microheterobrochate (ACCTRAN assumptions; Fig. 10C).

The microheterobrochate ornamentation evolved at least four times within the tribe (Fig. 9-10), once in Cipurinae in the clade H (incl. *Hebertia*), and three times within Tigridiinae, once in the clade J (incl. *Alophia*), another time in *Eleutherine* (incl. in clade K), and in *Tigridia lutea* (incl. in clade L). All charater reconstructions inferred that the microheterobrochate ornamentation condition of the Cipurinae H clade evolved from an ancestor that presented pollen with reticulate ornamentation (Fig. 10-11). However the ancestor for the Tigridiinae with microheterobrochate sculpture varies between methods and clades observed. The rugulate pollen type evolved independently one time in each of the subtribes (Fig. 9-11).

4. DISCUSSION AND CONCLUSIONS

A palynological study and patterns of aperture types and sculpture evolution of Tigridieae are presented using the most up-to-date molecular phylogeny of the tribe. In this study the tribe's sampling is greatly expanded with respect to previous palynological studies of the group (Rudall and Wheeler 1988; Goldblatt and Le Thomas 1992). Most notable additions include species of *Calydorea, Cardenanthus*, *Cypella, Herbertia,* and South American *Tigridia.*

Traditionally *Alophia*, *Cardenanthus*, *Ennealophus*, *Hesperoxiphion*, *Mastigostyla* and the South Americana Tigridieae have been classified in the

subtribe Cipurinae as they all possess monosulcate pollen. Phylogenetic results now show that these genera belong to four basal clades of Tigridiinae. Patterns of aperture type evolution do not correlate with the traditional subtribal circumscription in Tigridieae. The characters reconstructions showed that although the monosulcate pollen is ancestral for the whole tribe it evolved within both subtribes. Analyses also suggest that the disulcate pollen evolved indeed only within the Tigridieae but it is not a synapomorphy for the subtribe and a rare derived condition within it.

Pollen sculpture optimized as a categorical character appears to be a constant feature for the tribe (Fig. 9-11). However it would be valuable to study the evolution of pollen sculpture variation as a continuous character using lumen area and muri width data. The patterns of exine sculpture treated as a continuous character is currently being re-evaluated using phylogenetically generalized least square (PGLS) algorithms in a Bayesian framework (Celis & Fuentes in prep).

The number of pollen apertures did not support the recognition of subtribe Tigridiinae as traditionally circumscribed. However the presence of disulcate pollen supports the expansion of Tigridia to include seven other genera (*Ainea*, *Cardiostigma*, *Cobana*, *Colima*, *Fosteria*, *Rigidella*, *Sessilanthera*).

Our preliminary results indicate that pollen morphology is potentially useful for understanding phylogenetic relationships within the tribe Tigrideae and should be further investigated.

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6. TABLES

Table 1-2: Summary of pollen morphology in Tigridieae.

The summary includes information taken from Rudall & Wheeler (1988), Goldblatt & Le Thomas (1992) and data generated in this study. *Alophia* (3/6), *Calydorea* (4/16), *Cipura* (5/9), *Cypella* (12/30 spp, 5 subspp), *Eleutherine* (1/2), *Ennealophus* (2/5), *Gelasine* (2/6), *Herbertia* (4/7), *Hesperoxiphion* (2/4), *Larentia* (1/4), *Mastigostyla* (4/20), *Tigridia* (6/55).

Abbreviations: C= Cipurinae, T= Tigridiinae (*Goldblattt, 1982); A= Lineage A, Lineage B (**Celis & García). Circumscription proposed in Chapter 1). zn= zonasulcate; ve= aperture membrane covered with a verrucate exine; gr= aperture membrane usually granular; s= aperture membrane smooth (psilate); ei= with exine islands; N/A= data not available.

GENERA	*TRIBE	**LINEAGE	MURI (Average)	SULCUS	APERTURE MEMBRANE	GRAIN LENGHT (µm)			GRAIN BREADTH (µm)		
						min	max	Average	min	max	Average
Alophia	C	B	0,34	1,(2?)	ve	31,94	41,00	38,00	23,06	37,50	30,25
Calydorea	C	A	0,4	1	s	21,11	48,60	35,18	19,73	36,80	27,59
Cipura	C	A	1,01	1,2,3,zs	s	45,35	68,10	54,89	39,39	59,00	47,17
Cypella	C	A	0,53	1	s	26,05	56,40	35,05	21,10	48,10	30,97
Eleutherine	C	B	n/a	1	s	24,51	35,20	27,87	n/a	n/a	n/a
Ennealophus	C	B	0,55	1	s (gr, ei)	36,03	46,18	41,58	28,93	37,83	33,19
Gelasine	C	B	0,33	1	s	30,19	58,80	35,36	27,41	43,80	32,23
Herbertia	C	A	0,36	1	s	25,97	49,80	33,78	22,64	41,80	29,14
Hesperoxiphion	C	B	0,57	1	gr	37,32	57,50	42,79	34,10	49,90	38,59
Larentia	C	A	0,56	1	s	44,87	49,69	47,18	n/a	n/a	n/a
Mastigostyla	C	B	0,33	1 (2?)	s	30,51	69,31	54,26	26,00	30,88	28,44
Tigridia	T	B	0,38	(1) 2	N/A	42,00	55,30	48,02	29,70	43,90	36,54

Table 2-2: List of species and pollen characters studied for Tigridieae.

Abbreviations used for describing exine structure: general sculpture (r= heterobrochate, mr= microheterobrochate, ru= rugulate), muri walls facing the lumina (s= straight; ns= not straight), and lumina endexine (ps= psilate; g= granulate). ve= aperture membrane covered with a verrucate exine; gr= aperture membrane usually granular; s= aperture membrane smooth (psilate); ei= with exine islands; N/A= data not available. References: 1. Datas obtained in this study; 2= Goldblatt & Le Thomas (1992); 3: Rudall & Wheeler (1988).

SPECIES	SULCUS	EXINE SCULPTURE	APERTURE MENBRANE	AVERAGE GRAIN SIZE		P/E index	POLLEN SHAPE (Erdtman 1952)	POLLEN SIZE	Pollen SIZE CATEGORY (Kremp 1965)	ACCESSION	ORIGEN	Ref.
				Length (µm) = P	Breadth (µn) = E							
Alophia drummondii (Graham) R.C.Foster	1	r-s-ps	Ve	39,20	33,00	1,19	Subprolate	118,79	vrl	Eggert s.n. (MO)	N/A	2
Alophia silvestris (Loes.) Goldblatt	1	mr-ns-ps	Ve	37,43	28,78	1,30	Subprolate	130,06	vrl	Roitman 23p	Mexico	1
Alophia silvestris (Loes.) Goldblatt	1	N/A	Ve	40,20	33,50	1,20	Subprolate	120,00	vrl	Dwyer 11034 (MO)	Belize, Belize	2
Alophia veracruzana Goldblatt & T.M.Howard	1	mr-(s+ns)-ps	Ve	41,00	37,50	1,09	Prolate-spheroidal	109,33	vrl	Howard s.n. (MO)	Mexico, Veracruz	2
Calydorea amabilis (Ravenna) Goldblatt & Henrich	1	mr-(s+ns)-ps	Sm	36,95	29,71	1,24	Subprolate	124,39	vrl	Roitman 12p	Brazil	1
Calydorea amabilis (Ravenna) Goldblatt & Henrich	1	N/A	Sm	37,80	30,50	1,24	Subprolate	123,93	vrl	Castillo s.n. (MO)	Argentina, Misiones	2
Calydorea approximata R.C.Foster (18)	1	mr-s-ps	Sm	22,72	20,97	1,08	Prolate-spheroidal	108,35	vrl	Roitman 18p	Argentina, Entre Rios	1
Calydorea campestris (Klatt) Baker	1	r-(s+ns)-g	Sm	46,60	33,10	1,41	Prolate	140,79	vrl	Donbrowski 55-344	Brazil	3
Calydorea pallens Griseb.	1	N/A	Sm	48,60	36,80	1,32	Subprolate	132,07	vrl	Castillo s.n. (MO)	Argentina, Misiones	2
Calydorea undulata	1	r-s-g	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1

Continued

Cipura campanulata Ravenna	2	mr-(s+ns)-ps	N/A	59,80	55,00	1,09	Prolate-spheroidal	108,73	vrl	Keton 33-201	Mexico	3
Cipura campanulata Ravenna	2	N/A	N/A	47,20	41,20	1,15	Subprolate	114,56	vrl	Keton 50-342	Mexico	3
Cipura campanulata Ravenna	2	N/A	N/A	61,70	51,60	1,20	Subprolate	119,57	vrl	Keton 33-195	Mexico	3
Cipura campanulata Ravenna	(1-2) 3s-zs	N/A	Sm	60,20	52,30	1,15	Subprolate	115,11	vrl	Henrinch 143 (MO)	Nicaragua	2
Cipura formosa Ravenna	1	N/A	Sm	68,10	56,80	1,20	Subprolate	119,89	vrl	Irwin 12718 (P)	N/A	2
Cipura paludosa Aubl.	zs	r-ns-g	Sm	66,20	59,00	1,12	Prolate-spheroidal	112,20	vrl	Plowman 9305 (MO)	Brazil, Maranhão, Imperatriz	2
Cipura rupicola Goldblatt & Henrich	1	N/A	Sm	60,70	59,00	1,03	Prolate-spheroidal	102,88	vrl	Davidse 26437 (MO)	Venezuela, Amazonas	2
Cipura xanthomelas subsp. *xanthomelas* (36)	1 (3z)	r-ns-g	N/A	50,48	43,10	1,17	Subprolate	117,12	vrl	Roitman 36p	N/A	1
Cypella aquatilis Ravenna	1	(r+p)-(s+ns)-ps	Sm	33,95	29,05	1,17	Subprolate	116,87	vrl	Rotman 14p	Argentina	1
Cypella armosa Ravenna (21)	1	r-s-ps	N/A	38,34	36,64	1,05	Prolate-spheroidal	104,64	vrl	Ritman 21p	N/A	1
Cypella brasiliensis (Baker) Ravenna	1	N/A	N/A	39,50	29,10	1,36	Prolate	135,74	vrl	Sello s.n.	N/A	2
Cypella brasiliensis (Baker) Ravenna (16)	1	mr-ns-ps	N/A	31,44	22,81	1,38	Prolate	137,79	vrl	Roitman 16p	N/A	1
Cypella coelestis (Lehm.) Diels	1	(mr+p)-ns-g	Sm	N/A	N/A	N/A	N/A	N/A	N/A	Roitman 34p	Brazil.Paraná	1
Cypella crenata (Vell.) Ravenna (43)	1	r-ns-g	N/A	N/A	38,11	N/A	N/A	N/A	N/A	Roitman 43p	N/A	1

Continued

Cypella exilis Ravenna (24)	1	r-ns-ps	N/A	41,21	33,54	1,23	Subprolate	122,87	vrl	Roitman 24	N/A	1
Cypella hauthalii subsp. *hauthalii*	1	r-s-ps	Sm	33,32	30,45	1,09	Prolate-spheroidal	109,42	vrl	Roitman 13p	Argentina	1
Cypella hauthalii subsp. *opalina* Ravenna	1	r-ns-ps	Sm	35,35	31,20	1,13	Prolate-spheroidal	113,30	vrl	Rotman 40	Paraguay	1
Cypella herbertii (Lindl.) Herb.	1	N/A	N/A	56,40	48,10	1,17	Subprolate	117,26	vrl	Cutler s.n.	Argentina	3
Cypella herbertii (Lindl.) Herb.	1	N/A	Sm	45,70	40,50	1,13	Prolate-spheroidal	112,84	vrl	N/A	N/A	2
Cypella herbertii subsp. *brevicristata* Ravenna	1	N/A	Sm	45,20	37,50	1,21	Subprolate	120,53	vrl	Castillo s.n. (MO)	N/A	2
Cypella herberti subsp. *herbertii* (27)	1	(r+p)-s-ps	N/A	39,13	30,12	1,30	Subprolate	129,91	vrl	Roitman 27p	Argentina, Entre Rios	1
Cypella herbertii subsp. *wolffhuegelii* (Hauman) Ravenna (7)	1	(ru+r)-ns-ps	N/A	38,68	32,69	1,18	Subprolate	118,33	vrl	Roitman 7	Argentina. Buenos Aires: Tandil	1
Cypella laxa Ravenna	1	mr-(s+ns)-ps	Sm	32,87	29,98	1,10	Prolate-spheroidal	109,65	vrl	Roitman 22p	Brazil	1
Cypella osteniana Beauverd	1	mr-s-ps	Sm	29,17	27,30	1,07	Prolate-spheroidal	106,85	vrl	Roitman 35	Uruguay	1
Cypella pabstiana Ravenna (20)	1	r-s-ps	N/A	40,72	34,97	1,16	Subprolate	116,43	vrl	Roitman 20p	Argentina. Misiones: Cerro Azul	1
Cypella sp.	1	N/A	N/A	41,30	39,60	1,04	Prolate-spheroidal	104,29	vrl	Cutler 26-207	Argentina	3
Eleutherine latifolia (Standl.& Will.) Ravenna	1	N/A	Sm	35,20	27,10	1,30	Subprolate	129,89	vrl	Castillo s.n. (MO)	N/A	2

Continued

Eleutherine latifolia (Standl.& Will.) Ravenna (26)	1	(mr+p)-ns-ps	Sm	27,54	N/A	N/A	N/A	N/A	N/A	Goldblatt 9072 (MO)	Argentina, Tucumán	1
Ennealophus euryandrus (Griseb.) Ravenna	1	r-ns-ps	N/A	36,70	34,90	1,05	Prolate-spheroidal	105,16	vrl	Cutler 68-357	Argentina	3
Ennealophus euryandrus (Griseb.) Ravenna	1	N/A	Gr	40,30	35,30	1,14	Prolate-spheroidal	114,16	vrl	Solomnon 9972 (MO)	N/A	2
Ennealophus euryandrus (Griseb.) Ravenna	1	r-ns-ps	Sm	41,94	31,85	1,32	Subprolate	131,67	vrl	Roitman 28	NW Argentina	1
Ennealophus foliosus (Kunth) Ravenna	1	N/A	Ei	46,10	39,30	1,17	Subprolate	117,30	vrl	Dillon 4514 (MO)	N/A	2
Ennealophus foliosus (Kunth) Ravenna	1	mr-ns-ps	Sm	38,59	36,10	1,07	Prolate-spheroidal	106,91	vrl	Roitman 17p	Peru	1
Gelasine coerulea (Vell.) Ravenna	1	N/A	Sm	58,80	43,80	1,34	Prolate	134,25	vrl	Muller 228 (P)	N/A	2
Gelasine elongata (Graham) Ravenna (2,50)	1	mr-ns-ps	N/A	32,12	29,34	1,09	Prolate-spheroidal	109,48	vrl	Roitman 50	Argentina, Misiones	1
Gelasine elongata (Graham) Ravenna (57)	1	mr-ns-ps	N/A	34,87	36,29	0,96	Oblate-spheroidal	96,09	lp	Roitman 57p	Argentina, Misiones	1
Gelasine elongata (Graham) Ravenna	1	N/A	Sm	45,10	37,50	1,20	Subprolate	120,27	vrl	Goldblatt s.n. (MO)	N/A	2
Gelasine elongata (Graham) Ravenna	1	N/A	N/A	43,50	39,10	1,11	Prolate-spheroidal	111,25	vrl	Goldblatt s.n. cultivated (MO)	Mexico	3
Herbertia lahue (Molina) Goldblatt (10)	1	mr-ns-ps	N/A	36,52	28,95	1,26	Subprolate	126,17	vrl	Roitman 10p	Argentina, Entre Rios	1
Herbertia lahue (Molina) Goldblatt	1	N/A	Sm	35,20	27,80	1,27	Subprolate	126,62	vrl	Goldblatt s.n. (MO)	Brazil, Rio Grande do Sul	2
Herbertia lahue (Molina) Goldblatt	1	N/A	Sm	49,80	41,80	1,19	Subprolate	119,14	vrl	Tubergen s.n.	N/A	3

Continued

Herbertia lahue subsp. *intermedie (Ined.)* (5-13)	1	(ru+r+p)-ns-ps	Sm	30,51	25,69	1,19	Subprolate	118,76	vrl	Roitma 5p	Argentina	1
Herbertia pulchella Sweet (17)	1	mr-ns-ps	Sm	34,66	29,28	1,18	Subprolate	118,37	vrl	N/a	Argentina. Buenos Aires: Tandil	1
Herbertia quareimana Ravenna (4)	1	mr-ns-ps	Sm	33,63	N/A	N/A	N/A	N/A	N/A		N/A	1
Herbertia tigridioides (Hicken) Goldblatt (14)	1	ru-ns-ps	Sm	33,10	29,69	1,11	Prolate-spheroidal	111,47	vrl	Roitman 14p	Argentina, Salta	1
Hesperoxiphion huilense Ravenna	1	N/A	Sm	50,00	39,60	1,26	Subprolate	126,26	vrl	Porter 144	Ecuador	3
Hesperoxiphion peruvianum (Baker) Baker (67)	1	r-(s+ns)-g	Sm	37,99	36,63	1,04	Prolate-spheroidal	103,72	vrl		N/A	1
Hesperoxiphion peruvianum (Baker) Baker	1	N/A	Gr	57,50	46,20	1,24	Subprolate	124,46	vrl	Goldblatt s.n. (MO)	Peru	2
Hesperoxiphion peruvianum (Baker) Baker	1	N/A	N/A	54,00	49,90	1,08	Prolate-spheroidal	108,22	vrl	Rudall 84-646	Peru	3
Hesperoxiphion peruvianum (Baker) Baker	1	N/A	N/A	48,00	44,10	1,09	Prolate-spheroidal	108,84	vrl	Rudall 118-725	Peru	3
Larentia linearis (Kunth) Klatt (37)	1	r-ns-ps	Sm	47,18	N/A	N/A	N/A	N/A	N/A			1
Mastigostyla boliviensis (R.C.Foster) Goldblatt (34)	1	mr-ns-ps	Sm	45,93	N/A	N/A	N/A	N/A	N/A			1
Mastigostyla sp. nov (99)	1	r-ns-ps	Sm	31,57	28,44	1,11	Prolate-spheroidal	111,01	vrl			1
Mastigostyla tunariensis (R.C.Foster) Ravenna (98)	1	mr-ns-ps	Sm	61,00	N/A	N/A	N/A	N/A	N/A		Bolivia, Cochabamba	1

Continued

Tigridia dugesii S.Watson	2	N/A	R	48,4	32,5	1,49	Prolate	148,92	vrl	Pringle 4400 (P)	Mexico	2
Tigridia galanthoides Molseed	2	mr-ns-ps	N/A	46,4	37,4	1,24	Subprolate	124,06	vrl	Walker 74.008	Mexico	3
Tigridia lutea Link	2	mr-ns-ps	lm-p	N/A	N/A	N/A	N/A	N/A	N/A	Dombey s.n. (P)	N/A	2
Tigridia meleagris (Lindl.) G.Nicholson	2	N/A	N/A	42	29,7	1,41	Prolate	141,41	vrl	Keton 52-367	Mexico	3
Tigridia molseediana Ravenna	2	ru-(s+ns)-ps	R	47,9	33,8	1,42	Prolate	141,72	vrl	Pringle 4771 (P)	N/A	2
Tigridia oaxacana (Molseed) Goldblatt	2	N/A	N/A	44	36,6	1,20	Subprolate	120,22	vrl	Keton 21-135	Mexico	3
Tigridia orthantha (Lem.) Ravenna	2	N/A	lm-lh	55,3	43,9	1,26	Subprolate	125,97	vrl	Keton 18-98	Mexico	3

Table 3-2: Exine sculpture variation in Tigridieae (Iridaceae).

Abbreviations used for describing exine structure: general sculpture (r= heterobrochate, mr= microheterobrochate, ru= rugulate), muri walls facing the lumina (s= straight; ns= not straight), and lumina endexine (ps= psilate; g= granulate).

Species	Exine sculpture formula	General sculpture		Small lumina intermixed with largest lumina		Muri sculpture		Muri		Muri walls facing lumina		Muri breadth (µm)	Endexine in lumina		Area lumen (µm^2)		
		Rugulate	Heterobrocate	Present	No present	Psilate	Perforate	Continuos	Discontinuos	Straight	Not straight		Granulate	Psilate	Mean	Minimum-maximum values	25-75% quantiles
Alophia drummondii (Graham) R.C.Foster	r-s-ps		X		X	X		X		X		N/A		X			
Alophia silvestris (Loes.) Goldblatt	mr-ns-ps		X-MICRO		X			X			X	0,34		X	0,09	0.01-0.7	0.061-0.118
Alophia veracruzana Goldblatt & T.M.Howard	mr-(s+ns)-ps		X-MICRO		X	X		X		X	X	0,44		X	0,33	0.079-0.551	0.250-0.401
Calydorea amabilis (Ravenna) Goldblatt & Henrich	mr-(s+ns)-ps		X-MICRO		X			X		X	X	0,41		X	0,23	0.003-0.652	0.145-0.298
Calydorea approximata R.C.Foster (18)	mr-s-ps		X-MICRO		X	X		X			X	0,35		X	0,06	0.01-0.172	0.041-0.082
Calydorea campestris (Klatt) Baker	r-(s+ns)-g		X		X	X		X		X	X	N/A	X		1,74	1.057-2.443	1.193-2.308
Calydorea undulata	r-s-g		X		X	X		X		X			X				
Cipura campanulata Ravenna	mr-(s+ns)-ps		X-MICRO		X	X		X		X	X			X	NA		
Cipura paludosa Aubl.	r-ns-g		X		X	X		X			X	1,33	X		NA		
Cipura xanthomelas subsp. *xanthomelas* (36)	r-ns-g		X		X	X		X			X	0,99	X		6,50		

Continued

Cypella aquatilis Ravenna	(r+p)-(s+ns)-ps		X	X: The smallest lumina (0.016-0.5 µm) less obvious and sparcelly disposed, the largest ones more obvious (0.5-0.6 [-3.5] µm)	X	X	X	X		X	X	0,55		X	0,23	0.002-3.147	0.016-0.208
Cypella armosa Ravenna (21)	r-s-ps		X		X open	X		X		X predominantly		0,41		X	2,22	0.831-4.735	1.119-2.67
Cypella brasiliensis (Baker) Ravenna (16)	mr-ns-ps		X-MICRO		X	X		X			X	0,25		X	0,23	0.048-0.498 (0.666)	0.149-0.282
Cypella coelestis (Lehm.) Diels	(mr+p)-ns-g		X-MICRO-PERFORATE	X: The smallest lumina less obvious (0.03-1.7 µm), the largest ones more obvious (1.7-5.234 µm)		X	X	X			X	0,58	X		0,98	0.03-5.234	0.107-1.709
Cypella crenata (Vell.) Ravenna (43)	r-ns-g		X		X	X		X			X	0,7	X		0,88	0.139-3.323	0.393-1.166
Cypella exilis Ravenna (24)	r-ns-ps		X		X	X		X			X	0,32		X	0,84	0.093-3.1	0.44-1.09
Cypella hauthalii subsp. *hauthalii*	r-s-ps		X		X	X		X		X		0,6		X	0,79	0.018-1.705	0.366-1.137
Cypella hauthalii subsp. *opalina* Ravenna	r-ns-ps		X		X	X		X			X	0,54		X	0,90	0.058-2.032	0.51-1.278
Cypella herberti subsp. *herbertii* (27)	(r+p)-s-ps		X-PERFORATE SPREADLY		X	X		X		X		0,53		X	1,07	0.023-3.58	0.59-1.42
Cypella herbertii subsp. *wolffhuegelii*	(ru+r)-ns-ps	X	X		X open	X			X		X	0,55		X	1,43	0.23-2.961	1.06-1.799

Continued

(Hauman) Ravenna (7)																
Cypella laxa Ravenna	mr-(s+ns)-ps		X-MICRO		X	X		X		X	X	0,53		X	1,02	0.008-1.015	0,54
Cypella osteniana Beauverd	mr-s-ps		X-MICRO		X	X		X			X	0,54		X	0,39	0.091-0.951	0.292-0.493
Cypella pabstiana Ravenna (20)	r-s-ps		X		X	X		X		X		0,45		X	1,65	0.157-5.569	0.72-2.268
Eleutherine latifolia (Standl.& Will.) Ravenna (26)	(mr+p)-ns-ps		X-MICRO-PERFORATE		X	X		X			X	0,28		X	0,07	0.014-0.209;	0.038-0.95
Ennealophus euryandrus (Griseb.) Ravenna	r-ns-ps		X		X	X		X			X			X			
Ennealophus euryandrus (Griseb.) Ravenna			X		X	X		X			X			X			
Ennealophus euryandrus (Griseb.) Ravenna	r-ns-ps		X		X	X		X			X	0,51		X	1,17	0.124-5.06	0.73-1.44
Ennealophus foliosus (Kunth) Ravenna	mr-ns-ps		X-MICRO		X	X		X			X	0,62		X	1,37	0.006-4.23	0.738-1.883
Gelasine elongata (Graham) Ravenna (2,50)	mr-ns-ps		X-MICRO		X	X		X			X	0,29		X	0,16	0.006-0.436	0.098-0.196
Gelasine elongata (Graham) Ravenna (57)	mr-ns-ps		X-MICRO		X	X		X			X	0,41		X	0,20	0.032-0.889	0.115-0.27
Herbertia lahue (Molina) Goldblatt (10)	mr-ns-ps		X-MICRO		X	X		X			X			X	0,17	0.078-0.417	0.111-0.215
Herbertia lahue subsp. *intermedie (Ined.)* (5-13)	(ru+mr+p)-ns-ps	X	X-MICRO		X	X		X			X	0,39		X	0,06	0.015-0.202	0.036-0.0725
Herbertia pulchella Sweet (17)	mr-ns-ps		X-MICRO		X			X			X			X	0,11	0.001-0.405	0.072-0.155
Herbertia quareimana Ravenna (4)	mr-ns-ps		X-MICRO		X	X		X			X	0,34		X	0,09	0.029-0.253	0.058-0.121
Herbertia tigridioides (Hicken) Goldblatt (14)	ru-ns-ps	X						X			X			X	0,09	0.032-0.182	0.070-0.112
Hesperoxiphion peruvianum (Baker) Baker (67)	r-(s+ns)-g		X	X: The smallest lumina (0.074-0.761 µm) less evident, the largest		X	X	X		X	X		X predominantly		0,62	0.004-4.894	0.074-0.761

Continued

				ones more obvious (2.046-4.89 µm)													
Larentia linearis (Kunth) Klatt (37)	r-ns-ps		X-OPEN		X	X		X			X			X	3,10	1.333-7.888	2.152-3.638
Mastigostyla boliviensis (R.C.Foster) Goldblatt (34)	mr-ns-ps		X-MICRO		X	X		X			X	0,33		X	0,42	0.085-0.951	0.311-0.519
Mastigostyla sp. nov (99)	r-ns-ps		X		X	X		X			X	0,34		X	0,89	0.117-2.284	0.479-1.219
Mastigostyla tunariensis (R.C.Foster) Ravenna (98)	mr-ns-ps		X-MICRO		X	X		X			X	N/A		X	0,79	0.115-2.632	0.44-1.07
Tigridia galanthoides Molseed	mr-ns-ps		X-MICRO		X	X		X			X			X			
Tigridia lutea Link	mr-ns-ps		X-MICRO		X	X		X			X	0,38		X	0,42	0.234-0.672	0.288-0.555
Tigridia molseediana Ravenna	ru-(s+ns)-ps	X				X		X			X			X			

Table 4-2: Comparison of proposals for classification of pollen exine in Tigridieae.

Rudall & Wheeler 1982	Goldblatt & Le Thomas 1992	Celis & Fuentes-Soriano 2012		
		GENERAL EXINE SCULPTURE	MURI WALLS	ENDEXINE
Small pisolumina (lumina without granules) ranging from polyhedral to rounded (as microreticulate) (e.g., *Eleutherine*, *Nemastylis*)	Microreticulate (less 1 µm) (e.g., *Eleutherine*)	Microhetereticulate or microheterobrochate	straight	psilate
Very large polygonal lumina (e.g., *Cipura flava*, *Tigridia pavonia*)	Reticulum very open (>2 µm, (3-4 µm)) (e.g., Cipura)	Heteroreticulate or heterobrochate	straight	psilate
Large polygonal psilolumina (e.g., *Calydorea campestris*, *Cypella* sp., *Herbertia pulchella*)	Reticulate exine with the lumina 1-2 µm	Heteroreticulate or heterobrochate	straight	psilate
Lumina similar in *Ennealophus euryandrus*, *Catila amabilis* y *Cipura campanulata* but with granules,				
Large polygonal lumina with small rounded psilolumina,	Reticulum very heterogeneous, with two sizes of lumina (ca. 2 µm or ca. 0.5) (e.g., *Hesperoxihion peruviana*)	Heterobrochate + Perforate; Heterobrochate + microheterobrochate	straight + not straight	
Rounded psilolumina (e.g., *Mastygostyla*)				
Large circular psilolumina (e.g., *Cypella herberti*)				
Narrow pisilolumina (e.g., *Herbertia lahue*, G*elasine azurea*, *Herbertia pulchella*)	Reticulo-rugulate	Rugulate		

Table 5-2: Characters and character states proposed to define exine sculpture in Tigridieae

1. General exine sculpture
• Heterobrochate
• Microheterobrochate
• Porate
• Rugulate
2. Small lumina intermixed with larger lumina
• Present
• Absent
3. Muri continuity
• Continuous
• Discontinuous
4. Muri walls toward lumina
• Straight
• Not straight
5. Endexina in Lumina
• Psilate
• Granulate

7. FIGURES

Figure 1-2: Floral variation in Tigridieae (Iridaceae). **A.** *Sessilanthera latifolia* (Weath.) Molseed & Cruden. **B.** *Alophia veracruzana* Goldblatt & T.M.Howard. **C.** *Nemastylis tenuis* (Herb.) S. Watson. **D.** *Tigridia pavonia* (L.f.) DC. **E.** *Tigridia multiflora* (Baker) Ravenna. **F.** *Cipura paludosa* Aublet. **G.** *Tigridia sp.* (South America). **H.** *Tigridia flammea* (Lindl.) Ravenna. **I.** *Cardiostigma hintonii* (R.C.Foster) Ravenna. **J.** *Tigridia oaxacana* (Molseed) Goldblatt. **K.** *Herbertia sp.*

Photos. **A, B, C, E, H, I, J:** Aarón Rodríguez. **D, F:** Julio Betancur. **G:** Marcela Celis. **K:** Germán Roitman

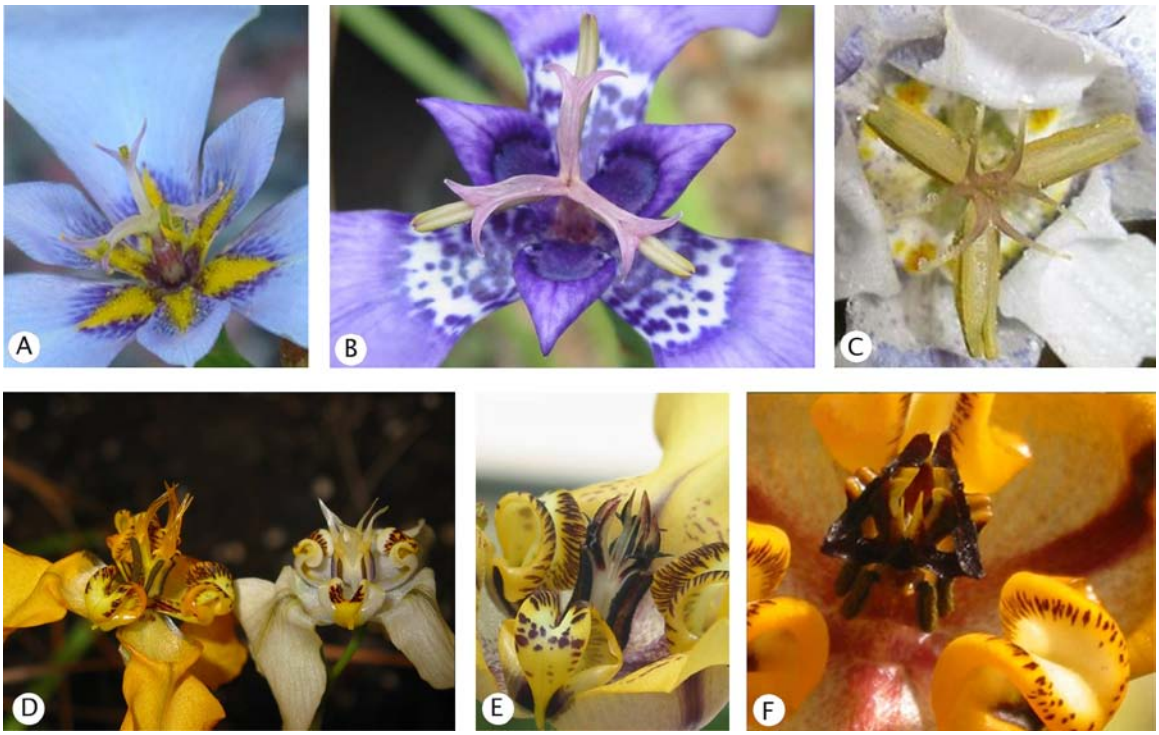

Figure 2-2: Stigma-Style complex variation in Tigridieae (Iridaceae). **A.** *Herbertia furcata* (Klatt) Ravenna, anthers opposite to the forked style branches as long as the style branches. **B.** *Herbertia lahue* (Molina) Goldblatt, anthers opposite to the forked style branches longer than the style branches. **C.** *Tigridia* sp., anthers alternate to the forked style branches longer than the style branches. (South American). **D.** *Cypella osteniana* subsp. *aurantiaca* Roitman & J.A.Castillo (left side); Cypella osteniana subsp osteniana (right side). **E.** *Cypella herbertii* subsp. *wolffhuegelii* (Hauman) Ravenna. **F.** *Cypella herbertii* subsp. *brevicristata* Ravenna. D, E and F with anthers opposite to the forked style branches shorter and adpressed to the style branches

Photos. **A, B, D, E, F:** Germán Roitman. **C:** Marcela Celis.

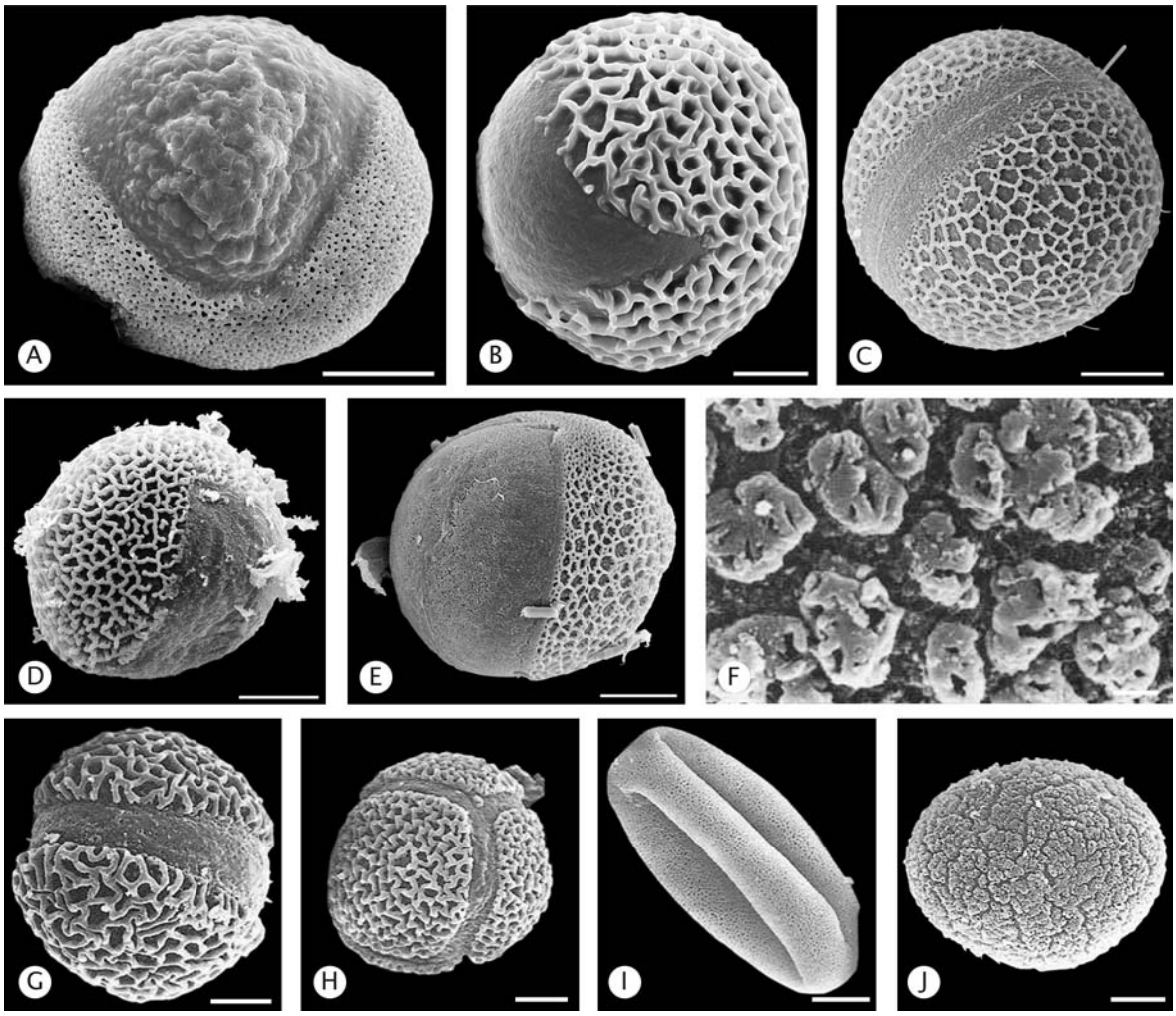

Figure 3-2: Aperture types in Tigridieae (Iridaceae). A-E Monosulcate grains. A *Alophia silvestris* (Loes.) Goldblatt. **B** *Cipura xanthomelas* subsp. *xanthomelas*. **C** *Cypella pabstiana* Ravenna. **D** *Cypella herbertii* subsp. *wolffhuegelii* (Hauman) Ravenna. **E** *Hesperoxiphion peruvianum* (Baker) Baker. **F**. **Inaperturate grain with intectate exine**- *Diplarrena latifolia* Benth. **G**. **Zonasulculate grain**-*Cipura paludosa* Aublet. **H. Trichotomosulcate grain**-*Cipura campanulata* Ravenna. **I. Disulcate grain**-*Tigridia lutea* Link. **J. Inaperturate grain**- *Diplarrena latifolia* Benth. **A-E, G-H** represent the Cipurinae subtribe and **I** represents the Tigridiinae subtribe. Scale bars = 10 µm.

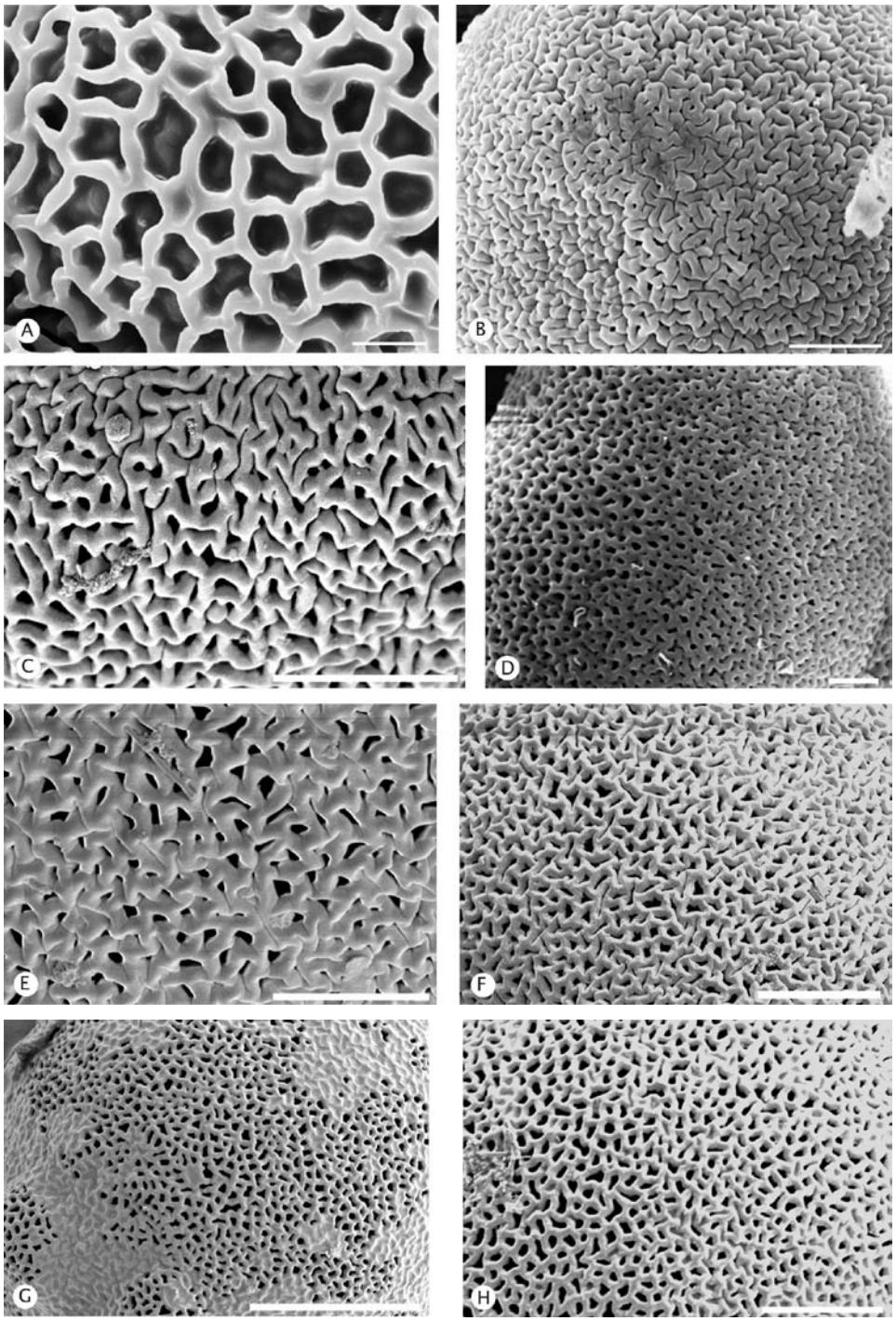

Figure 4-2: Polen sculpture variation in Tigridieae (Iridaceae). A. Reticulate - *Cipura xanthomelas* subsp. *xanthomelas*. **B-C. Rugulate exine. B.** *Calydorea undulata* Ravenna. **C.** *Gelasine elongata* (Graham) Ravenna. **D-H. Microreticulate. D.** *Eleutherine latifolia* (Standl. & L.O.Williams) Ravenna. **E-F** *Herbertia lahue* (Molina) Goldblatt. **G** *Herbertia pulchella* Sweet. **H** *Herbertia quareimana* Ravenna**.** scale bars in A, B, C, F, H = 5 µm; in D = 2 µm; in E = 3 µm; in G = 10 µm

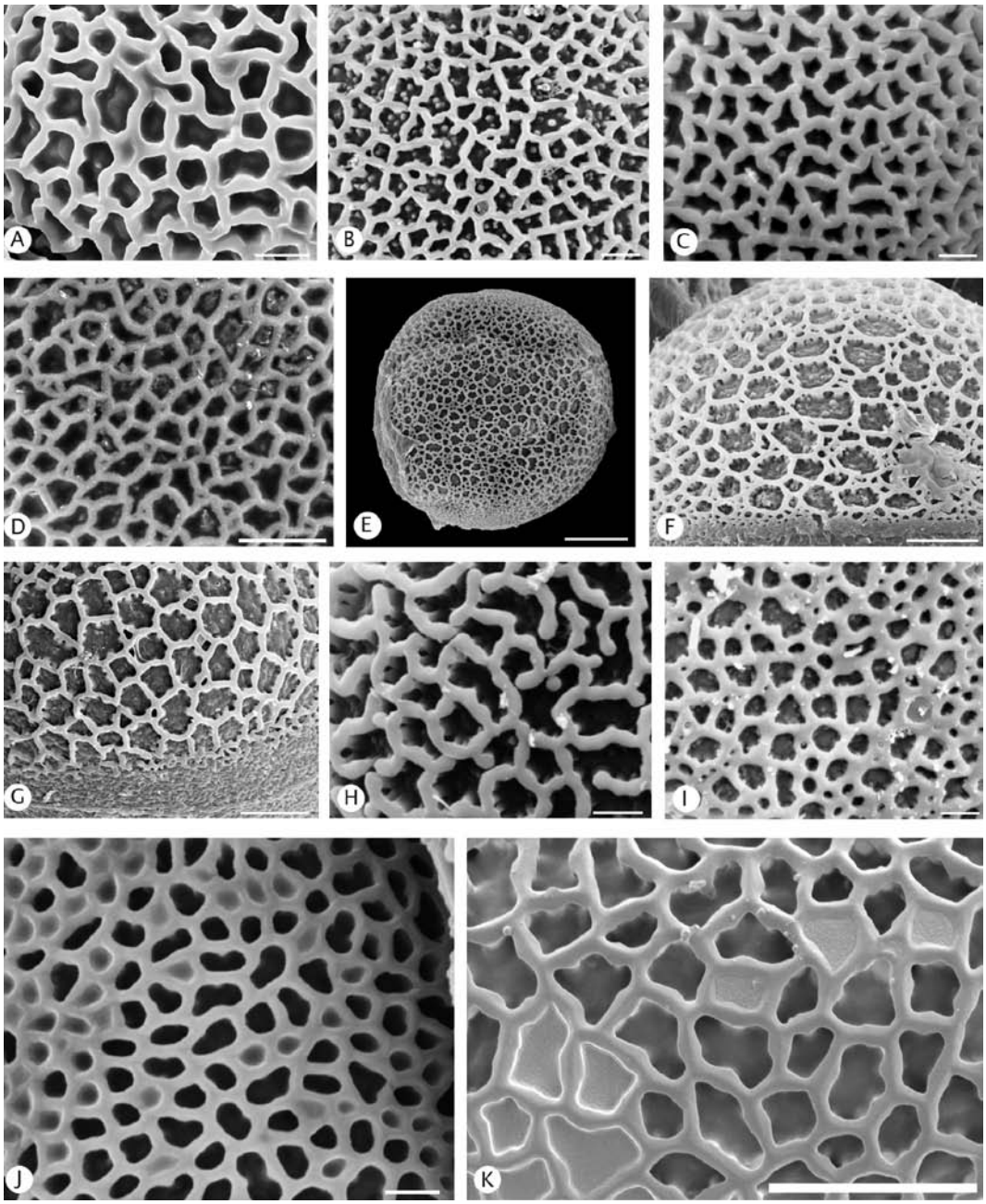

Figure 5-2: Reticulate pollen sculpture variation in Tigridieae (Iridaceae). A. *Cipura xanthomelas* subsp. *xanthomelas.* **B.** *Calydorea undulata* Ravenna. **C** *Cypella hauthalii* subsp. *hauthalii*. **D.** *Cypella laxa* Ravenna. **E-F.** *Hesperoxiphion peruvianum* (Baker) Baker. **G.** *Cypella pabstiana* Ravenna. **H.** *Cypella herbertii* subsp. *wolffhuegelii* (Hauman) Ravenna. **I.** *Cypella coelestis* (Lehm.) Diels. **J.** *Mastigostyla tunariensis* (R.C. Foster) Ravenna. **K.** *Ennealophus euryandrus* (Griseb.) Ravenna. Scales bars in B, C, H, I, J = 2 µm; in A, D, F, G, K = 5 µm; in E = 10 µm.

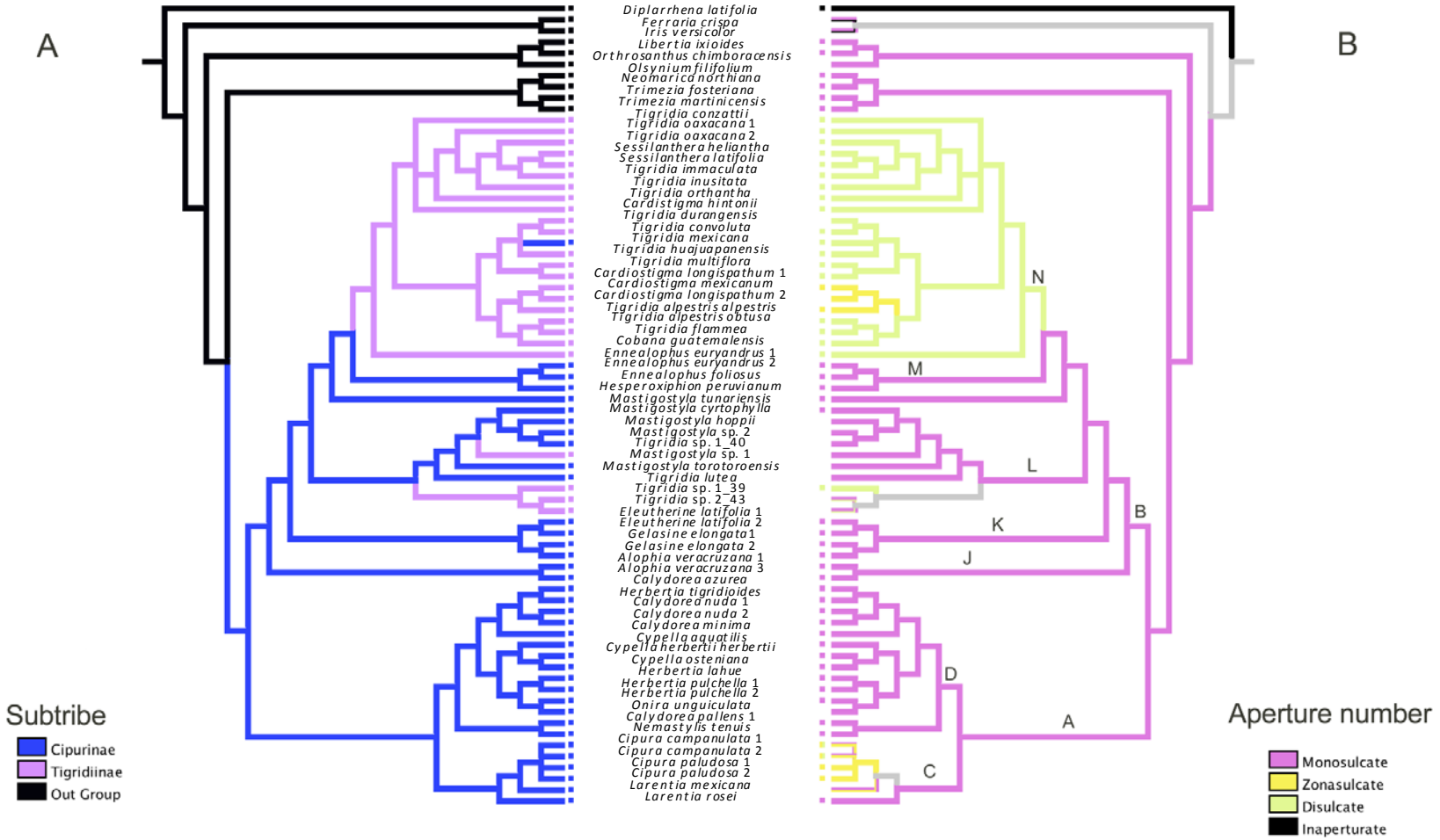

Figure 6-2: Phylogeny of Tigridieae (from Celis & García, Chapter 1) with parsimony ancestral state reconstruction of the characters "subtribe". (A) Subtribes follow Godblatt 1982. (B) aperture number showing all ambiguous reconstructions in gray. Clade names follow Celis & García (Chapter 1).

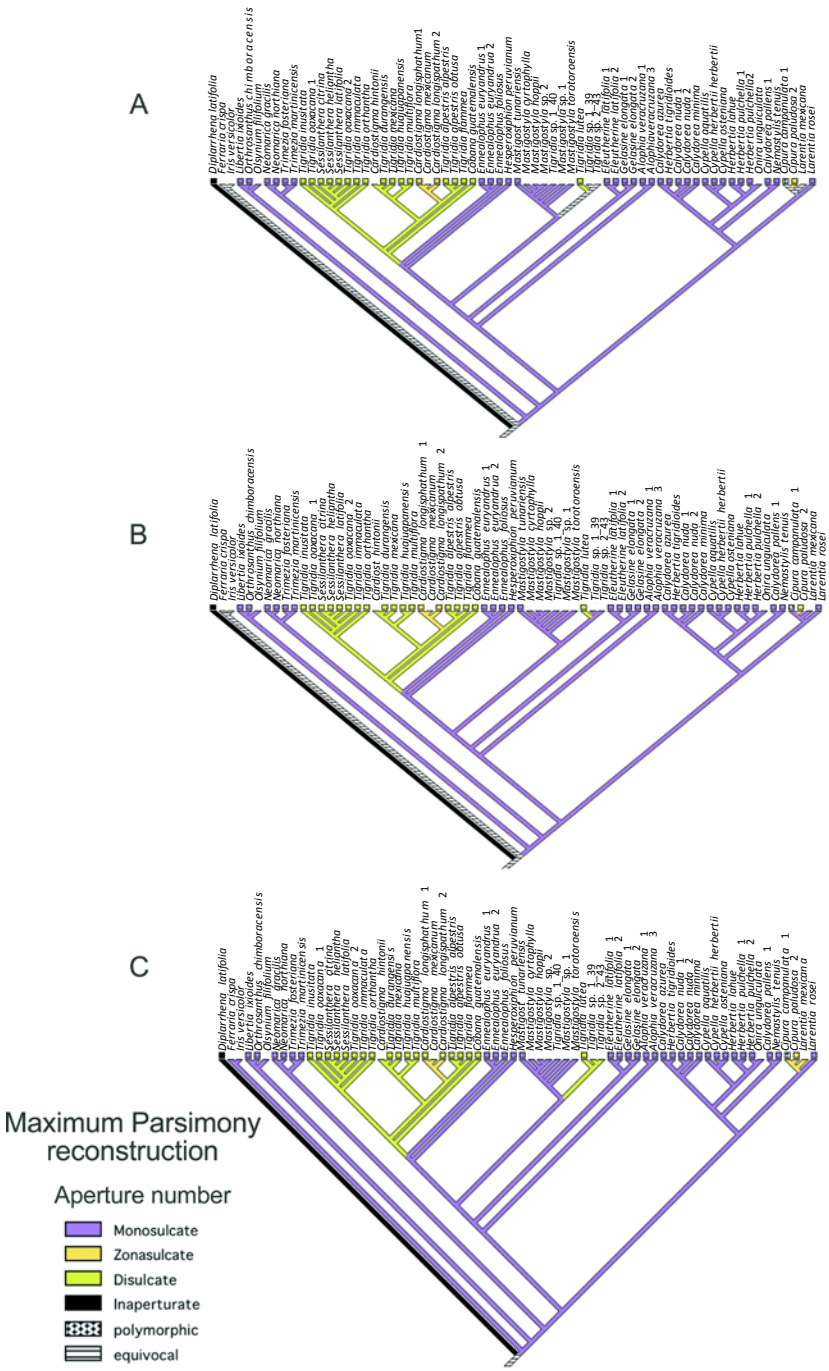

Figure 7-2: Phylogeny of Tigridieae (from Celis & García, Chapter 1) with parsimony ancestral state reconstruction of the character "aperture number." A. Reconstructions under the "all most-parsimonious states" trace option. B. DELTRAN optimization scheme. C. ACCTRAN optimization scheme.

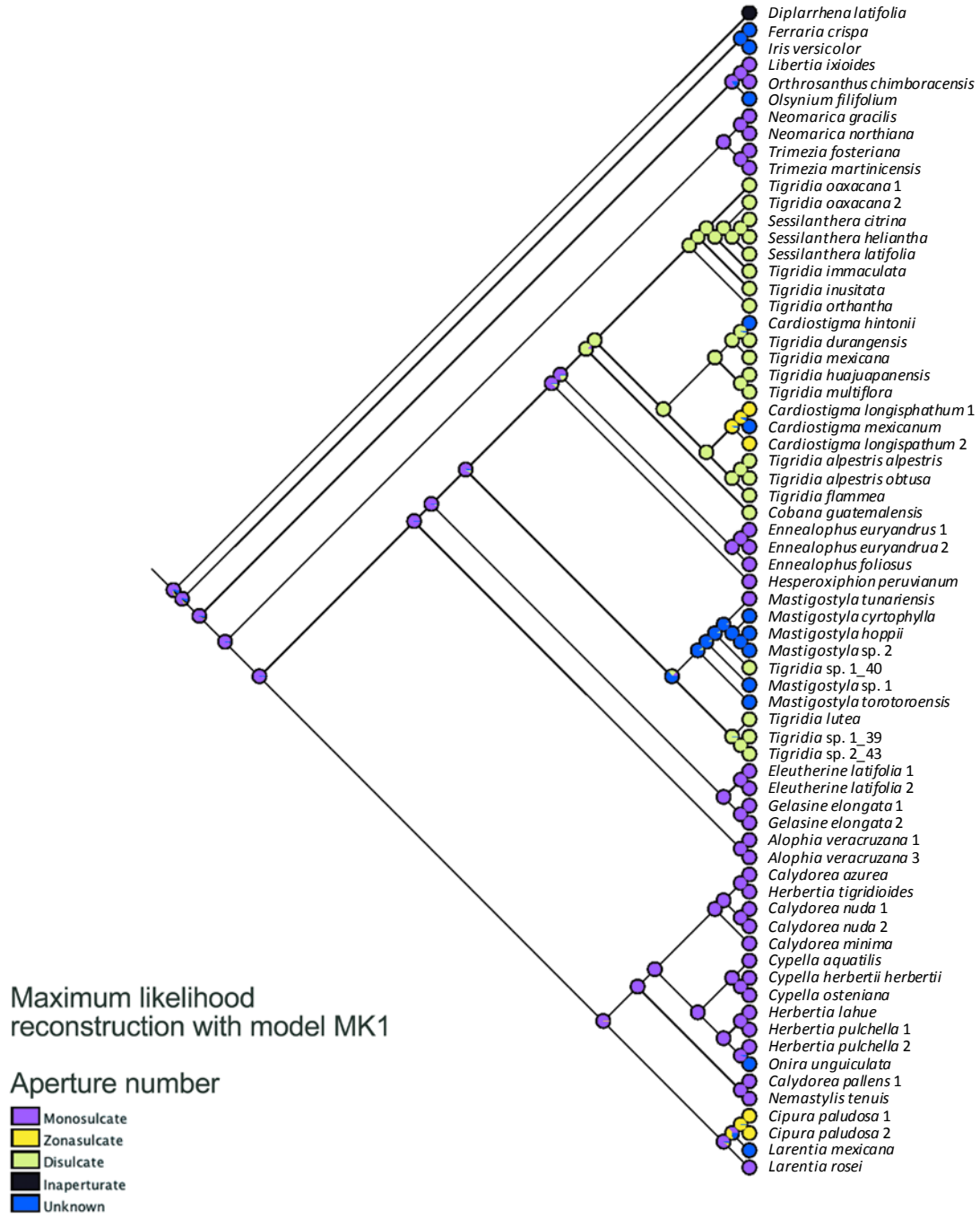

Figure 8-2: Phylogeny of Tigrideae (from Celis & García, Chapter 1) with Maximum likelihood ancestral state reconstruction of the character "aperture number."

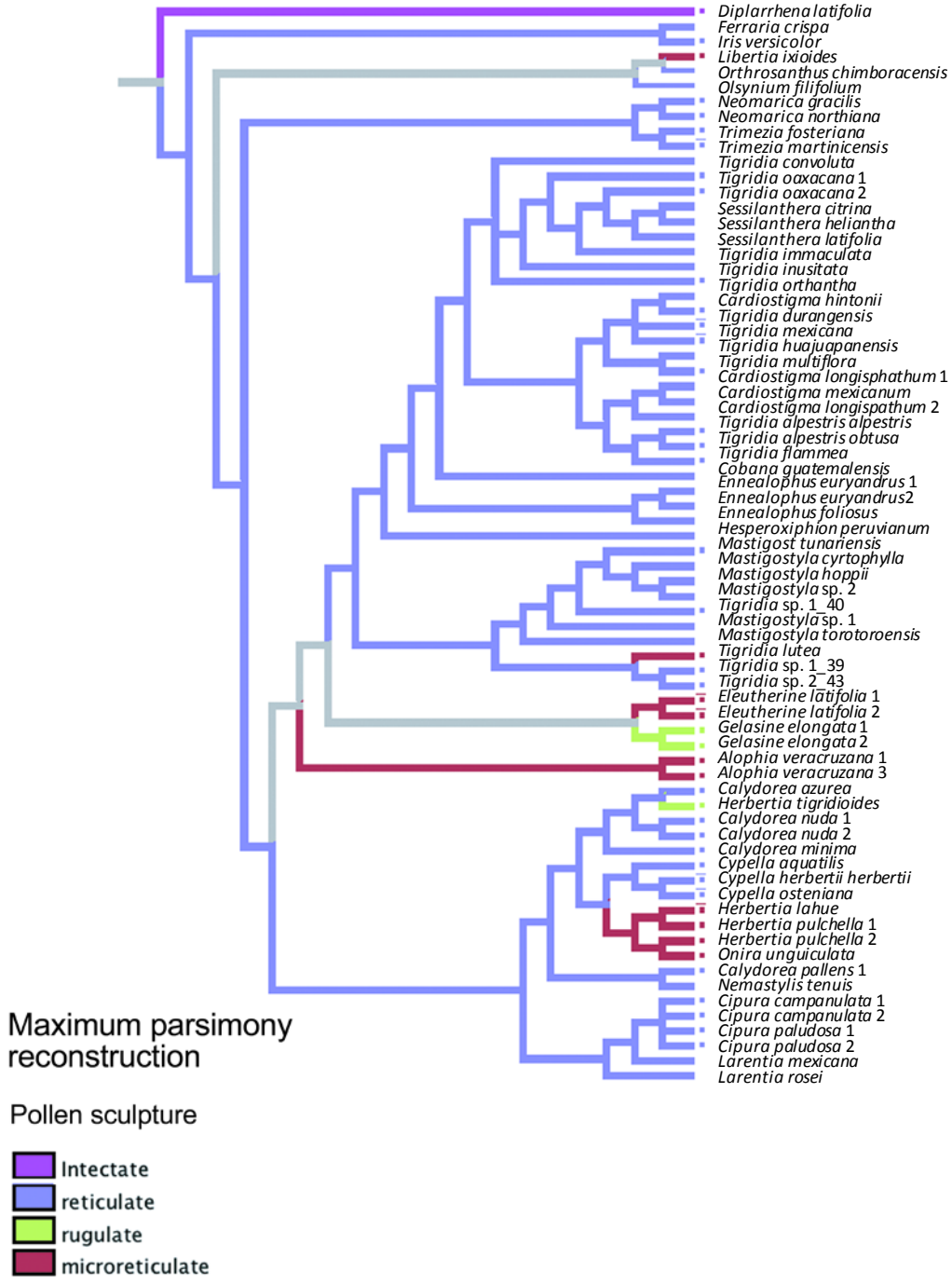

Figure 9-2: Phylogeny of Tigridieae (from Celis & García, Chapter 1) with parsimony ancestral state reconstruction of the character "pollen sculpture." Reconstruction under the "all most-parsimonious states" trace option. All ambiguous reconstructions in gray.

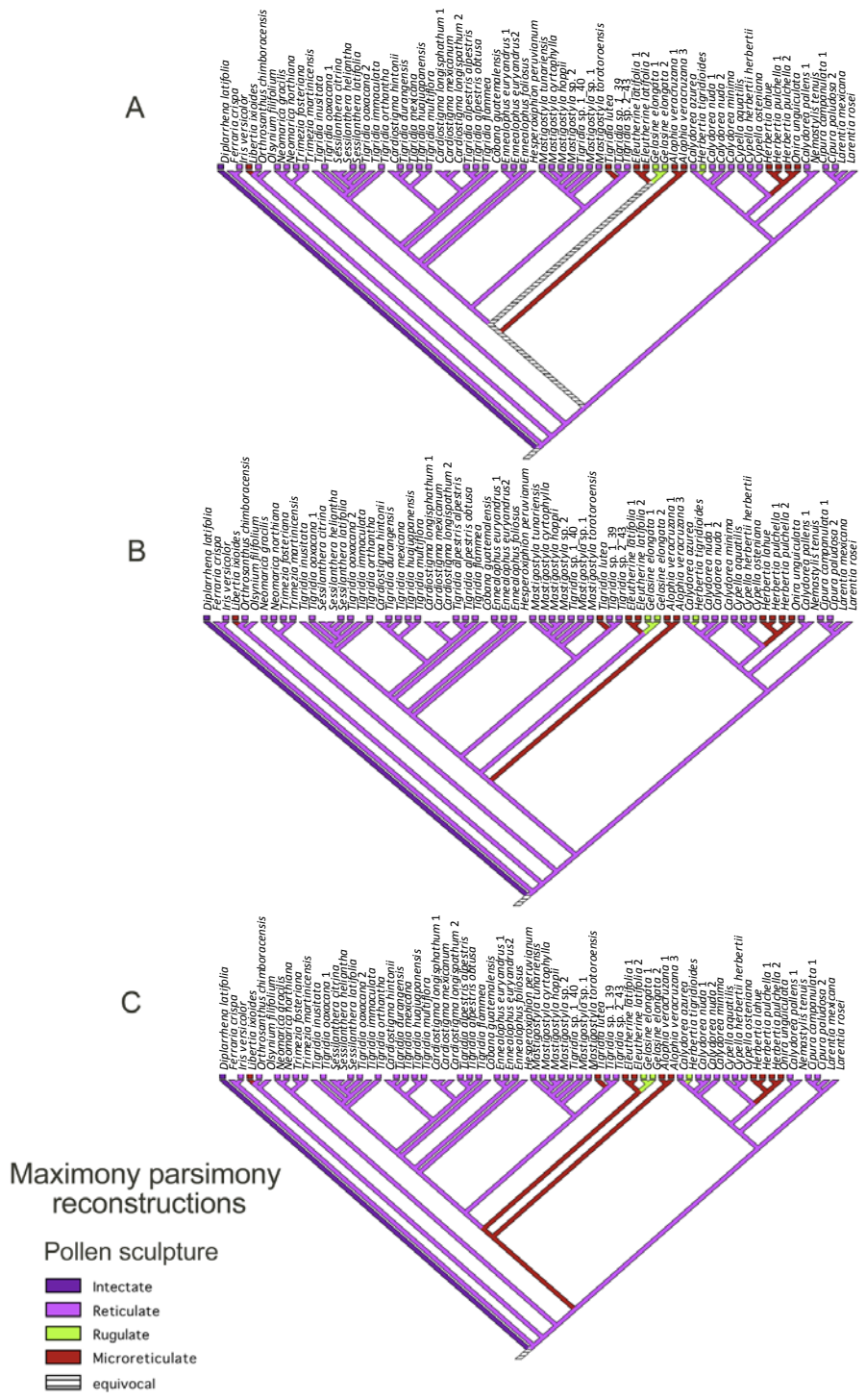

Figure 10-2: Phylogeny of Tigridieae (from Celis & García, Chapter 1) with parsimony ancestral state reconstruction of the character "pollen sculpture." A.reconstruction under the "all most-parsimonious states" trace option. B. DELTRAN optimization scheme. C. ACCTRAN optimization scheme.

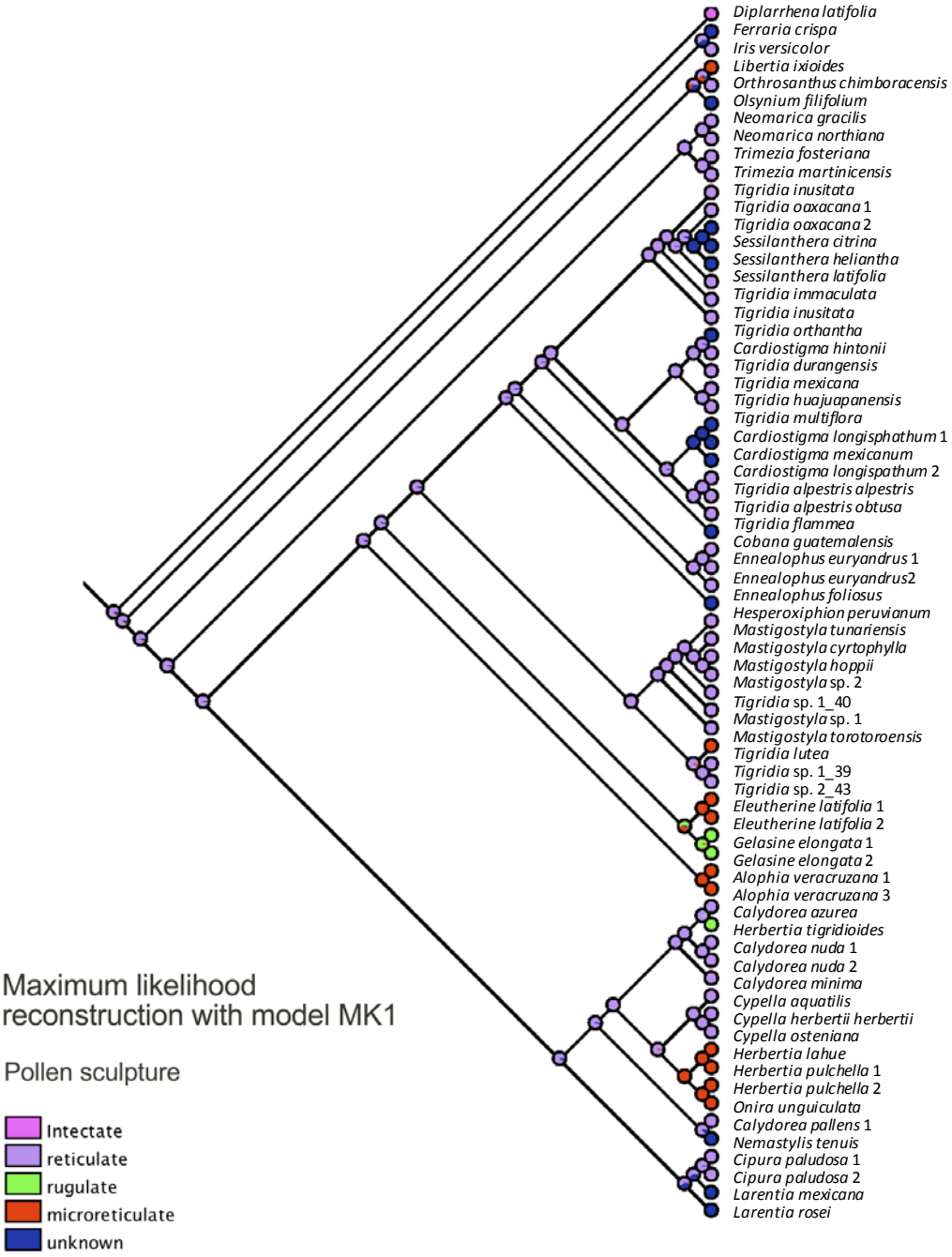

Figure 11-2: Phylogeny of Tigridieae (from Celis & García, Chapter 1) with Maximum likelihood ancestral state reconstruction of the character "aperture number."

8. APPENDICES

APPENDIX 1-2: List of species and pollen characters of Tigridieae.

Abbreviations: C= Cipurinae, T= Tigridiinae (*Goldblatt, 1982); A= Lineage A, Lieneage B (Celis & García). Circumscription proposed in Chapter 1). N/A = data not available. References: 1. Datas obtained in the current work; 2= Goldblatt & Le Thomas (1992); 3: Rudall & Wheeler (1988).

SPECIES	Tribe	Lineage	SULCUS	GRAIN LENGTH (µm)			GRAIN BREADTH (µm)			LUMINA AREA				MURI BREADTH (µm)			SULCUS BREADTH (µm)			Refe-rence
				max	min	average	max	min	average		max	min	average	max	min	average	max	min	average	
Alophia drummondii (Graham) R.C.Foster	C	B	2	N/A	N/A	39,20	N/A	N/A	33,00		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Alophia silvestris (Loes.) Goldblatt	C	B	1	39,79	31,94	37,43	36,67	23,06	28,78		0,17	0,02	0,09	0,50	0,22	0,34	26,89	9,42	18,44	1
Alophia silvestris (Loes.) Goldblatt	C	B	1	N/A	N/A	40,20	N/A	N/A	33,50		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Alophia veracruzana Goldblatt & T.M.Howard	C	B	1	N/A	N/A	41,00	N/A	N/A	37,50		0,55	0,08	0,33	0,47	0,39	0,44				2
Calydorea amabilis (Ravenna) Goldblatt & Henrich	C	A	1	39,45	33,54	36,95	31,15	27,97	29,71		0,65	0,003	0,23	0,57	0,21	0,41	19,63	12,90	15,72	1
Calydorea amabilis (Ravenna) Goldblatt & Henrich	C	A	1	N/A	N/A	37,80	N/A	N/A	30,50		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Calydorea approximata R.C.Foster (18)	C	A	1	24,58	21,11	22,72	21,84	19,73	20,97		0,17	0,01	0,06	0,41	0,26	0,35	N/A	N/A	N/A	1
Calydorea campestris (Klatt) Baker	C	A	1	N/A	N/A	46,60	N/A	N/A	33,10		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3
Calydorea pallens Griseb.	C	A	1	N/A	N/A	48,60	N/A	N/A	36,80		2,44	1,06	1,74	0,64	0,33	0,46	N/A	N/A	N/A	2
Cipura campanulata Ravenna	C	A	2	N/A	N/A	59,80	N/A	N/A	55,00		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3
Cipura campanulata Ravenna	C	A	2	N/A	N/A	47,20	N/A	N/A	41,20		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3
Cipura campanulata Ravenna	C	A	2	N/A	N/A	61,70	N/A	N/A	51,60		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3
Cipura campanulata Ravenna	C	A	(1-2) 3-zs	N/A	N/A	60,2	N/A	N/A	52,3		4,35	2,4	3,3	1,17	0,78	0,95	N/A	N/A	N/A	2
Cipura formosa Ravenna	C	A	1	N/A	N/A	68,1	N/A	N/A	56,8		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Cipura paludosa Aubl.	C	A	zs	N/A	N/A	66,2	N/A	N/A	59		N/A	N/A	3	1,6	1,05	1,33	N/A	N/A	N/A	2

Continued

Cipura rupicola Goldblatt & Henrich	C	A	1	N/A	N/A	60,7	N/A	N/A	59		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Cipura xanthomelas subsp. *xanthomelas* (36)	C	A	1 (3z)	45,35	50,48	45,88	39,39	43,10	43,11		20,34	2,15	6,54	1,36	0,63	0,99	17,10	15,00	15,91	1
Cypella aquatilis Ravenna	C	A	1	35,90	32,31	33,95	31,97	25,18	29,05		3,15	0,00	0,00	0,71	0,39	0,55	22,70	14,09	18,40	1
										large	3,15	1,40	2,07							
										medium	1,07	0,13	0,28							
										small	0,10	0,00	0,03							
Cypella armosa Ravenna (21)	C	A	1	38,34	38,34	38,34	36,64	36,64	36,64		6,06	0,83	2,22	0,55	0,27	0,41	n/a	n/a	n/a	1
Cypella brasiliensis (Baker) Ravenna (16)	C	A	1	32,70	29,20	31,44	24,55	21,08	22,81		0,67	0,05	0,23	0,31	0,18	0,25	15,38	11,98	13,64	1
Cypella brasiliensis (Baker) Ravenna	C	A		39,50	N/A	N/A	N/A	N/A	29,10		0,28	0,05	0,12	0,31	0,18	0,25	N/A	N/A	N/A	2
Cypella coelestis (Lehm.) Diels	C	A	1	33,45	N/A	N/A	31,41	n/a	31,41		5,20	0,03	0,97	0,84	0,37	0,58	N/A	N/A	N/A	1
										large	5,20	1,73	2,64				N/A	N/A	N/A	
										medium	1,68	0,61	1,00				N/A	N/A	N/A	
										small	0,52	0,03	0,10				N/A	N/A	N/A	
Cypella crenata (Vell.) Ravenna (43)	C	A	1	N/A	N/A	N/A	41,36	34,86	38,11		3,32	0,14	0,88	0,85	0,47	0,68	N/A	N/A	N/A	1
Cypella exilis Ravenna (24)	C	A	1	44,84	35,50	41,21	33,96	32,73	33,54		3,10	0,09	0,86	0,39	0,25	0,32	N/A	N/A	N/A	1
Cypella hauthalii subsp. *hauthalii*	C	A	1	35,94	32,25	34,12	29,00	28,86	28,93		1,71	0,02	0,65	0,81	0,42	0,60	25,90	18,70	22,10	1
										large	1,71	1,03	1,38							
										medium	0,98	0,19	0,53							
										small	0,14	0,02	0,07							
Cypella hauthalii subsp. *opalina* Ravenna	C	A	1	40,87	31,27	35,35	34,71	26,23	31,20		3,22	0,01		0,79	0,39	0,53	25,24	14,23	19,50	1
										large	3,22	1,06	1,64							
										medium	1,05	0,01	0,51							
Cypella herbertii (Lindl.) Herb.	C	A	1	N/A	N/A	56,40	N/A	N/A	48,10		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Cypella herbertii (Lindl.) Herb.	C	A	1	N/A	N/A	45,70	N/A	N/A	40,50		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Cypella herbertii subsp. *brevicristata* Ravenna	C	A	1	N/A	N/A	45,20	N/A	N/A	37,50		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Cypella herberti subsp. *herbertii* (27)	C	A	1	40,51	37,36	39,13	32,12	27,97	30,12		3,50	0,02	1,07	0,76	0,40	0,53	n/a	n/a	n/a	1

Continued

Cypella herbertii subsp. *wolffhuegelii* (Hauman) Ravenna (7)	C	A	1	38,68	38,68	38,68	34,77	30,55	32,69		2,96	0,23	1,43	0,73	0,43	0,55	n/a	n/a	n/a	1
Cypella laxa Ravenna	C	A	1	40,26	26,10	32,87	35,88	23,99	29,98		3,70	0,08	1,00	0,75	0,36	0,53	21,07	9,83	16,54	1
Cypella osteniana Beauverd	C	A	1	32,00	26,05	29,17	29,30	24,80	27,30		0,95	0,25	0,46	0,69	0,38	0,54	23,50	18,50	20,70	1
Cypella pabstiana Ravenna (20)	C	A	1	43,76	36,84	40,72	37,36	32,19	34,97		5,57	0,16	1,66	0,65	0,29	0,45	6,65	6,29	6,49	1
Cypella sp.	C	A		41,30	N/A	N/A	39,60	N/A	N/A		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3
Eleutherine latifolia (Standl.& Will.) Ravenna (26)	C	B	1	31,10	24,51	27,54	n/a	n/a	n/a		0,21	0,01	0,07	0,33	0,24	0,28	n/a	n/a	n/a	1
Eleutherine latifolia (Standl.& Will.) Ravenna	C	B	1	N/A	N/A	35,20	N/A	N/A	27,10		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Ennealophus euryandrus (Griseb.) Ravenna	C	B	1	N/A	N/A	36,70	N/A	N/A	34,90		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3
Ennealophus euryandrus (Griseb.) Ravenna	C	B	1	N/A	N/A	40,30	N/A	N/A	35,30		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Ennealophus euryandrus (Griseb.) Ravenna	C	B	1	46,20	37,90	41,90	N/A	N/A	n/a		2,40	1,03	1,50	0,80	0,35	0,51	19,90	19,90	19,90	1
Ennealophus foliosus (Kunth) Ravenna	C	B	1	41,05	36,03	38,59	37,83	34,27	36,10		4,23	0,06	1,30	0,84	0,47	0,60	25,84	21,82	23,58	1
										large	4,23	1,71	2,54							
										medium	1,53	0,80	1,12							
										small	0,79	0,06	0,46							
Ennealophus foliosus (Kunth) Ravenna	C	B	1	N/A	N/A	46,10	N/A	N/A	39,30		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Gelasine coerulea (Vell.) Ravenna	C	B	1	N/A	N/A	58,80	N/A	N/A	43,80		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Gelasine elongata (Graham) Ravenna	C	B	1	N/A	N/A	45,10	N/A	N/A	37,50		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Gelasine elongata (Graham) Ravenna	C	B	1	N/A	N/A	43,50	N/A	N/A	39,10		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3
Gelasine elongata (Graham) Ravenna (57)	C	B	1	37,73	31,45	34,87	37,72	34,86	36,29		0,89	0,03	0,20	0,58	0,30	0,41	n/a	n/a	n/a	1
Gelasine elongata (Graham) Ravenna (2,50)	C	B	1	34,11	30,19	32,12	31,29	27,41	29,34		0,44	0,01	0,16	0,45	0,22	0,29	26,57	22,39	24,89	1
Herbertia lahue (Molina) Goldblatt (10)	C	A	1	39,97	30,53	36,52	28,95	28,95	28,95		0,42	0,08	0,17	N/A	N/A	N/A	N/A	N/A	N/A	1
Herbertia lahue (Molina) Goldblatt	C	A	1	N/A	N/A	35,20	N/A	N/A	27,80		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Herbertia lahue (Molina) Goldblatt	C	A	1	N/A	N/A	49,80	N/A	N/A	41,80		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3
Herbertia lahue subsp. *intermedie* (Ined.) (5-13)	C	A	1	39,39	25,97	30,51	27,68	22,64	25,69		0,20	0,02	0,06	0,50	0,29	0,39	N/A	N/A	N/A	1

Continued

Herbertia pulchella Sweet (17)	C	A	1	36,94	30,97	34,66	31,25	27,69	29,28		0,41	0,001	0,11	N/A	N/A	N/A	N/A	N/A	N/A	1
Herbertia quareimana Ravenna (4)	C	A	1	34,41	32,00	33,63	N/A	N/A	N/A		0,25	0,03	0,09	0,46	0,25	0,34	N/A	N/A	N/A	1
Herbertia tigridioides (Hicken) Goldblatt (14)	C	A	1	37,01	29,28	33,10	31,63	27,63	29,69		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1
Hesperoxiphion huilense Ravenna	C	B	1	N/A	N/A	50,00			39,60											3
Hesperoxiphion peruvianum (Baker) Baker (67)	C	B	1	39,22	37,32	37,99	39,42	34,10	36,63		4,89	0,004	0,61	N/A	N/A	N/A	N/A	N/A	N/A	1
Hesperoxiphion peruvianum (Baker) Baker	C	B	1	N/A	N/A	57,5	N/A	N/A	46,2	large	2,23	0,650	1,60	0,77	0,43	0,57	N/A	N/A	N/A	2
										small	0,33	0,060	0,17	N/A	N/A	N/A	N/A	N/A	N/A	2
Hesperoxiphion peruvianum (Baker) Baker	C	B	1	N/A	N/A	54,00	N/A	N/A	49,90		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3
Hesperoxiphion peruvianum (Baker) Baker	C	B	1	N/A	N/A	48,00	N/A	N/A	44,10		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3
Larentia linearis (Kunth) Klatt (37)	C	A	1	49,69	44,87	47,18	n/a	n/a	n/a		7,89	1,33	3,10	0,71	0,40	0,56	n/a	n/a	n/a	1
Mastigostyla boliviensis (R.C.Foster) Goldblatt (34)	C	B	1	51,62	40,49	45,93	n/a	n/a	n/a		0,95	0,09	0,42	0,47	0,20	0,33	11,88	11,88	11,88	1
Mastigostyla sp. nov (99)	C	B	1	32,71	30,51	31,57	30,88	26,00	28,44		2,28	0,12	0,89	0,46	0,19	0,34	n/a	n/a	N/A	1
Mastigostyla tunariensis (R.C.Foster) Ravenna (98)	C	B	1	69,31	44,94	61,00	n/a	n/a	n/a		2,63	0,12	0,79	N/A	N/A	N/A	N/A	N/A	N/A	1
Tigridia dugesii S.Watson	T	B	2	N/A	N/A	48,4	N/A	N/A	32,5		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Tigridia galanthoides Molseed	T	B	2	N/A	N/A	46,4	N/A	N/A	37,4		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3
Tigridia lutea Link	T	B	2	N/A	N/A	N/A	N/A	N/A	N/A		0,6	0,23	0,4	0,4	0,36	0,38	N/A	N/A	N/A	2
Tigridia meleagris (Lindl.) G.Nicholson	T	B	2	N/A	N/A	42	N/A	N/A	29,7		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3
Tigridia molseediana Ravenna	T	B	2	N/A	N/A	47,9	N/A	N/A	33,8		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Tigridia oaxacana (Molseed) Goldblatt	T	B	2	N/A	N/A	44	N/A	N/A	36,6		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2
Tigridia orthantha (Lem.) Ravenna	T	B	2	N/A	N/A	55,3	N/A	N/A	43,9		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3

Appendix 2-2: Evolution of pollen morphology in Tigridieae (Iridaceae) in a new molecular philogenetic context.

Evolution of pollen morphology in Tigridieae (Iridaceae) in a new molecular phylogenetic context

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Fig. 1. Floral variation in Tigridieae. A. *Sessilanthera fatifolia*, **B.** *Alophia veracruzana*, **C.** *Nemastylis tenuis*, **D.** *Tigridia pavonia*, **E.** *Tigridia multiflora*, **F.** *Cipura paludosa*, **G.** *Tigridia sp.* (South America), **H.** *Rigidella flammea*, **I.** *Cardiostigma hintonii*.

Tigridieae, a diverse tribe of ca. 15 genera and 170 species, occurs in the Americas. Although vegetatively uniform, the group exhibits sizeable variation in floral morphology, including differences in characters such as tepal orientation, color patterning, type of nectar produced, and structure of the stamens and style branches (Fig. 1). Two subtribes are recognized: 1) Tigridiinae, with disulcate pollen grains, a chromosome number of n=14, and a geographic distribution centered in Mexico and Guatemala, 2) Cipurinae, with monosulcate pollen grains, n=7, and a center of diversification in South America with some species extending into the Southern US. Using the molecular phylogenetic framework of Celis et al. (in prep.), we conducted a palynological analysis of Tigridieae, which greatly expanded the sampling scheme employed in previous palynological studies of the group (Rudall & Wheeler 1988; Goldblatt & Le Thomas 1992). Most notable additions include species of *Cardenanthus*, *Calydorea*, *Cypella*, *Herbertia*, and South American *Tigridia*.

OBJECTIVES

The two goals of this study were to:

1) evaluate micromorphological variation in the tribe and to assess the phylogenetic utility of several palynological characters.

2) study pollen evolution in Tigridieae using new molecular-based phylogenies.

MATERIALS & METHODS

Molecular phylogenetic analyses:
An unpublished Bayesian tree was estimated using a total of 72 accessions representing 69 taxa (including 10 species in the outgroup). The phylogeny was based on six markers: nuclear ITS, plastid *trn*L-*trn*F, *trn*L intron, *trn*H-*psb*A, *mat*K, *trn*K3 markers (Celis et al., in prep.).

Palynological study:
The pollen of 87 species was investigated. Information for several unavailable species was taken from other published palynological reports (e.g., Rudall & Wheeler 1988, Goldblatt & Le Thomas 1992).

Pollen material was gathered from herbarium specimens (HSB, MO), live plants in field, and from Germán Roitman's living collection in Buenos Aires University, Buenos Aires, Argentina. Observations using Scanning Electronic Microscopy (SEM) were made at Royal Botanic Gardens, Kew (K) and the Missouri Botanical Garden (MO).

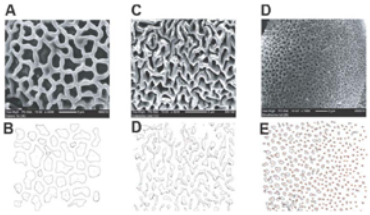

Fig 2. SEM images and digital maps of the lumen area showing the ornamentation pattern. A-B. reticulate. **C-D.** rugulate. **E-F.** microreticulate.

Measurements and image analyses (Fig. 2) were made for six pollen characters (sulci number, pollen size, aperture size, ornamentation type, lumina size, muri size).

Character optimizations of sulci number and exine type were done using parsimony approaches in Mesquite.

Palynological variation was evaluated with univariate, bivariate, and multivariate statistical methods (Principal Component Analysis, PCA) using JMP software.

RESULTS

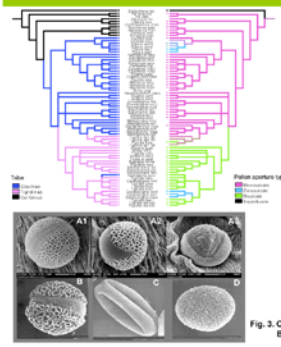

Fig. 3. Optimization of sulci number. A1—A3. monosulcate; **B.** zonasulcate; **C.** disulcate; **D.** inaperturate.

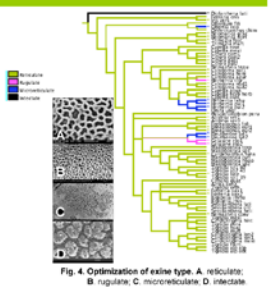

Fig. 4. Optimization of exine type. A. reticulate; **B.** rugulate; **C.** microreticulate; **D.** intectate.

Traditionally *Alophia*, *Cardenanthus*, *Ennealophus*, *Hesperoxiphion*, *Mastigostyla* and the South Americana Tigridieae have been classified in subtribe Cipurinae as they all possess monosulcate pollen.

Phylogenetic results show that those genera belong to basal clades of Tigridiinae (Fig. 3). Optimization of aperture number suggests that the character cannot be used to distinguish subtribes. Our SEM observations, image analyses, and statistical tests indicate that features of the exine ornamentation may be taxonomically useful and phylogenetically informative (Fig. 5), but further analysis of pollen ornamentation is needed.

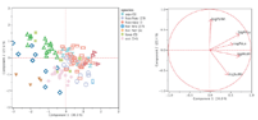

Fig. 6. PCA of pollen traits in species of *Cypella*.

PCA analyses show that pollen length, muri width, and area lumen describe 30% of the total pollen variation in species of *Cypella*, confirming the palynotaxonomic utility of these characters in our analysis (Fig. 6).

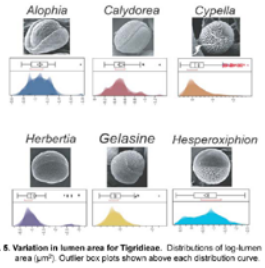

Fig. 5. Variation in lumen area for Tigridieae. Distributions of log-lumen area (µm²). Outlier box plots shown above each distribution curve.

Although pollen ornamentation when optimized as a categorical character lacks a phylogenetic signal (Fig. 4), it is more informative when treated as a continuous variable. Differences in continuous variation of the lumen area and muri width, both defining ornamentation type, appear to be helpful to trace pollen evolution and to distinguish species with more certainty (Figs. 5-6).

CONCLUSIONS

The number of pollen apertures did not support the recognition of subtribe Tigridiinae as traditionally circumscribed, but the presence of disulcate pollen supported a broader circumscription of *Tigridia* to include seven other genera (*Ainea*, *Cardiostigma*, *Cobana*, *Colima*, *Fosteria*, *Rigidella*, *Sessilanthera*).

Our preliminary results indicate that pollen morphology is potentially useful for understanding phylogenetic relationships within the tribe Tigridieae and should be further investigated.

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CHAPTER 3.

ZYGELLA S. MOORE, A SYNONYM OF *LARENTIA* KLATT (IRIDACEAE)

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ZYGELLA S. MOORE, A SYNONYM OF *LARENTIA* KLATT (IRIDACEAE)

ABSTRACT

The identity of *Zygella* S. Moore, a genus of Iridaceae of Mato Grosso, Brazil, has never been satisfactorily established. Plants described by S. M. Moore in 1895 as *Zygella graminea* appear to us to be conspecific with *Larentia linearis* (Kunth) Klatt, type species of Larentia Klatt, a plant well represented in herbaria, mostly from grasslands of Venezuela. We reduce Zygella to synonymy in Larentia, and *Z. graminea* becomes a synonym of *L. linearis*. A second species, *Z. mooreana* Hoehne, described in 1910 and also from Mato Grosso, is likewise conspecific with *L. linearis* and a lectotype is designated for that species. *Cypella mexicana* C. V. Morton and R. C. Foster, which shares the characters of Larentia, is transferred to the genus as *L. mexicana* (C. V. Morton and R. C. Foster) Goldblatt; with this addition, Larentia now includes three species.

Key words: Brazil, Iridaceae, Larentia, Mato Grosso, Tigridieae, Venezuela, Zygella.

The genus *Zygella* was described by S. M. Moore in 1895 for a species of Iridaceae collected in Mato Grosso in interior Brazil two years previously, and at the time consisted of only one species, *Z. graminea* S. Moore. Both genus and species appear to have been overlooked by systematists dealing with the Iridaceae of South America and have remained, at least technically, recognized until now. The characteristics of *Zygella* include a bulbous rootstock; linear, unifacial leaves with pleated blades; and an inflorescence of the *Iris*-type, a rhipidium, enclosed in a pair of large opposed leafy bracts. The flowers have three free stamens with the anthers appressed to narrow, compressed style branches that each bear a pair of small crests, below which lies a bilobed stigmatic lip. These features place the genus squarely in tribe Tigridieae of subfamily Iridoideae (Goldblatt, 1990), an exclusively New World tribe of some 15 genera and over 175 species Goldblatt and Manning, 2008; Goldblatt *et al.*, 2008).

Illustrations accompanying the protologue show a plant with a branched stem; a distinctive, narrow, attenuate leaf subtending the lowermost branch; narrow rhipidia with the outer spathe about half as long as the inner; and flowers with subequal, laxly spreading, unmarked tepals. *Zygella mooreana* Hoehne (Hoehne, 1910), also from Mato Grosso, is evidently conspecific with *Z. graminea*, but the illustrations of the flowers in Hoehne's publication are more carefully drawn and show the tepals to be markedly clawed, with the narrow claws and limb bases darkly speckled. The illustrations and type specimen at the Natural History Museum, London, are a close match to the Venezuelan *Larentia linearis* (Kunth) Klatt (Klatt, 1882). We have not been able to locate the syntypes of *Z. mooreana*. Differences in the flowers illustrated in Moore's and Hoehne's publications are probably not significant. More likely those in the former illustration are simply poorly rendered, perhaps because they were drawn from dried flowers revived in water, whereas the latter appears to have been drawn from life.

Larentia Klatt (Klatt, 1882) was described with the single species *L. linearis* of grassland habitats of Venezuela. The genus was included in *Cypella* Herb., a

genus mainly (possibly exclusively) of temperate South America, by Baker (1892), now including ca. 30 species. The American specialist of the systematics of New World Iridaceae, R. C. Foster (1945), followed Baker's taxonomy in this instance. In contrast, Ravenna (1977) regarded *Larentia* as separate from *Cypella* and added one species, *L. rosei* (R. C. Foster) Ravenna, to the genus.

A molecular study using five plastid DNA regions (Goldblatt *et al.*, 2008) shows one that justifies Ravenna's recognition of *Larentia* as separate from *Cypella*. We infer that *L. linearis* plus two Mexican species, *L. rosei* and *C. mexicana* (here transferred to *Larentia*), constitute a genus sister to *Cipura*. Rodriguez and Sytsma (2006), using both nuclear and chloroplast genes, likewise showed *Larentia* sister to *Cipura* (using *L. rosei* as *C. rosei* R. C. Foster), but their study did not include any true *Cypella* species; their analysis thus provides no evidence directly relevant to this discussion. *Larentia* now includes three species.

For the reasons outlined above, Goldblatt and Manning (2008) followed Ravenna in recognizing *Larentia*, pending a more extensive molecular systematic analysis of Tigridieae. We therefore reduce *Zygella* to synonymy in *Larentia*. We also refer two species of *Zygella* to *L. linearis*. Until now it has not been clear in the literature that *L. linearis* occurs in Brazil, but apart from the collections included in the two species of *Zygella*, we have found specimens identified as *L. linearis* from Brazil, e.g., Hatschbach 33300 (MO) from Goias, and several more. The Brazilian plants seem to us in no way different from those from Venezuela. Location of the type material of Hoehne's *Z. mooreana* remains problematic, as it has not been located at SP where expected. For this reason, we have chosen a lectotype for the species, the illustration accompanying the protologue.

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Larentia linearis (Kunth) Klatt, Abh. Naturf. Ges. Halle 15: 362. 1882. Basionym: *Moraea linearis* Kunth, in Humb., Bonpl. and Kunth, Nov. Gen. Sp. (quarto ed.) 1: 321. 1815 [1816]. TYPE: Venezuela. ‘‘Crescit in humidis calidisque Guayanae prope El Trapiche de Farreras,’’ June, *Humboldt and Bonpland* s.n. (type, P).

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Zygella mooreana Hoehne, Commiss. Linhas Telegr. Estrate´g. Matto Grosso Amazonas 1: 19. 1910, syn. nov. TYPE: Brazil. Mato Grosso: Porto Esperidiao (lectotype, designated here, fig. 58 in Hoehne, 1910).

Larentia mexicana (C. V. Morton and R. C. Foster)

Goldblatt, comb. nov. Basionym: *Cypella Mexicana* C. V. Morton and R. C. Foster, Contr. Gray Herb. 171: 22. 1950. TYPE: Mexico. Guerrero: Montes de Oca, 15 June 1937, *G. Hinton et al., 10322* (holotype, US; isotypes, GH, MO, NY).

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GENERAL CONCLUSIONS

The Family Iridaceae is a monophyletic group of plants, successfully diversified with a worldwide distribution, particularly in Africa. It includes between 65-70 genera and about 1800 species. Morphologically they have been defined by several synapomorphies and a few phylogenetic approaches have proposed four subfamilies and seven tribes. Thus, although the family is considered a monophyletic group, some doubt remains for the inclusion of a few genera, and the generic relationships. The tribe Tigridieae, with 15 genera and about 140 species, is restricted to the New World and its classification has been interpreted under different criteria by various authors. Recently, and based on the pollen form, the branches of the style, filaments and chromosome number, Goldblatt (1982) divided the Tigridieae into two subtribes, Tigridiinae and Cipurinae.

Since the evolutionary relationships among members of the tribe Tigridieae has not been explored, this work used chloroplast DNA regions (*psbA-trnH*, *trnL* intron, *trnL-F* and *matK*) and the nuclear gene ITS in order to reconstruct the phylogeny of the tribe and other members of the Iridoideae. We also explored the monophyletic status of Tigridiinae and Cipurinae when more species of their distribution are included, particularly the South American ones. We used genes from both nuclear and chloroplast genomes because they have proved to be useful at different levels of resolution to infer the relationships among different groups of plants. Additionally and by using pollen morphology, this

work includes significant morphological characters for the generic delimitation of Tigridiinae and Cipurinae. In order to obtain these goals, we used different methods of reconstruction methods as maximum parsimony, maximum likelihood and Bayesian inference. Different combination of genes as well as single gene analysis were designed in order to have a complete view of the phylogenetic history of Tigridieae.

This work allow us to conclude:

- Levels of sequence divergence were low as expected. For example, *psbA-trnH* and *matK* had the lowest levels of divergence in most comparisons and *trnL-F* and ITS had moderate levels of sequence divergence. We did not find signs of saturation, so all analyses were performed by weighting all characters equally. Although single gene topologies did not reveal good resolution at the different levels, given in part by sampling limitations, they did suggest that a combination of the different datasets would improve the phylogenetic resolution at all levels. Thus, a combination that included all species that had sequences of at least 3 of the total of 6 genes studied (72 taxa: 10 for the outgroup, 62 for the ingroup) was used to make the main inferences about the phylogeny of the tribe Tigridieae.

- The different analyses show a monophyletic tribe Tigridieae that includes both monophyletic subtribes Trigridiinae (clade A) and Cipurinae (clade B), all with the maximum support values for the three methods of reconstruction used. The tribe Trimezieae appears as the sister clade of the cluster formed by Trigridiinae and Cipurinae. The tribes Sisyrinchieae, Irideae, and Diplarreneae are basal in the tree with other members of the subfamily.

- Tigridiinae consists of both Mesoamerican and South American genera. Five out of ten genera from which we had more than one species resulted as monophyletic groups (*Sessilanthera, Ennealophus, Eleutherine, Gelasine* and *Alophia*) whereas two genera turned paraphyletic in this analysis (*Tigridia* and *Mastigostyla*). In this clade, it was also evident the existence of several subclades (N, L, K and J).

- The second largest clade includes the Cipurinae, in which two out of the five genera, from which we had more than one species turned out monophyletic (*Cypella* and *Cipura*). In the Cipurinae clade, it is also evident the presence of two subclades: Clade C and D.

- The current circumscription of subtribes, based on molecular data and phylogenetic analysis, is not consistent with the traditional circumscription proposed by Goldblatt (1982). Therefore:

- We propose a new circumscription of Cipurinae and Tigridiinae, transferring five Cipurinae genera (*Eleutherine*, *Ennealophus*, *Gelasine*, *Hesperoxiphion* and *Mastigostyla*) to Tigridiinae.

- We strongly suggest that the original name on which *Herbertia ungiculata*, was first described, should be maintained.

- We strongly suggest to transfer the South American species of the genus *Mastigostyla* to *Tigridia*

- We suggest to include samples of *Larentia linearis* in future studies, in order to evaluate whether or not *Larentia mexicana* and *Larentia rosei* should be transferred to *Cipura***.**

- In the context of the molecular results, the characters traditionally used to define Tigridiinae and Cipurinae (sulci number and style morphology), do not represent synapomorphies.

- Our results indicate that diversification of Tigridieae occurred about 38,9 MA, and its ancestral region is inferred as corresponding to the north of Mexico and Mesoamerica. The Cipurinae and Tigridiinae subtribes began diversifying ca. 26,41 and 25,11 MA respectively, from their common ancestral area in Mesoamerica during the late Oligocene and early Miocene long before North and South America where finally joined via Central America. The ancestral lineage of the Cipurinae differentiated into two groups, the *Cipura-Larentia* and the Clade D.

- The ancestor of *Cipura-Larentia* occured in Mesoamerica about 13 MA, later *Cipura* diverged in *C. campanulata* and *C. paludosa*) whose current distributions are in northern South America, particularly the high cordilleras of Venezuela, Colombia, Ecuador, and Peru; the Orinoco plains and Amazonia (Areas A, G, H; see Fig 5A). This migration or long distance dispersal happened long before the formation of the Isthmus of Panama around 3 MA ago.

- In these analyses 50 molecular synapomorphies at different levels were found for the different clades in the different genes. A total of 13 synapomorphies defined the Subtribe Cipurinae where as six defined the subtribe Tigridiinae.

Different clusters are well supported with the three methods of reconstruction used, including subgroups within the Cipurinae (clades C and D), and subgroups within Tigridiinae (clades K, L, N).

- Our pollen results indicate that pollen morphology is potentially useful for understanding phylogenetic relationships within the tribe Tigridieae and should be further investigated. Traditionally *Alophia*, *Cardenanthus*, *Ennealophus*, *Hesperoxiphion*, *Mastigostyla* and the South American Tigridieae have been classified in the subtribe Cipurinae as they all possess monosulcate pollen; instead, our phylogenetic results show that these genera belong to four basal clades of Tigridiinae. Patterns of aperture type do not support the traditional subtribal circumscription in Tigridieae. The characters reconstructions showed that the monosulcate pollen is ancestral for the whole tribe. Analyses also suggest that the disulcate pollen indeed evolved only within the Tigridieae; however it is not a synapomorphy for the subtribe and it appears as a rare derived condition within the subtribe.

- Pollen sculpture optimized as a categorical character appears to be a constant feature for the tribe. However, when the sculpture variation is studied as a continuous character defined by the lumen area and muri width, it seems to be helpful to separate species and to trace pollen evolution with more certainty. PCA analyses show that pollen length, muri width, and area lumen describe 30% of the total variation in species of *Cypella*, confirming the palynotaxonomic utilility of these characters.

- The number of pollen apertures did not support the recognition of subtribe Tigridiinae as traditionally circumscribed, however the presence of disulcate

pollen supports the expansion of *Tigridia* to include seven other genera (*Ainea*, *Cardiostigma*, *Cobana*, *Colima*, *Fosteria*, *Rigidella*, *Sessilanthera*).

- Molecular phylogenetic, character optimizations and statistical analyses suggested that differences in number of sulci, lumina size, muri size, and patterns of ornamentation are taxonomically and phylogenetically informative and can be used to distinguish some taxa and clades in Tigridieae.

- The use of genes from both genomes has proved to be useful in providing good phylogenetic signal at different levels. ITS showed low sequence divergence, but defined the main groups studied. We did not find evidence of paralogous genes in these plants. On the other hand, the chloroplast genes provided the best resolution when combined with ITS sequences in the different analysis. It is important to include a larger taxonomic sampling and larger data sets, including other genes in order to better understand the evolutionary scenario of this largely diversified group of plants.

RECOMMENDATIONS

• To evaluate the phylogenetic information of the exine as a quantitative character in other species of Tigridieae.

• Under this new phylogenetic scenario, to study genera and species complexes using molecular and morphological evidence. The genera priority are: *Cypella*, *Mastigostyla* and *Tigridia*