

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE BIOCÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA

**Morfologia, anatomia e evolução em Tigridieae
(Iridoideae: Iridaceae)**

Tamara Pastori

Porto Alegre, março de 2018



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Morfologia, anatomia e evolução em Tigridieae (Iridoideae: Iridaceae)

Tamara Pastori

Tese apresentada ao Curso de Doutorado do Programa de Pós Graduação em Botânica da Universidade Federal do Rio Grande do Sul, como requisito parcial para a obtenção do Título de Doutora em Ciências (Botânica).

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e irmãos Mateus e Erick,

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“aprendi com as primaveras a deixar-me cortar

e a voltar sempre inteira”

(Cecília Meireles)

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RESUMO

Morfologia, anatomia e evolução em Tigridaeae (Iridoideae: Iridaceae)

Iridaceae apresenta distribuição cosmopolita e constitui uma das famílias mais diversas pertencentes à ordem Asparagales. Atualmente, estima-se que existam 2025 espécies e 66 gêneros, e a África subsaariana e a área neotropical são os prováveis centros de diversidade. Crocoideae e Iridoideae são as duas subfamílias mais diversas em Iridaceae, compreendendo 95% da riqueza de espécies. Iridoideae é formada por quatro grandes tribos e uma quinta tribo constituída exclusivamente pelo gênero australiano *Diplarrena*, grupo-irmão das demais tribos. Tigridaeae compreende de 15 a 20 gêneros e 172 espécies, que ocorrem no Sul da América do Norte, América Central e América do Sul. Tigridaeae foi subdividida em duas subtribos: Cipurinae e Tigridiinae, com base em caracteres citogenéticos, palinológicos e morfológicos. No entanto, a filogenia da subfamília Iridoideae envolvendo a tribo Tigridaeae, mostrou que tanto Cipurinae quanto Tigridiinae não são monofiléticas e propôs a divisão de Tigridaeae em dois clados (A e B). Com relação aos gêneros pertencentes ao Clado A, os dois gêneros mais representativos em número de espécies, *Cypella* e *Calydorea*, são não-monofiléticos e os caracteres tradicionalmente utilizados para a separação destes gêneros, como a fusão dos estames e a ramificação do estilete, não têm se mostrado eficientes para a separação genérica. O objetivo geral desta tese é fornecer dados para elucidar questões referentes à evolução e diversificação de Tigridaeae (Iridoideae: Iridaceae) utilizando abordagens morfológicas, anatômicas, filogenéticas, químicas e evolutivas. Para este trabalho foram delimitadas diversas abordagens. A delimitação de espécies de *Cypella* foi realizada através da utilização de dados morfológicos e análises multivariadas, combinadas com análises filogenéticas de marcadores nucleares e plastidiais (Capítulo II). Posteriormente foram realizadas análises filogenéticas, anatomia da seção transversal das folhas de Tigridaeae e evolução de caracteres (Capítulo III). Análises da composição química dos óleos florais foram realizadas a fim de compreender a relação dos mesmos com as estratégias de polinização (Capítulo IV). Os resultados obtidos com as diversas abordagens possibilitaram a delimitação de espécies de *Cypella*, e a sinonimização de *Cypella gloriana* em *Cypella pusilla*. Além disso, os caracteres de anatomia foliar, principalmente relacionados ao esclerênquima, possibilitaram a indicação de caracteres diagnósticos e uma nova circunscrição para Cipurinae e Tigridiinae. Resultados das análises químicas dos óleos florais possibilitaram a identificação de lipídios e revelaram uma ampla gama de variações na composição de ácidos graxos livres entre espécies. Este trabalho forneceu uma caracterização importante para estudos futuros em biologia de polinização e para a

utilização deste óleo para as abelhas coletoras. O conjunto de resultados finais contribuiu para a compreensão dos processos de diversificação de Tigridae e poderão ser utilizados em estudos futuros, principalmente para a revisão taxonômica dos principais gêneros de Cipurinae.

Palavras chave: filogenia, delimitação de espécies análises estatísticas multivariadas, anatomia foliar, caracteres diagnósticos, Cipurinae, Tigridae, recursos florais, óleos florais.

ABSTRACT

Morphology, anatomy and evolution in Tigridieae (Iridoideae: Iridaceae)

Iridaceae presents cosmopolitan distribution and constitutes one of the most diverse families belonging to the order Asparagales. Presently, there are an estimated 2025 species and 66 genera, and sub-Saharan Africa and the Neotropical area are likely centres of diversity. Crocoideae and Iridoideae are the two most diverse subfamilies in Iridaceae, comprising 95% of species richness. Iridoideae is formed by four large tribes and a fifth tribe constituted exclusively by the Australian genus *Diplarrena*, sister group of the other tribes. Tigridieae comprises 15 to 20 genera and 172 species, occurring in southern North America, Central and South America. Tigridieae was subdivided into two subtribes: Cipurinae and Tigridiinae, based on cytogenetic, palynological and morphological characters. However, the phylogeny of the subfamily Iridoideae involving the tribe Tigridieae, showed that both Cipurinae and Tigridiinae are not monophyletic and proposed the division of Tigridieae into two clades (A and B). In relation to the genera belonging to Clade A, the two most representative genera in number of species, *Cypella* and *Calydorea*, are not monophyletic and the characters traditionally used for the separation of these genera, such as the fusion of the stamens and the branching of the style, are not efficient for generic delimitation. The aim of this thesis is to provide data to elucidate questions regarding the evolution and diversification of Tigridieae (Iridoideae: Iridaceae) using morphological, anatomical, phylogenetic, chemical and evolutionary approaches. For this study several approaches were delimited. The species delimitation of *Cypella* was performed through the use of morphological data and multivariate analyses, combined with phylogenetic data of nuclear and plastid markers (Chapter II). Later, phylogenetic analyzes, the leaf anatomy of Tigridieae and evolution of characters were performed (Chapter III). Analyses of the chemical composition of the floral oils were carried out in order to understand the relationship between them and the pollination strategies (Chapter IV). The results obtained with the different approaches allowed the delimitation of species of *Cypella*, and the synonymization of *Cypella gloriana* in *Cypella pusilla*. In addition, leaf anatomy characters, mainly related to sclerenchyma, allowed for the designation of diagnostic characters and a new circumscription for Cipurinae and Tigridiinae. Results of the chemical analyses of the floral oils allowed the identification of lipids and revealed a wide range of variations in the composition of free fatty acids between species, provided an important characterization for future studies in pollination biology and for the use of this for oil collecting bees. The set of final results contributed to the

understanding of the processes of diversification of Tigridieae and could be used in future studies, mainly for the taxonomic revision of the main genera of Cipurinae.

Key words: phylogeny, species delimitation, multivariate statistical analyses, morphology, leaf anatomy, diagnostic characters, Cipurinae, Tigridineae, floral rewards, floral oils.

APRESENTAÇÃO

A presente tese está organizada em quatro capítulos: o primeiro fornece uma introdução geral sobre a família Iridaceae e os assuntos abordados na tese, bem como os objetivos gerais e específicos desta tese de doutorado. Os conteúdos dos capítulos seguintes são artigos científicos que serão ou estão submetidos a periódicos B1 ou superior. São estes: Capítulo II) “Iterative taxonomy based on morphological and molecular evidence to estimate species boundaries: a case study in *Cypella* Herb. (Tigridaeae: Iridaceae)” (submetido para o periódico: Plant Systematics and Evolution), no qual o objetivo é a delimitação de cinco espécies do gênero *Cypella* utilizando análises filogenéticas e análises morfológicas multivariadas. Capítulo III) “Phylogeny, leaf anatomy and evolution of characters in Tigridaeae (Iridoideae: Iridaceae)”, a ser submetido para o periódico American Journal of Botany, onde o objetivo foi investigar caracteres de anatomia foliar entre espécies de Tigridaeae e testar se estes podem ser utilizados como caracteres diagnósticos. O capítulo III) “Chemical of floral rewards: the role of non-volatile lipids on evolution of the Tigridaeae (Iridoideae: Iridaceae)” a ser submetido para o periódico Phytochemistry, onde o objetivo do manuscrito é caracterizar quimicamente os óleos florais e identificar os lipídios presentes nas espécies de Tigridaeae. O capítulo IV) Considerações finais, trata das conclusões obtidas nesta tese de doutorado. O capítulo V) consiste no o artigo “Overlooked diversity in Brazilian *Cypella* (Iridaceae, Iridoideae): four new taxa from the Río de la Plata grasslands” já publicado na revista Phytotaxa, onde estão descritas quatro táxons novos para o gênero *Cypella*, que foram utilizados nas análises desta tese.

CAPÍTULO I



INTRODUÇÃO GERAL

Iridaceae Juss.

Iridaceae é uma das famílias mais diversas pertencentes à ordem Asparagales (APG IV), com cerca de 2.025 espécies e 66 gêneros (Goldblatt e Manning, 2008). Iridaceae possui distribuição cosmopolita, a maior parte das espécies está distribuída na África subsaariana e na área neotropical, regiões indicadas como prováveis centros de diversidade (Goldblatt, 1990; Goldblatt e Manning, 2008). As espécies desta família possuem importância econômica principalmente no setor de paisagismo (por exemplo, os gêneros *Neomarica* Sprague, *Iris* L. e *Gladiolus* L.) e alimentação (*Crocus sativus* L., açafrão).

As Iridáceas são, em sua maioria, plantas herbáceas e de pequeno porte, podem possuir bulbos, rizomas, cormo ou caule lenhoso quando arbustivas (exemplo *Klattia* Baker, *Nivenia* Vent. e *Witsenia* Thunb.) (Goldblatt *et al.*, 1998). São reconhecidas principalmente pela grande variedade na morfologia floral e foliar (Rudall, 1994; Goldblatt e Manning, 2006). Em Iridaceae, as folhas são alternas, dísticas, com presença de cristais prismáticos de oxalato de cálcio nas bainhas dos feixes vasculares (Prychid e Rudall, 1999; Rudall, 1995). As flores são geralmente actinomorfas, formadas por seis tépalas distribuídas em dois verticilos, apresentam três estames, grãos de pólen com exina reticulada e o ovário é ínfero (Goldblatt, 1990; Goldblatt e Manning, 2008).

Atualmente, Iridaceae está dividida em sete subfamílias: Isophysidoideae, Patersonioideae, Geosiridoideae, Aristeoideae, Nivenioideae, Crocoideae e Iridoideae (Fig. 1). Crocoideae e Iridoideae são as mais diversas, compreendendo 95% da riqueza de espécies (Goldblatt *et al.*, 2008), havendo cerca de 29 gêneros e 1.032 espécies em Crocoideae e pelo menos 20 gêneros e 900 espécies em Iridoideae (Goldblatt e Manning, 2008). Os demais 5% das espécies estão distribuídas em cinco subfamílias: Isophysidoideae e Geosiridoideae são monoespecíficas, Aristeoideae e Patersonioideae são monogenéricas e representadas pelos gêneros *Aristea* Aiton e *Patersonia* R.Br., respectivamente. Nivenioideae possui cerca de 15 espécies, distribuídas em três gêneros *Klattia*, *Nivenia* e *Witsenia*.

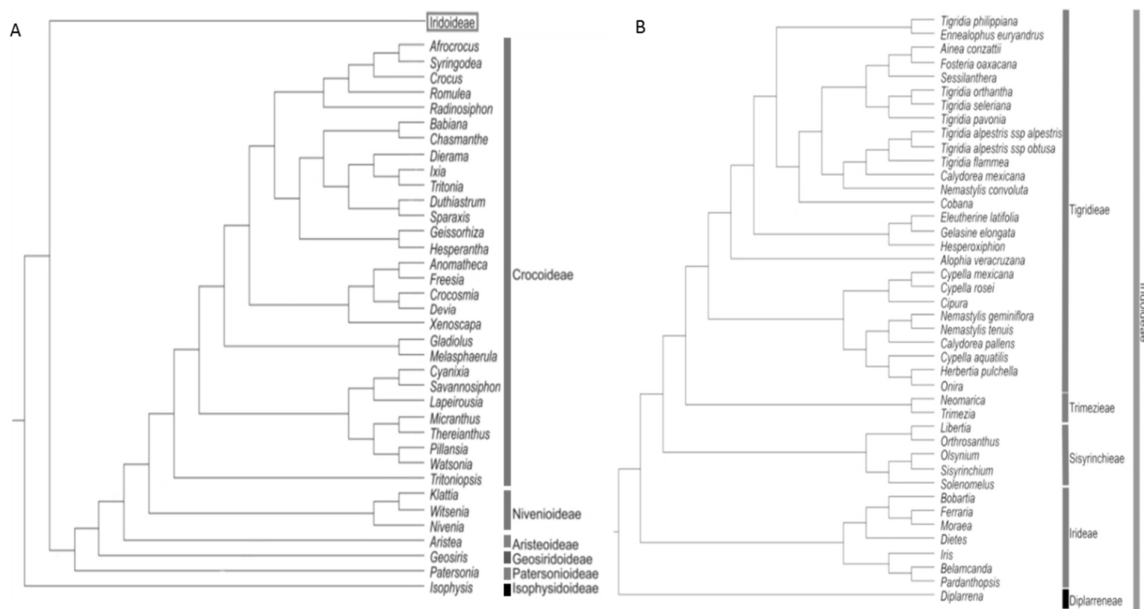


Figura 1 - Árvore filogenética das subfamílias e tribos de Iridaceae (A) e da subfamília Iridoideae (B), mostrando as cinco tribos. Adaptada de Goldblatt *et al.* (2008).

A subfamília Iridoideae é formada por cinco grandes grupos circunscritos ao nível taxonômico de tribo: Irideae, Sisyrinchieae, Trimezieae, Tigrídieae e Diplarreneae, cujo gênero australiano *Diplarrena* Labill., único representante de Diplarreneae, é grupo irmão das demais tribos (Goldblatt e Manning, 2008). A tribo Irideae, predominantemente originária do Velho Mundo (com algumas espécies de *Iris* na América do Norte) e a tribo Sisyrinchieae (cujos gêneros *Libertia* Spreng. e *Orthrosanthus* Sweet apresentam ocorrência Austral-asiática e Americana) são grupos irmão das tribos Trimezieae e Tigrídieae. Trimezieae e Tigrídieae formam uma linhagem monofilética definida por sinapomorfias moleculares e morfológicas (Reeves *et al.*, 2001; Rodriguez e Sytsma, 2006; Goldblatt *et al.*, 2008; Goldblatt e Manning, 2008) e ocorrem exclusivamente no continente americano. A separação destas duas tribos ocorreu provavelmente no Eoceno tardio, há cerca de 35 milhões de anos (Goldblatt *et al.*, 2008).

As espécies de Tigrídieae estão distribuídas desde o sul da América do Norte, até América do Sul (Rodriguez e Sytsma, 2006; Goldblatt *et al.*, 2008). Tigrídieae compreende de 15 a 20 gêneros e 172 espécies, caracterizadas principalmente por possuírem bulbos, folhas plicadas ou foliadas, flores actinomorfas, tépalas geralmente livres e ramos do estilete desde achatados a petaloides, simples ou bifurcados (Goldblatt e Manning, 2008).

Tigridieae foi subdividida em 1982 em duas subtribos: Cipurinae e Tigridiinae, com base em caracteres citogenéticos, palinológicos e morfológicos. A subtribo Cipurinae foi caracterizada por possuir número cromossômico $x = 7$, grãos de pólen monosulcados e ramos do estilete petaloides a cilíndricos, bifurcados ou simples, enquanto a subtribo Tigridiinae foi diferenciada pelo número cromossômico $x = 14$, grãos de pólen bissulcados e ramos do estilete cilíndricos, profundamente bifurcados (ou simples) (Goldblatt, 1982). No entanto, a filogenia da subfamília Iridoideae, publicada por Chauveau *et al.* (2012) envolvendo a tribo Tigridieae, mostrou que tanto Cipurinae quanto Tigridiinae não são monofiléticas e propôs a divisão de Tigridieae em dois clados (A e B). O Clado A englobou alguns gêneros incluídos anteriormente na subtribo Cipurinae (*Ainea* Ravenna, *Calydorea* Herb., *Catila* Ravenna, *Cipura* Aubl., *Cypella* Herb., *Herbertia* Sweet, *Nemastylis* Nutt, *Larentia* Klatt, *Kelissa* Ravenna e *Onira* Ravenna) (Fig. 2) e o Clado B, os gêneros restantes da subtribo Cipurinae (*Cardenanthus* R.C. Foster, *Eleutherine* Herb., *Ennealophus* N.E. Br., *Gelasine* Herb. (Fig. 3j), *Hesperoxiphion* Baker, *Phalocallis* Herb. (Fig. 3k) e *Mastigostyla* I.M. Johnst.) e todos os gêneros da subtribo Tigridiinae (*Cobana* Ravenna, *Fosteria* Molseed, *Tigridia* Juss., *Alophia* Herb., *Sessilanthera* Molseed & Cruden).

Com relação aos gêneros pertencentes ao Clado A, os dois gêneros mais representativos em número de espécies, *Cypella* e *Calydorea*, são não-monofiléticos (Chauveau *et al.*, 2012). Na filogenia recentemente publicada, *Kelissa* e *Onira* formam um primeiro agrupamento não-monofilético com *Cypella hauthalii* (Kuntze) R.C. Foster, sendo pouco diferenciadas de *Cypella* (Chauveau *et al.*, 2012). Goldblatt e Manning (2008) consideraram que os caracteres tradicionalmente utilizados para a separação destes gêneros, como a fusão dos estames e a ramificação do estilete, não têm se mostrado eficientes para a separação genérica, conforme proposição de inclusão de *Kelissa* e *Onira* em *Cypella*, elaborada por Roitman e Castilho (2007). As variações observadas na organização dos estames e estiletos em *Catila*, *Itysa* Ravenna, *Lethia* Ravenna e *Tamia* Ravenna também foram consideradas insuficientemente discriminantes por Goldblatt e Manning (2008) para a manutenção dos gêneros, sendo incluídos em *Calydorea* por estes autores. Ravenna (2009), por sua vez, propôs a revalidação dos gêneros *Kelissa*, *Onira*, *Catila*, *Itysa* e *Tamia*, reafirmando a distinção com base na análise dos caracteres citados.



Figura 2: Espécies de *Cypella* pertencentes ao Clado A de Tigridieae (Iridaceae) a) *C. hauthalii* (Kuntze) R.C.Foster subsp. *hauthalii*; b) *C. hauthalii* subsp. *opalina* Ravenna; c) *C. hauthalii* subsp. *minuticristata* Chauveau & L.Eggers; d) *C. ravenniana* Deble & F.S.Alves; e) *C. armosa* Ravenna; f) *C. rivularis* Chauveau & L.Eggers; g) *C. luteogibbosa* Deble; h) *C. discolor* Ravenna; i) *C. osteniana* Beauverd; j) *C. fucata* Ravenna; k) *C. herbertii* (Lindl.) Herb.; l) *C. amplimaculata* Chauveau & L.Eggers. (a-j): Scale bar = 1 cm; (k-l) Scale bar = 5 mm.



Figura 3: Espécies de Tigridae (Iridaceae) pertencentes aos Clados A e B a) *Calydorea approximata* R.C. Foster (foto de L.Eggers); b) *Calydorea alba* Roitman & J.A.Castillo; c) *Catila amabilis* Ravenna; d) *Cipura paludosa* Aubl.; e) *Kelissa brasiliensis* (Baker) Ravenna; f) *Herbertia zebrina* Deble; g) *Herberia pulchella* Sweet; h) *Herbertia furcata* (Klatt) Ravenna; i) *Herbertia darwinii* Roitman & J.A.Castillo; j) *Gelasine uruguayensis* Ravenna, k) *Gelasine elongata* (Graham) Ravenna, l) *Phalocallis coelestis* (Lehm.) Ravenna (foto de M. Verdi). Scale bar = 1cm.

Com relação ao Clado B, os resultados obtidos por Goldblatt *et al.* (2008) e Chauveau *et al.* (2012) sugerem a inclusão de *Cardiostigma* Baker, *Colima* (Ravenna) Aarón Rodr. & Ortiz-Cat., *Fosteria*, *Rigidella* Lindl. e *Sessilanthera* em *Tigridia*. Além destes, *Phalocallis* também apresenta divergências taxonômicas. Este gênero foi sinonimizado em *Cypella*, com base na morfologia floral (Roitman e Castillo, 2007; Goldblatt e Manning, 2008). Os caracteres utilizados para circunscrição de *Phalocallis* e *Cypella* não são distintivos e análises filogenéticas e cromossômicas recentes sugerem que estes gêneros devem ser separados (Chauveau *et al.*, 2012; Moraes *et al.*, 2015). Assim, a ausência de diferenças morfológicas claras entre gêneros de Tigridieae sugere que as circunscrições genéricas devem ser revistas (Chauveau *et al.*, 2012).

Delimitação de espécies

O conhecimento taxonômico é complexo e peculiar, considerando que muitas espécies ainda são desconhecidas, outras foram descritas muitas vezes com nomes científicos diferentes, fazendo com que o número de espécies varie, particularmente em grupos menos estudados (Isaac *et al.*, 2004). A delimitação de espécies é uma tarefa complexa, principalmente em um cenário evolutivo (Sites e Marshall, 2003). Idealmente, a caracterização das espécies deveria utilizar diferentes informações (morfológicas, anatômicas, fisiológicas, ecológicas, filogenéticas, geográficas, etc.) através de uma abordagem integrativa para a delimitação e identificação de *taxa* (Dayrat, 2005; Schlick-Steiner *et al.*, 2010).

A ausência de limites claros entre as linhagens dificulta estratégias para a conservação e distribuição das espécies, principalmente para a formulação de políticas públicas e compilação de dados de diversidade atualizados. A taxonomia integrativa é capaz de fornecer melhores inferências sobre os limites das espécies (Dayrat, 2005; Padial e De la Riva, 2006; Padial *et al.*, 2010). Entretanto, esta delimitação entre as linhagens envolve questões como o conceito de espécie a ser utilizado. A questão sobre “O que é uma espécie?” é muito antiga, já que muitos conceitos de espécie já foram propostos baseados em diferentes propriedades biológicas, como por exemplo, morfologia, isolamento reprodutivo, filogenia, ecologia, etc., e isso se deve provavelmente ao fato de que cada pesquisador aplica o conceito que entende como o mais adequado (De Queiroz, 2007). De fato, quanto maior o número de critérios de espécies atendidos por um grupo, mais provável é que ele seja uma linhagem distinta, sendo menos questionável o seu reconhecimento como espécie (De Queiroz, 2007). Apesar de existirem diversos conceitos de espécies, praticamente todos convergem em um elemento comum, que as

espécies são linhagens de metapopulações evoluindo separadamente, e este é o conceito de linhagem generalizada (GLC), amplamente adotado (Padial e De La Riva, 2006) e flexibilizado (Naciri e Linder, 2015; Freudenstein *et al.*, 2017). No entanto, de acordo com Carstens *et al.* (2013), é um erro assumir que o GLC é um pré-requisito para a delimitação de espécies. Então, parece que o mais apropriado para a delimitação de espécies é analisar os dados com diferentes abordagens e, em seguida, delimitar as linhagens consistentes (Carstens *et al.*, 2013; Naciri e Linder, 2015). Entretanto, a maioria das discordâncias entre diferentes autores sobre a ideia de espécie, não está relacionado ao conceito em si, mas sim em como reconhecer uma espécie.

Nas últimas décadas, os pesquisadores desenvolveram uma série de métodos para reconhecer novas espécies ou testar hipóteses de espécies (Wiens, 2007, Naciri e Linder, 2015). Entretanto, essas ferramentas para a delimitação de espécies envolvem muitas vezes métodos moleculares e computacionais dispendiosos e exigentes em mão de obra especializada. Além disso, nem todos os trabalhos que utilizam esses métodos propõem considerações taxonômicas, ou descrição de espécies novas, o que evidenciou a revisão realizada por Carstens *et al.* (2013): menos de 30% dos estudos avaliados propuseram recomendações taxonômicas e apenas 25% descrevem novas espécies. Segundo os autores, isso poderia indicar uma falta de confiança nos resultados, possivelmente ocasionada pela falta de treinamento taxonômico ou uma incapacidade de conciliar incongruências entre diferentes métodos. Nesse sentido, a obtenção de recursos e a formação de mão-de-obra especializada são essenciais para a compreensão dos limites entre as espécies e da diversidade como um todo.

Grupos de diversificação recente, ou grupos cientificamente pouco estudados, geralmente são os mais difíceis em taxonomia e sistemática. Iridaceae é uma das maiores famílias de monocotiledôneas e é considerado “taxonomicamente” difícil por diversos fatores, desde a duração efêmera das flores, dificuldade no processo de herborização, até processos de hibridação e evolução recente, o que dificulta a identificação e delimitação das espécies.

A taxonomia em Iridaceae, especialmente para os representantes americanos, está baseada principalmente na utilização de medidas morfológicas clássicas, principalmente de exsiccatas e onde poucos indivíduos são amostrados. Nos últimos oito anos, somente para o Clado A de Tigridieae foram descritos 19 novos *taxa*: dois novos *taxa* de *Herbertia* (Deble, 2010; Deble, 2013), três de *Calydorea* (Deble, 2011, 2013, 2016) e 14 de *Cypella* (Deble *et al.*, 2012; Chauveau *et al.*, 2014; Deble *et al.*, 2015a, b; Deble e Alves, 2017). Dentre estes novos *taxa* descritos, 100% foram baseados somente em análises morfológicas clássicas e, destes,

78,9% foram baseados na utilização de medidas de exsicatas e/ou através da utilização de uma ou duas populações.

De fato as diferenças morfológicas são úteis na delimitação de espécies, e têm sido utilizadas durante séculos. Entretanto, em alguns grupos, processos evolutivos recentes dificultam a delimitação, e muitas vezes a descrição de novas *taxa* baseada somente variação morfológica intraespecífica pode gerar uma superestimação no número de espécies (*inflated species delimitation*) (Duminil e Di Michele, 2009). No caso de Tigridaeae, a diversidade pode ter sido negligenciada até poucos anos atrás, mas, por outro lado, se deve considerar a hipótese que talvez a diversidade tenha sido superestimada.

Os caracteres morfológicos permitem a identificação taxonômica, embora as inferências sobre os limites das espécies sejam melhor feitas usando uma abordagem que integre a taxonomia em diferentes tipos de dados e análises (Dayrat, 2005; Padial *et al.*, 2010; Carstens *et al.*, 2013).

Anatomia foliar

Iridaceae é uma das poucas famílias onde estão disponíveis diversos estudos sobre anatomia, principalmente anatomia foliar, e, além disso, muitos caracteres foram utilizados para circunscrições taxonômicas na família (Rudall, 1984; 1986; 1990; 1993; Rudall e Burns, 1989; Rudall e Goldblatt, 1991; 1993). Em Tigridaeae, os caracteres vegetativos são muito semelhantes e a distinção de gêneros está basicamente indicada pela morfologia floral. A subdivisão de Tigridaeae nos Clados A e B, com base em análises filogenéticas (Chauveau *et al.*, 2012) evidenciou que caracteres anteriormente utilizados para a separação das duas subtribos (Cipurinae e Tigridiinae) não são homólogos e, portanto, uma investigação mais profunda é necessária, tendo em vista que outros atributos não evidentes, como a morfologia interna de estruturas vegetativas, poderia auxiliar na identificação de caracteres homólogos para os Clados A e B.

Estudos envolvendo anatomia foliar em Tigridaeae foram realizados há cerca de três décadas atrás, e inexistência de filogenias moleculares bem resolvidas dificultou as considerações taxonômicas. Análises anatômicas realizadas por Rudall (1991, 1994) evidenciaram dois tipos de morfologia foliar, caracterizadas como folhas “plicadas” e “foliadas”, e os gêneros de Tigridaeae foram classificados de acordo com esta. No entanto, ambos os tipos foliares ocorrem em Cipurinae e em Tigridiinae. Outras características anatômicas também foram consideradas por Rudall (1991; 1995), como o aspecto da margem

foliar e a presença de esclerênquima marginal. A presença, posição e constituição deste esclerênquima nas folhas mostra-se um caráter promissor para a separação de grupos em Tigridieae e, muito provavelmente, útil para a identificação de gêneros da tribo.

Oferta de recursos florais

A ampla variação na morfologia floral observada em Iridaceae esta relacionada com a diversidade dos sistemas de polinização, e é relatada principalmente para os gêneros africanos (Goldblatt e Manning, 2006). Na maioria das espécies de Iridaceae, o sistema de cruzamento preponderante é a fecundação cruzada, isto torna a presença do polinizador fundamental. Além disso, nas espécies africanas de Iridaceae, por exemplo, a grande maioria é polinizada por uma ou por poucas espécies de insetos, e somente 3% são visitadas por polinizadores generalistas (Goldblatt e Manning, 2006).

A oferta de recursos florais aos polinizadores é um fator chave a ser considerado para a evolução e a diversidade na morfologia floral. Neste sentido, Iridaceae é uma das poucas famílias de angiospermas a oferecer uma ampla gama de recursos florais. O néctar é o recurso mais comumente oferecido (Rudall *et al.*, 2003), em Crocoideae e Nivenioideae o néctar é produzido por células especializadas, localizadas entre as paredes ou septos no ovário e são chamados nectários septais (Rudall, 2003; Goldblatt e Manning, 2008). Em Iridoideae, os nectários septais são ausentes (exceto em Diplarreneae), o néctar é produzido em glândulas na superfície das tépalas (ex. *Tigridia*, *Moraea* Mill., *Ferraria* Burm. ex Mill.) ou dentro do tubo floral (Daumann, 1970; Rudall *et al.*, 2003). Em Trimezieae, os nectários são do tipo tricomáticos, presentes nas tépalas internas do gênero *Neomarica* (Rudall, 2003) e em Sisyrinchieae o único registro de nectários é em *Olsynium* Raf. Já em Tigridieae, a presença de nectários inclui o gênero *Tigridia* (Clado B), que possui nectários tricomáticos nas tépalas internas, e *Cypella* (Clado A) que possui nectários no conectivo das anteras (Vogel, 1974; Devoto e Medan, 2008, Pastori *et al.*, 2013, Pastori, 2014).

Os óleos florais constituem o terceiro tipo de recurso oferecido em Iridaceae. Este recurso é restrito a um conjunto de 11 famílias botânicas e dentre estas, Iridaceae é uma das únicas onde esse recurso surgiu independentemente diversas vezes (Orquidaceae é a outra família) (Renner e Schaefer, 2010). Em Iridaceae, os elaióforos são exclusivamente observados em representantes de Iridoideae e estão localizados geralmente nas tépalas ou no tubo estaminal (Vogel, 1974, Chauveau *et al.*, 2011, Silvério *et al.*, 2012). A única exceção é uma espécie pertencente à subfamília Crocoideae, *Tritoniopsis parviflora* (Jacq.) G.J. Lewis, que é

conhecida como produtora de óleos (Manning e Goldblatt, 2002, 2005). Segundo Buchmann (1987), possuem elaióforos os gêneros *Alophia*, *Cypella*, *Ennealophus*, *Sisyrrinchium* L., *Sphenostigma* Baker, *Tigridia* e *Trimezia* Salisb. ex Herb. No entanto, Chauveau *et al.* (2012) mostraram, a partir de dados bibliográficos, que elaióforos estão presentes também nas espécies *Cardenanthus vargasii* R.C. Foster, *Catila amabilis* Ravenna, *Cipura paludosa* Aubl., *Ennealophus euryandrus* (Griseb.) Ravenna, *Hesperoxiphion*, *Mastigostyla* e nos gêneros monotípicos *Kelissa* e *Onira*. Apesar da existência de elaióforos ter sido relatada para estes gêneros, apenas algumas espécies de *Sisyrrinchium* foram testadas através de análises histoquímicas para a detecção destas estruturas (Silvério *et al.*, 2012), os demais gêneros necessitam de testes de confirmação.

As relações entre as flores secretoras de óleo e abelhas coletoras constituem exemplo de uma especialização funcional e de uma interação incomum entre plantas e polinizadores, já que apenas polinizadores especializados são capazes de coletar estes óleos florais (Chauveau *et al.*, 2011, 2012). Estudos sugerem que a seleção mediada por polinizadores constitui a principal força motriz para a diversidade floral (Valente *et al.*, 2012; Van der Niet e Johnson, 2012; Forest *et al.*, 2014). Adaptações a diferentes agentes polinizadores adicionados a fatores evolutivos seriam responsáveis por padrões de diversidade encontrados em espécies de plantas com flores (Goldblatt e Manning, 2008; Givnish, 2010; Valente *et al.*, 2012).

O padrão de diversificação floral observado em espécies de Iridaceae parece estar diretamente relacionado com a ampla oferta de recursos florais aos polinizadores. Alguns gêneros africanos da família, como *Tritoniopsis* L. Bolus e *Lapeirousia* Pourr. (Manning e Goldblatt, 2002, 2005; Goldblatt e Manning, 2008), já são reconhecidos por possuir polinização bimodal, oferecendo combinações de recursos florais aos polinizadores, e estudos recentes tem demonstrado a especiação direcionada por essa interação (Valente *et al.*, 2012; Forest *et al.*, 2014). Os gêneros ocorrentes no continente americano são desconhecidos quanto a sua interação com os polinizadores. Estudos recentes tem demonstrado que em Tigridieae ocorre uma ampla oferta de recursos florais e com diferentes combinações como, por exemplo, *Calydorea* e *Catila* oferecem somente pólen, *Herbertia* e *Kelissa* oferecem pólen e óleos florais, *Cypella* e *Onira* oferecem pólen, óleos florais e néctar (Fig. 4) (Pastori *et al.*, 2013, Pastori, 2014). A ampla oferta e as diferentes combinações de recursos florais pode ter sido a força motriz para diversificação e a evolução da família na América do Sul, especialmente nas regiões Sul e Sudeste do Brasil (Pastori, 2014). A compreensão dos fatores envolvidos na diversificação das espécies é fundamental para a formulação de hipóteses sobre a evolução de um grupo

vegetal. Nesse contexto Iridaceae se destaca das demais famílias de angiospermas por ser uma das poucas que incluem ampla oferta de recursos florais e síndromes de polinização aliadas à alta diversidade de espécies (Manning e Goldblatt, 2005; Devoto e Medan, 2008; Goldblatt e Manning, 2008).

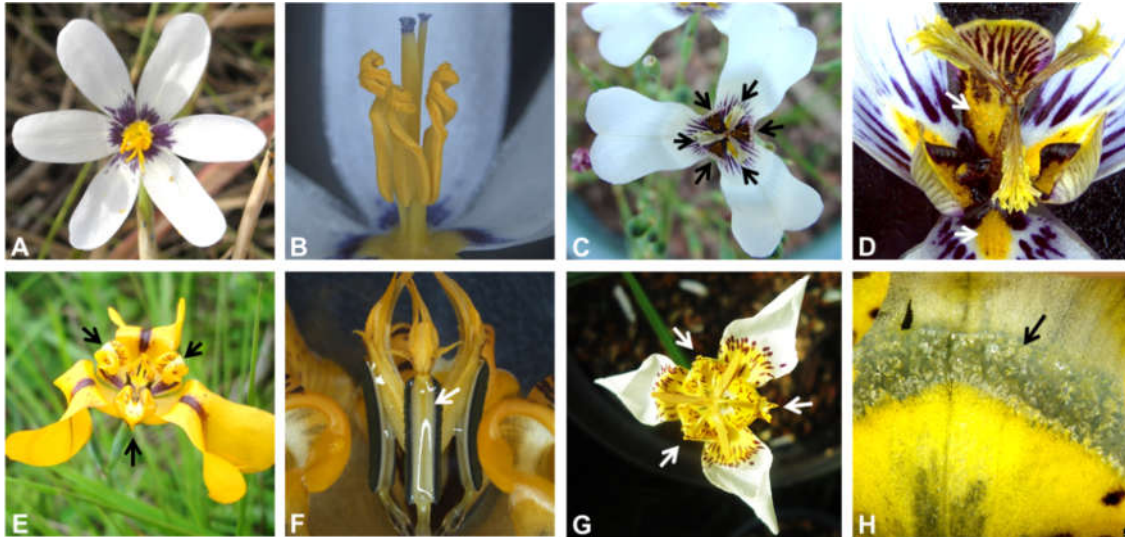


Figura 4: Espécies e diversidade de recursos florais oferecidos aos polinizadores em Tigridaeae. A-B) *Calydorea alba* oferece como recurso somente pólen; C-D) *Herbertia zebrina*, pólen e óleos florais, que são produzidos em elaióforos localizados nas tépalas externas e internas (C) (localização indicada por setas); E-F) *Cypella amplimaculata*, pólen, óleos florais em elaióforos localizados somente nas tépalas internas (E), e néctar produzido no nectário localizado no conectivo das anteras (F); G-H) *Tigridia chiapensis* Molseed ex Cruden, pólen e néctar, o nectário tricomático, está localizado nas tépalas internas (H).

OBJETIVOS

O objetivo geral desta tese é fornecer dados para elucidar questões referentes à evolução e diversificação de Tigridaeae (Iridoideae: Iridaceae) utilizando abordagens morfológicas, anatômicas, filogenéticas, químicas e evolutivas.

Objetivos específicos

- 1) Contribuir para a circunscrição e descrição de espécies de *Cypella*, através de uma abordagem iterativa, utilizando caracteres morfológicos e moleculares;
- 2) Gerar uma filogenia para a tribo Tigridaeae baseada em marcadores plastidiais, focada especialmente no Clado A e utilizar uma ampla amostragem, a fim de testar as relações filogenéticas entre os gêneros;
- 3) Gerar uma matriz de dados anatômicos para os gêneros de Tigridaeae;
- 4) Inferir a evolução dos caracteres de anatomia foliar de Tigridaeae a partir da filogenia obtida com marcadores moleculares;
- 5) Caracterizar quimicamente os óleos florais em espécies de Tigridaeae.

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CAPÍTULO II



CAPÍTULO III



CAPÍTULO IV



CAPÍTULO V



CAPÍTULO VI (ANEXOS)



Overlooked diversity in Brazilian *Cypella* (Iridaceae, Iridoideae): four new taxa from the Río de la Plata grasslands

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Abstract

Three new species and one subspecies of *Cypella* are described for Rio Grande do Sul (RS), Brazil: *Cypella altouruguaya* from northern RS, *C. amplimaculata* widely distributed across the state and *C. rivularis* restricted to southern RS, in grassland streams of the Pampa biome. *Cypella hauhalii* subsp. *minuticristata* is found in a central area of Rio Grande do Sul. The different taxa are described, illustrated and compared with related species. The resulting taxonomic framework shows that most of the species described for *Cypella* occur in the Río de la Plata grasslands, with various infrageneric taxa characterised by a high level of endemism, especially in the Subtropical Grasslands of Southern Brazil.

Key words: Campos eco-region, endemism, Rio Grande do Sul, Subtropical Grasslands, taxonomy

Introduction

Iridaceae is divided into seven subfamilies and comprise about 2030 species distributed among 65 to 75 genera (Goldblatt *et al.* 2008). The Iridoideae, with more than 900 species, represent one of the two major evolutionary branches of the family and make up about 44% of the species richness of the Iridaceae (Goldblatt & Manning 2008). Among the five tribes of this subfamily, the New World tribe of Tigridieae forms a monophyletic lineage of about 15 genera and 160 species (Goldblatt & Manning 2008, Chauveau *et al.* 2012). *Cypella* Herbert (1826: t. 2637), with 30 species and four subspecies accepted by the World Checklist of Iridaceae (WCI), is one of two largest genera of the tribe in South America (Goldblatt & Manning 2008, Barker 2014). The taxonomic delimitation of this genus phylogenetically closely related to *Calydorea* and *Herbertia* remains controversial (Chauveau *et al.* 2012, Deble *et al.* 2012). Indeed, among the species accepted by the WCI in *Cypella*, three species present the distinctive morphological features of *Phalocallis* Herbert (1839: t. 3710): *C. boliviana* Huaylla (2012: 297), *C. geniculata* (Klatt 1871: 517) Ravenna (1964: 53) and *C. oreophila* Spegazzini (1917: 44). The former species is considered morphologically strictly related to the type species of *Phalocallis*, *P. coelestis* (Lehmann 1826: 17) Ravenna (1977: 9) and is only distinguished by small variations of floral traits (Huaylla & Wood 2012). *Cypella geniculata* and *C. oreophila* were included by Ravenna (2009) in *Phalocallis* based on the same distinctive floral traits than the type species of the genus. Furthermore, the latest comprehensive phylogeny of Tigridieae confirmed that *Phalocallis* should be regarded as a separate genus from *Cypella* (Chauveau *et al.* 2012). The resulting circumscription of *Cypella* shows that 80% of the species and subspecies are found in the Río de la Plata grasslands (RPG), one of the most extensive biogeographic units of the grassland biome in the world (Medan *et al.* 2011). Indeed, this is the largest complex of subtropical and temperate grassland ecosystems in South America (Soriano *et al.* 1992, Miñarro & Bilencia 2008). These grasslands include the eco-regions of Pampas in North Eastern Argentina, and the Campos eco-regions in Uruguay, Northern Argentina, South East Paraguay and Southern Brazil, where most of the *Cypella* species are distributed (Di Giacomo & Krapovickas 2005, Overbeck *et al.* 2007, Paruelo *et al.* 2007). In Southern Brazil, the grassland vegetation is included in two separate biomes according to the current official classification (IBGE 2004): the Pampa and the Atlantic Forest (Overbeck *et al.* 2007). The RPG is perhaps one of the regions in the world with highest rates of land use and land cover changes related to human activities

(Vega *et al.* 2009). Taxonomic and ecological studies are essential to understand how these changes will impact the biodiversity and to identify appropriate actions of conservation.

This study aims to describe and illustrate three new species and one new subspecies of *Cypella*, endemic to the Subtropical Grasslands of Southern Brazil (Campos eco-regions). The new taxonomic framework provided by this study is used to assess the species richness and the level of endemism of *Cypella* species in the Río de la Plata grasslands.

Description of the new taxa

The terminology used for the descriptions follows Goldblatt & Manning (2008) and Beentje (2010). Observations and measurements are based on fresh specimens.

Cypella altouruguaya Chauveau & L.Eggers, *sp. nov.* (Figs. 1A and 2)

Cypella altouruguaya differs from two closely related species with similar yellow flowers, *C. armosa* and *C. pabstiana*, by the cuneate proximal half of the inner tepals (*vs. unguiculate*). It reminds *C. armosa* in flower size, but differs by its wider outer tepals, slightly retuse connective apex, adaxial crests not twisted basally and longer abaxial crests. The gross morphology of *C. altouruguaya* comes close to *C. pabstiana*, which is distinguished by its smaller flower, longer and totally free filaments, longer anthers and slightly excurrent connective apex.

Type—BRAZIL. Rio Grande do Sul: Rio Grande do Sul: Trindade do Sul, estrada Trindade do Sul - Pinhalzinho, 610 m, 03 December 2011 (fl, fr), T.B. Guimarães & L. Dal Ri 64 (holotype, ICN!)

Perennial herb, up to (32–)39–70 cm high above the soil, underground stem up to (3.5–)4.8–8.3(–8.7) cm long. Bulb ovoid, outer cataphylls dark brown, 14–15(–16.8) × (8–)12–15 mm, prolonged in a collar up to (1.8–)3.5–5 (–5.6) cm. Basal leaves green at anthesis 1–3(–4), blades linear-attenuate, plicate, (16.8–)22.5–42(–47) × (0.15–)0.3–0.45(–0.7) cm. Flowering stem cylindrical, (28–)40–63.4 cm long, proximally foliate (one reduced cauline leaf, rarely absent), then bracteose; first internode (4.2–)10–17.2(–19.5) cm long; cauline leaf (11–)16.1–32.7(–39) × (0.15–)0.3–0.7(–0.75) cm. Synflorescence cymosely branched, branches usually 2(–3), each subtending 2–4 pedunculate inflorescences arising from the same point, peduncles (1.0–)3–10.5(–11.5) cm long. Inflorescence one-flowered (rhipidium like); spathes herbaceous, bivalved, lower valve (2–)2.4–3.9 cm long, the upper (3.5–)4–6.3(–6.7) cm long, both with narrow membranous edges. Pedicel filiform, generally shorter than the upper valve with the ovary usually partly to sometimes entirely exserted. Flowers predominantly bright yellow, 45–55 mm diameter. Tepals unequal, shortly fused proximally for 0.5 mm. Outer tepals pandurate, (32–)37–42(–46) × (21–)24–31(–33) mm; the proximal part concave, pale yellow to bright yellow, slightly translucent, broadly marked with a red-brown irregular spot at the base, the distal edge of the concave part sparsely marked by an area of yellow glandular trichomes along the central vein; the distal part reclinate, bright yellow, obovate, retuse and acuminate. Inner tepals reduced, assurgent proximally, then incurved and abruptly reclinate distally, (10–)10.5–12.5(–13) × (8.5–)9–12(–13) mm; the proximal half cuneate, not unguiculate, bright yellow, broadly streaked with red-brown; the distal half bright yellow, longitudinally depressed, except at the distal end, with a dense oblong orange-yellow area of oil-producing trichomes (elaiophore) marked with red-brown spots, the lateral sides firmly revolute, striated transversely with red-brown, the apex acute, spotted with red-brown. Filaments free, erecto-patent and abruptly incurved at the distal end, whitish to whitish-yellow, obclavate, thick, 0.8–0.9 mm wide at mid-length, striated with purple on the inflated base, rarely on the whole length, 3–3.5(–4) mm long. Anthers oblong, 6–7(–7.9) × 1.4–1.8(–2) mm, adnate to the style arms for half of the length; connective apically slightly retuse, whitish-yellow to yellow towards the distal end, 0.8–1.2(–1.5) mm wide, usually covered with a viscous and transparent secretion; locules black; pollen dark yellow-green. Ovary subclavate, (6–)7–9.5(–11) × 2–2.9(–3.1) mm. Style whitish to yellow, 5–6 (–6.2) mm long. Style arms bright yellow, conduplicate, 5–6.5(–7) mm long; crests at the apex, bright yellow, adaxial crest 2, erect, falcate inwards, (3.5–)4–6(–6.5) mm long, abaxial crest triangular, lobed, (1–)1.3–2.6(–3) mm long; stigmatic surfaces transverse, 2, on each side at the base of the abaxial crest, bright yellow, (1.1–)1.2–1.6(–1.8) mm long. Capsule obovate-truncate, (10–)11.7–16.8(–21.8) × (3–)3.5–5(–5.4) mm. Seeds obconical, triangular in adaxial view, sharply angulate, epidermis verrucose areolate, 1.3–1.5 mm long.



FIGURE 1. Habits of new species of *Cypella*. A. *C. altouruguayana* Chauveau & L.Eggers. From T.B. Guimarães & L. Dal Ri 6 (ICN!) B. *C. amplimaculata* Chauveau & L.Eggers. From *A.M. Aita 49* (ICN!), drawings by Anelise Scherer.

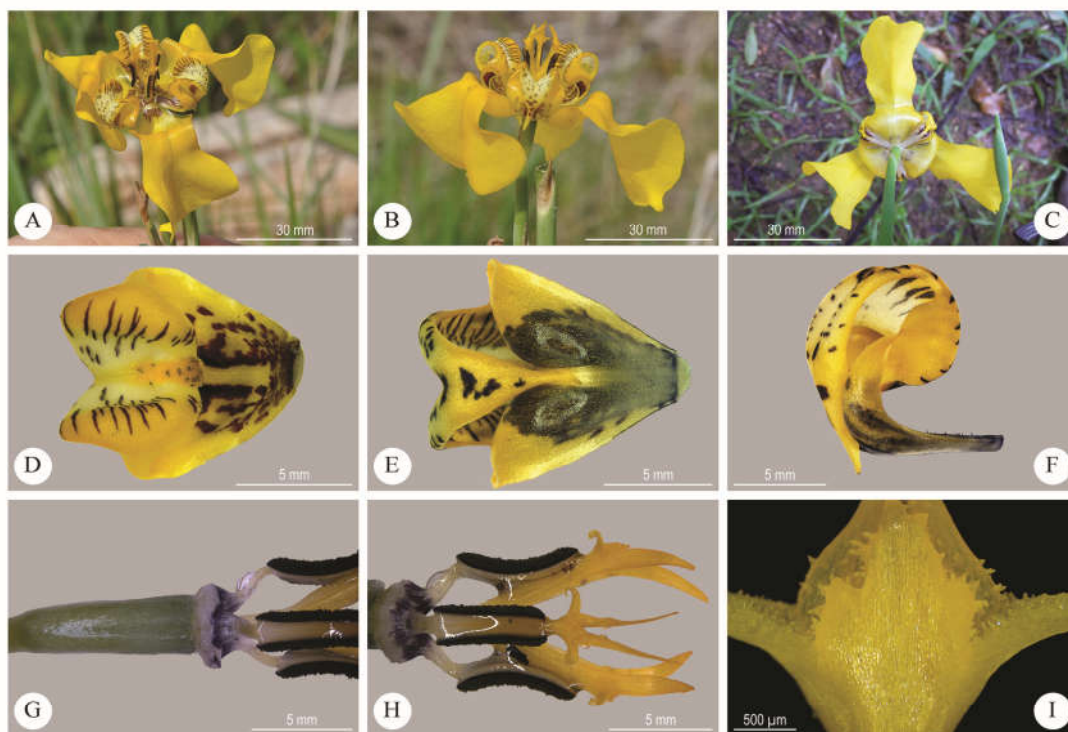


FIGURE 2. *Cypella altouruguaya* Chauveau & L.Eggers. A–C. Flower. A. apical view B. lateral view C. basal view D–F. Inner tepal D. adaxial view E. abaxial view F. lateral view G. Ovary H. Stamens and style in lateral view I. Style crests and stigmatic replicatures. From *L. Eggers & O. Chauveau 716* (ICN!).

Distribution and Habitat:—*Cypella altouruguaya* was collected in the northern part of the state of Rio Grande do Sul, Southern Brazil (Fig. 3), in herbaceous vegetation along roadsides and in contiguous grasslands. The elevation records range from 592 to 615 m. The geographical distribution of the species is markedly reduced, but the populations are dense and consist of numerous individuals. The range of the species falls within the Subtropical Highland Grasslands (Iganci *et al.* 2011), included in the Atlantic Forest biome.

Phenology:—Flowering and fruiting from August to December.

Conservation Status:—According to the IUCN Red List guidelines (IUCN 2001), the species is considered Critically Endangered (CR), with subcriteria B1 (a) and (biii): continuing decline of extent of occurrence and a decline of quality of habitat due to agricultural expansion.

Etymology:—Named after the Alto Uruguai, a physiographic area bounded by the Uruguai and Ijuí rivers where the species was encountered. This region lies in the northern part of Rio Grande do Sul.

Additional specimens examined (paratypes):—BRAZIL. Rio Grande do Sul: Trindade do Sul, estrada secundária entre Trindade do Sul e Rodeio Bonito, beira de estrada, 615 m, 18 August 2012 (fl), *L. Eggers & O. Chauveau 716* (ICN!); Trindade do Sul, estrada secundária entre Trindade do Sul e Rodeio Bonito, campo baixo, 592 m, 5 December 2013 (fl, fr), *L. Eggers & O. Chauveau 884* (MBM!).

Taxonomic relationships:—We first studied one herbarium specimen of the new species collected in 2011 and kept at ICN; thereafter, further specimens were obtained by ourselves at the type locality in August 2012. Only one plant had flowers at this time of the year, but bulbs of similar plants were collected from a large population and cultivated for identification, illustration and measurements. Additional field observations were conducted in 2013. The species is here compared with two species of *Cypella* with yellow flowers: *C. armosa* Ravenna (1981a: 20) and *C. pabstiana* Ravenna (1981a: 18). *Cypella armosa* is readily distinguished from the new species by the overall shape of the flower, marked by the laxly hanging outer tepals, and its long and twisted adaxial crests. *Cypella pabstiana* may be more easily confused with *C. altouruguaya*, and detailed observations of the stamens are needed to discriminate

between the two species. In *C. pabstiana*, the filaments are connate for more than two-thirds of their length and both filaments and anthers are distinctly shorter. The character states retained to compare and separate the different species are presented in Table 1.

TABLE 1. Morphological characters retained to compare *Cypella altouruguaya* and closely related species.

Character/Species	<i>C. altouruguaya</i>	<i>C. armosa</i> *	<i>C. pabstiana</i> **
Plant height (cm)	(32–)39–70	to 58	to 33
Flower diameter (mm)	45–55	50–65	40–45
Size of outer tepals (mm)	(32–)37–42(–46) × (21–)24–31(–33)	38–40 × 12–16	near to 30 × 26.5
Size of inner tepals (mm)	(10–)10.5–12.5(–13) × (8.5–)9–12(–13)	near to 15 long	near to 8 × 7
Inner tepal shape	proximal half cuneate, not unguiculate	narrowly unguiculate along the proximal two-thirds	proximally unguiculate on more than half
Filament length (mm)	3–3.5(–4), totally free	3.2, totally free	near to 2.5, connate for about 1.9
Anther length (mm)	6–7(–7.9)	7	near to 4.4
Connective apex	not excurrent, slightly retuse	excurrent, apiculate	slightly excurrent
Adaxial crests length (mm)	(3.5–)4–6(–6.5)	5.5–7.5	N/A
Adaxial crests shape	not twisted basally	strongly twisted basally	N/A
Abaxial crests length (mm)	(1.1–)1.2–1.6(–1.8)	near to 0.8	N/A
Geographical distribution	Southern Brazil (northern part of RS)	South Paraguay, Northeast Argentina (CC, CR, FO, MN, SF), and Southern Brazil (western border of RS)	Southern Brazil (PR)

*Data obtained from Ravenna (1981a) and the following measured specimens:—PARAGUAY. Cordillera: San Bernardino, February 1966, *P.F. Ravenna 462* (isotype, K!); ARGENTINA. Corrientes: Capital, 12 October 1967, *A. Krapovickas & C.L. Cristobal 13590* (CTES!), Santo Tomé, 16 November 1994, *M.M. Arbo et al. 6286* (CTES!); 18 November 1994, *M.M. Arbo et al. 6455* (CTES!); BRAZIL. Rio Grande do Sul: São Borja, 8 November 2012, *L. Eggers et al. 761* (ICN!).

**Data obtained from Ravenna (1981a).

Notes: N/A = not available; Provinces of Argentina: CC = Chaco, CR = Corrientes, FO = Formosa, MN = Misiones, SF = Santa Fe; States of Brazil: PR = Paraná, RS = Rio Grande do Sul.

Cypella amplimaculata Chauveau & L.Eggers, *sp. nov.* (Figs. 1B and 4)

Cypella amplimaculata is comparable to *C. fucata* and *C. herbertii*, two species with orange flowers; however, it is distinguished by a broad red brown central line extended longitudinally on the outer tepals and much longer style arms, the distance between the anthers being distinctly greater. The new species strongly reminds *C. fucata* in general aspect, but differs by a greater flower diameter, longer and erecto-patent filaments, longer anthers, and by the twisted adaxial crests. It is distinct from *C. herbertii* by the narrower leaf width, the shorter connate part of the filaments, the narrower width and lighter colour of the connective (vs. dark red-brown to dark violet), the longer adaxial crests and lighter colour of crests base (vs. dark red-brown to dark purple).

Type:—BRAZIL. Rio Grande do Sul: Piratini, BR 293, direção Bagé, 140 m, 25 October 2011 (fl, fr), *A.M. Aita 49* (holotype, ICN!).

Perennial herb, up to (16.5–)27–64(–70) cm high above the soil, underground stem up to (2.2–)3.5–7(–14) cm long. Bulb ovoid, outer cataphylls dark brown, 10–14(–17) × 10–13(–17) mm, prolonged in a short collar. Basal leaves green at anthesis (0–)2–3(–5), blades linear-attenuate, plicate, (8.5–)25–56.5(–65.5) × (0.15–)0.45–0.7(–0.9) cm. Flowering stem cylindrical, (11.5–)21.1–58(–64) cm long, proximally foliate (one reduced cauline leaf), then bracteose; first internode (0.4–)7.5–16.7(–24.5) cm long; cauline leaf (4.7–)12.7–24.5(–40.5) × (0.15–)0.45–0.8 cm. Synflorescence cymose, simple or 2(–3) branches, each subtending 2–5 pedunculate inflorescences arising from the same point, peduncles (3–)3.6–11.2 cm long. Inflorescence one-flowered (rhpidium like); spathes herbaceous, bivalved, lower valve 2–4 cm long, the upper (3.6–)4.1–5.6(–6.4) cm long, both with membranous edges. Pedicel filiform, generally

shorter than the upper valve with the ovary usually partly exerted. Flowers predominantly orange, 45–60 mm diameter. *Tepals* unequal, shortly fused proximally for 0.5–1 mm. Outer tepals pandurate, (27–)32–36(–39) × (19–)22–28 mm; the proximal part concave, pale yellow to orange-yellow, slightly translucent, finely purple veined mainly on the abaxial side, broadly marked with a purple spot at the base and a conspicuous purple to purplish-red central line extended longitudinally beyond the constricted region, the distal edge of the concave part sparsely marked by an area of glandular trichomes on the central line; the distal part reclinate, orange, obovate, slightly retuse and acuminate. Inner tepals reduced, assurgent proximally, then incurved and abruptly reclinate distally, 10–11(–12) × 7–9 mm; the proximal half cuneate, not unguiculate, whitish, broadly streaked with purple; the distal half orange, longitudinally depressed, except at the distal end, and white in the middle with a dense oblong orange-yellow area of oil-producing trichomes (elaiophore) marked with purple spots, the lateral sides firmly revolute, striated transversely with purple, the apex acute, spotted with purple. Filaments connate basally for 0.1–0.2 mm, porrect, whitish, obclavate, 0.5–0.6 mm wide at mid-length, striated with purple on the inflated base, rarely on the whole length, (2–)2.2–3.5(–4) mm long. Anthers oblong, (5–)5.2–6.2(–7.5) × 1.1–1.5 mm, adnate to the style arms for half of the length; connective apically slightly retuse, whitish to pale orange-yellow towards the distal end, 0.7–1 mm wide, usually covered with a viscous and transparent secretion; locules dark brown to black; pollen ochraceous. Ovary subclavate, (7–)8–9.5(–11) × 2.1–2.5(–3) mm. Style whitish to pale yellow, rarely finely striated with purple on the whole length, (4.1–)5–6(–6.8) mm long. Style arms pale yellow to orange towards the distal end, conduplicate, (4–)4.5–5(–5.5) mm long; crests at the apex, orange, adaxial crest 2, erect, longitudinally twisted at the base, slightly falcate inwards, (3.8–)4–5(–5.8) mm long, abaxial crest triangular, lobed, (0.6–)1–1.9(–2.1) mm long; stigmatic surfaces transverse, 2, on each side at the base of the abaxial crest, usually dark red-brown, (0.6–)1–1.3(–1.8) mm long. Capsule obovate-truncate, 11–20 × 4–6.5 mm. Seeds obconical, triangular in adaxial view, sharply angulate, epidermis verrucose areolate, 1.2–1.5 mm long.



FIGURE 3. Distribution map of *Cypella altouruguaya* (triangle inside circle), *C. hauthalii* subsp. *minuticristata* (star inside circle) and *C. rivularis* (rhombus inside circle) in Southern Brazil.

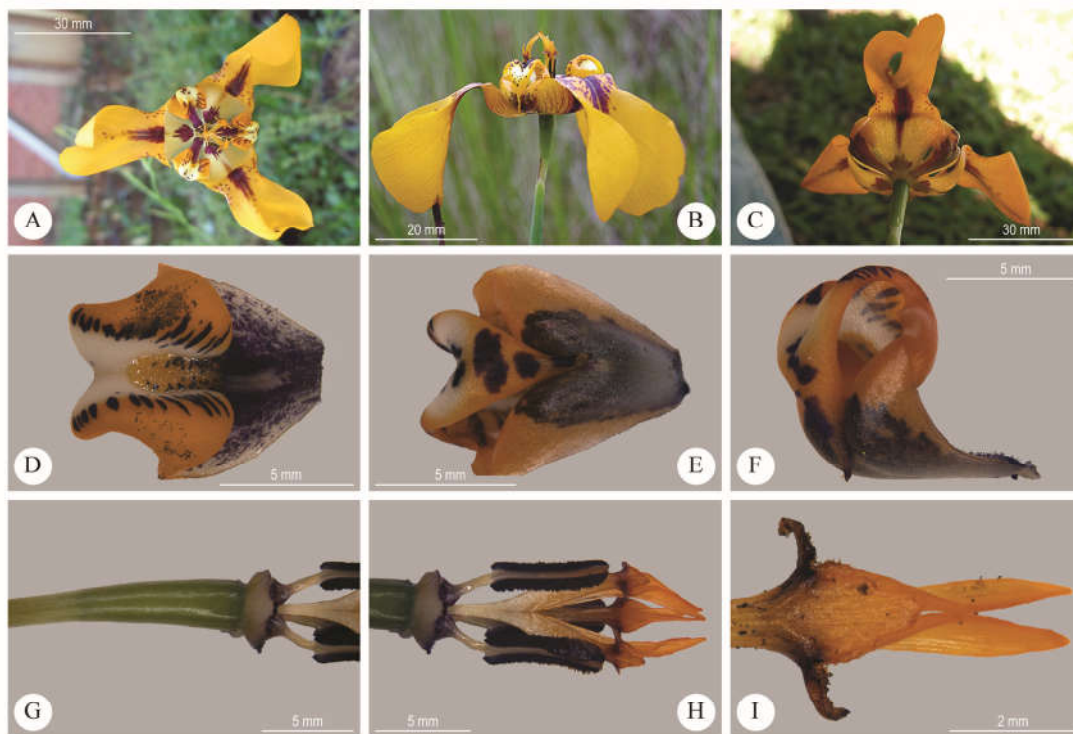


FIGURE 4. *Cypella amplimaculata* Chauveau & L.Eggers. A–C. Flower. A. apical view B. lateral view C. basal view D–F. Inner tepal D. adaxial view E. abaxial view F. lateral view G. Ovary H. Stamens and style in lateral view I. Style crests and stigmatic replicatures. From *A.M. Aita 49* (ICN!).

Distribution and Habitat:—*Cypella amplimaculata* was collected in the state of Rio Grande do Sul, Southern Brazil (Fig. 5), in grassland vegetation of low to moderate elevation (87 to 661 m). The populations usually consist of few individuals scattered in dry grasslands. The geographical distribution of the species overlaps the Pampa biome and the southern part of the Subtropical Highland Grasslands included in the Atlantic Forest biome.

Phenology:—Flowering and fruiting from September to December, March and June.

Conservation Status:—According to the IUCN Red List guidelines (IUCN 2001), the species can be considered as Nearly Threatened (NT), but may qualify for a higher threat category in the future, mainly because of decline quality or loss of habitat through substitution of natural grasslands by agricultural areas.

Etymology:—Named after the broad red-brown central line extended longitudinally on the outer tepals, since the species can be easily distinguished by this floral feature.

Additional specimens examined (paratypes):—BRAZIL. Rio Grande do Sul: Cerrito, BR 293, beira de estrada, 87 m, 18 November 2006 (fl), *L. Eggers & T.T. Souza-Chies 191* (ICN!); Capão do Leão, 92 m, 18 November 2006 (fl, fr), *L. Eggers & T.T. Souza-Chies 192* (ICN!); Livramento, Cerro do Armour, 210 m, 17 October 2009 (fl), *L. Eggers & T.T. Souza-Chies 508* (ICN!); Porto Alegre, Morro Santana, trilha para lado sul do Morro, 287 m, 23 September 2011 (fl), *L. Eggers & O. Chauveau 664* (MBM!); Porto Alegre, Morro Teresópolis, Praça Dr. Dario Rodrigues da Silva, 192 m, 05 October 2011 (fl), *T.L.S. Alves 81* (ICN!); Porto Alegre, Morro São Pedro, em campo próximo à antena, 15 December 2011 (fl, fr), *T.L.S. Alves 174* (SI!); Porto Alegre, Morro Santana, em campo recentemente queimado, face norte, 01 June 2012 (fl), *T.L.S. Alves 220* (ICN!); Viamão, Morro Santana, 286 m, 19 March 2013 (fl), *L. Eggers et al. 819* (ICN!); Júlio de Castilhos, estrada Júlio de Castilhos para Quevedos, campo pastejado, 468 m, 17 October 2013 (fl), *L. Eggers et al. 823* (ICN!); Júlio de Castilhos, estrada para baragem Kotzian, 307 m, 18 October 2013 (fl.), *L. Eggers & O. Chauveau 824* (MBM!); Fontoura Xavier, BR 386, aproximadamente Km 258, antes do Parque das Tuias, campo nativo perto de um córrego de água, 661 m, 04 December 2013 (fl, fr), *L. Eggers & O. Chauveau 883* (P!).

Taxonomic relationships:—*Cypella amplimaculata* has been collected since 2006 by ourselves, but has been erroneously neglected because of its close similarity to *C. fucata* Ravenna (1981a: 18). Most of the time, *C. amplimaculata*

is a higher plant with longer and broader basal leaves as well as larger flowers than *C. fucata*. However, these characters are not discriminant for some samples of the new species and the serious differences observed in relation to the original description of *C. fucata* have been initially attributed to a higher phenotypic plasticity. Nevertheless, further detailed observations were carried out during various field expeditions and diagnostic characters were identified to distinguish the new species from *C. fucata*. Beyond the broad central line present on the outer tepals and the length of the style arms, morphological characters such as the perigon diameter, the length of the different parts of the androecium, the way the filaments diverge from the main axis of the flower and the conformation of the adaxial crests were retained to differentiate both species. In this context, the specimens used by Marco *et al.* (2009) to study the genetic variability within *C. fucata* have been misidentified and belong definitely to *C. amplimaculata*.

The new species shares superficial similarities with *C. herbertii* (Lindley 1826: t. 949) Herbert (1826: supra t. 2599), but it can be easily distinguished by the characteristic connective and style crests base colour of the latter. Additionally, the length of adaxial crests is much shorter and the leaves are much broader in *C. herbertii*. Cross-comparisons of relevant character states between the three species are presented in Table 2.

TABLE 2. Morphological characters retained to compare *Cypella amplimaculata* and closely related species.

Character/Species	<i>C. amplimaculata</i>	<i>C. fucata</i> *	<i>C. herbertii</i> **
Plant height (cm)	(16.5–)27–64(–70)	10–35	30–100
Basal leaf length (cm)	(8.5–)25–56.5(–65.5)	8–22	16–35
Basal leaf width (mm)	(1.5–)4.5–7(–9)	0.6–3	20–25
Lower valve length (cm)	2–4	1.4–2.4	near to 1.9
Upper valve length (cm)	(3.6–)4.1–5.6(–6.4)	2.8–4.5	near to 3.8
Flower colour	orange	dull orange	orange
Flower diameter (mm)	45–60	25–33	60–70
Size of outer tepals (mm)	(27–)32–36(–39) × (19–)22–28	17–24 × 10–12	40–45 × 19–20
Size of inner tepals (mm)	10–11(–12) × 7–9	7–8 × 8–10	14 × 9
Filament length (mm)	(2–)2.2–3.5(–4), connate for 0.1–0.2	near to 1.8, connate for 0.1–0.2	near to 3.4, connate for about 1.4
Anther length (mm)	(5–)5.2–6.2(–7.5)	3.6–5	near to 5.5
Style arms length (mm)	(4–)4.5–5(–5.5)	1.2–2.5	1–1.9
Connective colour	whitish to pale orange-yellow	whitish to pale orange-yellow	dark red-brown to dark violet
Connective width (mm)	0.7–1	0.8–1.4	1.5–1.8
Adaxial crests length (mm)	(3.8–)4–5(–5.8)	3.8–5.3	1.8–2
Abaxial crests length (mm)	(0.6–)1–1.9(–2.1)	0.8–2	0.9–1
Stigmatic surfaces colour	dark red-brown	dull orange	dark red-brown to dark purple
Geographical distribution	Southern Brazil (RS)	Southern Brazil (RS, SC), Northeast Argentina (CR, ER), Uruguay	Southern Brazil, Northeast Argentina (BA, CR, ER, MN), South Paraguay, Uruguay

*Data obtained from Ravenna (1981a) and the following measured specimens:—BRAZIL. Santa Catarina: Lajes, 4 February 1963, *P.R. Reitz 6579* (paratype, HBR!); Rio Grande do Sul: Pinheiro Machado, 20 November 2008, *L. Eggers & T.T. Souza-Chies 442* (ICN!); Piratini, 21 November 2008, *L. Eggers & T.T. Souza-Chies 444* (ICN!); Porto Alegre, 09 November 2010, *L. Eggers & T.T. Souza-Chies 559* (ICN!); Uruguaiana, 06 November 2012, *L. Eggers et al. 746* (ICN!); Quarai, 22 November 2012, *L. Eggers et al. 790* (ICN!).

**Data obtained from Ravenna (1968) and the following measured specimens:—BRAZIL. Rio Grande do Sul: São Borja, 30 October 2009, *L. Eggers & T.T. Souza-Chies 547* (ICN!); Quarai, 27 October 2011, *A.M. Aita 077* (ICN!); Aceguá, 13 November 2013, *L. Eggers et al. 866* (ICN!); URUGUAY. Flores: Cerro Colorado, 10 November 2013, *L. Eggers et al. 850* (ICN!); Cerro Largo: Melo, 13 November 2013, *L. Eggers et al. 862* (ICN!).

Notes: Provinces of Argentina: BA = Buenos Aires, CR = Corrientes, ER = Entre Ríos, MN = Misiones; States of Brazil: RS = Rio Grande do Sul, SC = Santa Catarina.



FIGURE 5. Distribution map of *Cypella amplimaculata* (circle) in Southern Brazil.

Cypella hauthalii subsp. *minuticristata* Chauveau & L.Eggers, *subsp. nov.* (Figs. 6 and 7)

Cypella hauthalii subsp. *minuticristata* reminds *C. hauthalii* subsp. *opalina* and *C. hauthalii* subsp. *hauthalii* in gross morphology, but differs from both subspecies by the much shorter or even obsolete adaxial and abaxial crests and narrower inner tepals.

Type:—BRAZIL. Rio Grande do Sul: Soledade, propriedade particular do Sr. Waldemar Freitag, 534 m, 2 November 2013 (fl, fr), *L. Eggers et al.* 833 (holotype, ICN!; isotypes, MBM!, P!, SI!)

Perennial herb, up to (10–)11.4–20.6(–22.5) cm high above the soil, underground stem up to (2.9–)4.5–7.3(–9) cm long. Bulb subglobose to ovoid, outer cataphylls dark brown, (17–)19.3–24.1(–28.2) × (10.7–)13.5–20.2(–25) mm, prolonged in a short collar. Basal leaves green at anthesis (0–)1–4(–7), blades linear-attenuate, plicate, (13.9–)15.2–19.7(–22.5) × (0.3–)0.45–0.65 cm. Flowering stem cylindrical, (4.3–)5.5–12.3(–15) cm long, proximally foliate (one reduced cauline leaf), then bracteose; first internode (obsolete–)0.2–3.5(–4) cm long; cauline leaf (6.1–)8.5–12.5(–15.1) × (0.25–)0.35–0.6(–0.8) cm. Synflorescence cymosely branched, branches 2–3, each subtending 2–4 pedunculate inflorescences arising from the same point, peduncles 2.2–5.5(–6.5) cm long. Inflorescence two-flowered (rhpidium like); spathes herbaceous, bivalved, lower valve subventricose (2.3–)2.5–3.7(–3.9) cm long, the upper (3.6–)3.8–5.4(–5.6) cm long,

both with membranous edges. Pedicel filiform, generally shorter than the upper valve with the ovary usually partly exerted, the top of the ovary 1.7–4.4 mm above the top of the upper valve, but sometimes up to 2 mm below. Flowers predominantly white, subtly tinged with blue, 35–45(–50) mm diameter. Tepals unequal, conspicuously unguiculate, shortly fused proximally for 0.7–1(–1.5) mm. Outer tepals (26.9–)29–35(–38) × 20–28(–33) mm; the claw erecto-patent, attenuate towards the proximal end, marked with red-brown spots forming sometimes transversal and irregular stripes at the base, the distal edge sometimes marked in the middle with a yellow area extended longitudinally; the

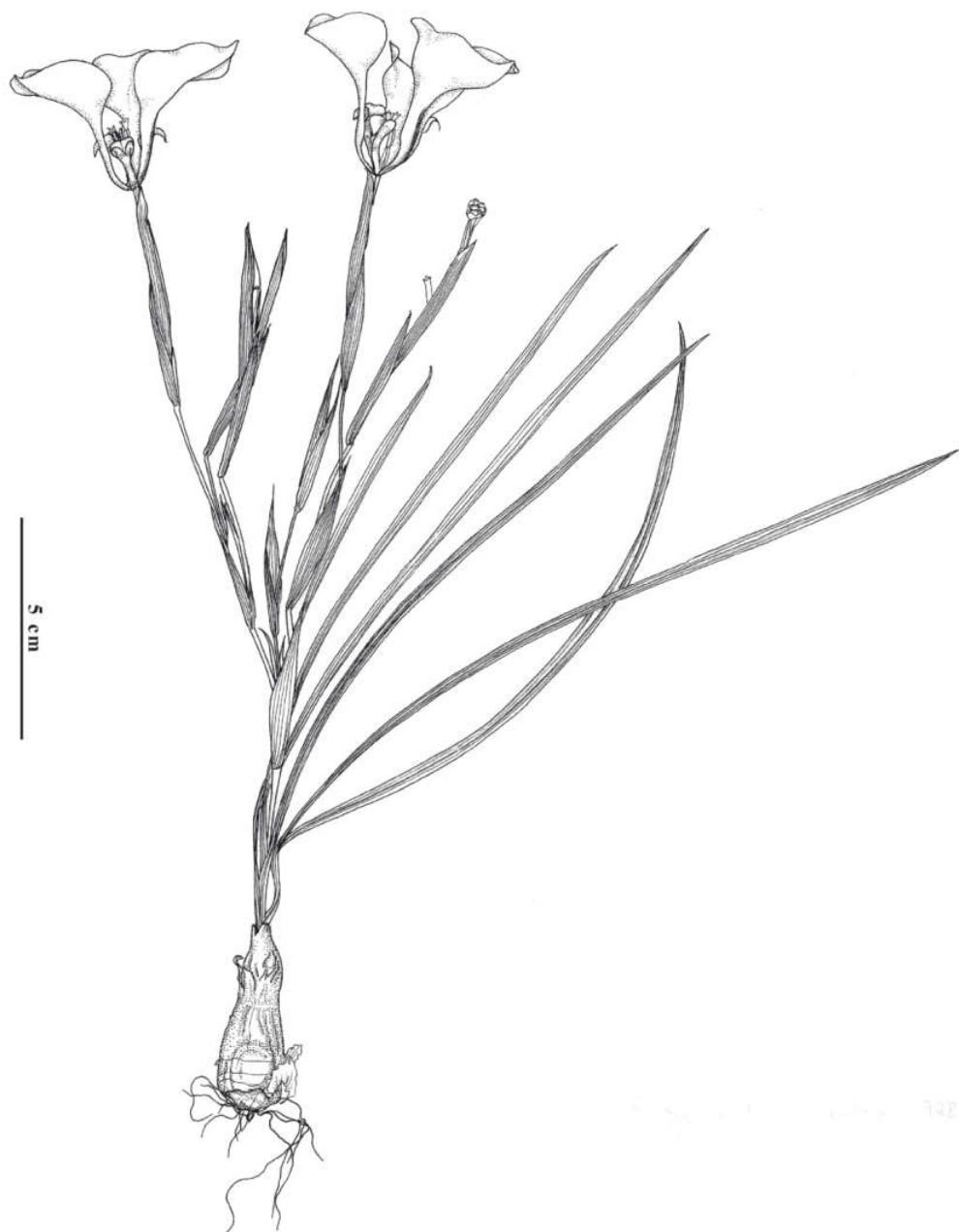


FIGURE 6. Habit of *Cypella hauthalii* subsp. *minuticristata* Chauveau & L.Eggers. From *L. Eggers & O. Chauveau 728* (ICN!), drawing by Anelise Scherer.

blade flabellate, spreading, slightly apiculate to acuminate. Inner tepals reduced, conspicuously unguiculate, (13.5–)14–16(–17) × 4–5(–6) mm; the claw whitish-blue, porrect, sublirate, extending to three-fourths of the tepal length, the distal one-third slightly widened, the proximal half densely marked with red-brown spots; the blade whitish-blue, ovate, curved upward proximally, then usually reclinate at the distal end, centrally depressed with a dense ovate to cordate yellow area of oil-producing trichomes (elaiophore) in the middle, the lateral sides firmly revolute, each with a yellow area spotted with red-brown, the apex acute. Filaments free, erect, whitish, filiform, 0.2–0.25 mm wide at mid-length, densely striated with purple on the slightly inflated base, sparsely on the whole length, (5–)6–6.5 mm long. Anthers oblong, (4.6–)5–6 × (0.7–)0.9–1.3(–1.4) mm, adnate to the style arms for two-thirds to 3/4 of the length; connective apically excurrent, slightly retuse, whitish to pale yellow, (0.2–)0.4–0.8(–1) mm wide, usually covered with a viscous and transparent secretion; locules yellow to black; pollen yellow. Ovary subclavate, (5.1–)6–7.5(–8.4) × (2–)2.2–2.8(–3.1) mm. Style whitish, sometimes finely striated with purple on the whole length, (7.6–)8.4–10(–10.2) mm long. Style arms whitish to pale blue towards the distal end, conduplicate, (2.7–)3–3.5(–3.9) mm long; crests at the apex, whitish to pale blue, adaxial crest 2, erect, rounded or sometimes lobed, (obsolete–)0.1–1(–1.5) mm long, abaxial crest often absent, rounded when present, obsolete to 0.1(–0.3) mm long; stigmatic surfaces transverse, 2, on each side at the base of the abaxial crest, usually pale blue, (0.3–)0.4–0.8(–1) mm long. Capsule obovate-truncate, 6.8–7.3 × 2.8–3.1 mm. Seeds obovoid, obovate in adaxial view, slightly angulate, epidermis reticulate, 1.6–2 mm long.



FIGURE 7. *Cypella hauthalii* subsp. *minuticristata* Chauveau & L.Eggers. A–C. Flower. A. apical view B. lateral view C. basal view D–F. Inner tepal D. adaxial view E. abaxial view F. lateral view G. Ovary H. Stamens and style in lateral view I. Style crests and stigmatic replicatures. From L. Eggers & O. Chauveau 728 (ICN!).

Distribution and Habitat:—*Cypella hauthalii* subsp. *minuticristata* was collected in the central region of the state of Rio Grande do Sul, Southern Brazil (Fig. 3), in grassland vegetation. The elevation records range from 308 to 534 m. The species usually forms large populations. Its geographical distribution overlaps the northern limit of the Pampa biome and the southern part of the Subtropical Highland Grasslands included in the Atlantic Forest biome.

Phenology:—Flowering and fruiting from October to December.

Conservation Status:—According to the IUCN Red List guidelines (IUCN 2001), the species is considered to be Critically Endangered (CR), with subcriteria B1 (a) and (biii): decline quality or loss of habitat through substitution of natural grasslands by agricultural areas.

Etymology:—Named after the outstanding short crests of the style, when compared to the other subspecies of *Cypella hauthalii*.

Additional specimens examined (paratype):—BRAZIL. Rio Grande do Sul: Salto do Jacuí, trevo da BR 481 para baragem, 397 m, 20 October 2012, (fl), *L. Eggers et al.* 727 (ICN!); Porto Alegre, planta cultivada desde 2010, proveniente de Salto do Jacuí, 18 October 2012 (fl), *L. Eggers & O. Chauveau* 728 (ICN!); Salto do Jacuí, 308 m, 19 October 2013 (fl), *L. Eggers et al.* 827 (ICN!). The new subspecies was also recorded from the municipality of Júlio de Castilhos (29°19'32.22"S - 53°48'44.82"W) but without specimen collection (Azambuja, B. 2012, Universidade Federal do Rio Grande do Sul, pers. comm.).

Taxonomic relationships:—This taxon is regarded as a subspecies of *Cypella hauthalii* (Kuntze 1898: 304) Foster (1950: 23) because it is strikingly similar to *Cypella hauthalii* subsp. *opalina* Ravenna (1981a: 2), another subspecies described from Northeast Argentina and western Rio Grande do Sul. The typical subspecies was transferred from *Alophia* Herbert (1840: t. 3779) and occurs in Southern Paraguay and Northeast Argentina. It differs from the other subspecies by the larger and pale lilac-blue flowers and by the long and whitish blue adaxial crests. *Cypella hauthalii* subsp. *opalina* is characterised by its white flowers, slightly tinged with yellow, and its long white adaxial crests. Both subspecies have much longer adaxial and abaxial crests than *C. hauthalii* subsp. *minuticristata* and this characteristic is easily discernible to the naked eye. Furthermore, the localities where occurrences of the new subspecies were identified suggest that its range is distinct from the distribution areas of the other subspecies. The character states retained to compare and separate the different subspecies are presented in Table 3.

TABLE 3. Morphological characters retained to compare *Cypella hauthalii* subsp. *minuticristata* and closely related species.

Character/Species	<i>C. hauthalii</i> subsp. <i>minuticristata</i>	<i>C. hauthalii</i> subsp. <i>opalina</i> *	<i>C. hauthalii</i> subsp. <i>hauthalii</i> **
Flower colour	white, subtly tinged with blue	White, subtly tinged with yellow	pale lilac-blue
Size of outer tepals (mm)	(26.9–)29–35(–38) × 28(–33)	20–25–34.5 × 17–23	38–43.7 × 23–24.5
Size of inner tepals (mm)	(13.5–)14–16(–17) × 6)	4–5(–14.5–16.5 × 5.2–5.9	23–24.6 × 7–8
Filament length (mm)	(5–)6–6.5	5.2–6.5	7–9
Anther length (mm)	(4.6–)5–6	5–6	6.5–7.5
Style arms length (mm)	(2.7–)3–3.5(–3.9)	3.5–4	7–7.4
Adaxial crests length (mm)	(obsolete–)0.1–1(–1.5)	3.7–4.5	4.6–4.8
Abaxial crests length (mm)	obsolete to 0.1(–0.3)	0.5–2.3	1.8–2
Stigmatic surfaces colour	whitish blue	white	whitish blue
Geographical distribution	Southern Brazil (central RS)	Northeast Argentina (CR, MN), Southern Brazil (western RS)	Southern Paraguay, Northeast Argentina (CR, MN)

*Data obtained from Ravenna (1981a) and the following measured specimens:—ARGENTINA. Corrientes: Santo Tomé, 20 September 1974, *A. Krapovickas et al.* 25807 (paratype, CTES!); BRAZIL. Rio Grande do Sul: Santo Antônio das Missões, 14 October 2005, *L. Eggers & T.T. Souza-Chies* 113 (ICN!); Unistalda, 30 October 2009, *L. Eggers & T.T. Souza-Chies* 553 (ICN!); São Borja, 08 November 2012, *L. Eggers et al.* 764 (ICN!).

**Data obtained from Kuntze (1898), Foster (1950) and the following measured specimens:—PARAGUAY. Paraguari: Ybytymi, October 1892, *R. Hauthal s.n.* (isotype, CTES!); ARGENTINA. Misiones: Posadas, 16 October 2013, *L. Eggers et al.* 820 (ICN!).

Notes: Provinces of Argentina: CR = Corrientes, MN = Misiones; States of Brazil: RS = Rio Grande do Sul.

Cypella rivularis Chauveau & L.Eggers, *sp. nov.* (Figs. 8 and 9)

Cypella rivularis differs strongly from related species by its habitat characterised by small streams running through the pampean grasslands (vs. well drained places for *C. laeta* and sandy, stony soils for *C. suffusa*). Morphologically, it differs from *C. laeta* by its uniflorous spathes and longer adaxial crests. It is distinguished from *C. suffusa* by its wider flower diameter, longer outer and inner tepals and longer style arms.

Type:—BRAZIL. Rio Grande do Sul: Uruguaiana, BR 290, aproximadamente Km 645, campo bem preservado, embaixada, em borda de pequenos riachos, 172 m, 25 November 2013 (fl, fr), *L. Eggers et al.* 869 (holotype, ICN!; isotypes, MBM!, P!).



FIGURE 8. Habit of *Cypella rivularis* Chauveau & L.Eggers. From L. Eggers *et al.* 869 (ICN!), drawing by Anelise Scherer.

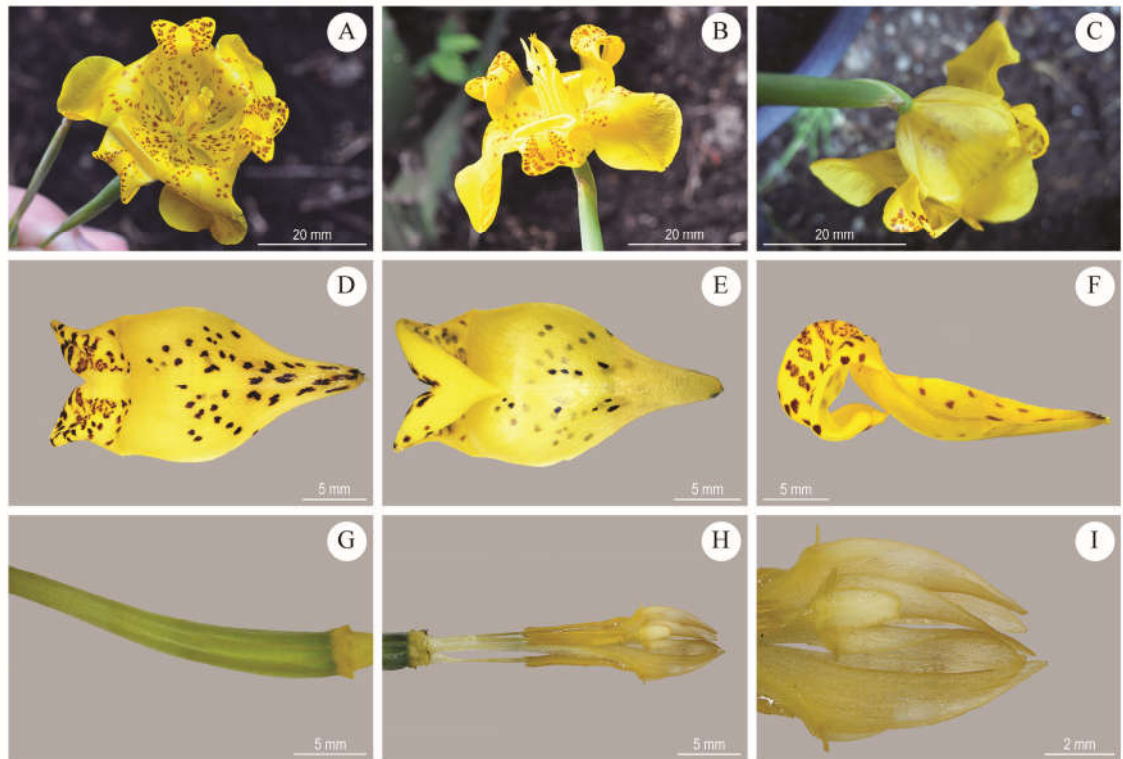


FIGURE 9. *Cypella rivularis* Chauveau & L.Eggers. A–C. Flower. A. apical view B. lateral view C. basal view D–F. Inner tepal D. adaxial view E. abaxial view F. lateral view G. Ovary H. Stamens and style in lateral view I. Style crests and stigmatic replicatures. From L. Eggers *et al.* 869 (ICN!).

Perennial herb, up to (23.5–)33.5–49(–70) cm high above the soil, underground stem up to (8–)12.5–19(–22) cm long. Bulb obovoid, outer cataphylls dark brown, (28–)32.6–38(–41.4) × (18–)20.7–27.1 mm, prolonged in a long collar. Basal leaves green at anthesis (2–)4–5(–7), blades linear-attenuate, plicate, (15–)21–51(–59.5) × (0.3–)0.4–0.7(–0.75) cm. Flowering stem cylindrical, (16.8–)26.3–39(–63) cm long, proximally foliate (one reduced cauline leaf, rarely absent), then bracteose; first internode (3–)5.3–13.5(–19.5) cm long; cauline leaf (6.5–)7.2–33.7(–45.7) × 0.2–0.46(–0.6) cm. Synflorescence cymosely branched, branches 2–3(–5), each subtending 2–7 pedunculate inflorescences arising from the same point, peduncles (1.2–)1.9–6(–9) cm long. Inflorescence one-flowered (rhipidium like); spathes herbaceous, bivalved, lower valve (1.3–)1.5–2(–2.2) cm long, the upper (3.2–)3.5–4.7(–7) cm long, both with narrow membranous edges. Pedicel filiform, usually slightly shorter than the upper valve with the ovary partly to entirely exerted, the top of the ovary (3.5–)6.7–13.6 mm above the top of the upper valve. Flowers predominantly bright yellow, (46–)50–58(–61) mm diameter. Tepals unequal, shortly fused proximally for (1.2–)1.6–2.1(–2.4) mm. Outer tepals pandurate, 35–39(–42) × (17–)18–22(–25) mm; the proximal part concave, bright yellow, marked with red-brown dots scattered on the whole surface, the distal edge of the concave part devoid of trichomes; the distal part reclinate, bright yellow, obovate, slightly retuse and shortly apiculate. Inner tepals reduced, the proximal two-thirds erecto-patent and lastly curved upward, the distal one-third incurved and abruptly reclinate, 21–24(–25.5) × 9–10.5(–14) mm; the proximal part shortly unguiculate, then distinctly cuneate and slightly constricted lastly, bright yellow, marked with red-brown dots scattered usually on the whole surface; the distal part bright yellow, longitudinally depressed, except at the distal end, with a dense lanceolate yellow area of oil-producing trichomes (elaiophore), the lateral sides firmly revolute, each densely spotted with red-brown, the apex acute. Filaments 6.4–7.8(–9.3) mm long, usually connate basally for (0–)1–2(–3.2) mm, free for (5–)5.6–6.5(–7.5) mm, erect to porrect, whitish-yellow to pale yellow, filiform, 0.2–0.25 mm wide at mid-length, slightly inflated at the base. Anthers narrowly oblong, (7.6–)7.8–8.5(–9) × (1–)1.4–1.7(–2) mm, adnate to the style arms for two-thirds to three-fourths of the length; connective apically excurrent, acuminate, pale yellow, (0.7–)0.9–1.2(–1.4) mm wide, usually covered with a viscous and transparent secretion; locules pale yellow; pollen yellow. Ovary narrowly subclavate, 9–11(–12) × (2.1–)2.4–2.7(–3) mm. Style whitish-yellow to pale

yellow, (9.9–)11–15.8(–18) mm long. Style arms pale-yellow, conduplicate, (3.7–)4–4.5(–4.9) mm long; crests at the apex, pale yellow to whitish-yellow; adaxial crest 2, erect, falcate inwards, (5–)5.9–6.8(–7.5) mm long; abaxial crest ovate, obtuse, (1.2–)2.2–3.4(–3.8) mm long; stigmatic surfaces transverse, 2, on each side at the base of the abaxial crest, pale yellow, (0.21–)0.5–1(–1.3) mm long. Capsule obovate-truncate, 18.9–23.1 × 4.8–5.5 mm. Seeds irregularly obovate to conical, sharply angulate, epidermis verrucose, 2.5–4 mm long.

TABLE 4. Morphological characters retained to compare *Cypella rivularis* and closely related species.

Character/Species	<i>C. rivularis</i>	<i>C. laeta</i> *	<i>C. suffusa</i> **
Plant height (cm)	(23.5–)33.5–49(–70)	20–35	10–40
Bulb size (mm)	(28–)32.6–38(–41.4) × (18–)20.7–27.1	22–28 × 15–22	33–47 × 26–30
Basal leaf length (cm)	(15–)21–51(–59.5)	15–20	12–16
Basal leaf width (mm)	(3–)4–7(–7.5)	2–5	1.5–2.5
Lower valve length (cm)	(1.3–)1.5–2(–2.2)	1.4–2.2	1.2–1.9
Upper valve length (cm)	(3.2–)3.5–4.7(–7)	3.4–3.8	3–4
Flowers number/spathe	one-flowered	two-flowered	one-flowered
Flower colour	bright yellow	yellow	yellow
Flower diameter (mm)	(46–)50–58(–61)	45–51	35–37
Outer tepals size (mm)	35–39(–42) × (17–)18–22(–25)	34–37 × 18–20	22–25 × 16–20
Inner tepals size (mm)	21–24(–25.5) × 9–10.5(–14)	16–23 × 7–10.5	18 × 12
Filament length (mm)	6.4–7.8(–9.3), connate for (0–)1–2(–3.2)	near to 6	near to 6
Anther length (mm)	(7.6–)7.8–8.5(–9)	near to 7	near to 7
Style arms length (mm)	(3.7–)4–4.5(–4.9)	near to 3.6	1.5–1.9
Adaxial crests length (mm)	(5–)5.9–6.8(–7.5)	3.5–4	3–4
Abaxial crests length (mm)	(1.2–)2.2–3.4(–3.8)	near to 2	N/A
Habitat	grassland streams	well drained places	sandy, stony soils
Geographical distribution	Southern Brazil (south-west RS)	Northeast Argentina (ER, MN), western Uruguay (P)	Northeast Argentina (CR, MN)

*Data obtained from Ravenna (1981a) and the following measured specimens:—ARGENTINA. Misiones: Apóstoles, February 1907, *C. spagazzini* s.n. (paratype, LPS!); URUGUAY. Paysandú: Chapicuy, 9 November 2013, *L. Eggers et al.* 843 (ICN!).

**Data obtained from Ravenna (2009) and the following measured specimens:—ARGENTINA. Misiones: Cainguaús, 15 March 2000, *F. Biganzoli et al.* 830 (holotype, SI!); Bonpland, October 1906, *Van de Verme* s.n. (Paratype: SI!).

Notes: N/A = not available; Provinces of Argentina: CR = Corrientes, ER = Entre Ríos, MN = Misiones; States of Brazil: RS = Rio Grande do Sul; Departments of Uruguay: P = Paysandú.

Distribution and Habitat:—*Cypella rivularis* was collected in the south-western part of the state of Rio Grande do Sul, Southern Brazil (Fig. 3), along banks and in the bed of narrow and stony grassland streams. The elevation records range from 103 to 226 m. The geographical distribution of the species is markedly reduced, but the populations are large and scattered along the small streams. The range of the species falls within the Pampa biome.

Phenology:—Flowering and fruiting from November to December.

Conservation Status:—According to the IUCN Red List guidelines (IUCN 2001), the species is considered to be Critically Endangered (CR), with subcriteria B2 (a) and (biii): continuing decline of area of occurrence and a decline of quality of habitat. The grassland streams where the species occurs are threatened by land-use changes or environmental degradations of contiguous areas.

Etymology:—Named after the specific grassland habitat of the species, which is not shared by any other member of the genus.

Additional specimens examined (paratypes):—BRAZIL. Rio Grande do Sul: Uruguaiana, estrada secundária para Santana do Livramento a partir da BR 290, campo bem preservado, em borda de pequeno riacho pedregoso, 211 m, 25 November 2013 (fl), *L. Eggers et al.* 872 (ICN!); Alegrete, estrada secundária a partir da BR 290, aproximadamente Km 611, bulbos entre as pedras em borda de riacho, 103 m, 25 November 2013 (fl), *L. Eggers et al.* 873 (ICN!);

Alegrete, estrada secundária a partir da BR 290, aproximadamente Km 620, campo preservado, em borda de pequeno riacho pedregoso, 226 m, 26 November 2013 (fl), *L. Eggers et al.* 874 (ICN!).

Taxonomic relationships:—*Cypella rivularis* has beautiful big yellow flowers and could be superficially mistaken with *C. laeta* Ravenna (1981a: 13) and *C. suffusa* Ravenna (2009: 1) (Table 4). However, its restricted geographical range and typical habitat are so singular that it can be easily identified. Plants grow in stony soils and between rocks along and in narrow streams of the Pampa grassland. During the flowering time, populations offer dazzling sceneries with dozens of bright yellow flowers scattered along the streams. To date, *C. laeta* and *C. suffusa* were not collected in Brazil, but both species occur in the following border provinces of Argentina: Corrientes and Misiones.



FIGURE 10. Distribution of the Río de la Plata Grasslands in southern South America, between 25 and 38°S. The Pampas eco-region is indicated in clear grey and the Campos eco-region in dark grey. The map is adapted from Di Giacomo & Krapovickas (2005), Overbeck *et al.* (2007) and Paruelo *et al.* (2007).

Discussion

The description of four new taxa of *Cypella* suggests that the overall diversity of the genus has been underestimated in the Subtropical Grasslands of Southern Brazil. Actually, with the addition of the taxa described in the present study, five new species and one subspecies were discovered in Southern Brazil since the past two years (Deble *et al.* 2012) and that number accounts for one third of the taxa already known for this region. Except *C. amplimaculata*, these new taxa are considered Critically Endangered (CR) according to the IUCN Red List. The extent of occurrence of *C. altouruguaya* and *C. hauthalii* subsp. *minuticristata* is less than 100 km², while the area of occupancy of *C. luteogibbosa* Deble in Deble *et al.* (2012: 60), *C. magnicristata* Deble in Deble *et al.* (2012: 63) and *C. rivularis* is smaller than 10 km². These results testify for a high level of local endemism among the taxa recently discovered and may explain why this unexpected diversity has been ignored until now. Our observations strongly suggest that the taxa newly described range exclusively in the Campos eco-region of the Río de la Plata grasslands (Fig. 10). All species and subspecies belonging to *Cypella* are found in the RPG biogeographic unit, except *C. amambaica* Ravenna (2009: 4), *C. craterantha* Ravenna (1964: 52), *C. crenata* (Velloso 1827: t. 67) Ravenna (1965: 312), *C. elegans* Spegazzini (1917: 43) and *C. mandonii* Rusby (1896: 125). Among the taxa distributed in the RPG, 20 species and three subspecies are endemic to the Campos eco-region; *C. yatayphila* Ravenna (2009: 3), *C. herbertii* subsp. *reflexa* Ravenna (1981a: 22) and *C. herbertii* subsp. *wolffhuegelii* (Hauman in Hauman-Merck 1909: 84) Ravenna (1965: 312), are endemic to the Pampas eco-region, while *C. herbertii* subsp. *herbertii* and *C. laeta* are found exclusively in both eco-regions of the RPG. Furthermore, *C. exilis* Ravenna (1981b: 492) and *C. laxa* Ravenna (1981a: 15) are mainly distributed in the Campos eco-region, but their range area extends further to the North in nearby regions (Eggers 2014). Therefore,

more than 85% of the taxa currently described for the genus occur in the Río de la Plata grasslands, 80% are endemic to this biogeographic unit and 65% are only found in the Campos eco-region, mainly in the state of Rio Grande do Sul (Southern Brazil) where 14 species and two subspecies are presently registered. These observations suggest that the centre of diversity of *Cypella* is located in the Río de la Plata grasslands, mostly in the subtropical Campos grasslands, and that local endemism is not uncommon at the infrageneric level.

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