

## TAXONOMY

# One species with a disjunct distribution or two with convergent evolution? Taxonomy of two South American garlics

Agostina B. Sassone,<sup>1,2</sup>  Mary T.K. Arroyo,<sup>3</sup>  Silvia C. Arroyo-Leuenberger,<sup>2</sup>  Nicolás García<sup>4</sup>  & María José Román<sup>4</sup> 

1 Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstraße 3, 06466 Gatersleben, Germany

2 Instituto de Botánica Darwinion, CONICET-ANCFEN, Labardén 200, CC 22, San Isidro, B1642HYD Buenos Aires, Argentina

3 Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Santiago; Instituto de Ecología y Biodiversidad, Las Palmeras 3425, Ñuñoa, Santiago, Chile

4 Herbario EIF & Laboratorio de Sistemática y Evolución de Plantas, Facultad de Ciencias Forestales y de la Conservación de la Naturaleza, Universidad de Chile, Av. Santa Rosa 11315, La Pintana, Santiago, Chile

Address for correspondence: Agostina B. Sassone, sassone@ipk-gatersleben.de

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**Abstract** The establishment of species boundaries has been a difficult task for biologists since the beginning of classifications. Within South American Allioideae (= Alliaceae), the delimitation of genera and species has long been a major challenge. Consequently, species-level nomenclature in these groups has been difficult to elucidate. *Ipheion* and *Tristagma* are two closely related South American genera for which the delimitation and circumscription of several species continue to be unclear. *Tristagma sessile*, a species inhabiting the Andes and the Coast Range in Chile, and *Ipheion recurvifolium*, a species native to Uruguay only found close to sea level, have been considered as one species with an intracontinental disjunct distribution. The divergent habitats and distributions of these taxa have stirred the question of whether they are a single species. The present study aims to elucidate the evolutionary relationships and status of the Chilean and Pampean taxa. We also analysed the name *Tristagma leichtlinii*, which has long been a source of confusion. Based on their phylogenetic relationships, geographical distributions, flowering periods, and DNA content, we conclude that the two taxa are different species and assignable to separate genera. We propose that the morphological similarity of *Ipheion recurvifolium* and *Tristagma sessile*, amply corroborated in this study, might be the sum of shared synapomorphies (between *Ipheion* and *Tristagma*) and convergent features. We update the biogeographic history of the two genera and propose that similar pollinators might underpin the convergence in floral traits. We outline the differences among genera and species, present a taxonomic review with a new lectotype and consider the conservation status of the studied species.

**Keywords** Alliaceae; *Ipheion*; molecular phylogeny; phenology; systematics; *Tristagma*

**Supporting Information** may be found online in the Supporting Information section at the end of the article.

## ■ INTRODUCTION

Species are the study units of biogeography, ecology, and evolutionary and conservation biology (Camargo & Sites, 2013). However, the establishment of species boundaries and interpretation of species concepts has been a difficult task since the early history of classification (de Queiroz, 2007). The delimitation of genera and species within Amaryllidaceae (Meerow & al., 1999) and South American Allioideae (= Alliaceae) has long represented a major challenge (Escobar, 2012; Escobar & al., 2020). Consequently, the associated nomenclature in these groups has been difficult and an ongoing task. Taxonomic history reveals that *Tristagma* Poepp. and *Ipheion* Raf. were originally treated as part of *Brodiaea* Sm., *Milla* Cav., or *Triteleia* Douglas ex Lindl. Even though there are evident morphological

similarities between these genera, currently *Brodiaea*, *Milla*, and *Triteleia* are restricted to North America and placed within Asparagales in Asparagaceae subfam. Brodiaeoidae (= Themidaceae, Stevens, 2001–; APG III, 2009). *Tristagma* and *Ipheion* form part of the South American tribe Leucocoryneae (Sassone & al., 2014; = subtribe Leucocoryninae, Pellicer & al., 2017). Currently recognized species in both genera have long been unstable regarding their generic affiliation. Indeed, several species have been described or placed under one or the other genus at different times, by the same authors (e.g., Traub, 1953; Traub & Moldenke, 1955). *Ipheion* and *Tristagma* share several synapomorphies: fused tepals forming a tube around the ovary, the presence of staminal filaments arranged in two series and fused to the tepal tube, and flowers generally white (although other colours are also found) but never yellow (differing from

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species in the close genus *Nothoscordum* Kunth). The phylogenetic relationships of the tribe have been studied by several authors (Souza & al., 2016; Pellicer & al., 2017; Sassone & Giussani, 2018), and the monophyly of *Ipheion* is not doubted; however, depending on the combination of the molecular markers and the sampling, *Ipheion* is found nested within *Tristagma* (Sassone & Giussani, 2018) or as a sister clade to the latter (Souza & al., 2016; Pellicer & al., 2017). The identity of *Ipheion* is also supported by morphological characters (spathe formed by one bifid bract [= two bracts fused for more than ¾ of their length] and unifloral inflorescences), distribution (if restricted to the Pampean region), and karyological features (Souza & al., 2010; Sassone & al., 2018).

*Ipheion sessile* (Phil.) Traub or *Tristagma sessile* (Phil.) Traub, native to Chile, is found from 2000 to 2800 m a.s.l. and *Ipheion recurvifolium* (C.H. Wright) Traub is found close to the sea level in Uruguay. These two entities have been considered the same species with a widely disjunct distribution. Based on their morphological similarities (under the accepted paradigm at that moment), Guaglianone (1972) was the first author to consider them under the same species in a taxonomic and nomenclatural study of *Ipheion* and *Nothoscordum* that included descriptions, the examination of type material and keys to genera and species. Traub & Moldenke (1955) treated the two entities as different taxa and placed them under *Ipheion*. On the other hand, Traub (1963) and Ravenna (1971), who also considered them as different entities, placed them both under *Tristagma*. Ravenna (2001) pointed out that *T. recurvifolium* and *T. sessile* are similar in the length of the bracts and degree of tepal fusion but can be differentiated by their geographic ranges and elevational occurrence. Hoffmann & al. (1998) described and illustrated *T. sessile* as a high-elevation plant growing in the central Chilean Andes. García (2010) accepted the name *T. sessile* and suggested phenotypic convergence between the Chilean (*T. sessile*) and the Pampean taxa (*I. recurvifolium*). Sassone & al. (2013) were unable to distinguish the two entities based on a multivariate analysis of morphological characters. The recent catalogue of the flora of vascular plants of Chile (Rodríguez & al., 2018; García, 2019) cited the Chilean populations under *Ipheion sessile*, following Guaglianone (1972).

Furthermore, the name *Tristagma leichtlinii* (Baker) Ravenna has been a source of confusion. Ravenna (1971) placed the species for the first time under *Tristagma* (described under *Milla* Cav.) mentioning its similarity with *T. sessile* but presenting 2 or 3 flowers per inflorescence (following Baker's original description). The Republic of Chile, based on the recommendation of the Ministry of the Environment of Chile, declared *T. leichtlinii* as extinct (Diario Oficial de la República de Chile, 2012). García (2010) cited the presence of *T. leichtlinii* in Altos de Chicauma (Metropolitan Region, Chile) and Flores Toro (2012) in "Cerro Caquis" in the "Cordillera del Melón" (Region of Valparaíso, Chile), both in the Chilean Coast Range, and proposed its re-categorization under the Critically Endangered category. Arroyo-Leuenberger & Sassone (2016), when revising the genus *Tristagma*, defined *T. leichtlinii* as a doubtful species with

insufficient data to synonymize. Later, the catalogue of the flora of vascular plants of Chile (Rodríguez & al., 2018; García, 2019) placed *T. leichtlinii* under the synonymy of *Ipheion sessile*. The characters described in the protologue and the illustration published one year later in *Curtis's botanical magazine* (Baker, 1876) are somewhat confusing because of the mention of a "corm", a plurifloral inflorescence, and the drawing showing a robust plant. More recently, Arroyo & al. (2019) included a population from the Andean cordillera in a multispecies study of ovule numbers in high-elevation habitats in central Chile.

Within the tribe Leucocoryneae, *Nothoscordum* Kunth and *Latace* Phil. (= *Zoellnerallium* Crosa) provide a good example of convergent floral morphology, but when multiple sources of evidence were considered, recognition of two different genera was warranted (Crosa, 2004; Sassone & al. 2015; Souza & al., 2016). The lack of good specimens of *T. sessile* (from Chile) for morphological and molecular studies has hindered testing a similar hypothesis in the case of the two species of interest. This study evaluates the specific status of the Chilean and Pampean taxa based on a consideration of morphological, phylogenetic, phenological, environmental data, and DNA content, and elucidates their synonyms.

## ■ MATERIALS AND METHODS

**Plant material.** — Fresh material was collected in the field in central Chile. Additionally, herbarium specimens stored at BAF, BAL, BM, CONC, EIF, GAT, HAL, ICN, JE, K, MVFA, P, RB, SGO, SI and UCVA were studied.

**Phylogenetic analysis.** — Genomic DNA was isolated from silica-dried leaf tissue following a modified CTAB protocol (Doyle & Doyle, 1987; Sassone & Giussani, 2018). Based on the resolution obtained by previous studies and the sequences available, we amplified the plastid genes, *matK* and *ndhF* and rDNA internal transcribed spacer (ITS). The amplification of the fragments followed the protocols described in Sassone & Giussani (2018). We generated 6 new sequences during this study of *Tristagma sessile*: García, N. 5129: MT929269, MT946696, MT946698; Arroyo, M.T.K. 29124: MT929270, MT946697, MT946699 (for voucher details, see "Additional specimens examined"); the remaining sequences were downloaded from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) and detailed in the Appendix 1. The quality of sequences was assessed by visual inspection of the chromatograms. Editing and assembling of sequences were performed in Geneious Prime v.2020.0 and then revised and edited manually. Based on the previous results of Sassone & Giussani (2018), Bayesian inference was used to reconstruct phylogenetic trees for the ITS and the combined dataset (cpDNA, ITS) using MrBayes v.3.2.2 (Huelsenbeck & Ronquist, 2001) implemented in CIPRES Science Gateway (Miller & al., 2010). To perform the Bayesian inference analysis, the best-fit substitution model was selected as GTR+I+ $\Gamma$  for ITS and GTR+ $\Gamma$  for plastid regions. The comparison of the tree topologies was performed with the package "phytools" (Revell, 2012) implemented on the

R environment (R Core Development Team, 2019) and used with the graphical interface Rstudio (RStudio Team, 2020). The aligned matrices were deposited in TreeBASE under submission ID 27950.

**Morphological studies.** — A total of 34 specimens covering the two taxa of interest were considered, including intra-population variation when possible. Following previous works, the morphological analysis was limited to 12 quantitative reproductive characters (Guaglianone, 1972; Sassone & al., 2013; Sassone, 2017; refer to supplementary Table S1). A principal component analysis (PCA) was also performed with the 12 quantitative characters using R basic packages and plotted with the “ggfortify” (Tang & al., 2016). Univariate analyses were performed in selected characters using the R package “DataExplorer” (Cui, 2019). One-way ANOVA (R basic package “stats”) was performed to facilitate the identification of diagnostic characters. All the cited packages are implemented in the R environment, and the script is available as supplementary Appendix S1.

**Flow cytometry.** — The DNA content of *Tristagma sessile* was estimated using a fresh leaf (from one specimen) preserved in cultivation at IPK Gatersleben, using a CyFlow Space (Sysmex Partec, Germany) flow cytometer against *Vicia faba* L. as internal standard (26.5 pg 2C), and following the procedure described by Bernhardt & al. (2017). We used the “CyStain PI Absolute P kit” (Sysmex Partec, Görlitz, Germany) nuclei isolation buffer following the manufacturer’s instructions. The ploidy level was inferred based on previous chromosome counts and estimation of DNA content for the genus *Tristagma* (Crosa, 1981; Souza & al., 2016; Pellicer & al., 2017, Sassone & al., 2018).

**Phenology.** — Information on flowering times was retrieved from labels of 30 herbarium specimens and plotted using packages “car” (Fox & al., 2012) and “circular” (Lund & al., 2017) implemented in R. The database JSTOR (<https://plants.jstor.org/>) was used to collect phenological and distributional information from specimens’ labels.

**Distribution and conservation status.** — Distribution maps were constructed from georeferences obtained from the field, and from herbarium specimens (when absent, by assignment based on locality data). This resulted in a total of 11 occurrences for the Chilean and 22 for the Pampean taxon. We retrieved data for the 19 bioclimatic variables and altitude (alt) from WorldClim 1.0 (Hijmans & al., 2015) with 2.5 min (~5 km<sup>2</sup>) spatial resolution. Variation of the environmental variables for the two taxa was studied using PCA and plotted with the R package “ggplot2” (Ginestet, 2011). The distribution map was assembled from the georeferences using the package “raster” (Hijmans & al., 2015). All cited packages are implemented in the R environment.

Conservation status assessments were made for the two taxa using the IUCN Categories and Criteria (Bland & al., 2017) employing the same geographical coordinates used for the distribution analyses. The Area of Occurrence (AOO) and the Extent of Occurrence (EOO) were estimated using the software GeoCAT v.BETA (Bachman & al., 2011).

All the figures presented were edited for publication using Inkscape v.0.92 (free open-source SVG graphics editor).

## ■ RESULTS

**Phylogenetic analysis.** — The aligned ITS sequence data matrix consisted of 63 accessions including outgroup taxa and 573 characters of which 357 were parsimony informative. The aligned plastid data matrix consisted of 3132 characters of which 330 were parsimony informative. Individual analyses of ITS and plastid markers topologies were compared (suppl. Fig. S1) and concatenated into a single matrix that resulted in 3705 characters of which 687 were parsimony informative. In the combined analysis, *Ipheion* species and the genus resolved as monophyletic (PP: 1.0, Fig. 1, suppl. Fig. S1). *Ipheion recurvifolium* is related to *I. tweedieanum* and *I. uniflorum*. Specimens of *Tristagma sessile* from two localities (Farellones and Altos de Chicauma) resolved as monophyletic (PP: 1.0) and part of a clade comprising *Tristagma ameghinoi* (Speg.) Speg., *T. bivalve* (Hook. ex Lindl.) Traub and *T. circinatum* (Sandwith) Traub. Overall, these phylogenetic results suggest that the two taxa are not the same species.

**DNA content.** — The genus *Tristagma* has a base chromosome number of  $x = 4$  and a karyotype of 3 metacentrics plus 1 acrocentric, varying from diploid to hexaploid (Crosa, 1981; Souza & al., 2016; Pellicer & al., 2017). *Tristagma* species present a monoploid genome size (1Cx) ranging between 15 and 21 pg among the different ploidy levels (Pellicer & al., 2017; Sassone & al., 2018). Our result shows a DNA amount of 76.49 pg for *T. sessile*. Based on the previous karyotype and DNA content reported for the genus *Tristagma*, we hypothesized that *T. sessile* is tetraploid, which points to four monoploid genomes around 19.12 pg in their somatic tissue ( $2n = 4x = 76.49$  pg). This result differs from the monoploid genome size previously reported for *Ipheion* species: 1Cx between 9.07 and 10.70 pg. *Ipheion recurvifolium* is a tetraploid species presenting a base chromosome number  $x = 5$  and a karyotype of 4 acrocentrics plus 1 submetacentric, and a genome size of  $2C = 4x = 36.29$  to 40.58 pg (Pellicer & al., 2017; Sassone & al., 2018, please note that in both papers this species is referred as *I. sessile*).

**Morphological studies.** — The ordination of OTUs in the PCA (suppl. Fig. S2) resulted in an overlap between the specimens of *Ipheion recurvifolium* and the specimens of *Tristagma sessile*. Univariate analysis resulted in three statistically different characters for these groupings: bract length ( $P = 0.0025$ ), bract fusion length ( $P = 0.035$ ), and ovary width ( $P = 0.0001$ ). The spathe, which is formed by two bracts fused only at the base in *Tristagma* and formed by one bifid bract (= two bracts fused for more than  $\frac{3}{4}$  of their length) in *Ipheion*, is a stable morphological character to differentiate both genera and, in this case, the two focal species (Fig. 1). Our results show that *I. recurvifolium* tends to have narrower ovaries. However, this difference is presently not considered to be a reliable diagnostic character and must wait for the examination of additional collections of *T. sessile*.

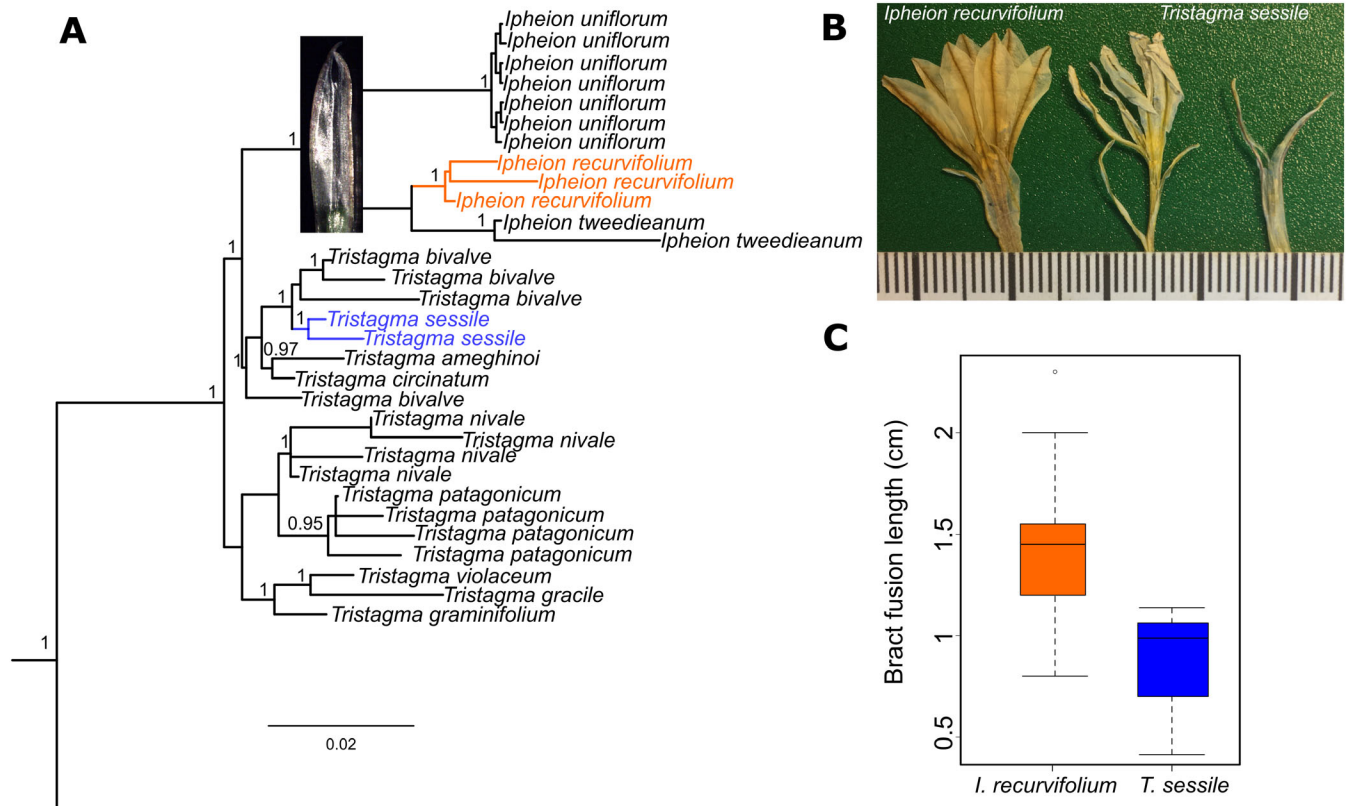


**Phenology.** — The flowering frequencies were plotted as circular histograms. *Ipheion recurvifolium* flowered from May to September, most frequently in May (Fig. 2). *Tristagma sessile* flowered after the snowmelt at high elevations in September and October, but there are records of flowering in November and January, associated with different altitudinal and/or latitudinal conditions (Fig. 2). As regards flower longevity, flowers of *T. sessile* appear to be fairly long-lived flowers for a species capable of autogamy. However, flower longevity under natural conditions needs to be accurately determined.

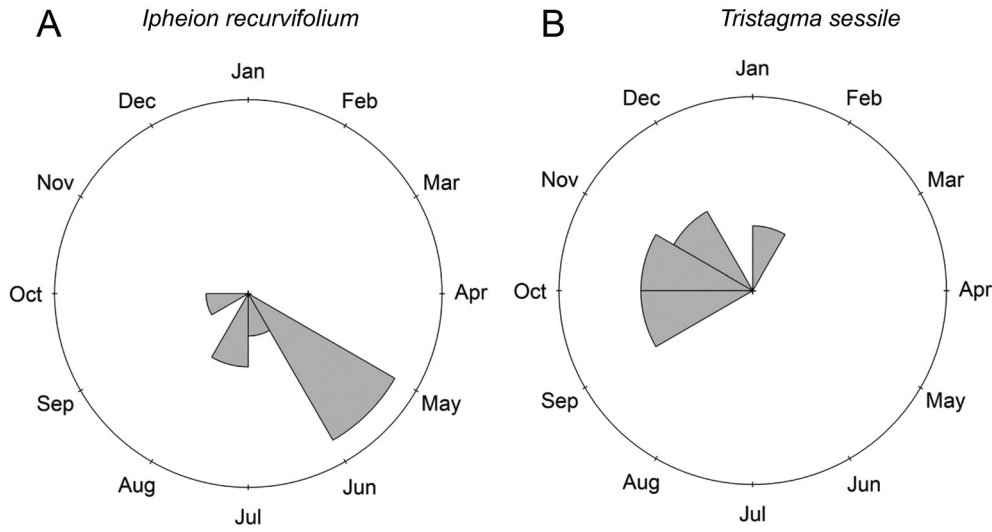
**Distribution and conservation status.** — The species of interest occupy different ecoregions and different habitats in southern South America (Fig. 3A). *Tristagma sessile* is distributed in the high Andes and Coast Range of central Chile, mostly above treeline on snow waterlogged soils and edges of slow-moving boggy streams. It inhabits areas characterized by a minimum and maximum temperature range between  $-5^{\circ}\text{C}$  and  $+28^{\circ}\text{C}$  and annual precipitation between 458 and 1151 mm, with the precipitation concentrated in the winter, principally as snowfall. *Ipheion recurvifolium* occurs in the Pampas region where it is currently found in fields with rocky

outcrops, but it can also be found in highly human-modified environments like parks, squares, and near houses. The minimum and maximum temperature range occupied by *I. recurvifolium* is  $6^{\circ}\text{C}$ – $31^{\circ}\text{C}$  and annual precipitation between 891 and 1483 mm, and the rainfall exhibits ample seasonal and annual variation. The PCA on bioclimatic variables plus altitude revealed a high degree of niche divergence between taxa with altitude resulting in the principal explanation for the different positions occupied in the environmental space. *Tristagma sessile* occurs in high-elevation areas from 2000 to 2800 m a.s.l. in contrast with the sea level or low-elevation habitat of *I. recurvifolium*. Upon removing the altitude, the PCA continued to retrieve two groups of specimens occupying different positions in the bioclimatic space (Fig. 3B).

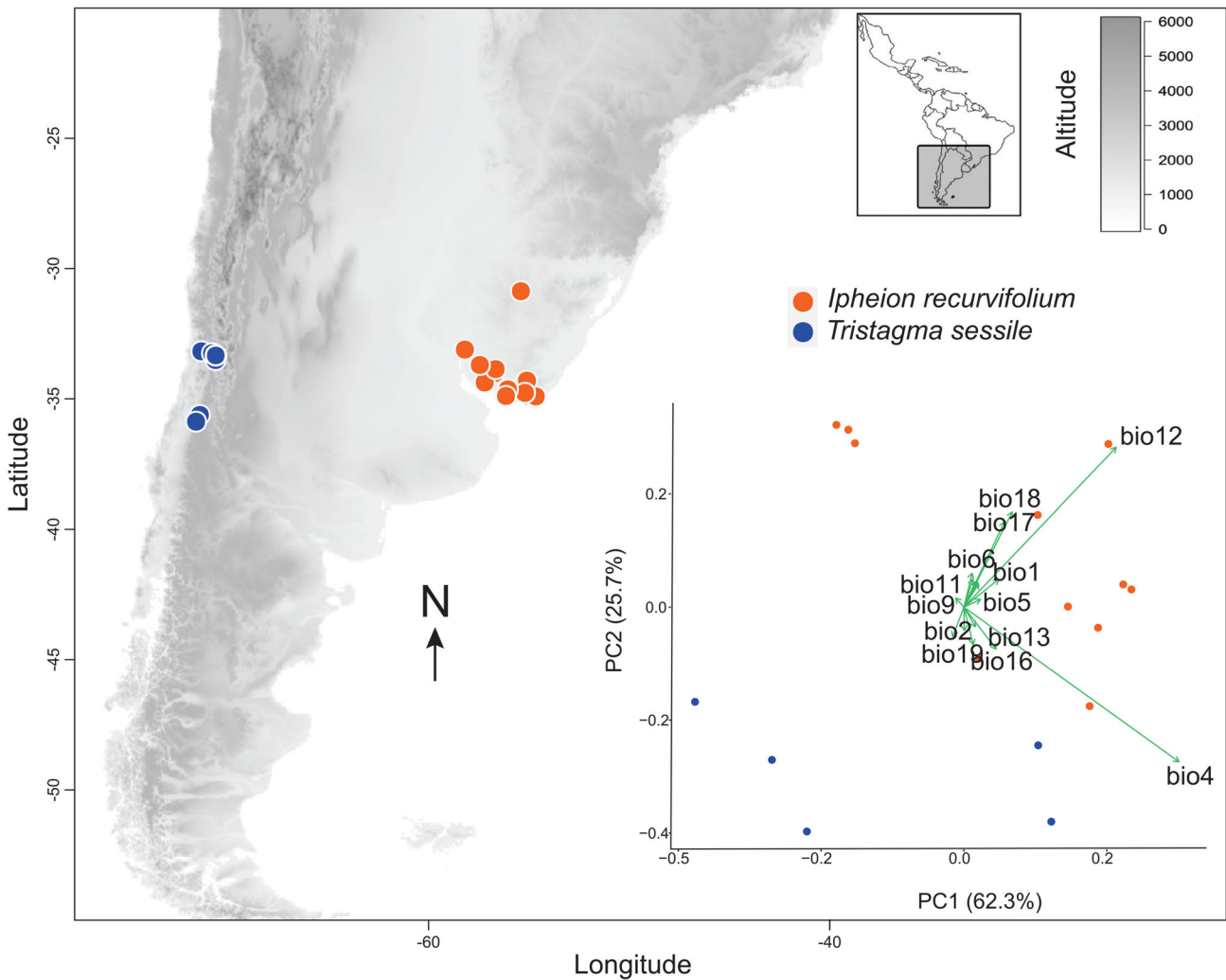
We retrieved 11 georeferences of *Tristagma sessile* corresponding to three different localities as defined by Bland & al. (2017). The Extent of Occurrence resulted in 1741 km<sup>2</sup> and the Area of Occupancy is 3636 km<sup>2</sup>. All known locations of *T. sessile* are outside of state-protected areas and several are near large ski centres where there is cattle grazing during the spring and summer months. Each currently known population occupies no more than an estimated 150 m<sup>2</sup> and it has rarely been collected



**Fig. 1.** A, Bayesian inference tree of tribe Leucocoryneae showing only the *Tristagma* Poepp. + *Ipheion* Raf. clade based on a combined dataset of nrDNA ITS and the plastid genes *matK* and *ndhF* (for the complete tree refer to suppl. Fig. S1). Posterior probability values  $\geq 0.8$  are indicated above branches. Above the basal branch of the *Ipheion* clade, a photo of the one bifid bract (= two bracts fused for more than  $\frac{3}{4}$  of their length), autapomorphy of this genus. B, Flowers and spathe of *Ipheion recurvifolium* (C.H. Wright) Traub (notice that the photo was taken from an unusual 7-tepal flower) and *Tristagma sessile* (Phil.) Traub. C, Boxplot of the Bract fusion length (cm) between *Ipheion recurvifolium* and *Tristagma sessile* ( $P = 0.035$ ). — Photos: A.B. Sassone.



**Fig. 2.** Circular histograms of flowering phenology of: **A,** *Ipheion recurvifolium* (C.H.Wright) Traub; **B,** *Tristagma sessile* (Phil.) Traub.



**Fig. 3.** Geographical distribution of the investigated specimens. The elevation is depicted in grayscale from sea level (white) to over 4000 m above sea level (dark grey). Biplot of the two principal components extracted in the PCA of the 19 climatic variables data matrix, for the investigated specimens.

by botanists. Accordingly, we suggest recognizing *T. sessile* as Vulnerable (VU) under criterion B1ab (i and ii). Regarding *I. recurvifolium*, the Extent of Occurrence resulted in 81,932 km<sup>2</sup> and the Area of Occupancy is 20,772 km<sup>2</sup>, suggesting that there are no major risks for the conservation of this species.

## ■ DISCUSSION

As previously reported (Souza & al., 2016; Pellicer & al., 2017; Sassone & Giussani, 2018), a *Tristagma* + *Ipheion* clade is strongly supported (PP: 1.0) and *Ipheion* is recovered as monophyletic (PP: 1.0, Fig. 1, suppl. Fig. S1). Previous molecular studies defined *Ipheion* as the sister clade to *Tristagma* (Souza & al., 2016; Pellicer & al., 2017). However, when more exhaustive sampling of *Tristagma* is considered, combined analyses of cpDNA and ITS suggest that *Ipheion* is embedded within a paraphyletic *Tristagma* (Fig. 1, suppl. Fig. S1; Sassone & Giussani, 2018). Based on the presence of the single bifid bract, different basic chromosome numbers, karyotype formula, and DNA content (Pellicer & al., 2017; Sassone & Giussani, 2018), together with the present corroboration of their geographical disjunction, we will propose to continue recognizing these two genera, at least until additional molecular and karyological data are available (for a deeper discussion see Sassone & Giussani, 2018).

The two focal taxa share unique morphological characters that differentiate them from the remaining species within the

tribe Leucocoryneae, such as the presence of a subterranean scape, a short pedicel (<1 mm), an extremely long perigone (1.5–4.5 cm) associated with a partially subterranean long tepal tube and a long style (Fig. 4). The style is the longest among *Ipheion/Tristagma* species (>10 mm). These morphological similarities of *I. recurvifolium* and *T. sessile*, amply verified in this study, has led these two species being lumped in the same taxon and genus in the past (Guaglianone, 1972; Sassone & al., 2013; Rodríguez & al., 2018; García, 2019). However, the new molecular data support the differentiation of *T. sessile* (Chile) from *I. recurvifolium* (Uruguay) at the species level (Fig. 1). Differentiation of these two taxa is further supported by their different flowering periods (Fig. 2), DNA content (Pellicer & al., 2017; Sassone & al., 2018) and geographical distributions (Fig. 3). Arroyo & al. (2019), when studying the effect of elevation and phylogenetic relationships on ovule number in the southern Andes, included material from Valle Nevado (Region Metropolitana, Chile) in a phylogenetic analysis using ITS, which was identified as *T. leichtlinii* (based on the illustration in Baker, 1876). These plants correspond with other collections made by the authors in the same general area, here identified as *T. sessile*. There is absolutely no doubt that all material belongs to the same species. We examined the sequence (GenBank accession: MH781262) to check the position within an extended phylogenetic inference and corroborated that it was consistent with that of the newer sequences of *T. sessile* (not shown).



**Fig. 4.** Morphological similarities between the studied taxa. **A**, *Tristagma sessile* (Phil.) Traub from Cordillera de Santiago (between Farellones and La Parva); **B**, *T. sessile* from Altos de Chicauma; **C**, *Ipheion recurvifolium* (C.H. Wright) Traub from the department of Rio Negro; **D**, *I. recurvifolium* from Punta del Este. — Photos: A, M.T.K. Arroyo; B, N. García; C & D, A.B. Sassone.



*Tristagma sessile* is ecologically restricted to areas of deep snow accumulation and on soils that retain water after snowmelt or to the edge of sluggish boggy streams and hence it is not a common species from the typical high Andean vegetation found on dry slopes. *Tristagma sessile* was reported as the third-earliest species to begin flowering at 2500 m a.s.l. in the central Chilean Andes, after *Barneoudia chilensis* Gay and *Microsteris gracilis* (Hook.) Greene (Arroyo & al., 1981). The early flowering under the snow and the habitat of *T. sessile* have led to a paucity of botanical collections (but see Arroyo & al., 2019). Furthermore, the confusion associated with *T. leichtlinii*, in which the number of flowers per bract was described as plurifloral seems to be erroneous (*T. sessile* is always unifloral), has contributed to taxonomic misidentifications of material that belongs to *T. bivalve* (we corroborated the identification of herbaria specimens). Given our current knowledge of the high-altitude species and the presented bibliography, we propose synonymizing *T. leichtlinii* to *T. sessile* (see below under Taxonomic Treatment).

We propose that the morphological similarity of *Ipheion recurvifolium* and *Tristagma sessile* might be the sum of shared synapomorphies (between *Ipheion* and *Tristagma*: filaments arranged in two series, the fused tepals forming a tube around the ovary, and the presence of white flowers) and convergent features (e.g., size of the flower, long style, null or short pedicel, etc.). Now, a discussion of pollinator activity in these genera is necessarily speculative because of the lack of detailed observations in the field. However, our results and available field observations allow us to hypothesize that the shared morphological traits could have been triggered by co-evolution with similar pollinators. Flowers of *I. recurvifolium*, like those of *T. sessile*, are fragrant (sweet-scented). Diptera have been recorded in the field as flower visitors on *T. sessile* in a limited number of observations made in a community-wide pollination study (Arroyo & al., 1982); as well as on specimens of *I. recurvifolium* in the field and the greenhouse (A.B. Sassone, pers. obs.). However, the fragrant, white, long-tubular flowers and septal nectaries suggest that both species are more likely to be pollinated principally by moths. Indeed, *T. nivale* Poepp. is known to be visited by moths (Arroyo & al., 1982). Sphingidae tend to be sporadic visitors and thus are difficult to record without observations over several days and in the late evening hours. Sphingidae have been recorded visiting other plant species (M.T.K. Arroyo, pers. obs.) in the high-alpine habitat of *T. sessile*. Interestingly, specialized Sphingid pollination is known to be combined with a capacity for autonomous self-pollination, with the latter acting as a reproductive insurance mechanism when animal pollination fails (Pérez & al., 2009). *Tristagma sessile* is known to be self-compatible and capable of autonomous self-pollination (suppl. Table S2: hand self-pollination vs. autonomous self-pollination treatments). In unmanipulated pollinator-excluded flowers and flowers that are not naturally cross-pollinated, autonomous self-pollination probably takes place late in the flower lifespan. *Tristagma sessile* presents a higher number of seeds under open pollination than the number obtained under autonomous

self-pollination (suppl. Table S2), suggesting a combination of cross-pollination and autonomous selfing. Limited evidence (suppl. Table S2; emasculated flowers) suggests that it does not present autonomous (obligate) apomixis. However, as in all crossing experiments of this kind, where seed set occurs in the selfing treatment, but not under emasculation, without further detailed anatomical and cytological studies, it is difficult to discard the possibility of apomixis of the pseudogamous kind in which the development of viable agamosperous seeds requires pollination for endosperm development. D'Amato (1949) showed that adventitious embryony in the related genus *Nothoscordum* failed to occur when flowers were emasculated, indicating the need for pollination and thus a case of pseudogamy. If adventitious embryony exist in *T. sessile*, it could constitute an alternative or supplementary reproductive insurance mechanism to autonomous selfing when animal pollination is poor since adventitious embryony tends to occur in parallel with sexual megagametophyte development which in this case is mechanically possible through selfing (Whitton & al., 2008). More detailed work on the breeding systems and pollination of both species is highly desirable.

The assumption of a single species with disjunct distribution on either side of the South American continent, assumed a long-distance dispersal or a combination of different biogeographic events, as proposed by other authors for similarly disjunct distributed groups (e.g., Murillo-A. & al., 2016; Luebert & al., 2020). Our results corroborate *Ipheion recurvifolium* and *Tristagma sessile* to be different lineages, adding support to the differentiation of *Ipheion* from the rest of *Tristagma* species, with a unique karyological (Souza & al., 2016) and biogeographic history. *Ipheion* is limited to the Pampean region and *Tristagma* to central Chile and Patagonia (Chile and Argentina). It has been proposed that the tribe Leucocoryneae originated ca. 37–31 Mya and that *Tristagma* originated ca. 20 Mya (Sassone & Giussani, 2018, Escobar & al., 2020). The divergence of *Ipheion* occurred ca. 17–10 Mya during the Middle to Late Miocene. During this period, it is proposed that Atlantic marine transgressions (informally known as the “Paranean Sea”; Pascual & al., 1996, Magnussen Saffer, 2005; Albino, 2011) occurred in southern South America. The “Paranean Sea” was followed by likewise spread plains, extending northward from northern Patagonia, reaching central and northern Argentina, and Uruguay (Ortiz-Jaureguizar & Cladera, 2006, and references therein). Although much controversy still exists considering the exact timing of the Andean uplift at different latitudes, it appears, based on the ages of east-west vicariant plant groups, that heights of biogeographical significance would not have been achieved in the central and southern Andes before the Middle–Late Miocene (see Luebert & Weigend, 2014). We hypothesize that *Tristagma* has been distributed across southern South America before the significant uplift of the Andean range, with *T. sessile* likely evolving from Andean representatives of the lineage. Other lineages of *Tristagma* have probably been distributed in southern Patagonia, and *Ipheion* could have originated in the southern part of the Pampean region in Argentina from the northern part of the distribution

of one of the *Tristagma* lineages (Sassone & Giussani, 2018, Sassone & al., in prep.) as result of isolated populations in the emerged lands during the marine transgressions.

The availability of new collections of the rare and early flowering high-elevation *Tristagma sessile* has finally enabled solving the long-standing puzzle of its relationship with *Ipheion recurvifolium*. The phylogenetic relationships, geographical distributions, flowering periods, and DNA content of the two entities corroborate that they do not belong to the same species and can be ascribed to different genera. We verified morphological similarities between both species and attribute them to the sum of shared synapomorphies (between genera) and convergent features. Furthermore, we hypothesize that similar pollinators might underpin the convergence in floral traits. We hope that clarification of the Chilean taxon stimulates the location of additional populations of this rare and attractive species.

## ■ TAXONOMY

*Ipheion recurvifolium* (C.H.Wright) Traub in Pl. Life 9: 69. 1953 ≡ *Brodiaea recurvifolia* C.H.Wright in Bull. Misc. Inform. Kew 3: 117. 1915 ≡ *Beauverdia recurvifolia* (C.H.Wright) Herter in Estud. Bot. Región Uruguay 24: 210. 1956 ≡ *Tristagma recurvifolium* (C.H.Wright) Traub. in Pl. Life 19: 61. 1963 – Lectotype (designated by Guaglianone in Darwiniana 17: 178. 1972): Uruguay. Montevideo, Jun–Jul 1894, *Arechavaleta, J. 19* (K barcode K000523796!).

*Description.* – Herbs, perennial. Bulbs simple, without alliaceous odor; outer coats membranaceous, whitish. Leaves present at flowering time, basal, imbricate, sheathing basally 1.5–5 cm long; blade linear, 1.5–10 cm × 2–4 mm. Scape included in the neck; unifloral inflorescences subtended by one bifid spathe, cylindrical, membranaceous-papery, plurinerved; 1–2.5 cm long, fused for most of its length (0.8–2.3 cm long), pedicels absent or less than 1 mm long. Flowers white, 2–4.5 cm long, tepals 6 (rarely 7), 2-whorled, connate 10–27 mm long, with central purplish longitudinal stripe. Stamens 6, adnate to tepals, arrange in 2 series, filaments linear 0.15–0.5 × 0.1 cm; anthers dorsifixed, oblong, introrse. Ovary superior, sessile, 3-locular, 0.2–0.4 × 0.1–0.2 cm; ovules 10–15 per locule; style filiform 10–24 mm long; stigma terminal, trilobed. Fruits capsular, humifuse. Seeds polyhedral; tegument black. Fig. 4C,D.

*Chromosome number.* –  $2n = 4x = 20$  (16SM+4A). DNA content,  $2C = 36.2$ – $41.5$  pg (Crosa, 1975; Souza & al., 2010; Sassone & al., 2018).

*Conservation status.* – Least Concern (LC).

*Distribution.* – Uruguay, reaching the border with Argentina (but not reported yet in this country) and southern Brazil (Rio Grande do Sul).

*Iconography.* – Guaglianone, 1972: 177, fig. 6a–b.

*Phenology.* – Flowering from May to September (Fig. 2).

*Diagnostic features.* – *Ipheion recurvifolium* can be distinguished from the other two species of *Ipheion* by the longest tepal tube and style, together with the pedicel, and the

subterranean scape, as well as by karyotypic features (Crosa, 1975; Souza & al., 2010; Sassone & al., unpub.). Although the presence of a humifuse fruit (the scape becoming recurved after anthesis) has been found in other species of *Ipheion* and *Beauverdia*, it is always absent in *Tristagma* and plurifloral species of *Nothoscordum*.

*Additional specimens examined.* – **Brazil.** Rio Grande do Sul, Dpto. Cacapava do Sul, Santana do Livramento, 17 Jul 2009, *Boldrini, I.J. 1570* (ICN). **Uruguay.** 1918, *Felippone, F. 2205 bis* (SI); Cerro, Jun 1925, *Felippone, F. 3808* (SI); *Chase, M.W. 1596* (K 1985-2641); Maldonado, Punta del Este, *Guaglianone, E.R. s.n.* (SI 26507); Canelones, Canelón Chico, Apr–May 1910, *Berro 5898* (K barcode K000523797, MVFA); Maldonado, Sierra de las Ánimas, Ruta Interbalnearia km 89, *Forzza, R.C. 3389* (RB, K), *Forzza, R.C. 3390* (RB, K); Cuchilla Pereyra, Sep 1920, *Felippone, F. 3495 b* (SI); Punta Ballena, 23 May 2019, *Sassone, A.B. 69* (SI, BAL); La Barra, Punta del Este, 24 May 2019, *Sassone, A.B. 73* (SI, BAL); La Barra, Punta del Este, 24 May 2019, *Sassone, A.B. 77* (SI); Punta del Este, en veredas y plazoletas, 25 May 2019, *Sassone, A.B. 88* (SI, BAL); Punta del Este, 03 Jul 1971, *Guaglianone, E.R. s.n.* (SI 26507); Montevideo, Cerro Montevideo, *Herter, G. 152* (SI 78457); Cerro in Saxosis, *Osten, C. 5221* (SI, MVM); *Felippone, F. 3160* (SI 35509); 17 May 1908, *Sassone, A.B. 98* (SI, BAL); 03 May 1959, *Crosa, O. s.n.* (MVFA 9261); 01 Jul 1971, *Crosa, O. s.n.* (SI 26506); Minas, Cerro Verdún, En campito junto al estacionamiento, 20 May 2012, *Giussani, L.M. 469* (SI); Lavalleja, Cerro Arequita, 20 May 2012, *Giussani, L.M. 480* (SI); San José, 21 May 2012, *Giussani, L.M. 487* (SI); Río Negro, Aduana de Fray Bentos, 19 May 2012, *Giussani, L.M. 458* (SI); Soriano, Rodó, Parque los 33 Orientales, 19 May 2012, *Giussani, L.M. 460* (SI).

*Tristagma sessile* (Phil.) Traub in Pl. Life 19: 61. 1963 ≡ *Trieteleia sessilis* Phil. in Linnaea 29: 72. 1858 ≡ *Brodiaea sessilis* (Phil.) F.Meigen. in Bot. Jahrb. Syst. 17: 225. 1893 – Holotype: Chile. Cord. Arañas, Nov 1855, *Philippi, R.A. s.n.* (SGO barcode SGO000001126!).

= *Milla sessiliflora* Baker in Bot. J. Linn. Soc. 11: 382. 1870 – **Lectotype (designated here):** Chile. Cord. De Maule, Ann. 1856 et 1857, *Rec. Ph. Germain s.n.* (K barcode K000523798!; isolectotypes: BM barcode BM001122330!, P barcode P01855190!).

= *Tristagma leichtlinii* (Baker) Ravenna in Pl. Life 27: 85. 1971 ≡ *Milla leichtlinii* Baker in Gard. Chron. 3: 234. 1875 – Holotype: Chile. Región Andina, without data (K barcode K000524644!).

*Description.* – Herbs, perennial. Bulbs simple, without alliaceous odor; outer coats membranaceous, whitish. Leaves present at flowering time, basal, imbricate, sheathing basally 4–5 cm long; blade linear, 1.5–5 cm × 4–5 mm. Scape included in the neck; inflorescences subtended by one spathe formed by 2 bracts, membranaceous papery, plurinerved; 1.9–2.5 cm long, connate 0.9–1.4 cm, pedicels absent or less than 1 cm. Flowers 1, white, 1.5–3.5 cm long, tepals 6, 2-whorled, connate 1–2.2 cm long, with central green longitudinal stripe. Stamens 6, adnate to tepal, arranged in 2 series, filaments linear



0.3–0.4 × 0.1–0.2 cm; anthers dorsifixed, oblong, introrse. Ovary superior, sessile, 3-locular, 0.2–0.4 × 0.2–0.4 cm; ovules 10 per locule; style filiform 13–20 mm long; stigma terminal, trilobate. Fruits capsular, erect. Seeds polyhedral; tegument black. Fig. 4A,B.

*Chromosome number.* – A chromosome count is still needed. However, based on the DNA content ( $2C = 76.49$  pg) and previous reports for the genus, we hypothesize that *T. sessile* is a tetraploid.

*Conservation status.* – Vulnerable (VU) under criterion B1ab (i and ii). Refer to results.

*Distribution.* – Andes and Coastal Range (“Cordillera de la Costa”) in Chile, 2000–2800 m a.s.l. Found in areas of deep snow and waterlogged soils and at edges of streams.

*Iconography.* – Hoffmann & al., 1998: 245, fig. 6.

*Phenology.* – Flowering from September to December, one record in January (Fig. 2).

*Diagnostic features.* – *Tristagma sessile* can be differentiated from the rest of the *Tristagma* species by its unusually long tepal tube and style, together with the small or null pedicel, and the presence of a subterranean scape. Among *Tristagma* species, *T. sessile* comes closest to *T. patagonicum*, but this species bears 1–3 flowers per inflorescence. A key to other *Tristagma* species, besides *T. sessile*, can be found in Arroyo-Leuenberger & Sassone (2016, English) and Sassone & Arroyo-Leuenberger (2018, Spanish).

*Note.* – We place *Tristagma leichtlinii* under *T. sessile* principally based on the type specimen. Even though the specimen is incomplete (only consisting of flowers), the morphology of the flowers and description in the accompanying Leichtlin’s letter (pinned with the Kew herbarium sheet) refer it to *T. sessile*. This conclusion is further supported by our knowledge of species of *Tristagma* present in the high-altitude flora of central Chile (*T. bivalve*, *T. nivale*) both of which have different flowers than *T. leichtlinii*. Baker’s description presents two inconsistencies: the mention of a corm (instead of a bulb) and the 2–3-flowered inflorescence (instead of one-flowered inflorescence). The corm is reflected in the illustration in Baker (1876) as well as the robust nature of the whole plant. Evaluating the high-altitude Chilean flora and resident *Tristagma* species, there is no doubt that *T. leichtlinii* is a synonym of *T. sessile*. The inconsistencies previously described can be explained by the circumstances under which it was described. It is likely that Baker described *Milla leichtlinii* without the complete specimen, only with the flowers currently present in the type specimen. The description of a corm is possibly a mistake because even Leichtlin mentions the presence of bulbs (there is a letter accompanying the specimen). Furthermore, the illustration could have been based on a plant growing in a greenhouse in England, one year later. After reviewing fresh material and herbarium specimens of *T. bivalve* (a common species at high elevations in the central Chilean Andes) there is no doubt that Baker’s original publication was not referring to this multiflowered species (this species differs in size of the plant and flower, and it is always multiflowered). The confusion probably originated due to the

close occurrence of both species in the Andean region of Chile (for instance both have been recorded in Farellones, Santiago, Chile).

*Additional specimens examined.* – **Chile.** Región Metropolitana, Prov. Santiago, Cord. Santiago, *Philippi, R.A. s.n.* (JE barcode JE00008728); Cord. Santiago, *Philippi, R.A. s.n.* (HAL barcode HAL0109683); Cord. Santiago, *Philippi, R.A. s.n.* (BAF 7256); Cord. Santiago, no data (LP 20461); Cordillera de Santiago, *Philippi, R.A. s.n.* (K barcode K000523798); Cord. de Santiago, Arañas, Nov 1861, no data (SGO 38052); Cordillera de Santiago, Jan 1892, *Philippi, R.A.* (SGO 61065); Chacabuco, Altos de Chicauma, Lampa, 10 Sep 2007, *García, N. 4169* (CONC); Chacabuco, Altos de Chicauma, Lampa, 21 Sep 2018, *García, N. 5129* (EIF); Lo Barnechea, Road to Valle Nevado, 20 Oct 2013, *Arroyo, M.T.K. 28591* (CONC); Small stream on west side of road to Valle Nevado, 20 Oct 2013, *Arroyo, M.T.K. 28592* (CONC); Road between Farellones and La Parva, on side of small swampy stream in damp soil to edge of stream, 15 Sep 2019, *Arroyo, M.T.K. 29124* (SI, GAT); Between Farellones and La Parva, 03 Oct 2009, *Arroyo, M.T.K. 28508* (CONC); VII Región, Cordillera de Linares, 1856, *Germain, P. s.n.* (SGO 46616).

#### Key to genera

1. Spathe formed by one bifid bract (= two bracts fused for more than  $\frac{3}{4}$  of their length). Flowers white, light blue, light pink or light purple. Inflorescence 1-flowered. Fruits humifuse.....*Ipheion*
1. Spathe formed by two bracts fused only at the base (less than  $\frac{1}{2}$  of their length). Flowers white, green or dark purple. Inflorescence (1–)2–5-flowered. Fruits aerocarpic....  
.....*Tristagma*

#### ■ AUTHOR CONTRIBUTIONS

ABS and MTKA conceived the idea and wrote the manuscript with input from SCAL and NG. ABS performed all the analyses and produced the figures. SCAL collaborated with the taxonomic revision. MJR produced new sequences of *T. sessile*. MTKA and NG contributed specimens and conserved material of flowers. — ABS, <https://orcid.org/0000-0001-6295-1483>; MTKA, <https://orcid.org/0000-0001-6315-0426>; SCAL, <https://orcid.org/0000-0002-8670-496X>; NG <https://orcid.org/0000-0001-9003-1510>; MJR, <https://orcid.org/0000-0002-5084-3159>

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#### Appendix 1. Taxon and GenBank accession number of specimens included in this study, in the following order nrDNA ITS, *ndhF*, *matK*.

**Tribe Leucocoryneae:** *Beauverdia dialystemon* (Guagl.) Sassone & Guagl.: MH159821, MH159892, MH159944; *Beauverdia hirtella* (Kunth) Herter: MH159825, MH159891, MH159943; MH159824, MH159890, MH159942; *Beauverdia sellowiana* (Kunth) Herter: MH159827, MH159894, MH159945; MH159828, MH159895, MH159946; MH159829, MH159896, MH159947; *Beauverdia vittata* (Griseb.) Herter: MH159830, MH159897, MH159948; MH159831, MH159898, MH159949; MH159832, MH159899, MH159950; *Ipheion recurvifolium* (C.H.Wright) Traub: MH159850, MH159900, MH159951; MH159853, MH159901, MH159952; LT718334, LT718396, LT718272; *Ipheion tweedeanum* (Griseb.) Traub: MH159854, MH159902, MH159953; MH159855, MH159903, –; *Ipheion uniflorum* (Graham) Raf.: MH159857, MH159904, MH159954; MH159858, MH159905, MH159955; LT718338, LT718400, LT718276; LT718341, LT718403, LT718279; LT718340, LT718402, LT718278; LT718335, LT718397, LT718273; LT718339, LT718401, LT718277; *Leucocoryne coquimbensis* F.Phil.: LT718353, LT718415, LT718291; *Leucocoryne ixioides* Lindl.: MH159817, MH159888, MH159940; *Latace andina* (Poepp.) Sassone: MH159820, MH159889, MH159941; *Nothoscordum* sp.: MH159849, MH159927, MH159974; *Nothoscordum andicola* Kunth: MH159833, MH159928, MH159975; *Nothoscordum arenarium* Herter: MH159834, MH159929, MH159976; *Nothoscordum bivalve* (L.) Britton: MH159835, MH159930, MH159977; *Nothoscordum bonariense* Beauverd: MH159836, MH159931, MH159978; MH159837, MH159932, MH159979; MH159841, MH159936, MH159983; *Nothoscordum gracile* (Aiton) Stearn: MH159839, MH159934, MH159981; *Nothoscordum minarum* Beauverd: MH159842, MH159937, MH159984; *Nothoscordum montevidense* Beauverd: MH159840, MH159935, MH159982; *Nothoscordum nudicaule* (Lehm.) Guagl.: MH159845, MH159938, MH159985; *Tristagma ameghinoi* (Speg.) Speg.: MH159875, MH159907, MH159956; *Tristagma bivalve* (Lindl.) Traub: LT718383, LT718443, LT718321; MH159876, MH159919, MH159966; MH159881, MH159920, MH159967; MH159879, MH159921, MH159968; MH159880, MH159922, MH159969; *Tristagma circinatum* (Sandwith) Traub: MH159863, MH159910, MH159958; *Tristagma gracile* (Phil.) Traub: MH159883, MH159926, MH159973; *Tristagma graminifolium* (Phil.) Ravenna: MH159878, MH159923, MH159970; *Tristagma nivale*



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**Appendix 1.** Continued.

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Poepp.: MH159867, MH159912, MH159960; MH159870, MH159913, MH159961; MH159871, MH159914, MH159962; *Tristagma patagonicum* (Baker) Traub: MH159862, MH159909, MH159957; MH159866, MH159911, MH159959; MH159874, MH159906, MH159963; MH159872, MH159916, MH159964; *Tristagma violaceum* (Kunth) Traub: MH159877; MH159924, MH159971. — **Outgroup:** *Gilliesia graminea* Lindl.: LT718327, LT718389, LT718265; *Miersia chilensis* Lindl.: LT718373, –, LT718311; *Solaria atropurpurea* (Phil.) Ravenna: LT718325, LT718387, LT718263; *Solaria miersioides* Phil.: MH159816, MH159887, MH159939; LT718378, LT718439, LT718316; *Tulbaghia ludwigiana* Harv.: LT718384, LT718444, LT718322; *Tulbaghia simmleri* Beauverd: LT718385, LT718445, LT718323; *Tulbaghia violacea* Harv.: KT373964, JQ276781, JQ276393.

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