

# Is the southern South American genus *Tweedia* (Apocynaceae: Asclepiadoideae) monophyletic? Molecular phylogenies, distribution and taxonomy

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**Abstract** *Tweedia* is a genus traditionally regarded as composed of seven species distributed on both sides of the Andes in southern South America. Recent molecular analyses of two of its species have shown that they are found in different lineages within the MOOG clade of Apocynaceae subfam. Asclepiadoideae, which questions its monophyly and generic affinities. Whereas *Tweedia australis* falls within the *Diplolepis* clade of MOOG, *T. brunonis* appears within tribe Oxypetalinae. This work provides a molecular phylogeny of all species of *Tweedia* including its type, *T. birostrata*, to test the monophyly of the group and assess its relationships with *Diplolepis* and other genera of the MOOG clade. Phylogenetic analyses of cpDNA *trnT-trnF* and *rps16* regions of 68 accessions of Apocynaceae using maximum parsimony, maximum likelihood, and Bayesian inference methods show that *Tweedia* species form a well-supported monophyletic group that is morphologically distinct, allied to *Araujia* and *Philibertia* of the Oxypetalinae. The study also supports the recent transfer of the former *T. australis* to *Diplolepis* as *D. australis*. Within *Tweedia*, two clades divided by the Andes can be recognized, one from the west (central Chile) and one from the east (western Argentina). The geographical and environmental characteristics of the genus and of these two groups are discussed in an evolutionary context.

**Keywords** Asclepiadoideae MOOG clade; cpDNA; Oxypetalinae; phylogeny; *rps16*; southern Andes; *trnT-trnF*; *Tweedia*

## ■ INTRODUCTION

Southern South America is an area where the Apocynaceae subfam. Asclepiadoideae are highly diversified, including more than 35 genera and 250 species (Meyer, 1944; Ezcurra, 1999a; Ezcurra & al., 2008). In this area, establishing limits among genera of Asclepiadoideae is particularly complex. This has been stated for, e.g., *Tweedia* Hook. & Arn. (Rua, 1989), *Philibertia* Kunth (Goyder, 2004), *Matelea* Aubl. (Ezcurra & Belgrano, 2007), *Diplolepis* R.Br. (Hechem & al., 2011), *Jobinia* E.Fourn. and *Orthosia* Decne. (Liede-Schumann & Meve, 2013), and several other genera from this region. During the 20th century, following Woodson (1941), the taxonomy of Asclepiadoideae has been simplified and many small and ill-defined genera have been reduced to synonymy of larger ones (see, e.g., Rapini, 2002; Goyder, 2003, 2004; Liede-Schumann & al., 2005; Goyder & al., 2007; Meve & Liede-Schumann, 2007), but many genera of southern South America still remain little studied and difficult to distinguish morphologically. Only recently, molecular phylogenies have provided additional information to support their monophyly and help establish their limits and relationships (e.g., Liede-Schumann & al., 2005; Hechem & al., 2011; Rapini & al., 2011; Liede-Schumann & Meve, 2013).

*Tweedia* is a genus of erect or prostrate, frequently twining suffruticose perennial herbs with small, pale flowers (Fig. 1),

in which seven species from Argentina, southern Bolivia and central Chile have generally been recognized (Rua, 1989; Rua & Liede, 1994; Ezcurra, 1995, 1999b; Ezcurra & al., 2008). Recent phylogenetic studies of Asclepiadoideae from South America based on molecular data have shown that the species formerly known as *Tweedia australis* (Malme) C.Ezcurra (= *Tweedia odonellii* T.Meyer) belongs to a monophyletic group along with species previously treated as *Diplolepis* R.Br., *Grisebachiella* Lorentz, and *Cynanchum* L. from southern Argentina and Chile (Hechem & al., 2011). Consequently, all species of this clade including *Tweedia australis* have been included in *Diplolepis* (Hechem & al., 2011). As such, *Diplolepis* currently comprises a monophyletic although morphologically diverse taxonomic group from Chile and Argentina, with 14 species from temperate forests, high-elevation regions, semi-deserts and deserts of southern South America (Hechem & al., 2011, 2012).

Since its first description, the limits between *Tweedia* and related genera have been unclear. The genus was described by Hooker & Arnott (1834) with three species: *T. birostrata* (Hook. & Arn.) Hook. & Arn. from Chile, *T. macrolepis* Hook. & Arn. from southern Brazil, and *T. brunonis* Hook. & Arn. from central Argentina, without designating a type and doubting if *T. brunonis* should really be considered within *Tweedia*. Decaisne (1844) treated these three species in *Oxypetalum* R.Br.

and also created a new genus, *Turrigera* Decne., from other material of *Tweedia brunonis*. Malme (1904) was the first to produce a revision of *Tweedia* following Hooker & Arnott's (1834) concept and considered it different from *Oxypetalum* (although related), but he accepted Decaisne's (1844) exclusion of the Brazilian *T. macrolepis* as *Oxypetalum*, and this species has been treated within *Oxypetalum* until now (Rapini & al., 2010). Malme (1904) also added other Chilean and a south Andean species from Argentina to those originally described by Hooker & Arnott (1834), recognizing in total six species in *Tweedia*. In addition, Malme (1904) stressed that an important difference between *Tweedia* and *Oxypetalum* is their geographic distribution, *Tweedia* being found on both sides of the Andes of central Chile and western Argentina, and *Oxypetalum* mainly in central and southern Brazil and eastern Argentina. Meyer (1944, 1950) revised the species of *Tweedia* from Argentina and published a new species. Rua (1989) performed a detailed revision of *Tweedia* which resulted in six species, to which a new species was added a few years later (Rua & Liede, 1994). The revision of Rua (1989) differed from Malme's (1904) in the circumscription of some Chilean species and the inclusion of other species from Argentina (e.g., Rua, 1986). In addition, Rua (1989) rejected previous lectotypifications and selected a lectotype (*T. birostrata*) that agrees with the protologue and preserves current usage of the name *Tweedia*.

Thus, until recently, *Tweedia* had been regarded as a genus of seven species distributed in central Chile and western Argentina (Ezcurra, 1995; Ezcurra & al., 2008). Nevertheless, in his revision, Rua (1989) stated that the limits of the genus and its affinities were not clear, as not all of its species seemed morphologically related to *Oxypetalum*. In addition, recent molecular analyses that included two species of *Tweedia* (*T. australis*, *T. brunonis*) placed them in different lineages within the clade of Apocynaceae subfam. Asclepiadoideae that includes Metastelmatinae Endl. ex Meisn., Orthosiinae Liede & Rapini, Oxypetalinae E.Fourn., and Gonolobinae G.Don ex Liede (MOOG; Rapini & al., 2003, 2006; Liede-Schumann & al., 2005; Liede-Schumann & Meve, 2013). Whereas *T. brunonis* fell within Oxypetalinae, *T. australis* fell within the *Diplolepis* clade (Hechem & al., 2011), and this latter species consequently

has been transferred to *Diplolepis* (Hechem & al., 2011). The position of *T. australis* within *Diplolepis* casts doubts on the monophyly of *Tweedia* as currently conceived (Hechem & al., 2011). However, the phylogenetic positions of the remaining five species of *Tweedia* are unknown, including the type *T. birostrata*, because none of them have been analyzed in previous molecular studies.

This paper analyses the cpDNA *rps16* intron and *trnT* (UGU)-*trnF*(GAA) region (hereafter called *trnT-F*) of all species of *Tweedia* to estimate phylogenetic relationships among these species, verify the monophyly of *Tweedia*, and reconstruct the evolutionary history of this southern South American group. The phylogeny is also used together with morphology and distribution to assess the relationships of *Tweedia* with *Diplolepis* and other genera of the diverse and species-rich New World MOOG clade of Apocynaceae subfam. Asclepiadoideae. In addition, the geographical and environmental characteristics of *Tweedia* are discussed in an evolutionary context.

## ■ MATERIALS AND METHODS

**Accessions examined.** — In total 68 accessions of Apocynaceae were examined for cpDNA *trnT-F* and *rps16* sequence variation. These accessions included one species of subfamily Secamonoideae, two genera and two species from outside the MOOG clade of subfamily Asclepiadoideae, and 17 genera and 54 species representing the tribes and major lineages within the MOOG clade. DNA sequences for 16 accessions were obtained specifically for this study (Appendix 1); data for the remaining accessions were obtained from Liede-Schumann & al. (2005) (<http://purl.org/phylo/treebase/phyloWS/study/TB2:S1181>), and from our previous study on *Diplolepis* (Hechem & al., 2011). New accessions include the six species currently accepted as *Tweedia* (i.e., *T. andina* (Phil.) G.H.Rua, *T. aucaensis* G.H.Rua, *T. birostrata*, *T. brunonis*, *T. echegarayi* Malme, and *T. stipitata* G.H.Rua & Liede; nomenclature as in Ezcurra & al., 2008 and Hechem & al., 2011).

All phylogenetic trees were rooted with *Pervillaea tomentosa* Decne. (Secamonoideae) as a previous study revealed a



**Fig. 1.** Morphology of *Tweedia*. **A**, habit of *Tweedia aucaensis*; **B**, flower of *Tweedia aucaensis*; **C**, flower of *Tweedia birostrata*. — Scale bars: A = 1 cm; B, C = 1 mm. — Photographs by Daniel Testoni (A, B) and Bernardo Segura-Silva (C).



sister-group relationship between subfamilies Secamonoideae and Asclepiadoideae (Potgieter & Albert, 2001).

**DNA extraction, amplification and sequencing.** — Leaf material for DNA extraction was obtained from herbarium specimens or from plants collected in the field. Total genomic DNA was obtained from 20 mg of dried leaf tissue using the Wizard SV Genomic DNA Kit (Promega, Madison, Wisconsin, U.S.A.) or the Purelink Plant Total DNA Purification Kit (Invitrogen, Carlsbad, California, U.S.A.). The *trnT-F* region was PCR-amplified using the six universal primers designed by Taberlet & al. (1991). The protocols used to obtain the *trnT-F* and *rps16* intron sequence data were presented elsewhere (Calviño & al., 2006). All sequencing was done using an ABI (Applied Biosystems, Carlsbad, California, U.S.A.) 23 3730XL high-throughput DNA capillary sequencer at Macrogen (Seoul, Korea). Simultaneous consideration of both DNA strands across the entire cpDNA regions for most taxa permitted unambiguous base determination. All newly obtained cpDNA sequences have been submitted to GenBank (Appendix 1). The aligned data matrix can be obtained from TreeBase (study accession URL: <http://purl.org/phylo/treebase/phyloids/study/TB2:SI6173>).

**Sequence comparisons and phylogenetic analyses.** — Sequence chromatograms were edited manually using BioEdit v.6.0.7 (Hall, 1999). DNA sequences were aligned manually. Gaps were positioned to minimize nucleotide mismatches. A matrix of binary-coded indels was constructed for each locus (i.e., *trnT-F* and *rps16* intron) to incorporate length-mutational information into the phylogenetic analysis. Gap coding was according to Downie & Katz-Downie (1999); for several regions, gap coding was problematic because of homopolymers or indirect duplications of adjacent elements in two or more taxa. These gaps were not scored and these ambiguous regions were excluded from subsequent analysis.

Some regions of the alignments were scored as missing. The *trnT-L* intergenic spacer could not be obtained for *Tweedia birostrata* 158, and *trnL* intron could not be obtained for *T. birostrata* 159, 160, 161 and 162. Overall, missing data represented 3.5% of the entire cpDNA matrix.

The determination of boundary sequences for non-coding regions within the cpDNA *trnT-F* and *rps16* loci was based on corresponding boundaries inferred previously for Apocynaceae (Liede-Schumann & al., 2005). Characterization of the four non-coding cpDNA regions (i.e., the *trnT*(UGU)-*trnL*(UAA) 5' exon intergenic spacer, *trnL* intron, *trnL*(UAA) 3' exon-*trnF*(GAA) intergenic spacer, and *rps16* intron) was facilitated using BioEdit v.6.0.7 (Hall, 1999) and PAUP\* v.4.0b10 (Swofford, 2002). Uncorrected pairwise nucleotide distances of unambiguously aligned positions were determined using the distance matrix option of PAUP\*.

Combined *trnT-F* and *rps16* intron data matrices, with and without their scored indels, were analyzed using maximum parsimony (MP) as implemented by PAUP\*; 1000 heuristic searches were initiated using random addition starting trees with tree-bisection-reconnection (TBR) branch swapping and MulTrees selected, but saving no more than five trees from each search. These trees were subsequently used as starting trees for further TBR branch swapping. The maximum number of

saved trees was set to 20,000 and these were permitted to swap to completion. The strict consensus of these 20,000 minimal-length trees was then used as a topological constraint in another round of 1000 random addition replicate analyses in which only those trees that did not fit the constraint tree were saved. No additional trees as long as the initial shortest trees were found, suggesting that the strict consensus tree adequately summarizes the available evidence, even though the exact number of trees of that length is not known. Bootstrap values (BS) were calculated from 10,000 replicate analyses using the heuristic search strategy and random addition of taxa; only those values compatible with the majority-rule consensus tree were recorded.

Bayesian inference of the combined *trnT-F* and *rps16* intron matrix including and excluding scored indels was conducted using MrBayes v.3.2.2 (Huelsenbeck & Ronquist, 2001). Prior to analysis, MrModeltest v.2.3 (Nylander, 2004) was used to select an evolutionary model of nucleotide substitution that best fits each of the four non-coding cpDNA data partitions, as selected by the Akaike information criterion estimator (Posada & Buckley, 2004). The best-fit models selected were GTR+G for the *trnT-L* and *trnL-F* intergenic spacers and *trnL* intron, and GTR+I for the *rps16* intron. For the indels partition, the restriction (binary) evolutionary model of MrBayes was used, assuming gamma shape rate variation across sites, and specifying that the characters sampled are all variable to inform the coding bias and calculate the probability of the data correctly (Ronquist & al., 2011). From different random starting trees, four independent analyses with four chains each were run for 10 million generations; in some instances the analyses were stopped earlier when the average standard deviation of the split frequencies between the runs dropped to less than 0.01 using a relative burn-in of 25% (indicating that convergence in topology between the runs has already been reached for the posterior samples estimated so far). Overall mutation rate was allowed to vary among the four partitions. Trees and branch lengths were saved every 100 generations. Stationarity and additional convergence search strategies were the same as employed by Hechem & al. (2011). The states of the chain that were sampled before stationarity (i.e., the “burn-in” of the chain) were discarded and posterior probability values (PP; expressed as percentages) for each bipartition of the phylogeny were determined from the remaining trees.

The *trnT-F* and *rps16* intron matrix (without scored indels) was also analyzed using maximum likelihood (ML), as implemented in RAxML v.7.0.3 (Stamatakis, 2006). The best-scoring ML tree was searched under a GTR+G model and bootstrap values were calculated from 1000 replicate analyses using the rapid BS algorithm in a single run; only those values compatible with the majority-rule consensus tree were recorded.

**Morphology and distribution.** — Morphological information and geographical distribution of the genus and species were obtained from herbarium material and from recent treatments (Rua, 1989; Rua & Liede, 1994; Ezcurra, 1999b; Hechem & Ezcurra, 2006; Ezcurra & al., 2008; Hechem & al., 2012; Ezcurra & Hechem, in press). Generic limits of the genera to which *Tweedia* is related follow recircumscriptions recently accepted in Oxypetalinae (Goyder, 2004; Liede-Schumann & al., 2005; Rapini & al., 2006; Rapini & al., 2011).

## RESULTS

### Sequence comparisons and phylogenetic analyses. —

Sequence characteristics of the four non-coding cpDNA regions of the *trnT-F* and *rps16* loci are presented in Table 1. Of the 68 sequences compared, the regions varied in size from 774 to 854 bp in the *trnT-L* intergenic spacer, from 498 to 527 bp in the *trnL* intron, from 360 to 388 bp in the *trnL-F* intergenic spacer, and from 757 to 783 bp in the *rps16* intron. Alignment of these sequences resulted in a combined matrix of 2790 positions. Of these, 356 were excluded from subsequent analysis because of alignment ambiguities. The remaining 2434 aligned positions yielded 226 parsimony-informative characters. In addition, 19 unambiguous parsimony-informative indels were inferred, of which 11 occurred within the *trnT-L* intergenic spacer, 2 within the *trnL* intron, and 6 within the *rps16* intron. Informative indels ranged in size from 1 to 25 bp. Maximum pairwise sequence divergence across all sites among all taxa was 5.17% (between *Pervillaea tomentosa* and *Tweedia birostrata* 161; numbers after species names refer to DNA accessions). Pairwise distances between species of *Tweedia* ranged from 0.23% to 2.97% (between *T. andina* 155 and *T. birostrata* 158, and *T. aucaensis* 182 and *T. birostrata* 161, respectively). Maximum pairwise sequence divergence was greater within *Tweedia* than within any other genus of Oxypetalinae (*Funastrum* E.Fourn.: 1.37%, *Oxypetalum*: 1.22%, *Araujia* Brot.: 1.03%, *Philibertia* Kunth: 0.67%). MP analysis of 2434 unambiguously aligned *trnT-F* and *rps16* intron nucleotide positions resulted in the preset maximum tree limit of 20,000 trees, each of 658 steps (consistency indices, CIs = 0.8116 and 0.6998, with and without uninformative characters, respectively; retention index, RI = 0.8936). The relationships inferred in the strict consensus of these trees are largely identical to those resolved using Bayesian inference (Fig. 2). Repeating the MP analysis without indels also resulted in the preset limit of 20,000 trees, each of 634 steps (CIs = 0.8123 and 0.6941, with and without uninformative characters, respectively; RI = 0.8887). The topology of the strict consensus tree (not shown) was identical to that when indels were included, and bootstrap support values were slightly higher for some nodes (e.g., 62% vs. <50% without

and with indels, respectively, for *T. aucaensis*, *T. echegarayi*, and *T. brunonis*; and 51% vs. <50% for *Philibertia globiflora* (Goyder) Malme to *P. parviflora* Goyder) and slightly lower for others (e.g., 71% vs. 96% for species of *Philibertia*; <50% vs. 64% for *Oxypetalum*; 66% vs. 74% for the Oxypetalinae clade).

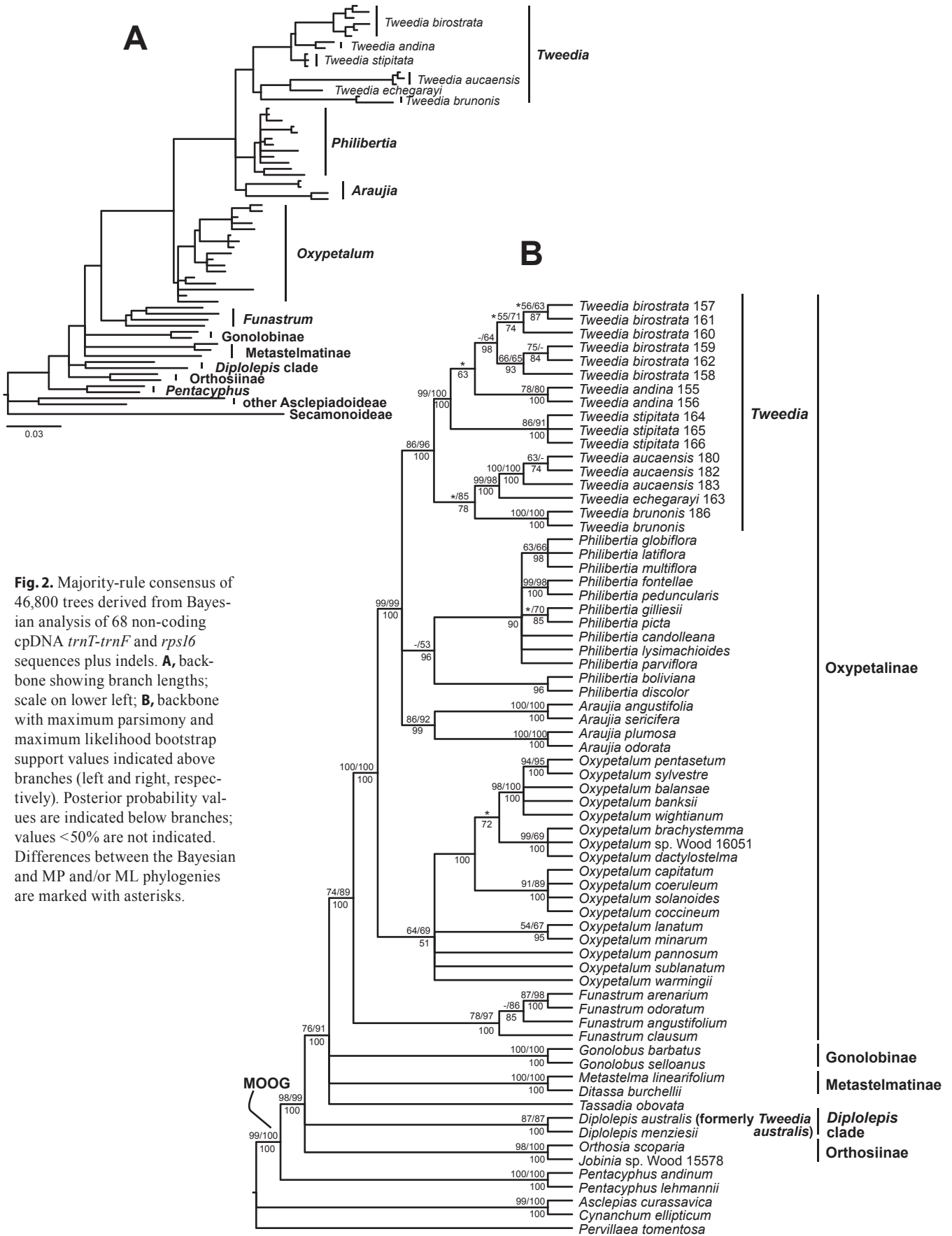
Bayesian analysis was stopped at 1.17 million generations because at that point the average standard deviation of the split frequencies between the four runs dropped to less than 0.01, indicating convergence in topologies. The first 2925 trees of each run were discarded as “burn-in” and majority-rule consensus trees that summarize topology and branch length information were calculated based upon the remaining 46,800 trees (Fig. 2). Repeating the analysis without indels resulted in the same topology as the majority-rule consensus tree with indels (convergence was reached after 2.24 million generations, and the majority-rule consensus tree was calculated based upon 50,400 trees), and with the only nodes differing for more than 5% PP being the clade of *Philibertia globiflora* to *P. parviflora* (51% PP vs. <50% PP without and with indels, respectively) and *Tweedia aucaensis* to *T. brunonis* (89% PP vs. 78% PP).

The phylogenies estimated using MP, Bayesian and ML analyses of *trnT-F* and *rps16* intron data are almost completely congruent with each other. The MP strict consensus tree is slightly less resolved than the Bayesian and ML trees (Fig. 2). In all cpDNA-derived trees, the 17 accessions of *Tweedia* examined form a strongly supported monophyletic group (86% MPB [maximum parsimony bootstrap], 96% MLB [maximum likelihood bootstrap], 100% PP) within the Oxypetalinae core group (100% MPB, 100% MLB, 100% PP). Within the latter, *Tweedia*, *Philibertia*, and *Araujia* form a clade (99% MPB, 99% MLB, 100% PP) that is sister to *Oxypetalum*. *Diplolepis australis* (Malme) Hechem & C.Ezcurra, formerly *Tweedia australis*, falls within the *Diplolepis* clade (87% MPB, 87% MLB, 100% PP), far away from the Oxypetalinae. All species of *Tweedia* are monophyletic (<50%–100% MPB, 64%–100% MLB, 98%–100% PP). Each species of *Tweedia* is subtended by significantly longer branches relative to the length of the branches of the species of other genera of Oxypetalinae ( $P < 0.05$ , Wilcoxon test; mean number of substitutions per site of crown: *Tweedia*, 0.20; *Funastrum*, 0.14; *Araujia*, 0.12; *Oxypetalum*,

**Table 1.** Sequence characteristics of the non-coding regions of the cpDNA *trnT-F* (*trnT-trnL*, *trnL* intron, *trnL-trnF*) and *rps16* intron loci for 68 accessions of Apocynaceae.

	<i>trnT-trnL</i> intergenic spacer	<i>trnL</i> intron	<i>trnL-trnF</i> intergenic spacer	<i>rps16</i> intron
Length variation (range in bp)	774–854	498–527	360–388	757–783
Aligned positions	971	552	430	837
Positions eliminated	187	39	60	70
Positions not variable	608	441	301	625
Positions autapomorphic	84	46	33	70
Positions parsimony informative	92	26	36	72
Unambiguous alignment gaps parsimony informative	11	2	0	6
Total number of parsimony-informative characters <sup>a</sup>	103	28	36	78

<sup>a</sup> Number of parsimony informative nucleotide substitutions plus number of parsimony-informative gaps.



**Fig. 2.** Majority-rule consensus of 46,800 trees derived from Bayesian analysis of 68 non-coding cpDNA *trnT-trnF* and *rps16* sequences plus indels. **A**, backbone showing branch lengths; scale on lower left; **B**, backbone with maximum parsimony and maximum likelihood bootstrap support values indicated above branches (left and right, respectively). Posterior probability values are indicated below branches; values <50% are not indicated. Differences between the Bayesian and MP and/or ML phylogenies are marked with asterisks.

0.10; *Philibertia*, 0.06; Fig. 2A). *Tweedia* is divided into two clades, one with the Chilean species *T. birostrata*, *T. andina* and *T. stipitata* (99% MPB, 100% MLB, 100% PP), and the other clade with the Argentinean species *T. aucaensis*, *T. echeagarayi*, and *T. brunonis* (<50% MPB, 85% MLB, 78% PP). The relationships of the widespread *T. brunonis* as sister to the other Argentinean species finds weak to moderate support depending on the phylogenetic method, and the inclusion of indels weakens this relationship.

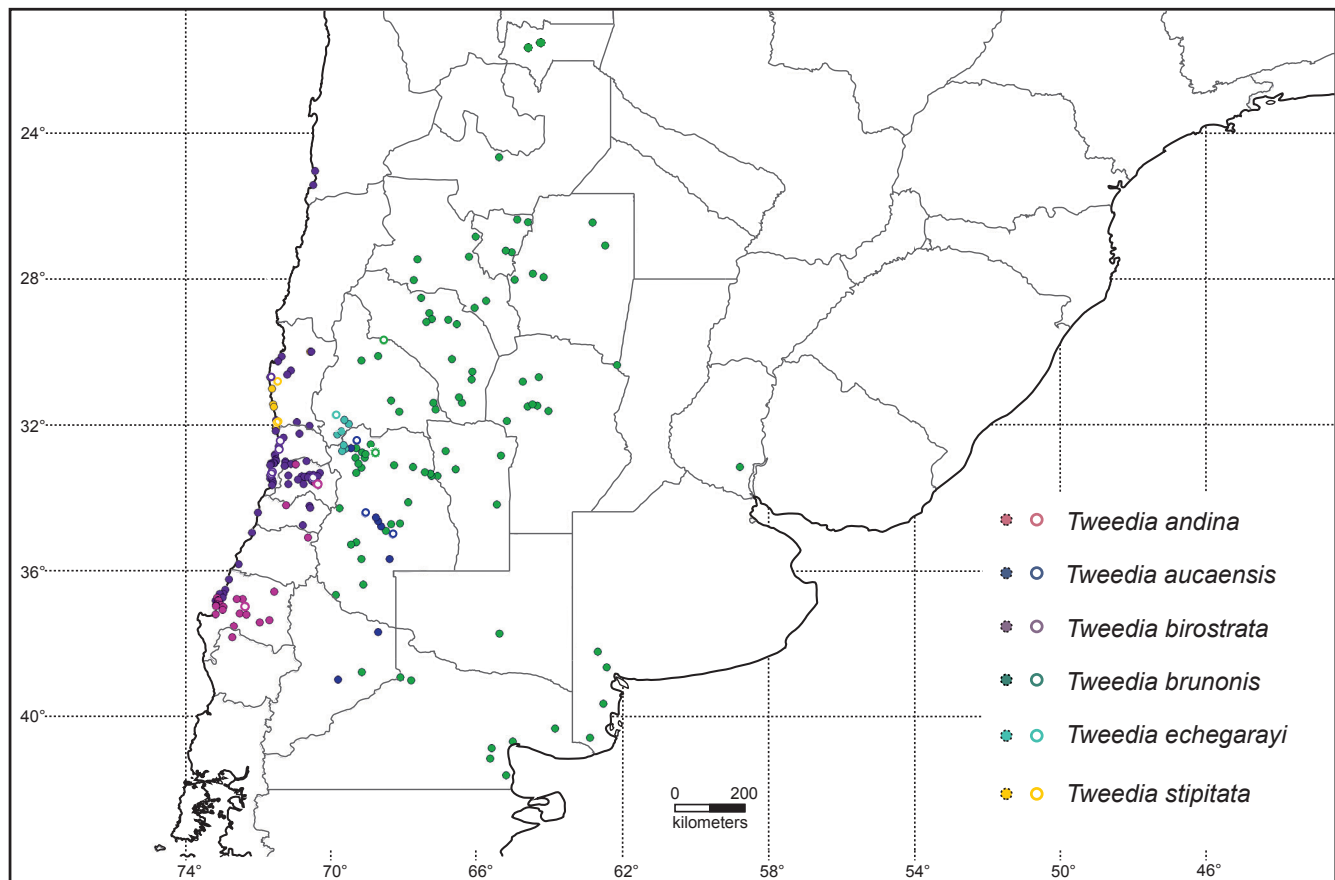
Based on the molecular phylogeny, *Tweedia* includes six species whose revised distributions are presented in Fig. 3.

## DISCUSSION

**Circumscription of *Tweedia*.** — Despite the historically controversial circumscription of *Tweedia*, the results of our work strongly confirm the morphological integrity and monophyly of the genus comprising six species: *T. andina*, *T. aucaensis*, *T. birostrata*, *T. brunonis*, *T. echeagarayi*, and *T. stipitata*. Based on cpDNA sequences, all these species form a highly supported clade that is characterized by a corolline corona with free lobes (rarely united) arising high on the corolla tube. The species until recently accepted as *Tweedia australis* (= *Diplolepis australis*), with a very shortly tubular gynostegial

corona, is the only one that does not fall in this group, confirming the validity of the new combination in *Diplolepis* based on chloroplast and morphological data (Hechem & al., 2011). It would be desirable to corroborate these estimations with additional independent markers, such as nuclear or mitochondrial data, in future studies of *Aclepiadoideae*.

**Relationships of *Tweedia* to other genera.** — Our results also contribute to assess relationships among genera of the taxonomically complex New World MOOG clade of *Asclepiadoideae*. *Tweedia* has been classified in *Oxypetalinae*, comprising the mostly South American *Araujia* Brot., *Funastrum* E.Fourn., *Oxypetalum* R.Br., *Philibertia* Kunth, *Tweedia* Hook. & Arn., and *Widgrenia* Malme (e.g., Goyder, 2004; Rapini & al., 2011; Endress & al., 2014). Here, *Tweedia* falls within the *Oxypetalinae* core group, in a clade together with *Philibertia* and *Araujia* which is sister to *Oxypetalum*. This supports Malme's (1904) early observations that although *Tweedia* seemed related to *Oxypetalum* it also had clear affinities with *Araujia*, and does not support suggestions to merge *Tweedia* and *Oxypetalum* (e.g., Decaisne, 1844; Mabberley, 2008). Although differences between the genera of *Oxypetalinae* are not clearcut (e.g., Goyder, 2003, 2004; Rapini & al., 2011), the combination of detailed morphological studies, molecular phylogenies and information on the distribution of the species helps to unravel their limits and relationships.



**Fig. 3.** Distribution of *Tweedia*. Solid circles represent localities of herbarium specimens, empty circles represent accessions of cpDNA used for this study.



Most genera of Oxypetalinae from southern South America have distribution ranges with little overlap (Malme, 1904; Goyder, 2004; Rapini & al., 2007). Following classical biogeographic divisions of South America (Cabrera & Willink, 1980), *Araujia* (currently including *Morrenia*: Rapini & al., 2011; ca. 15 species) has a subtropical Chaco distribution (Goyder, 2003, 2004; Ezcurra & al., 2008). *Oxypetalum* (ca. 120 species) is a lowland genus mostly of the Cerrado, Atlantic region and Pampas of central and southern Brazil and eastern Argentina (Ezcurra & al., 2008; Rapini & al., 2010, 2011). *Philibertia* (ca. 40 species) is centered in the dry east-Andean valleys of Bolivia and northern Argentina in the southern Yungas, and only a few of its species extend north to Peru and Ecuador, west to Chile, and south to Patagonia (Goyder, 2003, 2004; Ezcurra & al., 2008). The distribution of *Tweedia* is different from all other genera of Oxypetalinae as it is found more to the southwest, in semiarid areas on both sides of the Andes, i.e., mostly in the Monte from southern Bolivia to central Argentina, in the central Andes of western Argentina, and in the Desert and Matorral of central Chile (Rua, 1989; Ezcurra & al., 2008; Fig. 3).

**Evolution of *Tweedia*.** — *Tweedia* species are similar morphologically and sometimes difficult to distinguish. In general, when this is the case, one expects to find poor molecular phylogenetic resolution, too. However, it is noteworthy that the branches that support each of the species of *Tweedia* are on average significantly longer than the branches to the species of other genera of Oxypetalinae (also maximum pairwise sequence divergence is greater within *Tweedia* than within any other genus of Oxypetalinae), suggesting a negative correlation between the evolution of the genome and the phenotype. An increase in substitution rates for a certain lineage could be determined by population size, generation time, DNA repair efficiency, or life history of the species (Bromham, 2009). In order to understand these or other possible causes it is necessary to take into account time as an extra variable, and test the correlation between molecular and morphological rates of change (Seligmann, 2010). Until now, the only attempt to date a molecular phylogeny of the Asclepiadoideae has been within a study of the diversification of this group in the New World (Rapini & al., 2007). However, a comprehensive dated phylogeny of the Asclepiadoideae remains a difficult task because of the absence of fossils and of a detailed phylogeny of the whole subfamily (Liede-Schumann, pers. comm.).

Our phylogeny shows two clades within *Tweedia*, one from Argentina and the other from Chile. The former is found east of the Andes and comprises the geographically restricted semiarid Precordilleran *T. aucaensis* and *T. echeagarayi*, plus (albeit with weak to moderate support) the extended subtropical Monte and southwestern Chaco *T. brunonis*. The other is found west of the Andes and comprises the species from Mediterranean climate, i.e., *T. birostrata*, *T. andina*, and *T. stipitata*. The Monte and Chaco regions have semiarid Monsoon climates with summer rains, whereas central Chile has a Mediterranean climate with winter rains (Armesto & al., 2007). The division into these two lineages of different semiarid climates was probably related to climatic and geologic changes that occurred in South America during the Neogene. The Oxypetalinae have been suggested to

have had a primary diversification in central South America between 15° and 30°S in mid Miocene (Rapini & al., 2007). The ancestor of *Tweedia* therefore probably originated in the Neogene less than 15 million years ago east of the Andes, where the great majority of the species of Oxypetalinae occur (Rapini & al., 2011). As the Apocynaceae subfam. Asclepiadoideae are poorly represented in Chile (Marticorena & Quezada, 1985) and much more diverse in Argentina (Ezcurra, 1999a), the rise of the Andes could have been an important barrier to their dispersal.

Since the middle Miocene, the climate became increasingly cold and arid in South America because of extensive glaciation in Antarctica, the establishment of the cold Humboldt current along the western coast of the continent, and the increase in rain-shadow effects due to the rising of the southern Andes (Gregory-Wodzicki, 2000; Gengler-Nowak, 2002; Ortiz Jaureguizar & Cladera, 2006; Armesto & al., 2007). These events resulted in new arid and semiarid climates such as the Mediterranean of central Chile, with winter rain, that was probably established in the late Neogene (Armesto & al., 2007; Barreda & Palazzesi, 2007). Therefore, the evolution and diversification of *Tweedia*, with species on either side of the Andes of central Chile and Argentina, like many other plant genera of arid regions of the area (e.g., Ezcurra, 2002) could be the results of dispersal across the Andean barrier and the invasion of new habitats that provided novel opportunities for speciation.

## ■ TAXONOMIC TREATMENT

### Key to genera of Oxypetalinae

1. Corolline corona absent or forming a ring around the base of gynostegium only; gynostegial corona present or absent ..... 2
1. Corolline corona present, well-developed; gynostegial corona absent, or corona fused to both base of staminal column and base of corolla, or corona gynostegial with prominent tooth or ligule on ventral face ..... 3
2. Corolla globose, urceolate or campanulate, rarely rotate, shallowly lobed. Gynostegial corona without interstaminal parts. Andean dry valleys from southern Ecuador to northern Argentina, extending into temperate Argentina, Uruguay and SE Brazil ..... *Philibertia*
2. Corolla rotate, deeply five-parted or lobed. Gynostegial corona with interstaminal parts. Warm areas of southern United States and Mexico to northern Argentina ..... *Funastrum*
3. Corona lobes arising high on corolla tube, free, rarely united into a tube; central Chile, southern Bolivia, and western Argentina ..... *Tweedia*
3. Corona lobes arising at or near base of corolla tube .... 4
4. Corona lobes free; erect herbs, sometimes scrambling or twining. Widespread in seasonally dry parts of Central and South America, especially diverse in Pampas of eastern Argentina, southern Brazil and Paraguay .. *Oxypetalum*
4. Corona lobes united into a tube, sometimes united only very shortly at base; scrambling or twining robust creepers,

slightly succulent, frequently glaucous and/or discoloured. Chaco and surrounding regions, principally northern Argentina, Paraguay, SE Bolivia and adjacent parts of southern Brazil ..... *Araujia*

*Tweedia* Hook. & Arn. in J. Bot. (Hooker) 1: 291. Jan 1835, nom. cons. prop. – Type: *Tweedia birostrata* (Hook. & Arn.) Hook. & Arn., typ. cons. prop.  
= *Turrigera* Decne. in Candolle, Prodr. 8: 590. 1844 – Type: *Turrigera inconspicua* Decne. (= *Tweedia brunonis* Hook. et Arn.)

Perennial herbs, erect or prostrate from a thick, woody base, stems relatively thin and frequently twining. Leaves opposite, shortly petiolate or sessile, hastate, sagittate, trullate or linear, more or less cordate, margin generally revolute, glabrous to pubescent. Inflorescence umbelliform, extra-axillary, with 2 to numerous flowers. Calyx with or without glands on inside between lobes. Corolla imbricate in bud, with short tube and elongate, usually twisted lobes. Corolline corona present and well developed, united to basal tube of corolla, composed of 5 more or less free appendices (united in a tube only in *T. brunonis*), bilobed, bifid or emarginate at apex. Gynostegial corona on back of stamens absent. Gynostegium subsessile or stipitate. Stamens with widely ovate or triangular apical membrane. Pollinaria with elliptical corpusculum and generally flattened and subhorizontal or somewhat descending translator arms, pollinia pendant in anther cells. Styler head appendage more or less long and thick, bilobed, or bifid, sometimes entire. Follicles single, fusiform, generally glabrous. Seeds flat and verrucose, with terminal coma of silky hairs.

The genus commemorates John Tweedie (1775–1862), a Scottish botanist and gardener that was born in Edinburgh and lived in Buenos Aires collecting plants in Argentina, Uruguay and Brazil.

Observation: We reject Meyer's (1944) selection of *Tweedia brunonis* and Bullock's (1958) selection of *T. macrolepis* as type of *Tweedia*, and accept Rua's selection of *T. birostrata* as the type. However, we acknowledge that others would accept either Meyer's (1944) or Bullock's (1958) typification, thus rendering Rua's (1989) typification superfluous. Thus, we present a proposal to conserve the name *Tweedia* with the conserved type of *T. birostrata* (Calviño & al., 2014).

### Key to the species of *Tweedia*

1. Leaves narrowly linear, subsessile ..... 2
1. Leaves sagittate, hastate or trullate, shortly petiolate . 3
2. Decumbent suffruticose herb. Corona with 5 bifid, narrowly lanceolate appendices, free. Western Argentina, from Mendoza to Neuquén, in rocky terrain ..... *T. aucaensis*
2. Climbing vine, rarely decumbent herb. Corona with 5 emarginate, oblong appendices, fused and forming a tube. Western Argentina, in the Monte desert .... *T. brunonis*
3. Leaves trullate or hastate (cuneate or truncate at base). Corolla lobes adaxially pubescent at base ..... 4
3. Leaves sagittate, rarely hastate (with a deep sinus or

- rarely truncate at base). Corolla lobes adaxially glabrous ..... 5
4. Leaves densely pubescent. Corona lobes without adaxial protuberance. Mountains of western Argentina, from San Juan to Mendoza ..... *T. echeagarayi*
4. Leaves glabrous. Corona lobes with adaxial protuberance. South central Chile ..... *T. andina*
5. Gynostegium stipitate, styler head with short and inconspicuous appendix ..... *T. stipitata*
5. Gynostegium subsessile; styler head with well-developed, generally bifid appendix ..... *T. birostrata*

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**Appendix 1.** New accessions of *Tweedia* (Apocynaceae) from which non-coding cpDNA *trnT-F* and/or *rps16* sequences were obtained. All other accessions, from Liede-Schumann & al. (2005) and Hechem & al. (2011).

**Taxon**, accepted name, DNA accession no., voucher information, and GenBank reference no. for each data partition (*trnT-L* intergenic spacer, *trnL* intron, *trnL-F* intergenic spacer, and *rps16* intron).

*Tweedia andina* (Phil.) G.H.Rua, DNA no. CC-155, Chile, Reg. VIII, Prov. Bío Bío, camino entre Cabrera y General Cruz a 5 km de Cabrera, 25-Nov-99, *Baeza 1691* (CONC), KJ205515, KJ205487, KJ205499, KJ205471; *Tweedia andina* (Phil.) G.H.Rua, DNA no. CC-156, Chile, Reg. Metrop., Prov. Cordillera, Pirque, Reserva Río Clarillo, Dec-00, *Teillier 4736* (CONC), KJ205516, KJ205488, KJ205500, KJ205472; *Tweedia aucaensis* G.H.Rua, DNA no. CC-180, Argentina, Prov. Mendoza, Depto. Las Heras, Paramillo de Uspallata, 10-Feb-64, *Roig 4938* (MERL 23335), KJ205517, KJ205489, KJ205501, KJ205473; *Tweedia aucaensis* G.H.Rua, DNA no. CC-182, Argentina, Prov. Mendoza, Depto. San Carlos, entre Ao. El Hondo y Ao. La Faja, 1-Dec-82, *Ambrossetti & Del Vito s.n.* (MERL 34303), KJ205518, KJ205490, KJ205502, KJ205474; *Tweedia aucaensis* G.H.Rua, DNA no. CC-183, Argentina, Prov. Mendoza, Depto. San Rafael, cuadro Benegas, borde S del cráter del volcán Diamante, 3-Dec-82, *Ambrossetti & Del Vito s.n.* (MERL 34564), KJ205519, KJ205491, KJ205503, KJ205475; *Tweedia birostrata* Hook. & Arn., DNA no. CC-157, Chile, Reg. IV, Prov. Choapa, Pichidanguí, playa, en matorral de borde, 10-Dec-10, *Ezcurra 3704* (BCRU), KJ205520, KJ205492, KJ205504, KJ205476; *Tweedia birostrata* Hook. & Arn., DNA no. CC-158, Chile, Reg. V, Prov. San Antonio, entre Algarrobo y Mirasol, 11-Nov-87, *Matthei 324* (CONC), –, KJ205493, KJ205505, KJ205477; *Tweedia birostrata* Hook. & Arn., DNA no. CC-159, Chile, Reg. V, Valparaíso, Tunquén, estero Casablanca, 1-Nov-01, *Teillier 5882* (CONC), KJ205521, –, KJ205506, KJ205478; *Tweedia birostrata* Hook. & Arn., DNA no. CC-160, Chile, Reg. IV, Provincia Limari, PN Fray Jorge, few kms before entrance, 4-Nov-06, *Tepe 1741* (CONC 167803), KJ205522, –, KJ205507, KJ205479; *Tweedia birostrata* Hook. & Arn., DNA no. CC-161, Chile, Reg. V, Provincia Petorca, S of Cachahua, N of Quintero, 10-Nov-06, *Tepe 1954* (CONC 168017), KJ205523, –, KJ205508, KJ205480; *Tweedia birostrata* Hook. & Arn., DNA no. CC-162, Chile, Reg. Metrop, Comuna La Reina, Quebrada de Ramón, 9-Sep-00, *Tomé 312* (CONC), KJ205524, –, KJ205509, KJ205481; *Tweedia brunonis* Hook. & Arn., DNA no. CC-186, Argentina, Prov. San Juan, Depto. Jachal, Río Gualcamayo, 14-Feb-00, *Kiesling 9491* (SI), KJ205525, KJ205494, KJ205510, KJ205482; *Tweedia echagarayi* Malme, DNA no. CC-163, Argentina, Prov. San Juan, Calingasta, camino al Pachón, 1-Dec-10, *Kiesling 10414* (BCRU), KJ205526, KJ205495, KJ205511, KJ205483; *Tweedia stipitata* G.H.Rua & Liede, DNA no. CC-164, Chile, Reg. IV, Limari, Termas de Socos, 4 km S de Co. Lumi Lumi, 8-Oct-96, *Heyne 105* (CONC), KJ205527, KJ205496, KJ205512, KJ205484; *Tweedia stipitata* G.H.Rua & Liede, DNA no. CC-165, Chile, Reg. IV, Prov. Choapa, Los Vilos, 2 km S de la ciudad, 11-Oct-96, *Heyne 108* (CONC), KJ205528, KJ205497, KJ205513, KJ205485; *Tweedia stipitata* G.H.Rua & Liede, DNA no. CC-166, Chile, Reg. IV, Prov. Choapa, Los Vilos, Qda. El Boldo, 10-Oct-96, *Heyne 107-A* (CONC), KJ205529, KJ205498, KJ205514, KJ205486.