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## *Oxypetalum marchesii* (Apocynaceae, Asclepiadoideae), an Endemic New Species from Uruguay

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**Abstract**—A new species, *Oxypetalum marchesii*, from rocky grassland environments of Uruguay, is here described, illustrated, and compared to morphologically similar species. Data on its habitat, distribution, ecology, and conservation status are also provided. Based on its cpDNA, *O. marchesii* clearly corresponds to the recently re-circumscribed and morphologically diverse genus *Oxypetalum*. This species is mostly characterized by its creeping, non-twining habit, widely ovate and shortly petiolate hirsute leaves, relatively large flowers with pale greyish corollas, and short stylar-head appendage that is included within a corona formed of oblong-lanceolate, bifurcate lobes. The conservation status of this species is considered vulnerable because of its small geographic range and restricted population size.

**Keywords**—cpDNA, conservation, flower morphology, grasslands, Oxypetalinae, Pampas flora.

In the last ten years, several publications on Apocynaceae have brought greater resolution to the phylogeny of this large and complex family, allowing better understanding of the relationships among genera and finding their position in an updated classification (e.g., Endress et al. 2014 and references therein). In this recent classification, *Oxypetalum* R. Br. is placed in subtribe Oxypetalinae E. Fourn. of tribe Asclepiadeae Duby, together with ca. six other genera whose circumscriptions are currently being revised (e.g. Endress et al. 2014; Calviño et al. 2014; Liede-Schumann and Meve 2015).

Within the Oxypetalinae, *Oxypetalum* is the largest genus with approximately 120 species, distributed from Mexico to central Argentina (Farinaccio 2005; Farinaccio and Keller 2014; Farinaccio and Goyder 2016). Morphologically, this genus is very diverse, but can generally be characterized by showy flowers, corollas with lanceolate lacinia, a corona of free lobes arising at the base of the corolla tube, a rostrate stylar-head, well developed corpusculum (about the size of the pollinia or larger), and lateral teeth on the caudicles (Meyer 1943, 1944; Goyder 2004b; Farinaccio 2008; Rapini et al. 2011; Farinaccio and Mello-Silva 2013). It has an important center of diversification in the Cerrado, Atlantic region, and Pampas (sensu Cabrera and Willink 1973) of central and southern Brazil, Argentina, and Uruguay (Meyer 1944; Farinaccio 2005; Rapini et al. 2010, 2011). In recent catalogues, approximately 96 species have been reported from Brazil (Rapini et al. 2010), 41 from Argentina (Farinaccio and Keller 2014), 31 from Paraguay (Fontella-Pereira et al. 2010), 20 from Bolivia (Goyder et al. 2014), and 17 from Uruguay (Ezcurra et al. 2008).

Although the species of *Oxypetalum* from Uruguay were only treated as a whole at the beginning of the twentieth century (in *Flora Uruguaya*, Arechavaleta 1909), many of its species were also included in classical works on Brazilian Asclepiadoideae (e.g. Fournier 1885; Hoehne 1916), and also in later regional floras of nearby regions. For example, Meyer (1943, 1944) performed an important taxonomic treatment of the species present in Argentina that comprises most of the species from Uruguay. Later, other revisions that include species from Uruguay were performed in Argentina (e.g. Fabris 1965; Meyer and Bacigalupo 1979; Pontiroli 1983; Hechem and Ezcurra 2006), Brazil (e.g. Fontella Pereira et al.

2004; Farinaccio 2005; Marquete Ferreira da Silva et al. 2007), Paraguay (Fontella Pereira et al. 2010), and Bolivia (Farinaccio and Goyder 2016). Also, several new species from Brazil, Argentina, and Bolivia have been discovered and described in recent years (e.g. Farinaccio and Mello-Silva 2006; Farinaccio and Keller 2014; Keller 2015; Farinaccio and Goyder 2016; Martín et al. 2017; Keller and Funez 2017). In addition, information provided by recent phylogenetic studies within the Oxypetalinae have resulted in a re-circumscription of *Oxypetalum* and related genera (e.g. Goyder 2004a, b; Liede-Schumann et al. 2005; Rapini et al. 2006; Rapini et al. 2011; Calviño et al. 2014; Liede-Schumann and Meve 2015). Also, a recently accomplished phylogenetic study of the genus provides information on species relationships (Farinaccio 2008). Although we still are far away from a complete and detailed knowledge of this large and diverse genus, all these studies allow the ongoing revision and identification of species present in Uruguay.

Floristic studies on the vegetation of Uruguay performed by the first author resulted in the finding of a new species of *Oxypetalum*, which we here describe, illustrate, and compare to morphologically similar species. Data on its habitat, distribution, ecology, and conservation status are also provided. To verify its placement within the recently re-circumscribed *Oxypetalum*, we also include a molecular analysis of cpDNA *trnT-trnF* and *rps16* regions which places the species within the phylogeny of Oxypetalinae.

### MATERIALS AND METHODS

**Taxonomy**—Our study included fieldwork in grasslands of Uruguay plus analyses of Apocynaceae-Asclepiadoideae material from herbaria pertinent to the flora of the Pampas region (MVFA, MVJB, MVM, and SI), as well as consideration of the relevant literature (see Introduction). We also studied nomenclatural types of *Oxypetalum* from these herbaria, plus high definition digital images of types in other herbaria from JSTOR (<http://plants.jstor.org>). Morphological observations and measurements of vegetative and floral characters were performed on live plants in the field, and by using a stereoscopic microscope with camera lucida on hydrated material from dry herbarium specimens. The conservation status of the new species was assessed using the IUCN Red List Criteria (IUCN 2017).

**Molecular Analyses**—Leaf material for DNA extraction was obtained from plants collected in the field. The protocols used to obtain the *trnT-trnF* and *rps16* intron were presented in Calviño et al. (2014). The newly obtained cpDNA sequences correspond to the type specimen (C. Ezcurra & A. González 3774 (SI), DNA no. CC-561) and have been submitted to GenBank

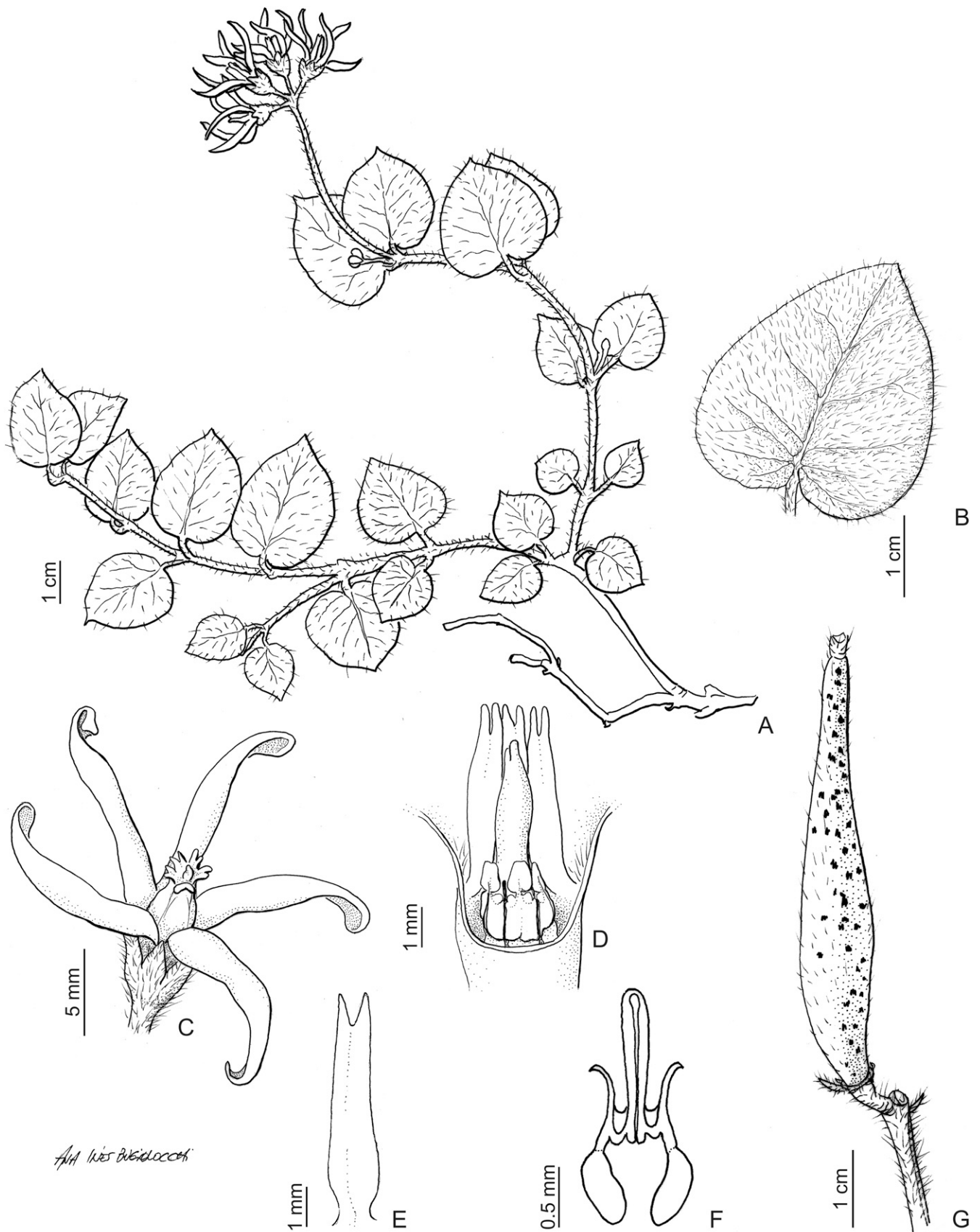


FIG. 1. *Oxypetalum marchesii*. A. Flowering stem. B. Detail of leaf pubescence. C. Flower with corona. D. Flower, longitudinal section showing calyx, corolla, corona, and gynostegium, with stamens and thick stylar-head included within corona. E. Corona appendix. F. Pollinarium with corpusculum, caudicles with free teeth, and pollinia. G. Fruit. Based on: C. E. 3774 (isotype: SI). Drawn by Ana Inés Bugliolochi.

(*trnT-L* intergenic spacer MG840945, *trnL* intron MG840943, *trnL-F* intergenic spacer MG840944 and *rps16* intron MG840942). Combined *trnT-F* and *rps16* intron data matrices, with and without their scored indels, were analyzed using maximum parsimony (MP), Bayesian inference (BS), and maximum likelihood (ML) methods using the settings described in Calviño et al. (2014).

#### TAXONOMIC TREATMENT

*Oxypetalum marchesii* C. Ezcurra & A. González *sp. nov.* TYPE: URUGUAY. Dep. Maldonado: Punta Ballena, pastizales rocosos del lado oeste, rastrera, flor grisácea, 04 Nov 2017, C. Ezcurra & A. González 3774 (holotype MVM 23414!; isotype SII!).

Similar in prostrate, creeping habit and ovate, shortly cordate leaves to *O. patulum* E.Fourn., but differs in the flowers with calyx lobes generally shorter than the corolla tube (vs. much longer), the long, bifurcate corona lobes (vs. short and rounded), and the almost undivided and thick stylar appendix included within the corona (vs. divided into two slender arms, long-exserted from the corona). In its creeping habit and leaf shape it is also similar to *O. tomentosum* Hook. & Arn., from which it differs in the flowers with bifid corona segments (vs. truncate and slightly emarginate), without an internal appendix (vs. with an appendix in the adaxial side), and in its included and only slightly divided stylar-head appendix (vs. long-exserted beyond the corona and markedly bifid). In floral characteristics it is similar to *O. crispum* Wight, from which it

principally differs by the deeply divided corona lobes (vs. shortly bifurcate), creeping habit (vs. erect), and widely ovate, flat leaves (vs. less wide and marginally undulate).

Plant suffrutescent, creeping, branches horizontal, 15–30(50) cm long, lactescent. Stems terete, glabrous near the base, hirsute-pubescent towards the apex, the trichomes 0.5–1 mm long, internodes 2–4 cm, with obsolete interpetiolar colleters. Leaves opposite, petiole 0.4–1 cm long, hirsute, with trichomes 0.5–1 mm long, blades 1.5–3.5 × 1.5–2.5 cm, ovate, flat, and parallel to the ground, the basal-most smaller, all adaxially and abaxially densely hirsute, more or less coriaceous, apex obtuse and slightly acuminate, base cordate, with 2 tiny colleters near the insertion of the petiole on the adaxial face, obscured by the dense pubescence. Inflorescence extra-axillary, appearing terminal, umbelliform, 4–6-flowered, peduncule 4–5 cm long, hirsute-pubescent, pedicels 0.5–1 cm long, densely pubescent. Calyx divided almost to the base, lobes 4–5 × 2–3 mm, ovate-lanceolate, apex acute, densely pubescent abaxially and glabrous adaxially, with 4–5 colleters in clusters below each sinus, the centrals longer. Corolla pale gray, sometimes slightly tinted with pink, tube 3.8–4.2 mm long, campanulate, outside hirsute-pubescent, inside hairy under the throat, lobes 11–13 × 1.6–2 mm, narrowly oblong-lanceolate, patent and markedly contorted, densely pubescent abaxially and glabrous adaxially, apex acute. Corona corolline, the lobes approx. 5.8–6 × 1.2 mm, free and arising near the base of the corolla tube, narrowly oblong-lanceolate, without internal appendices, the apex bifid with each portion approx. 1 mm long. Gynostegial

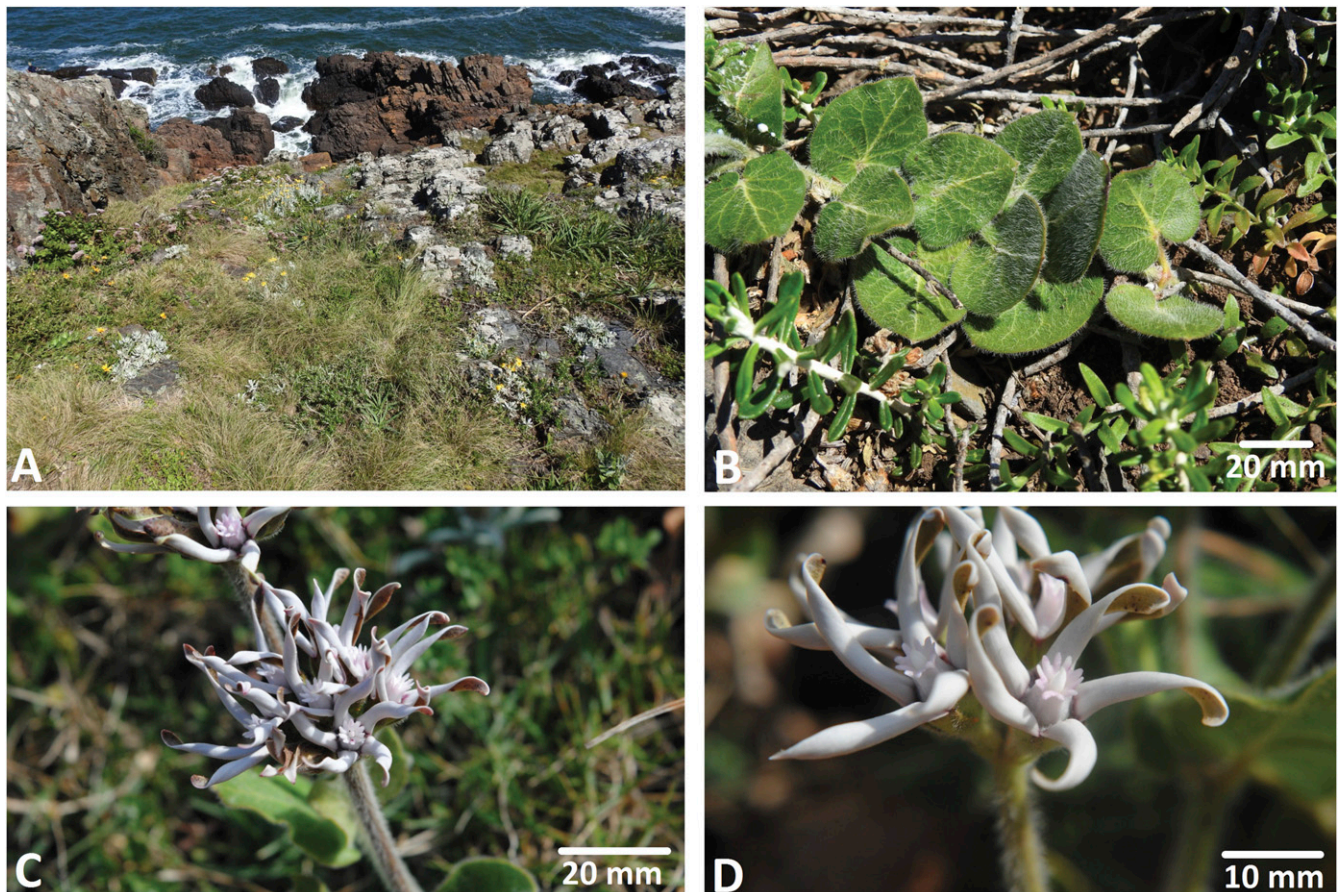


FIG. 2. *Oxypetalum marchesii*. A. Habitat in rocky grasslands of Punta Ballena, Maldonado, Uruguay. B. Creeping stem with leaves. C. Inflorescence. D. Mature flower with corolla, corona, and included stylar-head appendage. Photographs by Andrés González.



corona absent. Gynostegium sessile. Anthers  $2.5\text{--}3 \times 1\text{--}1.2$  mm, rectangular, terminal appendage approx.  $0.8 \times 1$  mm, narrowly triangular-ovate. Corpusculum  $1.1\text{--}1.2 \times 0.18\text{--}0.22$  mm, narrowly oblong, with a frontal slit, apex obtuse, caudicles

$0.26\text{--}0.28$  mm long, horizontal, flattened, translucent in part, with free prominent recurved teeth  $0.5$  mm, pollinia  $0.55\text{--}0.65 \times 0.24\text{--}0.25$  mm, ovoid, shorter than corpusculum. Styler-head extended beyond the anthers forming a thick cylindrical

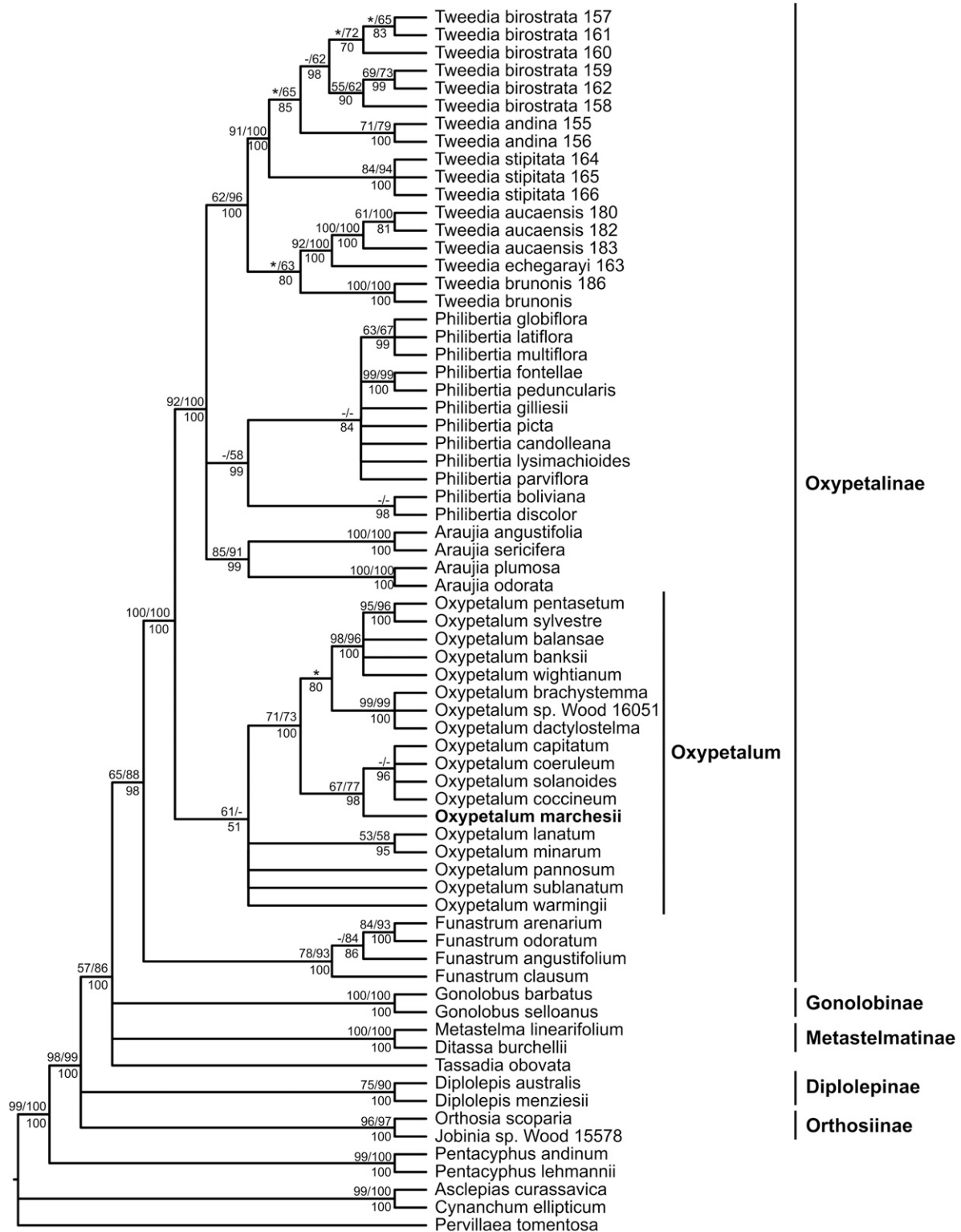


FIG. 3. Position of *Oxypetalum marchesii* in subtribe Oxypetalinae. Tree from a majority-rule consensus of 24,300 trees derived from Bayesian analysis of 69 non-coding cpDNA *trnT-trnF* and *rps16* sequences plus indels. Except for this taxon, the tree is identical to Fig. 2 in Calviño et al. (2014). Backbone with maximum parsimony (MP) and maximum likelihood (ML) 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.

appendage 2.9–3.1 × 1 mm, slightly divided in the end, included within the corona. Immature follicles erect, narrowly ovoid, 6–7 × 1–1.5 cm, seeds not seen (Figs. 1, 2).

**Etymology**—The name of this species is given in honor of Eduardo H. Marchesi, a collector of the species, for his great knowledge and deep interest in the flora of Uruguay.

**Phenology**—Flowering from October to December, fruiting from November to January.

**Distribution, Habitat, and Ecology**—*Oxypetalum marchesii* is known only from southeastern Uruguay, departments of Maldonado and Treinta y Tres. It has been found growing on grasslands on rocky ground. Explorations of the region since the beginning of the last century have resulted in collections from only three localities until now (Sierra de Ánimas, Punta Ballena, and Quebrada de los Cuervos, see Specimens Examined) dispersed in a narrow strip along a distance of approx. 200 km. At the type locality, *O. marchesii* grows in a rich herbaceous community along with *Grazielia brevipetiolata* (Sch.Bip. ex Baker) R.M.King & H.Rob., *Schlechtendalia luzulaefolia* Less., and *Senecio ostenii* Mattf. (Asteraceae), *Dyckia remotiflora* Otto & A.Dietr. (Bromeliaceae), *Croton lanatus* Lam. (Euphorbiaceae), *Lupinus multiflorus* Desr. (Fabaceae), *Dorstenia brasiliensis* Lam. (Moraceae), and *Bromus auleticus* Trin. ex Nees and *Elionurus muticus* (Spreng.) Kuntze (Poaceae).

**Conservation**—Following the criteria proposed by IUCN (2017) to evaluate if a taxon is threatened, the species appears vulnerable because of several characteristics related to geographic range and population size. Based on the evidence of current herbarium collections and our personal observations taking into account IUCN red list criteria (2017), the whole extent of occurrence of the species is probably < 5000 km<sup>2</sup>, its area of occupancy seems < 500 km<sup>2</sup>, and the species has been found in < 5 localities (see herbarium collections). In the locality where it has been observed recently (Punta Ballena, Ezcurra and Gonzalez pers. obs.), the species is not abundant, being represented by few individuals. In addition, in Punta Ballena, the plants of *O. marchesii* could be affected by the numerous visitors to this touristic site, and the local population of the species could be reduced if construction continues in the area. Therefore, following the IUCN criteria, the conservation of this species appears vulnerable.

**Additional Specimens Examined**—Uruguay.—MALDONADO: Cerro de las Ánimas, rastrera en lugar pedregoso, 24 Marzo 1899, *M. C. Berro* 2397 (MVFA); Sierra de las Ánimas, en ladera este, pétalos grises, 6 Diciembre 1964, *E. H. Marchesi* 1312 (MVFA); Sierra de las Ánimas, en ladera sur, 400 m, pétalos blancos, 22 Noviembre 1981, *E. H. Marchesi* 16846 (MVFA); Maldonado, Punta Ballena, flores grises claro, 22 Noviembre 1971, *A. Lombardo et al.* 10950 (MVFA); —TREINTA Y TRES: Quebrada de los Cuervos, 12 Noviembre 1965, *O. Crossa*, *O. Del Puerto* & *E. H. Marchesi* 5475 (MVFA).

**Molecular Analyses**—All MP, Bayesian, and ML analyses of *trnT-F* and *rps16* intron data place *Oxypetalum marchesii* in a group that includes *O. banksii* Schult., type species of the genus, with high support (Fig. 3).

## DISCUSSION

The genus *Oxypetalum* belongs to the Oxypetalinae together with *Araujia* Brot., *Funastrum* E. Fourn., *Philibertia* Kunth, and *Tweedia* Hook. & Arn. (e.g. Calviño et al. 2014; Liede-Schumann and Meve 2015). It has recently been re-circumscribed taking into account molecular characters, and currently comprises several species that were previously assigned to other genera because of their morphological characteristics (e.g. Rapini et al. 2011; Liede-Schumann and

Meve 2015). Even though morphological differences between genera of Oxypetalinae are not clear cut, which makes the generic assignment of some of its species difficult, the combination of morphology with molecular information has shown to be very useful for this object (e.g. Calviño et al. 2014). Therefore, this work includes molecular analyses of cpDNA sequences of *O. marchesii* to verify its placement within the re-circumscribed *Oxypetalum*. Results of these analyses (Fig. 3) show its robust phylogenetic position within the genus, allied to the type species of the genus. Also, morphologically, *O. marchesii* can be included within *Oxypetalum* because of its showy flowers, corolla with lanceolate lacinia, corona of free lobes arising near the base of the corolla tube, rostrate stylar-head, well developed corpusculum larger than the pollinia, and prominent lateral teeth on the caudicles (e.g. Farinaccio 2008; Rapini et al. 2011; Farinaccio and Mello-Silva 2013).

Within the genus, it is outstanding in its creeping, non-twining habit, widely ovate, shortly petiolate hirsute leaves, relatively large flowers with pale greyish corollas, and short stylar-head appendage that is included within the corona. In its habit and leaf shape, it is similar to *Oxypetalum patulum* E. Fourn. from southern Brazil, from which it differs in the flowers with calyx lobes generally shorter than the corolla tube (vs. much longer), the long, bifurcate corona lobes (vs. short and rounded), and the short, thick, stylar appendix, nearly undivided, included within the corona (vs. long and divided into two slender arms, long-exserted from the corona). In these floral characteristics, *O. marchesii* is similar to the widely distributed and variable *O. crispum* Wight ex Hook., also found in Uruguay, from which it principally differs by the deeply divided corona lobes (vs. shortly bifurcate), creeping habit (vs. erect), and widely ovate, flat leaves (vs. less wide and marginally undulate). An *Oxypetalum* species with habit and leaf shape similar to *O. marchesii* that is present in Uruguay is *O. tomentosum* Hook. & Arn., which differs from *O. marchesii* because it presents creeping but longer and frequently twining branches, and flowers that are smaller, greenish-white, and with a longer and divided stylar-head appendix that is bifid from its central part and clearly exserted from the corona.

*Oxypetalum marchesii* is a showy plant with its relatively short, creeping branches, wide, pubescent leaves, and large, grayish flowers, but it is relatively under-collected. It appears to be a rare and vulnerable species whose conservation may be threatened because of its restricted distribution in a small area of southeastern Uruguay, which in some instances is being modified by human pressure. Future collections will be useful to acquire more information on its biology, abundance, and conservation status.

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## AUTHOR CONTRIBUTIONS

AG found and photographed the species, collected material, studied additional material, and wrote part of the manuscript. MF performed the

laboratory molecular analyses and phylogenetic study, and wrote part of the manuscript. CE collected material, studied additional material, and conceived, designed, and wrote the manuscript.

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