



Petunia toropiensis (Solanaceae): A surprising new species endemic from Toropi river basin in southern Brazil

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ABSTRACT

A new species of *Petunia* (Solanaceae) is described and illustrated for the Brazilian flora. *Petunia toropiensis* Stehmann & Larocca is known only from a few sites along the middle Toropi river basin, in the central region of the Rio Grande do Sul state. The new species is easily recognizable by its erect or ascending habit, infundibuliform corolla with yellow tube and purple limb, yellow pollen, and erect peduncle in fruiting. The species was assessed as Critically Endangered due to the narrow geographic distribution, the few populations known, and the loss of habitat quality. A key for identification of *Petunia* species from Rio Grande do Sul is given. We also discuss the impact of the dams on the populations of the new species and the conservation efforts needed.

Keywords: Conservation status, endemism, IUCN, melithophilly, short-thonged bees.

Introduction

Petunia Juss. (Solanaceae) is one of the most important genera of the Solanaceae, especially for its ornamental use (Hawkes 1999; Stehmann *et al.* 2009), but also as a model for plant genetic studies (Bombarely *et al.* 2016; Vandebussche *et al.* 2016). It is broadly known from the hybrids widely cultivated as ornamentals all over the world. The origin of the cultivated plants dates back to the 19th century, when seeds of two phenotypically different species with relatively wide distribution in subtropical South America, *P. axillaris* (Lam.) Britton, Sterns & Poggenb. (white) and *P. integrifolia* (Hook.) Schinz & Thell. (purple), were sent to Glasgow by

Tweedie in 1827 (Stehmann *et al.* 2009). There, the species were firstly crossed, producing fertile hybrids that were spread to other countries of Europe in the following decades (Hooker 1831; 1837; Vilmorin 1863).

The genus has 15 species, with known distribution in Argentina, Bolivia, Brazil, Paraguay and Uruguay, half of them narrowly endemic (Stehmann *et al.* 2009; Greppi *et al.* 2019). The greatest richness is found in Brazil, where 13 species (87%) occur, with centers of diversity in the grasslands of southern Brazil (Stehmann *et al.* 2009; Stehmann 2022).

Phylogenetic studies using molecular markers have reconstructed its evolutionary history, which is now better understood. There are two main clades: one distributed

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predominantly in the pampas and sub-Andean region (mostly from lowlands), characterized by the tubular corolla and yellow pollen, and another in the grasslands of the southern Brazilian plateau (predominantly from highlands), with flowers that are usually short, infundibuliform or campanulate, and bluish pollen (Fregonezi *et al.* 2013). Endemisms are frequent in both the lineages, being generally associated with particular environments, especially rocky outcrops, as is the case of three species restricted to the Brazilian territory: *P. saxicola* L.B. Sm. & Downs, which occurs in the state of Santa Catarina, and *P. exserta* Stehmann and *P. secreta* Stehmann & Semir in Serra do Sudeste, at Rio Grande do Sul state (Lorenz-Lemke *et al.* 2006; Stehmann *et al.* 2009). Adaptive radiation for different groups of pollinators played an important role in the diversification process within the lineage with tubular corolla (Rodrigues *et al.* 2018).

Petunia can be considered a well-studied genus of Solanaceae due to several articles published on its systematics, taxonomy, reproductive biology and evolution in last decades (e.g., Ando *et al.* 2001; 2005; Gübitz *et al.* 2009; Kulcheski *et al.* 2006; Stehmann *et al.* 2009; Stehmann & Greppi 2013; Reck-Kortmann *et al.* 2014; Segatto *et al.* 2014a; b; Teixeira *et al.* 2019; 2021; Turchetto *et al.* 2015a; b; 2016). Most species of *Petunia* have showy and large flowers, making them easy to collect by attracting the attention of botanists, with the exception of the narrow endemic ones. That was the case of *Petunia secreta*, a species found only in the rocky outcrops in central Rio Grande do Sul (Stehmann & Semir 2005).

During floristic studies in the Toropi river basin at the central region of the state of Rio Grande do Sul, where four small hydroelectric dams are under construction, we found populations of a showy *Petunia* morphologically distinct from other species described for the genus. In this work we accept it as a new species, and present a full morphological description, illustrations, preliminary conservation status, and a key to the species from Rio Grande do Sul state. We also discuss the diagnostic characters of this new taxon, as well as its relationship with related species and conservation efforts needed.

Material and methods

The exsiccatae of *Petunia* from the following Brazilian herbaria were revised (acronyms following Thiers 2022 onwards): BHC, FURB, HAS, ICN, MBM, PACA, R, RB and SPF. We studied four populations of the species in the field for two consecutive years (2020–2021), when five flowers per population were fixed in alcohol for further morphological description. Plant samples representatives from each population were deposited in the BHC herbarium of the Centro de Coleções Taxonômicas at the Universidade Federal de Minas Gerais (CCT-UFMG), where the morphological

description was done. We also occasionally observed diurnal floral visitors, sampling these insects for later identification and depositing them in the entomological collection of the CCT-UFMG.

The occurrence records were plotted in the map generated in the Quantum GIS 3.10.14 software (QGIS 2022) using layers available at IBGE (2022). The conservation status of the new species was assessed through the guidelines of the International Union for Conservation of Nature (IUCN 2019). For this purpose, we visited the occurrence area of the species twice aiming to evaluate the habitat quality and size of the populations. The area of occupancy (AOO) and extent of occurrence (EOO) were calculated using Geospatial Conservation Assessment Tool, GeoCAT (Bachman *et al.* 2011), with grid resolution of 2km.

Results

Taxonomic treatment

Petunia toropiensis Stehmann & Larocca, *sp. nov.* **Type:** BRAZIL. Rio Grande do Sul: São Martinho da Serra, estrada para a antena, 29°27'08.44" S, 54°05'21.96" W, 197m, 9 November 2021 (fl., fr.), J.R. Stehmann, J. Larocca & R. Vasconcelos 6557 (holotype BHC206043!, isotypes ICN!, MBM!). (Figs. 1A–F, 2A–C, 3D).

Diagnosis: *Petunia toropiensis* has a unique set of morphological traits: erect and ascendant habit, infundibuliform corolla with purple limb and yellow throat, yellow pollen, and erect fruiting peduncle. The new species shares the yellow pollen, the erect peduncles in fruiting and the small seeds (< 0.6 mm long) with *P. axillaris*, *P. exserta* and *P. secreta*, but differs from these species by its infundibuliform corolla (*vs.* hypocrateriform corolla).

Annual herbs up to 1.5 m high, erect or ascendant, viscose; trichomes 0.4–1.2 mm long, simple, multicellular, with glandular apex; stems up to 1 cm diam., cylindrical, hollow, erect, later very ramified, producing basal ascendant secondary branches, pilose; internodes 20–70 (–100) mm long. Leaves evenly distributed along the stem, the blade 58–120 x 24–50 mm, deltate-ovate, ovate to elliptic, acute at apex, long-attenuate at base, the base forming sometimes a winged petiole in the basal leaves, with up to 2 cm long, shortening towards the apex, pilose, brochidodromous, midrib sunken adaxially and slightly prominent abaxially, secondary veins 3 or 4 pairs, the first pair rising near to the base and slightly divergent. Inflorescence with numerous sympodial units, each one composed of 1 flower associated with 2 opposite leaflike bracts; bracts 15–30 mm long, 5–11 mm wide, ovate, sometimes deltoid, with base short-attenuate or sessile, cuneate, rounded or subcordate; internodes 15–50 mm long, generally shorter than vegetative ones, pilose. Flowers without perceptible fragrance; peduncle 11–18 mm long, pilose; calyx deeply





Figure 1. *Petunia toropiensis* Stehmann & Larocca. A. Habit of a mature plant, with many branches; B. Young plant; C. Flower, in lateral view; D. Flower, in front view, showing the yellow throat and anthers; E. Flower with ripped corolla; in the detail, the stamens arrangement (two longer, two middle, one shorter), and the bilobed stigma placed between the longer and middle stamens pairs. F. Flower in front view, visited by *Hexanthera missionica* (Colletidae). A-B, E: without vouchers; D: Stehmann *et al.* 6557 (BHCB); C, F: Stehmann *et al.* 6552 (BHCB). Photographs 1A, C-F by J.R.Stehmann; B by J. Larocca.

lobed, tube 3–4 mm long, lobes 6.3–10 mm long, 2–2.5 mm wide, narrow-oblong to narrow-oblongate, apex obtuse; corolla infundibuliform, tube 15–20 mm long, base subcylindrical, then gradually expanded towards the apex, externally yellow, with marked veins, throat semilunar, 7–8 mm wide, yellow, purple net-veined, limb 30–45 mm diam., purple, lobes 13–17 mm long, 17–21 mm wide, obtuse; stamens 5, included, arranged in three distinct lengths; filaments with the free portion 4–5.2 mm, 4.5–5.7 mm, and 7.5–8 mm long, apex of the filaments curved, the adnate portion 4.5–6 (–7) mm long; anthers yellow, ca. 1.5 mm

long; nectariferous disc reduced to two lobes; ovary 3–4 mm long, conic or ovoid, style 11–12 mm long, stigma bilobed, located located between the middle and longer stamens. Capsule 2-valved, 9–14 mm long, 4–7 mm wide, ovoid, calyx with enlarged, unequal lobes, lobes 10–17 mm long, 2–3 mm wide, longer than the length of the capsule; fruiting peduncle 14–29 mm long, erect; many seeds, 0.48–0.55 mm long, subglobose. Chromosome number unknown.

Etymology: The specific epithet is a reference to the place of occurrence of the species, only found in the Toropi river basin, in Rio Grande do Sul state.

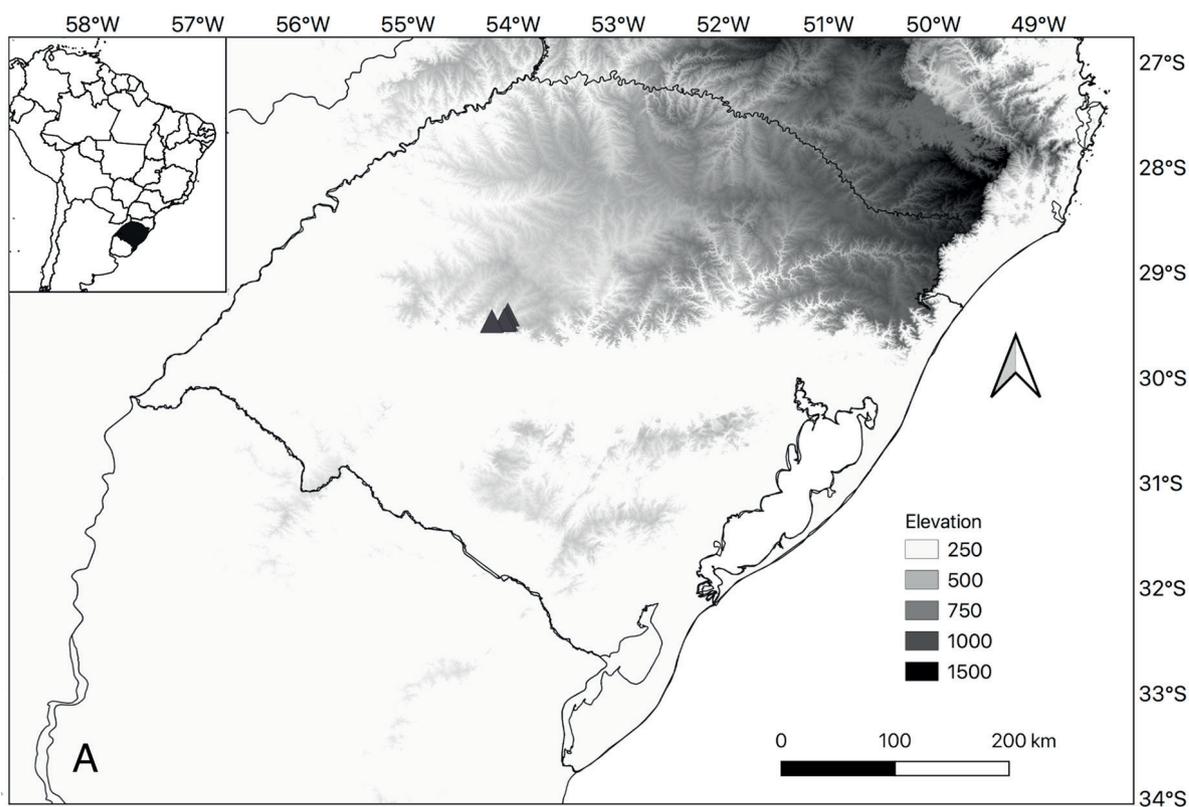


Figure 2. Distribution and habitat of *Petunia toropiensis* Stehmann & Larocca. A. Sites of occurrences (triangles) in Rio Grande do Sul state, Brazil. B-C. Original habitat, the rocky escarpments along the Toropi river. Photographs 2B-C by J. Larocca.

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Pollination and dispersal: The flowers were visited by bees, which were active only when the sunshine reflected on the corolla. The following species, *Hexanthera missionica* (Colletidae), *Callonychium* cf. *petuniae* (Andrenidae) and *Pseudagapostemon* sp. (Colletidae) were recorded visiting the flowers. Females of *H. missionica* (Fig. 1F) and *Pseudagapostemon* sp. actively collected pollen, while *Callonychium* was only observed patrolling the flowers. The capsular fruit produces hundreds of small seeds, which are dispersed by autochory.

Distribution, habitat and phenology: *Petunia toropiensis* is endemic to the Toropi river basin, at the municipalities of Quevedos, São Martinho da Serra and Toropi, in the Rio Grande do Sul state, southern Brazil (Fig. 2A). The main habitat of the species is the sunny rocky outcrops or escarpments along the riverbank (Fig. 2B-C), but it can also be found in disturbed places, where the soil was revolved (Fig. 1A-B). Flowering and fruiting specimens have been collected during the spring, from November to December.

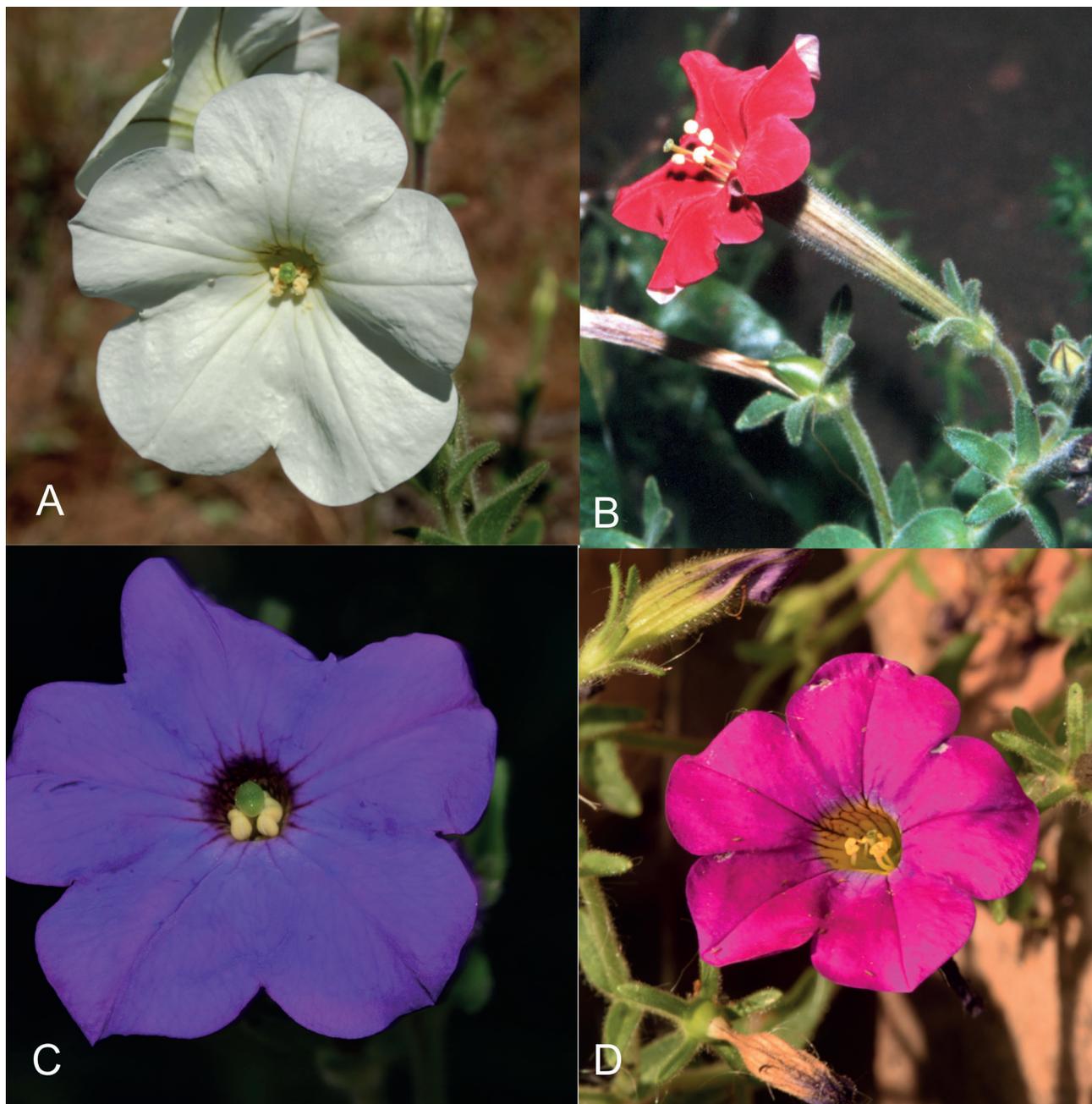


Figure 3. *Petunia* Juss. species with yellow pollen: A. *P. axillaris* (Lam.) Britton, Stern & Poggenb.; B. *P. exserta* Stehmann; C. *P. secreta* Stehmann & Semir; D. *P. toropiensis* Stehmann & Larocca. A: Stehmann *et al.* 4458; B: Stehmann *et al.* 3300; C: Stehmann *et al.* 6481; D: Stehmann *et al.* 6552; vouchers at BHC herbarium. All photographs by J.R. Stehmann.

Preliminary Conservation status: Based on the IUCN (2019) criteria, we assessed the species as Critically Endangered [B1, B2a,b (I, ii, iii, iv), D]. EOO 36.2 km² (CR); AOO 16.0 km² (EN). The new species is only known from four localities, distant at most circa 20 km each other, none included in protected areas. We estimate the occurrence of few mature individuals (< 10) in three of them, but more than 50 at PCH São Miguel, a disturbed site. Because of the building of four dams along the Toropi river, some areas where populations are currently inhabiting can potentially be flooded by the reservoir. However, some sites of occurrence will be not affected by the dams. We recommend searching for new populations and monitoring of the known ones, as well as the maintenance of *ex-situ* populations in order to prevent the extinction of the species. The habitat losses caused by the dams in the Toropi river

are the most significant threat (Marchiori *et al.* 2014), but the disruption of its genetic integrity caused by the contact with other *Petunia* species must not be neglected.

Additional specimens examined (Paratypes): BRAZIL. Rio Grande do Sul: Quevedos, 29°24'43" S, 54°03'03" W, 10 December 2020 (fl., fr.), J. Larocca s.n. (HAS 98384, BHC210792); *ibidem*, Cachoeira Cinco Veados, margem do rio Toropi, 29°23'56.3"S, 54°03'11.74" W, 197 m, 9 November 2021 (fl., fr.), J.R. Stehmann, J. Larocca & R. Vasconcelos 6552 (BHC206038). São Martinho da Serra, 10 December 2020 (fl., fr.), J. Larocca s.n. (BHC210793); *ibidem*, PCH São Miguel, margem do rio Toropi, 29°26'44.02" S, 54°04'5.51" W, 153 m, 9 November 2021 (fl., fr.), J. R. Stehmann, J. Larocca & R. Vasconcelos 6555 (BHC206041).

Key to the species of Petunia from Rio Grande do Sul state, Brazil

1. Corolla hypocrateriform, with subcylindrical tube; filaments adnate up to the half of the corolla tube; pollen yellow 2
- 1'. Corolla campanulate, narrow-infundibuliform or infundibuliform, filaments adnate to the base of the corolla tube; pollen yellow, lilac or violet4
2. Corolla reddish, anthers and stigma exerted from the corolla tube; sciophilous plants *P. exserta* Stehmann
- 2'. Corolla white, pink, magenta or purple, anthers and stigma opened at the mouth of the corolla tube; heliophilous plants 3
3. Corolla white, with strong fragrance perceptible at night *P. axillaris* (Lam.) Britton, Stern & Poggenb.
- 3'. Corolla pink, magenta or purple, without perceptible fragrance *P. secreta* Stehmann & Semir
4. Corolla with yellow throat; pollen yellow *P. toropiensis* Stehmann & Larocca
- 4'. Corolla with magenta or purple throat; pollen blue or violet 5
5. Corolla campanulate; stigma exerted above anthers of the longest stamens *P. bonjardinensis* T. Ando & Hashim.
- 5'. Corolla infundibuliform or tubular-infundibuliform; stigma placed at the same level or below the anthers of the longest stamens 6
6. Plant repent, rooting at the nodes; calyx halfway lobed *P. alti plana* T. Ando & Hashim.
- 6'. Plant erect, ascending, decumbent, procumbent or prostrate, not rooting at the nodes; calyx deeply lobed (except in coastal populations) (*P. integrifolia* complex) 7
7. Anthers with channeled thecae at anthesis *P. interior* T. Ando & Hashim.
- 7'. Anthers with flat thecae at anthesis 8
8. Plants viscid; leaves with prominent venation; throat of the corolla showing a reniform outline in front view *P. bajeensis* T. Ando & Hashim.
- 8'. Plants not evidently viscid; leaves with not prominent venation; throat of the corolla showing an elliptical outline in front view 9
9. Stem usually decumbent; capsule subglobose, with deflexed peduncle *P. integrifolia* (Hook.) Schinz & Thell.
- 9'. Stem usually erect or ascending; capsule ovoid, with upward peduncle *P. inflata* R.E. Fr.



Discussion

Petunia toropiensis is an annual herb, with erect or ascendant stems, easily recognizable by its showy infundibuliform corolla with purple (magenta) limb and yellow tube, this last trait not common in native *Petunia*. These features give it a great potential for ornamental use. The yellow pollen, the upward peduncle in fruiting and the small seeds (< 0.6 mm long) reveal that the species should be related with the species belonging to the long tube clade, that includes *P. axillaris*, *P. exserta* and *P. secreta* (Reck-Kortmann *et al.* 2014; Rodrigues *et al.* 2018). *Petunia toropiensis* differs from these species by its infundibuliform corolla with enlarged apex that shows the yellow throat (*vs.* hypocrateriform corolla with subcylindrical tube), not found in any taxon of the clade. The remaining *Petunia* species with infundibuliform corolla belong to the short tube clade and are easily distinct from the new species by the blue or violet pollen.

During the fieldwork, we observed bees often visiting the flowers, an indicative of melitophilous pollination syndrome. Two species of short-tongued bees, *Hexanthera missionica* (Collectidae) and *Pseudagapostemon* sp. (Halictidae), can be considered as potential pollinators due to their behaviour of entering the infundibuliform corolla tube to actively collect pollen or reach the short nectariferous camera. These behaviours were reported for the bees visiting the majority of species of the short corolla tube clade (Rodrigues *et al.* 2018), as well as some species of *Calibrachoa* (Stehmann & Semir 2001), a closely related genus included in *Petunia* in the past with similar flower morphology (Stehmann *et al.* 2009; Fregonezi *et al.* 2013).

By comparing the flower morphology of the two melitophilous species with yellow pollen, *P. secreta* and the new species, we observed that the shape and size of the corolla as well as the anthers position are the two major differences. The first species has subcylindrical corolla tube, longer than 4 cm, and androecium arranged in two levels (4+1), with the anthers placed just below the stigma at the mouth of the corolla tube (Stehmann *et al.* 2009; Rodrigues *et al.* 2018). *Petunia toropiensis*, conversely, has infundibuliform corolla tube, up to 3.5 cm in length, the androecium arranged in three levels (2+2+1) with anthers included in the corolla tube, and the stigma placed between the middle and longer stamens (Fig. 1E). *Petunia secreta* is morphologically and genetically related to *P. axillaris* subsp. *axillaris*, a sphingophilous taxon (Reck-Kortmann *et al.* 2014; Rodrigues *et al.* 2018), but the lack of genetic information about the new species does not permit to speculate if melitophily originated once or twice in the evolutionary history of the group.

The geographic distribution of the new species seems to be restricted to the rugged terrain crossed by the Toropi River, from the edge of the Serra Geral (about 300 m a.s.l.) to the beginning of the lower lands (ca. 150 m a.s.l.) where

it flows towards the Ibicuí River (Fig. 2A). Considering the large and showy flowers of *P. toropiensis*, the fact that the species had not been collected up until now is intriguing. We hypothesize that its original habitat corresponds to the sunny escarpments along the sinuous valley of the river (Fig. 2B-C). As an annual plant, massive amounts of seeds were released in a soil (seed bank) later disturbed by soil turning and logging during the installation of the hydroelectric dams, promoting a population explosion and putting the new species in evidence (Fig. 1A-B). This can explain why we have found for consecutive years many individuals along the banks of the Toropi River when we visited it in 2020 and 2021.

If the dams contributed momentarily for the expansion of some populations of *P. toropiensis*, on the other hand the reservoir will flood some sites of natural occurrence of the species. Another problem could be the genetic disruption led by the contact with populations of other melitophilous species, such as *P. inflata* R.E.Fr., collected along the Toropi river where the original forest was cleared. For these reasons, it will be important to monitor the populations and investigate if introgressive hybridization is happening (Allendorf *et al.* 2001) and affecting the gene pool of the new species, putting it at extinction risk.

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