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Morphological comparison between *Targionia hypophylla* L. and *T. stellaris* (Marchantiophyta) in subtropical Argentina with novel description of the sporophyte of *T. stellaris*

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Abstract – A morphological comparison between *Targionia hypophylla* L. and *T. stellaris* Hässel is carried out based on fertile material from northern Argentina. The two species differed in a large number of gametophytic traits, including: epidermal pores, antheridia location and ventral scales shape. The spores of *T. stellaris* are also described in detail and compared to those of *T. hypophylla*; their potential taxonomic value is discussed in depth. The first photomicrographs of *T. stellaris* are provided.

Morphology / *Targionia stellaris* / *Targionia hypophylla* / Taxonomy / South America

INTRODUCTION

Targionia L. stands as one of the earliest generic names proposed to include complex thalloid liverworts (Schuster, 1992). The genus is easily distinguished from remaining liverworts by the bivalved involucre below the thallus apex and its frequent dark-purple colour. Along with the development of dark pigments, the thallus characteristically rolls up to avoid desiccation and is rarely found with its margins fully extended (Bischler *et al.*, 2005; Hässel de Menéndez, 1963).

Although several species were ascribed to the genus (Söderström *et al.*, 2016), many are often treated as synonyms of *T. hypophylla* L. (e.g., *T. bifurca* Nees & Mont.; Bischler *et al.*, 2005). In the New World, the genus includes two highly similar species, *T. hypophylla* and *T. stellaris* Hässel. The former is distributed worldwide, being particularly frequent in dry or seasonal habitats. In the Neotropics, it is found at relatively high altitudes (> 2000 m) from Mexico (Baja California) to Argentina (Buenos Aires). *Targionia stellaris*, in contrast, occurs exclusively in exposed high-mountain environments (>2800 m) of northern Argentina. As Bischler *et al.* (2005) pointed out, *T. stellaris* is a rare species and, besides its type location

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(Jujuy, Argentina), it has been recorded only from the Tucuman province (Hässel de Menéndez & Rubies, 2009).

At present, the morphological distinction between both species lies primarily in the epidermal pores *Targionia hypophylla* exhibiting 2-3 concentric rings of 9-16 cells whereas *T. stellaris* shows a single ring of 6-7 cells. In addition, epidermal cells height, pore diameter, ventral scale morphology and antheridia location have been suggested as differentiating characters (Hässel de Menéndez, 1963; Gradstein *et al.*, 2001; Bischler *et al.*, 2005). Spore morphology has been considered as a valuable trait to discriminate complex thalloid liverworts at the genus or species level (Gupta & Udar, 1986). As the sporophyte of *T. stellaris* is unknown further potential diagnostic characters of the species, such as spore morphology, remain undescribed.

Recently, several fertile specimens with sporophytes of *Targionia stellaris* were collected in the high grassland "El Infiernillo" in the province of Tucuman, Argentina. In the present paper, the sporophyte of *T. stellaris* is fully described for the first time. Fertile material from successive fieldtrips allowed us to study the spores, including spore ornamentation, from different time periods, corroborating the stability of its traits. A detailed comparison between *T. hypophylla* and *T. stellaris*, with a special focus on the spore morphology, is performed. The first microphotographs of the main features of *T. stellaris* are also provided.

MATERIALS AND METHODS

Morphological survey

Specimens of *Targionia stellaris* and *T. hypophylla* were studied following the standard techniques applied to bryophytes (Gradstein *et al.*, 2001; Suárez & Schiavone, 2010; Flores & Suárez, 2015; Suarez *et al.*, 2017), using Hoyer's solution as mounting medium (Anderson, 1954). Observations of gametophytic traits were mainly performed under Leica DMLS light microscope. Gametophytic soft tissue was additionally studied under SEM after being dehydrated in graded ethanol series and subjected to critical point desiccation and gold layered. In order to study the morphology and ornamentation of spores, mature capsules were dissected and spores were mounted on aluminium stubs and coated with gold. Measures on spore size and observation on spore ornamentation were evaluated on distal face, equatorial face and proximal face as well. The terminology of spore morphology and ornamentation follows Punt *et al.* (2007). Terms commonly employed for bryophyte spores, according to Magill *et al.* (1990), are indicated within brackets where appropriate.

RESULTS AND DISCUSSION

Taxonomic treatment

Targionia stellaris (Müll. Frib.) Hässel, *Opera Lilloana* 7: 74. 1963

Figs 1-2; 5-6; 7-9

Thallus 1.5-3 mm wide, dark green, usually tinged with purple on both sides. Branching mainly ventral, less frequently dichotomous; margins entire, usually

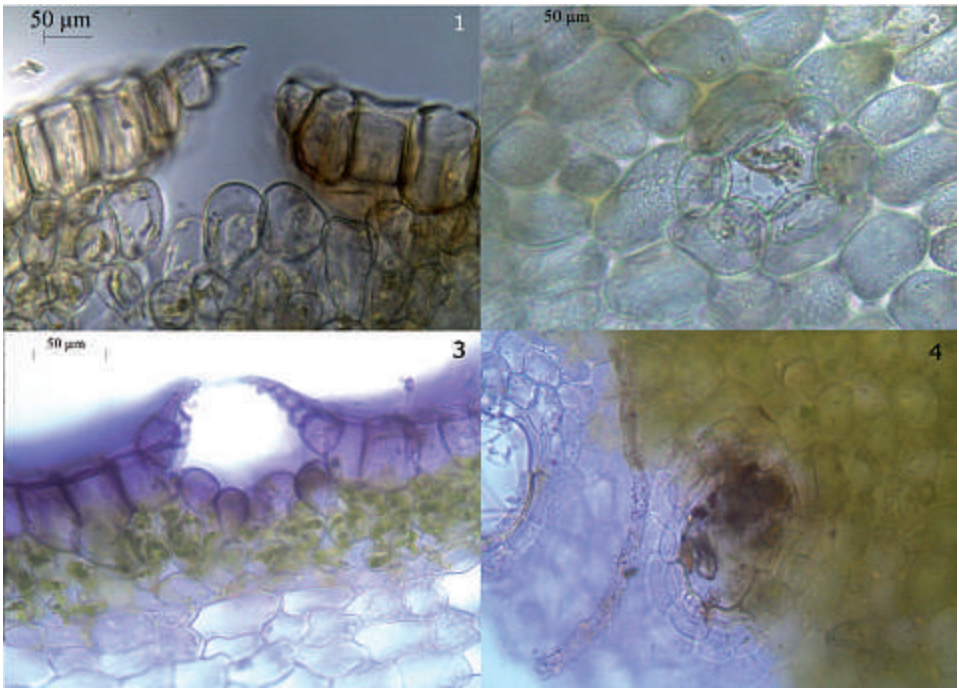
rolled up under stress conditions, 4-7 mm long. Dorsal surface smooth, concave at the middle and progressively flat towards the margins. Epidermal cells walls slightly thickened, striolate, trigones present. Epidermal pores simple, hardly elevated, 45-50 μm in diameter, with a single ring of 6-7 thin-wall cells; inner membrane present. Air chambers in a single layer, with 3(4)-cell filaments. Basal tissue extending 75% the thallus height. Ventral scales in two rows, dark purple, characteristically extending beyond thallus margins; 1 appendage, lanceolate-triangular, not constricted basally. Specialised asexual reproduction absent. Monoicous. Antheridia usually in irregular groups on dorsal surface of main or elongated ventral branches. Archegonia terminal, several per cavity. Involucre ventral terminal on thallus ventral surface, multi-layered, margins entire. Calyptra hyaline. Capsule brownish, globose; wall with annular thickenings. Spores large, 63-66 μm in diameter (equatorial view); distal face incompletely areolate, muri (= ridges) with microreticulum, cingulum (= wing) absent; proximal face concave, muri arranged as filament-like structures with strongly differentiated microreticulum; trilete scar indistinct. Elaters acute, with a single helical band.

Studied specimens: ***T. stellaris***. Argentina. Tucumán, Tafí del Valle, "El Infiernillo" (km 78), dry channel, under shrubs on a rocky slope, 3047 m, October 2013, *J Flores* 32 (LIL), ibid., August 2016 (locality covered by snow), *J Flores* 60 (LIL). ***T. hypophylla***. Argentina. Salta, Baritú, road to "Termas", on a slope alongside the road, May 2013, *J Flores* 15, 40-42 (LIL). Tucumán, Tafí del Valle. Near "El Infiernillo", on a slope besides the road, 3020 m, October 2013, *J Flores* 29-30 (LIL), ibid., 3020 m, August 2016, *J Flores* 56-57 (LIL). Tucumán, road to "Quebrada del Portugués" (motorway 325). Yungas rainforest, on exposed rocks under canopy gap, 772 m, July 2014, *J Flores* 48 (LIL).

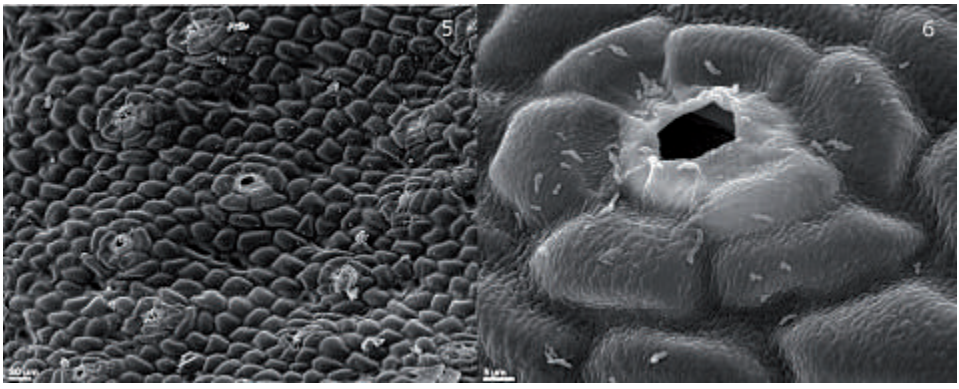
Targionia stellaris is endemic to Northwest Argentina, where it occurs in exposed sites in high-altitude habitats. This species is commonly found in the Prepuna and Monte regions of Jujuy (> 4000 m) and Tucuman (> 2800 m), respectively (Cabrera, 1971). These areas are characterised by long periods of drought and strong light radiation. In these sites, *T. stellaris* grows under shrubs or in loosely arranged patches on bare soil. *Targionia hypophylla* is widely distributed in South America (Bischler *et al.*, 2005; Hässel de Menéndez & Rubies, 2009) and grows under broader habitat conditions than the former. In northern Argentina, it is usually found in high-mountain environments above 4000 m but also in low-altitude rainforest areas (Yungas region), below 1000 m (Hässel de Menéndez, 1963; Bischler *et al.*, 2005). As compared with *T. stellaris*, *T. hypophylla* forms dense patches, sometimes intermixed with *Plagiochasma*, and is barely linked with vascular plants.

Morphological comparison

It is widely accepted that dissimilar ecological conditions may lead to morphological differentiation (Zimmerman *et al.*, 2007; Schluter, 2009). In this regard, the environmental constraints shown by both species may account for the observed differences between the continuous features (Table 1). Some of these characters, such as pore diameter and height of epidermal cells, were already noted by Hässel de Menéndez (1963) and others. In *Targionia stellaris*, epidermal cells tend to be higher and prismatic (Fig. 1) whereas in *T. hypophylla* they are shorter and slightly rectangular (Fig. 3). Pore diameter is considerably wider in *T. hypophylla* than in *T. stellaris* (Figs 2, 4; Table 1). Thallus dimensions were strikingly contrasting in both species (Table 1). *Targionia stellaris* exhibits a conspicuously thicker thallus (cross section) than *T. hypophylla* and thalli are wider in the latter species. Aside from the number of cells in the fundamental tissue, the remaining vegetative



Figs 1-4. **1.** Cross section of thallus showing prismatic epidermal cells of *Targionia stellaris* photosynthetic and fundamental tissues. **2.** Epidermal pore (surface view) with a single 6-cell ring and hyaline membrane. **3.** Cross section of the thallus of *T. hypophylla* showing short epidermal cells. **4.** Epidermal pore (surface view) of *T. hypophylla* with several rings of 7-9 cells.



Figs 5-6. *Targionia stellaris*. SEM photographs showing the number of pores on surface (**4**) and detail of the concentric cells and hyaline membrane (**5**).

characters were similar in both species (Table 1). Besides the differences in size and shape, epidermal cells contrasted in discrete traits as well. The walls of the epidermal cells were striolate (Figs 1, 5-6) which agrees with Bischler *et al.* (2005) description of the cell walls as roughened. In comparison, epidermal cells of *T. hypophylla* were

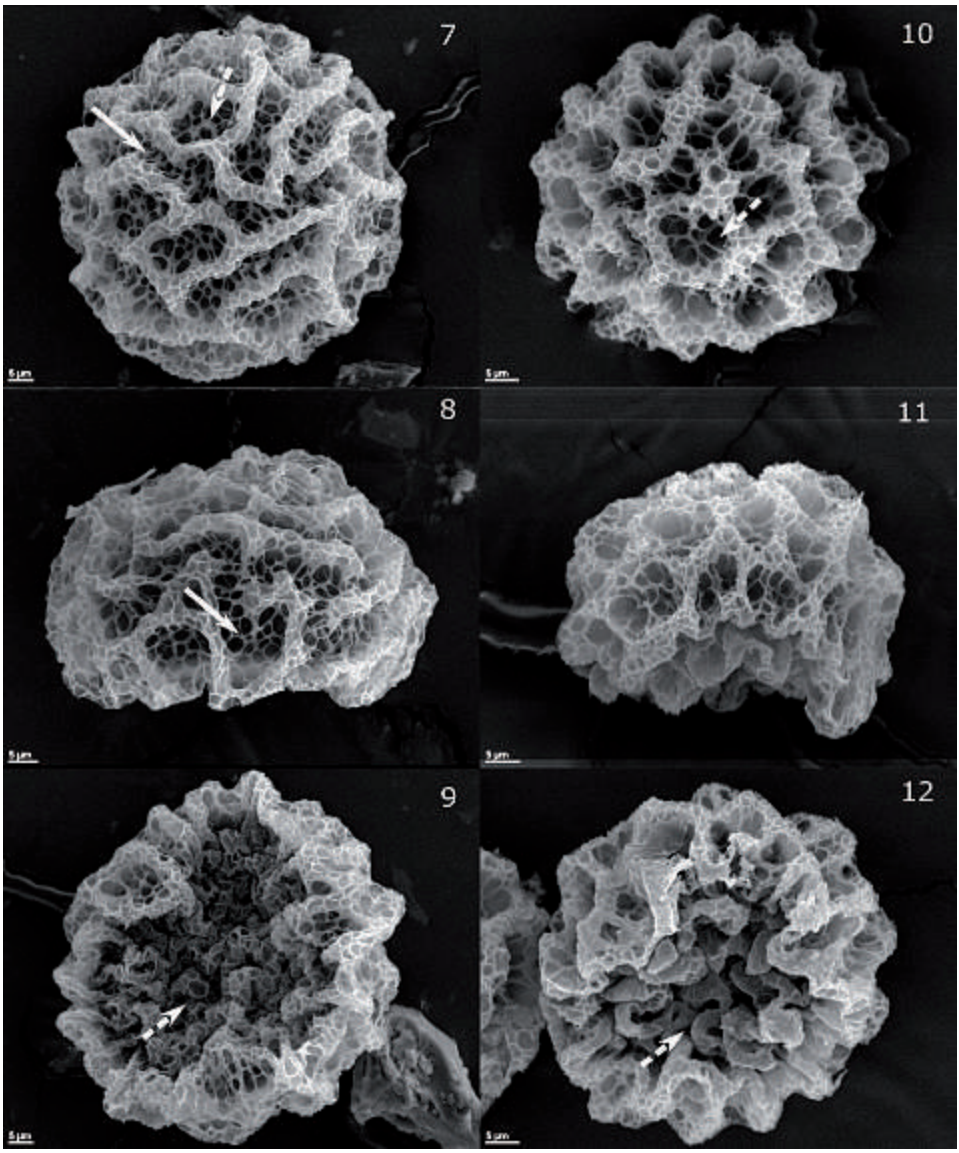
Table 1. Morphological differences between neotropical species of *Targionia*

	Targionia stellaris	*Targionia hypophylla*
Thallus thickness	0.8- 1.5 mm	0.3 mm- 0.45 (0.5) mm
Thallus width	1-2.5 mm	3-5 mm
Epidermal cells height	43.75 -50 μm	34 - 39 μm
Photosynthetic tissue	3- 4 cells	2- 3 cells
Fundamental tissue	≈ 25 cells	≈ 20 cells
Pore diameter	50-72μ	90-170μ
Pore cells	1 concentric ring of 6-7 cells	> 2 concentric rings; external ring of 7-9 cells
Ventral scales	Exceeding thallus margins	Not exceeding thallus margins
Scales width	0.3 mm - 0.5 mm	0.4 mm- 0.7 mm
Antheridia location	Main thallus	Ventral branches
Spore ornamentation	reticulate with partially opened areolae; muri with micro-reticulum of minor order in the distal face. Micro-reticulum conspicuous in the proximal face.	reticulate with closed areolae, muri with micro-reticulum of minor order in the distal face. Micro-reticulum hardly developed in the proximal face.

consistently smooth (Fig. 3; Bischler *et al.*, 2005). Drawings of the lectotype of *T. stellaris* depicted bulging trigones (Bischler *et al.*, 2015) though the current material did not account for these (Figs 1, 5-6).

Likewise, sporophyte gross morphology and spore dimensions were almost identical in both taxa. A difference was observed in the ornamentation of the distal face of spores. In *Targionia hypophylla* the distal face reticulum tends to delimit closed and well-defined areolae (Figs 10, 11) whereas in *T. stellaris* areolae are incompletely formed, constituting a continuum space (Figs 7, 8). In equatorial view, spores of *T. hypophylla* are slightly curved towards the proximal face, as opposed to the flat spores of *T. stellaris* (Figs 8, 11).

The taxonomic value of the spore fine sculpture in the genus *Targionia* has been also highlighted by previous authors (Scott & Pike, 1988; Schuster, 1992; Perold, 1993, 1999). In agreement with previous studies, the microreticulum in the distal face of *T. hypophylla* follows a 'loose' pattern defining wide spaces (Schuster, 1992; Perold, 1993; Fig. 10). In contrast, the distal face of *T. stellaris* shows a narrower microreticulum (Fig. 7). The studied material has also shown differences as to the development of the microreticulum in the proximal face of the spores. In *T. hypophylla* the microreticulum was observed to be weakly developed or even absent as compared with the conspicuously and homogenously developed microreticulum of *T. stellaris* (Figs 9, 12). Discussion in this regard is ambiguous in literature (Scott & Pike, 1988; Schuster, 1992; Perold, 1993; Paton, 1999). Schuster (1992, p. 76), while studying American liverworts, stated that the proximal faces ("*inner faces*") of *T. hypophylla* may be covered by a "*low, locally incomplete, feeble reticulum*" (=microreticulum). Although fine sculpturing was not



Figs 7-12. Comparison of spores between *Targionia stellaris* and *T. hypophylla* in distal (**7, 10**), equatorial (**8, 11**) and proximal view (**9, 12**). **7-9.** *Targionia stellaris* spores with opened-like areolae (solid arrow). **10-12.** *Targionia hypophylla* spores with closed areolae and curved outline (equatorial view). Note the microreticulum (dashed arrow) delimiting narrow spaces in *T. stellaris* as compared with *T. hypophylla* in the distal face (**7**, **10**). In the proximal face, a conspicuous microreticulum is present in *T. stellaris* while it is hardly developed in *T. hypophylla* (**9**, **12**; dashed arrow).

comprehensively discussed by Scott & Pike (1988), they assigned fully-microreticulate spores to *T. hypophylla* (Plate 1.1; p.161). Perold (1993) argued that the spores of *T. hypophylla* illustrated by Scott and Pike (1988) are rather assignable to

T. lorbeeriana Müll. Frib. If so, the presumed spores of *T. hypophylla* presented by Scott and Pike (1988; Plate 1.2 according to Perold, 1993) resemble the spores of the examined specimens in that the microreticulum in the proximal face is weakly or ill-defined (Fig. 12). Furthermore, the spores of the current material of *T. hypophylla* are highly similar to those provided by Perold (1999) concerning the hardly-developed fine sculpture of the proximal face (Plate 1.B, p. 19; Fig. 12). Regardless of the microreticulum development in the proximal face of the spores of *T. hypophylla* formerly studied (Scott & Pike 1988; Schuster, 1992; *see discussion in* Perold, 1993), the present subtropical specimens of *T. hypophylla* and *T. stellaris* clearly differ in their proximal face fine sculpturing (Figs 9, 12). Therefore, this character could at least be useful to discriminate southern South American specimens.

Many of the characters studied in this contribution provided useful means to discriminate between the neotropical species of the genus *Targionia*. Measures performed over thallus width and pore diameter are particularly valuable to distinguish *T. stellaris* from *T. hypophylla* (Table 1). In addition, discrete characters were seen to be reliable to separate species. Epidermal pores, for instance, contrasted in both species (Figs 1-4). The ornamentation of the spores shown differences in the current specimens as well (Figs 7-12; Table 1) though further studies might be undertaken in order to address the variability of this features.

So far, *Targionia stellaris* has shown an extremely restricted distribution. A previous record from Galapagos Islands (Gradstein & Weber, 1982) was verified to be *T. hypophylla* (Bischler *et al.*, 2005). However, species formerly unregistered for southern South America were recently found along high-mountain localities (Flores & Suárez, 2015; Flores *et al.*, 2017): *Cephaloziella hampeana* (Nees) Schiffn. *ex* Loeske (Flores *et al.*, 2017), *Pohlia chilensis* (Mont.) A.J. Shaw, (Suárez & Schiavone, 2008), *Saitobryum lorentzii* (Müll. Hal.) Ochyra (Suárez *et al.*, 2010), *Dicranella lorentzii* (Müll. Hal.) Broth. (Suárez *et al.*, 2013), *Neosharpiella aztecorum* H. Rob. & Delgad. (Jimenez *et al.*, 2015), and *Grimmia trinervis* R.S.Williams (Ellis *et al.* 2018). On this basis, it would be reasonable to expect a broader distributional range for *T. stellaris*. Such an extension could represent an opportunity to further evaluate the taxonomic value of the characters studied in this paper.

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