

## Epidermal features of *Lotus oroboides* = *Ottleya oroboides* (Leguminosae: Loteae)

SEBASTIÁN A. STENGLEIN<sup>1</sup> and [ANA M. ARAMBARRI](#)<sup>2\*</sup>

<sup>1</sup> CONICET Fellow. *Morfología Vegetal, Departamento de Ciencias Biológicas, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, C.C. 31, (1900) La Plata, Argentina.*

<sup>2</sup> Professor of Botany. *Morfología Vegetal, Departamento de Ciencias Biológicas, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, C.C. 31, (1900) La Plata, Argentina.*

\*Corresponding author

### Introduction

*Lotus oroboides* (Humb., Bonpl. and Kunth) Ottley ex Kearney and Peebles is distributed from southern United States to north-central part of Mexico. It is a polymorphic species adapted to a broad range of habitats. Since was described by the first time as *Tephrosia oroboides* by Kunth (1823) a considerable disagreement has been existed among systematists about the generic and specific delimitation, and if this species should be treated as one or two species and/or varieties.

The polymorphism of *L. oroboides* was attributed by Ottley (1944) to the existence of hybrids, and she created two varieties: (i) *Lotus oroboides* var. *plebeius* (Brandege) Ottley, and (ii) *L. oroboides* var. *ramulosus* (M.E. Jones) Ottley. Later, Isely (1978) cited two new combinations for this species (i) *L. oroboides* var. *nanus* (A. Gray) Isely, and (ii) *L. oroboides* var. *nummularius* (M.E. Jones) Isely. Isely (1981) reported the possibility that this species would include several ecotypes influenced by local environmental conditions or perhaps it may be a consequence of genetic infiltration, as was interpreted by Ottley (1944). Barneby (1989) on the basis of his observations included all (species and varieties), into two species (i) *L. plebeius* (Brandege) Barneby, with dimorphism between the lower and upper leaves, and frequently leaves 3-4 foliolate, and (ii) *L. oroboides* (Humb., Bonpl. and Kunth) Ottley ex Kearney and Peebles with upper leaves 7-9 foliolate. Later, Sokoloff (1999) suggested that the New World taxa should be excluded from the genus *Lotus* L., and referred to the genera *Acmispon* Raf., *Hosackia* Benth., and *Syrmatium* Vog., and created the genus *Ottleya* D.D. Sokoloff to contains the species of *Simpetaria* Ottley, including *L. oroboides* as *Ottleya oroboides* (Humb., Bonpl. and Kunth) D.D. Sokoloff, however the author did not mention the varieties.

We suppose that the polymorphism exhibited by this species may be present in its anatomical characters. In fact, the aim of this study was to establish the probable existence of relationships among the exomorphology polymorphism and variability in epidermal characteristics.

## **Materials and methods**

The study was performed by using specimens from the Herbario Nacional de Mexico (MEXU), Instituto de Botánica, Departamento de Biología, Universidad Nacional Autónoma de Mexico. Dried plant materials are deposited in the Herbario del Área de Botánica, Facultad de Ciencias Agrarias y Forestales (LPAG), Universidad Nacional de La Plata, Argentina. The six specimens studied from Chihuahua (C) and Durango (D), their collections and growth sites with altitude (m a.s.l.), collection date and vouchers are detailed in Table 1.

Three mature, fully expanded and unshaded leaves positioned on the middle of each specimen were selected for the study. Dried leaves were reconstituted in water with a drop of detergent and dried in oven at 30-35 °C for 24 h (D' Ambrogio de Argüeso 1986). To avoid alterations of the leaf samples each one was fixed in formalin: glacial acetic acid: 50% ethanol (FAA). The leaflets become transparent after treatment according to the method of Dizeo de Strittmatter (1973). Epidermal tissue was surveyed with a light microscope. The leaflet area examined was 1 cm<sup>2</sup> located in the centre of the mid-lamina, and in the intervenial area, on both the adaxial and the abaxial leaflet surfaces. Trichome density was established by counting the number of trichome basal cell. Trichomes located above leaf veins and all the trichomes, stomata and epidermal cells that were intersecting the edges of the observational area were not counted. Results were expressed per unit of leaf area (mm<sup>-2</sup>). Stomatal index was calculated as: [number of stomata / (number of stomata + number of epidermal cells)] x 100 (Salisbury, 1927). Measurements were taken with an ocular micrometer. Stomatal length (guard cells length) and width (interdorsal wall distance) were determined based on measurements performed on 25 replicates, respectively. Comparison of the trichome density, stomatal index and stomata size were performed by means of the *t*-test at a probability level of 0.05. Images were obtained with a colour PAL CCD camera attached to a light microscope and they were captured and digitalized by using Photo Express 1.0 software.

**Table 1.** *Ottleya oroboides* (Humb., Bonpl. and Kunth) D.D. Sokoloff (Leguminosae: Loteae)

Identity	Collection site	Growth site	Collection altitude (m a.s.l.)	Collection date	Vouchers
C 1641	Chihuahua 'Casas Grandes' (Mexico)	yellow lime soil; slopes with grasses	1450	1982-09-23	P. Tenorio 1641 and C. Romero (MEXU)
C 1903	Chihuahua 'El Molinito' (Mexico)	yellow soil; in pine forest area	2600	1982-09-30	P. Tenorio 1903 and C. Romero (MEXU)
C 10016	Chihuahua W de 'Ocampo' (Mexico)	yellow clay soil; in pine forest area	1750	1985-09-29	P. Tenorio 10016 C. Romero, J. Ignacio, and Patricia Davila (MEXU)
D 649	Durango N de 'El Salto' (Mexico)	in black soil; slopes in pine forest area	2200	1982-06-27	P. Tenorio 649 C. Romero, and R. Hernández (MEXU)
D 4169	Durango W 'Tepehuanes' (Mexico)	white lime soil; in pine forest area	2590	1983-08-27	P. Tenorio 4169 R. Torres, and Eva Torrecillas Nevarez (MEXU)
D 6047	Durango W 'El Salto' (Mexico)	yellow clay soil; in pine forest area	2100	1984-06-29	P. Tenorio 6047 C. Romero, and T. Ramamoorthy (MEXU)

## Results and discussion

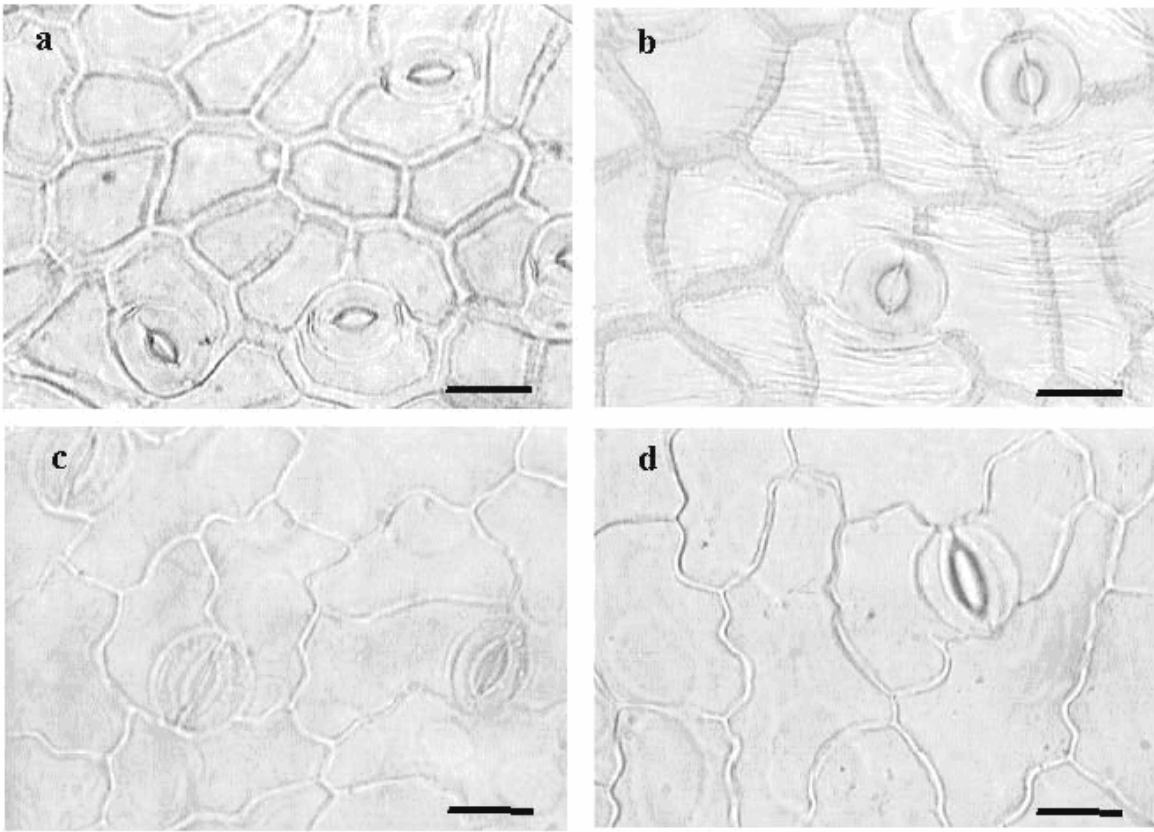
Anticlinal cell walls thickness in surface view, in the *O. oroboides* sample D 649 was statistically different on both the adaxial and the abaxial epidermis respect to the other specimens (Table 2; Figure 1a-d). Anticlinal cell wall patterns were straight to curved (C 1641, C 10016, D 649, and D 6047), curved to undulate U-shaped (D 4169) and undulate U-V-shaped (C 1903) (Figure 1a-d). This result coincides with the relationships found among the variability of anticlinal cell wall patterns with the environmental conditions where the species grow, and this waviness of cell walls have been found associated with environmental factors such as latitude, altitude and combined temperature and precipitation (Baas, 1975; Steiner, 1999).

Trichome density on the adaxial and the abaxial leaf surfaces was different among *O. oroboides* specimens investigated (Figure 2a). As a general rule, the abaxial leaf surface presented more trichomes than the adaxial one (except for C 10016). Furthermore, the *O. oroboides* samples from Durango always presented more trichomes than the samples from Chihuahua (Table 2; Figure 2a). Although Stace (1965) reported that trichomes density vary within leaves of plants that are grown under different environmental conditions, there are currently few evidences upon the environmental effect on trichome number (Bird and Gray,

2003). Since *O. oroboides* samples were collected from different sites, differences in trichomes density on both adaxial and abaxial epidermis might have been due to the environmental influence on trichome development and/or to genetic diversity among samples.

**Table 2.** Leaf epidermal characteristics of *Ottleya oroboides* (Humb., Bonpl. and Kunth) D.D. Sokoloff (Leguminosae: Loteae). Values in parentheses are means.

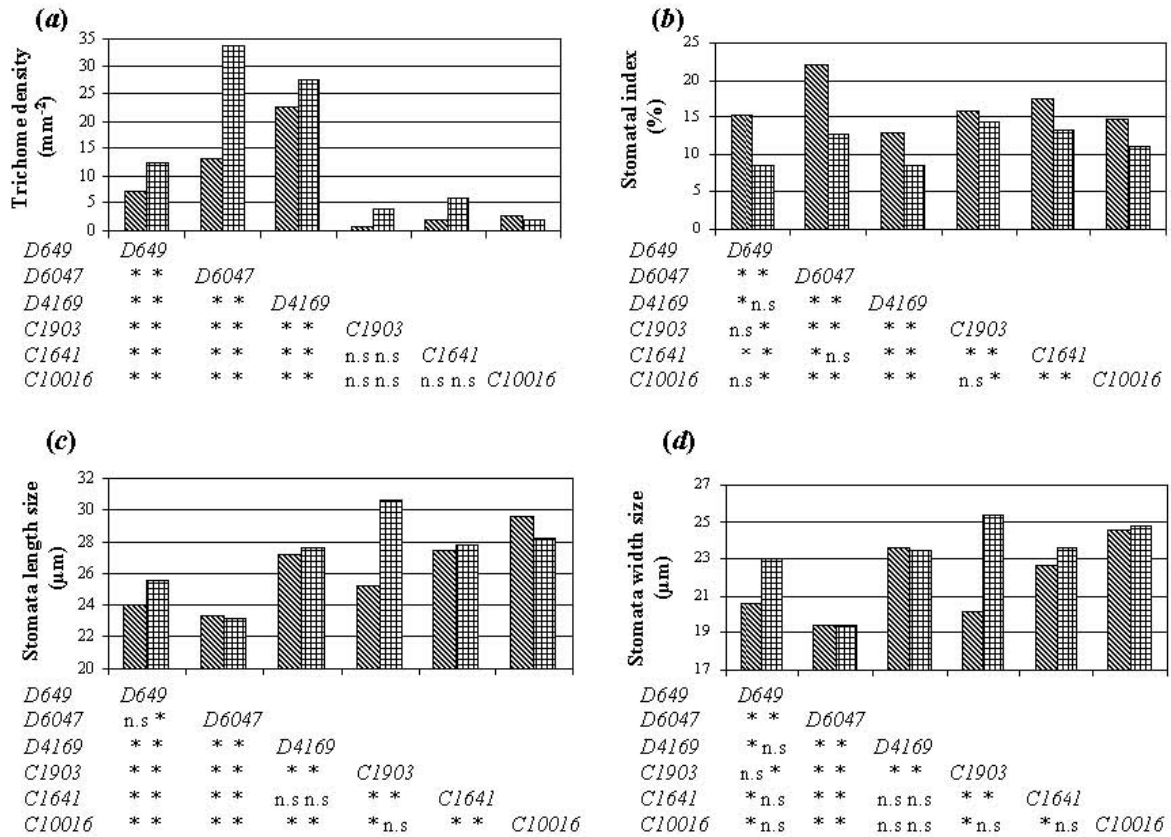
Identity	Leaflet Surface	Anticlinal cell Wall patterns	Anticlinal cell thickness (µm)	Stomatal index (%)	Stomata length (µm)	Stomata width (µm)	Trichome density (mm <sup>-2</sup> )
<b>C 1641</b>	adaxial	straight to curved	1-3 (1.3)	17-19 (17.4)	25-30 (27.4)	20-25 (22.6)	0-4 (2.0)
	abaxial	straight to curved	1-3 (1.3)	13-14 (13.2)	25-35 (27.8)	20-25 (23.6)	0-8 (6.0)
<b>C 1903</b>	adaxial	undulate U-V-shaped	1-2 (1.1)	15-16 (15.8)	25-30 (25.2)	20-25 (20.2)	0-1 (0.8)
	abaxial	undulate U-V-shaped	1-2 (1.3)	14-15 (14.4)	25-35 (30.6)	20-30 (25.4)	0-8 (4.0)
<b>C10016</b>	adaxial	straight to curved	1-3 (1.4)	14-16 (14.9)	25-35 (29.6)	20-30 (24.6)	0-8 (2.8)
	abaxial	straight to curved	1-3 (1.6)	10-12 (11.2)	25-35 (28.2)	20-30 (24.8)	0-8 (2.0)
<b>D 649</b>	adaxial	straight to curved	4-8 (5.4)	15-16 (15.3)	20-25 (24.0)	20-25 (20.6)	0-16 (7.2)
	abaxial	straight to curved	4-8 (5.6)	8-9 (8.6)	25-30 (25.6)	20-25 (23.0)	8-20 (12.4)
<b>D 4169</b>	adaxial	curved to undulate U-shaped	1-1 (1.0)	12-13 (12.9)	25-30 (27.6)	20-25 (23.4)	16-32 (22.4)
	abaxial	curved to undulate U-shaped	1-1 (1.0)	8-9 (8.6)	25-30 (27.2)	20-25 (23.6)	20-40 (27.6)
<b>D 6047</b>	adaxial	straight to curved	1-1 (1.0)	22-23 (22.2)	20-25 (23.4)	15-20 (19.4)	8-20 (13.2)
	abaxial	straight to curved	1-1 (1.0)	12-13 (12.9)	20-25 (23.2)	15-20 (19.4)	28-40 (33.6)



**Figure 1.** Light microscope images of epidermis in surface view of *Ottleya oroboides* (Humb., Bonpl. and Kunth) D.D.Sokoloff. **(a, b)** sample **D 649**: anticlinal epidermal cell walls straight to curved and thick, periclinal cell walls exhibiting cuticular ornamentation on the abaxial surface. **(c, d)** sample **C 1903**: anticlinal epidermal cell walls undulate U-V-shaped and thin, periclinal cell walls without cuticular ornamentation. **a** and **c**: adaxial epidermis; **b** and **d**: abaxial epidermis. Bars: 20  $\mu\text{m}$ .

Stomatal index of the *O. oroboides* leaflets ranged from 12.9% to 22.2% on the adaxial surface and from 8.6% to 14.4% on the abaxial surface (Table 2). Statistically significant differences were observed in stomatal index among *O. oroboides* samples (Figure 2b). In addition, the stomatal index value was always higher on the adaxial epidermis than on the abaxial one.

Stomatal measurements were 20-30  $\mu\text{m}$  long and 15-30  $\mu\text{m}$  wide on the adaxial surface, and 20-35  $\mu\text{m}$  long and 15-30  $\mu\text{m}$  wide on the abaxial surface (Table 2). The stomatal size differed significantly among *O. oroboides* samples (Figure 2c-d). Although, the biological significance of the existence of different stomata sizes remains to be determining, it might be related to the plant's ability to withstand water stress and/or differences in gas-exchange.



\*statistically significant differences at a 0.05 probability level.  
n.s.= non significant differences at a 0.05 probability level.

**Figure 2.** Statistical results of data from leaf epidermal characteristics of *Ottleya oroboides* (Humb., Bonpl. and Kunth) D.D.Sokoloff on both the adaxial (striped) and abaxial (cross-hatched) surfaces. **(a)** trichome density; **(b)** stomatal index; **(c)** stomata length; **(d)** stomata width. Each pair of columns corresponds to the measurements performed on the *O. oroboides* sample, indicated underneath. The tables presented below the figures correspond to the comparison of the means by the *t*-test. Each column shows the mean compared with the correspondent mean of the *O. oroboides* sample indicated in the column on the left of the table.

### Conclusion

Our results demonstrated that exomorphological differences correlates with the variability of epidermal microcharacters. We believe that *Lotus oroboides* = *Ottleya oroboides* have been evolved given varieties or subspecies.

### Acknowledgments

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