

Functional variation of leaf succulence in a cold rainforest epiphyte

Oscar Godoy^{1,*} & Ernesto Gianoli^{2,3}

¹Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, California 93106, USA

²Departamento de Biología, Universidad de La Serena, Casilla 554 La Serena, Chile

³Departamento de Botánica, Universidad de Concepción. Casilla 160-C Concepción, Chile

*Author for correspondence: godoy@msi.ucsb.edu

Background and aims – Succulence, a common attribute of floras in dry regions and of species living in microenvironments with transient water shortage, has been typically viewed as an adaptive plant feature for surviving in (semi-)arid conditions. The existence of leaf succulence in a temperate cold rainforest challenges the view of its adaptive value. We studied leaf functional variation in *Sarmienta repens* Ruiz & Pav. (Gesneriaceae), an epiphyte living in the Valdivian forest of southern Chile.

Material and methods – We measured leaf thickness, absolute leaf water content, specific leaf area and leaf anatomy (epidermis, palisade parenchyma, and spongy parenchyma) in two distinct light micro-environments: shaded understory versus border of canopy gaps. We also characterized micro-environmental conditions in terms of light availability, temperature and water evaporation.

Key results – We show that leaves from sun conditions, the environment with higher water demand, have lower SLA (specific leaf area), thicker epidermis and store more water due to a thicker spongy parenchyma, than leaves from shade conditions.

Conclusions – We found high phenotypic variation in *S. repens* at intraspecific level in response to contrasting environmental conditions. This variation reflects a two-fold strategy common in epiphytes: increase water storage and reduce water loss. Furthermore, it suggests that leaf succulence has an adaptive value even in a temperate cold rainforest. We discuss that the occurrence of succulence on a cold rainforest might be explained by a combination of ecological, biogeographic and phylogenetic factors.

Key words – Puyehue National Park, functional trait, leaf thickness, *Sarmienta repens* Ruiz & Pav., SLA, water storage, water loss.

INTRODUCTION

Plant features commonly observed in ecological communities are often the result of current selective pressures and prove their adaptive value when they are associated with plant success in function, distribution or abundance (Niklas 2007). For instance, deep roots for soil water uptake have been fairly documented in desert perennial plants (e.g. Caldwell & Richards 1989). In contrast, apparently adaptive characters or syndromes that are rare in a community may be explained by past evolutionary processes that took place long time before and/or faraway from the current plant environment, where the selective pressure is no longer operating (Larson & Losos 1996). For instance, overly long spines and large fruits with sweet pulps, thought to be suitable for defence against and dispersion by extinct megafauna, respectively, have been interpreted as evolutionary anachronisms (Janzen & Martin 1982, Janzen 1986, Bond & Silander 2007, Zaya & Howe 2009). It is not easy to elucidate whether rare

phenotypic traits are evolutionary anachronisms or actual adaptive features because a large amount of ecological, biogeographic and phylogenetic information is needed (Larson & Losos 1996). However, there are relatively simple approaches to current phenotypic variation that may shed light on this question (Rose & Lauder 1996). If it is shown that (i) there is field-based evidence of a functional relation between the observed phenotype and the putative selective factor, supported by ecophysiological knowledge (e.g. Saldaña et al. 2005) and/or (ii) variation in the candidate trait is significantly associated with plant fitness in the field (e.g. Saldaña et al. 2007), then it may be inferred that such a plant feature has an adaptive value in the current ecological scenario.

Several plant physiological and morphological characteristics are associated with adaptation to life in stressful environments (Schulze et al. 2005, Valladares & Niinemets 2008). Succulence, which allows water storage for further use when its availability decreases and when its demand increases, is one of those plant features typically viewed as

adaptive to arid environments (Eggli & Nyffeler 2009). Indeed, there is quantitative evidence of the adaptive value of leaf succulence in natural populations, as shown by phenotypic selection analysis on desert sunflowers (Donovan et al. 2007). This conspicuous feature is present in 60% of plant orders and has evolved independently over 30 times (Eggli & Nyffeler 2009). Leaf succulence is common in dry regions (Willert et al. 1990). It is also common in microenvironments where light/temperature or salinity strongly increase water demand seasonally such as those occupied by epiphytic plants (Benzing 1987) and halophytes (Breckle 2004). Succulent species are less frequent in cold regions. They are typically rosette plants with Arctic-alpine distribution; vascular epiphytes are extremely rare (Nieder & Barthlott 2001, Drennan 2009).

The Gesneriaceae family comprises about 3000 species, largely distributed in the tropics with a few temperate species (Smith et al. 1997). In the temperate rainforests of southern South America, Gesneriaceae are represented by three species within the tribe Coronanthereae (Salinas et al. 2010), which includes nine genera and twenty species distributed in the South Pacific, Australia and Southern South America (Smith et al. 2006). The three South American species, which belong to three monotypic genera (*Sarmienta*, *Asteranthera*, and *Mitraria*), are likely descendants of ancient tropical floras that arrived to southern South America during the Lower Tertiary (Villagrán & Hinojosa 1997). *Sarmienta repens* Ruiz & Pav. is a climbing plant endemic to the southern temperate rainforest (Gianoli et al. 2010); it is the only holoeipiphyte in Coronanthereae, i.e. it never roots in the ground and spends its whole life cycle along tree trunks (Salinas et al. 2010), using adhesive roots as attachment mechanism (Carrasco-Urra & Gianoli 2009). *S. repens* ('*Sarmienta*' hereafter) is the only species in the South American Coronanthereae that shows leaf succulence, an attribute that is not uncommon in the tropical Gesneriaceae (Guralnick et al. 1986, Benzing 1987, Medina et al. 1989, Chautems 2002). This is somewhat puzzling, considering that the cold temperate rainforest where *Sarmienta* grows is far from being an arid environment, with a combination of annual precipitations close to 3000 mm, mean annual temperatures around 9°C and potential evapotranspiration only 1/8 of precipitation rates (Dorsch 2003, Salinas et al. 2010).

Our research question was whether the uniqueness of leaf succulence exhibited by *Sarmienta* is a rather fixed trait, and hence a likely evolutionary anachronism, or it is a plant trait of current value, showing functional variation with the prevailing environmental factors that presumably exert local selection on plant traits. Specifically, in an old-growth temperate forest in Southern Chile we evaluated whether leaf succulence and related traits varied between two distinct light micro-environments: low-height under close canopy (shaded understory) vs. mid-height at full sun exposure (border of canopy gaps). Forest plants under high irradiances show higher water demand to avoid desiccation and to maintain an optimal physiological performance (Bassow & Bazaz 1998, Valladares & Niinemets 2008). Therefore, if leaf succulence in *Sarmienta* is currently of functional value, we expected that leaves under the full sun exposure environment would store more water.

MATERIAL AND METHODS

We studied leaf succulence variation in *Sarmienta repens* populations from the old-growth temperate rainforest at Parque Nacional Puyehue (40°39'S 72°11'W; 350 m a.s.l.), in the western piedmont of the Andes, southern Chile. Broad-leaved evergreen tree species are the most dominant component of the mature forest (Lusk et al. 2003, Saldaña & Lusk 2003) and woody vines are very common (Gianoli et al. 2010). *Sarmienta* is mainly found growing along the trunk of old trees, at 0.5 to > 30 m high, behaving as a holoeiphytic plant that climbs with the aid of adhesive roots (Carrasco-Urra & Gianoli 2009, Salinas et al. 2010). Opposite, fleshy leaves are ovate-orbulate (1–2.5 cm long, 1–1.5 cm wide) with entire to bidentate margin towards the apex and hairy petioles 1.5 mm long (Muñoz Schick 1980).

Leaf succulence was estimated as leaf thickness at the central area, measured with a digital calliper (0.01 mm resolution; Mitutoyo, Kanagawa, Japan). This was recorded in two contrasting micro-environments in terms of light availability: low-height (from 0.5 to 1.5 m) in a shaded understory and mid-height (from 4 to 7 m) in the border of canopy gaps. In both micro-sites, one fully expanded leaf per ramet was sampled in five different ramets growing on the same tree. This was replicated six times in different forest sites with the same light conditions (total n = 30 leaves per environment). To verify this assumption we characterised light environmental conditions (direct and diffuse radiation) using hemispherical photographs taken with a Nikon Coolpix 900 camera and processed with HemiView 2.1 (Delta-T Devices, Cambridge, U.K.). To roughly characterize the covariance between light availability and water evaporation in these two light micro-environments, we registered every 30 min available photosynthetic photon flux density (PPFD) and soil moisture on the ground litter (low light: forest understory) and on the litter of a tree hole (high light: gap edge), both close to several *Sarmienta* individuals, from October to December 2008. We could not find any tree hole below 1.5 m height that would make measurements strictly comparable. Importantly, these measurements do not reflect precisely water content available for *Sarmienta*, as the mechanisms of water uptake in this species are yet to be studied. Local air temperature was also recorded with the same frequency during the same time period with external sensors (Li-Cor, Lincoln, NB, USA; ThetaProbe sensors (Delta-T Devices, Cambridge, U.K.) attached to a data logger (HOBO model H08-006-04, Onset, Pocasset, MA).

We transported leaf samples to the laboratory of Universidad de Concepción (36°49'S 73°02'W) to make further measurements. This was done under humid storage in order to keep field values of leaf thickness constant, which was verified in the lab. Leaf size (area) was estimated by means of digital photography and later analysis with Sigma-Scan Pro5 software (SPSS Inc, Chicago, IL, USA). Afterwards, leaves were weighed and a leaf slide in their broader part was carefully cut to measure epidermis (cuticle + upper and lower epidermis), palisade parenchyma, and spongy parenchyma thickness; these measurements were done with a microscope. Epidermis thickness is functionally associated with reduced water loss in succulents and epiphytes (Riederer &

Schreiber 2001, Egli & Nyffeler 2009, Hao et al. 2010), and the spongy parenchyma serves for water storage as it has been previously described in another Gesneriaceae epiphyte (Kleinfeldt 1978). Accordingly, the spongy parenchyma of *Sarmienta* leaves, regardless of its light environment of origin, consisted of white irregularly-shaped cells full of water with no signs of photosynthetic activity.

Leaves were oven-dried at 60°C during 3 days and weighed to calculate absolute leaf water content (aLWC, g), relative leaf water content (rLWC = 100*(leaf fresh weight – leaf dry weight)/leaf fresh weight) and their specific leaf area (SLA = leaf area / leaf dry mass). Differences in leaf traits and environmental conditions between sites were evaluated with a one-way ANOVA, using Statistica 6.1 (StatSoft, Tulsa, OK, USA).

RESULTS

Significant differences in leaf succulence and associated traits were found in *Sarmienta* between shade and sun microsites. Leaf thickness and aLWC were greater on plants exposed to sun conditions (fig. 1). Although the three ana-

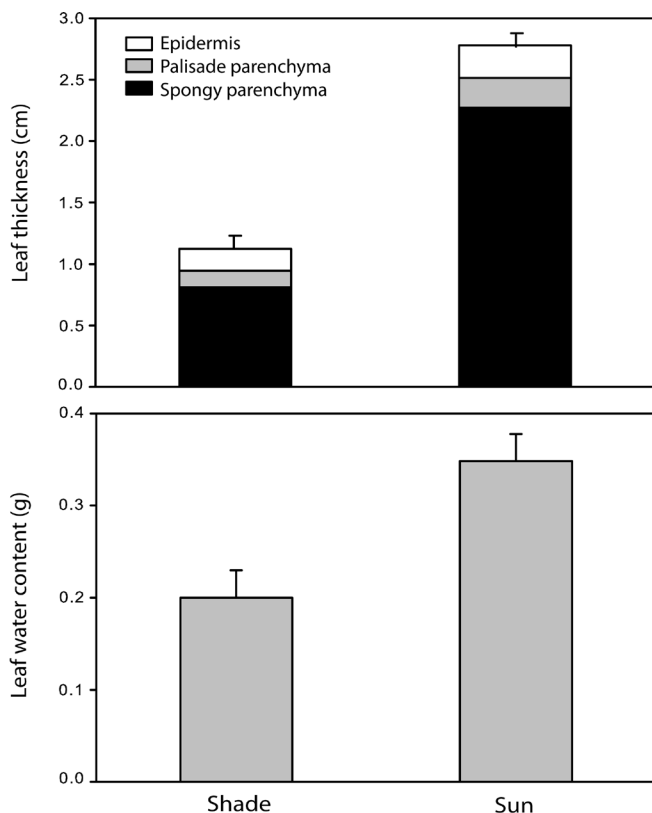


Figure 1 – Leaf thickness and leaf water content of *Sarmienta repens* in ramets growing at shade and sun conditions. Upper graph shows differential contribution of the epidermis (cuticle + upper and lower epidermis), the palisade parenchyma, and the spongy parenchyma to leaf thickness. Bars indicate mean ± SE (n = 30 leaves per environmental condition). Significant differences between traits were observed for leaf thickness, epidermis, palisade parenchyma, spongy parenchyma and leaf water content ($F_{1,58} = 50.4; 15.23; 47.1; 47.8; \text{ and } 12.7$ respectively). In all cases, $p < 0.001$.

Table 1 – Mean ± standard deviation values of relative leaf water content and associated leaf traits of *Sarmienta repens*.

One-way ANOVA revealed significant differences between plants from the forest understory (shade) and borders of canopy gaps (sun). For each analysis, F-values are shown ($df = 58$). *** $p < 0.001$, ns non-significant.

	Relative leaf water content (%)	Leaf area (cm ²)	SLA (cm ² /g)
Shade	95±1	2.79 ± 0.18	361.32 ± 26.86
Sun	94±1	2.38 ± 0.18	152.79 ±26.86
F-value	1.3 ns	2.56 ns	30.1***

tomical layers studied were significantly thicker at sun conditions, the spongy parenchyma contributed the most to the observed leaf succulence patterns (fig. 1). In contrast, rLWC was similar in both environments (table 1). Leaf area did not differ between shade and sun environments but specific leaf area (SLA) was greater in leaves developed in the shaded understory (table 1).

As expected, the shade and sun sites differed in environmental variables. Light availability (photosynthetic photon flux density (PPFD) and direct radiation) and substrate moisture were higher in the gap border (table 2). No differences between sites in diffuse radiation and mean temperature were found (table 2). Micro-environmental differences on direct radiation, but not on diffuse radiation, suggest that *Sarmienta* leaf variation was not only due to differences in light availability but also to differences in water demand (i.e. lower moisture under the sun exposure conditions, table 2).

DISCUSSION

We found that leaf succulence in *Sarmienta* in the cold rainforest of southern Chile was greater at the border of canopy gaps, compared to the shaded understory. Considering that leaf succulence is a water-saving attribute to offset water demand under high irradiances, this pattern suggests that it is a plant trait of current value. Thus, leaf succulence in *Sarmienta* shows functional variation with the prevailing environmental factors that presumably exert selection in this forest: light availability, in the first place, and the associated environmental moisture. The agreement of plant phenotypic variation in contrasting environments with predictions from ecophysiological knowledge likely indicates the occurrence of adaptive processes (Sultan 1995, Saldaña et al. 2005). Likewise, because we did not find that leaf succulence in *Sarmienta* is a fixed trait, whose expression does not vary with the environment, we consider unlikely that it is an evolutionary anachronism.

Since a detachment from the ground usually entails an abrupt change in the syndrome of attributes conferring drought tolerance, epiphytic growth form and water-saving attributes are two characteristics closely linked (Hao et al. 2010). Compared to their non-epiphytic relatives, epiphytes and hemi-epiphytes show higher SLA, thicker epidermis, tighter stomatal control, lower stomatal conductance, and lower leaf water flux rates (Patiño et al. 1995, Hao et al.

Table 2 – Mean \pm standard deviation values of environmental conditions from contrasting micro-sites where *Sarmienta repens* occurs. One-way ANOVA reveals significant differences between forest understory (shade) and borders of canopy gaps (sun). For each analysis, F-values are shown ($df > 1000$). *** $p < 0.001$, ns non-significant.

	Daily photosynthetic photon flux density ($\text{mol m}^{-2} \text{day}^{-1}$)	Temperature ($^{\circ}\text{C}$)	Moisture (% relative water content)	Proportion of direct radiation	Proportion of diffuse radiation
Shade	9 ± 1	11.4 ± 3.5	78 ± 0.6	0.15 ± 0.01	0.22 ± 0.01
Sun	17 ± 2	10.3 ± 3.4	65 ± 0.7	0.28 ± 0.01	0.23 ± 0.01
F-value	42.8***	3.68 ns	40.1***	36.3***	3.62 ns

2010). Changes in leaf size and leaf succulence, stomatal conductance, and water tank storage have been interpreted as niche differentiation to different pulses of fog and precipitation in epiphytic bromeliads (Reyes-García et al. 2011). Likewise, we found high phenotypic variation linked to microenvironmental differences in epiphytes at the intraspecific level. Thus, responses to differences in light exposure, likely mediating differences in water demand (Valladares & Ninemets 2008), resulted in *Sarmienta* plants that in the sun exhibited 148% thicker leaves that accumulated 70% more water, had 53% thicker epidermis and 130% smaller SLAs than plants in the shade.

Although our sampling was limited to 7 m height, *Sarmienta* may reach the canopy of exceptionally large, emergent trees (Díaz et al. 2010), where irradiation, temperature and evapotranspiration should be higher than those recorded here (Lorenzo et al. 2010). Therefore, we expect that those canopy *Sarmienta* plants should show even greater leaf succulence, unless a selective factor, other than the abiotic environment, counteracts the expected trend. It has been found in this forest that SLA – which is closely associated with succulence – may be selected by the light environment and invertebrate herbivores, in the case of a small tree (Salgado-Luarte & Gianoli 2012), and by the abiotic environment, in the case of woody vines (Gianoli & Saldaña 2013). Ideally, further research should address the relationship between leaf succulence and reproductive fitness in *Sarmienta*. However, the logistic challenges to undertake such a task are considerable, including enough sampling of canopies (see Díaz et al. 2010) and the rampant vegetative reproduction of these climbing plants that may prevent the distinction between true genets (see Gianoli et al. 2010).

A previous study, conducted at a similar site (old-growth forest), found that *Sarmienta* is one of the most common epiphytes in the Valdivian forest (Díaz et al. 2010). This result agrees with the positive relationship between epiphyte abundance and succulence found for montane forests in Bolivia (Krömer et al. 2007). It is likely that the unique capacity of *Sarmienta* to develop such a high degree of leaf succulence has promoted the exploitation of the trunk of tall trees. If leaf succulence in *Sarmienta* has an adaptive value, and this feature may be further related to its relative abundance, the question of why succulence is extremely rare in cold temperate rainforests still remains. Two complementary explanations seem plausible. First, *Sarmienta* may be part of the species pool arrived to southern regions presumably due to the lack of strong biogeographic barriers between tropical and temperate regions in ancient South America (Villagrán & Hinojosa 1997, Díaz et al. 2010). This hypothesis is somewhat supported by the lack of succulent epiphytes in cold

forests of western North America and New Zealand where geographical barriers made migration impossible (Díaz et al. 2010 and references therein). Second, *Sarmienta* might be among the few epiphyte species with the ability to cope with the general trade-off between leaf succulence and cold tolerance. With low temperatures, succulent organs are sensitive to freezing as their large cell sizes and high water content increase the potential for intracellular freezing and cell rupture (Nobel 2005). Moreover, in succulent epiphytes with C_3 -CAM metabolism, as we presume is the case for *Sarmienta*, water flow occurs at night when temperatures are lower and vessels are more prone to cavitation and embolism (Lüttge 2008). Further research should address cold tolerance in *Sarmienta* and its physiological basis.

CONCLUSION

We have reported that leaf succulence in epiphytes, an extremely rare feature in cold rainforests, occurs in the evergreen temperate rainforest of southern South America, and probably has an adaptive value for *Sarmienta* plants. Specifically, *Sarmienta* developed thicker spongy parenchyma and thicker epidermis under high irradiance, a common plant response to augment water storage and reduce water loss when evaporative demands are greater. This report is a first approximation to *Sarmienta*'s singularity, which may be used as a model system for further research addressing the trade-off between cold tolerance and succulence in epiphytes, and its implications for epiphytes' distribution patterns.

ACKNOWLEDGEMENTS

O.G. acknowledges the financial support provided by REMEDINAL II (CM-S2009/AMB-1783), the Spanish Ministry for Education and Science, and Fulbright Commission (FU-2009-0039). We are grateful to Alfredo Saldaña and Fernando Carrasco for their valuable help in the field. This study was funded by FONDECYT grant 1070503. We thank CONAF (National Forestry Corporation) for granting permits to work in Puyehue National Park.

REFERENCES

- Bassow S.L., Bazzaz F.A. (1998) How environmental conditions affect canopy leaf-level photosynthesis in four deciduous tree species. *Ecology* 79: 2660–2675. <http://dx.doi.org/10.2307/176508>
- Benzing D.H. (1987) Vascular epiphytism: taxonomic participation and adaptive diversity. *Annals of Missouri Botanical Garden* 74: 183–204. <http://dx.doi.org/10.2307/2399394>

- Bond W.J., Silander J.A. (2007) Springs and wire plants: anachronistic defences against Madagascar's extinct elephant birds. *Proceedings of the Royal Society B* 274: 1985–1992. <http://dx.doi.org/10.1098/rspb.2007.0414>
- Breckle S.W. (2004) Salinity, halophytes and salt affected natural ecosystems. In: Lauchli A., Luttge U. (eds) *Salinity: Environment- Plants- Molecules*: 53–77. Dordrecht, Kluwer Academic Publishers.
- Carrasco-Urra F., Gianoli E. (2009) Abundance of climbing plants in a southern temperate rainforest: host–tree characteristics or light availability? *Journal of Vegetation Science* 20: 1155–1162. <http://dx.doi.org/10.1111/j.1654-1103.2009.01115.x>
- Caldwell M., Richards J. (1989) Hydraulic lift: water efflux from upper roots improves effectiveness of water uptake by deep roots. *Oecologia* 1989: 1–5. <http://dx.doi.org/10.1007/BF00378231>
- Chautems A. (2002) Gesneriaceae. In: Eggl U. (ed.) *Illustrated Handbook of Succulent Plants: Dicotyledons*. 272–286. Berlin, Springer Verlag.
- Diaz I.A., Sieving K.E., Pena-Foxon M.E., Larrain J., Armesto J.J. (2010) Epiphyte diversity and biomass loads of canopy emergent trees in Chilean temperate rain forests: A neglected functional component. *Forest Ecology and Management* 259: 1490–1501. <http://dx.doi.org/10.1016/j.foreco.2010.01.025>
- Donovan L.A., Dudley S.A., Rosenthal D.M., Ludwig F. (2007) Phenotypic selection on leaf water use efficiency and related ecophysiological traits for natural populations of desert sunflowers. *Oecologia* 152: 13–25. <http://dx.doi.org/10.1007/s00442-006-0627-5>
- Dorsch K. (2003) *Hydrogeologische Untersuchungen der Geothermalfelder von Puyehue und Cordon Caulle Chile*. PhD thesis, Ludwig-Maximilians-Universitat, Munich, Germany.
- Drennan P.M. (2009) Temperature influences on plant species of arid and semi-arid regions with emphasis on cam succulents. In: Dela Barrera E., Smith W.K. (eds) *Perspectives in Biophysical plant ecophysiology*: 57–94. Mexico D.F., UNAM.
- Eggl U., Nyffeler R. (2009) Living under temporarily arid conditions succulence as an adaptive strategy. *Bradleya* 27: 13–36.
- Gianoli E., Saldana A. (2013) Phenotypic selection on leaf functional traits of two congeneric species in a temperate rainforest is consistent with their shade tolerance. *Oecologia: Online First*. <http://dx.doi.org/10.1007/s00442-013-2590-2>
- Gianoli E., Saldana A., Jimenez-Castillo M., Valladares F. (2010) Distribution and abundance of vines along the light gradient in a southern temperate rainforest. *Journal of Vegetation Science* 21: 66–73. <http://dx.doi.org/10.1111/j.1654-1103.2009.01124.x>
- Guralnick L.J., Ting I.P., Lord E.M. (1986) Crassulacean acid metabolism in the Gesneriaceae. *American Journal of Botany* 73: 336–345. <http://dx.doi.org/10.2307/2444076>
- Hao G.-Y., Sack L., Wang A.-Y., Cao K.-F., Goldstein G. (2010) Differentiation of leaf water flux and drought tolerance traits in hemiepiphytic and non-hemiepiphytic *Ficus* tree species. *Functional Ecology* 24: 731–740. <http://dx.doi.org/10.1111/j.1365-2435.2010.01724.x>
- Janzen D.H. (1986) Chihuahuan desert nopaleras: defaunated big mammal vegetation. *Annual Review of Ecology Evolution and Systematics* 17: 595–636. <http://dx.doi.org/10.1146/annurev.ecolsys.17.1.595>
- Janzen D.H., Martin P.S. (1982) Neotropical anachronisms: the fruits the gomphotheres ate. *Science* 215: 19–27. <http://dx.doi.org/10.1126/science.215.4528.19>
- Kleinfeldt S.E. (1978) Ants-gardens: the interaction of *Codonanthe crassifolia* (Gesneriaceae) and *Crematogaster longispina* (Formicidae). *Ecology* 59: 449–456. <http://dx.doi.org/10.2307/1936574>
- Kromer T., Kessler M., Gradstein S.R. (2007) Vertical stratification of vascular epiphytes in submontane and montane forest of the Bolivian Andes: the importance of the understory. *Plant Ecology*: 189: 261–278. <http://dx.doi.org/10.1007/s11258-006-9182-8>
- Larson A., Losos J.B. (1996) *Phylogenetic Systematics of Adaptation*. In: Rose M.R., Lauder G.V. (eds) *Adaptation*: 187–220. San Diego, Academic Press.
- Lorenzo N., Mantuano D.G., Mantovani A. (2010) Comparative leaf ecophysiology and anatomy of seedlings young and adult individuals of the epiphytic aroid *Anthurium scandens* (Aubl.) Engl. *Environmental and Experimental Botany* 68: 314–322. <http://dx.doi.org/10.1016/j.envexpbot.2009.11.011>
- Lusk C.H., Jara C., Parada T. (2003) Influence of canopy tree size on stand basal area may reflect uncoupling of crown expansion and trunk diameter growth. *Austral Ecology* 28: 216–218. <http://dx.doi.org/10.1046/j.1442-9993.2003.01279.x>
- Luttge U. (2008) Tropical Forest. IV. Lianas, Hemi-epiphytes, epiphytes and mistletoes. In: Luttge U. (ed) *Physiological ecology of tropical forest*. 165–226. Berlin, Springer Verlag.
- Medina E., Delgado M., Garca V. (1989) Cation accumulation and leaf succulence in *Codonanthe macradenia* J.D. Smith (Gesneriaceae) under field conditions. *Amazoniana* 11: 13–22.
- Munoz Schick M. (1980) *Flora del Parque Nacional Puyehue*. Santiago de Chile, Editorial Universitaria.
- Nieder J., Barthlott W. (2001) *Epiphytes and Canopy Fauna of the Otonga Rain Forest (Ecuador)*. Bonn, Books on Demand.
- Niklas K.J. (2007) *The Evolutionary Biology of Plants*. Chicago, The University of Chicago Press.
- Nobel P.S. (2005) *Physicochemical and Environmental Plant Physiology*. San Diego, Academic Press.
- Patino S., Tyree M.T., Herre E.A. (1995) Comparison of hydraulic architecture of woody plants of differing phylogeny and growth form with special reference to free-standing and hemiepiphytic *Ficus* species from Panama. *New Phytologist* 129: 125–134. <http://dx.doi.org/10.1111/j.1469-8137.1995.tb03016.x>
- Reyes-Garca C., Meja-Chang M., Griffiths H. (2011) High but not dry: diverse epiphytic bromeliad adaptations to exposure within a seasonally dry tropical forest community. *New Phytologist* 193: 745–754. <http://dx.doi.org/10.1111/j.1469-8137.2011.03946.x>
- Riederer M., Schreiber L. (2001) Protecting against water loss: analysis of the barrier properties of plant cuticles. *Journal of Experimental Botany* 52: 2023–2032. <http://dx.doi.org/10.1093/jexbot/52.363.2023>
- Rose M.R., Lauder G.V. (1996) *Adaptation*. San Diego, Academic Press.
- Saldana A., Gianoli E., Lusk C.H. (2005) Ecophysiological responses to light availability in three *Blechnum* species (Pteridophyta Blechnaceae) of different ecological breadth. *Oecologia* 145: 252–257. <http://dx.doi.org/10.1007/s00442-005-0116-2>
- Saldana A., Lusk C.H. (2003) Influencia de las especies del dosel en la disponibilidad de recursos y regeneracion avanzada en un bosque templado lluvioso del sur de Chile. *Revista Chilena de Historia Natural* 76: 639–650. <http://dx.doi.org/10.4067/S0716-078X2003000400008>
- Saldana A., Lusk C.H., Gonzales W.L., Gianoli E. (2007) Natural selection on ecophysiological traits of a fern species in a temperate rainforest. *Evolutionary Ecology* 21: 651–662. <http://dx.doi.org/10.1007/s10682-006-9143-7>

- Salgado-Luarte C., Gianoli E. (2012) Herbivores modify selection on plant functional traits in a temperate rainforest understory. *The American Naturalist* 180: 42–53. <http://dx.doi.org/10.1086/666612>
- Salinas M.F., Arroyo M.T.K., Armesto J.J. (2010) Epiphytic growth habits of Chilean Gesneriaceae and the evolution of epiphytes within the tribe Coronanthereae. *Annals of the Missouri Botanical Garden* 97: 117–127. <http://dx.doi.org/10.3417/2006210>
- Schulze E.-D., Beck E., Müller-Hohenstein K. (2005) *Plant Ecology*. Berlin, Springer Verlag.
- Smith J.F., Funke M.M., Woo V.L. (2006) A duplication of geyc predates divergence within tribe Coronanthereae (Gesneriaceae): Phylogenetic analysis and evolution. *Plant Systematics and Evolution* 261: 245–256. <http://dx.doi.org/10.1007/s00606-006-0445-6>
- Smith J.F., Wolfram J.C., Brown K.D., Carroll C.L., Denton D.S. (1997) Tribal relationships in the Gesneriaceae: evidence from DNA sequences of the chloroplast gene *ndhF*. *Annals of Missouri Botanical Garden* 84: 50–66. <http://dx.doi.org/10.2307/2399953>
- Sultan S.E. (1995) Phenotypic plasticity and plant adaptation. *Acta Botanica Neerlandica* 44: 363–383.
- Valladares F., Niinemets U. (2008) Shade tolerance, a key plant feature of complex nature and consequences. *Annual Review of Ecology, Evolution and Systematics* 39: 237–257. <http://dx.doi.org/10.1146/annurev.ecolsys.39.110707.173506>
- Villagrán C., Hinojosa L.F. (1997) Historia de los bosques del sur de Sudamérica II: Análisis fitogeográfico. *Revista Chilena de Historia Natural* 70: 241–267.
- Willert D.J.v., Eller B.M., Werger M.J.A., Brinckmann E. (1990) Desert succulents and their life strategies. *Vegetatio* 90: 133–144. <http://dx.doi.org/10.1007/BF00033023>
- Zaya D.N., Howe H.F. (2009) The anomalous Kentucky coffeetree: megafaunal fruit sinking to extinction? *Oecologia* 161: 221–226. <http://dx.doi.org/10.1007/s00442-009-1372-3>

Manuscript received 27 Sep. 2012; accepted in revised version 18 Apr. 2013.

Communicating Editor: Pierre Meerts.