

Arbuscular mycorrhizas and dark septate endophytes in bromeliads from South American arid environment

M.A. Lugo^{1*}, M.G. Molina², and E.M. Crespo¹

¹Diversidad Vegetal I and ²Morfología Vegetal, FQByF, UNSL, Ejército de los Andes 1148, 5700 San Luis, Argentina, Emails: lugo@unsl.edu.ar, mmolina@unsl.edu.ar and ecrespo@unsl.edu.ar

(Received October 16, 2007; Accepted May 5, 2008)

Abstract

Most plant roots are associated with glomalean fungi forming arbuscular mycorrhizas (AM) and a wide range are also colonized by ascomycetous dark septate endophytes (DSE). Bromeliaceae species can be epiphytic, rupicolous or terrestrial but their mycorrhizal status is poorly studied. We examined the AM and DSE status of 5 epiphytic and 4 terrestrial Bromeliaceae from an arid area of Central Argentina. The terrestrial species were either dually associated (AM and DSE) or non-associated whereas the epiphytes were only DSE colonized. Terrestrial Bromeliaceae that formed AM-DSE associations were likely responding to the arid conditions of the area and the availability of AM fungal (AMF) spores in the soil. The terrestrial *Bromelia ubaniana* was not colonized either by AMF or DSE. This could reflect its root morphology and high number of root hairs. DSE are endosymbiotic in the stressful ecosystems experienced by canopy epiphytes in the studied environment. The different fungal associations are discussed in relation to the three Bromeliaceae subfamilies and we suggest that environmental features determine the type of association formed by species in this plant family.

Keywords: Bromeliaceae, arbuscular mycorrhiza, dark septate, epiphytic bromeliads, terrestrial bromeliads, arid environment

1. Introduction

Mycorrhizal associations occurring on the roots of most plants are important for nutrient acquisition and assimilation by hosts. Arbuscular mycorrhizas (AM) (Phylum Glomeromycota) are the most widespread mycorrhizal type being present in 80% of higher land plants (Schubler et al., 2001). AM are found in a wide range of habitats, from deserts to tropical forest (Allen, 1991, 1996), mainly those that are characterized by nutrients deficient soils (Read, 1991; Smith and Read, 1997). Another widespread type of symbiotic association between roots and fungi is the “dark septate endophyte” (DSE), established by pigmented and anamorphic endophytic Ascomycetes. These associations are mainly found in extremely stressed environments. DSE develop interfaces with the host that could facilitate a form of biotrophic nutrition via which carbon exchange between host and fungus may occur.

Furthermore, the fungal continuum from vascular cylinder to cortex to epidermis to hyphae outside root

allows the possibility of bidirectional nutrient exchange between plant and soil (Barrow and Aaltonen, 2001; Barrow, 2003). It has been suggested that DSE enables enhanced phosphorus absorption by the host, but the association can fluctuate from mutualism to parasitism (Jumpponen and Trappe, 1998; Jumpponen, 2001).

The family Bromeliaceae includes over 50 genera and ca. 3.000 species, most of which are native in America, only one species is found in Africa (Benzing, 2000; Haro-Carrion, 2004). These plants are generally rosette-forming herbs, which can be terrestrial, rupicolous or epiphytic on bark. They are found in hot tropical, subtropical and temperate regions. They mainly exhibit a crassulacean acid metabolism (CAM) which is adapted to such habitats. Numerous other families of Angiosperms, e.g. Araceae, Orchidaceae, Piperaceae, Gymnosperms and ferns constitute the epiphytic community of vascular plants that abound in the tropics (Zotz and Hietz, 2001). In Argentina, the family Bromeliaceae is represented by 13 native genera and 110 species, 42 of which are endemic (Zuloaga et al., 1999). Some are terrestrial and others are epiphytic.

The “Sierras de las Quijadas” National Park constitutes an arid region in central Argentina, characterised by a rich

*The author to whom correspondence should be sent.

variety of xerophytic species which are exposed to water-stress and nutrient deficiency. The mycorrhizal status of native plants in this region is poorly studied. The objectives of this work were to: (1) analyze quantitatively the mycorrhizal and dark septate endophytes colonization in Bromeliaceae living in a habitat of extreme aridity; (2) compare colonization in the terrestrial and epiphytic species; and (3) assess differences in colonization between the various bromeliad species.

2. Materials and Methods

Location of the sample site

The Sierras de las Quijadas National Park, is located in the NW of San Luis province (between 32°20', 32°47' Lat. S and 67°10', 66°58' Long. W), at 800 meters above sea level comprises 150,000 ha of dry lands. It is located in an arid region between the biogeographic provinces of Monte and Chaco (Cabrera, 1976). Rainfall is scarce and irregular, presenting a mean annual precipitation of 200 mm. Rainfall tends to occur in summer, with the highest values up to 250 mm in the rainy season (December and January) and 0 mm in the dry season (February to November). In the Park, mean temperatures range from 27°C in summer to 9°C in winter. During summer, temperatures reach a maximum value of 45°C while in winter they fall to a minimum of -12°C, representing large seasonal, daily, and yearly thermal amplitudes. The mean annual thermal amplitude is 13.7°C (Rivarola, 1997). This weather is a characteristic of continental situations and in this case may be called hilly-arid. It is included in the temperate-dry type of weather typical of hills and prairies (Capitanelli, 1989). In the Park, water and wind sculpts the clay and sandstones forming a strange landscape named "huayquerias" or bad-lands. The flora is rich in xerophytic species many of which are shrubs. A total of 45 vascular plant families are represented in the Park, among which Bromeliaceae is dominant with a total of 10 native species (Del Vitto et al., 2001).

Hosts

Nine native species of Bromeliaceae were studied: five epiphytes (*Tillandsia angulosa* Mez, *T. bryoides* Griseb., *T. castellanii* L. B. Sm., *T. rectangula* Baker, *T. xiphioides* Ker Gawl.) and four terrestrial (*Bromelia urbaniana* (Mez) L. B. Sm., *Deuterocohnia longipetala* (Baker) Mez, *Dyckia floribunda* Griseb., *D. velascana* Mez), all of them occurring in the National Park.

Methods

The plants were randomly collected, 5 individuals per species (the low number was due to restrictions related to

the protected nature of the National Park) in summer and early autumn when plants and fungi are most active. The roots were separated from the flowering stems and were dried for voucher specimens that have been accessioned at the Vegetal Diversity Herbarium, Ecology Area, University of San Luis. The roots were frozen until they were washed and fixed in FAA.

The roots were then cleared, stained (Grace and Stribley, 1991) and mounted on semi-permanent preparations using polyvinyl alcohol. Root colonization was quantified using an optical microscope at 400× (McGonigle et al., 1990). For the quantification of arbuscular mycorrhizas, the following data were collected: colonized roots (% AM RC), arbuscules (% A) and vesicles (% V). For DSE, the method of McGonigle et al. (1990) was modified. The number of times in which these fungi were observed during quantification of the roots was counted and their frequency was calculated as the percentage of root colonized by DSE (% DSE RC). We considered as DSE only those hyphae that exhibited cortical colonization, were dark and formed microsclerotia.

For each species 5 individuals (i.e. replicates) were studied. The roots from each individual plant were mixed and 4 sub-samples were randomly extracted from each batch. Each sub-sample was mounted on a separate one slide and then observed under the microscope. One hundred roots segments were examined on each slide.

Data analysis

Kruskal-Wallis test was used with $\alpha=0.05$, when the "species" factor was considered, a test *a posteriori* of multiple comparisons (Marascuilo and McSweeney, 1977) was carried out when significant differences were found; whereas, for the "habitat" factor (terrestrial and epiphytic) the test performed was Mann-Whitney. The variables analyzed were: percentage of root dark septate fungi colonization (% DSE RC), percentage of arbuscular mycorrhizas fungi colonization (% AM RC), percentage of arbuscules (% A) and vesicles (% V). The analyses were performed using Infostat program version 2.0. 2002.

3. Results

Epiphytic Bromeliaceae were only associated with DSE (Table 1). Among the terrestrial species, *Bromelia urbaniana* was the only species in which no fungal associations were found in its roots, though occasionally DSE hyphae were observed externally in the rhizoplane. All the other terrestrial species formed dual associations involving both AM and DSE. In relation to the AM colonization (Table 1), the arbuscules were scarce (1%) and were only observed in one terrestrial species (*D. velascana*). Vesicles were found in *Dyckia velascana*

Table 1. Colonization of arbuscular mycorrhiza and dark septate endophytes in terrestrial and epiphytic bromeliads.

Host	% A	AM colonization % V	% AM RC	DSE colonization % DSE RC
Terrestrial bromeliads				
<i>Bromelia urbaniana</i>	0	0	0	0
<i>Deuterocohnia longipetala</i>	0 a	22.9 ± 8.0 bc	63.5 ± 12.9 ab	16.7 ± 33.5 ab
<i>Dyckia floribunda</i>	0 a	7.3 ± 3.9 ab	84.5 ± 10.4 bc	62.9 ± 10.5 c
<i>D. velascana</i>	0.5 ± 0.6 a	37.3 ± 2.9 c	100.0 ± 0.0 c	19.3 ± 14.1 abc
Epiphytic bromeliads				
<i>T. angulosa</i>	0	0	0	27.1 ± 18.3 ab
<i>T. bryoides</i>	0	0	0	69.4 ± 13.6 cd
<i>T. castellanii</i>	0	0	0	29.5 ± 23.6 a
<i>T. rectangula</i>	0	0	0	90.6 ± 10.43 d
<i>T. xiphioides</i>	0	0	0	36.5 ± 27.3 abc

The data are mean values (n=5) ± standard deviations. Different letters denote significant differences at $p < 0.05$ for the *a posteriori* Kruskal-Wallis test. % A (percentages of arbuscules), % V (percentages of vesicles), % AM RC (percentage of roots colonized by arbuscular mycorrhiza), % DSE RC (percentages of roots colonized by dark septate endophytes).

(34–41%), *Deuterocohnia longipetala* (15–32%) and *Dyckia floribunda* (3–11%). The percentage of V varied significantly with species (H: 14.12; p : 0.00). The differences between *Dyckia floribunda* and *D. velascana* were significant. The % AM RC, ranged from 100% in *D. velascana*, 78–100% in *Dyckia floribunda* and 52–77% in *Deuterocohnia longipetala* and the differences between the last two host species were found to be significant (H: 13.50; p : 0.00; Kruskal-Wallis and *a posteriori* test of multiple comparisons).

DSE associations occurred in both terrestrial and epiphytic habitats (Table 1), the % of DSE RC varying significantly between the habits (U: 215.50; p : 0.01). DSE were, in general, more frequent in the epiphytic Bromeliaceae (with average values of 27 to 91% of DSE RC), than in terrestrial (with mean of 0 to 63%). There were significant differences between the epiphytic Bromeliaceae species studied (H: 14.30; p : 0.01). Frequencies of colonization were high in *T. rectangula* (91%) and *T. bryoides* (69%) and they diminished in *T. xiphioides* (36.5%), *T. castellanii* (29.5%) and *T. angulosa* (27%). The last three species did not differ significantly in colonization, but they differed significantly from those with greater colonization (*T. bryoides* and *T. rectangula*).

In terrestrial Bromeliaceae, significant differences were also observed in the frequencies of DSE colonization among species (H: 9.15; p : 0.02). Colonization frequencies were greatest in *Dyckia floribunda* (63%), intermediate in *D. velascana* (19%) and *Deuterocohnia longipetala* (17%), while there was no colonization in *Bromelia urbaniana*. Differences in % DSE were significant between *B. urbaniana*, *Deuterocohnia longipetala* and *Dyckia floribunda*.

4. Discussion

In cold and nutrient-poor environments where it is difficult for AM-forming fungi (AMF) to disperse, DSE are commonly found to colonize plant roots (Barrow, 2003; Kohn and Stasovski, 1990). In extreme habitats such as those in alpine ecosystems and arid regions, DSE associate with a wide range of different host species such as Chenopodiaceae (Barrow and Aaltonen, 2001), Poaceae (Barrow, 2003) and Cyperaceae (Jumpponen and Trappe, 1998). AMF also occur in these arid zones (Allen, 1996; Smith and Read, 1997) and dual colonization has been found in grass roots (see Newsham, 1999). The presence of DSE in stressed environments may reflect the fact that the hyphae have high chitin and melanin concentrations in their walls. This makes the walls more rigid and reduce their permeability, protecting the fungal cells from extreme environmental conditions, so allowing them to be the first colonizers of a range of plants. DSE are ubiquitous, anamorphic or sterile, and may be polymorphic. They include rhizospheric fungi as well as soil fungi and their nutrition pattern may be biotrophic, saprophytic or parasitic (Schulz and Boyle, 2005).

In the present study epiphytic Bromeliaceae from the Park were associated with DSE and with the absence of AM. This is consistent with the results reported by other authors for the “seasonal tropical forests” of Mexico and “Atlantic tropical forests” of Brazil (Allen et al., 1993; Grippa et al., 2007). Although Allen et al. (1993) considered that DSE do not have the characteristics of fungal symbionts, in alpine environments, they concluded that the DSE might act as a “potential mycorrhizas”. Anatomical considerations lead us to think that the fungi

studied by these authors were DSE with hyaline structures and may be, functioning as systemic endophytic fungi or SEF (Barrow, 2003).

The epiphytic Bromeliaceae from Sierra de las Quijadas do not accumulate suspended soils; instead, their roots only function as an anchorage to the host plant. Thus neither organic matter accumulation nor the presence of spores or AMF hyphae, are favored. We suggest that in both the Brazilian tropical Atlantic forests (Grippa et al., 2007), and in our Park with its conditions of extreme aridity, the association of epiphytic Bromeliaceae with DSE allows them to survive. We hypothesize that the fungi assist in drought-resistance mechanisms and facilitate phosphorus capture, as happens when these fungi associate with other hosts (Jumpponen, 2001; Newsham, 1999). The lack of AM association in epiphytic Bromeliaceae in the Park may result from the absence of a dispersal mechanism for Glomerales that could transport spores from the soil to the branches of host plants. In tropical rain forests AMF spores have been found in bromeliad mats and climbing rodents seem to be the main spore dispersers though birds and ants may assist in dispersal, since they feed on the soil and return to the trees, carrying spores to their nests and anthills (Janos, 1993).

In Sierra de las Quijadas Park, colonization of the terrestrial Bromeliaceae by AMF is similar to data reported for tropical areas (Grippa et al., 2007; see Janos, 1993). In this Park, the association with Glomerales fungi may be the result of the presence of fungal propagules and a low concentrations of nutrients in the soil, both of which would enhance the establishment of AM in the terrestrial Bromeliaceae (Read, 1991; Smith and Read, 1997). Dual colonization of AMF and DSE types has not been reported, up to now, in terrestrial Bromeliaceae in extreme arid ecosystems. However, this type of dual association has been observed in *Gaultheria poeppigii* (Ericaceae) (Urcelay, 2002), and it has also been observed in native Poaceae in high grasslands in Central Argentina and Puna (Lugo personal observations).

Bromelia urbaniana is the only member of the terrestrial Bromeliaceae which did not show arbuscular mycorrhiza or DSE colonization. The absence of any fungal association may be related to its root morphology since it has abundant root hairs (Lugo personal observation). These could effectively provide the plant with essential nutrients and water, a theory previously postulated by Baylis (see Fitter, 2004). *B. urbaniana* also has branched roots whereas the rest of the terrestrial species have thick roots on which root hairs are scarce or absent. *Bromelia urbaniana* was found to have a few DSE fungal hyphae on the surface in the rhizoplane. This may indicate an allelopathic effect in *B. urbaniana* which should be studied. In *B. penguin*, a citotoxic effect resulting from flavonoid production in roots and stems has already been documented (Raffauf et al., 1981). It is interesting that flavonoides and isoflavonoid

compounds may either inhibit or stimulate AMF colonization (Harrison, 1997).

This is the first time that epiphytic and terrestrial habits have been compared in the three Bromeliaceae subfamilies growing under similar environmental conditions at the same site. The species studied were *Bromelia urbaniana* (Bromelioidea), *Deuterocohnia longipetala*, *Dyckia floribunda* and *D. velascana* (Pitcairnoidea) and *Tillandsia angulosa*, *T. bryoides*, *T. castellanii*, *T. rectangula* and *T. xiphoides* (Tillansioidea). Species belonging to Bromelioideae were nonmycorrhizal, in Pitcairnoidea there were both, species associated with AMF and species associated with DSE. Lastly, in Tillansioidea, only DSE associations were observed. We assume that the AM association is ancestral to other mycorrhizas (Trappe, 1987; Wang and Qiu, 2006) and that dual associations might function to improve nutrient absorption in poor soils or be a form of “insurance” or back-up when the inoculum of the AM fungal symbiont is not available (Brundrett, 2002). We suggest that in Sierra de las Quijadas, Pitcairnoidea would be more primitive and that the presence of dematiaceous endophytic fungi may be a more recent acquisition, as in Tillansioidea. Likewise, the absence of associations in Bromelioideae may coincide with a late divergence from the group, and, in the case of *Bromelia urbaniana*, related to its peculiar root morphology.

Some apparently contradictory results have been found for the subfamily Bromelioideae with respect to the presence of AM e.g. in *Ananas*, *Aechnea*, *Bilbergia* and *Nidularium* (Grippa et al., 2007; Wang and Qiu, 2006). This may be due to a difference in their habitat (terrestrial versus epiphytic), and is consistent with the presence of AM in the rest of the terrestrial species of the Pitcairnoidea. The absence, in our study, of AM in Bromelioideae and the presence of DSE in Tillansioidea agree with the findings of others and may be associated with the evolution of a capacity to conquer the canopy and exist as epiphyte (Maffia et al., 1993; Michelsen, 1993; Nadarajah and Nawawi, 1993).

Finally, the fact that different fungal associations are established in the subfamilies of Bromeliaceae contributes to our understanding of evolution in the family. Previously this has been based on metabolic types (CAM and C₃-carbon dioxide fixation by Calvin Cycle-) and the epiphytic habit. Together these suggest a monophyletic origin of the Bromelioideae and Tillansioidea and a possible paraphyletic origin of the Pitcairnoidea (Crayn et al., 2004).

Although the evidence is scarce, we speculate that the presence or absence of different types of mycorrhizal associations is related to patterns of evolution within the family. Further studies on bromeliads and their fungal partners would be desirable to provide a more complete evolutionary picture.

Acknowledgements

This work was supported by PROIPRO 2-0302 (SECyT, FQBYF-UNSL). The authors wish to thank the Argentinean National Park Administration for having allowed the implementation of the field work in Sierras de las Quijadas and, in particular, Horacio López, the forest guard for his kind attitude during the sampling processes. Professor D.H.S. Richardson and two anonymous reviewers for helpful comments on the grammar, English and content of an early draft of this manuscript. Also, the authors are very grateful to A.M. Mangione, E.R. Nouhra and C. Urcelay for their critical revision of a later version of this manuscript.

REFERENCES

- Allen, M.F. 1991. *The Ecology of Mycorrhizae*. Cambridge University Press, New York, 184 pp.
- Allen, M.F. 1996. The ecology of arbuscular mycorrhizas: a look back into the 20 th century and a peek into the 21st. *Mycological Research* **100**: 769–782.
- Allen, M.F., Rincon, E., Allen, E.B., Huante, P., and Dunn, J.J. 1993. Observation of canopy bromeliad roots compared with plants rooted in soils of seasonal tropical forest, Chamela, Jalisco, México. *Mycorrhiza* **4**: 21–25.
- Barrow, J.R. 2003. Atypical morphology of dark septate fungal root endophytes of *Bouteloua* in arid southwestern USA rangelands. *Mycorrhiza* **13**: 239–247.
- Barrow, J.R. and Aaltonen, R.E. 2001. Evaluation of the internal colonization of *Atriplex canescens* (Pursh) Nutt. Roots by dark septate fungi and the influence of host physiological activity. *Mycorrhiza* **11**: 199–205.
- Benzing, D.H. 2000. Introduction. In: *Bromeliaceae: Profile of an Adaptive Radiation*. Cambridge University Press, Cambridge, pp. 1–15.
- Brundrett, M.C. 2002. Coevolution of roots and mycorrhizas of land plants. *New Phytologist* **154**: 275–304.
- Cabrera, A.L. 1976. Territorios fitogeográficos de la República Argentina. In: *Enciclopedia Argentina de Agricultura y Jardinería*, 2da. Edición, II (1), ACME, Argentina, pp. 1–85.
- Capitanelli, R. 1989. *Geografía de San Luis. Gobierno de la Prov. de San Luis*. Ministerio de Cultura y Educación, pp. 205–216.
- Crayn, D.M., Winter, K., and Smith, A.C. 2004. Multiple origins of crassulacean acid metabolism and the epiphytic habit in the Neotropical family Bromeliaceae. *Proceedings of the National Academy of Sciences of the USA* **101**: 3703–3708.
- Del Vitto, L.A., Petenatti, E.M., and Petenatti, M.E. 2001. Catálogo preliminar de la Flora Vasculare, Parque Nacional "Sierra de las Quijadas" San Luis, Argentina. *Serie Técnica del Herbario UNSL* **8**: 1–13.
- Fitter, A.H. 2004. Magnolioid roots-hairs, architecture and mycorrhizal dependency. *New Phytologist* **61**: 15–16.
- Grace, C. and Stribley, D.P. 1991. A safer procedure for routine staining of vesicular-arbuscular mycorrhizal fungi. *Mycological Research* **95**: 1160–1162.
- Haro-Carrion, X. 2004. Bromeliad distribution in two plots in the Sumaco Biosphere Reserve. *Lyonia* **7**: 57–62.
- Harrison, M.J. 1997. The arbuscular mycorrhizal symbiosis: an underground association. *Trends in Plant Science* **2**: 54–60.
- Grippa, C.R., Hoeltgebaum, M.P., and Stürmer, S.L. 2007. Occurrence of arbuscular mycorrhizal fungi in bromeliad species from the tropical Atlantic forest biome in Brazil. *Mycorrhiza* **17**: 235–240.
- Janos, D.P. 1993. Vesicular-arbuscular mycorrhizae of epiphytes. *Mycorrhiza* **4**: 1–4.
- Jumpponen, A. 2001. Dark septate endophytes – are they mycorrhizal?. *Mycorrhiza* **11**: 207–211.
- Jumpponen, A. and Trappe, J.M. 1998. Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytologist* **140**: 295–310.
- Kohn, L.M. and Stasovski, E. 1990. The mycorrhizal status of plants at Alexandra fiord, Ellesmere island, Canada, a high Arctic site. *Mycologia* **82**: 23–35.
- Maffia, B., Nadkarni, N.M., and Janos, D.P. 1993. Vesicular-arbuscular mycorrhizae of epiphytic and terrestrial Piperaceae under field and greenhouse conditions. *Mycorrhiza* **4**: 5–9.
- Marascuilo, L.A. and McSweeney, M. 1977. Post-Hoc Multiple Comparisons in sample preparations for test of homogeneity. In: *Non-Parametric and Distribution Free Methods the Social Sciences*. McSweeney, M. and Marascuilo, L.A., eds. Cole Books, California, pp. 141–147.
- McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, D.L., and Swam, J.A. 1990. A new methods which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **115**: 495–501.
- Michelsen, A. 1993. The mycorrhizal status of vascular epiphytes in Bale Mountains National Park, Ethiopia. *Mycorrhiza* **4**: 11–15.
- Nadarajah, P. and Nawawi, A. 1993. Mycorrhizal status of epiphytes in Malaysian oil palm plantations. *Mycorrhiza* **4**: 21–25.
- Newsham, K.K. 1999. *Phialophora graminicola*, a dark septate fungus beneficial associate of the grass *Vulpia ciliata* ssp *ambigua*. *New Phytologist* **144**: 517–524.
- Raffauf, R.F., Menachery, M.D., Le Quesne, P.W., Arnold, E.V., and Clardy, J. 1981. Antitumor plants. 11. diterpenoid and flavonoid constituents of *Bromelia pinguin* L. *Journal of Organic Chemistry* **46**: 1094–1098.
- Read, D.J. 1991. Mycorrhizas in ecosystems. *Experientia* **47**: 376–391.
- Rivarola, D. 1997. *El Parque Nacional Sierra de las Quijadas y sus recursos naturales*. Printers Impresores, San Luis, Argentina, 48 pp.
- Schubler, A., Schwarzott, D., and Walker, C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* **105**: 1413–1421.
- Schulz, B. and Boyle, C. 2005. The endophytic continuum. *Mycological Research* **10**: 661–686.
- Smith, S.E. and Read, D.J. 1997. The symbionts forming VA mycorrhizas. In: *Mycorrhizal Symbiosis*. Smith, S.E. and Read, D.J., eds. Academic Press, San Diego, pp. 11–32.
- Trappe, J.M. 1987. Phylogenetic and ecological aspects of mycotrophy in the Angiosperms from an evolutionary standpoint. In: *Ecophysiology of VA Mycorrhizal Plants*. Safir, G.R., ed. RC Press, USA, pp. 5–25.
- Urcelay, C. 2002. Co-occurrence of three fungal root symbionts in *Gaultheria poeppigii* DC in Central Argentina. *Mycorrhiza* **12**: 89–92.
- Wang, B. and Qiu, Y-L. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* **16**: 299–363.
- Zotz, G. and Hietz, P. 2001. The physiological ecology of vascular epiphytes: current knowledge, open questions. *Journal of Experimental Botany* **52**: 2067–2078.
- Zuloaga, F.O., Morrone, O., and Rodríguez, D. 1999. Análisis de la biodiversidad en plantas vasculares de la Argentina. *Kurtziana* **27**: 17–167.

