Journal of Arid Environments 115 (2015) 19–26

Contents lists available at ScienceDirect

Journal of Arid Environments

journal homepage: www.elsevier.com/locate/jaridenv

Occurrence of *Epichloë* fungal endophytes in the sheep-preferred grass *Hordeum comosum* from Patagonia

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ARTICLE INFO

Article history:
Received 10 October 2014
Received in revised form
22 December 2014
Accepted 23 December 2014
Available online 7 January 2015

Keywords:
Native forage species
Patagonia
Epichloë
Endophytes
Phylogeny
Incidence

ABSTRACT

Grazing by exotic herbivores on native vegetation in Patagonian steppes has led to the deterioration of forage resources, where grasses are replaced by shrubs and preferred grasses by non-preferred ones. In this region, *Hordeum comosum* is one of the widely spread native-grasses highly preferred by sheep. Contrary to other preferred grasses, *H. comosum* establishes symbiosis with vertically-transmitted fungi of genus *Epichloë*. However, the level of incidence of the fungus and the phylogenetic diversity of the endophyte remained unclear. We found that endophyte incidence ranged from 0 to 100%, with higher incidence in populations from more arid sites. This would suggest an endophyte-conferred drought tolerance to host. Although the isolates presented several morphological differences, phylogenetic analyses of *tubB* and *tefA* genes separated them into only two lineages. One of these lineages was *Epichloë tembladerae*, the most common endophyte in temperate grasses of southern South America. Strikingly, the other lineage was a hybrid between *Epichloë typhina* and *Epichloë amarillans* detected for the first time in this part of the world and opening new questions about the grass-endophyte co-evolution. These results represent a starting-point in the potential use of fungal endophytes in breeding programs and natural grassland restoration in marginal environments.

1. Introduction

Water-limited ecosystems cover about 40% of the global land and still they provide support for human production activities and endemic biodiversity (Verón et al., 2006; Reynolds et al., 2007). In South America, one of the most extensive arid regions is represented by Patagonia (Soriano et al., 1983; León et al., 1998; Fernandez and Busso, 1997). As other similar arid ecosystems (Verón et al., 2006; Reynolds et al., 2007), the Patagonian steppes exhibit serious symptoms of deterioration as consequence of continuous and extensive grazing by sheep (Soriano et al., 1983; León et al., 1998). At the plant community level, one of these symptoms is the decline in the abundance of sheep-preferred species and the increase of non-preferred ones (Perelman et al., 1997) resulting in a reduction in their carrying capacity. The success of any attempt at restoration of natural forage resources must take into consideration not only the management practices and the standard breeding methods but also the ecology of the species (Golluscio et al., 1998). For example, current breeding programs of forage grasses are considering the symbiosis with benefic microorganisms in order to increase the persistence and productivity of plants (Gundel et al., 2013; Johnson et al., 2013; Card et al., 2014).

Certain cool season grasses form highly specific, persistent symbiosis with vertically transmitted fungal endophytes of the genus *Epichloë* which presumably improve their fitness (Clay and Schardl, 2002; Iannone et al., 2012). The symbiosis between grasses and endophytes has been extensively studied in two temperate forage grasses: tall fescue (*Schedonorus arundinaceus* Schreb Dumort) and perennial ryegrass (*Lolium perenne* L.), where it is usually associated with improved tolerance to drought and resistance to herbivory (Malinowski and Belesky, 2000; Clay and Schardl, 2002; Saikkonen et al., 2006). Despite the scarce information gathered from wild species, it is suggested that those effects may be highly variable – eventually turning to negative–,

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http://dx.doi.org/10.1016/j.jaridenv.2014.12.008
0140-1963/

depending on partners genotype and environmental conditions (Faeth and Sullivan, 2003; Saikkonen et al., 2006). Nonetheless, in some undomesticated species such as *Bromus auleticus* from South America, the endophytes have shown to be clearly beneficial by increasing plant production and protection against pathogens (Faeth and Sullivan, 2003; Saikkonen et al., 2006; Iannone et al., 2012; Vignale et al., 2013). From an agronomic point of view, a negative aspect of this symbiosis is that some grass-endophyte associations are highly toxic for livestock due to the fungal alkaloids ergot and indole-diterpenes that are produced by the endophyte (Clay and Schardl, 2002). The variability in the profile and level of alkaloids has allowed the inoculation of plant cultivars with selected fungi (so-called "safe endophytes") that are not toxic to cattle but still beneficial for host plants becoming a key strategy for breeding forage (Bouton et al., 2002; Johnson et al., 2013; Gundel et al., 2013). Alternatively, this strategy would not be necessary in some valuable forage species since the associated wild endophytes do not produce any alkaloid detrimental for livestock (*i.e.*: *E. uncinata* in *Festuca pratense* and *E. occultans* in *Lolium multiflorum*). The discovery of non-toxic 'safe' endophytes associated with grass species or populations that are locally adapted to marginal environments may become a useful strategy for managing breeding programs of native forage species and restoration plans.

Many grass species from arid and semiarid environments are associated with *Epichloë* species (Rudgers et al., 2009; Iannone et al., 2011). The knowledge about these associations in arid environments is mostly restricted to reports of their natural occurrence and incidence in the populations (Wei et al., 2006; Rudgers et al., 2009; Iannone, 2010). However, very few studies have established the way in which the symbiosis outcome is regulated by genetic factors and ecological conditions (Novas et al., 2007; Wilson, 2007; Sullivan and Faeth, 2008; Iannone et al., 2013; Afkhami et al., 2014, Semmartín et al., 2014). The results of these surveys are contradictory, for example in some host grasses the incidence of the endophyte decreases as stress conditions increases while in other host species the opposite is observed (Novas et al., 2007; Iannone et al., 2013; Afkhami et al., 2014). Considering the variability of responses observed in grass–endophytes associations with respect to stress tolerance, more surveys are necessary to understand the importance of fungal endophytes for the adaptation of grasses to arid environments.

In Patagonia, endophytes of grasses are preceded by a negative reputation since *Epichloë tembladerae* has been associated to the "*huecú*" disease in sheep and cattle grazing on *Festuca argentina* (Speg.) Parodi and *Poa huecu* Parodi (Parodi, 1950; Cabral et al., 1999). However, for many other Patagonian grasses the association with endophytes did not result in any harm to livestock (Novas et al., 2007; Wilson, 2007; Iannone et al., 2011). The diversity of grass–endophyte associations detected in Patagonia offers a diversity of model systems to study the role of this symbiosis in the adaptation of plants to arid environments.

In this study, we explored the association between the wild perennial grass *Hordeum comosum* J. Presl and fungal endophytes in Northwest Patagonia across a gradient of mean annual precipitation. Although the effect of the endophytes from *H. comosum* on herbivores has not been studied up to the moment, previous ecological studies showed a negative correlation between the abundance of *H. comosum* and grazing pressure by sheep (León and Aguiar, 1985; Perelman et al., 1997), suggesting no toxicity. Therefore, among the proposed two main controls of symbiosis incidence in grass populations (i.e. herbivory resistance and tolerance to drought; Malinowski and Belesky, 2000; Clay and Schardl, 2002; Gundel et al., 2011; Afkhami et al., 2014), we focused on the putative positive effect of the endophyte for host plants in facing the water deficit. As a first approximation, we surveyed the natural incidence of fungal endophytes in *H. comosum* populations across different environments covering a range of precipitations from ≈100 to ≈500 mm in an area of 20,000 km^2. Secondly, we isolated and described the fungi in terms of growing parameters in culture. Finally, we determined the fungus species through molecular phylogenetic analyses.

2. Materials and methods

2.1. Study area and plant collection

Patagonia occupies nearly 60 million ha in southern Argentina covering an extensive area of South America between 39° and 55° south latitude, and from the western limit of the Andes Mountains to the Atlantic Ocean (Soriano et al., 1983; León et al., 1998). The collection sites of *H. comosum* populations belonged to two different districts of the Patagonian Phytogeographic Province (Soriano, 1956): the Occidental District, with annual precipitation ranging from 300 to 600 mm and dominated by tussock grasses and shrubs, and the Sub-Andean District with precipitation ranging from 600 to 800 mm and grasslands dominated by *Festuca pallescens* (Soriano, 1956; Nicora, 1978; León and Aguiar, 1985; Jobbágy et al., 1995; Perelman et al., 1997) (Fig. 1).

The sites of collection were situated in two private ranches called Leleque (populations 1, 3–8) and Montoso (populations 2, 10–14), and in an experimental field station at Río Mayo (population 9), Chubut, Argentina, that belongs to the Instituto Nacional de Tecnología Agropecuaria (INTA) (Table 1, and Fig. 1). Mean annual precipitation is 400 mm, 300 mm and 150 mm for Leleque, Montoso and Río Mayo, respectively. The three ranches are actively managed for sheep production. In the first two ranches sheep follow a grazing management plan, while at Río Mayo site sheep graze in the same paddocks year-round.

2.2. Endophyte incidence in population

In each population, mature seeds were hand-harvested from at least 20 plants, in 2009. The amount of mature seeds produced by each plant was very low and just four to six seeds were collected from each plant. Therefore, seeds were pooled in order to increase the sample size and have a reliable estimation of the endophyte incidence at population level. Half of the seeds were stored at −4 °C for endophyte isolation. The remaining seeds were used to establish the incidence of endophytes in the population. To achieve this, 20–30 seeds were softened in KOH 10%, stained with aniline blue (0.1% w/v aqueous) and observed under microscope (see Iannone et al., 2011). To establish the existence of any relationship between endophyte incidence and precipitation a linear correlation analysis was performed between incidence and mean annual precipitation (MAP) in each site of collection. MAP was calculated as a function of the distance from the "cordillera" (Andes Mountains) to a given population accordingly to the mathematical models described by Jobbágy et al. (1995). These models allow establishing the MPA using the longitude (LON) of each collection site and have shown to be highly accurate for this area of South Argentina (Jobbágy et al., 1995).

2.3. Endophytes isolation, morphological and molecular characterization

Endophytes were isolated on potato dextrose agar (PDA) medium from superficially sterilized seeds from the collected plants (Iannone et al., 2009). Isolates were single spore isolated and grown on PDA in darkness at 24C for morphological characterization and DNA isolation as described in Iannone et al. (2009).

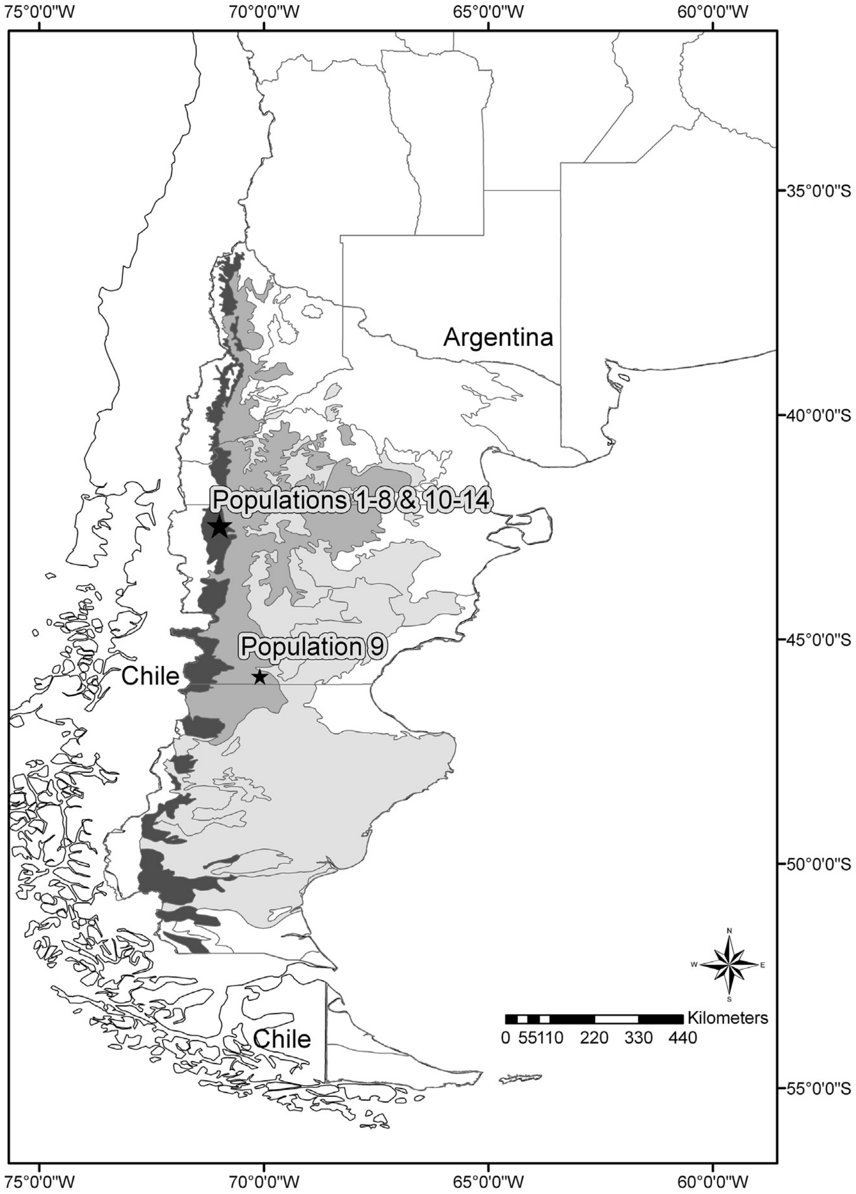

Fig. 1. Map showing the location and distribution of the studied populations of *Hordeum comosum* in the Patagonian Phytogeographic Province (Soriano, 1956): Sub-Andean District (Dark shaded area) and Occidental District (mild-dark shaded area), Argentina.

Phylogenetic analyses were achieved to identify the endophytes from *H. comosum* and to establish its relationship with other endophytes from southern South America, hosted in different grass species, and with endophytes from other *Hordeum* species from other Continents. One isolate of each population was selected for DNA sequencing. Intron rich regions of the genes encoding β-tubulin (*tubB*) gene of thirteen isolates and *tefA* gene of three isolates were amplified and sequenced. Sequences of these two genes have been extensively used to establish the phylogenic relationships between *Epichloë* species (Moon et al., 2004; Iannone et al., 2009). DNA isolation and PCR reactions were performed as described by Moon et al. (2004) and Mc Cargo et al. (2014). To establish the possibility of a hybrid origin, both genes were firstly amplified using non selective primers. Since hybrids present at least two different alleles of the gene, a two-dye-terminator peaks are observed at polymorphic single nucleotide position (Moon et al., 2004). All of the isolates presented double peaks. Thus, a selective amplification of each allele of *tubB* and *tefA* was performed. Sequencing reactions and sequence alignments were conducted following the methodology described in Iannone et al. (2009). Maximum Parsimony analyses (MP) were performed using Winclada (Nixon, 1999) and Maximum Likelihood analyses (ML) were conducted with Mega 6. The evolutionary model for ML was K2 + G (gamma = 0.37) for *tubB* and K2 for *tefA*, established with Mega 6 (Tamura et al., 2013). Reference sequences from other endophytes from Argentina, published by Iannone et al. (2009, 2012) and Mc Cargo et al. (2014), and from sexual and asexual *Epichloë* species and isolates from other *Hordeum* and close related species, published in Moon et al. (2004) were included in the analyses.

Table 1
Characterization of the sites where *Hordeum comosum* populations were collected, incidence of fungal endophytes (%) and isolates obtained from each population. Together with the geographic coordinates of the site, an ecological description of the system and values of mean annual precipitation are provided.

Location	Pop.	Ecological description	Geographic coordinates		Altitude	Dist. Cord.	MAP	Endophyte incidence		Isolates
			LAT	LON	(m.a.s.l)	(km)	(mm)	(%)	N	
Leleque	1	Grass Steppe (SD)	42° 25′ 38	70° 57′ 04	1044	40.9	214.54	80	20	2732, 2733
Montoso	2	Humid meadow (SD)	42° 35′ 41	71° 17′ 34	770	6.7	459.83	0	27	
Leleque	3	Shrub Steppe (OD)	42° 31′38	71° 03′ 28	814	30.2	272.17	79.41	34	2708, 2729, 2730
Montoso	4	Shrub Steppe (OD)	42° 37′ 23	70° 59′ 47	804	36.3	237.39	100	30	B1, 2705, 2724
Leleque	5	Grass Steppe (SD)	42° 27′ 12	71° 09′ 57	855	19.0	349.50	0	27	
Leleque	6	Grass Steppe (SD)	42° 27′49	71° 00′ 55	977	34.5	247.53	75.86	29	2707
Leleque	7	Grass Steppe (SD)	42° 26′ 2	70° 57′ 26	1049	40.3	217.52	74.07	27	2706
Leleque	8	Grass Steppe (SD)	42° 31′14	71° 10′ 46	903	17.0	365.45	0	33	
Río Mayo	9	Shrub steppe (OD)	45° 24′ 39	70° 17′ 34	536	106.7	49.40	90.91	22	2709, 2731, 2793
Montoso	10	Grass Steppe (SD)	42° 39′ 59	71° 02′ 55	972			ND		2704, 2722, 2723
Montoso	11	Grass Steppe (SD)	42° 40′ 10	71° 03′ 33	980			ND		2725
Montoso	12	Grass Steppe (SD)	42° 48′16	71° 07′17	871			ND		2700, 2701, 2726, 2727, 2728
Montoso	13	Shrub Steppe (OD)	42° 48′16	71° 06′58	864			ND		2702
Montoso	14	Shrub Steppe (OD)	42° 52′48	71° 06′48	772			ND		2703

Pop.: Populations, (OD): Occidental district, (SD): Sub-Andean district, LAT: latitude, LON: longitude, Dist. Cord.: Distance to the Andes Mountains, MAP: mean annual precipitation N = number of seeds used to determine the endophyte incidence in each population. m.a.s.l: meters above sea level. Underlined isolates were used for sequencing: *tubB* single underlined, *tefA* single underlined, *tubB* and *tefA* double underlined. ND: not determined.

3. Results

3.1. Endophyte incidence

Endophytic fungi were present in eleven of the fourteen explored populations with an average incidence across infected populations of 83.38% (Table 1). Infected populations were found in all the environments but endophyte-free populations (2, 5 and 8) were restricted to humid meadow and grass steppes from Sub-Andean District. Irrespective of the Phytogeographic Province, all populations collected in shrub steppes presented a high proportion of endophyte-infected individuals (≈90.10%) (Table 1). Endophyte incidence was inversely associated with MAP ($R^2 = 0.67$, $p = 0.007$) (Fig. 2).

3.2. Characterization of the endophytes

One to five isolates were obtained from each population. There were morphological differences among isolates; both in colony morphology and in microscopic characteristics (Table 2; Fig. 3).

However, these differences were not consistent enough to distinguish the endophytes from different populations (Table 2). Some isolates presented velvety colonies and slow radial growth on PDA, reaching only 10 mm in diameter after 30 days (Fig. 3A), other isolates presented fast growing cottony or slightly waxy colonies that reached 26–35 mm in diameters after 30 days (Fig. 3B and C). All isolates presented aculeate and unbranched phialidic conidiogenous cells which arise from hypha coils (Fig. 3D). Each conidiogenous cell produced one or two, rarely three, conidia. The length of the conidiogenous cell ranged from 14 to 27 μm, however some longer (45–50 μm long) conidiogenous cells were observed in some isolates. Conidial size varied among isolates, some isolates produced small conidia (6.1–7.8 μm long), other isolates presented middle sized conidia (7.2–9.5 μm long) and other presented large conidia (8–11 μm long) (Fig. 3E and F).

Two different copies of *tub*B and *tef*A genes were present in each isolate, indicating a hybrid origin (Fig. 4). Maximum Parsimony and ML phylogenetic analyses rendered the same clades.

For twelve of the isolates, one copy was grouped with reference sequences of *Epichloë typhina* and the other with the reference sequences of *Epichloë festucae*, indicating that these isolates are hybrids between *E. typhina* and *E. festucae*. In addition, for these isolates the sequences of each copy of *tubB* presented a 100% homology with the respective copy of *E. tembladerae* reference sequences, thus these isolates were considered to be *E. tembladerae*. For isolate Hco2702 from population 13, copy-1 of this gene was grouped with sequences from *Epichloë amarillans* and copy-2 with *E. typhina*. The same results were observed in *tefA* phylogeny (Fig. 4) and indicate that two different endophytic lineages are present in *H. comosum*. The whole set of *tubB* gene sequences is deposited in genBank under accession numbers: KP208987–KP209012. GenBank accession number of each allele of *tefA* sequences of isolate Hco2702 are KP202390 and KP202391.

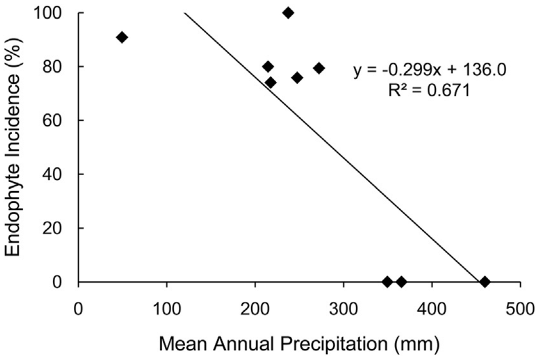

Fig. 2. Relationship between endophyte incidence and mean annual precipitation in each population.

4. Discussion

Hordeum comosum is a wide-spread forage grass from Patagonia that represents a suitable candidate for local domestication in order to restore land degradation caused by extensive domestic grazing in arid environments from Patagonia (Gundel et al., 2012). Here we described for the first time two aspects of the symbiosis between *H. comosum* and *Epichloë* fungal endophytes. Firstly we showed the existence of variability in the incidence of endophytes among wild populations of this grass species and that the incidence is associated with annual precipitation. Secondly we found a striking phenotypic and genotypic variability in the isolated fungi. In particular, genetic characterization indicated that there are at least

Table 2
Morphological characteristics of epichloae endophytes isolated from *Hordeum comosum* plants from Argentina.

Isolate	Population	Colony diameter (mm)	Colony aspect	Conidiogenous cell (μm)	Conidia length (μm)	Conidia wide (μm)
B1	4	27	Cottony	22 ± 1.5	8.8 ± 0.2	3.2 ± 0.11
2657	Unknown	26	Waxy	21.9 ± 1.6	7.9 ± 0.18	3.7 ± 0.16
2702	13	28	Cottony	22.3 ± 1.9	8.5 ± 0.18	3.4 ± 0.15
2704	10	15	Velvety	19.8 ± 0.73	8.7 ± 0.17	3.2 ± 0.09
2705	4	21	Cottony	22.3 ± 1.9	9.7 ± 0.14	3.1 ± 0.08
2706	7	27	Waxy	21.5 ± 1.8	9.6 ± 0.21	3.4 ± 0.06
2708	3	28	Cottony	24.2 ± 1.6	8.5 ± 0.18	3.2 ± 0.09
2709	9	33	Waxy	23.4 ± 1.2	8.7 ± 0.16	2.9 ± 0.06
2725	11	34	Waxy	22.8 ± 1.3	7.1 ± 0.13	3.3 ± 0.09
2726	12	24	Cottony	22.0 ± 1.3	8.1 ± 0.19	3.0 ± 0.08
2732	1	33.5	Waxy	18.3 ± 1.1	6.8 ± 0.11	3.1 ± 0.06

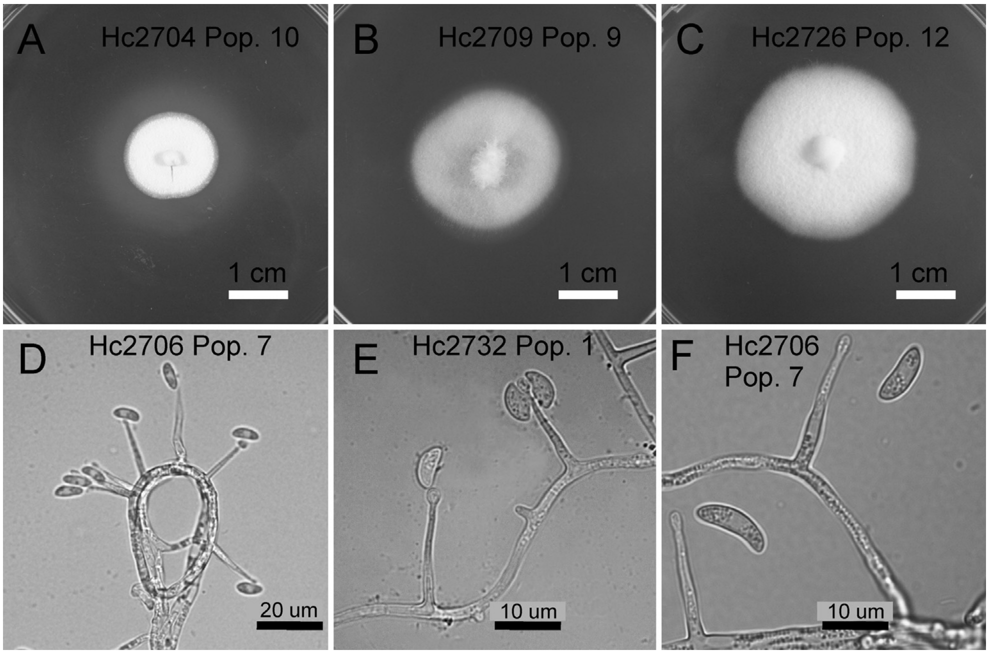

Fig. 3. Morphology of the fungal endophytes isolated from *Hordeum comosum*. Panels A–C: morphology of the colony in PDA medium. Panels D–F: conidia and conidiogenous cells. Representative isolates were chosen to show the morphological diversity. The ID number and population of the isolates are shown in each panel.

two different endophytic lineages associated with this host grass species.

Based on the negative correlation found between endophyte incidence and mean annual precipitation, the symbiotic relationship between *H. comosum* and endophytes seems to be controlled by environmental factors related to water availability. Improved tolerance to drought and resistance to herbivory are proposed to be the underlying mechanisms by which fungal endophytes are present at higher rates in grass populations (Malinowski and Belesky, 2000; Clay and Schardl, 2002; Gundel et al., 2011). Thus, our results would be in accordance with this general idea that fungal endophytes improve plant tolerance to drought. In *Poa lanuginosa* and *Poa bonariensis* populations with endophytes are located in areas that present water limitations in some periods of the year (Iannone et al., 2013). However, contrasting with this general idea are results from certain case-specific studies and recent reviews. For example, the incidence of fungal endophytes was positively correlated with mean annual precipitation in populations of *Bromus setifolius*, another palatable grass occurring in southernmost Patagonia (Novas et al., 2007). A very recent global review showed that sites with low aboveground net primary productivity (ANPP), likely as a result of water shortage, presented higher probability of populations with no-endophyte or low incidence of endophytes compared with sites with high ANPP (Semmartin et al., 2014). In addition, is worth mentioning that studies that experimentally addressed the hypothesis of improved tolerance to drought mediated by endophytes have been carried out with the mesophytic species tall fescue and perennial ryegrass exposed to water shortage for short periods of time (Saikkonen et al., 2006). Therefore, although the variability in the response of endophyte-infected plants to water availability may be idiosyncratic depending on multiple factors such as environmental condition, grass population and endophyte genotypes, our results suggest that more studies, including a broader range of grass-endophyte associations, are needed in order to unveil endophyte dynamics in wild grass populations from arid and semiarid environments.

Traits conferring tolerance to drought can converge and also confer resistance to grazing (Chapin et al., 2002), and domestic grazing may also be an important selection pressure on the incidence of endophytes in grass populations (Clay and Schardl, 2002). In this sense, a more detailed and extensive survey is needed in order to get a clearer picture on the association between the endophyte incidence in populations and local environmental variables in *H. comosum*. Therefore, we propose that a complete

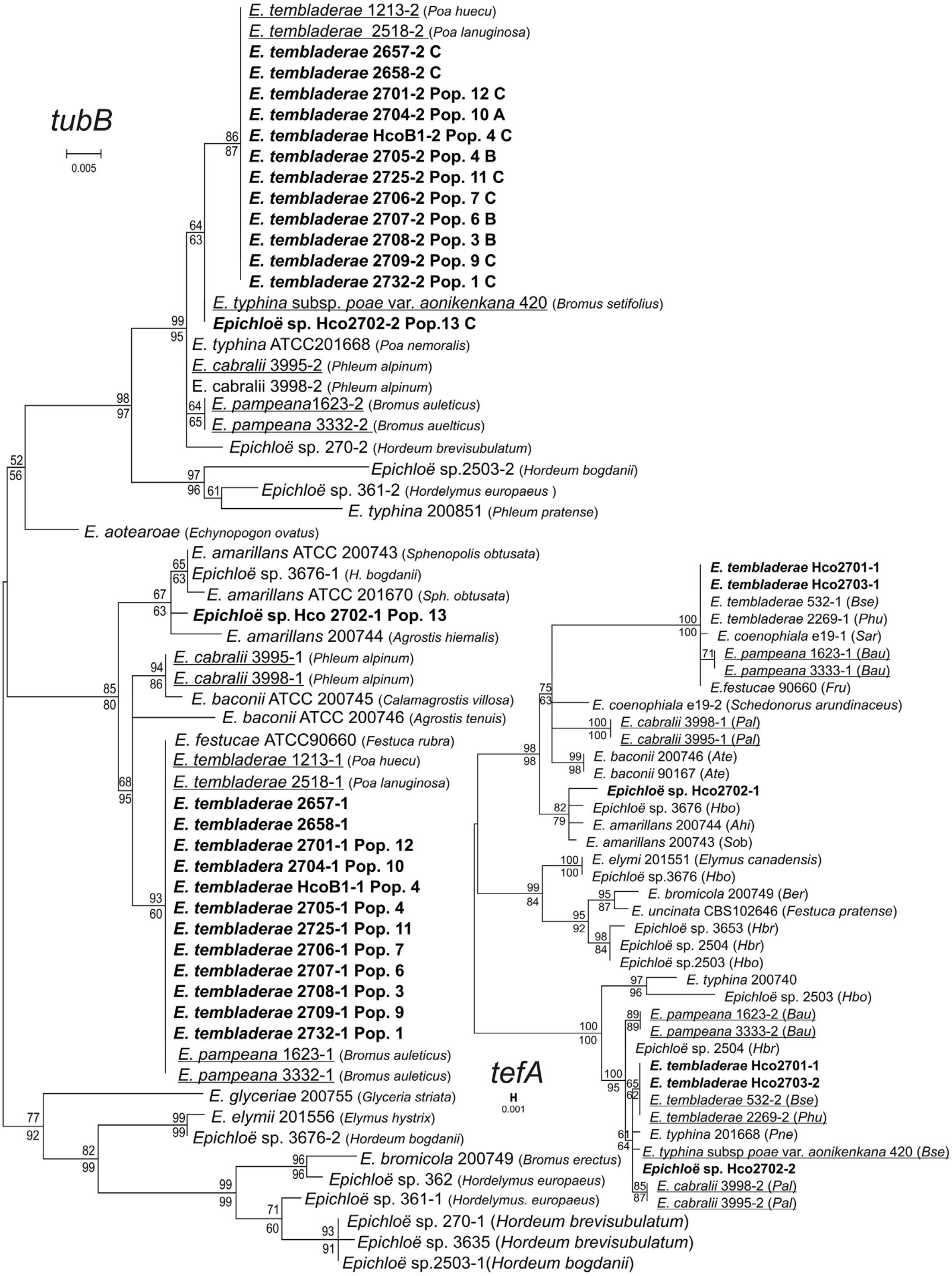

Fig. 4. Phylogenetic trees of *tubB* and *tefA* gene sequences obtained by ML or MP showing the relationships between fungal endophytes isolated from *Hordeum comosum* and other *Epichloë* species from other grass species described for Argentina or from other parts of the world. Isolates from *H. comosum* are shown in bold; isolates from other host from Argentina are underlined. The name of the host plant of each isolate is shown between parentheses. Numbers in the nodes indicate bootstrap support for ML above, MP below. On *tubB* tree, the population from each isolate is indicated by "Pop. Number" and the capital letter indicate the morphology of the colony as in Fig. 2.

understanding of the processes behind the incidence of endophytes in arid and semiarid environments needs further studies combining exclosures to grazers along precipitation gradients.

The characterization of the endophytes performed in this study reveals an even wider host range for *Epichloë tembladerae*, which has been reported to be associated with 19 grass species in Argentina (Iannone et al., 2012) and *Festuca arizonica* in USA (Moon et al., 2004). The detection of a new lineage (the isolate Hco2702) indicates the existence of at least five asexual lineages of *Epichloë* (*E. tembladerae*, *Epichloë pampeana*, *Epichloë cabralii*, *Epichloë typhina* subsp. *poae* var. *aonikenkana* and Hco2702) in South America.

The hybrid lineage Hco2702, which was until now unknown, presents some interesting features with respect to the evolutionary origin of the epichloae. This lineage may have evolved as the result of hybridization events between ancestors of *Epichloë amarillans* and *E. typhina* subsp. *poae* var. *aonikenkana*. The relevance of this finding lies on two remarkable facts. Firstly, *E. amarillans* × *E. typhina* hybrids have not been previously described in Argentina or elsewhere. Secondly, neither sexual nor asexual states of *E. amarillans* have been reported in South America. More studies should be conducted in order to know the incidence and distribution of this lineage in *H. comosum* or even in other grasses from the region.

The presence of *E. tembladerae* in a highly preferred grass is not unusual since most grasses associated with this endophyte species have been reported as non toxic (Iannone et al., 2012). This suggests that endophytes confer wider spectrum of benefits to their hosts, besides anti-herbivore defenses. For example, in other native grasses from Argentina, *E. tembladerae* confers resistance to the smut disease caused by *Ustilago bullata* (Vignale et al., 2013), a pathogen of *Hordeum* species. In addition, the symbiosis with endophytes has been found to protect other *Hordeum* species from herbivory by aphids (Clement et al., 1997). These characteristics are of capital importance in the use of endophytes in breeding programs and must be studied in the *H. comosum*-endophyte system. The exploitation of the benefits delivered by the symbionts is not limited to the particular case of *H. comosum* domestication, since it can also be inoculated in other agronomic valuable host species (Gundel et al., 2013; Johnson et al., 2013; Card et al., 2014).

The distribution range of *H. comosum* extends along the Andes Mountains from southern Patagonia in Argentina and Chile, to arid lands in the Bolivian highlands (Seberg and Petersen, 2012). This area comprises a diversity of arid environments which are mostly devoted to domestic grazing by sheep and goat. Given that the surveyed area represents a small part of the whole range covered by *H. comosum*, a more extensive study is required in order to establish distribution patterns of endophyte incidence in relation to environmental variables. The morphological diversity detected among endophytes isolated from the host *H. comosum* suggests the existence of a higher diversity of endophytes associated with this plant. More studies should be conducted in order to evaluate if this diversity is linked to the potential capacity of endophytes to produce (or not) alkaloids toxic to domestic cattle as, ergot alkaloids or indole-diterpenes.

5. Conclusions

Our work shows, for the first time, the existence of inter-population variability in the association of the native grass *H. comosum* and *Epichloë* fungal endophytes in Patagonia, South America. Contrary to what can be expected for an asexual and vertically transmitted microorganism, we found morphological and genetic diversity, which opens the possibility for finding candidates that can be cross-inoculated in other forage cultivars for their improvement. The results of our research are important to understand the role of symbiotic associations with fungal endophytes in the adaptation of the plants to arid environments and represent a starting point for the study of the diversity of fungal endophytes and their effects on host grass performance particularly, in highly stressing environments.

Acknowledgment

This research was supported by the University of Buenos Aires (grant 20020090300118), ANPCyT (PICT-2008-670), CONICET (grant PIP1482), ANPCyT (PICT-2011-1527). PROPLAME-PRHIDEB-CONICET. Publication n° 206.

References

Afkhami, M.E., Mc Intyre, P.J., Strauss, S.Y., 2014. Mutualist-mediated effects on species' range limits across large geographic scales. Ecol. Lett. 17, 1265–1273.

Bouton, J.H., Latch, G.C.M., Hill, N.S., Hoveland, C.S., McCann, M.A., Watson, R.H., Parish, J.A., Hawkins, L.L., Thompson, F.N., 2002. Reinfection of tall fescue cultivars with non-ergot alkaloid-producing endophytes. Agron. J. 94, 567–574.

Cabral, D., Cafaro, M.J., Saidman, B., Lugo, M., Reddy, P.V., White Jr., J.F., 1999. Evidence supporting the occurrence of a new species of endophyte in some South American grasses. Mycologia 91, 315–325.

Card, S.D., Faville, M.J., Simpson, W.R., Johnson, R.D., Voisey, C.R., de Bonth, A.C., Hume, D.E., 2014. Mutualistic fungal endophytes in the Triticeae – survey and description. FEMS Microbiol. Ecol. 88, 94–106.

Chapin, F.S., Matson, P.A., Mooney, H.A., 2002. Principles of Terrestrial Ecosystem Ecology. Springer, New York.

Clay, K., Schardl, C.L., 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. Am. Nat. 160 (S4), S99–S127.

Clement, S.L., Wilson, A.D., Lester, D.G., Davitt, C.M., 1997. Fungal endophytes of wild barley and their effects on *Diuraphis noxia* population development. Entomol. Exp. Appl. 82, 275–281.

Faeth, S.H., Sullivan, T.J., 2003. Mutualistic asexual endophytes in a native grass are usually parasitic. Am. Nat. 161, 310–325.

Fernandez, O.A., Busso, C.A., 1997. Arid and Semi-arid Rangelands: Two Thirds of Argentina. RALA Report 200, pp. 41–60.

Golluscio, R.A., Deregibus, V.A., Paruelo, J.M., 1998. Sustainability and range management in the Patagonian steppes. Ecol. Austral 8, 265–284.

Gundel, P.E., Rudgers, J.A., Ghersa, C.M., 2011. Incorporating the process of vertical transmission into understanding of host-symbiont dynamics. Oikos 120, 1121–1128.

Gundel, P.E., Irisarri, J.G.N., Sorzoli, N.S., Mosso, C.E., García-Martínez, G., Golluscio, R., 2012. Basic germination requirements of two palatable grasses (*Hordeum comosum* and *Koeleria vurilochensis* var. patagonica) from semiarid Patagonian steppes. J. Arid Environ. 78, 183–186.

Gundel, P.E., Pérez, L.I., Helander, M., Saikkonen, K., 2013. Symbiotically modified organisms: non-toxic fungal endophytes in grasses. Trends Plant Sci. 18, 420–427.

Iannone, L.J., Cabral, D., Schardl, C.L., Rossi, M.S., 2009. Phylogenetic divergence, morphological and physiological differences distinguish a new *Neotyphodium* species endophyte in the grass *Bromus auleticus* from South America. Mycologia 101, 340–351.

Iannone, L.J., Novas, M.V., Pinget, A., De Battista, J., Young, C., Schardl, C.L., 2012. Endophytes of native grasses from South-America: biodiversity and ecology. Fungal Ecol. 5, 357–363.

Iannone, L.J., White Jr., J.F., Giussani, L.M., Novas, M.V., 2011. Diversity and distribution of *Neotyphodium*-infected grasses in Argentina. Mycol. Prog. 10, 9–19.

Iannone, L.J., Mc Cargo, P.D., Giussani, L.M., Schardl, C.L., 2013. Geographic distribution patterns of vertically transmitted endophytes in two native grasses in Argentina. Symbiosis 59, 99–110.

Jobbágy, E., Paruelo, J.M., León, R.J.C., 1995. Estimación del régimen de precipitación a partir de la distancia a la cordillera en el noroeste de la Patagonia. Ecol. Austral. 5, 47–53.

Johnson, L.J., de Bonth, A.C.M., Briggs, L.R., Caradus, J.R., Finch, S.C., Fleetwood, D.J., Fletcher, L.R., Hume, D.E., Johnson, R.D., Popay, A.J., Tapper, B.A., Simpson, W.R., Voisey, C.R., Card, S.D., 2013. The exploitation of epichloae endophytes for agricultural benefit. Fungal Divers. 60, 171–188.

León, R.J.C., Aguiar, M.R., 1985. El deterioro por uso pasturil en estepas herbáceas patagónicas. Phytocoenologia 13, 181–196.

León, R.J.C., Bran, D., Collantes, M., Paruelo, J.M., Soriano, A., 1998. Grandes unidades de vegetación de la Patagonia extra andina. Ecol. Austral 8, 125–144.

Malinowski, D.P., Belesky, D.P., 2000. Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. Crop Sci. 40, 923–940.

Mc Cargo, P.D., Iannone, L.J., Vignale, M.V., Schardl, C.L., Rossi, M.S., 2014. Species diversity of *Epichloë* symbiotic with two grasses from Southern Argentinean Patagonia. Mycologia 106, 339–352.

Moon, C.M., Craven, K.D., Leuchtmann, A., Clement, S.L., Schardl, C.L., 2004. Prevalence of interspecific hybrids amongst asexual fungal endophytes of grasses. Mol. Ecol. 13, 1455–1467.

Nicora, E., 1978. Gramineae. In: Correa, M.N. (Ed.), Flora Patagónica Colección Científica del INTA, Buenos Aires, p. 563.

Nixon, K.C., 1999. Winclada (Beta) Ver. 0.9.9.

Novas, M.V., Collantes, M., Cabral, D., 2007. Environmental effects on grass-endophyte associations in the harsh conditions of south Patagonia. FEMS Microbiol. Ecol. 61, 164–173.

Parodi, L.R., 1950. Las gramíneas tóxicas para el ganado en la república Argentina. Rev. Argent. Agron. 17, 163–232.

Perelman, S.B., León, R.J.C., Bussacca, J.P., 1997. Floristic changes related to grazing intensity in a Patagonian shrub steppe. Ecography 20, 400–406.

Reynolds, J.F., Stafford Smith, D.M., Lambin, E.F., Turner II, B.L., Mortimore, M., Batterbury, S.P.J., Downing, T.E., Dowlatabadi, H., Fernández, R.J., Herrick, J.E., Huber-Sannwald, E., Jiang, H., Leemans, R., Lynam, T., Maestre, F.T., Ayarza, M., Walker, B., 2007. Global desertification: building a science for dryland development. Science 316, 847–851.

Rudgers, J.A., Afkhami, M.E., Rúa, M.A., Davitt, A.J., Hammer, S., Huguet, V.M., 2009.

A fungus among us: broad patterns of endophyte distribution in the grasses. Ecology 90, 1531–1539.

Saikkonen, K., Lehtonen, P., Helander, M., Koricheva, J., Faeth, S.H., 2006. Model systems in ecology: dissecting the endophyte-grass literature. Trends Plant Sci. 11, 428–433.

Seberg, O., Petersen, G., 2012. Hordeum L.. In: Zuloaga, F.O., Rúgolo, Z.E., Anton, A.M. (Eds.), Flora vascular de la República Argentina, vol. 3(2). Gráficamente ediciones, Córdoba, pp. 65–82.

Semmartin, M., Omacini, M., Gundel, P.E., Hernández-Agramonte, I.M., 2014. Broad-scale variation of fungal-endophyte incidence in temperate grasses. J. Ecol. http://dx.doi.org/10.1111/1365-2745.12343.

Soriano, A., 1956. Los distritos florísticos de la provincia patagónica. Rev. Investig. Agrícolas 10, 323–347.

Soriano, A., Movia, C.P., León, R.J.C., 1983. Deserts and semi-deserts of Patagonia. In: West, N.E. (Ed.), Temperate Deserts and Semi-deserts of the World, Ecosystems of the World. Elsevier Publ. Co., New York, pp. 440–454.

Sullivan, T.J., Faeth, S.H., 2008. Local adaptation in *Festuca arizonica* infected by hybrid and non-hybrid *Neotyphodium* endophytes. Microb. Ecol. 55, 697–704.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.

Verón, S.R., Paruelo, J.M., Oesterheld, M., 2006. Assessing desertification. J. Arid Environ. 66, 751–763.

Vignale, M.V., Astiz-Gassó, M., Novas, M.V., Iannone, L., 2013. *Epichloid* endophytes confer resistance to the smut *Ustilago bullata* in the wild grass *Bromus auleticus* (Trin.). Biol. Control 67, 1–7.

Wei, Y.K., Gao, Y.B., Xu, H., Su, D., Zhang, X., Wang, Y.H., Lin, F., Chen, L., Nie, L.Y., Ren, A.Z., 2006. Occurrence of endophytes in grasses native to northern China. Grass Forage Sci. 61, 422–429.

Wilson, A.D., 2007. Clavicipitaceous anamorphic endophytes in *Hordeum* germplasm. Plant Pathol. J. 6, 1–13.