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46	Abstract	The incidence of epichloid endophytes in populations of wild grasses is usually variable, and the knowledge about distribution patterns and how environmental factors affect such an incidence is limited. Here we performed a broad scale survey data to study whether the distribution patterns and the incidence of vertically-transmitted endophytes in populations of two native grasses from South-America, *Poa lanuginosa* Poir. and *Poa bonariensis* (Lam.) Kunth., are associated with environmental characteristics. We also characterized the endophytes from different populations to establish if the genotype of the endophytes is also correlated with environmental variables. The incidence of endophytes ranged from 0 to 100 % in both host species. In *P. lanuginosa*, endophytes were only found in populations on sandy coastal dunes and their incidence was positively associated with winter regime rainfall and soil water availability in the growing season. In *P. bonariensis*, endophytes were only found in populations in xerophytic forests and their incidence was highly associated with plant community. The

distributions of infested populations suggested that the endophytes are not found in those areas with the most favorable or most stressing growth conditions accordingly to climatic or edaphical characteristics. Only the vertically transmitted hybrid endophyte species *Neotyphodium tembladerae* was detected in both host species. Under the hypothesis of vertical transmission, these results suggested that the endophyte should have been lost in endophyte free populations but is maintained in populations established in environments presenting moderate stress as salinity or short drought periods.

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Geographic distribution patterns of vertically transmitted endophytes in two native grasses in Argentina

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7 Liliana M. Giussani · Christopher L. Schardl

10

Abstract The incidence of epichloid endophytes in populations of wild grasses is usually variable, and the knowledge about distribution patterns and how environmental factors affect such an incidence is limited. Here we performed a broad scale survey data to study whether the distribution patterns and the incidence of vertically-transmitted endophytes in populations of two native grasses from South-America, *Poa lanuginosa* Poir. and *Poa bonariensis* (Lam.) Kunth., are associated with environmental characteristics. We also characterized the endophytes from different populations to establish if the genotype of the endophytes is also correlated with environmental variables. The incidence of endophytes ranged from 0 to 100 % in both host species. In *P. lanuginosa*, endophytes were only found in populations on sandy coastal dunes and their incidence was positively associated with winter regime rainfall and soil water availability in the growing season. In *P. bonariensis*, endophytes were only found in populations in xerophytic forests and their incidence was highly associated with plant community. The distributions of infested populations suggested that the endophytes are not found in those areas with the most favorable or most stressing growth conditions accordingly to climatic or edaphical characteristics. Only the vertically transmitted hybrid endophyte species *Neotyphodium tembladerae* was detected in both host species. Under the hypothesis of vertical transmission, these results suggested that the endophyte should have been lost in endophyte free populations but is maintained in populations established in environments presenting moderate stress as salinity or short drought periods.

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1 Introduction

Most if not all plants in natural ecosystems are symbiotic with mycorrhizal fungi or fungal endophytes (Petrini 1986; Rodriguez et al. 2009). Many grass species, in the subfamily Pooideae, establish particular symbiotic associations with endophytic fungi in the genus *Epichloë* Tul. (Hypocreales, Clavicipitaceae), and with their evolutionary derivative species of the anamorphic genus *Neotyphodium* Glenn, Bacon and Hanlin.

Epichloid endophytes systemically and asymptomatically colonize the apoplast (Schardl et al. 2004) and the phylloplane (White et al. 1996; Moy et al. 2000; Christensen et al. 2012) of the aboveground tissues of their hosts. *Epichloë* species produce perithecia on stromata that choke the inflorescences, causing total or partial sterility of the host plants (White et al. 1993; Chung and Schardl 1997), and the ascospores produced in the perithecia are responsible for horizontal transmission of these fungi. All asexual epichloae and some sexual species are vertically transmitted within the

caryopses, colonizing the seedling as seeds germinate (Schardl et al. 2004). Although some asexual epichloae appear incapable of horizontal transmission (Latch and Christensen 1985), recently, it has been demonstrated that the asexual (yet stroma-forming) species *Epichloë poae* Tadych, Ambrose, Belanger and White is capable to be horizontally transmitted via conidia (Tadych et al. 2012).

In general, epichloid endophytes have been considered to be strong mutualistic symbionts of their hosts (Schardl 1996; Clay and Schardl 2002). They produce a battery of alkaloids detrimental for insect and vertebrate herbivores (Schardl et al. 2004). These endophytes also promote plant growth and confer resistance to different stresses (Malinowski and Belesky 2000; Iannone et al. 2012a). However, in recent years, evidence has indicated endophyte effects ranging from beneficial to detrimental, depending on host genotype and environmental factors (Hesse et al. 2003; Cheplick 2004; Faeth et al. 2004; Faeth et al. 2010).

The incidence of epichloid endophyte infections in natural populations of wild grasses is very variable, ranging from 0 to 100 % (Lewis et al. 1997; Schulthess and Faeth 1998; Saikkonen et al. 2000; Novas et al. 2007; Rudgers et al. 2009; Iannone et al. 2011). In addition, the same host species may be associated with different endophytes and different endophyte genotypes could be found through different environments (Hamilton et al. 2009; Iannone et al. 2009).

In some grass species the incidence of endophyte is associated with such environmental characteristics as altitude, abundance of herbivores, plant community and soil or climate (Schulthess and Faeth 1998; White et al. 2001; Bazely et al. 2007; Granath et al. 2007; Novas et al. 2007; Hamilton et al. 2009; Lembicz et al. 2011). However, considering the great diversity of host grass species, the association of endophytes with wild grasses has been poorly studied in terms of geographical distribution patterns of endophyte-infested populations and the incidence of endophytes in wild populations. To better understand the ecology and biology of these symbioses, it is necessary to increase the range of studied host species to determine the distribution pattern and incidence of endophytes in natural populations.

In Argentina, only asexual epichloae have been detected infecting many native grass species, covering a wide range of environments with different degrees of incidence on natural populations (0–100 %) (Iannone et al. 2011). The hybrid (*Epichloë poae* x *E. festucae*) *Neotyphodium tembladerae* Cabral and White is the most common endophyte, infecting more than 10 host species in the genera *Briza*, *Bromus*, *Festuca*, *Melica*, *Phleum* and *Poa* (Iannone et al. 2012b). In a preliminary study we reported that the incidence of endophytes in *Poa bonariensis* would be associated with climatic conditions and plant communities (Iannone et al. 2012b) but the identity of the endophyte was not established. In the same way, and in order to establish if endophyte incidence is associated with environmental characteristics, we expanded our studies to new populations of *P. bonariensis* and extended them to populations of *Poa lanuginosa*. These two host species inhabit a wide range of environments in Argentina, most of them herein sampled. In addition, in order to establish if different endophytes were associated with each host species or with a particular environment, we performed phylogenetic analyses of DNA sequences of the intron-rich regions of the β-tubulin (*tub*B) and translation elongation factor 1-α (*tef*A) genes.

2 Materials and methods

2.1 Host species

Poa lanuginosa and *Poa bonariensis* are perennial, dioecious and rhizomatous species of the subfamily Pooideae (Poaceae). These two species can be differentiated according to the size of the spikelets, leaf blade width and size of ligules (Giussani et al. 2012).

Poa lanuginosa inhabits grasslands and steppes on sandy soils in southern Argentina from the Atlantic coast in the east to the Andes Mountains in the west, and from parallel 35°S southward to Tierra del Fuego. *Poa bonariensis* inhabits grasslands and xeric forests of the Pampean and Mesopotamic regions between 30°S and 38°S (Giussani 2000).

2.2 Geography and ecology

The surveyed area extends between 35°S and 42°S, and from the Atlantic coast in the east to the Andes in the west, in Argentina; the sampled area is shown in Fig. 1. Hence, sampling was performed in almost the totality of the distribution area of both host species in Argentina. This area includes the Humid Pampa (a vast plain of temperate subhumid grasslands (Soriano 1991)) (Fig. 1), the phytogeographical province of xeric forests known as Espinal (Cabrera 1976) which extents as a bow from the north to the southwest surrounding the grasslands of the Pampa region (these forests are also found on the banks of the Paraná and Río de la Plata rivers (Cabrera 1976; Ribichich 2002)) (Fig. 1), and the Monte phytogeographical province in the western and southwestern region of the surveyed area characterized by shrubby dry forests and scrubby dry steppes in northern Patagonia and the Andes foothills (Cabrera 1976) (Fig. 1).

2.3 Population sampling

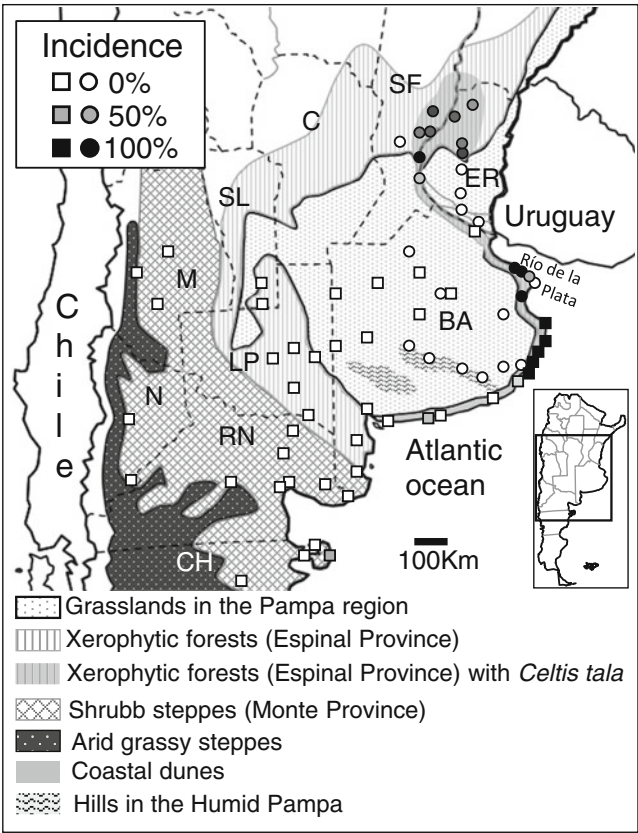Field collections were performed during spring and summer of 2005, 2006, 2007 and 2008. Seventy-four collection sites

Fig. 1 Map of the surveyed area showing the populations of *Poa lanuginosa* (*squares*) and *Poa bonariensis* (*circles*). Symbols are colored differently according to the incidence of endophytes in the population, ranging from white (0 %) to black (100 %). *Shaded* areas in the map represent different ecological regions (phytogeographical provinces) and environments. The name of the states (Provinces) are referred as follows: *BA* (Buenos Aires); *C* (Córdoba); *CH* (Chubut); *ER* (Entre Ríos); *LP* (La Pampa); *M* (Mendoza); *N* (Neuquén); *RN* (Río Negro); *SF* (Santa Fe)

were selected to represent most of the diversity of ecosystems in the ranges of *P. lanuginosa* and *P. bonariensis*. Collection sites were in pasture fields, forests with cattle, or sides and shoulders of country roads usually grazed by domestic cattle. Each collection site was considered as a community, and, if present, the set of individuals of each species was considered as a local population. Forty-six populations of *Poa lanuginosa* and 28 populations of *Poa bonariensis* were studied (Table 1) (Fig. 1). Twenty plants—10 male and 10 female—were collected 10 m apart from each population. Plants were stored in nylon bags at 5 °C until endophyte detection and isolation in the laboratory.

2.4 Endophytes detection and incidence

The frequency of endophyte-infected plants in each population was established by testing 2–5 culms or 20 seeds of each plant as follows. Parenchymal tissues scraped from culm pith, or seeds previously softened in 10 % NaOH for 8 h at 20 °C, were stained with aniline blue (0.1 % aqueous) (Clark et al. 1983) and observed under a light microscope. Plants were considered as endophyte-infected if characteristic unbranched hyphae were observed in parenchymal tissues or when a mass of hyphae stained dark blue was observed in the aleurone cell layer.

2.5 Environmental metadata

At each collection site, the geographical coordinates and the elevation above sea level (m) were registered with a global positioning system (GPS). Populations were characterized according to plant community (dominant plant species), ecological characteristics of the environment and following characterizations performed by Parodi (1940), Cabrera (1976) and Soriano (1991). The communities identified were: a) Grasslands in the inland Pampa, on sandy fertile soils and dominated by *Poa ligularis* and *Stipa* spp.; b) Grasslands in the humid Pampa, on soils rich in humus and mainly composed by *Bromus catharticus* and the introduced species *Lolium* spp. and *Festuca arundinacea*; c) Grasslands in the flooding Pampa (a region with flood periods) dominated by *Stipa* spp., *Paspalum* sp. and *Distichlis* spp.; d) Grasslands in hills between 200 and 600 m, in Tandilia and Ventania hills systems where rocky soils predominate and the dominant species are *Stipa* spp., *Bromus auleticus*, *Briza subaristata* and *Eringyum* sp. (Soriano 1991); three communities of xerophytic forests: e) Xerophytic forest with *Prosopis* spp. as dominant species on sandy or clayey soils in the north and west of the grasslands region in the phytogeographical "Espinal" province (Fig. 1), f) Xerophytic forest of *Prosopis* spp. and *Celtis tala* which are located on neutral or acidic soils along the Paraná river and g) Xerophytic forests with *Celtis tala*, *Prosopis* sp. and *Scutia buxifolia* on alkaline soils (pH=8) in ridges of shell debris rich in $CaCO_3$ along the banks of Río de la Plata River also considered in the "Espinal" phytogeographical province; h) Shrub steppes with *Larrea* spp., *Chuquiraga* sp. and *Stipa* sp. as dominant species in Northern Patagonia and Andes foothill in the "Monte" phytogeographical province; i) Salt flats mainly with *Atriplex lampa*, *Psila spartioides*, *Suaeda divaricata* and *Distichlis* sp.; and j) Coastal dunes communities dominated by *Panicum racemosum* and *Poa lanuginosa* or *Poa bergii* depending on the latitude and proximity to the sea, and also in some places *Pinus* sp. and *Acacia* sp. that were introduced to fix the dunes.

Eight climatic variables were recorded or recalculated from De Fina (1992), a publication that consists of the average values of a 10–30 years compilation of climatic data obtained from the meteorological stations nearest the sampled localities. The recorded variables were: (1) the warmest month average temperature (wmat) (°C), (2) the coldest month average temperature (cmat) (°C), (3) the annual average rainfall (aar) (mm), (4) the summer average rainfall (sar) (mm), (5) the

t1.1 **Table 1** Surveyed populations of *Poa lanuginosa* and *Poa bonariensis*, other hosts of *Neotyphodium tembladerae* living in sympatry, environ-
Q5 mental and floristic characteristics of the community, endophyte incidence in the population (%) and identification number of the isolates studied

	Poa lanuginosa	Population	Sympatric hosts	Environment	Incidence	Isolates
t1.2						
t1.3	San Clemente (BA)	1	*Bau*		100	2471
t1.4	Pta. Medanos (BA)	2			100	2474
t1.5	Pinamar (BA)	3	*Bau*		100	2589
t1.6	V. Gessell (BA)	4	*Bau*		100	2516-2517
t1.7	Mar Azul (BA)	5	*Bau*		100	2476-2775-2776-2777
t1.8	Mar Chiquita (BA)	6	*Bau*		10	2515
t1.9	Mar del Sur (BA)	7		Coastal dunes	0	
t1.10	Reta (BA)	8	*Pbe*		30	2518
t1.11	Claromecó (BA)	9	*Pbe*		0	
t1.12	Monte Hermoso (BA)	10			0	
t1.13	El Condor (RN)	11	*Pbe*		0	
t1.14	S.A.Oeste (RN)	12	*Pbe*		0	
t1.15	Las Grutas (RN)	13	*Pbe*		0	
t1.16	Caleta Valdez (CH)	14			50	2477
t1.17	Puerto Pirámides (CH)	15			0	
t1.18	Bahia Blanca (BA)	16			0	
t1.19	Salitral Vidriera (BA)	17			0	
t1.20	Buratovich (BA)	18		Salt flats	0	
t1.21	S.Colorada Grande (RN)	19			0	
t1.22	RN251-(RN)	20			0	
t1.23	Península Valdez (CH)	21			0	
t1.24	Florentino Ameghino (CH)	22			0	
t1.25	Maquinchao (RN)	23	*Far*	Shrub steppes in North Patagonia	0	
t1.26	RN251 & RN 22 (RN)	24			0	
t1.27	RN 251 Km160 (RN)	25			0	
t1.28	RN 251 Km192 (RN)	26			0	
t1.29	R3 km1025 (RN)	27			0	
t1.30	Arizona-Road 47 (SL)	28			0	
t1.31	Gonzales Moreno (BA)	29			0	
t1.32	Trenque Lauquen (BA)	30		Grasslands on dunes in inland Pampa or delta of Paraná river	0	
t1.33	Pehuajo (BA)	31			0	
t1.34	Bolivar (BA)	32			0	
t1.35	Est. Bonifacini (BA)	33			0	
t1.36	Carhue (BA)	34			0	
t1.37	Ibicuy (ER)	35			0	
t1.38	Rivera (BA)	36			0	
t1.39	General Hacha (LP)	37			0	
t1.40	Valle Utracan (LP)	38		Xerophytic forests. of *Prosopis* spp on sandy soils	0	
t1.41	Hucal (LP)	39			0	
t1.42	Fortuna (SL)	40			0	
t1.43	Unión (SL)	41			0	
t1.44	Agua del Toro (M)	42			0	
t1.45	Diamante (M)	43		Shrub steppes in Andes foothills	0	
t1.46	Confluencia (N)	44			0	
t1.47	Las Lajas (N)	45			0	
t1.48	Pareditas (M)	46			0	

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Table 1 (continued)

		Population	Sympatric hosts	Environment	Incidence	Isolates
t1.49	*Poa bonariensis*					
t1.50	Punta Indio (BA)	47*	*Bau*		100	2468-2469-2495-2498-2780
t1.51	Punta Piedras (BA)	48*	*Bau*	Xerophytic forest of *Celtis tala, Scutia* sp. and *Prosopis* sp. on ridges of shell debris	50	2497
t1.52	Est. San Jerónimo (BA)	49*			0	
t1.53	Magdalena (BA)	50			100	2470
t1.54	Esquina de Croto (BA)	51			100	2563-2564
t1.55	Sevigne (BA)	52*		Grasslands in the flooding Pampa	0	
t1.56	Coronel Vidal (BA)	53	*Bau*		0	
t1.57	Mar Azul (BA)	54*	*Bau*		0	
t1.58	Sierra de los Padres (BA)	55			0	
t1.59	Laguna Brava (BA)	56		Grasslands in hills between 200 and 600 masl	0	
t1.60	Balcarce (BA)	57*			0	
t1.61	Tandil (BA)	58	*Bau*		0	
t1.62	Saladillo (BA)	59*		Grasslands on humus-rich soils	0	
t1.63	Junín (BA)	60			0	
t1.64	Ibicuy (ER)	61		Grasslands in delta of Paraná river	0	
t1.65	Médanos (ER)	62*			0	
t1.66	Gualeguay (ER)	63*		Xerophytic forests of *Prosopis* spp. on clayey soils	0	
t1.67	Arroyo Obispo (SF)	64			0	
t1.68	Progreso (SF)	65*			0	
t1.69	Villaguay (ER)	66*			75	
t1.70	Paso La Laguna (ER)	67*			90	2590
t1.71	Arroyo Feliciano (ER)	68*		Xerophytic forests of *Prosopis* spp. and *Celtis tala* on neutral or slightly acidic soils	62	2591-2779
t1.72	Arroyo Feliciano (ER)	69			77	
t1.73	Cayastacito (SF)	70*			76	2592-2696
t1.74	Cayastá (SF)	71			89	2697
t1.75	South of Cayastá (SF)	72*			82	2593-2698
t1.76	Coronda (SF)	73			100	2699
t1.77	Arroyo Monje (SF)	74*			28	

Letters between parentheses are the abbreviations of the provinces as in Fig. 1. *BA* Buenos Aires; *CH* Chubut; *ER* Entre Ríos; *LP* La Pampa; *M* Mendoza; *N* Neuquén; *RN* Rio Negro; *SF* Santa Fe; *SL* San Luis. *Asterisks* indicate populations that were selected for soil parameters analyses. *Bau*: *Bromus auleticus*, *Far*: *Festuca argentina*, *Pbe*: *Poa bergii*

winter average rainfall (war) (mm). The recalculated variables were: (6) the ratio between average rainfall in winter and summer (war/sar). Variables of water availability in soil in winter (7) and in summer (8), (wasw) and (wass) respectively, were calculated accordingly to Thornthwaite (1948), a model that considers the rainfall and the potential evapotranspiration of the soil as a variable of the latitude.

Poa bonariensis populations were additionally characterized according to soil properties. Soil samples of the upper horizon (10–30 cm) were taken in 16 populations representative of the different environments from all ecological areas (populations: 47–49, 52, 54, 57, 59, 62–63, 65–68, 70, 72, 74). Soil samples were subjected to the following analyses,

according to Jackson (1982): pH in water solution 1:25; electric conductivity (E.C.); total Carbon (C) (Walkley-Black); total Nitrogen (N) (Kjeldahl); cation exchange capacity (C.E.C.) in ammonium acetate, 1 N, pH7; and the macronutrients: P, Ca, Mg, Na and K by the Laboratory of Geological and Edaphological Chemistry, CONICET, Argentina.

2.6 Numerical analyses

Principal Component Analysis (PCA) was used to characterize sampling sites according to climatic variables or soil variables. The PCA was performed on a standardized character matrix. Variables were standardized accordingly to Matteucci and

Colma (1982), Crisci and Lopez Armengol (1983). Correlation matrices derived from the standardized matrix were then used to obtain the principal components. To study the association between the environmental data of the population (collection site) and endophyte incidence, an external validation was made by means of the Pearson correlation coefficient to correlate the site scores of the first axis of the PCA with the scores of incidence in each population. Statistical analyses were performed as described by Novas et al. (2007) with the statistical package PC-Ord (McCune 1991).

2.7 Endophyte isolation and morphologic characterization

Endophytes were isolated from surface-disinfested leaf pieces accordingly to previously published methods (Clark et al. 1983). One to five isolates from each population were morphologically characterized accordingly to colony and growth rate on PDA at 23 °C (Iannone et al. 2009). Microscopic characteristics of conidia and conidiogenous cells were also studied accordingly to Iannone et al. (2011).

2.8 Endophyte characterization—tubB and tefA phylogenies

Nine isolates from *P. bonariensis* and four from *P. lanuginosa*, collected from different populations representing different environments, were chosen for gene sequencing. Total genomic DNA isolation, PCR of *tubB* and *tefA* segments, and DNA sequencing were performed as described by Iannone et al. (2009; 2012b). Gene sequences were deposited in GenBank under the following accession numbers: JX470369-JX470394 for *tubB* gene and JX470395 - JX470420 for *tefA* gene. Sequences were aligned using ClustalW for multiple alignment of the BioEdit v7.0.5 program as described in Iannone et al. (2009). Sequences of *Neotyphodium* species isolated from different host species from Argentina, and sequences from sexual and asexual endophytes from the Southern and Northern Hemispheres were included in the analyses. Phylogenetic analyses using Maximum parsimony (MP) and Bayesian algorithms were performed using WINCLADA ver. 0.9.9 (Nixon 1999) and Mr. Bayes ver. 3.2 (Ronquist et al. 2012) respectively, as described in Iannone et al. (2009).

3 Results

3.1 Endophyte incidence and distribution pattern

3.1.1 Poa lanuginosa

Endophyte-infected plants were found only in 8 of 46 populations (Fig. 1, Table 1). These populations are located on coastal dunes in the north of the Atlantic coast in Buenos

Aires province between 36°18′S and 37°42′S and at the eastern extreme of Península Valdez in Chubut province. No endophytes were found in plants inhabiting grasslands on fertile soils in the Humid Pampa, steppes in northern Patagonia, Andes foothills or in populations growing on coastal dunes south of 37°42′S.

PCA based on climatic variables (Fig. 2) indicated that the first three components accounted for the 93.2 % of the total variability. Principal Component I explained 57.5 % of the total variance and was mainly associated negatively with annual average rainfall, the average rainfall in summer and the available water in the soil in summer. Principal Component II explained 23.5 % of the variance and was mainly positively associated with the ratio of rainfall between winter and summer, water availability in soil in winter and the winter average rainfall. The Pearson correlation coefficient between the incidence of endophytes and the scores of populations on the Principal Component I was ($r=-0.63$; $r^2(\%)=$ 39.3, $P<0.001$) and between the scores of the populations on Principal Component II and endophyte incidence was ($r=$ 0.62; $r^2(\%)=37.9$; $P<0.001$). These values of correlation are important considering the extent of the area studied.

Pearson correlation analyses between the vector incidence and variables that more strongly contribute to the first
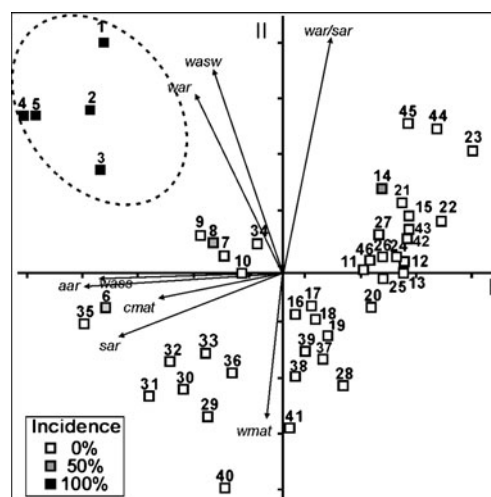

Fig. 2 Principal Component Analysis (PCA) ordination diagram of 46 populations of *Poa lanuginosa* according to climatic variables data. The *numbers* above the symbols represent the population number as in Table 1. The colour of the *squares* differs according to the incidence of endophytes in each population, ranging from white (0 %) to black (100 %). Vectors show how each variable contributed to each axis: the annual average rainfall (*aar*), the summer average rainfall (*sar*), the winter average rainfall (*war*), the ratio between average rainfall in the winter and summer (*war/sar*), water availability in soil in the winter and in summer, (*wasw*) and (*wass*) respectively and the average temperature in the coldest (*cmat*) and the warmest (*wmat*) months respectively. Populations inside of the ellipse are established on coastal dunes that presented the higher incidences of endophytes and are associated with the higher scores of available water in soil in winter, annual rainfall level and wintry rainfall regime

and second components showed a highly significant positive correlation with soil water availability in winter (r= 0.88, r^2(%)=77.5) and rainfall in winter (r=0.87; r^2 (%)= 75.9). Accordingly to this ordination, in the plane established by the first and the second axis of the PCA (Fig. 2), populations with the higher incidence of endophytes (black squares) are mostly clustered in the quadrant defined by the negative semi-axis of Component I and the positive semi-axis of Component II. These results indicate that endophyte-infested populations (black-gray squares) tend to be located in those coastal dune environments with the highest annual rainfall level (except for population 14, see Discussion section), and that the incidence of endophytes highly correlates positively with a winter rainfall regime and with a higher availability of water in soil during winter. These populations also present the highest temperatures in winter (*cmat*) and the coldest temperatures in summer (*wmat*) (Fig. 2).

3.1.2 Poa bonariensis

Thirteen out of 28 *Poa bonariensis* populations surveyed were endophyte-infested. These populations were found in xerophytic forests with *Celtis tala* and *Prosopis* spp., in the northern limit of the distribution area for this species (Fig. 1), and in populations in communities with *C. tala* forests on shell debris banks of the Río de la Plata coast of Buenos Aires province (Fig. 1) (Table 1). These forests are included in the "Espinal" phytogeographical province

(Cabrera 1976; Ribichich 2002), (Fig. 1). Endophyte-infected plants were not found in plains or hills of the Humid Pampa, in the Paraná river delta or in the xerophytic forest of *Prosopis* spp. on sandy or clayed soils (Fig. 1).

PCA based on climatic variables (Fig. 3a) indicated that the first three components accounted for the 95 % of the total variance. Component I (70.9 % of the total variance), was negatively associated with the ratio between average rainfall in winter and summer, the availability of water in winter and winter average rainfall, and it was positively associated with rainfall in summer and the average temperature in the coldest and warmest month. Component II (17.5 % of the total variability), was mainly negatively associated with the water availability in soil in summer and positively associated with the annual average rainfall. Thus, in the PCA on climatic variables (Fig. 3a) infested populations of the northern region (pops. 66–74) were distributed on the positive extreme of Principal Component I, being characterized mainly by the below one ratio in average rainfall between winter and summer (summer rainfall regime) and low availability of water in winter. Populations from shell debris banks on the coast of Río de la Plata (pops. 47–51) characterized mainly by the homogeneous distribution of rainfall over the year (rainfall in summer / rainfall in winter≈1), its higher availability of water in soil in winter, the highest average rainfall in the winter, and high average annual rainfall were grouped in the negative extreme of Component I and positive semi-axis of Component II.
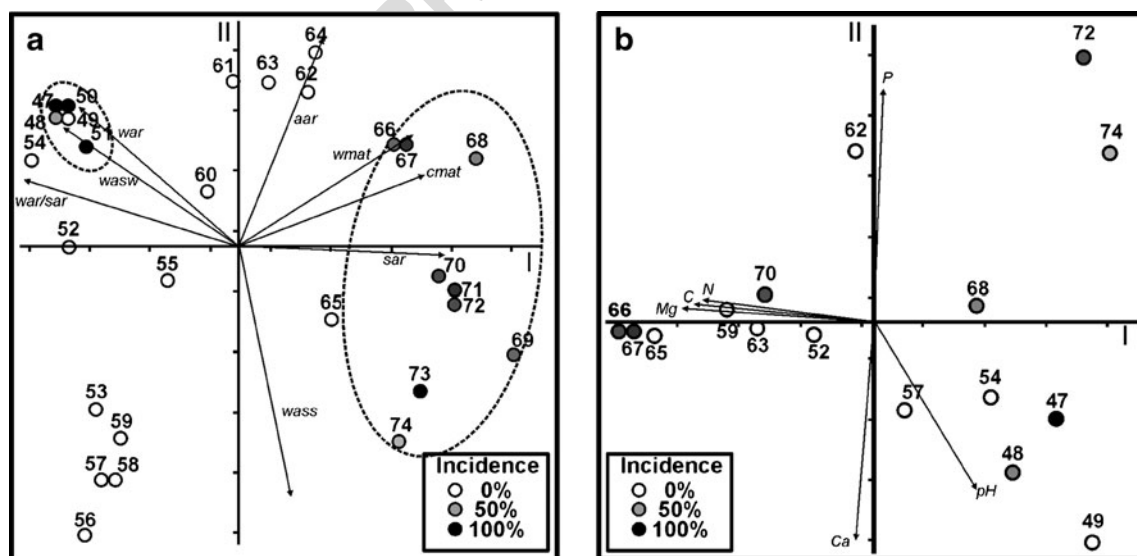

Fig. 3 Principal Component Analysis (PCA) ordination diagram of 28 populations of *Poa bonariensis* based on climatic variables (**a**), and PCA ordination diagram of 16 populations of *Poa bonariensis* based on soil variables data (**b**). The *numbers* above the symbols represent the population number as in Table 1. The colour of the *circles* differs according to the incidence of endophytes in each population, ranging from white (0 %) to black (100 %). In panel (**a**) vectors show how each variable contributed to each component: the annual average rainfall (*aar*), the summer average rainfall (*sar*), the winter average rainfall (*war*), the ratio between average rainfall in winter and summer (*war/sar*), water availability in soil in winter and in summer, (*wasw*) and (*wass*) respectively. In panel (**b**) only the most important variables are shown: total Carbon (C), total Nitrogen (N), Magnesium (Mg), Calcium (Ca), Phosphorous (P) and pH. Populations inside of the ellipses are those established in xerophytic forests with *Celtis tala*

The first three components of the PCA based on soil parameters (Fig. 3b), accounted for 72.9 % of the total variability. However, the endophyte-infested populations were dispersed in the planes defined by the Principal Components I and II (Fig. 3b); I and III (not shown), or II and III (not shown), indicating no clear association between endophyte incidence and those soil parameters analyzed. Populations in soils poor in Nitrogen (N) and Carbon (C) are distributed in the positive extreme of Component I, among them, populations from *Celtis tala* forests on ridges of shell debris (pops. 47–49) are distributed along the vector pH since they also presented alkaline soils (pH=8). Populations on soils rich in N and C (pops. 59, 63, 65–67, 70) are placed in the negative extreme of Component I. Pearson correlation analyses between the vector incidence and variables that more strongly contribute to the first and second components of both PCA (Fig. 3a, b) did not show significant correlation.

These results indicate that endophyte incidence is not clearly associated with climatic or soil characteristics, but infested populations are associated with those xerophytic forests characterized by the presence of *Celtis tala* (pops. 47–51 and pops. 66–74) (Table 1).

3.2 Endophyte characterization

3.2.1 Morphology

Twelve isolates were obtained from *P. lanuginosa* and 18 from *P. bonariensis* (Table 1). Colonies on PDA were white, felted to velvety, and the rate of growth on PDA ranged from 0.6 to 1.1 mm/day. Conidiogenous cells were solitary, sometimes branched, smooth, 15–45 μm long from base to tip, tapering gently from 1.5 to 2.5 μm at the base to 0.5–1.0 μm at the tip. A basal septum was rarely present. Conidial ontogeny was enteroblastic and two (rarely three) alantoid conidia were produced by each conidiogenous cell. Conidia measures ranged from 6.5 to 10 μm long and from 2 to 4 μm wide.

3.2.2 Phylogeny of tubB and tefA genes

Partial sequences of *tub*B and *tef*A genes, mainly comprising intron sequences, were obtained from endophytes of *Poa lanuginosa* and *P. bonariensis*. Two different alleles from each of *tub*B and *tef*A genes, which differed in nucleotide substitutions and indels, were amplified from all of the isolates. Aligned *tub*B sequences totaled 433 positions, of which 22 were parsimony-informative sites. The aligned *tef*A sequences totaled 688 positions, of which 73 were parsimony-informative sites.

Aligned sequences of each allele of both gene sequences showed 99.5–100 % identity among all the isolates of both host species, respectively, and with previously characterized *Neotyphodium tembladerae* isolates (Gentile et al. 2005; Iannone et al. 2009).

Results from Maximum parsimony and Bayesian phylogenetic analyses for each gene were congruent, and the tree obtained for the *tef*A gene is shown in Fig. 4. One of the alleles of all of the isolates, placed in the *Epichloë typhina* clade, was phylogenetically derived from *E. poae* (Fig. 4, lower clade), and sequences from the other allele were derived from *E. festucae* (Fig. 4, upper clade). This result indicates a hybrid origin for all the isolates of these hosts. The phylogeny inferred from each allele of *tef*A and *tub*B genes, grouped the endophytes from *P. bonariensis* and *P. lanuginosa* in a well supported clade that includes *N. tembladerae*.

4 Discussion

In this work we show that the incidence of the epichloid endophyte *Neotyphodium tembladerae* is highly variable among populations of the two wild grasses *Poa lanuginosa* and *P. bonariensis*. The geographic distribution of infested populations is strongly associated with ecological and environmental characteristics; so that the presence of endophytes is largely restricted to particular environments. However we have not found any differences among the endophytes associated with each host species or with the different environments.

Endophyte-infested populations of *Poa lanuginosa*, were only found on some coastal dunes. This environment is characterized by constant winds, salt spray, nutrient deficiency, sand movements and low water capacity that can lead to water and saline stresses (Van der Maarel 1981). Sand dunes are very dynamic ecosystems; species composition and cover vegetation may change rapidly and be drastically driven by changes in environmental factors (Van der Maarel 1981). In this environment, endophyte-infested populations were located in areas with the highest average rainfall in winter. Only one population (pop 14) on the coast of Península Valdez, with a low rainfall level, was endophyte-infested; however, this population is located in the most humid extreme of this dry region. Considering this, in these coastal populations the stressing conditions could be partially mitigated by the rainfall in winter, the vegetative

Fig. 4 Phylogenetic tree for *tef*A gene sequences showing the same hybrid origin for the endophytes of *Poa lanuginosa* and *Poa bonariensis* (in **bold**). Both species are infested with *Neotyphodium tembladerae*. Mr. Bayes posterior probabilities and Maximum parsimony bootstrap support values are shown above and below of each node, respectively. The *numbers* after *P. lanuginosa* and *P. bonariensis* indicate the isolate identification number and the population as in Table 1

tefA

0.1

0.95
62
- *N. pampeanum* 3334-1 *Bromus auleticus*
- *N. pampeanum* 3332-1 *Bromus auleticus*
- *N. pampeanum* 1623-1 *Bromus auleticus*
- *Neotyphodium* sp. 642-1 *Poa spiciformis*
- *E. festucae* ATCC 90660 *Festuca rubra*
- *N. tembladerae* 3329-1 *Bromus auleticus*
- *N. tembladerae* 3330-1 *Bromus auleticus*
- *N.tembladerae*355-1 *Bromus auleticus*
- *N. tembladerae* 353-1 *Festuca magellanica*
- *N. tembladerae* 532-1 *Bromus setifolius*
- *N. tembladerae* 1213-1 *Poa huecu*

1.0
100
- **Poa bonariensis 2469-1 Pop. 49**
- **Poa bonariensis 2563-1 Pop. 51**
- **Poa bonariensis 2696-1 Pop. 70**
- **Poa bonariensis 2697-1 Pop. 71**
- **Poa bonariensis 2698-1 Pop. 72**
- **Poa bonariensis 2699-1 Pop. 73**
- **Poa bonariensis 2592-1 Pop. 70**
- **Poa bonariensis 2590-1 Pop. 66**
- **Poa bonariensis 2591-1 Pop. 68**
- **Poa lanuginosa 2471-1 Pop. 1**
- **Poa lanuginosa 2477-1 Pop. 14**
- **Poa lanuginosa 2518-1 Pop. 8**
- **Poa lanuginosa 2515-1 Pop. 6**
- *N. tembladerae* 734-1 *Festuca hieronymii*

0.95
59

1.0
90

1.0
100
- *E. amarillans* ATCC 200743 *Sphenohpolis obtusata*
- *E. baconii* ATCC 200745 *Calamagrostis villosa*

1.0
100
- *E. bromicola* ATCC 200749 *Bromus erectus*
- *N.uncinatum* CBS 102646 *Lolium pratense*

0.94
62

1.0
98
- *N. pampeanum* 3332-2 *Bromus auleticus*
- *N. pampeanum* 3334-2 *Bromus auleticus*
- *N. pampeanum* 1623-2 *Bromus auleticus*
- *Neotyphodium* sp. 642-2 *Poa spiciformis*
- *E. poae* ATCC201668 *Poa nemoralis*

0.98
67
- *N. tembladerae* 3329-2 *Bromus auleticus*
- *N. tembladerae* 3330-2 *Bromus auleticus*
- *N. tembladerae* 355-2 *Bromus auleticus*
- *N. tembladerae* 1213-2 *Poa huecu*
- *N. tembladerae* 353-2 *Festuca magellanica*
- *N. tembladerae* 532-2 *Bromus setifolius*

1.0
98

0.82
62
- **Poa bonariensis 2469-2 Pop. 49**
- **Poa bonariensis 2563-2 Pop. 51**
- **Poa bonariensis 2696-2 Pop. 70**
- **Poa bonariensis 2697-2 Pop. 71**
- **Poa bonariensis 2698-2 Pop. 72**
- **Poa bonariensis 2699-2 Pop. 73**
- **Poa bonariensis 2590-2 Pop. 66**
- **Poa bonariensis 2591-2 Pop. 68**
- **Poa bonariensis 2592-2 Pop. 70**
- **Poa lanuginosa 2477-2 Pop. 14**
- **Poa lanuginosa 2515-2 Pop. 6**
- **Poa lanuginosa 2471-2 Pop. 1**
- **Poa lanuginosa 2518-2 Pop. 8**
- *N. tembladerae* 734-2 *Festuca hieronymii*

1.0
100
- *N. australiense* 937-2 *Echinopogon ovatus*
- *E. typhina* E432 *Lolium perenne*

growing period of these early flowering species and by the moderate temperatures in winter and summer. Zabalgogeazcoa et al. (2006) found high level of infection by *Epichloe festucae* in *Festuca rubra* growing in cliffs in Galicia (Spain). Although they did not find beneficial effects of the endophyte on plants growing under saline stress, Gundel et al. (2011a) found that under low water potential *E. festucae*-infected seedlings presented higher survival than their endophyte-free counterparts. Unfortunately we were unable to get germinated seeds of *Poa lanuginosa* to study the effect of the endophyte on plant fitness under controlled conditions. However if the incidences of endophytes were only explained by the resistance to salinity we should have found infested populations in other coastal environments as well as in salt flats.

In *Poa bonariensis*, infested populations were clearly located in two separate regions with different climatic and edaphic conditions. However, these regions are similar due to their short drought periods in part of the year and in their floristic composition, being xerophytic forests with *Celtis tala* that were considered in the same phytogeographical region by Cabrera (1976) and Ribichich (2002). In Buenos Aires province, endophytes were found in forests on banks of shell debris characterized by well-drained alkaline soils (pH=8) (Ribichich and Protomastro 1998). In the northern area of its distribution, endophytes were found in forests with drought periods in the growing season but with water availability in summer when the temperatures reach the highest values. However, the distribution of infested population was not associated with soil parameters analyzed. This result contrasts with those obtained by Hamilton et al. (2009) who found that in *Festuca arizonica* the incidence of hybrid endophytes was higher in populations with low nutrients in soil.

In both host species, populations located in the most favorable and productive environments from an agronomic point of view (with respect to the soil and climatic conditions) (Soriano 1991; De Fina 1992), as those in grasslands of the Humid Pampa, were apparently endophyte-free. The same result was observed in populations located in the most stressing environments of the distribution areas of each host; as dry steppes in Patagonia and in the mountains and salt flats for *P. lanuginosa*, or some xerophytic forests for *Poa lanuginosa* and *Poa bonariensis*. Thus, considering the distribution area of these two hosts, endophyte-infested populations are located in those regions that present moderate environmental stress levels and moderate agronomic capacity (De Fina 1992), i.e. poor soils with some saline stress but with mild temperatures and water availability in the growing season (*P. lanuginosa*) or those environments with xerophytic forest dominated by *Celtis tala* in the case of *P. bonariensis*.

This pattern of distribution has also been reported for three other host species from South America (Novas et al. 2007) and, at a first sight these results suggest that endophytes would be beneficial under moderate stress situations, becoming detrimental or unnecessary for the host in very stressing or very favorable conditions. Some authors suggest that the effect of the endophyte on host fitness is not enough to explain distribution patterns, and that the efficiency of the transmission of the endophyte via seeds should be considered even more important (Ravel et al. 1997; Saikkonen et al. 2002; Gundel et al. 2008). Imperfect transmission of the endophyte from the mother plant to the seed may occur if the endophytes fail to colonize all the tillers, all the flowers or if the endophyte dies during seed dormancy. We cannot establish if these distribution patterns are explained by the benefits that the endophytes confer to these hosts or by imperfect endophyte transmission. However, considering that the two studied host species are perennial, the persistence of infected plants and their capability to produce infected rhizomes could play a more important role on endophyte incidence than endophyte vertical transmission.

The interaction between the genotypes of the host plant and the endophyte alters plant fitness and could also affect endophyte vertical transmission by incompatibilities between both partners (Gundel et al. 2011b) which could have an effect on endophyte incidence (Ravel et al. 1997; Saikkonen et al. 2002; Afkhami and Rudgers 2008) and distribution patterns. In addition, in some host species, different genotypes of the endophyte could be found associated with particular environmental characteristics (Wäli et al. 2007; Hamilton et al. 2009). Unfortunately, the molecular markers used in this work do not allow detecting intraspecific variability in the endophytes, and no molecular markers have been developed to identify genetic variability in the host species.

The morphological characteristics and molecular phylogeny of the endophytes of *Poa lanuginosa* and *P. bonariensis* confirmed that both hosts are associated with *Neotyphodium tembladerae* regardless of the environmental characteristics. This result is also contrasting with those obtained by Hamilton et al. (2009) and Iannone et al. (2009). These authors found that populations of *Festuca arizonica* and *Bromus auleticus* respectively, were associated with different endophytes accordingly with the environmental characteristics. The presence of *N. tembladerae* in two different grass species is not surprising, since this endophyte seems to be ubiquitous in many grasses from South America (Gentile et al. 2005; Iannone et al. 2012b). An explanation for the presence of this seed transmitted fungus in these two close related species of grasses could be that *P. lanuginosa* and *P. bonariensis* underwent speciation from a common ancestor infected with *N. tembladerae*. Considering that *N. tembladerae* is a hybrid between *E. festucae* and *E. poae*, and the former species has not been detected in the distribution area of these hosts, a likely explanation for the existence of

endophyte free populations is loss of the endophytes in some environments. The asexual *Epichloë poae* may also be horizontally transmitted (Tadych et al. 2012), therefore, we cannot discard the possibility that these two host species acquired the endophytes from other plant species living in sympatry, infected with *N. tembladerae*.

Although our results clearly show a distribution pattern of endophyte-infested populations, more experiments are necessary to study the genetic intra-specific variability of the host and *N. tembladerae* in the different environments, in order to establish whether the association between environmental characteristics with distribution patterns is explained by the effect of the endophyte on host fitness or by the effects of environmental conditions on endophyte transmission.

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