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Population genetic structure of the South American species *Hypochaeris lutea* (Asteraceae)

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Abstract

The genus *Hypochaeris* has a recent evolutionary history caused by long-distance dispersal in conjunction with adaptive radiation in the South American continent. *Hypochaeris lutea* is a perennial herb that grows mostly at altitudes of around 1000 m in cold swamps of the southern regions of Brazil. We investigated the amplified fragment length polymorphism (AFLP) in 270 individuals representing 11 Brazilian populations of *H. lutea* to elucidate the population genetic structure of this species. The frequencies of polymorphic loci and gene diversity ranged from 83.42% to 91.66% and from 0.26 to 0.34, respectively. Analysis of molecular variance revealed that most of the genetic variability was found within (76.67%) rather than among (23.3%) populations, agreeing with the pattern of genetic distribution within and among populations observed in other allogamous species of *Hypochaeris*. A Mantel test showed no correlation between genetic and geographic distances when all populations were considered. Simulations performed using a Bayesian approach consistently identified two clusters with different admixture proportions of individuals, as also revealed by a UPGMA dendrogram of populations. The pattern of genetic structure observed in *H. lutea* is consistent with a process of successive colonization events by long-distance dispersal resembling the rapid and recent radiation that has been proposed to explain the origin of the South American species of *Hypochaeris*.

Keywords: AFLP, founder effect, *Hypochaeris*, population genetics, South American species.

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Introduction

Hypochaeris L., Asteraceae, tribe Cichorieae Lam. and DC., subtribe Hypochaeridinae Less, consists of more than 60 species of annual or perennial herbs displaying a disjunct distribution, with 15 species scattered in the Mediterranean region, central Europe and Asia and about 50 species in South America (Stebbins 1971; DeFillips 1976; Cerbah *et al*. 1998; Samuel *et al*. 2003; Ruas *et al*. 2005; Tremetsberger *et al*. 2005). This interesting biogegraphic pattern, with a broad intercontinental distribution, is known for only few genera of Asteraceae (Riggins & Seiger 2012). Major plate tectonic and global climatic events affecting the South American continent (formation of the Amazon basin and coastal ranges, uplifting of the Andes, drying of intermountain valleys, local vulcanism, and Pleistocene glaciation) may have directly contributed to the diversification of plant species, making this continent particularly attractive for examining the impacts of environmental change on genetic variation in natural plant populations (Tremetsberger *et al*. 2003b). Hence, interpreting the evolutionary history of *Hypochaeris* is of great importance. As suggested by molecular clock dating, the South American group diversified only 0.25–1.00 mya from a common ancestor that arrived in the continent by long-distance dispersal (Tremetsberger *et al*. 2005). Enrichment of the environment provided areas for colonization, allowing the genus to radiate and diverge into the continent (Samuel *et al*. 2003; Tremetsberger *et al*. 2005, 2006, 2009). Studies have provided evidence of the

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potential of Asteraceae species for long-distance dispersal via light fruits and attached bristles (pappus; Sheldon & Burrows 1973; Anderson 1993). Moreover, a connection between long-distance dispersal events and the phylogenetic relationships of groups of species that present the potential for this type of dispersal has also been reported (Vijverberg *et al*. 1999; Coleman *et al*. 2003).

Recent investigation based on DNA sequencing of internal transcribed spacer (ITS), *matK* and *trnL-F* regions (Samuel *et al*. 2003; Tremetsberger *et al*. 2005), and amplified fragment length polymorphism (AFLP) (Tremetsberger *et al*. 2006) revealed that the South American *Hypochaeris* is monophyletic and closely related to *H. angustifolia*, an endemic species to the altiplano of the Atlas Mountains in Morocco. Studies using AFLPs data have provided insights to elucidate the mechanisms of dispersal and colonization of *Hypochaeris* in South America. In a population genetics approach, AFLP data reflected that there is no clear pattern of genetic differentiation between survivor and founder populations of *H. tenuifolia* established after the eruption of the Volcán Lonquimay in Chile (Tremetsberger *et al*. 2003a). The AFLP data have also been successfully used to analyze genetic diversity in many other populations of *Hypochaeris* species from South America, including *H. acaulis* (Tremetsberger *et al*. 2003b), *H. palustris* (Muellner *et al*. 2005), and *H. incana* (Tremetsberger *et al*. 2009).

Hypochaeris lutea (Vell.) Britton (syn. *H. rosengurttii* var. *rosengurttii*; Azevêdo-Gonçalves & Matzenbacher 2005, 2006, 2007) is a perennial herb with a distribution range that extends from southern to southeastern Brazil, with some reports of occurrence in Argentina and Uruguay (Azevêdo-Gonçalves & Matzenbacher 2007). Within this distribution area, *H. lutea* developed characteristics that allowed the species to colonize and be restricted to a wetland environment.

In this study we test the hypothesis regarding the consequence of the recent radiation of *Hypochaeris* on genetic structuring of *H. lutea* populations. Specifically, we wish to examine if there was enough time for population differentiation. We focus on this question by examining multiple populations of *H. lutea* using AFLP markers to provide useful information to understand the successful establishment of the genus in the South American continent.

Material and methods

Species and sampling

Hypochaeris lutea is a diploid (Fiorin *et al*. 2013), perennial, and insect- or wind-pollinated herb with a distribution range that covers part of the temperate and subtropical regions of the South American continent. This species is particularly represented at altitudes around 1000 m in cold swamps of southern Brazil, with few scattered individuals reported in southeastern states. Morphologically and ecologically *H. lutea* is clearly distinguishable from other South American taxa. These characteristics have favoured the development of pivotant roots, a long and thin flower stem, and very narrow long leaves that assist this species in searching for photosynthetic resources (Matzenbacher 1998; Azevêdo-Gonçalves & Matzenbacher 2007). Interestingly, the ecological characteristics of *H. lutea* are shared with the Moroccan *H. angustifolia* (growing in wetlands and swamps), which is considered the sister species of South American *Hypochaeris* (Tremetsberger *et al*. 2005). Recent phylogenetic studies based on AFLP markers and detailed cytogenetic data placed *H. lutea* into a new group among the South American species (i.e., the Lutea group; Reck *et al*. 2011).

The study was carried out in 11 populations of *H. lutea* obtained from field expeditions in the years of 2006, 2007, and 2010. Since *H. lutea* appears only in specific habitats (swamps), extensive field expedition combed suitable areas to verify the occurrence o *H. lutea* populations. Sampling was oriented to locate populations distributed along a south-southeastern transect, to represent, as much as possible, the distribution range of the species. Fresh young leaves were collected from individual plants at least 3 m apart, placed in porous bags and preserved in silica-gel. Collection areas included the states of Paraná, Santa Catarina, and Rio Grande do Sul, resulting in 25 plants per population, except for the population of Serra do Rio do Rastro (FUEL-40654), which was represented by 20 plants totalling 270 individuals. The karyotypes from five of the 11 populations investigated here were determined by Fiorin *et al*. (2013), one of which (population Serra do Rio do Rastro; RR), showed poliploidy, a phenomenon reported for only few species of *Hypochaeris* (Weiss-Schneeweiss *et al*. 2007, 2008). Voucher specimens, from each population (Table 1, Fig. 1), were deposited in the Herbarium of Universidade Estadual de Londrina (FUEL).

DNA extraction and AFLP

Total genomic DNA was extracted from silica-dried leaves using the CTAB protocol (Doyle & Doyle 1987) with few modifications. The quality of the extracted DNA was checked on 1% agarose gels and DNA concentrations were measured using a fluorometer (DyNA Quant 200, Höfer-Pharmacia). The extracted DNA was stored at –20°C or at –80°C for long-term storage.

For AFLP procedures we followed pre-established protocols of Vos *et al*. 1995. Approximately 0.8 µg of genomic

Table 1 Localities and their details for the 11 populations of *H. lutea* collected in the three states of southern Brazil, coordinates, collectors and number collection, number of individuals analyzed (*N*), fixed fragment (F_f), total number of loci per population (P_L), percentage of polymorphic loci (P_P) and average gene diversity (H_S)

States	Populations	Localities and collection number	Collectors	Coordinates	N	Ff	P_L	P_P	H_S
Paraná	BN	Balsa Nova. (FUEL44451).	CR, PR, LR, MR	25°31′16″ 49°35′15″W	25	15	169	87.56	0.26
	CS	Campina Grande do Sul. (FUEL44452).	CR, PR, LR, MR	25°18′77″S 48°55′90″W	25	15	170	88.08	0.27
Santa Catarina	BJ	Road between São Joaquim and Bom Jardim da Serra. (FUEL43)	CR, PR, EU	28°17′S 49°56′W	25	8	184	95.34	0.34
	RR	Rio do Rastro Mountain Range in Bom Jardim da Serra. (FUEL40654).	PR, CR, TN	28°16′S 49°52′W	20	24	161	83.42	0.28
	RQ	Rancho Queimado. (FUEL42).	CR, PR, EU	27°40′S 49°01′W	25	11	179	92.75	0.28
	SJ	São Joaquim. (FUEL40652)	PR, CR, TN	28°17′S 49°55′W	25	18	169	87.56	0.29
Rio Grande do Sul	CF	Fortaleza Canyon between Santa Catarina and Rio Grande do Sul States next to Cambará do Sul. (FUEL42233).	CR, PR, NM, MO	29°2′86″S 50°8′W	25	12	177	91.71	0.29
	FCM	São Francisco de Paula next to Capão do Muniz Farm. (FUEL42225).	CR, PR, NM, MO	29°33′S 51°51′W	25	6	186	96.37	0.31
	GUA	São Maximiniano Farm in Guaíba. (FUEL42235).	NM	30°10′S 51°23′W	25	14	172	89.12	0.27
	SR	Rocinha Mountain Range in Cambará do Sul. (FUEL42235).	CR, PR, NM, MO	29°01′S 50°09′W	25	2	191	98.96	0.33
	SM	Montenegro Mountain in São José dos Ausentes. (FUEL42241).	CR, PR, NM, MO	28°37′S 49°48′W	25	5	188	97.41	0.34
Average					**25**	**11.82**	**176.91**	**91.66**	**0.30**

CR, C.F. Ruas; EU, E. Urtubey; LR, L.A. Rodrigues; MO, M.A. Ortiz; MR, M. Reck; PR, P.M. Ruas; NM, N.I. Matzenbacher; TN, T.J. Nakayama. FUEL – Herbário da Universidade Estadual de Londrina.

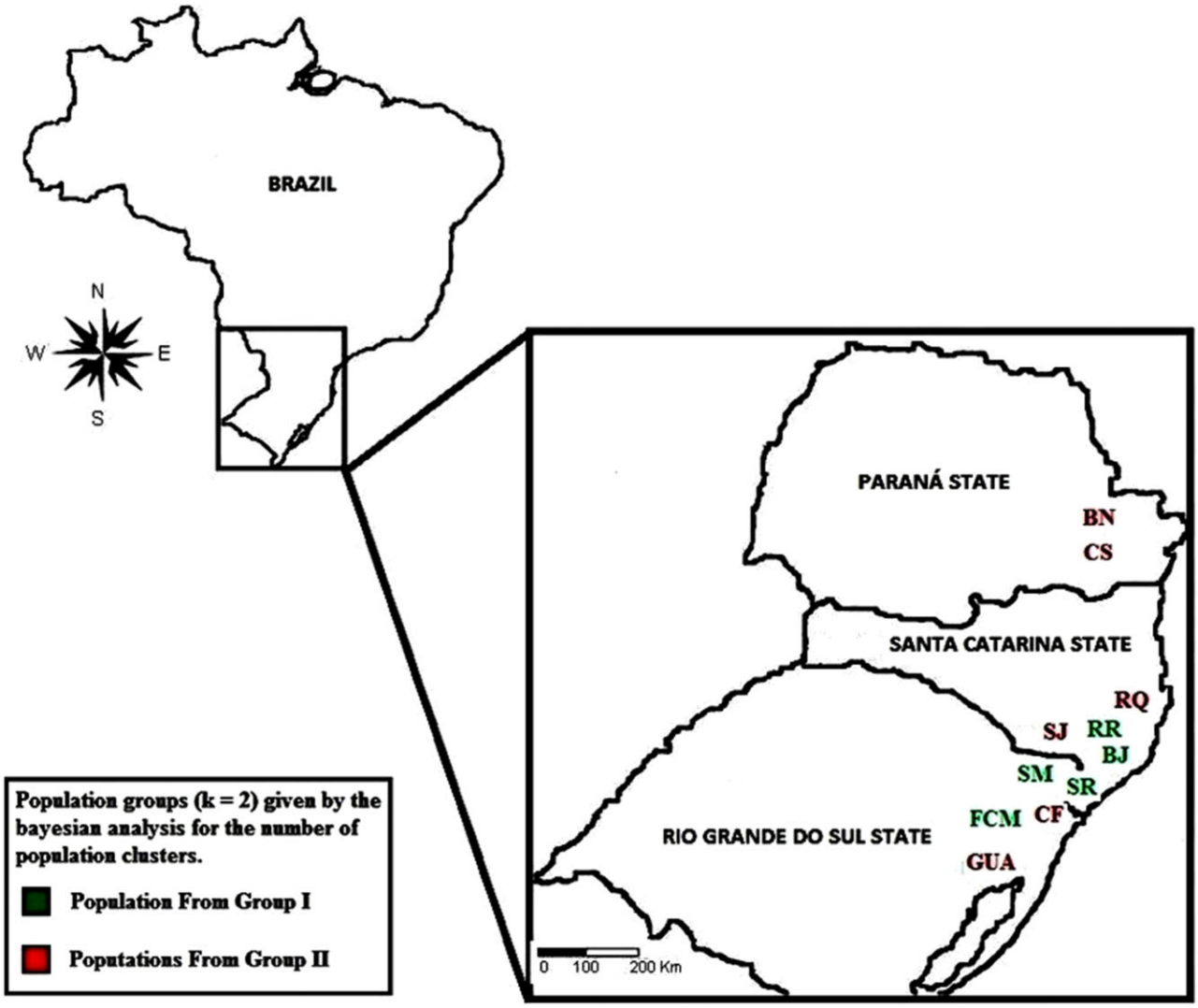

Fig. 1 Sampling localities of 11 populations of *Hypochaeris lutea* in southern Brazil, used for the AFLP study. For details of populations, see Table 1. Population groups ($K = 2$) given by the Bayesian analysis for the number of population clusters. (■) Population from Group I, (■) Population from Group II.

DNA was digested with *Eco*RI and *Mse*I endonucleases and ligated to double-stranded *Eco*RI and *Mse*I adaptors at 37°C for 16 h. The DNA fragments were then diluted fivefold with TE (Tris and ethylenediaminetetraacetic acid (EDTA)) buffer and used for the pre-selective and selective amplifications (PTC-200™, MJ Research, Inc.). Pre-selective primers based on the sequences of *Eco*RI and *Mse*I adaptors with the addition of a single nucleotide (*Eco*RI-A and *Mse*I-C) were used to amplify a subset of fragments having the matching nucleotide downstream from the restriction sites, resulting in ≈ 16-fold reduction in the number of amplified fragments. Pre-selective products were diluted fivefold with TE buffer (0.1 mM) and used for amplification, with selective primers having two or three additional selective nucleotides. For selective amplification, an initial screen using 16 primer combinations was performed on three individuals of two populations. Six primer combinations (*Eco*RI-AGC/*Mse*I-CAG, *Eco*RI-AGC/*Mse*I-CAAG, *Eco*RI-AGC/*Mse*I-CTAG, *Eco*RI-ACT/*Mse*I-GTCG, *Eco*RI-ACT/*Mse*I-CAAG, *Eco*RI-AGC/*Mse*I-CTCG) were then used for the selective PCR. The amplification products were separated on a 7% polyacrylamide gel along with a molecular ladder (Ludwig Biotecnologia, Ltd). The results were scored for presence/absence of amplified fragments and used to create a binary matrix.

Data analysis

The coefficient of variation for the number of AFLP fragments was calculated using the dBoot software version 1.1 (Coelho 2000). The percentage of polymorphic loci, Nei's (1978) gene diversity (H_S) for each population assuming Hardy–Weinberg (HW) equilibrium, was calculated using the software POPGENE version 1.31 (Yeh *et al*. 2000). Partitioning of genetic variation within population, between groups of populations and among populations, and the pairwise F_{st}, used to estimate the genetic divergence among populations, were performed using analysis of variance for molecular data (AMOVA), implemented in the software Arlequin version 3.11 (Excoffier *et al*. 2005). Significance was confirmed following 1023 randomizations. A comparison between genetic and geographical distances matrix was applied using the TFPGA (Tools For Population Genetic Analysis) software v. 1.3 (Miller 1997). Fixed alleles were calculated using the software Fingerprint Analysis with Missing Data (FAMD) version 1.2 (Schlüter & Harris 2006). The UPGMA (unweighted pair group method of arithmetic means) dendrogram among populations was constructed using the Population Genetic Analysis (POPGENE) computer program version 1.31 (Yeh *et al*. 2000). We also constructed a dendrogram based on the neighbor joining method using the software Splits Tree 4 (Huson & Bryant 2006). The trustworthiness of these clusters was tested using a Bayesian-based analysis with the software STRUCTURE version 2.3.1 (Pritchard *et al*. 2000). This analysis was performed based on an admixture model, and the number of subpopulations (K) was set from 1 to 11. After preliminary runs, the optimal number of groups (K) was determined using a 50 000 cycle burn-in period and 500 000 Monte-Carlo Markov Chains, using the admixture model (Falush *et al*. 2003, 2007; Hubisz *et al*. 2009) and assuming correlated allele frequencies among subpopulations without any prior information on clustering of samples. Simulations for each value of K were repeated 20 times to provide stable probability estimates. The optimal number of groups was determined using the second-order rate of change approach of Evano *et al*. (2005).

Results

AFLP scoring and genetic diversity

The scoring of six AFLP primer combinations yielded 193 markers, ranging from 50 to 1000 base pairs (bp). The total number of amplified fragments per primer pair ranged from 24 (*Eco*RI-AGC/*Mse*I-CTAG) to 45 (*Eco*RI-AGC/*Mse*I-CAAG), with an average of 32.17 markers. The coefficient of variation (Coelho 2000) calculated for the total number of markers was 8.7% and revealed the high consistency of the AFLP data for estimation of the genetic parameters in *H. lutea*. Whereas some monomorphic AFLP loci were identified within certain populations, none of them were monomorphic across all individuals throughout the populations. No private alleles were identified in all examined populations and there were no individuals that were exactly alike within or among any of the populations.

When considering all populations the percentage of polymorphic loci was 100%, although when we analyzed only single populations the percentage of polymorphic loci ranged from 83.42% (RR) to 98.96% (SR), with an average of 91.66%. Average gene diversity across all populations was 0.30 and ranged from 0.26 (BN) to 0.34 (BJ and SM), while the number of fixed alleles ranged from 2 (SR) to 24 (RR) for individual populations (Table 1).

Genetic structure

Pairwise F_{st} values were examined in order to identify possible correlation between geographic distance and population differentiation. Estimates of pairwise F_{st} among populations of *H. lutea* identified the smallest genetic distance (0.078) between SR and FCM and between SM and BJ populations, while the greatest distance (0.33) was observed between CS and RR populations (Table 2). There was no positive correlation between the geographical and genetic distances, as showed by the Mantel test ($r = 0.087$ $P > 0.05$). We also performed a Mantel test for the geographic and genetic distances within each state, nearby regions and within both groups of populations ($K = 2$), however, the lack of correlation was still maintained. The AMOVA showed that genetic variation was higher within populations (76.67%) rather than within groups of populations (12.51%) and among groups of populations (10.81%) of *H. lutea* (Table 3). The Bayesian analysis was applied to determine the most likely number of clusters (K) according to the statistics described by Evano *et al*. (2005). Simulations performed using STRUCTURE consistently identified $K = 2$ groups of populations (Fig. 2). The first group comprised six populations (CS, BN, CF, GUA, RQ, and SJ) and the remaining five populations (SR, FCM, BJ, SM, and RR) formed the second group, with the RR population more isolated from the others within this group. This result was further substantiated by the dendrogram, when applying the UPGMA method, which also distributed the populations into two major groups (Fig. 2). However, when we used the neighbor joining method, the group formation did not agree with the Bayesian analysis given by the software STRUCTURE (not shown). Moreover, the number of fixed fragments differed between the two groups, in which one group averaged 14.17 fixed fragments, 89.9% of polymorphic loci and gene diversity of 0.27, while the other

Table 2 Pairwise geographical distance (in km) between populations (values above diagonal), F_{st} pairwise (values below diagonal) in *H. lutea* based on analysis of 193 AFLP fragments

	Paraná		Santa Catarina				Rio Grande do Sul				
*Populations	BN	CS	BJ	RR	RQ	SJ	CF	FCM	GUA	SR	SM
BN	–	71	308	305	244	308	389	499	544	391	344
CS	0.14177	–	346	342	262	345	427	553	591	430	378
BJ	0.26525	0.27337	–	7	113	2	82	234	252	84	39
RR	0.31558	0.3308	0.1869	–	107	5	85	240	257	88	39
RQ	0.1306	0.12435	0.22262	0.28886	–	112	184	347	361	186	130
SJ	0.15312	0.18647	0.22756	0.27965	0.17459	–	82	235	253	84	39
CF	0.17556	0.18843	0.24299	0.30282	0.12064	0.17297	–	178	177	3	53
FCM	0.21554	0.24653	0.13495	0.18707	0.1791	0.22284	0.18372	–	82	175	225
GUA	0.14435	0.14055	0.24676	0.30631	0.1083	0.20239	0.11257	0.20606	–	175	230
SR	0.19038	0.21105	0.09206	0.17975	0.16512	0.17172	0.17321	0.07846	0.17152	–	56
SM	0.25186	0.26822	0.07806	0.17003	0.20276	0.21212	0.21787	0.10204	0.22775	0.08192	–

*Population names in Table 1.

Table 3 Analysis of Molecular Variance (AMOVA) of 11 populations of *Hypochaeris lutea*

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation index
Among groups	1	779.238	4.61601 Va	10.81	$F_{st} = 0.23$**
Among populations within groups	9	1473.93	5.34066Vb	12.51	
Within population	259	8476.01	32.72591 Vc	76.67	
Total	269	10729.18	42.68258		

**$P < 0.01$.

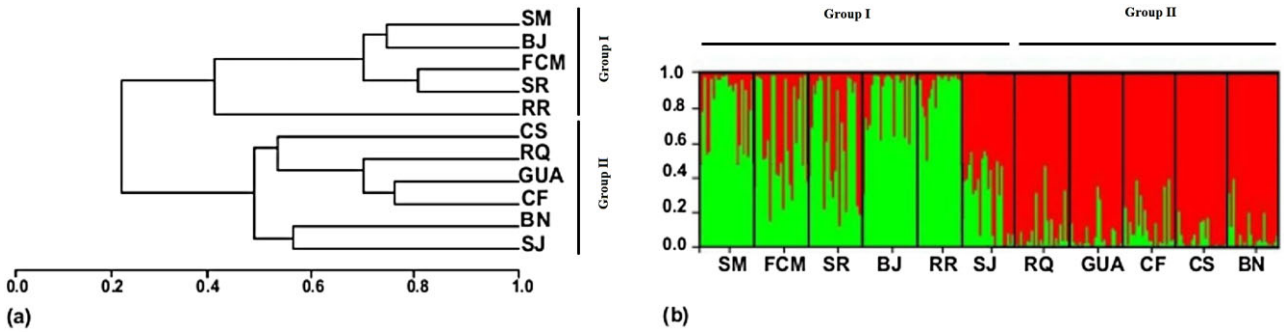

Fig. 2 (a) Dendrogram (UPGMA) showing genetic relationship among the 11 populations based on AFLP markers using Nei's 1978 coefficient. (b) Plots of group membership for the 270 individuals in *H. lutea* for $K = 2$, with colors representing proportion of ancestry derived from each group. Black lines indicate the division between populations.

averaged nine fixed fragments, 93.18% of polymorphic loci and gene diversity of 0.32 (Table 1).

Discussion

Hypochaeris lutea is morphologically and ecologically distinguishable from other South American species occurring in different physiographic regions, where it is associated with other plant species that are adapted to deep and damp soils (Azevêdo-Gonçalves & Matzenbacher 2007; Matzenbacher 1998). This species is widespread, with a distribution range that extends from southern to southeastern Brazil and some reports of occurrence in Argentina and Uruguay (Azevêdo-Gonçalves & Matzenbacher (2007). Furthermore, the high within-population genetic diversity and moderate differentiation among populations, evidenced by AFLPs, could be associated with high levels of gene flow among populations.

In contrast, in other South American *Hypochaeris* species, particularly those with a narrow distribution and that had their genetic structure affected by ecological variables, a much lower level of genetic polymorphism has been observed. For instance, the percentage of polymorphic loci ranged from 1.0 to 24.0% in *H. acaulis* (Tremetsberger *et al*. 2003a), from 0.7 to 20.7% in *H. palustris* (Muellner *et al*. 2005) and from 8.2 to 23.6% in *H. incana* (Tremetsberger *et al*. 2009).

Nei's gene diversity (H_S, Nei 1978) for *H. lutea* ranged from 0.26 (population BN) to 0.34 (population BJ), with an average of 0.30 (Table 2). These values are also higher than those observed in other *Hypochaeris* species. For example, the gene diversity ranged from 0.01 to 0.15 in *H. angustifolia*, the Moroccan sister species of the South American *Hypochaeris* (Terrab *et al*. 2009), from 0.0426 to 0.1675 in *H. salzmaniana*, an endemic species from south-eastern Europe (Ortiz *et al*. 2007), and from 0.002 to 0.056 in the South American *H. acaulis* (Tremetsberger *et al*. 2003b). *Hypochaeris lutea* is a rhizomatous herb with the ability for sexual reproduction, with flowering occurring from September to February (Azevêdo-Gonçalves & Matzenbacher 2007; Matzenbacher 1998), when the plants are visited by bees that possibly respond for the pollination, as observed during the fieldwork. The high genetic variability found within its populations suggests that the most frequent breeding system for *H. lutea* is allogamy, agreeing with the pattern of genetic distribution within and among populations observed in other allogamous species of *Hypochaeris* (Tremetsberger *et al*. 2003a).

Similar results were found in other South American species, such as the allogamous *H. tenuifolia* (Tremetsberger *et al*. 2003a) that displays higher variation within (69.11%) than among (30.89%) populations, and *H. uniflora* (Mra'z *et al*. 2007), a sporophytic self-incompatible species with higher values (75%) of within population genetic differentiation. Cornuet and Luikart (1996) and Piry *et al*. (1999) suggest that populations experiencing recent reduction of its effective size exhibit a decrease in the percentage of polymorphic loci and gene diversity. The values of gene diversity and the high percentage of polymorphic loci suggest that populations of *H. lutea* were unaffected by any recent event that could reduce the effective population size. It is also possible that the widespread distribution of the species and its intrinsic characteristics, such as the reproductive system and life cycle, have contributed to maintain its high genetic diversity.

Conversely, a different pattern is observed in the autogamous species *H. acaulis* and in the facultative autogamous species *H. palustris*, in which the genetic variability was higher among (67.9% and 78%) than within (32.1% and 22%) populations, respectively (Tremetsberger *et al*. 2003b; Muellner *et al*. 2005).

The index of population differentiation showed a F_{st} of 0.23 for *H. lutea*, in agreement with high levels of intraspecific variation, following the parameters of Hartl and Clark (2007). The level of within (76.67%) population differentiation evidenced by *H. lutea* was higher than observed in other South American species such as *H. tenuifolia* (69.11%; Tremetsberger *et al*. 2003a), *H. acaulis* (32.1%; Tremetsberger *et al*. 2003b), *H. palustris* (75%; Muellner *et al*. 2005), and *H. incana* (75%; Tremetsberger *et al*. 2009). Some of these species, such as the Andean *H. palustris*, *H. acaulis*, and *H. incana*, were under direct impact of the climate changes that occurred during the last glaciations in the Pleistocene, which may have influenced the genetic differentiation and population structuring. The characteristic pattern of geographic distribution found in populations of *H. lutea* suggests that the extreme climatic changes did not influence distribution of the genetic structure of this species. *Hypochaeris lutea* is broadly distributed, occurring in large populations of cold regions at altitudes around 1000 m. In Brazil the occurrence of *H. lutea* is reported mostly in mountain areas of the south, in the states of Rio Grande do Sul, Santa Catarina, and Paraná, with few reports of scattered individuals in the southeast, in the states of Minas Gerais, São Paulo, and Rio de Janeiro (Azevêdo-Gonçalves & Matzenbacher 2007). However, the species is ecologically restricted, occurring only in moist environments such as swamps and wetlands. The distribution of population genetic variation suggests that *H. lutea* has invaded moist environments through a sequence of events of long-distance dispersal. Although such events are particularly difficult to be monitored, because they are relatively rare and not completely understood, they are essential for the colonization of new areas, particularly for species whose natural habitats occur in patches (Ouborg *et al*. 1999).

Members of the Asteraceae family have strong potential for long-distance dispersal via light fruits and attached bristles (Sheldon & Burrows 1973; Anderson 1993), reinforcing the pattern of distribution of the genetic variability within populations of *H. lutea*. Furthermore, during field collection we documented the occurrence of several bird species that could, at least in part, be the responsible agents for the dispersal of *H. lutea*. Several bird species have been found in different wetlands along the distribution area of *H. lutea* (26 resident breeder species), most of them being intertropical migrants between these, thus providing a crucial link that favors the dispersal of *H. lutea*. Also, this species has feathery seeds, very common in Asteraceae, which are easily dispersed by wind (Soons *et al*. 2004), and, as Holmes (1995) describe, seeds may be carried by air currents for long distances. Cavalli and Wing (2003) state that populations that present seed dispersion by wind, animal ingestion, or adhesion

commonly present lower levels of genetic variation between rather than within populations, as observed in *H. lutea*.

There was no obvious correlation between geographic and genetic distances among populations of *H. lutea*, suggesting that this species does not follow a clear pattern of isolation by distance, as reported for *H. acaulis* (Tremetsberger *et al*. 2003b) and for the Moroccan species *H. angustifolia* (Terrab *et al*. 2009). The lack of geographic pattern is supported by the UPGMA tree constructed from genetic distances among populations. *Hypochaeris* species share a recent evolutionary history in South America (Samuel *et al*. 2003; Tremetsberger *et al*. 2005, 2006), which could explain the pattern of genetic differentiation among populations of *H. lutea*. Furthermore, it is also possible that the low genetic structuring is a consequence of selection for ecological and morphological characters, although not for AFLPs, resulting from similar environmental stresses as suggested in *Plantagus* (Dunbar-Co *et al*. 2009). Conversely, it is likely that differences in the AFLP markers observed among populations are the results of founder effect and genetic drift that may have occurred during the colonization of new sites. Furthermore, the recent massive range expansion of *H. lutea* in southern Brazil has possibly prevented an association of the genetic and geographic distance from arising between these populations. Founder effect was also suggested when we analyzed the occurrence of fixed fragments among populations, which varied from two (pop SR) to 24 (pop RR).

Founder events during colonization followed by population growth may promote considerable changes in genetic diversity relative to the source population, as for instance, the rate of genetic drift changes from high to low, generating novel variance on which selection can act (Slatkin 1996; Templeton 2008). Such events may have had an important role in generating and shaping genetic differentiation in *H. lutea*. *Hypochaeris lutea* populations occur only in swamp areas that are, in general, isolated from one another and by many kilometres distance. Such distribution indicates that events of long-distance dispersal and colonization of these areas were vital for shaping the current genetic variation and geographic distribution of this species.

The dendrogram generated from AFLP data for the populations of *H. lutea* using the UPGMA method identified two clusters that were not grouped by population origin (Fig. 2a). However when the neighbor joining method was employed, populations did not present the formation of these two clusters and neither were grouped by population origin. The levels of among-population genetic differentiation shown by the UPGMA method was substantiated by the Bayesian analysis that indicated $K = 2$ number of groups (Fig. 2). The first group comprised six populations (CS, BN, CF, GUA, RQ and SJ) and the second was formed by the remaining five populations (SR, FCM, BJ, SM, and RR), with the RR population more isolated from the others within this group. Cytogenetic studies revealed the polyploid nature of the RR population (Fiorin *et al*. 2013). However, the authors were not able to define the type o polyploid (autopoliploid or allopolyploid). Our results suggest that this population could be allopolyploid, which could explain, at least in part, the degree of differentiation between the RR and the other populations of *H. lutea*, which are diploid.

In conclusion, the populations of *H. lutea* have high values in the percentage of polymorphic loci, gene diversity and intrapopulation genetic diversity. When compared with other *Hypochaeris* species, the value of F_{st} indicates that *H. lutea* has a pattern of genetic distribution characteristic of species with outcrossing and a mixed system of reproduction, but predominantly allogamous. The level of divergence among populations of *H. lutea* may be considered high, however, there is no genetic structure by sampling geographic areas among populations, probably due to the recent process of diversification present in the South American Group of *Hypochaeris* and successive colonization events by long-distance dispersal being very common in the Asteraceae family.

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