ISSN 0373-580 X
Bol. Soc. Argent. Bot. 50 (3): 377-384. 2015

No differences in genetic diversity of *Cotoneaster franchetii* (Rosaceae) shrubs between native and non-native ranges

IRENE LETT[1*], ISABELL HENSEN[2,3], HEIDI HIRSCH[2,4] and DANIEL RENISON[1,5]

Summary: It is commonly assumed that plants have more genetic diversity in their native range than in areas where they have been introduced due to founder effects. However, few studies have proven this assumption and included the comparison between non-native and native ranges. We analyzed AFLP fingerprint patterns of 149 individuals from five native (China) and five non-native (Argentina) populations of *Cotoneaster franchetii*, a shrub which successfully invades different habitats and forms extensive monospecific stands. We compared genetic diversity estimates and assessed genetic differentiation among populations by inspecting FST values and conducting a PCoA, an AMOVA and a Mantel test. No evidence was found for reduced genetic diversity in non-native populations while the PCoA revealed two distinct groups, reflecting their Chinese and Argentine origin. The exceptions were ten individuals from two Chinese populations that clustered within the Argentine populations, supporting the idea of multiple introductions from China to Argentina.

Key words: AFLP, Argentina, biological invasion, China, genetic differentiation, multiple introductions.

Resumen: No hay diferencias en la diversidad genética entre arbustos de *Cotoneaster franchetii* (Rosaceae) de rangos nativos y no nativos. La diversidad genética de los arbustos de *Cotoneaster franchetii* es similar entre los rangos de distribución nativo y no nativo. Debido al efecto fundador comúnmente se asume que las plantas tienen mayor diversidad genética en su rango nativo que en las áreas donde fueron introducidos. Sin embargo, pocos estudios han probado este supuesto incluyendo la comparación entre los rangos nativos y no nativos. Nosotros analizamos marcadores de AFLP en 149 individuos de *Cotoneaster franchetii* pertenecientes a cinco poblaciones nativas (China) y cinco no nativas (Argentina) donde este arbusto invade exitosamente diferentes ambientes, y forma rodales extensos y monoespecíficos. Además comparamos los estimadores de diversidad genética y evaluamos la diferenciación genética entre las poblaciones examinando los valores de Fst y realizando un ACoP, un AMOVA y una prueba de Mantel. No se encontró evidencia de diversidad genética reducida en las poblaciones no nativas, mientras que el ACoP reveló dos grupos distintos, reflejando sus orígenes argentinos y chinos. Diez individuos de dos de las poblaciones chinas fueron la excepción, debido a que se agruparon dentro de las poblaciones argentinas, apoyando la idea de introducciones múltiples desde China hacia Argentina.

Palabras clave: AFLP, Argentina, invasión biológica, China, diferenciación genética, introducciones múltiples.

[1] Centro de Ecología y Recursos Naturales Renovables - Dr. Ricardo Luti (CERNAR - FCEFyN - UNC), Av. Vélez Sarsfield 1611, X5016GCA Córdoba, Argentina.
[2] Institute of Biology/Geobotany and Botanical Garden, Martin Luther University Halle-Wittenberg, Am Kirchtor 1, D-06108 Halle, Germany.
[3] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, D-04103 Leipzig, Germany.
[4] Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa.
[5] Instituto de Investigaciones Biológicas y Tecnológicas (IIByT - CONICET - UNC), Av. Vélez Sarsfield 1611, X5016GCA Córdoba, Argentina.
* irelett@hotmail.com

Introduction

Most non-native plant populations derive from a few introduced individuals, and such initially small populations are generally assumed to have low genetic diversity due to founder effects (Dlugosch & Parker, 2008; Harris *et al.,* 2012). However, the extent of these effects depends on several factors such as the species' reproductive system (Barrett *et al.,* 2008; Ebeling *et al.,* 2012; Kettenring & Mock, 2012) or a species' pre-adaptations to abiotic influences (Schlaepfer *et al.,* 2010). In some cases, invasive species can be very successful at colonizing new habitats, in spite of their low levels of genetic variability (Zimmermann *et al.,* 2010; Harris *et al.,* 2012). Likewise, where multiple introductions from differing sites of origin have occurred, genetic diversity of the introduced populations can equal or be even higher than that of the native populations (Bossdorf *et al.,* 2005; Harris *et al.,* 2012; Wolf *et al.,* 2012). Detailed insights into these aspects of invasion can often be gained by comparing non-native and native populations of the invasive species (Bossdorf *et al.,* 2005; Hierro *et al.,* 2005; Ebeling *et al.,* 2008; Hirsch *et al.,* 2011; Harris *et al.,* 2012; Cahill & Viard, 2014).

The shrub *Cotoneaster franchetii* Bois. is native to China and was introduced to South America and other continents for ornamental reasons (Richardson & Rejmánek, 2011). It is listed on plant watch lists in several countries (Alston & Richardson, 2006), and recently in Central Argentina (Giorgis & Tecco, 2014). The introduction history of this species is still poorly known. We suppose several *C. franchetti* generations have elapsed since their introduction to the region because a survey in the higher mountains of Central Argentina has revealed its widespread presence in a variety of habitats including native grasslands and forests (Giorgis *et al.,* 2011). Genetic analyses could determine relatedness of introduced populations where records of species introduction are unavailable (Atwood & Meyerson, 2011). As such, in order to explore the ecological and evolutionary processes that underlie the successful spread of *Cotoneaster franchetii* in Argentina, we initially examined individuals from five sites (hereafter referred to as "populations") at the molecular scale in the native range in China against five non-native populations in Argentina. In particular, we asked whether there are differences among native and non-native populations in 1) genetic diversity, and 2) genetic differentiation. We expected both genetic diversity and genetic differentiation to be lower in the non-native range in Argentina.

Materials and Methods

Study species

Cotoneaster franchetii Bois. (Rosaceae, Maloideae) is a multi-stemmed, 0.5 m to 3 m tall shrub with pendent twigs and year-round green leaves. The species is insect pollinated (Diptera, Hymenoptera) and thus probably exogamous; its orange elliptic fruits are 6 x 7 mm in diameter, contain three to five pyrenes and are dispersed by birds (Zheng-yi *et al*., 2003). The species is native to south-western China and northern Thailand, where it grows on sunny and rocky mountain slopes at altitudes between 1600 m and 2900 m a. s. l. (Zheng-yi *et al*., 2003). It was initially introduced to several countries as an ornamental garden plant due to its attractive fruits, but it later spread from gardens and has since become naturalized or invasive in Europe, South America, North America, South Africa, Australia and New Zealand (Krüssman, 1976). As a consequence of its broad environmental tolerance, high amount of seed production, fast development to maturity and vigorous re-sprouting rate following damage, *C. franchetii* has the potential to become a serious problem species, even in areas where it is not yet recognized as an environmental weed (Alston & Richardson, 2006).

Study sites

In 2007, we collected fresh leaf material from five native populations in China and in five non-native populations in Argentina, which we dried in silica gel (Table 1). Populations were defined as entities of individuals occurring at least 0.5 km apart, and the distance between studied populations ranged from 1 to 91 km in Argentina and from 22 to 427 km in China. Estimated population areas varied between 1.9 and 10 hectares in the native range and between 56 and 208 hectares in the non-native range. Argentinean populations were spread over highlands dominated by grasslands and forests (Giorgis *et al*., 2011). In China, *C.*

franchetii populations were confined to remnant mixed deciduous and secondary subtropical evergreen forests (Zheng-yi *et al*., 2003). Although our choosing sampling areas of 30 x 30 m within populations may have led to underestimations for total genetic diversity in large populations, it allowed for comparisons to be made across the two regions on a similar spatial scale. We sampled between eight and (mostly) fifteen individuals per population (Table 1), with the total sample size amounting to 149 samples (74 Argentinean and 75 Chinese). Collected specimens were deposited at the Herbarium CERNAR FCEFyN of the National University of Córdoba in Argentina.

DNA extraction, AFLP analysis and genotyping

We applied the same extraction protocol and AFLP procedure as described in Hensen *et al*., (2011) with the following modifications: pre-amplification was performed in a 20 µl volume containing 0.1 µl BioTaq DNA Polymerase (5 U/µl), 2.0 µl PCR 10x reaction buffer, 0.6 µl $MgCl_2$ (50 mM), 1.6 µl of each dNTP (2.5 mM; all Bioline, Luckenwalde, Germany), 1.0 µl of each pre-primer (5pmol), and 4 µl of the ligation product with the following temperature profile: 5 min initial denaturation at 94°C, 20 cycles of 20 s denaturation at 94°C, 30 s annealing at 56°C and 120 s elongation at 72°C. The pre-amplification product was diluted tenfold with sterile demineralised water. Selective amplification was carried out in a 20 µl volume containing 0.1 µl BioTaq DNA Polymerase (5 U/µl), 2.0 µl PCR 10x reaction buffer, 0.6 µl $MgCl_2$ (50 mM), 1.6 µl of each dNTP (2.5 mM; all Bioline, Luckenwalde, Germany), 1.0 µl *Mse*I selective primer (5 pmol), 1.0 µl *Eco*RI selective primer (1 pmol), both fluorescence labelled, as well as 3 µl pre-amplification product with the following temperature profile: 1 min initial denaturation at 95°C, 10 cycles of 20 s denaturation at 94°C, 30 s annealing at 65°C (decreasing by 1°C per cycle), 120 s elongation at 72°C, followed by 25 cycles of 20 s denaturation at 94°C, 30 s annealing at 56°C and 120 s elongation at 72°C (increasing by 4 s per cycle). For the selective amplification, 21 different primer combinations were tested on 12 samples for their level of variability within and among species, and five primer combinations were chosen for fingerprinting each of the samples. The five combinations were 5´-*Eco*RI+AAC*FAM-3´ / 5´-*Mse*I+CCA-3´, 5´-*Eco*RI+AGC*HEX-3´ / 5´-*Mse*I+CCA-3´, 5´-*Eco*RI+AAC*FAM-3´ / 5´-*Mse*I+CAA-3´, 5´-*Eco*RI+AGC*HEX-3´ / 5´-*Mse*I+CAA-3´ and 5´-*Eco*RI+AGC*HEX-3´ / 5´-*Mse*I+CTT-3´.

Table 1. Sampled populations of *Cotoneaster franchetii*. Geographic coordinates (Lat = latitude; Long = longitude) are provided in decimal degrees. For each population, sample number (N), expected heterozygosity (He), proportion of polymorphic loci ($PLP_{5\%}$) and band richness ($B_r[8]$) are shown. The latter two parameters were rarefied to eight individuals.

Population	Country	Lat	Long	N	He	$PLP_{5\%}$	$B_r[8]$
Non-native							
AR1	Argentina	-318,951	-647,709	16	0.227	0.697	1,582
AR2	Argentina	-316,256	-646,708	15	0.167	0.526	1,427
AR3	Argentina	-316,286	-646,814	17	0.142	0.448	1,339
AR4	Argentina	-316,309	-646,742	17	0.154	0.494	1,388
AR5	Argentina	-311,051	-644,980	9	0.177	0.542	1,522
Native							
CH1	China	249,572	1,026,268	17	0.179	0.628	1,482
CH2	China	253,993	1,027,163	17	0.137	0.470	1,354
CH3	China	246,556	1,036,012	8	0.125	0.376	1,376
CH4	China	267,705	1,002,761	16	0.191	0.589	1,487
CH5	China	269,496	1,001,885	17	0.185	0.616	1,498

Data analysis

Polymorphic DNA bands were scored as present (1) or absent (0) for each DNA sample and smeared and weak bands were excluded through visual inspection. The three primer combinations used in the AFLP analysis of *C. franchetii* yielded 734 reliable bands, of which 696 (94.82%) were polymorphic and consequently used in the analysis. The number of polymorphic bands per primer pair of *C. franchetii* ranged between 77 and 192.

Genetic diversity

Genetic diversity per population was calculated as Nei's expected heterozygosity (He) with the software GenAlEx (version 6.5b3; Peakall & Smouse, 2012). Since sample sizes per population were unbalanced, band richness (B_r) along with the proportion of polymorphic loci (PLP) at the 5% level were calculated, with a rarefaction to the minimal sample size of eight individuals using AFLPDiv (version 1.1; Petit *et al*., 1998, Coart *et al*., 2005). To test whether genetic diversity measures (He, Br-1 and PLP; all arscsine-square-root-transformed) differed between native and non-native populations, we applied analyses of molecular variance (AMOVA) using R software (version 2.15.0; R Development Core Team 2012).

Genetic differentiation

We applied a principal coordinate analysis (PCoA) based on square-root-transformed Jaccard dissimilarities (equivalent to Jaccard distance, which is calculated by subtracting the Jaccard similarity from 1; package vegan version 2.0-5 in R; Oksanen *et al*., 2012) to visualize the genetic relationships between individuals. An AMOVA was used to describe genetic structure and to measure the amount of variation found within and between populations; Φ statistics (analogues of F statistics) were extracted and significance levels were tested with 999 permutations for each analysis. Populations examined in the AMOVA procedure were assigned to the two groups based on their geographic origins (Argentina and China, Table 1). The AMOVA was performed with GenAlEx. Mantel tests (Mantel, 1967), performed with the vegan package in R, were used to examine whether the matrix of genetic differentiation among populations (pairwise Φ_{ST} -values) was correlated with the matrix of geographical distance (log transformed).

Results

Genetic diversity

Native Chinese populations and non-native Argentinean populations did not differ significantly in estimates of genetic diversity (AMOVA He: $F_{1,8} = 0.26$, $P > 0.05$; PLP: $F_{1,8} = 0.01$, $P > 0.05$; B_r: $F_{1,8} = 0.05$, $P > 0.05$). The mean values of genetic diversity measures for the native range were He: 0.16 ± 0.03; PLP: 0.53 ± 0.11; B_r: 1.44 ± 0.07 and for the non-native range He: 0.17 ± 0.03; PLP: 0.54 ± 0.09; B_r: 1.45 ± 0.09 (Mean ± standard deviation).

Genetic differentiation

In the PCoA (Fig. 1), two distinct groups could be recognized reflecting their geographical origin, with the first two axes explaining 21.5% of the overall genetic variability. Axis 1 (14.1%) separated the native Chinese populations from the non-native Argentinean populations. Axis 2 (7.4%) clearly isolated seven out of nine individuals from one Argentinean population (AR5). Remarkably, ten out of 33 individuals of two Chinese populations (CH1 and CH4) clustered among the Argentinean samples.

When Chinese and Argentinean populations were combined in a single data set, the AMOVA showed that 63.1% of the genetic variation was found within populations, 18.4% among populations and 18.5% among ranges. Within population variation increased when ranges were analyzed separately as compared to the combined data set (native: 76.5%, non-native: 78.4%; Table 2).

Pairwise Φ_{ST} values among native Chinese populations were, on average, slightly larger (mean: 0.24, standard deviation: 0.07) than among Argentinean populations (mean: 0.22, standard deviation: 0.18; Table 3). However, excluding the one Argentinean population (AR1) along with the separated individuals in the PCoA led to a clear decline in the mean pairwise Φ_{ST} value among non-native Argentinean populations (mean: 0.09, standard deviation: 0.06). Genetic distance did not significantly correlate with geographic distance in the native range (only China: Mantel statistic $r = 0.34$; $P = 0.13$) but was significantly correlated in the non-native range (only Argentina: Mantel statistic $r = 0.81$; $P = 0.01$).

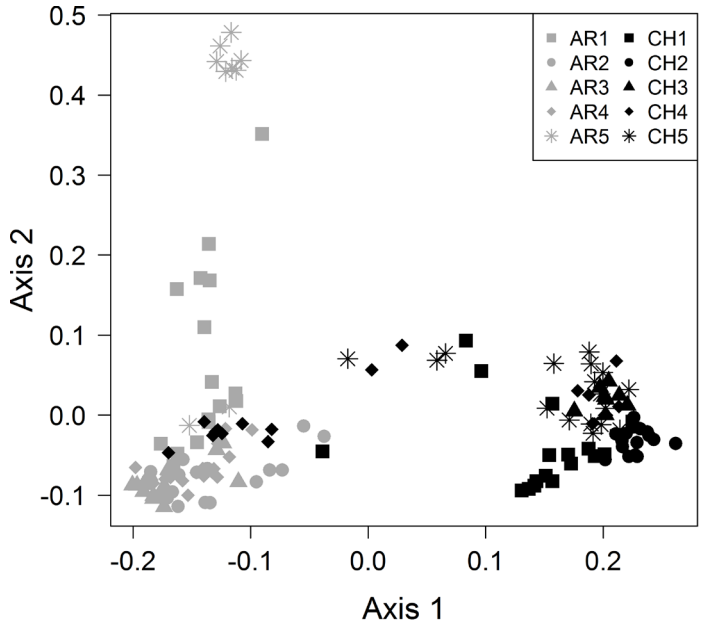

Fig 1. Principal coordinates analysis (PCoA) for the investigated *Cotoneaster franchetii* samples (based on square-root-transformed Jaccard dissimilarities). Native populations are characterized by black symbols and non-native populations by grey symbols. Explained variance: axis 1 = 14.1%, axis 2 = 7.4% and axis 3 = 4.5%.

Table 2. Analyses of molecular variance (AMOVA) among and within the native and non-native ranges of *Cotoneaster franchetii.*

Source of variation	Over all Φ_{ST}	Percentage of variation		
		Among ranges	Among populations	Within populations
Both ranges pooled	0.37	18.50	18.39	63.11
Argentina	0.22	-	21.58	78.42
China	0.24	-	23.50	76.50

Table 3. Pairwise Φ_{ST}-values per population.

	AR1	AR2	AR3	AR4	AR5	CH1	CH2	CH3	CH4
AR2	0.134	0.000							
AR3	0.170	0.039	0.000						
AR4	0.121	0.018	0.032	0.000					
AR5	0.292	0.437	0.488	0.443	0.000				
CH1	0.305	0.270	0.360	0.308	0.492	0.000			
CH2	0.411	0.434	0.512	0.454	0.588	0.208	0.000		
CH3	0.389	0.420	0.513	0.453	0.536	0.239	0.335	0.000	
CH4	0.193	0.153	0.206	0.167	0.365	0.215	0.342	0.272	0.000
CH5	0.330	0.325	0.401	0.351	0.455	0.180	0.274	0.180	0.138

Discussion

Non-native *C. franchetii* populations in Argentina showed similar levels of genetic diversity to native Chinese populations. This was unexpected and inconsistent with data on other invasive Rosaceae populations in Argentina, such as e.g. *Rosa rubiginosa* (Zimmermann *et al*., 2010; Hirsch *et al*., 2011). Nevertheless, *Rosa rugosa* populations introduced to Europe did not differ in genetic diversity with its native ranges (Kelager *et al*., 2013), and similar genetic diversity differences have also been found in other plants species between their natural and introduced origins (e.g. *Centaurea solstitialis*, Andonian & Hierro, 2011) and also in marine invertebrates species, where it is very common to find no differences between both ranges (Harris *et al*., 2012). The reason for no reduced genetic variation could be due to non-native populations being characterized by multiple introductions. If multiple introductions from several original populations had occurred in Central Argentina, the resulting interbreeding may have compensated possible bottlenecks during each introduction. Unfortunately, there are no records on the introduction history of *C. franchetii* in Argentina, but we were able to find information on other Asian species, such as *Ulmus pumila* (Ulmaceae). This elm was introduced into Argentina on several occasions and always from regions of the world where the species had already been introduced, such as the US or Italy, with no records indicating direct introductions from the species' countries of origin (Moore, 1960; Poduje, 1972). A similar scenario involving *C. franchetii* being introduced from several native sites for cultivation purposes in several foreign countries including Argentina would explain the similar genetic diversity between Chinese and Argentinean populations.

Within both *C. franchetii* study regions, we only found moderate differentiation between populations and, strikingly, there was an overlap between populations of Argentina and China, indicating that two of the study populations from China may indeed represent the origin populations of some of the non-native populations in Argentina. As with *Ulmus pumila*, these could have been introduced either directly or via secondary introductions.

There was no correlation between genetic and geographic distance for the Chinese populations, indicating that the differentiation among populations cannot be explained by distance. Instead, in the Argentine range we found that differentiation among populations was explained by distance, perhaps due to deficient pollen transfer or strong selective pressure in their new range. Geographical structuring among Argentine and Chinese ranges at least indicates that gene flow is not high between ranges, and that multiple introductions may have occurred for Argentina, perhaps from other regions of China or northern Thailand, where *C. franchetii* is also native.

The existing genetic diversity and lack of equal AFLP phenotypes also indicates that neither apomixes nor vegetative reproduction play an important part in the reproduction of *C. franchetii*. This is also in contrast to Zimmermann *et al*., (2010) and Amsellem *et al*., (2000), both of whom revealed an increase in asexual reproduction in non-native populations of the Rosaceae *Rosa rubiginosa* and *Rubus alceifolius* in comparison to populations in the native range.

We therefore conclude that *C. franchetii* in its introduced range within Argentina has similar genetic diversity to that of native populations in China, although the populations of both countries are, in part, genetically differentiated. This result does not support the general assumption that introductions are associated with a loss in genetic diversity and more native vs. non-native genetic comparisons are therefore required to inform any general conclusions. Understanding the relations among invasion history (e.g. number of introduction events), genetic variation and invasion success may be helpful for understanding the evolutionary outcomes of invasions and how to incorporate them into management and control strategies for invasive species (Harris *et al*., 2012).

Acknowledgements

We are grateful to numerous people and groups who were of enormous help during field work, laboratory analysis and compilation of the manuscript, in particular, Birgit Müller, Sandra Weber, Ende Liu, Gerhard Klotz, Weijia Xie, Yanchun Liu, Yongpeng Ma, Zhao Hui Dong and the Quebrada del Condorito National Park. We also thank two anonymous reviewers for helpful

suggestions and comments. This study was funded by BMZ – DFG. We thank Danny McCluskey for checking our written English.

Bibliography

AMSELLEM, L., J. L. NOYER, T. LE BOURGEOIS & M. HOSSAERT-MCKEY. 2000. Comparison of genetic diversity of the invasive weed *Rubus alceifolius* Poir. (Rosaceae) in its native range and in areas of introduction, using amplified fragment length polymorphism (AFLP) markers. *Mol. Ecol.* 9: 443-455.

ALSTON, K. P. & D. M. RICHARDSON. 2006. The roles of habitat features, disturbance, and distance from putative source populations in structuring alien plant invasions at the urban/wildland interface on the Cape Peninsula, South Africa. *Biol. Conserv.* 132: 183-198.

ANDONIAN, K. & J. L. HIERRO. 2011. Species interactions contribute to the success of a global plant invader. *Biol. Invasions* 13: 2957-2965.

ATWOOD, J. P. & L. A. MEYERSON. 2011. Beyond EICA: understanding post-establishment evolution requires a broader evaluation of potential selection pressures. *NeoBiota* 10: 7-25.

BARRETT, S. C. H., R. I. COLAUTTI & C. G. ECKERT. 2008. Plant reproductive systems and evolution during biological invasion. *Mol. Ecol.* 17: 373-383.

BOSSDORF, O., H. AUGE, L. LAFUMA, W. E. ROGERS, E. SIEMANN & D. PRATI. 2005. Phenotypic and genetic differentiation between native and introduced plant populations *Oecologia* 144: 1-11.

CAHILL, A. E. & F. VIARD. 2014. Genetic structure in native and non-native populations of the direct-developing gastropod *Crepidula convexa. Mar. Biol.* 161: 2433-2443.

COART, E., S. VAN GLABEKE, R. J. PETIT, E. VAN BOCKSTAELE & I. ROLDÁN-RUIZ. 2005. Range wide versus local patterns of genetic diversity in the hornbeam (*Carpinus betulus* L.). *Conserv. Genet.* 6: 259-273.

DLUGOSCH, K. M. & I. M. PARKER. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Mol. Ecol.* 17: 431-449.

EBELING, S. K., I. HENSEN & H. AUGE. 2008. The invasive shrub *Buddleja davidii* performs better in the introduced area. *Divers. Distrib.* 14: 225-234.

EBELING, S. K., S. SCHREITER, I. HENSEN, W. DURKA & H. AUGE. 2012. Outcrossing breeding system does not compromise invasiveness in *Buddleja davidii. Flora* 207: 843-848.

GIORGIS, M., P. TECCO, A. M. CINGOLANI, D. RENISON, P. MARCORA & V. PAIARO. 2011. Factors associated with woody alien species distribution in a newly invaded mountain system of central Argentina. *Biol. Invasions* 13: 1423-1434.

GIORGIS, M. A. & TECCO, P. A. 2014. Árboles y arbustos invasores de la Provincia de Córdoba (Argentina): una contribución a la sistematización de bases de datos globales. *Bol. Soc. Argent. Bot.* 49: 581-603.

HARRIS, C. J., E. E. DORMONTT, J. J. LE ROUX, A. LOWE & M. R. LEISHMAN. 2012. No consistent association between changes in genetic diversity and adaptive responses of Australian acacias in novel ranges. *Evol. Ecol.* 26: 1345-1360.

HENSEN, I., I. TEICH, H. HIRSCH, H. VON WEHRDEN & D. RENISON. 2011. Range-wide genetic structure and diversity of the endemic treeline species *Polylepis australis* (Rosaceae) in Argentina. *Am. J. Bot.* 98: 1825-1833.

HIERRO, J. L., J. L. MARON & R. M. CALLAWAY. 2005. A biogeographical approach to plant invasions: The importance of studying exotics in their introduced and native range. *J. Ecol.* 93: 5-15.

HIRSCH, H., H. ZIMMERMANN, C. M. RITZ, V. WISSEMANN, H. VON WEHRDEN, D. RENISON, K. WESCHE, E. WELK & I. HENSEN. 2011. Tracking the origin of invasive *Rosa rubiginosa* populations in Argentina. *Int. J. Plant Sci.* 172: 540-550.

KELAGER, A., J. S. PEDERSEN & H. H. BRUUN. 2013. Multiple introductions and no loss of genetic diversity: invasion history of Japanese Rose, *Rosa rugosa*, in Europe *Biol. Invasions* 15: 1125-1141.

KETTENRING, K. M. & K. E. MOCK. 2012. Genetic diversity, reproductive mode, and dispersal differ between the cryptic invader, *Phragmites australis*, and its native conspecific. *Biol. Invasions* 14: 2489-2504.

KRÜSSMAN, G. 1976. Manual of cultivated broad-leaved trees and shrubs. Volume 1, A-D. In cooperation with the American Horticultural Society, Timber Press, Beaverton, OR, 448 pp.

MANTEL, N. 1967 The detection of disease clustering and a generalized regression approach. Cancer Res. 27: 209-220. http://cancerres.aacrjournals.org/content/27/2_Part_1/209 [Access: 10th September 2013]

MOORE, G. 1960 El cultivo de Ulmus pumila ofrece buenas perspectivas en la Argentina. *Revista Forest. Argent.* 4: 77-81.

OKSANEN, J., F. G. BLANCHET, R. KINDT, P. LEGENDRE, P. R. MINCHIN, R. B. O´HARA, G. L. SIMPSON, P. SOLYMOS, M. H. H. STEVENS & H. WAGNER. 2012. vegan: community ecology package. R package version 2.0-5. Available in: http://CRAN.R-project. Org/package=vegan

PEAKALL, R. & P. E. SMOUSE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics* 28: 2537-2539.

PETIT, R. J., A. EL MOUSADIK & O. PONS. 1998. Identifying populations for conservation on the basis of genetic markers. *Conserv. Biol.* 12: 844-855.

PODUJE, L. 1972. Contribución al conocimiento del cultivo del olmo siberiano (*Ulmus pumila* var. *arborea* Litw.) en La Pampa. *Revista Forest. Argent.* 16: 137-141.

R DEVELOPMENT CORE TEAM. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, Available in: http://www.R-project.org/

RICHARDSON, D. & M. REJMÁNEK. 2011. Trees and shrubs as invasive alien species - a global review. *Divers. Distrib.* 17: 788-809.

SCHLAEPFER, D.R., M. GLÄTTLI, M. FISCHER & M. VAN KLEUNEN. 2010. A multi-species experiment in their native range indicates pre-adaptation of invasive alien plant species. *New Phytol.* 185: 1087-1099.

WOLF, V. C., A. GASSMANN, B. M. CLASEN, A. G. SMITH & C. MÜLLER. 2012. Genetic and chemical variation of *Tanacetum vulgare* in plants of native and invasive origin. *Biol. Control* 61: 240-245.

ZIMMERMANN, H., C. M. RITZ, H. HIRSCH, D. RENISON, K. WESCHE & I. HENSEN. 2010. Highly reduced genetic diversity of *Rosa rubiginosa* L. populations in the invasive range. *Int. J. Plant Sci.* 171: 435-446.

ZHENG-YI, W., P. H. RAVEN & H. DEYUAN. 2003. Flora of China 9:1–496. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis. http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=108169 [Access: 23rd January 2011]

Recibido el 31 de diciembre de 2014, aceptado el 3 de junio de 2015.