Extensive analysis of native and non-native *Centaurea solstitialis* L. populations across the world shows no traces of polyploidization

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*Centauera solstitialis* L. (yellow starthistle, Asteraceae) is a Eurasian native plant introduced as an exotic into North and South America, and Australia, where it is regarded as a noxious invasive. Changes in ploidy level have been found to be responsible for numerous plant biological invasions, as they are involved in trait shifts critical to invasive success, like increased growth rate and biomass, longer life-span, or polycarpy. *C. solstitialis* had been reported to be diploid (2n = 2x = 16 chromosomes), however, actual data are scarce and sometimes contradictory. We determined for the first time the absolute nuclear DNA content by flow cytometry and estimated ploidy level in 52 natural populations of *C. solstitialis* across its native and non-native ranges, around the world. All the *C. solstitialis* populations screened were found to be homogeneously diploid (average 2C value of 1.72 pg, SD = ± 0.06 pg), with no significant variation in DNA content between invasive and non-invasive genotypes. We did not find any meaningful difference among the extensive number of native and non-native *C. solstitialis* populations sampled around the globe, indicating that the species invasive success is not due to changes in genome size or ploidy level.
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ABSTRACT

Background. *Centaurea solstitialis* L. (yellow starthistle, Asteraceae) is a Eurasian native plant introduced as an exotic into North and South America, and Australia, where it is regarded as a noxious invasive. Changes in ploidy level have been found to be responsible for numerous plant biological invasions, as they are involved in trait shifts critical to invasive success, like increased growth rate and biomass, longer life-span, or polycarpy. *C. solstitialis* had been reported to be diploid (2n = 2x = 16 chromosomes), however, actual data are scarce and sometimes contradictory. Methods. We determined for the first time the absolute nuclear DNA content by flow cytometry and estimated ploidy level in 52 natural populations of *C. solstitialis* across its native and non-native ranges, around the world. Results. All the *C. solstitialis* populations screened were found to be homogeneously diploid (average 2C value of 1.72 pg, SD = ± 0.06 pg) with no significant variation in DNA content between invasive and non-invasive genotypes.

Discussion. We did not find any meaningful difference among the extensive number of native and non-native *C. solstitialis* populations sampled around the globe, indicating that the species invasive success is not due to changes in genome size or ploidy level.

Subjects Plant Science, Biogeography, Evolutionary Studies

Keywords Yellow starthistle, Invasiveness, Genome size, Flow cytometry, Ploidy level, Hybridization
INTRODUCTION

Changes in ploidy level have been reported to be important for the invasive success of some plants species (te Beest et al., 2011), by altering morphological, physiological and ecological parameters which can confer hybrid vigor, stress resistance, competitive advantages, or increased phenotypic plasticity, like in the case of the North American tetraploids of *Centaurea stoebe* L. (Hahn et al., 2012). Additionally, there are a series of associated “genome size constrained traits”, related mostly to reproduction and dispersal, which dictate the ecological niche a species can access (te Beest et al., 2011). In contrast, several studies support the hypothesis that a smaller genome can contribute to some species invasive potential by boosting early plant growth and enhancing competitive ability (Bennett et al., 1998; Grotkopp et al., 2004; Beaulieu et al., 2006; Lavergne et al., 2010; Suda et al., 2015). For instance, *Phalaris arundinacea* L. (reed canary grass, Poaceae) in the USA underwent a quick and significant reduction in genome size compared to the native European genotype, which was correlated with some advantageous phenotypic effects and enhanced aggressiveness (Lavergne et al., 2010). A list comparing the ploidy level of 128 worst invasive plant species worldwide, was recently made available by te Beest et al. (2011), indicating that a quarter of them possess at least two different ploidy levels. An interesting example is *C. stoebe* (spotted knapweed) which occurs both as a diploid and tetraploid, with only the latter cytotype becoming invasive in the Western parts of the USA (Mráz et al., 2011). Although there is still ongoing debate about the prevalence of polyploids among plant species, it has been recently inferred that polyploidy has had a key role in the evolution of most angiosperm lineages (Soltis et al., 2009). However, for many invasive species, ploidy levels and genome size are unknown or have not been thoroughly investigated.
Centaurea L. is one of the most species rich genera in the Asteraceae (Bremer, 1994). Numerous Centaurea species have been introduced into new non-native regions, where many of them have become invasive. For instance, the US Federal Noxious Weeds list (USDA NRCS, The PLANTS Database, 2017), includes no fewer than 13 taxa, but ploidy level for many of these is unknown or uncertain. In particular, C. solstitialis is a Eurasian native annual herb which was introduced into the Americas and Australia during the last two centuries (Barker et al., 2017) and became an impactful invader in the former case. In the invaded ranges, C. solstitialis forms dense stands that displace native plants species and reduce considerably livestock grazing capacity and forage value (Eagle et al., 2007). It alters ecosystem functions by depleting soil water and nutrients through an extensive root system (DiTomaso, 2000), and can cause a neurological disorder in horses similar to human Parkinson (Chang et al., 2011). As an economically important plant, the species has been the subject of intensive research, and significant divergences between native and non-native ranges have been reported for plant size (Eriksen et al., 2012; Graebner et al., 2012; García et al., 2013; Dlugosch et al., 2015), growth rates (Graebner et al., 2012), germination (Hierro et al., 2009), competitive ability (Montesinos & Callaway, 2017), and reproduction (Montesinos et al., 2012), among others. Such changes suggest diverging local adaptation occurring among native and non-native ranges, and hypothetical changes in genome size and ploidy level could be potentially responsible for at least some of the observed trait-shifts.

Until now, only three genome size estimates were available in the literature for C. solstitialis: two from the native range (Bulgaria: 1.74 pg/2C, one accession, in Bancheva & Greilhuber 2006; and Croatia: 1.95 pg/2C, five accessions, in Carev et al., 2017) and another from an invasive population in western USA: 1.66 pg/2C, thirty accessions (Miskella, 2014).
Based on these few studies, *C. solstitialis* had been reported to be diploid (Dlugosch et al., 2013; Rice et al., 2015) with 2n = 2x = 16 chromosomes. However, records of 2n = 2x = 18 chromosomes were published more than 30 years ago from the native range of Bulgaria (Jasiewicz & Mizianty, 1975; Kuzmanov & Georgieva, 1990) and recently from one accession from Sicily and the other one from Sardinia (Widmer et al., 2007). Furthermore, Inceer et al. (2007) reported tetraploids in seeds (single accession) sampled in northern Turkey, but none of those observations, made in only a handful of individuals, have been confirmed since then. Consequently, it was still unclear whether ploidy could have played a role in at least some of the *C. solstitialis* invaded ranges. To fill this knowledge gap for such an important species, we aimed to thoroughly sample and assess *C. solstitialis* ploidy level and genome size in a representative number of populations from around the world, including native Turkey, the ancestral origin of the species; native Spain, the main source of American populations; and all the known non-native regions represented by Argentina, Chile, USA and Australia.

**METHODS**

**Seed collection**

A total of 477 accessions from 52 natural populations (Table S1) of *C. solstitialis* were investigated in this study, for genome size and ploidy level assessment. Within the native area, we sampled ten populations from Turkey, near the Caucasus region, where high genetic diversity has been detected, and is regarded as the site of origin of the species (Wagenitz, 1955; Gerlach, 1997a; Uygur et al., 2004; Dlugosch et al., 2013; Eriksen et al., 2014), and ten populations from Spain, considered as the primary source of seeds to have colonized Chile and Argentina (Hijano & Basigalup, 1995; Eriksen et al., 2012, 2014; Dlugosch et al., 2013; Barker et al., 2017) in the
nineteenth century (Gerlach, 1997b). For the non-native regions, we included ten populations from Argentina and California, eight from Australia and four from Chile. Seeds were extracted from mature flower heads collected in the wild from ten individuals per population between 2009 and 2014. Ten seeds from each individual were germinated in plant growing trays, under common greenhouse conditions, in early spring 2016 at the Botanical Garden of the University of Coimbra, Portugal.

Flow cytometry

Young and intact leaves of 4-6 weeks-old plants were sampled and screened by flow cytometry. Since analyses were based on leaves of small plants, which were destroyed by leaf sampling, no voucher specimens could be collected. Nuclei were isolated following the chopping method of Galbraith et al., 1983. Briefly, about 1 cm\(^2\) of leaf tissue was co-chopped with a razor blade together with the same amount of reference standard (Raphanus sativus L. ‘Saxa’, 2C = 1.11 pg, Doležel et al., 1992) in 1 mL of woody plant buffer (WPB): 0.2 M Tris.HCl, 4 mM MgCl\(_2\).6H\(_2\)O, 2 mM EDTA Na\(_2\).2H\(_2\)O, 86 mM NaCl, 10 mM sodium metabisulfite, 1% PVP-10, 1% (v/v) Triton X-100, pH 7.5 (Loureiro et al., 2007). The resulting homogenate was filtered through a 50 μm nylon filter into a sample tube to remove large debris. Nuclei were stained with 50 mg/mL propidium iodide (PI, Fluka, Buchs, Switzerland), and 50 mg/ml of RNAse (Fluka, Buchs, Switzerland) was added to prevent the staining of double stranded RNA. Samples were kept at room temperature and analyzed immediately on a Partec CyFlow Space flow cytometer (Partec GmbH, Görlitz, Germany) equipped with a 532 nm green solid-state laser, operating at 30 mW.
Data collection and analysis

Results were acquired using Partec FloMax software (v2.4d) (Partec GmbH, Münster, Germany) in the form of six graphics: fluorescence pulse integral in linear scale (FL); forward light scatter (FS) vs. side light scatter (SS), both in logarithmic (log) scale; FL vs. time; FL vs. fluorescence pulse height; FL vs. FS in log scale and FL vs. SS in log scale. Mean fluorescence values and coefficient of variation (CV value) of the fluorescence of both sample and standard were obtained for at least 1300 nuclei in each G1 peak, whenever possible. Samples with CV values above 5% were discarded, prepared and ran again. At least three individuals from every population were used to estimate genome size (Table S2), in different days, to account for the variation generated by the flow cytometer. The remaining individuals were analyzed in pool (three or four individuals) to determine ploidy level, only. The absolute DNA content of a sample was calculated based on the following formula: 2C nuclear DNA content of the sample = (sample G1 peak mean) / (standard G1 peak mean) × 2C DNA content of standard. Descriptive statistics were calculated for genome size data (mean, standard deviation of the mean, standard error, coefficient of variation and minimum and maximum values) using Microsoft Excel 2016. Differences in average genome size values among regions were assessed by means of Linear Mixed-Effect Models with the formulation of Laird and Ware (1982), with a region as fixed factor and population within region as a random nested factor, in R-3.2.0 (R Development Core Team, 2010). Data was plotted in BoxPlotR (Spitzer et al., 2014).

RESULTS

Analysis of fresh leaf tissue sampled from seedlings germinated from wild seeds of individuals from 52 populations from Turkey, Spain, Argentina, Chile, USA and Australia (Table S1),
showed no significant differences in genome size ($F_{5,44}=0.58; p=0.716$) among regions (Fig. 1).

All individuals ($N = 477$) were found to be diploid, presumably with $2n = 16$ chromosomes.

Average genome size ranged from 1.70 pg/2C (SD = 0.06 pg) in Australia and Spain (SD = 0.06 pg) to 1.71 pg/2C (SD = 0.06 pg) in Chile, 1.72 pg/2C (SD = 0.06 pg) in Argentina and

California (SD = 0.07 pg) and 1.73 pg/2C (SD = 0.07 pg) in Turkey (Table 1).

**Figure 1**: Comparison of genome size among native and non-native genotypes of *Centaurea solstitialis*. Black center lines represent the medians, crosses indicate sample means, box limits indicate the 25th and 75th percentiles, whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, bars show 95% confidence intervals of the means and outliers are represented by empty dots. Width of the boxes is proportional to the square root of sample size, $n=26, 28, 29, 12, 30, 24$ sample points.
Table 1: Genome size estimations in *Centaurea solstitialis* across the six sampled regions.

| Region  | Mean  | SD    | SE    | Min  | Max  | N  |
|---------|-------|-------|-------|------|------|----|
| Argentina | 1.727 | 0.067 | 0.012 | 1.53 | 1.84 | 29 |
| Australia | 1.705 | 0.061 | 0.012 | 1.59 | 1.83 | 24 |
| California | 1.727 | 0.074 | 0.013 | 1.59 | 1.85 | 30 |
| Chile | 1.717 | 0.065 | 0.018 | 1.59 | 1.81 | 12 |
| Spain | 1.709 | 0.069 | 0.013 | 1.57 | 1.83 | 28 |
| Turkey | 1.737 | 0.070 | 0.013 | 1.60 | 1.88 | 26 |
| **Total** | **1.720** | **0.068** | **0.014** | **1.57** | **1.84** | **149** |

Note: Values are given as mean, standard deviation and standard error of the mean. The minimum and maximum values and the number of analyzed individuals (N) for genome size estimations are also provided.

Genome size variation among populations within regions (Table S1) was also not significantly different, as indicated by very small standard deviations for the intercept and the residual obtained for the random effects ($SD_{\text{intercept}}=0.024$; $SD_{\text{residual}}=0.063$).

**DISCUSSION**

We found no traces of polyploidization events in the *C. solstitialis* populations investigated and geographic differences in genome size were negligible.

A previous record of isolated tetraploids (one accession) in Northern Turkey (Inceer et al., 2007) is intriguing, since further genomic sampling in the area (e.g., less than 40 km from the initial site, Barker et al., 2017) did not validate the findings. Further investigation is also required to clarify the reported putative hybridization (Barker et al., 2017) with *Centaurea nicaensis* L. (2n = 20 chromosomes, Guinochet & Foissac 1962), since inter-specific hybridization does not seem to have played a significant role in the past invasion history of *C. solstitialis* (Barker et al., 2017). Formerly, a single natural hybrid of *Centaurea × moncktonii* C.E.
Britton and *C. solstitialis* was described from Oregon, USA (Roché & Susanna 2010) and found to be a sterile triploid (Miskella 2014).

The genome size value we obtained for California (1.72 pg/2C, SD = 0.07 pg) was similar to the one previously reported for Southwestern Oregon (1.66 pg/2C, SD = 0.07 pg,) by Miskella (2014) and, overall, genome sizes were similar among the six world regions.

In conclusion, our thorough sampling of the most representative native and non-native populations across the world’s distribution of *C. solstitialis* indicates that its invasive success is not due to changes in genome size or ploidy level. We cannot discard that some individuals in some unsampled populations could present some degree of polyploidy, but their role in invasive success, to date, would have been of minor importance.

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REFERENCES

Bancheva S, Greilhuber J. 2006. Genome size in Bulgarian *Centaurea* s.l. (Asteraceae). *Plant Systematics and Evolution* 257:95–117. DOI 10.1007/s00606-005-0384-7.

Barker BS, Andonian K, Swope SM, Luster DG, Dlugosch KM. 2017. Population genomic analysis reveal a history of range expansion and trait evolution across the native and invaded range of yellow starthistle (*Centaurea solstitialis*). *Molecular Ecology* 26(4):1131–1147. DOI: 10.1111/mec.13998.

Beaulieu JM, Moles AT, Leitch IJ, Bennett MD, Dickie JB, Knight CA. 2007. Correlated evolution of genome size and seed mass. *New Phytologist* 173:422–437. DOI 10.1111/j.1469-8137.2006.01919.x.

Bennett MD, Leitch IJ, Hanson L. 1998. DNA amounts in two samples of angiosperm weeds. *Annals of Botany* 82(Supplement A):121–134. DOI 10.1006/anbo.1998.0785.

Bremer K. 1994. Asteraceae – cladistics and classification. Portland: Timber Press.
Carev I, Ruščić M, Skočibušić M, Maravić A, Silijak-Yakovlev S, Politeo, O. 2017. Phytochemical and cytogenetic characterization of Centaurea solstitialis L. (Asteraceae) from Croatia. *Chemistry and Biodiversity* **14**, e1600213. [DOI:10.1002/cbdv.201600213](https://doi.org/10.1002/cbdv.201600213).

Chang HT, Rumbeiha WK, Patterson JS, Puschner B. 2011. Toxic equine Parkinsonism: an immuno-histochemical study of 10 horses with nigropallidal encephalomalacia. *Veterinary Pathology* **49**(2):398-402. [DOI 10.1177/0300985811406885](https://doi.org/10.1177/0300985811406885).

DiTomaso JM. 2000. Invasive weeds in rangelands: species, impacts, and management. *Weed Science* **48**(2):255-265. [DOI 10.1614/0043-1745(2000)048[0255:IWIRSI]2.0.CO;2](https://doi.org/10.1614/0043-1745(2000)048[0255:IWIRSI]2.0.CO;2).

Doležel J, Sgorbati S, Lucretti S. 1992. Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. *Physiologia Plantarum* **85**:625–631. [DOI 10.1111/j.1399-3054.1992.tb04764.x](https://doi.org/10.1111/j.1399-3054.1992.tb04764.x).

Dlugosch KM, Lai Z, Benin A, Hierro JL, Rieseberg LH. 2013. Allele identification for transcriptome based population genomics in the invasive plant Centaurea solstitialis. *G3 Genes | Genomes | Genetics* **3**:359–367. [DOI 10.1534/g3.112.003871](https://doi.org/10.1534/g3.112.003871).

Dlugosch KM, Cang FA, Barker BS, Andonian K, Swope SM, Rieseberg LH. 2015. Evolution of invasiveness through increased resource use in a vacant niche. *Nature Plants* **1**:15066. [DOI 10.1038/nplants.2015.66](https://doi.org/10.1038/nplants.2015.66).

Eagle AJ, Eiswerth ME, Johnson WS, Schoenig SE, van Kooten CG. 2007. Costs and losses imposed on California ranchers by yellow starthistle. *Rangeland Ecology & Management* **60**:369-377. [DOI 10.2111/1551-5028(2007)60[369:CALIOC]2.0.CO;2](https://doi.org/10.2111/1551-5028(2007)60[369:CALIOC]2.0.CO;2).

Eriksen RL, Desronvil T, Hierro JL, Kesseli R. 2012. Morphological differentiation in a common garden experiment among native and non-native specimens of the invasive weed yellow starthistle (Centaurea solstitialis). *Biological Invasions* **7**:1459–1467. [DOI 10.1007/s10530-012-0172-6](https://doi.org/10.1007/s10530-012-0172-6).

Eriksen RL, Hierro JL, Eren O, Andonian K, Török K, Becerra PI, Montesinos D, Khetsuriani L, Diaconu A, Kesseli R. 2014. Dispersal pathways and genetic differentiation among worldwide populations of the invasive Centaurea solstitialis L. (Asteraceae). *PLoS One* **9**:e114786. [DOI 10.1371/journal.pone.0114786](https://doi.org/10.1371/journal.pone.0114786).

García Y, Callaway RM, Diaconu A, Montesinos D. 2013. Invasive and non-invasive congeners show similar trait shifts between their same native and non-native ranges. *PLoS One* **8**:e82281. [DOI 10.1371/journal.pone.0082281](https://doi.org/10.1371/journal.pone.0082281).

Gerlach JD. 1997a. How the west was lost: reconstructing the invasion dynamics of yellow starthistle and other plant invaders of western rangelands and natural areas. *California Exotic Pest Plant Council, Symposium Proceedings* 3:67–72.

Gerlach JD. 1997b. The introduction, dynamics of geographic range expansion and ecosystem effects of yellow starthistle (Centaurea solstitialis). *Proceedings of Californian Weed Science Society* **49**:236–241.

Graebner RC, Callaway RM, Montesinos D. 2012. Invasive species grows faster, competes better, and shows greater evolution toward increased size and growth than exotic non-invasive congeners. *Plant Ecology* **213**:545–553. [DOI 10.1007/s11258-012-0020-x](https://doi.org/10.1007/s11258-012-0020-x).

Grotkopp E, Rejmánek M, Sanderson MJ, and Rost TL. 2004. Evolution of genome size in Pines (Pinus) and its life-history correlates: supertree analyses. *Evolution* **58**:1705–1729.
Guinochet M, Foissac J. 1962. Sur les Caryotypes de quelques espèces du genre *Centaurea* L. et leur signification taxonomique. *Bulletin de la Société Botanique de France* 109:373–389. DOI 10.1080/00378941.1962.10838114.

Hahn MA, Buckley YM, Müller-Schärer H. 2012. Increased population growth rate in invasive polyploid *Centaurea stoebe* in a common garden. *Ecology Letters* 15(9):947–954. DOI 10.1111/j.1461-0248.2012.01813.x.

Hierro JL, Eren O, Khetsuriani L, Diaconu A, Török K, Andonian K, Kikodze D, Janoian L, Villarreal D, Estanga-Mollica ME, Callaway, RM. 2009. Germination responses of an invasive species in native and non-native ranges. *Oikos* 118:529–538. DOI 10.1111/j.1600-0706.2008.17283.x.

Hijano EH, Basigalup DH. 1995. El cultivo de la alfalfa en la República Argentina. In: Hijano EH and Navarro A, ed. *La Alfalfa en la Argentina*. INTA, Buenos Aires. 13-18.

Inceer H, Hayirlioglu-Ayaz S, Ozcan M. 2007. Chromosome numbers of the twenty-two Turkish plant species. *Caryologia* 60:349–357. DOI 10.1080/00087114.2007.10797958.

Jasiewicz A, Miziantsy M. 1975. Chromosome numbers of some Bulgarian plants. *Fragmenta Floristica et Geobotanica* 21(3):277–288.

Kuzmanov BA, Jurukova-Grancarova PD, Georgieva, SB. 1990. Chromosome numbers of Bulgarian angiosperms. *Fitologia* (Sofia) 38:92.

Laird NNM, Ware JJH 1982. Random-effects models for longitudinal data. *Biometrics* 38(4):963–974.

Lavergne S, Muenke NJ, Molofsky J. 2010. Genome size reduction can trigger rapid phenotypic evolution in invasive plants. *Annals of Botany* 105:109–116. DOI 10.1093/aob/mcp271.

Louéire J, Rodriguez E, Doležel J, Santos C. 2007. Two new nuclear isolation buffers for plant DNA flow cytometry: a test with 37 species. *Annals of Botany* 4:875–488. DOI 10.1093/aob/mcm152.

Miskella J. 2014. Hybridization between yellow starthistle (*Centaurea solstitialis*) and meadow knapweed (*Centaurea×moncktonii*). Master’s Thesis. Oregon State University.

Montesinos D, Santiago G, Callaway RM. 2012. Neo-allopatry and rapid reproductive isolation. *American Naturalist* 180:529–533. DOI 10.1086/667585.

Montesinos D, Callaway, RM. 2017. Inter-regional hybrids of native and invasive *Centaurea solstitialis* display intermediate competitive ability. *Ecography*. DOI 10.1111/eccg.02653.

Mráz P, García-Jacas N, Gex-Fabry E, Susanna A, Barres L, Müller-Schärer H. 2011. Allopolyploid origin of highly invasive *Centaurea stoebe* s.l. (Asteraceae). *Molecular Phylogenetics and Evolution* 62:612–623. DOI 10.1016/j.ympev.2011.11.006.

R Development Core Team. 2010. R: A Language and Environment for Statistical Computing. [R Foundation for Statistical Computing](https://www.r-project.org/) (accessed 6 June 2017).

Rice A, Glick L, Abadi S, Einhorn M, Kopelman NM, Salman-Minkov A, Mayzel J, Chay O, Mayrose I. 2015. The Chromosome Counts Database (CCDB) - a community resource of plant chromosome numbers. *New Phytologist* 206:19–26. DOI 10.1111/nph.13191.

Roché CT, Susanna A. 2010. New habitats, new menaces: *Centaurea×kleinii* (*C. moncktonii×C. solstitialis*), a new hybrid species between two alien weeds. *Collectanea Botanica* 29
Soltis DE, Albert VA, Leebens-Mack J, Bell CD, Paterson AH, Zheng C, Sankoff D, dePamphilis CW, Wall PK, Soltis PS. 2009. Polyploidy and angiosperm diversification. American Journal of Botany 96(1):336–348. DOI 10.3732/ajb.0800079.

Spitzer M, Wildenhain J, Rappsilber J, Tyres M. 2014. BoxPlotR: a web tool for generation of box plots. Nature Methods 11:121–122. DOI 10.1038/nmeth.2811.

Suda J, Meyerson LA, Leitch IJ, Pyšek P. 2015. The hidden side of plant invasions: the role of genome size. New Phytologist 205: 994–1007. DOI 10.1111/nph.13107.

te Beest M, Le Roux JJ, Richardson DM, Brysting AK, Suda J, Kubešová M, Pyšek P. 2011. The more the better? The role of polyploidy in facilitating plant invasions. Annals of Botany 109:19–45. DOI 10.1093/aob/mcr277.

USDA, NRCS. The PLANTS Database. 2017. National Plant Data Team, Greensboro. Available at http://plants.usda.gov (accessed 10 January 2017).

Uygur S, Smith L, Nezhi Uygur F, Cristofaro M, Balciunas J. 2004. Population densities of yellow starthistle (Centaurea solstitialis) in Turkey. Weed Science 52:746–753. DOI 10.1614/WS-03-150R1.

Wagenitz C. 1955. Pollenmorphologie and Systematik in der gattung Centaurea L. s.l. Flora 142:213–275.

Widmer, TL, Guermache FG, Dolgovskaia MY, Reznik SY. 2007. Enhanced growth and seed properties in introduced vs. native populations of yellow starthistle (Centaurea solstitialis). Weed Science 55:465-473. DOI 10.1614/WS-06-211R1.