A genome size and phylogenetic survey of Mediterranean *Tripleurospermum* and *Matricaria* (Anthemideae, Asteraceae)

Huseyin Inceer¹, Teresa Garnatje², Sema Hayırlıoğlu-Ayaz¹, Joan Pere Pascual-Díaz², Joan Vallès³, Sonia Garcia²*

¹ Karadeniz Technical University, Faculty of Science, Department of Biology, Trabzon, Turkey, ² Institut Botànic de Barcelona (IBB, CSIC-ICUB), Passeig del Mídia s/n, Parc de Montjuïc, Barcelona, Catalonia, Spain, ³ Laboratori de Botànica (UB)–Unitat associada al CSIC, Facultat de Farmàcia i Ciències de l’Alimentació, Universitat de Barcelona, Barcelona, Catalonia, Spain

* soniagarcia@ibb.csic.es

Abstract

The study of genome size variation can contribute valuable information on species relationships as well as correlate to several morphological or ecological features, among others. Here we provide an extensive report on genome sizes on genus *Tripleurospermum* and its closely related genus *Matricaria*, which are two typically Mediterranean genera particularly widespread and diverse in Turkey, the origin of most of the populations here studied. We analyse and discuss genome size variation in the first relatively complete molecular phylogenetic framework of *Tripleurospermum* (based on ITS and ETS ribosomal DNA–rDNA–regions). We find cases of intraspecific genome size variation, which could be taxonomically significant. Genome downsizing is also detected as the typical response to polyploidisation in *Tripleurospermum* taxa, being most conspicuous at the tetraploid level. Several positive correlations with genome size, including those with pollen and stomatal size or cypsela length, among others, are also found. Remarkably, taxa presenting rhizomes tend to present higher genome sizes, confirming a trend to accumulate nuclear DNA in such species, which could be explained by the nutrient reserves availability in their storage organs, allowing genome expansion, or by the lower rates of sexual reproduction in rhizomatous taxa.

Introduction

*Tripleurospermum* Sch.Bip. is a genus of family Asteraceae, tribe Anthemideae, comprising between 30 to 40 species, depending on the authors and systematic treatments. Distributed in the Northern hemisphere, mainly in the Mediterranean basin including Europe, temperate Asia and North Africa [1, 2, 3], some taxa are also present in North America. It is particularly abundant and diverse in Turkey, its main center of diversity, where around 30 *Tripleurospermum* taxa can be found half of which endemic [4]. One of its species, *T. inodorum* (L.) Sch. Bip., is widespread as a weed [1]. Another Anthemideae genus with an important presence in the Mediterranean basin and closely related to *Tripleurospermum* is *Matricaria* L. The genus
comprises six species [2, 5], three of them present in Turkey. Its species *M. chamomilla* L., a part from being widely known for its medicinal properties, is also a widespread weed [6, 7].

As in many Anthemideae and mostly due to close morphological affinity *Tripleurospermum* (subtribe Anthemidinae), *Matricaria* (subtribe Matricariinae) and, to a lesser extent, *Anthemis* L. (subtribe Anthemidinae), among others, have been confused both taxonomically and nomenclaturally [4, 8, 9, 10]. However, although some *Tripleurospermum* species were formerly assigned to *Matricaria*, later it was recognized as a different genus based on fruit features. On the one hand, the morphology of *Tripleurospermum* cypsela differ from that of those of *Matricaria* in its shape and ornamentation [2]. Besides, *Tripleurospermum* species have a tetrasporic embryo sac, a character shared with the genus *Anthemis*, while *Matricaria* and other Anthemideae present monosporic embryo sac [11, 12]. Molecular phylogenetic researches based on tribe Anthemideae are also consistent with this difference and both genera appear well separated in different and supported clades [13, 14].

Although karyology can also contribute to clarify relationships between closely related taxa [15] such as *Matricaria* and *Tripleurospermum*, little differences have been found until present regarding their chromosomes. Karyological knowledge on both genera is mostly based on chromosome counts and in some (few) karyotypes. Species of *Tripleurospermum* and *Matricaria* have the same and exclusive base chromosome number, *x* = 9, which is also the most common in tribe Anthemideae and in family Asteraceae as a whole, where it is considered the ancestral condition [16]. In certain *Tripleurospermum* species, triploid, tetraploid and pentaploid populations have been detected [17, 18], whereas in *Matricaria* only the diploid level has been recorded [19].

The study of genome size variation has shown its utility in systematic and evolutionary research in many plant groups. A first flow cytometric approach in *Tripleurospermum* was performed by our research group [17], where we measured genome sizes by flow cytometry for seven of its species and related them with features such as ploidy level, life cycle and environmental factors. Until then, only Nagl and Ehrendorfer [20] had estimated, through Feulgen cytodensitometry, the genome size for *Tripleurospermum maritimum*, the most widespread species of the genus. Very recently, Certner et al. [21] studied genome size variation by flow cytometry in mixed ploidy populations of *T. inodorum*. As for *Matricaria*, previous studies have contributed genome size information, using either Feulgen cytodensitometry [20, 22] or flow cytometry [23, 24].

The main purpose of this study is to address genome size variation in the two closely related genera *Tripleurospermum* and *Matricaria*, increasing the sample to embrace most of the species of both genera, for many of which we will provide first estimates. We will test relationships between genome size and morphological, karyological and ecological features of the species in a phylogenetic framework constructed for this purpose. This may also help clarifying systematic relationships within and between these genera and other closely related Anthemideae, as genus *Anthemis*.

**Materials and methods**

**Plant materials**

Cypsela of 64 populations, corresponding to 30 *Tripleurospermum* species and subspecies (42 populations), four *Matricaria* species and subspecies (21 populations) and one *Anthemis* species were collected from the wild for genome size assessments and molecular phylogenetics. We have included the species *Anthemis macrotis* (Rech.f.) Oberpr. & Vogt in the analysis since (i) it was relevant as a genus closely related to both genera and had been previously considered a member of *Matricaria* (*M. macrotis* Rech.f.), although a more recent taxonomic study based
on molecular markers best placed it as a member of *Anthemis* [9], and (ii) no previous genome size estimate was available for the species. None of the studied species is listed as endangered or protected in any national or international legal regulation. The accessions are listed in Table 1 following the taxonomic names in the Flora of Turkey the East Aegean Islands [25], Inceer and Hayırlıoğlu-Ayaz [4] and Inceer [26]. The map in Fig 1 indicates the districts of provenance of the *Tripleurospermum*, *Matricaria* and *Anthemis* populations here studied. Specimen vouchers of the studied materials have been deposited in the herbaria of either the Karadeniz Technical University, Department of Biology (KTUB) (including the H. Inceer collection), the Centre de Documentació de Biodiversitat Vegetal, Universitat de Barcelona (BCN) and the Botanical Institute of Barcelona (BC).

**Molecular techniques: DNA extraction, amplification and sequencing**

Total genomic DNA was extracted using either the CTAB method [27] as modified by [28] or the Nucleospin Plant (Macherey-Nagel, GmbH et Co., Düren, Germany), depending on the quality of the vegetal material. Polymerase chain reaction (PCR) was performed by using an MJ Research Inc. thermal cycler (Watertown, Massachusetts, USA) in a 25 μL volume. Direct sequencing of the amplified DNA segment was performed with the Big Dye Terminator Cycle Sequencing v3.1 (PE Biosystems, Foster City, California, USA). Nucleotide sequencing was carried out at the Serveis Científics i Tecnològics (Universitat de Barcelona) on an ABI PRISM 3700 DNA analyzer (PE Biosystems, Foster City, California, USA). *ITS region*—Double-stranded DNA of the ITS region (including ITS1, 5.8S gene, and ITS2) was amplified by PCR with ITS1f and ITS4r primers [29]. The PCR profile used for amplification was 94˚C 3 min; 30 × (94˚C 20 s; 55˚C 1 min; 72˚C 1 min); 72˚C 10 min. Only the ITS4 primer was used for sequencing in most cases. *ETS region*—Double-stranded DNA of the ETS region was amplified with the ETS1f and 18 SesTr primers [30]. The PCR profile used for amplification was 94˚C 3 min; 30 × (94˚C 20 s; 50˚C 1 min s; 72˚C 1 min); 72˚C 10 min. Both ETS1f and 18 SesTr were used as sequencing primers, and also the internal primers AST1F and AST1R [31] were used occasionally.

**Flow cytometric measurements**

For flow cytometric measurements of leaf tissue were obtained from seeds grown in pots in the greenhouse of the Faculty of Pharmacy and Food Sciences, Universitat de Barcelona. Five individuals per population of each species were studied, and two samples of each were individually processed. *Petunia hybrida* Vilm. 'PxPc6' (2C = 2.85 pg) and *Pisum sativum* L. 'Express Long' (2C = 8.37 pg) were used as the internal standards [32]. Fresh leaf tissue for the standard and the target species were chopped together in 600 μl of LB01 buffer (8% Triton X-100) [33] supplemented with 100 μg/ml ribonuclease A (RNase A, Boehringer, Meylan, France) and stained with 36 μl of 1 mg/ml propidium iodide (Sigma-Aldrich, Alcobendas, Madrid) to a final concentration of 60 μg/ml, and kept on ice for 20 min. The fluorescence measurements were performed using an Epics XL flow cytometer (Coulter Corporation, Miami, FL, USA) at the Centres Científics i Tecnològics, University of Barcelona. More details about the method are in [34]. The data were submitted to the Genome Size in Asteraceae Database (GSAD) [35].

**Phylogenetic analyses and reconstruction of character evolution**

The nuclear ribosomal DNA dataset (1075 concatenated bp) includes ITS (excluding the 5.8S) and 5’ ETS sequences (636 and 439 bp, respectively) for 45 taxa out of which 34 belong to *Tripleurospermum*, four to *Matricaria* and the remaining seven to different species of genera *Achillea* (GenBank accession number—hereafter GB:: AY603251), *Anacyclus* L. (GB::
Table 1. Provenance and voucher number of the populations here studied, together with ploidy level, chromosome number, holoploid and monoploid genome size and internal standard used. All genome sizes are first estimates in the taxa concerned with the exception of *M. chamomilla* var. *chamomilla*, *T. callosum*, *T. elongatum*, *T. maritimum*, *T. melanolepis*, *T. oreades* var. *oreades*, *T. oreades* var. *teihatchewi*, *T. repens* and *T. sevanense*. (1) Information of locality and collection date.

| Taxon | Locality¹ | Voucher | PL² | 2n³ | 2C (SD)⁴ | 1Cx⁵ | 1C (Mbp)⁶ | Internal standard |
|-------|------------|---------|-----|------|----------|--------|-----------|-------------------|
| *Anthemis macrotis* (Rech.f.) Oberprieler & Vogt * (E) | C2 Muğla, Turkey: Near Köyceğiz, roadsides, 10 m a.s.l., 13.iv.2009 (1) | Inceer 711 | 2x | 18 | 5.55 | (0.19) | 2.78 | 2713.95 | Petunia hybrida |
|          | *A. chia* L.* | C3 Antalya, Turkey: Aksu, at the Perge ruins. 27.iii.2010 (2) | BNC 70405 | 2x | 18** | 7.4 | (0.15)** | 3.70** | 3618.6** | Petunia hybrida *² |
| *Matricaria aurea* (Loefl.) Sch.Bip.* | C6 Gaziantep/Şanurfa, Turkey: Between Nizip and Birecik, Dutlu mevkii, roadsides, near cultivated area, 440 m a.s.l., 08.v.2007 (3) | Inceer 322 | 2x | 18*** | 5.04 | (0.07) | 2.52 | 2464.56 | Petunia hybrida |
| *M. aurea* | C6 Gaziantep/Şanurfa, Turkey: Between Nizip and Birecik, roadsides, near cultivated area, 500 m a.s.l., 08. v.2007 | Inceer 323 | 2x | 18 | 4.91 | (0.08) | 2.46 | 2400.99 | Petunia hybrida |
| *M. chamomilla* var. *chamomilla* | A1(E) Çanakkale, Turkey: Korus Dağ, near Pinus brutia forest, 70 m a.s.l., 11.v.2007 | Inceer 332 | 2x | 18 | 5.13 | (0.07) | 2.57 | 2508.57 | Petunia hybrida |
|          | *M. chamomilla* var. *chamomilla* | C1 Muğla, Turkey: Bodrum, Ortakent, roadsides, 50 m a. s.l., 16.iv.2007 | Inceer 279 | 2x | 18 | 5.17 | (0.08) | 2.59 | 2528.13 | Petunia hybrida |
|          | *M. chamomilla* var. *chamomilla* | C2 Muğla, Turkey: Köyceğiz, near Dalyan, cultivated area, 23 m a.s.l., 17.iv.2007 | Inceer 298 | 2x | 18 | 5.17 | (0.06) | 2.59 | 2528.13 | Petunia hybrida |
|          | *M. chamomilla* var. *chamomilla* | C2 Muğla, Turkey: Marmaris, between Marmaris and Köyceğiz, 20 m a.s.l., 18.iv.2007 | Inceer 304 | 2x | 18 | 5.33 | (0.09) | 2.67 | 2606.37 | Petunia hybrida |
|          | *M. chamomilla* var. *chamomilla* | C3 Antalya, Turkey: Elmali, Sarkaya, roadsides, 220 m a. s.l., 19.iv.2007 | Inceer 312 | 2x | 18 | 5.39 | (0.04) | 2.70 | 2635.71 | Petunia hybrida |
|          | *M. chamomilla* var. *chamomilla* | C1 Muğla, Turkey: Bodrum, Ortakent, roadsides, 16. iv.2007, 132 m. | Inceer 283b | 2x† | 18† | 5.30 | (0.19) | 2.65 | 2591.7 | Petunia hybrida |
|          | *M. chamomilla* var. *chamomilla* | C1 Muğla, Turkey: Bodrum, Görece, roadsides, 117 m a.s. l., 16.iv.2007 | Inceer 285 | 2x† | 18† | 4.96 | (0.03) | 2.48 | 2425.44 | Petunia hybrida |
|          | *M. chamomilla* var. *chamomilla* | C1 Muğla, Turkey: Bodrum, Ortakent, roadsides, 89 m a. s.l., 16.iv.2007 | Inceer 289 | 2x† | 18† | 5.13 | (0.10) | 2.57 | 2508.57 | Petunia hybrida |
|          | *M. chamomilla* var. *chamomilla* | C3 Antalya, Turkey: Elmali road, Yalnız village, roadsides, 450 m a.s.l., 19.iv.2007 | Inceer 313 | 2x† | 18† | 5.33 | (0.11) | 2.67 | 2606.37 | Petunia hybrida |
|          | *M. chamomilla* var. *chamomilla* | A1(E) Çanakkale, Turkey: Korus Dağ, near Pinus brutia forest, 70 m a.s.l., 11.v.2007 | Inceer 332 | 2x† | 18† | 5.12 | (0.02) | 2.56 | 2503.68 | Petunia hybrida |
|          | *M. chamomilla* var. *chamomilla* | C1 Muğla, Turkey: Bodrum, Ortakent roadsides, 50 m a.s.l., 16.v.2007 (4) | Inceer 281 | 2x | 18 | 5.18 | (0.12) | 2.59 | 2533.02 | Petunia hybrida |
|          | *M. chamomilla* var. *recutita* (L.) Fiori | B2 İzmir, Turkey: Bozdağ, roadsides, 307 m a.s.l., 14. iv.2007 | Inceer 270 | 2x | 18 | 5.27 | (0.04) | 2.64 | 2577.03 | Petunia hybrida |
|          | *M. chamomilla* var. *recutita* | B1 İzmir, Turkey: Yamanlar Dağ, roadsides, 750 m a.s.l., 15.iv.2007 | Inceer 278 | 2x | 18 | 5.18 | (0.04) | 2.59 | 2533.02 | Petunia hybrida |
|          | *M. chamomilla* var. *recutita* | C1 Muğla, Turkey: Bodrum, Ortakent, roadsides, 60 m a.s.l., 16.iv.2007 | Inceer 288 | 2x | 18 | 5.13 | (0.11) | 2.57 | 2508.57 | Petunia hybrida |
|          | *M. chamomilla* var. *recutita* | A1 Balıkesir, Turkey: Near Bandirma-Gönen road, roadsides, 150 m a.s.l., 12.v.2007 | Inceer 341 | 2x | 18 | 5.14 | (0.10) | 2.57 | 2513.46 | Petunia hybrida |
|          | *M. chamomilla* var. *recutita* | A1 Balıkesir, Turkey: Near Bandirma-Fevzi Paşa, roadsides, 50 m a.s.l., 13.v.2007 | Inceer 344 | 2x† | 18† | 5.22 | (0.37) | 2.61 | 2552.58 | Petunia hybrida |
|          | *M. chamomilla* var. *recutita* | A1(E) Tekirdağ, Turkey: Near Köseilas village, roadsides, 130 m a.s.l., 10.v.2007 | Inceer 324 | 2x† | 18† | 5.24 | (0.02) | 2.62 | 2562.36 | Petunia hybrida |
|          | *M. chamomilla* var. *recutita* | A1(E) Edirne, Turkey: From Tekirdağ to Keşan, near Keşan, roadsides, 100 m a.s.l., 11.v.2007 (5) | Inceer 326 | 2x | 18 | 5.13 | (0.07) | 2.57 | 2508.57 | Petunia hybrida |
|          | *M. matricarioides* (Less.) Porter ex Britton * | A9 Ardahan, Turkey: Kars-Ardahan, Göle road, 1800 m a. s.l., 18.vii. (6) | Inceer 420 | 2x | 18*** | 4.6 | (0.07) | 2.30 | 2249.4 | Petunia hybrida |
| *Tripleurospermum baytopianum* E. Hossain (E) | A1(E) Çanakkale, Turkey: Korus Dağ, near Pinus brutia forest, 70 m a.s.l., 11.v.2007 | Inceer 329 | 2x | 18 | 4.96 | (0.28) | 2.48 | 2425.44 | Petunia hybrida |

(Continued)
| Taxon                  | Locality¹                  | Voucher         | PL²  | 2n³  | 2C (SD)⁴  | 1C ⁵ | Internal standard |
|-----------------------|----------------------------|-----------------|------|------|-----------|------|------------------|
| T. baytopianum (E)    | A1(E) Çanakkale, Turkey: Koru Dağı, near Pinus brutia forest, 70 m a.s.l., 11.v.2007 | Inceer 330 | 2x    | 18   | 4.8 (0.10) | 2.41 | 2356.98 Petunia hybrida |
| T. baytopianum (E)    | A1(E) Çanakkale, Turkey: Koru Dağı, near Pinus brutia forest, 350 m a.s.l., 11.v.2007 | Inceer 333 | 2x    | 18   | 4.65 (0.27) | 2.33 | 2273.85 Petunia hybrida |
| T. baytopianum * (E)  | A1 Çanakkale, Turkey: Koru Dağı, between Keşan and Evreşe, near Pinus brutia forest, 70 m a.s.l., 11.v.2007 (7) | Inceer 327 | 2x    | 18   | 5.02 (0.04) | 1.26 | 2454.78 Petunia hybrida |
| T. callosum (Boiss. & Heldr.) E. Hossain * (E) | B3 Eskisehir, Turkey: Çatakci, near Pinus forest, roadsides,1304 m a.s.l., 27.vi.2007 (8) | Inceer 369a | 4x    | 36   | 7.71 (0.14) | 1.93 | 1885.095 Petunia hybrida |
| T. caucasicum (Willd.) Hayek * | A8 Rize, Turkey: Ayder, Kavrun, alpine meadows, 2000 m a.s.l., 11.vii.2009 (9) | Inceer 765 | 4x    | 36   | 8.05 (0.92) | 2.01 | 3936.45 Psismat sativum |
| T. caucasicum *       | A7 Gümüşhane, Turkey: Near Köse Dağı pass, 1852 m a.s.l., 13.vi.2009 (10) | Inceer 730 | 2x    | 18   | 5.16 (0.14) | 2.58 | 2523.24 Petunia hybrida |
| T. concolinum (Boiss. & Bal.) Hayek * (E) | B2 Izmir, Turkey: Bozdağ, cultivated area, 1178 m a.s.l., 14.iv.2007 (11) | Inceer 260 | 2x    | 18   | 5.98 (0.11) | 2.99 | 2924.22 Petunia hybrida |
| T. concolinum * (E)   | B2 Izmir, Turkey: Bozdağ, cultivated area, 1178 m a.s.l., 14.iv.2007 (12) | Inceer 262 | 2x    | 18   | 5.18 (0.06) | 2.59 | 2533.02 Petunia hybrida |
| T. corymbosum E. Hossain * (E) | B9 Ağrı, Turkey: Suluçem, 1791 m a.s.l., 30.vi.2009 (13) | Inceer 757 | 2x†   | 18†  | 5.29 (0.02) | 1.32 | 1293.405 Petunia hybrida |
| T. decipiens (Fisch. & C.A.Mey.) Bornm. * | C5 Niğde, Turkey: Ulúksla, Bolkar mountains, near mine, 1650 m a.s.l., 14.vii.2007 (14) | Inceer 395 | 4x†   | 36†  | 8.18 (0.11) | 4.09 | 4000.02 Petunia hybrida |
| T. disciforme (C.A.Mey.) Sch.Bip. * | B1 Manisa, Turkey: Between Manisa and Izmir, roadsides, M 7076, 06.vii.2008 (15) | Inceer 592 | 2x    | 18*** | 4.93 (0.10) | 2.47 | 2410.77 Petunia hybrida |
| T. elongatum (D.C.) Bornm. * | A9 Ardahan, Turkey: Between Ardahan and Göle, roadsides, 1800 m a.s.l., 18.vii.2007 (16) | Inceer 423 | 2x    | 18*** | 4.68 (0.06) | 2.34 | 2288.52 Petunia hybrida |
| T. fisculare (Sosn.) E.Hossain * (E) | A8 Artvin, Turkey: Yusufeli, between Yusufeli and Ispir, roadsides, rocky slopes, 617 m a.s.l., 04.vi.2007 (17) | Inceer 351 | 2x    | 18*** | 5.33 (0.18) | 2.67 | 2606.37 Petunia hybrida |
| T. heterolepis (Freyn. & Sint.) Bornm. * (E) | A8 Bayburt, Turkey: Kop Dağı, roadsides, damp alpine meadows, 2494 m a.s.l., 05.viii.2007 (18) | Inceer 467 | 4x    | 36   | 8.42 (0.26) | 2.11 | 2058.69 Petunia hybrida |
| T. heterolepis * (E)  | A7 Gümüşhane, Turkey: Keçikel Village, roadsides, 1618 m a.s.l., 04.vii.2007 (19) | Inceer 382b | 4x    | 36*** | 8.21 (0.18) | 2.05 | 2007.345 Petunia hybrida |
| T. hygrophilum (Bornm.) Bornm. (E) | B1 Izmir, Turkey: Yamanlar Dağı, near Pinus forest, open places, 887 m a.s.l., 15.v.2007 | Inceer 274 | 2x    | 18   | 4.94 (0.07) | 2.47 | 2415.66 Petunia hybrida |
| T. hygrophilum (E)    | B1 Izmir, Turkey: Yamanlar Dağı, roadsides, 730 m a.s.l., 15.v.2007 | Inceer 277 | 2x    | 18   | 4.96 (0.02) | 2.48 | 2425.44 Petunia hybrida |
| T. hygrophilum * (E)  | B1 Izmir, Turkey: Yamanlar Dağı, above Karagöl, meadows, 820 m a.s.l., 14.vi.2007 (20) | Inceer 271 | 2x    | 18   | 4.95 (0.05) | 2.48 | 2420.55 Petunia hybrida |
| T. inodorum (L.) Sch.Bip. * | A9 Erzurum, Turkey: Pasinler, cultivated area, 1635 m a.s.l., 19.vi.2007 (21) | Inceer 754 | 4x    | 36   | 8.61 (0.15) | 2.15 | 2105.145 Petunia hybrida |
| T. inodorum *         | Münster, Germany: Wolbeck Berler Kamp. 1.vi.2012 | BCN 75281 | 4x    | 36   | 9.32 (0.18) | 2.33 | 2278.74 Petunia hybrida |
| T. insulare Inceer & Hayırlioğlu-Ayaz * (E) | A1 (E) Çanakkale, Turkey: Gököçeada, 30 m a.s.l., 24. iv.2010 (22) | Inceer 789 | 2x    | 18   | 5.68 (0.12) | 2.84 | 2777.52 Pismat sativum |
| T. kotschyi (Boiss.) E.Hossain * (E) | C5 Niğde, Turkey: Ulúksla, Bolkar mountains, near Karagöl, 2600 m a.s.l., 2008 (23) | Inceer 702 | 4x    | 36*** | 8.29 (0.14) | 2.07 | 2026.905 Petunia hybrida |
| T. maritimum (L.) W.D.I.Koch * | Barcelona Spain: near plaça Cerdà, Spain. 9 m a.s.l., 09.v.2005 | BC 906990 | 2x†   | 18†  | 5.28 (0.10) | 2.64 | 2581.92 Petunia hybrida |
| T. melanocephal (Boiss. & Buhse) Pobel. * | A9 Artvin, Turkey: Şavşat, near Çamlıbel passs, 2550–2600 m a.s.l., 20.06.2009 (24) | Inceer 741 | 2x    | 18   | 4.88 (0.09) | 2.44 | 2386.32 Petunia hybrida |
| T. microcephalum (Boiss.) Bornm. * | B8 Muş, Turkey: Fallow fields, banks, roadsides, 1323 m a.s.l., 09.vii.2008 (25) | Inceer 594 | 2x    | 18*** | 5.49 (0.07) | 2.75 | 2684.61 Petunia hybrida |
| T. monticolum (Boiss. & A.Huet) Bornm.* (E) | A9 Artvin, Turkey: Şavşat, alpine meadows, 2185 m a.s.l., 17.vii.2007 (26) | Inceer 416 | 4x    | 36   | 9.65 (0.02) | 2.41 | 2359.425 Petunia hybrida |

(Continued)
Table 1. (Continued)

| Taxon | Locality¹ | Voucher | PL² | 2n³ | 2C (SD)⁴ | 1C⁵ | 1C (Mbp)⁶ | Internal standard |
|-------|-----------|---------|-----|-----|----------|-----|-----------|------------------|
| _T. oreades_ (Boiss.) Rech.f. var. oreades | A8 Rize, Turkey: Anzer, roadsides, 1370 m a.s.l., 19. vii.2007 | Inceer 469 | 4x | 36 | 8.36 (0.13) | 2.09 | 2044.02 | Petunia hybrida |
| _T. oreades_ (Boiss.) Rech.f. var. tchatchewii (Boiss.) E.Hossain* | A9 Artvin, Turkey: Şavşat, alpine meadows, 2185 m a.s.l., 17.vii.2007 (27) | Inceer 414 | 4x | 36 | 9.62 (0.15) | 2.41 | 2352.09 | Petunia hybrida |
| _T. oreades_ * | A7 Giresun, Turkey: Kümbet, near Şehitler pass, roadsides, meadows, 1719 m a.s.l., 21.vii.2008 (28) | Inceer 658 | 4x | 36 | 8.9 (0.17) | 2.23 | 2176.05 | Petunia hybrida |
| _T. parviflorum_ (Willd.) Pobed. | C3 Antalya, Turkey: Korkuteli, 965 m a.s.l., 19.iv.2007 | Inceer 315 | 2x | 18 | 6.01 (0.07) | 3.01 | 2938.89 | Petunia hybrida |
| _T. parviflorum_ | B2 Izmir, Turkey: Bozdağ, roadsides, 1154 m a.s.l., 14.iv.2007 (29) | Inceer 266 | 2x | 18 | 6.15 (0.04) | 3.08 | 3007.35 | Petunia hybrida |
| _T. pichleri_ (Boiss.) Bornm.* (E) | A2 Bursa, Turkey: Uludağ, meadows, 1828 m a.s.l., 11.vi.2008 (30) | Inceer 553 | 4x | 36*** | 8.56 (0.20) | 2.14 | 2092.92 | Petunia hybrida |
| _T. repens_ (Freyn & Sint.) Bornm.* (E) | A7 Gümrüšhane, Turkey: Geze Village, meadows, 1987 m a.s.l., 08.vii.2007 (31) | Inceer 385 | 4x | 36 | 8.56 (0.16) | 2.14 | 2092.92 | Petunia hybrida |
| _T. roselli (Boiss. & Orph.) Hayek var. album E.Hossain* (E) | A3 Bolu, Turkey: Near Abant Lake, alpine meadows, 1313 m a.s.l., 12.vii.2008 (32) | Inceer 555 | 2x | 18*** | 4.65 (0.07) | 2.33 | 2273.85 | Petunia hybrida |
| _T. sevanense_ (Manden.) Pobed.* | B3 Eskişehir, Turkey: Çatakicion, near Pinus forest, roadsides, 1304 m a.s.l., 27.vi.2007 (33) | Inceer 369b | 4x | 36 | 8.40 (0.13) | 2.10 | 2053.8 | Petunia hybrida |
| _T. subminale_ Pobed.* | A8 Rize, Turkey: Ayder, Kavrun, alpine meadows, 2278 m a.s.l., 23.vii.2008 (34) | Inceer 672b | 5x | 42– 48 | 13.11 (0.33) | 2.62 | 2564.316 | Petunia hybrida |
| _T. tempysayanum_ (Freyn & Sint.) Hayek* (SE) | A2 Bursa, Turkey: Uludağ, near hotels, meadows, open places, 1815 m a.s.l., 25.vi.2009 (35) | Inceer 751 | 4x | 36 | 8.94 (0.10) | 2.24 | 2185.83 | Petunia hybrida |
| _T. tenfoofolium_ (Kit.) Freyn* | A1 Balikesir, Turkey: Erdek, Kapu Dağı, roadsides, 437 m a.s.l., 16.v.2009 (36) | Inceer 722 | 4x | 36 | 9.11 (0.08) | 2.28 | 2227.395 | Petunia hybrida |
| _T. transcaucasicum_ (Manden.) Pobed. | A9 Ardahan, Turkey: Between Göle and Karş, near Balçesme, 2115 m a.s.l., 18.vi.2007 | Inceer 429 | 2x | 18 | 4.98 (0.08) | 2.49 | 2435.22 | Petunia hybrida |
| _T. transcaucasicum_ * | A9 Ardahan, Turkey: Between Ardahan and Göle, roadsides, 2115 m a.s.l., 18.vi.2007 (37) | Inceer 427 | 2x | 18 | 5.16 (0.07) | 2.58 | 2523.24 | Petunia hybrida |
| _T. ziganae_ Inceer & Hayırlıoğlu- Ayaz* (E) | A7 Gümüşhane, Turkey: Zigana Dağ, between Zigana pass and Torul, 1300 m a.s.l., 02.iv.2009 (38) | Inceer 723 | 2x | 18 | 4.82 (0.09) | 2.41 | 2356.98 | Petunia hybrida |

(1) Numbers in brackets correspond to the location of each population in the map of Fig 1, and codes at the beginning correspond to the grid system classifying the different districts as it appears in the Flora of Turkey [25].

(2) Ploidy level.

(3) Somatic chromosome number.

(4) Holoploid genome size and standard deviation in brackets.

(5) Monoploid genome size.

(6) Genome size in Mbp. (E) endemic to Turkey. (SE) subendemic to Greece and Turkey.

(*) Taxa used for molecular phylogenetic analyses.

(**) Data from [56].

(****) Data from [18].

(*) Ploidy level and chromosome number inferred from genome size data.

https://doi.org/10.1371/journal.pone.0203762.t001

AY603258, GU818490, GU818112) and _Tanacetum_ L. (GB: EF577323, AB359894), known to occupy an intermediate position between _Tripleurospermum_ and _Matricaria_ in several phylogenetic treatments of tribe Anthemideae [2] and in [36]. One species of _Artemisia_ was used as outgroup (GB: HQ019060, HQ019018). The newly sequenced regions of species from _Tripleurospermum_ (34 taxa), _Matricaria_ (four taxa) and _Anthemis_ (two taxa) are deposited in GenBank under the accession numbers MG740672-MG740711 (ITS) and MG725262-MG725301 (ETS) (release date 1st January 2019 if not published before).
The two sequence matrices obtained with the nuclear molecular markers were manually edited and concatenated (S1 File) with BioEdit v. 7.1.3.0 [37] and MAFFT [38]. 54 gaps or indel characters were coded with FastGap [39] as 0/1 (absence/presence) and added to the data matrix as a separate partition.

The phylogenetic analyses were performed in the CIPRES Science Gateway [40]. Bayesian Inference phylogenetic analysis was performed in MrBayes v.3.2.6 [41] using the GTR+I+G model previously determined from jModeltest v.2.1.6 [42] under the Akaike information criterion [43]. The Markov chain Monte Carlo (MCMC) sampling approach was used to calculate posterior probabilities (PP). Four consecutive MCMC computations were run for 100,000,000 generations, with tree sampling every 10,000 generations. The first 25% of tree samples were discarded as the burn-in period. PP were estimated through the construction of a 50% majority rule consensus tree.

**Statistical analyses**

All data manipulations and statistical analyses were performed with RStudio, v.0.98.1078, a user interface for R [44]. The phylogenetic generalised least squares (PGLS) algorithm as implemented in the *nlme* package for R (Version 3.1–118) was used to analyse variation of genome size with respect to karyological (chromosome number, ploidy level) and morphological traits (pollen polar axis and equatorial diameter, size of the stomata, length and width of the cypsela, size of the plant, capitulum type (homogamous, heterogamous), presence of mucilage in cypsela, presence of rhizome), as well as other features such as life cycle, altitude, habitat and invasive behavior, following Olanji *et al.* [45]. The information used for the analyses is presented in S1 Table, together with genome sizes of the accompanying species of other genera, which have been obtained from the GSAD database [35]. Additionally, regular statistical
analyses of regression, one-way analysis of variance (ANOVA) and Shapiro–Wilk test for normality were performed, without considering phylogenetic relationships between taxa. The packages ape and geiger were also required for the phylogenetic-statistical analyses, as well as the package agricolae for LSD tests. Since in most cases datasets were not normally distributed, we also performed non-parametric tests such as Spearman rank correlation, the Kruskal-Wallis test by ranks and multiple comparison tests after Kruskal-Wallis (using the pgirmess package).

Results

Phylogenetic relationships between *Tripleurospermum* and *Matricaria*

Here we contribute a preliminary molecular phylogenetic framework for *Tripleurospermum* and related genera, in which to analyse genome size variation together with other traits of the species. Genus *Tripleurospermum* appears as monophyletic and strongly supported (PP = 1.00) in our phylogenetic reconstruction (Fig 2). All *Tripleurospermum* taxa possess a 106 bp insertion in the 5’ ETS which clearly defines the group. Relationships within the genus point to two large groups highly supported (PP = 0.99 and 0.94), and several other clades at lower hierarchical levels also show strong statistical support (PP = 1.00). Although the support is moderate (PP = 0.83) the sister taxa for *Tripleurospermum* could be species from genus *Anacyclus*. Species from genera *Anthemis*, *Tanacetum* and *Achillea* occupy intermediate positions between *Tripleurospermum* and *Matricaria*. The latter genus also appears as monophyletic and strongly supported (PP = 1.00). Both genera, *Tripleurospermum* and *Matricaria*, are clearly independent in this phylogenetic framework, although both grouped with the remaining genera in a highly supported clade whose outgroup is *Artemisia*.

Genome size variation

The present study expands genome size knowledge in *Tripleurospermum* up to 83% of the accepted taxa (25 out of 30 taxa according to the Euro+Med PlantBase [46]). There are also some species, such as *T. melanolepis* (Boiss. & Buhse) Pobed. and *T. transcaucasicum* (Manden.) Pobed., that do not appear in this database but are included as accepted names in The Plant List ([47], accepting 38 species in the genus), for which we also contribute new genome sizes. Our contribution to *Matricaria* expands to 50% the genome size knowledge on the genus, while for *Anthemis* it is much smaller, since we only add one species to the about 150 accepted species. The basic chromosome number of all taxa (*Tripleurospermum*, *Matricaria* and *Anthemis*) is $x = 9$ and ploidy level of the 64 populations here studied ranges from diploid (46 populations, 71.9%), to pentaploid (albeit only one population of *T. subnivale*), being the remaining 17 populations (26.6%) tetraploid.

Table 1 provides holoploid genome sizes (2C), ranging from 4.65 to 13.11 pg in *Tripleurospermum* and from 4.6 to 5.39 pg in *Matricaria*. The average half peak coefficient of variation (HPCV) corresponding to 10 samples of five individuals was 4.5% for the problem plant and 3.2% for the standards, indicating the good quality of the flow cytometric assessments. We found low intraspecific genome size differences in several cases in which two or more populations had been assessed in this study: 0.4% between three populations of *T. hygrophilum* (Bornm.) Bornm., 2.34% between two populations of *T. parviflorum* (Willd.) Pobed., 2.55% between two populations of *T. heterolepis* (Frey. & Sint.) Bornm. and 2.64% between two populations of *Matricaria aurea*. Moderate intraspecific differences were detected between 11 populations of *Matricaria chamomilla* (8.66%) and seven populations of *M. chamomilla* var. *recutita* (L.) Fiori (5.18%), four populations of *T. baytopianum* E. Hossain (7.95%) and two populations of *T. inodorum* (8.25%). The highest percentage difference was found
between two populations of *T. conoclinum* (15.44%) and also between three populations and two varieties of *T. oreades* (15.07%). Within *Tripleurospermum*, two supported clades can be clearly distinguished (A and B in Fig 1). Ploidy levels are significantly different between these clades (Pearson’s Chi-squared test $X^2 = 18.514, p = 1.656 \times 10^{-5}$), in which species in clade A are all tetraploid except six (out of 21), while species in clade B are all tetraploid with the exception of one (out of 12). This is also reflected as significantly different holoploid genome sizes between both clades ($p = 6.47 \times 10^{-5}$). However, holoploid genome sizes (2C) of diploid species in both clades are not significantly different ($p = 0.221$).

Table 2 summarizes the main results of the statistical analyses performed. Using the phylogenetically generalized least squares method (PGLS) we found that holoploid genome size
(2C) is significantly and positively correlated with ploidy level \((p = 0.0000)\) and chromosome number \((p = 0.0000)\), which points to certain degree of genome downsizing. Other morphological characters that have produced significant relationships with genome size, considering phylogenetic relationship between taxa, are: size of the plant \((p = 0.0216)\), presence of mucilage in cypselas \((p = 0.0436)\) and presence of rhizome \((p = 0.0034)\). Regarding the habitat, species living in alpine environments present significantly higher genome sizes than those living in the remaining habitats \((2C = 9.23, p = 0.0007)\) while species inhabiting lower, ruderal areas, show significantly lower C-values \((2C = 5.51, p = 0.043)\). As for the geographical distribution, differences are not significant (though nearly) between species with a large \((2C = 6.26, p = 0.0568)\), medium \((2C = 6.45,\)

| Table 2. Results of the statistical analyses and comparisons, using the ordinary tests (mostly analysis of regression and ANOVA) and the phylogenetically based generalized least squares (PGLS) algorithms. |
|---------------------------------------------|------------------|------------------|
| Ordinary tests                              | PGLS test        |
| 2C vs. ploidy level                         | \(p < 0.0001\) (positive correlation) |
| 1Cx vs. ploidy level                        | \(p < 0.0001\) (negative correlation) |
| 2C vs. pollen polar axis                    | \(p = 0.02\) (positive correlation)    | Nonsignificant |
| 2C vs. pollen equatorial diameter           | \(p = 0.03\) (positive correlation)    | Nonsignificant |
| 2C vs. cypsel length                        | \(p < 0.0001\) (positive correlation)  | Nonsignificant |
| 2C vs. cypsel width                         | Nonsignificant   |
| 2C vs. plant size                           | Nonsignificant   |
| 2C vs. stomatal size                        | \(p < 0.0001\) (positive correlation)  |
| 2C vs. altitude                             | \(p = 0.00709\) (positive correlation) | Nonsignificant |
| 2C vs. mucilage                             | \(p = 0.008939\) | \(p = 0.0436\) |
|                                              | presence \((2C \bar{x} = 5.66)\)         |
|                                              | absence \((2C \bar{x} = 7.72)\)           |
| 2C vs. habitat type                         | alpine \((2C \bar{x} = 9.74)\) \((p = 0.00874 / p = 0.0007)\) |
|                                              | miscellaneous \((2C \bar{x} = 4.85)\) \((nonsignificant / p = 0.0218)\) |
|                                              | open places \((2C \bar{x} = 5.31)\)       |
|                                              | roadsides \((2C \bar{x} = 6.59)\)         |
|                                              | meadows \((2C \bar{x} = 6.73)\)           |
| 2C vs. rhizome                              | \(p = 0.000179\)                               |
|                                              | presence \((2C \bar{x} = 8.36)\)           |
|                                              | absence \((2C \bar{x} = 6.35)\)            |
| 2C vs. life cycle                           | Nonsignificant differences between groups   |
|                                              | annual \((2C \bar{x} = 6.53)\)             |
|                                              | biennial \((2C \bar{x} = 7.41)\)           |
|                                              | perennial \((2C \bar{x} = 7.19)\)          |
| 2C vs. distribution range                   | Nonsignificant differences between groups   |
|                                              | large \((2C \bar{x} = 7.22)\)              |
|                                              | medium \((2C \bar{x} = 7.05)\)             |
|                                              | small \((2C \bar{x} = 6.97)\)              |
| 2C vs. capitulum type                       | Nonsignificant differences between groups   |
|                                              | discloid capitulum \((2C \bar{x} = 5.88)\) |
|                                              | discliform capitulum \((2C \bar{x} = 6.55)\) |
|                                              | radiate capitulum \((2C \bar{x} = 6.97)\)  |

https://doi.org/10.1371/journal.pone.0203762.t002
$p = 0.063$) or restricted ($2C = 6.50, p = 0.0523$) area. The other features tested against genome size (pollen polar axis and equatorial diameter, dimensions of cypsela, size of stomata, capitulum type, altitude and life cycle) did not show any phylogenetically significant correlation. However, when the correlations were performed without considering the phylogenetic relationships between taxa, pollen polar axis and equatorial diameter were significantly and positively correlated with genome size ($p = 0.002$ and $p = 0.003$, respectively), as it was the length of the cypsela ($p < 0.0001$), the size of the stomata ($p < 0.0001$) and the altitude ($p = 0.0071$).

**Discussion**

**Phylogenetic placement of *Tripleurospermum* and *Matricaria*, and a mention to *Anthemis macrotis***

Understanding phylogenetic relationships within tribe Anthemideae has always been problematic. As Oberprieler et al. [2] stated, while the circumscription of the tribe is clear [1], the subtribal classification has caused considerable difficulties through its taxonomic history. Both *Tripleurospermum* and *Matricaria* appear as monophyletic and highly supported, and independent from each other, confirming previous works on morphological [48] and molecular bases [2]. Our results allow, in this respect, confirming the placement of *Tripleurospermum* and *Matricaria* in different subtribes, as proposed previously (Anthemidinae and Matricariinae, respectively) [5]. Phylogenetic relationships above *Tripleurospermum* are overall consistent with the recent work of Vitales et al. [49], placing species from genus *Anacyclus* as the likely sister group of the former genus. Besides, the species *Anthemis macrotis* occupies an intermediate position between *Tripleurospermum* and *Matricaria*, confirming [9], now with an additional molecular marker (ETS), which best placed the former *Matricaria macrotis* as a member of *Anthemis* on molecular bases. The former inclusion of this “rare and enigmatic species of the East Aegean” in *Matricaria* was based on certain morphological features (absence of receptacular scales) yet others (e.g. indumentum, achene traits) pointed to its best treatment as *Anthemis*, which was later confirmed [9].

As for intraspecific structuring, despite extensive research in the genus, in particular from the morphological [50, 51], anatomical [12, 52], palynological [53], chemical [54, 55] and karyological [18, 56] points of view, little is known about phylogenetic relationships between *Tripleurospermum* species. The reconstruction here presented has been performed with the purpose of providing a phylogenetic framework in which to analyse genome size variation, although it gives, in addition, a quite resolved picture of the interspecific relationships within the genus. As indicated previously, two large and significantly supported clades are apparent (Fig 1A and 1B) and these appear to differ on ploidy levels of their species (see the different sizes of the bars representing genome sizes in both clades in Fig 1). It is also observed that clade A is the largest and most diversified, containing 19 taxa and 18 species as compared to clade B with only 13 taxa and 11 species. Polyploidy has been linked to higher diversification rates [57] and it is likely that this has been the case in *Tripleurospermum*, in which, additionally, the polyploid taxa are more widely distributed geographically than the diploid.

Several clades observed in this phylogenetic reconstruction reflect what previous work (mostly floras) had already stated for certain species. In particular, species like *T. repens*, *T. heterolepis*, *T. sevanense* and *T. callosum*, inhabiting similar areas in North East Anatolia, had been considered closely related [25, 51]. They appear in the phylogeny in a strongly supported clade (Fig 2A); similarly, *T. melanolepis*, *T. subnivale*, *T. caucasicum* and both varieties of *T. oreades* are allied in the Flora of USSR, in the Flora of Turkey and in other taxonomic treatments [8, 25]; besides, *T. caucasicum* and *T. oreades* are usually considered as synonyms [46, 47], whereas these species are not considered as synonyms by some authors [8, 25, 26] [8].
These taxa are also members of the same highly supported clade in the molecular phylogeny (Fig 2B). Species like *T. maritimum* and *T. inodorum* (Fig 2C) also form a moderately supported clade, which is consistent with the fact that they have also been considered as synonyms of each other in several occasions (indeed one of the populations was recorded as *T. perforatum*, considered synonym of *T. inodorum*; similarly, *T. maritimum* has sometimes been quoted as *T. inodorum* ssp. *maritimum*). Other consistent groupings, such as the one formed by both populations of *T. conoclinum* and *T. parviflorum*, are probably explained by the sympatric habitat of both species (the three populations inhabit in close proximity in the area of Izmir Province). Other groupings may also respond to sympatry, such as the one uniting *T. corymbosum*, *T. elongatum*, *T. transcaucasicum* and *T. microcephalum* (Fig 2D): the studied populations are found in the close areas of Ağrı, Ardahan and Kars Provinces, respectively, from East Anatolia; in the Flora of Turkey [25] also a close relationship between *T. corymbosum* and *T. transcaucasicum* had previously been suggested on a morphological basis.

**Genome size variation and genome downsizing in polyploids**

The present study contributes new genome size data for 22 taxa of *Tripleurospermum*, for the species *Matricaria aurea* and one variety of *M. chamomilla* (*M. chamomilla* var. *recutita*) and for the species *Anthemis macrotis*, for whose genus only five genome size estimates were previously available. Genome sizes of seven *Tripleurospermum* species and one variety (*T. callosum*, *T. elongatum*, *T. maritimum*, *T. melanolepis*, *T. oreades*, *T. oreades* var. *tchihatchewii*, *T. repens* and *T. sevanense*) have been reassessed with respect to previous results [17], which are consistent through both research works. As for *Matricaria*, the values reported for *M. chamomilla* var. *chamomilla* are also consistent with previous genome size estimates of diploid populations of the species [58]. Regarding *M. discoidea* (synonym of *M. matricarioides*) there is a remarkable difference (23%) with one of the populations measured previously [22] with Feulgen microdensitometry (4.6 vs 5.66 pg). Average genome sizes of *Tripleurospermum* (at diploid level) and *Matricaria* are not significantly different (5.19 and 4.98, respectively), however the average genome size of *Anthemis* species (6.47) is significantly different from both genera (*p = 0.0057*), which adds evidence to the separation of *Anthemis macrotis* (2C = 5.55 pg) from *Matricaria*.

Although the C of the term “C-value” stands for constancy of genome size within a species [59], examples accumulate which show that, many times, this is not the case [29, 60, 61]. Although generally attributed to karyotype variations (e.g., polyploidy, aneuploidy or presence of B-chromosomes) many research works have observed intraspecific genome size variation beyond chromosomal features. However, well-known methodological variation or presence of staining inhibitors in plants must always be taken into account when discussing intraspecific genome size variation. Intraspecific genome size changes were explained by Ceccarelli et al. [62] as a result of quantitative modulations of DNA repeats and transposable elements. Genuine intraspecific variation, even within a population, has been documented for several species [63]. As discussed earlier [45], it is difficult to set a cut-off point from which a given percentage should be considered significant. In the previous genome size study on some *Tripleurospermum* taxa we found low genome size differences between most populations [17]. Here, with a much larger sampling in terms of both taxa and populations, we detect a moderate to high degree of variation between the two varieties of *T. oreades*, two populations of *T. inodorum* and two populations of *T. conoclinum*. Murray [64] related intraspecific genome size variations to microevolutionary differentiations, which could be taxonomically significant. This could be the case of the two varieties of *T. oreades*, whose distinct phylogenetic placement (Fig 2) would also support their separation as distinct species beyond genome size, as proposed previously.
on the basis of morphological and karyological characters [8, 56]. The case of *T. inodorum* could be explained by its widespread distribution and possible introgression or hybridization with its congeners [65]. Interestingly, however, one of the populations analysed of *T. inodorum* was formerly given the name of *T. perforatum*, considered as a synonym of *T. inodorum*, which is indeed the valid name for the species [47]. The fact that both populations of *T. inodorum* appear closely related in the phylogenetic tree would support this consideration; yet, genome size of the population named as *T. inodorum* is 8.25% lower than that formerly named as *T. perforatum*. So this difference might also be interpreted in terms of microevolutionary differentiations indicating certain speciation processes, still not enough manifested morphologically. The same could have happened between the two populations analysed of *T. conoclinum*: remarkably, the genome size of population 264 is closer to *T. parviflorum* than to that of the other *T. conoclinum* population 262, forming a highly supported clade in the phylogeny.

As for *Matricaria*, the interpopulational differences detected in this study between *M. chamomilla* var. *chamomilla* and *M. chamomilla* var. *recutita* are moderate (below 10%), despite having assessed a relatively large number of populations (11 and seven, respectively). Our results are almost 50% lower than previously reported (2C = 7.7 pg) [20] for a population of the same ploidy level. Similarly, our genome size assessment of *M. matricarioides* is about 23% lower than previously reported by the same authors (2C = 5.66 pg). The different techniques used in our study (flow cytometry) and in the previous (Feulgen densitometry) could partly explain the discordance between data. However, results obtained for *T. maritimum* in the present study (2C = 5.28 pg) are almost equal to the previous data provided by Nagl and Ehrendorfer [20] (2C = 5.25 pg) so we cannot discard genuine interpopulational differences.

Genome downsizing is a well-known and common finding in polyploid systems [66]. It is a widespread biological response to polyploidisation, which may lead to the further diploidisation of the polyploid genome. In the case of *Tripleurospermum*, there is significant genome downsizing between diploids and tetraploids, which have around 20% less than expected monoploid genome size on average. However, the only pentaploid species of our dataset apparently does not undergo downsizing, as its monoploid genome size (1Cx = 2.62) is equivalent to that of the diploids (1Cx = 2.60). Genome downsizing has been detected in genera closely related genera to *Tripleurospermum* such as *Artemisia* [67], although the highest levels of reduction in the monoploid genome were usually found at higher ploidy levels contrary to our findings in the pentaploid *T. subnivale*. Maybe, the likely allopolyploidisation process responsible for the appearance of the pentaploid cytotype could imply the expansion of certain genomic repeats, which would increase its size.

**Genome size and phenotypic traits**

Correlations between genome size at the phenotype scale are supported by many research works pointing to a positive relationship with traits such as seed and leaf mass [68, 69, 70], pollen size [45], and stomatal size [71], among others. In this regard, we have also detected a positive correlation between pollen dimensions, stomatal size and length of the cypsela, which support the direct effect of genome size on cell size (nucleotype theory, i.e. the indirect influence of DNA in development by the physical-mechanical effects of its mass) [72]. At a higher phenotype scale, we found comparable results with the height of the plant. Knight and Beau lieu [73] showed that the strength of the correlations between genome size decreased in predictive power with increasing phenotypic scale, yet our findings argue for such correlations also at higher scales in *Tripleurospermum*. Closely related to this argument, rhizomatous *Tripleurospermum* species show significantly higher genome sizes than those without rhizomes, as pointed previously from a more limited sampling on the genus [17]. Also in the Asteraceae
genus *Artemisia*, the largest genome sizes are found in plants showing vegetative multiplication [74]. Much earlier, Rees and Jones [75] had also observed larger genome sizes in species with vegetative reproduction than in those presenting sexual reproduction from genus *Lolium*. We had previously argued that the presence of rhizomes could be related with higher rates of asexual reproduction and therefore lower incidence of meiosis as a controlling mechanism of genome size expansion, which may explain higher genome sizes in species showing such vegetative organs. Besides, Veselý et al. [76] explained that the universal tendency of geophytes (to which rhizomatous species belong) to possess higher genome sizes than their non-geophytic relatives could be related with the nutrient reserves availability in their storage organs. Polyploidy has also been found strongly associated with vegetative reproduction than diploids [77] and this is consistent with our findings since many rhizomatous species of our sample are polyploid (though not all).

The negative correlation between the presence of slime in cypselas (a mucilaginous layer that protects the seed and promotes its germination) and genome size/ploidy level in *Tripleurospermum* is consistent with the observations of [78, 79], which did not detect any slime production in certain polyploid *Artemisia*, while most diploids showed it. It was argued that the ability to form slime may depend on ploidy level [79]. In high *Artemisia* polyploids developmental abnormalities have been observed, such as disturbances in cypsela production or germination. Differences in gene expression have also been found in potato autopolyploid series at higher ploidies [80], so it might be possible that there is some dysfunction within the expression of genes responsible for mucilage production in cypselas of polyploid taxa, possibly explaining the usual absence of slime in polyploid *Tripleurospermum* taxa.

**Genome size, environment and ecology**

The genome sizes of the diploid plants studied fall within the category of small genomes (2C values between 2.8 and 7 pg), whereas those of polyploids belong to the category of intermediate genomes (2C values between 7 and 28 pg) [81], and no large and very large genomes are present in the current set. This is consistent with the absence of extremophilous taxa among those considered, in agreement with the large genome constraint hypothesis [82], postulating that plants with large genomes are rare in extreme environments.

It has been suggested [83, 84] that an increased DNA amount was an adaptation to altitude. In the same line [85, 86, 87] found a high rate of polyploid taxa in different alpine systems. The selective advantages that polyploidy may confer could explain a better colonizing ability of alpine habitats by polyploids. Although polyploidy is probably not essential in determining species adaptation to alpine environments [34], we found that alpine *Tripleurospermum* taxa are usually polyploid and present higher genome sizes. Consistently, species inhabiting ruderal areas present lower C-values and genome size is positively correlated with altitude. However, this correlation may not be true in all systems and the relationship between genome size, polyploidy and altitude is probably multifactorial. For example, Mas de Xaxars et al. [34] found mostly diploid species in the clade comprising high mountain *Artemisia*. Indeed, our previous work on *Tripleurospermum*’s genome size variation [17] detected a negative correlation with altitude, although the sampling was much more reduced.

Some of the taxa here studied have a wide distribution, and are even considered as invasive (e.g. the weed *T. maritimum*), while some others have a more restricted geographical range, being endemic to very narrow areas (e.g. *T. baytopianum, T. corymbosum, T. heterolepis*). Although we failed to find significant differences, species with a wider distribution tend to present smaller genome sizes than those from restricted areas. Similar findings were reported previously in other groups [67]. There is evidence that a small genome size could be related to
invasiveness [69, 88] and hence a wider distribution, although the reasons of this relationship are badly understood. Perhaps, a small(er) genome size would favor faster generation times that may contribute to wider geographical distributions.

Concluding remarks

Molecular phylogenetic data, not always in agreement with classical classifications, have proved themselves here useful to confirm the monophyly of both genera addressed, *Tripleurospermum* and *Matricaria*. This was already suggested by morphological traits, irrespective of the fact that several taxa have been the object of nomenclatural combinations under both genera. We have also shown here that the study of genome size variation within a genus, or closely related genera, can provide interesting hypotheses to understand certain morphological or ecological traits observed in plants, such as the results obtained on e.g. higher genome sizes in rhizomatous taxa or absence of slime in polyploids. Our interpretations may be backed, and may be applied in a more general scope, if similar studies in other genera raise similar conclusions, but such works are still scanty in the literature.

Supporting information

S1 File. DNA sequence matrix of ITS+ETS rDNA regions for the studied species. (FAS)

S1 Table. Data used for the statistical analyses together with source references. (DOCX)

Acknowledgments

This work was supported by the Dirección General de Investigación Científica y Técnica, Government of Spain (CGL2016-75694-P), the Generalitat de Catalunya, Government of Catalonia ("Ajuts a grups de recerca consolidats" 2017SGR1116) and the Scientific and Technological Research Council of Turkey (TUBITAK, project no. 106T162). SG benefited from a Ramón y Cajal contract from the Government of Spain (RYC-2014-16608). SYNTHESYS provided to HI the funding for the visit to the Royal Botanic Garden, Edinburgh (GB-TAF-1175). We thank Daniel Vitales for assistance on phylogenetic analyses, Ernst Vitek for the procurement of herbarium material of certain *Tripleurospermum* species used in this work, and Spencer C. Brown for supplying internal standards for flow cytometry. The curators of the herbaria B, E and G are also acknowledged for the herbarium studies, and Faik Ahmet Ayaz, Melahat Ozcan and Murat Bal for field trips.

Author Contributions

**Conceptualization:** Huseyin Inceer, Joan Vallès, Sònia Garcia.

**Data curation:** Huseyin Inceer, Sema Hayırlıoğlu-Ayaz, Sònia Garcia.

**Formal analysis:** Huseyin Inceer, Teresa Garnatje, Sema Hayırlıoğlu-Ayaz, Sònia Garcia.

**Funding acquisition:** Teresa Garnatje, Joan Vallès, Sònia Garcia.

**Investigation:** Huseyin Inceer, Teresa Garnatje, Joan Pere Pascual-Díaz, Joan Vallès, Sònia Garcia.

**Methodology:** Huseyin Inceer, Teresa Garnatje, Sema Hayırlıoğlu-Ayaz, Joan Pere Pascual-Díaz, Joan Vallès, Sònia Garcia.
Project administration: Sónia Garcia.

Resources: Joan Pere Pascual-Díaz, Sónia Garcia.

Software: Sónia Garcia.

Supervision: Joan Vallès, Sónia Garcia.

Validation: Huseyin Inceer, Teresa Garnatje, Sónia Garcia.

Visualization: Sónia Garcia.

Writing – original draft: Huseyin Inceer, Sónia Garcia.

Writing – review & editing: Huseyin Inceer, Teresa Garnatje, Joan Pere Pascual-Díaz, Joan Vallès, Sónia Garcia.

References
1. Bremer K, Humphries C. Generic monograph of the Asteraceae-Anthemideae. Bull. Nat. Hist. Mus. London (Bot.). 1993; 23(2): 71–177.
2. Oberprieler C, Himmelreich S, Vogt R. A new subtribal classification of the tribe Anthemideae (Compositae). Willdenowia. 2007; 37(1): 89–114.
3. Himmelreich S, Källersjö M, Eldenäs P. Oberprieler C. Phylogeny of southern hemisphere Compositae-Anthemideae based on nrDNA ITS and cpDNA ndhF sequence information. Plant Syst Evol. 2008; 272 (1): 131–153.
4. Inceer H, Hayriioğlu-Ayaz S. Tripleurospermum insularum (Asteraceae, Anthemideae), a new species from Turkey. Ann Bot Fennici. 2003; 39(2): 49–53.
5. Oberprieler C, Himmelreich S, Källersjö M, Vallès J, Watson LE, Vogt R. Anthemideae. In: Systematics, evolution and biogeography of Compositae (Funk VA, Susanna A, Stuessy TF, Bayer RJ, eds.). Vienna, International Association for Plant Taxonomy. 2009; pp. 631–666.
6. Mennan H, Isik D. Invasive weed species in onion production systems during the last 25 years in Amasya, Turkey. Pak. J. Bot. 2008; 35(2): 155–160.
7. Abe T, Fukami M, Ogasawara M. Cadmium accumulation in the shoots and roots of 93 weed species. Soil Sci. Plant Nutr. 2008; 54(4): 566–573.
8. Pobedimova EG. Tripleurospermum Sch. Bip. In: Flora U.S.S.R. (Shishkin BK, Bobrov EG, eds.). Bishen Singh Mahendra Pal Singh, Dehra Dun and Koeltz Scientific Books, Koenigsten, Germany, v. 26, 181–213: 1995.
9. Oberprieler C, Vogt R. The taxonomic position of Matricaria macrotis (Compositae-Anthemideae). Willdenowia. 2006; 36: 329–338.
10. Applequist WL. A reassessment of the nomenclature of Matricaria L. and Tripleurospermum Sch. Bip. (Asteraceae). Taxon. 2002; 51(4): 757–761.
11. Harling G. Embryological studies in the Compositae. III. Astereae. Acta Horti Berg. 1951; 16: 73–120.
12. Inceer H, Ozcan M. Leaf anatomy as an additional taxonomy tool for 18 taxa of Matricaria L. and Tripleurospermum Sch. Bip. (Anthemideae-Asteraceae) in Turkey. Plant Syst Evol. 2011; 296(3–4): 205–215.
13. Oberprieler C. Phylogenetic relationships in Anthemis L. (Compositae, Anthemideae) based on nrDNA ITS sequence variation. Taxon. 2001; 50: 745–762.
14. Sonboli A, Oberprieler C. Insights into the phylogenetic and taxonomic position of Tanacetum semenovi Herder (Compositae, Anthemideae) based on nrDNA ITS sequences data. Biochem Syst Ecol. 2012; 45: 166–170.
15. Guerra M. Chromosome numbers in plant cytotaxonomy: concepts and implications. Cytogenet Genome Res. 2008; 120(3–4): 339–350. https://doi.org/10.1159/000121083 PMID: 18504363
16. Semple JC, Watanabe K. A review of chromosome numbers in the Asteraceae with hypotheses on chromosome base number evolution. In: Systematics, Evolution and Biogeography of the Compositae (Funk VA, Susanna A, Stuessy T, Bayer R, eds.). Vienna, International Association for Plant Taxonomy, 21–32: 2009.
17. García S, Inceer H, Garnatje T, Vallés J. Genome size variation in some representatives of the genus Tripleurospermum. Biol Plant. 2005; 49(3): 381–387.
18. Inceer H, Hayırlıoğlu-Ayaz S. Chromosome number of Tripleurospermum Sch.Bip. (Asteraceae) and closely related genera: relationships between ploidy level and stomatal length. Plant Syst Evol. 2010; 285: 149–157. https://doi.org/10.1007/s00606-009-0266-5 PMID: 20725644
19. The Chromosome Counts Database (CCDB). Published on internet http://www.ccdb.tau.ac.il/home/ [22/01/2018].
20. Nagl W, Ehrendorfer F. DNA content, heterochromatin, mitotic index, and growth in perennial and annual Anthemideae (Asteraceae). Plant Syst Evol. 1974; 123(1): 35–54.
21. Certner M, Fenclova E, Kur P, Kolar F, Koutecky P, Krahulcová A, Suda J. Evolutionary dynamics of mixed-ploidy populations in an annual herb: dispersal, local persistence and recurrent origins of polyploids. Ann Bot. 2017; 120: 303–315. https://doi.org/10.1093/aob/mcx032 PMID: 28398545
22. Bennett MD. Nuclear DNA Content and Minimum Generation Time in Herbaceous Plants. Proc R Soc Lond B.1972; 181: 109–135. PMID: 4403285
23. Kovačič J, Klejduš B, Hedbavný J, Mártonti P, Štôrk F, Mártontiová L. Copper uptake, physiology and cytogenetic characteristics in three Matricaria chamomilla cultivars. Water Air Soil Pollut. 2011; 218(1–4): 681–691.
24. Kubšová M, Moravcová L, Suda J, Jarošík V, Pyšek P. Naturalized plants have smaller genomes than their non-invading relatives: a flow cytometric analysis of the Czech alien flora. Preslia. 2010; 82(1): 81–96.
25. Enayet Hossain ABM. Tripleurospermum Schultz Bip. In: Flora of Turkey and the East Aegean Islands (Davis PH, ed.). Edinburgh, Edinburgh University Press, v. 5, 295–311: 1975.
26. Inceer H. Achene slime content in some taxa of Matricaria L. Acta Bot Croat. 2012; 70:109–114.
27. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull. 1987; 19: 11–15.
28. Soltis DE, Soltis PS, Collier TG, Edgerton ML. The Heuchera group (Saxifragaceae): Evidence for chloroplast transfer and paraphyly. Am J Bot. 1991; 78:1091–1112.
29. White TJ, Bruns TD, Lee SB, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ [eds.], PCR protocols: A guide to methods and applications. 1990. Pp: 315–322. Academic Press, San Diego, California, USA.
30. Baldwin BG, Markos S. Phylogenetic utility of the external transcribed spacer (ETS) of 18S-26 rDNA: congruence of ITS and ETS trees of Calycadenia (Compositae). Mol Phy Evol. 1998; 10: 449–463.
31. Markos S, Baldwin BG. Higher-level relationships and major lineages of Lessingia (Compositae, Asteraceae) based on nuclear rDNA internal and external transcribed spacer (ITS and ETS) sequences. Syst Bot. 2001; 26: 168–183.
32. Marie D, Brown SC. A cytometric exercise in plant DNA histograms, with 2C values for 70 species. Biol Cell. 1993; 78: 41–51. PMID: 8220226
33. Loureiro J, Rodriguez E, Doležel J, Santos C. Comparison of four nuclear isolation buffers for plant DNA flow cytometry. Ann Bot. 2006; 98:679–89. https://doi.org/10.1093/aob/mcl141 PMID: 16820407
34. Mas de Xaxars G, Garnatje T, Pellicer J, Siljak-Yakovlev S, Vallès J, Garcia S. Impact of dysploidy and polyploidy on the diversification of high mountain Artemisia (Asteraceae) and allies. Alp Bot. 2016; 126 (1): 35–48.
35. Garnatje T, Canela MA, Garcia S, Hidalgo O, Pellicer J, Sánchez-Jiménez I, Siljak-Yakovlev S, Vitales D, Vallès J. GSAD: A genome size in the Asteraceae data base. Cytometry Part A. 2011; 79A: 401–404.
36. Vitales D, D’Ambrosio U, Gálvez F, Kovačič A, García S. Third release of the plant rDNA database with updated content and information on telomere composition and sequenced plant genomes. Plant Syst Evol. 2017; 303: 1115–1121.
37. Hall T. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999; 41:95–98.
38. Katoh K, Rozewicki J, Yamada KD. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics. 2017.
39. Borchsenius F. FastGap 1.2. Department of Biosciences, Aarhus University, Denmark. Published online at 2009; http://www.aubot.dk/FastGap_home.htm
40. CIPRES. Published on internet http://www.phylo.org.
41. Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 2003; 19: 1572–1574. PMID: 12912839
42. Darriba D, Taboada GL, Doallo R, Posada D. JModelTest 2: more models, new heuristics and parallel computing. Nat Methods. 2012; 9: 772–772.
43. Akaike H. A Bayesian extension of the minimum AIC procedure of autoregressive model fitting. Biometrika. 1979; 66: 237–242.

44. Rstudio. Published on internet http://www.rstudio.com.

45. Olanj N, Garnatje T, Sonboli A, Válles J, García S. The striking and unexpected cytogenetic diversity of genus Tanacetum L. (Asteraceae): a cytometric and fluorescent in situ hybridisation study of Iranian taxa. BMC Plant Biol. 2015; 15(1): 174–174.

46. Euro+Med PlantBase—the information resource for Euro-Mediterranean plant diversity. Published on the Internet http://ww2.bgbm.org/EuroPlusMed/ [03/01/2018].

47. The Plant List. Published on the Internet http://www.theplantlist.org/ [03/01/2018].

48. Bremer K. Asteraceae. Cladistics and classification. Portland, OR: Timber Press, 1994.

49. Vitales D, Feliner GN, Válles J, Garnatje T, Fírat M, Álvarez I. A new circumscription of the Mediterranean genus Anacyclus (Aphanthaceae, Asteraceae) based on plastid and nuclear DNA markers. Phyto-taxa. 2018; 349(1): 1–17.

50. Inceer H. Doğu Karadeniz Bölgesi Tripleurosperrum Sch.Bip. (Asteraceae) türlerinin morfolojik ve sito-taksonomik yönden incelenmesi. Doctoral thesis, Karadeniz Technical University, Trabzon, Turkey (in Turkish). 2003.

51. Inceer H. Redescription of Tripleurosperrum heterolepis (Asteraceae), endemic to Turkey. Phytotaxa. 2015; 202(3): 214–218.

52. Inceer H, Bal M, Ceter T, Pinar NM. Fruit structure of 12 Turkish endemic Tripleurospermum Sch. Bp. (Asteraceae) taxa and its taxonomic implications. Plant Syst Evol. 2012; 298(4): 845–855.

53. Ceter T, Pinar NM, Inceer H, Hayriloğlu-Ayaz S, Yaparak AE. The comparative pollen morphology of genera Matricaria L. and Tripleurospermum Sch.Bip. (Asteraceae) in Turkey. Plant Syst Evol. 2013; 299: 959–977.

54. Ayaz FA, Inceer H, Hayriloğlu-Ayaz S, Aksu Kalmuk N. Achene Fatty Acid Composition in the Tribe Anthemideae (Asteraceae). Rom Biotechnol Lett. 2016; 21(3): 205–208.

55. Colak N, Inceer H, Gruz J, Strnad M, Hayriloğlu-Ayaz S, Aksu Kalmuk N, Ayaz FA. Antioxidant capacity of phenolics in some representatives of the tribe Anthemideae (Asteraceae) from Turkey. Int J Pharm Sci Res. 2017; 8(8): 3265–3277.

56. Inceer H, Beyazoğlu O. Karyological studies in Tripleurospermum (Asteraceae, Anthemideae) from north-east Anatolia. Bot J Linn Soc. 2004; 146(4): 427–438.

57. Tank DC, Eastman JM, Pennell MW, Soltis PS, Soltis DE, Hinchliff CE, Harmon LJ. Nested radiations and the pulse of angiosperm diversification: increased diversification rates often follow whole genome duplications. New Phytologist. 2015; 207(2): 454–467. https://doi.org/10.1111/nph.13491 PMID: 26053261

58. Kováčik J, Klejduš B, Hedbavný J, Mártonti P, Štork F, Mártontiová L. Copper uptake, physiology and cytogenetic characteristics in three Matricaria chamomilla cultivars. Water, Air, & Soil Pollution. 2011; 218(1–4): 681–691.

59. Swift H. The constancy of deoxyribos e nucleic acid in plant nuclei. P Natl Acad Sci- Biol. 1950; 36:643–654.

60. Biemont C. Within-species variation in genome size. Heredity. 2008; 101: 297–298. https://doi.org/10.1038/hdy.2008.80 PMID: 18665185

61. Garcia S, Hidalgo O, Jakovljević I, Siljak-Yakovlev S, Vigo J, Garnatje T, et al. New data on genome size in 128 Asteraceae species and subspecies, with first assessments for 40 genera, three tribes and two subfamilies. Plant Biosyst. 2013; 147: 1219–1227.

62. Ceccarelli M, Sarri V, Caceres ME, Cionini PG. Intraspecific genotypic diversity in plants. Genome. 2011; 54(9): 701–709. https://doi.org/10.1139/G11-039 PMID: 21888597

63. Šmarda P, Bureš P. Understanding intraspecific variation in genome size in plants. Preslia. 2010; 82 (1): 41–61.

64. Murray B. When does intraspecific C-value variation become taxonomically significant. Ann Bot. 2005; 95(1): 119–125. https://doi.org/10.1093/aob/mci007 PMID: 15596461

65. Kay OON. Tripleurospermum Inodorum (L.) Schultz Bip. J Ecol. 1994; 82(3): 681–697.

66. Leitch IJ, Bennett MD. Genome downsizing in polyploid plants. Biol J Linn Soc. 2004; 82(4): 651–663.

67. García S, Canela MÁ, Garnatje T, Mcarthur ED, Pellicer J, Sanderson SC, Valls J. Evolutionary and ecological implications of genome size in the North American endemic sagebrushes and allies (Artemisia, Asteraceae). Bot J Linn Soc. 2008; 94(3): 631–649.

68. Knight CA, Ackerly DD. Variation in nuclear DNA content across environmental gradients: a quantile regression analysis. Ecol Lett. 2002; 5(1): 66–76.
69. Grotkop E, Rejmánek M, Sanderson MJ, Rost TL. Evolution of genome size in pines (Pinus) and its life-
    history correlates: supertree analyses. Evolution. 2004; 58(8): 1705–1729. PMID: 15446425
70. Morgan HD, Westoby M. The relationship between nuclear DNA content and leaf strategy in seed
    plants. Ann Bot. 2005; 96(7): 1321–1330. https://doi.org/10.1093/aob/mci284 PMID: 16230323
71. Hodgson JG, Sharafi M, Jalili A, Diaz S, Montserrat-Martí G., Palmer C, Jamzad Z. Stomatal vs.
    genome size in angiosperms: the somatic tail wagging the genomic dog. Ann Bot. 2010; 105(4): 573–
    584. https://doi.org/10.1093/aob/mcq011 PMID: 20375204
72. Bennett MD. Nuclear DNA content and minimum generation time in herbaceous plants. P Roy Soc
    Lond B Bio. 1972; 181(1063): 109–135.
73. Knight CA, Beaulieu JM. Genome size scaling through phenotype space. Ann Bot. 2008; 101(6): 759–
    766. https://doi.org/10.1093/aob/mcm321 PMID: 18222911
74. Vallès J, Canela MA, Garcia S, Hidalgo O, Pellicer J, Sánchez-Jiménez I, Siljak-Yakovlev S, Vitales D,
    Garnatje T (2013). Genome size variation and evolution in the family Asteraceae. Caryologia. 2013; 66
    (3): 221–235.
75. Rees H, Jones GH. Chromosome evolution in Lolium. Heredity. 1967; 22(1): 1–18.
76. Veselý P, Bureš P, Šmarda P. Nutrient reserves may allow for genome size increase: evidence from
    comparison of geophytes and their sister non-geophytic relatives. Ann Bot. 2013; 112(6): 1193–1200.
    https://doi.org/10.1093/aob/mct185 PMID: 23960044
77. Herben T, Suda J, Klimešová J. Polyploid species rely on vegetative reproduction more than diploids:
    a re-examination of the old hypothesis. Ann Bot. 2017; 120(2): 341–349. https://doi.org/10.1093/aob/
    mcx009 PMID: 28334206
78. Kreitschitz A, Vallès J. Achene morphology and slime structure in some taxa of Artemisia L. and Neo-
    pallasia L. (Asteraceae). Flora. 2007; 202(7): 570–580.
79. Kreitschitz A. Muclilage formation in selected taxa of the genus Artemisia L. (Asteraceae, Anthemideae).
    Seed Sci Res. 2012; 22(3): 177–189.
80. Stupar RM, Bhaskar PB, Yandell BS, Rensink WA, Hart AL, Ouyang S, Jiang J. Phenotypic and trans-
    criptomic changes associated with potato autopolyploidization. Genetics. 2007; 176(4): 2055–2067.
    https://doi.org/10.1534/genetics.107.074286 PMID: 17565939
81. Leitch IJ, Soltis DE, Soltis PS, Bennett MD. Evolution of DNA amounts across land plants (embryo-
    phyta). Ann Bot–Oxford. 2005; 95: 207–217.
82. Knight CA, Molinari NA, Petrov DA. The large genome constraint hypothesis: evolution, ecology and
    phenotype. Ann Bot–Oxford. 2005; 95: 177–190.
83. Bennett MD. DNA amount, latitude, and crop plant distribution. Environ Exper Bot. 1976; 16(2–3): 93.
84. Rayburn AL, Auger JA. Genome size variation in Zea mays ssp. mays adapted to different altitudes.
    Theor Appl Genet. 1990; 79(4): 470–474. https://doi.org/10.1007/BF00226155 PMID: 24226450
85. Løve A, Løve D. Polyploidy and altitude: Mt. Washington. Biol Zentralbl Suppl. 1967; 86: 307–312.
86. Morton J. Chromosome numbers and polyploidy in the flora of Cameroons Mountain. Op Bot. 1993;
    121: 159–172.
87. Vamosi J, McEwen J. Origin, elevation, and evolutionary success of hybrids and polyploids in British
    Columbia, Canada. Botany. 2013; 91:182–188.
88. Rejmánek M, Richardson DM, Higgens SJ, Pitcairn MJ, Grotkop E. Ecology of invasive plants: state of
    the art. In: Invasive alien species: searching for solutions (Mooney HA, McNeely JA, Neville L, Schei
    PJ, Waage JK, eds.). Washington, DC.: Island Press; 2005. pp. 104–161.