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Chromosome numbers of selected species of *Elatine* L. (Elatinaceae)

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Abstract

The paper reports chromosome numbers for 13 taxa of *Elatine* L., including all 11 species occurring in Europe, namely *E. alsinastrum*, *E. ambigua*, *E. brachysperma*, *E. brochonii*, *E. californica*, *E. campylosperma*, *E. gussonei*, *E. hexandra*, *E. hungarica*, *E. hydropiper*, *E. macropoda*, *E. orthosperma*, *E. triandra* originating from 17, field-collected populations. For seven of them (*E. ambigua*, *E. californica*, *E. campylosperma*, *E. brachysperma*, *E. brochonii*, *E. hungarica*, *E. orthosperma*) the chromosome numbers are reported for the first time. With these records, chromosome numbers for the whole section *Elatinella* Seub. became available. Although $2n = 36$ was reported to be the most common and the lowest chromosome number in the genus, our data show that out of thirteen species analyzed, six had 36 chromosomes but five species had 54 chromosomes, and the lowest number of chromosomes was 18. These data further corroborates that the basic chromosome number in *Elatine* is $x = 9$.

Keywords: ephemeral taxa; polyploids; diploid; Europe; Asia; North America; *Elatine*; chromosome number

Introduction

Elatine L. is one of the two genera of the family Elatinaceae belonging to Malpighiales [1,2] and containing ca. 15–25 ephemeral, amphibious species occurring mainly in temperate regions of both hemispheres [3]. Most of the species are facultative autogamous. In recent years, they are of interest to European researchers because of their rarity throughout the range, relatively poorly known distribution and taxonomy, erratic temporal appearance that depends mainly on environmental factors, as well as ecology [4–14].

Chromosomes in *Elatine* L. are very small and thus difficult to count and analyze. So far only few taxa have been studied: *E. hydropiper* L. [15,16], *E. triandra* subsp. *americana* (Pursh) Á. Löve & D. Löve [17], *E. americana* (Pursh) Arn. [18], *E. macropoda* Guss. [19], *E. hexandra* (Lapíerre) DC [20], *E. gratioloides* A. Cunn. [21], *E. alsinastrum* L. [22] and *E. gussonei* (Sommier) Brullo, Lanfr., Pavone & Ronsisv. [23]. Some of these data, especially the oldest ones, are contradictory, as these are not supported with good quality pictures making the counting in many cases to be equivocal. Nevertheless, the previous records hint at a basic number of 9 [15,24,25], implying tetraploid, hexaploid, octoploid and even dodecaploid ploidy levels for the species [26].

The aim of this study was to count the chromosome numbers for 13 *Elatine* species, originated from 17 populations. This was done to support our ongoing work to unravel phylogenetic relationship within the European members of the genus. For seven of the taxa studied, namely: *E. ambigua* Wight, *E. californica* A. Gray, *E. campylosperma* Seub., *E. brachysperma* A. Gray, *E. brochonii* Clav. , *E. hungarica* Moesz and *E. orthosperma* Dueb., the chromosome numbers have not been reported yet.

Material and methods

Plants studied were collected in field across Europe, in Asia and North America (Tab. 1). The field-collected plants were cultivated at both the Center for Molecular Biology at the University of Szczecin and/or Department of Botany at the University of Debrecen. Once the plants reached maturity and produced seeds, these were either sent to Szczecin (in case of plants cultivated in Debrecen), or processed locally. Seeds were sown in 12.5 × 8.5 cm plastic boxes on sterilized soil, which was continuously wetted with distilled water. Plants were grown in climate controlled culture chamber with 12 h/day light and 30 000 LUX light intensity, temperatures: under light 22 ±2°C and under darkness 18 ±2°C. Roots were immersed in 0.05% colchicine solution (Sigma-Aldrich, St. Louis, USA) for 3 hours at 16°C, then washed in ice-cold distilled water for

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10 min. Pretreated roots were fixed in Carnoy's solution (absolute ethanol : glacial acetic acid 3:1 v/v) for 24 hours at 4°C. Roots were washed in distilled water, and the root tips were dissected under a stereoscopic microscope. Each root tip was macerated directly on a microscope slide in a mixture of 4% (w/v) pectinase (Fluka, Buchs, Switzerland), 6% (w/v) hemicellulase (Sigma-Aldrich, St. Louis, USA) and 4% (w/v) cellulase (Sigma-Aldrich, St. Louis, USA) in 0.01 M citric acid – sodium citrate buffer (pH 4.8), for 4 hours at 37°C in a humidity chamber. Root tips were carefully washed with 45% acetic acid. Each preparation was covered with a cover glass, heated for 20 min at 47°C, and then the root tips were gently squashed. The cover slip was removed after freezing over dry ice, and the slides were air-dried overnight. Slides were dehydrated in a graded ethanol series (70%, 96%, and 99.8%) at room temperature, air-dried and stained with DAPI (1 µg/ml; Sigma-Aldrich, St. Louis, USA) for 15 min. The slides were rinsed briefly in distilled water, air-dried and mounted in Vectashield® Hard Set mounting medium for fluorescence (Vector Laboratories, Burlingame, USA) and analyzed with the epifluorescence microscope Axio Imager Z2 (Carl Zeiss, Oberkochen, Germany). The resulting images were captured and analyzed using the GenASIs software (Applied Spectral Imaging). From each species/population about 50 slides were prepared and analyzed. In order to confirm the results chromosomes were counted in 30 well-spread metaphase plates.

Results

Altogether, we have analyzed chromosome numbers in 17 populations representing 13 species of *Elatine* (Tab. 1). For seven of the taxa studied, namely: *E. ambigua* Wight, *E. californica* A. Gray, *E. campylosperma* Seub., *E. brachysperma* A. Gray, *E. brochonii* Clav., *E. hungarica* Moesz and *E. orthosperma* Dueb., the chromosome numbers have not been reported yet. Probably the most unexpected record is the finding that *E. campylosperma* has $2n = 18$ chromosomes (Fig. 1a), and hence – as the basic number for *Elatine* genus is $x = 9$ – is a diploid species. Populations from both localities (Italy, Spain) had the same chromosome number, and neither in this study, nor in the literature a diploid species of this genus was ever reported before. All other species had chromosome number corresponding to the tetraploid level ($2n = 4x = 36$) or higher (Tab. 1). We found that *E. alsinastrum* (Fig. 1b), *E. brochonii* (Fig. 1c), *E. californica* (Fig. 1d), *E. hungarica* (Fig. 1e), *E. hydropiper* (Fig. 1f) and *E. orthosperma* (Fig. 1g) are tetraploids with $2n = 4x = 36$ chromosomes. Also, the ploidy level of $2n = 6x = 54$ seems to be common among species of the genus *Elatine*; such hexaploids are: *E. ambigua* (Fig. 1h), *E. brachysperma* (Fig. 1i), *E. gussonei* (Fig. 1j), *E. macropoda* (Fig. 1k) and *E. triandra* (Fig. 1l). For all of these species tested, there was no variation in chromosome number between populations from various localities. Unique number of chromosomes $2n$

Tab. 1 Species included in the study; population origins, number of chromosomes found.

Species	Locality	Lat. (°N)	Long. (°E)	Number of chromosomes
Elatine alsinastrum L.	Poland: Staw Noakowski	50.82	23.02	$2n = 36$
E. ambigua Wight	Nepal: Aadarsh Nagar Tou	27.72	52.08	$2n = 54$
E. brachysperma A. Gray	USA: Fallbrook	33.46	−117.37	$2n = 54$
E. brochonii Clav.	Spain: San Silvestre de Guzmán	37.40	−7.36	$2n = 36$
E. californica A. Gray	USA: Los Angeles	33.82	−118.34	$2n = 36$
E. campylosperma Seub.	Italy: Sardegna: Gesturi	39.73	9.03	$2n = 18$
E. campylosperma Seub.	Spain: El Rocío	37.12	−6.49	$2n = 18$
E. gussonei (Sommier) Brullo, Lanfr., Pavone & Ronsisv.	Spain: Casar de Cáceres	39.33	−6.25	$2n = 54$
E. gussonei (Sommier) Brullo, Lanfr., Pavone & Ronsisv.	Italy: Lampedusa	35.51	12.56	$2n = 54$
E. gussonei (Sommier) Brullo, Lanfr., Pavone & Ronsisv.	Italy: Sicily: Modica	36.76	14.77	$2n = 54$
E. hexandra DC.	Spain: San Silvestre de Guzmán	37.40	−7.36	$2n = 108$
E. hexandra DC.	Poland: Poznań (Milicz)	51.55	17.35	$2n = 108$
E. hungarica Moesz	Hungary: Konyár	47.31	21.67	$2n = 36$
E. hydropiper L.	Hungary: Tiszagyenda	47.36	20.52	$2n = 36$
E. macropoda Guss.	Italy: Sardegna: Olmedo	40.63	8.41	$2n = 54$
E. orthosperma Dueb.	Finland: Oulu	65.06	25.47	$2n = 36$
E. triandra Schkuhr	Hungary: Karcag	47.27	20.90	$2n = 54$

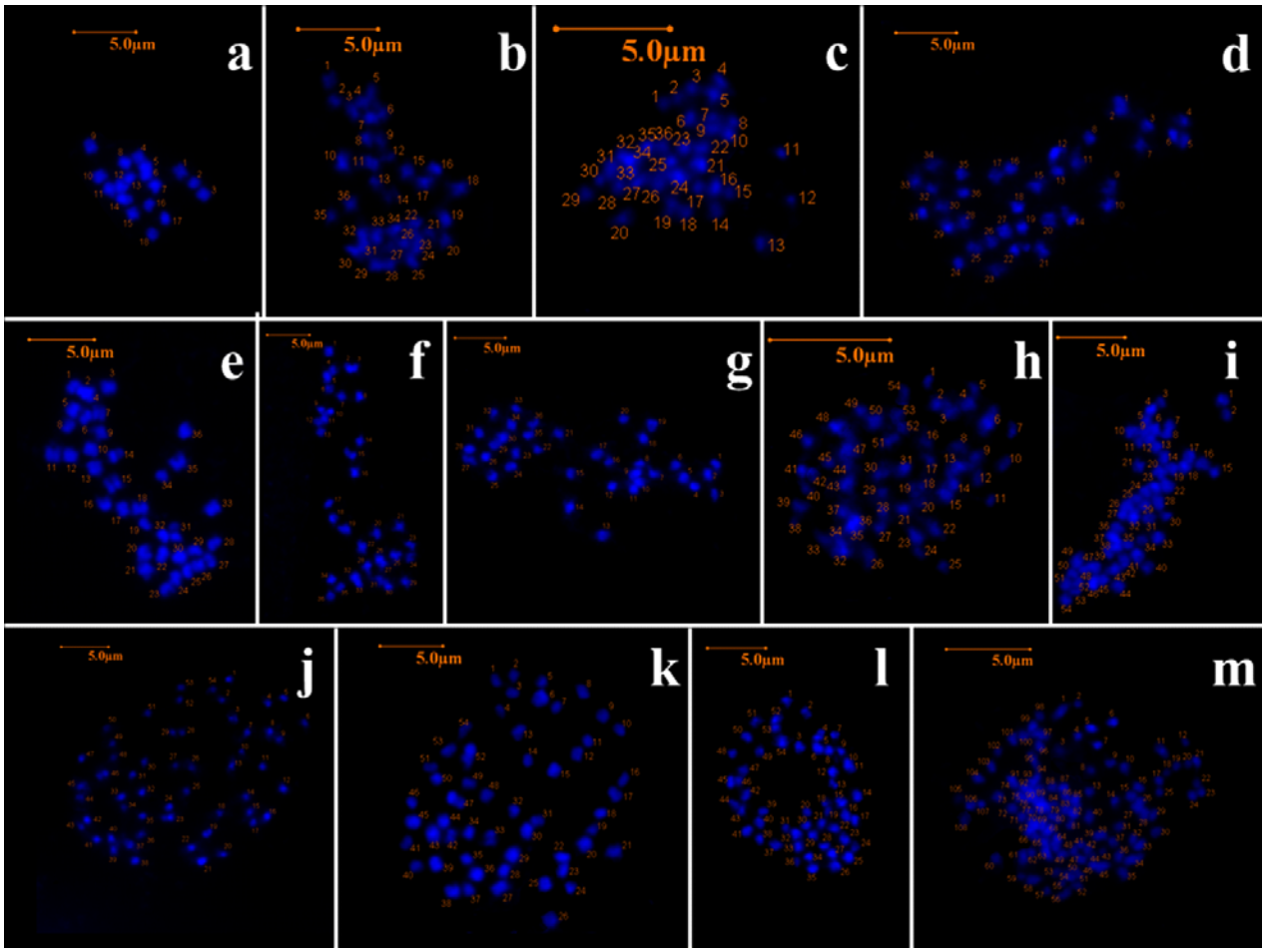

Fig. 1 Chromosomes of the analyzed *Elatine* species stained with DAPI (blue). **a** *E. campylosperma* $2n = 18$. **b** *E. alsinastrum* $2n = 36$. **c** *E. brochonii* $2n = 36$. **d** *E. californica* $2n = 36$. **e** *E. hungarica* $2n = 36$. **f** *E. hydropiper* $2n = 36$. **g** *E. orthosperma* $2n = 36$. **h** *E. ambigua* $2n = 54$. **i** *E. brachysperma* $2n = 54$. **j** *E. gussonei* $2n = 54$. **k** *E. macropoda* $2n = 54$. **l** *E. triandra* $2n = 54$. **m** *E. hexandra* $2n = 108$.

$= 12x = 108$ was found in *E. hexandra* (Fig. 1m), collected both from Poland and Spain (Tab. 1).

Discussion

According to literature data [15,24,25] the basic number of chromosomes for the genus *Elatine* is $x = 9$. This study strongly supports this view as all of the analyzed species had chromosome number dividable by 9. Moreover, we report for the first time the existence of a diploid species, *E. campylosperma*, in the genus. No other diploid species in *Elatine* was reported so far. It was claimed [26] that different ploidy levels, such as tetraploids, octoploids and dodecaploids can exist within *Elatine*. In general, there are some dubious reports for chromosome numbers in the literature. One of the first chromosome number counts for an *Elatine* species was reported in 1974 [15], determining the chromosome number of *E. hydropiper* as equaling to $2n = 40$. However, subsequent studies showed that chromosome number in this taxon is $2n = 36$ [16]. Also, our study confirmed that the number of chromosomes of *E. hydropiper* counted in two populations is $2n = 36$. We also confirmed the same chromosome number ($2n = 36$) for *E. alsinastrum*, in line of what was previously reported [22]. Actually, it was suggested that $2n = 36$ is the most common number and the lowest number of chromosomes in species of *Elatine* genus, as it was also found in the recently described species *E. gratioloides* [21], and the species with the widest distribution range in the genus, *E. triandra* [17]. In this study we showed that *E. triandra* can also have $2n = 54$ chromosomes, thus the tetraploid and hexaploid level can coexist in the genus. Examples of plant species with different ploidy-levels (i.e., coexistence of different cytotypes) is numerous [27–32], especially when they are autopolyploids.

Similarly doubtful is the record of Contandriopoulos et al. [19], who reported $2n = 40$ chromosomes in *E. macropoda*. Although this record much deviates from our counting, as we found $2n = 54$ in this species (Tab. 1), we cannot exclude that the material analyzed by the above authors was in fact $2n = 36$. Another example of coexistence of different ploidy-level cytotypes is *E. hexandra*, for which two different chromosome numbers are reported, $2n = 72, 108$ [20]. In this paper we found that our Spanish and Polish material of

E. hexandra had $2n = 12x = 108$, but we cannot rule out that some populations of the species have different chromosome number. This view is further corroborated by the finding of Probatova and Sokolovskaya [18], who reported that other species, i.e., *E. americana* can also have $2n = (70–)72$. The differences in the chromosome number counts in *Elatine* clearly demonstrate how difficult is to determine the number of such small chromosomes, and that there is a need to perform a number of tests and optimization to obtain accurate results. It should be noted that when the chromosomes are small in size and there are many of them in one cell, it is extremely difficult to obtain well-spread metaphase plates as, depending on the procedure of preparation, chromosomes may overlap or the metaphase plate may be incomplete. Both cases can lead to counting errors with a tendency to underreporting the number of chromosomes.

In our previous study we reported that *E. gussonei* collected from the Maltese Archipelago has the number of $2n = 8x = 54$ chromosomes [23]. Here, we have confirmed this result by adding two more populations from geographically distant locations. It is important to analyze the chromosome number from different population as some chromosome rearrangements may lead to the change of the chromosome number in genetically isolated populations. Unfortunately, we were not able to distinguish the types of chromosomes because of their minute size. Karyotyping could shed some light on the possibilities of chromosome rearrangements, which can be the reason for varying chromosome number. Consequently, we cannot totally exclude the existence of some populations of the same species with different chromosome number (e.g., $2n = 40$ in case of a $2n = 36$ species), but there is a slightly higher likelihood of incorrectly counting more chromosomes due to their small size.

Another aspect of chromosome number changes is the phenomenon of polyploidization itself. This aspect may explain different number counts in different reports. Most of the *Elatine species* are polyploids and it is a well-known fact that meiosis in newly formed polyploids is unstable [33,34]. For example, several studies found that 30–40% of the progeny of autotetraploid maize is aneuploid [35]. If aneuploids became isolated, they might give rise to a population with different chromosome number. Polyploid meiosis frequently produces aneuploid gametes, although their frequency varies between species or ploidy level. There is a relationship between polyploidy and aneuploidy: eupolyploids produce frequently aneuploids, which in turn can produce euploids [33].

It is believed, that polyploidization is a major driving force of plant evolution [36,37]. Obtained chromosome number data clearly indicate that polyploidization was one of the most important phenomena accompanying the evolution of the genus *Elatine*. Further studies should bring the answer to the question if *E. campylosperma* is one of the ancestors of polyploids. Another question is whether these species are auto- or allopolyploids, which originated through hybridization event between different species, accompanied or followed by genome duplication. Also interesting is to correlate ploidy level in *Elatine* to geographic latitude the species most commonly inhabit. As it is demonstrated in Europe and North America [38], polyploids are more frequent at high latitudes. If we examine our list of chromosome numbers, we found that the European species found exclusively in the temperate climate zone (*E. hungarica*, *E. hydropiper* and *E. orthosperma*) have $2n = 4x = 36$. Two of three Mediterranean species (*E. gussonei*, *E. macropoda*) have $2n = 6x = 54$. This is an apparent contradiction to the above rule, and requires the reconstruction of phylogenetic relationships for a better understanding. Whatever the pattern behind this finding, the large chromosome number differences between different *Elatine* species suggests that the genome of *Elatine* is very tolerant to redundancy of genetic information, and its evolution was accompanied by multiple polyploidization events.

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Authors' contributions

The following declarations about authors' contributions to the research have been made: conducting experiments, writing the manuscript: AK; field sampling, manuscript preparation: GS; field sampling, cultivating plants: OH; research idea and coordination in Debrecen, field sampling, manuscript preparation: AM; research idea and coordination in Szczecin, field research, manuscript preparation: AP.

Competing interests

No competing interests have been declared.

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