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Short communication

Chromosome number of *Elatine gussonei* (Sommier) Brullo (Elatinaceae) and its distribution on the Maltese islands

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Abstract – *Elatine gussonei* (Sommier) Brullo is an endemic species, with a distribution restricted to the central part of the Mediterranean Basin (Maltese islands, Lampedusa, southern part of Sicily). This hydrophyte grows in rainwater pools and cavities in karstic limestone. Although the morphology has been well studied, no karyological study has been carried out, and hence this work brings the first chromosome data for the Maltese-pelago endemic *E. gussonei*. We have found a diploid number of 54 chromosomes in *E. gussonei*, which differs from the chromosome number of most of *Elatine* species (2n = 36). Additionally, this account gives a recent distribution of the species on the Maltese islands.

Key words: *Elatine gussonei*, chromosome number, phytogeography

Introduction

Elatinaceae Dum. is a small cosmopolitan family of herbaceous aquatic and semi-aquatic plants and terrestrial shrubs composed of two genera, i.e. *Elatine* L., comprising about 15–20 taxa, and occurring in areas of moderate temperatures in both hemispheres, and *Bergia* L., with about 25 species occurring mostly in tropical areas of the Old World, primarily in Africa, and also in Australia (TUCKER 1986, LEACH 1989). With regards to the genus *Elatine*, in Europe 10 species are acknowledged: *E. triandra* Schuhr., *E. alsinastrum* L.; *E. hydropiper* L.; *E. orthosperma* Dueben; *E. hungarica* Moesz.; *E. macropoda* Guss.; *E. campylosperma* Seub.; *E. hexandra* (Lapierre) DC; *E. brochonii* Clavaud and *E. gussonei* (Sommier) Brullo. Based on the arrangement of the leaves along the stem, NIEDENZU (1925, after SEUBERT 1845) proposed the subdivision of the genus *Elatine* into lower sub-

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genera: the subgenus *Potamopithys* (Adanson) Seub. (whorled leaves) and the subgenus *Elatine (Hydropiper* Moesz.) Seub. (opposite leaves). The latter subgenus includes two sections: *Elatinella* Seub., where the number of stamens is twice that of petals, and *Triandra* Seub. (= *Crypta* (Nutt. Seub.), characterised by an equal number of stamens and petals (TUCKER 1986). *Elatine gussonei* is one of eight species of the section *Elatinella*, one of which is found in Euroasia, six are found in Europe (three of them also in North Africa) and one in North America (California).

Elatine gussonei was first found and described by SOMMIER (1908) on the island of Lampedusa, under the taxon of *Elatine hydropiper* L. var. *gussonei* Sommier, based on color of corolla, the ratio in sizes of the flower parts and general seed morphology. Later, similar plants were also found in Malta. Both populations were reported to grow in rainwater pools contained in superficial small basins and cavities in karstic limestone (SOMMIER and CARUANA GATTO 1915), commonly known as rock pools. Much later, detailed morphological studies were carried out by BRULLO et al. (1988) resulted in the promotion of the taxon to species rank: *Elatine gussonei* (Sommier) Brullo, Lanfranco, Pavone and Ronsisvale. However the first record for the Maltese islands should be attributed to GRECH DELICATA (1853) who recorded *Elatine macropoda* Guss. from Wied Balluta, San Ġiljan. Since all local *Elatine* populations have been identified as *E. gussonei*, it is most probable that GRECH DELICATA (1853) also found *E. gussonei* – a species which is closely related to *E. macropoda*. Recently *E. gussonei* was also found in three localities in the south-eastern part of Sicily (MOLNÁR et al. 2013).

The number of chromosomes in *Elatine gussonei* was not previously reported, in contrast to the unequivocal results of chromosome number for other species of the *Elatine* genus. The aim of this paper is to present results of the study on chromosome number of the taxon, as well as the new records of its distribution for the Maltese islands.

Materials and methods

The list of sites of *Elatine gussonei* and the distribution map for the Maltese are based on published (BORG 1927) and unpublished records by one of the authors.

Specimens were collected from 5 different populations in the Maltese islands and were immediately delivered to Department of Biology, University of Szczecin for karyological analysis supervised by three of the authors. Because it is a protected species, the necessary permits were obtained from the Malta Environment and Planning authority.

Roots were treated with 0.05% colchicine (Sigma-Aldrich, St. Luis, USA) solution for 3 hours, followed by a wash of 10 min with ice-cold distilled water. Then they were fixed in ethanol : glacial acetic acid, 3 : 1 (v / v) for 24 hours at 4 °C. Fixed roots were stored at 4 °C in fresh fixing solution. Roots were washed in distilled water, and under stereoscopic microscope the root tips were dissected. Each root tip was macerated directly on a microscope slide in a 10 μL mixture of 4% (w/v) pectinase (Fluka, Buchs, Switzerland), 6% (w/v) hemicellulase (Sigma-Aldrich, St. Luis, USA) and 4% (w/v) cellulase (Sigma-Aldrich, St. Luis, USA) in 0.01 M citric buffer for 2 hours at 37 °C in a humidity chamber. Root tips were carefully transferred with hypodermic needles to a drop of 45% acetic acid onto a new slide, and 10 μL of 2 % (w / v) aceto-orcein (Sigma-Aldrich, St. Luis, USA) were added. Each preparation was covered with a cover glass and stained at 47 °C for 1 hour. During this time aceto-orcein was added 3–5 times. The stain under a cover glass was

replaced with 45% acetic acid. Root tips were squashed, and this was followed by gentle heating (5–10 min at 47 °C). Preparations were analyzed under an ECLIPSE E600 microscope (Nikon, Tokyo, Japan). Images were captured at 1000 × magnification with CCD DS-Fi1c camera (Nikon, Tokyo, Japan) and analyzed by means of NIS-Elements AR3.1 (Nikon, Tokyo, Japan) software.

Results and discussion

Phytogeographic study

Elatine gussonei is in general well scattered throughout the rural areas of the Maltese islands, and therefore is not considered a locally rare species (Tab. 1, Fig. 1). It is specifically

Tab. 1. The list of localities of *Elatine gussonei* (Sommier) Brullo in the Maltase islands.

Locality	References
Mainland Malta:	
Attard – Wied Incita	SOMMIER and CARUANA GATTO 1915
Birżebbuġa – Wied Hal-Saptan	SM, 2006*
East of Għar Hasan	SM, 2012*
Għargħur – in-Nigret	SM, 2006*
Mellieħa – Qammieħ	SM, 2006*
Il-Miżieb	SM, 2006*
l-Imġiebaħ	SM, 2006*
Coastal cliffs near Mistra area	SM, 2008*
Mosta – Wied il-Għasel	SOMMIER and CARUANA GATTO 1915
Tal-Wej	SM, 2006*
Wied Filep	BORG 1927 [1]
Rabat – Buskett Gardens	BORG 1927
Wied il-Girgenti	HASLAM and BORG 1998
Mtaħleb	SM, 2006*
Sliema – Wied tal-Balluta	GRECH DELICATA 1853
San Ġiljan – Tal-Minsija	SOMMIER and CARUANA GATTO 1915
Ħarq ħammiem	STEVENS 1995
Pembroke	SM, 2006*
San Pawl il-Baħar – Rocky areas near Salini	SM, 2006*
Siġġiewi – Tal-Għolja	BORG 1927
San Ġorġ and Ġebel Ciantar area, limits of Fawwara	SM, 2010*
Żurrieq – Wied il-Bassasa	SM, 2011*
Gozo:	
Munxar – Xlendi	SOMMIER and CARUANA GATTO 1915
Ras il-Fekruna	SM, 2007*
Ta' Sannat – Ta' Ċenċ	SOMMIER and CARUANA GATTO 1915
Wied Mġarr ix-Xini	SM, 2006*

* New records (= sites of *E. gussonei* not found in present literature)
[1] Demolished to make space for a stone quarry (from Wikipedia.org, accessed Jun 2012)

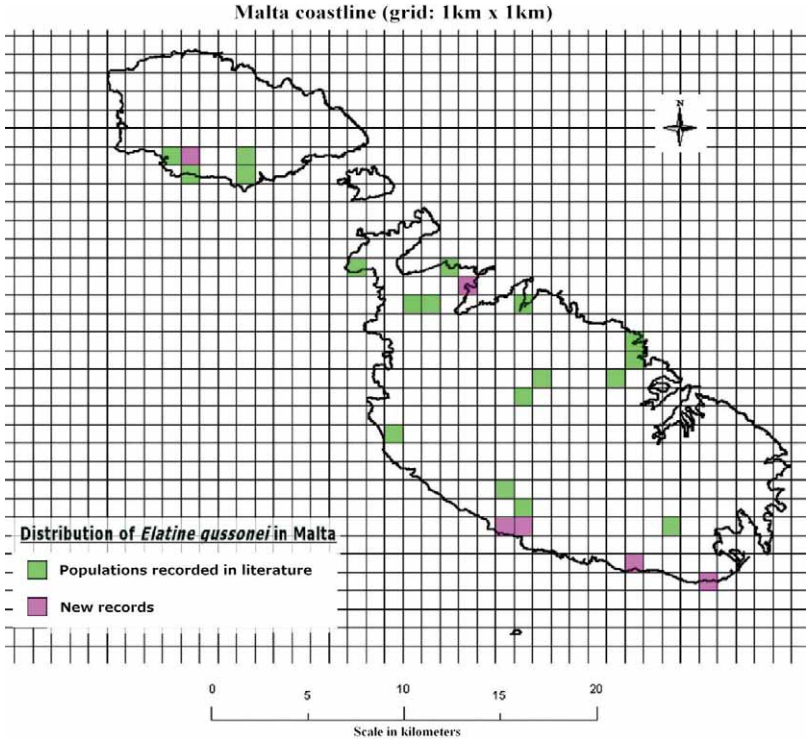

Fig. 1. Distribution of *Elatine gussonei* in the Maltese islands. (1 grid box = 1km).

and restrictedly found in rock pools scattered on coralline limestone mostly situated in rocky valley sides, borders of escarpments or coastal cliffs, and sometimes exposed, sloped rocky ground. Most natural rock-basins are not suitable to hold water for long and hence provide the habitat for *E. gussonei* because water seeps out through cracks and as a result they become void after few days without rain. However, the species often dominates suitable rock pools, forming a dense population composed of thousands of specimens. Among the species included in the section *Elatinella, Elatine gussonei* is the one most closely related to *E. macropoda*; a species mainly found in the western and central part of Mediterranean basin (POPIELA and ŁYSKO 2010). Many of the morphological differences used since SOMMIER (1908) to distinguish the two species appear to be somewhat unreliable. It turned out that the distinctive characters used by SOMMIER (1908) and SOMMIER et al.(1915) vary considerably according to environmental conditions and as a result they intersect between the species. It was proposed that more important distinguishing differences are the presence of a conspicuous semilunar membrane at the acute curvature of the seed and the shape and number of 'pits' in the reticulation of the seed testa (MOLNÁR et al. 2013).

Karyological analysis

A total of 100 metaphase plates were analysed. Only well-spread metaphases were included in the study as overlapping chromosomes are the main cause of errors in determining the chromosome number (Fig. 2A). The chromosome number in *E. gussonei* amounts to

2n = 54 (Fig. 2B). This count was confirmed through the detailed analysis of 30 metaphase plates. Chromosomes of *Elatine gussonei* are very small in size and for this reason the analysis was much more difficult than expected. Chromosomes of *Elatine gussonei* contain appreciable amount of heterochromatin, which is perfectly visible in the nuclei (Fig. 2C). However, the chromosomes are so small that it can hardly be stated if the condensed fraction of chromatin occupies the centromeric or telomeric part of chromosomes. It seems likely that at least in some chromosome pairs there are large blocks of heterochromatin in the subcentromeric region (Fig. 2D). This is the first report on chromosome number for this species. It was proposed that the basic number for *Elatine* genus is x = 9 (LÖVE and LÖVE 1974, GOLDBLATT 1981, 1985). There are also several polyploid levels in different *Elatine* spp. such as tetraploids, octoploids and dodecaploids (UOTILA 1974). However, there are contradictory reports for chromosome numbers in the literature. For instance, CONTANDRIOPOULOS et al. (1987) reported that there are 2n = 40 chromosomes in *Elatine macropoda* Guss. The same chromosome number of 2n = 40 was reported for *Elatine hydropiper* L. (LÖVE and LÖVE 1974). However, subsequent studies showed that chromosome number in this taxon is actually 2n = 36 (KRAHULCOVÁ 1990). There is an indication that reports of 2n = 40 probably refer to 2n = 36 (UOTILA 1974) and the counting error arises from the fact that the chromosomes of *Elatine* are very small and hence difficult to count precisely. In present paper the measurements of chromosomes from 30 metaphase spreads were made. The mean length of the chromosome was 0.69 μm, with a minimum value of 0.26 μm and maximum

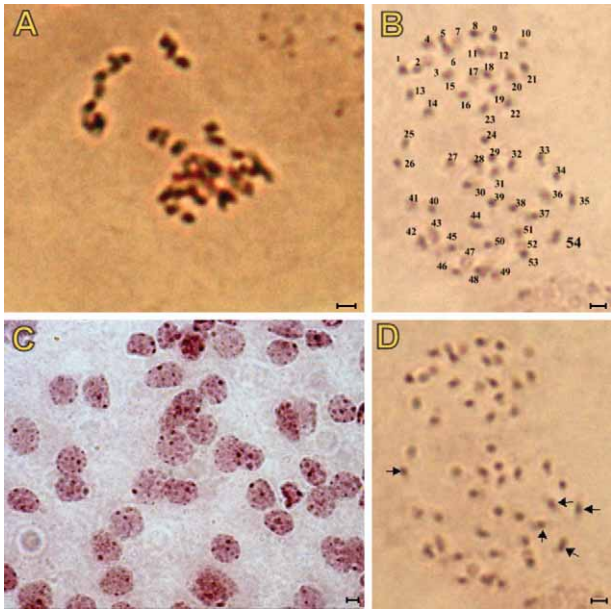

Fig. 2. Chromosomes and interphase nuclei of *Elatine gussonei* stained with aceto-orcein; **A** – metaphase spread with overlapping chromosomes, giving the false impression of 36 visible chromosomes; B – metaphase spreads with 54 chromosomes; C – interphase nuclei; dark spots correspond to condensed chromatin; D – metaphase spread; the condensed chromatin at centromeric / subcentromeric regions of some chromosomes are marked with arrows. Scale bar = 1 μm.

value of 1.12 μm (σ = 0.17 μm). The same discrepancies appear in other species: *Elatine americana* (Pursh) Arn. 2n = 70–72 (PROBATOVA and SOKOLOVSKAYA 1986), *Elatine hexandra* (Lapíerre) DC 2n = 72, 108 (JANKUN 1989, POGAN et al. 1990). The most common, as well as the lowest number of chromosomes in species of *Elatine* genus is 2n = 36 as was found in *Elatine gratioloides* A. Cunn. (DE LANGE et al. 2004) and *Elatine triandra* subsp. *americana* (Pursh) (LÖVE and LÖVE 1982).

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