

A Comparative Study between *Elatine gussonei* (from Malta) and *Elatine macropoda* (from Majorca)

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Abstract

This study compared differences between the closely related *Elatine macropoda* Gussone and *Elatine gussonei* (Sommier) Brullo, Lanfranco, Pavone & Ronsisvalle as published in literature to differences between the two species as observed in the wild and in vitro. Literature indicated that main characteristics of *E. gussonei* that distinguish it from *E. macropoda* are its pink flowers, petal size equal to sepal's and its strongly curved seed. However observations in the field showed that environmental conditions as well as flowering stage significantly change the appearance of the flowers of both species. Mean seed curvature was shown to be significantly different between the species and unaffected by environmental factors. However the range of seed curvature in both species is wide and overlaps each other, thus also making seed curvature an unreliable diagnostic feature.

The most important characteristic, discovered during the course of this study, which easily differentiates the two species is the reticulation of the seed testa. The pit shape in *E. gussonei* is ~regular hexagonal with 15 ± 3 pits per row while in *E. macropoda* the pit shape is ~long-hexagonal with 21 ± 3 pits/row. This important difference substantiates the uniqueness of *E. gussonei* and emphasizes the necessity for the conservation of its highly vulnerable habitat in the Maltese islands.

Introduction

Elatine macropoda Guss. and *E. gussonei* (Sommier) Brullo, Lanfranco, Pavone & Ronsisvalle are both small Mediterranean plants inhabiting temporary winter rainwater pools. *E. gussonei* is restricted to Malta, Gozo and Lampedusa while *E. macropoda* is much more widely distributed around the Mediterranean basin.



Figure 1 Temporary rain water rock pools at Wied Sabtan. Vulnerable natural habitat of *Elatine gussonei*





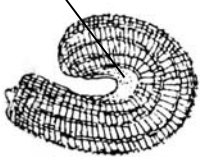
In Malta, environmental conditions have been observed to significantly influence the appearance of *E. gussonei* both in the vegetative and the flowering stage, while photographs of *E. macropoda* flowers in Majorca showed no significant difference to flowers observed in *E. gussonei* in Malta. These observations and the fact that many other *Elatine* species and varieties (eg *E. major*, *E. campylosperma* and *E. fabri*) have been reduced to synonyms of *E. hydropiper* or *E. macropoda* has led to the hypothesis that the two are environment-induced 'morphs' of the same species. This study aims to answer this question.

Literature review

The first time *E. gussonei* was described was as *Elatine hydropiper* L. var. *gussonei* by Sommier¹ in 1908 in his description of the flora in Lampedusa¹. He defined it as a different variety of *Elatine hydropiper* than the varieties *macropoda*, *hydropiper* and *pedunculata*. This was based on observations regarding differences in flower structure, petal colour and seed shape. In previous publications on the flora of Lampedusa by other botanists it had been listed as *E. macropoda* or *E. campylosperma*.

Sommier found this same variety of *Elatine* also in Malta and described it again to a lesser degree in a publication on the flora of Malta, coauthored with A Caruana Gatto in 1915². As in Lampedusa he found the plant growing in rain water rock pools, 'abundantly in various locations such as 'Wied Incita, Minsija, Wied Balluta and Wied il-Ghasel'.

Table 1 Differences between *Elatine macropoda* and *E. gussonei* as described in literature (Sommier's description in **Bold**, Flora Europeae's in *Italic*)

	<i>Elatine macropoda</i> Gussone (<i>Elatine hydropiper</i> L. var. <i>macropoda</i> (Guss.))	<i>Elatine gussonei</i> (Sommier) Brullo, Lanfranco, Pavone & Ronsisvalle (<i>Elatine hydropiper</i> L. var. <i>gussonei</i> (Sommier))
Sepal to Petal ratio	<ul style="list-style-type: none"> • Petals half as long as sepals^{1,6}  <p>Figure 3 from Flora Italiana illustrata⁵</p>  <p>Figure 2 from Abbé Coste⁴</p> <ul style="list-style-type: none"> • <i>Petals shorter than sepals</i>⁷ 	<ul style="list-style-type: none"> • Petals equal in length to sepals^{1,2,6}
Flower colour	<ul style="list-style-type: none"> • White^{1,6} • <i>Pale red</i>⁷ 	<ul style="list-style-type: none"> • pulchre roseis¹ (A beautiful pink)
Length of pedicel (flower stalk)	<ul style="list-style-type: none"> • 1-5 times longer than leaves⁶ • <i>up to 23mm long</i>⁷  <p>Figure 4 from Flora Italiana illustrata⁵</p>	<ul style="list-style-type: none"> • less than or equal to leaves^{1,6} • flowers reach level of green mat¹ • in deep water are longer²
Seed shape	<ul style="list-style-type: none"> • Slightly curved^{1,6} • No apparent semilunar membrane¹  <p>Figure 5 from Flora illustrata⁵</p> <ul style="list-style-type: none"> • <i>Almost straight or asymmetrically horseshoe shaped with long arm 2-2.5 times longer than short arm</i>⁷ 	<ul style="list-style-type: none"> • Strongly curved, looking like a horse shoe or hook^{1,2,6} • apparent semilunar membrane as in <i>E. hydropiper</i> seeds¹  <p>Figure 6 <i>Elatine hydropiper</i> seed⁵</p>

During the mid 20th century, *E. macropoda*, *E. hexandra*, and *E. triandra*, all formerly varieties of *E. hydropiper* were elevated to species status. Similarly Brullo et al.³ basing arguments raised by Sommier elevated the Maltese/Lampedusan variety to species status in 1988.

Methods used for the comparative study.

1) Observations on *E. gussonei* in the wild have been carried out in a number of locations in Malta and Gozo for many years. The plant lives in temporary rainwater pools that form in karstic coralline limestone in certain localities (see Figure 7).

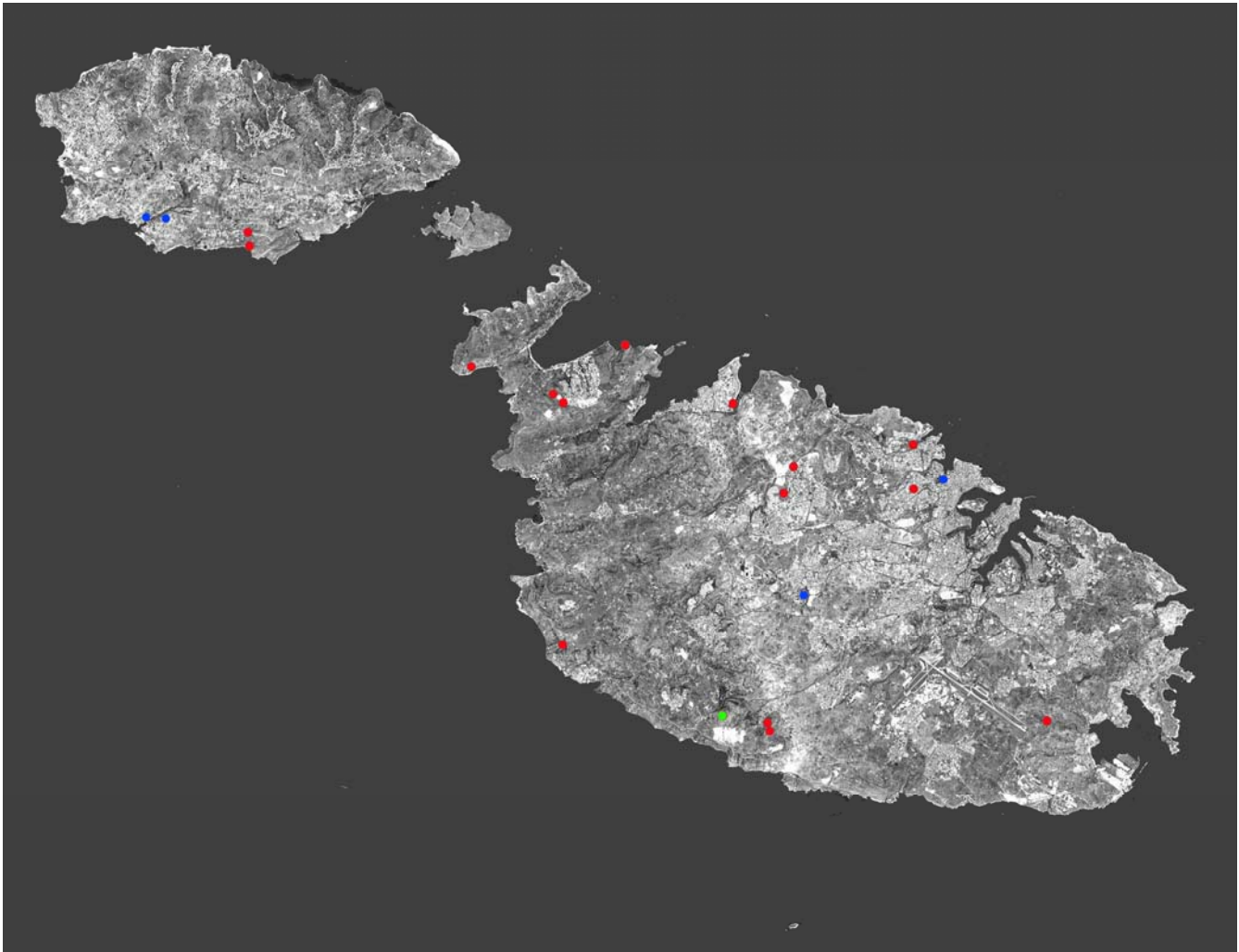


Figure 7 Distribution of *E. gussonei* in the Maltese Islands (red-existing populations, blue- recorded but not confirmed, green- recently destroyed by vandals)

2) Details of Photographs of live material of *E. macropoda* in Majorca as well as seed obtained from these plants were studied using a light microscope and imaging software..

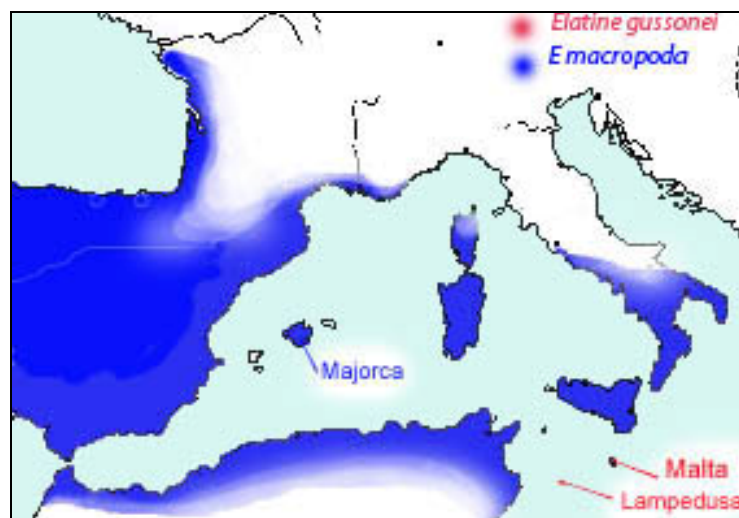


Figure 8 Distribution of *Elatine macropoda* and *E. gussonei* in west Mediterranean^{12,6,7,8}

3) Seeds from *E. gussonei* (from various locations in Malta) and *E. macropoda* (from Majorca) were used to start in-vitro cultures of the two plants. The plants were grown in identical situations but in 2 separate shallow containers containing a few

centimeters of sterile substrate and covered with a few centimeters of water, and left in daylight to replicate natural conditions as far as possible. The experiment started in late September 2005 (corresponding to the start of the rainy season) and ended in early April 2006. Observations and measurements were taken as time progressed. Seeds collected were photographed and compared using imaging and statistics software.



Figure 9 Containers with cultures of *E. macropoda* and *E. gussonei* (Feb 2006)

Results.

1. Seed germination

Time taken for seed germination was 1 to 2 weeks for both species after inundation. No significant differences were observed.

2. Vegetative growth.

In nature *Elatine gussonei* has been observed to have vegetative growth forms that can be markedly different according to the pools in which they grow. (See Figure 10)



Figure 10 Difference in sizes of *E. gussonei* collected from different localities on the same day

This difference appeared to be correlated to shade and nutrient status of the pools. Larger leaves and longer petioles were produced in deeper shaded pools (as in Mellieha). Sommer had also noted differences between plants growing in deep pools and shallow pools.

In nutrient rich pools the green was darker and the thick growth resulted in vertical stems as plants competed for light. However, in all cases, when plants of different forms were placed in the same in-vitro conditions their new growth was identical.

In vitro the stem of both species grew horizontally with pairs of spatulate leaves and roots originating at the nodes. At later stages nodes gave rise to branches.



Figure 11 Usual growth pattern of *E. gussonei* in shallow rock pools

Both species grown in vitro had very similar vegetative growth and could not be distinguished from each other. However it was observed that *E. macropoda* grew at a more rapid pace and covered ground at more than twice the rate of *E. gussonei*.

3. Flower bud formation

In natural conditions, the usual time for flower buds initiation in *E. gussonei* is late January with lengthening daylight period, but this depends on weather conditions and depth of pools. If rains fail to keep the pool full, plants can even flower in October before the substrate dries completely, while in deeper or shaded pools bud formation can be extended to Mar.

No data was collected for flower bud initiation of *E. macropoda* while in Majorca. However cultivated *E. macropoda* started to form buds 1 – 2 weeks later than *E. gussonei*.

4. Pedicel length

In his original latin description of *Elatine hydropiper* L. var. *gussonei* in Lampedusa, Sommier distinguished it from var. *macropoda* by having a short flower stalk¹. However in Malta he appears to have noticed that the length of the pedicel is influenced by the depth of the pool, and did not repeat the differentiation²



Figure 12 Pedicel length in *E.gussonei* is influenced by depth of pool.

This latter observation has been confirmed in both natural and in vitro situations (see Figure 12). If anything, the pedicel length of *E. gussonei* in the in-vitro comparison was somewhat longer, but given the fact that bud initiation started earlier, this observation was not considered significant.

5. Flowers – Petal:Sepal sizes

According to the original description by Sommier, *E. gussonei* and *E. macropoda* differ markedly in the flower structure. Whereas *E. gussonei* has petal lengths equal to sepals, *E. macropoda* has petals half as long as sepals. Other authors^{4,7} agreed that *E. macropoda* has petal size less than sepal's, but not apparently to the same extent (see Table 1).

Observations showed that naturally growing *E. gussonei* does have a petal:sepal size ratio of 1:1, but only when the flower is exposed to full sun, and only soon after opening (see fig Figure 13, top flower).



Figure 13 *E. gussonei* flowers at Mtahleb

However once the flowers open, the ratio changes as the sepals continue growing (Figure 13, bottom flower), so the 1:1 ratio description is only true for the initial stages of flowering.

In nature flowers of *E. gussonei* at various stages were observed with varying petal to sepal ratios (see Figure 14).



Figure 14 Flowers of *E. gussonei* in a pool at Wied Sabtan

- 1 Flower buds opening
- 2 Flowers partly opened
- 3 Flowers fully opened
- 4 Ovary maturing (Sepal:Petal ration not 1:1)

Surprisingly, photographs of flowers of *E. macropoda* growing in Majorca showed no difference to those of *E. gussonei*. Petal to Sepal length ratio is similarly 1:1 while opening and upon opening. However the sepals continue growing as the ovary matures, so that the petal:sepal ratio reduces to a similar one to that in Sommier’s description.



Figure 15 *Elatine macropoda*
(photographed in Majorca)

- 1 Flower buds opening
- 2 Flowers partly opened
- 3 Flowers fully opened (note
Sepal:Petal ration is 1:1)
- 4 Ovary maturing

To complicate matters, when the buds are not exposed to full sunlight, the petal:sepal size ratio of both species is small even at the initial stages, as happens when growing in the partial shade of nearby plants or rocks. In such instances opening flowers have small petals (see Figure 16)



Figure 16 *E. gussonei* growing in partial shade with petal:sepal ratio < 1

6. Flowers - Petal Colour

According to Sommier¹ *E. hydropiper* var. *gussonei* has nice pink petals while var. *macropoda* has white petals. The photographs from Majorca and the description of *E. macropoda* in Flora Europea⁷ contradicted the note regarding *E. macropoda*, but to see if there is a real difference in petal colour, flowers of plants growing in vitro were compared and photographed (see Figure 17). Although the same camera was used to reduce error, lighting conditions could have affected the colours to a certain degree. However, what is still apparent, is the high variability in flower colour of both species according to growing conditions. Petal colour in *E. gussonei* growing in shade is practically white! To give credit to Sommier however, the petals of *E. gussonei* growing in full sun, especially while opening, are in the authors' opinion truly a 'beautiful pink'.

E. gussonei in shade



E. macropoda in partial shade



E. gussonei in sun



E. macropoda in sun

Figure 17 Petal colour(s) of *Elatine gussonei* and *E. macropoda* as affected by lighting conditions.

7. Seed - Curvature

The most important distinction Sommier¹ made between *E. hydropiper* var. *gussonei* and var. *macropoda* was in the curvature of the seed, with var. *macropoda* having only slightly curved seed. On the other hand Flora Europea⁷ gives a much wider range of seed curvature to *E. macropoda* (see Table 1), that in effect would encompass seed curvature of *Elatine gussonei*.

The present study involved calculating the angle of curvature of random samples of *Elatine gussonei* (from Malta) and *E. macropoda* (from Majorca). Subsequently statistical calculations using the t-test were performed to determine if the difference in means between the different sets was actually significant or not.

The angle of curvature of seeds was determined by photographing randomly scattered seeds, after shaking, so that seeds lied on their sides (with the few exceptions causing similar sources of error in each set). The photographs of a large number of seeds (to make the statistical test valid) were processed using imaging software and the angle of curvature determined (see fig 18 for an example).

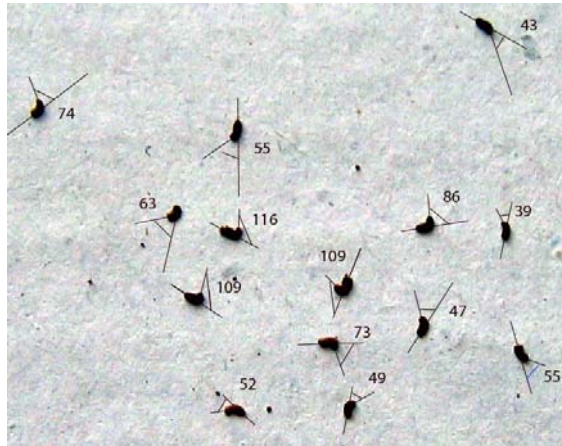


Figure 18 Example of determining angle of curvature in *E. macropoda*

Table 2 Statistics on sets of *Elatine* seeds

Set	Seeds from	Sample size	Mean seed angle of curvature (MSAOC)	SD
A	<i>Elatine macropoda</i> (from Majorca)	26	64	20
B	<i>Elatine macropoda</i> (from invitro culture Malta) sun	105	59	17
C	<i>Elatine gussonei</i> (from invitro culture Malta) sun	105	101	22
D	<i>Elatine gussonei</i> (from invitro culture Malta) shade	44	98	25

T-test between Sets:	P	Ho	Significance
A & B	0.183 > 0.1 (two tails)	Not rejected	No significant difference between MSAOC of <i>E. macropoda</i> from Majorca and growing in vitro Malta.
B & C	1.18E-36 << 0.05 (one tail)	Rejected	Very significant difference in MSAOC between <i>E. macropoda</i> and <i>E. gussonei</i> , as cultivated in vitro.
C & D	0.532 > 0.1 (two tails)	Not rejected	No significant difference in MSAOC between <i>E. gussonei</i> growing in sun and shade

The mains findings of this exercise were:

- There was a significant difference between the mean angle of curvature (MSAOC) in the seeds of *E. macropoda* (Majorca) and *E. gussonei* (Malta). Hence *E. gussonei* seeds were generally more curved.
- Environment does not appear to affect seed shape in both *Elatine* species.
- There is a considerable overlap in the SAOC of both *Elatine* species (see Figure 19).

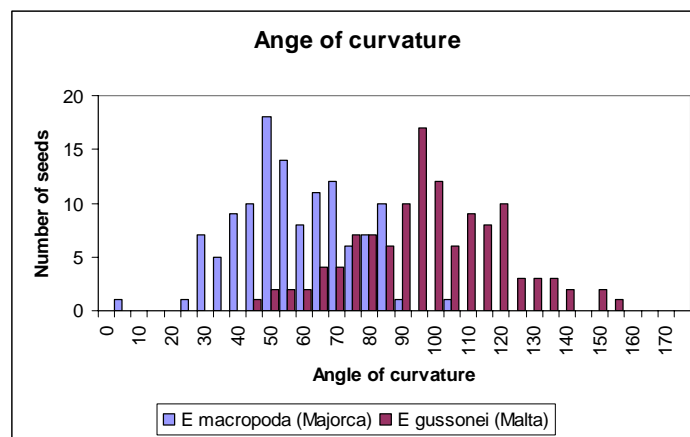


Figure 19 Angle of curvature (degrees) in seeds

8. Seed coat reticulation

Seed coat reticulation is a significant diagnostic characteristic in *Elatine* species. Unfortunately Sommer did not give any kind of description for *E. hydropiper* var. *gussonei*, and apparently there are no published images of its seeds.

There is more published information with regard to *E. macropoda*. Flora Europae⁷ describes the reticulations on the testa as 'usually hexagonal at base of seed'. Figures 2 and 5 indicate pits which appear to be long-hexagons, with appr. 20 pits per row.

Seeds of *E. macropoda* obtained from Majorca, as expected, showed similar reticulation to those published. Seeds of *E. macropoda* grown in Malta also had similar reticulation. A number of seeds were viewed. These had 21 ± 3 pits/row with pits being mainly long-hexagons in outline as expected.

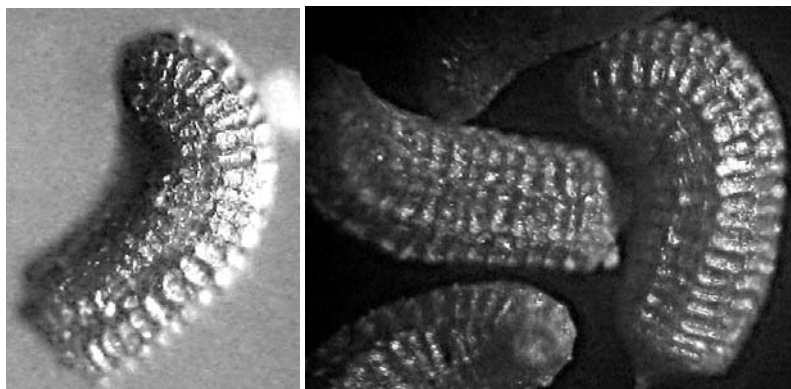
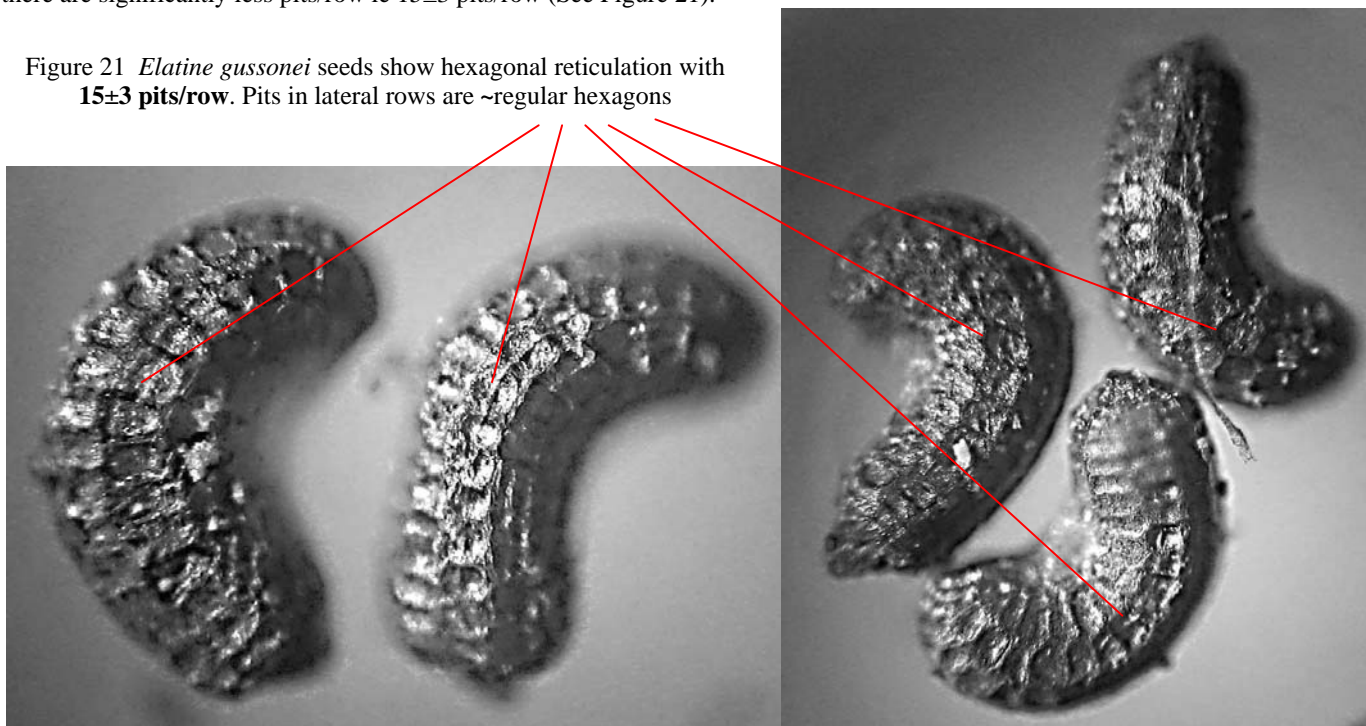


Figure 20 *E. macropoda* seeds (a) from Majorca on left (b) from invitro culture (Malta) right. Both with flattened hexagonal reticulation with **21 ± 3 pits/row**

Seed reticulation of *E. gussonei* (Malta) seeds appears to be significantly different. Pits in the lateral rows, although hexagonal are more regular than long, with a larger surface area per pit. Since seeds are of a similar size to those of *E. macropoda*, this means that there are significantly less pits/row ie 15 ± 3 pits/row (See Figure 21).

Figure 21 *Elatine gussonei* seeds show hexagonal reticulation with **15 ± 3 pits/row**. Pits in lateral rows are ~regular hexagons



The difference between the reticulation of the two species is very evident when seen side by side as in Fig 22 below. There is very little difficulty identifying the two species by just noting this pattern.

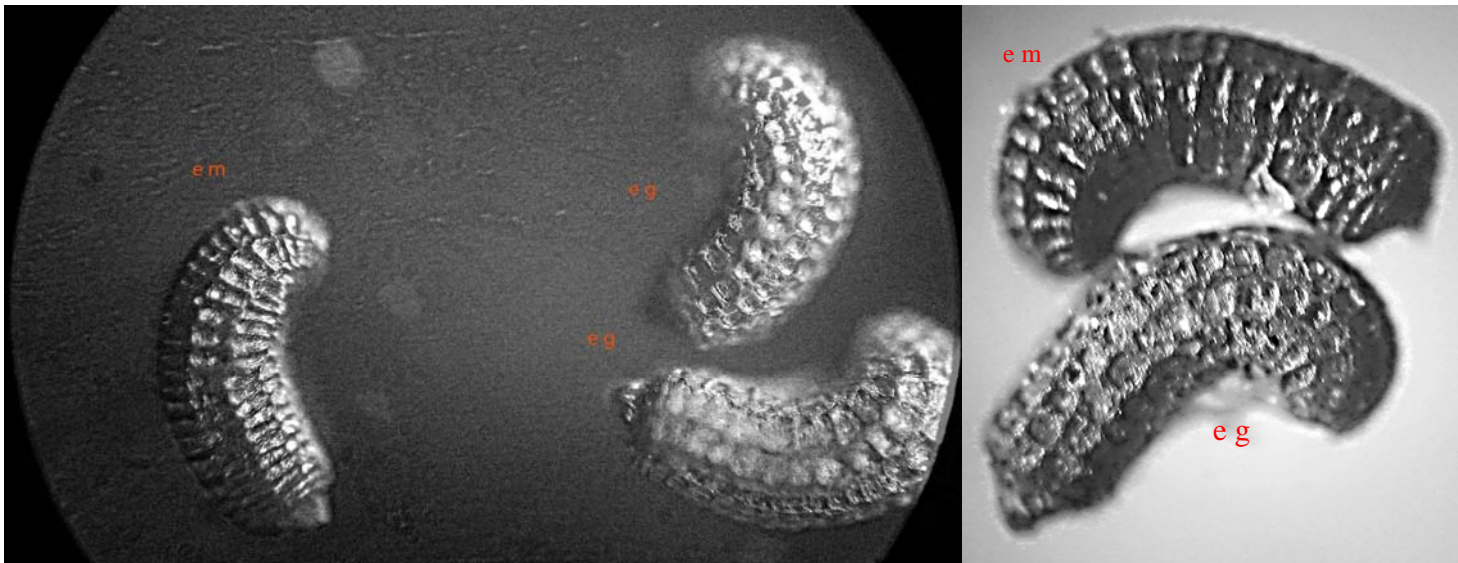


Figure 22 Difference between *E. gussonei* and *E. macropoda* seed coat reticulation is very evident

- The seed coat reticulation of *E. gussonei* seed is also very different from *E. hydropiper* seed as described and depicted in literature (see Figure 6).
- Seeds from *E. gussonei* in various pools in Malta, from Mellieha to Birzebugia, showed the same seed coat reticulation pattern.

9. Seed ‘semi lunar’ membrane

Sommier describes *E. gussonei* seeds as having a ‘semilunar’ membrane in the concave side of the seed¹ (as in Fig 6). This membrane is correlated to the angle of curvature. The greater the curvature, the more evident the ‘membrane’. This appears to be a thickening of the testa on the concave side of the seed. Since seeds of *E. gussonei* tended to show a higher degree of curvature, they also tended to have a very apparent ‘semilunar membrane’. However strongly curved seeds of *E. macropoda* (as in Figure 20) also exhibited this ‘membrane’ and so this feature could not be used to differentiate the two species.

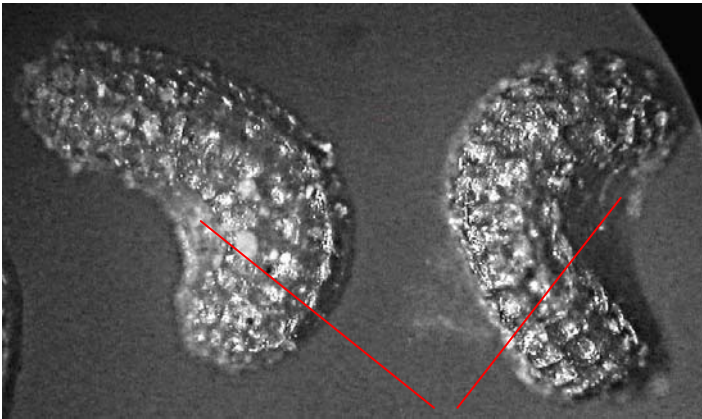


Figure 23 Seeds of *E. gussonei* exhibiting ‘semilunar membrane’

Conclusions

This study has shown that with most regards *E. gussonei* (Malta) is not easily distinguished from *E. macropoda* (Majorca). The usual defining characteristics for flowers of *E. gussonei* (pink flowers, petals as long as sepals) are highly variable according to environmental conditions and stage of flowering. The important distinction of a greater seed curvature in *E. gussonei* compared to *E. macropoda*, although true - if averages are used, has much overlap, and could lead to confusion if only a few seed curvatures are recorded.

However there is a more important diagnostic characteristic of *E. gussonei*, (as yet unpublished,) and that is the distinctive reticulation of the seed testa. The wide hexagonal shape of pits in *E. gussonei* and smaller number of pits/row (15 ± 3) is very difficult to confuse with *E. macropoda*'s 21 ± 3 narrow pits/row. On its own this is enough to distinguish the two species. It is also this significant characteristic which backs the status of species to Malta's only *Elatine*.

This support to the uniqueness of *Elatine gussonei* should also be taken into account for the conservation of the few scattered and highly vulnerable temporary pools in which populations still exist.

Note: This study is only relevant to *Elatine macropoda* from Majorca and *Elatine gussonei* from Malta. Further study on the same *Elatine* species in other countries may have differing results. It would therefore be very interesting to confirm the seed-testa reticulation pattern of *E. gussonei* from Lampedusa, while DNA studies should provide further insights as to the relationships between the various *Elatine* species and varieties worldwide.

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