This manuscript is contextually identical with the following published paper:
Takács A; Molnár VA; Horváth O; Sramkó G; Popiela A; Mesterházy A; Lovas-Kiss Á; Green AJ; Löki V; Nagy T; Lukács BA (2017) The rare aquatic angiosperm Elatine gussonei (Elatinaceae) is more widely distributed than previously thought. - AQUATIC BOTANY 141: pp. 47-50.
The original published PDF available in this website:
http://www.sciencedirect.com/science/article/pii/S0304377016302509?via%3Dihub

The rare aquatic angiosperm *Elatine gussonei* (Elatinaceae) is more widely distributed than previously thought

Attila Takács[a,b#], Attila Molnár V.[a, #], Orsolya Horváth[a], Gábor Sramkó[a,b], Agnieszka Popiela[c], Attila Mesterházy[d], Ádám Lovas-Kiss[a], Andy J. Green[e], Viktor Löki[a], Timea Nagy[f] & Balázs A. Lukács[g*]

[a] Department of Botany, University of Debrecen, Egyetem tér 1., H-4032, Debrecen, Hungary;
[b] MTA-DE "Lendület" Evolutionary Phylogenomics Research Group, Egyetem tér 1., H-4032, Debrecen, Hungary;
[c] Department of Botany and Nature Conservation, University of Szczecin, Felczaka 3c, 71-412 Szczecin, Poland;
[d] Hunyadi u. 55., H-9500 Celldömölk, Hungary;
[e] Estación Biológica de Doñana, EBD-CSIC, C/ Américo Vespucio, 26. 41092, Seville, Spain;
[f] Department of Plant Sciences and Biotechnology, University of Pannonia, Georgikon Faculty, Festetics u. 7., H-8360 Keszthely, Hungary;
[g] Department of Tisza River Research, MTA Centre for Ecological Research-DRI, Bem tér 18/C. H-4026, Debrecen, Hungary.

[#] first two authors contributed equally to this work.

*Corresponding author. E-mail: lukacs.balazs@okologia.mta.hu

Abstract

Elatine gussonei (Sommier) Brullo et al. is a rare freshwater plant previously assumed to be endemic to Lampedusa and the Maltase archipelago. Taxonomic uncertainties within the *Elatine* genus may have caused E. gussonei populations to be overlooked in the Mediterranean region. To clarify the distribution of *E. gussonei*, we reviewed

Elatine specimens from 12 herbaria and conducted eight field surveys in Mediterranean countries. Through our herbarium review we documented previously unknown occurrences of E. gussonei from Portugal, Spain, France, Algeria, Egypt, Cyprus and Israel. Additionally, in field studies we found populations from Cyprus, Morocco and Spain. *Elatine gussonei* is therefore more widespread than previously assumed, although the species still has a scattered distribution around the Mediterranean and many of the records are old. We found intact *E. gussonei* seeds in the faeces of migratory Greylag Geese (*Anser anser*) collected in southern Spain, suggesting that *E. gussonei* has a high capacity for long distance dispersal.

Keywords: aquatic plant, macrophyte, conservation, endozoochory, distribution, overlooked species, herbaria, Mediterranean temporary ponds, natural history collections, waterwort

1. Introduction

Plant species of the class Isoëto-Nanojuncetea are rare elements of the European flora, and many are threatened by extinction (Bilz et al., 2011; Lukács et al., 2013). The genus *Elatine* L. suffers from taxonomic uncertainty due to a high degree of phenotypic plasticity (Mason, 1956; Molnár et al., 2015), and the distribution and habitat requirements of different species are unclear. The different species are distributed mostly in the northern hemisphere, predominantly in areas with a temperate climate, but the size of their known geographical range varies from extensive (e.g. *E. alsinastrum* L.) to very restricted (e.g. *E. gussonei* (Sommier) Brullo et al.). The clarification of taxonomic status and the reliable assessment of distribution is required for the effective conservation of any species (Primack, 2010). Mapping distributions also allows us to answer fundamental questions in ecology, such as patterns of abundance, rarity, or species richness at different spatial scales (Baselga et al., 2012).

Elatine gussonei (Fig. S2) was first described as *E. hydropiper* var. *gussonei* Sommier based on the shape of the seed and the length of flower pedicels (Sommier, 1908), and was later classified as a separate species (Brullo et al., 1988). It is rare and threatened, and was considered to be endemic to the archipelago of the central part of the Mediterranean Basin (Lampedusa and Malta; Sommier, 1908; Mifsud, 2006; Kalinka et al., 2014) until new locations were recently discovered in Sicily (Molnár et al., 2014; Minissale and Sciandrello, 2014). According to the IUCN Red List (Lansdown, 2011), the population trend of *E. gussonei* is decreasing and the total area of occupancy is unlikely to exceed 100 km2. As part of ongoing research into the taxonomy, biology and distribution of *Elatine* species in Europe we visited several suitable habitats and reviewed existing herbarium sheets. Here, we report new records of *E. gussonei* around the Mediterranean region which indicate a major extension of the previously recognized distribution. According to our recently published molecular phylogeny (Sramkó et al., 2016), *E. gussonei* is clearly a separate species. Samples from Sicily, Morocco and Spain form a monophyletic group, whereas samples from

Lampedusa and Malta are clustered together with *E. macropoda* Guss. due to recent hybridisation between the two species (Sramkó et al., 2016). Unfortunately, Sramkó et al. (2016) failed to point out this hybrid lineage *E. gussonei* × *macropoda* in their paper. If this hybrid lineage is excluded, *E. gussonei* is delimited as a monophyletic entity.

The main aims of our study were 1) to clarify the distribution range of *E. gussonei* in the Mediterranean Basin and 2) to report evidence for long-distance dispersal capacity which helps to explain its scattered distribution over an extensive range.

2. Materials and methods

Distribution data *of E. gussonei* were obtained from field sampling and the examination of a total of 293 specimens (Table 1) deposited in 12 herbaria (Table S1). We studied herbarium sheets of *Elatine* taxa with opposite leaves and tetramerous flowers that were collected in the Mediterranean.

Field sampling campaigns were conducted in Morocco, Portugal, Spain, Malta, Sicily, Sardinia, Lampedusa and Cyprus. During field sampling, we visited those sites of *E. gussonei* identified from the reviewed herbarium sheets, and we searched suitable habitats (temporary and permanent ponds) for the species in surrounding areas.

Because vegetative characteristics show high overlap between *Elatine* species (Mifsud, 2006; Molnár et al., 2015), we paid particular attention to the seeds, to ensure correct identification. The main differentiation characters between the tetramerous *Elatine* species in Europe are observable in the seed curvature and the reticulation on the testa (Molnár et al., 2015) (Fig. S1). These characters were evaluated according to the identification key presented by Popiela et al. (2017). Namely, the specimens which have seeds with (80–)180–247(–347)° curvature on average, and have 17–23(–32) hexagonal pits in the middle row of the seed reticulation, were recorded as *E. gussonei*.

In late winter (12 February 2016), we collected 10 faecal samples of Greylag Goose (*Anser anser*) from Caño de Rosalimán in Doñana National Park (N 37.07513°, W 06.39077°) in southern Spain, where *E. gussonei* was recorded according to our reassessment of herbarium sheets. The mean mass of each sample was 1.99 g (range 0.69–6.53 g). Samples were closely inspected to avoid contamination from soil or plant parts before placing them in individual plastic bags and storage at 5 °C until processing. Faeces were washed in a 100 μm sieve with deionised water. Sieved contents were then inspected with a Zeiss microscope to locate seeds. After the separation, we immediately conducted germination tests by placing all seeds on moistened filter paper in Petri-dishes. The paper was regularly irrigated during the tests. The Petri-dishes were placed in a germination chamber with a 12 h:12 h photoperiod. During the light period the temperature was 22 °C and during the dark period it was 18 °C. Seeds were checked daily for germination for 30 days.

3. Results and Discussion

Taxonomic and biogeographical research of phenotypically plastic species is particularly important for species of conservation importance such as *E. gussonei*. We reviewed 293 sheets of the tetramerous species of the *Elatinella* section in 12 herbaria (Table S1). For 85 sheets (29%), we could not make a reliable identification mainly due to the lack of seeds. We found *E. gussonei* in 79 sheets with seeds, but most of them (74 specimens, 94%) had been misidentified. Altogether we identified 59 localities (Table S2). 27% of reviewed herbarium sheets (38% of the sheets with seeds) were classified as *E. gussonei*. Table 1 provides the taxon-names under which *E. gussonei* had been misclassified. We detected mixed specimens (*E. gussonei* + *E. macropoda*) in two cases (Table S2).

Our study indicates that *E. gussonei* has a considerably wider geographic distribution than formerly assumed and is quite widespread around the Mediterranean (Fig. 1). Beyond the previously known distribution (Lampedusa, Malta, Sicily) we found current populations in Morocco (Ben Slimane, N 33.61388°, W 07.10129°), Spain (Casar de Cáceres, N 39.55333°, W 06.42000°) and Cyprus (Peyia, N 34.88473°, E 32.35952°; Neo Chorio, N 35.01505°, E 32.30002°), and through the revision of herbarium material, we identified previously unknown sites from Portugal, Spain, France, Algeria, Egypt, Cyprus and Israel. Since most of the herbarium data originated before 1950, the current status of *E. gussonei* in these sites should be assessed. The extent to which *E. gussonei* has been under-recorded is illustrated by the fact that it has until now been overlooked in the Doñana wetlands in SW Spain, despite the fact that this area has been extensively studied (Green et al., 2016b).

In line with previous statements (Sommier, 1908; Mifsud, 2006; Molnár et al., 2014; Minissale and Sciandrello, 2014), *E. gussonei* mainly prefers limestone rock pools (Fig. S3 A–C). Besides that, we observed the presence of *E. gussonei* along muddy lakeshores (Fig. S3 D) and in temporary pools (Fig. S3 E). Based on the labels of the herbarium sheets, *E. gussonei* also prefers marshes (Algeria, Egypt), oxbows, ditches and temporarily inundated depressions (France) (Table S2). Extensive overlap in the distribution, ecology and morphology of *E. macropoda* (Mifsud, 2006; Popiela and Łysko, 2010) and *E. gussonei* has obviously contributed to the high frequency of misidentifications. At the same time, the single suitable distinctive feature, namely the seed morphological characters identified by Sommier (1908), have been widely ignored until now. Unfortunately, identification keys for Mediterranean *Elatine* taxa erroneously suggest that *E. macropoda* may have strongly curved (like a horseshoe) seeds (e.g. Cirujano and Velayos, 1993), leading to general confusion of the two species. Although seed curvature of *E. gussonei* can vary within certain limits, hybridisation increases the level of variability observed. For example, the Maltese samples, which are clearly hybrids between *E. gussone*i and *E. macropoda* (Sramkó et al., 2016) have less curved seeds (see Fig. 5 of Sramkó et al., 2016; Fig. S1). Although Sramkó et al. (2016) clearly demonstrated monophyly and lack of hybridization for Spanish, Moroccan and Italian samples of *E. gussonei*, molecular studies are required to confirm that there are no

hybrids in the new *E. gussonei* localities we have identified during the herbarium revision.

These new distribution data of *E. gussonei* bring into question the presence of *E. hungarica* in Portugal, where it is reported as an introduced species (Uotila, 2009). This report from Portugal originated from Cook (1968) and Greuter et al. (1986) but the source of the data is unknown. Our results suggest that this record refers to *E. gussonei*. These two taxa are morphologically very similar and distinguishable almost exclusively by the reticulation of the seed surface (Molnár et al., 2015; Popiela et al., 2017). Moreover, molecular analyses revealed that *E. hungarica* represents a northern Eurasian clade (subsect. *Hydropiperia*), while *E. gussonei* are set in a Mediterranean clade (subsect. *Macropodae*) within the *Elatinella* section (Sramkó et al., 2016).

There is a shortage of previous information on how plants of the genus *Elatine* disperse, partly because these small seeds are so easily overlooked. Water (hydrochory) is likely to be a major means of local dispersal. *E. triandra* can disperse by hydrochory during flooding within a restored floodplain (Hayashi et al., 2012). At a broader scale, birds are likely to be the most important means of dispersal. Kerner von Marilaun and Oliver (1895) collected mud from nests of swallows, snipes, wagtails, jackdaws and found seeds of *E. hydropiper* embedded within the mud. Molodovsky (1971) found the same species in the digestive tract of Eurasian teal *Anas crecca*. Migratory waterbirds are likely to be particularly effective vectors for *Elatine* species (Green et al., 2016a).

In Doñana, an average of 50,000 Greylag Geese spend the winter, making frequent daily flights of 5–20 km between feeding and roosting sites (Rendón et al., 2008). At the time of our sampling in February, the geese were already beginning their return migration, making non-stop flights to central and northern Spain (Ramo et al., 2015). The geese we sampled were eating mainly Cyperaceae (*Bolboschoenus maritimus* and *Schoenoplectus litoralis*) tubers (Amat and Varo, 2008) within the temporary marshes of Doñana (Green et al., 2016b) and they readily swallow small seeds within the sediments in an incidental manner. Three of the ten faecal samples from geese from Spain had *E. gussonei* seeds, with a combined total of seven seeds. In addition, two Charophyceae oogonia and one seed of *Callitriche truncata* were found. None of the propagules germinated within 30 days. The seeds and oogonia from the seed bank may have been old, of low viability, or with a dormancy that is hard to break, and this may explain why no *E. gussonei* seeds germinated. On the other hand, each sample represented only a small fraction of daily faeces produced by each bird (Hahn et al., 2008). Since there are so many geese and ducks in Doñana (Rendón et al., 2008), our results suggest that *E. gussonei* seeds are frequently dispersed by migratory waterbirds. There are still relatively few studies of seed dispersal by waterfowl (Green et al., 2016a) and we have no data to compare the dispersal potential of other *Elatine* species.

3.1. Conclusions

Previous underestimation of the wide geographical range of *E. gussonei* may be explained by several factors. Firstly, Mediterranean temporary rock pools are understudied habitats. *Callitriche pulchra*, which grows in similar habitats, has also recently been shown to have a much wider geographic area and lower extinction risk than previously reported by Lansdown et al. (2016). Secondly, the high degree of morphological variability (i.e. environmentally induced phenotypic plasticity) of *Elatine* species contributes to taxonomic uncertainties and can cause misidentification by researchers using out-of-date identification keys (Molnár et al., 2015). Therefore, the great number of misidentifications and the belief that such a rare species cannot grow far from areas within the known range can lead to a bias in the known distribution pattern. The revision of the most similar species, as we conducted in several herbaria, can resolve such issues and reveal new sites. Thirdly, the strong potential for seed dispersal by waterbirds supports the likelihood for a more extensive distribution area. This suggests that E. gussonei can readily disperse over long distances, therefore further biogeographical investigations are required in its potential habitats across the Mediterranean, especially in North Africa.

4. Acknowledgments

The authors are grateful to Anna E. Vojtkó, Ágnes Mosolygó-Lukács, Edvárd Mizsei, Endre Bajka, Judit Sonkoly, Kristóf Süveges and Réka Fekete for their assistance during field work and to Nikola Rahmé (Budapest) for seed photographs. We especially thank Robert Vogt (B), Lajos Somlyay (BP), Mihai Puscas (CL), Alexander Sennikov and Pertti Uotila (H), Georges Barale (LY), Mauricio Velayos and Rosario Noya Santos (MA), Otakar Sida (PR), Patrik Mráz (PRC), Montserrat Arista (SEV), Trinidad Ruiz Téllez (UNEX) and Bruno Wallnöfer (W) for their very kind help in herbarium revision work.

Faeces were collected from Doñana under permit from the Doñana Natural Space (N 2014/31). Sample processing and germination tests were initiated in the Aquatic Ecology Laboratory (LEA-EBD). The work was financially supported by the European Union and the State of Hungary and co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/2-11/1-2012-0001 “National Excellence Program”. The instrumental and infrastructural support of OTKA K108992 is also highly appreciated. BAL was supported by the GINOP-2.3.2-15-2016-00019 project and the OTKAPD120775 Grant. ALK was supported by the Tempus Public Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aquabot.2017.05.004.

5. References

Amat, J.A., Varo, N., 2008. Grit ingestion and size-related consumption of tubers by Greylag Geese. Waterbirds 31, 133–137.

Baselga, A., Lobo, J.M., Svenning, J.C., Araújo, M.B., 2012. Global patterns in the shape of species geographical ranges reveal range determinants. J. Biogeogr. 39, 760–771.

Bilz, M., Kell, S.P., Maxted, N., Lansdown, R.V., 2011. European Red List of Vascular Plants. Publication Office of the European Union, Luxembourg.

Brullo, S., Lanfranco, E., Pavone, P., Ronsisvalle, G., 1988. Taxonomical notes on the endemic flora of Malta. Giorn. Bot. Ital. 122, 45.

Cirujano, S., Velayos, M., 1993. Elatine L. In: In: Castroviejo, S. (Ed.), Flora Iberica, vol. 3. Real Jardín Botanico, CSIC, pp. 155–156.

Cook, C.D.K., 1968. Elatine L. In: In: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), Flora Europaea, vol. 2. Rosaceae–Umbelliferae, Cambridge, pp. 295–296.

Green, A.J., Brochet, A.L., Kleyheeg, E., Soons, M.B., 2016a. Dispersal of plants by waterbirds. In: Şekercioğlu, C.H., Wenny, D.G., Whelan, C.J. (Eds.), Why Birds Matter: Avian Ecological Function and Ecosystem Services. University of Chicago Press, pp. 147–195.

Green, A.J., Bustamante, J., Janss, G.F.E., Fernández-Zamudio, R., Díaz-Paniagua, C., 2016b. Doñana wetlands. In: Finlayson, C.M., Milton, G.R., Prentice, R.C., Davidson, N.C. (Eds.), The Wetland Book: II: Distribution, Description and Conservation. Springer, Netherlands. http://dx.doi.org/10.1007/978-94-007-6173-5_139-1.

Greuter, W., Burdet, H.M., Long, G., 1986. Quantification of allochthonous nutrient input into freshwater bodies by herbivorous waterbirds. Freshwater Biol. 53, 181–193 Med-Checklist 3, Genève & Berlin.

Hahn, S., Bauer, S., Klaassen, M., 2008. Quantification of allochthonous nutrient input into freshwater bodies by herbivorous waterbirds. Freshwater Biol. 53, 181–193.

Hayashi, H., Shimatani, Y., Shigematsu, K., Nishihiro, J., Ikematsu, S., Kawaguchi, Y., 2012. A study of seed dispersal by flood flow in an artificially restored floodplain. Landscape Ecol. Eng. 8, 129–143.

Kalinka, A., Mifsud, S., Popiela, A., Achrem, M., 2014. Chromosome number of *Elatine gussonei* (Sommier) Brullo (*Elatinaceae*) and its distribution on the Maltese islands. Acta Bot. Croat. 73, 267–273.

Kerner von Marilaun, A., Oliver, F.W., 1895. The Natural History of Plants: Their Forms, Growth, Reproduction and Distribution. Blackie Son, Ltd.

Lansdown, R.V., Kefalas, K., Bazos, I., 2016. New information on the status and distribution of *Callitriche pulchra* (*Plantaginaceae*), including a first record from Cyprus. Willdenowia 46, 379–385.

293 Lansdown, R.V., 2011. *Elatine Gussonei*. The IUCN Red List of Threatened Species.
294 e.T162252A5564512. http://www.iucnredlist.org/details/162252/0 (Accessed 18
295 November 2016).
296 Lukács, B.A., Sramkó, G., Molnár, V.A., 2013. Plant diversity and conservation value
297 of continental temporary pools. Biol. Conserv. 158, 393–400.
298 Mason, H.L., 1956. New species of Elatine in California. Madrono 13, 239–240.
299 Mifsud, S., 2006. A comparative study between *Elatine gussonei* (from Malta) and
300 *Elatine macropoda* (from Majorca). Aquat. Gard. https://www.aquatic-gardeners.org/
301 Articles/elatine/Comparitive_study.pdf (Accessed 15 May 2017).
302 Minissale, P., Sciandrello, S., 2014. Ecological features affect patterns of plant
303 communities in Mediterranean temporary rock pools. Plant Biosyst. 150, 171–179.
304 Molnár, V.A., Popiela, A., Lukács, B.A., 2014. *Elatine gussonei* (Sommier) Brullo et al.
305 (*Elatinaceae*) in Sicily. Plant Biosyst. 27–30.
306 Molnár, V.A., Tóth, J.P., Sramkó, G., Horváth, O., Popiela, A., Mesterházy, A., Lukács,
307 B.A., 2015. Flood induced phenotypic plasticity in amphibious genus *Elatine*
308 (*Elatinaceae*). PeerJ 3, e1473.
309 Molodovsky, A.O., 1971. Feeding of *Anas crecca* L. and A. querquedula L. of the
310 gorky reservoirs. Biol. Nauki 11, 20–25.
311 Popiela, A., Łysko, A.R., 2010. The distribution of *Elatine macropoda* guss.
312 (*Elatinaceae*). Acta Soc. Bot. Pol. 79, 81–86.
313 Popiela, A., Łysko, A., Białecka, B., Bihun, M.M., Sramkó, G., Staroń, W., Wieczorek,
314 A., Molnár, V.A., 2017. Seed morphometric characteristics of European species of
315 *Elatine* (*Elatinaceae*). PeerJ. http://dx.doi.org/10.7717/peerj.3399. 5:e3399.
316 Primack, R.B., 2010. Essentials of Conservation Biology, 5th ed. Sinauer Associates,
317 Sunderland (538 pp).
318 Ramo, C., Amat, J.A., Nilsson, L., Schricke, V., Rodriguez-Alonso, M., Gomez-Crespo,
319 E., Jubete, F., Navedo, J.G., Masero, J.A., Palacios, J., Boos, M., Green, A.J., 2015.
320 Latitudinal-related variation in wintering population trends of Greylag geese (*Anser*
321 *anser*) along the Atlantic flyway: a response to climate change? PLoS One 10,
322 e0140181.
323 Rendón, M.A., Green, A.J., Aquilera, E., Almaraz, P., 2008. Status, distribution and
324 longterm changes in the waterbird community wintering in Donana south-west
325 Spain. Biol. Conserv. 141, 1371–1388.
326 Sommier, S., 1908. Le isole Pelagie, Lampedusa, Linosa, Lampione e la loro flora con
327 un elenco complete delle piante di Pantelleira. Firenze, Pellas 345 pp.
328 Sramkó, G., Molnár, V.A., Tóth, J.P., Laczkó, L., Kalinka, A., Horváth, O., Skuza, L.,
329 Lukács, B.A., Popiela, A., 2016. Molecular phylogenetics, seed morphometrics,
330 chromosome number evolution and systematics of European *Elatine* L. (*Elatinaceae*)
331 species. PeerJ 4, e2800. http://dx.doi.org/10.7717/peerj.2800.
332 Uotila, P., 2009. Elatinaceae. – In: Euro. http://ww2.bgbm.org/EuroPlusMed/(Accessed
333 21 January 2015).
334

Figure captions

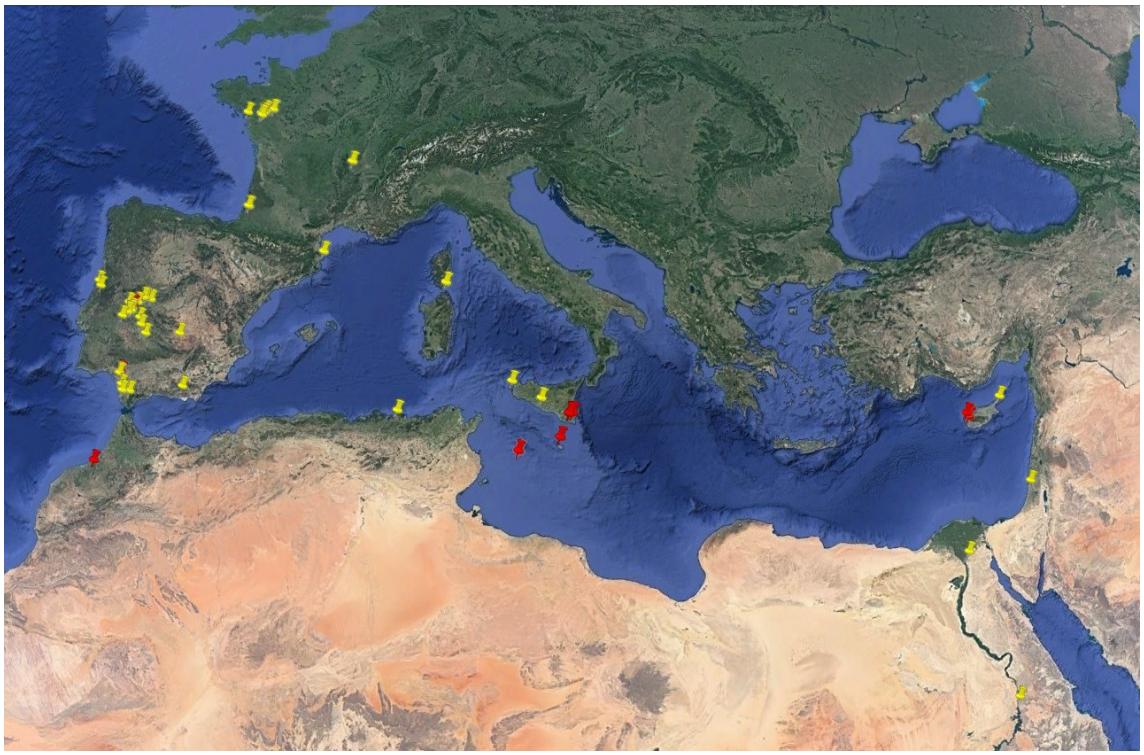

Figure 1. Revised distribution of *Elatine gussonei* based on herbarium and field records (highlighted with yellow and red marks, respectively)..

347 **Tables**

348

349 **Table 1.** The number of reviewed sheets and the number of sheets where *Elatine*
350 *gussonei* was found, sorted by the original taxon name on the labels.

351

Taxa name on the label	Number of reviewed sheets	Number of sheets with seeds	Number of *E. gussonei* sheets	Proportion of misidentified *E. gussonei* sheets (%)
E. macropoda	190	128	36	28
E. campylosperma (incl. *hydropiper* var. *pedunculata; hydropiper* f. *campylosperma*)	74	63	33	52
E. gussonei (incl. *hydropiper* var. *gussonei*)	6	5	5	0
E. major	8	5	2	40
E. aquatica	1	1	1	100
E. hydropiper	7	2	1	50
E. fabri	6	3	1	33
E. hardyana	1	1	0	0

352