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Rapid Communication

Morphological and molecular data confirm the first European record of *Sagina maxima* (Caryophyllaceae) in Bassano del Grappa (Veneto region, northeastern Italy)

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Abstract

A species belonging to the genus *Sagina*, that has been known since at least 2016 from several localities in the city of Bassano del Grappa (Veneto region, northeastern Italy), was identified as *S. maxima*. It is a species from (predominantly) coastal areas in western North America and Far Eastern Asia. Morphological and molecular data confirmed this identification, although this finding raises some questions about the ecology and morphology of *S. maxima* and some of its relatives and/or similar-looking species. These records appear to be the first in Europe. A concise morphological description and illustrations are provided along with a comparison with related and similar-looking *Sagina* species. The status of *S. maxima* in Italy is also briefly discussed and a key for the identification of the Italian species from the genus is presented.

Key words: adventive, East Asia, naturalization, North America, urban flora

Introduction

Sagina L. is a relatively small genus with about 30–33 species (Alban et al. 2022) or even much less according to some authors, e.g. Crow (2005), depending on species delimitation. It predominantly occurs throughout the temperate regions of the northern hemisphere. A few species are confined to the southern hemisphere (e.g. Australia, South America and New Guinea) whereas in the tropics it is exclusively found at higher altitudes (Crow 1979; Timaná et al. 2019; Dillenberger and Kadereit 2022; Iamonico et al. 2023). The center of diversity of the genus is in Europe where about 16 species are found (Clapham and Jardine 1993; Marhold 2011+). Out of these, 10 species—all native—are found in Italy (Iamonico 2016; Iamonico et al. 2023).

A comprehensive monographic study of *Sagina* is lacking but relatively recent accounts are available for most parts of the northern hemisphere: Europe (Clapham and Jardine 1993), North America (Crow 2005), China (Lu and Rabeler 2001), Russia (Shteinberg 1970) and Japan (Mizushima 1960).

The monophyly of the genus *Sagina* was recently confirmed in a comprehensively sampled molecular phylogeny (Alban et al. 2022).

In the course of recent floristic studies, aimed at establishing a thorough inventory of the urban flora of Bassano del Grappa (Veneto region, Italy), an unknown species of *Sagina* was found. It turned out to belong to *S. maxima* A. Gray, a species from temperate East Asia, the Aleutian Islands and the western U.S.A., that had not been recorded before in Europe.

Materials and methods

Sampling and morphological identification

Since 2016 an unknown species of *Sagina* has been known from Bassano del Grappa (Veneto region, northeastern Italy) (Figure 1). The plants stood out because they were coarser than their native congeners and, in a way, looked more like *Spergularia* (Pers.) J. Presl & C. Presl than to *Sagina*. The collected specimens were obviously alien to the area and were, after consulting numerous relevant literature sources and online herbarium specimens, eventually identified as *S. maxima*, a species from fairly natural habitats in its area of origin and therefore rather unexpected as an alien species. In addition, owing to taxonomic difficulties in the genus and a striking resemblance of this species to others such as *S. japonica* (Sw.) Ohwi, we decided to verify this identification by means of a molecular phylogenetic analysis. Voucher herbarium specimens are preserved in the personal herbarium of G. Busnardo and in the Civic Museum of Rovereto, Italy (ROV) and duplicates were deposited at the herbaria of Meise Botanic Garden, Belgium (BR) and the Natural History Museum of Florence University, Italy (FI).

DNA extraction, amplification and sequencing

The nuclear rDNA internal transcribed spacer (ITS) region and the plastid atpB-rbcL intergenic spacer were selected as markers for phylogenetic analysis and for guiding species identification. The selection of these markers was based on their widespread availability from previous studies (Alban et al. 2022; Dillenberger and Kadereit 2022). Total genomic DNA was extracted from silica-dried leaf material using a modified CTAB protocol (Doyle and Doyle 1987; Verloove et al. 2020). The ITS region was amplified using primers ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GAAG TAAAAGTCGTAACAAGG-3') (White et al. 1990), and the atpB-rbcL spacer was amplified using primers rbcL1 (5'-AACACCAGCTTTRAAT CCAA-3') and atpB1 (5'-ACATCKARTACKGGACCAATAA-3') (Chiang et al. 1998). PCR conditions are described in Verloove et al. (2020), and PCR quality control was performed with a BioAnalyzer (Agilent Inc.). After purification using ExoSAP-IT (ThermoFisher Scientific), PCR products were sequenced by Macrogen (Seoul, South Korea). Forward and reverse sequences were assembled using Geneious Prime v2023.2.1. (Biomatters,

Auckland, New Zealand). Sequences have been deposited in EMBL/GenBank under study number PRJEB70470 and accession numbers OY805160 (ITS) and OY805161 (atpB-rbcL).

Sequence alignment and phylogenetic analysis

Two datasets of 37 ITS (719 positions) and 29 atpB-rbcL sequences (847 positions) were constructed, including the sequences generated in this study and publicly available sequences obtained from GenBank (https://www.ncbi.nlm.nih.gov/genbank/), including the sequences from Alban et al. (2022) and closest BLAST hits (Supplementary material Table S1). Sequences were aligned using MUSCLE (Edgar 2004). Phylogenetic relationships were reconstructed using maximum likelihood and 1000 ultrafast bootstrap replicates in IQ-TREE 2.2.2.7 (Nguyen et al. 2015) on the CIPRES Science Gateway portal (Miller et al. 2010).

Results

Since 2016, *Sagina maxima* has been found in eight localities in Bassano del Grappa (Figure 1):

1. Several individuals on the corner between Vicolo Margnan and the wall of Palazzo Bombardini (25 April 2016);
2. On the pavement in Via Margnan (5 May 2020);
3. Several individuals between the pavement slabs of the "new bridge" over the Brenta river (7 April 2023);
4. A couple of individuals in Via Roma at the exit of the shopping center (27 April 2023);
5. A few individuals between the cobblestones at Piazza Garibaldi in front of the haberdashery shop (29 April 2023);
6. A couple of individuals in cracks in the asphalt at the bottom of the Margnan slope, at the corner of the old gate of the castle (29 April 2023);
7. A couple of individuals on the edge of the pavement/wall in the middle of Via Gamba (1 May 2023);
8. Three individuals on pavement slabs in Via del Museo, at the library entrance (2 May 2023).

Over the years it has been noted that the species persists very well in the oldest locations (nrs. 1 and 2 in the above list). Moreover, the species produces a lot of ripe seeds and has since spread to nearby parts of the city (location nrs. 3 to 8), as the crow flies up to more than 600 meters away from the place where the species was discovered in 2016. Only in locality nr. 3 the species has disappeared after resurfacing the bridge. It is found in typical urban, anthropogenic habitats such as between cobble stones, at the foot of walls and in cracks in concrete and asphalt.

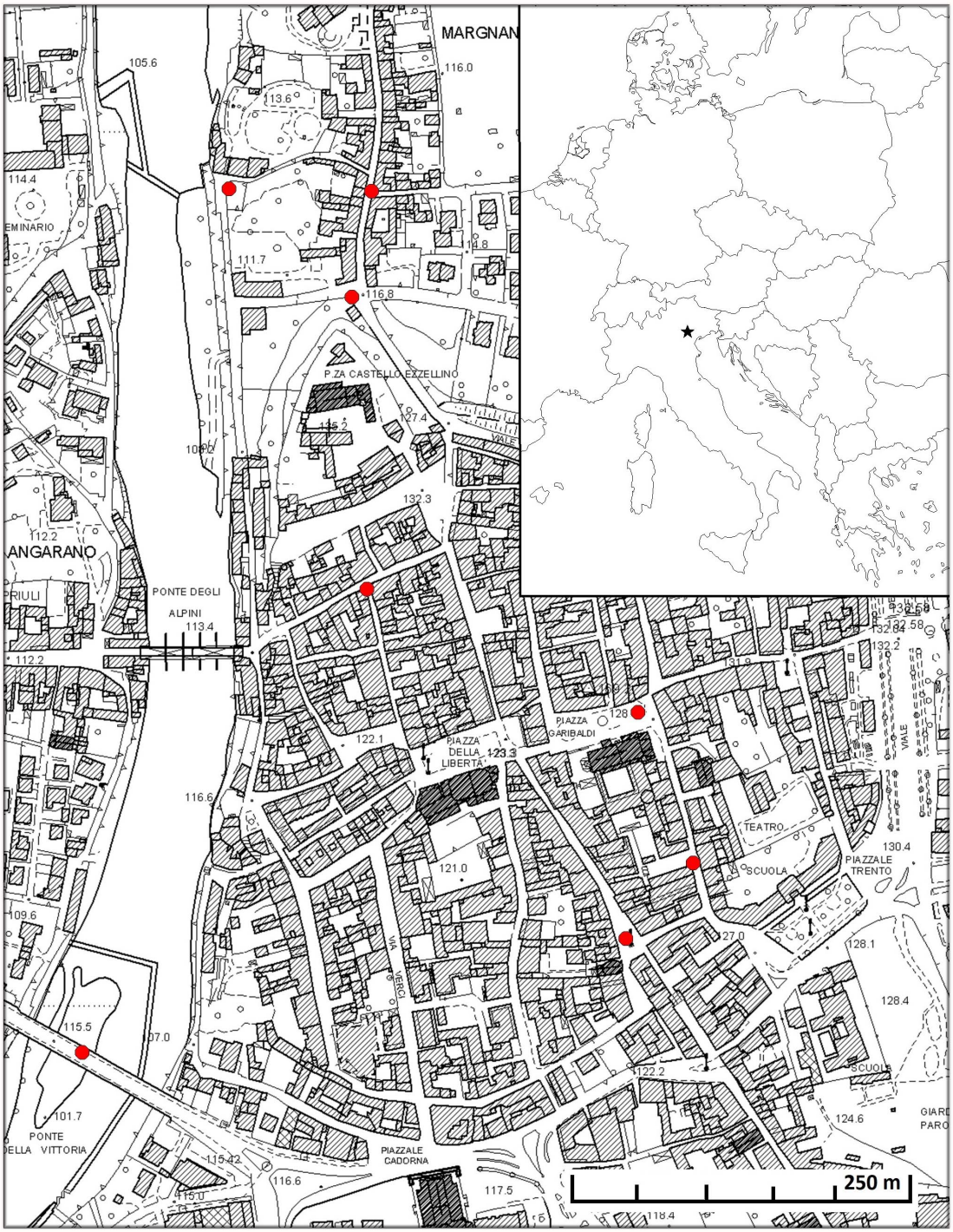

Figure 1. Localities (red dots) where *Sagina maxima* was found in Bassano del Grappa (Veneto region, Italy) between 2016 and 2023.

Sagina maxima A. Gray in Mem. Amer. Acad. Arts, n.s., 6: 382 (1859)

≡ *Sagina linnaei* C. Presl var. *maxima* (A. Gray) Maxim. in Bull. Acad. Imp. Sci. Saint-Pétersbourg, sér. 3, 18: 372 (1873).

Type: Japan: Hakodadi, Cape Sangar, *C. Wright* s.n. 1853–1856 (holotype GH-00037841, image available at: https://kiki.huh.harvard.edu/databases/specimen_search.php?mode=details&id=81893; isotype NY-00342601, image available at: https://sweetgum.nybg.org/science/vh/specimen-details/?irn=329117).

= *Sagina litoralis* Hultén in Kongl. Svenska Vetensk. Acad. Handl., ser. 3, 5(2): 78 (1928)

Type: South Kamchatka: Toporkof Island, Sarannaja Bay, *Hultén* 789 (holotype S G-7161, image available at: https://herbarium.nrm.se/specimens/S-G-7161).

Figure 2. Pressed ROV 80190 specimen of *Sagina maxima* from Bassano del Grappa. Because of the relative robustness and the short flower peduncles, the plants somehow resemble *Spergularia rubra*.

Morphology (Figures 2–4). A detailed description of *Sagina maxima* is available in Mizushima (1960), Crow (1978), Lu and Rabeler (2001) and Crow (2005).

The principal distinguishing characteristics of *S. maxima*, as observed in Bassano del Grappa, are: the annual life form; stems prostrate, densely glandular hairy; leaves without stipules, mucronate at apex, basal leaves long (up to 27 mm); flowering peduncles clearly shorter than to slightly longer than the subtending leaf (but see below); flowers 5-merous, with sepals rounded at apex with a hyaline margin, with petals slightly shorter than the sepals; five stamens; capsule opening with 5 (very rarely 6, but never 4) valves; seeds small (about 0.5 mm across or slightly less), pebbled, brown, without a dorsal groove (Figure 4).

Interestingly, in gross morphology and at first glance, *Sagina maxima* from Bassano del Grappa looks more like *Spergularia rubra* (L.) J. Presl & C. Presl than to any native *Sagina* species. An image of a dried specimen was even identified as such by the online plant identification app Pl@ntNet (https://identify.plantnet.org/)! This undoubtedly relates to its more robust

Figure 3. *Sagina maxima* in Bassano del Grappa. Plants are found in typical urban habitats, such as in cracks of concrete, between cobble stones, etc. Photo by Giuseppe Busnardo.

appearance and the short-peduncled flowers. Only upon closer inspection it is recognizable as a species of *Sagina*. It then clearly differs in seed shape from the native species of that genus: species from section *Maximae* Crow have reniform, almost globular seeds that lack a dorsal groove whereas species from section *Saginae* have obliquely triangular seeds with a dorsal groove (Crow 1978, 1979). In addition, in the native annual species flowers are 4-merous. However, morphologically, *S. maxima* is very similar to the only other member of section *Maximae*, *S. japonica*. The latter species has seeds that are distinctly tuberculate, not merely pebbled (Crow 1979; Figure 4).

It should be noted that in almost all the specimens and photographs of *Sagina maxima* found on the internet, including the type material, the flower peduncles are much longer than the corresponding leaf. In the Italian plants, in turn, the peduncles are clearly shorter than to slightly longer than the corresponding leaf. Plants determined as *S. maxima* with such short peduncles

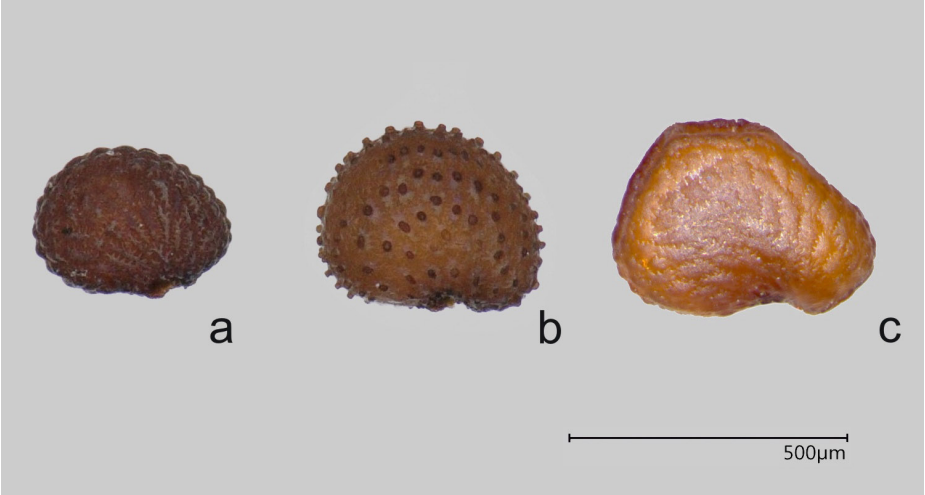

Figure 4. Seeds of (a) *Sagina maxima* (Bassano del Grappa: the seed surface is merely pebbled), (b) *S. japonica* (Antwerp port area, Belgium: the seed surface is clearly tuberculate) and (c) a species from section *Saginae*. In the latter species, the seeds are somewhat triangular in shape and a dorsal groove is discernible, in contrast with the reniform seeds that lack a dorsal groove in the first two species (section *Maximae*). Photo by Iris Van der Beeten and Marco Nave.

were seen from Japan, for example https://www.gbif.org/occurrence/4413407729 (also growing in pavement cracks, even if near the sea) and https://www.gbif.org/occurrence/912560912 (sub *S. taquetii* H. Lév., a heterotypic synonym of *S. maxima* subsp. *maxima* according to POWO 2024).

Based on the ornamented seeds and glandular pubescence, the plants found in Italy almost certainly originate in East Asia, since in populations from North America and the Aleutian Islands seeds have smooth surfaces and plants tend to be less glandular (Crow 1978, 2005). Such Asian plants were formerly segregated as *S. litoralis* Hultén, although some controversy surrounds this binomial (e.g. Mizushima 1960; Shteinberg 1970). However, they intergrade with other plants from East Asia and are therefore of no taxonomic relevance (Crow 1978).

At present two subspecies are accepted in *S. maxima* (POWO 2024): the entirely glabrous and strictly coastal subsp. *crassicaulis* (S. Watson) G.E. Crow and the pubescent subsp. *maxima*, to which the Italian populations belong.

In his review of *Sagina japonica* and *S. maxima*, Mizushima (1960) noted that these two species show great morphological similarity to *S. humifusa* (Cambess.) Fenzl ex Rohrb., a species native to the southern hemisphere (Argentina, Brazil and Uruguay). It shares the annual habit, glandular pubescence and papillose seeds with *S. japonica* and *S. maxima*, and required further study. Using the recent key to Brazilian species (Carneiro 2023), the Italian plants indeed easily key out to a couplet that includes two species, *S. humifusa* and *S. chilensis* Gay, both glandular annuals with 5-merous flowers. It seems that these two species, at least in gross morphology, are not easily distinguished from *S. maxima*, as already observed by Mizushima l.c. However, their seeds are somewhat triangular in shape and have a dorsal groove, unlike

the species in section *Maximae*. Moreover, according to the phylogeny, both ITS and plastid markers confirm that *S. chilensis*/*humifusa* clearly differ from *S. maxima*/*japonica*; they are placed in a southern hemisphere clade and are also distinct from *S. apetala* and relatives (Alban et al. 2022).

Molecular phylogeny. The ITS alignment comprised 37 sequences and 719 positions, with 105 variable positions. The atpB-rbcL alignment included 29 sequences and 847 positions, with 53 variable positions.

The ITS-based phylogeny (Figure 5) revealed that the Italian plant is positioned in a clade (bootstrap value = 100) together with specimens identified as *S. hookeri* Timaná, *S. japonica*, and *S. maxima* from various regions, including China, South Korea, Japan, Amsterdam Island (southern Indian Ocean) and North America. Within this clade, the Italian specimen was sister to a specimen identified as *S. japonica* from Aichi, Nagoya, Japan (GenBank accession number MT415640). Unfortunately, no publication or voucher is linked to this sequence, precluding a morphological examination of the plant.

The atpB-rbcL-based phylogeny (Figure 5) yielded less conclusive results regarding the phylogenetic placement of the Italian plant. It was positioned sister (bootstrap value = 84) to a specimen identified as *Sagina japonica* from Qimen County, Anhui Province, China (MT534488), a sequence published by Alban et al. (2022) with a voucher deposited in the herbarium of Oregon State University (OSC 177365). Unfortunately, the identity of this specimen could not be verified. In contrast to the ITS-based phylogeny, *S. hookeri*, *S. japonica*, and *S. maxima* did not form a well-supported clade.

Examined specimens. **ITALY, Veneto**: Bassano del Grappa sull'angolo tra vicolo Margnan e il muro di Palazzo Bombardini (45.770886°N; 11.731708°E), 110 m, sul selciato, 25 April 2016, leg. *G. Busnardo s.n.*, det. F. Prosser and F. Verloove (ROV 80190, BR0000027058602V). Bassano del Grappa, in via Margnan (45.770836°E; 11.733163°N), 115 m, sul selciato, 15 May 2020, leg. *G. Busnardo s.n.*, det. F. Prosser and F. Verloove (FI, ROV 80189). Bassano del Grappa, sul "Ponte nuovo" sul F. Brenta (45.764899°E; 11.729987°N), 115 m, diversi individui tra le lastre del marciapiede, 7 April 2023, *G. Busnardo s.n.* (Herb. Busnardo).

Phenology in Italy. Flowering and fruiting from April to May.

Habitat and ecology. On the Pacific coast in North America, *Sagina maxima* grows on rocky or sandy bluffs, along rocky shores and on gravelly-sandy beaches (Crow 1978, 2005). In Japan too, it mostly is a seashore plant (Mizushima 1960), whereas in China it also occurs further inland in rocky mountain slope meadows and in more disturbed habitats such as fields (Lu and Rabeler 2001). In Italy, it is found in anthropogenic habitats: between cobble stones, at the foot of walls and in cracks in concrete. Plants accompanying *S. maxima* are *Eleusine indica* (L.) Gaertn., *Eragrostis minor* Host, *Plantago major* L., *Poa annua* L., *Polycarpon tetraphyllum* (L.) L.,

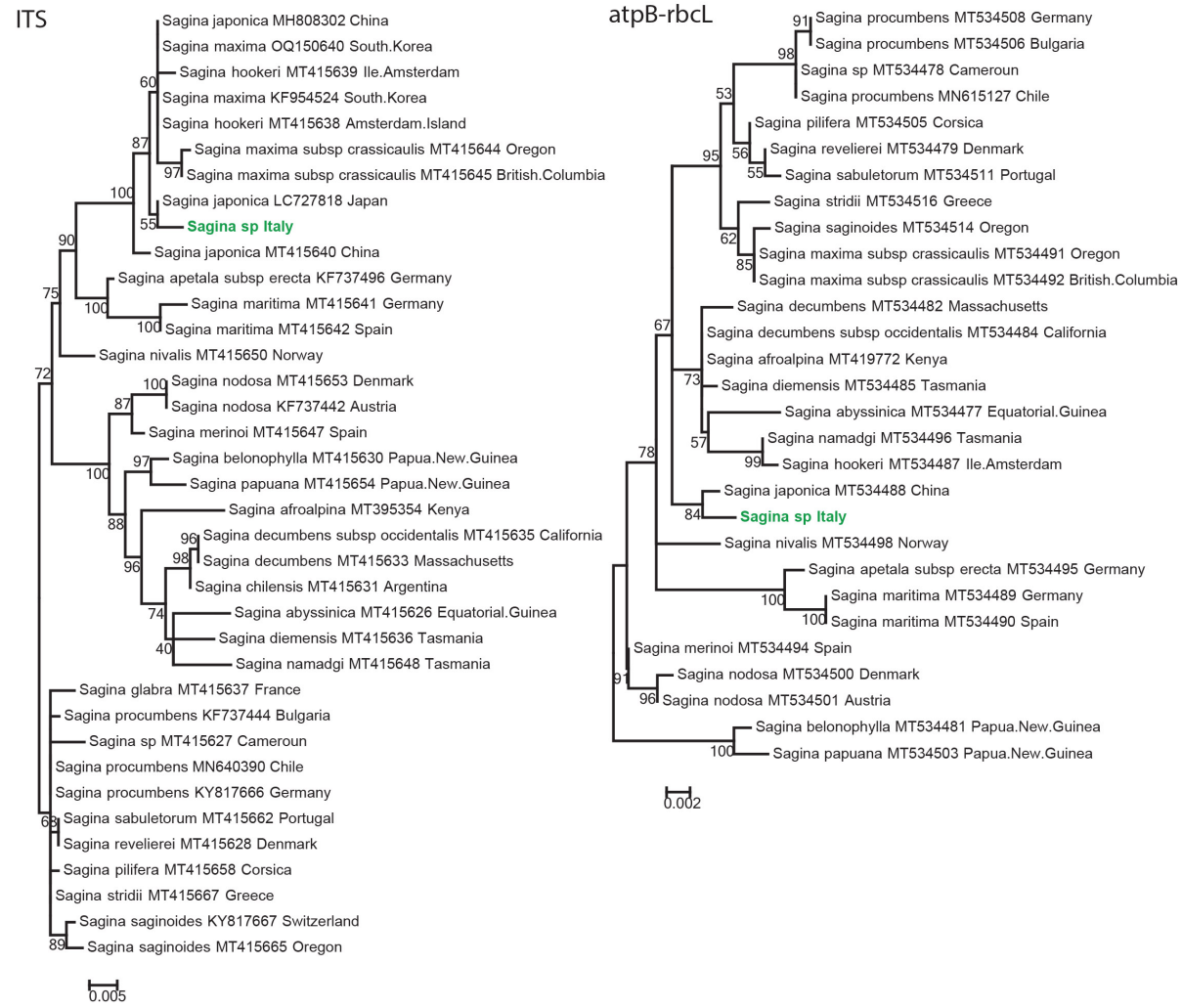

Figure 5. Maximum likelihood trees (IQ-TREE) inferred from nuclear rDNA internal transcribed spacer (ITS) region and the plastid atpB-rbcL intergenic spacer sequences, showing the position of the Italian *Sagina* plant. Values at branches correspond to UFBoot2 supports (1000 replicates). Scale represents estimated substitutions per site.

Sagina apetala Ard., *S. procumbens* L., *Stellaria media* (L.) Vill. and *Taraxacum* sect. *Taraxacum* F.H. Wigg., species typical of the class Polygono arenastri-Poetalia annuae Tüxen in Géhu, Richard & Tüxen 1972 corr. Rivas-Martínez, Báscones, T.E. Díaz, Fernández-González & Loidi 1991.

Introduction and current naturalization status in Italy. The exact introduction mode of *Sagina maxima* remains unknown. There are local trade activities that are more or less in connection with the Far East, but there is no clear source. The species seems to be a recent introduction and is only known from eight localities in a single city (although it cannot be excluded that the species is overlooked elsewhere). Although it has been known since at least 2016, with sustaining self-replacing populations without direct human intervention, according to the terminology of Pyšek et al. (2004), this species still has to be considered a casual alien (the threshold being at least 10 years of establishment). This status may change, however, in the near future if further observations become available in the next years. Based on Blackburn et al. (2011), moreover, the Italian populations are assessed as C3 (Individuals surviving in the wild in location where introduced,

reproduction occurring, and population self-sustaining) or perhaps even D2 (Self-sustaining population in the wild, with individuals surviving and reproducing a significant distance from the original point of introduction). According to Kornaś' classification, *S. maxima* can be assessed as a xenophyte-epecophyte in Bassano del Grappa (Kornaś 1990).

General distribution. *Sagina maxima* is naturally distributed on the Pacific coast of North America (Canada, United States of America), the Aleutian Islands and East Asia (China, Japan, Korea, Taiwan and Russian Far East). Outside its native range, the species has been reported from eastern North America where it occurs sporadically as an adventive that does not show signs of aggression or spreading (Crow 1978). Our record represents the first of this species in Europe.

Key to the *Sagina* species in Italy

Adapted from Pignatti et al. (2019).

1. Annual plants
    2. Flowers with 4 sepals; seeds obliquely triangular, with a dorsal groove
        3. Ripe fruits with patent sepals ..... *Sagina micropetala* Rauschert
        3. Ripe fruits with sepals close to the capsule
            4. Leaves with a clearly distinct awn at the apex; seeds on average < 0.4 mm..... *Sagina apetala* Ard.
            4. Leaves obtuse at the apex, acute or mucronulate, never with a true awn; seeds bigger..... *Sagina maritima* Don
    2. Flowers with 5 sepals; seeds reniform, almost globular, without a dorsal groove ..... *Sagina maxima* A. Gray
1. Perennial plants
    5. Flowers with 4 sepals ..... *Sagina procumbens* L.
    5. Flowers with 5 sepals
        6. Leaves with a clearly distinct awn at the apex (long 0.75–1.5 times the maximum leaf diameter)
            7. Petals 0.8–1.5 times as long as the sepals .... *Sagina alexandrae* Iamonico
            7. Petals 1.5 times longer than sepals... *Sagina revelierei* Jord. & Fourr.
            7. Petals 1.5–2 times as long as the sepals or more ....*Sagina pilifera* (DC.) Fenzl
        6. Leaves without awn at the apex, sometimes with a mucro shorter than ¾ of the maximum leaf diameter
            8. Leaves of the 2 highest nodes of the stem short (< 2.5 mm) and with dense bundles of axillary leaves; lower leaves more elongated..... *Sagina nodosa* (L.) Fenzl
            8. Lower and upper leaves of slightly different length
                9. Petals 0.8–1.5 times as long as the sepals..... .....*Sagina saginoides* (L.) H. Karst.
                9. Petals 1.5–2 times as long as the sepals..... ..... *Sagina glabra* (Willd.) Fenzl

Discussion

The genus *Sagina* is reputed for being taxonomically challenging, especially since many species look very similar in gross morphology. The recent records of an alien species from this genus in the city of Bassano del Grappa in Italy confirmed this. As the geographic origin of these populations was unknown and a comprehensive worldwide monograph of the genus is lacking, the quest for a correct name was not straightforward. Although the plants were identified—from the very beginning—as *S. maxima* (subsp. *maxima*), some doubt remained about this identification. Because of the species' ecological preferences in the area of origin, it is not very susceptible of being transported as an adventitious species to another part of the world. However, at least in China, *S. maxima* is also found in inland, disturbed habitats (in addition to the more natural coastal ones). Based on seed sculpturing and overall pubescence, the Italian plants indeed likely originate in Eastern Asia, not in North America where the species seems to be restricted to non-anthropogenic habitats. This is also confirmed in our molecular phylogenetic analysis which shows that the Italian plants are sister to a Chinese and Japanese specimen in the ITS- and atpB-rbcL-based phylogenies, respectively. Interestingly, it has been suggested recently that the ancestral habitat of several widespread ruderal species of *Sagina* in fact might have been coastal sites (Dillenberger and Kadereit 2022). In addition to some ecological concerns, the fact remains that *S. maxima* is not always easily separated from *S. japonica*, a more weedy species from the same section (and which therefore rather can be expected as an inadvertent introduction). The latter species is regularly found as a stowaway in bonsai pots (van Valkenburg et al. 2021; Rainer Otto, December 2022, *pers. comm.*) and it was recently also found in a shipment of rice in the Antwerp port area in Belgium (FV *pers. obs.*). In the absence of ripe seeds, these two species cannot be told apart according to Mizushima (1960). Nonetheless, ripe seeds were observed in the Italian plants and these correspond in all details with *S. maxima*: the seed surface is pebbled, not tuberculate. During our search for a suitable name, we further came across a number of species from the southern hemisphere that were similar in many respects to the Italian plants, in particular *S. humifusa* and *S. chilensis*. Based on just morphological grounds, these two species hardly seem to differ from *S. maxima*, except for their seed shape. In general, they also seem to be much more tiny plants, especially as compared to the relatively robust plants found in Italy. Genetically, however, the southern hemisphere representatives of the genus are clearly separated from both *S. maxima* and *S. japonica* on the one hand and *S. apetala* and relatives on the other, leaving no doubt about the genuine identity of the Italian plants. Yet, it should be noted that ITS markers clearly suggest a very close relationship between *S. maxima*, *S. japonica* and *S. hookeri*, a species that was recently described from Île Amsterdam (French Southern and Antarctic Lands) (Timaná et al. 2019).

The genus *Sagina* includes several species that are classified as weeds. Holm et al. (1979) mentioned four species [*S. apetala* Ard., *S. japonica*, *S. nodosa* (L.) Fenzl and *S. procumbens* L.], Randall (2017) even 13 [in addition to the previous also *S. alexandrae* Iamonico, *S. chilensis*, *S. decumbens* (Elliott) Torr. & A. Gray, *S. humifusa*, *S. maritima* Don, *S. maxima* (both subspecies), *S. micropetala* Rauschert, *S. sabuletorum* (J. Gay) Lange and *S. saginoides* (L.) H. Karst. – nomenclature updated by the authors], although their degree of weediness varies greatly. In any case, it is clear that the genus includes a number of weeds. This only partly applies to *S. maxima*: in its native area the species is primarily associated with more natural habitats near the coast and is only secondarily found in more disturbed habitats. Such a shift has previously been suggested for *Sagina* species that have now become cosmopolitan weeds (Dillenberger and Kadereit 2022). In terms of behavior, *S. maxima* in Bassano del Grappa shows similarities with, for example, *S. procumbens*, one of the best-known weeds of the genus worldwide. It appears that the species will become permanently established, at least locally, but its impact may be limited to anthropogenic environments.

Authors' contribution

Research conceptualization: FV and FP. Sample design and methodology: FV and FP. Investigation and data collection: GB. Molecular analysis and interpretation: FL. Writing: FV, FP and FL.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Taxon sampling for the phylogenetic analysis, including details on locality, vouchers and GenBank accession numbers.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2024/Supplements/BIR_2024_Verloove_etal_SupplementaryMaterial.xlsx