The distinction between Sagina apetala and S. micropetala (Caryophyllaceae: Sagineae), their phylogenetic relationships, and a note on the coastal origin of some widespread ruderals

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The distinction between *Sagina apetala* and *S. micropetala* (*Caryophyllaceae*: *Sagineae*), their phylogenetic relationships, and a note on the coastal origin of some widespread ruderals

Markus S. Dillenberger1,2 & Joachim W. Kadereit1

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**Abstract:** The distinction of the annual *Sagina apetala* and *S. micropetala (= *S. apetala* subsp. *erecta*, *S. filicaulis*) is based mainly on the position of sepal in fruit and shape and colour of the sepals, but identification of the two species is difficult. A molecular phylogeny of material identified as *S. apetala* and *S. micropetala* as well as other species of the genus using two nuclear and two plastid markers showed that there exist two lineages that are sister to each other and can be unambiguously distinguished molecularly. Although many of the morphological characters used in the literature proved useful in distinguishing these two lineages, sepal indumentum is the most reliable character to discriminate between them in Germany. Whereas *S. micropetala* usually has glabrous sepals, the sepals of *S. apetala* usually are glandular-pubescent. The chromosome number of $2n = 12$ for *S. micropetala*, here determined for the first time, is identical to that of *S. apetala*, supporting the close relationship between the two species. *Sagina apetala* and *S. micropetala* are sister to *S. maritima*, an annual species from European coasts, which may imply a coastal origin of the two species. A brief review of the possible origin of other European ruderals from coastal relatives is provided.

**Keywords:** Caryophyllaceae, chromosome count, phylogeny, *Sagina*, *Sagina apetala*, *Sagina filicaulis*, *Sagina maritima*, *Sagina micropetala*, *Sagineae*

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**Introduction**

*Sagina* L. of tribe *Sagineae* (*Caryophyllaceae*) is a predominantly northern hemisphere genus of c. 33 species (Bittrich 1993; Alban & al. 2022). The genus occurs on all continents except Antarctica, and has its centre of diversity in Europe. The closest relative of *Sagina* is *Colobanthus* Bartl. (Harbaugh & al. 2010; Greenberg & Donoghue 2011), and the monophyly of these two genera was recently confirmed in a comprehensively sampled molecular phylogeny (Alban & al. 2022). Species of *Sagina* are small, erect or procumbent, sometimes caespitose or cushion-forming herbs. Most species have white petals while some are apetalous (Clapham & Jardine 1993; Jonsell 2001; Crow 2005). In Germany, seven species and one hybrid are currently recognized (Jäger 2011). Of these seven species, three have pentameric flowers with white petals, and the other four have tetramerous flowers and often lack petals. The only perennial species among the tetramerous taxa is *S. procumbens* L., the type species of the genus. This species is a common weed typically growing in pavement cracks and has been introduced to different parts of the world (e.g. the Americas; Crow 2005). Apart from being perennial, *S. procumbens* can be distinguished from other tetramerous species by the presence of a persistent basal leaf rosette, its procumbent growth, and pedicels curved downward after anthesis (Jäger 2011). Petals are often present and, when the flowers open, the anthers are still closed (pers. obs.). In contrast, the remaining tetramerous species, i.e. *S. apetala* Ard., *S. maritima* Don and *S. micropetala* Rauschert, are erect, have basal leaf rosettes that wither quickly, pedicels that remain erect after anthesis (Jäger 2011), petals present only rarely, and anthers open when flowers open (pers. obs.).

*Sagina maritima* is a coastal species of Europe occurring from the North Sea to the Mediterranean Sea (Clapham & Jardine 1993) in open sandy to clayey salt marshes (Jäger 2011). It has slightly succulent leaves that are shortly mucronate, and its sepal are obtuse. *Sagina apetala* and *S. micropetala* are never succulent and do not grow in coastal habitats. Their leaves have a long mucro, and at least the outer sepals (i.e. the two sepals

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overlapping the other two sepals) have been described as cucullate, mucronate or acute (Jonsell 2001; Jäger 2011; Duistermaat 2020).

The distinction between *Sagina apetala* and *S. micropetala* is more difficult to characterize. Both species occur in human-influenced habitats such as pavement cracks, open and disturbed habitats along roads and sandy dry grassland or sandy farmland (Jäger 2011). *Sagina apetala* has been reported to occur in more natural habitats such as sandy dry grasslands of Europe, western Asia and northern Africa (POWO 2021), whereas *S. micropetala* has mostly been reported from more strongly human-influenced habitats such as pavement cracks of Europe (POWO 2021). Both species seem to be more vulnerable to trampling than *S. procumbens* and avoid central parts of sidewalks (pers. obs.).

While some floras do not distinguish the two species (e.g. Crow 2005), other floras give different characters to discriminate between them. Parolly & Rohwer (2019) used only the position of sepals in fruit in most flowers, which are appressed to the capsule in *Sagina apetala* vs horizontally spreading in *S. micropetala*. This trait has also been used by Clapham & Jardine (1993), Duistermaat (2020) and Jäger (2011). All of these authors also stated that the outer sepals are (sub)acute and more or less flat in *S. apetala* vs (sub)obtuse, hooded or cucullate with a mucro in *S. micropetala*. Furthermore, Duistermaat (2020) described *S. micropetala* as having reddish sepal margins; these are white in *S. apetala*. Duistermaat (2020) and Jäger (2011) also stated that the sepals are much shorter than the capsules in *S. micropetala* but almost equal the capsule length in *S. apetala*.

In contrast to the above treatments, Jonsell (2001) gave different frequencies for the above traits. According to Jonsell (2001), *Sagina micropetala* has at least some flowers with spreading sepals (not necessarily spreading in most), the reddish margin of the sepals has been described for at least the bud stage, and the outer sepals are obtuse or cucullate. In contrast, *S. apetala* was distinguished by having acuminate to acute sepals with a white margin appressed to the fruit (Jonsell 2001).

In conclusion, position and shape of the (outer) sepals are the most commonly used traits to distinguish *Sagina apetala* and *S. micropetala*, sometimes complemented by other characters. Unfortunately, some of these characters are difficult to observe for various reasons. For example, sepal position depends on the developmental state of flowers and can be assessed correctly only at complete fruit maturity. Another example is the shape of sepals. While distinction between (sub)acute vs (sub)obtuse sounds fairly straightforward, this distinction refers only to the outer sepals, and the observation of white or hyaline margins and tiny mucros is very difficult in dry sepals at fruiting stage. For both species a large number of varieties have been described (e.g. 13 synonyms at varietal level for *S. apetala* incl. *S. micropetala* are given in Montserrat Martí & Montserrat Martí 1990) and are still used for both species by some authors (e.g. Sell & Murrell 2018), indicating substantial morphological variation. All this raises the question whether distinction of *S. apetala* and *S. micropetala* at whatever rank is meaningful at all.

We use here the names *Sagina apetala* and *S. micropetala* according to Jäger (2011), although the name *S. micropetala* has some taxonomic problems, which we will discuss below. Although some of the floras cited above treat these two taxa at subspecific rank (i.e. *S. apetala* subsp. *apetala* and subsp. *erecta* (Hornem.) F. Herm.; e.g. Clapham & Jardine 1964, 1993; Parolly & Rohwer 2019), they use the same set of characters to distinguish between them.

In this study we will investigate the following questions: (1) Are there, among material provisionally identified as *Sagina apetala* and *S. micropetala*, two clades that can be distinguished with standard molecular markers? (2) If there are two clades, can these be unambiguously characterized morphologically? (3) Which names should be used for these clades? We will investigate these questions using a broad sample of *S. apetala* and *S. micropetala* from Germany and elsewhere in Europe for the observation of morphological characters and for phylogenetic reconstruction using standard DNA markers, i.e. the Internal and External Transcribed Spacers (ITS/ETS) and the two plastid spacers *atpB-rbcL* and *trnQ-rps16*. As we found that *S. apetala* and *S. micropetala* are closest relatives of the coastal *S. maritima*, we will briefly discuss the possibly coastal origin of *S. apetala* and *S. micropetala* as well as of other widespread European ruderals.

**Material and methods**

We used 76 samples of *Sagina* (Table 1), including *S. apetala* and *S. micropetala* (48 samples), *S. maritima* (14 samples) and *S. procumbens* (8 samples). We sampled all tetramerous species from Germany, but did not sample other tetramerous species of the genus because either these are perennials and of extra-European distribution, or they were sampled by Alban & al. (2022) and were found not to be closely related to the species of interest in this study. The sampling was complemented by the closest relatives of *S. apetala*, *S. micropetala* and *S. maritima* (i.e. *S. hookeri* Timán, *S. japonica* (Sw. ex Steud.) Ohwi and *S. maxima* A. Gray) identified in a recent phylogenetic analysis (Alban & al. 2022). Samples were collected and dried on silica-gel, or herbarium specimens were used. The sampling was focused on Germany for the species of interest, but also included samples from other European countries and one introduced population from New Zealand.

DNA extraction and amplification of the nuclear ribosomal Internal Transcribed Spacer (ITS) and the two plastid spacers *atpB-rbcL* and *trnQ-rps16* were carried out as described in Alban & al. (2022). Primer design for
the External Transcribed Spacer (ETS) was performed for three samples of Sagina, i.e. Min209, Min319 and Sab141 (Table 1). The whole Intergenic Spacer region (IGS) was amplified using primer 18S-2L (Linder & al. 2000) and 26S-II (Ochsman 2000). Amplified fragments were sequenced unidirectionally with primer 18S-2L.

Based on these sequences, two reverse primers in conserved regions were designed: Sam1 5′-GGT AGT TCG CTC GCG GTA C-3′ and Sam2 5′-AAG GAT GCT CGC GTT A-3′. Most samples were amplified with primers 18S-2L and Sam2, which produced better results in the PCR. Sam1 was only used for some Sagina maritima samples where Sam2 did not result in any amplification. The PCR was carried out in 25 µL reactions containing 1× reaction buffer, 3.2 mM MgCl2, 0.2 mM dNTPs, 80 µg/mL bovine serum albumin, 0.8 µM of each primer, and 0.04 U/µL NEB-Taq-polymerase. PCR cycles consisted of 94 °C for 1 min, followed by 35 cycles of 94 °C for 20 s, 62 °C for 30 s and 72 °C for 1 min, and finished by 94 °C for 20 s, 62 °C for 1 min 20 s and 72 °C for 8 min. PCR products were sequenced with both primers used in the PCRs by StarSeq (Mainz, Germany). Sequences were checked, edited and aligned manually and were made available on GenBank (Table 1).

Phylogenetic reconstructions using maximum likelihood were carried out using RAxML v.8.2.12 (Stamatakis 2014). Each of the four DNA regions was run individually under the GTRGAMMA model and bootstrapping was stopped automatically. To combine datasets, phylogenies of individual markers were checked manually for supported conflict. There was no supported conflict between the plastid and the nuclear phylogenies, except for the position of Sagina hookeri, while it was sister to Sagina procumbens in the ITS dataset. To combine the ITS and ETS dataset, Sagina maxima was sister to Sagina hookeri, while it was sister to Sagina procumbens in the ITS dataset. To combine the ITS and ETS dataset, Sagina maxima was removed from both datasets. The phylogenies of the plastid spacers atpB-rbcL and trnQ-rps16 were found to be congruent and the datasets were combined into the plastid dataset. Phylogenetic reconstructions were carried out for both combined datasets as described above. The resulting phylogenies were again checked for supported conflict. There was no supported conflict between the plastid and the nuclear phylogenies, but Sagina maxima was removed from the plastid dataset before combination with the nuclear dataset. Phylogenetic reconstruction with RAxML was carried out for the complete dataset including all markers as described for all other datasets.

Morphology was investigated using specimens from MJG (Table 1; herbarium codes according to Index herbariorum http://sweetgum.nybg.org/science/ih/). We contacted herbarium staff. Specimens were seen as digital images, and specimens not seen were indicated as “n.v.”.

**Results**

All phylogenetic reconstructions using the individual markers were congruent, except for the position of Sagina maxima. While Sagina maxima was sister to Sagina hookeri in the ITS phylogeny, it was sister to Sagina procumbens in all other individual phylogenies (not shown). Therefore, Sagina maxima was removed from the complete dataset. The
Table 1. List of specimens used for phylogenetic analyses, including lab abbreviations (Abbr.), locations, vouchers and GenBank accession numbers (n.a. = not amplified/available).

<table>
<thead>
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<th>Abbr.</th>
<th>Locality</th>
<th>Coordinates</th>
<th>Voucher</th>
<th>ITS</th>
<th>ETS</th>
<th>apB-rbcL</th>
<th>trnQ-rps16</th>
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**Sagina maxima subsp. crassicaulis** (S. Watson) G. E. Crow

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**Sagina micropetala** Rauschert

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<td>MT534495</td>
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<td>Locality</td>
<td>Coordinates</td>
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<td>Sab175</td>
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<td>C. D. Preston s.n. (MJG 028558)</td>
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<td>I. Timmermann-Trosiener ITT-1 (MJG 028395)</td>
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<td>OK446604</td>
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<td>M. S. Dillenberger 20005 (MJG 028312)</td>
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<td>Sab223</td>
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<td>M. S. Dillenberger 20016 (MJG 028386)</td>
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</table>

**Sagina procumbens L.**

| Min183 | Bulgaria, Kyustendil, Rila | 42°07'48"N, 23°10'48"E | J. Klein & S. Gencheva 29.8.7.2 (MJG 004137) | KF737444 | OK505986 | MT534506 | KY700826 |
| Min208 | Germany, Rheinland-Pfalz, Mainz | 49°59'24"N, 08°14'24"E | M. S. Dillenberger 20127 (MJG 009791) | KF737495 | OK505987 | MT534507 | KY817729 |
| Min319 | Germany, Hessen, Hofheim-Diedenbergen | 50°03'36"N, 08°25'12"E | M. S. Dillenberger 15002 (MJG 016484) | KY817666 | OK505988 | MT534508 | KY817731 |
| Sab72 | Greece, Pieria, Skotina | 40°12'00"N, 22°10'48"E | Andersen 11368 (C s.n.) | MT415659 | OK505991 | MT534509 | MT671714 |
| Sab99 | Germany, Rheinland-Pfalz, Mainz | 49°59'24"N, 08°14'24"E | M. S. Dillenberger 14047 (MJG 014940) | MT415660 | OK505992 | MT534510 | MT671715 |
| Sab201 | Germany, Schleswig-Holstein, Hallig Langeneß | 54°36'N, 08°30'E | J. W. Kadewit, T. Messerschmidt s.n. | OK446625 | OK505989 | OK505916 | OK505855 |
| Sab208 | United Kingdom, Middlesex, Ruislip | G. A. Matthews s.n. (C s.n.) | OK446626 | OK505990 | OK505917 | OK505856 |
| Sab226 | Germany, Nordrhein-Westfalen, Borken | 51°50'37"N, 06°51'40"E | M. S. Dillenberger 15022 (MJG 017807) | OK446627 | n.a. | OK505918 | OK505857 |
Fig. 1. Phylogenetic reconstruction with maximum likelihood obtained with RAxML of the complete dataset, including the markers ITS, ETS, \textit{atpB-rbcL} and \textit{trnQ-rps16}. Values at branches are bootstrap support values; only values ≥ 70 are shown. For sample abbreviations see Table 1.
results of the phylogenetic reconstruction of the complete dataset of all DNA markers is shown in Fig. 1. Based on Alban & al. (2022), S. procumbens was used as root. Sagina japonica and S. hookeri were sister to each other and together were sister to the group containing S. maritima, S. apetala and S. micropetala. The latter group was supported with bootstrap support (BS) of 99. The existence of a well-supported clade containing S. maritima, S. micropetala (as S. apetala subsp. erecta) and S. apetala had already been shown by Alban & al. (2022) in their phylogenetic analysis of Sagina including 25 species of the genus. We consider it unlikely that any of the species not sampled by Alban & al. (2022) fall into the clade of S. maritima, S. apetala and S. micropetala identified here. Of the six species not sampled by Alban & al. (2022) only one is tetramerous but is found only on a southern Indian Ocean island. Of the five pentamerous species, three are from New Guinea and one is from South America. The only unsampled species that occurs geographically close to Europe is S. libanotica Rech. f., from Lebanon, which is a perennials with pentamerous flowers and morphologically most similar to S. saginoides (L.) H. Karst. (Rechinger 1952).

Sagina maritima (BS 100) and the clade comprising S. apetala and S. micropetala (BS 92) were both monophyletic. The samples containing the two species of interest clearly fell into two clades. The larger clade contained 35 samples (clade I; BS 97), the smaller clade 13 samples (clade II; BS 100). Within clade II, one subclade (BS 98) contained six samples from Germany, and the other subclade (BS 79) contained seven samples from Greece, Cyprus and the introduced sample from New Zealand. Within clade I, no supported groups (BS ≥ 70) could be found.

For clades I and II, we obtained the following morphological results (Table 2). Unless indicated otherwise, percentage values refer to the percentage of flowers, leaves and axes with the relevant trait. In specimens of clade I, sepals were horizontally spreading in 56% of the flowers. While 87% of the individuals had at least some flowers at flowering time showing this trait, 13% of the individuals had only flowers with sepals appressed to the capsule. Flowers had sepals equaling the capsule length in 87% (sepal length > 4/5 of capsule length). The capsule was more often broadly ovoid (capsule almost as wide as long; 60%) than narrowly ovoid (capsule width ≤ 4/5 of capsule length; 40%). Sepal tips were usually obtuse (93%) and cucullate (62%), and a sepal mucro was visible in only 9% of the flowers. Mostly (97%) there were no glandular hairs on the sepals. The 3% of flowers with glandular-pubescent sepals represented 6% of the individuals investigated (94% of individuals had only glabrous sepals). Of these, three individuals were from the United Kingdom (all specimens from the United Kingdom investigated) and only two from Germany. The sepal margin was more often red (69%) than white (31%). Most individuals (87%) had at least some flowers with a red sepal margin. The leaf margin was ciliate (84%), and half of the leaves had cilia extending to the upper half of the leaf (46%), while 38% were ciliate only at the base of the leaves. The axes were often glabrous (65%), non-glandular-pubescent in 31% of the individuals, and glandular-pubescent in 4%. Highest density of hairs, when present, could be found at the base of plants and decreased toward the inflorescence.

In clade II, sepals were appressed to the capsule in 91% of the flowers. Plants had sepals that were equaling the capsule length in 88%. The capsules were more often narrowly (65%) than broadly ovoid (35%). The sepal tip was acute in 61% of the flowers and usually flat (78%), and a sepal mucro was visible in only 11% of the flowers. The sepals were glandular-pubescent in 91% of the flowers, and all individuals had at least a few sepals with glandular hairs, especially in young flowers. The sepal margin was almost always white (96%). Half of the leaves were not ciliate (54%), while 45% were ciliate at the base. Only 1% of the leaves showed cilia on the upper half of the leaf. The axes were often glabrous (85%), never eglandular-pubescent, and glandular-pubescent in 15%. Indumentum density decreased strongly toward the base of plants where the axis was usually glabrous.

Chromosome counts are summarized in Table 3. For Sagina micropetala, a chromosome number of 2n = 12 was counted for six populations from Germany. Sagina apetala was counted with 2n = 12 for one population from Germany.

Discussion

Phylogeny of annual European Sagina species

Our phylogeny clearly shows that there exist three different clades among the tetramerous annual species from Germany. Sagina maritima, from across its distribution range from the North Sea to the Mediterranean Sea including a sample from the Azores, was found to be monophyletic. It is sister to all samples that were assigned to S. apetala and S. micropetala. These samples form two well-supported clades which, given the taxonomic history of the samples, can be recognized as two taxa (Fig. 1). We will treat these two taxa here as species because they are clearly separated molecularly, they occur sympatrically and even next to each other in pavement cracks (e.g. Sab177/178 from Hamburg, Germany; Table 1), and because we found no evidence for hybridization between them, even when growing in a mixed population.

Morphological characterization of Sagina apetala and S. micropetala

Recent floras (Clapham & Jardine 1993; Jöger 2011; Jonsell 2001; Stace 2010; Tison & Foucault 2014; Pignatti 2017; Sell & Murrell 2018; Parolly & Rohwer 2019; Duistermaat 2020) have used a common set of
As is evident from our detailed results (Table 2), none of these characters is unambiguous, and we saw plants of one species that showed several of the character states typical for the other species. The most reliable character to distinguish the two species is the presence of glandular hairs on the sepals, a character that has not been used before in *Sagina* but has been found useful in other genera of the Caryophyllaceae (e.g. *Cerastium* L.; Rabeler & Hartman 2006, 2020). Whereas *S. apetala* had glandular hairs in 91% of the flowers, and at least some flowers of all individuals had glandular hairs, *S. micropetala* had no glandular hairs in 97% of the flowers and in 94% of the individuals examined. Red sepal margins were similarly reliable for *S. micropetala*. Whereas 69% of *S. micropetala* flowers (and 87% of individuals) showed this character, only 4% of *S. apetala* flowers had red sepall margins. In contrast, white sepal margins were not a reliable character for *S. apetala*. Although most flowers of *S. apetala* showed white margins (96%), white margins were also found in 31% of *S. micropetala* flowers.

<table>
<thead>
<tr>
<th>Character</th>
<th>Percentage of flowers/leaves/axes</th>
<th>Percentage of individuals with only this character state</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepals appressed to capsule</td>
<td>Clade II (Sagina apetala) 91.25</td>
<td>Clade II (Sagina micropetala) 62.5</td>
</tr>
<tr>
<td>Sepals horizontally spreading</td>
<td>8.75 44.15 55.85 0</td>
<td></td>
</tr>
<tr>
<td>Sepals equalling capsule length</td>
<td>87.5 86.76 50.0</td>
<td></td>
</tr>
<tr>
<td>Sepals distinctly shorter than capsule (sepal length ≤ 4/5 capsule length)</td>
<td>12.5 13.24 0</td>
<td></td>
</tr>
<tr>
<td>Outer sepals acute</td>
<td>61.25 6.86 31.25</td>
<td>0</td>
</tr>
<tr>
<td>Outer sepals obtuse</td>
<td>38.75 93.14 6.25</td>
<td>72.94</td>
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<tr>
<td>Outer sepals flat</td>
<td>78.25 37.83 50.0</td>
<td>4.71</td>
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<tr>
<td>Outer sepals cucullate</td>
<td>21.25 62.17 0</td>
<td>16.47</td>
</tr>
<tr>
<td>Outer sepals mucronate</td>
<td>11.25 8.51 0</td>
<td>0</td>
</tr>
<tr>
<td>Outer sepals not mucronate</td>
<td>88.75 91.49 56.25</td>
<td>68.24</td>
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<tr>
<td>Sepals glandular-pubescent</td>
<td>91.25 2.84 75.0</td>
<td>1.18</td>
</tr>
<tr>
<td>Sepals glabrous</td>
<td>8.75 97.16 0</td>
<td>94.12</td>
</tr>
<tr>
<td>Sepal margins red</td>
<td>3.75 68.87 0</td>
<td>49.41</td>
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<tr>
<td>Sepal margins white-hyaline</td>
<td>96.25 31.13 87.5</td>
<td>12.94</td>
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<td>Leaves not ciliate</td>
<td>53.75 16.04 31.25</td>
<td>9.41</td>
</tr>
<tr>
<td>Leaves ciliate only at base</td>
<td>45.0 37.5 25.0</td>
<td>21.18</td>
</tr>
<tr>
<td>Leaves ciliate beyond their middle</td>
<td>1.25 46.46 0</td>
<td>31.76</td>
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<td>Axis glabrous</td>
<td>85.0 65.09 75.0</td>
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<tr>
<td>Axis eglandular-pubescent</td>
<td>0 31.17 0</td>
<td>22.35</td>
</tr>
<tr>
<td>Axis glandular-pubescent</td>
<td>15.0 3.54 0</td>
<td>2.35</td>
</tr>
<tr>
<td>Capsule narrowly ovoid (capsule width ≤ 4/5 capsule length)</td>
<td>65.0 39.62 25.0</td>
<td>9.41</td>
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<tr>
<td>Capsule broadly ovoid</td>
<td>35.0 60.38 6.25</td>
<td>23.53</td>
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</table>

Table 2. Morphological characters investigated in specimens of *Sagina apetala* and *S. micropetala*. All characters except two appear to be suitable for this distinction based on our observations. Plants of clade I typically showed at least some of the following characters: sepals horizontally spreading in fruit, capsules broadly ovoid, sepal tips obtuse and cucullate, sepals not glandular-pubescent, sepal margins red, and leaf margins ciliate. Therefore, clade I can be identified as *S. micropetala*. We did not observe that the capsule was much longer than the sepals. Sepal tip mucros were observed in only very few flowers. This could be related to the fact that mucros are curved inward in *S. micropetala* and are hidden when sepals are withered.

Plants of clade II usually had sepals appressed to the capsule, and the capsules were narrowly ovoid. Sepals were usually acute and flat, but some had a small erect mucro at the tip. The plants always had at least some flowers with glandular-pubescent sepals and the sepal margin was white. Leaves were ciliate at the base or without cilia. Therefore, clade II can be identified as *Sagina apetala*.
and 13% of individuals had only flowers with white sepal margins. In combination, sepal pubescence and sepal margin colour are highly (but not completely) reliable in distinguishing *S. apetala* and *S. micropetala*. All other characters discussed can further support identification based on these two characters.

Some of the characters discussed appear to be influenced by the environment. For example, plants of *Sagina micropetala* collected at shady localities had a smaller percentage of red sepal margins, and some did not have a single flower with red sepals margins. Similar variation of red bands at the base of the calyx has been observed in *Stellaria pallida* ( Dumort.) Piré by Rabeler (1988).

Our findings are mostly based on German material and will have to be carefully tested in other parts of their native distribution ranges as well as in introduced populations around the world. For example, in contrast to the German material, all material of *Sagina micropetala* from the United Kingdom had glandular-pubescent sepals.

### Taxonomy and nomenclature of *Sagina apetala* and *S. micropetala*

The taxonomy of *Sagina apetala* and *S. micropetala* as used in several floras (e.g. Jäger 2011) harbours some problems. *Sagina apetala* was described by Ardouin (1764: 22) and is clearly the first validly published name for clade II. A supposed later homonym attributed to Linnaeus (1771: 559) is not a new name because Linnaeus clearly cited Arduin (1764) as the author. The name was lectotyphified by Crow (1978: 73) with a Linnaean specimen (Herb. Linn. No. 177.2 in LINN; http://linnean-online.org/2016/).

For the material falling into clade I, three names are currently used: *Sagina micropetala* Rauschert (e.g. Jäger 2011; Duistermaat 2020). *S. apetala* subsp. *erecta* (Hornem.) F. Herm. (e.g. Clapham & Jardine 1993; Parolli & Rohwer 2019) and *S. filicaulis* Jord. (e.g. Tison & Foucault 2014; Sell & Murrell 2018).

*Sagina micropetala* was described by Rauschert (1969: 413) as a replacement name at species rank for *S. apetala* subsp. *erecta* (Hornem.) F. Herm. because *S. erecta* L. (= *Moenchia erecta* (L.) G. Gaertn. & al.) is blocking the epithet. Therefore, *S. micropetala* is a homotypic synonym of *S. apetala* subsp. *erecta*. Unfortunately, the combination for subsp. *erecta* by Hermann (1912: 182) was made in an identification key and lacks further information about the basionym except for giving "*erecta Lam.*", which does not fit with the basionym author (Hornemann) that is usually given. The study of all available publications of Lamarck in the Biodiversity Heritage Library (https://www.biodiversitylibrary.org/) offers a possible explanation for this incorrect authorship. Lamarck used the name "*Sagina erecta* Lin. Sp. 185 […] β. *Sagina apetala*. Lin. mant. 559" (Lamarck 1778: 9), best interpreted as *S. erecta var. apetala*. This implies the existence of a var. *erecta*, which consequently is a synonym of *Moenchia erecta* (≡ *S. erecta*). Later, Lamark cited his own work as "*S. apetala*. Linn. Mant. 559. – *S. erecta*, β. Lam. Fl. fr. 3. p. 9" (Lamarck & Candolle 1815: 769). The second part might incorrectly imply a var. *erecta* published by Lamark. Considering all of this, there is no validly published name by Lamark in the entire *Caryophyllaceae* at species rank or below called "*erecta*", so that the authorship of Lamark for this name seems to be incorrect. The first traceable valid publication of the name *S. apetala" var. *a erecta" can be found in Hornemann (1834), who illustrated this variety in comparison to "var. *β decumbens*. The growth form of var. *decumbens* clearly excludes it from *S. apetala* and *S. micropetala* so that var. *decumbens* most likely represents *S. procumbens*. Hermann (1912) stated that subsp. *erecta* has spreading sepals at fruit maturity, and in the illustration of var. *erecta* by Hornemann (1834) one of more than 20 flowers shows this trait. The lectotype has obtuse sepals spreading horizontally without glandular hairs (Olof Ryding, Copenhagen, pers. comm.), confirming its identification as *S. micropetala*.

For the third name, *Sagina filicaulis*, Jordan (1849) stated “Elle est très-rapprochée des *Sagina apetala* L. […] mais elle se distingue de la première par ses sépales toujours appliqués sur le fruit et non étalés en croix [It is very close to *S. apetala* L. […] but it is distinct from the former by its sepals always appressed to the fruit and not spread in a cross]”. This trait can clearly be attributed to *S. apetala*. In the Jordan collection at LY two specimens of *S. filicaulis* can be found. Only specimen [LY 0826452](https://biodiversitylibrary.org/page/72571948)
was possibly collected before the publication date (date missing on sheet), and this specimen fits the protologue information. It also fits the description by Jordan (1849) of a filiform plant with sepals appressed to the capsule, but does not have any glandular hairs on the sepals. The sepal margin appears to be white-hyaline, and the leaves are ciliate not only at the base but have cilia beyond their middle. Although typical glandular hairs are missing, several characters imply that the specimen belongs to *S. apetala*. As shown for British material of *S. micropetala*, indumentum of sepals can vary, and the most important character used in its description to distinguish *S. filicaulis* (sepals appressed to the capsule, Jordan 1849) is a character that is most common in *S. apetala*. Our data show that appressed sepals also occur in *S. micropetala*, but only 9% of individuals had appressed sepals in all flowers, and not a single population (with more than two individuals on the specimen) showed that character for all individuals. The type specimen together with all other specimens cited by Jordan (1849) indicates that this character is common in his material, supporting our decision to consider *S. filicaulis* a synonym of *S. apetala*. In the future, DNA sequencing of material with similar morphology from the type locality might further support our treatment.

**Chromosome number variation in *Sagina***

Chromosome numbers reported for *Sagina* are highly variable. Although only available for about half of the species, chromosome numbers include $2n = 12, 18–22, 20, 22, 28, 36, 46, 56, c. 60, 64, 66, 84$ and $c. 88$ (Crow 1978; Goldblatt & Johnson 1979–2021). For *S. apetala*, $2n = 12$ has been counted (Petrova 1995; Runemark 1996; Lökvist & Hultgård 1999) in material from the Mediterranean region and from Sweden. Our chromosome count of $2n = 12$ for *S. apetala* confirms previous findings from other regions and provides the first count of this taxon from Germany/Central Europe. No chromosome counts have been published until now for material that can be assigned to *S. micropetala*. Our count of $2n = 12$ from mitotic root tip cells of *S. micropetala* confirms the close relationship of this species to *S. apetala*. It clearly sets both apart from their closest relative *S. maritima* with $2n = 24/28$. The chromosome number of *S. maritima* is not entirely clear because Wulff (1937) counted $n = 11–12$ (“annäherungsweise”, i.e. approximately) for material from Schleswig-Holstein (Germany) and Runemark (1996) counted $2n = 28$ for material from the Mediterranean region.

**A note on the coastal origin of some widespread ruderals**

As shown above, *Sagina apetala* and *S. micropetala* are the closest relatives of *S. maritima*. This may imply, irrespective of chromosome number variation, that the ancestral habitat of these two species might have been coastal sites, i.e. thin soil on cliff tops, the spray zone, open places in salt marshes, damp sandy places behind beaches, dune slacks and pavement cracks on seaside promenades, where *S. maritima* occurs. Interestingly, an origin from coastal habitats such as salt marshes, dunes and particularly tidal drift vegetation has been suggested for a number of important ruderals (Nordhagen 1939/1940; Baker 1974; Willerding 1986; Sukopp & Scholz 1997; Ellenberg & Leuschner 2010). Of those species commonly found in tidal drift vegetation, ruderal *Senecio vulgaris* L. var. vulgaris (Kadereit 1984a, 1984b; Ellenberg & Leuschner 2010), *Tripleurospermum maritimum* subsp. inodorum (L.) Appleq. (Ellenberg & Leuschner 2010) and ruderal forms of *Atriplex prostrata* DC. (Taschereau 1985; Grime & al. 1988) have been suggested to probably have originated from tidal drift populations. For *T. maritimum* subsp. *inodorum*, however, Kay (1972) did not discuss this possibility and later (Kay 1994) hypothesized that the type of natural habitat of the taxon before colonization of man-made habitats may no longer exist (in the British Isles). In *Beta vulgaris* L., ruderal beets in SW France (Desplanque & al. 1999; Fénart & al. 2008) and Morocco (Leys & al. 2014), to be distinguished from weedy beets, which are probably the result of hybridization between ruderal and coastal beets, have been postulated to be most closely related to the coastal *B. maritima* L. subsp. *maritima*. Other ruderal species commonly found in tidal drift habitats and partly on primary and white dunes include *Polygonum aviculare* L., *Rumex crispus* L. and *Sonchus arvensis* L. Interestingly, among the species sampled by Kim & al. (2007) and Mejías & al. (2018), *S. arvensis*, with subsp. *uliginosus* (M. Bieb.) Nyman in coastal and other habitats, is most closely related to *S. crassifolius* Willd., and *S. maritimus* L., which are species of damp saline and calcareous soils. Such relationship may imply that a coastal or at least saline habitat is ancestral in *S. arvensis*, which would support the idea of an origin of ruderal populations of the species from the coast, as already speculated by Hegi (1929). *Rumex crispus* commonly grows in tidal drift vegetation when left undisturbed (Tüxen 1950), and also in dunes. Populations from such habitats have been referred to as *R. crispus* subsp. *littoreus* (J. Hardy) Akeroyl. Although no explicit suggestion has been made that inland ruderal populations originated from coastal forms, Cavers & Harper (1964) and Akeroyl & Briggs (1983), in cultivation experiments, found that whereas inland plants often flower in their first year, this can never be observed in coastal plants, which flower in their second or later years. This observation may imply evolutionary directionality from the coast to inland habitats. However, different from the findings for *Senecio vulgaris*, where the coastal subsp. *denticulatus* (O. F. Müll.) P. D. Sell shows substantial seed dormancy, which is absent from ruderal var. *vulgaris* (Kadereit 1984a), *R. crispus* subsp. *littoreus* lacks seed dormancy, which is present in ruderal populations (Cavers & Harper 1966). Finally, ruderal *Polygonum aviculare* may also be of coastal origin, although this possibility has never been
discussed (e.g. Styles 1962). Coastal forms have been referred to as, e.g., *P. aviculare var. littorale* (Link) Mert. & W. D. J. Koch (Rechinger 1958), *P. neglectum* Besser (Scholz 1959) or *P. aviculare* subsp. *turbivagum* (Boreau) Berher (Jäger 2011). In each case discussed, the direction of habitat shift – either from coastal to ruderal sites or *vice versa* – is not unambiguously clear. However, the ecological similarity between particularly tidal drift and ruderal sites, i.e. irregular disturbance, low competition and high nutrient contents, make tidal drift a probable starting point for the evolution of the above ruderals, and indeed all of them (except *Sagina apetala, S. micropetala* and *Senecio vulgaris*) are known as subfossils from glacial times in the British Isles (Godwin 1975). Interestingly, the oldest finds (Iron age) of *Tripleurospermum maritimum* associated with human activity appear to belong to subsp. *maritimum* and have been recorded from sites near the North Sea coast (Willerding 1986).

**Taxonomic treatment**

The taxonomic treatment is focused on the *Sagina apetala–S. micropetala* group. Numerous synonyms exist that have usually been assigned to *S. apetala*. We did not check all of these names in detail but only compared descriptions with the characters we observed. Some synonyms (e.g. *S. patula* Jord.) clearly fit our circumscription of *S. apetala*, while others are less clear. Only one younger name can clearly be assigned to the synonymy of *S. micropetala*, but further investigation especially of type material might lead to a different result. If any other existing name belongs to *S. micropetala*, it would have priority because all of these names are older than *S. micropetala*. We did not consider further intraspecific synonyms.

**Identification key to annual *Sagina*-species of Germany**

1. Leaves (at least slightly) succulent, upper leaves with a short mucro < 0.18 mm long; sepals obtuse; plants in coastal habitats (e.g. salt marshes) ............. 2. *S. maritima*

   – Leaves never succulent, upper leaves with a longer mucro > 0.2 mm long; outer sepals obtuse, mucronate, acuminate or acute; plants not in coastal habitats .................................. 2

2. Sepals usually glabrous, often with red margin; other traits: sepals often spreading horizontally at fruiting time at least in some flowers, outer sepals usually obtuse or cucullate, sometimes with an incurved mucro; leaves usually ciliate, often beyond their middle; capsule more often broadly ovoid (width \( \leq 4/5 \) of length) than not ................................. 3. *S. micropetala*

   – Sepals usually glandular–pubescent (at least at base), usually with white–hyaline margin; other traits: sepals usually appressed to mature capsule, outer sepals often acute and flat, sometimes with an erect mucro; leaves ciliate at base or without cilia; capsule more often narrowly ovoid (width \( \leq 4/5 \) of length) than not


   – Note: Linnaeus (1771) did not publish a new name but cited Arduino’s name. Nevertheless, Linnaeus is often incorrectly cited as author of the name. Rossmann (1860) considered the epithet “apotæla” as meaningless for a plant with (minute) petals and provided a nomenclaturally superfluous (and hence illegitimate) replacement name for *S. apetala*. Schlosser & Vukotinović (1869) cited “*Moenchia quaternella* Alsch.” as a synonym of *S. quaternella*. However, they were not citing a basionym but rather what they evidently regarded as a misapplied name – *M. quaternella* sensu Alschinger, non Ehrh. – because on p. 356 “*Moenchia quaternella* Ehrh.” was cited as a synonym of *M. erecta* (L.) Gaertn. & al. *Sagina quaternella* was nomenclaturally superfluous when published, and is an illegitimate replacement name for *S. apetala*, because the latter name was also cited in its synonymy.

   = *Sagina ciliata* Fr., Utaksv. Fl., ed. 3: 713. 1816

   = *Sagina apetala* subsp. *ciliata* (Fr.) Hook. f., Student Fl. Brit. Isl.: 61. 1870. – Type: not designated. – Protologue: “V. på åkerfält vid Nefelöf nära Ystad”.


   = *Sagina patula* Jord., Observ. Pl. Nov. 1: 23. 1846


   = *Sagina filicaulis* Jord., Observ. Pl. Nov. 7: 16. 1849


   = *Sagina lamyi* F. W. Schultz in Jahresber, Pollichia 8: 30. 1850

Florent, sable du Cher (Tourangin). — Haute-Vien. Magne-Bourg, roches de Serpentine (Lamy)”. — Note: Schultz based S. lamyi on S. depressa sensu Boreau (1849), non Schultz. The lectotype was originally in the herbarium of Alfred Déséglise, who was a student of Gustave Tourangin (Briquet 1940: 206–209). The lectotype is now in P.

≡ Sagina ambigua J. Lloyd, Fl. Ouest France: 74. 1854. – Type: not designated. – Protologue: “c. golfe du Morbihan, murs, lieux secs; je l’ai revu à Cاديئل (char.-Inf.), sur les côteaux du Guessonant près لامب بال (C.-Nord), et M. Pontarlier me l’a donné de خلالان (Vend.) localités qui sont supposer que cette espèce croit sur plusieurs points intermédiaires”.


≡ Sagina urbica Phil. in Linnaea 28: 613. 1857 = Sagina apetala var. urbica (Phil.) Reiche, Fl. Chile 1: 186. 1896. – Lectotype (designated here): [Chile], Curacasí, Sep 1853, Philippi s.n. (SGO 000001986). – Protologue: “In plateis urbis Santiago frequens, ad Quillota, Curacasí etc.”. – Note: Other original material from Santiago is available at HAL (HAL 0117866).


≡ Sagina ambigua J. Lloyd, Fl. Ouest France: 74. 1854. – Type: not designated. – Protologue: “c. golfe du Morbihan, murs, lieux secs; je l’ai revu à Cاديئل (char.-Inf.), sur les côteaux du Guessonant près لامب بال (C.-Nord), et M. Pontarlier me l’a donné de خلالان (Vend.) localités qui sont supposer que cette espèce croit sur plusieurs points intermédiaires”.


≡ Sagina urbica Phil. in Linnaea 28: 613. 1857 = Sagina apetala var. urbica (Phil.) Reiche, Fl. Chile 1: 186. 1896. – Lectotype (designated here): [Chile], Curacasí, Sep 1853, Philippi s.n. (SGO 000001986). – Protologue: “In plateis urbis Santiago frequens, ad Quillota, Curacasí etc.”. – Note: Other original material from Santiago is available at HAL (HAL 0117866).


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≡ Sagina urbica Phil. in Linnaea 28: 613. 1857 = Sagina apetala var. urbica (Phil.) Reiche, Fl. Chile 1: 186. 1896. – Lectotype (designated here): [Chile], Curacasí, Sep 1853, Philippi s.n. (SGO 000001986). – Protologue: “In plateis urbis Santiago frequens, ad Quillota, Curacasí etc.”. – Note: Other original material from Santiago is available at HAL (HAL 0117866).


≡ Sagina ambigua J. Lloyd, Fl. Ouest France: 74. 1854. – Type: not designated. – Protologue: “c. golfe du Morbihan, murs, lieux secs; je l’ai revu à Cاديئل (char.-Inf.), sur les côteaux du Guessonant près لامب بال (C.-Nord), et M. Pontarlier me l’a donné de خلالان (Vend.) localités qui sont supposer que cette espèce croit sur plusieurs points intermédiaires”.

Fig. 2. Lectotype of *Sagina micropetala* – specimen no. 3 on the sheet: [Germany], Heiligenhaven, Aug 1825, *Nolte s.n.* (C 10024083). – Reproduced with permission of the Natural History Museum of Denmark.
“Patria ignota. Semina ex horto Gottingensi nomine Sagina saxatilis a’ 1849 et 1851 accepiimus”.


= *Sagina emporitana* Sennen in Treb. Inst. Catalana Hist. Nat. 3: 84. 1917. – **Type: not designated.** – Protologue: “champs sauvages entre Port de Molins et Figueres”. – Note: Sennen (1917: 84) wrote: “Nous ne devons pas omettre une forme probablement am- pondanaise des champs sauvages entre Port de Molins et Figueres, que nous croyons avoir distribuée comme variété emporitana du *S. maritima* Don. et qui serait plutôt une race *S. emporitana* Sennen, forme du *S. Rodriguezzii* Willk.” [We must not omit a probably ampondanais form from the sandy fields between Port de Molins and Figueres that we believe we have distributed as a variety *emporitana* of *S. maritima* Don. and which would seem to be rather a race of *S. emporitana* Sennen, a form of *S. Rodriguezzii* Willk.; translated from French by Ellen Lévy/Toulouse]. It is not clear if the terms “race” and “forme” indicate Sennen’s intention to designate a taxonomic rank. If yes, this would be in conflict with the species rank indicated by the name used by him, i.e. *S. emporitana*.


3. *Sagina micropetala* Rauschert in Feddes Repert. 79: 413. 1969 ≡ *Sagina apetala* var. *erecta* Hornem., Fl. Danica [Hornem.] 12: 3, tab. MC1L. 1834 ≡ *Sagina apetala* subsp. *erecta* (Hornem.) F. Herm., Fl. Deutschl. Fennskand.: 182. 1912. – **Lectotype (designated here):** [Germany], Heiligenhaven, Aug 1825, Nolte s.n. (C 10024083 specimen no. 3 on sheet; Fig. 2). – Protologue: “Inter segetes solo pingui ad Heiligenhaven, Blankenese, Altonam et Buchholz Lauenburgise legit celeberr. Professor Nolte”. – Note: The protologue does not provide separate information for var. *erecta* and var. *decumbens*. Specimen no. 3 on the sheet consists of three large plants all belonging to *S. micropetala* and has the correct location, collector and date. Specimen no. 1 seems to include var. *decumbens*, no. 2 lacks a date, and label information of no. 4 does not fit the protologue.

= *Sagina schiraevskii* Tzvelev in Bot. Zhurn. (Moscow & Leningrad) 87(3): 122. 2002. – Holotype: Ukraine, proper opp. Starobiljsk, in area humida, Aug 1904, J. Schiraevski s.n. (LE n.v.; isotype: LE n.v.). – Note: Tzvelev (2002) gave as main differences to *S. apetala* and *S. micropetala* the indument of the plant and the shorter apex of the leaves. Considering the morphological variability of the group, we treat this name as a synonym of *S. micropetala*, based on its obtuse, cucullate and mucronate (mucro incurved) sepals, which are spreading horizontally in fruit.

**Conclusions**

Our phylogenetic analysis of material provisionally identified as *Sagina apetala* and *S. micropetala* revealed the existence of two distinct and well-supported lineages that are sister to each other and closest relatives of the morphologically similar *S. maritima*. Morphological investigation of mainly German material showed that the two lineages correspond to the morphological species *S. apetala* and *S. micropetala* and helped in identifying characters useful for their discrimination. Although our results highlight that no morphological character is unambiguous in discriminating the two species, indumentum of sepals and colour of the sepals margin are most reliable. Several other characters should be considered for their correct identification. The close relationship of *S. apetala* and *S. micropetala* is further supported by their shared chromosome number of 2n = 12, first reported in this study for *S. micropetala*. Future studies will have to show whether the characters identified by us are useful in other parts of the distribution range of the two species.

**Author contributions**

MSD and JWK designed the study. MSD generated the datasets and analysed the data. MSD and JWK wrote the manuscript.

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Annotated alignments in Nexus format for ITS, ETS, atpB-rbcL and trnQ-rps16.