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ORIGINAL RESEARCH PAPER

Is the presence of simple or glandular hairs a good trait for distinguishing species in Caryophyllaceae? A case study of *Arenaria serpyllifolia* sensu lato in southern Poland

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Abstract

The presence of different types of indumentum is regarded as a valuable taxonomic trait for describing and differentiating between species in many different families and genera. In Caryophyllaceae, however, this character is sometimes a subject of discussion and scientific conflicts. For instance, within *Arenaria serpyllifolia* sensu lato (s. l.), two or three taxa have been distinguished based on the presence or absence of glandular and/or eglandular hairs on the calyx and uppermost leaves, namely *A. serpyllifolia*, *A. viscida*, and *A. serpyllifolia* var. *intermedia*. The most common, based on material from Poland, is the glandular morphotype of *Arenaria serpyllifolia* s. l. (315 specimens), which is in contrast to the eglandular and intermediate morphotypes represented by 174 and 24 specimens, respectively; however, the ranges of distribution of these morphotypes fully overlap. Based on our macro- and micromorphological examination of specimens belonging to the group, as well as numerical and molecular studies, we conclude that the occurrence and abundance of eglandular and glandular hairs may vary in particular parts of specimens of *Arenaria serpyllifolia* s. l., i.e., on their calyces, bracts, and uppermost and middle cauline leaves. The width of the capsule was the only character for which significant differences between the SERP and VIS morphotypes were found; however, considering all studied morphotypes together, the capsule characters overlap considerably. Moreover, morphological variation expressed by different types of indumenta is not paralleled by internal transcribed spacer (ITS) polymorphism patterns. The probable lack of genetic barriers between populations of individuals with glandular or eglandular hairs supports the hypothesis that the type of indumentum is of minor taxonomic importance. However, it is possible that we are dealing with sampling in the hybrid zones of both taxa, and that individuals demonstrating morphological traits typical of both taxa (eglandular and glandular) do not in fact represent pure taxa but only different kinds of hybrids, backcrosses, or introgressive forms.

Keywords
Arenaria; calyx indumentum; ITS; morphometric analysis; SEM observation

Introduction

The genus *Arenaria* L., widely distributed in the northern temperate and arctic regions of Eurasia and the Americas [1], comprises over 270 species worldwide [2], 126 of which occur in Europe [3]. In keeping with this wide geographical distribution, the genus expresses a high level of morphological variability, including various forms such as small annuals, biennials, perennials, spiny shrubs, or cushion plants [1,4].

Tab. 1 Classification of taxa grouped in *Arenaria serpyllifolia* s. l. according to different taxonomic arrangements.

Poland			Germany	Czech Republic	Europe	NW Russia	Asian Russia	Europ. part of Russia	Siberia	Kirgistan	Caucasus	Former SSSR	Russia	China	Aghanistan
Stengl-Rejthar [7]	Rutkowski [15]	Mirek et al. [19]	Jäger and Werner [14]	Danihelka et al. [36]	Chater and Halliday [8]	Tzvelev [12]	Vlasova [18]	Tzvelev [5]	Vlasova [9] / Ebel [13]	Lazkov [10]	Lazkov [17]	Schischkin and Knorring [37]	Czerepanov [38]	Wu et al. [9]	Breckle et al. [39]
A. serp. subsp. *serp.*	*A. serp.* subsp. *serp.*	*A. serp.*	*A. serp.* subsp. *serp.*	*A. serp.*	*A. serp.*	*A. serp.*	*A. serp.*	*A. serp.*	-	*A. serp.*	*A. serp.*	*A. serp.* var. scabra	*A. serp.*	*A. serp.*	*A. serp.*
A. serp. subsp. *gland.*	*A. serp.* subsp. *gland.*	-	*A. serp.* subsp. *gland.*	-	-	*A. viscida*	-	*A. viscida*	*A. viscida*	-	-	*A. serp.* var. *viscida*	*A. uralensis*, *A. zozii*	-	-

Abbreviations: serp. – serpyllifolia; gland. – glandulosa.

In the genus *Arenaria*, especially in sect. *Arenaria*, some species have been described and differentiated in terms of indumentum characters. *Arenaria serpyllifolia* L. sensu lato (s. l.) can serve as an example in which two or three taxa have been distinguished based on the presence or absence of glandular hairs on the calyx and uppermost leaves. These taxa have been distinguished at the level of species, subspecies, or variety. Plants covered by simple eglandular hairs are treated as *A. serpyllifolia* sensu stricto (s. s.), whereas plants covered with glandular hairs, sometimes with an admixture of a small number of straight hairs, are classified as *A. viscida* [syn. *A. serpyllifolia* subsp. *viscida* (Hall. f. ex Lois.) Dostál; *A. serpyllifolia* var. *viscida* (Loisel.) DC.; *A. serpyllifolia* subsp. *glutinosa* (Mert. & W. D. J. Koch) Arcang.; *A. serpyllifolia* var. *glutinosa* W. D. J. Koch; *A. serpyllifolia* subsp. *sarmatica* Zapał.], *A. zozii* Kleop., or *A. uralensis* Pall. ex Spreng. (Tab. 1). Eglandular plants with a small admixture of glandular hairs are distinguished either as *A. serpyllifolia* [syn. *A. serpyllifolia* subsp. *scabra* Fenzl.; *A. serpyllifolia* var. *intermedia* Zapał.] [5] or *A. serpyllifolia* subsp. *sarmatica* var. *intermedia* Zapał. [6]. Some authors have stressed that *A. serpyllifolia* and *A. viscida* also differ in terms of general distribution range, and regard the former as primarily a western European species and the latter as a Eurasian taxon [5,7]; however, both taxa have been recorded throughout the distribution range of *Arenaria serpyllifolia* s. l. [8–10]. It is worth emphasizing that there is still no consensus among taxonomists dealing with this group of taxa; some treat *A. serpyllifolia* and *A. viscida* as two clearly-identified species [5,11–13], subspecies, or varieties within *A. serpyllifolia* [7,14,15], whereas others [8,16–18] regard the presence or absence of glandular hairs as a character of minor taxonomic value and treat both morphotypes as belonging to one taxon, namely *A. serpyllifolia* s. s.

In Poland, the genus *Arenaria* is represented by three native species – *A. serpyllifolia*, *A. graminifolia* Schrad. [syn. *A. procera* Spreng. subsp. *glabra* (F. N. Williams) Holub], and *A. tenella* Kit. (syn. *A. ciliata* L.) [7,15,19,20] – as well as the non-native *A. leptoclados* Guss., occasionally observed mainly in the southwestern part of the country [19]. Of the three above-mentioned native species, *A. serpyllifolia* is the most common in Poland [21], growing in dry and ruderal areas, fields, fallows, and grasslands, in sandy and calcareous soils [7,20,22]. Two subspecies of *A. serpyllifolia* have been distinguished in Poland, namely *A. serpyllifolia* subsp. *serpyllifolia* and *A. serpyllifolia* subsp. *glandulosa* [7,15]. Since both glandular and eglandular morphotypes have been reported within *A. serpyllifolia*, we would like to answer the following questions in the present study: (***i***) Can these morphotypes be distinguished by any morphological character other than the indumentum? (***ii***) Are there any differences between the two morphotypes in the micromorphology of capsules and seeds? (***iii***) Is there any geographical distributional pattern characterizing these morphotypes in Poland? (***iv***) Does morphological differentiation based on indumentum type coincide with molecular variation?

Material and methods

Morphological analysis

The study is based on herbarium material of *Arenaria serpyllifolia* s. l., preserved in two major Polish herbaria, KRA and KRAM. Altogether, 454 herbarium sheets were examined. On selected sheets, measurements of three complete and undamaged specimens (when available) were made. In total, 525 specimens of *Arenaria* were analyzed. When different morphotypes were available within a single herbarium sheet, each specimen was treated as a separate individual. Based on available keys for identification, three quantitative and four qualitative characters were selected for further examination: calyx length, capsule length, and capsule width (quantitative); calyx indumentum, bracts indumentum, uppermost cauline leaf indumentum, and middle cauline leaf

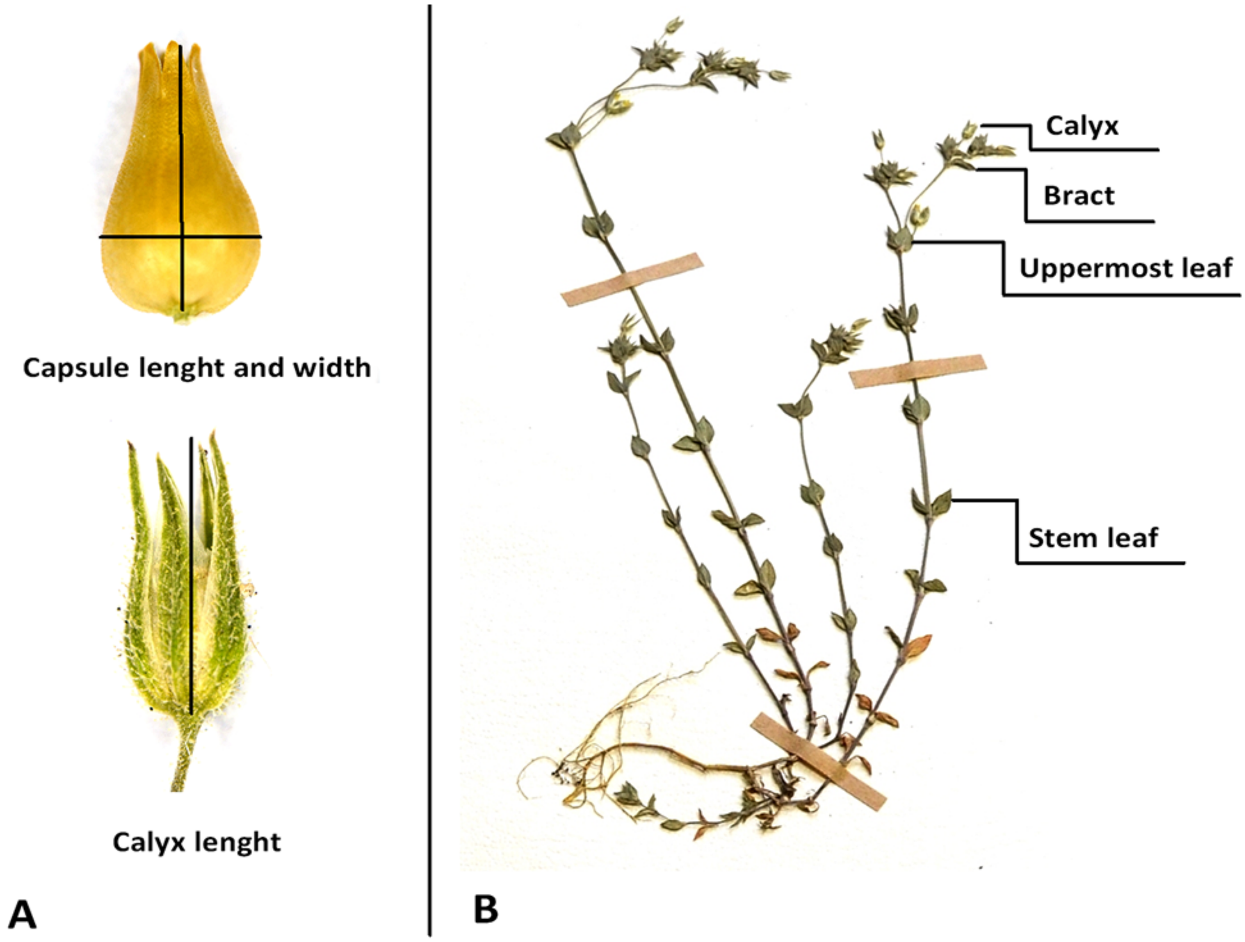

Fig. 1 Quantitative (**A**) and qualitative (type of indumentum on selected part of the plant) (**B**) characters used for examination.

indumentum (qualitative) (Fig. 1). The qualitative characters were assessed on the basis of three separate estimates for each specimen. The examined specimens were then assigned to one of three groups (morphotypes) based on the predominant type of calyx indumentum, namely VIS, SERP, and MIX. Specimens with glandular indumentum were assigned to VIS and eglandular specimens to SERP; those with both types of hairs were assigned to MIX.

Micromorphological analysis

Using scanning electron microscopy (SEM) observations, we analyzed a total of eight specimens of VIS, MIX, and SERP. A list of voucher specimens used in the study is given in Tab. 2. Samples of seeds and calyces were coated with a thin layer of gold using a JFC-1100E ion sputter (JEOL), then observed and photographed using a Hitachi S-4700 scanning electron microscope.

Molecular analysis

A total of nine herbarium specimens representing three morphotypes (VIS, MIX, SERP) were selected for molecular analyses. Dried plant material (10–15 mg) was used for DNA isolation. The plant tissue was ground to a fine powder using an MM 400 mixer mill (Retsch) and 3-mm tungsten beads. Total genomic DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's protocol. The internal transcribed spacer region (ITS) of the 18S-5.8S-26S nuclear ribosomal cistron, consisting of ITS 1 and ITS 2, was amplified using the universal primers ITS1A and ITS 4 [23,24]. Mix composition and PCR conditions were in accordance

Tab. 2 Samples of *Arenaria serpyllifolia* s. l. included in SEM observation.

Morphotype and part of the plant	Herbarium No.	Geographical origin
SERP-calyx	KRA0140663	Gorzyce vill.
SERP-seed	KRA0247057	Bażanowice vill.
SERP-seed	KRA0321322	Kozy vill.
VIS-calyx	KRA0393467	Kanice Nowe vill.
VIS-seed	KRA0278827	Czajków Południowy vill.
VIS-seed	KRA0393468	Niecka Włoszczowska region
MIX-calyx	KRA0239830	Brama Bolechowicka stone
MIX-seed	KRA0247892	Śnietnica vill. near Gorlice

with those presented by Zalewska-Gałosz et al. [25]. PCR products were purified using a High Pure PCR Product Purification Kit (Roche Diagnostics) according to the manufacturer's protocol and sequenced in two directions with the primers used for amplification. Sequencing was performed using a BigDye Terminator ver. 3.1 cycle sequencing kit (Applied Biosystems) with supplied sequencing buffer according to the manufacturer's manual. Sequencing products were purified using the ethanol/EDTA protocol, resuspended in 12 μL formamide, and separated on an ABI 3100-Avant Genetic Analyzer using 50-cm capillaries and POP-6 polymer (Applied Biosystems). Raw sequencing profiles were analyzed with DNA Sequencing Analysis Software ver. 5.1 (Applied Biosystems). The sequences were manually verified/adjusted using the software FinchTV ver. 1.4.0 (Geospiza Inc.). Alignments of sequences for all regions were conducted manually using BIOEDIT ver. 5.0.9. (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Nucleotide polymorphisms were examined using two strands to ensure their consistency and coded using the IUPAC nucleotide ambiguity codes. The origin of the studied samples and the GenBank accession numbers of sequences obtained in the study are provided in Tab. 3.

Tab. 3 Samples of *Arenaria serpyllifolia* s. l. included in DNA study.

Morphotype and sample No.	Herbarium No.	Geographical origin	Long. (E)	Lat. (N)	Collector	Date	GB accession numbers
SERP-1	KRA0293958	Muszyna	20.8968	49.3566	A. Tyc	2004-05-28	MG101844
SERP-2	KRA0313310	Skała village	19.8541	50.2304	M. Zarzyka-Ryszka	2001-05-03	-
SERP-3	KRA0140668	Zarzecze vill. near Jasło	22.5144	49.9824	K. Oklejewicz	1990-04-28	MG101845
VIS-4	KRA0348596	Bzinek-Bór vill. near Skarżysko-Kamienna	20.8478	51.1143	M. Podgórska	2007-04-29	MG101846
VIS-5	KRA0316823	Dobczyce	20.0891	49.8812	K. Stawowczyk	2004-05-25	MG101847
MIX-6	KRA0312267	Kółko Żabieckie vill.	21.0925	50.3444	M. Zarzyka-Ryszka	2004-05-02	MG101848
VIS-7	KRA0314075	Opole	17.9213	50.6751	M. Kozak	2004-05-16	MG101849
VIS-8	KRA0278827	Czajków Południowy village	21.2987	50.5705	I. Skrzynecka	2001-06-26	MG101850
VIS-9	KRA0262680	Wieliczka	20.0648	49.9871	M. Wayda	2004-05-31	MG101851

To check for potential intraspecific sequence polymorphisms within *Arenaria serpyllifolia* s. l., all accessions of the ITS region obtained from this taxon and deposited in GenBank were retrieved and included in the analysis (Tab. 4).

Statistical analyses

The proportion of different types of indumentum on particular parts of the plant was calculated for the predefined morphotypes (i.e., VIS, MIX, SERP). This analysis was made on the basis of at least three separate estimates for a single individual. After

Tab. 4 Polymorphisms in the ITS sequences from analyzed samples and accessions of *Arenaria serpyllifolia* s. l. retrieved from GenBank. Polymorphic nucleotide sites are coded using the IUPAC code.

Taxon (sample code)	Source of data	GB number	Position in the alignment																						
			10	89	114	118	119	153	340	343	474	475	476	477	478	479	481	483	489	490	491	492	496	504	508
A. serpyllifolia	GB	KX167467	?	?	?	?	?	?	T	C	C	C	C	G	T	C	C	C	T	T	T	G	T	T	A
A. serpyllifolia	GB	KX166753	?	?	?	?	?	?	T	C	C	C	C	G	T	C	C	C	T	T	T	G	T	T	A
A. serpyllifolia	GB	KX166482	?	?	?	?	?	?	T	C	C	C	C	G	T	C	C	C	T	T	T	G	T	T	A
A. serpyllifolia	GB	KP148896	T	C	G	T	G	C	A	C	C	C	C	G	T	C	C	C	T	T	T	G	T	T	A
A. serpyllifolia	GB	FJ869024	T	C	G	T	G	C	A	C	C	C	C	G	T	C	C	C	T	T	T	G	T	T	A
A. serpyllifolia	GB	KX166752	T	C	G	C	G	C	A	C	C	C	C	G	T	C	C	C	T	T	T	G	T	T	A
A. serpyllifolia	GB	AY936279	T	C	G	C	G	C	A	C	C	C	C	G	T	C	C	C	T	T	T	G	T	T	A
A. serpyllifolia	GB	JN589113	T	C	G	C	G	C	A	C	C	C	C	G	T	C	C	C	T	T	T	G	T	T	A
A. serpyllifolia (MIX-6)	This study	MG101848	T	C	G	C	G	C	A	C	C	C	C	G	T	C	C	C	T	T	T	G	T	T	A
A. serpyllifolia (SERP-3)	This study	MG101845	T	C	G	C	G	C	A	C	Y	C	C	G	T	C	C	C	T	T	T	G	T	T	A
A. serpyllifolia (VIS-7)	This study	MG101849	T	C	G	C	G	C	A	Y	Y	C	C	C	T	C	C	C	T	T	T	G	T	T	A
A. serpyllifolia (VIS-5)	This study	MG101847	W	Y	K	C	R	Y	W	C	T	C	S	G	T	C	C	C	T	T	T	G	T	T	A
A. serpyllifolia (VIS-9)	This study	MG101851	W	Y	K	C	R	Y	W	C	C	C	C	C	T	C	C	C	T	T	T	G	T	T	A
A. serpyllifolia (SERP-1)	This study	MG101844	W	Y	K	C	R	Y	W	C	Y	C	C	C	T	C	C	C	T	T	T	G	T	T	A
A. serpyllifolia (VIS-8)	This study	MG101850	W	Y	K	C	R	Y	W	C	C	G	G	A	A	G	A	A	A	A	C	–	G	A	G
A. serpyllifolia (VIS-4)	This study	MG101846	W	Y	K	C	R	Y	W	C	C	G	G	A	T	G	M	C	T	W	C	–	G	T	G

? – lack of data due to different length of compared GB accessions.

using Levene's test to assess equality of variance, one-way ANOVA, followed by Tukey's HSD test, were performed for unequal sample frequencies to assess the significance of differences between the means of characters across all examined groups of specimens representing particular morphotypes (i.e., VIS, SERP, MIX). Variables that did not meet the assumptions of normality were Box–Cox transformed to find the optimal normalizing transformation in each case. Nonmetric multidimensional scaling (NMDS) was used to find the pattern of similarities between studied specimens of *A. serpyllifolia* s. l. in terms of all quantitative and qualitative characters, except the calyx indumentum, which was treated as a grouping variable. The analysis, performed using the Bray–Curtis similarity coefficient, was based on the averaged values of each character for specimens examined on a single herbarium sheet. Statistical calculations were made using PAST 3.10 [26] and STATISTICA 12.

Results

Morphological analyses

Based on herbarium material, three morphotypes of *Arenaria serpyllifolia* s. l. in Poland were recorded. The first (VIS) was represented by 315 specimens, the second (SERP) by 174, and the third (MIX) by 24. The proportion of indumentum types in the defined morphotypes varied across individual parts of the plants (Fig. 2). The VIS morphotype was characterized by glandular hairs on the calyx, an increase in the proportion of mixed or eglandular hairs on bracts and the uppermost leaf, and over 80% of leaves on the stem leaves being eglandular. The SERP morphotype was characterized by the consistent presence of eglandular hairs on each part of the plant from the calyx to stem leaves. Rarely, single glandular hairs at the base of the calyx were observed in this morphotype. In the MIX morphotype, eglandular and glandular hairs were present, albeit in different proportions, on the calyx within a single specimen; however, in the lower part of the plant, the proportion of glandular hairs significantly declined, and on the stem leaves the share of glandular hairs was less than 5%. The calyx length and ratio of capsule length to width failed to distinguish particular taxa (ANOVA; $p > 0.05$;

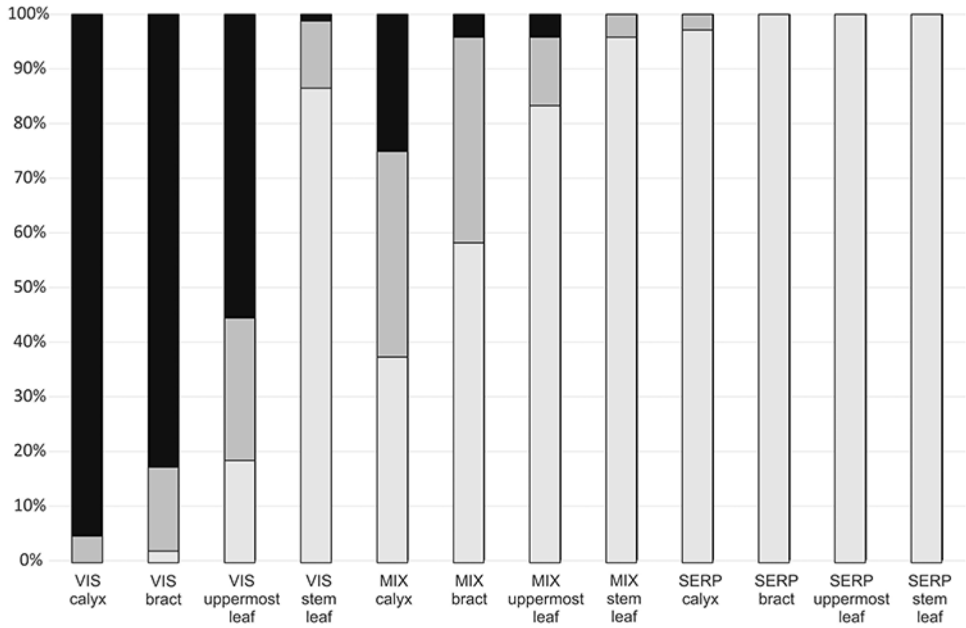

Fig. 2 The share of glandular and eglandular hairs in particular parts of studied specimens representing different plant of predefined morphotypes (i.e., VIS, MIX, SERP); grey – domination of eglandular hairs, dark-grey – mix of eglandular and glandular hairs with equaling proportion; black – domination of glandular hairs.

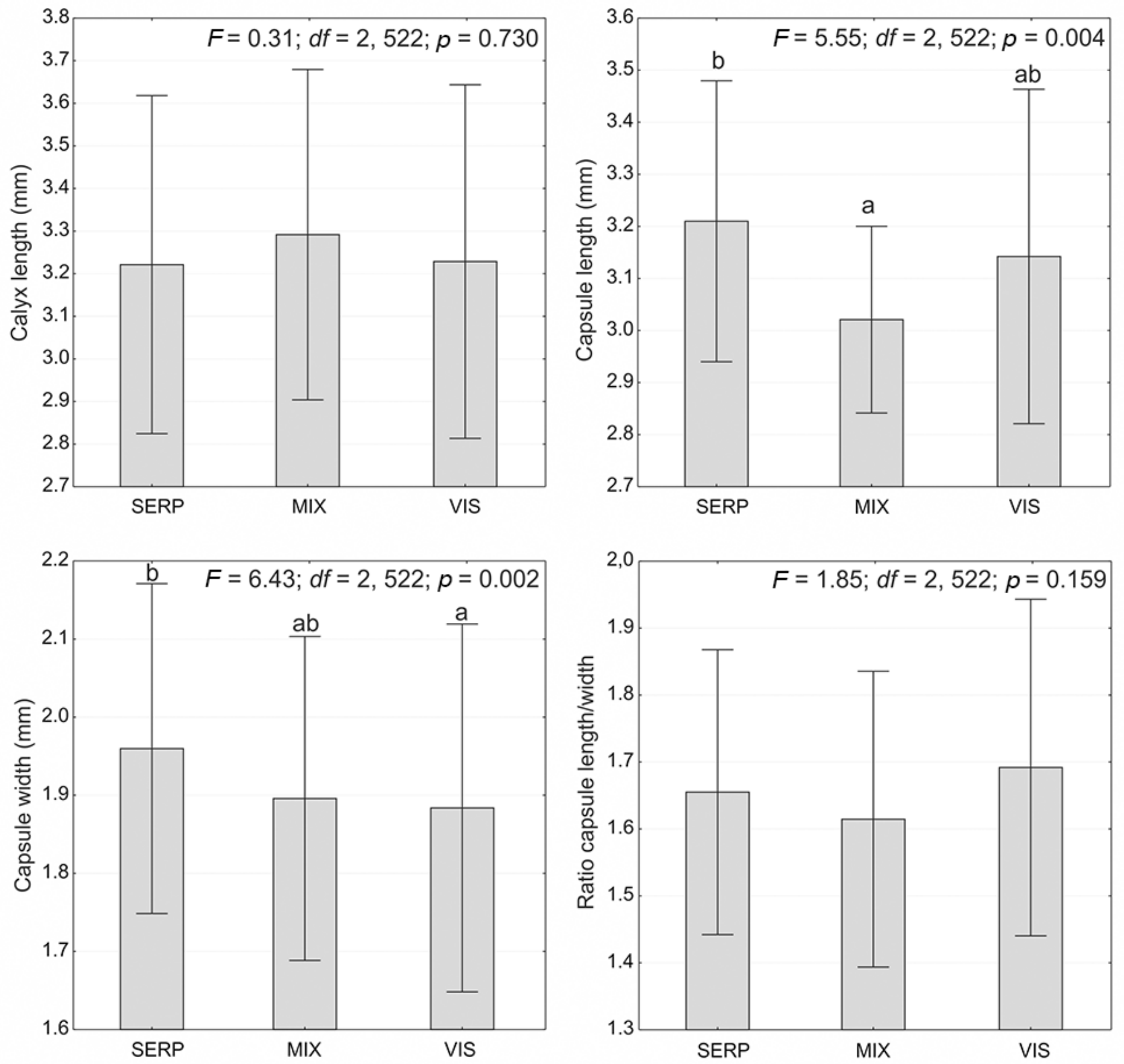

Fig. 3 Quantitative characters (means ±*SD*) of studied morphotypes (VIS, MIX, SERP). Results of ANOVA ($p < 0.05$): *F* and *p* values are provided. Letters denote the results of Tukey's (HSD) test; different letters indicate significant differences at the $p < 0.05$ level.

Fig. 3). The width and length of the capsule differed significantly between morphotypes (ANOVA; $F = 6.34$, $df = 2, 522$, $p = 0.002$ and $F = 5.55$, $df = 2, 522$, $p = 0.004$, respectively). Tukey's post hoc test revealed significantly wider capsules in the case of SERP compared to VIS, whereas the length of the capsule proved to be significantly higher in SERP compared to MIX, but comparable with VIS.

NMDS ordination showed similarities between the studied specimens (Fig. 4). The MIX morphotype was not distinguished as a separate group, as it remained within the range of morphological variability between VIS and SERP. The VIS morphotype tended to be concentrated on the right side of the diagram, SERP on the left side. This result suggested that the analyzed characters were capable of clearly distinguishing only the VIS and SERP morphotypes.

Micromorphological analysis

The general pattern of seed and calyx micromorphology was typical of *Arenaria serpyllifolia*; the micromorphology of the seeds and calyces of the three studied morphotypes – eglandular, mixed, and glandular – were similar. There were no differences in the shapes of seeds or their cells in the examined morphotypes. The shapes of cells

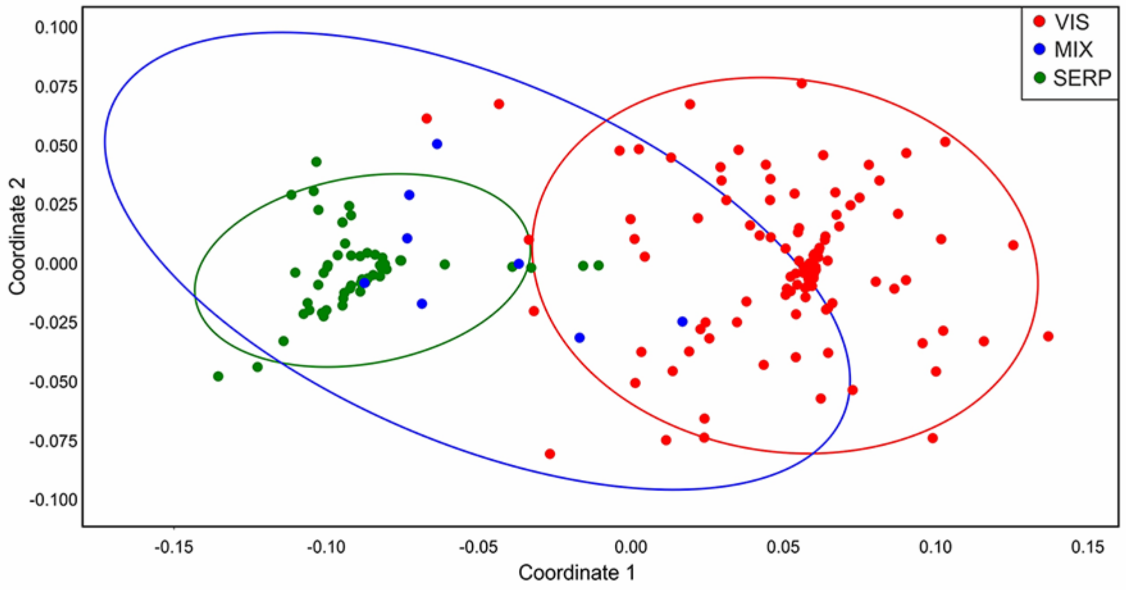

Fig. 4 Nonmetric multidimensional scaling (NMDS) scatterplot of studied specimens representing *Arenaria serpyllifolia* s. l. morphotypes (VIS, MIX, SERP).

with deeply sinuous side walls were generally also very similar in the three examined morphotypes. The only difference was the presence of different types of trichomes in particular morphotypes: two–three cellular, straight, and/or slightly falcate hairs in SERP (Fig. 5C); two–four cellular, straight or slightly falcate, glandular hairs, with an admixture of a few eglandular hairs, in MIX (Fig. 5G); and three–four(–five) cellular, straight, glandular hairs in VIS (Fig. 5K).

Variation in ITS region sequence

ITS sequences obtained from the studied samples were 691–711 bp long and their alignment together with the sequences retrieved from GenBank covered 509 bp. Twenty-three polymorphic sites were detected in the data set, including 22 substitutions and one insertion/deletion (indel; Tab. 4). Sample SERP-2 was excluded from the study because the quality of the ITS sequence obtained was insufficient. Within the sequences retrieved from GenBank, two polymorphic sites were detected (118, 340), differentiating two ribotypes within *A. serpyllifolia*. One sequence obtained in this study, MIX-6, closely matched the ribotype distinguished based on GB accessions. The remaining studied samples were not identical and showed an additive polymorphism pattern in at least one position (Tab. 4). The polymorphism pattern detected in the ITS sequences did not correspond with the morphotypes defined based on the presence or absence of glandular hairs.

Geographic distribution of morphotypes in southern Poland

The distribution ranges of the three morphotypes overlap (Fig. 6). We found no clear geographical pattern of distribution for particular morphotypes. Specimens with glandular, eglandular, and mixed hairs were scattered throughout southern Poland; however, morphotype SERP seems to be more frequent in southwestern Poland, whereas morphotype VIS is more common in the central and eastern parts of the examined area (Fig. 6). Different morphotypes were also frequently collected in mutual proximity, and, moreover, occurred within the same populations in several localities. VIS and SER morphotypes were found in six sites: Myślenice, Maków Podhalański, Kraków, Piotrówka, Kędzierzyn-Koźle, and Rycerka Dolna. VIS and MIX morphotypes occurred in Kraków and Pętkowice, while SER and MIX occurred only in Kraków (Appendix S1).

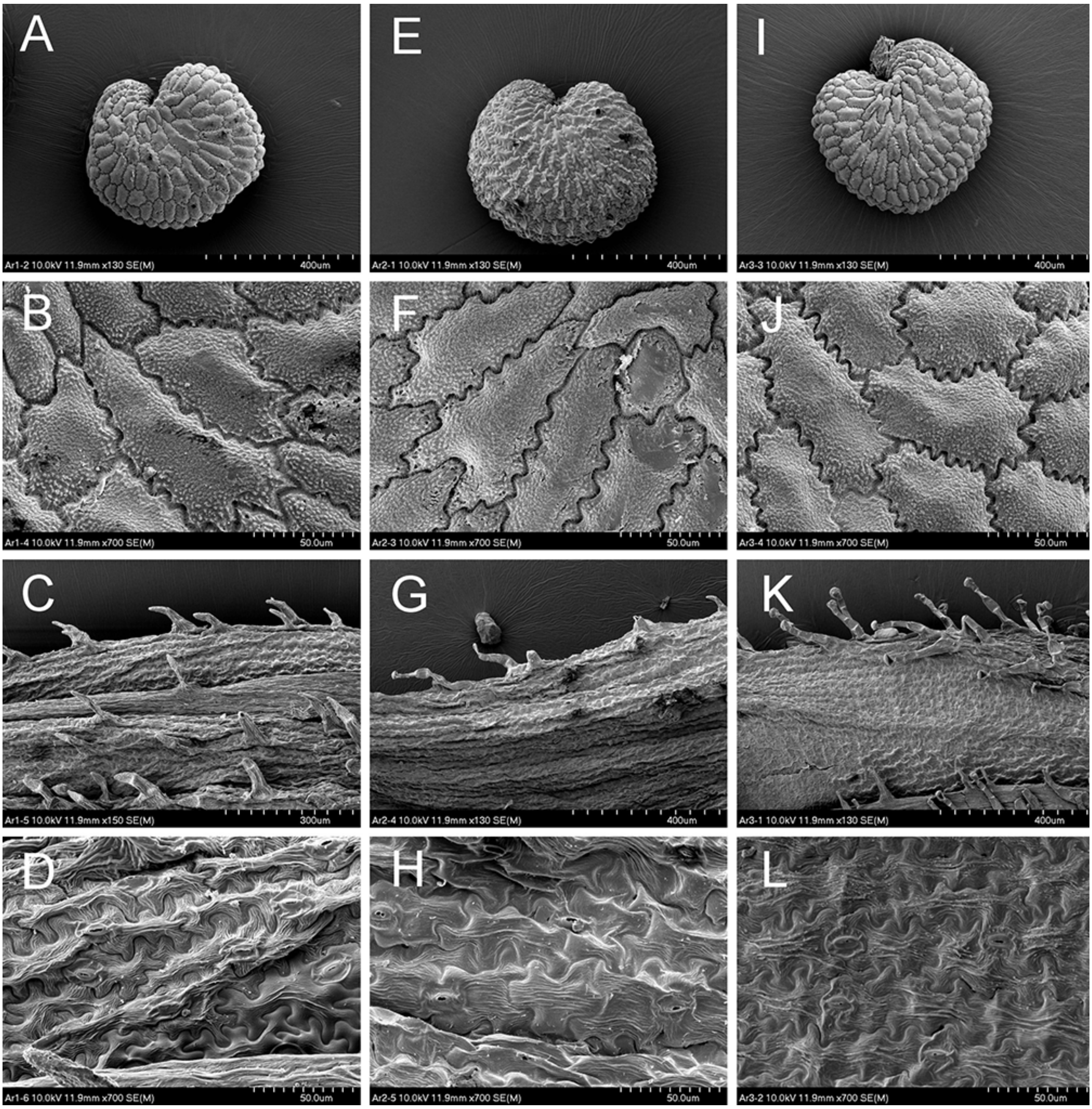

Fig. 5 Micromorphology of seeds [shape of seed (**A,E,I**); sinuous side walls of the basal cells (**B,F,J**)] and calyxes [eglandular, mixed, and glandular hairs on the abaxial surface of calyx (**C,G,K**); deeply sinuous slide walls of the calyx basal cells (**D,H,L**)] in *Arenaria serpyllifolia* s. l.: (**A–D**) morphotype SERP; (**E–H**) morphotype MIX; (**I–L**) morphotype VIS.

Discussion

The presence of various types of indumentum is regarded as a useful taxonomic trait in describing and differentiating species in many different families and genera. In Caryophyllaceae, however, this character is sometimes a subject of discussion and scientific conflicts because the presence of glandular and eglandular (simple) hairs on plants is regarded by some as simply the result of morphological variability in the taxon, while others use it to distinguish new species or taxa of lower ranks. A good example is *Arenaria serpyllifolia* s. l., in which some taxa have been distinguished based on differences in indumentum as well as on the range of their general geographical distribution, e.g., *A. viscida* (plants with glandular hairs, distributed in Central-Eastern Europe and Asia) and *A. serpyllifolia* (plants with simple eglandular hairs, distributed in Western Europe) [5,7,12,15]. However, it is worth emphasizing that, even in Central Asia [Kyrgyzstan, Nobis et al. (KRA 0475129)], eglandular specimens have sometimes

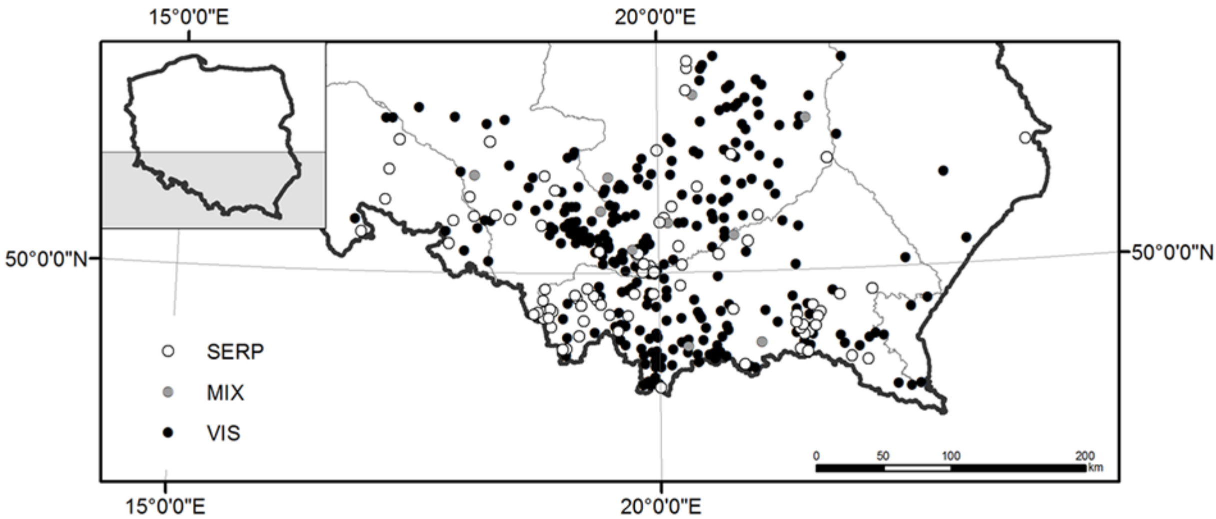

Fig. 6 Distribution map of different morphotypes of *Arenaria serpyllifolia* s. l. in southern Poland, based on material analyzed. white dots – SERP; grey dots – MIX; black dots – VIS.

been recorded along with glandular plants in the same population [10]. Moreover, these two morphotypes also occur sympatrically in Europe, and thus are treated by some researchers as a single taxon, *A. serpyllifolia* [8].

The ranges of many Western European and Euro-Asian species reach their distribution limits in Central Europe, including Poland [21,27,28]. Some researchers include eglandular *Arenaria serpyllifolia* and glandular *A. viscida* among these species; however, in Poland the ranges of these taxa overlap and representatives of both may co-occur in the same area or locality, and even within a single population (Fig. 6, Appendix S1). This is extremely interesting, since gene flow could take place in the contact zones of two species with different ranges [29–33]. The presumed hybrids are often first noticed when individuals are found with morphological characters between those of the parent taxa. Thus, based on the characters of indumentum, within *Arenaria serpylifolia* s. l. we distinguished, in addition to SERP and VIS, the MIX morphotype, which may suggest the presence of natural hybrids in the contact zone of both of the basic morphotypes mentioned above. The occurrence and abundance of eglandular and glandular hairs, however, may vary in parts of *Arenaria serpyllifolia* individuals. In the VIS and MIX morphotypes, the share of glandular hairs increases in the upper part of the plant and is highest on the calyx. The VIS morphotype may exhibit a small admixture of eglandular hairs on calyces, whereas in MIX, the abundance of glandular and eglandular hairs on calyces is comparable (Fig. 2). In contrast, the SERP morphotype is covered only by simple eglandular hairs; an admixture of few scattered glandular hairs may also be observed sporadically on the calyx (Fig. 2). Tzvelev [12] pointed out that, besides the indumentum, these two morphotypes also differ in capsule length (3.0–3.6 mm in *A. serpyllifolia* vs. 2.5–3.2 mm in *A. viscida*). However, we found no significant difference in capsule length between SERP and VIS morphotypes (Fig. 3); for both morphotypes, the 25–75% percentile ranges were 3.0–3.5 mm. It is noteworthy that the only character that differed significantly between the SERP and VIS morphotypes was capsule width; however, the differences were rather subtle (mean capsule width values of 1.96 and 1.88 mm for the SERP and VIS morphotypes, respectively). Moreover, the variability range of this character for the MIX morphotype considerably overlaps with those of SERP and VIS. Although the mixed indumentum type of the MIX morphotype may suggest its hybrid origin, it is not excluded that it merely represents a variant of the VIS morphotype (as also suggested by Tzvelev [12]), with simple hairs representing a minor admixture along with frequently occurring glandular hairs. Our SEM analysis of seeds taken from capsules of the three studied morphotypes revealed no differences between them (Fig. 5). Genetic evidence, however limited, also shows that morphological variation expressed by different types of indumentum is not paralleled by the ITS polymorphism pattern. A relatively high level of polymorphism and the partly additive character detected in various samples of *A. serpyllifolia* s. l. provide evidence that gene flow exists between populations of this taxon. The probable lack of genetic barriers between populations represented by individuals with glandular and eglandular hairs supports the hypothesis that differences in the type of indumentum represent a taxonomically less important character.

Nor is it out of the question that our sampling was conducted within the hybrid zone, i.e., the area where species overlap in range and interbreed, producing viable hybrid offspring of mixed ancestry [30,33]. This phenomenon is common in many plant and animal organisms and can cause various consequences, including hybrid zone movement [32–35]. A lack of both clear morphological delimitation and the convergence of an ITS polymorphism pattern with indumentum type may suggest genetic introgression between parental populations. Bearing in mind that southern Poland is an area characterized by the co-occurrence of both morphotypes, we cannot exclude the possibility that glandular and eglandular individuals do not, in fact, represent pure taxa, but rather different kinds of hybrid offspring, backcrosses, or even introgressive forms in which morphological characters can be differentiated independently of the ITS genetic marker.

In Caryophyllaceae, there are a number of taxa that, as in the *Arenaria serpyllifolia* complex, were described based on the type of indumentum, e.g., *Arenaria leptoclados* (Rchb.) Guss. (with glandular hairs) vs. *A. viscidula* Tzvelev nom. illeg. (with eglandular hairs); *Cerastium tauricum* Spreng. (with glandular hairs) vs. *C. brachypetalum* Desp. ex Pers. (with eglandular hairs); *Cerastium triviale* Link (with glandular hairs) vs. *C. holosteoides* Fr. (with eglandular hairs). Other examples of glandular and glabrous pairs of taxa include: *Holosteum glutinosum* (M. Bieb.) Fisch. & C. A. Mey. vs. *H. umbellatum* L. or *Silene nutans* L. subsp. *nutans* and *S. nutans* subsp. *glabra* (DC.) Rothm. As in the case of *A. serpyllifolia* s. l., either the taxa listed above are considered to be at different taxonomic levels, or the differences in indumentum characterizing them are treated as morphological variability within a single taxon [2]. To resolve this issue, samples of both morphotypes from populations collected throughout their distribution ranges are needed, along with the use of modern molecular analyses, e.g., next-generation sequencing, gene expression analysis, and experimental planting of both morphotypes in different climatic conditions. Such research may help answer the question of whether the observed variability has a genetic background or represents phenotypic variation only, and, if the latter is true, what causes this variation.

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Supplementary material

The following supplementary material for this article is available at http://pbsociety.org.pl/journals/index.php/asbp/rt/suppFiles/asbp.3630/0:

Appendix S1 Herbarium specimens examined.

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