

Article

New Techniques for Seed Shape Description in *Silene* Species

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Abstract: Seed shape in *Silene* species is often described by means of adjectives such as reniform, globose, and orbicular, but the application of seed shape for species classification requires quantification. A method for the description and quantification of seed shape consists in the comparison with geometric models. Geometric models based on mathematical equations were applied to characterize the general morphology of the seeds in 21 species of *Silene*. In addition to the previously described four models (M1 is the cardioid, and M2 to M4 are figures derived from it), we present four new geometric models (model 5–8). Models 5 and 6 are open cardioids that resemble M3, quite different from the flat models, M2 and M4. Models 7 and 8 were applied to those species not covered by models 2 to 6. Morphological measures were obtained to describe and characterize the dorsal view of the seeds. The analyses done on dorsal views revealed a notable morphological diversity and four groups were identified. A correlation was found between roundness of dorsal view and the geometric models based on lateral views, such that some of the groups defined by seed roundness are also characterized by the similarity to particular models. The usefulness of new morphological tools of seed morphology to taxonomy is discussed.



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1. Introduction

Silene L., with ca. 850 species, is the largest genus in the family Caryophyllaceae Juss. According to a comprehensive DNA sequence analysis, the genus has been split in three subgenera: *S. subg. Lychnis* (L.) Greuter, *S. subg. Behenantha* (Otth) Torr. & A.Gray, and *S. subg. Silene sensu stricto* [1]. *Silene subg. Lychnis* is weakly supported as sister to the other subgenera and includes four sections: *Agrostemma* (DC.) Greuter, *Lychnis* (L.) Greuter, *Coccyganthe* (Rchb.) Greuter and *Uebelina* (Hochst.) F. Jafari, Oxelman & Rabeler. *Silene subg. Behenantha* is poorly resolved and contains eighteen sections: *Psammophilae* (Talavera) Greuter, *Elisanthe* (Fenzl ex Endl.) Ledeb., *Melandrium* (Röhl.) Rabeler, *Conoimorpha* Otth, *Cryptoneuræ* Aydin & Oxelman, *Sedoïdes* Oxelman & Greuter, *Cucubalus* (L.) Greuter, *Cucubaloides* Edgeworth & Hook, *Cordifoliae* Chowdhuri, *Erectorefractae* Chowdhuri, *Acutifoliae* Oxelman & F. Jafari, *Anotites* (Greene) Oxelman, F. Jafari & Rabeler, *Odontopetalae* Chowdhuri, *Fimbriatae* (Boiss.) Bornm., *Dichotomae* (Rohrb.) Chowdhuri, *Saponarioides* (Boiss.) Schischk., *Behenantha* Otth, and *Physolychnis* (Benth.) Bocquet. *Silene subg. Silene* contains the following eleven sections named: *Silene*, *Petrocoma* (Rupr.) F. Jafari, Oxelman & Rabeler, *Lasiocalycinae* (Boiss.) Chowdhuri, *Auriculatae* (Boiss.) Schischk., *Rigidulae* (Boiss.) Schischk., *Sclerocalycinae* (Boiss.) Schischk., *Siphonomorpha* Otth, *Pulvinatae* (Chowdhuri) F. Jafari, Oxelman & Gholipour, *Sclerophyllae* (Chowdhuri) F. Jafari, Oxelman & Rabeler, *Muscipula* (Tzvelev) Oxelman, F. Jafari & Gholipour, and *Portenses* F. Jafari & Oxelman.

Finally, and no less important, a number of *Silene* species remain without a clear adscription to any of these sections [1].

The history of seed morphology in *Silene* has two reference dates in 1867 and 1869, when Boissier and Rohrbach respectively, published their books containing a series of characters for seed shape description [2,3]. Some of the adjectives used refer to overall shape in the lateral view, such as reniformia (kidney-shaped), or in the dorsal view, such as compressa, while other terms describe the details of the lateral and dorsal faces. The lateral face was described by Boissier and Rohrbach as plane (or planiuscule), concave, or sub-concave. The dorsal side was termed plane (dorso plana), convex, or deepened (dorso canaliculate; dorso obtuse canaliculata) [2,3]. In his treatment, Boissier described 31 sections for the genus *Silene*, some of which are maintained today, such as sections *Conoimorpha*, *Lasiocalycinae*, and *Sclerocalycinae* [1,2]. According to the last taxonomic version [1], these three sections still include some of their original species by Boissier [2]: (i) for sect. *Conoimorpha*, the species *S. conica* L., *S. coniflora* Nees ex Otth, *S. conoidea* L., *S. lydia* Boiss., *S. macrodonta* Boiss. and *S. subconica* Friv.; (ii) for sect. *Lasiocalycinae*, *S. crassipes* Fenzl; and (iii) for sect. *Sclerocalycinae*, *S. armena* Boiss., *S. bupleuroides* L., *S. chlorifolia* Sm., *S. laxa* Boiss. & Kotschy, *S. peduncularis* Boiss., *S. phrygia* Boiss. and *S. swertiifolia* Boiss. Similarly, some infrageneric treatments have been changed in relation to *S.* subg. *Behenantha*. For example, the current sect. *Melandrium*, named as sect. *Melandrifformes* by Boissier [2], includes the widespread species *S. latifolia* Poir., and *S. dioica* (L.) Clair., together with some other less restricted or endemic species as *S. astrachanicum* (Pacz.) Takt., *S. diclinis* (Lag.) M.Laínz, *S. heuffelii* Soó, *S. marizii* Samp. and *S. physocalycina* (Hauskn. & Bornm.) Melzh. [1,4]. However, the previous inclusion of *S. noctiflora* L. within this section [2] is currently considered as a member of sect. *Elisanthe* [1].

The morphological characters used by Boissier and Rohrbach [2,3] for seed description have survived the era of molecular biology and they are currently used one and a half century latter. Some degree of consistency in seed morphological features is noted for the above mentioned sections *Conoimorpha*, *Melandrium*, and *Sclerocalycinae* [3]. Nevertheless, up to date, there is not a clear relationship between the descriptions of seed shape for each species, and their taxonomic position in the currently recognized sections. It remains to be explored whether the general gap between taxonomy and seed morphological description is due to a real lack of correspondence between both aspects or, alternatively, if this responds to an insufficient or inaccurate description of seed shape. On the one hand, a high degree of homoplasy hampers the application of a number of morphological characters in *Silene* taxonomy; on the other hand, it is also clear that the correct identification of those characters less submitted to homoplasy may contribute to explore the relationship between seed shape and taxonomic position [1]. Related to this, there is a need to give accurate definitions of the morphological characters under study. For example, general seed shape descriptions, that are frequently based on adjectives such as “reniform”, “circular”, “globular”, or “semi-globular” [5–11], may be of increased precision when based on new mathematical applications with the identification of the objects of geometry that may resemble the shapes of seeds.

On the basis of previous studies on seeds of the model plant *Arabidopsis thaliana* (L.) Heynh. [12,13] and the model legumes *Lotus japonicus* (Regel) K. Larsen and *Medicago truncatula* Gaertn. [14,15], we have developed seed shape descriptions based on the comparison with geometrical objects [16–18]. Interestingly, the similarity of the seeds of *Silene* species with the cardioid is remarkable; in particular, for species within *S.* subg. *Behenantha* (e.g., *S. noctiflora*, *S. conica*, *S. latifolia*) [19]. The *J* index represents the percent of similarity between the geometric figure used as a model (cardioid) and the seed image. Values of *J* index superior to 90 were obtained in eleven species analyzed corresponding to *S.* subg. *Behenantha* (*S. acutifolia* Link ex Rohrb., *S. conica*, *S. diclinis*, *S. dioica*, *S. latifolia*, *S. noctiflora*, *S. pendula* L., *S. uniflora* Roth, *S. viscosa* (L.) Pers, *S. vulgaris* (Moench) Garcke and *S. zawadzki* Herbich.) [19]. In addition to the cardioid (Model 1), three other models have already been described [19]. Model 2 corresponded to a flattened cardioid that gave maximum

similarity with the species *S. noctiflora* (*J* index 94.4) and *S. latifolia* (*J* index 93.0). Model 3 was defined as an open cardioid curve that gave maximum similarity with *S. gallica* L. (*J* index 90.4). Finally, Model 4 was a flattened and elongated cardioid curve that gave maximum similarity with *S. latifolia* (*J* index 92.5) and *S. declinis* (*J* index 91.5). *J* index values were more stable than other typical morphological measures (i.e., length, width), hence the association between seed morphology and geometrical figures was stated as robust [19], and it could be used for classification purposes [20].

The objectives of this work have been: (1) to develop the description of seed shape based on the comparison with geometric models to include new species of *Silene*, and to describe new geometric models that better fit the shape of seeds in *Silene* species; and (2) to describe the dorsal views of the seeds as a further step in the search for quantification methods of seed shape in *Silene* species. In addition, the quantification of dorsal views would allow to define morphological seed differences between the species in order to identify any morphological pattern. Consequently, the proposed objectives would enhance the knowledge of the morphology of the *Silene* seeds and contribute to assess their value in the infrageneric classification of this genus, especially about *S. subg. Behenantha* and *S. subg. Silene*.

2. Experimental Section

2.1. Seeds of *Silene* Analyzed

Twenty-one seed stocks belonging to different species of *Silene* of the subgenera *S. subg. Behenantha* and *S. subg. Silene* were analyzed. The species were chosen to confirm or discard the hypothesis that there are differences in shape between both subgenera as reported before [19]. Species are listed in Table 1 with the locations of the populations used, and the indication of the corresponding subgenus and section according to the infrageneric classification of *Silene* based on Jafari et al. [1] and Oxelman et al. [4]. At least ten specimens were available from each population.

Table 1. A summary of the species of *Silene* analyzed in this work.

Species	Population Name	Origin	Subgenus and Section
<i>S. conica</i> L.	Villena	Alicante, Spain	<i>S. subg. Behenantha</i> sect. <i>Conoimorpha</i>
<i>S. coutinhoi</i> Rothm. & P.Silva	Larouco	Ourense, Spain	<i>S. subg. Silene</i> sect. <i>Siphonomorpha</i>
<i>S. crassipes</i> Fenzl.	Hania	Kriti, Greece	<i>S. subg. Silene</i> sect. <i>Lasiocalycinae</i>
<i>S. disticha</i> Willd.	Alcari	Estremadura, Portugal	<i>S. subg. Silene</i> sect. <i>Silene</i>
<i>S. diversifolia</i> Otth.	Elda	Alicante, Spain	<i>S. subg. Silene</i> sect. <i>Silene</i>
<i>S. foetida</i> Link ex Spreng.	Muiños	Ourense, Spain	<i>S. subg. Behenantha</i> sect. <i>Acutifoliae</i>
<i>S. gallica</i> L.	Corse	Corse, France	<i>S. subg. Silene</i> sect. <i>Silene</i>
<i>S. latifolia</i> Poir.	Pego	Alicante, Spain	<i>S. subg. Behenantha</i> sect. <i>Melandrium</i>
<i>S. littorea</i> Brot.	Melides	Baixo Alentejo, Portugal	<i>S. subg. Behenantha</i> sect. <i>Psammophilae</i>
<i>S. mellifera</i> Boiss. & Reut.	Calatayud	Zaragoza, Spain	<i>S. subg. Silene</i> sect. <i>Siphonomorpha</i>
<i>S. micropetala</i> Lag.	Nador	Nador, Morocco	<i>S. subg. Silene</i> sect. <i>Silene</i>
<i>S. muscipula</i> L.	Granada	Granada, Spain	<i>S. subg. Silene</i> sect. <i>Muscipula</i>
<i>S. nicaeensis</i> All.	Benicarló	Castellón, Spain	<i>S. subg. Silene</i> sect. <i>Silene</i>
<i>S. nocturna</i> L.	L'Abdet	Alicante, Spain	<i>S. subg. Silene</i> sect. <i>Silene</i>
<i>S. portensis</i> L.	Gea	Teruel, Spain	<i>S. subg. Silene</i> sect. <i>Portenses</i>
<i>S. scabriflora</i> Brot.	Cavado	Minho, Portugal	<i>S. subg. Silene</i> sect. <i>Silene</i>
<i>S. sclerocarpa</i> Dufour	Nador	Nador, Morocco	<i>S. subg. Silene</i> sect. <i>Silene</i>
<i>S. stricta</i> L.	Barbate	Cádiz, Spain	<i>S. subg. Silene</i> sect. <i>Muscipula</i>
<i>S. tridentata</i> Desf.	Villena	Alicante, Spain	<i>S. subg. Silene</i> sect. <i>Silene</i>
<i>S. vivianii</i> Steud.	Saidia	Morocco	<i>S. subg. Silene</i> sect. <i>Silene</i>
<i>S. vulgaris</i> (Moench) Garcke	Elda	Alicante, Spain	<i>S. subg. Behenantha</i> sect. <i>Behenantha</i>

2.2. Seed Images

Photographs were taken with a Nikon Stereomicroscope Model SMZ1500 equipped with a camera Nikon DS-Fi1 of 5.24 megapixels. Composed images containing 20 seeds per accession were prepared with Corel Photo Paint for the lateral views of the seeds. The images are stored in: <https://zenodo.org/record/5536569#.YVRnrgzAM8> (accessed on 22 November 2021).

2.3. General Morphological Description

Area (A), perimeter (P), length of the major axis (L), width (W), aspect ratio (AR is the ratio L/W), circularity (C), and roundness (R) were obtained for the lateral and dorsal views of seeds of each species with ImageJ program [21]. A total of 20 seeds were used for each species. For the lateral view, the seeds were oriented with the micropyle to the right. A ruler was the reference for the conversion of pixel units to length or surface units (mm or mm²). The circularity index and roundness were calculated as described [22]. Circularity is the ratio $(4\pi \times A)/P^2$, while roundness is $(4 \times A)/\pi L^2$, in consequence circularity decreases with irregularities of seed surface that increase the perimeter, but roundness is not affected.

2.4. Obtention of an Average Silhouette

The average silhouette is a representative image of seed shape for each group of seeds. A total of 20 seeds were used for each studied species. The silhouette was obtained in Corel Photo Paint, by the protocol described in [23] (a detailed video is available at Zenodo: <https://zenodo.org/record/4478344#.YBPOguhKiM8>, accessed on 2 November 2021). The layers containing the seeds are superimposed and the opacity is given a value of 3 in all layers. All the layers are combined, and the brightness is adjusted to a minimum value. From this image, we are interested in the inner region representing the area where most of the seeds coincide, which is the darkest area. To select it, we use the magic wand tool and with tolerance equal to 10, this selection is copied and pasted as a new layer.

2.5. Comparison with Geometric Models: Calculation of the J Index

The images containing twenty seeds were used for calculation of J index. J index is defined by:

$$J \text{ index} = (\text{area } S) / (\text{area } T) \times 100 \quad (1)$$

where *S* is the area shared between the seed and the model and *T*, the total area occupied by both figures. J index ranges between 0 and 100, reaching maximum values when the geometric model and the seed image areas coincide. A high value of J index, i.e., high similarity with a given model, means a precise definition of seed shape for a particular species. A good adjust to the model is considered when J index values were superior to 90 [19].

Area calculation was performed by superimposing the model on each seed image in groups of 20 seeds, searching a maximum adjustment between the shapes of the seeds and the geometric model (Figure 1). Three files were kept for each composition: (1) A document (PSD format) with the seeds and the geometric figure adapted to each of them, in which it is possible to make corrections; (2) a file (JPG format) with the geometric models in black, that is useful to obtain total area (*T*) with the software ImageJ; and (3) another file in JPG format with the geometric models in white, useful to obtain the values of area shared between the geometric figure and the seed image (*S*) in ImageJ (Figure 1). Image composition with seeds and models was done in Corel PHOTO-PAINT X7, and area quantification in ImageJ. Figure 1 illustrates the examples of the adjustment between seed images and the geometric models with indication of the areas measured for the calculation of the J index.

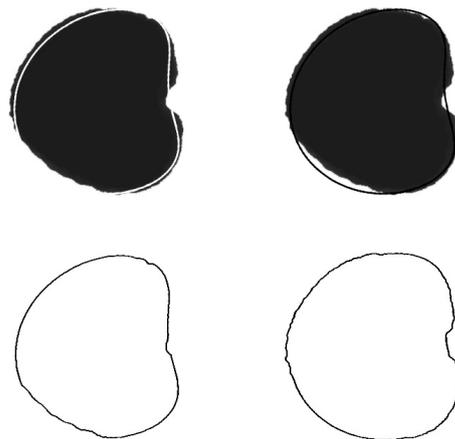


Figure 1. Representation of the method used to calculate J index. Top: Seed images with the model superimposed. Left: The model is in white to obtain the shared area (S). Right: The model is in black to obtain the total area (T). Below: Interpretation of the images by ImageJ. The estimated areas, shared (S) and total (T), are of 159479 and 177362 pixel, respectively. The J index of this example equals to 89.9.

The basic geometric model, the cardioid (Model 1 in [19]), was initially compared to each lateral view of 20 seeds per species to get the J index per each seed image, and hence mean, minimum, and maximum values and standard deviation per species were calculated. The obtained values of J index correspond to the percent of similarity of each species with the cardioid. New calculations were done also with alternative modified geometric models (cardioid derived models), already described and named as models 2 (flattened cardioid), 3 (open cardioid), and 4 (flattened and elongated cardioid) [19], and with new models proposed in this work. In addition, the comparison of the models was also done to the average silhouette of the seed, and therefore, an additional value of J index was obtained for each species.

2.6. Statistical Analysis

The values of A , P , L , W , AR , C , and R of the lateral and dorsal views of the seeds are given as mean values. The J index for the obtained models are given as mean, minimum, and maximum values with the number of seeds indicated in each case, and the standard deviation. According to Kolmogorov and Shapiro–Wilk tests, it cannot be rejected that the data came from a normally distributed population. One-way ANOVA was used to infer significant differences between species for the measured variables, followed by Scheffé post-hoc tests to provide specific information on which means were significantly different from one another. In addition, a Student's t test was calculated to compare the mean values of A , P , L , W , AR , C , and R between the subgenera. Those p values inferior to 0.05 were considered significant. The analyses were conducted using the software IBM SPSS statistics v25 (SPSS 2017). Hierarchical clustering analysis was performed in R Studio, V.1.2.1335 [R statistics]. Complete-linkage was used to calculate the distance matrix and the hierarchical clustering method was McQuitty's similarity analysis [24].

3. Results

3.1. Description of the Lateral Views of Seeds. Comparison between *Silene* Species

Figure 2 and Table 2 show the average silhouettes and the main morphological features, respectively, of the lateral views of seeds in the species analyzed. The minimum values of area, length of major axis, and width were obtained in the species *S. disticha* and of perimeter in *S. stricta* (both belonging to *S.* subg. *Silene*) whereas the maximum values of these four measurements corresponded to *S. vulgaris* (*S.* subg. *Behenantha*). The values of aspect ratio were between 1.12 in *S. portensis* and 1.37 in *S. mellifera* (both in *S.* subg. *Silene*),

and these two same species had the minimum and maximum values of roundness but in inverted order. Circularity values were between 0.68 and 0.86 corresponding to *S. vulgaris* and *S. littorea*, both species in *S. subg. Behenantha*. Roundness was between 0.74 (*S. mellifera*) and 0.90 (*S. portensis*) for both species in *S. subg. Silene*.

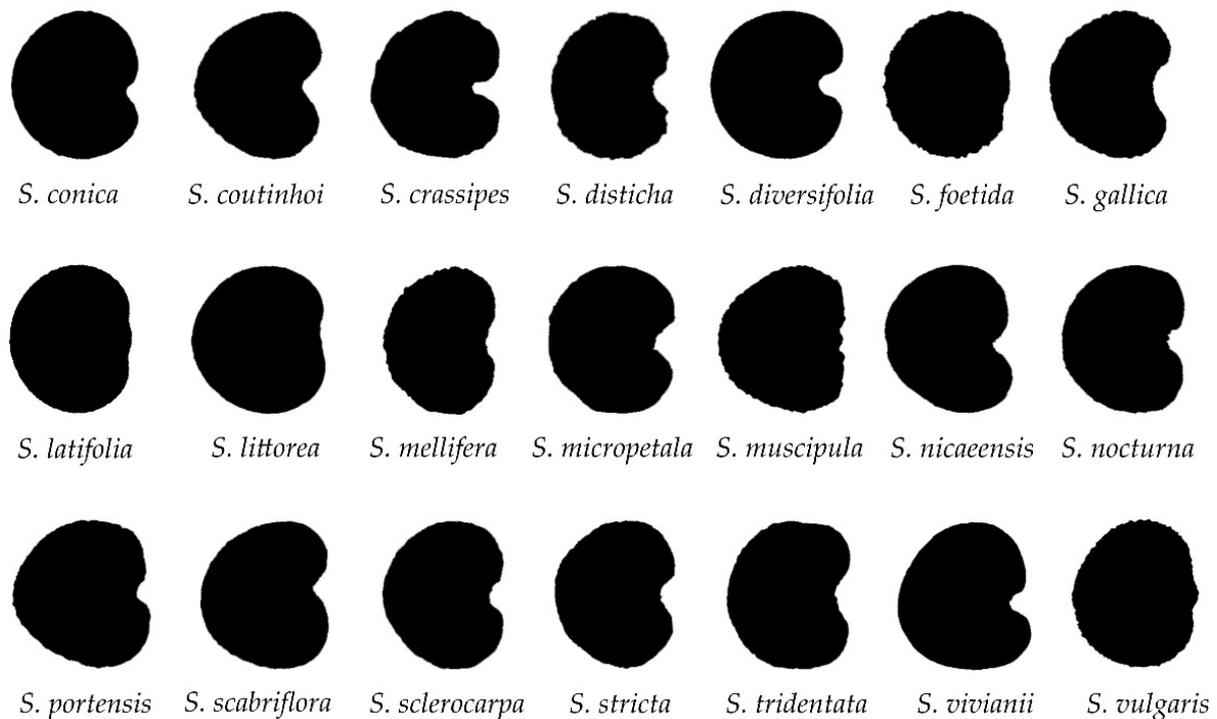


Figure 2. Average silhouettes obtained for the lateral views of the seed in the 21 species analyzed.

Table 2. Mean values of the area (A), perimeter (P), length of the major axis (L), length of the minor axis (W), aspect ratio (AR), circularity (C), and roundness (R) in the lateral view of seeds of *Silene*. Values of A are given in mm²; P, L and W, in mm. Superscript letters indicate the results of Scheffé test: the mean values marked with the same letter in each column do not differ significantly at $p < 0.05$. N is the number of seeds analyzed.

Species	N	A	P	L	W	AR	C	R
<i>S. conica</i>	20	0.64 ^{fg}	3.15 ^f	0.98 ^{efg}	0.83 ^{efg}	1.18 ^{abcd}	0.81 ^{jk}	0.85 ^{efgh}
<i>S. coutinhoi</i>	20	0.71 ^g	3.46 ^g	1.05 ^g	0.86 ^{fg}	1.21 ^{bcde}	0.74 ^{def}	0.83 ^{cdef}
<i>S. crassipes</i>	20	1.02 ^j	4.31 ^k	1.24 ⁱ	1.04 ⁱ	1.20 ^{abcde}	0.69 ^{ab}	0.84 ^{defgh}
<i>S. disticha</i>	20	0.26 ^a	2.19 ^a	0.66 ^a	0.51 ^a	1.31 ^{fg}	0.70 ^{abc}	0.77 ^{abc}
<i>S. diversifolia</i>	20	0.57 ^{ef}	3.11 ^{ef}	0.92 ^{def}	0.79 ^e	1.17 ^{abc}	0.74 ^{def}	0.86 ^{fgh}
<i>S. foetida</i>	20	0.86 ^{hi}	3.98 ^{hi}	1.14 ^h	0.96 ^h	1.18 ^{abcd}	0.69 ^a	0.85 ^{efgh}
<i>S. gallica</i>	20	0.48 ^{cd}	2.88 ^{cd}	0.89 ^{cd}	0.68 ^c	1.31 ^{fg}	0.72 ^{cde}	0.77 ^{ab}
<i>S. latifolia</i>	20	1.17 ^k	4.23 ^{jk}	1.32 ^j	1.13 ^j	1.18 ^{abcd}	0.82 ^k	0.85 ^{efgh}
<i>S. littorea</i>	20	0.58 ^{ef}	2.92 ^{cde}	0.92 ^{de}	0.81 ^{ef}	1.13 ^{ab}	0.86 ^l	0.89 ^{gh}
<i>S. mellifera</i>	20	0.84 ^h	3.82 ^h	1.20 ^{hi}	0.89 ^g	1.37 ^g	0.72 ^{bcd}	0.74 ^a
<i>S. micropetala</i>	20	0.94 ^{ij}	3.94 ^{hi}	1.19 ^{hi}	1.00 ^{hi}	1.20 ^{abcde}	0.76 ^{fgh}	0.84 ^{defg}
<i>S. muscipula</i>	20	0.93 ^{ij}	4.07 ^{ij}	1.19 ^{hi}	1.00 ^{hi}	1.19 ^{abcde}	0.71 ^{abc}	0.84 ^{efgh}
<i>S. nicaeensis</i>	20	0.56 ^{def}	2.99 ^{def}	0.92 ^{def}	0.77 ^{de}	1.20 ^{abcde}	0.78 ^{hij}	0.84 ^{defg}
<i>S. nocturna</i>	20	0.51 ^{cde}	2.90 ^{cde}	0.90 ^{cd}	0.72 ^{cd}	1.26 ^{def}	0.76 ^{fgh}	0.80 ^{abcde}
<i>S. portensis</i>	20	0.69 ^g	3.39 ^g	0.99 ^{fg}	0.88 ^g	1.12 ^a	0.75 ^{efg}	0.90 ^h
<i>S. scabriflora</i>	20	0.47 ^c	2.73 ^c	0.85 ^c	0.71 ^c	1.21 ^{abcde}	0.79 ^{ijk}	0.83 ^{defg}

Table 2. Cont.

Species	N	A	P	L	W	AR	C	R
<i>S. sclerocarpa</i>	20	0.37 ^b	2.45 ^b	0.77 ^b	0.61 ^b	1.28 ^{efg}	0.77 ^{fghi}	0.79 ^{abcd}
<i>S. stricta</i>	20	0.28 ^{ab}	2.18 ^a	0.67 ^a	0.54 ^a	1.26 ^{cdef}	0.75 ^{fg}	0.80 ^{bcdef}
<i>S. tridentata</i>	20	0.29 ^{ab}	2.23 ^a	0.68 ^a	0.54 ^a	1.26 ^{def}	0.74 ^{def}	0.79 ^{abcde}
<i>S. vivianii</i>	20	0.95 ^{ij}	3.92 ^{hi}	1.19 ^{hi}	1.01 ^{hi}	1.18 ^{abcd}	0.77 ^{fghi}	0.85 ^{efgh}
<i>S. vulgaris</i>	20	1.97 ^l	6.03 ^l	1.73 ^k	1.44 ^k	1.20 ^{abcde}	0.68 ^a	0.84 ^{defg}

Significant differences were found for all parameters between *S. subg. Silene* and *S. subg. Behenantha*. The values of area, perimeter, length and width, circularity and roundness were superior in *S. subg. Behenantha* and aspect ratio was superior in *S. subg. Silene* ($p < 0.05$; Table 3).

Table 3. Comparison of the mean values of the area (A), perimeter (P), length of the major axis (L), length of the minor axis (W), aspect ratio (AR), circularity (C), and roundness (R) in the lateral views of the *Silene* seeds between species in *S. subg. Behenantha* and *S. subg. Silene*. Between parentheses: standard deviation. Superscript letters indicate the results of T-Student analyses: the mean values marked with different letters in each column differ significantly at $p < 0.05$. N is the total number of seeds analyzed per subgenera.

Subgenera	N	A	P	L	W	AR	C	R
<i>S. subg. Behenantha</i>	100	1.04 ^b (0.52)	4.06 ^b (1.12)	1.22 ^b (0.30)	1.03 ^b (0.24)	1.18 ^a (0.05)	0.77 ^b (0.08)	0.85 ^b (0.04)
<i>S. subg. Silene</i>	320	0.62 ^a (0.26)	3.16 ^a (0.71)	0.96 ^a (0.20)	0.78 ^a (0.18)	1.23 ^b (0.11)	0.74 ^a (0.04)	0.82 ^a (0.07)

3.2. Description of the Dorsal Views of Seeds. Comparison between *Silene* Species

The values obtained for area, perimeter, length of major axis and width, aspect ratio, roundness, and circularity are shown in Table 4. The minimum and maximum values of area, perimeter, and length of major axis were obtained in *S. tridentata* (*S. subg. Silene*) and *S. vulgaris* (*S. subg. Behenantha*), respectively. For the width, the minimum values corresponded to *S. vivianii* (*S. subg. Silene*) and the maximum values to *S. vulgaris* (*S. subg. Behenantha*). Circularity values varied between 0.44 and 0.79, corresponding to the species *S. micropetala* (*S. subg. Silene*) and *S. littorea* (*S. subg. Behenantha*), respectively. The aspect ratio values ranged from 1.12 (*S. disticha*) to 3.32 (*S. vivianii*), whereas roundness values varied from 0.30 to 0.89 for the same species (*S. vivianii* and *S. disticha*, respectively), both species in *S. subg. Silene*.

Table 4. Mean values of the area (A), perimeter (P), length of the major axis (L), width (W), aspect ratio (AR), circularity (C) and roundness (R) in the dorsal views of the *Silene* seeds. Values of A are given in mm²; P, L, and W, in mm. Superscript letters indicate the results of Scheffé test: the mean values marked with a different letter in each column differ significantly at $p < 0.05$. N is the number of seeds analyzed.

Species	N	A	P	L	W	AR	C	R
<i>S. conica</i>	18	0.56 ^h	3.04 ^{gh}	1.01 ^{ef}	0.70 ^{hi}	1.43 ^{bcd}	0.76 ^{fg}	0.70 ⁱ
<i>S. coutinhoi</i>	16	0.56 ^h	3.28 ^{ij}	1.09 ^g	0.65 ^{fg}	1.69 ^{ef}	0.65 ^{cd}	0.59 ^{ef}
<i>S. crassipes</i>	18	0.52 ^{gh}	3.70 ^l	1.25 ^h	0.53 ^c	2.41 ⁱ	0.48 ^{ab}	0.43 ^b
<i>S. disticha</i>	15	0.33 ^{abc}	2.43 ^{bc}	0.68 ^a	0.61 ^{efg}	1.12 ^a	0.70 ^e	0.89 ^l
<i>S. diversifolia</i>	19	0.43 ^{defg}	2.80 ^{def}	0.94 ^{cde}	0.58 ^{cdefg}	1.62 ^{def}	0.69 ^e	0.62 ^{efgh}

Table 4. Cont.

Species	N	A	P	L	W	AR	C	R
<i>S. foetida</i>	19	0.76 ⁱ	4.42 ⁿ	1.19 ^h	0.81 ^j	1.47 ^{cde}	0.49 ^b	0.68 ^{hi}
<i>S. gallica</i>	15	0.39 ^{cdef}	2.68 ^{de}	0.91 ^{cd}	0.55 ^{cdef}	1.66 ^{def}	0.69 ^e	0.60 ^{efg}
<i>S. latifolia</i>	20	1.01 ^j	4.04 ^m	1.35 ⁱ	0.95 ^k	1.43 ^{bcd}	0.77 ^{fg}	0.70 ⁱ
<i>S. littorea</i>	19	0.45 ^{fg}	2.67 ^{de}	0.94 ^{cde}	0.60 ^{defg}	1.56 ^{def}	0.79 ^g	0.64 ^{fg}
<i>S. mellifera</i>	15	0.71 ⁱ	3.60 ^{kl}	1.25 ^h	0.72 ⁱ	1.77 ^{fg}	0.68 ^{de}	0.58 ^{de}
<i>S. micropetala</i>	13	0.39 ^{bcdef}	3.29 ^{ij}	1.20 ^h	0.41 ^{ab}	3.04 ^j	0.44 ^a	0.34 ^a
<i>S. muscipula</i>	15	0.59 ^h	3.46 ^{jk}	1.24 ^h	0.61 ^{fg}	2.03 ^h	0.62 ^c	0.50 ^c
<i>S. nicaeensis</i>	16	0.34 ^{abcd}	2.64 ^{cd}	0.96 ^{de}	0.45 ^b	2.19 ^{hi}	0.61 ^c	0.47 ^{bc}
<i>S. nocturna</i>	17	0.55 ^h	2.99 ^{fgh}	0.92 ^{cd}	0.76 ^{ij}	1.21 ^{ab}	0.77 ^{fg}	0.83 ^{ijkl}
<i>S. portensis</i>	18	0.45 ^{efg}	2.87 ^{efg}	1.06 ^{fg}	0.54 ^{cde}	1.97 ^{gh}	0.68 ^{de}	0.51 ^{cd}
<i>S. scabriflora</i>	19	0.39 ^{cdef}	2.64 ^{cd}	0.88 ^c	0.56 ^{cdef}	1.60 ^{def}	0.70 ^e	0.63 ^{efgh}
<i>S. sclerocarpa</i>	18	0.44 ^{efg}	2.71 ^{de}	0.80 ^b	0.70 ^{hi}	1.15 ^a	0.75 ^f	0.87 ^{kl}
<i>S. stricta</i>	17	0.30 ^{ab}	2.23 ^{ab}	0.69 ^a	0.55 ^{cdef}	1.26 ^{abc}	0.75 ^{fg}	0.80 ^j
<i>S. tridentata</i>	17	0.28 ^a	2.16 ^a	0.66 ^a	0.54 ^{cd}	1.24 ^{abc}	0.75 ^{fg}	0.81 ^{jk}
<i>S. vivianii</i>	15	0.36 ^{abcde}	3.15 ^{hi}	1.22 ^h	0.37 ^a	3.32 ^k	0.45 ^{ab}	0.30 ^a
<i>S. vulgaris</i>	16	1.67 ^k	5.73 ^o	1.79 ^j	1.19 ^l	1.51 ^{de}	0.64 ^c	0.66 ^{ghi}

Differences were found for all parameters between *S. subg. Silene* and *S. subg. Behenantha* with values of area, perimeter, length and width, circularity and roundness notably superior in *S. subg. Behenantha* and mean values of aspect ratio were superior in *S. subg. Silene* ($p < 0.05$ in all cases; Table 5).

Table 5. Mean values of the area (A), perimeter (P), length of the major axis (L), width (W), aspect ratio (AR), circularity (C) and roundness (R) in the dorsal views of the *Silene* seeds between species in *S. subg. Behenantha* and *S. subg. Silene*. Numbers between parentheses: standard deviation. Superscript letters indicate the results of T-Student: the mean values marked with different letters in each column differ significantly at $p < 0.05$. N is the total number of seeds analyzed per subgenera.

Subgen.	N	A	P	L	W	AR	C	R
<i>Behenantha</i>	92	0.87 ^b (0.43)	3.93 ^b (1.08)	1.24 ^b (0.30)	0.84 ^b (0.20)	1.48 ^a (0.09)	0.69 ^b (0.12)	0.68 ^b (0.04)
<i>Silene</i>	263	0.44 ^a (0.13)	2.90 ^a (0.48)	0.98 ^a (0.21)	0.57 ^a (0.11)	1.80 ^b (0.65)	0.66 ^a (0.11)	0.62 ^a (0.19)

According to their dorsal view, and based on their roundness, the seeds were divided primarily into three groups, A, B, and C (Figure 3, Table 4). Group A is characterized by the seeds with highest roundness. It comprises five species: *S. disticha*, *S. nocturna*, *S. sclerocarpa*, *S. stricta*, and *S. tridentata*. Group B contains those species with the highest AR values, and lowest roundness. Four species were included in group B: *S. crassipes*, *S. micropetala*, *S. nicaeensis*, and *S. vivianii*. The twelve species remaining, of intermediate roundness values, are included in Group C. Among them, two groups were defined based on the convexity of their figures. Convexity is the property of a plane figure that consists in that any straight line resulting from the union of two points belonging to the figure, remains completely inside the figure. Group C1 of convex seeds includes *S. foetida*, *S. latifolia*, *S. littorea* and *S. vulgaris*. Group C of concave seeds is formed by *S. conica*, *S. coutinhoi*, *S. diversifolia*, *S. gallica*, *S. mellifera*, *S. muscipula*, *S. portensis*, and *S. scabriflora*.

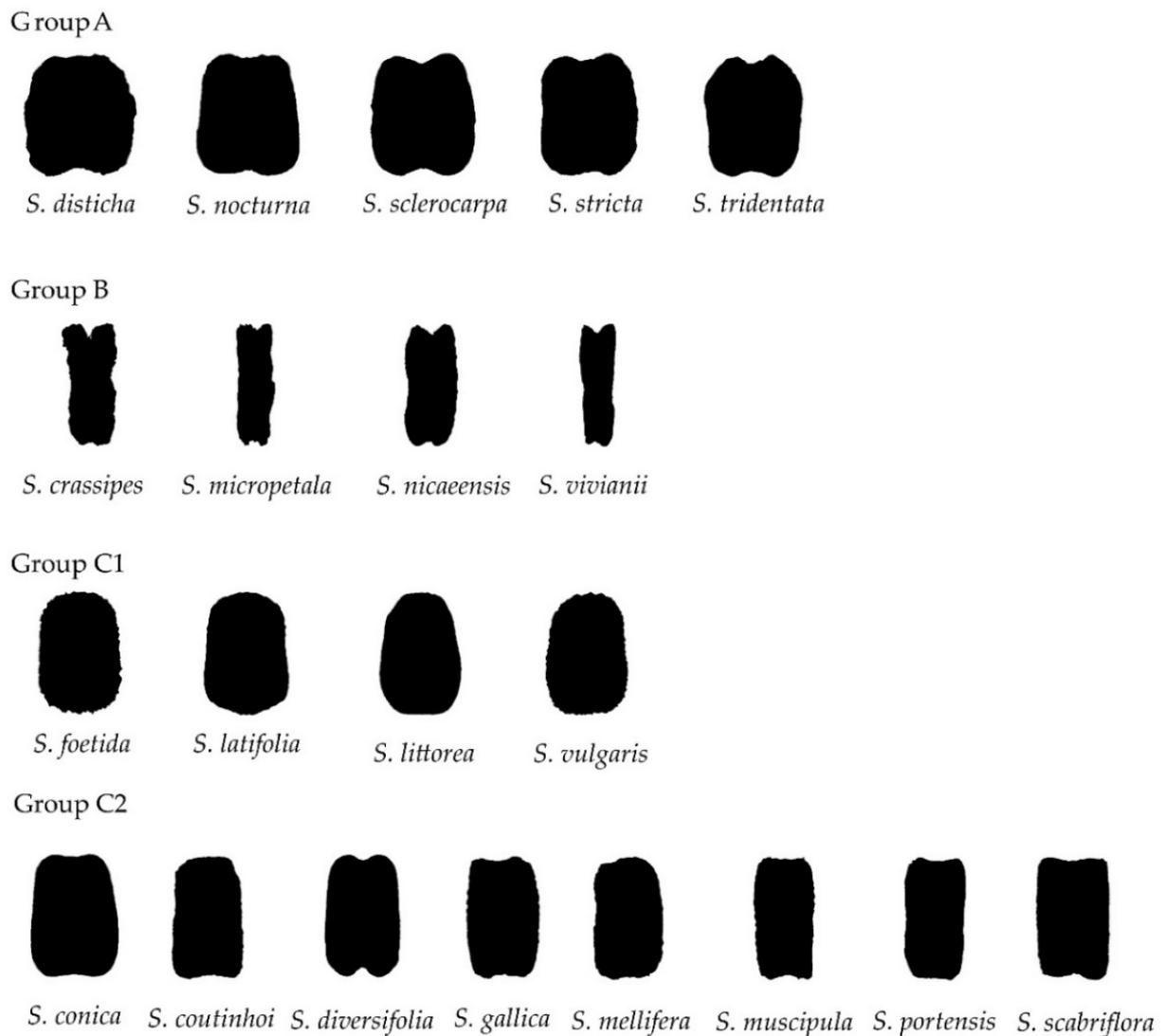


Figure 3. Average silhouettes obtained for the dorsal views of the seed in the 21 species of *Silene* analyzed. Groups A, B and C are different in their roundness and aspect ratio. The silhouettes in subgroup C1 are convex, while the silhouettes in C2 are concave.

Hierarchical clustering analysis based on the morphological parameters and including convexity as a categorical factor revealed four clusters (Figure 4). Cluster C1 includes convex seeds *S. foetida*, *S. latifolia*, *S. littorea*, and *S. vulgaris*. Cluster B contains those species with the highest AR values and lowest roundness. Three species were included in cluster B: *S. crassipes*, *S. micropetala*, and *S. vivianii*. Cluster C2 includes concave seeds *S. conica*, *S. coutinhoi*, *S. diversifolia*, *S. gallica*, *S. mellifera*, *S. muscipula*, *S. nicaeensis*, *S. portensis*, and *S. scabriflora*. Cluster A is characterized by the seeds with highest roundness. It comprises five species: *S. disticha*, *S. nocturna*, *S. sclerocarpa*, *S. stricta*, and *S. tridentata*.

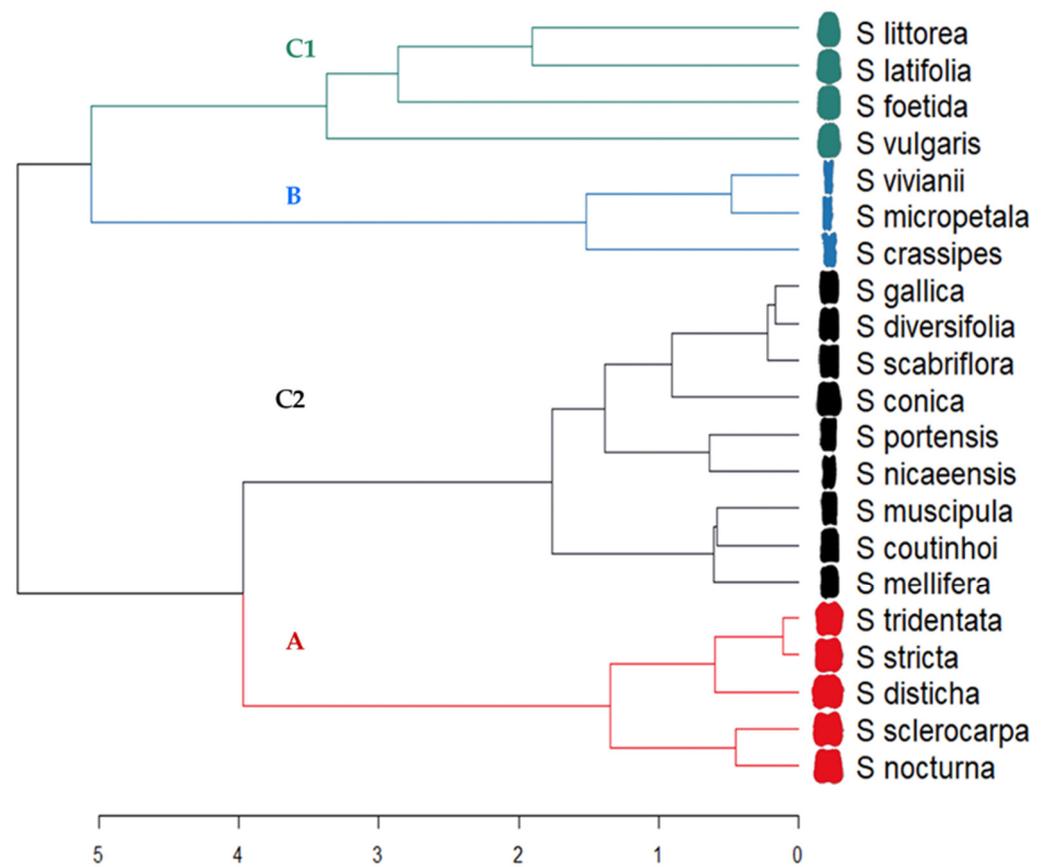


Figure 4. Dendrogram based on hierarchical clustering from morphological data including convexity as a binary factor (presence/absence). Four clusters are identified according to the color of branches. The average silhouettes obtained for the dorsal views of the different *Silene* species are represented based on the cluster/color.

3.3. Comparison of the Lateral Views with the Cardioid. *J* Index Values

The lateral views of all seeds (20 per species) were compared with the cardioid (Model 1, Figure 5). Once the values of *J* index for each species with the cardioid were obtained (percent of similarity of the seeds of each species with the cardioid), other models were also tested.

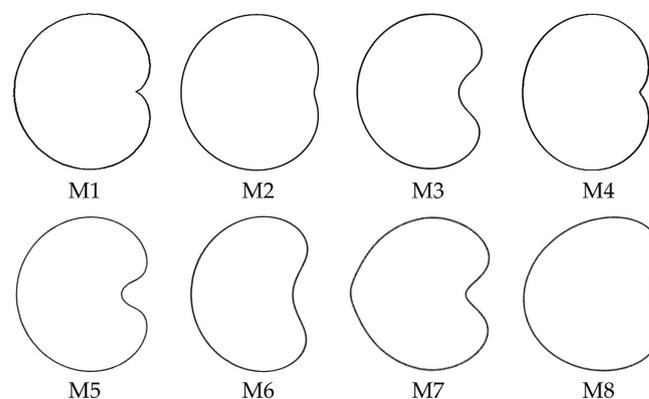


Figure 5. Left to right, top row: Model 1 (cardioid), Model 2 (flattened cardioid), Model 3 (open cardioid, Model 4 (flattened and elongated cardioid). Bottom row: Model 5 (open cardioid), similar to Model 3 but less open; Model 6 (open cardioid), similar to Model 3 but more open; Model 7, derived from the heart curve; Model 8 (slightly asymmetric figure).

Table 6 contains the mean *J* index values obtained for the lateral views of the seeds of the species tested. The values were comprised between 80.9 (*S. mellifera*) and 92.5 (*S. conica*). *J* index was superior to 90 in four species: *S. conica* (92.5), *S. littorea* (92.0), *S. portensis* (90.8), and *S. vivianii* (90.5). Of these, two (*S. conica* and *S. littorea*) belong to *S. subg. Behenantha* and other two belong to *S. subg. Silene* (*S. portensis* and *S. vivianii*). Similar to previous results [16], mean values of *J* index with the cardioid were higher in *S. subg. Behenantha* than in *S. subg. Silene* ($p \leq 0.01$).

Table 6. *J* index values obtained in the comparison of the seed images and average silhouette with the cardioid (Model 1). The mean values and standard deviation (between brackets) are shown in the third column. The fourth and fifth columns show minimum and maximum values. The sixth column presents data obtained with the average silhouette for each species (values in cursive). Superscript letters indicate the results of Scheffé test: the mean values marked with a different letter in each column differ significantly at $p < 0.05$. N is the total number of studied seeds per species.

Species	N	Mean <i>J</i> Index Cardioid (Standard Deviation)	Min	Max	<i>J</i> Index Value in Average Silhouette
<i>S. conica</i>	20	92.5 ^k (1.18)	90.0	94.4	94.2
<i>S. coutinhoi</i>	20	86.8 ^{bcdef} (1.81)	83.3	89.5	88.3
<i>S. crassipes</i>	20	87.3 ^{cdef} (3.04)	80.0	91.5	90.1
<i>S. disticha</i>	20	83.7 ^{ab} (4.26)	70.8	88.1	88.0
<i>S. diversifolia</i>	20	89.7 ^{efghijk} (1.62)	85.9	91.9	91.6
<i>S. foetida</i>	20	89.8 ^{fghijk} (1.10)	87.8	91.3	92.6
<i>S. gallica</i>	20	84.7 ^{bc} (2.82)	78.9	89.4	87.3
<i>S. latifolia</i>	20	89.7 ^{efghijk} (1.55)	86.4	93.2	91.1
<i>S. littorea</i>	20	92.0 ^{jk} (2.07)	86.7	94.7	93.3
<i>S. mellifera</i>	20	80.9 ^a (6.07)	70.4	89.0	92.9
<i>S. micropetala</i>	20	88.7 ^{defghi} (2.89)	80.3	91.7	91.7
<i>S. muscipula</i>	20	86.6 ^{bcde} (3.18)	79.8	90.4	90.2
<i>S. nicaeensis</i>	20	87.4 ^{cdefg} (3.16)	81.4	92.7	89.6
<i>S. nocturna</i>	20	86.3 ^{bcd} (4.14)	76.1	91.5	94.9
<i>S. portensis</i>	20	90.8 ^{hijk} (0.85)	88.8	92.1	90.3
<i>S. scabriflora</i>	20	89.1 ^{defghij} (2.10)	84.1	93.3	90.6
<i>S. sclerocarpa</i>	20	87.9 ^{defgh} (1.98)	84.3	92.2	89.8
<i>S. stricta</i>	20	87.5 ^{cdefg} (2.88)	82.6	91.3	90.1
<i>S. tridentata</i>	20	88.5 ^{defgh} (2.27)	82.8	91.6	89.1
<i>S. vivianii</i>	20	90.5 ^{ghijk} (1.36)	86.4	92.3	90.4
<i>S. vulgaris</i>	20	88.6 ^{defghi} (2.44)	82.1	91.9	92.2

The values of *J* index obtained for the comparison of the cardioid with the average silhouette for each species revealed some remarkable peculiarities (Table 6). In general, these values are higher than those obtained as the mean values of twenty seeds per species, and only six species gave values of *J* index below 90: *S. coutinhoi*, *S. disticha*, *S. gallica*, *S. nicaeensis*, *S. sclerocarpa*, and *S. tridentata*. This indicates that, behind all variation in shape, there is a trend to maintain a geometric “consensus” shape in the seeds of a population. The greatest differences between both estimations of *J* index were found for *S. mellifera* and *S. nocturna*, coincident with highest values of standard deviation in the estimations of *J* index with twenty seeds. To improve the description, the seeds were tested with alternative models, including models 2, 3, and 4 already described [16], and Models 5, 6, 7, and 8 obtained in the course of this work and described below (Figure 4).

3.4. New Models Obtained to Adjust Seed Shape in *Silene* Species

Models 5 and 6 are both open cardioids, based on Model 3, but less and more open, respectively (Figure 5). Model 5 was designed to fit the seed silhouettes of the species *S. crassipes* and *S. diversifolia* (both included in *S.* subg. *Silene*). It resembles Model 3, but it is more closed around the hilum zone. Model 6 was designed to adjust to the silhouettes of *S. mellifera* (*S.* subg. *Silene*). This model looks like Model 3 but it is more open around the hilum, and hence, this is notably less prominent.

Model 7 is a heart curve derived from Model ADE1, that was obtained to fit the seeds of *Ampelopsis delavayana* Planch. (Vitaceae) [25]. Model 7 was designed to fit the outline of the seeds of *S. coutinhoi*, *S. portensis*, and *S. scabriflora* (*S.* subg. *Silene*). The seeds of these species are less rounded presenting a wedge shape in the dorsal side (Figure 5), this resembling the waterdrop and heart curves described for the seeds of the Vitaceae.

Model 8 derives from the equation of the cardioid by searching for a slightly triangular and asymmetric figure (Figure 5), adapted to the seeds of *S. muscipula*, *S. nicaeensis*, and *S. vivianii* (all *S.* subg. *Silene*). The Mathematica code for models 1 to 8 is available at: <https://zenodo.org/record/5535612#.YVQvgLgzaM8> (accessed on 22 November 2021).

Equations for models 5 to 7 are as follows:

Model 5:

$$2x^4 + 4x^3 + 4x^2y^2 + 4xy^2 + 2y^4 - 2y^2 - y = 0 \quad (2)$$

Model 6:

$$x^4 + 4x^3 + 2x^2y^2 + 4xy^2 + y^4 - 4y^2 - 16y = 0 \quad (3)$$

Model 7:

$$\left(\sqrt{1-x^2} + \frac{1}{10+100x^2} - y \right) \left(\sqrt{1-x^2} - \frac{6}{10+100x^2} + y \right) = 0 \quad (4)$$

Model 8:

$$9x^4 + 12x^3 + 12x^2y^2 + 8xy^2 + 4y^4 - 4y^2 - 16y = 0 \quad (5)$$

3.5. J Index Values Obtained with Models 2 to 8 with Seeds of *Silene* Species

Table 7 presents the values of *J* index obtained with models 2 to 8, as the mean value of twenty seeds in different *Silene* species (numbers in bold), as well as the values obtained with the average silhouette for all the species with each model (numbers in cursive). When *J* index was scored as the mean value of 20 seeds, nine species had values superior to 90 with one or more models. Model 2 adjusted well to *S. conica*, *S. latifolia*, *S. littorea*, *S. portensis*, and *S. vivianii*. Four of these species (*S. conica*, *S. latifolia*, *S. littorea*, and *S. vivianii*) define a group that had the highest values of *J* index with model 2, while *S. portensis* had slightly better values of *J* index with model 8. According to the measurements of *J* index with 20 seeds, *S. diversifolia* and *S. tridentata* gave values superior to 90 with model 5; *S. nicaeensis*, *S. scabriflora*, and *S. portensis* with model 8. *S. diversifolia* and *S. portensis* gave values of *J* index superior to 90 with model 7, but in both cases the values obtained with other models were superior (*S. diversifolia* with model 5; *S. portensis* with model 8). In consequence, the measurement of *J* index as the mean of twenty seeds resulted in three groups defined according to their similarity with models 2, 5, and 8. The first group of seeds, resembling model 2, included *S. conica*, *S. latifolia*, *S. littorea*, and *S. vivianii*; the second group of seeds, similar to model 5, with *S. diversifolia* and *S. tridentata*. The third group composed of seeds similar to model 8 with *S. nicaeensis*, *S. scabriflora*, and *S. portensis*.

Table 7. Values of *J* index obtained with models 2 to 8 of the different *Silene* species. Numbers in bold correspond to mean values obtained as a mean value of 20 seeds. Numbers in cursive correspond to data obtained by comparison with the average silhouette for each species. Superscript letters indicate the results of Scheffé test: the mean values marked with a different letter in each column differ significantly at $p < 0.05$.

Models/Species	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8
<i>S. conica</i>	91.0 hi	86.0 bcdef	89.2 efgh	88.6 defg	84.1 cdefg	87.7 bcdef	88.6 cdef
	94.3	88.0	92.1	91.2	87.3	90.4	90.1
<i>S. coutinhoi</i>	84.7 bcde	84.5 abcd	83.2 b	86.3 cde	83.4 bcdef	87.2 bcdef	86.3 cd
	87.4	90.0	87.4	91.4	87.4	92.6	91.2
<i>S. crassipes</i>	84.2 abc	82.8 ab	83.9 bc	84.1 abc	80.3 ab	85.8 bc	86.3 cd
	90.9	88.0	87.7	91.9	84.9	92.0	90.6
<i>S. disticha</i>	82.8 ab	83.8 abc	79.2 a	82.3 a	84.1 cdefg	81.3 a	81.2 a
	87.8	91.3	92.2	91.2	90.1	88.8	88.3
<i>S. diversifolia</i>	89.9 ghi	84.8 abcd	86.2 bcde	91.0 g	83.4 bcdef	90.4 f	87.3 cde
	91.4	88.9	87.3	93.3	84.4	93.6	91.4
<i>S. foetida</i>	89.8 ghi	84.4 abcd	89.7 gh	87.8 defg	81.1 abcd	85.3 bc	88.1 cdef
	94.3	87.7	93.2	90.6	85.6	88.3	89.8
<i>S. gallica</i>	84.3 abcd	88.4 efg	87.2 defg	88.1 defg	86.5 fg	84.9 b	85.5 bc
	86.6	93.4	88.9	92.0	92.0	89.5	88.5
<i>S. latifolia</i>	91.1 hi	85.6 abcdef	90.8 h	87.8 defg	82.3 abcde	85.8 bc	87.1 cde
	91.3	86.7	92.5	88.2	86.4	85.3	86.9
<i>S. littorea</i>	92.4 i	84.2 abcd	89.2 efgh	88.5 defg	80.5 abc	89.4 ef	91.4 f
	95.1	85.7	90.4	89.9	81.0	91.1	91.3
<i>S. mellifera</i>	81.6 a	82.6 a	83.5 b	82.8 ab	82.7 abcde	81.4 a	82.3 ab
	85.2	91.4	89.4	89.5	92.0	87.3	85.3
<i>S. micropetala</i>	88.9 fgh	85.2 abcde	87.7 defgh	88.7 defg	81.8 abcde	88.5 cdef	87.9 cdef
	92.0	89.6	90.3	92.4	87.8	91.7	88.6
<i>S. muscipula</i>	87.3 defg	83.6 abc	85.5 bcd	85.5 bcd	80.0 ab	85.8 bcd	87.4 cde
	90.4	86.7	89.3	89.1	83.9	88.7	93.3
<i>S. nicaeensis</i>	87.9 fg	86.2 cdef	86.9 cdefg	88.3 defg	83.2 abcdef	88.4 cdef	90.1 ef
	89.8	88.1	89.9	90.9	85.5	89.3	91.0
<i>S. nocturna</i>	86.3 cdef	86.8 cdefg	87.1 cdefg	88.3 defg	85.3 efg	87.6 bcdef	87.4 cde
	90.3	92.2	90.4	94.3	89.7	91.9	90.3
<i>S. portensis</i>	90.2 ghi	84.3 abcd	86.4 bcdef	87.6 def	79.7 a	90.0 ef	90.4 ef
	90.4	84.5	88.4	89.1	80.9	90.8	92.7
<i>S. scabriflora</i>	88.6 fgh	87.2 defg	88.1 defgh	89.1 efg	84.0 cdefg	89.8 ef	91.3 f
	89.8	88.3	89.0	91.2	84.5	91.4	94.1
<i>S. sclerocarpa</i>	87.6 efg	88.6 fg	89.6 gh	89.3 efg	86.9 fg	88.3 bcdef	87.6 cde
	88.8	92.1	92.2	93.2	90.2	91.2	88.9
<i>S. stricta</i>	87.3 defg	86.6 cdefg	88.3 defgh	87.8 defg	84.4 defg	86.9 bcde	85.7 c
	87.9	91.5	90.9	92.8	89.8	90.2	88.5
<i>S. tridentata</i>	88.3 fgh	89.7 g	89.5 fgh	90.8 fg	87.5 g	89.5 ef	88.9 cdef
	89.6	92.9	90.8	93.2	90.2	90.5	88.3
<i>S. viviani</i>	90.1 ghi	86.0 bcdef	87.7 defgh	89.4 efg	82.4 abcde	89.2 def	89.8 def
	90.1	85.1	87.4	86.3	83.4	89.7	91.9
<i>S. vulgaris</i>	89.3 ghi	84.9 abcd	88.8 efgh	86.6 cde	82.8 abcde	85.1 bc	87.6 cde
	92.6	87.8	93.0	90.4	86.0	87.4	89.9

The results of the measurement of *J* index based on the mean silhouettes allowed to include two new species (*S. foetida* and *S. vulgaris*) in the group of species that gave their highest values of *J* index with model 2 (*S. conica*, *S. latifolia*, *S. littorea*, and *S. vivianii*). In addition, *S. vivianii* showed its highest *J* index value based on mean silhouette with the model 8, so this species was excluded and hence, the group was defined more broadly as those seeds resembling models 2 and 4 (Figure 6). The group is formed by five species: *S. conica*, *S. foetida*, *S. latifolia*, *S. littorea*, and *S. vulgaris* (Figure 6), four of them in Group

A of dorsal shape, and all them belong to *S.* subg. *Behenantha*. Among them, the mean silhouettes of *S. latifolia* and *S. vulgaris* had higher scores with model 4 than with model 2.

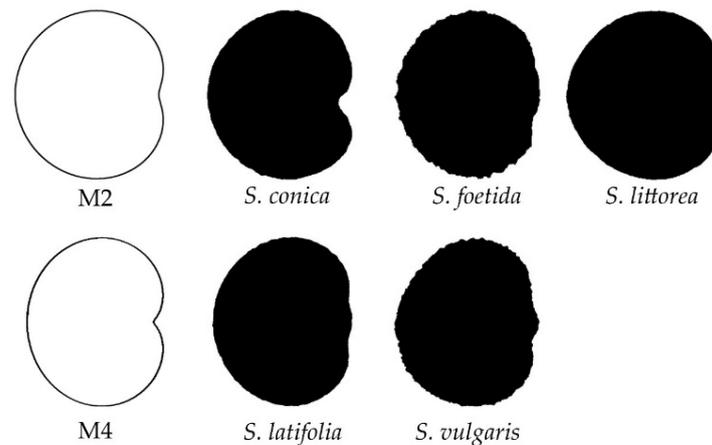


Figure 6. Models 2 and 4 based on the average silhouette and those *Silene* species with the best adjustment. The best fit for model 2 corresponds to *S. conica*, *S. foetida*, and *S. littorea*; for model 4, *S. latifolia*, and *S. vulgaris*.

Another group is formed by those species that gave their best scores based on mean silhouette with models 3, 5, and 6 (Figure 7): *S. disticha*, *S. diversifolia*, *S. gallica*, *S. mellifera*, *S. micropetala*, *S. nocturna*, *S. sclerocarpa*, *S. stricta* and *S. tridentata*. Among them, *S. gallica* showed the highest value for model 3 (*J* index 93.4), *S. nocturna* for model 5 (*J* index 94.3) and *S. mellifera* for model 6 (*J* index 92.0). Although *S. disticha* had higher scores with model 4, it was included in this group due to high values above 90 with the three models that define it (models 3, 5 and 6). Likewise, the combination of the highest values of *J* index based on the mean silhouette (93.3) and on the mean value of 20 seeds (91.0) supported the inclusion of *S. diversifolia* within this group instead of with model 7.

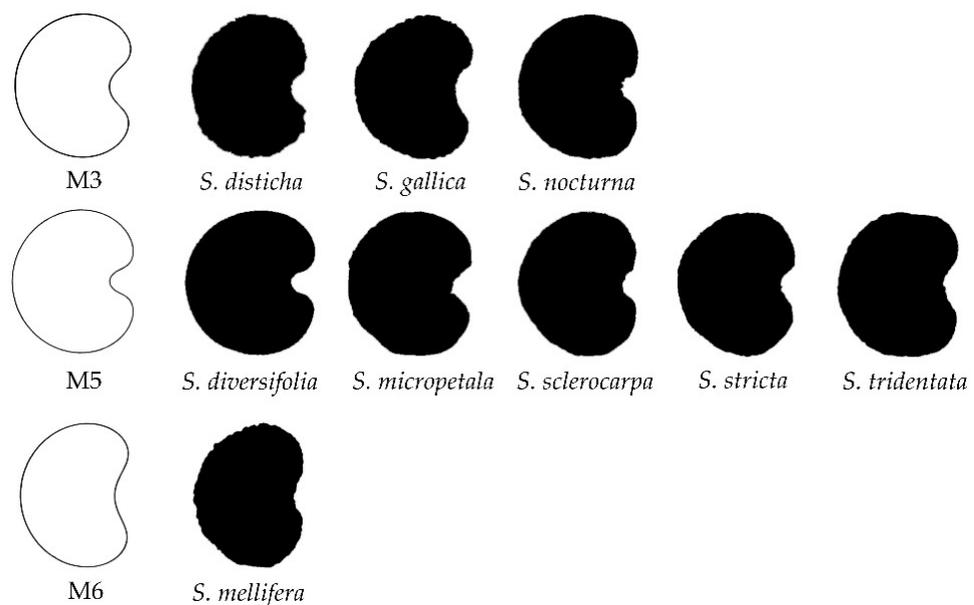


Figure 7. Models 3, 5, and 6 with the average silhouette of *Silene* species. Top row: Model 3, *S. disticha*, *S. gallica* and *S. nocturna*. Middle row: Model 5, *S. diversifolia*, *S. sclerocarpa*, *S. stricta*, *S. micropetala* and *S. tridentata*; Bottom row: Model 6, *S. mellifera*.

Finally, some species gave their best J index scores with models 7 and 8. On the one hand, only two species, *S. coutinhoi* and *S. crassipes*, obtained their highest score with model 7 (Table 7, Figure 8). On the other hand, *S. muscipula*, *S. nicaensis*, *S. portensis*, *S. scabriflora*, and *S. vivianii* gave the best score for a slightly triangular and asymmetric figure corresponding to model 8 (Figure 9), and among them, the best adjustment was obtained for the species *S. scabriflora* (J index = 94.1).

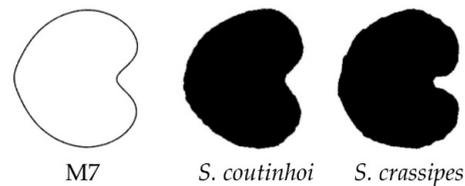


Figure 8. Model 7 with the average silhouette of *S. coutinhoi* and *S. crassipes*.

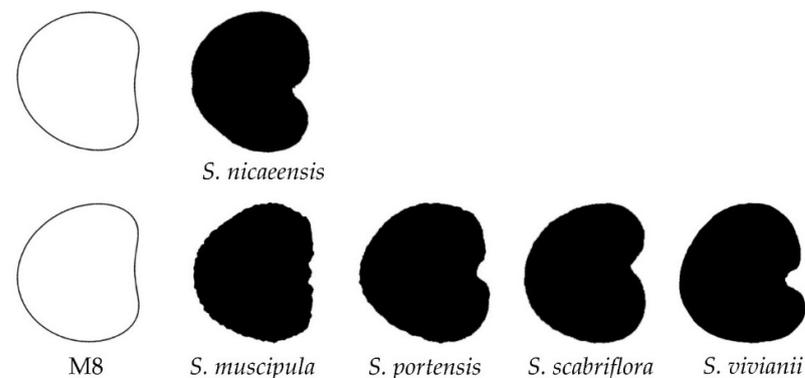


Figure 9. Seed silhouettes of *Silene* species resembling the geometric model M8. The typical asymmetric figure of this model is observable on the opposite area of the hilum, as exposed on the two lines of seeds.

4. Discussion

Developments in microscopy in recent decades have contributed to improved descriptions of seed morphology; especially, micromorphology has contributed to the description of seed surface features in the Caryophyllaceae [26] and in many species of *Silene* [5–11,27–29]. In the coming years, the availability of image databases and mathematic programs may open new opportunities for the macromorphological description of seeds. Our approach departs from previous works done in model plants and is based on the comparison of seed shape with geometric models [12–20].

The two-dimensional images of the lateral views of the seeds of *Silene* species adjust well to the cardioid figure (model 1), with values of J index superior to 90 for certain species, in particular *S. noctiflora*, *S. conica*, and *S. latifolia* belonging to *S. subg. Behenantha* [19]. In addition to the cardioid, other geometric figures proved to be useful in the description and quantification of seed shape in *Silene* species corresponding to the flattened (models 2 and 4) and the open cardioids (model 3) [19]. While in the former models, the region corresponding to the hilum is reduced and even almost plane, there is a clear opening in model 3. Previous studies stated that models 2 and 4 adjusted well to the seeds of *S. latifolia* and *S. declinis*, respectively (both belonging to *S. subg. Behenantha* sect. *Melandrium*), while model 3 described well the seeds of *S. gallica* (*S. subg. Silene* sect. *Silene*) [19]. Due to the morphological variation of the seeds [19,27–29], we have expanded the range of geometric models in this work with two new models of the “open” type (model 3), which complemented it. These have been named as model 5 and model 6, that were, respectively, less and more open than model 3. Additionally, two new models, 7 and 8 were also described, with modifications in the dorsal side of the seeds, with a certain

asymmetry in the latter. These four new geometric models (models 5–8) proved to be useful for the description of the lateral views for certain species. The need to describe new geometric models that better fit the morphological shape of the seeds would support the aforementioned morphological variability of this genus. The morphological descriptions based on geometric principles would reflect the result of a complex program of development and, in consequence, might be characters not submitted to homoplasy and suitable for their application in taxonomy.

In this study, we have investigated seed morphology by comparison with geometric models in 21 species of *Silene*, of which 16 were not already described, while the previous work included different populations of the other five species (*S. conica*, *S. gallica*, *S. latifolia*, *S. mellifera*, and *S. vulgaris*) [19]. Albeit a low number of species were coincident in both works, the comparative (previous data vs. new data) revealed a certain coincidence about the obtained models based on the seeds of the same species (e.g., *S. gallica*, *S. latifolia*) from different geographic localities. Nevertheless, some peculiar differences have been observed between the here-obtained data and those previously exposed by Martín-Gómez et al. [19], especially those related to the area, perimeter, and aspect ratio. Therefore, measurements of shape based on *J* index might be more stable in comparison with the size measurements. Seed heterogeneity may increase with the age of the samples, depending on the time and conditions of storage [30], which makes it difficult to find and describe a perfect model for all samples. Certain degree of variation in shape was observed in this work for the seeds of *S. disticha*, *S. mellifera*, *S. nocturna*, and *S. stricta*. In the case of *S. mellifera*, this heterogeneity might be due to peculiarities of the sample used, and not only to properties of the species itself. As a matter of fact, a different population sample of the same species used in a previous work [19] was more homogeneous, since the values of *J* index obtained for *S. mellifera* (89.8) [19] were notably higher than the obtained value of 80.9 in this work. In addition to *S. mellifera*, four more species (*S. conica*, *S. gallica*, *S. latifolia* and *S. vulgaris*) were previously analyzed [19]. The values of *J* index calculated here for these four species were, respectively, 92.5, 84.7, 89.7, and 88.6 (Table 3), while the values from the previous work [19] were 92.1, 90.0, 92.6, 91.2, respectively. The slightly reduced *J* index values obtained in this work for *S. gallica*, *S. latifolia*, and *S. vulgaris* would be in agreement with more heterogenous populations in this work.

The novelty of computing the values of *J* index based on the average silhouette for the genus *Silene* has constituted an interesting tool for the analysis of the seed shape, as reported for *Vitis* [23]. All these outcomes are of interest as a starting point for a further follow-up study on the characterization of the seed morphology and the stability of the geometric models, including, for this purpose, different geographic populations of the species.

In addition to the use of the mean silhouette, another relevant innovation of this work is the description of the dorsal views of the seeds. Three groups A, B, and C were defined based on their measurements, and group C was divided into C1 and C2 on the basis of the criterium of convexity, resulting in four different clusters in a dendrogram. Cluster C1 contains convex seeds and includes four of the five species analyzed of *S. subg. Behenantha*: *S. foetida*, *S. latifolia*, *S. littorea*, and *S. vulgaris*. These four species, with the addition of *S. conica*, constitute the group formed by similarity to M2 and M4 in the lateral views, thus matching the current taxonomical treatment on sections of the genus *Silene* based on DNA sequence analysis [1]. These results are also in agreement with the initial findings about the seed morphology and geometric models between the two studied subgenera [19] and present two new morphological properties of *S. subg. Behenantha*—similarity to models 2 and 4 in the lateral view and the predominance of convex forms in the dorsal view.

The species of *S. subg. Silene* were classified within three different groups (A, B, and C2), with different roundness and AR values. These groups showed a correspondence predominantly to geometric models 3, 5, 6, 7, and 8. In particular, group A contains five species with the thinnest and shortest seeds adjusting to geometric models 3, 5, and 6; group B includes three species with the thinnest seeds fitting to models 5, 7, and 8; while

group C2 contains nine species adjusting to models 3, 5, 6, 7, and 8, and *S. conica*, whose seeds adjust to model 2 and is the only case of convex seeds in *S. subg. Behenantha*. This seed diversity of groups based on lateral and dorsal views *S. subg. Silene* might be related to the high diversity of this specific subgenus, since it includes more than 500 different species [4].

The study of the dorsal view of the seeds increases the data available on morphological traits, which in combination with the detailed study of cell surface [9–11,26,27], will allow a more detailed characterization of the different species of the genus with a potential taxonomic use undescribed to date. In addition, a remarkable relationship between the groups resulting from geometric models (models 1–8) based on the lateral views and from the dorsal views is reported. Further detailed studies about more species from different sections are needed to increase the available data about the here-studied seed morphology, which might be considered as a useful morphological tool to support the infrageneric classification of the genus. The new analytical methods reported can be applied to scanning microscope photographs, because this technique does not result in major alterations of the overall morphology of the seeds [9–11].

In the genus *Silene*, seed morphological features have been useful for species identification and classification with consistent data for species in sections Conoimorphae, Melandriiformes, and Sclerocalycinae [27]. In combination with geometrical models, individual morphological traits, which may be homoplastic with very low informative value, were used to discriminate between *Silene* subgenera [19]. Combined with molecular phylogenetic data, geometric models could be used either, for phylogenetic classification or to study significant seed morphology changes during *Silene* evolution.

Although the dominant state of qualitative seed characters was reported as useful to differentiate among *Silene* taxa [28], a notable morphological variability of the *Silene* seeds has been reported [29]. Most of the taxonomical treatments of the genus described the seeds as reniform, but included certain level of variation. Therefore, the seeds have been described as rounded, ear-shaped, and reniform [31], reniform, globose or discoidal [32], reniform or reniform-circular [5], orbicular reniform or asymmetrical reniform [33], reniform with a notable variation (orbicular, rectangular, flagellate, heart-shaped, ovate-triangular) [28], or, merely reniform or orbicular [1]. However, the obtained data have revealed that under the same qualitative denomination (e.g., reniform), a wider morphological quantitative variation can be easily identified. The potential use of the geometrical models, especially based on the mean silhouette, together with the morphological study of the dorsal view would increase the morphological seed traits, which would be considered for taxonomical purposes.

5. Conclusions

In addition to previously described models (models 1 to 4), four new morphological models are described (models 5 to 8) that contribute to the description of lateral views in *Silene* species. The dorsal view showed differences between species that allowed a classification in three groups according to roundness (Groups A to C). Group C was divided in C1 and C2, based on the convexity or concavity of the seed images. A correlation between morphological classification based on the dorsal view and similarity to a model in the lateral view is reported. The seeds of morphological group C1, based in dorsal shape, resemble to models 2 and 4, and belong to *Silene subg. Behenantha*. The seeds of groups A, B, and C2 resemble models 3, 5, 6, 7, and 8, and belong to *Silene subg. Silene*, with the only exception of *S. conica*. The detailed study of the seeds with the transformation of qualitative information into quantitative data would be of interest for taxonomic purposes at different levels (i.e., subgenera, section), providing also a detailed description of the features of the seeds for each *Silene* species.

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