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Curvature Analysis in Seed Surface of SEM Images of *Silene* Species from Türkiye

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Abstract: Recently, based on light microscopy images, the tubercle structure on the seed surface of 100 *Silene* species was quantitatively described, including tubercle width, height, and curvature associated with general morphometric data. Curvature measures the rate of change of the tangent vector in a curve and can be calculated by the following methods described for *Arabidopsis* roots. Here, we apply curvature measurements to the SEM images of 40 *Silene* species from Türkiye, demonstrating that a quantitative analysis of tubercles can be made based on SEM images with similar results to optical photographs. The association of morphometric tubercle data allows for classification into six groups, five of them corresponding to described shapes: rugose (two groups), echinate, mammillate, and papillose, and a sixth group of tubercles plane on top. The curvature values vary between 20 and 200 mm^{-1} and differ among the morphological tubercle types described. The correlation of curvature values with other general measurements and morphological seed characteristics is investigated. Tubercle quantification not only is a useful tool for *Silene* taxonomy, but also provides the basis for the analysis of the genetic control and developmental effects on tubercle structure and shape in the seed surface.

Keywords: caryophylllaceae; curvature; development; morphological analysis; quantitative morphology; tubercles

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1. Introduction

The fruits and seeds of many plant species present distinct surface protrusions, often associated with various types of dispersion [1]. Thus, winged fruits and seeds occur in species of 93 families at least [2–4], being frequent in climbers such as the Aristolochiaceae [5] and Cucurbitaceae [6], and absent or not described in the Arecaceae. Seed surface formations may also contribute to zoochory or hydrochory, as the endocarp fibers of *Chamaedorea cataractarum* Mart. [7] and other outgrowths may play a role as mechanisms facilitating the establishment and support of seeds in rocks and cliffs [8–10].

In the Caryophyllaceae and other families of the order Caryophyllales, winged seeds are rare [11]. However, the name *Dipterospermae* (Rohrb.) was applied to a section in the *Silene* subgen. *Silene*, now ascribed to sect. *Silene* [12]. The lateral view of seeds in this section resembles a butterfly, and the two lateral projections are called wings, but there is no evidence for the role of these structures in wind transport. Nevertheless, the diversity of tubercle size and shape in the Caryophyllaceae has attracted the attention of botanists for

generations, and even the genera were named considering seed morphological peculiarities, such as *Heliosperma* (Rchb.) Rchb., due to long tubercles that expand from the central part of the seed, giving it a radiated shape akin to the rays of the sun (Helios). Tubercle structure has been proposed as a key character in the genera of the order Caryophyllales, for example, *Portulaca* L. (Portulacaceae) [13,14], and in many genera of the Caryophyllaceae, such as *Arenaria* Ruppius ex L. [15–18], *Cerastium* Tourn. ex L. [15,18,19], *Minuartia* Loefl. [18,20], *Moehringia* L. [21,22], *Sagina* L. [23,24], and *Stellaria* L. [15,18,19,25–28] in the Alsineae; *Acanthophyllum* C.A.Mey [29,30], *Dianthus* L. [23], and *Gypsophilla* L. [23,31,32] in the Caryophylleae; *Paronychia* Mill. (Paronichieae) [33,34] and *Silene* L. [35–61] (Sileneae). However, evidence for a physiological and/or ecological function of seed surface tubercles is very scarce in this order. After observation in many species, mainly with SEM (Scanning Electron Microscopy), and classification into morphological types, the ecological functions of the tubercles remain largely unexplained except for a general role of support and attachment of the seeds to the substrate in rocks and cliffs against dragging by water and wind [8–10].

Recent work based on the quantification of geometric measurements in light microscopy images proposed a broad classification of the tubercles in the genus *Silene* in four types: smooth, rugose, echinate, and papillose [62,63]. Smooth seeds are defined by the absence of visible protuberances and were described before in *Arenaria* L. [15–17], *Minuartia* L. [20], *Moehringia* L. [22], and *Silene* [37,38,49]. Some species belonging to the *S. subgen*. *Silene* sect. *Silene* presented smooth seeds. These seeds are characterized by high circularity and solidity values in their lateral views, while in their dorsal views, higher circularity values are shared by echinate and rugose seeds [62]. In contrast to smooth seeds, the papillose seed group in *Silene* is characterized by the largest protuberances, resulting in the lowest values of circularity and solidity in both lateral and dorsal views. It includes the species *S. holzmani* Heldr. ex Boiss. (sect. *Behenantha*), *S. laciniata* Cav. (sect. *Physolychnis*), *S. magellanica* (Desr.) Bocquet (sect. *Physolychnis*), *S. perlmanii* W.L.Wagner, D.R.Herbst & Sohmer (sect. *Sclerophyllae*) [63]. Rugose and echinate seeds are distinguished by their tubercle types, being more rounded in the former type and more triangular or acute in the second. Geometrically, these two types of tubercles are well differentiated by analysis of their curvature values, higher in echinate than in rugose tubercles [64]. Concerning the taxonomy of the genus, although there is not a clear-cut difference between subgenera, nevertheless, the smooth and rugose types are more frequent in the *S.* subgen. *Silene.* The echinate type of higher curvature values occurs more often in the *S.* subgen. *Behenantha* [64].

The curvature of a curve is the rate at which the unit tangent vector changes with respect to arc length [65–67]. The methods here applied for curvature measurements are based on those developed for the analysis of the root apex in *Arabidopsis* Heynh. (Brassicaceae) [65,66]. This methodology was also applied to the seeds of wheat, grape, Cucurbitaceae, and some species of *Silene* [64,67–71].

The first objective of this work was to implement a set of methods for the analysis of the curvature on the surface of *Silene* seeds from SEM images and to demonstrate that the methodology can be applied both to SEM images and to optical microscopy photographs, providing equivalent and consistent data. The second objective was to obtain an accurate measurement of the curvature of individual seed tubercles to analyze their distribution and types in the *Silene* species from the Turkish SEM image collection.

2. Materials and Methods

2.1. Seed Images

This work is based on the SEM images of the Turkish collection of images corresponding to 122 Silene species maintained by K. Yildiz. The collection contains seed images taken at 100× that were used in this work, making this approach more comparable to our work with light microscopy.

2.2. Image Selection

Two aspects are important for an optimal analysis of tubercle size and shape. Tubercles must be well defined and regularly shaped. Small, irregular, or densely compacted tubercles can make measurements of curvature, height, and width at the base difficult or confusing. Images were selected based on a good tubercle definition avoiding those containing irregularities or that were too densely compacted. Smooth seeds, such as those of *S. chaetodonta* Boiss. (*Arenosa*, *Silene*), *S. colorata* Poir. (*Silene*, *Silene*), *S. eremitica* Boiss. (*Siphonomorpha*, *Silene*), *S.erciyesdagensis* Aksoy & Hamzaoğlu (*Lasiostemones*, *Silene*), *S. laxa* Boiss. & Kotschy (*Sclerocalycinae*, *Silene*), *S. macrodonta* Boiss. (*Conoideae*, *Behenantha*), *S. nerimaniae* G.E.Genç, Kandemir & I.Genç (*Auriculatae*, *Silene*), and *S. sordida* Hub.-Mor. & Reese (*Sordidae*, *Silene*) were excluded from the analysis because they lacked tubercles or were too small. Table 1 contains a description of the species whose seeds were used in this present study.

Table 1. *Silene* seeds were used in this work with indication of taxa, collector, place and date of collection, and herbarium were these specimens were deposited.

Taxa	Collector and Reference Number	Locality and Collected Date	Specimens Deposited
S. anatolica Melzheimer & A. Baytop * *Behenantha* (*Silene*)	K. Yıldız 0153-2 and M.Y. Dadandı	Karaman; Ermenek-Bozkır 91. km, Korualan village-Hadim 1. km, 1495–1500 m, 13 vii 2006	M. Celal Bayar Univ. Herb.
S. birandiana Ekim. *Saxifragoideae* (*Silene*)	K. Yıldız 041-1 and M.Y. Dadandı	Amasya; Akdağ, 1800–1900 m, 25 vii 2005	M. Celal Bayar Univ. Herb.
S. brevicaulis Boiss. *Auriculatae* (*Silene*)	F. Holtz (Nr. 00.699), P. Hanel and T. Kesereciođlu	Kahramanmaraş; Kahramanmaraş-Göksun, 2000 m, 16 vii 1973	EGE
S. bupleuroides L. *Sclerocalycinae* (*Silene*)	K. Yıldız 058-1 and M.Y. Dadandı	Ardahan; Yalnızçam, NE slopes, rocky, 2045 m, 29 vii 2005	M. Celal Bayar Univ. Herb.
S. caramanica Boiss. & Heldr. *Sclerocalycinae,* (*Silene*)	K. Yıldız 026-3 and M.Y. Dadandı	Konya; Hadim, near Bozkır turn, rocky, 1470 m, 10 vii 2005	M. Celal Bayar Univ. Herb.
S. caryophylloides subsp. *masmenaea* (Boiss.) Coode & Cullen Auriculatae (Silene)	K. Yıldız 0347-1, E. Minareci and M. Kuh	Kayseri; Dereşimli-Hanyeri, Gezibeli pass, stony rocky areas, 2000–2250 m, 19 vii 2011	M. Celal Bayar Univ. Herb.
S. cephalantha Boiss. *Auriculatae* (*Silene*)	K. Yıldız 060-1 and M.Y. Dadandı	Ardahan; Çıldır and Aktaş lake, slopes meadows, 2140 m, 29 vii 2005	E; M. Celal Bayar Univ. Herb.
S. chlorifolia Sm. *Sclerocalycinae* (*Silene*)	K. Yıldız 020-3 and M.Y. Dadandı	Karaman; Ermenek-Mut, near Gezende dam, rocky, 1050 m, 09 vii 2005	M. Celal Bayar Univ. Herb.
S. confertiflora Chowdhuri *Siphonomorpha* (*Silene*)	K. Yıldız 0174 and M.Y. Dadandı	Hatay; Dörtyol, Topaktaş plateau, Mığır, 1100–1150 m, 19 viii 2006	E; M. Celal Bayar Univ. Herb.
S. conica L. *Conoimorpha* (*Behenantha*)	K. Yıldız 0345-1, E. Minareci and M. Kuh	Kayseri; Saraycıbeli pass, Eşelik-Dereşimli, 1748 m, 19 vii 2011	M. Celal Bayar Univ. Herb.
S. cserei Baumg. *Behenantha* (*Behenantha*)	K. Yıldız 1124	Çorum; İskilip, 700 m, 23 v 1991	M. Celal Bayar Univ. Herb.
S. densiflora *Otites* (*Silene*)	K. Yıldız 0179	Edirne; Edirne-Lalapaşa road, between 0.5–1 km, 100–150 m, 25 viii 2006	M. Celal Bayar Univ. Herb.
S. doganii A.Duran & Menemen *Sclerocalycinae* (*Silene*)	K. Yıldız 0172-1 and M.Y. Dadandı	Osmaniye; Zorkun plaeuto, valley, 1750–2000 m, 18 viii 2006	M. Celal Bayar Univ. Herb.
S. echinospermoides Hub.-Mor. *Rigidulae* (*Silene*)	K. Yıldız 089-2	Muğla: Datça-Marmaris 33–35 km, rocky, 300–400 m, 21 v 2006	M. Celal Bayar Univ. Herb.
S. eremitica Boiss. *Siphonomorpha* (*Silene*)	H. Demiriz 3303	Kars; Iğdır, border of Türkiye-İran, Subaşı, sandy soil, 800 m, 19 vii 1956	ISTF

Table 1. *Cont.*

Taxa	Collector and Reference Number	Locality and Collected Date	Specimens Deposited
S. euxina (Rupr.) Hand.-Mazz. *Dichotoma* (*Silene*)	K. Yıldız 0302-2, M. Kuh and E. Minareci	Sakarya; Karasu, İhsaniye village, s.l., 18 vii 2010	M. Celal Bayar Univ. Herb.
S. gallica L. *Silene* (*Silene*)	K. Yıldız 084	Aydın; Milas, Karpuzlu, rocky slopes, 400 m, 19 v 2006	M. Celal Bayar Univ. Herb.
S. gigantea (L.) L. *Siphonomorpha* (*Silene*)	K. Yıldız 0128-1	İzmir; Gümüldür-Menderes, rocky slopes, 02 vii 2006	M. Celal Bayar Univ. Herb.
S. haradjannii Chowdhuri *Sclerocalycinae*, (*Silene*)	K. Yıldız 0176 and M.Y. Dadandı	Hatay; Dörtyol, Topraktaş plateau, 1728 m, 19 viii 2006	M. Celal Bayar Univ. Herb.
S. isaurica Contandr. & Quézel *Lasiostemones* (*Silene*)	K. Yıldız 0160 and M.Y. Dadandı	Antalya; Akseki, Çimi village-Kuyu plateau 1550–1570 m, 15 vii 2006	M. Celal Bayar Univ. Herb.
S. italica (L.) Pers. *Siphonomorpha* (*Silene*)	K. Yıldız 042-1 and M.Y. Dadandı	Amasya; Akdağ, Taşlıyayla-Ladik 4–5 km, under *Pinus nigra*, 1600–1650 m, 25 vii 2005	M. Celal Bayar Univ. Herb.
S. leptoclada Boiss. *Siphonomorpha* (*Silene*)	K. Yıldız 0263, E. Minareci and M. Kuh	Antalya; Fethiye, Kemer-Korkuteli, lime cliffs, 850 m, 10 vi 2010	M. Celal Bayar Univ. Herb.
S. lydia Boiss. *Conoimorpha* (*Behenantha*)	K. Yıldız 066	Manisa; Sipil mountain, 900–1000 m, 14 v 2003	M. Celal Bayar Univ. Herb.
S. macrodonta Boiss. *Conoimorpha* (*Behenantha*)	K. Yıldız 0278-1, E. Minareci and M. Kuh	Karaman; Ermenek yolu, to Tekeçatı 1 km, 1520 m, 13 vi 2010	M. Celal Bayar Univ. Herb.
S. manissadjianii Freyn *Lasiostemones* (*Silene*)	K. Yıldız 045 and M.Y. Dadandı	Tokat; Niksar, Çamiçi, Özalan village, rocky, 1260 m, 25 vii 2005	M. Celal Bayar Univ. Herb.
S. marschalii C.A.Mey. *Sclerocalycinae* (*Silene*)	K. Yıldız 004-1 and M.Y. Dadandı	Nevşehir; Uçhisar, rocky, 1470–1500 m, 04 vii 2005	M. Celal Bayar Univ. Herb.
S. microsperma Fenzl *Arenosa* (*Silene*)	K. Yıldız 079	İzmir; Torbalı-Tire 20. km, rocky, Akkoyunlu village, 100 m, 19 v 2006	M. Celal Bayar Univ. Herb.
S. nocturna L. *Silene* (*Silene*)	K. Yıldız 0288-1	Manisa; M. Celal Bayar University, 100 m, 30 iv 2010	M. Celal Bayar Univ. Herb.
S. olympica Boiss. *Sclerocalycinae* (*Silene*)	K. Yıldız 037 and M.Y. Dadandı	Bursa; Uludağ, near hotels, rocky, *Abies* forest, 1870–1950 m, 14 vii 2005	M. Celal Bayar Univ. Herb.
S. oreophila Boiss. *Spergulifoliae* (*Silene*)	K. Yıldız 042-2 and M.Y. Dadandı	Amasya; Akdağ, Taşlı plateau-Ladik 4–5 km, SW slope, below *Pinus nigra*, 1600–1650 m, 25 vii 2005	M. Celal Bayar Univ. Herb.
S. papillosa Boiss. *Lasiocalycinae* (*Silene*)	K. Yıldız 0333-1, M. Kuh and E. Minareci	Antalya; Korkuteli, inside forest, 310–320 m, 09 vi 2011	M. Celal Bayar Univ. Herb.
S. pendula L. *Behenantha* (*Behenantha*)		İzmir; Narlıdere, flower shop, 05 v 2014	M. Celal Bayar Univ. Herb.
S. phrygia Boiss. *Sclerocalycinae* (*Silene*)	K. Yıldız 156-2 and M.Y. Dadandı	Konya; Seydişehir-Akseki, 1600–1650 m, 14 vii 2006	M. Celal Bayar Univ. Herb.
Silene portensis subsp. *Rigidula* *Portenses* (*Silene*)	K. Yıldız 0297-1, E. Minareci and M. Kuh	Antalya; Alanya, Güzelbağ-Gündoğmuş, W slopes, 650 m, 15 vii 2010	M. Celal Bayar Univ. Herb.
S. rhynchocarpa Boiss. *Auriculatae* (*Silene*)	K. Yıldız 033-4 and M.Y. Dadandı	Antalya; Elmalı, Tekke village, Dökergöl, *Cedrus-Juniperus*, 1450–1500 m, 13 vii 2005	M. Celal Bayar Univ. Herb.
S. saxatilis Sims *Lasiostemones* (*Silene*)	K. Yıldız 058-2 and M.Y. Dadandı	Ardahan; Yalnızçam, rocky, 2045 m, 29 vii 2005	E; M. Celal Bayar Univ. Herb.
S. spergulifolia (Willd.) M.Bieb. Spergulifoliae (*Silene*)	K. Yıldız	Tokat; Tokat-Sivas way, Killik-Tekneli road junction, roadside, 1100 m, 12 vi 1990	MUFE (MARA)

Table 1. *Cont.*

Taxa	Collector and Reference Number	Locality and Collected Date	Specimens Deposited
S. swertiifolia Boiss. *Sclerocalycinae* (*Silene*)	K. Yıldız 015-1 and M.Y. Dadandı	Kahramanmaraş; Ahir mountain, near Kucukgol, steppe, 1380 m, 07 vii 2005	M. Celal Bayar Univ. Herb.
S. tenuiflora Guss. *Arenosae* (*Silene*)	K. Yıldız 094-1	Muğla; Köyceğiz-Sandras mountain, 470–500 m, 21 v 2006	M. Celal Bayar Univ. Herb.
S. tunicoides Boiss. Tunicoidae (*Silene*)	K. Yıldız 090	Muğla; Köyceğiz-Fethiye, 10–15 km, S slopes, 180 m, 21 v 2006	M. Celal Bayar Univ. Herb.

*: *Silene anatolica* Melzheimer & A. Baytop are very similar to *S. cserei*. In the Flora of the Caucasus [72], *S. anatolica* is given as the synonym of *S. cserei*. However, *S. cserei* and *S. anatolica* are different species in terms of characteristics such as base and stem leaf sizes, calyx veining, and anthophore length. Therefore, the species *S. anatolica* is not a synonym for the species *S. cserei*, and both are separate species.

2.3. Curvature Measurement

The curvature was measured in pairs of tubercles to obtain the values in two adjacent peaks and in the curve between them. The coordinates of points along the tubercles were automatically derived from their images with Image J and processed with the Mathematica program designed first to give their Bézier curve and second to calculate the corresponding curvature values along the curve, as described in Refs. [64–67]. Tubercle images were oriented with their peaks upwards and opened with Image J. After size adjustment with the ruler of each image, selected regions were thresholded, and the corresponding (x,y) coordinates were copied and processed to Mathematica. Bézier curves and the corresponding curvature values resulted from published protocols [64–67] (see Supplementary Data). Curvature is given in mm^{-1}; thus, a curvature of 20 belongs to a circumference of 50 microns while a curvature of 2 is associated with a circumference of 500 microns (0.5 mm). For most species analyzed, four pairs of tubercles were measured. The images and corresponding Mathematica files are available as indicated in the Supplementary Materials section.

2.4. Statistical Analysis

For each group of seeds, the mean values and coefficients of variation were obtained for general morphological measurements and tubercle measurements (curvature, width, height). For morphological characteristics and curvature analysis, the data did not adjust to normal distributions, and the Kruskal–Wallis test was applied, followed by stepwise stepdown comparisons by the ad hoc procedure developed by Campbell and Skillings [73]; p values below 0.05 were considered significant. The coefficient of variation was calculated as CVtrait = standard deviationtrait/meantrait × 100 [74]. Statistical analyses were conducted with IBM SPSS statistics v29 (SPSS 2022).

The Euclidean distance and Ward algorithm for clustering were used to calculate the dendrogram. The matrix used for the analysis contained the data for general morphological measurements (Appendix A, Tables A1–A6) and tubercle measurements of curvature values, as well as tubercle width (W), height (H), and slope.

3. Results

The seeds were classified by visual observation into six groups: (1) seeds with small, rugose tubercles regularly distributed along the surface; (2) small seeds with broad, rugose tubercles; (3) seeds with rugose tubercles with nipple-shaped protuberances or mammillae (umbonated [35,51,55,64]); (4) seeds with tubercles plane on top; (5) seeds with echinate tubercles, and (6) seeds with papillose tubercles. Groups 1, 2, and 3 contain rugose (rounded) tubercles. The categories of rugose, echinate, and papillose follow the previous classification [62,63].

Sections 3.1–3.6 contain the description and examples of tubercle measurements in each of these six groups; Section 3.7 contains the comparison between groups.

3.1. Seeds with Small Tubercles Regularly Distributed along the Surface

This group includes seeds of the following species (10): *S. birandiana*, *S. bupleuroides*, *S. caramanica*, *S. caryophylloides*, *S. chlorifolia*, *S. doganii*, *S. eremitica*, *S. haradjiannii*, *S. marschalii*, and *S. swertiifolia.*, *S. birandiana* belongs to *S.* sect. *Saxifragoideae*; *S. caryophylloides* belongs to *S.* sect. *Auriculatae*; *S. eremitica* belongs to *S.* sect. *Siphonomorpha*; and the remaining seven species belong to sect. *Sclerocalycinae* (*S. bupleuroides*, *S. caramanica*, *S. chlorifolia*, *S. doganii*, *S. haradjiannii*, *S. marschalii*, *S. swertiifolia*). All of them belong to the *S.* subgen. *Silene.*

A summary of the general morphological measurements is presented in Table A1 (Appendix A) and tubercle measurements (curvature analysis, tubercle height, width and slope) are shown in Table 2. Figures 1 and 2 correspond, respectively, to *S. caryophylloides* and *S. chlorifolia* and contain examples of tubercles of this group.

Table 2. Tubercle measurements in seeds of Group 1. Mean values (coefficient of variation) for each species are given for the measurements of maximum tubercle curvature (MaxTubCurv), maximum inter-tubercle curvature (MaxIntCurv), tubercle width (W), tubercle height (H), and slope. N indicates the number of tubercles measured in each species.

Species	N	MaxTubCurv	MaxIntCurv	W	H	Slope
S. birandiana	8	30.6 (33.6)	30.1 (43.1)	67.4 (12.6)	23.0 (22.2)	68.6 (20.4)
S. bupleuroides	8	38.4 (27.2)	81.9 (42.1)	76.4 (14.7)	33.4 (7.2)	88.8 (13.4)
S. caramanica	8	30.9 (25.4)	37.2 (36.5)	111.8 (14.0)	42.8 (19.6)	76.4 (11.4)
S. caryophylloides	8	29.7 (13.6)	41.2 (6.8)	65.4 (7.0)	33.3 (12.7)	102.4 (17.0)
S. chlorifolia	8	24.0 (16.6)	53.0 (15.4)	90.4 (6.2)	24.6 (10.8)	54.4 (5.7)
S. doganii	8	24.7 (15.9)	40.4 (25.3)	98.1 (18.8)	26.0 (18.4)	53.3 (12.9)
S. eremitica	8	31.1 (41.1)	34.0 (46.5)	41.9 (23.2)	11.9 (33.5)	57.5 (29.0)
S. haradjiannii	8	40.8 (18.6)	62.0 (32.4)	62.6 (18.4)	19.0 (22.9)	61.3 (21.6)
S. marschalii	8	29.8 (25.5)	61.3 (15.8)	80.9 (8.7)	22.8 (9.6)	56.5 (9.9)
S. swertiifolia	8	36.5 (16.6)	95.6 (17.2)	78.0 (11.5)	30.9 (24.6)	79.8 (25.1)

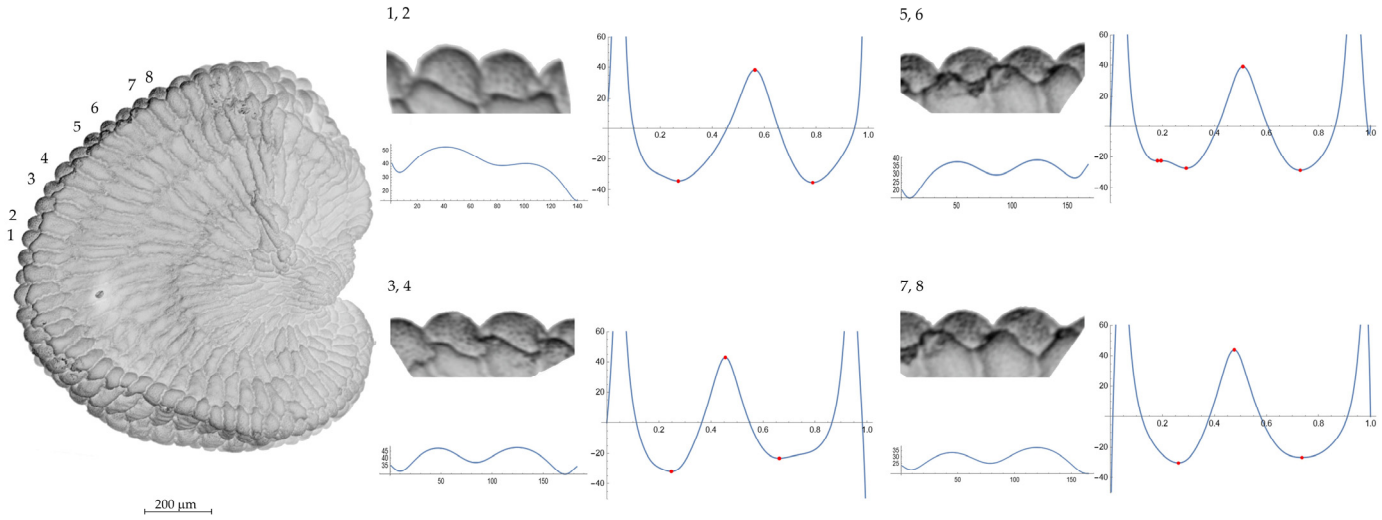

Figure 1. Left: a seed of *S. caryophylloides* with eight tubercles numbered (below, scale). **Right**: tubercle images, Bézier curves, and curvature plots corresponding to the four pairs of tubercles.

The tubercles of the seeds in this group are rounded and have mean curvature values comprised between 24 and 40.8 mm^{-1}. Inter-tubercle curvature is comprised between 30.1 and 95.6.

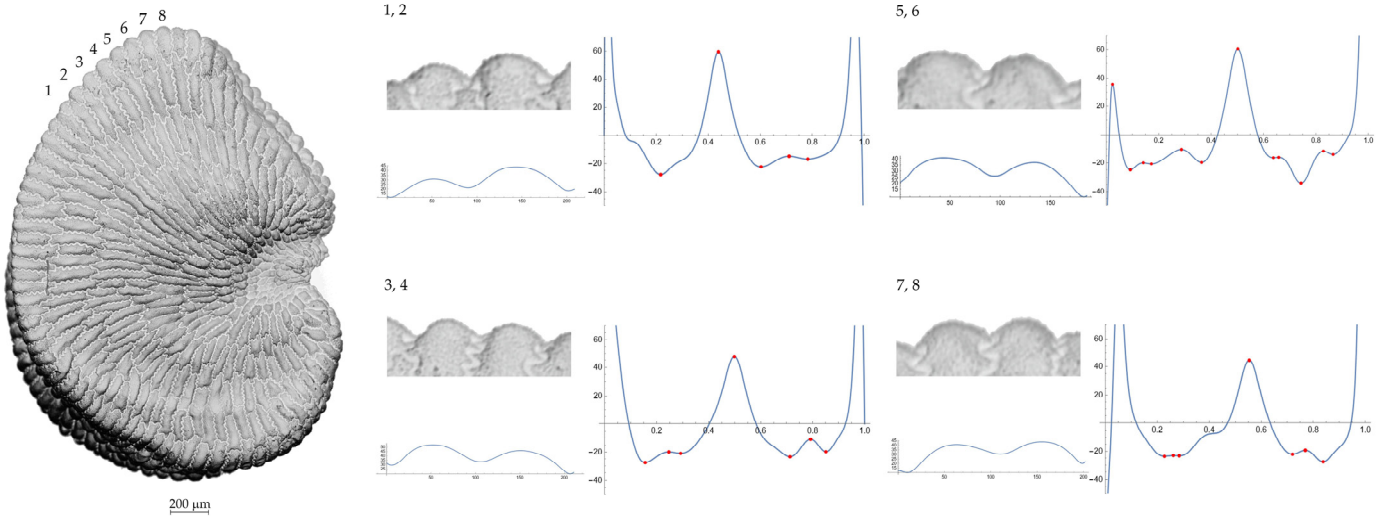

Figure 2. Left: a seed of *S. chlorifolia* with eight tubercles numbered (below, scale). **Right**: tubercle images, Bézier curves, and curvature plots corresponding to the four pairs of tubercles.

3.2. Small Seeds with Broad Tubercles

This group includes seeds of the following species (11): *S. cephalantha*, *S. densiflora*, *S. euxina*, *S. macrodonta*, *S. manissadjianii*, *S. olympica*, *S. oreophila*, *S. phrygia*, *S. saxatilis*, *S. spergulifolia*, and S. *tunicoides.* Three of these belong to *S.* sect. *Sclerocalycinae* (*S. olympica*, *S. phrygia*, and *S. tunicoides*); two to *S.* sect. *Spergulifoliae* (*S. cephalantha* and *S. spergulifolia*), and to *S.* sect. *Lasiostemones* (*S. manissadjanii* and *S. saxatilis*); and one to each of the following four sections: *Conoimorpha* (*S. macrodonta*), *Spergulifoliae* (*S. oreophila*), *Dichotomae* (*S. euxina*), *Otites* (*S. densiflora*).

A summary of the general morphological measurements is presented in Table A2 (Appendix A) and the tubercle measurements (curvature analysis, tubercle height, width, and slope) are shown in Table 3. Figures 3 and 4, correspond, respectively, to *S. oreophila* and *S. phrygia* contain examples of tubercles of this group.

Table 3. Tubercle measurements in seeds of Group 2. Mean values (coefficient of variation) for each species are given for the measurements of maximum tubercle curvature (MaxTubCurv), maximum inter-tubercle curvature (MaxIntCurv), tubercle width (W), tubercle height (H), and slope. N indicates the number of tubercles measured in each species.

Species	N	MaxCExt	MaxCInt	W	H	Slope
S. cephalantha	6	25.8 (6.2)	44.9 (9.7)	89.7 (4.7)	35.3 (11.7)	78.7 (9.4)
S. densiflora	8	41.6 (34.4)	83.7 (30.1)	63.9 (11.6)	18.1 (21.4)	57.8 (28.3)
S. euxina	8	18.7 (20.2)	26.3 (6.7)	99.9(22.3)	55.6 (43.6)	108.6 (31.5)
S. macrodonta	8	26.8 (25.6)	49.8 (39.9)	93.0 (24.3)	20.9 (25.8)	47.0 (35.9)
S. manissadjianii	8	45.6 (39.0)	65.7 (17.2)	75.9 (24.8)	30.3 (30.8)	81.2 (29.2)
S. olympica	8	24.4 (20.0)	30.8 (50.0)	69.2 (22.9)	15.5 (20.5)	45.1 (13.2)
S. oreophila	8	72.3 (14.2)	118.6 (26.9)	90.4 (13.2)	41.4 (14.7)	92.1 (12.3)
S. phrygia	8	46.7 (53.0)	133.7 (81.7)	89.5 (16.6)	45.5 (20.6)	104.5 (27.6)
S. saxatilis	8	30.3 (18.4)	46.1 (28.0)	83.4 (10.7)	24.9 (12.8)	60.1 (14.9)
S. spergulifolia	8	24.9 (13.1)	54.4 (25.6)	102.6 (18.9)	30.0 (15.4)	59.6 (16.3)
S. tunicoides	8	43.0 (21.2)	116.9 (11.2)	91.1 (9.2)	25.0 (13.9)	55.1 (13.9)

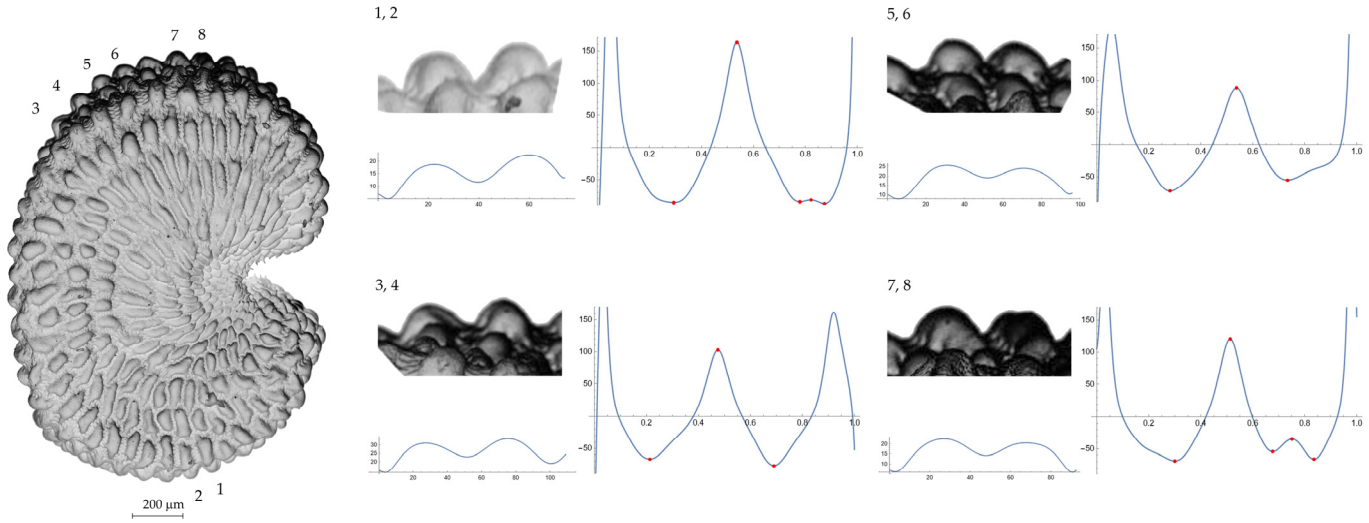

Figure 3. Left: a seed of *S. oreophila* with eight tubercles numbered (below, scale). **Right**: tubercle images, Bézier curves, and curvature plots corresponding to the four pairs of tubercles.

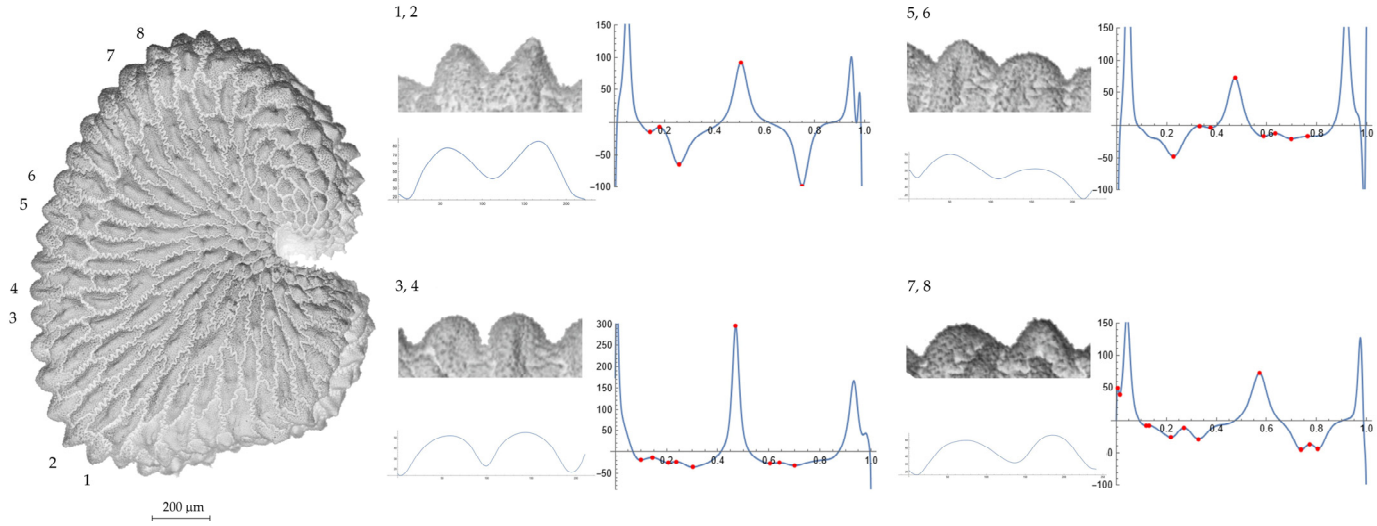

Figure 4. Left: a seed of *S. phrygia* with eight tubercles numbered (below, scale). **Right**: tubercle images, Bézier curves, and curvature plots corresponding to the four pairs of tubercles.

The tubercles of these seeds are mainly rounded and have mean curvature values comprised between 18.7 and 72.3 mm^{-1}. Inter-tubercle curvature is comprised between 26.3 and 133.7.

3.3. Seeds with Rounded Tubercles with Mammillae (Umbonated)

This group includes seeds of the following species (6): *S. gallica*, *S. gigantea*, *S. italica*, *S. microsperma*, *S. nocturna*, and *S. tenuiflora*. These species belong to the *S.* subgen. *Silene* in three sections: *Arenosa* (*S. microsperma* and *S. tenuiflora*), *Silene* (*S. gallica*, *nocturna)*, and *Siphonomorpha* (*S. gigantea*, *S. italica)*.

A summary of the general morphological measurements is presented in Table A3 (Appendix A) and the tubercle measurements (curvature analysis, tubercle height, width, and slope) are shown in Table 4. Figures 5 and 6, corresponding, respectively, to *S. italica* and *S. tenuiflora*, contain examples of tubercles of this group.

Table 4. Tubercle measurements in seeds of Group 3. Mean values (coefficient of variation) for each species are given for the measurements of maximum tubercle curvature (MaxTubCurv), maximum inter-tubercle curvature (MaxIntCurv), tubercle width (W), tubercle height (H), and slope. N indicates the number of tubercles measured in each species.

Species	N	MaxCExt	MaxCInt	W	H	Slope
S. gallica	8	93.4 (13.8)	102.9 (12.2)	41.1 (12.0)	23.1 (8.2)	113.6 (12.3)
S. gigantea	8	25.6 (50.6)	25.0 (16.4)	71.4 (12.4)	22.1 (34.6)	62.3 (33.3)
S. italica	8	71.7 (58.0)	71.0 (21.3)	97.0 (6.2)	41.1 (30.3)	85.6 (31.9)
S. microsperma	8	168.6 (54.7)	114.5 (36.5)	35.3 (14.4)	28.9 (132.6)	185.7 (151.5)
S. nocturna	8	92.3 (83.5)	81.6 (36.4)	41.9 (13.0)	12.4 (35.6)	58.5 (24.7)
S. tenuiflora	8	183.7 (36.3)	169.3 (19.9)	41.1 (8.1)	21.4 (20.6)	103.4 (16.6)

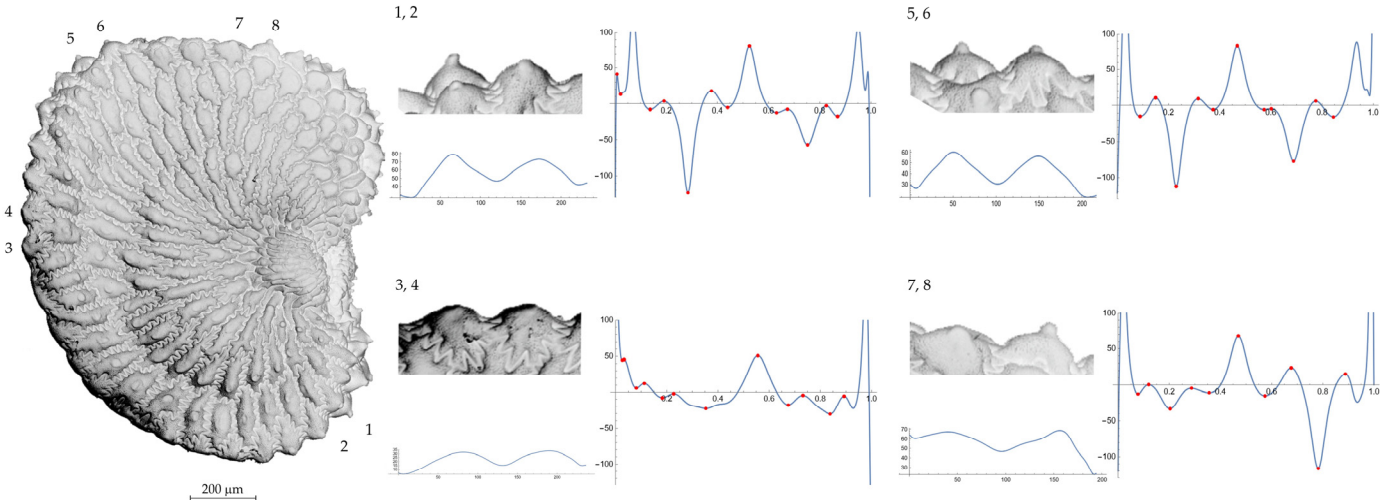

Figure 5. Left: a seed of *S. italica* with eight tubercles numbered (below, scale). **Right**: tubercle images, Bézier curves, and curvature plots corresponding to the four pairs of tubercles.

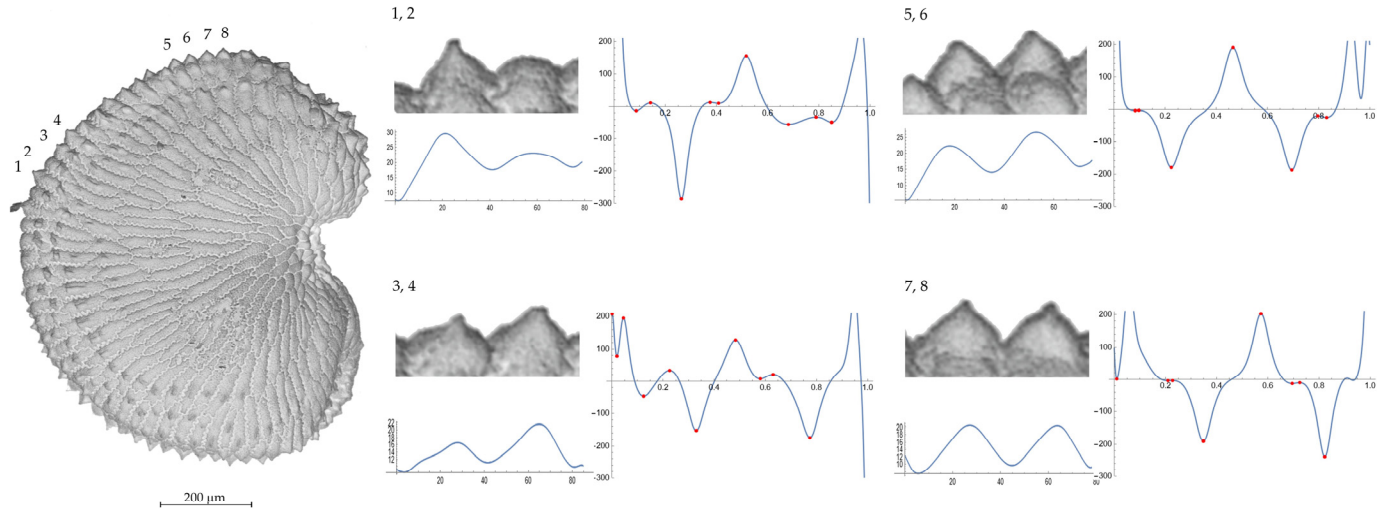

Figure 6. Left: a seed of *S. tenuiflora* with eight tubercles numbered (below, scale). **Right**: tubercle images, Bézier curves, and curvature plots corresponding to the four pairs of tubercles.

The seed tubercles in this group are rounded and umbonated (with mammillae). The curvature values, more variable than the other groups due to two types of tubercles (umbonated or not), are comprised between 25.6 and 183.7 mm^{-1}. Inter-tubercle curvature is comprised between 25.0 and 169.3.

3.4. Seeds with Tubercles Plane on Top

This group includes seeds of three species: *S. anatolica*, *S. cserei*, and *S. rynchocarpa.* These species belong to the *S.* subgen. *Behenantha*, sect. *Behen* (*S. anatolica* and *S. cserei*), and *S.* subgen. *Silene* sect. *Auriculatae* (*S. rhynchocarpa*).

A summary of the general morphological measurements is presented in Table A4 (Appendix A). Tubercle measurements (curvature analysis, tubercle height, width, and slope) are shown in Table 5. Figures 7–9, corresponding, respectively, to *S. anatolica*, *S. cserei*, and *S. rhynchocarpa*, contain examples of tubercles of this group.

Table 5. Tubercle measurements in seeds of Group 4. Mean values (coefficient of variation) for each species are given for the measurements of maximum tubercle curvature (MaxTubCurv), maximum inter-tubercle curvature (MaxIntCurv), tubercle width (W), tubercle height (H), and slope. N indicates the number of tubercles measured in each species.

Species	N	MaxCExt	MaxCInt	W	H	Slope
S. anatolica	8	56.4 (12.9)	261.4 (18.8)	73.4 (8.7)	41.5 (11.7)	114.2 (17.3)
S. cserei	8	53.7 (23.2)	81.9 (59.8)	94.3 (31.1)	55.5 (20.3)	122.3 (21.8)
S. rhynchocarpa	8	29.7 (18.9)	56.2 (29.5)	80.1 (16.7)	20.9 (9.0)	53.6 (21.9)

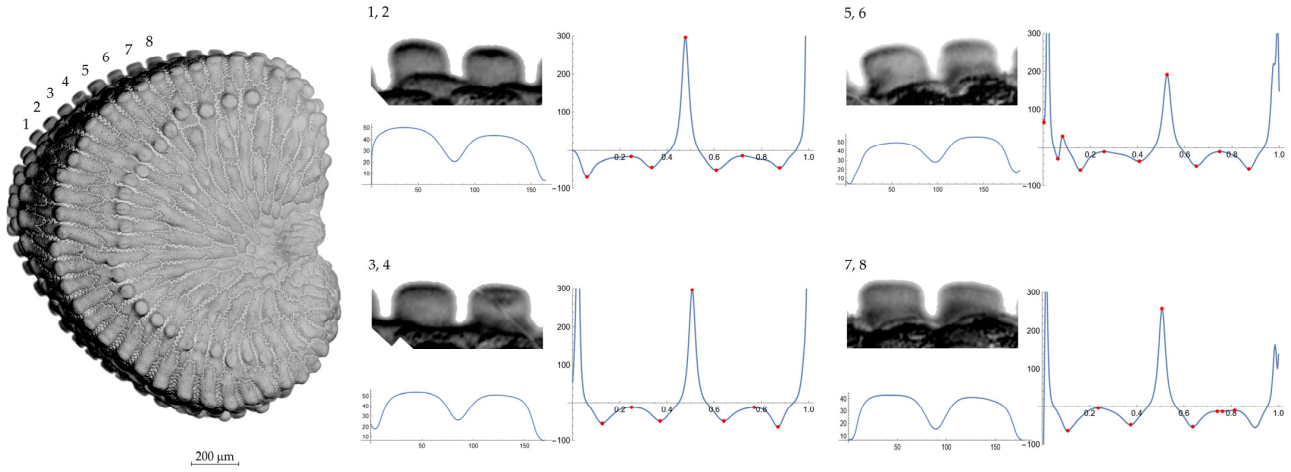

Figure 7. Left: a seed of *S. anatolica* with eight tubercles numbered (below, scale). **Right**: tubercle images, Bézier curves, and curvature plots corresponding to the four pairs of tubercles.

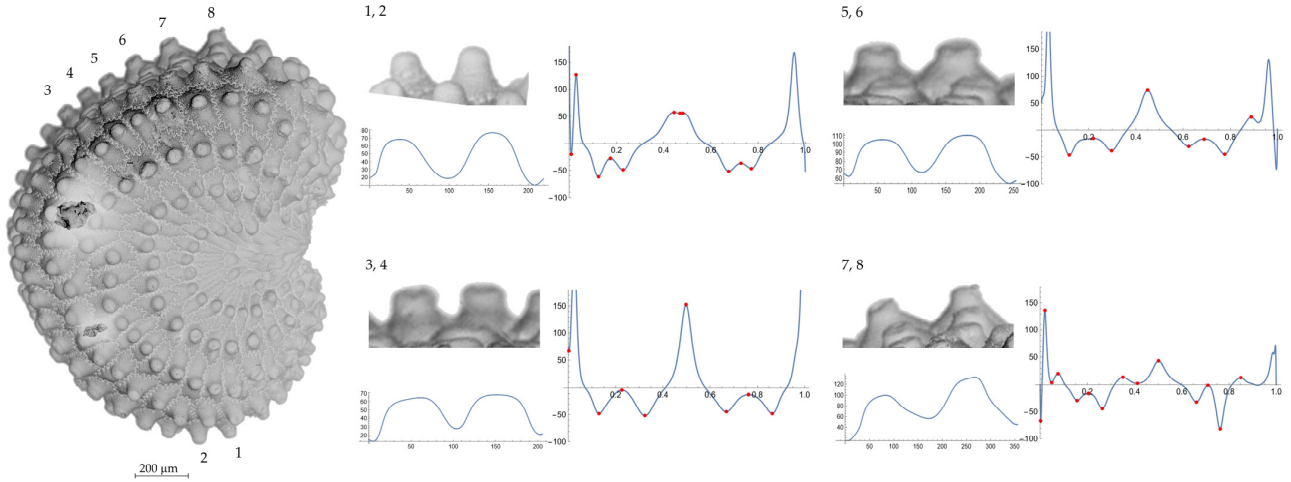

Figure 8. Left: a seed of *S. cserei* with eight tubercles numbered (below, scale). **Right**: tubercle images, Bézier curves, and curvature plots corresponding to the four pairs of tubercles.

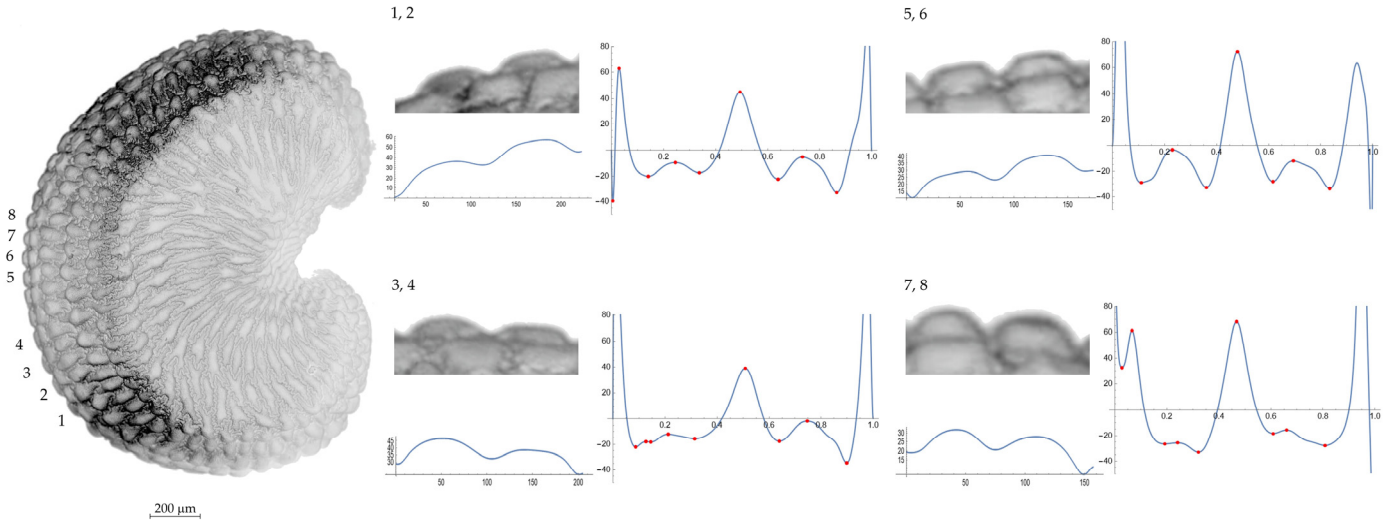

Figure 9. Left: a seed of *S. rhynchocarpa* with eight tubercles numbered (below, scale). **Right**: tubercle images, Bézier curves, and curvature plots corresponding to the four pairs of tubercles.

The curvature graphs of tubercles in this group are characteristic, with at least three peaks per tubercle, two peaks at the extremes of high curvature values, and at least one peak between them of a lower curvature. Tubercles have mean curvature values comprised between 29.7 and 56.4 mm^{-1}. Inter-tubercle curvature is comprised between 56.2 and 261.4.

3.5. Seeds with Echinate Tubercles

This group includes seeds of the following species (7): *S. brevicaulis, S. confertiflora, S. conica, S. isaurica, S. papillosa, S. pendula,* and *S. portensis* subsp. *rigidula.* Two of these species belong to the *S.* subgen. *Behenantha*, sections *Conoimorpha (S. conica) and Behenantha (S. pendula),* and the remaining to five different sections of *S.* subgen. *Silene: Auriculatae (S. brevicaulis*), *Lasiocalycinae* (*S. papillosa*), *Lasiostemones* (*S. isaurica*), sect. *Portenses* (*S. portensis* subsp. *rigidula*), and *Siphonomorpha* (*S. confertiflora*).

A summary of the general morphological measurements and curvature analysis is shown in Table A5 (Appendix A). Tubercle measurements (curvature analysis, tubercle height, width, and slope) are shown in Table 6. Figures 10 and 11, corresponding, respectively, to *S. isaurica* and *S. conica,* contain examples of tubercles of this group.

Table 6. Tubercle measurements in seeds of Group 5. Mean values (coefficient of variation) for each species are given for the measurements of maximum tubercle curvature (MaxTubCurv), maximum inter-tubercle curvature (MaxIntCurv), tubercle width (W), tubercle height (H), and slope. N indicates the number of tubercles measured in each species.

Species	N	MaxCExt	MaxCInt	W	H	Slope
S. brevicaulis	6	61.4 (19.3)	40.7 (7.1)	81.0 (9.0)	33.2 (10.8)	82.5 (26.3)
S. confertiflora	8	68.5 (16.2)	31.9 (9.3)	67.9 (7.3)	54.3 (6.4)	160.6 (17.2)
S. conica	6	68.4 (13.9)	56.9 (14.7)	57.7 (8.9)	24.7 (3.4)	86.9 (15.0)
S. isaurica	8	109.4 (8.0)	187.7 (54.4)	40.8 (3.5)	23.3 (2.3)	114.7 (13.2)
S. papillosa	6	66.3 (15.4)	115.5 (15.6)	71.0 (4.6)	31.8 (2.9)	89.7 (6.9)
S. pendula	6	63.6 (12.6)	92.9 (11.0)	68.5 (6.5)	36.8 (6.4)	109.0 (25.8)

Tubercles in this group have mean curvature values comprised between 61.4 and 109.4 mm^{-1}. Inter-tubercle curvature is comprised between 31.9 and 187.7.

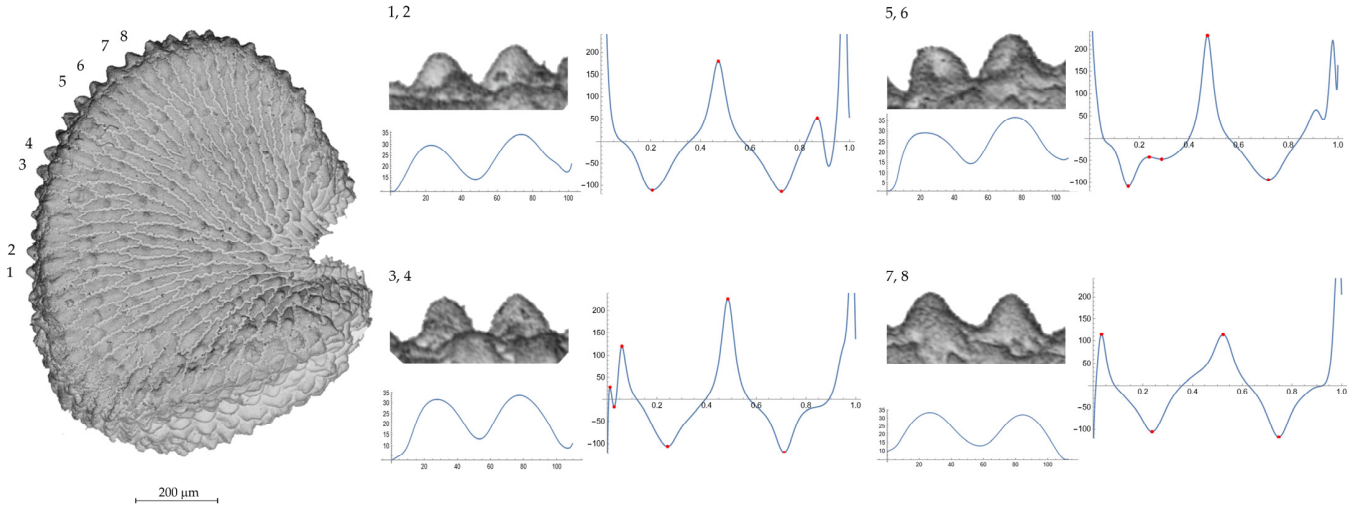

Figure 10. Left: a seed of *S. isaurica* with eight tubercles numbered (below, scale). **Right**: tubercle images, Bézier curves, and curvature plots corresponding to the four pairs of tubercles.

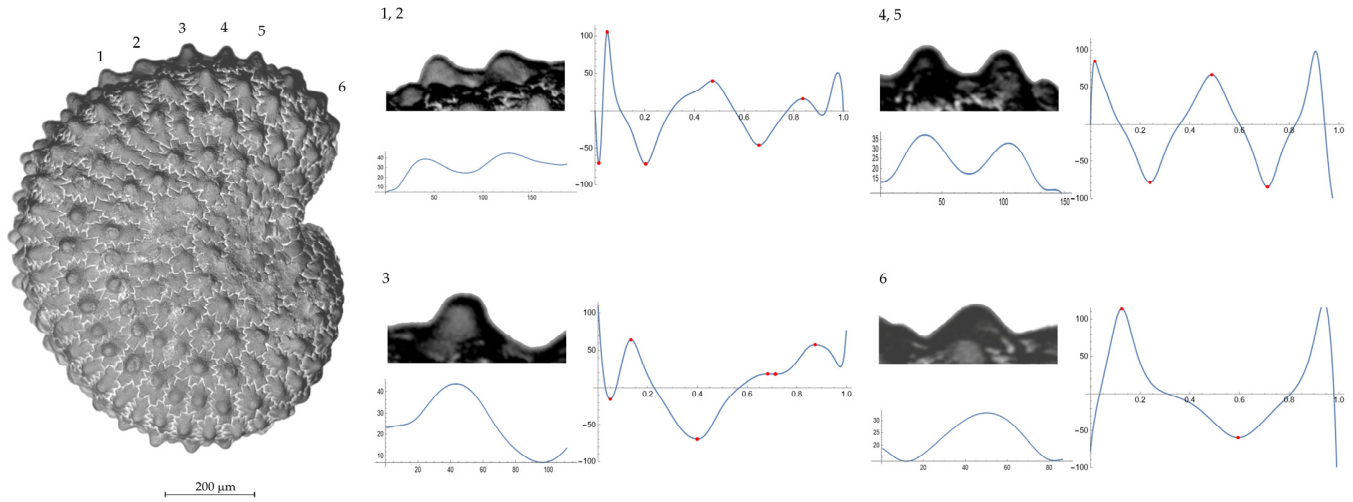

Figure 11. Left: a seed of *S. conica* with eight tubercles numbered (below, scale). **Right**: tubercle images, Bézier curves, and curvature plots corresponding to two pairs and two individual tubercles.

3.6. Seeds with Papillose Tubercles

This group includes seeds of three species, two of *S.* subgen. *Silene*: *S. echinospermoides* (*Rigidulae*), and *S. leptoclada* (*Siphonomoprpha*); and one to *S.* subgen. *Behenantha*: *S. lydia* (*Conoimorpha*). A summary of the general morphological measurements is shown in Table A6 (Appendix A). Tubercle measurements (curvature analysis, tubercle height, width, and slope) are shown in Table 7. Figure 12, corresponding to *S. echinospermoides*, contains examples of tubercles of this group.

Table 7. Tubercle measurements in seeds of Group 6. N is the number of tubercles analyzed per species. Mean values (coefficient of variation) for each species are given for the measurements of maximum tubercle curvature (MaxTubCurv), maximum inter-tubercle curvature (MaxIntCurv), tubercle width (W), tubercle height (H), and slope.

Species	N	MaxCExt	MaxCInt	W	H	Slope
S. echinospermoides	5	167.0 (18.0)	175.0 (43.2)	87.4 (12.8)	99.8 (16.9)	227.5 (5.8)
S. leptoclada	6	133.1 (18.4)	188.8 (94.6)	56.9 (12.1)	52.4 (12.9)	184.7 (8.8)
S. lydia	5	87.4 (15.0)	228.0 (72.5)	73.7 (9.9)	41.0 (7.7)	112.0 (10.1)

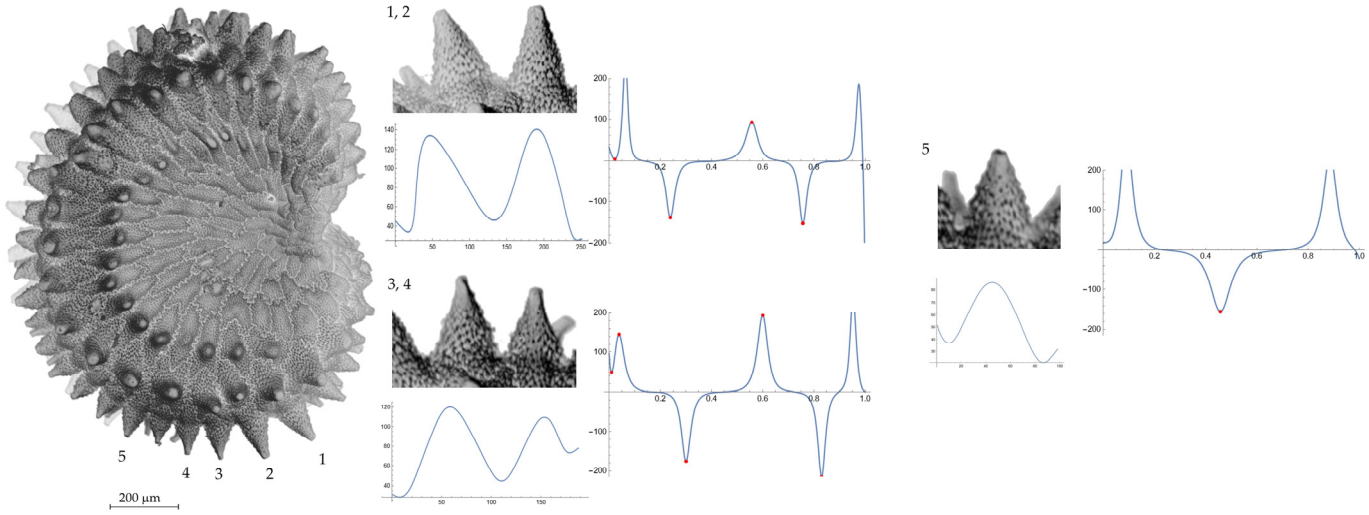

Figure 12. **Left**: a seed of *S. echinospermoides* with eight tubercles numbered (below, scale). **Right**: tubercle images, Bézier curves, and curvature plots corresponding to two pairs and one individual tubercle.

3.7. Comparison between Seed Groups Based on Tubercle Type

There were differences between groups for the morphological data (Table 8), as well as for tubercle measurements of width, height, and curvature (Table 9). In all distance-based measurements (A, P, L, W), a group of higher values corresponded to the first group of small tubercle seeds, and lower values were observed in the remaining groups, except plane tubercle seeds in area and width, plane and papillose tubercle seeds in perimeter, and plane and broad tubercle seeds for length. Circularity was higher in small and lower in papillose seeds. The aspect ratio was higher in broad and lower in mammillate seeds, and the inverse result was observed for roundness. Solidity was higher in the seeds of small tubercles and lower in the seeds with echinate and papillose tubercles.

Table 8. Morphological measurements for seeds. Mean values (coefficient of variation) for Area (A), Perimeter (P), Length (L), Width (W), Circularity (C), Aspect Ratio (AR), Roundness (R), and Solidity (S) in the seed types considered according to their tubercle shape. Different superscript letters in the same column indicate significant differences from Campbell and Skillings analysis ($p < 0.05$).

Seed Groups	N	A	P	L	W	C	AR	R	S
Rugose, small	10	2.35 ^{b} (45.1)	6.87 ^{b} (23.1)	1.92 ^{c} (23.8)	1.52 ^{b} (21.0)	0.62 ^{c} (12.4)	1.26 ^{ab} (9.3)	0.80 ^{ab} (8.4)	0.964 ^{b} (1.3)
Rugose, broad	11	1.09 ^{a} (28.4)	4.87 ^{a} (23.5)	1.35 ^{ab} (15.2)	1.01 ^{a} (13.5)	0.59 ^{bc} (17.5)	1.33 ^{b} (7.0)	0.76 ^{a} (6.9)	0.950 ^{ab} (1.4)
Mammillate	6	0.92 ^{a} (84.9)	4.28 ^{a} (35.4)	1.12 ^{a} (40.5)	0.92 ^{a} (38.6)	0.56 ^{abc} (19.0)	1.21 ^{a} (2.1)	0.83 ^{b} (2.0)	0.949 ^{ab} (1.4)
Plane on top	3	1.59 ^{ab} (8.3)	6.00 ^{ab} (3.3)	1.58 ^{bc} (5.1)	1.28 ^{ab} (6.5)	0.55 ^{abc} (10.0)	1.24 ^{ab} (7.3)	0.81 ^{ab} (7.5)	0.953 ^{ab} (1.4)
Echinate	7	0.87 ^{a} (42.1)	4.75 ^{a} (26.8)	1.15 ^{a} (20.9)	0.93 ^{a} (20.4)	0.48 ^{ab} (12.9)	1.25 ^{ab} (3.1)	0.80 ^{ab} (2.7)	0.938 ^{a} (1.1)
Papillose	3	0.71 ^{a} (44.9)	5.54 ^{ab} (41.0)	1.06 ^{a} (19.9)	0.83 ^{a} (24.1)	0.32 ^{a} (36.3)	1.28 ^{ab} (8.6)	0.78 ^{ab} (8.2)	0.912 ^{a} (3.0)

Table 9. Tubercle measurements. Mean values (coefficient of variation) for each seed type in the measurements of maximum tubercle curvature (MaxTubCurv), maximum inter-tubercle curvature (MaxIntCurv), tubercle width, tubercle height, and slope. Different superscript letters in the same column indicates significant differences from Campbell and Skillings analysis ($p < 0.05$). N indicates the number of tubercles measured in each group.

Tubercles in Groups of Seeds	N	MaxTubCurv	MaxIntCurv	W	H	Slope
Rugose, small	80	31.6 ^{a} (29.0)	53.7 ^{a} (47.3)	77.3 ^{c} (27.8)	26.8 ^{b} (35.5)	69.9 ^{a} (30.2)
Rugose, broad	86	36.6 ^{a} (50.5)	70.7 ^{ab} (69.2)	86.1 ^{d} (21.7)	31.0 ^{b} (47.6)	71.6 ^{a} (38.7)
Mammillate	48	105.9 ^{c} (74.2)	94.0 ^{bc} (53.8)	54.6 ^{a} (42.4)	24.8 ^{a} (73.7)	101.5 ^{b} (116.2)
Plane on top	24	46.6 ^{b} (32.0)	133.2 ^{bc} (76.9)	82.6 ^{cd} (24.4)	39.3 ^{c} (40.9)	96.7 ^{b} (38.1)
Echinate	48	78.5 ^{c} (33.0)	93.9 ^{bc} (62.6)	67.5 ^{b} (24.7)	39.9 ^{c} (42.6)	117.4 ^{c} (29.3)
Papillose	19	129.4 ^{d} (30.4)	197.3 ^{c} (65.7)	70.2 ^{bc} (21.4)	61.3 ^{d} (42.2)	173.0 ^{d} (27.8)

Differences between groups were also observed in curvature values as well as in tubercle width, tubercle height, and slope (Table 9).

The dendrogram in Figure 13 shows the relationship between the groups formed on the basis of tubercle morphology. Most species of *S.* subgen. *Silene* have lower curvature values and are grouped in the upper part of the diagram.

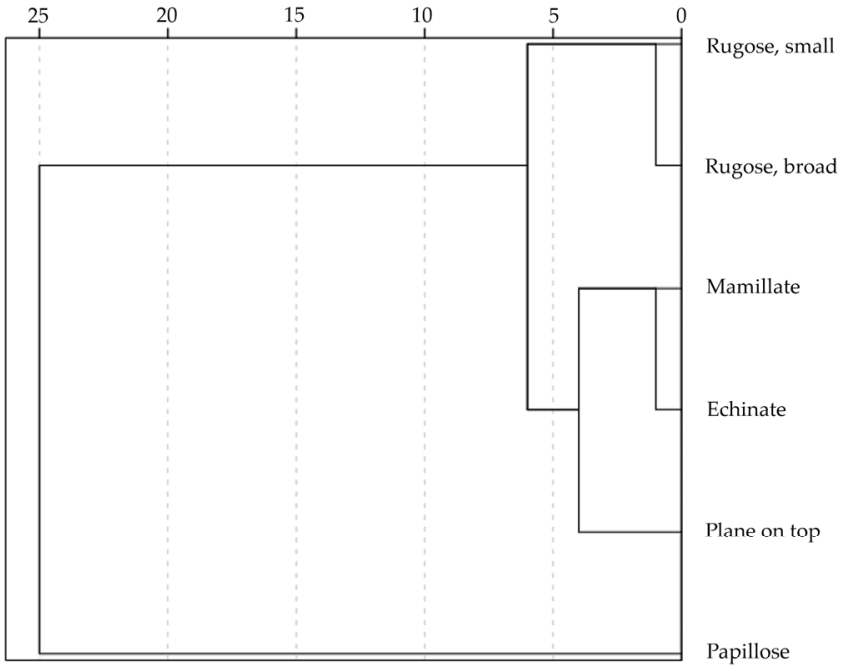

Figure 13. Dendrogram based on hierarchical clustering with the values of tubercle measurements indicated in Tables 2–7 and seed morphological measurements (Tables A1–A6).

4. Discussion

Silene L., with about 850 species, is the largest genus of Caryophyllaceae [12]. In addition to a model for infrageneric diversity, *S. latifolia*, *S. dioica*, and other species [75] have been proposed as models for the study of sex determination. Like humans, these species have heteromorphic chromosomes, with a rapidly evolving non-recombining Y region rich in repetitive DNA that provides a unique system for the study of the origin and modification of sex chromosomes [76]. *Silene* species also present variations in seed shape that can be quantified by different means. The overall seed shape can be compared with geometric objects, and in many species, the lateral seed views show striking similarities with the cardioid and derived models [68]. Tubercle size, shape, and distribution can also be studied using geometrical and statistical methods [68,77].

Most descriptions of the seed surface in *Silene* are based on SEM images, and the terms to describe the shape of tubercles include conical [51], convex [55], cylindrical [51], echinate [55], granulate [45], mammillate (umbonated) [51,55], papillose [48], rounded [55], and winkled [36]. In general, although there may be different representations of these types in the different sections, there is no ascription of these particular types to any sections or subgenus. Based on seed silhouettes derived from light microscopy images, we classified the tuberculated seeds into three types: rugose, echinate, and papillose. Curvature analysis revealed higher values in the echinate than in the rugose-type tubercles [64]. Following our previous terminology, in this work, the seeds were classified into six groups. Groups 1 and 2 consisted of rugose seeds, which were differentiated by seed size and tubercle width. Groups 3 and 4 were made up of mammillate seeds and seeds with flat tubercles on top. Groups 5 and 6 contained echinate and papillose seeds, respectively. In accordance with previous results [62,63], these groups are supported based on morphological measurements. Thus, papillose seeds had lower circularity than the two groups of rugose seeds, and both papillose and echinate seeds had lower solidity values than the rugose seeds. There is also a difference between the two rugose groups based on area, perimeter, length, and width. According to these measurements, mammillate seeds are closer to seeds with broad rugose tubercles than to seeds with small rugose tubercles. However, the mammillate group differs from the two rugose groups in tubercle dimensions, whereas it differs from the group of small rugose tubercles in tubercle curvature. Differences between groups are noticeable in the maximum curvature, with lower values in the two groups of rugose seeds, and higher in the papillose seed group.

Mammillate tubercles were reported for the first time for species in sections *Siphonomorpha* (*S. gigantea*, *S. mollissima* Pers., *S. pseudovelutina* Roth, *S. velutina* Pourr., *S. coutinhoi* Rothm. & P.Silva, *S. spinescens* Sm.) and *Spergulifoliae* (*S. arguta* Fenzl, *S. brahuica* Boiss., *S. erioclycina* Boiss.) [35], being described as umbonated. This was supported by our results in *S. spinescens* Sm. and *S. gigantea* (L.) L. [64]. Other authors indicated that this type of tubercle is very frequent in the section *Siphonomorpha* [51]. This work reported the presence of mammillae in some but not all populations of *S. gallica*, confirming the infraspecific variation reported before [77]. Hoseini et al. [55] found this character in three species of sect. *Lasiocalycinae* (*S. dichotoma* Ehrh., *S. gallica* L., *S. lagenocalyx* Fenzl ex. Boiss.) as well as in *S. meyeri* Fenzl ex Boiss. & Buhse (*Auriculatae*), *S. nana* Kar. & Kir. (sect. *Saponarioides*), *S. ovalifolia* (Regel and Schmalh.) Popov (sect. *Otites*), and *S. viscosa* (L.) Pers. (*Physolychnis*). Section *Otites* corresponds to *Siphonomorpha* in the classification of Jafari et al. [12], while the tubercles observed in *S. viscosa* under our concept could be better denominated as echinate because they are more triangular than having a central protuberance. In conclusion, this type is more abundant in *S.* subgen *Silene* than in *Behenantha*, and particularly frequent in sect. *Siphonomorpha*.

Unlike groups 1, 2, and 3, groups 4, 5, and 6 are more diverse. The tubercles of Group 4 have a very characteristic shape—plane at the top. Although this type is clearly distinguishable to the naked eye, it also has noteworthy geometrical features. The tubercles had a profile with two lateral maxima and a central point of lesser curvature, and the curve between tubercles had higher curvature values than in any of the other types. In addition to the examples reported here, some populations of *S. behen* also have such tubercles [57]. In the seeds of Group 4 studied here (see Table 5), higher curvature values and higher tubercles corresponded to *S. cserei*, and smaller ones to *S. rhynchocarpa*. The low-plane tubercles of *S. rhynchocarpa* seed resemble those of Groups 1 and 2.

The work reported here confirms a certain association between tubercle type and taxonomic sections. Groups 1 to 3 contain almost exclusively species of *S.* subgen *Silene*. Species in Groups 1 and 2 (rugose, or rounded tubercles) belong to the *Silene* subgen. *Silene*. The difference between these two groups lies in the size of the seeds and the width of the tubercles. While the seeds of Group 1 are large and have smaller tubercles, the seeds of Group 2 are smaller and have tubercles broader at the base.

5. Conclusions

Analysis of tubercles based on the SEM images obtained from the seed collection of Turkish species of *Silene* allowed us to delimit five principal morphological types that were reported before as smooth, rugose, mammillate, echinate, and papillose seeds, to which a sixth group of seeds with tubercles flat on top has been added. The rugose type is divided into two characterized by large seeds with small tubercles and smaller seeds with broader tubercles, respectively. Differences were found between groups both in seed and in tubercle morphology. The rugose and papillose groups differ both in circularity and maximum values of curvature of their tubercles. Both rugose and echinate groups have lower solidity than the rugose groups. The mammillate group is characterized by high curvature values in some of the tubercles, with mean values superior to rugose seeds. This work supports a certain association between tubercle type and sections within the genus *Silene*, with the groups of rugose and mammillate seeds composed mainly of species of *S.* subgen *Silene*.

Supplementary Materials: The following are available at Zenodo (https://zenodo.org/records/10983689; accessed on 17 April 2024): (1) JPG files with seed images, Bézier curves and curvature analysis for 40 seeds (one seed of each species); (2) Mathematica files with curvature analysis corresponding to 40 seeds (one seed of each species).

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Appendix A

Table A1. General morphological measurements corresponding to the 10 species of Group 1. A = Area; P = Perimeter; L = Length; W = Width; C = Circularity; AR = Aspect Ratio; R = Roundness; S = Solidity.

Species	A	P	L	W	C	AR	R	S
S. birandiana	1.30	5.81	1.60	1.36	0.63	1.17	0.85	0.967
S. bupleuroides	1.99	7.57	1.95	1.30	0.44	1.50	0.67	0.946
S. caramanica	3.94	8.74	2.50	2.01	0.65	1.20	0.81	0.976
S. caryophylloides	1.50	5.30	1.45	1.31	0.65	1.11	0.90	0.968
S. chlorifolia	3.23	7.83	2.36	1.75	0.66	1.35	0.74	0.973
S. doganii	3.77	8.79	2.48	1.94	0.61	1.28	0.78	0.957
S. eremitica	1.04	4.33	1.24	1.07	0.70	1.15	0.87	0.969
S. haradjiannii	1.40	5.24	1.48	1.20	0.64	1.23	0.81	0.966
S. marschalii	2.60	7.05	2.06	1.60	0.66	1.28	0.78	0.978
S. swertiifolia	2.70	7.99	2.12	1.61	0.53	1.36	0.76	0.942

Table A2. General morphological measurements corresponding to the 11 species of Group 2. A = Area; P = Perimeter; L = Length; W = Width; C = Circularity; AR = Aspect Ratio; R = Roundness; S = Solidity.

Species	A	P	L	W	C	AR	R	S
S. cephalantha	1.05	4.86	1.31	1.01	0.56	1.28	0.78	0.943
S. densiflora	1.02	4.80	1.37	0.95	0.56	1.45	0.69	0.949
S. euxina	1.28	4.88	1.49	1.09	0.67	1.37	0.73	0.965
S. macrodonta	0.95	4.30	1.23	0.99	0.65	1.25	0.80	0.963
S. manissadjianii	0.87	3.89	1.14	0.97	0.72	1.17	0.85	0.954
S. olympica	1.10	4.70	1.33	1.05	0.62	1.27	0.79	0.966
S. oreophila	1.77	6.93	1.78	1.26	0.47	1.41	0.71	0.945
S. phrygia	1.40	7.10	1.54	1.16	0.36	1.32	0.76	0.937
S. saxatilis	0.62	3.49	1.02	0.77	0.64	1.32	0.76	0.951
S. spergulifolia	1.08	4.47	1.34	1.03	0.68	1.30	0.77	0.920
S. tunicoides	0.83	4.19	1.25	0.84	0.59	1.49	0.67	0.958

Table A3. General morphological measurements corresponding to the six species of Group 3. A = Area; P = Perimeter; L = Length; W = Width; C = Circularity; AR = Aspect Ratio; R = Roundness; S = Solidity.

Species	A	P	L	W	C	AR	R	S
S. gallica	0.61	4.25	0.97	0.80	0.42	1.21	0.82	0.932
S. gigantea	2.41	6.85	1.94	1.57	0.65	1.24	0.81	0.961
S. italica	1.17	5.08	1.35	1.10	0.57	1.23	0.82	0.949
S. microsperma	0.41	2.68	0.78	0.66	0.71	1.18	0.85	0.967
S. nocturna	0.39	3.14	0.77	0.65	0.50	1.18	0.85	0.949
S. tenuiflora	0.54	3.67	0.91	0.76	0.51	1.20	0.83	0.937

Table A4. General morphological measurements corresponding to the two species of Group 4. A = Area; P = Perimeter; L = Length; W = Width; C = Circularity; AR = Aspect Ratio; R = Roundness; S = Solidity.

Species	A	P	L	W	C	AR	R	S
S. anatolica	1.69	6.21	1.57	1.37	0.55	1.15	0.87	0.969
S. cserei	1.44	5.99	1.51	1.21	0.50	1.25	0.80	0.943
S. rhynchocarpa	1.64	5.81	1.67	1.25	0.61	1.33	0.75	0.948

Table A5. General morphological measurements corresponding to the seven species of Group 5. A = Area; P = Perimeter; L = Length; W = Width; C = Circularity; AR = Aspect Ratio; R = Roundness; S = Solidity.

Species	A	P	L	W	C	AR	R	S
S. brevicaulis	0.59	3.83	0.96	0.78	0.51	1.22	0.82	0.930
S. confertiflora	1.42	6.45	1.50	1.20	0.43	1.26	0.80	0.923
S. conica	0.54	3.37	0.91	0.75	0.59	1.20	0.82	0.940
S. isaurica	0.65	4.17	1.02	0.81	0.47	1.26	0.79	0.949
S. papillosa	0.65	3.98	1.04	0.80	0.52	1.31	0.77	0.936
S. pendula	0.91	4.95	1.18	0.98	0.47	1.21	0.83	0.952

Table A6. General morphological measurements corresponding to the three species of Group 6. A = Area; P = Perimeter; L = Length; W = Width; C = Circularity; AR = Aspect Ratio; R = Roundness; S = Solidity.

Species	A	P	L	W	C	AR	R	S
S. echinospermoides	1.08	8.16	1.30	1.05	0.20	1.23	0.81	0.911
S. leptoclada	0.49	4.38	0.94	0.66	0.32	1.41	0.71	0.885
S. lydia	0.57	4.08	0.93	0.78	0.43	1.21	0.83	0.940

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