

A KEY TO 19 MICROSPECIES OF THE *PORTULACA OLERACEA* AGGREGATE

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Abstract: Recent investigations and publication of a few additional microspecies of the *Portulaca oleracea* aggregate brought their number to 19. A key for the identification of all microspecies known at present, with SEM images, is provided.

Keywords: seed coat morphology, key to 19 *Portulaca* microspecies, phylogeny

Introduction

The vegetative body of the taxa of the *Portulaca oleracea* aggregate does not show any useful diagnostic characters (Danin et al., 1978) but seed coat morphology does. Following previous investigations of the aggregate in Tenerife (Danin & Reyes–Betancort, 2006) and four Mediterranean islands (Danin et al., 2008), seeds of this group were collected in the summer of 2009 in Sardinia (Danin et al., 2011). Taking into account the description of a new microspecies from Socotra (Domina & Raimondo, 2009) and of a new cultivar (Danin & Bagella, 2012), and the typification of another microspecies from N. Africa (Domina et al., 2010), a key for the identification of the 19 microspecies found and published so far is presented. The key is based on seed-coat morphological characters and seed size. Chromosome number in most of the taxa is counted and presented here as well. The microspecies are illustrated by scanning electron micrographs (SEM). Hypothetical relationships in several lines, based on resemblance of seed coat morphology, are suggested.

Materials and methods

Danin et al. (1979) provided a first key to the microspecies of the *Portulaca oleracea* aggregate, covering the nine microspecies known at that time (and regarded then as subspecies). Ten taxa have since been added. For convenience the following artificial key includes all 19 taxa that are presently known. A few seeds, discovered by Knörzner in archaeological diggings in Germany (Knörzner 1970), were determined in 2010. The SEM images displayed in the key were presented in the previous publications mentioned above. Most images were prepared at the Geological Institute of Jerusalem. Seeds were fixed onto metal stubs and coated with a 20 nm gold layer or onto aluminium stubs with carbon tapes and coated with a 5-10 nm carbon layer in auto-sputter-coaters.

The main morphological features here referred to are: 1. Seed diameter, with 0.85 mm being the threshold between the hexaploids (>0.85) and the tetraploids and diploids (<0.85); 1.1 mm is the lower threshold for the two cultivated microspecies. – 2. Epidermis cells have the firm "interior" (Fig. 1ai) and "rays" (Fig. 1aii) projecting from it and interdigitating (Fig. 1aii) with the rays of the neighbouring cells (Figs. 1a, 2a, 2b, 2c, 2d). – 3. Epidermis cells may be smooth (Figs. 1a, 2b) or with protuberances, circular in cross-section, above the cells (Fig. 1biii); we use the term "tubercles" for large elements (0.03 mm diam; Fig. 1b) and "papillae" for small ones (0.01-0.02 mm diam; Figs. 1c, 1d). The latter look like small grains (granula), and were used in naming *P. granulostellulata* (Figs. 1d, 15). – 4. Papillae may be situated at the tips of the rays (Figs. 1d, 4b, 10, 14, 15) or at the base of the rays (Fig. 16b), or they may be found scattered on the interior (Figs. 4d, 17). – 5. Cells may be star-shaped (Figs. 1a, 2b). The line encircling the tips of the rays projecting from the interior (the main cell body) may be circular (Fig. 2a); or the cell may be elongated (Figs. 2c, 2d) where the line encircling the tips of the rays projecting from the main cell body is ellipse (Fig. 2d). The rays may be short (Fig. 2c) or long (Fig. 2b). – 6. The seed surface may vary in micromorphology when observed at high magnification (Fig. 3a-d).

Results

Key for the determination of 19 taxa by their seed features

For author names and one specimen per taxon, see Appendix 1.

1. Seeds bluish, few or many of any one specimen covered with wax; testa cells radially elongated; rays short (Fig. 5) *P. nicaraguensis*
1. Seeds not covered with wax 2
2. Major seed diameter > 1.1 mm 3
2. Major seed diameter < 1.1 mm 4
3. Seed surface covered with small papillae of almost equal size; 10-15 papillae on each cell (Fig. 4c, 4d) *P. edulis*
3. Testa cells isodiametric, star-shaped, with long rays and single or paired tubercles at the center (Fig. 6) *P. sativa*
4. Major seed diameter > 0.85 mm 5
4. Major seed diameter < 0.85 mm 14
5. Seed surface dull; surface with steeply sloping swellings that may appear as white dots, at a magnification of x100 to x1000 (Figs. 7c, 8d) 6
5. Seed surface shiny; if covered with swellings, their edge sloping gradually (Fig. 3d) 7
6. Testa cells isodiametric, with long rays, their centre convex, resembling a turtle shell (Fig. 7) *P. impolita*
6. Testa cells elongated, with short rays (Fig. 8) *P. canariensis*
7. Seed surface covered with small papillae of almost equal size (Fig. 9) *P. rausii*
7. Testa cells with 1-3 large tubercles at the center (Fig. 1b, 2a), with papillae at the cell centre, with papillae on the rays (Fig. 1d), or smooth, lacking protuberances and star-shaped cells (Fig. 1a) 8
8. Testa cells smooth, lacking protuberances, star-shaped (Fig. 1a, 2b).....*P. oleracea*
8. Testa cells with at least one kind of tubercle or papilla 9
9. Testa cells with tubercles 10
9. Testa cells with papillae 12
10. Testa cells isodiametric, star-shaped, with long rays and single or paired tubercles at the centre (Fig.13) *P. cypria*
10. Testa cells elongated 11
11. One or two peripheral lateral testa cells isodiametric, tuberculate, the central lateral cells smooth, without any papillae or tubercles, elongated more than in any other microspecies (Fig. 12)..... *P. sicula*
11. Most lateral testa cells elongated, with (2-)3 tubercles close to each other (Fig. 11) *P. trituberculata*
12. Cell interior with 2-7 papillae, rays short, many of them with terminal papillae (Fig 17) *P. macrantha*
12. Cell interior smooth (without papillae), rays long, with or without terminal papillae 13
13. Rays long, many with terminal papillae (Fig.10) but not all papillate *P. papillatostellulata*
13. Most rays papillate, forming circles or ellipses of papillae among neighbouring testa cells (Fig. 4a, 4b)..... *P. sardoa*
- 14 (4). Testa cells isodiametric, with long rays, star-shaped, smooth, with neither tubercles nor papillae (Fig. 1a, 2b) *P. nitida*
14. Testa cells elongated, often tuberculate or papillate 15
15. Testa cells papillate 16
15. Testa cells tuberculate, or with both tubercles and papillae 18

16. Seed surface evenly covered with small papillae of almost equal size; stellulate cell pattern hardly visible (Fig. 1c, 14) *P. zaffranii*
16. Cell pattern prominent, papillae at the tips of the rays or in a few cells at the bases of the rays..... 17
17. Testa cells star-shaped, the papillae emerging from the tips of the rays (Fig.15); rays 1.5-2 as long as wide..... *P. granulatostellulata*
17. Testa cells star-shaped, the papillae emerging from the base of each ray (Fig.16); rays as long as wide; cells with papillae only at the periphery of the lateral face *P. socotrana*
18. Testa cells with tubercles in their central part and papillae on some of the rays (Fig. 18) *P. tuberculata*
18. Peripheral lateral testa cells tuberculate and with long rays, all the others elongated and smooth, as in *P. sicula* (Fig. 13) *P. africana*

Discussion

The enigma of the evolutionary history of the *P. oleracea* aggregate

Many authors have discussed the cosmopolitan range of the *P. oleracea* aggregate. In our previous articles (Danin et al. 1978; Danin & Reyes Betancort 2006; Danin et al. 2008) we considered the New World to be the place of origin, and no new material in later articles has caused us to consider the Old World as a place of origin. However, microspecies that may be considered as endemic to relatively small areas, such as *P. sicula* and *P. sardoa*, may suggest local speciation. The finding of semi-fossil seeds of *P. trituberculata* in archaeological excavations in Germany (Knörzer, 1970) and in Italy (Bosi et al., 2009) in layers 1600-2000 years old prove that *P. trituberculata* was already present in the Old World long before the “discovery” of the New World took place. According to Bosi et al. (2009), *P. sativa* seeds are found in 14th and 15th century subfossils in Ferrara excavations. Their findings and determination were accepted in 2010-2011 by the first author (A.D.). Additional findings in Germany have shown that *P. trituberculata* was the only microspecies found in: 1. Roman (römisch, 16 BC-70 AD, location of excavation: Neuss), 2. Medieval (Hochmittelalter, 10th-13th centuries AD, location of excavation: Meerbusch), and 3. Modern Age times (Neuzeit, 1700-1740 AD, location of excavation: Neuss, Münsterschule).

Another case emphasizing the importance of studying seed morphology at high magnification in as many sites as possible is that of *P. rausii*. It is a hexaploid wild type which was known before now from the northeast Mediterranean area of Greece and Turkey (cf. Danin et al., 2008 and distribution maps in Euro+Med PlantBase.) Seeds collected in 2007 in Peru have the same ornamentation as in *P. rausii*, the same seed size and the same chromosome number (counted by J. Walter in 2012). We have not found a diagnostic character differentiating between specimens from the two continents. Other microspecies which could have been transported by natural agents, such as the Gulf Stream, did not need anthropogenic assistance for their transportation. We believe that human involvement in transportation of *P. rausii* took place in this case. Material from archaeological excavations may provide evidence for transportation between the continents. We speculate that *Portulaca* seeds of several South American taxa could be transported with crops such as tomatoes, potatoes and maize.

Similarity of taxa from different ploidy levels

The increasing number of micro-species found in the *Portulaca oleracea* aggregate calls for an attempt to construct the overall picture as revealed in the seed morphology of the aggregate. The following groups have similar seed surface morphology but differ in seed size and in ploidy level.

P. africana (2x) - *P. sicula* (4x)

P. nitida (4x) - *P. oleracea* (6x)

P. nicaraguensis (2x) - *P. canariensis* (?) - *P. granulatostellulata* (4x) - *P. papillatostellulata* (6x) - *P. sardoa* (2n=48)

P. tuberculata (?) - *P. trituberculata* (6x)
P. zaffranii (4x) - *P. rausii* (6x) - *P. edulis* (6x)
P. cypria (6x) - *P. sativa* (6x)

Morphological similarities of many taxa were dealt with at length by Danin et al. (1979). The scanning electron micrographs of the additional ten taxa discovered or studied since fit well into the hypothesis of phylogenetic links between the taxa. It is possible to see morphological similarities or links between 16 of the 19 known taxa. As in 1979, application of further cytogenetic and molecular-systematic methods is recommended. We assume that, in future, further detailed investigations will reveal additional taxa which will eventually enable the construction of the whole evolutionary tree of the *P. oleracea* aggregate.

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Specimens seen

Portulaca oleracea L.

Israel: N Negev, Arad, 15.10.1981, A. Danin (HUJ!).

2n = 54

Portulaca africana (Danin et H.G. Baker) Danin

Sudan Francais (Mali): Timbuktu, dry sand, 14.7.1927, Hagerup 262 (K!)

2n = 18

Portulaca canariensis Danin et Reyes-Betancort

Tenerife: Fasnía, La Hondura, 30.3.1996, Cruz Trujillo 39.452 (TFC!)

2n = ?

Portulaca cypria Danin

Cyprus: Fasouri forest nursery at Akrotiri Peninsula, 5 km west of Lemesos port,

1.8.2007, Hadjikyriakou 6976 (PAL, HUJ, B, herb. Hadjikyriakou!)

2n = 54

Portulaca edulis Danin et Bagella

Cyprus: Deftera, cultivated, 22.9.2004 Danin & Hadjikyriakou Cy2004-22a (HUJ)

2n = 54

Portulaca granulostellulata (Poelln.) C. Ricceri et P.V. Arrigoni

Egypt: Cairo, American University, 2.7.2009, M. Hassan EG-04 (B)

2n = 36

Portulaca impolita (Danin et H.G. Baker) Danin,

California: San Bernadino Co, New York Mountains, 1640 m, 14.9.1955, Burns (UC!)

2n = ?

Portulaca macrantha (Maire) C. Ricceri et P.V. Arrigoni in *Parlatorea* 4: 93 (2000)

Morocco: in rupestribus calcareis fino conspurcatis prope Agadir N'Ighir / 3.4" [manu Maire] (MPU001992).

conspurcatis prope Agadir N'Ighir / 3.4" [manu Maire] (MPU001992, Fig. 1, 2).

2n = ?

Portulaca nicaraguensis (Danin et H.G. Baker) Danin

Tenerife: Playa de Las Americas, gardens, 13. 10. 2003, Danin T33 (HUJ!)

2n = 18

Portulaca nitida (Danin et H.G. Baker) C. Ricceri et P.V. Arrigoni

Egypt: Cairo, American University, 2.7.2009, M. Hassan EG-08 (B!)

2n = 36

Portulaca papillatostellulata (Danin et H.G. Baker) Danin,

Italy: Lazio, Rome, "Ateneo Pontificio Regina Apostolorum", 15.9.2007, A. Danin (HUJ!)

2n = 54

Portulaca rausii Danin, Domina et Raimondo

Turkey-in-Europe: A1(E) Kirklareli, Luleburgaz, 1.7.2007, A. Danin (HUJ!)

Peru: Sacred Valley, Urubamba, 4.2.2007, Danin 07-K-01 (HUJ!)

2n = 54

Portulaca sardoa Danin, Bagella et Marrosu

Italy: Sardinia, Mores, 28.7.2009, Caterina Lippi S42D (SS!).

2n = 48

Portulaca sativa Haw.

Switzerland: Carouge, near Genève, 8.1899, Chenevard (G!)

2n = 54

Portulaca sicula Danin, Domina et Raimondo

Sicily: Termini Imerese c.da Sacchitello, 14.9.2005, Danin & Domina (HUI, PAL!).

2n = 54

Portulaca socotrana Domina et Raimondo

Photograph!

2n = 36

Portulaca trituberculata Danin, Domina et Raimondo

Sicily: Trapani, 18.9.2005, *Danin & Domina* (PAL!).

2n = 54

Portulaca tuberculata (Danin et H.G. Baker) Danin

Columbia: Dept. Santander, 70 km north of Velez, 680 m, Fassett 25307 (GH).

2n = ?

Portulaca zaffranii Danin

Greece: Mesogia, 10 km N of Athens airport, 7.10.2004, A. Danin (HUI).

2n = 36

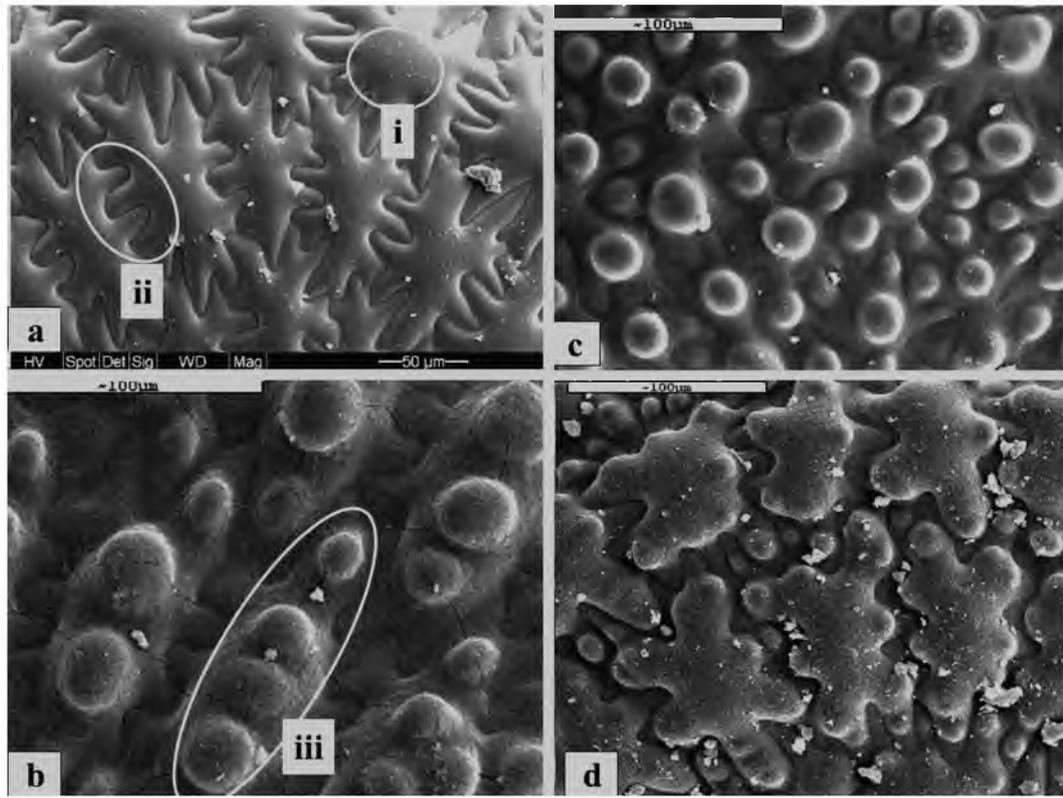


Fig. 1. Morphological elements: a. cells smooth; i. interior of a cell, ii. three rays interdigitating, *P. oleracea*, b. tubercles at cell centre, iii. four tubercles projecting, *P. trituberculata*, c. papillae throughout the cells, *P. rausii*, d. stellulate with papillae at the ray tips, *P. granulostellulata*.

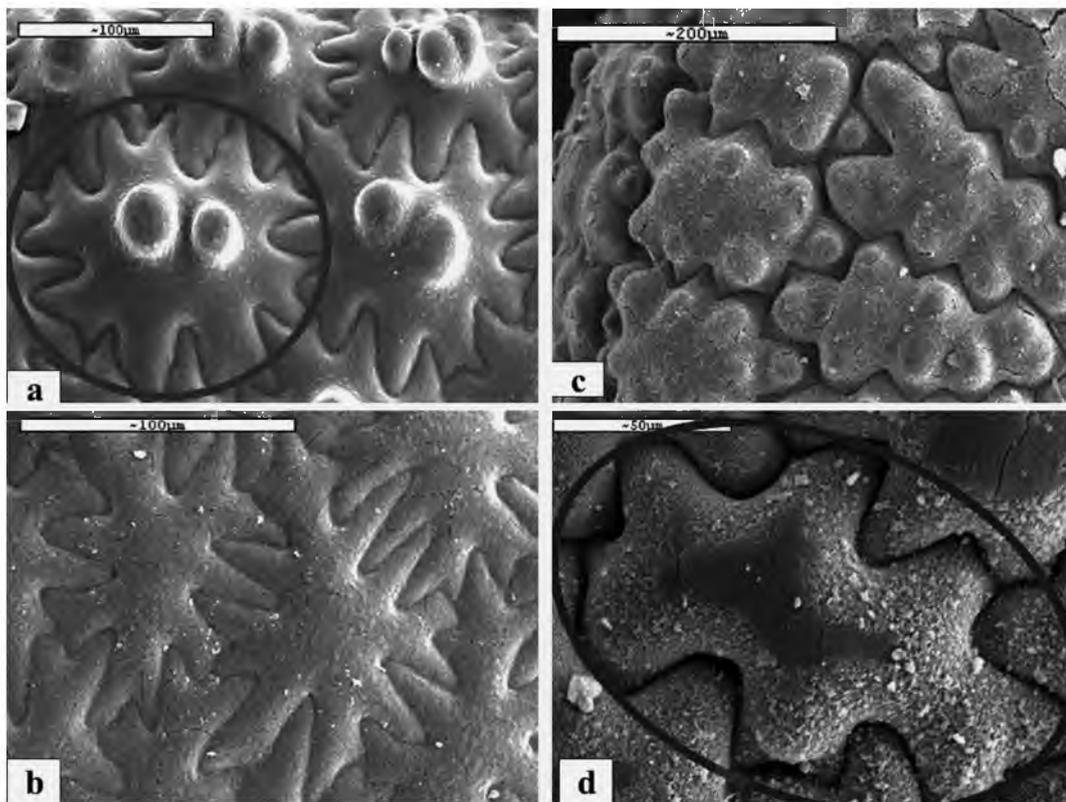


Fig. 2. Cells shape: a and b – isodiametric epidermal cells; c and d – elongated cells

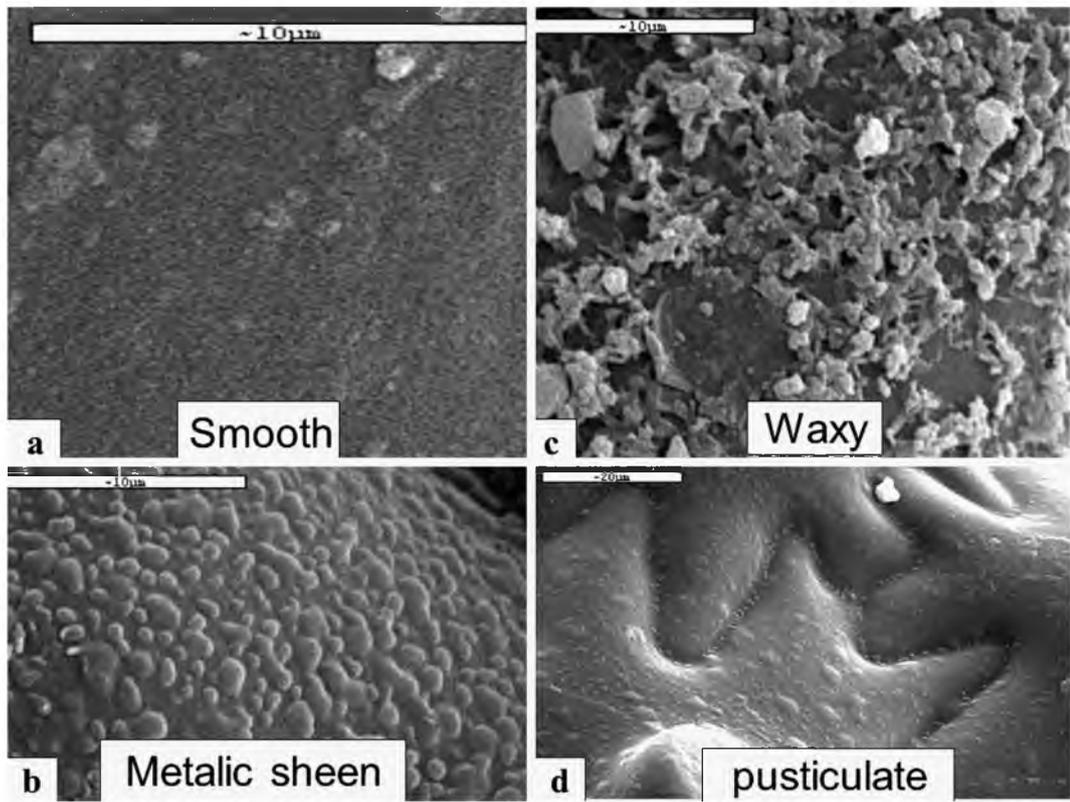


Fig. 3. Seed surface at high magnification: a. smooth, b. surface of seeds with metallic sheen, c. wax covered, d. pusticulate

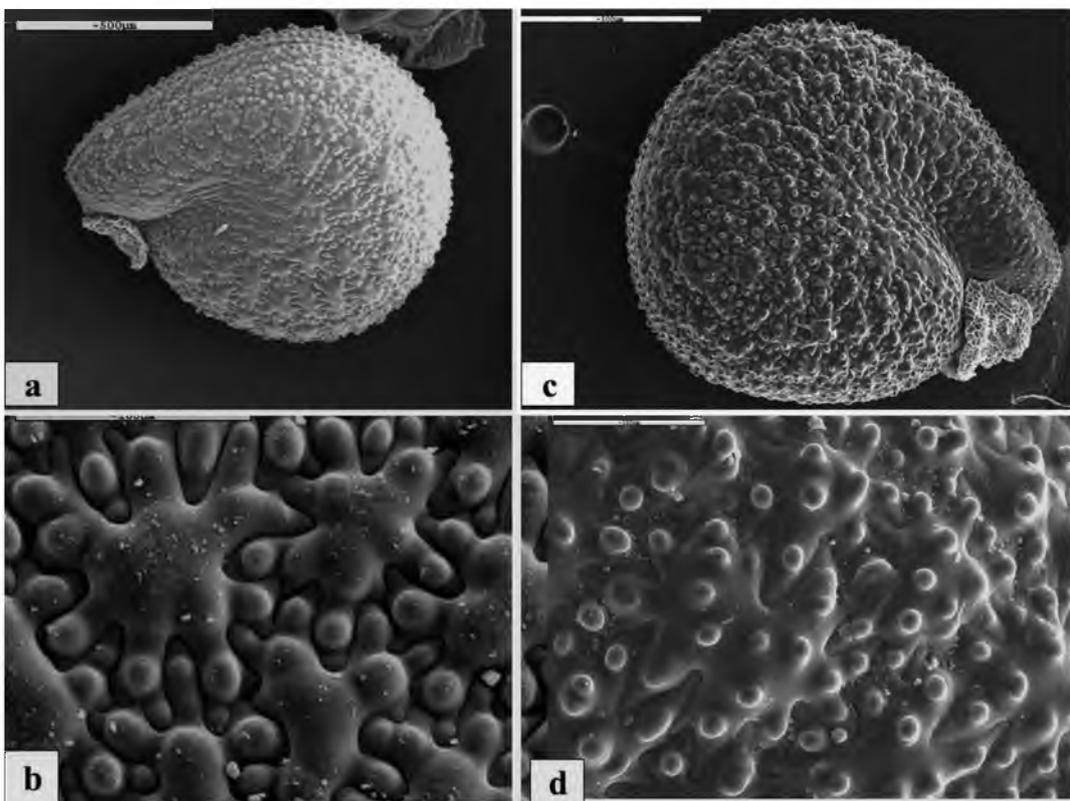


Fig. 4. Seed surface of recently described new microspecies: a. and b. *Portulaca sardoa*; c. and d. *Portulaca edulis*

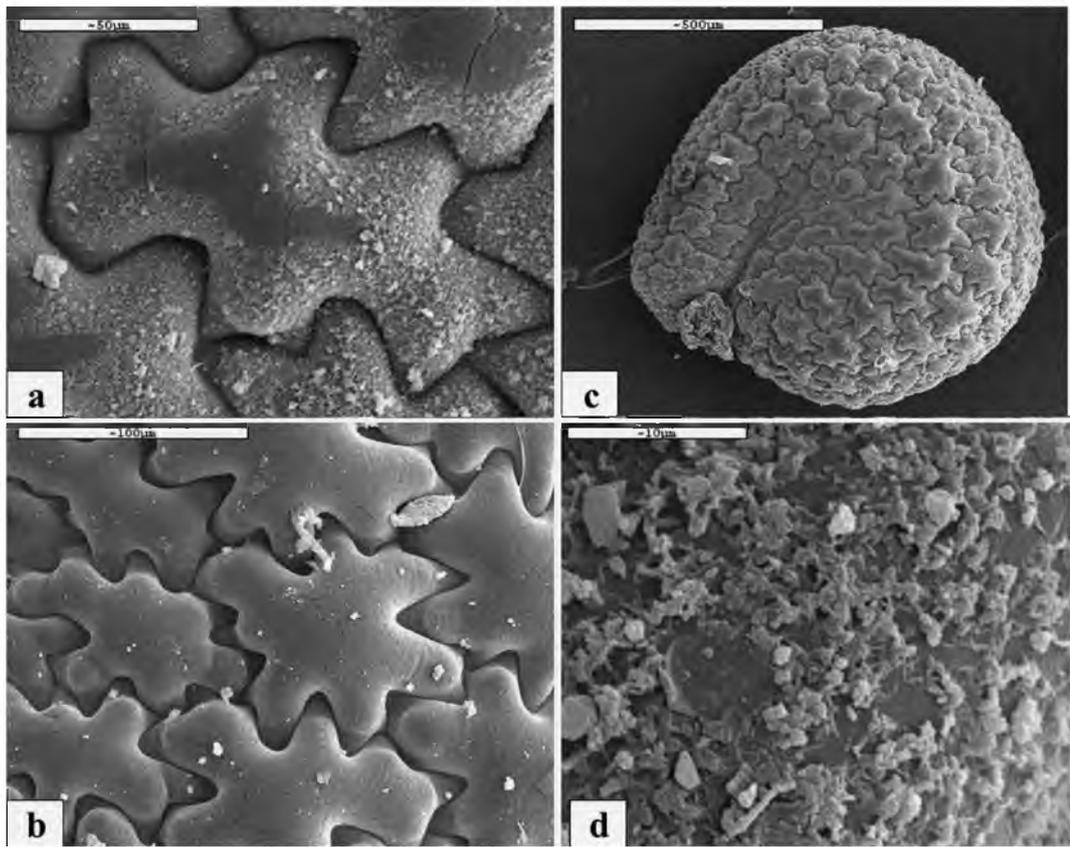


Fig. 5 *Portulaca nicaraguensis*: a. cells covered with wax, b. cells with no wax cover, c. a whole seed covered with wax, d. wax-covered seed surface

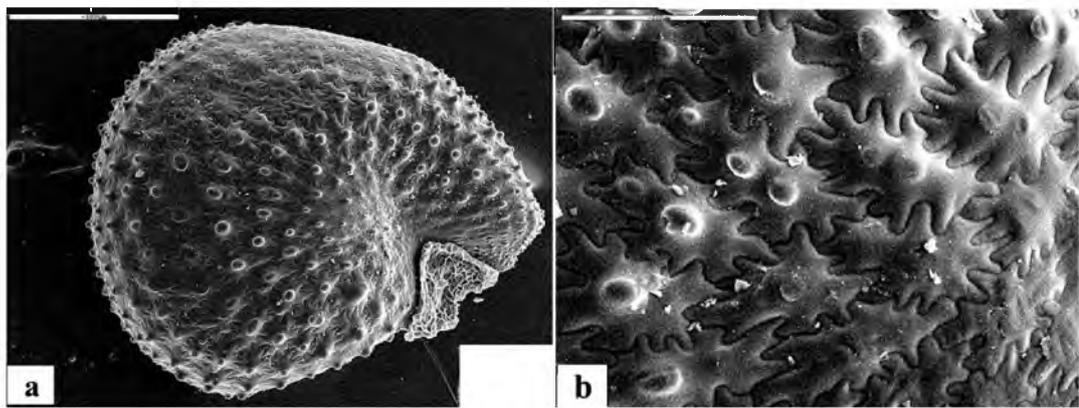


Fig. 6. *Portulaca sativa*

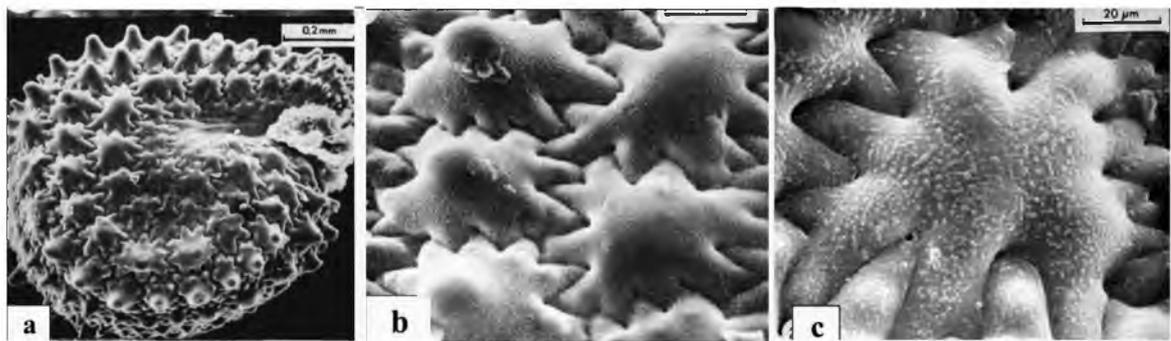


Fig. 7. *Portulaca impolita*

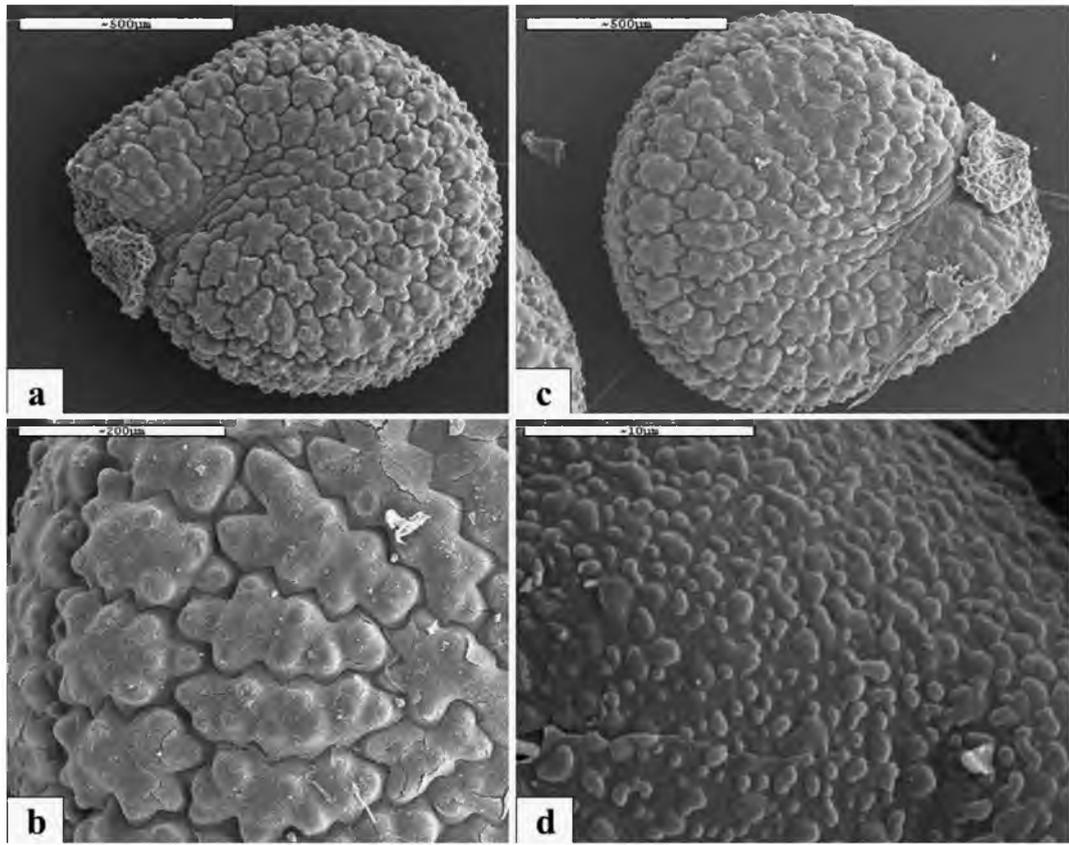


Fig. 8. *Portulaca canariense*

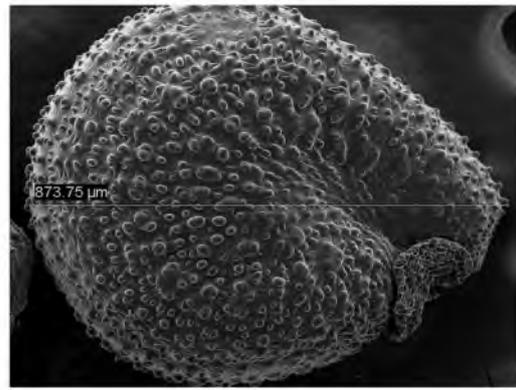


Fig. 9. *Portulaca rausii*

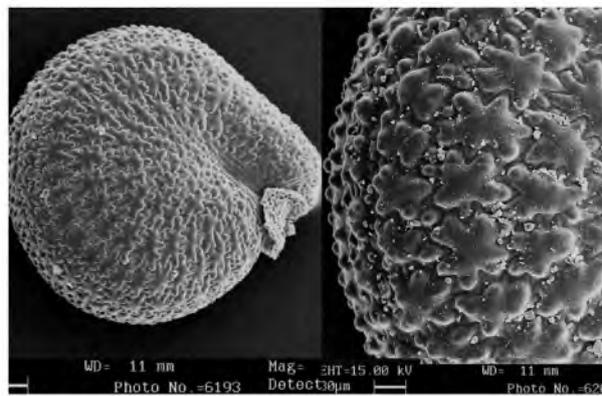


Fig. 10. *Portulaca papillatostellulata*

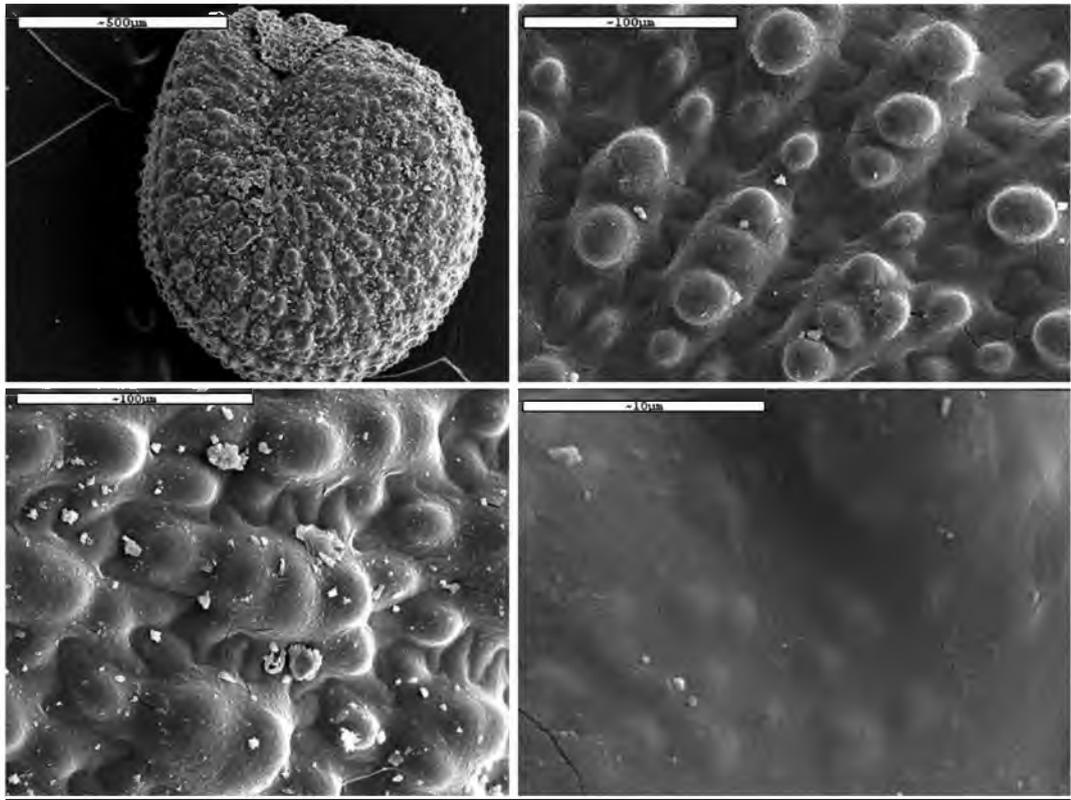


Fig. 11. *Portulaca trituberculata*

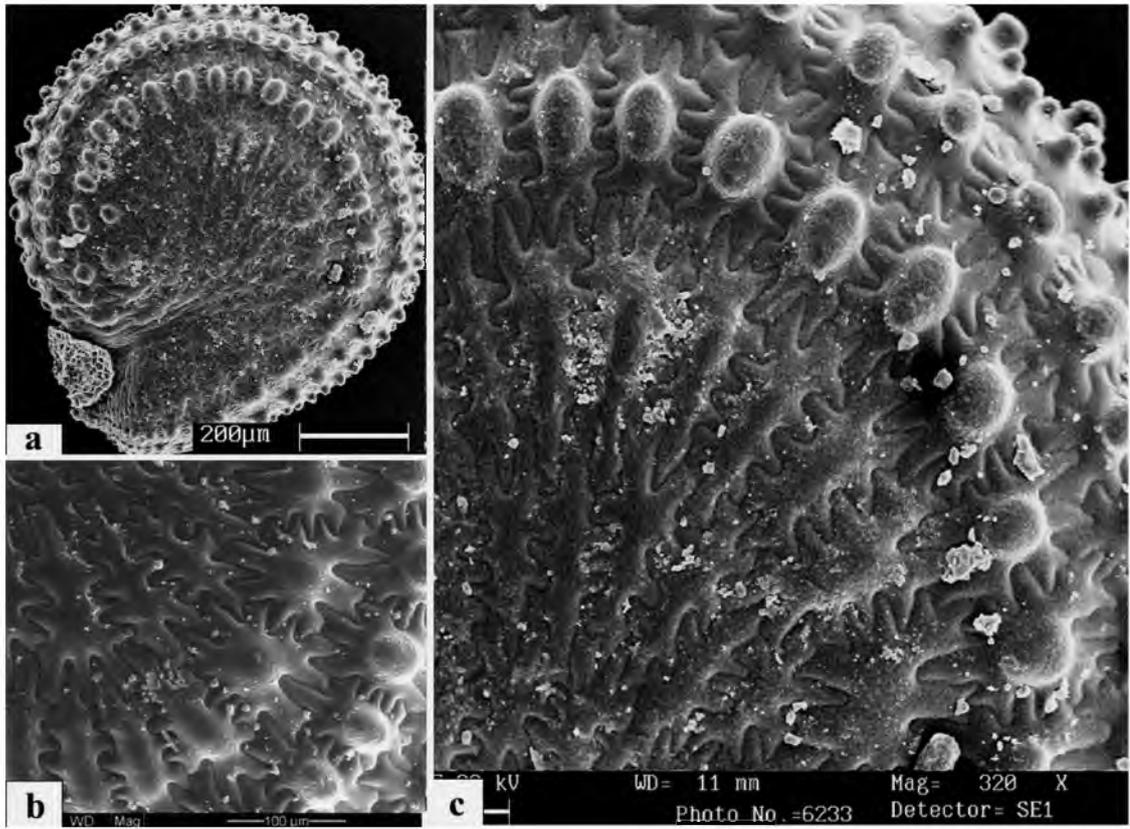


Fig. 12. *Portulaca sicula*

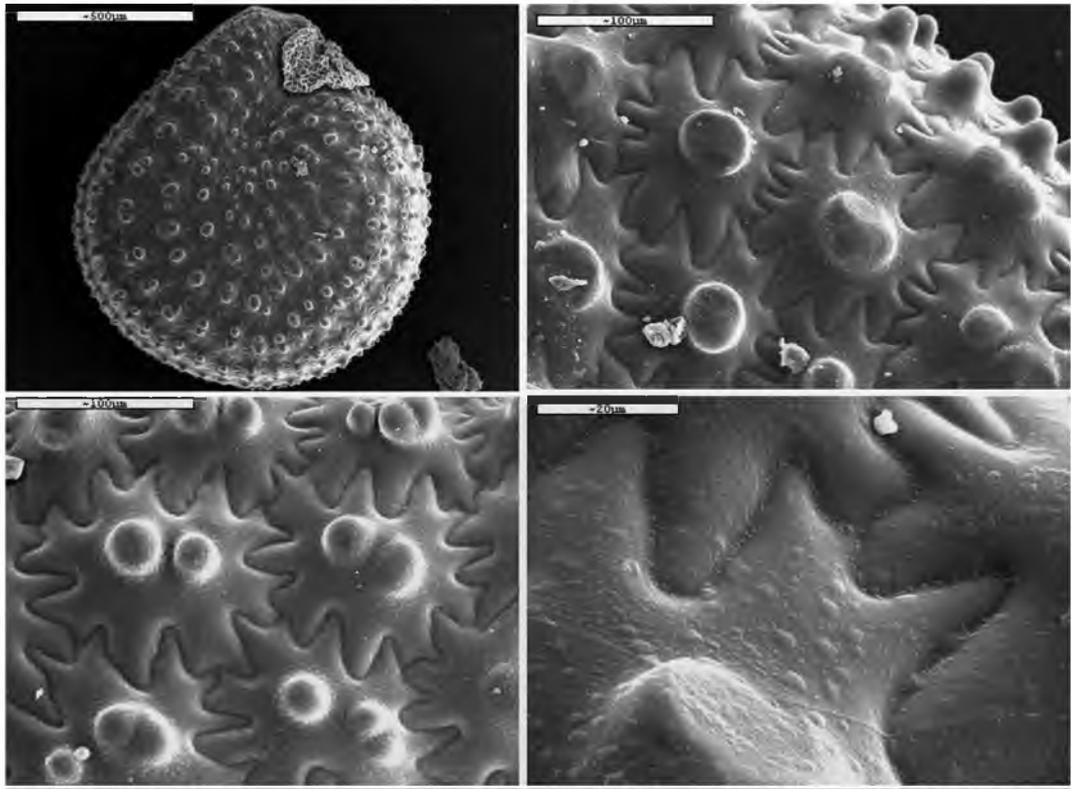


Fig. 13. *Portulaca cypria*

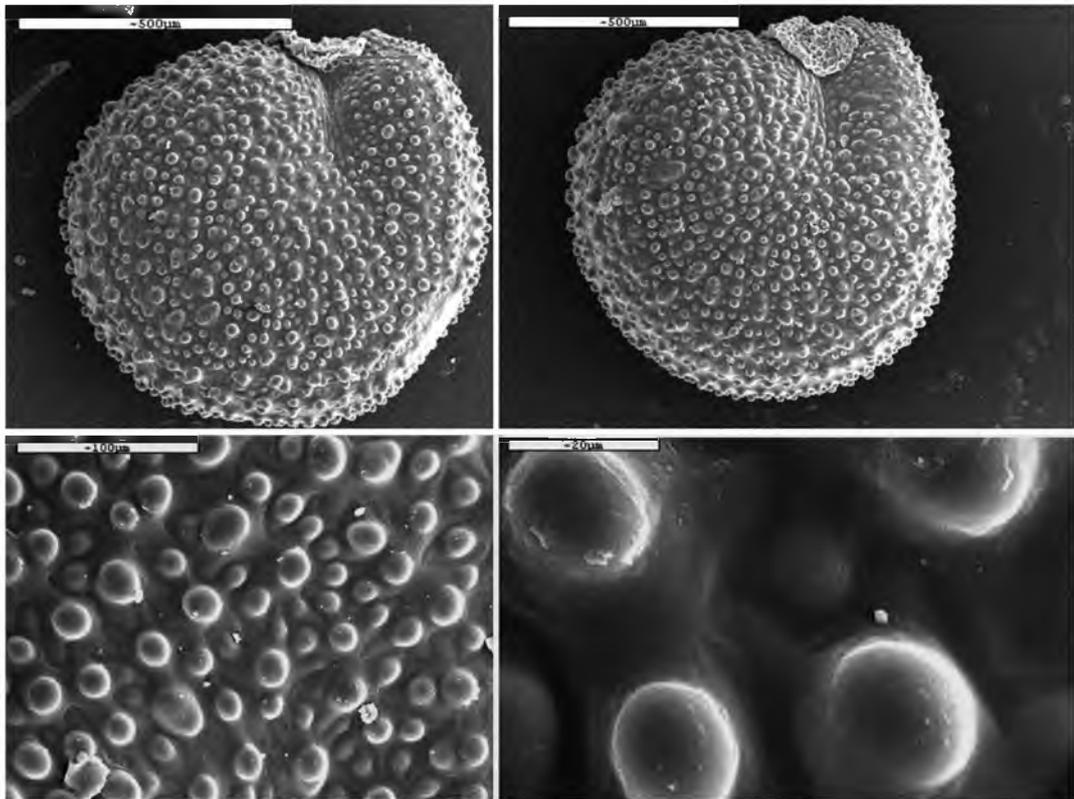


Fig. 14. *Portulaca zaffranii*

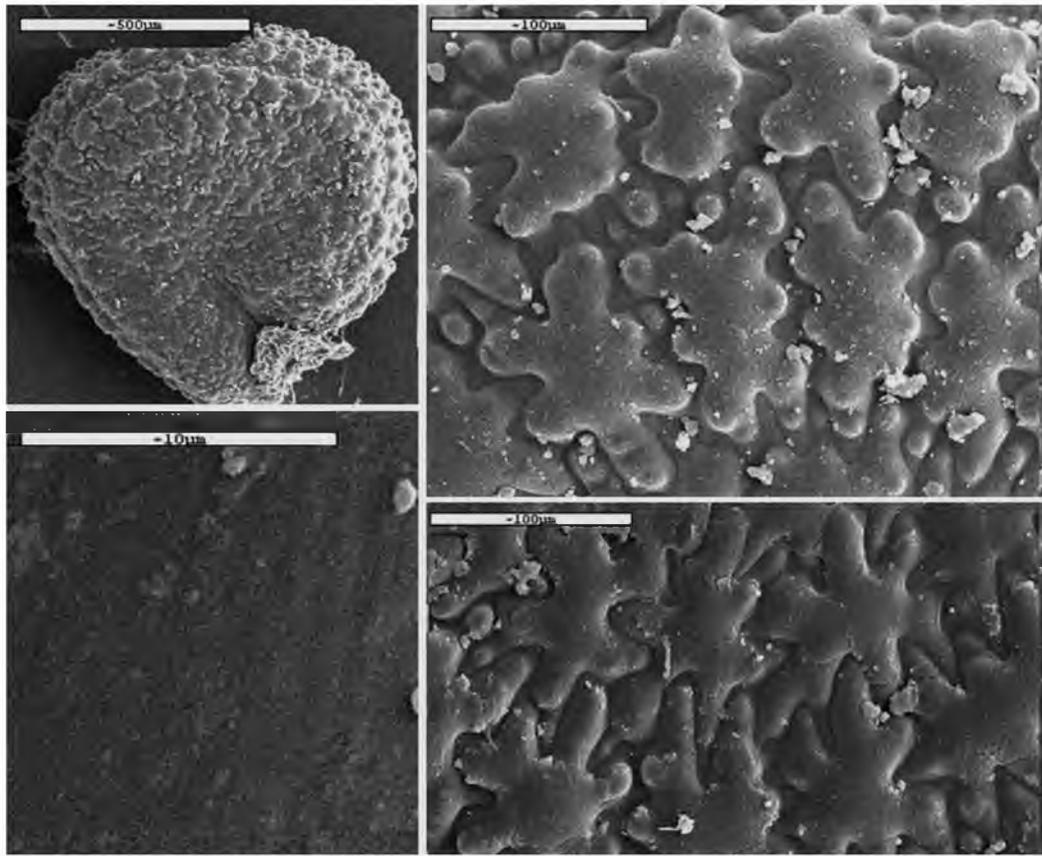


Fig. 15. *Portulaca granulostellulata*

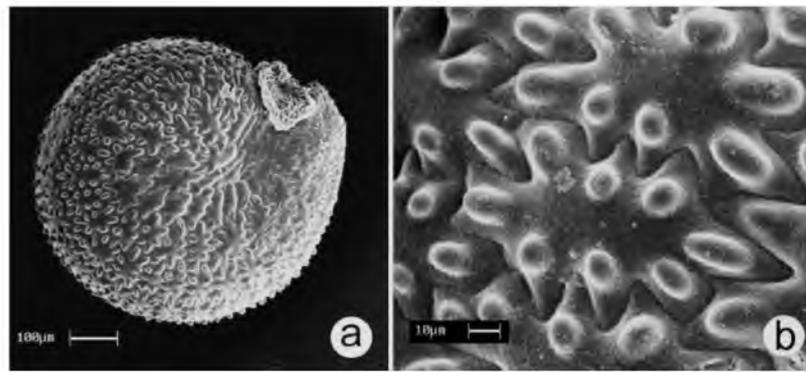


Fig. 16. *Portulaca socotrana*

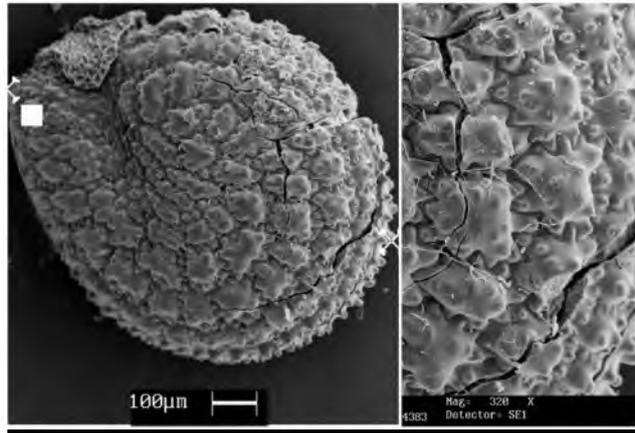


Fig. 17. *Portulaca macrantha*; type material from Agadir [Maire s.n., MPU001992] [©Herbarium of the University Montpellier II. Reproduced with permission]

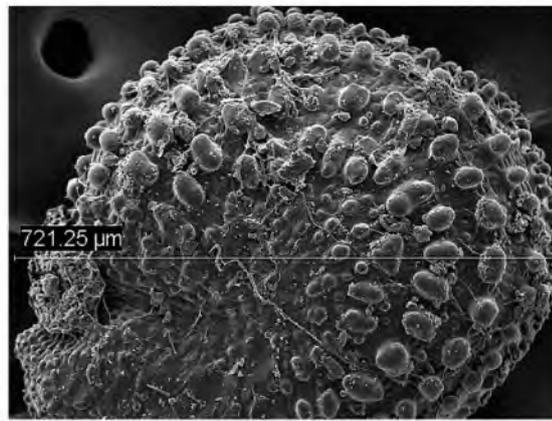


Fig. 18. *Portulaca tuberculata*

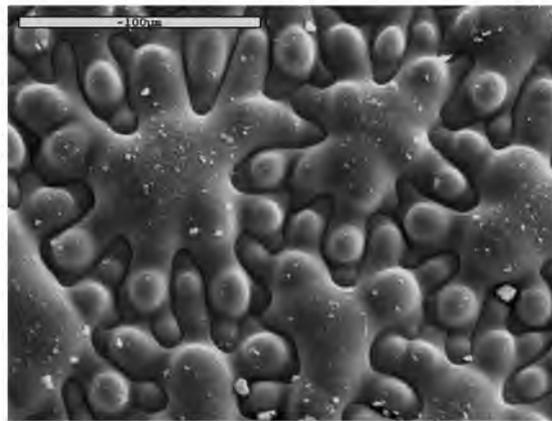


Fig. 19. *Portulaca sardoa*