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## Seed micromorphology and taxonomy in *Orobanche* (*Orobanchaceae*)

### Abstract

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Seed coat sculpturing in *Orobanche* sect. *Orobanche* and sect. *Trionychon* is discussed in relation to taxonomy. Four different groups are recognized, depending on outer seed wall ornamentation: interweaving fibrillar, smooth, pitted and papillate. The branched taxa of *O.* sect. *Trionychon* can be separated from members of *O.* sect. *Orobanche* by their distinct interweaving fibrillar sculpturing, although some non-branched variants have seed sculpturing similar to that found in *O.* sect. *Orobanche*. The presence of smooth and pitted sculpturing in *O.* sect. *Orobanche* suggests the recognition of two taxonomic groups.

### Introduction

Taxa of *Orobanche* L. occur as holoparasites on a wide range of vegetable crops and legume pulses. Many are superficially similar in morphology and general habit, such as the members of the *O. ramosa* group and some unbranched broomrapes such as *O. caesia*, *O. arenaria* and *O. purpurea*. Chater & Webb (1972), Felipo & Felipo (1958), Gilli (1982), Feinbrun-Dothan (1978), and Schiman-Czeika (1964) enumerate many taxa of *Orobanche* whose taxonomy is still controversial. Holub (1990) suggested the necessity of splitting the genus *Orobanche* into four distinct genera, viz.: *Aphyllon* Mitchell, *Myzorrhiza* Philippi, *Phelipanche* Pomel, and *Orobanche*, while Teryokhin (1991) placed *O. ramosa*, *O. aegyptiaca* and *O. mutellii* of *O.* sect. *Trionychon* within the genus *Phelipanche*. The use of seed surface characters, from gross morphology to subcellular level, is of great importance, particularly in *Orobanche* which possesses a very reduced vegetative growth and consequently provides few vegetative characters for taxonomic purposes. A literature search showed that seed morphology in *Orobanche*, particularly sect. *Trionychon*, has not been studied thoroughly in the past, although some members of the *Orobanchaceae* and related families have been examined (Tiagi & Sankhla 1963, Kuijt 1969, Granel de Solignac 1970, Musselman & Mann 1976, Joel 1987, Ungurean 1986, Teryokhin & Kravatsova 1987). Kuijt (1969) mentioned the presence of an inner and outer tangential testa wall on the seeds of *Orobanche*. According to Granel de Solignac (1970), it is possible to set up a classification of the *Orobanchaceae* using light microscopy, based on the ornamentation of the seed testa. Musselman & Mann (1976) observed seed reticulations in all the species examined of the families *Scrophulariaceae* and *Orobanchaceae*.

Table 1. Details of voucher specimens of *Orobanche* species studied.

<b><i>Orobanche</i></b>	<b>Locality &amp; Collector</b>	<b>Group</b>
<i>aegyptiaca</i> Pers.	Turkey, <i>Scott 113</i> (RNG), West Bank, <i>Musselman 10447</i> (RNG), Jordan, <i>Boulos &amp; El-Esawi 5353</i> (RNG).	A
<i>alba</i> Willd.	Spain, <i>Valdés 1880</i> (RNG).	C
<i>androssovii</i> Novopokr.	Iran, <i>Foroughi 8929</i> (RNG).	B
<i>arenaria</i> Borkh	Spain, <i>R. U. Bot. Dept. Exp. 823</i> (RNG), Turkey, <i>Bormuller 5416</i> (B).	B
<i>borissovae</i> Novopokr.	Iran, <i>Dini &amp; Arazm 14657</i> (RNG).	A
<i>bungeana</i> Beck	Turkestan, <i>Bornmüller 990</i> (B).	D
<i>caesia</i> Reichenb.	Spain, <i>Jury &amp; Ross 1020a</i> (RNG), USSR, <i>Gross s.n.</i> (BM).	B
<i>canesens</i> Presl.	Greece, <i>Bowen 1700</i> (RNG).	B
<i>caucasica</i> Beck	Turkey, <i>Balls 1523</i> (RNG).	A
<i>cernua</i> Loeffl.	West Bank, <i>Musselman 10267</i> (RNG).	C
<i>cilicica</i> Beck	Turkey, <i>Bornmüller 5417</i> (B).	A
<i>coelestis</i> (Reut.) Beck	Iran, <i>Bornmüller 7910, 7912</i> (B).	A
<i>crenata</i> Forsskål.	Spain, <i>Jury &amp; Ross 10202</i> (RNG).	C
<i>elatior</i> Sutton	U. K. <i>Wilson 790</i> (RNG).	B
<i>flava</i> Schultz.	U. K. <i>Bowen 6087</i> (RNG).	B
<i>gracilis</i> Sm.	Spain, <i>Jury &amp; al. 10282</i> (RNG).	B
<i>heldreichii</i> (Reut.) Beck	Turkey, <i>Bornmüller 10054</i> (B).	A
<i>lavandulacea</i> Reichenb.	Iran, <i>Lindsay 492</i> (BM), Italy, <i>Bornmüller 457</i> (B), Italy, <i>Davis &amp; Sutton 63347</i> (BM).	A
<i>longiflora</i> Pers.	Russia, <i>Bornmüller s.n.</i> (B).	A
<i>minor</i> Sm.	U. K. <i>Lousely s.n.</i> (RNG).	C
<i>mutelii</i> Schultz.	Tunisia, <i>Wilkin &amp; Wellens 221</i> (RNG), Canary Islands, <i>Bramwell 1538</i> (RNG), Spain, <i>R.U. M.Sc. Tax. Expd. 351</i> (RNG).	A
<i>nana</i> Noë.	Morocco, <i>Drennan s.n.</i> (RNG), Iran, <i>Gheisseri 2424</i> (RNG).	A
<i>orientalis</i> Beck	Armenia, <i>Czerepanov 4614</i> (B).	A
<i>oxyloba</i> (Reut.) Beck	Turkey, <i>Scott 156</i> (RNG), Iran, <i>Lindsay 800</i> (RNG), Iran, <i>Gabriel 80</i> (RNG).	A
<i>pubescens</i> D'Urv	Turkey, <i>Scott 117</i> (RNG).	B
<i>purpurea</i> Jacq.	Spain, <i>Carr 3350</i> (RNG), Gibraltar, <i>Heywood &amp; al. 20</i> (RNG), Austria, <i>Rumsey s.n.</i> (RNG).	A
<i>ramosa</i> L.	Canary Islands, <i>Aldridge 1399</i> (RNG), Morocco, <i>Jury &amp; al. 8893</i> (RNG), West Bank, <i>Musselman 10178, 10369, 10442</i> (RNG).	A
<i>rosmarina</i> Beck	Croatia, <i>Baschant 1766</i> (B).	A
<i>rubra</i> Sm.	U. K. <i>Alstee s.n.</i> (RNG).	B
<i>schultzii</i> Mutel.	West Bank, <i>Musselman 10415, 10348, 10464</i> (RNG).	A
<i>schwingenschussii</i> Gilli	Iran, <i>Makouii 10033</i> (RNG).	B
<i>teucris</i> Holandre	France, <i>Bowen 4722</i> (RNG).	B

<i>Orobanche</i>	Locality & Collector	Group
<i>trichocalyx</i> (Webb & Berth.) Beck	Spain, Cannon & al. 504 (RNG), R. U. Bot. Dept. Exp. 504 (RNG).	B
<i>tricholoba</i> Beck	Kurdistania, Bornmüller 1641 (B).	A

Table 2. Seed surface characters of *Orobanche* species studied.

Abbreviations: Sd L = Seed Length, Sd W = Seed Width, Alv L = Alveolae Length, Alv W = Alveolae Width, PD = Perforation Diameter, Wall orn. = outer wall ornamentation, int. fib. = interweaving fibrillar, pap. str. = papillate striate.

Note : All measurements are in  $\mu\text{m}$ .

<i>Orobanche</i>	Sd L	Sd W	Seed Shape	Alv L	Alv W	PD	Wall orn.
<i>aegyptiaca</i>	300	210	broadly ovate	95	45	4.0	int.fib.
<i>alba</i>	360	250	ovate	65	45	3.5	pitted
<i>androssovii</i>	230	170	ovate	55	35	2.5	smooth
<i>arenaria</i>	240	200	pear shaped to ovate	55	30	3.0	$\pm$ smooth
<i>borissovae</i>	380	290	ovate	70	45	3.5	int.fib.
<i>bungeana</i>	250	170	ovate	45	35	2.5	pap.str.
<i>caesia</i>	240	165	narrowly ovate	85	35	3.5	smooth
<i>canesens</i>	230	120	elongate elliptical	60	35	2.5	smooth
<i>caucasica</i>	290	240	ovate	65	45	3.5	int.fib.
<i>cernua</i>	350	220	$\pm$ ovate	55	35	4.0	pitted
<i>cilicica</i>	360	265	ovate	65	45	3.0	int.fib.
<i>coelestis</i>	360	280	ovate	80	45	6.0	int.fib.
<i>crenata</i>	320	200	$\pm$ ovate	75	55	3.5	pitted
<i>elatior</i>	330	250	pear shaped to ovate	65	50	3.5	smooth
<i>flava</i>	340	200	narrowly ovate	65	45	3.5	smooth
<i>gracilis</i>	340	220	narrowly ovate	80	45	4.0	smooth
<i>heldreichii</i>	340	200	pear shaped to ovate	95	60	4.0	int.fib.
<i>lavandulacea</i>	320	305	roundish ovate	70	45	3.5	int.fib.
<i>longiflora</i>	310	260	ovate	70	45	3.0	int.fib.
<i>minor</i>	290	180	ovate	65	50	3.0	pitted
<i>mutelii</i>	275	180	narrowly ovate	70	35	4.0	int.fib.
<i>nana</i>	300	230	pear shaped to ovate	85	35	5.0	int. fib.
<i>orientalis</i>	355	280	ovate	85	65	4.0	int.fib.
<i>oxyloba</i>	370	290	$\pm$ ovate	80	50	4.0	int.fib.
<i>pubescens</i>	360	290	ovate	60	45	4.0	smooth
<i>purpurea</i>	250	190	$\pm$ ovate	70	40	3.5	int.fib.
<i>ramosa</i>	270	190	pear shaped to ovate	60	45	4.0	int. fib.
<i>rosmarina</i>	280	185	$\pm$ ovate	55	50	3.0	int.fib.
<i>rubra</i>	240	170	ovate	55	50	3.5	smooth
<i>schultzei</i>	290	220	ovate	85	45	3.5	int.fib.
<i>schwingeri</i>	230	120	narrowly ovate	40	25	3.0	smooth
<i>schussii</i>							
<i>teucrici</i>	370	260	pear shaped to ovate	75	60	4.0	smooth
<i>trichocalyx</i>	330	290	roundish ovate	65	60	3.5	smooth
<i>tricholoba</i>	380	250	pear shaped to ovate	60	45	3.0	int.fib.

These reticulations are of a differential taxonomic value, especially when a secondary reticulum is involved (Ungurean 1986). Unfortunately, no-one has, as yet, undertaken any comprehensive studies.

The study described below was initiated with a view to clarify the taxonomic status of *Orobanche* sect. *Trionychon* within the genus and to find out whether seed micromorphology could be a useful taxonomic marker for subgeneric grouping. The seed sculpturing of 22 species of *O.* sect. *Trionychon* and 12 of *O.* sect. *Orobanche*, was examined by scanning electron microscopy.

## Material and methods

This study is based on dry herbarium specimens, loaned from the Natural History Museum, London (BM), Reading University (RNG), Botanisches Museum Berlin-Dahlem (B), and the Research Institute of Forests and Rangelands, Iran (TARI). One to five seed samples taken from different herbarium specimens (Table 1) were prepared for each taxon. The seeds were fixed onto metal stubs, coated with 30 nm of gold in a Polaron sputter coater, and then examined and photographed using a JEOL JSM-T20 scanning electron microscope.

## Results and discussion

The present study concentrated mainly on the outer seed wall ornamentation. Four different patterns were recognized. Group A, in which the outer seed wall shows an interweaving fibrillar ornamentation, was common in the branched broomrapes, including: *Orobanche ramosa*, *O. aegyptiaca*, *O. mutelii*, *O. schultzei*, *O. oxyloba*, *O. borissovae* and *O. orientalis*. Some members of *O.* sect. *Trionychon* which have more or less simple stems appeared to have the same sort of fibrillar pattern (*O. nana*, *O. purpurea*, *O. caucasica*, *O. heldreichii*, *O. tricholoba*, *O. cilicica*, *O. rosmarina* and *O. coelestis*) (Fig. 1e). *O. longiflora* appeared to have star-shaped fibrils arising up from the outer tangential wall (Figs. 1g). *O. lavandulacea* exhibits a more or less fibrillar ornamentation overlapped with a granular type of sculpturing (Fig. 1f). These two patterns of sculpturing were included under group A.

Group B includes some non-branched members of *Orobanche* sect. *Trionychon* (*O. caesia*, *O. trichocalyx*, *O. androssovii* and *O. schwingenschussii*) and some members of *O.* sect. *Orobanche* (*O. pubescens*, *O. gracilis*, *O. elatior*, *O. rubra*, *O. canesens*, *O. teucarii*, and *O. flava*). This group appeared to have seeds with a smooth surface (Fig. 1j). *O. arenaria* has a smooth surface, but with some tiny cracking scattered over the alveolae (Fig. 1m). This taxon is best placed within group B.

Group C includes *Orobanche alba*, *O. minor*, *O. crenata* and *O. cernua*, of *O.* sect. *Orobanche*. This group showed a pitted form of ornamentation on the seed surface (Fig. 2b). None of the members of *O.* sect. *Trionychon* has this feature.

Group D includes only one taxon *Orobanche bungeana* of *O.* sect. *Trionychon*. This taxon has been placed under *O. arenaria* by Chater & Webb (1972), although Felipo & Felipo (1958), Schiman-Czeika (1964), and Gilli (1982) recognize it as a separate taxon. In our study, *O. bungeana* was distinctive in having a papillate striate pattern distributed on the surface of all the alveolae (Fig. 2c, d). According to seed micromorphology, *O. bungeana* should perhaps be set apart as a separate group.

Seed shape in *Orobanche* (Table 2) was found to be a variable character, in the majority of cases even amongst individuals of the same species. The seeds were mostly ovate, narrowly ovate, pear-shaped, elliptic-elongate, or more or less roundish, with the micropylar region generally narrow, forming a neck, and the chalazal region broad and

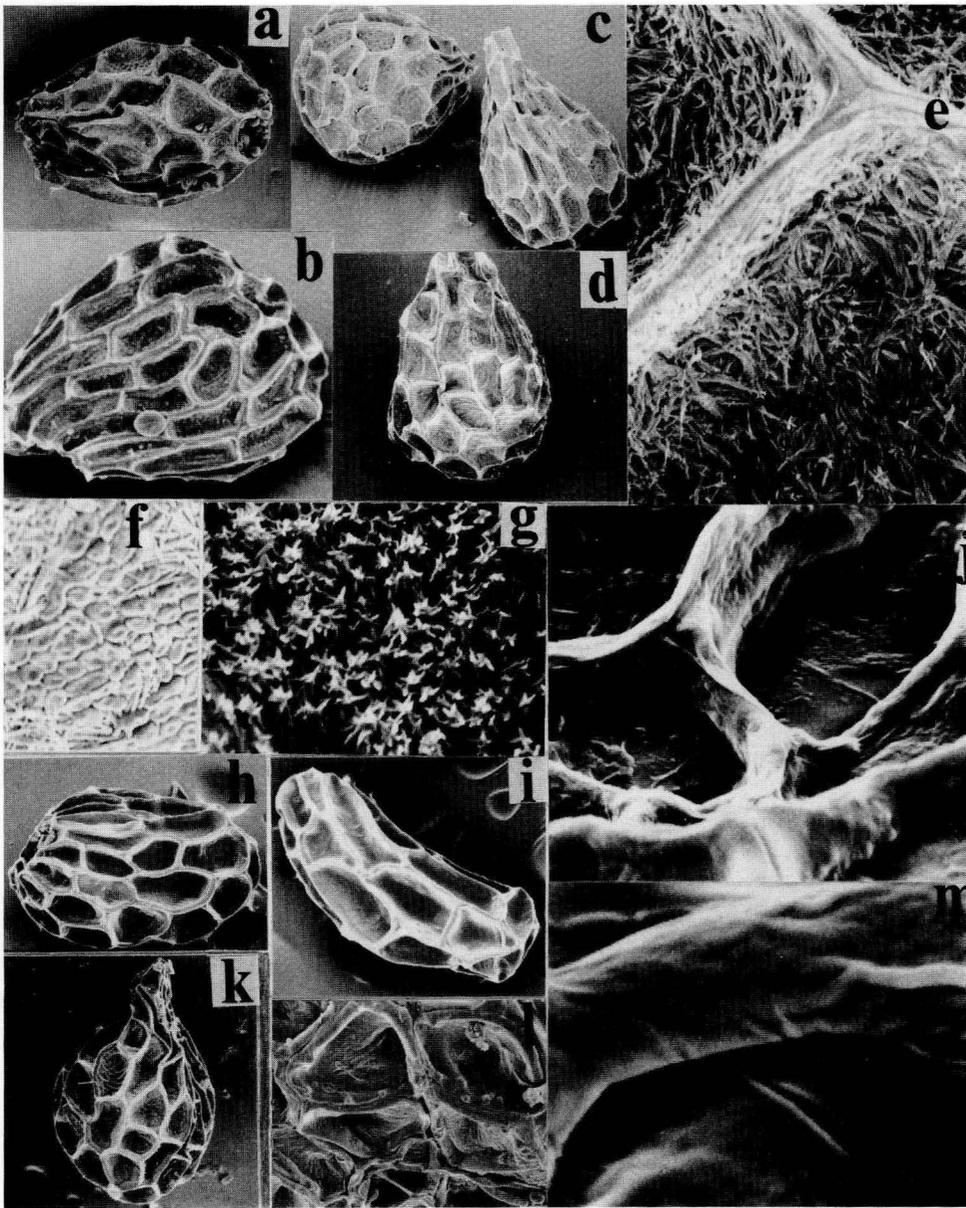


Fig. 1. Scanning electron micrographs of *Orobanche* seeds.- **a-d**, *O. ramosa*, with different seed shapes; **a**, roundish ovate; **b** broadly ovate; **c**, pear shape; **d**, narrowly ovate; **e**, *O. ramosa*, with an interweaving fibrillar sculpturing; **f**, *O. lavandulacea*, with fibrillar ornamentation overlapped with a granulate sculpturing; **g**, *O. longiflora*, showing a star-shaped fibrils arising from the outer tangential testa wall; **h**, *O. pubescens*, roundish ovate; **i**, *O. canesens*, elongate-elliptical; **j**, *O. canesens*, An outer tangential testa wall with a smooth sculpturing; **k**, *O. arenaria*, ovate seed shape **l**, *O. arenaria*, seed with a smooth surface; **m**, *O. arenaria*, with some tiny cracking scattered over the alveolae. Scale bar = 75  $\mu\text{m}$  (a-d, h-i, k), 5  $\mu\text{m}$  (e-g, j), 10  $\mu\text{m}$  (l), 2  $\mu\text{m}$  (m).

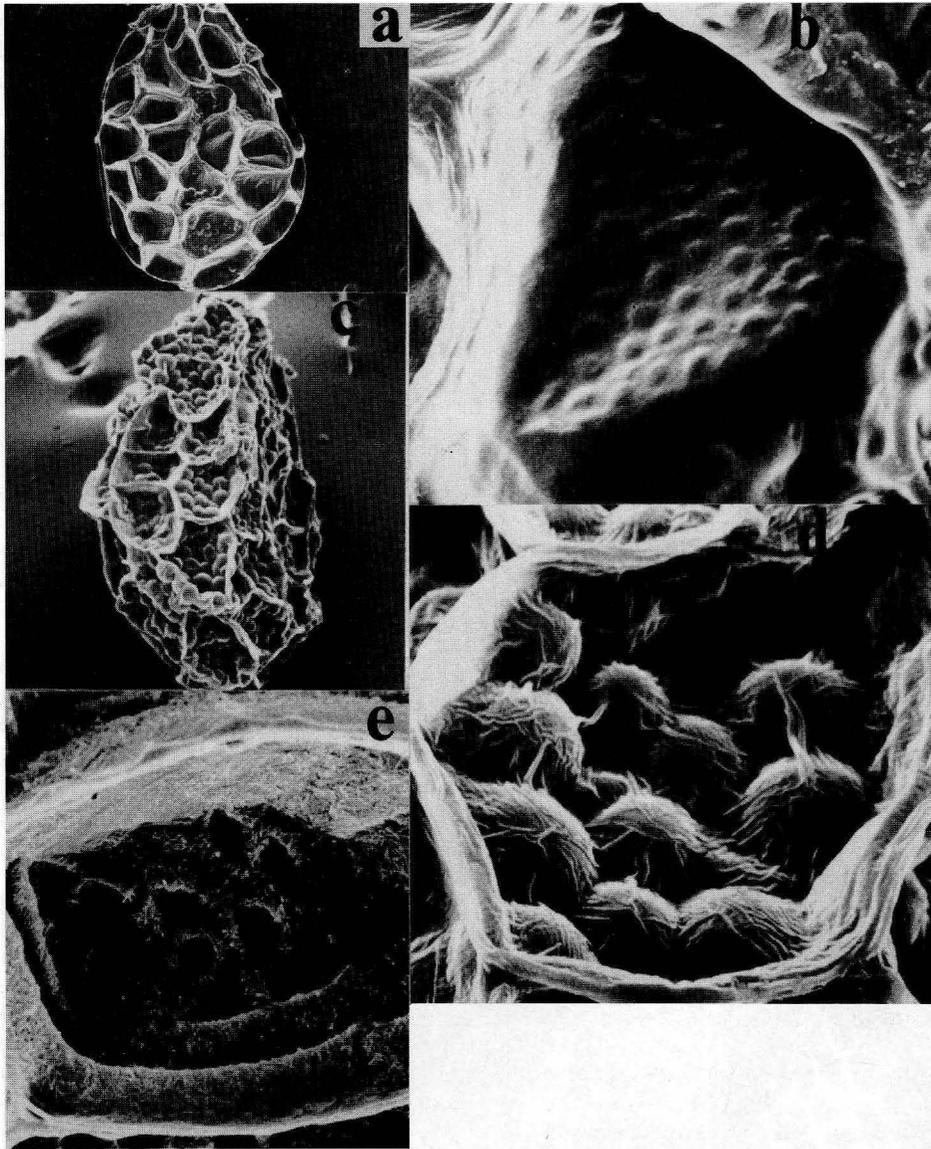


Fig. 2. Scanning electron micrographs of *Orobanche* seeds.- a, *O. alba*, ovate seed shape; b, *O. alba*, seed surface sculpturing with a pitted ornamentation; c, *O. bungeana*, ovate seed shape; d, *O. bungeana*, Papillate-striate surface sculpturing; e, *O. ramosa* showing inner wall perforations and outer tangential testa wall. Scale bar = 70  $\mu\text{m}$  (a, c), 8  $\mu\text{m}$  (b), 7  $\mu\text{m}$  (d), 5  $\mu\text{m}$  (e).

widened towards the funiculus. The length and width of the seeds, and the length and width of the alveolae, had measured ranges of 230-380 $\mu\text{m}$ , 120-290 $\mu\text{m}$ , 30-95 $\mu\text{m}$  and 25-65 $\mu\text{m}$ , respectively. These dimensions, due to their variability, did not show any taxonomic

significance, although some members of *O. sect. Trionychon* seem to have their alveolae larger than many members of *O. sect. Orobanche*. In all the species, the most frequent shape of the alveolae was more or less pentagonal, although some trigonal, tetragonal, and hexagonal alveolae were often present on the same seed. The longitudinal axis of the alveolae was always parallel to the longitudinal axis of the seed.

The inner tangential testa wall is reticulate-perforate in all species examined. Normally, it is attached to, or hidden below, the outer tangential testa wall, unless the latter is collapsed. The average diameter of each reticulum had a measured range of 2.5-6.0  $\mu\text{m}$ .

## Conclusions

The present study supports the taxonomic recognition and separation of members of *Orobanche sect. Trionychon* from *sect. Orobanche*. The occurrence of interweaving fibrillar sculpturing in the branched taxa of *O. sect. Trionychon* and smooth and pitted sculpturing in *O. sect. Orobanche* correlates with the two sections. Some non-branched members of *O. sect. Trionychon*, including *O. caesia*, *O. arenaria*, *O. schwingenschussii*, *O. trichocalyx*, and *O. androssovii*, are similar to *O. sect. Orobanche* in their seed morphology. Other non-branched members of *O. sect. Trionychon*, including *O. purpurea*, *O. caucasica*, *O. heldreichii*, *O. nana*, *O. tricholoba*, *O. cilicica*, *O. lavandulacea*, *O. coelestis*, *O. longiflora*, and *O. rosmarina*, appeared to have seeds with an interweaving fibrillar sculpturing pattern similar to that of the *O. ramosa* group. The presence of some taxa with a smooth surface and others with a pitted surface in *O. sect. Orobanche*, suggests the consideration of two taxonomic groups. *O. bungeana* is the only taxon in this study with a papillate-striate surface and, therefore, its recognition as a separate species should be accepted.

The shape and size of the seeds were very variable characters within all species, and appeared to be of no use for taxonomic grouping.

Earlier studies by us on the pollen (unpublished) have revealed a trend from a 'less advanced' tricolpate pollen (*O. sect. Trionychon*) to the 'more advanced' inaperturate one (*O. sect. Orobanche*). The different seed surface patterns cannot be qualified as "primitive" or "advanced" and, therefore, offer no clue as to the evolutionary history of the sections. In both palynological and seed micromorphological studies, the non-branched members of *O. sect. Trionychon* occupy a more or less intermediate position.

## Taxonomic groupings using seeds

**Group A.**- This group includes 17 species (*O. ramosa*, *O. aegyptiaca*, *O. mutellii*, *O. schultzei*, *O. oxyloba*, *O. orientalis*, *O. nana*, *O. purpurea*, *O. caucasica*, *O. heldreichii*, *O. tricholoba*, *O. cilicica*, *O. borissovae*, *O. longiflora*, *O. lavandulacea*, *O. rosmarina* and *O. coelestis*), described as follows: seeds mainly ovate, though sometimes broadly ovate, narrowly ovate, pear-shaped, or  $\pm$  roundish, 250-380 x 180-305  $\mu\text{m}$ ; alveolae variable, mostly pentagonal, 55-95 x 30-60  $\mu\text{m}$ , longitudinal axis parallel to the longitudinal axis of the seed; inner tangential wall reticulate-perforate, perforations 3-6  $\mu\text{m}$  in diameter; outer tangential testa wall with interweaving fibrils (Fig. 1a-g).

**Group B.**- This group includes 12 species (*O. flava*, *O. pubescens*, *O. elatior*, *O. rubra*, *O. schwingenschussii*, *O. teucris*, *O. canescens*, *O. caesia*, *O. trichocalyx*, *O. arenaria*, *O. androssovii* and *O. gracilis*), described as follows: seeds mainly roundish, though sometimes ovate, narrowly ovate, elongate-elliptical, 230-370 x 120-290  $\mu\text{m}$ ; alveolae variable, 40-85 x 25-60  $\mu\text{m}$ , more or less pentagonal (except in *O. canescens* which has elongate-tetragonal alveolae), longitudinal axis parallel to the longitudinal axis of the seed;

inner tangential wall reticulate-perforate, perforations  $\pm$  roundish, 2.5-4.0  $\mu\text{m}$  in diameter; outer tangential wall smooth (Fig. 1h-m).

**Group C.**- This group includes four species (*O. alba*, *O. minor*, *O. crenata* and *O. cernua*), described as follows: seeds  $\pm$  ovate, 290-360 x 180-250  $\mu\text{m}$ ; alveolae  $\pm$  pentagonal, 55-75 x 35-55  $\mu\text{m}$ , their longitudinal axis parallel to the longitudinal axis of the seed; inner tangential wall reticulate-perforate, perforations 3.0-4.0  $\mu\text{m}$  in diameter; outer tangential wall pitted and usually clinging to the inner tangential wall (Figs 2a, b).

**Group D.**- This group includes only one species (*O. bungeana*), with seeds ovate 230-270 x 130-200  $\mu\text{m}$ ; alveolae  $\pm$  pentagonal, 30-50 x 25-45  $\mu\text{m}$ , their longitudinal axis parallel to the longitudinal axis of the seed; inner tangential wall reticulate-perforate, perforations  $\pm$  2.5  $\mu\text{m}$ ; outer tangential wall papillate striate (Figs 2c, d).

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