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Seed micromorphology and its variation in Sicilian *Orobanche* (*Magnoliopsida*)

Abstract

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The seed micromorphology of all species and subspecies of *Orobanche* occurring in Sicily has been studied. A synthesis of the results is presented, and comments are made on its taxonomic relevance. In addition, the influence of different hosts on seed size and sculpture has been considered in four species, and intra-individual variation of seed dimensions in four other species has been assessed. Scanning electron micrographs illustrate the seed morphology of 9 species for which no published documents of this kind have been found.

Introduction

Species distinction in *Orobanche* L. is notoriously difficult because of the lack of clear cut morphological differences. Moreover, some features that are evident in live material are difficult to observe on dried specimens, e.g. the colour of corolla and stigma as well the hairiness at the base of stamen filaments. Musselman & Mann (1976), Granel de Solignac (1970), Plaza & al. (2004) and Joel (1970a), among others, have stressed the great importance that the sculpturing of the seed testa can have for *Orobanche* taxonomy. In that genus, where fruiting material is all but impossible to identify based on gross morphology, seed micromorphology can be the only reliable means to distinguish related taxa. Abu Sbaih & Jury (1994) and Ungurean (1986) made valuable contributions to the knowledge of the seed surface of European and Mediterranean taxa of *O*. sect. *Trionychon*, which they compared with some representatives of the *O*. sect. *Orobanche*.

We have ourselves studied the morphology of the 21 species and 3 subspecies of *Orobanche* occurring in Sicily. In addition, we present a statistical evaluation variability of intra-individual seed size of variation in four Sicilian species (*O. lavadulacea, O. litorea, O. chironii* and *O. variegata*). Furthermore, taking into account the fact that the host may influence the general morphology of *Orobanche* species, as shown by the large number of host-related varieties and forms recognised by previous authors (e.g., Beck, 1930), we have made a preliminary investigation, in four species, of possible correlations between the host plant and features of the seed.

Table 1. Voucher specimens of taxa studied.

Taxon	**Locality, host, date and collector**
Sect. *Trionychon*	
Orobanche ramosa L. subsp. *ramosa*	Siracusa c.da Milocca, on *Lycopersicon esculentum*, 18.4.2004, Domina (PAL)
O. ramosa subsp. *nana* (Reut.) Cout.	Palermo Orto Botanico, on *Oxalis pes-caprae*, 25.3.2004, Domina (PAL)
O. ramosa subsp. *mutelii* (F. W. Schultz) Cout.	Isnello c.da Mericola (Palermo), on *Sonchus tenerrimus*, 8.4.2004, Domina (PAL)
O. lavandulacea Rchb.	Portella Paglia (Palermo), on *Bituminaria bituminosa*, 9.6.2004, Domina (PAL)
O. schultzii Mutel	Marettimo, 4.1981, Catanzaro (PAL)
O. oxyloba (Reut.) G. Beck in L. Koch	Rocca Busambra (Palermo), on *Arabis alpina*, 20.06.2004, Domina (PAL)
Sect. *Orobanche*	
O. alba Steph. ex Willd.	Nicolosi (Catania), on *Calamintha nepeta*, 27.6.2004, Domina (PAL)
O. caryophyllacea Sm.	Poggio San Francesco (Palermo), on *Galium lucidum*, 9.6.2004, Domina (PAL)
O. crenata Forssk.	Monte Pellegrino (Palermo), on *Vicia villosa* subsp. *varia*, 24.4.2004, Domina (PAL); Campofelice di Roccella (Palermo), on *Vicia faba*, 23.3.2004, G. Domina (PAL)
O. cernua L. in Loefl.	Salina (Messina), on *Asteraceae*, 11.2002, Domina (PAL)
O. chironii Lojac.	Rocca Busambra (Palermo), on *Anthemis cupaniana*, 16.6.2003, Domina (PAL)
O. thapsoides Lojac.	Gela (Caltanissetta), 4.1869, Citarda (PAL 43238)
O. litorea Guss.	Balestrate (Palermo), on *Anthemis maritima*, 25.4.2004, Domina (PAL)
O. minor Sm.	Rocca di Mele (Palermo), on *Trifolium subterraneum*, 30.6.2002, Domina (PAL)
O. hederae Duby	Bosco di Ficuzza, on *Hedera helix*, 16.6.2003, Domina (PAL)
O. amethystea Thuill.	Noto (Siracusa), on *Daucus carota*, 1.5.2004, Domina (PAL); Portella Paglia (Palermo), on *Eryngium campestre*, 9.6.2004, Domina (PAL); pressi fiume San Leonardo (Catania) on *Scolymus grandiflorus*, 18.4.2004, Domina (PAL)
O. picridis F. W. Schultz	Portella delle Ginestre (Palermo), on *Picris hieracioides*, 20.6.2004, Domina (PAL)
O. canescens C. Presl	Rinella (Messina), on *Carlina* sp., 1.6.2004, Domina (PAL); San Martino delle Scale (Palermo) on *Carlina sicula*, 26.4.2004, Domina (PAL)
O. pubescens d'Urv.	Tusa (Messina) on *Crepis leontodotoides,* 31.3.2004, Domina (PAL)
O. sanguinea C. Presl	Isnello c.da Mericola (Palermo), on *Lotus cytisoides,* 5.6.2003, Domina (PAL); Campofelice di Roccella (Palermo), on *Lotus cytisoides*, 18.05.2003, Domina (PAL)
O. gracilis Sm.	Tortorici (Messina) on *Spartium junceum,* 14.5.2004, Domina (PAL)
O. variegata Wallr.	Pollina (Palermo) on *Calycotome infesta*, 3.4.2003, Domina (PAL); Giacalone (Palermo) on *Spartium junceum*, 18.4.2004, Domina (PAL)
O. rapum-genistae Thuill.	Nicolosi (Catania) on *Genista aetnensis*, 18.6.2004, Domina (PAL); Zafferana etnea (Catania) on *Spartium juceum*, 16.6.2003, Domina (PAL)
O. rapum-genistae subsp. *benthamii* (Timb.-Lagr.) P. Fourn.	Acquedolci (Messina) on *Spartium junceum*, 12.4.2003, Domina (PAL)

Material and methods

Seeds were either taken from herbarium specimens housed at the *Herbarium Mediterraneum Panormitanum* (PAL) or from individuals collected in field. To evaluate intra-individual variation, thee seeds were sampled from fruiting, dead plants identified with, and vouchered by, living plants growing nearby in the same population.

In order to characterise our taxa we have adhered to the same criteria as Abu Sbaih & Jury (1994): we prepared one to five seed samples per taxon, each of about a dozen seeds taken from one herbarium specimen (Table 1).

Seed variation in co-specific plants parasitising different hosts was examined for *Orobanche variegata* (on *Calicotome infesta* (C. Presl) Guss. and *Spartium junceum* L.), *O. rapum-genistae* Thuill. (on *Genista aetnensis* (Biv.) DC. and *Spartium junceum*), *O. crenata* Forssk. (on *Vicia faba* L. and on *Vicia villosa* subsp. *varia* (Host) Corb.) and *O. amethystea* Thuill. (on *Daucus carota* L., *Eryngium campestre* L. and *Scolymus grandiflorus* Desf.).

For study under a "Leica Cambridge LEO 420" S.E.M., seeds were fixed onto metal stubs and coated with a 20 nm gold layer in an "Agar" auto-sputter-coater.

Intra-individual size variation was surveyed for *Orobanche chironii, Orobanche lavandulacea, O. litorea* and *O. variegata*, using a sample of 50 seeds taken from a single individual of each species. The seeds were observed and measured under a light microscope. The range of both length and width is illustrated by box-and whiskers plots in which rectangles define 25 and 75 percentiles, horizontal lines show median, whiskers are from 10 to 90 percentiles and asterisks extreme values.

Results

Size and shape of seeds are extremely variable, both between and within individuals. Moreover, the ranges of variation of different species overlap, as exemplified by *Orobanche chironii, O. lavadulacea, O. litorea* and *O. variegata* (Fig. 1). In a general way,

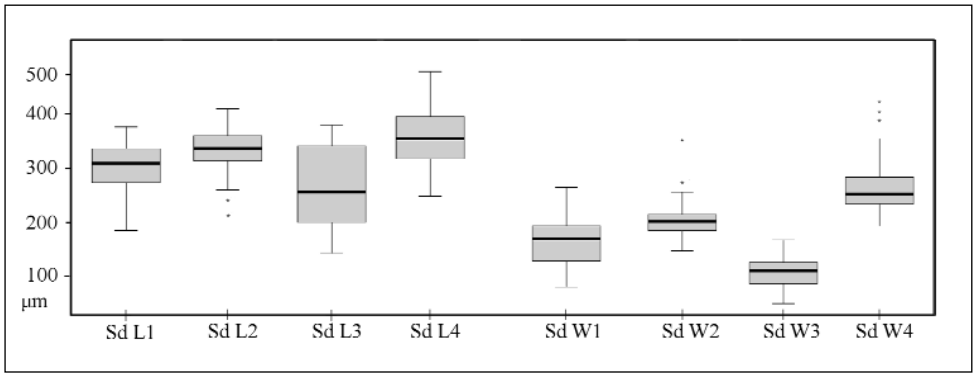

Fig. 1. Box-and-whiskers plots of seed length (Sd L) and width (Sd W) in *Orobanche chironii* (1), *O. lavandulacea* (2), *O. litorea* (3) and *O. variegata* (4). Rectangles define 25 and 75 percentiles; horizontal lines show median; whiskers are from 10 to 90 percentiles; asterisks extreme values.

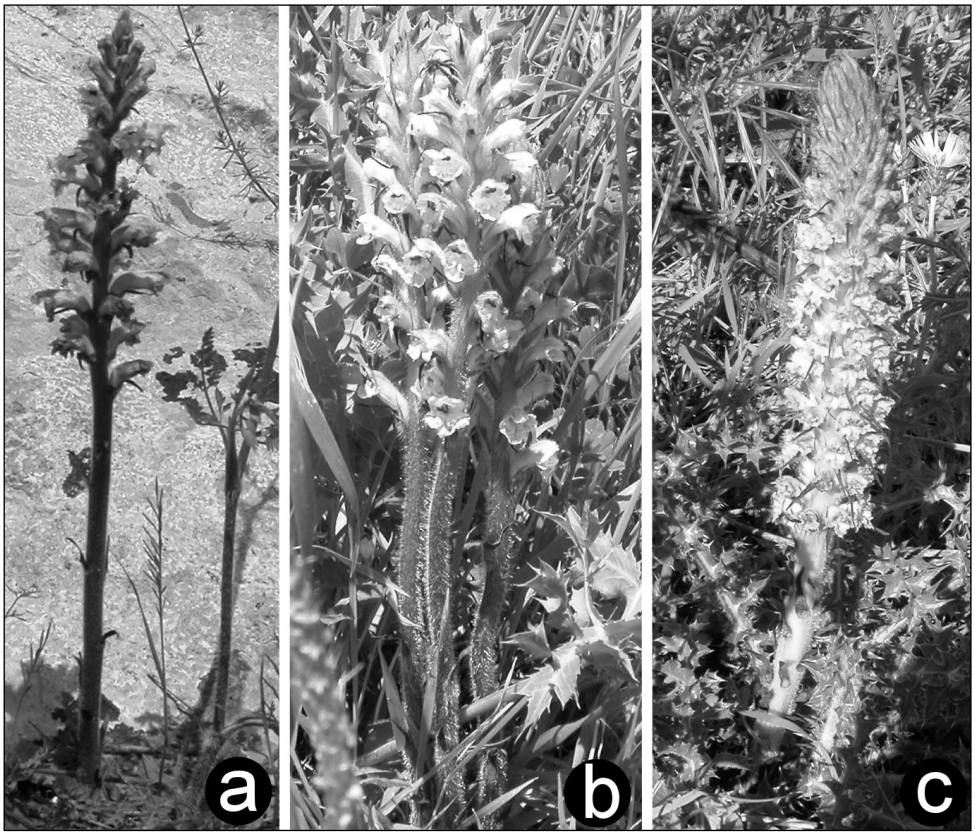

Fig. 2. *Orobanche amethystea* in the field, parasite on *Daucus carota* (a), *Eryngium campestre* (b) and *Scolymus grandiflorus* (c).

for none of the species investigated can seed length and width be used as a differential character.

The ornamentation of the testa is alveolar in all taxa, the longitudinal axis of the alveoles being parallel to the longitudinal axis of the seed. The shape of the alveoles can be of two types: roundish-pentagonal and relatively wide (Fig. 3h) or elliptical and elongate (Fig. 3e). This feature is quite uniform within species and has diagnostic value. Species of *Orobanche* sect. *Trionychon*, as well as some of *O*. sect. *Orobanche* (*O. alba, O. caryophyllacea, O. cernua, O. variegata, O. gracilis* and *O. rapum-genistae*) show the roundish-pentagonal pattern. The remainig species of *O*. sect. *Orobanche* (*O. crenata, O. chironii, O. thapsoides, O. litorea, O. minor, O. hederae, O. amethystea, O. picridis, O. canescens and O. pubescens*. and *O. sanguinea*) have the elongate-elliptical pattern. *O. sanguinea* has an almost regular elliptical pattern (Fig. 4e). This peculiarity distinguishes among the four Sicilian species of *O*. "grex" *Arcuatae* (Beck Mannagetta 1930: *O. sanguinea, O. variegata, O. gracilis* and *O. rapum-genistae*) only the first one shows elon-

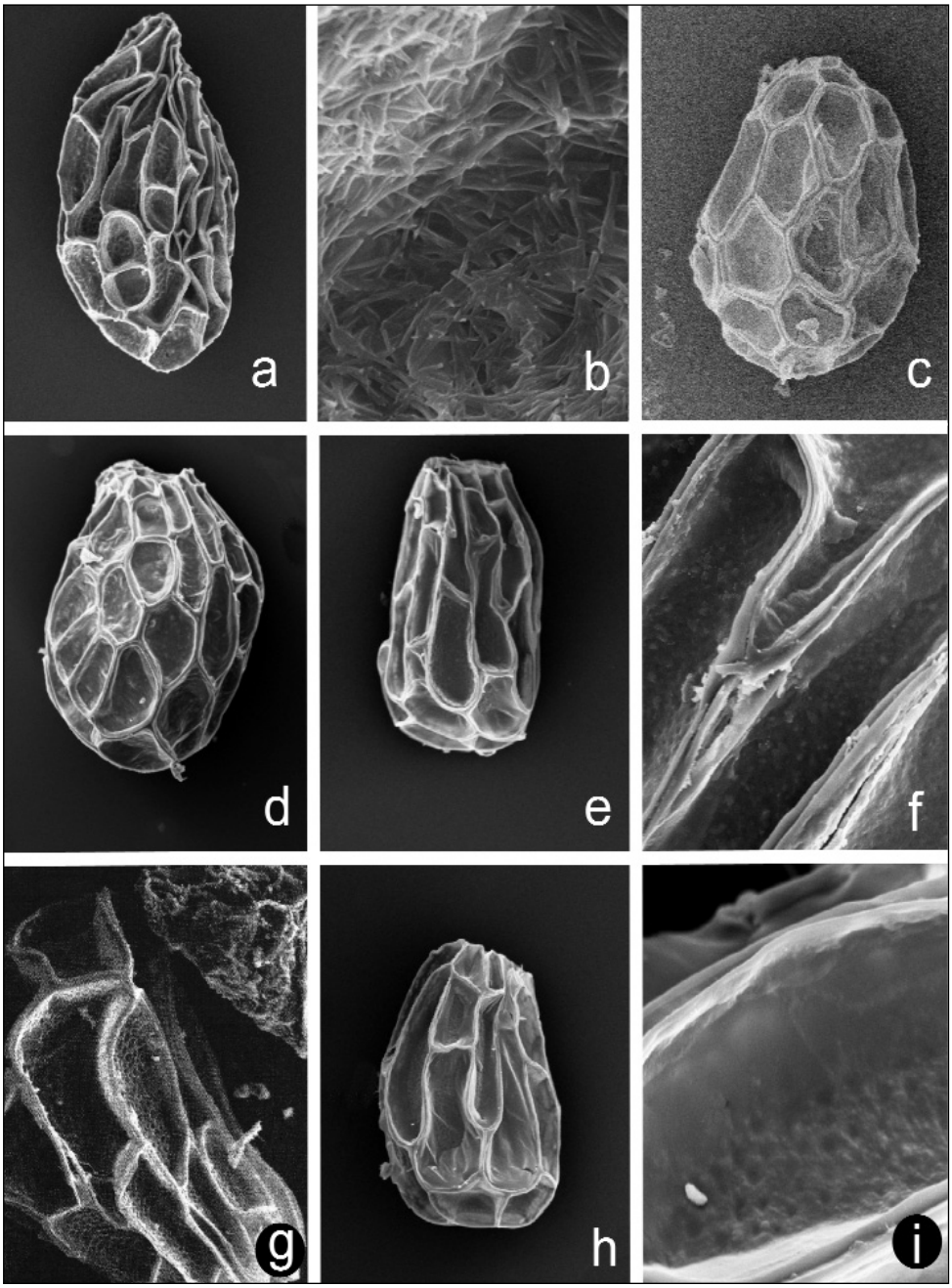

Fig. 3. Scanning electron micrographs of *Orobanche* seeds: **a-b,** *O. lavandulacea*; **c,** *O. alba*; **d,** *O. caryophyllacea*; **e-f,** *O. chironii*; **g,** *O. thapsoides*; **h-i,** *O. litorea*.

Table 2. Seed surface characters of *Orobanche* species studied. **Sd L**= Average seed length, **Sd W**= Average seed Width, **Seed Shape**= the most common seed shape observed, **Alv L**= Average alveole Length, **Alv W**= Average alveole Width, **Alv. pattern**= Alveolar pattern, **P Ø** = average perforation diameter, **Orn. fib.** = the reticulum on the bottom of the alveoles, as well as the side walls, are covered with a layer of interwoven fibrillae. (All measurements are in μm).

taxon	Sd L	Sd W	Seed Shape	Alv L	Alv W	Alv pattern	P Ø	Orn. fib.
Sect. *Trionychon*								
Orobanche ramosa L. subsp. *ramosa*	330	200	elliptical to ovate	100	40	roundish-pentagonal	5	yes
O. ramosa subsp. *nana* (Reut.) Cout.	300	190	ovate	50	30	roundish-pentagonal	5	yes
O. ramosa subsp. *mutelii* (F. W. Schultz) Cout.	280	200	ovate	70	30	roundish-pentagonal	4	yes
O. lavandulacea Rchb.	335	205	elliptical to ovate	75	40	roundish-pentagonal	4	yes
O. schultzii Mutel	420	330	ovate	90	45	roundish-pentagonal	4	yes
O. oxyloba (Reut.) G. Beck in L. Koch	370	240	≈ ovate	90	45	roundish-pentagonal	5	yes
Sect. *Orobanche*								
O. alba Steph. ex Willd.	310	250	≈ ovate	80	65	roundish-pentagonal	3	no
O. caryophyllacea Sm.	390	208	pear shaped to ovate	95	40	roundish-pentagonal	3,5	no
O. cernua L. in Loefl.	220	160	pear shaped to ovate	75	25	roundish-pentagonal	3,5	no
O. crenata Forssk.	350	210	≈ ovate	120	40	elliptical elongated	2	no
O. chironii Lojac.	300	165	narrowly ovate	120	30	elliptical elongated	1,5	no
O. thapsoides Lojac.	360	180	pear shaped to elongate	100	80	elliptical elongated	3,5	no
O. litorea Guss.	280	170	≈ ovate	90	40	elliptical elongated	4	no
O. minor Sm.	270	175	ovate	75	60	elliptical elongated	3	no
O. hederae Duby	380	290	ovate	120	80	elliptical elongated	1,5	no
O. amethystea Thuill.	270	160	narrowly ovate	90	30	elliptical elongated	3,5	no
O. picridis F. W. Schultz	300	230	≈ ovate	80	70	elliptical elongated	2,5	no
O. canescens C. Presl	260	150	narrowly ovate	120	45	elliptical elongated	2,0	no
O. pubescens d'Urv.	340	255	≈ ovate	120	55	elliptical elongated	1,5	no
O. sanguinea C. Presl	300	160	narrowly ovate	95	40	elliptical	4,0	no
O. gracilis Sm.	360	260	roundish ovate to pear shaped	110	65	roundish-pentagonal	3,0	no
O. variegata Wallr.	370	265	roundish ovate to	110	80	roundish-	2,5	no

gate-elliptical testa alveoles.

When the seeds are just barely mature, they are enveloped by a papery, smooth layer adhering to the inter-alveolar ridges and but loosely covering the alveoles themselves (Fig. 4e ed altre). As time goes by, this outer layer gradually disintegrates (Fig. 4h) so that the

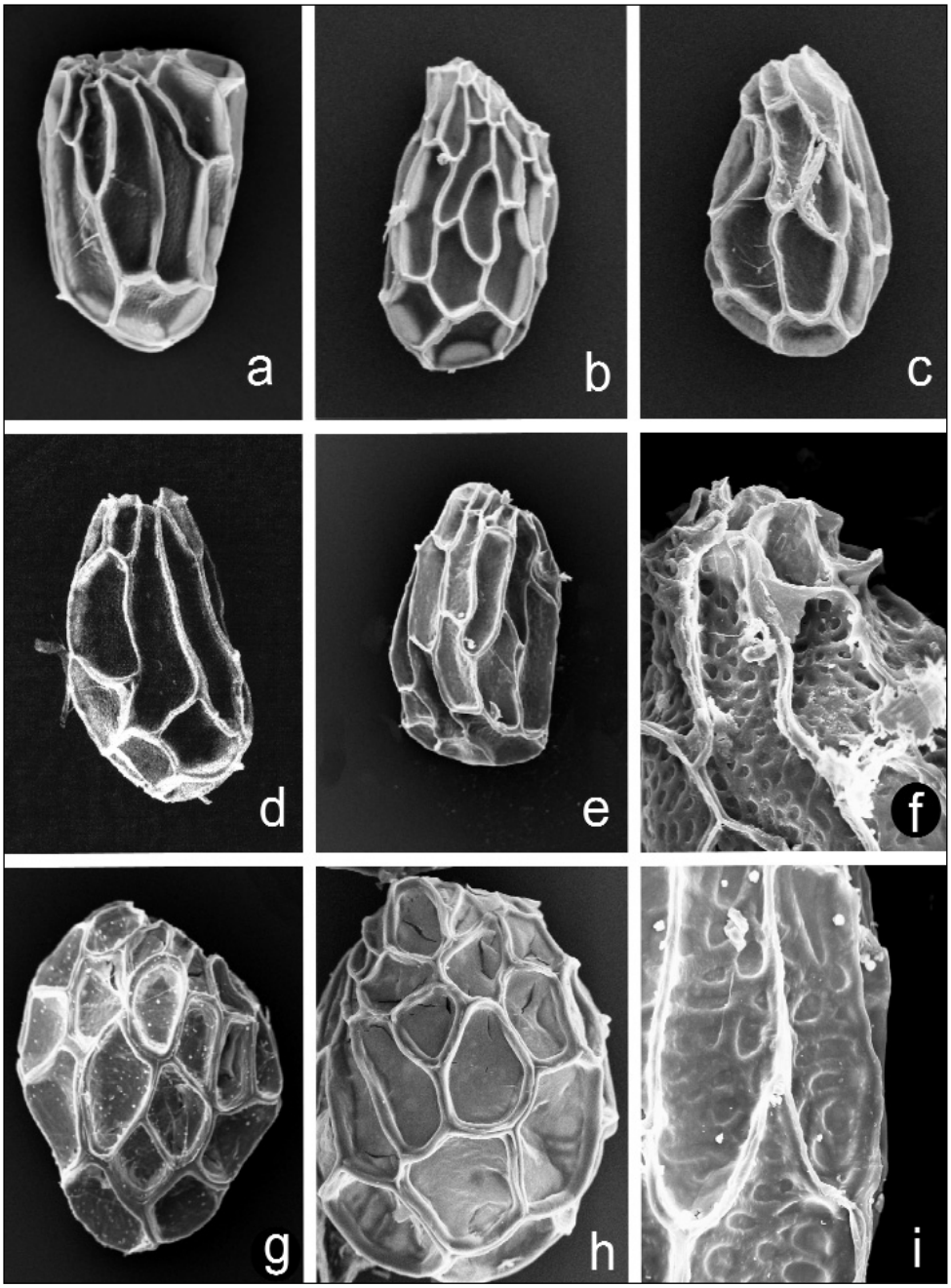

Fig. 4. Scanning electron micrographs of *Orobanche* seeds: **a-c,** *O. amethystea* [parasite on *Daucus carota* (a), *Eryngium campestre* (b) and *Scolymus grandiflorus* (c)]; **d,** *O. canescens*; **e-f,** *O. sanguinea*; **g,** *O. variegata*; **h-i,** *O. rapum-genistae*.

bottom of the alveoles becomes visible (Joel 1987b). In *Orobanche variegata* and *O. gracilis,* seeds may conserve the outer layer intact for a long time.

The bottom of the alveoles, once it becomes visible, is finely to coarsely reticulate. The mesh diameter is quite variable, even in seeds of one and the same plant, but it is generally larger in *Orobanche* sect. *Trionychon* than in *O*. sect. *Orobanche*. In addition, *O. crenata, O. chironii, O. hederae, O. pubescens* and *O. canescens* have narrower meshes than the other species of *O.* sect. *Orobanche* - a useful additional feature for distinguishing *O. canescens* from *O. litorea*, as well a *O. pubescens* from *O. thapsoides*, two species pairs that had been lumped together by Beck Mannagetta (1930) and have but recently been restored to independent specific rank (Domina & Mazzola, 2004a, 2004b).

There is a further structural difference apt to characterise the two sections (Table 2). As already noted by Abu Sbaih & Jury (1994), in *Orobanche* sect. *Trionychon* the reticulum on the bottom of the alveoles, as well as the side walls, are covered with a layer of interwoven fibrillae (Fig. 3b) that is lacking in *O.* sect. *Orobanche* (Fig. 3i).

Relating to host-dependent variation, we found no significant differences in any of the species surveyed (Fig. 4a,b,c). *Orobanche variegata* and *O. rapum-genistae* do not vary in their gross morphologically either, on different hosts (woody *Leguminosae*), whereas such differences have been observed in *O. crenata* when growing on different herbaceous *Leguminosae*, and individuals of *O. amethystea* growing on *Apiaceae* and *Asteraceae* are evidently dissimilar (Fig. 2).

Conclusions

Our study of the structure of seeds of the 24 *Orobanche* taxa occurring in Sicily adds new data to the knowledge of seed micro-morphology of the genus. For 9 species, no published studies of this kind have been found: for these, scanning electron micrographs illustrating the seeds have been included here (Figs 3-4). Four of them (*Orobanche chironii, O. thapsoides, O. litorea* and *O. variegata*) were first described from Sicily. Their seed morphology confirms their distinctness from the most closely related taxa.

Seed structure by itself is of course insufficient to distinguish between species or subspecies, in *Orobanche*; but it provides a useful additional criterion for identifying groups. Seed identification techniques suggested by Joel (1987a, 1987b) can assist in the analysis of agricultural soil samples, where the number of species involved is limited; and it can help in herbarium studies, in some cases, to distinguishing related taxa. This is of particular relevance with badly damaged specimens, when it is often still possible find some seeds among the debris. However, owing to the observed variation of seed size and structure (Joel, 1987b), unequivocal identification must rely on a set of seeds rather that on single seeds.

Finally *Orobanche* growing on different hosts, even when differing in their gross morphology, do not show seed significant differences in their characters.

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