Seed micromorphological characteristics in *Ochradenus* and *Reseda* and their taxonomic significance in Iran

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Abstract

Seed micromorphology of 16 taxa from *Ochradenus* and *Reseda* (*Resedaceae*) was examined using stereomicroscope and scanning electron microscope. In the present study, three quantitative (length, width, and ratio of seed length/seed width) and 10 qualitative micromorphological characteristics (color, brightness, the situation of surface, the state of hilum, shape, coat pattern sculpture, outer epidermal cell shape, the characteristics of anticlinal and periclinal walls, and the presence or absence of carunculoid tissue) of seed were examined. Results showed that, seed quantitative characteristics varied among studied taxa. In addition, significant diversity was found in all qualitative characteristics (especially seed color with 11 states and its coat pattern sculpture with nine states). Dendrogram of seed micromorphological characteristics constructed four seed types and two subtypes as follows: type 1 or *Ochradenus-Reseda* type with two subtypes, type 2 or core section *Reseda* type, type 3 or *Reseda-luteola* type, and type 4 or derived *Reseda* type. Seed micromorphology characteristics evidence partly verified the taxonomical and phylogenetical situation of the taxa from the above-mentioned family in Iran.

Keywords: Phylogeny, *Resedaceae*, scanning electron microscope, seed sculpture, seed type, stereomicroscope

ریزریخت‌شناسی دانه *Ochradenus* و *Reseda* و روابط آرایه‌شناسی آن‌ها در ایران

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خلاصه

خصوصیات ریزریخت‌شناسی دانه ۱۶ آرایه از *Ochradenus* Delile و *Reseda* Tourn. ex L. (اسپرکیان) به وسیله استریومیکروسکوپ و میکروسکوپ الکترونی نگاره مورد بررسی قرار گرفت. در مطالعه حاضر، سه صفت ریزریخت‌شناسی کمی (شامل طول و عرض و نسبت طول به عرض دانه) و ۱۰ صفت ریزریخت‌شناسی کیفی (شامل نوع رنگ، درخشندگی، وضعیت سطح، حالت ناف، شکل، الگوی تزیینات سطح، شکل یاخته‌های اپیدرم بیرونی، خصوصیات دیواره‌ها و حضور یا عدم حضور بافت زایده) در دانه مورد بررسی قرار گرفت. نتایج نشان داد که صفات کمی ریزریخت‌شناسی دانه در آرایه‌های مورد بررسی متفاوت بودند. همچنین، تنوع خاصی در صفات کیفی ریزریخت‌شناسی یافت شد (به ویژه رنگ دانه با ۱۱ حالت و تزیینات سطح آن با نُه حالت). دندروگرام به دست آمده از خصوصیات ریزریخت‌شناسی دانه، چهار تیپ و دو زیرتیپ را شامل تیپ ۱ یا *Ochradenus-Reseda* با دو زیرتیپ، تیپ ۲ یا core section *Reseda*، تیپ ۳ یا *Reseda-luteola* و تیپ ۴ یا derived *Reseda* ایجاد نمود. شواهد ریزریخت‌شناسی دانه تا حدودی با یافته‌های آرایه‌شناسی و تبارزایی مربوط به تیره موردنظر در ایران مطابقت داشت.

واژه‌های کلیدی: استریومیکروسکوپ، تبارزایی، تیپ دانه، تزیینات دانه، میکروسکوپ الکترونی نگاره، *Resedaceae*

Introduction

The *Resedaceae* contains about six genera (*Caylusea* A.St.-Hil., *Ochradenus*, *Oligomeris* Cambess., *Randonia* Coss., *Reseda*, and *Sesamoides* Ortega) that are widely distributed in the Old World, with a major center of species diversity in the Mediterranean basin (Nowroozi 1993, Martín-Bravo *et al.* 2007, Martín-Bravo & Amini Rad 2010, APG IV 2016, Ranjbar & Asgari Nematian 2021). Many species of the family grow on basic soils of arid habitats, others are ruderal weeds, and a few confined to high mountains (Martín-Bravo *et al.* 2007, Çilden *et al.* 2018). Some taxonomical attempts have been made to classify *Resedaceae* taxa based on morphological characters (Abdallah & de Wit 1978, Miller 1984). Traditional taxonomy divided the genus *Reseda* into three subgenera: *Reseda*, *Glaucoreseda*, and *Neoreseda*. *Reseda* subg. *Reseda* was divided into three sections as follows *Reseda*, *Leucoreseda*, and *Phyteuma*. In addition, phylogenetic relationships of this family based on molecular markers (ITS, trnL-F) were identified (Martín-Bravo *et al.* 2007). Recently, micromorphological data (pollen and seed) had valuable taxonomical understanding of the taxa from this family (De Leonardis *et al.* 1997, Al-Nowaihi *et al.* 2002, Asgari Nematian & Ranjbar 2021, Çilden & Yildirimli 2021). While seed micromorphology has been a valuable tool in systematic studies of various plant families (Arabi *et al*. 2017, Hoseini *et al*. 2017, Nejad Falatoury *et al*. 2021). Seed data for the *Resedaceae* family have not been previously examined in Iran. This study was conducted to investigate the potential utility of seed micromorphological characteristics for better understanding taxonomic relationships among members of this family, including sectional and infrageneric classification.

Materials and Methods

Seeds of 16 taxa belong to three genera of the *Resedaceae* (especially focused on genus *Reseda*) were provided Bu-Ali Sina University Herbarium (BASU) samples. The information for voucher specimens used in the present study, including herbarium deposition and some notes on the location of the plants was given in table 1. The mature seeds used in this study were removed from herbarium specimens. Qualitative and quantitative seed micromorphological characteristics were detected and listed in tables 2 & 3. Seed micromorphological characteristics were also examined by stereomicroscope (Olympus CX 41) and Scanning Electron Microscope (SEM) (JEOL JSM-840). Before the SEM observation, seeds were mounted directly on aluminium stubs using double-sided adhesive tape and were coated with a thin layer of gold. For recording the seed and size parameters, at least 10 seeds were measured. The descriptive terminology applied here is comparable to that used by Barthlott (1981), Stearn (1992), and Bojnanský & Fargašová (2007). Eight seed characteristics were determined (five qualitative characteristics and three quantitative characteristics). Color of seed had a high variation in the species (Figs 1 & 2) that were listed in table 2. MVSP software (Multi-Variate Statistical Package) along with the UPGMA method (Un Weighted Pair-Group Analysis) based on Euclidean distances was applied for constructing of dendrogram from the micromorphological seed characteristics. In addition, Principal Component Analysis (PCA) by MVSP software was used for the studied species (as PCA case scores) and seed characteristics (as PCA variable loadings) (Kovach 1999).

Table 1. List of the studied taxa used in this study from Iran along with related data

Taxon	Locality & voucher No. (BASU)
Ochradenus baccatus Delile.	Kish Island; Asgari 38429
O. ochradeni (Boiss.) Abdallah	Khorasan Prov.: Neyshabur; Asgari 35145
Reseda alba subsp. *alba* L.	Khuzestan Prov.: Omidieh; Asgari 53491
R. arabica Boiss.	Khuzestan Prov.: Omidieh; Ranjbar 3208
R. aucheri subsp. *afghanica* Rech.f.	Khorasan Prov.; Ranjbar 3209
R. aucheri subsp. *aucheri* Boiss.	Kerman Prov.: Bam to Iranshahr; Ranjbar 3207
R. aucheri subsp. *bracteata* (Boiss.) Rech.f.	Bushehr Prov.: Delvar to Tangestan; Joharchi 2430
R. aucheri subsp. *rechingeri* (Abdallah & De Wit) Rech.f.	Kerman Prov.: Bam; Ranjbar & Asgari 31358
R. aucheri subsp. *rotundifolia* (Kotschy ex. Müll. Arg.) Rech.f.	Kermanshah Prov.: Sumar-Tange Bijar; Ranjbar & Asgari 37594
R. aucheri subsp. *transitoria* Rech.f.	Fars Prov.: Shiraz, Kazeron; Ranjbar 14289
R. buhseana var. *buhseana* Mull. Arg.	Yazd Prov.: Taft to Dehbala; Ranjbar 803
R. buhseana var. *dshebeli* (Czerniak.) Abdallah & de Wit.	E. Azerbaijan Prov.: Mianeh; Joharchi & Zangooie 11371
R. hemithamnodes Czernjak.	Semnan Prov.: Damghan; Hossein-Zadeh & Joharchi 30931
R. lutea L.	Lorestan Prov.: Noorabad to Kenar Takhteh; Ranjbar & Asgari 41706
R. luteola L.	Khorasan Prov.: Bojnurd; Joharchi & Aydani 35718
R. microcarpa Mull. Arg.	W. Azerbaijan Prov.: Siahroad to Jolfa; Ranjbar 45833

* BASU (Bu-Ali Sina University Herbarium)

Results

- Stereomicroscopic observations

Based on the results derived from the present study, seed brightness was dull for *R. arabica* (Fig. 1D), *R. aucheri* subsp. *afghanica* (Fig. 1E), *R. aucheri* subsp. *rechingeri* (Fig. 1H), *R. buhseana* var. *dshebeli* (Fig. 2D), and *R. microcarpa* (Fig. 2H) and the other studied species had glossy state (Table 2 & Figs 1 & 2). Four surface states were found in the studied species (*Ochradenus baccatus* and *R. microcarpa* with warty state (Figs 1A, 2H), *O. ochradeni* and *R. alba* subsp. *alba* with slightly warty state (Figs 1B, 1C), *R. arabica* with rugose state (Fig. 1D), and the other species (Figs 1 & 2) with smooth state) (Table 2). In the present study, subcentral and central hilum was seen in *R. aucheri* subsp. *afghanica* (Fig. 1E) and the others had subcentral (Table 2 & Figs 1 & 2). The measurements of seed length and width (quantitative characteristics) were varied in the studied species (Table 2). In addition, this differentiation was seen in length and width ratio (L/W) as the other quantitative characteristic (Table 2). *Reseda luteola* had a minimum L/W ratio (1.06) among the studied species (Table 2). Maximum L/W ratio was measured for *R. arabica* (1.64) (Table 2). Three seed shapes were detected during the present study as follows: *Reseda lutea* with reniform-ovate (Fig. 2F), *R. luteola* with reniform-globose (Fig. 2G), and the others (Figs 1 & 2) had reniform seed shapes (Table 2).

Table 2. Seed micromorphology characters of studied species by stereomicroscope

Taxon	Color	Seed brightness	Surface	Hilum	Seed length (mm)	Seed width (mm)	L/W	Shape
Ochradenus baccatus	Brown to reddish black	Glossy	Warty	Subcentral	1.75(1.83 ± .08)1.96	1.39(1.48 ± .06)1.54	1.19	Reniform
O. ochradeni	Light brown	Glossy	Slightly warty	Subcentral	2.20(2.33 ± .11)2.55	1.48(1.59 ± 0.06)1.69	1.46	Reniform
Reseda alba subsp. *alba*	Greyish dark brown	Glossy	Slightly warty	Subcentral	1.33(1.41 ± .05)1.49	1.05(1.13 ± 0.05)1.20	1.24	Reniform
R. arabica	Light brown	Dull	Rugose	Subcentral	2.55(2.69 ± .09)2.83	1.45(1.64 ± 0.11)1.74	1.64	Reniform
R. aucheri subsp. *afghanica*	Yellowish light brown	Dull	Smooth	Subcentral-central	0.94(0.98 ± 04)1.09	0.72(0.80 ± 0.04)0.84	1.22	Reniform
R. aucheri subsp. *aucheri*	Yellowish dark brown	Glossy	Smooth	Subcentral	0.92(0.94 ± .02)0.99	0.76(0.78 ± 0.01)0.81	1.20	Reniform
R. aucheri subsp. *bracteata*	Yellowish dark brown	Glossy	Smooth	Subcentral	0.65(0.72 ± .05)0.80	0.52(0.55 ± 0.01)0.58	1.30	Reniform
R. aucheri subsp. *rechingeri*	Greenish light brown	Dull	Smooth	Subcentral	0.79(0.86 ± .04)0.91	0.67(0.70 ± 0.02)0.73	1.22	Reniform
R. aucheri subsp. *rotundifolia*	Greenish dark brown	Glossy	Smooth	Subcentral	0.77(0.90 ± .06)0.95	0.69(0.77 ± 0.04)0.82	1.16	Reniform
R. aucheri subsp *transitoria*	Greenish light brown	Glossy	Smooth	Subcentral	0.67(0.71 ± .03)0.76	0.51(0.55 ± 0.03)0.61	1.29	Reniform
R. buhseana var. *buhseana*	Dark brown	Glossy	Smooth	Subcentral	1.23(1.27 ± .03)1.33	0.89(0.98 ± 0.06)1.06	1.29	Reniform
R. buhseana var. *dshebeli*	Reddish brown	Dull	Smooth	Subcentral	1.08(1.15 ± 05)1.23	0.89(1.00 ± 0.09)1.13	1.15	Reniform
R. hemithamnodes	Light brown	Glossy	Smooth	Subcentral	0.83(0.92 ± .06)1.01	0.70(0.74 ± 0.04)0.82	1.24	Reniform
R. lutea	Dark brown	Glossy	Smooth	Subcentral	1.63(1.75 ± .08)1.85	1.18(1.28 ± 0.08)1.40	1.36	Reniform-ovate
R. luteola	Blackish dark brown	Glossy	Smooth	Subcentral	0.89(0.99 ± .09)1.10	0.90(0.93 ± 0.04)1.01	1.06	Reniform-globose
R. microcarpa	Brownish black	Dull	Warty	Subcentral	1.25(1.35 ± .14)1.49	1.21(1.25 ± 0.05)1.31	1.27	Reniform

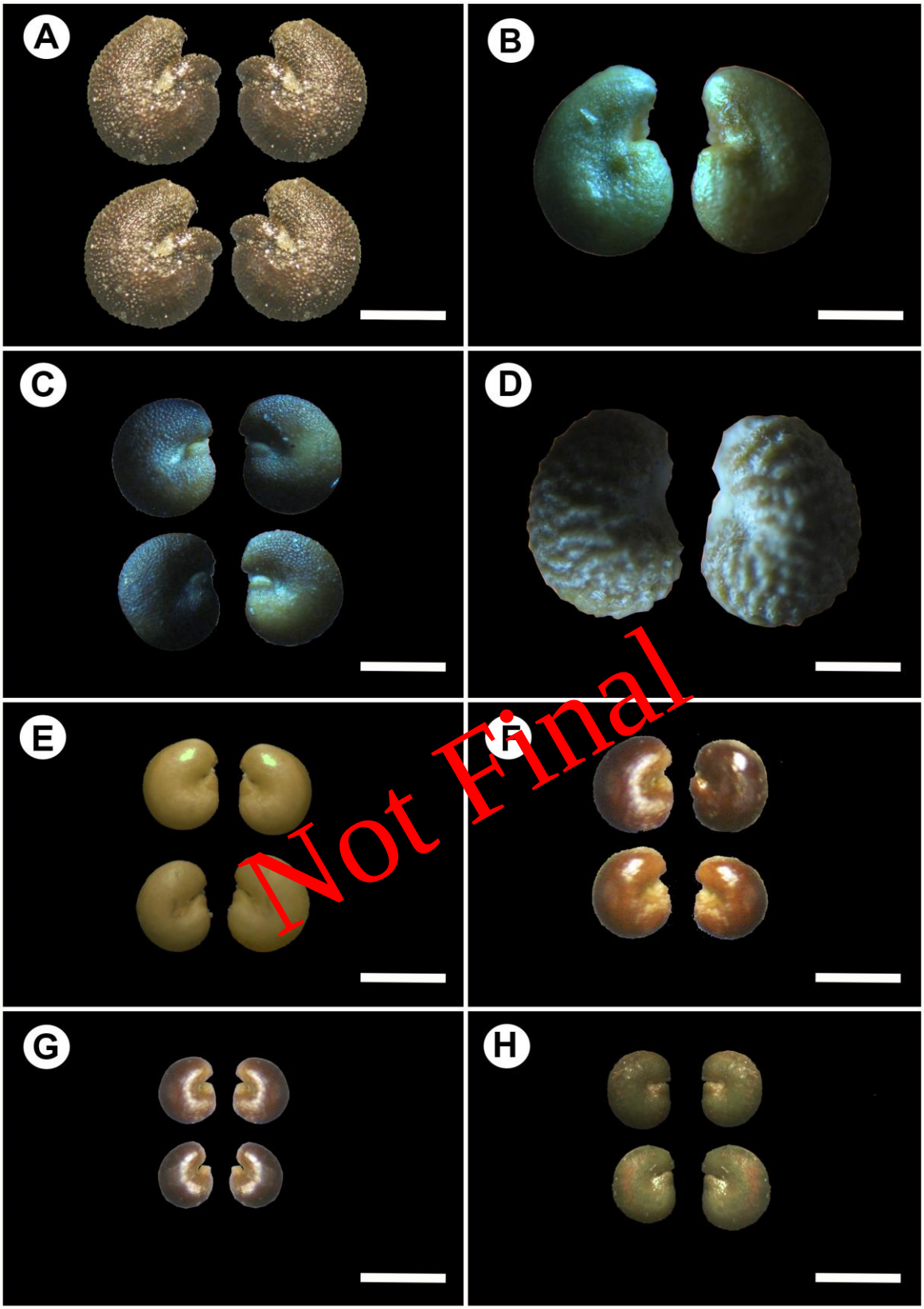

Fig. 1. Stereomicroscopic photos of seeds of the *Resedaceae* species in Iran: A. *Ochradenus baccatus*, B. *O. ochradeni*, C. *Reseda alba*, D. *R. arabica*, E. *R. aucheri* subsp. *afghanica*, F. *R. aucheri* subsp. *aucheri*, G. *R. aucheri* subsp. *bracteata*, H. *R. aucheri* subsp. *rechingeri* (Bar = 2 mm).

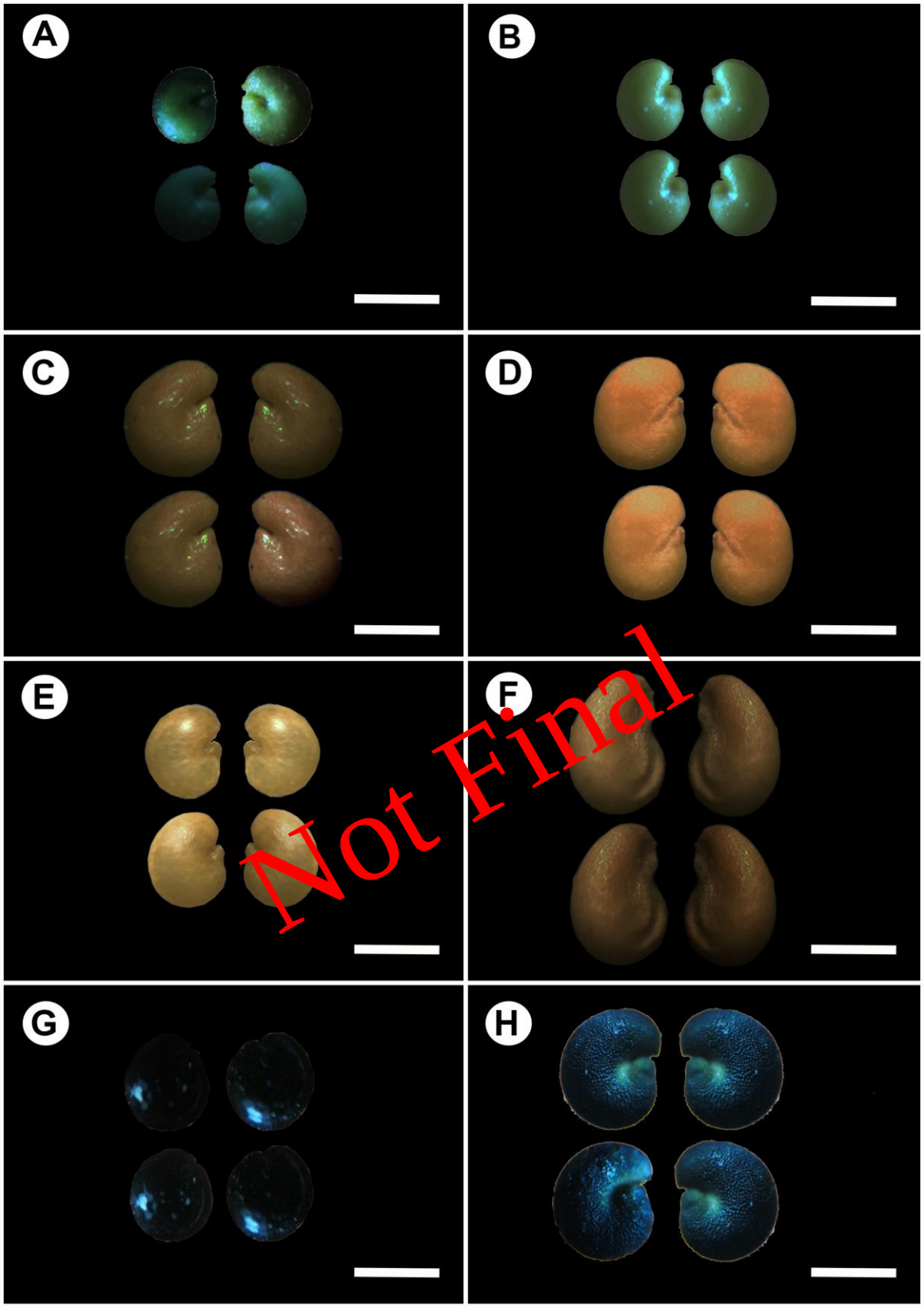

Fig. 2. Stereomicroscopic photos of seeds of the *Resedaceae* species in Iran: A. *Reseda aucheri* subsp. *rotundifolia*, B. *R. aucheri* subsp. *transitoria*, C. *R. buhseana* var. *buhseana*, D. *R. buhseana* var. *dshebeli*, E. *R. hemithamnodes*, F. *R. lutea*, G. *R. luteola*, H. *R. microcarpa* (Bar = 2 mm).

- Scanning electron microscopic observations

In the present study, nine qualitative seed micromorphological characteristics were investigated through scanning electron microscope (Table 3 & Figs 3–6). As a result, seed sculpture had high differentiation among these characteristics with nine patterns (*O. baccatus* and *R. microcarpa* with tuberculate pattern (Figs 3A2, 6D2), *O. ochradeni* and *Reseda alba* subsp. *alba* with colliculate pattern (Figs 3B2, 3C2), *R. arabica* and *R. buhseana* var. *dshebeli* with rugose pattern (Figs 3D2, 5D2), *R. aucheri* subsp. *afghanica* and *R. aucheri* subsp. *aucheri* with granulate pattern (Figs 4A2, 4B2), *R. aucheri* subsp. *bracteata* and *R. aucheri* subsp. *rotundifolia* with rugulose pattern (Figs 4C2, 5A2), *R. aucheri* subsp. *rechingeri* and *R. aucheri* subsp *transitoria* with undulate pattern (Figs 4D2, 5B2), *R. lutea* and *R. luteola* with smooth pattern (Figs 6B2, 6C2), *R. buhseana* var. *buhseana* with areolate-reticulate pattern (Fig. 5C2, and *R. hemithamnodes* (Fig. 6A2) with reticulate pattern) (Table 3). There were isodiametric polygonal and irregular polygonal cell shapes in the outer epidermal cells of *O. ochradeni* and *R. alba* subsp. *alba* (Table 3 & Figs 3–6), while, *O. baccatus*, *R. arabica*, *R. aucheri* subsp. *rotundifolia*, *R. aucheri* subsp *transitoria*, *R. buhseana* var. *buhseana*, *R. lutea*, and *R. microcarpa* had polygonal to elongated cell shapes (Table 3 & Figs 3A2, 3D2, 5A2, 5B2, 5C2, 6B2, 6D2). About this micromorphological characteristic, the rest species studied had polygonal cell shapes (Table 3 & Figs 3–6). In the anticlinal seed wall, four micromorphological characteristics were detected as follows: shape with five states (*R. aucheri* subsp. *bracteata* with sinuous shape (Fig. 4C2); *R. luteola* with straight to undulate shape (Fig. 6C2), *R. aucheri* subsp. *rotundifolia*, *R. lutea* and *R. microcarpa* with straight to slightly undulate shape (Figs. 5A2, 6B2, 6D2); *R. arabica*, *R. aucheri* subsp. *afghanica*, and *R. aucheri* subsp. *aucheri*, *R. aucheri* subsp. *rechingeri*, *R. aucheri* subsp *transitoria*, and *R. buhseana* var. *dshebeli* with undulate shape (Figs 3D2, 4A2, 4B2, 4D2, 5B2, 5D2) and the rest (Figs 3–6) with straight to slightly sinuous); texture with five states (*O. baccatus* and *R. luteola* with a smooth texture (Figs 3A2, 6C2); *R. alba* subsp. *alba* with smooth to folded texture (Fig. 3C2); *R. arabica*, *R. aucheri* subsp. *aucheri*, *R. buhseana* var. *dshebeli*, and *R. microcarpa* with striate texture (Figs 3D2, 4B2, 5D2, 6D2); *R. lutea* with smooth to slightly striate state (Fig. 6B2) and the other studied species (Figs 3–6) with smooth to striate texture) (Table 3); thickness and level (*R. arabica*, *R. hemithamnodes*, *R. lutea* with thickness and raised wall (Figs 3D2, 6A2, 6B2), *O. ochradeni* with thickness and grooved wall (Fig. 3A2) and the rest (Figs 3–6) with thinness and grooved wall) (Table 3). In this way, two micromorphological characteristics were examined such as texture and level of periclinal wall (Table 3). There were six kinds of textures in *O. ochradeni* and *R. luteola* with smooth texture (Figs 3B2, 6C2); *R. alba* subsp. *alba* with microreticulate-folded texture (Fig. 3C2); *R. aucheri* subsp. *rechingeri* with striate to fine folded texture (Fig. 4D2); *R. buhseana* var. *dshebeli* with linear striate texture (Fig. 5D2); *R. lutea* with smooth to fine striate texture (Fig. 6B2), and the other studied species (Figs 3–6) with striate texture) (Table 3). Level of the periclinal wall is categorized into 6 states as follows: convex with conical elevations (*O. baccatus* and *R. microcarpa*) (Figs 3A2, 6D2), convex (*O. ochradeni*, *R. alba* subsp. *alba*, and *R. aucheri* subsp. *afghanica*) (Figs 3B2, 3C2, 4A2), flat (*R. arabica*, *R. lutea* and *R. luteola*) (Figs 3D2, 6B2, 6C2), convex to flat (*R. aucheri* subsp. *bracteata*) (Fig. 4C2), flat to slightly convex (*R. buhseana* var. *buhseana*) (Fig. 5C2), and flat to convex (the other studied species) (Table 3 & Figs 3–6). Carunculoid tissue was absent in the seed of *O. baccatus*, *R. alba* subsp. *alba*, *R. lutea*, and *R. microcarpa* (Table 3 & Figs 3A3, 3C3, 6B3, 6D3). In the other studied species, this tissue was seen (Table 3 & Figs 3B3, 3D3, 4A3, 4B3, 4C3, 4D3, 5A3,5B3, 5C3, 5D3, 6A3, 6C3).

Table 3. Seed micromorphology characters of studied species by scanning electron microscope with code reference for qualitative characteristics

Taxon	Seed coat pattern sculpture	Outer epidermal cell shape	Anticlinal wall			Periclinal wall		Carunculoid tissue
			Shape	Texture	Thickness	Level	Texture	Level	
Ochradenus baccatus	Tuberculate	Polygonal to elongated cells	Straight to slightly sinuous	Smooth	Thin	Grooved	Striate	Conical elevations	Absent
O. ochradeni	Colliculate	Isodiametric polygonal cells	Straight to slightly sinuous	Smooth to striate	Thick	Grooved	Smooth	Convex	Present
R. alba subsp. *alba*	Colliculate	Irregular polygonal cells	Straight to slightly sinuous	Smooth to folded	Thin	Grooved	Microreticulate-folded	Convex	Absent
R. arabica	Rugose	Polygonal to elongated cells	Undulate	Striate	Thick	Raised	Striate	Flat	Present
R. aucheri subsp. *afghanica*	Granulate	Polygonal cells	Undulate	Smooth to striate	Thin	Grooved	Striate	Convex	Present
R. aucheri subsp. *aucheri*	Granulate	Polygonal cells	Undulate	Striate	Thin	Grooved	Striate	Flat to convex	Present
R. aucheri subsp. *bracteata*	Rugulose	Polygonal cells	Sinuous	Smooth to striate	Thin	Grooved	Striate	Convex to flat	Present
R. aucheri subsp. *rechingeri*	Undulate	Polygonal cells	Undulate	Smooth to striate	Thin	Grooved	Striate to fine folded	Flat to convex	Present
R. aucheri subsp. *rotundifolia*	Rugulose	Polygonal to elongated cells	Straight to slightly undulate	Smooth to striate	Thin	Grooved	Striate	Flat to convex	Present
R. aucheri subsp *transitoria*	Undulate	Polygonal to elongated cells	Undulate	Smooth to striate	Thin	Grooved	Striate	Flat to convex	Present
R. buhseana var. *buhseana*	Areolate-reticulate	Polygonal to elongated cells	Straight to slightly sinuous	Smooth to striate	Thin	Grooved	Striate	Flat to slightly convex	Present
R. buhseana var. *dshebeli*	Rugose	Polygonal cells	Undulate	Striate	Thin	Grooved	Linear striate	Flat to convex	Present
R. hemithamnodes	Reticulate	Polygonal cells	Straight to slightly sinuous	Smooth to striate	Thick	Raised	Striate	Flat to convex	Present
R. lutea	Smooth	Polygonal to elongated cells	Straight to slightly undulate	Smooth to slightly striate	Thick	Raised	Smooth to fine striate	Flat	Absent
R. luteola	Smooth	Polygonal cells	Straight to Undulate	Smooth	Thin	Grooved	Smooth	Flat	Present
R. microcarpa	Tuberculate	Polygonal to elongated cells	Straight to slightly undulate	Striate	Thin	Grooved	Striate	Conical elevations	Absent

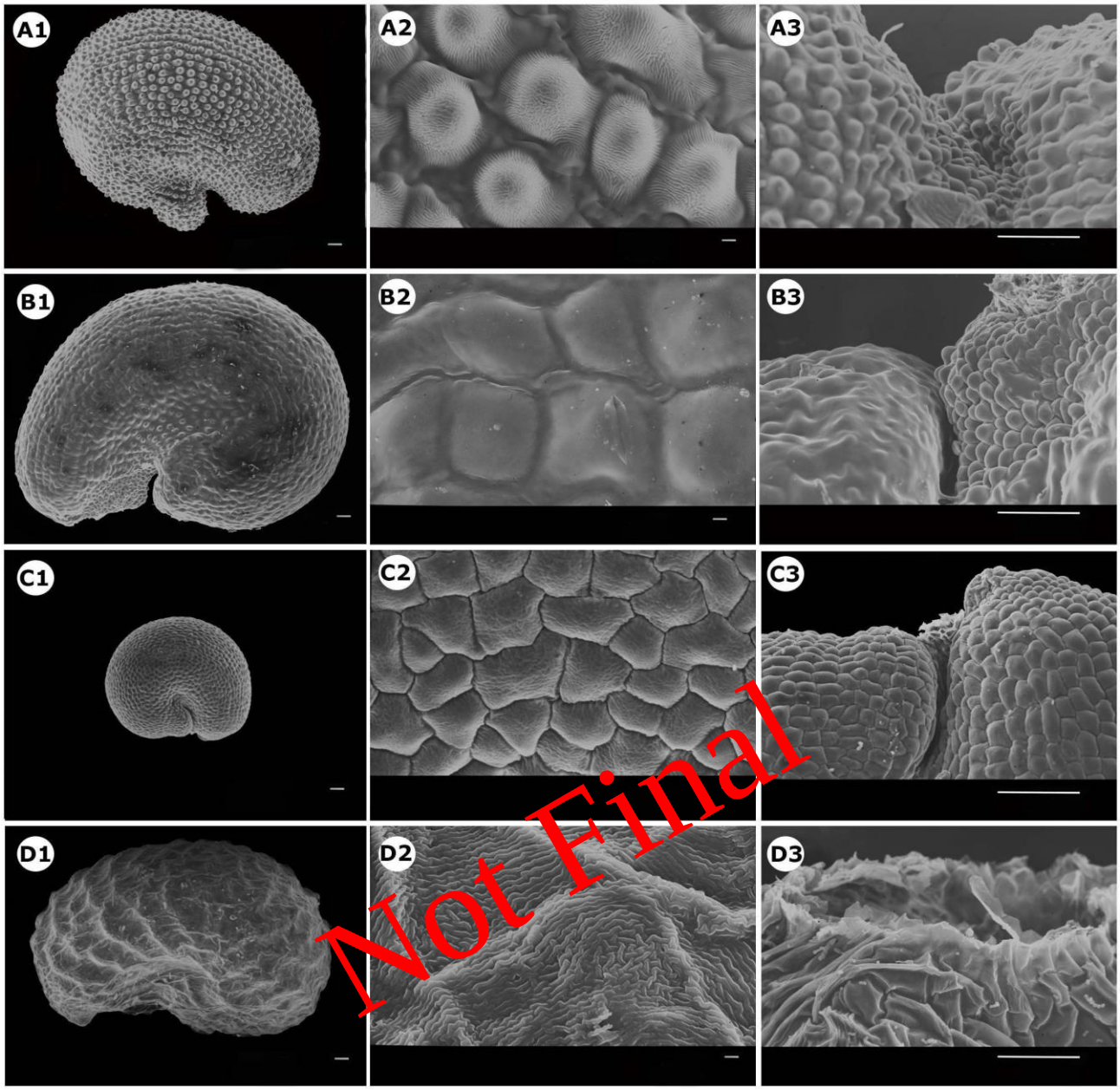

Fig. 3. Scanning electron microscope micrographs of seeds of the *Resedaceae* species in Iran: A1. General view of *Ochradenus baccatus*, A2. Seed coat pattern sculpture, A3, Hilum, B1. General view of *O. ochradeni*, B2. Seed coat pattern sculpture, B3, Hilum, C1. General view of *Reseda alba*, C2. Seed coat pattern sculpture, C3. Hilum, D1. General view of *R. arabica*, D2. Seed coat pattern sculpture, D3, Hilum (Bars: A1-D1 = 100 µm, A2-D2 = 10 µm, A3-D3 = 10 µm).

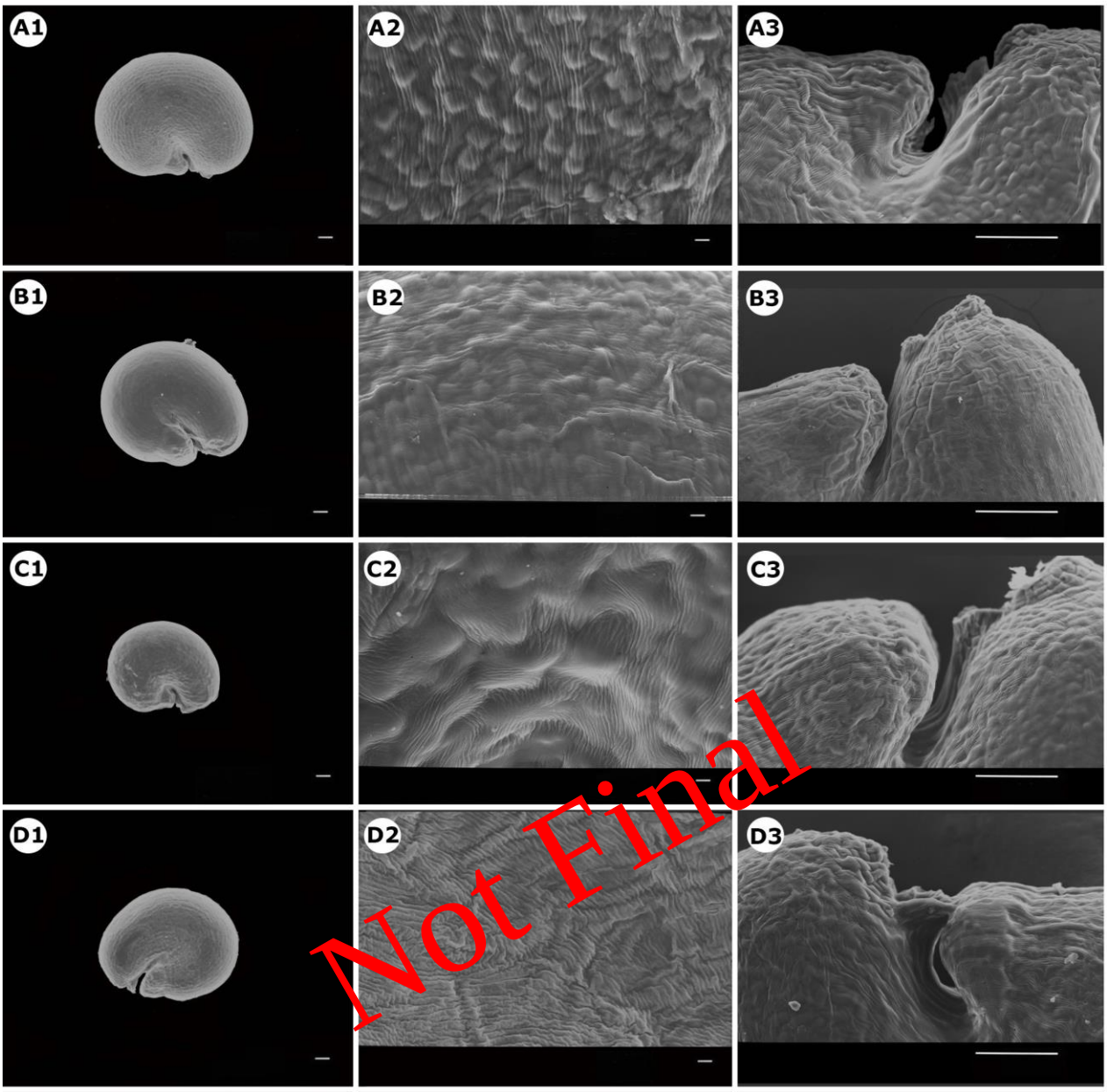

Fig. 4. Scanning electron microscope micrographs of seeds of the *Resedaceae* species in Iran: A1. General view of *Reseda aucheri* subsp. *afghanica*, A2. Seed coat pattern sculpture, A3. Hilum, B1. General view of *R. aucheri* subsp. *aucheri*, B2. Seed coat pattern sculpture, B3. Hilum, C1. General view of *R. aucheri* subsp. *bracteata*, C2. Seed coat pattern sculpture, C3. Hilum, D1. General view of *R. aucheri* subsp. *rechingeri*, D2. Seed coat pattern sculpture, D3. Hilum (Bars: A1-D1 = 100 µm, A2-D2 = 10 µm, A3-D3 = 10 µm).

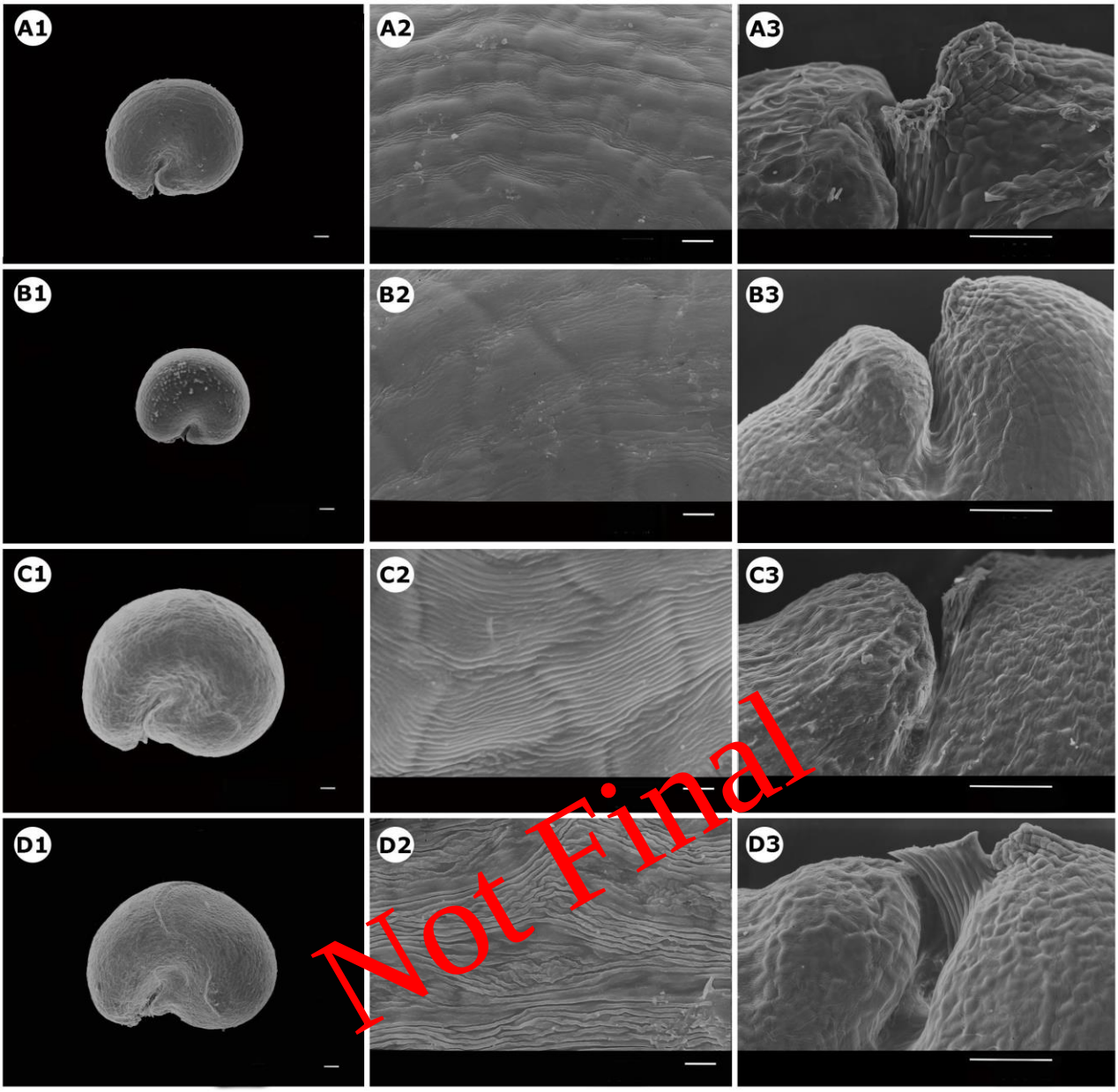

Fig. 5. Scanning electron microscope micrographs of seeds of the *Resedaceae* species in Iran: A1. General view of *Reseda aucheri* subsp. *rotundifolia*, A2. Seed coat pattern sculpture, A3. Hilum, B1. General view of *R. aucheri* subsp. *transitoria*, B2. Seed coat pattern sculpture, B3. Hilum, C1. General view of *R. buhseana* var. *buhseana*, C2. Seed coat pattern sculpture, C3. Hilum, D1. General view of *R. buhseana* var. *dshebeli*, D2. Seed coat pattern sculpture, D3. Hilum (Bars: A1-D1 = 100 µm, A2-D2 = 10 µm, A3-D3 = 10 µm).

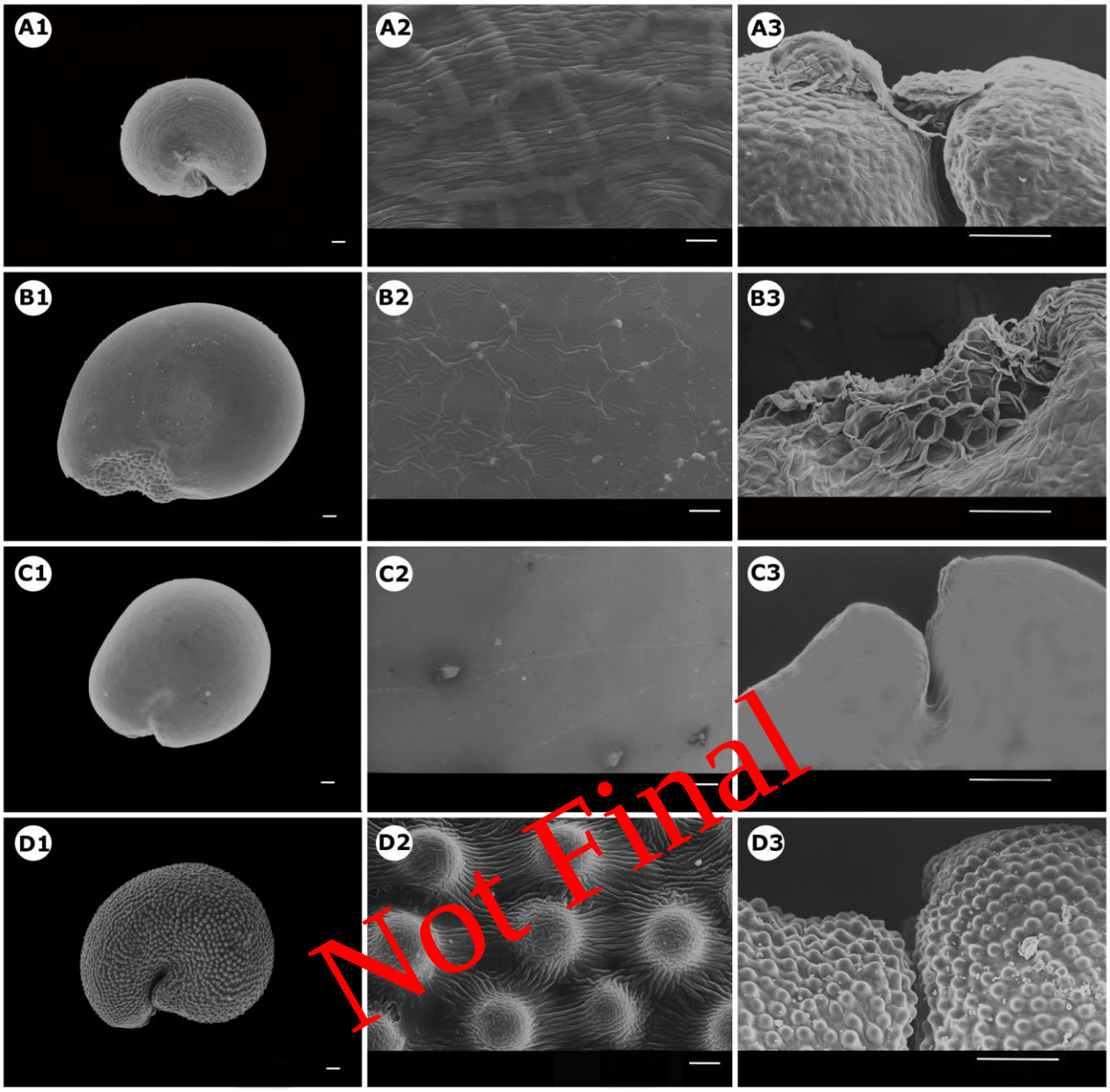

Fig. 6. Scanning electron microscope micrographs of seeds of the *Resedaceae* species in Iran: A1. General view of *Reseda hemithamnodes*, A2. Seed coat pattern sculpture, A3. Hilum, B1. General view of *R. lutea*, B2. Seed coat pattern sculpture, B3. Hilum, C1. General view of *R. luteola*, C2. Seed coat pattern sculpture, C3. Hilum, D1. General view of *R. microcarpa*, D2. Seed coat pattern sculpture, D3. Hilum (Bars: A1-D1 = 100 µm, A2-D2 = 10 µm, A3-D3 = 10 µm).

- Statistical analyses: Un-weighted Pair-Group Analysis (UPGMA) and Principal Components Analysis (PCA)

According to the UPGMA method based on Euclidean distances from the seed micromorphological characteristics under stereomicroscopic and scanning electron microscopic observation, four seed types and two subtypes were obtained which are given as follows: type 1 (*Ochradenus-Reseda* type) including two subtypes: subtype 1 (genus *Ochradenus* and *R. alba* subsp. *alba*) and subtype 2 (*R. arabica*); type 2 or core section *Reseda* type (*R. aucheri*, *R. buhseana*, and *R. hemithamnodes*), type 3 (*Reseda-luteola* type: *R. lutea* and *R. luteola*), and type 4 or derived *Reseda* type (*R. microcarpa*) (Fig. 7).

The results of PCA including eigenvalues, percentages, and cumulative percentages for both axes, PCA case scores (as the studied species), and PCA variable loadings (as the seed micromorphological characteristics) which were obtained from the present study, are shown in table 4 and figure 8. According to this, four types of case scores in both axes were present: type 1 includes two subtypes, subtype 1: genus *Ochradenus* and *R. alba* subsp. *alba* (negative in both axes) and subtype 2: *R. arabica* (positive in axis 2 and negative in axis 1) (Table 4 & Fig. 8). Type 2 includes *R. aucheri*, *R. buhseana*, and *R. hemithamnodes* (positive and negative in both axes) (Table 4 & Fig. 8). Type 3 includes *R. lutea* and *R. luteola* (positive in both axes), and type 4 includes *R. microcarpa* (positive in axis 1 and negative in axis 2) (Table 4 & Fig. 8).

The results of PCA analysis based on the studied species and their seed micromorphological characteristics overlapping showed that, seed color (characteristic A) and seed coat pattern sculpture (characteristic O) had a major role in the situation of types 3 and 4 in the seed micromorphological biplot (Fig. 8A). Shape of the anticlinal wall (characteristic Q), the texture of anticlinal wall (characteristic R), the texture of periclinal wall (characteristic U), seed surface (characteristic C), outer epidermal cell shape (characteristic P), and the level of periclinal wall (characteristic V) had major roles in the situation of type 2 (Fig. 8A). The rest studied seed characters in the central part of the biplot (characteristics group A) were important for the situation of all studied species (Table 4 & Fig. 8).

Table 4. The seed micromorphological characteristics analyzed by principal components analysis (PCA)

Principal component analysis (analyzing 23 variables x 16 cases)			
		Axis 1	**Axis 2**
	Eigenvalues	13.775	6.983
	Percentage	45.282	22.954
	Cum. percentage	45.282	68.237
	PCA variable loading		
		Axis 1	Axis 2
Color		0.725	-0.558
Surface		0.192	0.267
Seed coat pattern sculpture		0.526	0.624
Outer epidermal cell shape		0.054	0.204
Shape of anticlinal wall		0.244	-0.159
Texture of anticlinal wall		0.048	-0.189
Texture of periclinal wall		0.165	-0.08
Level of periclinal wall		0.183	0.284
	PCA case scores		
		Axis 1	**Axis 2**
Ochradenus baccatus	1	-1.798	-0.247
O. ochradeni	2	-1.349	-0.096
Reseda alba subsp. *alba*	3	-1.014	-0.231
R. arabica	4	-1.109	0.015
R. aucheri subsp. *afghanica*	5	-0.437	0.224
R. aucheri subsp. *aucheri*	6	-0.124	0.149
R. aucheri subsp. *bracteata*	7	0.12	0.46
R. aucheri subsp. *rechingeri*	8	0.446	0.351
R. aucheri subsp. *rotundifolia*	9	0.451	-0.117
R. aucheri subsp *transitoria*	10	0.291	0.287
R. buhseana var. *buhseana*	11	0.781	0.304
R. buhseana var. *dshebeli*	12	0.638	-0.709
R. hemithamnodes	13	-0.233	1.393
R. lutea	14	1.319	0.006
R. luteola	15	1.683	0.159
R. microcarpa	16	0.336	-1.948

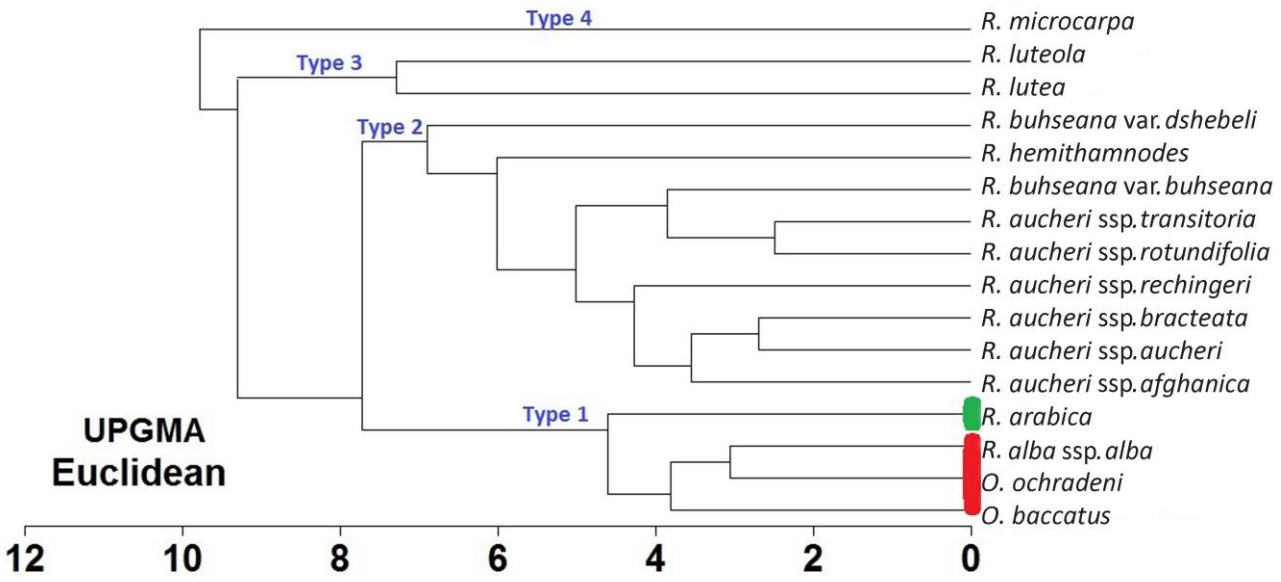

Fig. 7. Dendrogram of the studied species of *Reseda* and *Ochradenus* which was analyzed by MVSP software based on the UPGMA method (Euclidean distances) from seed micromorphological data and their taxonomic relationships. Four seed types and two seed subtypes (red and green color) were illustrated.

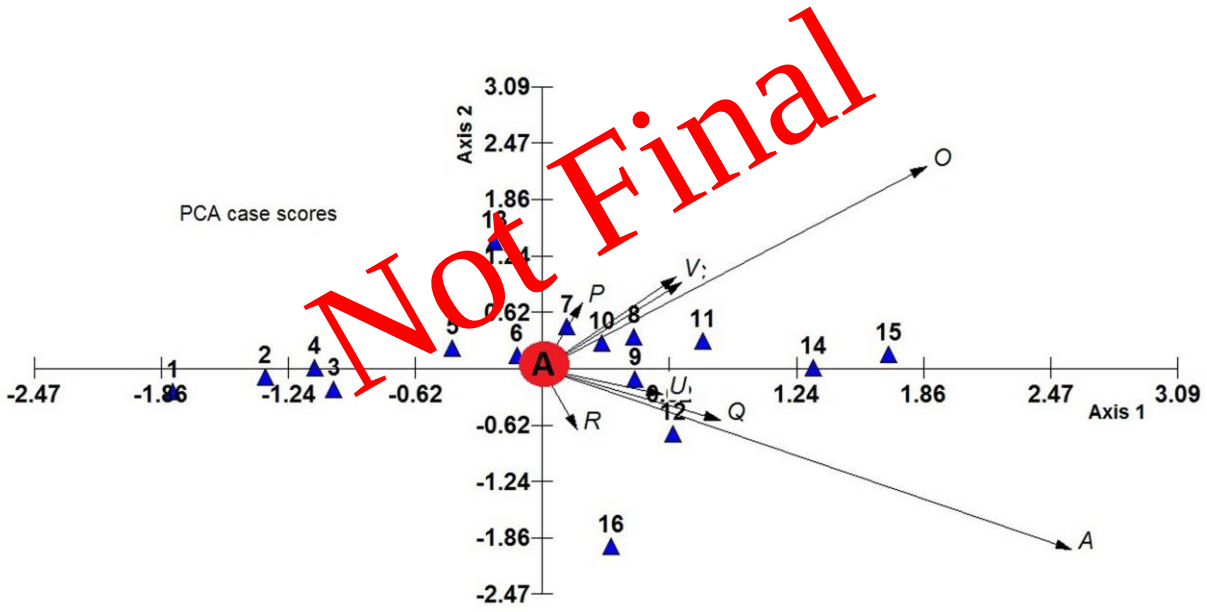

Fig. 8. Principal components analysis scatterplot obtained from overlapping of *Ochradenus* and *Reseda* species with seed characteristics (case scores and variable loadings) and the seed characteristics (variable loadings). Case scores (as species): *O. baccatus* (1), *O. ochradeni* (2), *R. alba* subsp. *alba* (3), *R. arabica* (4), *R. aucheri* subsp. *afghanica* (5), *R. aucheri* subsp. *aucheri* (6), *R. aucheri* subsp. *bracteata* (7), *R. aucheri* subsp. *rechingeri* (8), *R. aucheri* subsp. *rotundifolia* (9), *R. aucheri* subsp *transitoria* (10), *R. buhseana* var. *buhseana* (11), *R. buhseana* var. *dshebeli* (12), *R. hemithamnodes* (13), *R. lutea* (14), *R. luteola* (15) and *R. microcarpa* (16). Variable loading (as pollen grain characteristics): seed color (A), shape of anticlinal wall (Q), texture of anticlinal wall (R), texture of periclinal wall (U), seed surface (C), seed coat pattern sculpture (O), outer epidermal cell shape (P), level of periclinal wall (V), and characteristics group A (the other seed micromorphological characteristics).

Discussion

Recent studies have reported new records of *Resedaceae* in the Flora of Iran (Martín-Bravo & Amini Rad 2010, Ranjbar & Asgari Nematian 2021). Micromorphological characteristics, in conjunction with morphological features such as leaf size and shape, pedicel length, leaf, stem, and fruit indumentum, and petal shape and size, have been found to be valuable in species and subspecies delimitation within *Reseda* (Martín-Bravo *et al.* 2007, Çilden *et al.* 2018, Ranjbar & Asgari Nematian 2021). Additionally, micromorphological characteristics have recently demonstrated their effectiveness in distinguishing taxa within the *Resedaceae* family (Çilden & Yildirimli 2021).

- Seed micromorphological issues

Results of the present study showed that, the seed micromorphology was variable in the genus *Reseda*. Micromorphological characteristics had also minor contrast with the taxonomical and phylogenetical relations of this family in Iran. Previously, some studies had been done on this genus by De Leonardis *et al.* (1997), Al-Nowaihi *et al.* (2002), and Çilden & Yildirimli (2021) with valuable taxonomical data.

Based on De Leonardis *et al.* (1997), three studied species of *Reseda* were different in seed coat sculptures (*R. alba* with tuberculate sculpture, *R. luteola* with reticulate-foveolate sculpture, and *R. lutea* with reticulate sculpture). Moreover, there was a distinction in the presence or absence of aril between *R. luteola* and *R. lutea* (De Leonardis *et al.* 1997). Results of Al-Nowaihi *et al.* (2002) distinguished variable seed micromorphology such as four kind of seed shape (reniform, ovoid, ovoid-pear, and spherical), combination of seed testa color for each taxon (except *Randonia africana* and *Reseda phyteuma* with brown color), varied measurement on seed dimension especially in the taxa of *Reseda*, presence or absence of seed aril (presence in the genus of *Reseda*), three states of seed sinus (closed, narrow opened or slightly opened, and fairly wide slit), six kinds of sculpture on seed surface in the studied species of *Reseda* (tuberculate, areolate-scalariform, areolate-globulate, favulariate- rugose to luminate with different striations, reticulate-smooth to finely granulate, and colliculate-rugose), and spermoderm anatomy included characteristics of seed exotesta region (shape of the cell in this region, thickness and shape of outer with inner and anticlinal periclinal wall); mesotesta region (presence in *Caylusea hexagyna*); endotesta region (with one to multi layers), exotegmen region (with one layer in all studied taxa); mesotegmen region (with different cell shapes in one layer in all studied taxa); and endotegmen region (one layer in all studied taxa except *Oligomeris linifolia* with two or three) (Al-Nowaihi *et al.* 2002).

According to recent seed micromorphology research, the studied species of *Reseda* varied in Turkey (Çilden & Yildirimli 2021). Based on their study, seed color, seed brightness, seed shape, presence of carunculoid tissue (in *R. armena*, *R. coodei*, *R. balansae*, *R. orientalis*, *R. minoica*, and *R. anatolica*), testa surface, and quantitative characteristics measurements such as length and width of seed, and sinus width had higher variation (with four states) (Çilden & Yildirimli 2021).

In the present investigation, eight kinds of seed color and two kinds of seed brightness were determined in the taxa of *Ochradenus* and *Reseda* which accepted the variation of these characteristics with the previous research by Çilden & Yildirimli (2021). In addition, three seed shapes were seen in the studied taxa during the present survey. This result partly agreed with the previous seed studies (Al-Nowaihi *et al.* 2002, Çilden & Yildirimli 2021). In the present study, for a better understanding of the differentiation of studied species, five qualitative characteristics were examined (surface and hilum state, outer epidermal cell shape, situation of anticlinal, and periclinal wall). In this way, the seed surface had the best variation among those confirmed by the result of Al-Nowaihi *et al.* (2002). In addition, anticlinal

and periclinal walls had valuable seed information and there was a differentiation in seed length and width measurement in the results derived from the present study similar to the previous studies (Al-Nowaihi *et al.* 2002, Çilden & Yildirimli 2021). Moreover, the L/W ratio was measured for the first during this research. As a whole, the results of Al-Nowaihi *et al.* (2002) showed that, high variation in seed micromorphology in the *Resedaceae*. The results of the present study on the sculpture issues differ from previous researches (Al-Nowaihi *et al.* 2002, Çilden & Yildirimli 2021, De Leonardis *et al.* 1997). It seems that, this characteristic varied in this family based on ecological conditions. By comparison of both previous seed studies about the presence or absence of aril, there was variety in the taxa of *Reseda* (De Leonardis *et al.* 1997, Al-Nowaihi *et al.* 2002). However, the presence or absence of carunculoid tissue of the two taxa (*R. alba* and *R. lutea*) differs from previous researches (Çilden & Yildirimli 2021). The Absence of this tissue was similar to the results of Çilden & Yildirimli (2021) in *R. aucheri* subsp. *rotundifolia* and *R. luteola*. The other species studied in the present research, were reported for the first time about this tissue. Therefore, the results of this study and previous researches showed variation in the presence or absence of carunculoid tissue.

- Taxonomical issues

In Iran, the *Resedaceae* is represented by 14 species to three genera including *Ochradenus* (three species), *Oligomeris* (one species), and *Reseda* (10 species, eight subspecies and two varieties) (Abdallah & de Wit 1978, Nowroozi 1993). The genus *Reseda* includes 12 species, eight subspecies, and four varieties in the Flora Iranica areas (Abdallah *et al*. 1982). *Reseda* species belong to two subgenera (*Luteola*, *Reseda*). While, subgenus *luteola* incudes one taxon *(R. luteola*) (Abdallah & de Wit 1978). Subgenus *Reseda* has been divided into three sections (*Reseda*, *Leucoreseda* with *R. alba*, and *Phyteuma* with *R. arabica*) (Abdallah & de Wit 1978). Section *Reseda* is categorized into two series (*Reseda* and *Asperula*). In *Aperula* series, there is *R. microcarpa* and *R. buhseana* var. *asperula* (Abdallah & de Wit 1978). Series *Reseda* segregated into subseries *Reseda* with *R. lutea* and *Multilaciniata* series with taxa included *R. aucheri* (with six subspecies), *R. bungei*, *R. hemithamnodes*, *R. macrobotrys*, and *R. buhseana* (with two varieties) (Abdallah & de Wit 1978). Taxonomical issues of *Ochradenus* are considered by Miller (1984).

Recently, molecular data supported taxonomical issues based on morphological characteristics (Martín-Bravo *et al.* 2007). Based on this study, the monophyly of the *Resedaceae* and the situation of three tribes in this family were accepted (Martín-Bravo *et al.* 2007). In addition, the monophyly situation of genera *Caylusea* and *Sesamoides* was determined in the cladogram based on nuclear and plastid markers (Martín-Bravo *et al.* 2007). The other genera of this family include *Ochradenus*, *Oligomeris*, *Randonia*, and *Reseda* gathered in the cladogram as a paraphyletic core group (Martín-Bravo *et al.* 2007). In addition, phylogenetic properties obtained the monophyly placement from six sections of the genus *Reseda* (Martín-Bravo *et al.* 2007).

In the present study, the taxa of genus *Ochradenus* partly distinguished from taxa of *Reseda*. This result corroborated with the core group situation obtained from the cladogram (Martín-Bravo *et al.* 2007). In this way, the studied subspecies belongs to *R. aucheri* and the studied varieties of *R. buhseana* partly followed the taxonomical properties of Abdallah & de Wit (1978). Although the seed dendrogram situation of section *Phyteuma* accepted the monophyly of this section. The studied taxon of section *Leucoreseda* did not support this situation of phylogram by Martín-Bravo *et al.* (2007). In the present study, *R. lutea* (section *Reseda* and from subgenus *Reseda*) and *R. luteola* (subgenus *luteola*) were placed in one seed micromorphological type. Although, phylogenetical and taxonomical issues do not support this result (Abdallah & de Wit 1978, Martín-Bravo *et al.* 2007). Both mentioned taxa were found to be

closely related in the previous micromorphological studies (De Leonardis *et al.* 1997, Çilden & Yildirimli 2021). Subgenus *luteola* had close relations with section *Reseda* of subgenus *Reseda* (Çilden & Yildirimli 2021). *Reseda luteola* and *R. lutea* were placed in one seed cluster here and the seed micromorphological placement was similar to the results derived from the present study. *Reseda microcarpa* from *Asperula* series segregated from the other studied taxa of section *Reseda* based on taxonomical research (Abdallah & de Wit 1978). There was a similar result about *Asperula* series in the dendrogram drawn in the present article. The paraphyletic placement of this section was strongly supported by seed micromorphological data (Martín-Bravo *et al.* 2007). Based on the results derived from the present study, seed micromorphological data overlapped with morphological variety characteristics. In comparison, between the present study in Iran and the recent seed micromorphological study in Turkey (Çilden & Yildirimli 2021), all *Reseda* taxa are grouped with high-resolution clarified subgeneric and sectional taxonomical ranks. However, seed micromorphology in the flora of Egypt had distinct data to clear the generic and specific levels of the *Resedaceae* based on each micromorphological characteristic (Al-Nowaihi *et al.* 2002). All characteristics in this research were not evaluated together for the interpretation of seed taxonomical relations. Only, *R. alba* and *R. decursiva* were introduced as separate species (Al-Nowaihi *et al.* 2002). The similarity between *R. lutea*, *R. luteola*, and the dissimilarity between *R. alba* and *R. lutea* was found by the use of micromorphological characteristics in the Sicily region (De Leonardis *et al.* 1997). The seed dendrogram drawn here confirmed the results of De Leonardis *et al.* (1997).

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