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POLLEN MORPHOLOGY OF EGYPTIAN *ONONIS* L. (FABACEAE) AND ITS TAXONOMIC VALUE

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Pollen morphology of 10 taxa of genus *Ononis* was examined and photographed by SEM to discuss the relationship among them. The taxonomic relationships among taxa were analyzed by means of numerical analysis using UPGMA clustering as well as PCA analysis based on 24 morphological and palynological characters. Shape of pollen, aperture characters and exine sculpturing were described. Based on pollen shape in the equatorial view, four classes were recognized: prolate, subprolate, perprolate and spheroidal–prolate. All the studied pollen grains have tricolporate apertures. The results revealed five patterns of exine sculpturing: reticulate heterobrochate, reticulate-verrucate, punctate, fossulate and microreticulate, the later considered the most dominant type of exine sculpture among the taxa under investigation. *Ononis pubescens* has the largest pollen grains among the taxa under investigation. Pollen characters proved that it can be useful to differentiate taxa on the sub-sectional level, for example *O. diffusa* and *O. serrata* (the representing taxa of sect. *Ononis* sub-sect. *Diffusae*) can be easily differentiated from *O. mitissima* (the representing taxon of sect. *Ononis* sub-sect. *Mitissimae*) by shape of pollen grains which is prolate in the former and spheroidal in the latter.

Keywords

Exine sculpture; Fabaceae; Leguminosae; *Ononis*; Palynology; Taxonomy; Trifolieae

INTRODUCTION

The family Fabaceae (or Leguminosae) is the third largest family of Angiosperms; its members are morphologically, physiologically and ecologically very diverse (Judd, 2002; Lavin *et al.*, 2005; Schwarz *et al.*, 2015; Werner *et al.*, 2015). Therefore, a new sub-familiar classification was suggested to represent their diverse phylogenic history based on matK sequences. Now, Fabaceae is divided into six subfamilies (i.e. Cercidoideae, Detarioideae, Duparquetioideae, Dialioideae, Caesalpinioideae and Papilionoideae). Papilionoideae De Candolle (1825: 94) is the largest subfamily with 30 tribes, 455 genera and about 12,000 species (LPWG, 2017). It is the most widely distributed and their members are adapted to a wide variety of environments.

Fabaceae is stenopalynous family (Luz, *et al.* 2013), with vastly diverse androecium and pollen characters (Kavanagh & Ferguson, 1981; Sørensen, 1989; Pavlova & Manova 2000; Taia , 2004; Lashin 2006). Some examples for the varying androecial characters in Fabaceae are: Number of stamens (1–) 9–10 vs. many; free vs. coherent (monoadelphous, or diadelphous); 1 whorled vs. 2–6 whorled; fertile stamens vs. staminodes; dorsifixed vs. basifixed vs. dorsifixed and basifixed; anthers dehiscing via pores vs. via longitudinal slits; Introrse vs. latrorse; unilocular vs. bilocular and appendaged vs. unappendaged (Prenner, 2004; Marazzi *et al.*, 2007). Some examples for the variation in the pollen characters of Fabaceae are: Pollen shed in monads vs. tetrads or polyads; Pollen grains aperturate vs. inaperturate; (2–) 3(–4) aperturates vs. 6 aperturates; colporate vs, porate vs. colpate (Taia, 2004; Clifford *et al*., 2009; Gunes & Cirpicim 2010; Banks *et al.*, 2017). Thus, Androecium and Pollen features are paramount traits for the classification of legumes at different taxonomic levels (Kavanagh & Ferguson, 1981; Sørensen, 1989; Pavlova & Manova, 2000;

Taia , 2004; Lashin 2006, Galloni *et al.*, 2007; Pinar *et al.*, 2009; Avci *et al.*, 2013; Ceter *et al.*, 2013; Ghadiri *et al.*, 2014; Pinar *et al.*, 2014).

Ononis L. is a large genus belonging to subfamily Papilionoideae. The monophyly of *Ononis* was proved by a comprehensive phylogenetic study done by Turini *et al.*, (2010). The genus was eventually divided into five major lineages. The tribal position of *Ononis* was always considered as a debatable subject. *Ononis* was traditionally treated under tribe Trifolieae (Bentham & Hooker, 1865; Boissier, 1872; Taubert, 1891; Meikle, 1977 and Polhill & Raven, 1981). However, *Onions* has been always regarded to be only marginally related to the other genera in Trifolieae. Ascherson & Graebner (1907) subdivided tribe Trifolieae on the basis of habit (shrubs or subshrubs vs. herbs), androecium (monadelphous vs. diadelphous) and keel (beaked vs. blunt) characters into two subtribes, namely: Ononidinae (including only *Ononis*) and Trifoliinae (including five genera: *Trifolium*, *Trigonella*, *Medicago*, *Melilotus* and *Parochetus*). Hutchinson (1964: 454) proposed a separated tribe named Ononideae to include *Ononis* and its small segregate *Passaea* Adanson (a monotypic genus), this opinion has been followed by some authors (i.e. Huber-Morath 1970, Zohary 1972, Townsend 1974 and Rechinger 1984).

In Egypt, the genus *Ononis* was represented by 9 species (Boulos, 1999); recently, a new species was recorded in south Sinai, namely *O. viscosa* subsp. *breviflora* (Fayed *et al*., in press.).

This study aims to: first describe pollen morphology for ten taxa of *Ononis*, second estimate the taxonomic significance of pollen characters to phenetically divided taxa into different groups using multivariate statistical analysis; and finally, to discuss the significance of pollen features in taxonomy of the genus in Egypt.

MATERIAL AND METHODS

Pollen materials were obtained from the herbarium specimens kept in ASTU, CAI and CAIM. Acronyms according to Thiers (2017) for the palynological investigations. For SEM, dry pollen grains were mounted directly on the stubs and covered with gold in a JEOL JFC 1100E ion sputtering device, then examined and photographed with JEOL JSM 5400LV scanning electron microscopy that is operated at accelerated voltage of 15 kv. at the Electron Microscopy Unit (EMU) in Assiut University, Egypt. Pollen terminology was according to Hesse *et al.*(2009). According to Erdtman's system (1969) and based on the P/E ratio, different classes of pollen shapes are recognized. Numerical analysis was carried out by using NCSS 12 statistical software (Hintze, 1998).

RESULTS

The description of pollen grains morphology of ten species of *Ononis*, is presented in Table 2 and Figures 1, 2, 3 and 4.

1. *Ononis diffusa* Tenore (1811: XLI).

Pollen grains are 3-zonocolporate, prolate, (P/E ratio 1.41), Polar axis 20-23 µm, Equatorial diameter 14-16 µm, concave triangular in polar outline, ovate in equatorial outline. Apocolpium 4-7 µm in diameter. Ectoaperture 16.8-18 µm in length, approximately 1 µm in width with granulate membrane. Endoaperture (ora) lalongate. Mesocolpium 8-10 µm in width. Exine with reticulate sculpture. Lumina 0.55-1 µm in diameter, angular or rounded in shape, heterobrochate (Figures 1: A,B)

Voucher specimen: EGYPT. North Sinai: Rafah, April 1921, *Hefnawy M.* s.n., (CAIM).

2. *Ononis serrata* Forsskål (1775: 130).

Pollen grains are 3-zonocolporate, prolate, (P/E ratio 1.36), Polar axis 17.68-19.46 µm, Equatorial diameter 12.17-15.35 µm, concave triangular in polar outline, ovate in equatorial outline. Apocolpium 8-10.5 µm in diameter. Ectoaperture 14.75-16.45 µm in length, approximately 1 µm in width with granulate membrane. Endoaperture (ora) lolongate. Mesocolpium 9-11.5 µm in width. Exine with microreticulate to fossulate sculpture. Lumina 0.27-0.5 µm in diameter, rounded or irregularly in shape, often contiguous forming irregular fossulae (Figures 1: C,D)

Voucher specimen: EGYPT. El-Garawla, 15 Km before Mersa Matruh, 31º 15' 34" N, 27º 22' 12" E, Alt. 11m, 07 April 2015, *Faried A., Olwey A. & Hassan M.* s.n., (ASTU).

3. *Ononis mitissima* Linnaeus (1763: 1007).

Pollen grains are 3-zonocolporate, spheroidal–prolate, (P/E ratio 1.02), Polar axis 11.56-10.64 µm, Equatorial diameter 10.31-11.09 µm, circular in polar outline, elliptic in equatorial outline. Apocolpium 7-9 µm in diameter. Ectoaperture 8.67-10.5 µm in length, approximately 1 µm in width with granulate membrane. Endoaperture (ora) lolongate. Mesocolpium 5-7 µm in width. Exine with foveolate to microreticulate sculpture. Lumina 0.2-0.5 µm in diameter, rounded or angular in shape (Figures 2: A,B)

Voucher specimen: EGYPT. Cairo: Shubra, on wayside of fields, 10 April 1940, *Shabetai J. R.* z6248, (CAIM).

4. *Ononis variegata* Linnaeus (1753: 717).

Pollen grains are 3-zonocolporate, prolate, (P/E ratio 1.32), Polar axis 15.04-13.54 µm, Equatorial diameter 9.32-12.22 µm, concave triangular in polar outline, oblong in equatorial outline. Apocolpium 7-10 µm in diameter. Ectoaperture 9.81-13.19 µm in length, approximately 1 µm in width with granulate membrane. Endoaperture (ora) lolongate. Mesocolpium 6-10 µm in width. Exine with microreticulate sculpture. Lumina 0.2-0.5 µm in diameter, rounded or angular in shape (Figures 1: E,F)

Voucher specimen: ALGERIA. La Macta: prés de Mostaganem sables maritimes, 07 May 1916, *Faure A.* s.n., (CAI).

***5. Ononis natrix* L. subsp. *stenophylla* (Boiss.) Širjaev (1932: 470).**

Pollen grains are 3-zonocolporate, subprolate, (P/E ratio 1.204), Polar axis 12.88-14.17 µm, Equatorial diameter 14.17-10.82 µm, concave triangular in polar outline, ovate in equatorial outline. Apocolpium 10-12 µm in diameter. Ectoaperture 10.73-11.56 µm in length, approximately 1 µm in width with smooth to slightly granulate membrane. Endoaperture (ora) lolongate. Mesocolpium 5.5 -9.5 in width. Exine with punctate to fossulate sculpture. Lumina (or puncta) very narrow, 0.1-0.25 µm in diameter, rounded to irregularly shaped (Figures 2: C,D).

Voucher specimen: EGYPT. Rafah, near the station, 22 March 1928 *Täckholm G.* s.n., (CAI)

6. *Ononis vaginalis* Vahl (1790: 53).

Pollen grains are 3-zonocolporate, perprolate, (P/E ratio 2.01), Polar axis 23.31-25.64 µm, Equatorial diameter 11.82-12.94 µm, concave triangular in polar outline, oblong in equatorial outline. Apocolpium 8-10 µm in diameter. Ectoaperture18.23-22. 93 µm in length, less than 1 µm in width, with smooth membrane. Endoaperture (ora) lalongate. Mesocolpium 6.5-8.5 µm

in width. Exine with microreticulate. Lumina 0.5-0.9 μm in diameter, angular (Figures 2: E,H).

Voucher specimen: EGYPT. Mersa Matruh: Al Kasr road, 31º 21' 49" N,27º 7' 55" E,3 m alt., 07 April 2015, *Faried A., Olwey A. & Hassan M.* s.n., (ASTU).

7. *Ononis reclinata* L. subsp. *mollis* (Savi) Béguinot (1912: 134).

Pollen grains are 3-zonocolporate, perprolate, (P/E ratio 2.12), Polar axis 21.09-21.49 μm, Equatorial diameter 9.69-10.28 μm, concave triangular in polar outline, oblong in equatorial outline. Apocolpium 6-10 μm in diameter. Ectoaperture 17.89-19.29 μm in length, less than 1 μm in width, with smooth membrane. Endoaperture (ora) lalongate. Mesocolpium 5-8 μm in width. Exine with reticulate. Lumina 0.5-1.36 μm in diameter, polyangular, heterobrochate (Figures 3: A,D).

Voucher specimen: EGYPT. East Sallum: Saret Retama, sandy soil, 16 April 1934, *Shabetai J. R.* z3384, (CAIM).

8. *Ononis pubescens* Linnaeus (1771: 267–268).

Pollen grains are 3-zonocolporate, perprolate, (P/E ratio 2.27), Polar axis 43.97-44.95 μm, Equatorial diameter 19.46-19.77 μm, concave triangular in polar outline, oblong in equatorial outline. Apocolpium 13.5-16 μm in diameter. Ectoaperture 38.19- 40.13 μm in length, less than 1 μm in width, with smooth membrane. Endoaperture (ora) lalongate. Mesocolpium 8-10 μm in width. Exine with reticulate. Lumina 0.63-1.77 μm in diameter, polyangular with small verrucae inside, heterobrochate (Figures 3: E,H).

Voucher specimen: JORDON. 7 km east of Al-Hemma, 13 May 1976, *Täckholm V., El-Hadidi M. N., Lahham J. & Boulos L*. 8907, (CAI).

9. *Ononis sicula* Gussone (1821: 78).

Pollen grains are 3-zonocolporate, prolate, (P/E ratio 1.38), Polar axis 14.18-18.13 μm, Equatorial diameter 10.86-12.51 μm, concave triangular in polar outline, ovate in equatorial outline. Apocolpium 10-12 μm in diameter. Ectoaperture 10.09-16.04 μm in length, about 1 μm in width, with granulate membrane. Endoaperture (ora) lolongate. Mesocolpium 8-10 μm in width. Exine with microreticulate. Lumina very narrow, 0.1-0.4 μm in diameter, rounded to oval (Figures 4: A,B).

Voucher specimen: EGYPT. King Mariut: Champs Caleaur, 15 April 1908, *Lwans* 282, (CAI).

10. *Ononis viscosa* L. subsp. *breviflora* (DC.) Nyman (1878: 161).

Pollen grains are 3-zonocolporate, prolate, (P/E ratio 1.43), Polar axis 13.23-18.34 μm, Equatorial diameter 10.32-13.11 μm, circular in polar outline, ovate in equatorial outline. Apocolpium 8-10 μm in diameter. Ectoaperture 13.01-15.45 μm in length, about 1 μm in width, with smooth membrane. Endoaperture (ora) lolongate. Mesocolpium 7-11 μm in width. Exine with microreticulate. Lumina narrow, 0.3-0.5 μm in diameter, rounded to oval (Figures 4: C,D).

Voucher specimen: EGYPT. Southern Sinai: Wadi Gebal region: Al-Sheq, 28.3226 N,33.5623 E,1940 m Alt., 13 May 2004, *Fayed A., El Garf I., Abdel-Khalik K. & Osman A. s.n.*, (ASTU).

DISCUSSION

According to (Kavanagh & Ferguson, 1981), Papilionoideae have two main types of pollen grains: primitive and advanced. The primitive type has three apertures, colporate, the

exine structure is columellate, the endexine is thin and the foot layer is absent. The advanced type characterized by many apertures, transitions from colporate to simple porate, thick endexine, and the columellae layer of the infratectum is replaced by well-developed spherical sporopollenin bodies known as granular interstitium. Here, we find that pollen of *Ononis* belong to the less advanced type.

Ononis is a stenopalynous genus; different shapes in the pollen outline were recorded (prolate, perprolate, spheroidal, subprolate), P/E ratio ranges from 1.02 to 2.27, and there are different exine sculpture patterns that can be recognized in the pollen of studied taxa (i.e. reticulate, microreticulate, fossulate, foveolate, punctate, verrucate). Our SEM analysis clearly revealed that pollen of *Ononis* is all trizonocolporate in contrast with a previous study by Taia (2004) that reported tricolpate pollen for *O. sicula.*

The length of polar axis (PA) was exceptionally interesting, the variation in length can be assembled into three groups: short (< 20 μm) , medium (21-40 μm) and long (> 40 μm); taxa with short polar axis are (*O. serrata, O. mitissima, O. variegata, O. natrix* subsp. *stenophylla, O. sicula* and *O. viscosa* subsp. *breviflora*), taxa with medium polar axis are (*Ononis diffusa, O. vaginalis* and *O. reclinata* subsp. *mollis*), while *O. pubescens* is the only taxon with long polar axis (see Figure 5).

Despite the genus *Ononis* represents a monophyletic group (Turini *et al.*, 2010), the traditional sectional classification (sensu Širjaev, 1932) has been proved to be unnatural (Turini *et al.*, 2010). Our palynological evidence agrees with this suggestion; there is no evidence from pollen morphology that can split our taxa into two major sections: sect. *Ononis* and sect. *Natrix* (Figure 7). On the other hand, pollen characters can be useful to differentiate taxa on the sub-sectional level, for example *O. diffusa* and *O. serrata* (the representing taxa of sect. *Ononis* sub-sect. *Diffusae*) can be easily differentiated from *O. mitissima* (the representing taxon of sect. *Ononis* sub-sect. *Mitissimae*) by the shape of pollen which is prolate in the former and spheroidal in the latter.

The multivariate analysis was applied to test the significance of pollen morphology in taxonomy of *Ononis* taxa in Egypt. We performed two clustering analysis using the UPGMA method and the Euclidean distance. In the first cluster analysis, only the palynological characters were employed, a phenogram with cophenetic correlation 0.79 was resulted (Figure 6). In the second cluster analysis, combination of exo-morphological and palynological characters were employed, a phenogram with cophenetic correlation 0.78 was resulted (Figure 7). Then, PCA was carried out using combination of exo-morphological and palynological characters to confirm our results (Figure 8).

In the first phenogram (Figure 6), where we employed only palynological characters, three closely allied taxa ere separated into two different groups; in the first group: *O. diffusa* and *O. serrata* (the representing taxa of sect. *Ononis* subsect. *Diffusae*), they have been grouped together at distance of 0.567, they both have prolate pollen. In the second group, *O. mitissima* (the representing taxon of sect. *Ononis* subsect. *Mittisimae*) was separated alone, it can be recognized by possessing spheroidal pollen. Whereas, in the second phenogram (Figure 7) where a combination of exo-morphological and palynological characters was employed, all the three taxa were grouped together in one group at distance 0.466, these taxa were also grouped in the PCA scatter plot (Figure 8), they are belonging to clade V- subclade C sensu Turini *et al.*, (2010): *O. diffusa, O. serrata* and *O. mitissima*, they share many external morphological characters such as, herbaceous habit, most of leaves are trifoliate, adnate stipules, reduced peduncle not elongating to produce arista, white to pink flowers and ovoid or globose pods with few seeds.

Ononis sicula and *O. viscosa* subsp. *breviflora* are very closely allied species, that the former was used to be treated as a subspecies under the latter by some previous authors (i.e. Huber-Morath, 1970). They were both grouped together in the first phenogram (Figure 6**)** at distance of 0.357, and in the second phenogram (Figure 7**)** at distance of 0.259, and in PCA (Figure 8); they have many characters in common, such as the herbaceous habit, the adnate stipules, the yellow flowers, well-developed peduncles that elongate to produce arista, linear pods with many seeds, prolate pollen with short polar axis and microreticulate exine.

Three taxa belonging to sect. *Natrix* sensu Širjae**v** (1932): *Ononis natrix* subsp. *stenophylla*, *O. vaginalis* and *O. reclinata* subsp. *mollis*; they were very distant from one another in the first phenogram (Figure 6) where only pollen characters were employed, they were grouped at a distance of 0.725, because they are not sharing many palynological features, this agrees with Turini *et al.* (2010), who separate these species in different clades (i.e. *O. reclinata* subsp. *molllis* in clade V - subclade B, while *O. natrix* subsp. *stenophylla* and *O. vaginalis* in clade III – subclade A). However, these taxa have been grouped together in the second phenogram (Figure 7**)** at distance of 0.557 and in PCA (Figure 8); because they all have yellow flowers and linear many-seeded pods.

Although, *O. pubescens* was separated from the remaining taxa at distance of 0.61 in the second phenogram (Figure 7) and in PCA (Figure 8), because it exhibits a combination of distinct exo-morphological characters: dense raceme; peduncle not pendent nor forming aristum; calyx as long as or shorter than corolla; corolla yellow with reddish-purple veins on standard and deep yellow notch on top of the keel; standard glabrous, base of style hairy, subglobose pod and smooth seeds. However, its pollen does not exhibit as much uniqueness as the exo-morphology.

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القيمة التصنيفية للشكل الظاهري لحبوب اللقاح في جنس الأُنونس (الفصيلة القرنية) في مصر

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تم في هذه الدراسة مناقشة العلاقات التقاربية بين عشر وحدات تصنيفية من جنس الأُنونس في مصر باستخدام الشكل الظاهري لحبوب اللقاح. تم فحص وتصوير حبوب اللقاح باستخدام الميكروسكوب الإلكتروني الماسح. شملت الدراسة تحليل العلاقات التصنيفية بين الوحدات محل الدراسة باستخدام كل من UPGMA و PCA للتحليل العددي لعدد أربع وعشرون صفة مورفولوجية، تشمل الشكل الظاهري للنبات وكذلك شكل حبوب اللقاح. بالإضافة الي ذلك، تم وصف فتحة الإنبات وكذلك زخرفة الجدار الخارجي لحبوب اللقاح. اسفرت النتائج عن وجود أربعة طرز لشكل حبوب اللقاح: prolate, subprolate, perprolate and spheroidal–prolate. بينت الدراسة ان جميع حبوب اللقاح التي شملتها الدراسة لها ثلاث فتحات شقية ثقبيه. وأظهرت النتائج وجود خمسة أنماط من زخرفة الجدار الخارجي لحبوب اللقاح: شبكي غير منتظم، شبكي به ثأليل، مثقب، محفر، وشبكي مصغر، حيث كان الأخير هو الطراز السائد بين حبوب لقاح الوحدات التصنيفية قيد الدراسة. بالإضافة الي ما سبق، فقد كشفت الدراسة ان *Ononis pubescens* يمكن تميزه عن باقي الأنواع لاحتوائه علي أكبر حبوب اللقاح حجماً. وأثبتت الدراسة أن لصفات حبوب اللقاح أهمية تصنيفية عالية للتفرقة علي مستوي تحت القسم، على سبيل المثال، يمكن التمييز بسهولة بين *O. diffusa* و *O. serrata* (النوعان الممثلان لقسم *Ononis*، تحت – قسم *Diffusae*) عن *O. mitissima* (النوع الممثل لقسم *Ononis*، تحت – قسم *Mitissimae*) باستخدام شكل حبة اللقاح، حيث انها prolate في النوعين المذكوران أولاً و spheroidal في النوع الأخير.

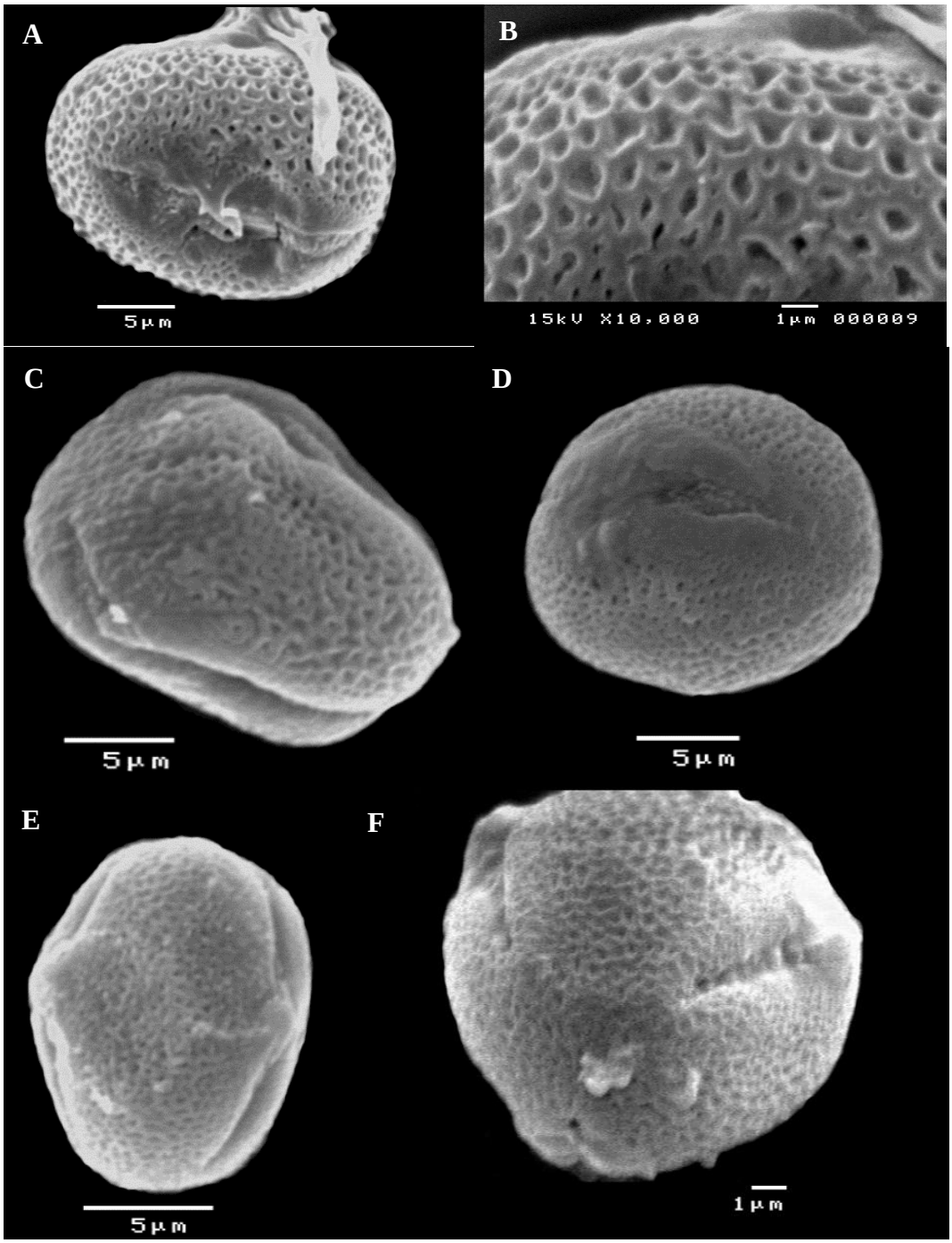

Figure 1: SEM micrographs of pollen grains: A & B, *Ononis diffusa* in the equatorial view showing tricolporate grain and reticulate heterobrochate exine at the mesocolpium with lumina round or angular. C & D, *O. serrata* in the equatorial view showing colporate grain, microreticulate to fossulate exine at the mesocolpium. E, *O. variegata* in the equatorial view showing colporate grain, lolongate endoaperture and microreticulate exine at the mesocolpium. F, *O. variegata* in the polar view showing three crassimarginate colpi and microreticulate exine at the apocolpium**.**

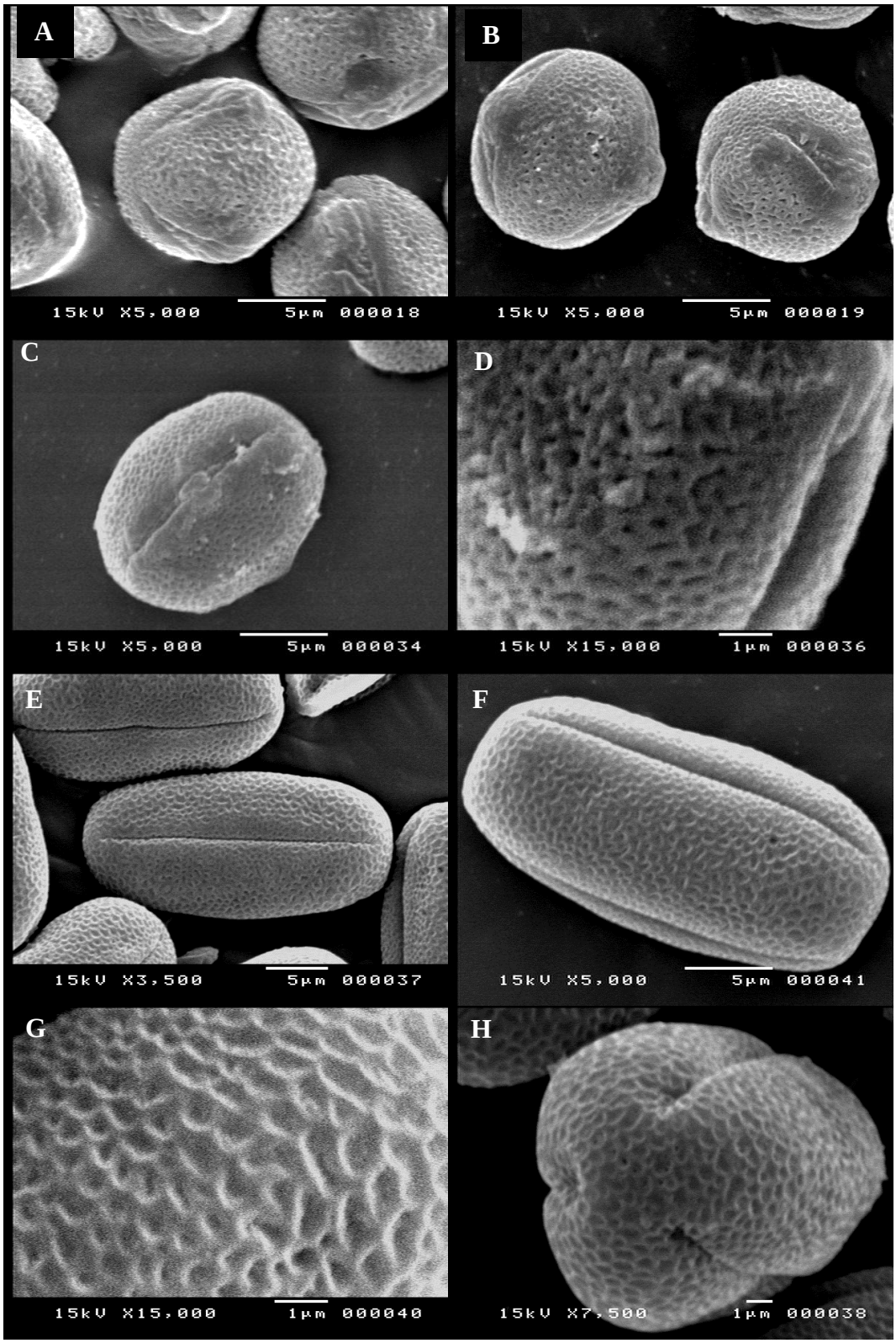

Figure 2: SEM micrographs of pollen grains: A, *Ononis mitissima* in the equatorial view. B, *O. mitissima* in the equatorial (left) and polar (right) views, showing tricolporate grains with foveolate to microreticulate exine and lolongate endoaperture. C & D, *O. natrix* subsp. *stenophylla* in the equatorial view showing tricolporate pollen and punctate to fossulate exine at the mesocolpium with rounded to irregular lumina. E, F & G, *O. vaginalis* in equatorial view showing tricolporate pollen with microreticulate exine at the mesocolpium. H, *O. vaginalis* in the polar view showing concave triangular pollen with crassimarginate colpi and microreticulate exine at the apocolpium.

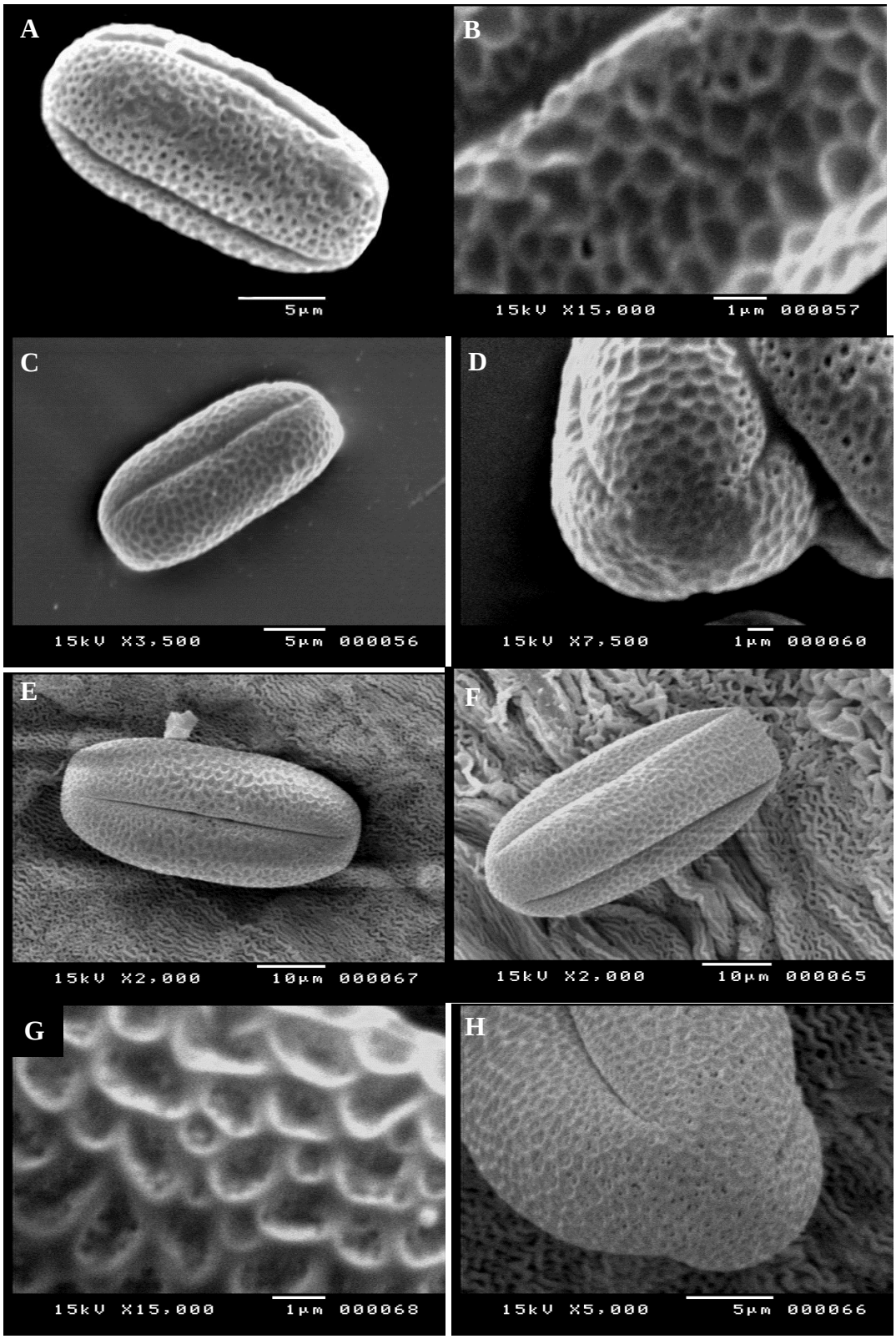

Figure 3: SEM micrographs of pollen grains: A, B & C*, Ononis reclinata* subsp. *mollis* in the equatorial view showing tricolporate pollen and reticulate exine at the mesocolpium. D, *O. reclinata* subsp. *mollis* in polar view showing concave triangular pollen with crassimarginate colpi with reticulate exine at the apocolpium. E, F & G, *O. pubescens* in the equatorial view showing tricolporate pollen with lalongate endoaperture and reticulate exine at mesocolpium, lumina with small verrucae inside. H, *O. pubescens* in the polar view showing reticulate-verrucate exine on apocolpium.

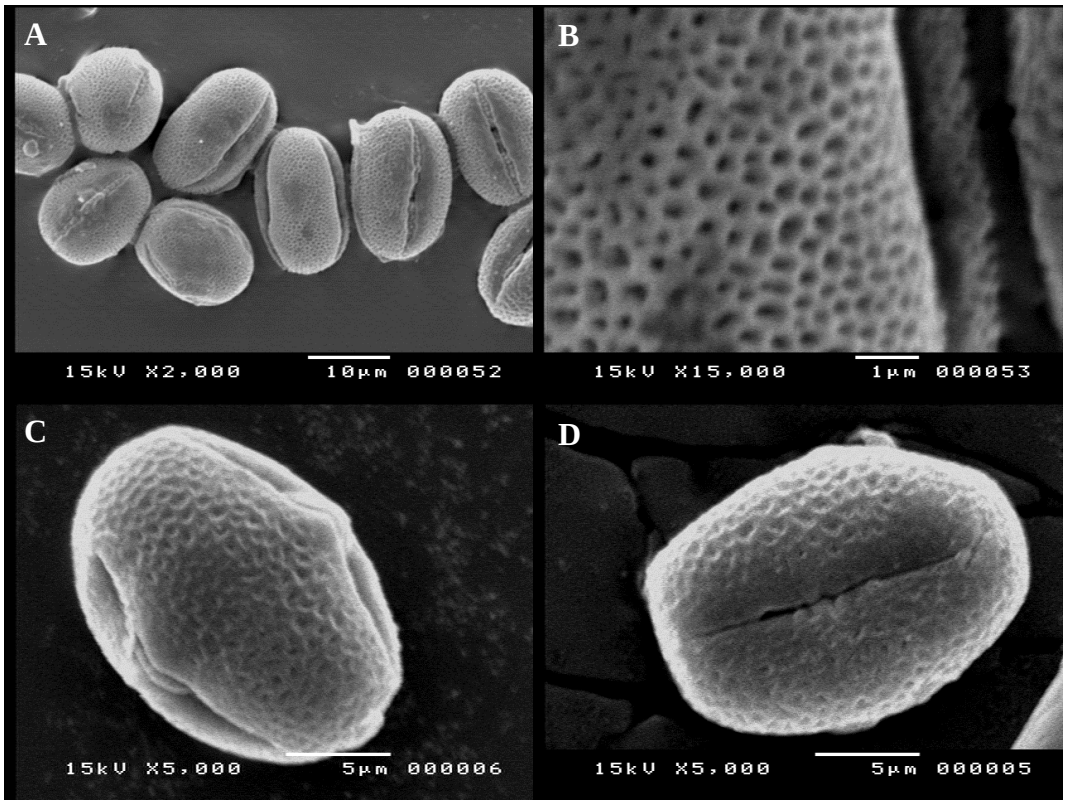

Figure 4: SEM micrographs of pollen grains: A & B, *Ononis sicula* showing tricolporate pollen with microreticulate exine. C & D, *O. viscosa* subsp. *breviflora* in the equatorial view showing tricolporate pollen with lolongate endoaperture and microreticulate exine at the mesocolpium

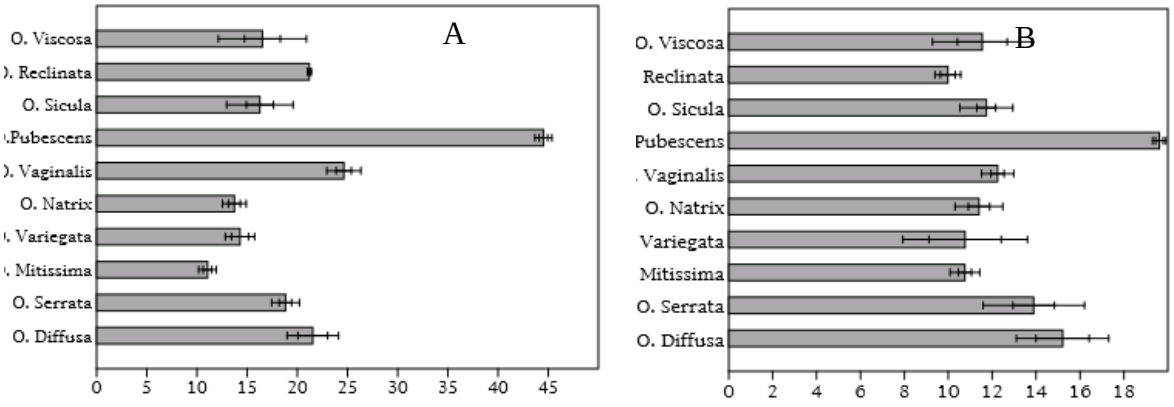

Figure 5: Bar-chart showing: A, length of polar axis. B, length of equatorial diameter in studied taxa of *Ononis*.

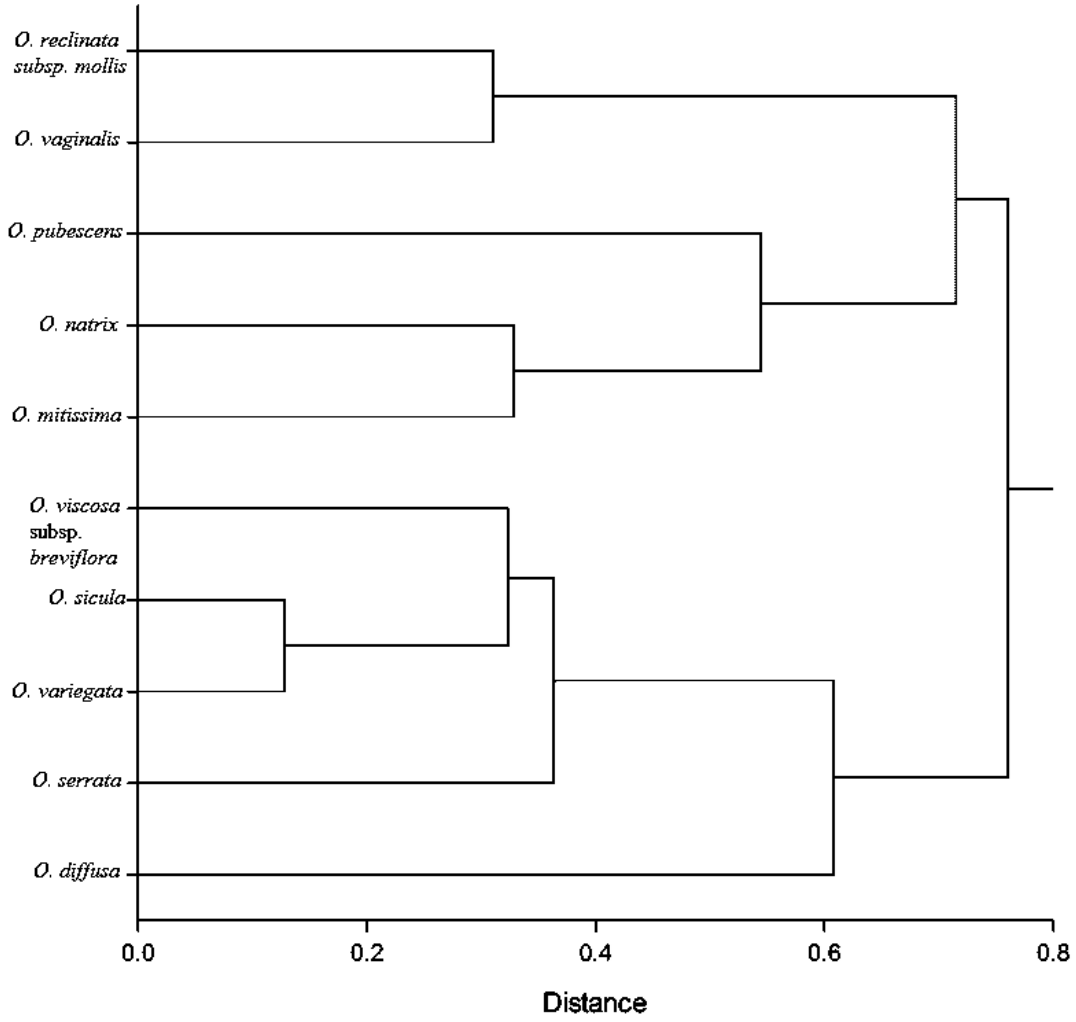

Figure 6: UPGMA phenogram using Euclidean distance of 10 palynological characters of the studied taxa of *Ononis*.

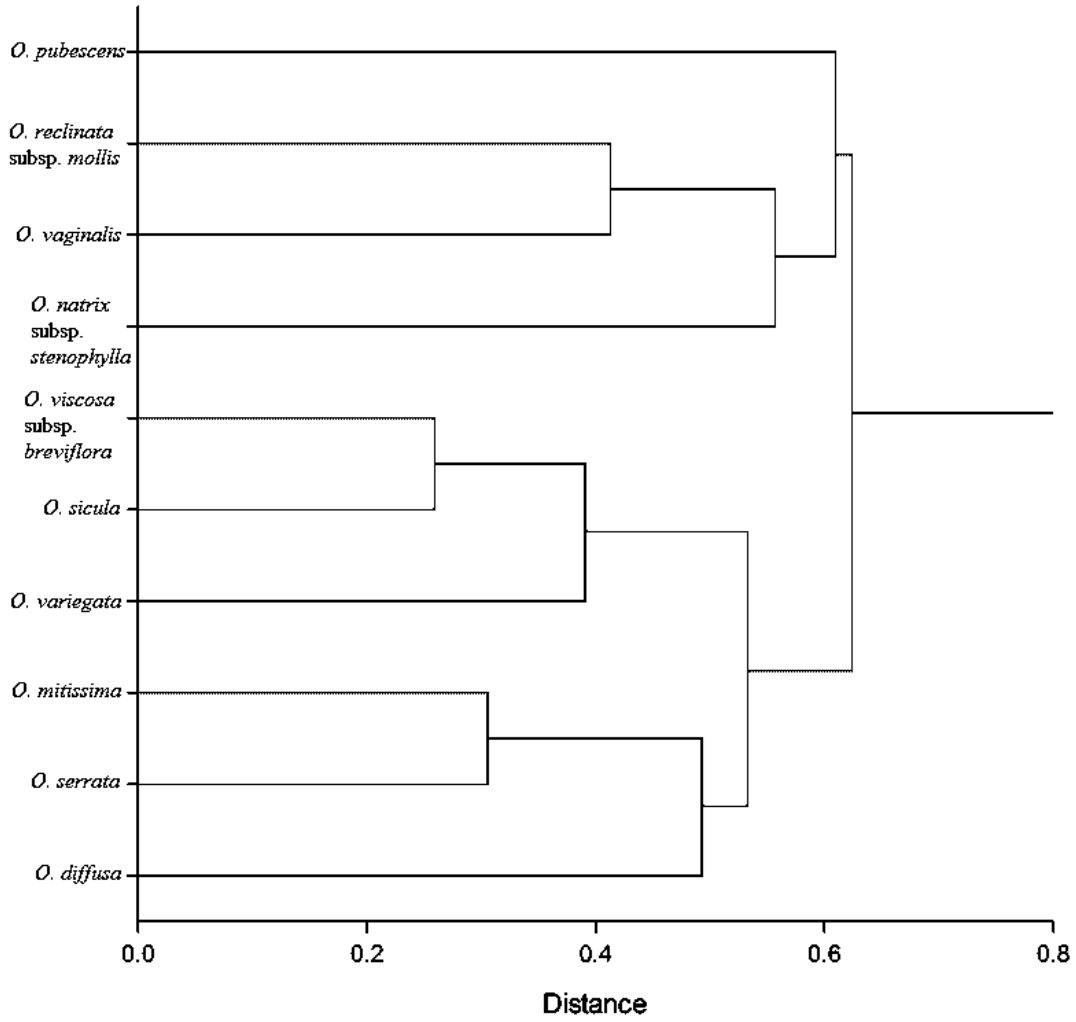

Figure 7: UPGMA phenogram using Euclidean distance of 24 morphological and palynological characters of the studied taxa of *Ononis.*

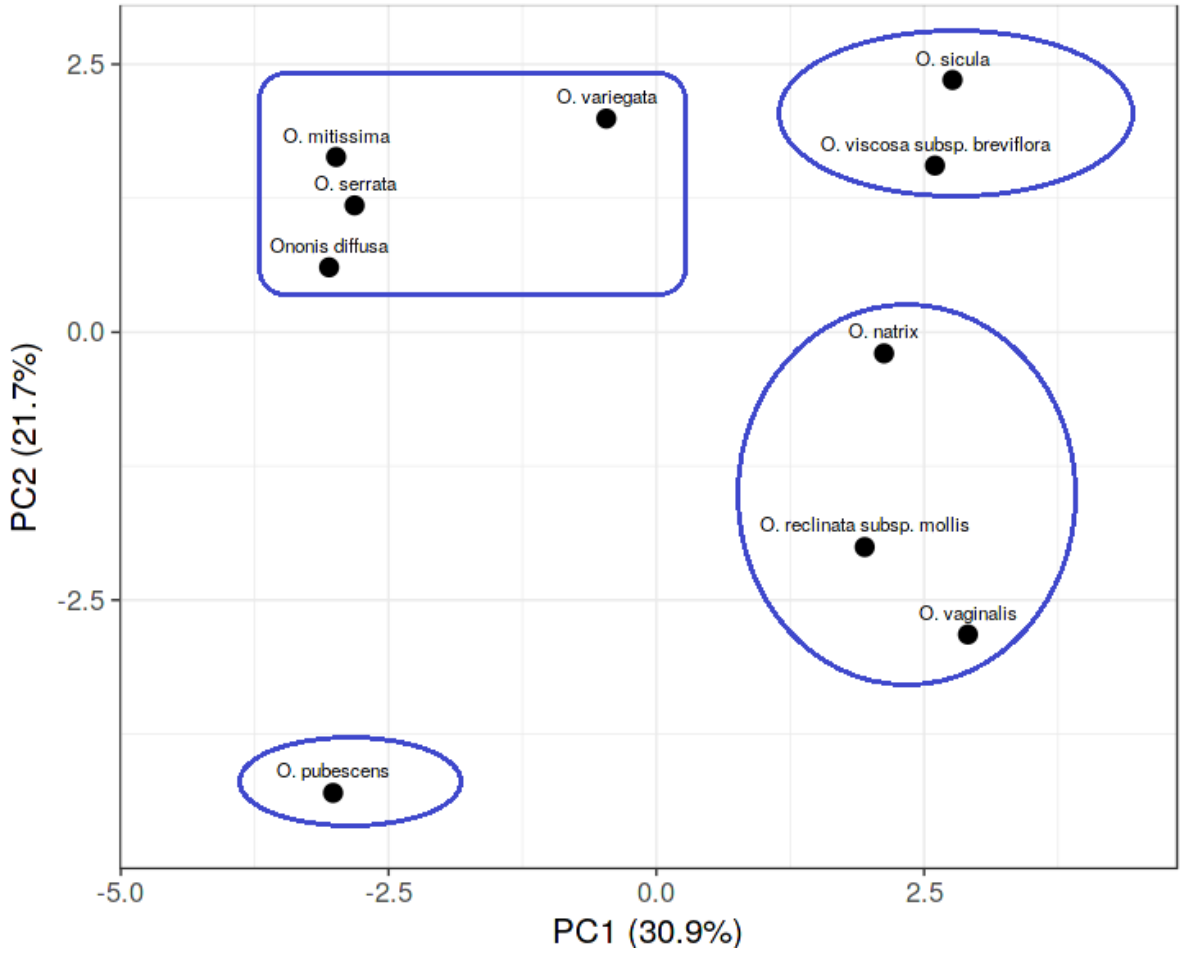

Figure 8: Scatter plot of the first two principal components of the studied taxa of *Ononis* hat explain 30.9% and 21.7% of the total variance, respectively.

Table 1: Morphological characters of pollen of *Ononis* taxa in Egypt

Taxon	**Polar axis (PA) µm**			**Equatorial diameter (ED) µm**			**Length of ectoaperture (L) µm**			**P/E**	**Pollen shape**	**Exine ornamentation**
	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD			
Ononis diffusa	20.18	22.78	21.54 ± 1.3	14.11	16.25	15.21 ± 1.07	16.86	18.14	17.5 ±0.64	1.41	prolate	reticulate, heterobrochate
O. serrata	17.68	19.46	18.82 ± 0.7	12.17	15.35	13.89 ± 1.32	14.75	16.45	15.6 ± 0.85	1.36	prolate	microreticulate to fossulate
O. mitissima	10.64	11.56	11.05 ± 0.45	10.31	11.09	10.76 ± 0.39	8.67	10.5	9.59 ± 1.29	1.02	spheroidal – prolate	foveolate to microreticulate
O. variegata	15.04	13.54	14.29 ± 0.75	9.32	12.22	10.77 ± 1.45	9.81	13.19	11.5 ± 1.69	1.32	prolate	microreticulate
O. natrix subsp. *stenophylla*	12.88	14.17	13.73 ± 0.6	10.82	12.01	11.39 ± 0.64	10.73	11.56	11.17 ± 0.41	1.204	subprolate	punctate to fossulate
O. vaginalis	23.31	25.64	24.63 ± 0.86	11.82	12.94	12.25 ± 0.37	18.23	22.93	20.77 ± 1.69	2.01	perprolate	microreticulate
O. reclinata subsp. *mollis*	21.09	21.49	21.29 ± 0.2	9.69	10.28	9.97 ± 0.42	17.89	19.29	18.49 ± 0.72	2.12	perprolate	reticulate, heterobrochate
O. pubescens	43.97	44.95	44.52 ± 0.37	19.46	19.77	19.61 ± 0.15	38.19	40.13	39.34 ± 0.83	2.27	perprolate	reticulate-verrucate
O. sicula	14.18	18.13	16.27 ± 1.54	10.86	12.51	11.73 ± 0.61	10.09	16.04	13.75 ± 2.16	1.38	prolate	microreticulate
O. viscosa subsp. *breviflora*	13.23	18.34	16.52 ± 2.3	10.32	13.11	11.55 ± 1.16	13.01	15.45	14.13 ±1	1.43	prolate	microreticulate

Table 2: Studied taxonomic characters, character states and their taxonomic codes

Code	Taxonomic characters	Character states and taxonomic codes
1	Habit	herb (1); subshrub (2)
2	Leaves	mostly trifoliate (1); mostly unifoliate (2)
3	Stipules	adnate to leaf base (1); sheathed (2)
4	Type of inflorescence	solitary flowers (1); flowers arranged in raceme (2)
5	Flower	pendulous (1); not pendulous (2)
6	Colour of flower	white with pink veins (1); yellow with purple veins (2).
7	Aristum	present (1); absent (2)
8	Length of corolla in relation to length of calyx	corolla obviously shorter than calyx (1); corolla sub-equaling calyx, slightly longer or slightly shorter than calyx (2); corolla longer than calyx (3)
9	Conjunctive tooth	present (1); absent (2)
10	Style	glabrous (1); hairy at the base (2)
11	Pod length	short (4-8 mm) (1); long (9-23 mm) (2)
12	Pod shape	globose, elliptic or ovoid (1); oblong (2); linear (3)
13	Number of seeds per pod	few (1 or 2) (1); medium (2-5) (2); many (up to 25) (3)
14	Seed surface	smooth (1); tuberculate (2)
15	Pollen shape in equatorial view	prolate (1); spheroidal – prolate (2); subprolate (3); perprolate (4)
16	Pollen shape in polar view	concave triangular (1); circular (2)
17	Ectoaperture length	< 15 μm (1); > 15 μm (2)
18	Length of polar axis	< 20 μm (1); 21-40 μm (2); > 40 μm (3)
19	Colpus membrane	granulate (1); smooth (2)
20	Endoaperture	lalongate (1); lolongate (2); circular (3)
21	Exine sculpture on mesocolpium	reticulate (1); microreticulate (2); fossulate (3); foveolate (4);punctate (5); verrucate (6)
22	Exine sculpture on apocolpium	reticulate (1); microreticulate (2); fossulate (3); foveolate (4);punctate (5); verrucate (6)
23	Exine lumina diameter	0.5 μm or less (1); more than 0.5 μm (2)
24	Exine lumina shape	angular (1); rounded (2); irregular (3)
25	Apocolpium diameter	< 10 μm (1); ≥10 μm (2)
25	Mesocolpium width	≤ 7 μm (1); > 7 μm (2)