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Phylogenetic and Palynological Study of the Genera *Agrimonia* L., *Alchemilla* L., *Geum* L. and *Poterium* L. (Rosaceae) in Kurdistan Region-Iraq

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Abstract

The present study involved seven species of four genera within the family Rosaceae that are available in Kurdistan Region–Iraq: *Ag. eupatoria* L., *Al. kurdica* Rothm., *Al. persica* Rothm., *G. urbanum* L., *P. lasiocarpum* Boiss. et Hausskn. ex Boiss., *P. sanguisorba* L. (involved two subspecies: *P. sanguisorba* subsp. *sanguisorba* and *P. sanguisorba* subsp. *muricatum* (Spach) Rouy et Fouc.) and *P. verrucosum* Ehrenb. The objective of this study was to investigate phylogenetic relationships among the studied genera, as well as in detail pollen grains study of the species. Molecular phylogenetic of the genera were studied using nrDNA ITS and 28SrRNA regions, phylogenetic analysis was performed using maximum parsimony approach as implemented in PAUP*, Bayesian method using MrBayes program for both regions. Palynological study were studied by using Light Microscope (LM) and Scanning Electron Microscope (SEM). The results from molecular study showed that three clades of both regions with the minimum differences in the locations of the species. Furthermore, pollen grains were tri-colporate, monads with different shapes. It is concluded that the genera are divided on three tribes Agrimonieae, Potentllaea and Coluriea.

keywords: palynological, phylogenetic, ITS, 28SrRNA, *Agrimonia*, *Alchemilla*, *Geum*, *Poterium*

1. Introduction Rosaceae family involves 3200 species throughout the world which distributed on 115 genera (Core, 1955); 2800 species on 95 genera (Sipson, 2006) and in Iraq, involves 53 species distributed on 19 genera (Al-Rawi, 1964). In Iraq, the genera *Agrimonia*, *Alchemilla*, *Geum* and *Poterium* have not been dealt with extensive studies as the other Iraqi plant genera which they also in need to phylogenetic and palynological studies, where the Flora of Iraq is still in need to this type of study.

The first practical use of molecular data in Rosaceae was done by Takhtadzhian et al., (1997). Integrating some of the possible results of the first molecular phylogenetic study of proper relationships across Rosaceae, traditionally recognized twelve subfamilies (Morgan et al., 1994). However, several reliable sources of molecular data adequately support the monophyly of Rosaceae (Potter et al., 2007, Evans et al., 2000, Morgan et al., 1994, Faghir et al., 2014).

Potter et al., (2002), showed the phylogenetic relationships within 37 genera of Rosaceae, which are traditionally divided into 4 subfamilies and 3 related genera based on two specific regions of chloroplast DNA, the *matK* and the *trn*L-F genes. In addition, Potter *et al.* (2007), accurately classified 88 distinct genera of Rosaceae constructed on phylogenetic relationships based on nucleotide sequence data of six nuclear (18S, *gbssi1*, *gbssi2*, ITS, *pgip*, and *ppo*) and four chloroplasts (*matK, ndhF, rbcL, and trnL-F*) regions. Faghir et al., (2018), revealed that phylogenetic relationship of the 5 species of *Geum* were strongly under the influence of hybridization and polyploidy (allopolyploidy).

The genus *Alchemilla* L., known for its medicinal and ornamental value, is widely distributed in the Holarctic regions with a few species found in Asia and Africa. Delimitation

of species within *Alchemilla* is difficult due to hybridization, autonomous apomixes, and polyploidy, necessitating efficient molecular-based characterization. The availability of complete chloroplast genome in the genus *Alchemilla* will contribute to species delineation and further phylogenetic and evolutionary studies in the family Rosaceae (Rono et al., 2020).

The genus *Agrimonia* L. typifies the tribe Sanguisorbeae of the sub-family Rosoideae in Rosaceae. The genus is herbaceous and composed of 15 species distributed in Northern and Southern temperate regions of the world (Santapau and Henry, 1973). In India, the genus is represented by 2 species as *A. eupatoria* and *A. pilosa*. *A. eupatoria* L. is popularly known as "Agrimony" (Kumar et al., 2011).

Phylogeny and biogeography of subtribe Agrimoniinae (Rosoideae) composed of four monotypic endemics (*Aremonia*, *Hagenia*, *Leucosidea*, and *Spenceria*) and a worldwide genus (*Agrimonia*) are constructed from nuclear and plastid sequences (GBSSI-1, trnL-trnF, trnS-trnG-trnG). All nucleotide data support the monophyly of subtribe Agrimoniinae with a basal group of the Asian monotypic *Spenceria*, the sister relationship of the African genera *Hagenia* and *Leucosidea*, and the monophyly of *Agrimonia* and *Aremonia*, a European monotypic genus (Chung, 2008).

Sanguisorba, commonly known as burnet, is widespread in the Northern Hemisphere and consists of ten to 30 herbaceous species (Mabberley, 1997, Li, 2007), depending on the circumscription of the genus. *Sanguisorba* is traditionally known for its various medical applications (Cheng and Cao, 1992, Shin et al., 2002, Park et al., 2004, Cai et al., 2012). There has been much taxonomic confusion concerning the genus since its description by Linnaeus in 1753, with the recognition of several taxa as independent genera or as species of *Sanguisorba*. Recent phylogenetic and biogeographic analyses show that *Sanguisorba* in its traditional sense is paraphyletic and should be divided into three genera (*Poteridium* Spach, *Poterium* L. and *Sanguisorba*). Phylogenetic studies based on molecular data indicated that *Sanguisorba* and *Poterium* form the earliest branching taxa of Sanguisorbinae (Wang et al., 2020).

Pollen grains are important character for taxonomic purposes. In addition to the taxonomic value of the pore structure the variations in the pollen grain sculptures are important features for species classification (Reitsma, 1966). Comparative studies of pollen morphology have provided useful characters for demarcating genera and species and determining relationships in several ancestries of Rosaceae (Reitsma, 1966, Eide, 1981, Hebda et al., 1991, Hebda and Chinnappa, 1994, Dönmez, 2008, Wrońska-Pilarek, 2011).

Faghir et al., (2015) by using both light microscope (LM) and scanning electron microscope (SEM), showed that the pollen grains of genus *Alchemilla* are monad, radially symmetrical, isopolar, or subisopolar; small to medium in size; tri- and tetracolporate; rectangular to cylindrical (from equatorial view) and triangular to circular (from polar view) in outline; and prolate-spheroidal to subprolate and prolate in shape. The exine ornamentation is psilate and microechinate.

Perveen and Qaiser, (2014) showed that the pollen grains of the genus *Geum* are usually free, radially symmetrical, isopolar, prolate-spheroidal to subprolate or oblate-spheroidal rarely perprolate, tricolporate rarely tricolpate. Tectum mostly coarsely-finely striate, rarely striate-rugulate, scabrate or spinulose often reticulate. Saffari et al., (2021) exposed that the pollen grains of *Ag. eupatoria* are monad, radially symmetrical and tricolporate with striate exine.

Chung et al., (2010) revealed that the pollen grains of sanguisorbeae are generally subprolate to spheroidal in shape, had operculate or pontoperculate apertures, and had three apertures. Exine sculpturing within Sanguisorbinae represented variations of striate, verrucate, rugulate, and perforate patterns often with microechinate sculpturing.

The aim of the present study is to investigate the relativeness among the genera *Agrimonia*, *Alchemilla*, *Geum* and *Poterium* based on the phylogenetic relationships and comparing with the nearest genus. For this purpose, two regions were selected, the first region is nuclear ribosomal DNA ITS and the second is 28SrRNA. Furthermore, to investigate the morphology of the pollen grains of the genera under study

2. Materials and Methods

2.1. Phylogenetic Study

2.1.1. Taxon Sampling

The plant taxa used in the present study were collected from the different districts of Kurdistan Region-Iraq, as well as the preserved specimens in the Herbaria of College of Education and College of Science/Salahaddin University. Nine distinct taxa consist of eight ingroup taxa and one out group *Rhamnus cathartica* were used in the analysis. The outgroup sequence was obtained from gene bank (Voucher IRAN: 36839, Accession numbers MT735328 and KX167927 for ITS region and 28SrRNA region respectively) (Table 1).

2.1.2. DNA Extraction

Total DNA was extracted from the collected specimens. The extraction method was based on the CTAB protocol of Doyle and Doyle (1990) with some modification (1X CTAB: 10 mL of 1.0 M Tris-HCl, PH 8; 4 mL of 0.5 M EDTA, PH 8; 28 mL of 5 M NaCl; 2% CTAB; 2 g PVP; and 158 ddH_2O), the washing process of the DNA pellet has been conducted twice with 0.5 mL of 80% ethanol, then DNA was dissolved in 25 µl TE-buffer.

2.1.3. PCR and DNA sequencing

The two noncoding regions of nrDNA and 28SrRNA were amplified by using the primers as shown in (Table 2). The primers were ordered from Macrogen Company, Seoul, Korea. The total volume of amplification reactions was 25 µL and Master Mix made up of 12.5 µL, 3 µL genomic DNA extract, 2 µL of each primer, 5.5 µL free nuclease water. The PCR-Thermal cycler for 28SrRNA gene started with 5 min for initial denaturation at 94 C° followed by 35 cycles: denaturation at 94 C° for 30 sec.; annealing at 54 C° for 60 sec.; extension at 72 C° for 60 sec. and the final extension at 72 C° for 5 min. While, the PCR program for ITS gene started with 5 min for initial denaturation at 94 C° followed by 35 cycles: denaturation at 94 C° for 30 sec.; annealing at 56 C° for 20 sec.; extension at 72 C° for 20 sec. and the final extension at 72 C° for 5 min. The resultant PCR products were checked on 1.5% agarose gel run in TAE buffer. The gel was stained with Safe red dye and photographed under UV transilluminator. PCR products were purified by using Kits (Promega Company-Madison-USA). The purified PCR products were sent to the National Science and Technology Development Agency (NSTDA) in Thailand for sequencing.

Table 1: Specimen numbers of *Agrimonia*, *Alchemilla*, *Geum* and *Poterium* species which their DNA and pollen grains have been studied, and their preserved locations in the Herbarium of College of Education/ Salahaddin University with collection date

Species	Specimen number & Herbarium symbol		Specimen location	Date of collection
Ag. eupatoria	8021	ESUH	Kory valley	7.11.2021
Al. kurdica	8028	ESUH	Halgurd M.	7.7.2011
Al. persica	8035	ESUH	Sakran M.	13.7.2018
G. urbanum	8042	ESUH	Rowanduz	1.5.2005
P. lasiocarpum	8049	ESUH	Hasarost M.	14.9.2021
P. sanguisorba	80568	ESUH	Halgurd M.	26.7.2018
P. verrucosum	00064	ESUH	Haji Omran	20.7.2007

Table 2**:** list of primers and their sequences that have been used in the study.

Primer name	Product size	Sequence 5'---- 3'		References
		Foreword	Reverse	
28S rRNA	700 bp	TCT GAC ATG TGT GCG AGT CA	GAT TCG GCA GGT GAG TTG TT	(Chen et al., 2010)
ITS	400 bp	ATG CGA TAC TTG GTG TGA AT	TCC TCC GCT TAT TGA TAT GC	(Taberlet et al., 1991)

2.1.4. Sequence Alignment and Phylogenetic Analysis

All the DNA sequences were edited and aligned with ClastalW option available in BioEdit, Version 7.0.4.1 (Hall, 2001) and manual adjustment, there are 9 accessions for each ITS, 28SrRNA, including the out group species. Bayesian inference (BI) and Maximum parsimony (MP) analyses were conducted for each dataset separately built from the two markers that included 9 terminal taxa with all sequences available. For MP, PAUP_ 4.0a164 (Swofford, 2000) was also used. Using heuristic search with 100 replicates of random taxon additions, Tree-Bisection-Reconnection (TBR) branch swapping, MulTrees on, and steepest decent off was performed. The maximum numbers of saved trees were 100 for each replicate. The bootstrap values were calculated from 100 replicates, the consistency index (CI), retention index (RI), rescaled consistency (RC), and homoplasy index (HI) were measured (Felsenstein, 1985).

Before running BI, the optimal substitution models were estimated using the Akaike information criterion (AIC) in MrModeltest2 version 2.3 (Nylander et al., 2004). The general time reversible model of nucleotide substitution with gamma-shaped rate variation and a proportion of invariable sites (GTR+I+G) was the estimated best-fit model for ITS region and (GTR+G) was the estimated best-fit model for 28SrRNA. For BI analyses we used MrBayes v.3.2 (Ronquist and Huelsenbeck, 2003). The priors on state frequencies and rates and variation across sites were estimated automatically by the program. Four Markov chains starting with a random tree were run simultaneously, two independent analyses were run with 2 million generations set for ITS and 5 million generations for 28SrRNA datasets with four chains (one cold and three heated) for each generation and the temperature parameter set to 0.1. Trees were sampled every 100th generations. After that (25% of initial tree sampled) were removed by burn-in period samples, a tree with maximum 50% (majority rule consensus tree) was plotted. The value of posterior probability (PP) was calculated and the final tree was plotted by using FigTree software version 1.4.3 (Rambaut, 2016).

2.2. Palynological Study

a. Light Microscope (LM)

For light microscope, a mature anther has been taken from a fresh specimen and has put in a clean hour glass, a drop of safranin-glycerine stain has added to it (Al-Mayah, 1983). The anther has been opened by two dissected needles, then the pollens have pull with the stain by using a special dropper for each species, and have put on a clean slide, then covered by the cover slip slightly, at this stage the slide was ready for examine.

In the present study, the data have been taken from (10-15) specimens for each species. The slides have examined under Olympus-compound microscope, and photographed by using A mobile camera (Sumsung-A5) in Education College/Salahaddin University.

b. Scanning electron microscope (SEM)

For scanning electron microscope, the anthers were washed with sterilized distil water in eppendorf tubes and then dehydrated with alcohol series 50%, 70%, 80%, 85%, 90%, 95% and three times 100%, followed by three times of 100% acetone for 30 min each time, then left to dry in the room temperature to eliminate the large amount of impurities that might hinder vision of the structural characteristic of the wall. Finally, the grains were mounted on metal stup with double-side cellophane tape and then coated with a film of gold palladium by the aid of sputtering chamber, after that the coated samples were viewed under scanning electron microscope (INSPECT S50) in (College of Science / Kufa University). The pollen terminology used in according of (Erdtman, 1952) and (Ueda and Tomita, 1989).

3. RESULT

3.1. Sequence alignment

The ITS length of all sequences was 364 bp. While, the 28SrRNA sequences was 593 bp. The sequence lengths were different in the numbers of characters included because of ambiguity at the beginning and end of sequences (Table 2).

3.2. ITS Region

The ITS data set consisted of eight ingroups and one outgroup taxa with 364 aligned DNA characters (including gaps) were used, from them 90 were parsimony informative. The maximum parsimony analysis showed 100 trees from which a single most parsimonious tree was retained with a tree length of 435 steps. The CI, RI, RC and HI were 0. 920, 0.739, 0.679 and 0.080, respectively, with topology identical between MP and BI analyses. The summary of the analysis showed in (Table 3).

Table 3: A summary of alignment and tree statistics of 28SrRNA and nrDNA ITS region analyses.

Parameters/Regions	ITS	28srRNA
Aligned length	364	593
Number of parsimony informative characters	90	95
Number of variable parsimony uninformative characters	221	416
Number of constant characters	53	82
Tree length (steps)	435	698
CI (Consistency Index)	0.920	0.974
RI (Retention Index)	0.739	0.833
RC (Rescaled Index)	0.679	0.812
HI (Homoplasy index)	0.080	0.026
Model	GTR+G+I	GTR+G

3.2.1. Maximum Parsimony (MP)

The strict consensus tree generated by summarizing the entire most parsimonious tree displays three clades divided on three tribes described by color Figure (1): Tribe Agrimonieae consists of *P. lasiocarpum*, *P. verrucosum*, *P. sanguisorba* subsp. *Sanguisorba*, *P. sanguisorba* subsp. *Muricatum* and *Ag. eupatoria*. with bootstrap support (bs=97%) which is

strongly supported; the tribe Potentilleae consists of *Al. kurdica* and *Al. persica* with highly supported (bs=93%); while the tribe Coluriea consists of only *G. urbanum* and is highly supported (bs=90%).

3.2.2. Bayesian Inference (BI) Analysis

Bayesian AIC majority rule consensus tree Figure (1) resulting from the analysis showed the same results as the MP tree. With different the posterior probability (pp) value ranges were between (0.84-1.00).

3.3. 28SrRNA Gene

The 28SrRNA gene consisted of eight ingroups and one outgroup taxa with 593 aligned DNA characters (including gaps) were used, from them 95 were parsimony informative. The maximum parsimony analysis showed 100 trees from which a single most parsimonious tree was retained with a tree length of 698 steps. The CI, RI, RC and HI were 0. 974, 0.833, 0.812 and 0.026, respectively, with topology identical between MP and BI analyses. The summary of the analysis showed in (Table 3).

3.3.1. Maximum Parsimony (MP)

The strict consensus tree generated by summarizing the entire most parsimonious tree displays three clades divided on three tribes described by color Figure (2): Tribe Agrimonieae consists of *P. lasiocarpum*; *P. verrucosum*; *P. sanguisorba* subsp. *Sanguisorba; P. sanguisorba* subsp. *Muricatum* and *Ag. eupatoria*. with bootstrap support (bs=97%) which is strongly supported; the tribe Potentilleae consists of *Al. kurdica* and *Al. persica* with highly supported (bs=93%); while the tribe Coluriea consists of only *G. urbanum* and is highly supported (bs=90%).

3.3.2. Bayesian Inference (BI) Analysis

Bayesian AIC majority rule consensus tree Figure (2) resulting from the analysis showed the same results as the MP tree. With different the posterior probability (pp) value ranges were between (0.84-1.00).

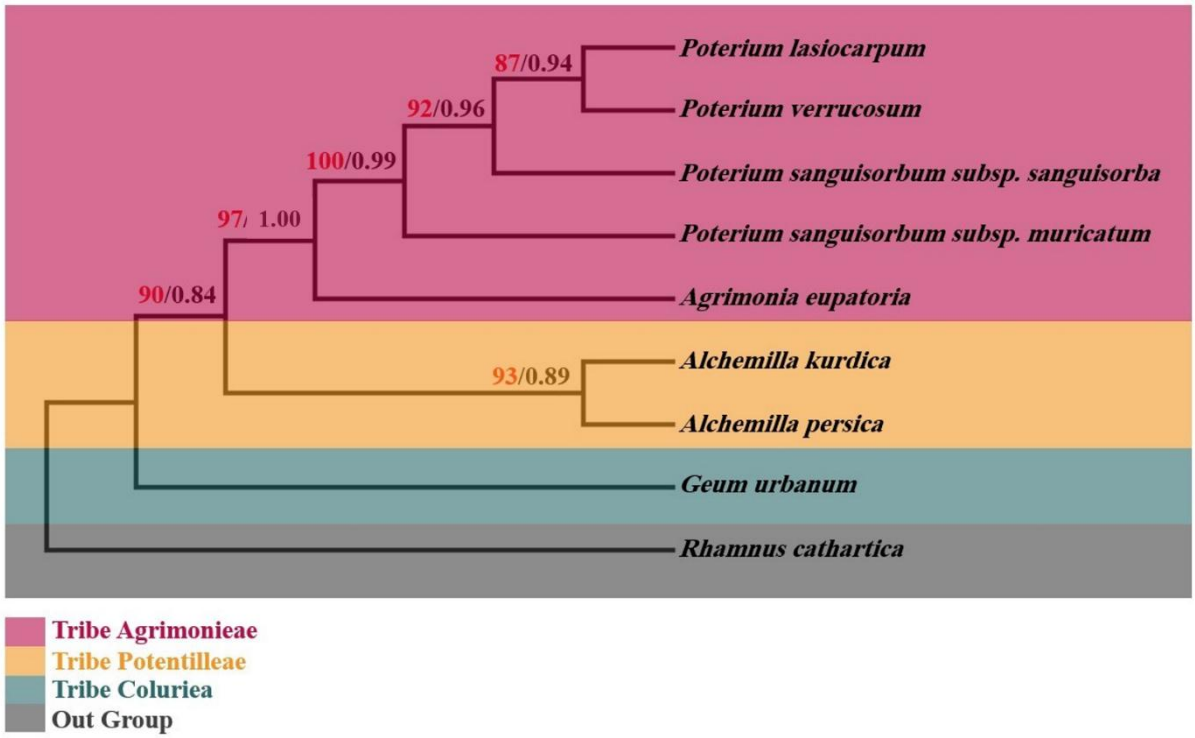


Figure 1. Strict consensus tree of most parsimonious tree resulting from phylogenetic analysis of the nrDNA ITS sequences with heuristic search using maximum parsimony analysis. (Tree length of 364 steps, CI = 0.920, RI = 0.739, RC = 0.679 and HI =0.080). Numbers on the branches which in red color indicate bootstrap support and numbers in black color are Bayesian posterior probability values and tribes are identified by colors.

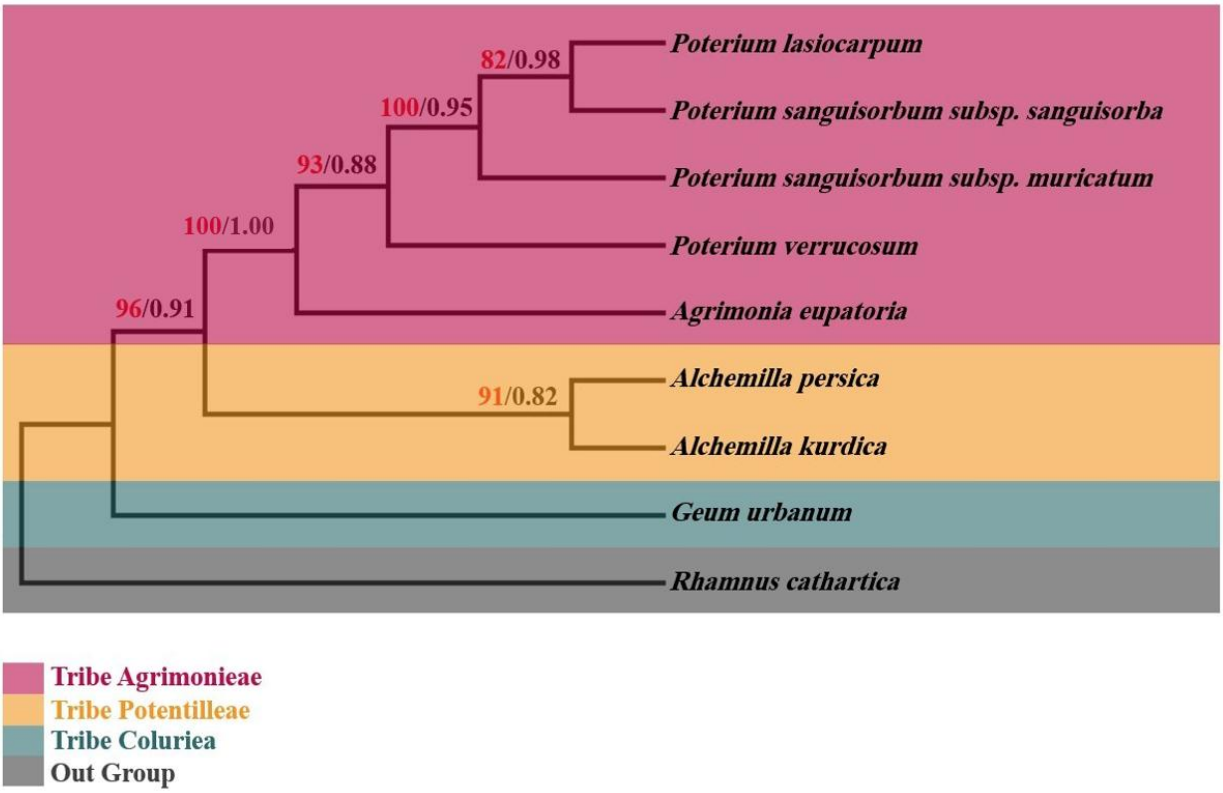


Figure 2. Strict consensus tree of most parsimonious tree resulting from phylogenetic analysis of the 28SrRNA sequences with heuristic search using maximum parsimony analysis. (Tree length of 698 steps, CI = 0.974, RI = 0.833, RC = 0.812 and HI =0.026). Numbers on the branches which in red color indicate bootstrap support and numbers in black color are Bayesian posterior probability values and tribes are identified by colors.

3.4 Palynological Study

The main pollen morphological features of the studied species are summarized in (Table 4). pollen grains of all genera are 3-colporate, radially symmetrical, monads. The shape classes of pollen grains are based on P/E ratio are studied, various shapes found in polar and equatorial view from oblate (0.681 µm) as in *Ag. euparoria* (Plate 1 E&F from LM, Plate 3 C&D from SEM), prolate-spheroidal (1.007,1.091 and 1.021 µm) as in *Al. kurdica*, *Al. persica* and *G. urbanum* (Plate 1 A&B, C&D and G&H from LM and plate 3 from SEM) Respectively, and subprolate (1.200 and 1.236 µm) as in *P. verrocosum* and *P. lasciocarpum* (Plate 2 E&F, A&B from LM and Plate 4 D&E , A from SEM) and finally prolate (1.336 µm) as in *P. sanguisorba* (Plate 2 C&D from LM and plate 4 B&C from SEM). The aperture includes three ectocolpi and three endopores. The size of the pollen grains ranged from small to medium. The exine sculpturing type includes striate pattern and consists of shallow, fingerprint-like ridges and predominately parallel arranged in all genera except *A. persica* which was reticulum.

Table 4: Pollen grain features of the studied species

Taxon	Polar Axis (µm) mean	Equatorial Axis (µm) mean	P/E	Pollen Shape (PS)	size	Sculpture	Number	Aperture
Ag. eupatoria	(12.50-25.00) 18.75 ± 6.25	(22.50-32.50) 27.50 ± 5	0.681	Oblate	small-medium	striate	numerous	3-colporate
Al. kurdica	(18.75-25.00) 21.25 ± 3.12	(20.00-22.50) 21.25 ± 1.25	1.007	Prolate spheroidal	small	striate microechinate	very few	3-colporate
Al. persica	(20.00-32.50) 26.25 ± 6.25	(20.00-30.00) 25.00 ± 5	1.091	Prolate spheroidal	small-medium	reticulate	very few	3-colporate
G. urbanum	(10.00-20.00) 15.00 ± 5	(12.50-17.50) 15.00 ± 2.5	1.021	Prolate spheroidal	small	striate	numerous	3-colporate
P. lasiocarpum	(17.50-25.00) 21.25 ± 3.75	(15.00-20.00) 17.5 ± 2.5	1.236	Subprolate	medium	striate microechinate	few	3-colporate
P. sanguisorba	(17.50-22.50) 20.46 ± 2.5	(12.50-17.50) 15.00 ± 2.5	1.336	prolate	medium	striate microechinate	few	3-colporate
P. verrucosum	(15.00-22.50) 18.75 ± 3.75	(12.50-18.75) 15.62 ± 3.125	1.200	Subprolate	medium	striate microechinate	few	3-colporate

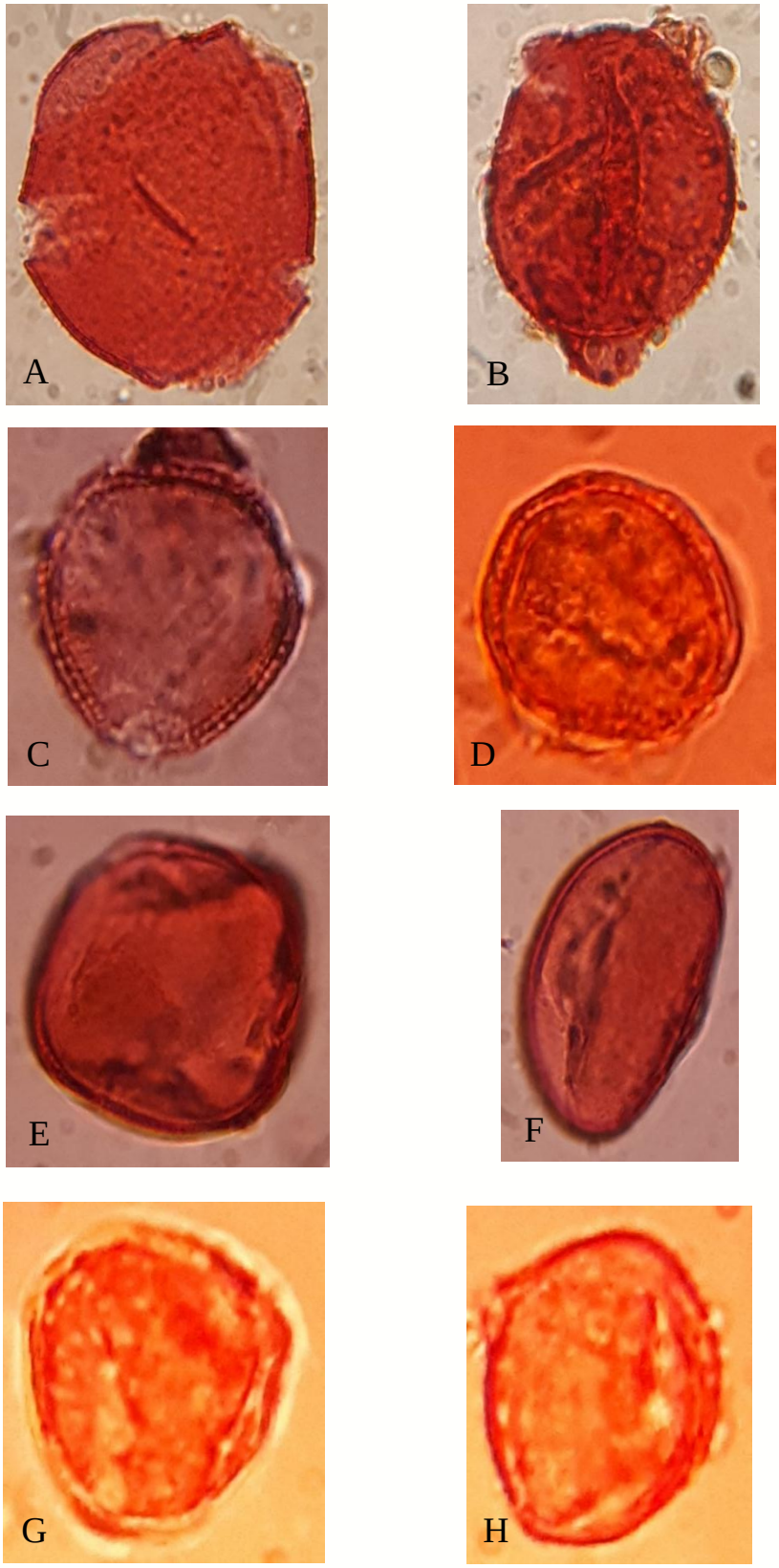


Plate 1: Pollen grains of the studied species (LM) x100: A&B. *Al. kurdica*; C&D. *Al. persica*; E&F. *Ag. eupatoria*; G&H. *G. urbanum* A, C, E & G = polar view; B, D, F & H = equatorial view

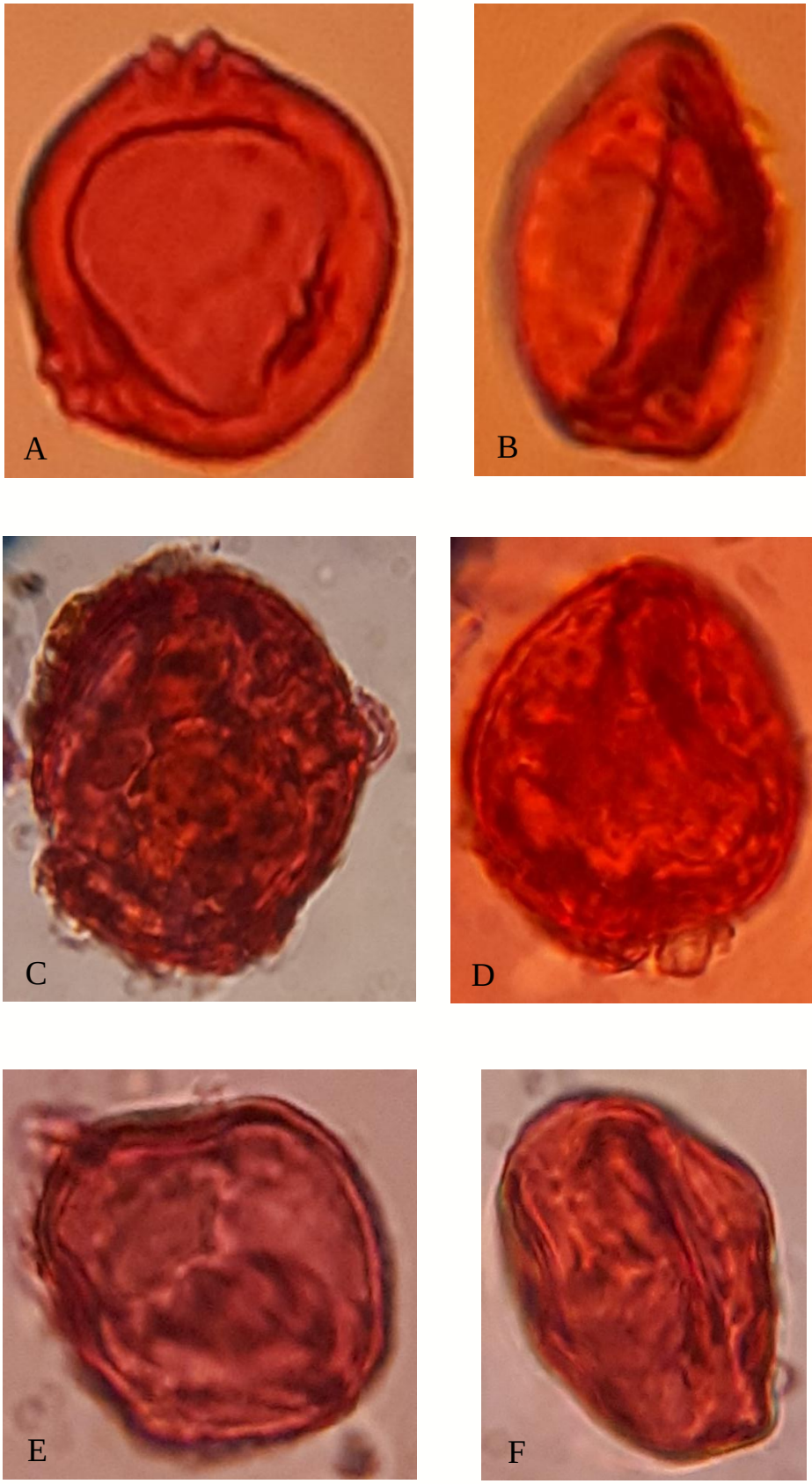


Plate 2: Pollen grains of the studied species (LM) x100: A&B. *P. lasiocarpum*; C&D. *P. sanguisorba*; E&F. *P. verrucosum*; A, C & E = polar view; B, D & F = equatorial view

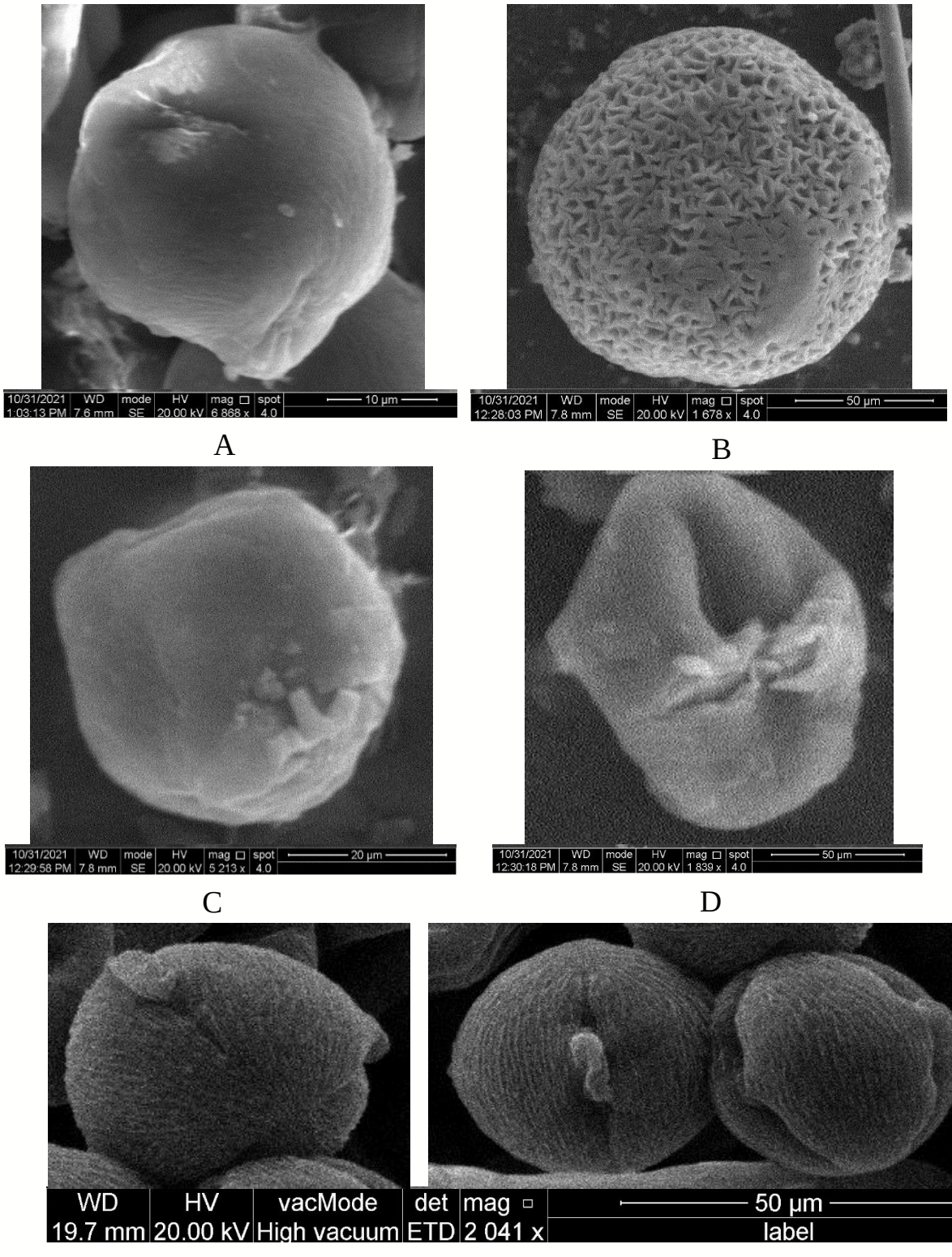
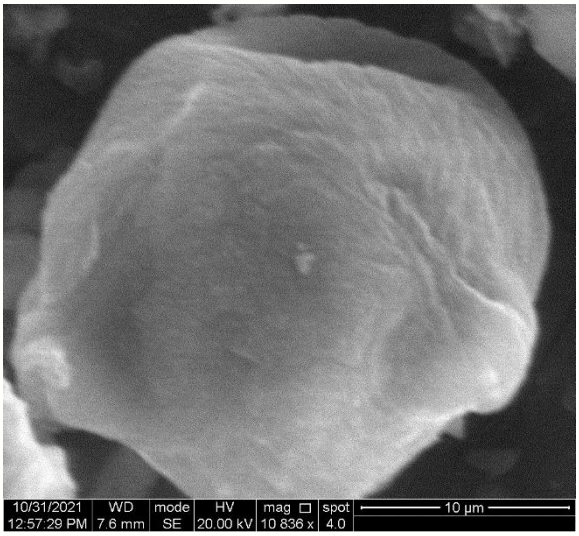
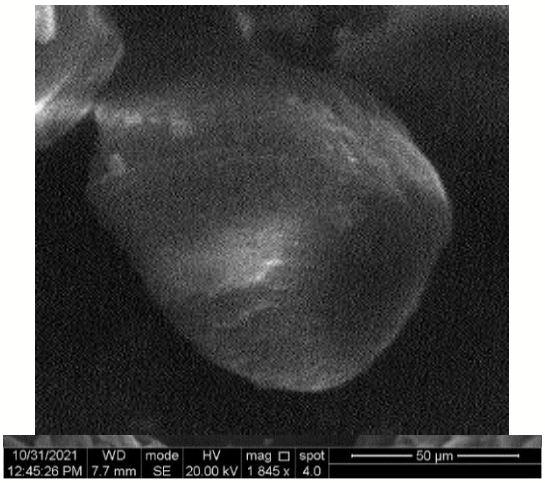


Plate 3: Pollen grains of the studied species (SEM): A. *Al. kurdica*; B. *Al. persica*; C&D. *Ag. eupatoria*; E&F. *G. urbanum*; A, B, C & E = polar view; D & F = equatorial view



A



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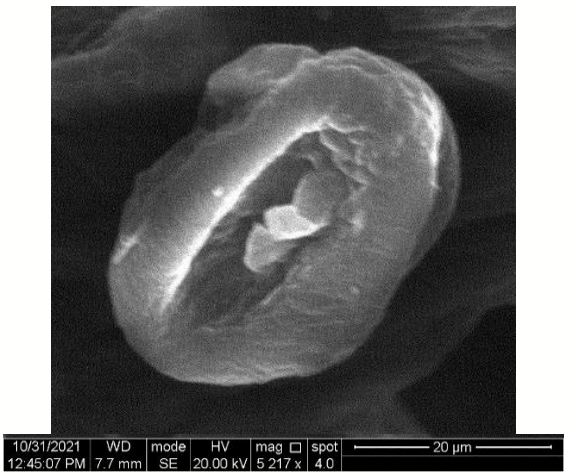

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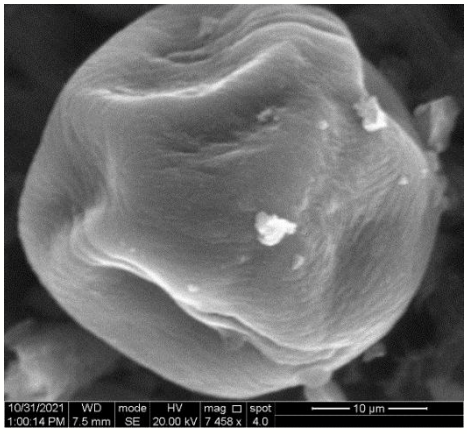

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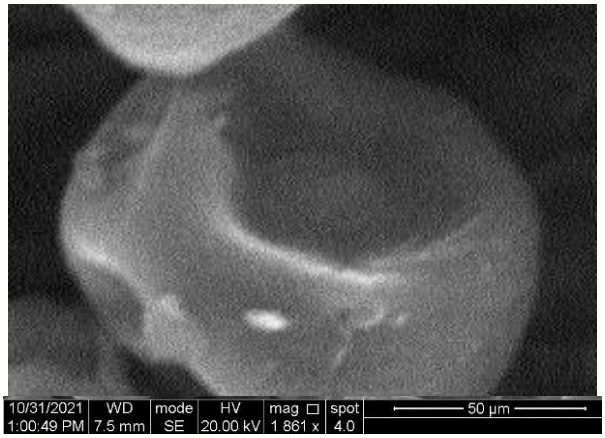

E

Plate 4: Pollen grains of the studied species (SEM): A. *P. lasiocarpum*; B&C. *P. sanguisorba*; D&E. *P. verrucosum*; A, B & D = polar view; C & E = equatorial view

4. Discussion

4.1.Phylogenetic analysis

The tree depending on nrDNA ITS gene sequences data in (Figure 1) showing three clades or tribes with high supports (bs=100%, pp=0.99): The tribe Agrimonieae in which pink in color consists of *Ag. eupatoria* with supports (bs=97%, pp=1.00) near to *P. sanguisorba* subsp. *muricatum* with high supports (bs=100%, pp=0.99), due to the special occurrence and similar habitat and *P. sanguisorba* subsp. *sanguisorba* with supports (bs=92%, pp=0.96) with sister species of *P. lasiocarpum* and *P. verrocosum* with supports (bs=87%, pp=0.94), due to their pollen grain shapes, similarity in morphology and their habitat also. while the tribe Potentilleae in which orange in color consists of sister species *Al. kurdica* and *Al. persica* with supports (bs=93%, pp=0.89), because they are similar in their pollen grain shapes and having small teeth at tip of their leaves and covered with soft hairs; And tribe Coluriea in which blue color consists of only *G. urbanum* with supports (bs=90%, pp=0.84), because its flower has a hypanthium, inflorescence is one-sided (the flowers are arrayed in a spiral around the inflorescence axis or branches, or occur singly, or in several ranks), both sepals and petals are separated and not fuse.

While phylogenetic tree of 28SrRNA gene sequence data in (Figure 2) showing also three tribes, with high supports (bs=100%, pp=0.100), and the results are slightly differing from ITS gene in which in Agrimonieae tribe the subspecies *P. sanguisorba* subsp. *sanguisorba* become a sister with *P. lasiocarpum*, due to their morphological similarity and habitat; And *Ag. eupatoria* become nearly to *P. verrocosum* due to their pinnate leaves, inflorescence is lax and branched and leaves are serrate.

The phylogenetic analysis based on 28SribosomalRNA and nuclear ribosomal DNA ITS regions are showed to be monophyletic (Faghir et al., 2018).

4.2. Pollen grains study

It is noticeable that the pollen grains of the four genera are generally tricolporate in aperture and oblate, prolate-spheroidal, subprolate and prolate in shapes. The size of the grains according to (Erdtman, 1952) are small to medium, the largest pollen grain seen in *P. sanguisorba*, while the smallest one seen in *Ag. eupatoria*. The outlines of species are varied from circular to ellipsoid.

Tricolporate pollen grains are founded in previous works of authors (Reitsma, 1966, Eide, 1981, Agudo et al., 1998, Faghir et al., 2012).

The aperture includes three ectocolpi and three endospores. Exine sculpture types are important characters for identification among the members of family Rosaceae (Ueda and Tomita, 1989). The exine sculpturing type includes striate pattern and consists of shallow, fingerprint-like ridges and predominately parallel arranged in all genera except *A. persica* which was reticulum (Plate 3B).

Any pores on the surface of exine sculpture have not seen due to the low resolution of the images of SEM. Finally, the study results shows that there is no deep difference among these four genera in pollen morphology.

4. Conclusions

In the current study three main tribes within the genera *Agrimonia*, *Alchemilla*, *Geum* and *Poterium* were identified. In the ITS tree the species *P. lasiocarpum*, *P. verrucosum*, *P. sanguisorba* subsp. *sanguisorba* and *P. sanguisorba* subsp. *muricatum*

placed in the same tribe, while in the 28SrRNA tree the species *P. verrucosum* replaced by the subspecies *P. sanguisorba* subsp. *sanguisorba*.

The pollen grains study by using both light and scanning electron microscope showed that palynological data of the four genera not represented the more variation among the genera, may be due to that they are morphologically similar.

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