



**International Journal of Biology, Pharmacy  
and Allied Sciences (IJBPAS)**

*'A Bridge Between Laboratory and Reader'*

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**THE POLLEN MORPHOLOGY STUDY ON SOME SPECIES OF HYPERICUM**

**L.GENUS IN THE NORTHERN IRAN**

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**ABSTRACT**

*Hypericum L.* genus with about 500 species in the world belongs to Hypericaceae family. The members of this family are present everywhere except for the Polar Regions, deserts, deep waters and low-height tropical areas. According to the latest studies in Iran, 19 species of this genus have been found in Iran. This study aims at comparing the pollen grains in 5 species of *Hypericum L.* genus through light and scanning electron microscopy (SEM) in three Northern provinces of Iran. The plant samples are collected from their natural habitats, and then studied after transferring to the laboratory. According to the quantitative and qualitative studies on the pollen grain of these plants, it is found that the three-groove pollen grains are often isopolar with Monad is the dispersion unit and some of them have different orientation models on surface of Exine. The relationship between these species is studied by the statistical cluster analysis and principal component analysis (PCA), and it is found that the results of pollen studies can properly separate the species of *Hypericum L.* genus in the northern Iran.

**Keywords: Hypericum, pollen morphology, Northern Iran, SEM**

**INTRODUCTION**

With about 500 species in the world, *Hypericum L.* plant is the largest genus out of 9 genera in Hypericaceae (Guttiferae) family and it belongs to Closioid clade

from Malpighiales Phylum according to global plant classification. (APG 2009; Nürk 2011; Carine and Christenhusz, 2012) This genus allocates 80% of diversity

in this family and is classified into 36 taxonomical sections. [16], [13], [4], [14].

This genus is composed of the small trees, large herbaceous plants or rhizome and sometimes one-year herbaceous plants, which have mostly fruit capsules and the yellow or orange petals, and sometimes the petals and leaves have glands. *Hypericum L.* has the global distribution and can be seen in every continent, but not in Antarctica, deserts and tropical lowlands. Despite a very good distribution in Northern Hemisphere, this plant is also widespread in the tropical highlands of southern hemisphere. *Hypericum L.* exists almost in all types of habitats, but rarely seen in water except for the too shallow areas (Nürk, 2011; Meseguer and Sanmartín, 2012), (Robson, 1977). Numerous studies with different approaches (Molecular, taxonomic, chemotaxonomic, phylogenetic, karyological and pollen morphology) have been recently conducted on this genus. (Khan, 1969; Thomas, 1970; Clarke, 1975, 1976, 1981; Barros and Ramos, 1984; Mártonfi et al, 2002; Crockett and Robson, 2011; Nürk, 2011; Meseguer and Sanmartín, 2012)

These studies have found that not only *Hypericum L.* genus, but also other members of Hypericeae Phylum (Triadenum, Santomasia and Thornea) are

not the members of a single ancestral group. The important pharmacological compounds such as Bioactive Secondary Metabolites, Flavonol, Glikoside, Biflavonoids, Xanthones, and Phloroglucinol derivatives, which play the important roles for treatment of diseases such as the depression, cancer, acute kidney failure play, etc, and some species of *Hypericum L.* have been widely used in traditional medicine since ancient times. [9], Avato, 2005; [10].

This plant has a great economic value in Europe and America and consumed as a drug or dietary supplement. (Linde, 2009) Iran is a country wherein there are 19 species, 4 subspecies and 4 varieties of 5 taxonomic sections according to the recent conducted studies by Mr. Azadi during 1997 to 1999. According to the previous studies, the pollen grain of *Hypericum L.* has Microreticulate, perforate orientation model and is from 3-zonocolporate type, but it is a little different with the pollen Tricolporate structure in other angiosperms. (Robson, 1958; Erdtman et al, 1961; Khan, 1969; Thomas, 1973; Aytuğ et al, 1971; Clarke, 1975, 1976, 1981; Barros and Ramos, 1984; Mártonfi et al, 2002; [12].

This genus also has the pollen grains with irregular shapes, but the regular pollen can be used for separation of genera (Clarke, 1975; Mártonfi et al, 2002; Meseguer and

Sanmartín, 2012). The pollen grain shape (the ratio of polar to equatorial axis), the type of exine ornamentation and Endo-aperture are among the main differentiation characteristics. (Meseguer and Sanmartín) 11 different pollen grains have been recorded for this genus by Clarke (1981) and it is revised by Meseguer and Sanmartín [13], Since the pollen studies are very detailed, they can help to resolve the ambiguities in plant systematics. Therefore, we have decided to conduct the pollen studies on some species of *Hypericum L.* genus in the northern Iran.

#### MATERIALS AND METHODS

The applied samples in this study have been all collected from their natural populations, and pressed and dried after transferring to the laboratory and then applied. The unopened flower and buds are utilized to extract the pollen grains to prevent pollen grains from being contaminated by pollen grains of other varieties. The acetolysed and dried pollen grains are used in a study by light microscopy (LM) and scanning electron microscopy (SEM). The measurements of pollen grains have been based on at least 30 pollen grains per population. In the study by light microscopy (LM), OLYMPUS microscope, CH40RF200 model is used and TK-C1380E camera, JVC model applied for taking the photograph.

Microscope KYKY from KYKY-EM 3200 model is used for a study by scanning electron microscopy (SEM). The pollen grains are not acetolysed in the study by scanning electron microscopy (SEM) and the dried ones are directly put on the Stub by double sided tape and put into the microscope after coating them with gold by Sputter coater machine model KYKY-SBC 12 to a diameter of approximately 100 Å (Angstrom). A drop of Acetocarmine or Safranin paint is poured on the pollen grains extracted from anther and distributed on the glass slide in order to stain the pollen grains, and then they are studied.

The numerical taxonomy is done through evaluation and measurement of 16 quantitative and qualitative characteristics (Table 2). Punt et al (2007) have described the terms of pollen grain.

The multivariate analysis methods including the cluster analysis and principal component analysis (PCA) are utilized in order to determine the relationships between studied species. In this study, the average rates of quantitative traits are utilized to measure them, and the qualitative traits are encoded by two or multi modes, and then applied. The square Euclidean distance is taken into account as the dissimilarity coefficient in evaluation. The statistical analyses are done by SPSS ver. 22 software.

Table 1: Studied populations and habitats

Taxon	Habitat	Altitude	Collectors samples
<i>Hypericum androsaemum</i> L.	Golestan province-zarringol village 36°52' 6.48N 54°47' 22.29E	430 m	Karim Alinattajomrani Mahdi Saffariyeh
<i>Hypericum perforatum</i> L.	Golestan province-Loveh village 37°21' 7.18N 55°39' 53.17E	530 m	Karim Alinattajomrani Mahdi Saffariyeh
<i>Hypericum fursei</i> N.Robson	Gilan province-between Viyeh and Aliva village 36°47' 47.72N 49°54' 40.68E	2150 m	Karim Alinattajomrani Mahdi Saffariyeh
<i>Hypericum hirsutum</i> L.	Mazandaran province-Sangechal village 36°10' 47.70N 52°30' 10.61E	1320 m	Karim Alinattajomrani Mahdi Saffariyeh
<i>Hypericum tetrapterum</i> Fries,Nov	Mazandaran province-Noor- Nargeskety village 36°32' 27.72N 36°13' 22.18E	6m	Karim Alinattajomrani

Table 2: Measured qualitative and quantitative traits in pollen grains

	Quantitative characters	Qualitative characters
1	Length of polar axis(p)	Shape of pollen grain from polar view
2	Length of equatorial axis(q)	Shape of pollen grain from equatorial view
3	P/E ratio	Type of exine ornamentation
4	Pollen size(according to the large axis)	Pollen type(class)
5	Ectocolpi interval(d)	Shape of pore on exine surface
6	Length of colpus	
7	Width of mesocolpium	
8	Apocolpium index (d/D)	
9	Diameter of pollen grain(D)	
10	Diameter pore on exine surface	
11	Distance between the pore exine	

## RESULTS

The data of studies on pollen grains are described in Table 3. This table represents the quantitative and qualitative characteristics of 5 species of *Hypericum* L. genus in three northern provinces of Iran (Golestan, Mazandaran, and Gilan).

The pollen grains of studied species are all from the Tricolporate type and have Monod distribution model. The pollen grains are small in *H.androsaemum* L. and *H. tetrapterum* Fries, Nov species and medium in the rest of species. *H.fursei* N.Robson species is heteropolar and has the prolate, irregular or folded shapes. *H.perforatum* L. species has

the Perprolate shape and the rest of species have prolate shapes. From the polar perspective, *H.androsaemum* L. is spherical, and *H.fursei* N.Robson triangular or irregular, and the remaining ones with three-lobed symmetric shapes. In terms of tropical view, the pollen grain of *H.androsaemum* L. species has the spherical type, and *H.hirsutum* L. and *H. tetrapterum* Fries, Nov. species prolate, and *H.perforatum* L. species has the perprolate shape; and *H.fursei* N.Robson species has the depressed and folded shape in the groove area and with irregular shapes. The pores on the exine surface have irregular and

different shapes in *H.androsaemum L.* and they are in the forms of irregular grooves in *H.fursei*<sub>N.Robson</sub>. The pores on exine surface have the circle (pore) shapes in the rest of species. The type of exine ornamentation in *H.androsaemum L.* species has Granium type and it is Regular in *H.fursei*<sub>N.Robson</sub> species with the form of wrinkles on human cerebral cortex. There are the pollens with

irregular shapes in all species with a very high frequency in *H.fursei*<sub>N.Robson</sub> species (more than 90%), but they are a few in other species. According to the conducted study on the use of multivariate statistical methods for determining the relationships between the studied species, the cluster analysis charts are drawn by WARD and Scree plot (Figure 2).

Table 3: Morphometrical parameters of investigated *Hypericum* taxa (µm)

characters taxon	Length of polar axis(p)	Length of equatorial axis(E)	P/E ratio	Ectocolp interval (d)	Length of colpus	Width of mesocolpium	Apocolpium index (d/D)	Diameter pore on exine surface	Diameter of pollen grain (D)	Shape of pollen grain from equatorial view	Shape of pollen grain from polar view	Pollen type(class)	Shape of pore on exine surface	Type of exine ornamentation	Pollen size (according to the large axis)	Distance between the pore exine
<i>H.androsaemum L.</i>	Min 17.363 Max 18.793	Min 16.190 Max 16.973	Min 1.045 Max 1.140	Min 3.360 Max 4.445	Min 12.906 Max 13.797	Min 9.428 Max 10.923	Min 0.198 Max 0.277	Min 0.184 Max 0.221	Min 15.094 Max 16.987	Spherical-isopolar	Spherical	Spherical-tricolporate	Irregular, Different	Granium	Small	Irregular, Different
<i>H.hirsutum L.</i>	Min 24.166 Max 32.787	Min 16.781 Max 18.140	Min 1.446 Max 1.900	Min 2.878 Max 3.157	Min 22.175 Max 30.830	Min 9.622 Max 12.730	Min 0.143 Max 0.192	Min 0.574 Max 0.673	Min 16.436 Max 17.456	Prolate-isopolar	3-lobed regular	Prolate-tricolporate	Pore	Microreticulate, perforate	Medium	Less than 0.6 µm
<i>H.fursei</i> <sub>N.Robson</sub>	Min 29.543 Max 36.687	Min 17.856 Max 22.090	Min 1.503 Max 1.632	Min 3.251 Max 3.589	Min 20.416 Max 25.520	Min 8.338 Max 10.892	Min 0.125 Max 0.147	Min 0.211 Max 0.314	Min 22.481 Max 27.862	Irregular-heteropolar	Triangle or irregular	Tricolporate, Pollen infolding, Irregular	Irregular grooves	Rugulae (like brain cortex)	Medium	Irregular, Different
<i>H.perforatum L.</i>	Min 24.886 Max 34.310	Min 12.647 Max 13.909	Min 1.789 Max 2.592	Min 3.383 Max 4.807	Min 21.300 Max 32.530	Min 7.740 Max 9.110	Min 0.244 Max 0.335	Min 0.443 Max 0.562	Min 12.647 Max 13.909	Perprolate-isopolar	3-lobed regular	Tricolporate perprolate	Pore	Microreticulate, perforate	Medium	Less than 0.6 µm
<i>H.tetrapterum</i> <sub>Fries.Nov</sub>	Min 17.747 Max 18.793	Min 12.769 Max 15.476	Min 1.177 Max 1.463	Min 2.400 Max 3.380	Min 15.900 Max 16.714	Min 8.448 Max 10.688	Min 0.157 Max 0.240	Min 0.210 Max 0.269	Min 15.261 Max 15.808	Prolate-isopolar	3-lobed regular	Tricolporate	Pore	Microreticulate, perforate	Small	Less than 0.6 µm

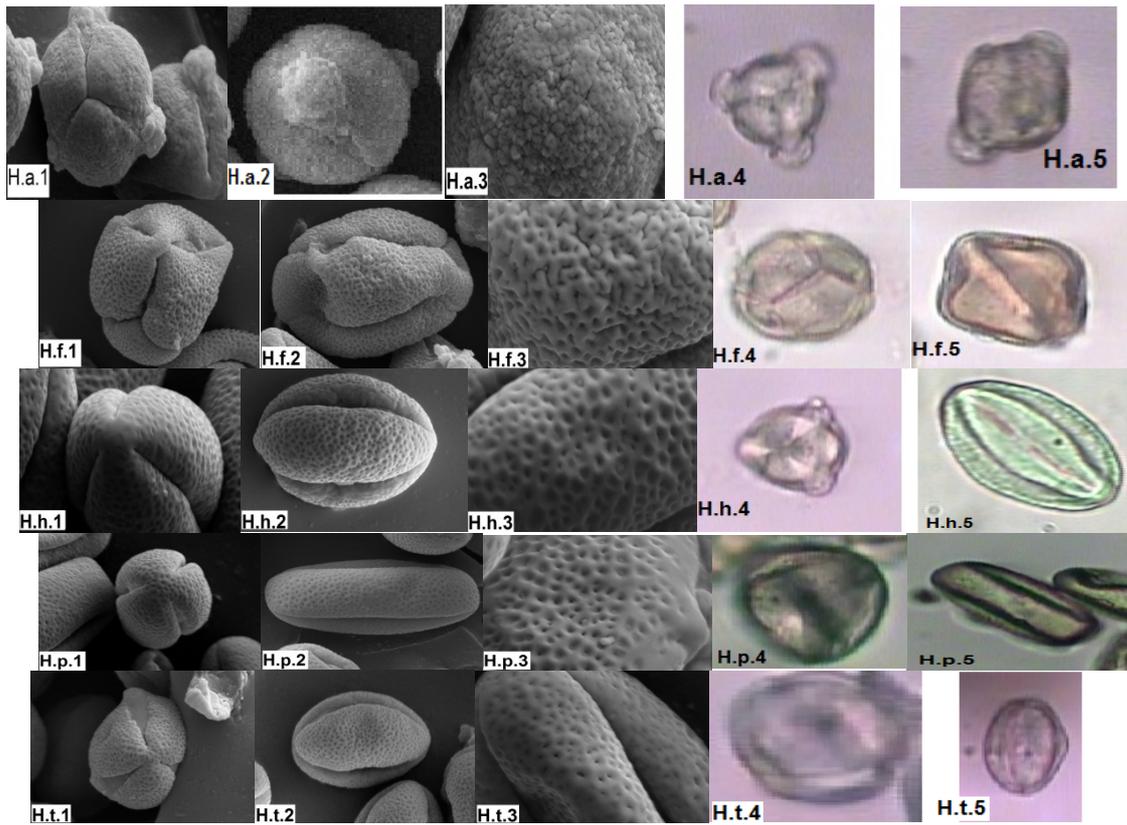


Figure 1: Images of pollen grains by scanning electron microscope (SEM) and light microscope (LM) Pollen microphotography of investigated Hypericum taxa. H.androsaemum (Ha1-5). H.fursei (Hf1-5). H.hirsutum (Hh1-5). H.perforatum (Hp1-5). H.tetrapterum (Ht1-5).

1-polar view of a non-acetolysed pollen in SEM, 2- equatorial view of a non-acetolysed pollen in SEM, 3-ornamentation type of a non-acetolysed pollen in SEM, 4-polar view of a non acetolysed pollen in LM, 5- equatorial view of a non acetolysed pollen in LM.

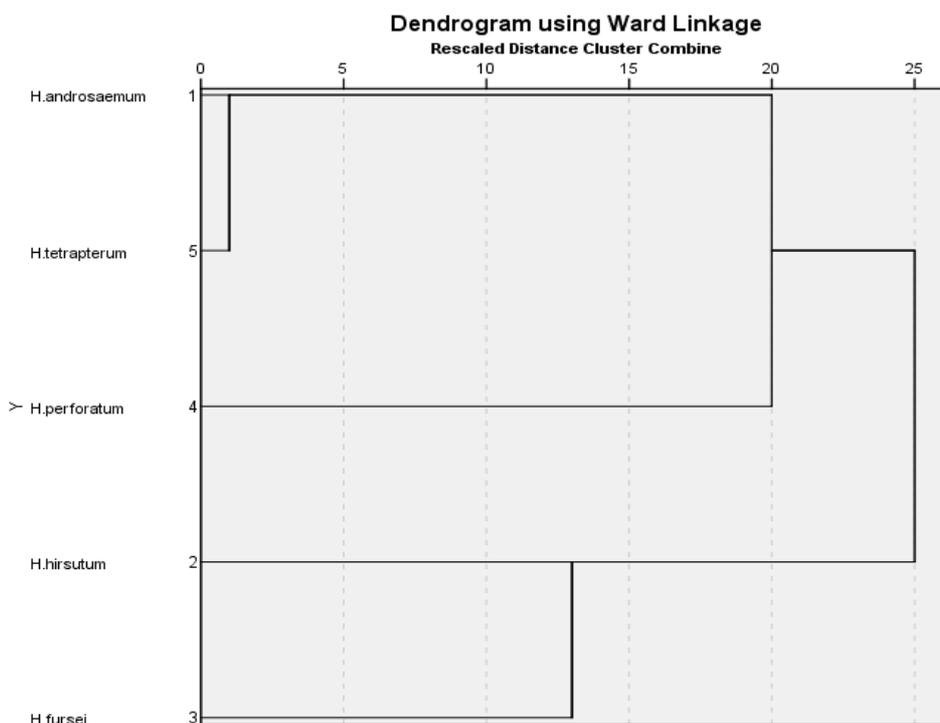


Figure 2: WARD cluster diagram

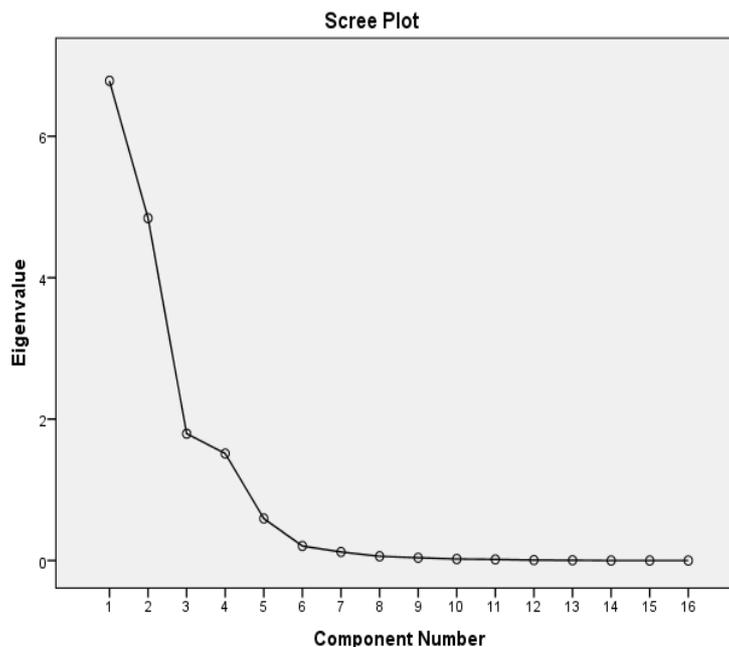


Figure 3: Scree plot  
Table 4: Table of variances

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	6.783	42.397	42.397	6.783	42.397	42.397	4.569	28.554	28.554
2	4.842	30.263	72.660	4.842	30.263	72.660	4.303	26.896	55.450
3	1.794	11.212	83.872	1.794	11.212	83.872	3.099	19.369	74.820
4	1.513	9.458	93.330	1.513	9.458	93.330	2.962	18.510	93.330
5	.594	3.712	97.043						
6	.206	1.285	98.328						
7	.121	.757	99.085						
8	.060	.373	99.458						
9	.039	.244	99.702						
10	.021	.133	99.835						
11	.016	.101	99.936						
12	.006	.037	99.973						
13	.004	.027	100.000						
14	9.348E-18	5.843E-17	100.000						
15	2.116E-18	1.322E-17	100.000						
16	-2.718E-16	-1.699E-15	100.000						

Extraction Method: Principal Component Analysis.

Table 5: Rotated Component Matrix  
Rotated Component Matrix<sup>a</sup>

	Component			
	1	2	3	4
B	.538	.801	-.161	.055
C	.294	.168	-.718	-.583
D	.163	.729	.295	.525
E	.463	.514	.588	.407
F	.939	.271	-.061	-.115
G	.939	.271	-.061	-.115
H	.451	.844	-.073	.075
I	.463	.514	.588	.407
J	-.335	.081	-.153	.852
K	.286	.920	.043	-.032
L	-.389	.157	-.224	-.678
M	-.396	.159	.273	.826
N	-.138	.819	.410	-.204
O	-.033	.225	.956	-.055
P	.605	.128	-.729	-.232
Q	.939	.271	-.061	-.115

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.<sup>a</sup>

a. Rotation converged in 8 iterations.

Based on the cluster method by WARD, it is found that the obtained dendrogram has two main clusters in which the main first and second clusters are divided into the other sub-clusters. The first sub-cluster is in turn divided into two sub-clusters. In the first cluster, *H.androsaemum L.* and *H.tetrapterum Fries, Nov* species are similar at the level 1; in other words they have the similarity percentage of 99%. *H.hirsutum L.* and *H.fursei N.Robson* species are similar at level 13 and put in a same cluster. *H.perfrpratum L.* species is similar to *H.androsaemum L.* and *H.tetrapterum Fries, Nov.* species at the level of 20 and put in the same cluster. Scree plot is drawn based on the pollen morphology data and it is found that four main factors have made the highest differences and separated the species. According to the table of variance (Table 4), it can be seen that the first factor includes approximately 42%, the second factor 30%, the third 11%, and the fourth

factor 9% of the total variance. According to the matrix table of rotated factors, it is found that the pollen grain shape from polar view, the type of exine surface orientation and the shape of exine pores have played the greatest impact and roles in the variance of first component. The size of pollen grain and groove length are put in the highest importance for the second component.

## DISCUSSION

This study, which is conducted in Iran for the first time, concludes that the pollen grains in *Hypericum L.* genus are all monad, tricolporate, and almost isopolar with small and medium sizes in mentioned species.

They have the prolate, perprolate, spherical and irregular shapes. Their Exine surface orientations are Regular; Microreticulate, perforate and Granium and this is consistent with the previously conducted studies. (Meseguer and Sanmartín, 2012;

Clarke 1981) However, *H.fursei* N.Robson species is only seen in Iran and the information of this pollen grain is reported for the first time. The irregular pollens are seen among these species and this is consistent with the previously conducted studies. (Meseguer and Sanmartín, 2012; Clarke 1981; Mártonfi et al, 2002) However, there are a high percentage of irregular pollen grains in *H.fursei* N.Robson species (more than 90%). According to the conducted studies in this research, the most effective traits in separation of these species from each other are as follows; The shape of pollen grain from the polar perspective, the type of orientation on exine surface, the shape of pores on exine, the length of groove and size of pollen grain. This information is largely done by taxonomic divisions in sections of *Hypericum* L. genus and presented by N.Robson. According to this study, *H.hirsutum* L. and *H.fursei* N.Robson species are in a cluster and also in *Taeniocarpium* section according to taxonomic divisions. *H.perfratum* L. and *H. tetrapterum* Fries, Nov. species are also in a cluster and in *Hypericum* section according to taxonomic classification. However, *H.androsaemum* L. species is very similar to *H. tetrapterum* Fries, Nov. species and are placed together in a cluster. According to taxonomic classification, *H.androsaemum* L. species is

seen in *Androsaemum* section, but it is placed with *H. tetrapterum* Fries, Nov species in a cluster in this study due to the numerous similar quantitative traits and since they have the same value characteristics in numerical taxonomy. These two species are clearly distinguishable due to different qualitative pollen traits.

Based on the conducted research on the pollen grains of these 5 species in three Northern provinces of Iran, it can be concluded that the pollen traits can clearly separate the above-mentioned species.

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