PALYNOLOGICAL STUDY OF GENUS *Pterocephalus* IN IRAQ

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ABSTRACT

This research was aimed to study the pollen morphology for the genus *Pterocephalus* (Vaill) from Dipsacaceae family in Iraq, and to utilize these feathers in isolating the species as valuable taxonomic traits for enriching Iraqi flora. The study included characteristics of the type, shape, size, sculpturing and apertures, as well as determining the full dimensions using light microscopy as well as numerical analysis of this species and draw polygonal shapes and denderogram convergence between species. The results of the study of pollen and polygonal forms showed significant differences in the characteristics at the level of each species, which helps to identification the genus species, as it was found that the pollen was a tricolpate, and shape was specified to be sub spheroidal, sub prolate, oblate spheroidal. and prolate spheroidal. The pollen grains varied in size between large, and very large size. However the most significant feature is that included four types of sculpture using to isolate the species to: Reticulate and spinate in *P. laxus*, *P. strictus*, and *P. nestorianus*, reticulate and spinulate in *P. brevis*, *P. plumosus*, Reticulate to striate and spinulate in *P. canus*, and Spinate in *P. kurdicus*, and *P. pyrethrifolius*. The dendritic scheme showed the convergence of some species among them and in varying convergence ratios.

Key words: Species, taxonomic, dipsaceae, morphology, isolation, flora, prolate

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INTRODUCTION
Dipsacaceae is one of the Iraqi flora families, represented by 280 species have been distributed all over the world; these are divided into 10 genera (14). In Iraq, the family includes 24 species divided into 4 genera (7). The genus Pterocephalus (Vaill) is a member of the family Dipsacaceae. The pollen morphology of the Dipsacaceae family was previously mentioned in some references (11,18,21). Morphological features of pollen have the ability to aggregate several distributed genera and resolved problems concerning to the ancestry and development of numerous taxa (17), and to derive classification of angiosperm (10). Clark & Jones (9) examined morphological features of pollen of the family Dipsacaceae of northwest Europe. Mayer and Ehrendorfer (15) published palynological studies in relevance to the classification of the genus Pterocephalus and founded a new genus Pterocephalodes on the basis of pollen morphology and fruit features. palynological study of several Egyptian species and its systematics importance has been studied by Khalik (13). Stuessy (20) they decided that the information of pollen grains are known to be helpful at different levels of taxonomical hierarchy (generic, subgeneric, interspecific, and even at intraspecific levels) and can oftentimes be useful in explain a relationship types, Pollen grains of the family Dipsacaceae in general radially uniform, isopolar rarely a polar. Shape commonly prolate-spheroidal, little spheroidal, porate or ticolpate, sexine thicker or thinner than nexine. Tectal generally spinulose, echinata or scabrate (19). Dipsacaceae are applied in traditional medicine of much countries e.g. the classical Chinese medicine "Xuduan" is made from roots of Dipsacus species (especially D. asper), which is utilized for the therapy of pain and tumefaction in lower back and knees, (16). There is no research on pollen morphology from the genus Pterocephalus of Iraq.. The objective of this study is to base on the pollen morphology of eight species belong to genus Pterocephalus by compound light microscope orderly to a prove their importance of futurity taxonomic work in Iraq.

MATERIALS AND METHODS
Plant samples were collected from the dried specimens which previously reserved at Iraqi herbarium, A mature floral bud was taken and placed on a glass slide to extract the pollen from the anther sacs by using a fine needle. A drop of dye was added and missed with pollen grains, remove anther residuen preparing and then gently covered with cover slide, the slide ready for examination. The dye used to examine the samples is Safranin - Glycerin, prepared by adding one volume of the Safranin dye to six volumes of Glycerin (1: 6), which was used for easy moving the cover of the slide at the examination and thus easy to move the sample and study it from all sides. The samples were examined using a compound light microscope and measurements were taken by using the Ocular micrometer for 30 pollen grains of each species. The pollen was photographed under the 40x lens and the surface ornamentation of the pollen was observed under the oil lens 100x. A high-resolution Sony (16.2 Megapixels) camera was used. Numerical analysis, Applied by firstly drawing the polygonal shapes after the arrangement of the pollen features as in Table 1, and analysis by using Microsoft office excel program, then the similarity ratio between each two species had been determined by using this formula: ab=S1*100/n after the arrangement of features occurring as in Table.2. and then represented in tree- diagram or dendrogram. (ab: similityt ratio between any two species, S1: summation of similar features, n: total numbers of studied features).

RESULTS AND DISCUSSION
The study of pollen grains for the species revealed several differences, whole pollen features and dimensions of each pollen type, shows in Table. 3, and represents in polygonal shapes Fig.1. which reveals the differences among all the species. The pollen study included the following aspects:
The present study was appeared that the pollen grain for all species is Tricolpate and this is identical to what was confirmed by other researchers (21,22) (Fig. 1 and 2). The shape is determined by the value of the ratio between the polar axis to the equatorial axis P / E., four shapes of pollen grains were identified as follows:

- **Subspheroidal** in the species *P. brevis*
- **Oblate spheroidal** in the species *P. pyrethrifolius*
- **Subprolate** in the species *P. nestorianus*
- **Prolate spheroidal** in the species *P. laxus*

According to the differences in polar views, the species can be dividing in to three grouped as follow:

- Pollen with circular polar view: as in *P. brevis*, *P. kurdicus*, and *P. nestorianus*.
- Pollen with lobed polar view: as in *P. canus*, and *P. laxus*.
- Pollen with lobed to circular polar view: as in *P. plumosus*, *P. pyrethrifolius*, and *P. strictus*.

While equatorial views of all species pollen appear widely elliptical to sub-circular.

**Size**

The following results were recorded for the size of the pollen grain:

- large size: as in the species *P. brevis*, *P. plumosus*, *P. laxus*, and *P. canus*, *P. nestorianus*
- very large size: as in the species *P. pyrethrifolius*, *P. strictus*, and *P. kurdicus*

### Table 1. pollen features arrangement as used in polygonal shapes drawings

<table>
<thead>
<tr>
<th>Feature</th>
<th>Features symbol</th>
<th>Features description</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape</td>
<td>A</td>
<td>Sub-Spheroidal</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prolate-spheroidal</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oblate -spheroidal</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sub -prolate</td>
<td>4</td>
</tr>
<tr>
<td>Size</td>
<td>B</td>
<td>large</td>
<td>1</td>
</tr>
<tr>
<td>Aperture width</td>
<td>C</td>
<td>Medium</td>
<td>2</td>
</tr>
<tr>
<td>Sculpture</td>
<td>D</td>
<td>Slit</td>
<td>3</td>
</tr>
<tr>
<td>Polar shape</td>
<td>E</td>
<td>Circular</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number</th>
<th>Width of colpus (11.7µm)</th>
<th>Maximum rate of length of pollen grain</th>
<th>Minimum rate of length of pollen grain</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. brevis</em>(A)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. canus</em>(B)</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>P. kurdicus</em>(c)</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>P. laxus</em>(D)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. nestorianus</em>(E)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. pyrethrifolius</em>(F)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. strictus</em>(H)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Polar axis (P):** The length of pollen grains were appeared great variances, ranging from the largest size for *P. pyrethrifolius* (105.3 µm) to the smallest size for *P. brevis* (53.56 µm).

**Equatorial axis (E):** The length of pollen grains were appeared great variances, ranging from the largest size for *P. pyrethrifolius* (96.2µm) to the smallest size for *P. brevis* (55.38µm).

**Colpi:** The study was appeared variance in the measurement of the colpi; maximum rate of length of colpus (66.3µm) in the species *P. pyrethrifolius*, and a minimum rate of length of it (19.76µm) in the species *P. brevis*. The maximum rate of a width of colpus (11.7µ) in the species *P. strictus*, and the minimum rate of a width of it (slit inside the cavity) in *P. canus*. So, according to the aperture features the species pollen grains isolated into:

- **Wide:** in the species: *P. brevis*, *P. plumosus*, *P. strictus*, and *P. Korducky*.
- **Medium:** in the species: *P. nestorianus* and *P. pyrethrifolius*.
- **Slit:** in the species: *P. Canus* and *P. laxus*

**Mesocolpium**

Maximum rate of mesocolpium (65µm) was record in the species *P. pyrethrifolius*, and the minimum rate of it (11.7µm) in the species *P. brevis*.
Figure 1. Polygonal shapes of studied species, A,B,C,D,E pollen features represented in table 1.
Figure 2. Showing the morphological characteristics of the pollen in the species under study
A-polar view (400x)  B-equatorial axis (400x)
C-Aperture (400x)  D-spinate (400x)
### Table 3. Measurement the morphological character for the pollen in studied species

<table>
<thead>
<tr>
<th>Species</th>
<th>Polar view</th>
<th>Polar axis/</th>
<th>Pollen shape</th>
<th>Equatorial (E)</th>
<th>P/E</th>
<th>Ratio</th>
<th>Apocolpium</th>
<th>Mesocolpium</th>
<th>Aperture width</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. brevis</em></td>
<td>55.9</td>
<td>53.56</td>
<td>Subspheroidal</td>
<td>55.38</td>
<td>0.96</td>
<td>36.4</td>
<td>11.7</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td><em>P. canus</em></td>
<td>70.2</td>
<td>68.9</td>
<td>Oblate spheroidal</td>
<td>70.2</td>
<td>0.9</td>
<td>20.8</td>
<td>41.6</td>
<td>Slit</td>
<td></td>
</tr>
<tr>
<td><em>P. kurdicus</em></td>
<td>83.2</td>
<td>104</td>
<td>Sub Prolate</td>
<td>84.5</td>
<td>1.23</td>
<td>41.6</td>
<td>55.9</td>
<td>10.4</td>
<td></td>
</tr>
<tr>
<td><em>P. laxus</em></td>
<td>79.3</td>
<td>83.2</td>
<td>Prolate spheroidal</td>
<td>61.1</td>
<td>1.36</td>
<td>48.1</td>
<td>41.6</td>
<td>slit</td>
<td></td>
</tr>
<tr>
<td><em>P. nestorianus</em></td>
<td>93.6</td>
<td>96.2</td>
<td>Prolate spheroidal</td>
<td>92.3</td>
<td>1.04</td>
<td>45.5</td>
<td>58.5</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td><em>P. plumosus</em></td>
<td>66.3</td>
<td>68.9</td>
<td>Prolate spheroidal</td>
<td>67.6</td>
<td>1.01</td>
<td>37.7</td>
<td>41.6</td>
<td>11.7</td>
<td></td>
</tr>
<tr>
<td><em>P. pyrethifolius</em></td>
<td>102.7</td>
<td>105.3</td>
<td>Prolate spheroidal</td>
<td>96.2</td>
<td>1.09</td>
<td>36.4</td>
<td>65</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td><em>P. strictus</em></td>
<td>62.4</td>
<td>101.4</td>
<td>SubProlate</td>
<td>81.9</td>
<td>1.23</td>
<td>31.2</td>
<td>45.5</td>
<td>9.1</td>
<td></td>
</tr>
</tbody>
</table>

![Dendrogram showing the relationship among studied species according to pollen features.](image)

**Figure 3.** Dendrogram showing the relationship among studied species according to pollen features.
Figure 3. Showing the morphological characteristics of the pollen in the species under study
A- polar view (400x)    B- equatorial axis (400x)    C- Aperture (400x)    D- spinate (400x)

Apocolpium
Maximum rate of apocolpium (48.1µm) was recorded in the species P. laxus, and a minimum rate of it (20.8µm) in the species P. canus.

Sculpture:
According to the sculpturing features the species were isolated into four groups shows in the scheme 1:
● Group I: Spinate in P. Kurdicus and P. pyrethrifolius
● Group II: Reticulate to striate and spinulate in P. canus
● Group III: Reticulate and spinulate in P. brevis, and P. plimusus
● Group IV: Reticulate and spinate in P. laxus, P. strictus, and P. nestorianus

This research is display the importance of properties of pollen grains, that help the other features for separate the species such as morphological and anatomical and environmental at classification (3,4,5). Dipsacaceae is a small family, it is a eurypalynous and the pollen morphology for the family is completely heterogenous (1,5). The research display polymorphism for pollen grain for the species in genus Pterocephalus, mostly, the interspecific variation in the genus are oftentimes small, however, there is noticeable variation among the different genera (13,16). Different palynological researches on various species of Dipsacaceae family assured the significance of pollen features for distinguishing taxa (2,19). Khalik (13) studied the pollen features in nine species return to four genera of Dipsacaceae family in Egypt and display that pollen feature can be applied to identify the species. Statistical analyses appeared that the qualitative features were helpful in the classification of studied species and possess its taxonomic value in addition to show the relationship among the studied species as represented in Fig.1,3. Aperture types, polar view, pollen size, sculpture, and pollen shape able to be applied as an identification guide for studied species and could be used as a taxonomic key for genus species as represented in Scheme.1. So, the sculpture and aperture type is the most important pollen features, as mentioned by Clark and Jones (9) which was split the northwest European species from the Dipsacaceae family for 4 pollen types on the bases of the aperture properties. This study was appeared for the first time the importance of pollen features of Pterocephalus in Iraq, further, could be which
employ this information in the process of updating the data of the Iraqi flora.

Scheme 1. Show possible isolated species according to pollen features.

REFERENCES
