Numerical taxonomy of *Galium* (*Rubiaceae*) in Egypt

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Abstract. On the basis of fifty morphological characters, including vegetative parts, flowers, fruits, seeds, pollen grains, and anatomical structure, a systematic study of 13 taxa belonging to genus *Galium* (*Rubiaceae*) from Egypt was conducted by means of numerical analysis. Four branches and clusters were distinguished. Representatives of these groups were clustered together according to characters with high factor loading in the principal coordinates analysis. The results showed congruence between the UPGMA clustering and principal coordinates analysis in suggesting four groups. There was some degree of similarity among the species of sect. *Aparine* (*Kolgyda*). The results indicated also that the sect. *Leiogalium* (*G. mollugo*) was a separate group, while *Aparine* (*Kolgyda*) was the most heterogeneous one.

Key words: *Galium*, numerical taxonomy, PCO, *Rubiaceae*, UPGMA cluster

Introduction

*Rubiaceae* form the fourth largest angiosperm family after *Asteraceae*, *Orchidaceae* and *Leguminosae*, comprising approximately 640 genera and over 10 000 species in about 10 tribes distributed across the world, chiefly in tropical regions (Robbrecht 1988). The family comprises a large number of monotypic genera, and there are also several very large genera, such as *Galium* (300 species), *Oldenlandia* (200 species), *Psychotria* (nearly 1200 species), etc. (Rendle 1963). A phylogenetic analysis has been recently carried out, using *rbcL* sequences from cpDNA of 48 different genera of *Rubiaceae*, representing 23 tribes and four subfamilies (Bremer & al. 1995).

One of the tribes with mostly herbaceous species is *Rubieae*, which accommodates 13 genera, with estimated 670 species altogether (Robbrecht 1988, 1994). The tribe is characterized by verticillate leaves and raphides. The leaf whorls are in fact whorls of true leaf blades and modified stipules (Rutishauer 1984). The terminal inflorescences have flowers with rudimentary calyces and valvate corolla lobes. The ovaries are bilocular, with a single, erect ovule in each locule that develops into mostly dry, or somewhat fleshy didymous fruits (Robbrecht 1988, 1994). In *Galium*, a restricted number of representatives are characterized by unisexual flowers (Dempster & Ehrendorfer 1965).

*Rubieae* appear monophyletic in the molecular studies of *atpB-rbcL* intergene region (Manen & al. 1994; Natali & al. 1995). The tribe is thus well characterized both morphologically and molecularly. Natali & al. (1996) studied seven species of the tribe *Rubieae* and 25 species belonging to 14 other tribes of *Rubiaceae*, using the DNA sequence of the chloroplast *atpB-rbcL* intergene region and concluded that the tribe *Rubieae* is monophyletic, while *Galium* and *Asperula* are polyphyletic in origin. Huysmans & al. (2003) studied six genera of *Rubieae* that occur in...
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NW Europe: *Asperula, Crucianella, Cruciata, Galium, Rubia,* and *Sherardia.* They observed that most genera of *Rubieae* had very similar pollen and concluded that the tribe *Rubieae* was unique among *Rubiaceae* in the combination of pollen features.

According to Boulos (1995, 2000), *Rubieae* is represented in Egypt by eight genera, viz. *Galium, Valantia, Callipeltis, Crucianella, Rubia, Kohautia, Oldenlandia,* and *Pterograillonia.* The first five genera, in addition to *Cruciata* represented in the Egyptian flora, belong to the tribe *Rubieae.*

*Galium* is one of the largest genera of *Rubiaceae,* with some 400 species distributed in both temperate and tropical regions of the world (Willis 1985; Mabberley 1987).

The genus *Galium* was described by Linnaeus (1753) who reported the occurrence of 26 species. He divided them into two groups according to fruit type (*glabro* = glabrous and *hispido* = hispid). Boissier (1881) reported 90 species of *Galium* and divided them into three sections (*Eugalium, Aparine* and *Cruciata*) and 11 subsections. Ehrendorfer (1976) recognized 145 species of genus *Galium,* classified into 10 sections, and the studied species were placed under three sections. Ehrendorfer & Schonbeck-Temesy (1982) recognized 145 species of genus *Galium,* classified into 10 sections, and the studied taxa were placed under three sections. Ehrendorfer & Schonbeck-Temesy (1982) listed for the flora of Turkey 101 species of *Galium* divided into 10 sections, and the studied taxa were placed into two sections.

Täckholm (1974) named 12 species for Egypt: *G. sinaicum, G. canum, G. mollugo, G. articulatum, G. murale, G. tricornutum, G. ceratopodum, G. aparine, G. spurium, G. nigricans, G. setaceum,* and *G. parisiense.* Boulos (1995, 2000) recognized only 10 species of *Galium* from Egypt: *G. sinaicum, G. canum, G. mollugo, G. murale, G. tricornutum, G. ceratopodum, G. aparine, G. spurium, G. setaceum,* and *G. parisiense.* Abdel Khalik & al. (2007) investigated the pollen morphology of 11 species and one subspecies of the genus *Galium* from Egypt and concluded that the pollen grains were zonocolpate, and the number of colpi ranges from 5 to 10. The pollen grains were used to distinguish closely related species within the genus *Galium.*

The purpose of this study is to use numerical taxonomy so as to better understand the phenetic relationships between species within the genus *Galium* in Egypt, and to verify whether these results correspond to the results of Boissier (1881), Ehrendorfer (1976) and Ehrendorfer & Schonbeck-Temesy (1982) for *Galium* sections, or not. This study is based on a large number of characters (50) of vegetative parts, pollen grains and seeds and uses UPGMA clustering and PCO analysis.

**Material and methods**

**Plant material**

The present study was largely based on herbarium material collections kept in the following herbaria: BR, CAI, CAIM, K, L, SHG, and WAG. In addition, fresh material of most of the taxa was studied, and field observations were made in several localities in Egypt.

In the analyses, the species constituted OTU (Operational Taxonomic Units, Table 1). In order to sample broadly the variations, the OTUs consisted of a number of collections/accessions (either herbarium specimens, or fresh material, or both) from different localities in Egypt. For some taxa, materials from Egypt were not available or limited, so specimens from other countries were used (e.g., OTU 2, 4, 6, and 7).

**Morphological character observations**

Table 2 shows the characters and character states scored for plant, seed, and pollen morphology, averaged for each OTU. A total of 50 characters were measured for each specimen: 12 quantitative and 38 qualitative. Twenty-seven of the qualitative characters were scored as binary and the rest were scored as multi-state characters.

**Vegetative parts, flower and fruit characters**

The measurements for all specimens of a taxon were averaged into one OTU score for each of the characters. OTU scores for quantitative characters were averages of measurements for at least 10 specimens (wherever possible). Bearing in mind that herbarium specimens cannot be regarded as a random sample of the species, we followed Wieringa (1999: 62-65) by calculating the mean value of the minimum and maximum measurements. For some of OTUs we lacked observations of certain characters, and these omissions were coded as missing data (-999). The complete data matrix is available on request at the Botany Department, Faculty of Science, Sohag University, Egypt.
Table 1. List of OTUs for the *Galium* species used for the studies arranged by section and subsections, according to Boissier (1881), Ehrendorfer (1976) and Ehrendorfer & Schonbeck-Temesy (1982).

|-----|-----------------------------------|---------------------------|--------------------|------------------|--------------------|----------------------------------------|

Table 2. Character and character states used in morphometric analysis of genus *Galium*.

<table>
<thead>
<tr>
<th>No.</th>
<th>Characters</th>
<th>Character state</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Life cycle</td>
<td>Annual</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perennial</td>
<td>2</td>
</tr>
<tr>
<td>2.</td>
<td>Plant length</td>
<td>Mean length in cm</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Plant nature</td>
<td>Scrambling</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prostrate to ascending</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Erect</td>
<td>3</td>
</tr>
<tr>
<td>4.</td>
<td>Plant surface</td>
<td>Glabrous</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hairy to sparsely hairy</td>
<td>2</td>
</tr>
<tr>
<td>5.</td>
<td>Stem type</td>
<td>Herbaceous</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Woody at the base</td>
<td>2</td>
</tr>
<tr>
<td>6.</td>
<td>Leaves arrangement</td>
<td>In pairs</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In whorls</td>
<td>2</td>
</tr>
<tr>
<td>7.</td>
<td>Number of leaves in whorls</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>More than 2</td>
<td>2</td>
</tr>
<tr>
<td>8.</td>
<td>Leaf shape</td>
<td>Linear oblongate</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oblongate</td>
<td>2</td>
</tr>
<tr>
<td>9.</td>
<td>Leaf length</td>
<td>Mean length in mm</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Leaf width</td>
<td>Mean width in mm</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Leaf margins</td>
<td>Flat to slightly revolute</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Strongly revolute</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Densely villous</td>
<td>2</td>
</tr>
<tr>
<td>12.</td>
<td>Leaf base</td>
<td>Not tapering</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tapering</td>
<td>2</td>
</tr>
<tr>
<td>13.</td>
<td>Inflorescence type</td>
<td>Cymes</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thyrses</td>
<td>2</td>
</tr>
<tr>
<td>14.</td>
<td>Inflorescence position</td>
<td>Axillary</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Terminal and axillary</td>
<td>2</td>
</tr>
<tr>
<td>15.</td>
<td>Inflorescence flower number</td>
<td>Only one</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1–3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>More than 3</td>
<td>3</td>
</tr>
<tr>
<td>16.</td>
<td>Peduncle length</td>
<td>Mean length in mm</td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>Peduncle diameter shape</td>
<td>Quadrangular</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slender</td>
<td>2</td>
</tr>
<tr>
<td>18.</td>
<td>Peduncle surface</td>
<td>Glabrous</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hairy to sparsely hairy</td>
<td>2</td>
</tr>
<tr>
<td>19.</td>
<td>Pedicel length</td>
<td>Mean length in mm</td>
<td></td>
</tr>
<tr>
<td>20.</td>
<td>Pedicel diameter shape</td>
<td>Quadrangular</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slender</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hairy to sparsely hairy</td>
<td>2</td>
</tr>
<tr>
<td>21.</td>
<td>Pedicel shape</td>
<td>Strongly recurved just under fruits</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Strongly recurved from the base</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Erect</td>
<td>3</td>
</tr>
<tr>
<td>22.</td>
<td>Flower diameter size</td>
<td>Mean size in mm</td>
<td></td>
</tr>
<tr>
<td>23.</td>
<td>Petal length</td>
<td>Mean length in mm</td>
<td></td>
</tr>
<tr>
<td>24.</td>
<td>Petal width</td>
<td>Mean length in mm</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Continuation.

<table>
<thead>
<tr>
<th>No.</th>
<th>Characters</th>
<th>Character state</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.</td>
<td>Petal surface</td>
<td>Glabrous</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glabrous or hairy</td>
<td>2</td>
</tr>
<tr>
<td>26.</td>
<td>Petal apex</td>
<td>Acute</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aristate</td>
<td>2</td>
</tr>
<tr>
<td>27.</td>
<td>Petal color</td>
<td>White</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Greenish–white</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yellow green</td>
<td>3</td>
</tr>
<tr>
<td>28.</td>
<td>Stamen length</td>
<td>Mean length in mm</td>
<td></td>
</tr>
<tr>
<td>29.</td>
<td>Style length</td>
<td>Mean length in mm</td>
<td></td>
</tr>
<tr>
<td>30.</td>
<td>Ovary shape</td>
<td>Globose to subglobose</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cylindrical</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reniform</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cylindrical</td>
<td>3</td>
</tr>
<tr>
<td>31.</td>
<td>Mericarp size (mm)</td>
<td>3–5 x 3–5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3–2.6 x 0.3–2.6</td>
<td>2</td>
</tr>
<tr>
<td>32.</td>
<td>Mericarp surface</td>
<td>Tuberculated</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Micropapillate</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Covered with hooked hairs, not tuberculated at the base</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Covered with hooked hairs arising from tubercle-like base</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Covered with long white simple straight hairs</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Covered with a depressed hairs</td>
<td>6</td>
</tr>
<tr>
<td>33.</td>
<td>Seed shape</td>
<td>Globose to subglobose</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reniform</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slender</td>
<td>3</td>
</tr>
<tr>
<td>34.</td>
<td>Seed size (mm)</td>
<td>2.5–4.5 x 2.5–3.7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.7–2.3 x 1.1–2.4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1–1.6 x 0.2–1.0</td>
<td>3</td>
</tr>
<tr>
<td>35.</td>
<td>Epidermal cell patterns</td>
<td>Isodiametric, 4, 5, 6 gonals or elongate in one direction</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Isodiametric, polygonal</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Polygonal or elongate in one direction</td>
<td>3</td>
</tr>
<tr>
<td>36.</td>
<td>Anticlinal walls</td>
<td>Straight</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sinuous</td>
<td>2</td>
</tr>
</tbody>
</table>

Data analysis

Two types of analyses were performed with NTSYS-pc 2.02k software (Applied Biostatistics Inc., Setauket, New York, USA). First, we performed a cluster analysis using average taxonomic distance and UPGMA clustering (procedures SIMINT, SAHN, and TREE). To reduce the effects of different scales of measurement for different characters, the values for each character were standardized with STAND procedure, according to the formula: yi,STD = (yi - AVGyi)/STDyi, where the default value in NTSYS-pc (STAND) for yi = the value to be standardized, AVGyi = the average of all values for the character, and STDyi = the standard deviation. The cophenetic correlation coefficient between the distance matrix and the tree matrix was calculated to examine how well the cluster analysis fits the distance matrix (procedures COPH and MX-COMP). Second, we performed a principal coordinates analysis (PCO), using the product-moment correlation as a coefficient. The procedure SIMINT was used to calculate the distance matrix based on STAND data, while the procedures EIGEN, PROJ and MX-PLOT were used to perform the PCO. We preferred PCO rather than PCA (Principal Components Analysis), because PCO performs better on data sets with missing data (Rohlf 1972).
Results

Cluster analysis

Figure 1 shows the UPGMA phenogram comprising all OTUs in the present work. The cophenetic correlation of the distance matrix and tree matrix was 0.90, indicating a good fit of the phenogram to the distance matrix (Rohlf 1993). Four branches and clusters can be distinguished: (1) a branch with *G. aparine*, *G. tricornutum*, *G. ceratopodium*, *G. spurium* subsp. *africanum* and *G. spurium* subsp. *spurium*; (2) a branch with *G. canum*, *G. nigricans* and *G. sinaicum*; (3) a branch with *G. mollugo*; and (4) a cluster is divided into two subgroups: subgroup (I) comprising *G. murale* and subgroup (II) comprising *G. parisienne*, *G. setaceum* subsp. *setaceum* and *G. setaceum* subsp. *decaisnei*.

Principal coordinates analysis (PCO)

Figures 2, 3 & 4 show the plot of 13 OTUs on the first three principal coordinate axes. These axes explain 56.14% of the total observed variation. Along the first axis (27.79% of the total variation, Figs 2 & 3), a segregation between two groups was demonstrated: (1) the group of *G. aparine*, *G. tricornutum*, *G. ceratopodium*, *G. spurium* subsp. *africanum* and *G. spurium* subsp. *spurium*, and (2) the group of *G. mollugo*. The main characters explaining this segregation (characters with high factor loading > 0.6) were plant surface, peduncle length, pedicel length, pedicel diameter shape, pedicel surface, mericarp size, sculpture of periclinal cell wall, xylem and pith.

The second axis (16.44% of the total variation, Figs 2 & 4) revealed a split between (1) the group of *G. aparine*, *G. tricornutum*, *G. ceratopodium*, *G. spurium* subsp. *africanum* and *G. spurium* subsp. *spurium*, (2) the group of *G. canum*, *G. nigricans* and *G. sinaicum*, and (3) the group of *G. murale*, *G. parisienne*, *G. setaceum* subsp. *setaceum* and *G. setaceum* subsp. *decaisnei*. This split was based mainly on plant length, plant nature, leaf length, leaf width, leaf margins, leaf base, peduncle diameter shape, pedicel diameter shape, pedicel shape, petal surface, mericarp size, seed shape, seed size, anticlinal walls, polar axis, equatorial axis, cross section shape, xylem and pith (Table 3).
Along the third axis (11.91% of the total variation, Figs 3 & 4), a clear separation of *G. mollugo* from the remaining groups was observed. This separation was based mainly on life cycle, plant nature, stem type, leaves arrangement, number of leaves in whorls, leaf length, peduncle surface, flower diameter size, petal width, petal surface, petal color, mericarp surface, sculpture of anticlinal boundaries, polar axis, exine perforation, and pith characters.

### Table 3. Morphological characters showing the highest factor loading on the first three principal coordinates axes.

<table>
<thead>
<tr>
<th>No.</th>
<th>Characters</th>
<th>Principal coordinates</th>
<th>Factors loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>26</td>
<td>Petal apex</td>
<td>0.32</td>
<td>0.44</td>
</tr>
<tr>
<td>27</td>
<td>Petal color</td>
<td>0.41</td>
<td>-0.58</td>
</tr>
<tr>
<td>28</td>
<td>Stamen length</td>
<td>-0.56</td>
<td>0.49</td>
</tr>
<tr>
<td>29</td>
<td>Style length</td>
<td>-0.29</td>
<td>0.18</td>
</tr>
<tr>
<td>30</td>
<td>Ovary shape</td>
<td>0.30</td>
<td>-0.39</td>
</tr>
<tr>
<td>31</td>
<td>Mericarp size (mm)</td>
<td>0.87</td>
<td>0.65</td>
</tr>
<tr>
<td>32</td>
<td>Mericarp surface</td>
<td>0.23</td>
<td>-0.43</td>
</tr>
<tr>
<td>33</td>
<td>Seed shape</td>
<td>0.15</td>
<td>-0.89</td>
</tr>
<tr>
<td>34</td>
<td>Seed size (mm)</td>
<td>0.19</td>
<td>0.89</td>
</tr>
<tr>
<td>35</td>
<td>Epidermal cell patterns</td>
<td>0.38</td>
<td>-0.28</td>
</tr>
<tr>
<td>36</td>
<td>Anticlinal walls</td>
<td>-0.38</td>
<td>-0.96</td>
</tr>
<tr>
<td>37</td>
<td>The sculpture of anticlinal boundaries</td>
<td>0.20</td>
<td>-0.11</td>
</tr>
<tr>
<td>38</td>
<td>Outer periclinal cell walls</td>
<td>0.54</td>
<td>-0.21</td>
</tr>
<tr>
<td>39</td>
<td>Sculpture of periclinal cell walls</td>
<td>-0.63</td>
<td>0.57</td>
</tr>
<tr>
<td>40</td>
<td>Pollen shape</td>
<td>0.38</td>
<td>-0.11</td>
</tr>
<tr>
<td>41</td>
<td>Polar Axis (P)</td>
<td>-0.17</td>
<td>-0.81</td>
</tr>
<tr>
<td>42</td>
<td>Equatorial Axis (E)</td>
<td>-0.14</td>
<td>-0.70</td>
</tr>
<tr>
<td>43</td>
<td>Number of colpi</td>
<td>-0.49</td>
<td>-0.51</td>
</tr>
<tr>
<td>44</td>
<td>Exine microspines</td>
<td>-0.25</td>
<td>0.35</td>
</tr>
<tr>
<td>45</td>
<td>Exine microspines</td>
<td>0.26</td>
<td>-0.28</td>
</tr>
<tr>
<td>46</td>
<td>Cross section shape</td>
<td>0.23</td>
<td>-0.70</td>
</tr>
<tr>
<td>47</td>
<td>Cortex</td>
<td>0.38</td>
<td>0.37</td>
</tr>
<tr>
<td>48</td>
<td>Xylem</td>
<td>0.80</td>
<td>0.60</td>
</tr>
<tr>
<td>49</td>
<td>Pith</td>
<td>-0.80</td>
<td>-0.60</td>
</tr>
<tr>
<td>50</td>
<td>Pith characters</td>
<td>-0.37</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>Percentage per PCO</td>
<td>27.79</td>
<td>16.44</td>
</tr>
</tbody>
</table>

Percentage total variation for the first three principal coordinates amount 56.14%


**Discussion**

Taxonomy must largely rely on morphological characters to define taxa. Problems in classification arise when the taxa display a large amount of variability, due to phenotypic plasticity (van den Berg & Groenendijk-Wilders 1999). Several authors have tried to provide a natural system to divide the genus *Galium* into sections and subsections (Linnaeus 1753; Boissier 1881; Ehrendorfer 1976; Ehrendorfer & Schonbeck-Temesy 1982; see Table 1). These studies were based on a small number of morphological characters such as pedicel and peduncle characters, fruit shape, and surface and leaf characters. In the present study, a large number of characters were scored and numerical methods (UPGMA and PCO) were applied to study the relationships between taxa and to approximate the level of variation between them. UPGMA gives insight into the degree of similarity among the OTUs and whether they form groups/clusters, and indicates the level of variation between species. PCO reflects which characters are important on the axes, and indicates the significant characters on the basis of the highest factor loading (Table 3). Thus it becomes clear which characters help differentiate between the groups and can be useful to distinguish taxa. Generally, our results demonstrated congruence between the UPGMA clustering and PCO analyses in suggesting four groups: (1) *G. aparine*, *G. tricornutum*, *G. ceratopodium*, *G. spurium* subsp. *africanum* and *G. spurium* subsp. *spurium*, (2) *G. canum*, *G. nigricans* and *G. sinaicum*, (3) *G. mollugo*, and (4) *G. murale*, *G. parisiense*, *G. setaceum* subsp. *setaceum* and *G. setaceum* subsp. *decaisnei*.

Boissier (1881) treated *G. aparine*, *G. tricornutum*, *G. ceratopodium*, *G. spurium* subsp. *africanum* and *G. spurium* subsp. *spurium* (sect. *Aparine* = *Kolgyda*) as a well-distinguished group, characterized by: (1) scrambling plant habit, (2) leaf width (2.5–4 mm), (3) flat to slightly revolute leaf margins, (4) tapering leaf base, (5) quadrangular peduncle and pedicel diameter shape, (6) glabrous petal surface, (7) globose to subglobose seed and mericarp shape, (8) sinuous antical walls, (9) mean of polar and equatorial axis, (10) quadrangular cross-section shape, (11) narrow xylem, (12) wide pith. Within this group, we can show that *G. aparine* and *G. tricornutum* form a subgroup, and another subgroup includes *G. ceratopodium* and *G. spurium*. These results are congruent with those of Ehrendorfer (1971), Natali & al. (1996) and Abdel Khalik & al. (2007).

Concerning the group of *G. canum*, *G. nigricans* and *G. sinaicum*, Boissier (1881) differentiated it into two sections on the basis of annual or perennial habit, flower hermaphrodite or polygamous, and peduncle erect or recurved: sect. *Aparine* and sect. *Eugalium*. He placed *G. nigricans* in the former, with annual habit, flower hermaphrodite or polygamous and peduncle erect or recurved, while *G. canum* and *G. sinaicum* were placed in the second section on the basis of perennial habit, flower hermaphrodite and erect peduncle. Furthermore, Ehrendorfer (1976) and Ehrendorfer & Schonbeck-Temesy (1982) placed *G. canum* and *G. sinaicum* in a separate section (*Jubogalium*) and *G. nigricans* in another section (*Kolgyda*). Abdel Khalik & al. (2007) indicated that *G. canum* has 5–7 colpi, *G. sinaicum* 5–6 colpi and *G. nigricans* 7–8 colpi. According to the cluster and principal coordinates analyses,
this group is distinct from the others by the strongly revolute leaf margins, erect pedicel shape, glabrous or hairy petal surface, reniform mericarp and seed shape, mericarp size (0.3–2.6 × 0.3–2.6 mm), seed size (0.1–1.6 × 0.2–1 mm), mean of polar and equatorial axis, wide xylem and narrow pith. These results disagree with those of Boissier (1881), Ehrendorfer (1976) and Ehrendorfer & Schönbeck-Temesy (1982), and partially agree with Abdel Khalik & al. (2007).

Regarding differentiation of *G. mollugo*, Boissier (1881) treated this species under sect. *Eugalium* and subsect. *Leiogalium*. Karyologically, *G. mollugo* can behave both as a diploid – 2n = 22 (Natali & Jeanmonod 2000), or as a tetraploid – 2n = 44 (Ehrendorfer 1961). On the other hand, Ehrendorfer (1976) considered *G. mollugo* a highly polymorphic taxon and a species aggregate with numerous specific and sub-specific segregates, and set it apart as a separate section (sect. *Leiogalium*). Natali & al. (1996) indicated that *G. mollugo* was bunched in a separate clade. In our results, both cluster and principal coordinates analysis, *G. mollugo* (*Leiogalium*) is distanced from other groups, and is distinct from the other *Galium* species by a rhizomatous perennial, erect plant habit, glabrous peduncle surface, large flower (diameter size, 3 mm), white petal color, micropapillate mericarp surface, folded sculpture of antical walls, narrow xylem and pith. These results are congruent with those of Boissier (1881) and Natali & al. (1995, 1996), and partially agree with those of Ehrendorfer (1976), Ehrendorfer & Schönbeck-Temesy (1982), Huysmans & al. (2003), and Abdel Khalik & al. (2007).

Conclusions

UPGMA and PCO analyses have been used to study the morphological variation among species within the genus *Galium* in Egypt, so as to determine the similarities between species. Our results indicated some degree of similarity among the species of section *Aparine* (*Kolgyda*). The section *Eugalium* or *Leiogalium* (*G. mollugo*) is considered as a separate group, while *Aparine* (*Kolgyda*) is the most heterogeneous and this is congruent with the results of Natali & al. (1995, 1996). Although this study has contributed new conclusions to literature, it is limited to the known genera, sections, species, and subspecies in Egypt. A comprehensive study covering all *Galium* species would be necessary to make a more thorough classification and it would be very useful for the further studies to use molecular data. It will make possible a comparison of morphological results with molecular results.

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