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

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Received: February 19, 2008 ▷ Accepted: March 16, 2008

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 (Rutishauser 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 monphyletic, while *Galium* and *Asperula* are polyphyletic in origin. Huysmans & al. (2003) studied six genera of *Rubieae* that occur in

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), *Rubiaceae* is represented in Egypt by eight genera, viz. *Galium*, *Valantia*, *Callipeltis*, *Crucianella*, *Rubia*, *Kohautia*, *Oldenlandia*, and *Pterogaillonia.* 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) 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 morphoogy, 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 calculaing 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).

No.	Taxon	Origin	No. of individuals	Boissier (1881)	Ehrendorfer (1976)	Ehrendorfer & Schonbeck-Temesy (1982)
1.	*Galium aparine* L.	Egypt, Palestine	5	sect. *Aparine* subsect. *Leucaprinea*	sect. *Kolgyda*	sect. *Kolgyda*
2.	*Galium canum* Req. ex DC.	Palestine, Turkey	5	sect. *Eugalium* subsect. *Chromogalia*	sect. *Jubogalium*	sect. *Jubogalium*
3.	*Galium ceratopodum* Boiss.	Egypt	15	sect. *Aparine* subsect. *Camptopoda*	–	–
4.	*Galium mollugo* L.	Netherlands, France	5	sect. *Eugalium* subsect. *Leiogalia*	sect. *Leiogalium*	–
5.	*Galium murale* (L.) All.	Egypt	6	sect. *Aparine* subsect. *Apera*	sect. *Kolgyda*	sect. *Kolgyda*
6.	*Galium nigricans* Boiss.	Iran	4	sect. *Aparine* subsect. *Xanthaparinea*	–	sect. *Kolgyda*
7.	*Galium parisiense* L.	Morocco, France	4	sect. *Aparine* subsect. *Xanthaparinea*	sect. *Kolgyda*	sect. *Kolgyda*
8.	*Galium setaceum* Lam. subsp. *setaceum*	Egypt	10	sect. *Aparine* subsect. *Xanthaparinea*	sect. *Jubogalium*	sect. *Jubogalium*
9.	*Galium setaceum* subsp. *decaisnei* (Boiss.) Ehrend.	Egypt	20	sect. *Aparine* subsect. *Xanthaparinea*	sect. *Jubogalium*	sect. *Jubogalium*
10	*Galium sinaicum* (Delile ex Decne) Boiss.	Egypt	25	sect. *Eugalium* subsect. *Chromogalia*	–	–
11.	*Galium spurium* L. subsp. *spurium*	Egypt	15	sect. *Aparine* subsect. *Leucaprinea*	sect. *Kolgyda*	sect. *Kolgyda*
12.	*Galium spurium* subsp. *africanum* Verdc.	Egypt	8	sect. *Aparine* subsect. *Leucaprinea*	sect. *Kolgyda*	sect. *Kolgyda*
13.	*Galium tricornutum* Dandy	Egypt, Morocco, Iran	5	sect. *Aparine* subsect. *Camptopoda*	sect. *Kolgyda*	sect. *Kolgyda*

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

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

Table 2. Continuation.

No.	Characters	Character state	Code
25.	Petal surface	Glabrous	1
		Glabrous or hairy	2
26.	Petal apex	Acute	1
		Aristate	2
27.	Petal color	White	1
		Greenish–white	2
		Yellow green	3
28	Stamen length	Mean length in mm	
29.	Style length	Mean length in mm	
30.	Ovary shape	Globose to subglobose	1
		Cylindrical	2
		Reniform	2
		Cylindrical	3
31.	Mericarp size (mm)	3–5 x 3–5	1
		0.3–2.6 x 0.3–2.6	2
32.	Mericarp surface	Tuberculated	1
		Micropapillate	2
		Covered with hooked hairs, not tuberculated at the base	3
		Covered with hooked hairs arising from tubercle-like base	4
		Covered with long white simple straight hairs	5
		Covered with a depressed hairs	6
33.	Seed shape	Globose to subglobose	1
		Reniform	2
		Slender	3
34.	Seed size (mm)	2.5–4.5 x 2.5–3.7	1
		1.7–2.3 x 1.1–2.4	2
		0.1–1.6 x 0.2–1.0	3
35.	Epidermal cell patterns	Isodiametric, 4, 5, 6 gonals or elongate in one direction	1
		Isodimetric, polygonal	2
		Polygonal or elongate in one direction	3
36.	Anticlinal walls	Straight	1
		Sinuous	2
37.	The sculpture of anticlinal boundaries	Smooth	1
		Folded	2
38.	Outer periclinal cell walls	Flat	1
		Flat to slightly concave	2
		Convex	3
39.	Sculpture of periclinal cell walls	Smooth	1
		Folded	2
		Radiate course folds	3
		Microreticulate	4
		Micropapillate	5
40.	pollen shape	Spheroidal	1
		Prolate spheroidal	2
		Oblate spheroidal	3
		Suboblate	4
41.	Polar Axis (*P*)	Mean length in µm	
42.	Equatorial Axis (*E*)	Mean length in µm	
43.	Number of colpi	5–8	1
		More than 8	2
44.	Exine microspienes	Lower density and large microspines	1
		Higher density and small microspines	2
45.	Exine perforation	High density and large perforation	1
		Lower density and small perforation	2
46.	Cross section shape	Quadrangular	1
		More or less circular	2
47.	Cortex	Narrow	1
		Wide	2
48.	Xylem	Narrow	1
		Wide	2
49.	Pith	Narrow	1
		Wide	2
50.	Pith characters	Hollow	1
		Solid above, hollow below	2
		Solid	3

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 MXCOMP). 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 MXPLOT 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. ceratopodum*, *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. parisiense, 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. ceratopodum*, *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. ceratopodum*, *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. parisiense*, *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).

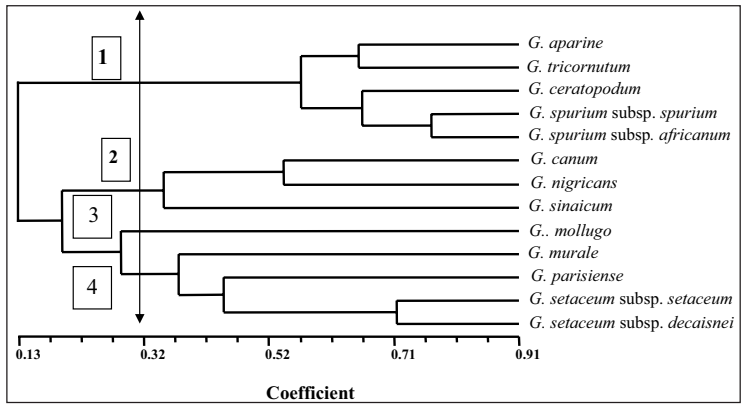

Fig. 1. Phenogram of the 13 OTUs studied, clustering with UPGMA method.

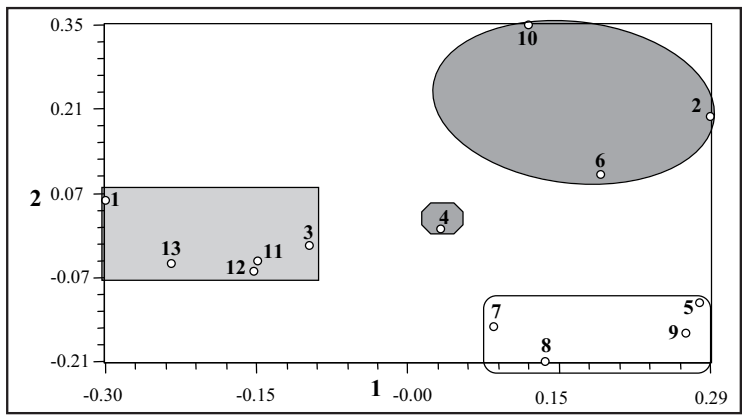

Fig. 2. Scatter plot of the 13 OTUs plotted against the first principal coordinate by the second principal coordinate.

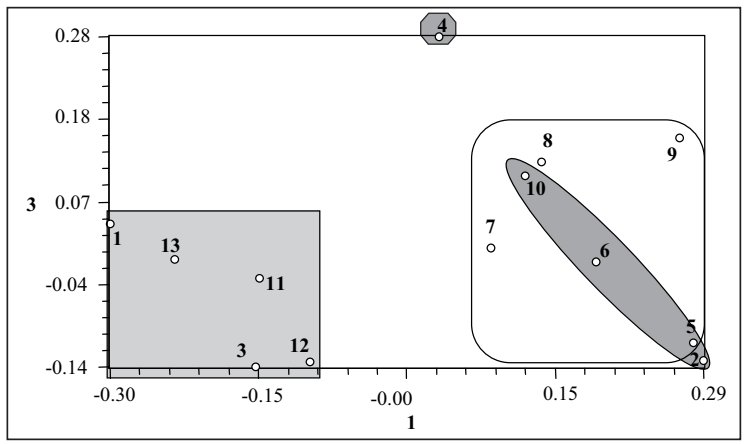

Fig. 3. Scatter plot of the 13 OTUs plotted against the first principal coordinate by the third principal coordinate.

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.

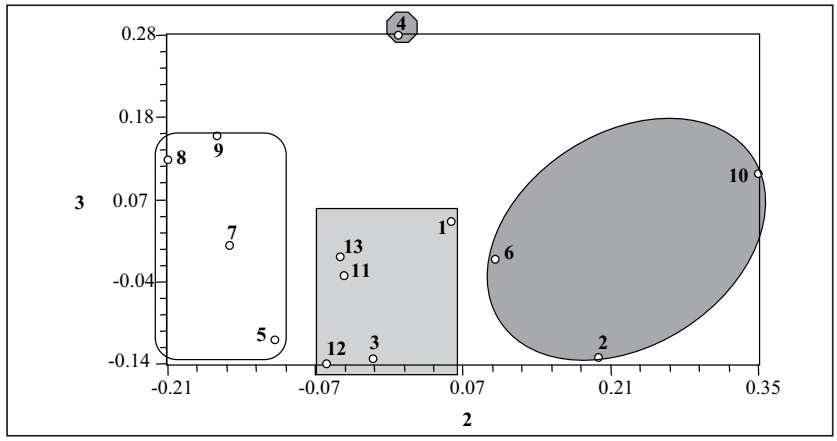

Fig. 4. Scatter plot of the 13 OTUs plotted against the second principal coordinate by the third principal coordinate.

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

No.	Characters	Principal coordinates		
		1	2	3
		Factors loading		
1	2	3	4	5
1.	Life cycle	0.31	0.29	0.72
2.	Plant length (cm)	-0.46	-0.69	-0.46
3.	Plant nature	0.39	0.86	0.96
4.	Plant surface	-0.73	0.28	-0.41
5.	Stem type	0.24	0.11	0.71
6.	Leaves arrangement	-0.24	-0.11	-0.71
7.	Number of leaves in whorls	-0.24	-0.11	-0.71
8.	Leaf Shape	-0.57	0.39	-0.15
9.	Leaf length	-0.21	0.77	0.83
10.	Leaf width	-0.26	0.77	-0.16
11.	Leaf margins	0.22	0.79	0.26
12.	Leaf base	-0.51	-0.66	-0.18
13.	Inflorescence type	0.44	0.14	0.16
14.	Inflorescence position	-0.33	-0.16	0.36
15.	Inflorescence flower number	0.59	0.33	0.20
16.	Peduncle length	-0.67	-0.48	0.20
17.	Peduncle diameter shape	0.15	-0.69	-0.33
18.	Peduncle surface	-0.41	0.47	-0.67
19.	Pedicel length	-0.98	-0.56	-0.22
20.	Pedicel diameter shape	-0.99	-0.80	-0.19
21.	Pedicel shape	0.31	0.86	0.36
22.	Flower diameter size	-0.23	0.11	0.85
23.	Petal length	0.31	-0.33	0.31
24.	Petal width	-0.51	0.26	0.78
25	Petal surface	0.27	0.63	-0.61
26.	Petal apex	0.32	0.44	-0.41
27.	Petal color	0.41	-0.58	-0.90
28.	Stamen length	-0.56	0.49	0.39
29.	Style length	-0.29	0.18	0.29
30.	Ovary shape	0.30	-0.39	-0.22
31.	Mericarp size (mm)	0.87	0.65	-0.38
32.	Mericarp surface	0.23	-0.43	0.67
33.	Seed shape	0.15	-0.89	0.47
34.	Seed size (mm)	0.19	0.89	0.23
35.	Epidermal cell patterns	0.38	-0.28	-0.57
36.	Anticlinal walls	-0.38	-0.96	-0.39
37.	The sculpture of anticlinal boundaries	0.20	-0.11	0.69
38.	Outer periclinal cell walls	0.54	-0.21	0.13
39.	Sculpture of periclinal cell walls	-0.63	0.57	0.28
40.	Pollen shape	0.38	-0.11	0.41
41.	Polar Axis (P)	-0.17	-0.81	0.66
42.	Equatorial Axis (E)	-0.14	-0.70	0.42
43.	Number of colpi	-0.49	-0.51	0.14
44.	Exine microspienes	-0.25	0.35	0.37
45.	Exine perforation	0.26	-0.28	-0.62
46.	Cross section shape	0.23	-0.70	-0.10
47.	Cortex	0.38	0.37	-0.38
48.	Xylem	0.80	0.60	0.54
49.	Pith	-0.80	-0.60	-0.54
50.	Pith characters	-0.37	0.46	0.76
Percentage per **PCO**		27.79	16.44	11.91
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 & Groendijk-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. ceratopodum*, *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. ceratopodum*, *G. spurium* subsp. *africanum* and *G. spurium* subsp. *spurium* as members in section *Aparine*, and classified them in different subsections. However, Ehrendorfer (1976) and Ehrendorfer & Schonbeck-Temesy (1982) considered these taxa as a good as members in section *Kolgyda* (synonym of sect. *Aparine*).

Natali & al. (1996) presented a phylogenetic analysis of 70 species from tribe *Rubieae* (32 species of *Galium*) using the DNA sequence of the chloroplast atpB-rbcL intergene region and indicated that *Galium* is a polyphyletic genus, and *G. aparine, G. spurium* and *G. tricornutum* were joined together in the same clade. Ehrendorfer (1971) considered the autogamous *G. aparine* complex as probably originating by allopolyploidy from three racial stocks of Southwest Asian origin. The species seems to include tetraploid, hexaploid and octaploid cytotypes; $2n$ = 42, 44, 48, 62, 66 and 68. Hanf (1983) considered that variants of *G. spurium* with setose fruits, apart from the flower characters and the diploid chromosome number are often not easy to distinguish from *G. aparine*. Abdel Khalik & al. (2007) investigated pollen morphology of *Galium* in Egypt and indicated that the genus is a stenopalynous and based on the number of apertures, which indicated that *G. aparine* has 7–9 colpi; *G. spurium* 6–8 colpi; *G. ceratopodium* 6–8, and *G. tricornutum* 8–9 colpi.

The results of both cluster and principal coordinated analysis confirmed that the group of *G. aparine*, *G. tricornutum, G. ceratopodum*, *G. spurium* subsp. *africanum,* and *G. spurium* subsp. *spurium* (sect. *Aparine* = *Kolgyda*) is a well-distinguished group, characteized 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 anticlinal 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 incongruent 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 & Schonbeck-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. *Leiogalia*. 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 subspecific 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 anticlinal boundaries, and solid above and hollow below pith characters. This result agrees with those of Ehrendorfer (1976) and Natali & al. (1996).

Boissier (1881) placed *G. murale, G. parisiense, G. setaceum* subsp. *setaceum,* and *G. setaceum* subsp. *decaisnei* in one section (sect. *Aparine*) and two subsections: *Apera* and *Xanthaparinea*. However, Ehrendorfer (1976) and Ehrendorfer & Schonbeck-Temesy (1982) regarded *G. murale* and *G. parisiense* as belonging to one and the same section (sect. *Kolgyda*), and *G. setaceum* to another section (sect. *Jubogalium*). Natali & al. (1995, 1996) presented a phylogenetic analysis of the tribe *Rubieae* using the DNA sequence of the chloroplast atpB-rbcL intergene region and indicated that *Galium* is polyphyletic genus and *G. murale* and *G. parisiense* are joined together in the same clade. Huysmans & al. (2003) reported that *G. parisiense* has 8 colpi, but *G. murale* and *G. setaceum* have 6–7colpi. Abdel Khalik & al. (2007) reported that *G. murale* and *G. setaceum* have 6–7 colpi, but *G. parisiense* has 8–10 colpi. On the other hand, Abdel Khalik & al. (2007) investigated the exine ornamentation of the pollen and found that *G. murale* and *G. parisiense* can be differentiated by their relatively larger and fewer microspines and overall density of spines. Our UPGMA and PCO analyses showed that this group (*G. murale, G. parisiense, G. setaceum* subsp. *setaceum,* and *G. setaceum* subsp. *decaisnei*) can be differentiated from the rest. The OTUs of this group are rather homogeneous. The group is distinct from the others by prostrate to ascending plant, strongly revolute leaf margins, erect pedicel, mericarp size (0.3–2.6 × 0.3–2.6 mm), sinuous anticlinal 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 & Schonbeck-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.

Acknowledgements. We are grateful to the Director and Curator of Brussels (Maeise) Herbarium (BR), Kew Herbarium (K), Leiden Herbarium (L), and Wageningen University Herbarium (WAG). Our great thanks also go to Prof. Dr van der Maesen, Professor of Plant Taxonomy in Biosystematics Group, Plant Science at Wageningen University, the Netherlands, for reading the manuscript and making some valuable suggestions. The authors are also indebted to the anonymous reviewer for the critical and useful notes and comments.

References

Abdel Khalik, K., Abdel Ghani, M. M. & El kordy, A. 2007. A palynological study of *Gaium* (*Rubiaceae*) in Egypt and its systematic implication. – Feddes Repert., **118**(7-8): 311-326.

Boissier, E. 1881. Flora Orientalis.Vol. 5. Geneva and Basel.

Boulos, L. 1995. Flora of Egypt, Checklist. Al Hadara Publishing, Cairo.

Boulos, L. 2000. Flora of Egypt. Vol. **2**. Al Hadara Publishing, Cairo.

Bremer, B., Andreasen, K. & Olsson, D. 1995. Subfamilial and tribal relationships in the *Rubiaceae* based on rbcL sequence data. – Ann. Missouri Bot. Gard., **82**: 383-397.

Dempster, L.T. & Ehrendorfer, F. 1965. Evolution of the *Galium multiflorum* complex in Western North America. II. Critical taxonomic revision. – Brittonia, **17:** 289-334.

Ehrendorfer F. 1961. *Rubiaceae*. – In: **Löve, A. & Löve, D**. (eds), Chromosome Number of Central and Northwest European Plant Species. – Opera Bot., **5:** 318-321.

Ehrendorfer, F. 1971. Evolution and ecogeographical differentiation in some Southwest Asiatic *Rubiaceae*.– In: **Davis, P.H., Harper, P.C. & Hedge, I.C.** (eds), Plant life of Southwest Asia. Pp. 195-215. Edinburgh Univ. Press, Edinburgh.

Ehrendorfer, F. 1976. *Galium* L. – In **Tutin, T.G. & al**. (eds), Flora Europaea. Vol. **4**, pp. 14-36. Cambridge Univ. Press, Cambridge.

Ehrendorfer, F. & Schonbeck-Temesy, E. 1982. *Galium* L. – In: **Davis, P.H.** (ed.), Fl. Turkey and the East Aegean Islands. Vol. **7**, pp. 767-823. Edinburgh Univ. Press, Edinburgh.

Hanf, M. 1983. The Arable Weeds of Europe. BASF UK Ltd, Ipswich.

Huysmans, S., Dessein, S., Smets, E. & Robbrecht, E. 2003. Pollen morphology of NW European representatives confirms monophyly of *Rubieae* (*Rubiaceae*). – Rev. Palaeobot. Palynol., **127**: 219-240.

Linnaeus, C. 1753. Species Plantarum. Laurentius Salvius, Holmiae.

Mabberley, D.J. 1987. The Plant Book. Cambridge Univ. Press, Cambridge.

Manen, J.F., Natali, A. & Ehrendorfer, F. 1994. Phylogeny of *Rubiaceae-Rubieae* inferred from the sequence of a cpDNA intergene region. – Plant Syst. Evol., **190:** 195-211.

Natali, A & Jeanmonod, D. 2000. *Rubiaceae*. – In: **Jeanmonod, D**. (ed.), Compléments au Prodrome de la Flore Corse. Conservatoire et Jardin Botaniques Ville de Genève.

Natali, A., Manen, J.F. & Ehrendorfer, F. 1995. Phylogeny of the *Rubiaceae-Rubioideae*, in particular the tribe *Rubieae*: evidence from a non-coding chloroplast DNA sequence. – Ann. Missouri Bot. Gard., **82**: 428-439.

Natali, A., Manen, J.F., Kiehn, M. & Ehrendorfer, F. 1996. Tribal generic and specific relationships in the *Rubioideae–Rubieae* (*Rubiaceae*) based on sequence data of a cpDNA intergene region. – Opera Bot. Belg., **7**: 193–203.

Rendle, A.B. 1963. The Classification of Flowering Plants, Vol. **2**, Dicotyledones. Cambridge Univ. Press, Cambridge.

Robbrecht, E. 1988. Tropical woody *Rubiaceae*. Characteristic features and progressions. Contributions to a new subfamilial classification. – Opera Bot. Belg. **1**: 1-271.

Robbrecht, E. 1994. Supplement to the 1988 outline of the classification of the *Rubiaceae* index to the genera. – In: **Robbrecht, E.** (ed.), Advances in *Rubiaceae* Macrosystematics. – Opera Bot. Belg., **6**: 173-196.

Rohlf, F.J. 1972. An empirical comparison of three ordination techniques in numerical taxonomy. – Syst. Zool., **21**: 271-280.

Rohlf, F.J. 1993. NTSYS-PC. Numerical Taxonomy and Multivariate Analysis System. Version 1.80. Applied Biostatistics Inc., New York.

Rutishauser, R. 1984. Blattquirle, Stipeln und Kolleteren beiden Rubieae (Rubiaceae) im Vergleich mit anderen Angiospermen. – Beitr. Biol. Pflanzen., **59**: 375-424.

Täckholm, V. 1974. Students' Flora of Egypt. Ed. 2. Publisher Cairo Univ., Beirut.

Van den Berg, R.G. & Groendijk-Wilders, N. 1999. Numerical analysis of the taxa of series *Circaefolia* (*Solanum* sect. *Petota*). – In: **Nee, M., Symon, D.E., Lester, R.N. & Jessop, J.P.** (eds.), *Solanaceae* IV. Advances in Biology and Utilization. Pp. 213-226. Royal Bot. Gard., Kew.

Wieringa, J.J. 1999. Monopetalanthus exit. A systematic study of *Aphanocalyx*, *Bikinia*, *Icuria*, *Michelsonia,* and *Tetraberlinia* (*Leguminosae, Caesalpinioideae*). – Wageningen Agric. Univ. Pap., **99-4**: 115-186.

Willis, J.C. 1985. A Dictionary of the Flowering Plants and Ferns. Ed. 8. Cambridge Univ. Press, Cambridge.