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NUMERICAL TAXONOMIC STUDY OF SOME TRIBES OF COMPOSITAE (SUBFAMILY ASTEROIDEAE) FROM EGYPT

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Abstract

A systematic study of 25 taxa belonging to 12 genera of tribes Gnaphalieae, Helenieae, Plucheeae and Senecioneae of Compositae from Egypt was conducted by means of numerical analysis based on 19 main pollen grains characters. On the basis of UPGMA (Unpaired Group Method off Averaging) clustering and PCO (Principal Component Analysis), two main groups and five subgroups are recognized.

Introduction

The Compositae (Asteraceae) is the largest family of plants, comprises of 1590 genera and around 23,600 known species arranged in 3 subfamilies, Asteroideae, Cichorioideae and Barnadesioideae and 17 tribes (Bremer, 1994; Bremer & Jansen 1992).

Tribe Gnaphalieae is one of the largest in the family, with more than 180 genera and 2000 species. Most Gnaphalieae are characterized by a two-layered pollen sexine with an outer baculae and an inner perforated layer. The Gnaphalieae are subdivided into five subtribes (Anderberg, 1991a). In subtribe Gnaphaliinae, the two largest genera are *Helichrysum* and *Gnaphalium*, with hundreds of species and with many closely related segregate genera. Classification problems within the tribe are dominated by the difficulties in generic delimitation of *Helichrysum* and *Gnaphalium.*

The Helenieae comprise a little more than 800 species in 110 genera, are often herbs. Some species have become naturalized as weeds in most parts of the world, eg., *Tagetes minuta* L. (Bierner, 1989). The widespread, familiar genera of the Helenieae are *Flaveria*, *Helenium*, *Pectis* and *Tagetes*. Most of the genera are monotypic or small- to medium-sized in species number; the average is about 7 species per genus (Robinson, 1981).

The Plucheeae are a small tribe of 28 genera and approximately 220 species. The tribe is widely distributed in the tropics and includes some widespread weedy species, eg., in the paleotropical genus *Sphaeranthus*. The pollen morphology and anatomy of the Plucheeae correspond to that of the Inuleae; thus, the pollen is echinate and caveate with one layer of baculae between the spines and two baculate layer in the spines bases (Leins, 1971; 1973).

The Senecioneae are the largest tribe, with more than 3000 species in 150 genera (Pelser *et al*., 2007), classified into three subtripes; Blennospermatinae, Senecioninae and Tussilagininae. In Senecioninae there is the largest genus of the family, *Senecio* that comprises well over 1000 species.

Materials and Methods

The present study dealt with the taxonomy of 25 species belonging to 12 genera of tribes Gnaphalieae, Helenieae, Plucheeae and Senecioneae of subfamily Asteroideae (Compositae) and these species are represented in flora of Egypt. Pollen materials were removed from living specimens collected in the field, or from herbarium specimens kept in the Herbarium of the Botany Department, University of Cairo (CAI), or from Herbarium of South Valley University Qena (QNA, proposed abbreviation) (Table 1). The nomenclature of the plants conforms to the following studies (El-Hadidi & Fayed, 1994/1995) and (Boulos, 1995, 2002).

In order to give a clear and complete picture of the morphological features of different pollen types, light microscopy (LM) in combination with scanning electron microscopy (SEM) are generally used. The LM studies were made with the aid of a Leitz light microscope equipped with an apochromatic oil immersion objective (x 100, N. A. 1.32) and periplan eyepieces (G. F. x 10). Measurements are based on 15-25 pollen grains perlide. SEM examinations were made with a Jeol-6300.

A total of 19 characters were measured for each species, comprising 9 morphometrical and 10 morphological characters. The characters were coded (Table 2) and subjected to UPGMA clustering method using a computer program (STATISTICA 5). Factor analysis and factor loading were performed to determine the major and specific characters that aid in separation using the same program. The relationships between the studied taxa have been expressed using the similarity coefficient proposed by Sokal & Sneath (1963). Construction of the tree illustrating the relationships between the studied samples was performed using Arithmetic Average (UPGMA) proposed by Sokal & Michener (1958). After that, we performed a principal component analysis (PCO). The terminology of Erdtman (1986) and Punt *et al*., (1974) were used to describe pollen grains.

Results

Clustering analysis of the 25 species belonging to 12 genera depending on 19 main pollen grains characters revealed the separation of these species into two major clads at 8 dissimilarity distance. The first clad comprised 16 species *Phagnalon nitidum*, *Phagnalon schweinfurthii*, *Helichrysum orientale*, *Filago contracta*, *Lasiopogon muscoides*, *Filago desertorum*, *Pseudognaphalium luteoalbum*, *Helichrysum conglobatum*, *Homognaphalium pulvinatum*, *Senecio glaucus subsp. coronopifolius*, *Filago mareotica*, *Filago prolifera*, *Gnaphalium uliginosum*, *Helichrysum glumaceum*, *Ifloga spicata* and *Sphaeranthus suaveolens*, while the second clad comprised 9 species *Phagnalon barbeyanum*, *Phagnalon rupestre*, *Flaveria bidentis*, *Senecio vulgaris*, *Senecio aegyptius*, *Senecio flavus*, *Senecio glaucus* subsp. *glaucus*, *Tagetes minuta* and *Senecio hoggariensis* (Fig. 1).

In the first major clad, we distinguished three subclads: 1) A subclad with *Sphaeranthus suaveolens* at 7.3 dissimilarity distance. 2) A subclad with *Helichrysum glumaceum* and *Ifloga spicata* at 6.8 dissimilarity distance, 3) and the third subclad comprised the rest of the 16 studied species at 5.9 dissimilarity distance, but in the second major clad, we distinguished only two subclads, one at 6.8 dissimilarity distance with *Tagetes minuta* and *Senecio hoggariensis* and the other at 6.3 dissimilarity distance with the rest of the 9 studied species.

Sculpture type (7) and collumellae type (9) characters (Table 2) were excluded in the analysis because they had only one code and caused no variation in the matrix.

Table 1. List of Taxa used for the study, arranged by tribe according to Bremer (1994).

No.	Species	Tribe	Herb.
	1. Unassigned Gnaphalieae		
1	*Phagnalon barbeyanum* Asch. & Schweinf.	Gnaphalieae	QNA
2	*P. nitidum* Fresen.	Gnaphalieae	QNA
3	*P. rupestre* (L.) DC.	Gnaphalieae	QNA
4	*P. schweinfurthii* Sch. Bip. ex Schweinf.	Gnaphalieae	CAI
	2. Subtribe Gnaphaliinae		
5	*Filago contracta* (Boiss.) Chrtek & Holub	Gnaphalieae	CAI
6	*F. desertorum* Pomel, Nouv.	Gnaphalieae	QNA
7	*F. mareotica* Delile, Descr.	Gnaphalieae	QNA
8	*F. prolifera* Pomel, Nouv.	Gnaphalieae	CAI
9	*Gnaphalium uliginosum* L.	Gnaphalieae	CAI
10	*Helichrysum conglobatum* (Viv.) Steud.	Gnaphalieae	CAI
11	*H. glumaceum* DC.	Gnaphalieae	CAI
12	*H. orientale* (L.) Gaertn.	Gnaphalieae	CAI
13	*Homognaphalium pulvinatum* (Delile) Fayed & Zareh	Gnaphalieae	CAI
14	*Ifloga spicata* (Forssk.) Sch.Bip.	Gnaphalieae	QNA
15	*Lasiopogon muscoides* (Desf.) DC.	Gnaphalieae	CAI
16	*Pseudognaphalium luteoalbum* (L.) Hilliard & B. L. Burtt	Gnaphalieae	CAI
	1. Subtribe Flaveriinae		
17	*Flaveria bidentis* (L.) Kuntze	Helenieae	CAI
	2.Subtribe Pectidinae		
18	*Tagetes minuta* L.	Helenieae	QNA
19	*Sphaeranthus suaveolens* (Forssk.) Dc.	Plucheeae	CAI
	1. Subtribe Senecioninae		
20	*Senecio aegyptius* L.	Senecioneae	CAI
21	*S. flavus* (Decne.) Sch. Bip.	Senecioneae	QNA
22	*S. glaucus* L. subsp. *coronopifolius* (Maire) C.	Senecioneae	QNA
23	*S. glaucus* L. subsp. *glaucus* L.	Senecioneae	CAI
24	*S. hoggariensis* Batt. & Trab.	Senecioneae	CAI
25	*S. vulgaris* L.	Senecioneae	CAI

Factor analysis using principal component analysis showed that the most intrinsic characters enhanced separations of the 25 species were 1-pollen class, 6-echinae type (both morphological characters), 12-exine thickness, 13-echinae length, 16-colpus width, 17-ora diameter and 19-mesocolpium diameter (morphometrical characters) and represented by factor 1 and responsible for 41.2% of the variation. On the other hand, factor 2 was responsible for 11.8% of species variation and represented by two characters, nexine state (morphological character) and P/E ratio (morphometrical character). Factor 3 was also excluded because there were no characters scored on it.

Discussion

In the Compositae, several authors have tried to provide natural system to divide this family into tribes (Bentham 1873a, Hoffmann 1890, Bremer 1994, Bremer & Jansen 1992, Anderberg 1991a, Bierner 1989, Robinson 1981, Pelser *et al*., 2007). These studies were based on a morphological and pollen grains characters, anatomy, phylogenetic analysis and DNA sequence. In the present study a large number of pollen morphology characters were scored and numerical methods (UPGMA and PCO) were applied to study the relationships between four Compositae tribes and estimate the level of variation within and between these tribes.

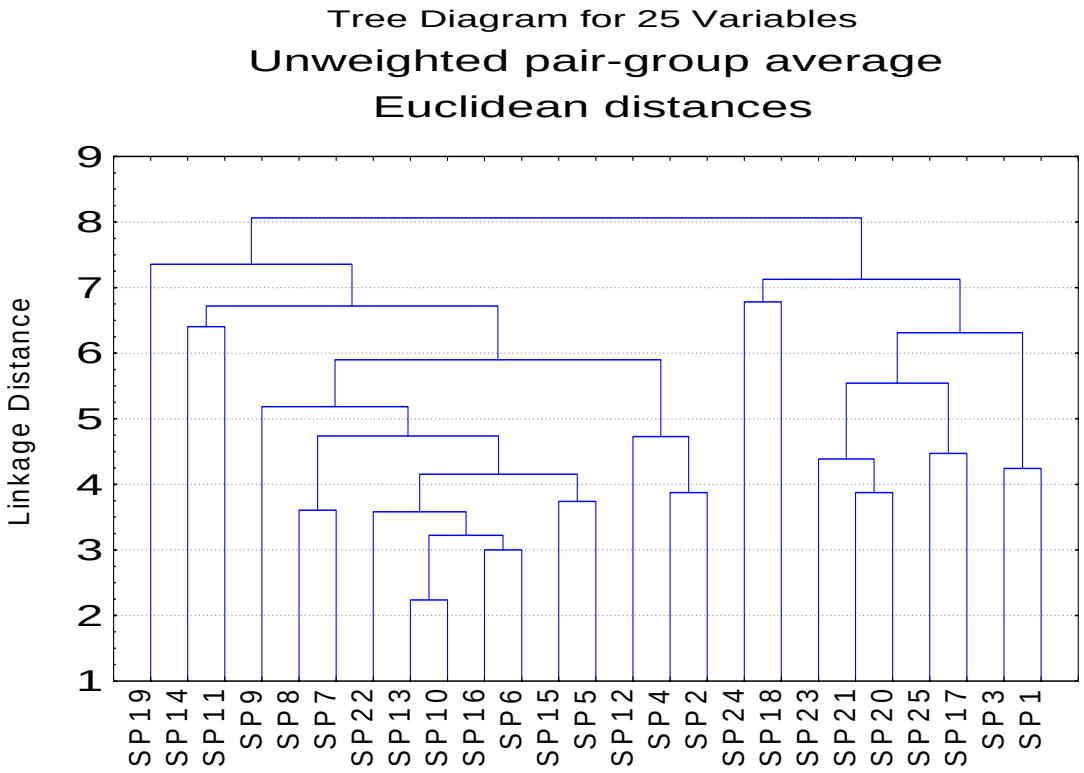

Fig. 1. Phenogram of the 25 studied species, clustering with the UPGMA method.

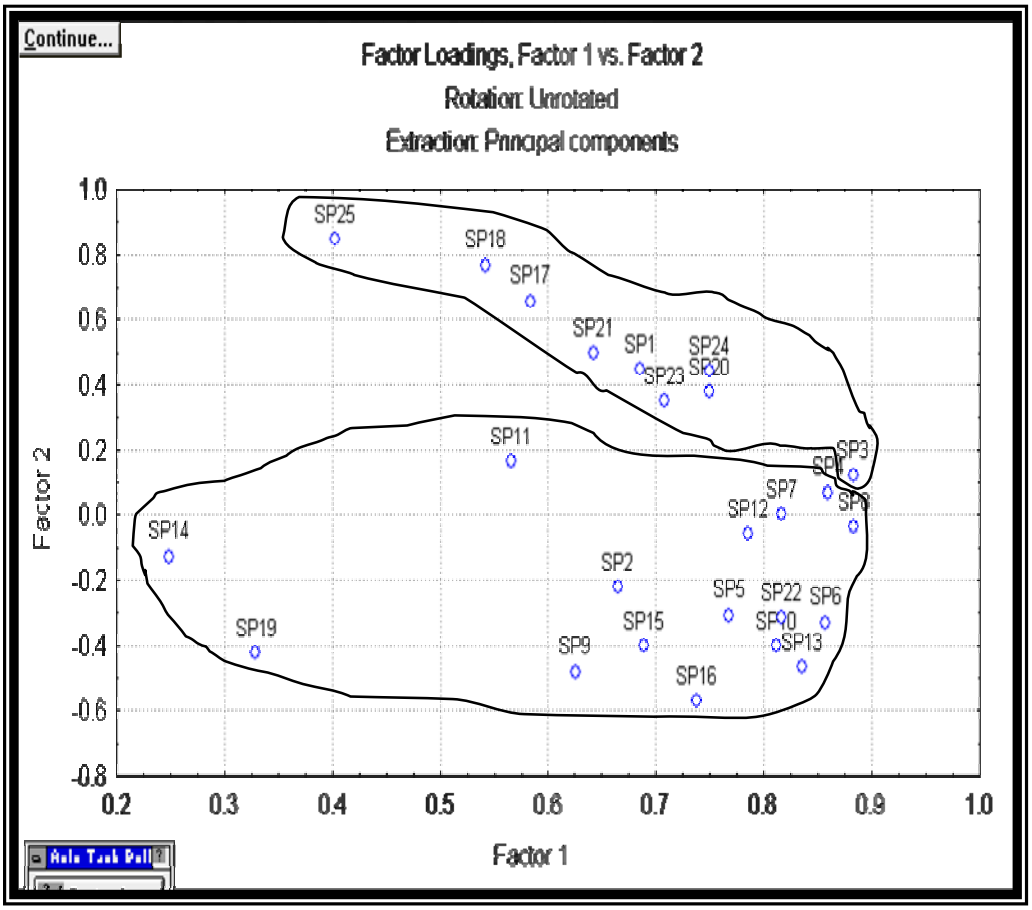

Fig. 2. Scatterplot of the 25 studied taxa plotted against the first principal coordinate by the second principal coordinate.

Table 2. Characters and character states used in morphometric analysis of Compositae tribes.

Character	Character state	Code
Morphological characters		
1. Pollen class	Pollen 3-zonocolporate (I)	1
	Pollen 3-zonocolpororate (II)	2
2. Pollen shape	Pollen suboblate	1
	Pollen oblate-spheroidal	2
	Pollen spheroidal	3
	Pollen prolate-spheroidal	4
3. Colpi width at equator	Slender	1
	Narrow	2
	Slightly wide	3
	Wide	4
4. Colpi ends	Tapered	1
	Pointed	2
5. Ora shape (Pores shape)	Lalongate, elliptic	1
	Lalongate, rectangular	2
	Lalongate, ovate	3
	Lolongate, elliptic	4
	Lolongate, rectangular	5
	Lolongate, ovate	6
	Circular	7
6. Echinae type (Spines type)	Spinules	1
	Spines	2
7. Sculpture type	Perforate	1
8. Sculpture state	Sculpturing diameter increases fairly towards the aperture margins	1
	Sculpturing diameter increases fairly towards the echinae bases	2
	Sculpturing restrict to the echinae bases	3
	Sculpturing diameter the same on whole of the pollen surface	4
	Sculpturing diameter increases towards the ecinae bases and the pollen pole	5
	Sculpturing diameter the same on the pollen surface and slightly increasing in number and diameter of pores present towards the apertures margins	6
	Sculpturing diameter the same on the pollen surface and slightly increasing in number and diameter of pores present towards the echinae bases	7
9. Columellae state	Columellae with thin, short and unbranched rods	1
10. Nexine state	Sexine as thick as Nexine	1
	Sexine thicker than Nexine	2
Morphometrical characters		
11. P/E (polar view/equatorial diameter)	P/E < 1	1
	P/E = 1	2
	P/E > 1	3

Table 2. (Cont'd.).

Character	Character state	Code
12. Exine thickness include echinae	Thickness = 3 µm	1
	Thickness = 4 µm	2
	Thickness = 5 µm	3
	Thickness = 6 µm	4
	Thickness = 7 µm	5
	Thickness = 8 µm	6
13. Echinae length	Length = 1 µm	1
	Length = 2 µm	2
	Length = 3 µm	3
	Length = 4 µm	4
	Length = 5 µm	**5**
14. Nexine thickness	Thickness = 1 µm	1
	Thickness = 2 µm	2
	Thickness = 3 µm	3
15. Colpus length	Length = 12 µm	1
	Length = 13 µm	2
	Length = 15 µm	3
	Length = 16 µm	4
	Length = 18 µm	5
	Length = 19 µm	6
	Length = 21 µm	7
	Length = 27 µm	8
16. Colpus width	Width = 2 µm	1
	Width = 3 µm	2
	Width = 4 µm	3
	Width = 5 µm	4
17. Ora diameter	Diameter = 4 µm	1
	Diameter = 5 µm	2
	Diameter = 6 µm	3
	Diameter = 7 µm	4
	Diameter = 8 µm	5
18. Apocolpium diameter	Diameter = 5 µm	1
	Diameter = 6 µm	2
	Diameter = 7 µm	3
	Diameter = 8 µm	4
	Diameter = 10 µm	5
	Diameter = 16 µm	6
19. Mesocolpium diameter	Diameter = 10 µm	1
	Diameter = 11 µm	2
	Diameter = 12 µm	3
	Diameter = 13 µm	4
	Diameter = 14 µm	5
	Diameter = 15 µm	6
	Diameter = 16 µm	7
	Diameter = 17 µm	8
	Diameter = 18 µm	9
	Diameter = 24 µm	10

Table 3. Data matrix used in the numerical analysis of the Compositae tribes.

No.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1.	*Phagnalon barbeyanum*	2	4	4	1	2	2	1	5	1	1	3	3	3	2	4	4	5	4	8
2.	*P. nitidum*	2	2	4	2	6	1	1	4	1	2	1	3	2	2	2	4	5	4	3
3.	*P. rupestre*	2	4	4	1	6	2	1	4	1	1	3	3	3	2	3	4	5	4	8
4.	*P. schweinfurthii*	2	2	4	2	6	2	1	4	1	2	1	3	3	1	3	4	4	5	6
5.	*Filago contracta*	1	3	3	2	4	1	1	3	1	1	2	1	1	2	3	3	2	3	3
6.	*F. desertorum*	1	3	3	2	6	1	1	4	1	1	2	1	1	1	4	2	2	2	4
7.	*F. mareotica*	1	4	3	2	4	1	1	4	1	1	3	1	1	2	4	3	3	3	6
8.	*F. prolifera*	1	3	3	2	6	1	1	4	1	1	2	2	1	2	4	3	2	1	7
9.	*Gnaphalium uliginosum*	1	3	4	1	6	2	1	6	1	2	2	3	3	1	2	1	1	1	3
10.	*Helichrysum conglobatum*	2	3	4	1	6	1	1	4	1	1	2	1	2	1	2	2	2	4	4
11.	*H. glumaceum*	2	3	4	2	2	2	1	7	1	1	2	3	3	2	5	3	2	1	5
12.	*H. orientale*	1	2	4	1	7	2	1	2	1	1	1	3	3	2	1	3	3	3	6
13.	*Homognaphalium pulvinatum*	1	3	4	2	7	1	1	4	1	1	2	1	2	1	2	3	2	3	4
14.	*Ifloga spicata*	2	3	3	1	1	1	1	4	1	2	2	2	2	1	2	2	1	3	2
15.	*Lasiopogon muscoides*	1	3	1	1	4	1	1	4	1	2	2	1	1	1	2	2	2	1	3
16.	*Pseudognaphalium luteoalbum*	1	3	4	2	6	1	1	4	1	1	2	1	2	1	2	2	1	1	3
17.	*Flaveria bidentis*	2	1	4	2	3	2	1	2	1	1	1	5	5	2	4	3	4	2	8
18.	*Tagetes minuta*	2	3	4	2	2	2	1	2	1	1	2	6	5	2	7	4	5	6	10
19.	*Sphaeranthus suaveolens*	2	3	4	1	7	2	1	1	1	2	2	2	5	1	1	3	3	4	1
20.	*Senecio aegyptius*	2	2	2	2	5	2	1	2	1	2	1	3	3	2	5	4	3	2	6
21.	*S. flavus*	2	3	4	2	3	2	1	2	1	2	2	4	3	1	4	4	4	2	5
22.	*S. glaucus* subsp. *coronopifolius*	2	3	3	2	6	1	1	4	1	2	2	3	2	1	3	3	2	1	4
23.	*S. glaucus* subsp. *glaucus*	2	2	3	2	4	2	1	4	1	2	1	3	4	2	6	3	2	4	5
24.	*S. hoggariensis*	2	2	1	1	6	2	1	4	1	2	1	4	3	1	8	3	5	5	9
25.	*S. vulgaris*	2	1	3	1	1	2	1	3	1	2	1	5	5	3	6	4	5	4	7

UPGMA gives insight into degree of similarity among the studied species and whether they form groups // clusters and gives an indication of the level of variation within and between tribes. PCO reflects which characters are important on the axes, and indicates the significant characters based on the highest factor score (Table 4). Therefore it becomes clear which characters cause the separation between groups and can be useful to distinguish taxa. Generally, our results show congruence between the UPGMA clustering and PCO analyses in suggesting two main groups and five subgroups.

Anderberg & Bremer (1991) and Anderberg & Freire (1991) classified the Gnaphalieae into 6 groups, Gnaphalieae unassigned to a subtribe group, subtribes Loricariinae, Relhaniinae, Cassiniinae, Angianthinae and Gnaphaliinae. They putted *Phagnalon* in Gnaphalieae unassigned to a subtribe group. Within the subtribe Gnaphaliinae, Anderberg (1991a) recognized 5 informal groups. From these groups, *Helichrysum* group that comprises from our genera, *Helichrysum***,** *Homognaphalium* and *Pseudognaphalium*, *Gnaphalium* group that comprises the genus *Gnaphalium* from our taxa, *Filago* group that comprises the genus *Filago*. In addition to the last five groups of Gnaphaliinae, a number of genera that are similar vegetatively were not placed in any group by Anderberg (1991a) from these genera, *Ifloga* and *Lasiopogon* that are still obscure. In our results two species of *Phagnalon* (*P. nitidum* and *P. schweinfurthii*) were in the third subclad of the first major clad and the another two species (*P. barbeyanum* and *P. rupestre*) were in the second subclad of the second major clad. All species of genera *Helichrysum***,** *Homognaphalium* and *Pseudognaphalium* were in the third subclad of the first major clad, except *Helichrysum glumaceum* was with *Ifloga spicata* in the second subclad of the first major clad. All species of genera *Gnaphalium, Lasiopogon* and *Filago* were in the third subclad of the first major clad.

In his cladistic, Karis (1993b) recognized 8 subtribes under the Helenieae, from these subtribes, subtribe Flaveriinae that comprises genus *Flaveria* and subtribe Pectidinae that comprises genus *Tagetes***.** In our results, both *Flaveria* and *Tagetes* were in the second major clad, but *Flaveria* was in the second subclad and *Tagetes* was with *Senecio hoggariensis* in the first subclad.

Anderberg (1991c) presented a cladistic analysis of the Plucheeae with some modifications on Bentham (1873a) and Hoffmann (1890) classifications. He put the *Pluchea* and *Sphaeranthus* groups together in the Plucheeae, comprising 12 genera and more than 150 species. In Anderberg classification the *Sphaeranthus* group comprises of 6 genera in addition to *Sphaeranthus*. In our results *Sphaeranthus* only was in the first subclad of the first major clad.

Approximately one-third of Senecioneae species are placed in the genus *Senecio*, making it one of the largest and the oldest genera of flowering plants (Willis 1918). Pelser *et al*. (2007) made phylogenetic analyses of nrITS and plastid DNA sequence data of tribe Senecioneae and a new, monophyletic, delimitation of *Senecio*.

In a preliminary cladistic analysis of the Senecioneae, Bremer (1994) recognized that all genera are readily assigned to the senecioid complex or to the cacalioid complex. In old world Senecioid genera, *Senecio* was included. In our results, all species of genus *Senecio* except *S. hoggariensis* (in the first subclad of the second major clad) and *S. glaucus L. subsp. coronopifolius* (in the third subclad of the first major clad) were in the second subclad of the second major clad.

Table 4. Factor loadings showed the most intrinsic characters enhanced separations of the studied species.

Factor Loadings **Rotation: Unrotated** **Extraction: Principal components**			
	Factor 1	**Factor 2**	**Factor 3**
1. Pollen class	**0.704594**	-0.10053	0.240509
2. Pollen shape	-0.52622	0.669851	0.239122
3. Colpus width at equator	0.05553	0.354384	0.61413
4. Colpi ends	-0.0321	0.121514	-0.47099
5. Ora shape	-0.40811	-0.25164	0.297195
6. Echinae type	**0.76094**	-0.04901	0.248048
7. Sculpture type			
8. Sculpture state	-0.37275	0.257576	-0.22823
9. Colpus state			
10. Nexine state	0.201808	**-0.75165**	0.047866
11. P/E	-0.43427	**0.757103**	0.224489
12. Exine thick includes echinae	**0.900116**	-0.03651	-0.01818
13. Echinae length	**0.802749**	-0.15833	0.338105
14. Nexine thick	0.55711	0.34943	-0.30883
15. Colpus length	0.651853	0.146722	-0.57976
16. Colpus width	**0.739537**	0.27478	0.022313
17. Ora diameter	**0.815435**	0.240725	0.131999
18. Apocolpium diameter	0.604641	0.124386	0.330522
19. Mesocolpium diameter	**0.704726**	0.444947	-0.26482
Percentage	**41.2%**	**11.8%**	**-**

References

Anderberg, A.A and S.E. Freire. 1991. A cladistic and biogeographical analysis of Lucilia group (Asteraceae–Gnaphalieae). *Botanical Journal of Linnean Society*, 106: 173-198.

Anderberg, A.A. 1991a. Taxonomy and phylogeny of the tribe Gnaphalieae (Asteraceae). *Opera Botanica*, 104: 1-195.

Anderberg, A.A. 1991c. Taxonomy and Phylogeny of the tribe Plucheeae (Asteraceae). *Plant Systematics and Evolution* 176: 145-177.

Anderberg, A.A. and K. Bremer. 1991. Parsimony analysis and cladistic reclassification of the *Relhania* generic group (Asteraceae - Gnaphalieae). *Annals of the Missouri Botanical Garden*, 78: 1061-1072.

Bentham, G. 1873. Compositae. In *Genera Plantarum*, vol. 2, no. 1. (Eds.): G. Bentham and J.D. Hooker. London: Lovell Reeve. 163-533.

Bierner, M.W. 1989. Taxonomy of Helenium sect. Amarum (Asteraceae). *Sida*, 13: 453-459.

Boulos, L. 2002. *Flora of Egypt*. Vol. 3. Verbenaceae-Compositae. Al-Hadara Publishing, Cairo.

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

Bremer, K. 1994. *Asteraceae. Cladistics and Classifications*. Timber Press Inc., Portland, OR.

Bremer, K. and R.K. Jansen. 1992. A new subfamily of the Asteraceae. *Annals of the Missouri Botanical Jarden*, 79: 414-415.

El-Hadidi, M.N. and A.A. Fayed. 1994/95. Materials for Excursion flora of Egypt. *Taeckholmia*, 15: 96-98.

Erdtman, G. 1986. *Pollen morphology and plant taxonomy, Angiosperms* (3rd ed.) Brill, Leiden.

Hoffmann, O. 1890. Compositae. In *Die Natürlichen Pflanzenfamilien*, vol. 4, no. 5. (Eds.): A. Engler and K. Prantl. Leipzig: Verlag von Wilhelm Engelmann. 87-391.

Karis, P.O. 1993b. The Heliantheae sensu lato (Asteraceae), clades and classification. *Plant systematics and Evolution*, 186: 69-93.

Leins, P. 1971. Pollensystematische studien an Inuleen, I: Tarchonanthinae, Plucheinae, Inulinae, Buphthalminae. *Botanishe Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie*, 91: 91-146.

Leins, P. 1973. Pollensystematische studien an Inuleen, II: Filagininae. *Botanishe Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 93: 603-611.

Mobberley, D.J. 2008. Mabberley's Plant-Book. *Apartable dictionary of plant, there classification and uses.* Ed.3, Cambridge.

Pelser, P.B., B. Nordenstam, J.W. Kadereit and L.E. Watson. 2007. An ITS phylogeny of tribe Senecioneae (Asteraceae) and a new delimitation of *Senecio* L. *Taxon (International Association for Plant Taxonomy* (IAPT)). 56 (4): pp. 1077–14E(-1062).

Punt, W., J.S. De Leeuw and W.A.P. Van Oostrum. 1994. The Northwest European pollen Flora, 3. Primulaceae. *Rev. Palaeobot. Palynol.*, 17: 31-70.

Robinson, H. 1981. A revision of the tribal and sub-tribal limits of the Heliantheae (Asteraceae). *Smithsonian Contributions to Botany*, 51: 1-102.

Sokal, R.R. and C.D. Michener. 1958. A statistical method for evaluating systematic relationships. *Univ. Kansas, Sci. Bull.*, 28: 1409-1438.

Sokal, R.R. and P.A. Sneath. 1963. *In*: *Principles of numerical taxonomy*. San Francisco: Freeman, p. 359.

Willis, J.C. 1918. The age and area hypothesis. *Science*, 47: 626.

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