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Phenetic and cladistic analyses of Boraginaceae Juss.

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Abstract

Background The systematics of family Boraginaceae draw attention of many botanists for many years. The current study's primary goals are to clarify phenetic and phylogenetic relationships within Boraginaceae according to morphology and molecular characteristics and to evaluate the morphological characters that can be applied in systematics of Boraginaceae.

Results The macromorphological characters of 39 species, 2 subspecies and 5 varieties of wild boraginaceous plants were extracted and subjected to phenetic and principal component analysis that was performed for detecting the most important characters differentiating the studied taxa. The generated dendrogram is divided into five clear groups; *Arnebia decumbens* var. *macrocalyx* and *Heliotropium curassavicum* are the most distantly related species, while *Echium angustifolium* subsp. *angustifolium* and *E. angustifolium* subsp. *sericeum* are the most closely related species. The phylogenetic relations among the examined taxa were determined using DNA barcoding of the *rbcl* gene. The phylogenetic analysis generated a cladogram showing that among the studied taxa of Boraginaceae there is a bolster for three clear lineages with resolved relationships.

Conclusions It is concluded that the chosen morphological characters were important in species delimitation, where more than half of the total morphological variations (67.94%) were explained by the first two principal components, indicating that the morphological characters showed high variability, which is useful for discrimination, and these characters, in addition to molecular characters, shared in drawing the phenetic and phylogenetic relationships within Boraginaceae that were considered not monophyletic groups. Boraginaceae contained some monophyletic genera such as *Heliotropium* and *Alkanna*, while the other studied taxa expressed a non-monophyletic relationships.

Keywords Cladogram, MrBayes, PAST, PCA, Phenogram

1 Background

Boraginaceae Juss. includes around 1600 to 1700 species in 90 genera [1] and is widely widespread in tropical (Northern and Central South America), subtropical, and temperate (Irano-Turanian and Mediterranean) regions of the world [2, 3]. This family is represented in the wild Egyptian flora by 15 genera [4] viz. *Adelocaryum*, *Alkanna*, *Anchusa*, *Arnebia*, *Cordia*, *Echiochilon*, *Echium*, *Heliotropium*, *Lappula* (=*Echinosperma*), *Lithospermum*, (=*Moltiopsis*), *Myosotis*, *Nonnea*, *Onosma* (=*Podonosma*), *Paracaryum* and *Trichodesma*. These genera include 44 species and 5 varieties. The largest

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genus is *Heliotropium* (11 species), *Echium* (7 species), and *Anchusa* (6 species). On the other hand, the genera *Adelocaryum*, *Echiochilon*, *Myosotis*, *Nonnea* and *Podonosma* are represented in Egypt by one species. [5] transferred *Cordia* species and *Coldenia* to the family Ehretiaceae and combined four additional genera: *Asperugo*, *Eritrichium*, *Gastrocotyle* and *Hormuzakia*, which include 52 species and 9 varieties.

Many botanists were interested in the systematic categorization of the family for many years, viz. [6–10].

The infrafamilial classification of Boraginaceae was traditionally divided into five subfamilies: Boraginoideae, Cordioideae, Ehretioideae, Heliotropioideae and Wellstedioideae. [11–19] accepted this subfamilial treatment although other scientists not. [20–22] moved Cordioideae, Heliotropioideae and Ehretioideae to Heliotropiaceae based on embryological criteria, while [17, 23–25] treated Wellstedioideae at familial level as Wellstediaceae. Conversely, Hoplestigmataceae, Hydrophyllaceae and Lennoaceae were widely recognized as different families. [3] recognize eight subfamilies, viz. Boraginoideae, Cordioideae, Ehretioideae, Heliotropioideae, Hydrophylloideae, Lennooideae, Namoideae and Wellstedioideae.

Boraginaceae comprises about 13 tribes divided into eight subtribes [26]. [27] recognizes six tribes Borageineae, Cynoglosseae, Eritrichieae, Lithospermeae, Myosotideae and Trigonotideae but molecular criteria of [10] nest Eritrichieae, Myosotideae and Trigonotideae within Cynoglosseae *s. l.* so support four tribes based on both molecular characteristics and morphology [28, 29] including Borageineae, Cynoglosseae, Echiochileae and Lithospermeae. Cynoglosseae *s. l.* is the largest and morphologically complex tribe that contains more than half of the family's species.

Boraginaceae is regarded as monophyletic due to morphological, molecular and phytochemical traits [30–34]. Other phylogenetic studies demonstrate that Boraginaceae traditionally is paraphyletic with regard to Hoplestigmataceae, Hydrophyllaceae and Lennoaceae [9, 10, 30, 32, 35, 36].

Multiple phylogenetic analyses on Boraginaceae are centered on connections inside a genus or among genera that are closely related [37, 38], although other studies carried on tribal level [28, 29, 35, 39] that resolve the interrelationship among tribes but the relationships inside each tribe still largely unsettled [37].

The main objectives of the present study were to clarify phenetic and phylogenetic relationships within Boraginaceae according to morphology and molecular characters and to evaluate the morphological characters that can be used in systematics of Boraginaceae.

2 Methods

2.1 Plant material

The current study was carried out on 46 taxa (39 species, 2 subspecies, 5 varieties) belonging to 14 genera (Table 1) representing more than 93% of the Boraginaceae in the flora of Egypt according to [4].

2.2 Macromorphological characters investigation

The easily observable character states of (42) morphological characters are summarized in Table 2. These characters were investigated from herbarium specimens deposited at the Herbaria of Ain Shams University, Faculty of Science (CAIA), Cairo University, Faculty of Science (CAI), Flora and Phytotaxonomy Research Department (CAIM) and Orman Botanical Garden, Giza. Published descriptions also were consulted [40]. The identification and nomenclature were authenticated using [5, 41] and International Plant Name Index [42].

2.3 Extraction of DNA and amplification of *rbcL* primers

In an Eppendorf tube, liquid nitrogen was used to crush 100 mg of leaves into a powder, and then, DNA was extracted with the aid of CTAB (cetyltrimethylammonium bromide) protocol of Doyle and Doyle (1987). The *rbcL* region of the purified DNA was amplified with the aid of PCR with the following universal primers:

Forward primer: 5′-ATG TCA ACA CAA ACA GAG ACT AAA GC-3′;

Reverse primer: 5′-GAA ACG GTC TAT CCA ACG CAT-3′.

The reactions of the amplification were performed in 25 µL as follows: 5×GoTaq® Flexi buffer 5 µL, MgCl2 (25 mM) 2.5 µL, dNTPs (10 mM each) 0.5 µL, forward primer (10 µM) 1.2 µL, reverse primer (10 µM) 1.2 µL, Go Taq™ (5 U/µL) 5 µL, DNA stock 2 µL, H_2O 7.6 µL up to make 25 µL total volume. The following were the reaction conditions: initial denaturation at 95 °C for 5 min, 40 cycles at 94 °C for 30 s, 58 °C for 30 s, 72 °C for 45 s and 72 °C for 10 min. The purification kit of the PCR product (Thermo PCR Purification Kit, USA) was used to separate all positive PCR amplicons from other unwanted materials such as dimers, RNA, free nucleotides, and unamplified DNA fragments. It is a necessary step prior to the automated DNA sequencing. Macrogen Korea, 6F, 172, Dolma-ro, Bundang-gu, Seongnam-si, Gyeonggi-do (Jeongja-dong, Seoul National University Bundang Hospital Healthcare Innovation Park) received the purified DNA for sequencing.

Table 1 Voucher specimens of Boraginaceae, their numbers that were kept in the public herbarium of Ain Shams University and their accession numbers in GenBank (46 taxa, 14 genera)

No.	Taxa	Voucher	*rbcl*
1.	*Alkanna orientalis* Boiss., Diagn. Pl. Orient. ser. 1, 4: 46 (1844)	UKA201	OP933830
2.	*Alkanna strigosa* Boiss. & Hohen., Diagn. Pl. Orient. ser. 1, 4: 46 (1844)	UKA202	OP933831
3.	*Alkanna tinctoria* Tausch, Flora 7(1): 234 (1824)	UKA203	OP933832
4.	*Anchusa aegyptiaca* DC., Prodr. [A. P. de Candolle] 10: 48 (1846)	UKA204	OP933833
5.	*Anchusa hispida* Forssk., Fl. Aegypt.-Arab. 40. (1775)	UKA205	OP933834
6.	*Anchusa humilis* I.M.Johnst., Contr. Gray Herb. 73: 55 (1924)	UKA206	OP933835
7.	*Anchusa milleri* Lam. ex Spreng., Bot. Gart. Halle Erster Nachtrag: 11 (1801)	UKA207	OP933836
8.	*Anchusa undulata* L., Sp. Pl. 1: 133 (1753)	UKA208	OP933837
9.	*Arnebia decumbens* var. *decumbens* Coss. & Kralik, Bull. Soc. Bot. France 4: 398, 402 (1857)	UKA200	OP933838
10.	*Arnebia decumbens* var. *macrocalyx* Coss. & Kralik, *Bull. Soc. Bot. France 4: 403 (1857)*	UKA210	OP933839
11.	*Arnebia hispidissima* DC., Prodr. [A. P. de Candolle] 10: 94 (1846)	UKA211	OP933840
12.	*Arnebia linearifolia* DC., Prodr. [A. P. de Candolle] 10: 95 (1846)	UKA212	OP933841
13.	*Arnebia tinctoria* Forssk., Fl. Aegypt.-Arab. 63. (1775)	UKA213	OP933842
14.	*Asperugo procumbens* L., Sp. Pl. 1: 138 (1753)	UKA214	OP933843
15.	*Buglossoides incrassata* (Guss.) I.M.Johnst., J. Arnold Arbor. 35(1): 43 (1954)	UKA215	OP933844
16.	*Buglossoides tenuiflora* (L.f.) I.M.Johnst., J. Arnold Arbor. 35(1): 42 (1954)	UKA216	OP933845
17.	*Coldenia procumbens* L., Sp. Pl. 1: 125 (1753)	UKA217	OP933846
18.	*Echiochilon fruticosum* Desf., Fl. Atlant. 1: 166, t. 47 (1798)	UKA218	OP933847
19.	*Echium angustifolium* subsp. *angustifolium* Mill., Gard. Dict., ed. 8. n. 6 (1768)	UKA219	OP933848
20.	*Echium angustifolium* subsp. *sericeum* (Vahl) Klotz, *Wiss. Z. Martin-Luther-Univ. Halle-Wittenberg, Math.-Naturwiss. Reihe 11: 298 1962*	UKA220	OP933849
21.	*Echium horridum* Batt., Bull. Soc. Bot. France 39: 336 (1893)	UKA221	OP933850
22.	*Echium rauwolfii* Delile, Descr. Egypte, Hist. Nat. 195, t. 19, f. 3 (1813)	UKA222	OP933851
23.	*Echium rubrum* Forssk., Fl. Aegypt.-Arab. 41 (1775)	UKA223	OP933852
24.	*Echium sabulicola* Pomel, *Nouv. Mat. Fl. Atl. 1: 90 1874*	UKA224	OP933853
25.	*Heliotropium aegyptiacum* Lehm., Ind. Sem. Hort. Hamburg. (1820) 8	UKA225	OP933854
26.	*Heliotropium arbainense* Fresen., Mus. Senckenberg. i. (1833) 168	UKA226	OP933855
27.	*Heliotropium bacciferum* var. *bacciferum* Forssk., Fl. Aegypt.-Arab. 38. (1775)	UKA227	OP933856
28.	*Heliotropium bacciferum* var. *erosum* (Lehm.) Hadidy, in L. Boulos, Fl. Egypt Checklist 118 (1995)	UKA228	OP933857
29.	*Heliotropium curassavicum* L., Sp. Pl. 1: 130 (1753)	UKA229	OP933858
30.	*Heliotropium digynum* Asch. ex C.Chr., Dansk Bot. Ark. iv. No. 3, 14 (1922)	UKA230	OP933859
31.	*Heliotropium hirsutissimum* Weber, Pl. Min. Cogn. Decuria 1 (1784)	UKA231	OP933860
32.	*Heliotropium lasiocarpum* Fisch. & C.A.Mey., Index Seminum [St.Petersburg (Petropolitanus)] iv. 38	UKA232	OP933861
33.	*Heliotropium ovalifolium* Forssk., Fl. Aegypt.-Arab. 38. (1775)	UKA233	OP933862
34.	*Heliotropium pterocarpum* Hockst. & Steud. ex Bunge, Bull. Soc. Imp. Naturalistes Moscou 42(1): 331, (1869)	UKA234	OP933863
35.	*Heliotropium ramosissimum* Sieber ex DC., Prodr. [A. P. de Candolle] 9: 536 (1845)	UKA235	OP933864
36.	*Heliotropium strigosum* var. *brevifolium* (Wall.) C.B.Clarke, Fl. Brit. India [J. D. Hooker] 4: 151 (1883)	UKA236	OP933865
37.	*Heliotropium supinum* L., Sp. Pl. 1: 130 (1753)	UKA237	OP933866
38.	*Heliotropium zeylanicum* Lam., Encycl. [J. Lamarck & al.] 3(1): 94 (1789)	UKA238	OP933867
39.	*Lappula sinaica* (A.DC.) Asch. & Schweinf., Mém. Inst. Égypt. 2: 111 (1887)	UKA239	OP933868
40.	*Lappula spinocarpos* (Forssk.) Asch. ex Kuntze, Trudy Imp. S.-Peterburgsk. Bot. Sada 10: 215 (1887)	UKA240	OP933869
41.	*Moltkiopsis ciliata* (Forssk.) I.M.Johnst., J. Arnold Arbor. 34: 3 (1953)	UKA241	OP933870
42.	*Nonea viviani* A.DC., Prodr. [A. P. de Candolle] 10: 31 (1846)	UKA242	OP933871
43.	*Paracaryum intermedium* Lipsky, Trudy Imp. S.-Peterburgsk. Bot. Sada xxvi. 487 (1910)	UKA243	OP933872
44.	*Paracaryum rugulosum* Boiss., Diagn. Pl. Orient. ser. 1, 11: 129 (1849)	UKA244	OP933873
45.	*Trichodesma africanum* (L.) Sm., Cycl. [A. Rees], (London ed.) 36: Trichodesma no. 2 (1817)	UKA245	OP933874
46.	*Trichodesma ehrenbergii* Schweinf. ex Boiss., Fl. Orient. [Boissier] 4(2): 281 (1879)	UKA246	OP933875

Table 2 The extracted morphological characters (42), their states (107) and codes of the studied taxa

No.	Character	Character state and its (code)
1.	Habit	Herb (0) Woody shrub (1)
2.	Texture	Glabrous (1) Hairy (0)
3.	Strength	Erect (0) Prostrate (1)
4.	Stem branching	From base (0) Above the base (1)
5.	Basal leaves arrangement	Alternate (1) Opposite (0)
6.	Upper leaves petioles	Pedicelled (0) Sessile to sub-sessile (1)
7.	Lamina composition	Simple (0) Pinnatipartite (1)
8.	Lamina shape	Lanceolate (0) Linear (1) Oblong (2) Ovate (3)
9.	Lamina surface	Wrinkled (0) Smooth (1)
10.	Base of lamina	Symmetric (0) Asymmetric (1)
11.	Leaf veins	Prominent (0) Not prominent (1)
12.	Leaf margin	Entire (0) Serrate (1) Undulate (2)
13.	Leaf apex	Acute (0) Obtuse (1)
14.	Hairs on leaves	Absent (1) Simple (0) Glandular (2) Bulbs (3) Hispid (4) Woolly (5) Simple & glandular (6) Simple & bulbs (7) Simple & hispid (8) Simple & wooly (9) Glandular & hispid (10) Bulb & hispid (11)
15.	Bracteoles	Absent (0) Enclosing calyx (1) Not enclosing calyx (2)
16.	Inflorescence type	Raceme (0) Circinnate (1)
17.	Inflorescence leaves	Leafy (0) Leafless (1)
18.	Number of flowers	Less than 8 (0) More than 10 (1)
19.	Flower	Pedicelled (0) Sessile to sub-sessile (1)
20.	Sepal fusion	Less than half the length (0) More than half the length (1)
21.	Apex of calyx lobes	Acute (0) Filiform (1)
22.	Hairs on sepals	Absent (0) Simple (1) Glandular (2) Hispid (3) Wooly (4) Simple & glandular (5) Simple & hispid (6) Simple & wooly (7) Glandular & hispid (8) Simple, glandular and wooly (9)
23.	Petal color	Blue, purple or pink (0) Yellow or white (1)
24.	Petal texture	Glabrous (0) Hairy (1)
25.	Petal apex	Acute (0) Obtuse (1)
26.	Petal fusion	Less than half the length (0) More than half the length (1)
27.	Petal lobes	Equal (0) Unequal (1)
28.	Corolla throat	With scales (0) Without scales (1)
29.	No. of stamens	Two (0) Five (1)
30.	Anthers level	Exerted (0) Included (1)
31.	Filaments texture	Glabrous (0) Hairy (1) Reduced (2)
32.	Appendix on anther	Absent (0) Present (1)
33.	Anthers shape	Sagittate (0) Not sagittate (1)
34.	Style texture	Glabrous (0) Hairy (1)
35.	Style origin	Terminal (0) Gynobasic (1)
36.	Style position	Inserted (0) Exerted (1)
37.	Style shape	Bifid (0) Undivided (1)
38.	Stigma shape	Conical (0) Capitate-globose (1)
39.	Stigma length	As long as style or shorter (0) Much longer than style (1)
40.	Ovary texture	Glabrous (0) Hairy (1)
41.	Nectar disk	Absent (0) Present (1)
42.	Gynophore	Absent (0) Present (1)

2.4 Phenetic analysis

Character states (107) by taxon (46) matrix (Additional file 1: Appendix A) were subjected to phenetic analysis by use of PAleontological STatistics version 3.23 [43]. PCA (principal component analysis) ordination and similarity matrix were created using the same software, based on the investigated morphological characters of the studied taxa.

2.5 Phylogenetic analysis

Phylogenetic analyses that are based on maximum parsimony were performed on the produced data matrix using MrBayes 3.2 [44] with Markov chain Monte Carlo simulation. The sample and print frequency is 500, the diagnostic frequency is 5000 and the run length is 1,000,000. *Vahlia digyna* (Vahliaceae) was used as an outgroup for rooting the cladogram.

3 Results

The phenetic analysis of the coded data matrix of the investigated morphological character states generated a dendrogram (Fig. 1) that is divided into five clear groups: The first one consists of *Alkanna strigosa*, *Arnebia tinctoria*, *Echiochilon fruticosum*, *Echium horridum*, *Lappula spinocarpos*, *Moltkiopsis ciliata* and *Nonea vivianii* at 0.6 taxonomic distance. The second group comprises *Alkanna orientalis*, *A. tinctoria*, *Anchusa aegyptiaca*, *A. hispida*, *A. humilis*, *A. milleri*, *A. undulata*, *Arnebia decumbens var. decumbens*, *A. decumbens var. macrocalyx*, *A. hispidissima*, *A. linearifolia*, *Asperugo procumbens*, *Buglossoides incrassata*, *B. tenuiflora*, *Paracaryum intermedium* and *P. rugulosum* at about 0.675 similarity index. The third group includes *Echium angustifolium* subsp. *Anugstifolium*, *E. angustifolium* subsp. *sericeum*, *E. rauwolfii*, *E. rubrum* and *E. sabulicolum* at taxonomic distance (0.675). All the studied taxa of genus *Heliotropium* are nested in the fourth group at about 0.45 taxonomic distance. The last group comprises *Trichodesma africanum and T. ehrenbergii* at about 0.375 similarity index. *Coldenia procumbens* and *Lappula sinaica* are separated as distinct identities at taxonomic distances 0.375 and 0.525, respectively.

PCA ordination and matrix of similarity that based on the investigated morphological characteristics of the studied taxa are presented in Fig. 2 and Additional file 1: Appendix B. Among the investigated taxa, the most distant and the closest species are determined. *Arnebia decumbens* var. *macrocalyx* and *Heliotropium curassavicum* are the species that are the most distantly linked (percentage dissimilarity: 13.49074), while *Echium angustifolium* subsp. *angustifolium* and *E. angustifolium* subsp. *sericeum* are the species that are most closely linked (percentage dissimilarity: 1.7320508). The outline of the analysis indicated that contributions for the first two principal components to total variation of 42 characters were (42.79%) and (25.15%) eigenvalues, respectively. The biological meaning of the components was analogized by the correlation between the component and character (Table 3). The first component is positively correlated with inflorescence type (0.29), petal color (0.23), stigma length (0.24) and ovary texture (0.23), and negatively with upper leaves petioles (−0.29), bracteoles (−0.29), style origin (−0.28) and stigma shape (−0.32). The second component is positively correlated with petal texture (0.29), petal fusion (0.26), style texture (0.24), style position (0.36) and nectar disk (0.23) and negatively with anthers level (−0.36), filament texture (−0.24) and style shape (−0.28).

The sequences were submitted to GenBank and assigned accession numbers from OP933830 to OP933875. The phylogenetic analysis generated a cladogram (Fig. 3) showing that among the studied taxa of Boraginaceae there is a bolster for three clear lineages with resolved relationships viz. *Heliotropium* lineage that included all studied taxa of genus *Heliotropium*, lineage II (*Anchusa undulata*, *Arnebia hispidissima*, *A. tinctoria*, *Echium angustifolium* subsp. *angustifolium*, *E. angustifolium* subsp. *sericeum*, *E. rubrum*) and lineage III (*Alkanna orientalis*, *A. strigosa*, *A. tinctoria*, *Anchusa aegyptiaca*, *A. hispida*, *A. humilis*, *A. milleri*, *Asperugo procumbens*, *Buglossoides tenuiflora*, *Lappula sinaica*, *Paracaryum intermedium*, *P. rugulosum*, *Trichodesma africanum* and *T. ehrenbergii*).

4 Discussion

From phenetic point of view as revealed in the produced phenogram, the studied taxa *Alkanna strigosa*, *Arnebia tinctoria, Echiochilon fruticosum*, *Echium horridum*, *Lappula spinocarpos*, *Moltkiopsis ciliata* and *Nonea vivianii* are grouped together in a single phenetic group. This is in accord with [28] where *Alkanna*, *Arnebia*, *Echiochilon* and *Echium* are included in the same tribe Lithospermeae. [45] agree with this but exclude *Echiochilon* in tribe Echiochileae and [15, 26, 38, 46] treated *Moltkiopsis* in tribe Lithospermeae along with *Alkanna*, *Arnebia* and *Echium*, while [27, 28] put it under tribe Trigonotideae. On the other hand, [10] put *Nonea* and *Lappula* in two distinct tribes: Borageineae and Cynoglosseae, respectively.

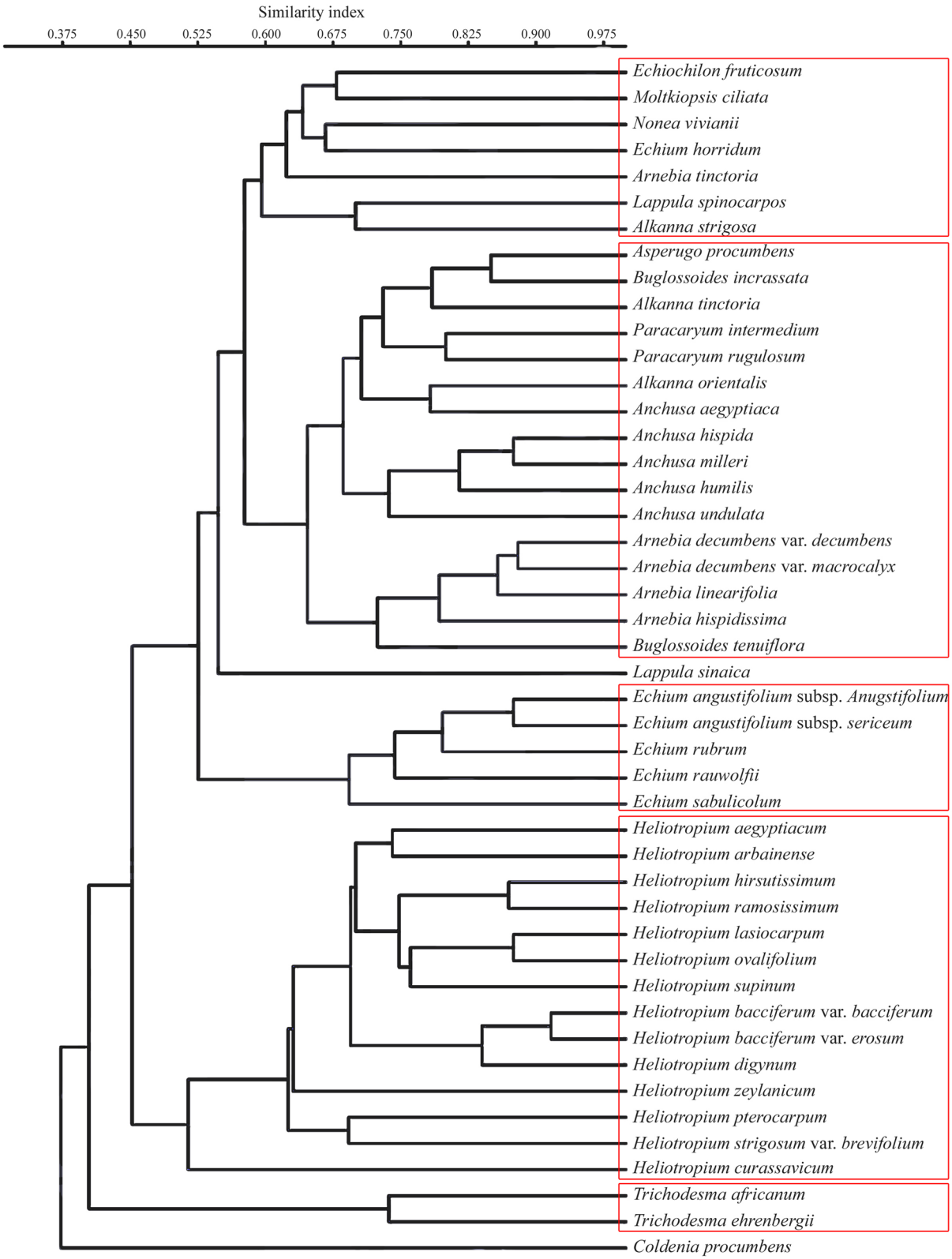

Fig. 1 UPGMA clustering of the studied boraginaceous taxa based on (42) morphological characters

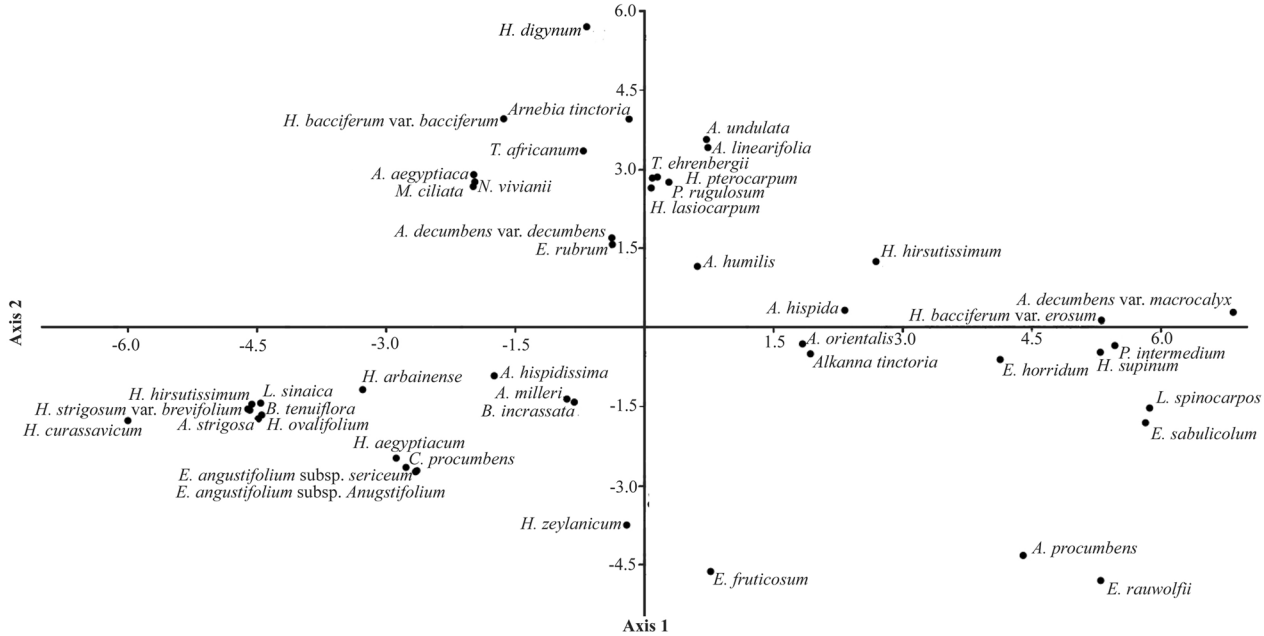

Fig. 2 Principal component analysis of the studied boraginaceous taxa based on (42) morphological characters

Alkanna orientalis, *A. tinctoria*, *Anchusa aegyptiaca*, *A. hispida*, *A. humilis*, *A. milleri*, *A. undulata*, *Arnebia decumbens var. decumbens*, *A. decumbens var. macrocalyx*, *A. hispidissima*, *A. linearifolia*, *Asperugo procumbens*, *Buglossoides incrassata*, *B. tenuiflora*, *Paracaryum intermedium* and *P. rugulosum* are grouped together in an exclusive group. This is consistent with the positioning of *Alkanna*, *Arnebia* and *Buglossoides* in the same tribe Lithospermeae [10, 27, 28, 45]. Taxonomic systems, viz. [10, 15, 27, 28, 46, 47], distribute *Asperugo*, *Anchusa* and *Paracaryum* in tribes Eritrichieae, Borragineae and Cynoglosseae, respectively.

Echium angustifolium subsp. *Anugstifolium*, *E. angustifolium* subsp. *sericeum*, *E. rauwolfii*, *E. rubrum* and *E. sabulicolum* are clustered together. This is consistent with the positioning of *Echium* in tribe Echieae according to [15, 26, 48] and in tribe Lithospermeae according to [10, 27, 28, 47].

The clustering of all the studied taxa of genus *Heliotropium* in a single group is confirmed by [26] classification system in placing all the taxa of *Heliotropium* at the same tribe Heliotropieae also [46] in placing them at the same subfamily Heliotropioideae.

T. africanum and *T. ehrenbergii* are grouped together in a single phenetic group. This is in accord with [10, 15, 28, 47, 48] where the present genera were included in the same tribe Cynoglosseae. [26, 27] placed it under tribe Trichodesmeae but [49] put it in subtribe Rindereae under the tribe Borragineae. *Coldenia procumbens* is separated as a distinct identity, and this is in accord with [46] in placing it under subfamily Cordioideae.

PCA can be useful in providing information on character variability [50]. The cumulative variance values of the main components obtained reveal the investigated features in boraginaceous taxa, because of their large variance value that can be useful in explaining discrepancies among taxa. Furthermore, among the examined specimens, the morphological features were chosen for PCA to assess the qualities that are relevant in description change.

PC1 explained 42.79% of total morphological variation which was positively and negatively determined by some floral characters, while PC2 explained 25.15% of total morphological variability that related to floral characters as the same as PC1; accordingly, more than half of total information (67.94%) could be explained by the first two principal components. This indicates that the component was determined by flower variables. So, the results indicate that floral structure showed variability, which is useful for discrimination. In this regard, [51] indicates that the morphological variability in Boraginaceae is explained to greater degree by floral variables.

From phylogenetic point of view, the produced cladogram showed that Boraginaceae are not monophyletic

Table 3 PCA variable loadings of a two-dimensions, eigenvalues, contributions and scores of the components for (42) morphological characters of the studied taxa of Boraginaceae

No.	Character	Axis 1	Axis 2
1.	Habit	0.22	−0.04
2.	Texture	−0.09	0.08
3.	Strength	−0.14	0.13
4.	Stem branching	−0.08	−0.04
5.	Basal leaves	0.02	−0.15
6.	Upper leaves petioles	−0.29	0.05
7.	Lamina composition	−0.05	−0.04
8.	Lamina shape	0.19	0.01
9.	Lamina surface	−0.08	0.17
10.	Base of lamina	−0.05	−0.04
11.	Leaf veins	−0.16	−0.15
12.	Leaf margin	0.1	−0.03
13.	Leaf apex	−0.02	0.09
14.	Hairs on leaves	−0.07	0.08
15.	Bracteoles	−0.29	−0.04
16.	Inflorescence type	0.29	0.13
17.	Inflorescence	0.1	0.04
18.	Number of flowers	0.13	0.08
19.	Flower	0.14	0.1
20.	Sepal fusion	−0.06	0.02
21.	Apex of calyx lobes	−0.02	0.02
22.	Hairs on sepals	−0.01	−0.03
23.	Petal color	0.23	−0.06
24.	Petal texture	0.19	0.29
25.	Petal apex	−0.04	0.11
26.	Petal fusion	0.13	0.26
27.	Petal lobes	−0.11	0.2
28.	Corolla throat	0.15	0.2
29.	No. of stamens	−0.05	0.03
30.	Anthers level	0.07	−0.36
31.	Filaments texture	0.11	−0.24
32.	Appendix on anther	0.04	−0.18
33.	Anthers shape	−0.13	0.17
34.	Style texture	0.12	0.24
35.	Style origin	−0.28	0.14
36.	Style position	−0.07	0.36
37.	Style shape	0.11	−0.28
38.	Stigma shape	−0.32	0.03
39.	Stigma length	0.24	0.01
40.	Ovary texture	0.23	0.05
41.	Nectar disk	0.13	0.23
42.	Gynophore	−0.01	−0.02
Eigenvalue		11.28	06.62
Contribution %		24.79	25.15
Cumulative contribution		67.94	93.09

group contrary to some previous studies based on the data of morphology, phytochemistry and molecular structure that indicate the monophyly of Boraginaceae within its specific boundaries [31, 33, 34, 52]. *Heliotropium* lineage included all exemplars of genus *Heliotropium* confirming that *Heliotropium* is a monophyletic group. Tribe Heliotropieae is now typically recognized as subfamily Heliotropioideae [2, 28, 52].

Some exemplars of *Anchusa*, *Arneba* and *Echium* were grouped together in lineage II, and this is in accordance with placement of *Arneba* and *Echium* in tribe Lithospermeae according to [10, 27, 28, 46, 47], while [53] placed *Arneba* and *Echium* in one tribe Boraginoideae. Some taxonomic systems, viz. [10, 15, 26–28, 46–48, 53], put *Anchusa* in tribe Boragineae. Previous phylogenetic studies found a sister relationship between Boragineae and Lithospermeae [10, 39].

Alkanna orientalis, *A. strigosa*, *A. tinctoria*, *Anchusa aegyptiaca*, *A. hispida*, *A. humilis*, *A. milleri*, *Asperugo procumbens*, *Buglossoides tenuiflora*, *Lappula sinaica*, *Paracaryum intermedium*, *P. rugulosum*, *Trichodesma africanum* and *T. ehrenbergii* were grouped together. This is in accord with placing *Alkanna* and *Anchusa* in the same tribe Boragineae [10, 15, 26–28, 46–48, 53] keep placing *Anchusa in tribe* Boragineae but place *Alkanna* in tribe Lithospermeae. Taxonomic systems viz. [10, 15, 27, 28, 46, 47] placed *Asperugo* in tribe Eritrichieae. [10, 48, 54] put it in tribe Cynoglosseae. [26] put it in tribe Asperugeae. Phylogenetic studies found that Cynoglosseae is closest relative to Boragineae and Lithospermeae [10, 39]. *Lappula* and *Trichodesma* were included in the same tribe Cynoglosseae. [15, 47] also placed *Trichodesma* and *Paracaryum* in the same tribe Cynoglosseae.

5 Conclusion

It is concluded that the chosen morphological characters were important in species delimitation, where more than half of total morphological variations (67.94%) were explained by the first two principal components, indicating that the morphological characters showed high variability, which is useful for discrimination, and these characters shared in drawing the phenetic relationships within Boraginaceae. In addition, the phylogenetic relationships clarified that Boraginaceae is not a monophyletic group, but it contained some monophyletic genera such as *Heliotropium* and *Alkanna*, while the other studied taxa expressed non-monophyletic relationships.

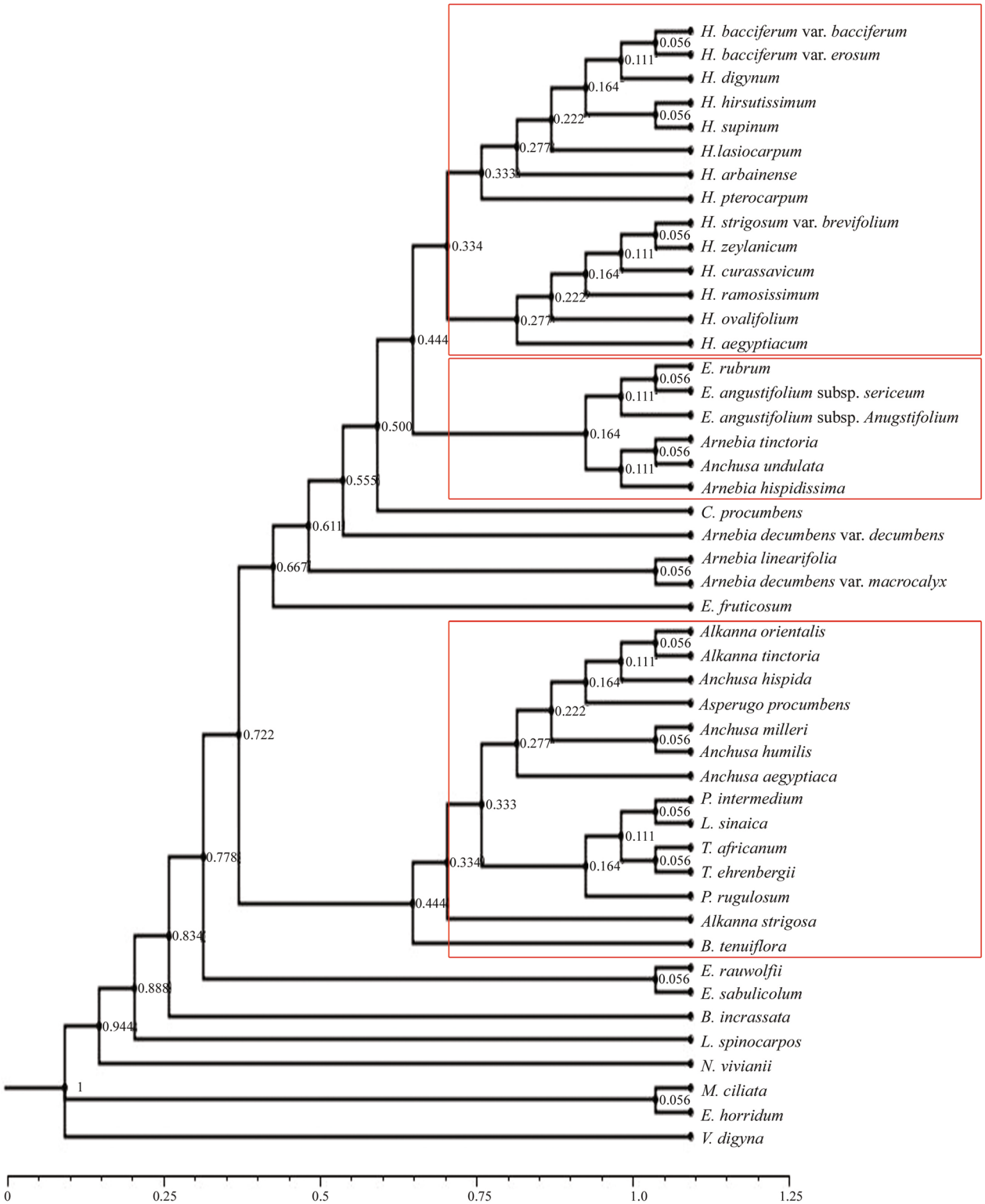

Fig. 3 Neighbor joining phylogenetic tree of the studied boraginaceous taxa based on chloroplast *rbcL* sequence

Abbreviations

CAI	Herbaria of Cairo University, Faculty of Science
CAIA	Herbaria of Ain Shams University, Faculty of Science
CAIM	Herbaria of Flora and Phytotaxonomy Research Department
CTAB	Cetyltrimethylammonium bromide
IPNI	International Plant Name Index
PCA	Principal component analysis
PCR	Polymerase chain reaction

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43088-023-00456-8.

Additional file 1. Appendix A. Data matrix of (42) morphological characters and their (107) states of the studied taxa of Boraginaceae and outgroup. **Appendix B.** Similarity matrix among the studied boraginaceous taxa based on (42) morphological characters.

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Author contributions
UA was involved in study conception and design, data curation, analysis and interpretation of data, writing—original draft preparation and supervision. SA was involved in acquisition of data, methodology, investigation and visualization. WO was involved in validation and resources. UA and SA were involved in formal analysis. WO, WH and NA were involved in critical revision and editing. WH and NA were involved in project administration. NA was involved in funding acquisition.

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Availability of data and materials
All data are available from corresponding author upon request.

Declarations

Ethics approval and consent to participate
All materials that were used in the current research do not need ethically approved permission, human or animal materials.

Consent for publication
Not applicable.

Competing interests
None. The authors declare no competing interests. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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