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Geometric morphometrics of leaves of *Cynanchum acutum* L. (Apocynaceae) from Egypt

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Abstract

This study aimed to revise *Cynanchum acutum* in Egypt and their relative subspecies that belonging to Section Cynanchum (Old World) and comparing it with an American group of genera *Cynanchum laeve* subgen. (Sect.) Mellichampia (New World) by using classical taxonomy and Modern geometric morphometric methods (GMMs). The description of 85 specimens depending on 36 quantitative traits was measured for all leaves by ImageJ Tool software. Data of leaf description was further processed using R program. The fan neighbor-joining topology revealed to the relationship between studied taxa and population, however, a PCA biplot showed a total variation 37.7% at first axis and 8.9% at the second axis. Significant variation in taxa and populations estimated using ANOVA and MANOVA. Canonical variate analysis showed a significant difference between taxa (P=0.001*), with a total variation of 67% between three taxa. The total variation checked between two taxa by using discriminant analysis, *C. acutum* subsp. *acutum* and *C. acutum* subsp. *sibiricum* calculated 77% variation, while as the total variation between then between *C. acutum* subsp. *acutum* and *C. laeve* showed 86% of the variation and significant value (P=0.002**). The study elucidated the situation of *Cynanchum acutum* in Egypt and confirmed the presence of two subspecies viz.: subsp. *acutum* and subsp. *sibiricum.***

Keywords: *Cynanchum acutum*, Modern geometric morphometric methods, leaf architecture, Egypt.

1. Introduction:

The *Cynanchum* L. genus formerly situated as part of Asclepiadaceae however according to the several phylogenetic studies, Asclepiadaceae treated as a subfamily of Apocynaceae to be Asclepiadoideae (Goyder *et al.* 2007; Rapini *et al.* 2007; Endress 2016; Nam & Chung 2018). *Cynanchum* has been worldwide distributed with more than 400 species over the Old and the New World and about 110 species extent in Africa, Madagascar considers the main center of diversity in Africa with nearly 90 species, in contrast, the minor centers are southern Africa and eastern Africa with 10 –15 species each (Liede 1997; Liede 1999; Liede & Kunze 2002; Alvarez Cruz & Schmelzer 2012; Khanum *et al.* 2016).

The last treatment of *Cynanchum* in Egypt was done by Boulos (2000) who recognized only one species of *Cynanchum* at Egypt *"Cynanchum acutum"*, with one subspecies "subsp. *acutum"*, in agreement with Täckholm (1974) and Greuter *et al.* Med-Checklist 1 1984. On the other hand, Boulos, in Flora Checklist (1995) revealed the presence of two subspecies in Egypt "subsp. *sibiricum"* and "subsp. *acutum"*, the main difference between two subspecies is the leaf shapes, lengths, and base lobe. Gilbert *et al.* (1995) were stated to *C. acutum* subsp. *acutum* is most common in the Mediterranean region where there is an overlapping area with *C. acutum* subsp. *sibiricum*.

The leaf morphology is the main discriminative power in plant taxonomy and systematics (Bell and Bryan 2008; Cope *et al.* 2012). Leaf morphometric is commonly used in several fields, such as morphology, systematic taxonomy, developmental biology, plant breeding, and agronomy (Vieira *et al.* 2014). Modern geometric morphometric methods (GMMs) contribute to increasing scientific description accuracy for the

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important traits of the phenotypic measurement at biodiversity and proper for differentiating complex taxa. In contrast to classical taxonomy requires handling and admission to specimens (Viscosi and Cardini 2011; Klein and Svoboda 2017; Liu *et al.* 2018).

Therefore, it is appropriate to apply the morphology of leaf to examine the presence of subspecies "*Cynanchum acutum*" at Egypt by using modern geometric morphometric methods with assessment of taxonomic treatment and leaf architecture. In additional to, analyzing the results by focusing on *Cynanchum acutum* populations, to decide suitable distinctive traits to distinguish between *Cynanchum acutum* subspecies by comparison both with *Cynanchum laeve* as outgroup.

2. Material and Methods

2.1. Study site and plant material

Field survey of the *Cynanchum acutum* location in Fayoum depression (FD) took place during summer 2017 (Fig. 1, Suppl.1). Fayoum depression (FD) is situated at 90 Kilometers to the southwest of Cairo, the coordinates: 28° 55′ - 29° 35′ N, 30° 23′ - 31° 05′ E (Effat, & El-Zeiny, 2017). Detailed investigation of samples and the population gathered from studying Herbarium sheet kept at Cairo University herbarium (CAI), collected samples from FD and Images from Florida Herbarium site for *Cynanchum laeve.* Description of 85 specimens (Suppl.2) with a total 105 observation from one to three leaves to each branch were measured. The quantitative measurement totally 36 summarized in table (1), leaf descriptors were carefully chosen concerning conclusions of studies by other authors (e.g. Zahidi *et al.* 2013; Baláš *et al.* 2016; Aykut *et al.* 2017; Lo Bianco and Mirabella 2018). Digital images were used to measure the different measured traits using ImageJ Tool program (Rasband 1997-2018).

2.2. Data analysis

Data from leaf descriptors were further processed in program R (R Development Core Team, 2016).

2.2.1. Testing variation in taxa and populations, using multivariate methods (Cluster analyses and ordination analyses):

For agglomerative clustering the standardization must be done to make variable comparable. Removing the lines with missing data and standardize the data without missing values. Firstly, to standardize the data, it should be converted to a numeric matrix as an input to complete the scaling on the column. Secondly, the Gower-dissimilarity is used, the data scaled and computed a Manhattan distance (Viscosi and Cardini 2011; Kassambara 2017). Selecting neighbor-joining (NJ) tree by calculating the cophenetic correlation which is better than that at Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

The "factoextra" and "ggplot2" packages in R were used for visualizing the distance matrices that used at the Principal Component Analysis. PCA was used to know the total variation of leaf traits and identified the most important trends in leaf shape (Vieira et al. 2014). PCA decreases the dimensionality of multivariate data, to two or three that can be visualized graphically with minimal loss of information. fviz_pca provides ggplot2- based stylish visualization of PCA (Kassambara 2017).

2.2.2. Testing variation in taxa and populations, using a modified Procrustes ANOVA

Differences in leaf descriptors can chart in several planes. A hierarchical analysis of variance (ANOVA) generated to measure and test the variation in taxa and geographical populations using (aov) function, and the measuring the size (t-value) was calculated by summary (lm) function, as well as testing the significant of variables by manova function of package "dplyr" for multivariate analysis of variance (MANOVA).

2.2.3. Testing taxa differences using permutation tests and discriminant analyses

By utilizing a series of R functions of morphometric analysis (MorphoTools) were performed descriptive statistics for populations and taxon, Box-and-whisker plots representing all leaf descriptors to display the variation range between three investigated

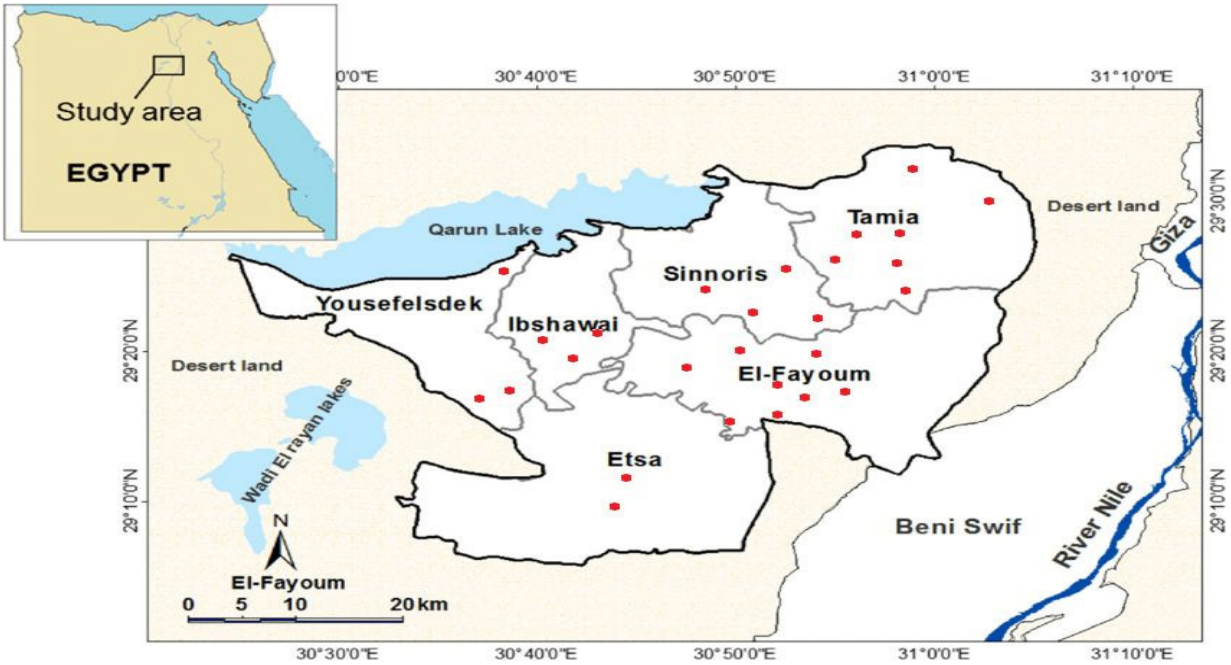


Fig.1. Distribution map of the collected Cynanchum *acutum* subsp. *acutum* in Fayoum depression during summer 2017.

taxa, Canonical Variate Analysis (CVA) intended for discriminant test to three taxa and Discriminant Analysis (DA) for comparison of the two subspecies (Koutecký 2015).

3. Results

3.1. Systematic treatment

***Cynanchum* L. Sp. PI. 212 (1753).**

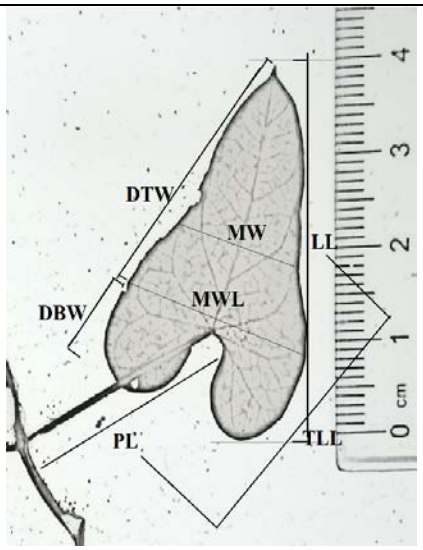
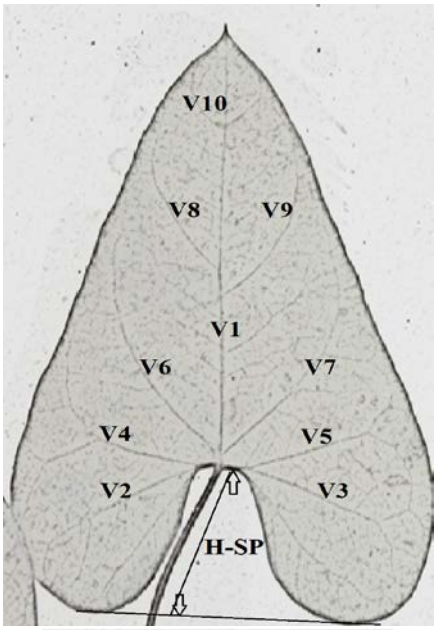
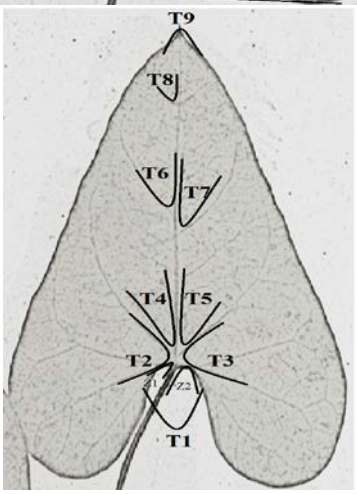
Type: *Cynanchum acutum* L. designated by E. Meyer (1837).

Perennial twining, climbing or erect, subshrubs or herbs. Multicellular Hair or glabrous. Yellow to white milky latex. Fibrous, fleshy or woody roots. Greenish stem. Leaves opposite, petiolate, usually cordate, elliptical, ovate or obovate with entire margin and acute apex. Leaves sometimes have small leafy stipule. Inflorescences extra-axillary, raceme-like, corymbose or umbel-like. Flowers 3-15 mm diameter; aestivation imbricate or contorted, dextrorse; nectariferous. Calyx free ± to the base with erect sepals, frequently have basal glands. Corolla deeply divided, contorted in the bud, rotate, sub-rotate to tubular, white, green, yellow or rarely reddish. The crown (corona) as high as, higher than or inserted on base the gynostegium; campanulate or tubular; white, rarely red; fleshy or membranous, divided into 10 free fringes; the 5 outer segments tridentate and longer than 5 inside and opposite to the outer ones. Filaments united in a tube, membranous Anther with apical appendages, ovate pollinia with 2 per pollinarium, divergent, pendulous. Pentagonal stigma with 2 apical tubercles. Follicle solitary lanceolate or fusiform smooth or with bulges, glabrous or hairy, winged or wingless, thin-walled. Seeds ovate or boat-shaped, smooth, brown, papillate or with trichomes, sculptured, winged or wingless, with coma. Chromosome number usually 2n = 22 (Davis, 1978; Rechinger, 1970; Forster, 1991; Li et al., 1995; Liede, 1999).

Speciose *Cynanchum* genus worldwide distribution; over than 400 species, here, the study focusses in literature and synonyms for Egyptian species *Cynanchum acutum* (Old World) belong to Sect. *Cynanchum* and linkage it with more resembling *Cynanchum laeve* (New World) *subgen.* Mellichampia by cordate (heart-shaped) leaves as out group taxa (Liede & Täuber 2002; Liede & Meve, 2003; Khanum *et al.* 2016).

Based on revision of the Herbarium sheet kept at (CAI) and study the description of *C. acutum* subspecies in different flora like Flora of Egypt, Flora of Iranica, Flora of

Table 1. List of investigated leaf measurements used for morphometric analysis with illustration

Leaf Trait	Abbreviation	Illustration
Lamina length	LL	
Maximal width of lamina	MWL	
Middle width of lamina	MW	
The distance between the widest point and the leaf tip	DTW	
The distance between the widest point and the leaf base	DBW	
Laminar ratio	LR=MWL/LL	
Area of leaf (laminar size)	LA	
Petiole length	PL	
Total leaf length	TLL=LL+PL	
Petiole length x 100/total leaf length	P%=PL x 100 /TLL	
Middle width of lamina x 100/total leaf length	MW%= MW x 100 /TLL	
Maximal width of lamina x 100/total leaf length	MWL%= MWL x 100 /TLL	
leaf surface as the product of the length and maximum width	SURF= LL x MWL	
Petiole sinus depth	H-SP	
Length of main vein	V1	
Length of vein 2 (left)	V2	
Length of vein 3	V3	
Length of vein 4	V4	
Length of vein 5	V5	
Length of vein 6	V6	
Length of vein 7	V7	
Length of vein 8	V8	
Length of vein 9	V9	
Length of vein 10	V10	
Petiole sinus angle (concave up)	T1	
Inner angle V2+4 (left)	T2	
Inner angle V3+5	T3	
Inner angle V6	T4	
Inner angle V7	T5	
Inner angle V8	T6	
Inner angle V9	T7	
Inner angle V10	T8	
Petiole vein angle (3rd order, left)	Z1	
Petiole vein angle (3rd order, right)	Z2	
Apex angle	T9	

Turkey and Flora of China have been showed that the main difference is the shape and base lobe of leaf between two subspecies with variable data for leaves as shown in table (2). The present study confirms of existence *C. acutum* subsp. *sibiricum* in Egypt at Nile Delta, Siwa Oasis and Baharia Oasis, is being in line with the first record of *C. acutum* subsp. *sibiricum* in Egypt Boulos, Flora Checklist (1995), Fig. (2).

The elementary venation pattern of *C. acutum* and *C. laeve* appear to the correspondence at the leaf architecture; which are pinnate, the secondary vein doesn't reach to margin (Camptodromous), these 2nd veins connected in a series of conspicuous curvatures (brochidodromous), with Irregular vein spacing and the vein angle abruptly increasing toward the base. The Inter-secondary veins present. Random reticulate is characterize to third vein category called tertiaries anastomose with acute vein and regular polygonal reticulate fourth vein. The arola was moderately or poorly, all these characters appear naked eye (Fig. 3).

Sect. Cynanchum (Old World)

Cynanchum acutum L., Sp. PI. 212 (1753).

Syn: *Cynanchum monspeliacum* L, Sp. Pl. ed.1: 212 (1753).

Stems terete, up to 3 m, herbaceous, slender, vine, branched, lower parts glabrous, sometimes hairy above. Petiolate leaves, glabrous, cordate, ovate, very variable in shape and size, 2-10 (-17) x l.5-8 (-16) cm apex sub-acute to acute, variable in base auriculate, deeply cordate to sagittate. Inflorescences Cymos, few- to many-flowered, peduncles 5-10 cm, pubescent. Calyx (1.5-2) mm long, triangular lobes. Corolla white or pink, 6-9 mm diameters, lobes oblong and obtuse. Follicles 6-16 x 1 cm, narrow, glabrous or papery when young, acuminate.

Artificial Key to subsp. of *Cynanchum acutum* based on morphological characters

1. The leaves one to double as long as broad, broadly ovate-cordate or narrow linear; basal lobes scarcely divergent, convex sides (broadly auriculate). Follicles 6-15cm ……. **subsp. *acutum***
2. The leaves one to triple as long as broad, broadly cordate to hastate-cordate, or oblong-hastate; basal lobes distinctly divergent concave sides (auriculate). Follicles 12.5-16cm ….. **subsp. *Sibiricum***

***1. Cynanchum acutum* subsp. *acutum*.**

The leaves one to double as long as broad, up to 9 x 7 cm; broadly ovate-cordate or narrow linear; basal lobes scarcely divergent, convex sides (broadly auriculate). Corona linguiform; appendages longer than lanceolate. Follicles 6 -15 cm.

Representative specimens:

Faculty of agriculture, farms, Alexandria; 28.8.1953; Loutfy Boulos; s.n. (CAI). Faculty of agriculture farm, Alexandria Univ., Alexandria; 28.8.1952; Nabil El Hadidy s.n. (CAI). Masraf El Gabaroti, El Aslah Mohatta, on the road Alexandria-Rosetta, Alexandria; 18.9.1970; Imam, Ibrahim, Mahdi;s.n. (CAI).Meshanded 25Km West of Siwa, Siwa Oasis; 26.10.1963; Loutfy Boulos s.n.(CAI).Siwa oasis/ Abu Sharour, Siwa Oasis; 1/4/1968; Gun Romeé & N. El Hadidy s.n.(CAI).Siwa oasis; 27.12.1969;M. A. Zahran & L. Boulos s.n. (CAI).Baharia Oasis, Zabou;11.9.1971;M. Imam s.n.(CAI).Baharia Oasis, Al-Zabw;4.10.1978;Monier Abd El Ghani; s.n.(CAI).Baharia Oasi, Al-Harra, Ain Boulol, on walls & hedges; 24.10.1979; Monier Abd El Ghani; s.n.(CAI). Baharia Oasis, Al-Zabw; 18.7.1978; Monier Abd El Ghani; s.n. (CAI). Giza near Cairo; 19.10.1910; E. Hartmann; s.n. (CAI). Orman Gardens, Giza; 6.10.1954; Soliman Sisi s.n. (CAI). Giza, 10.10.1967; M. El-Mahdi s.n. (CAI). At Parks and gardens, Orman Gardens, Giza; 27.8.1963; Mohammed El-Mahdi; s.n. (CAI). At Parks and gardens, Giza; 10.10.1967; Mohammed El-Mahdi s.n.(CAI). Beheira province, Rosetta; 16.7.1987; Alaa Amer; s.n. (CAI). Beheira; 29.9.1966; Nabil, Imam & V. Tackholm s.n. (CAI). Wadi El Natraun, in Olive orchard, Beheira; 18.7.2009; A. Farhat s.n. (CAI). Boghaz, Rosetta, Beheira; 30.10.1971; Imam, Ibrahim, Mahdi s.n. (CAI). Dakahlia- Aga, Dakahlia;

Table 2. Comparison between two subspecies of *C. acutum* based on information collected from different floras book, showed how much the variation of leaves between two subsp.

	C. acutum subsp. *acutum*	*C. acutum* subsp. *sibiricum*
Flora of Iranica (Rechinger 1970)	The leaves up to ± 9x7 cm, narrow linear, lobes of the basal of leaf separate oblong as well suborbiculate very divergent provided sinus wide open.	The leaves up to ±10x3 cm, broadly-cordata, usually a large one to double times longer than sinus and lobes of the basal diverging from their blade barely separated.
Flora of Turkey (Davis 1978)	Leaves 1-2(x) as long as broad, broadly ovate-cordate with scarcely divergent basal lobes.	Leaves 3 or more x as long as broad, narrowly sagittate with distinctly divergent basal lobes.
Flora of China (Li *et al*. 1995)	having ovate leaves with convex rather than concave	leaf blade hastate, hastate- cordate, or oblong-hastate, base auriculate, apex acute to long acuminate, basal lobes recurved, parallel or divergent, ciliate
Flora of Egypt (Boulos 2000)	The difference is subsp. *acutum* has base broadly auriculate	

Fig. 2. The figures compare *C. acutum* Herbarium specimen sheet kept at (CAI) (A, B and C) by illustration of Flora of China (D and E), and F pic downloaded from" https://images.app.goo.gl/VLFQPkWf1Uwm3P18A", to confirm the presence of *C. acutum* subsp. *sibiricum* by Image comparison method, and Written description.

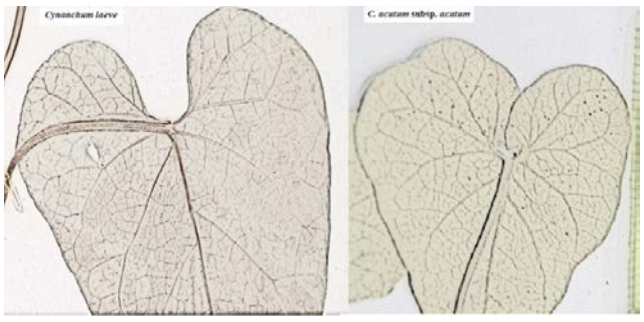


Fig. 3. the leaf architecture, show the venation pattern for *C. acutum* subsp. *acutum and C. laeve* seem to be similar.

11.10.1929; E. Greiss s.n. (CAI). Between Ras el Bar and Demietta, 13.9.1929; M.T. Hefnawy s.n. (CAI). East of the Nile Delta/ El-Maneyl, East; 17.6.1980; A. Bakry; s.n. (CAI). East of the Nile Delta, El-Mullok, 25.4.1980; A. Bakry; s.n. (CAI). Cairo: Ain Shams University; 1977; A. Gazzar; s.n. (CAI). KomAushim, Faiyum; 21.9.1959; V. Täckholm s.n. (CAI). El-Faiyum, 28.5.1954; M. El Shafay s.n. (CAI). Kom Aushim, Faiyum, 29.9.1967; V. Täckholm, M. Imam, I. Elsayed & M. El Mahdi s.n. (CAI). Kom Aushim, Faiyum, 21.9.1959; V. Täckholm s.n. (CAI). Kom Aushim,near Faiyum, ; Jan. 1953;Nabil El Hadidy s.n. (CAI). KomAushim, Faiyum, Fayoum; 15.7.1960; Loutfy Boulos s.n. (CAI). Wadi Wl. Rayan protected area herbarium, Fayoum; 1.2.2000; L.Boulos s.n. (CAI). Qasr Rashwan Tameyah, Fayoum, 29°27'30.4"N, 30°55'22.5"E; 25.8.2017; M. Sayed, M. Faker & F. Yousef (Fayium univ. Herbarium). Madinet Tameyah, Fayoum, 29°29'02.2"N, 30°56'28.1"E; 25.8.2018; M. Sayed, M. Faker & F. Yousef (Fayium univ. Herbarium). Ezbet El-Hariry, El-Rawda, Tameyah, Fayoum; 2927'38.8"N 30°58'41.5"E, 25.8.2019; M. Sayed, M. Faker & F. yousef (Fayium univ. Herbarium). Madinet Tameyah, Fayoum; 29°29'25.5"N, 30°58'41.9"E; 25.8.2020; M. Sayed, M. Faker & F. Yousef (Fayium univ. Herbarium). Kafr Al Maslat – Tamya, Fayoum,29°32'52.5"N 30°58'55.9"E; 25.8.2021; M. Sayed, M. Faker & F. Yousef (Fayium univ. Herbarium). Monshaat Doctor El-Gammal, Fayoum, 29°30'32.1"N 31°03'21.3"E; 25.8.2022; M. Sayed, M. Faker & F. Yousef (Fayium univ. Herbarium). Forqos, Tameyah, Fayoum,29°25'28.4"N, 30°58'50.4" E; 25.8.2023; M. Sayed, M. Faker & F. Yousef (Fayium univ. Herbarium). Al Gharq-Faiyum way Izbat Ad Daw, Minya, Etsa, Fayoum; 29°12'44.4" N 30°45'50.1"E; 16.9.2017; M. Sayed, M. Faker & F. Yousef (Fayium univ. Herbarium). Madinat Al Fayoum - Tersa way –Naqalifah, Fayoum, 29°24 '39.8" N 30°49'31.9"E; 16.9.2018; M. Sayed, M. Faker & F. Yousef (Fayium univ. Herbarium). Al Eelam Al Fayoum, Fayoum, 29°19'38.2"N 30°52'07.5"E;20.9.2017; M. Sayed, M. Faker & F. Yousef (Fayium univ. Herbarium). Damascus, Syria; 23.8.1959; Samir Ghabbour s.n. (CAI).

Flowering time: July- September

Distribution in Egypt: It has been reported from Nile Delta, Nile Vally, Oases of Siwa, Bahariya and Wadi Natrun, the Mediterranean coastal.

General distribution: Mediterranean area, S.W. Asia to N. Iran, S. Russia, Caucasus.

2. *Cynanchum acutum* subsp. *sibiricum* (Willd.) Rech. f, Fl. Iran. 73: 9. (1970).

Syn: *Cynanchum sibiricum* Willd. in Neue Schriften Ges. Naturf. Freunde Berlin 2: 124 (1799)

C. acutum L. var. *longifolium* (G.Martens) Ledeb., Fl. Ross. 3:548 (1847). Described from Siberia (holo. B-Willd. 5233, photo). The leaves one to triple as long as broad, up to 10x3 cm; broadly-cordata or narrowly sagittate; Basel lobes distinctly divergent concave sides (auriculate). Apex acute to long acuminate. Corona rarely short linguiformes; elongated appendages often lanceolate. Follicles 12.5-16 cm.

Representative specimens:

Siwa Oasis; Meshanded 25Km West of Siwa; 26.10.1963; Loutfy Boulos; s.n. (CAI). Kom Aushim, Faiyum; Fayoum; 13.10.1967; V. Täckholm - M. Imam- N. El Hadidi; s.n. (CAI). El-Heiz, Tabliun, Baharia Oasis; 15.5.1978; Monier Abd El Ghani; 320 (CAI). Wadi Digla, Eastern Desert; 25.4.2006; M. Abdel Aleem; s.n. (CAI). El-Manair, 12 km from Ismaila on the desert road; 17.6.1980; Leg. A. Bakry; 1229(CAI). East of the Nile Delta/ El-Adlyah company for land reclamation; 14.9.1981; Leg. A. Bakry; 3175 (CAI). Ezbet EL Dleba, on gawsah drain: Hefna; El-Sharqia; 3.9.1953; N. El Hadidi; s.n. (CAI). Giza, Olive orchard; 16.11.2010; Bennoba, s.n. (CAI).

Flowering time: May-August.

Distribution in Egypt: Nile Delta, Siwa Oasis and Baharia Oasis

General distribution: Afghanistan, Kashmir, Kazakhstan, Mongolia, Pakistan, Russia, Turkmenistan; SW Asia.

-Cynanchum subgen. Mellichampia (New World)

Cynanchum subgen. *Mellichampia* (A. Gray) Woodson, Ann. Missouri Bot. Gard. 28: 209

(1941).
Cynanchum sect. *Mellichampia* (A.Gray ex S. Watson) Sundell, Evol. Monogr. 5 (New World Sp. *Cynanchum* subgen. *Mellichampia*) 13 (1981): (1981).
=*Cynanchum laeve* (Michx.) Pers.
Syn. : *Ampelamus albidus* (Nutt.) Britton
Enslenia albida Nutt.
Gonolobus laevis Michx.
Ampelamus laevis (Michx.) Krings
Herbaceous stem, twining, climbing with whitish sap, with villous hairy at stem margins. Petiolate, opposite leaves. Petioles up to 7cm long, reddish-green. Glabrous blade, dark green with obvious veining top, typically cordate, to ovate, acute to apiculate or acuminate, up top 10 cm long and 8 cm broad. Inflorescence; Axillary umbellate cyme approximate 30 flowers, Peduncles up to 5 cm, hairy. Pedicels up to 1cm long, pubescent. Corolla intensely 5- whitish lobes, up to 7 mm long, and 3 mm broad, erect to patent, glabrous. Corona of 5 appendages. White appendages erect, up to 6 mm long, extended at base, 2 linear lobes pointed at apex. Calyx 5- green lobes, with nearly purple, ovate - lanceolate, up to 3mm long, sparse pubescent. Large follicle, up to 10-11 cm long and 4 cm wide at widest point, teardrop-shaped, glabrous. Winged seeds, up to +8 mm long.
Flowering; July to September.
Habitat: Disturbed sites, railroads, roadsides, waste ground, along fences.
Distribution: Native to U.S.
Representative specimens: Prince George Co., Illinois, USA; 10.8.1939; L. B. Smith; R. P. underlin; Barcode:13667 (USF). Halifax Co., North Carolina, USA;19.7.1956; H. E. Ahles; R. P.underlin; Barcode:20265(USF). Jasper Co., Missouri, USA; 8.8.1920; E. J. Palmer; R. P. Wunderlin; Barcode:29684, (USF). Fairfax Co., Virginia, USA; 7.8.1910; P. Dowell; R. P. Wunderlin; Barcode:29685, (USF). Hamilton Co., Ohio, USA;7.8.1882; C. G. Lloyd; R. P. Wunderlin; Barcode:33754, (USF). Sharkey Co., Mississippi, USA;11.7.1955; J. D. Ray, Jr.; R. P. Wunderlin; Barcode:41111, (USF). Mercer Co.,Kentuchy,USA;23.7.1955;M. E. Wharton; R. P. Wunderlin; Barcode:43698, (USF). Union Co., Illinois, USA;23.8.1958; R. F. Thorne; R. P. Wunderlin; Barcode:53192, (USF). Independence Co., Arkansas, USA;6.7.1968; R. D. Thomas; Barcode:86801, (USF). Macon Co., Tennessee, USA; 14.10.1968; K. E. Rogers; D. Drapalik; Barcode: 86937, (USF). Macon Co., Tennessee, USA; 14.10.1968; K. E. Rogers; D. Drapalik; Barcode: 94278, (USF). Cocke Co., Tennessee, USA; 29.7.1977; P. Genelle; Barcode: 95410, (USF).Lincoln Co., Missouri, USA; 10.8.1978; W. G. D'Arcy; B. Summers; Barcode: 214841, (USF).Westmoreland Co., Virginia, USA; 7.7.1990; T. R. Bradley; Barcode: 215064, (USF).Champaign Co., Illinois, USA; 10.8.2008;S. R. Hill; S. R. Hill;Barcode:277162, (USF).Jackson Co., Illinois, USA; 23.7.1998; S. R. Hill; S. R. Hill; Barcode: 283635, (USF). Wunderlin, et al., 2019. Atlas of Florida Plants (http://florida.plantatlas.usf.edu/).

3.2. Morphometric analysis

3.2.1. Testing variation in taxa and populations, using multivariate methods (Cluster analyses and ordination analyses)

After estimating how accurately the dendrograms represent the original Gower distance matrix, the cophenetic distances calculated from the NJ dendrogram (r=0.83) while UPGMA representing (r=0.80). Consequently, the NJ topology is applied to the present study (Saitou & Nei, 1987; Pankhurst 1991; Raff, 1966).

The NJ topology (Fig. 4) for multivariate analysis of 59 observation of 36 variables after removing lines with missing data. The relationship between studied taxa clarified at fan NJ, meanwhile, the most comparable leaf shapes and characters are on sister clades, and the most divergent cluster is insulated next to the root (Viscosi and Cardini 2011). However, the identified taxa of *C. acutum* subsp. *sibiricum* collected together at one node in the first cluster with some taxa from *C. acutum* subsp. *acutum* confirmed the more closely related between two subspecies. On the other hand, the second cluster gathered taxa from *C. acutum* subsp. *acutum* and *C. laeve* to show the relation and the similarity between two species.

PCA biplot (Fig. 5) showed a total variation 37.7% at first PC1 axis and 8.9% at second PC2, both designated the highest and moderate linkage and relationships between

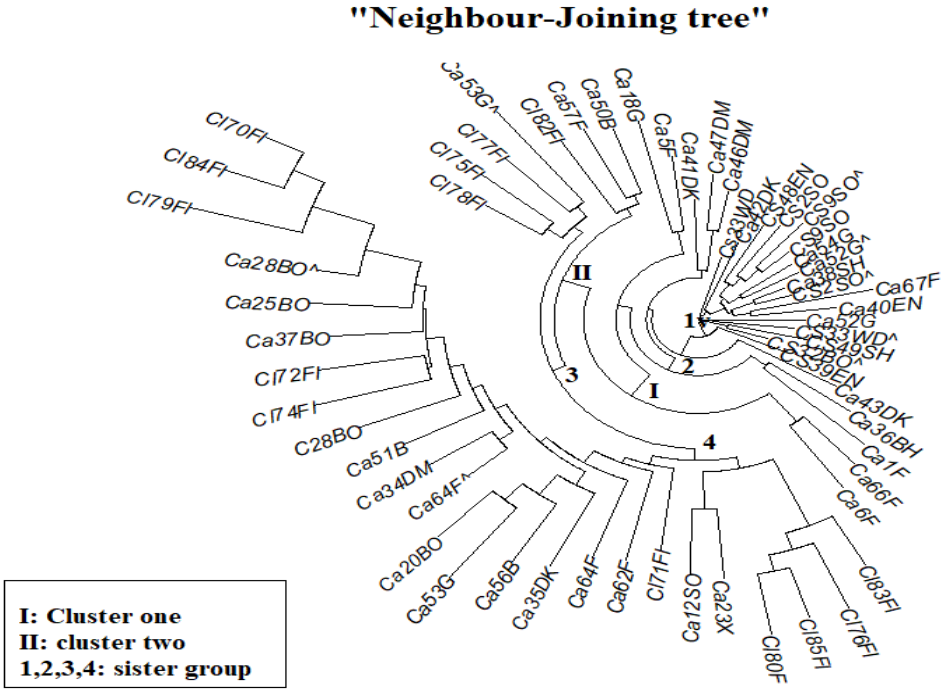


Fig. 4. Fan Neighbour-Joining tree for multivariate analysis of 59 observation of 36 variables.

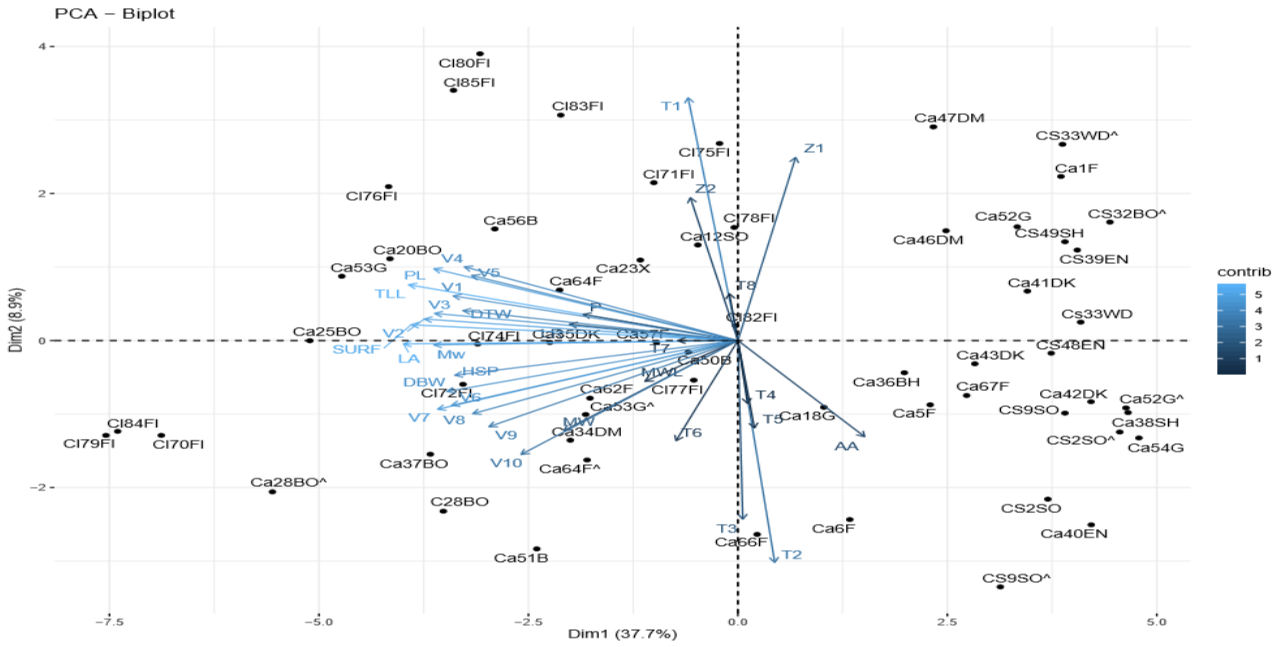


Fig.5. PCA biplot with total variation 37.7% for first PC1 axis separate *C. acutum* subsp. *sibiricum* and *C. acutum* subsp. *acutum* from larger leaf *C. acutum* subsp. *acutum* and *C. laeve*. Total variation 8.9% for second PC2 separate leaf *C. acutum* subsp. *acutum* and *C. laeve* according to the measurements of medium width and maximum width length.

examined taxa and also among the population from same taxa.

3.2.2. Testing variation in taxa and populations, using a modified Procrustes ANOVA

Results of the statistical estimation of variance in ANOVA and MANOVA, demonstrated in Table 3 and showed significant ($P < 0.05$) differences in leaf traits between three taxa (*C. acutum* subsp. *acutum*, *C. acutum* subsp. *sibiricum* and *C. laeve*). This result is also obviously seen in the Box-plot and CVA.

3.23. Testing taxa differences using permutation tests and discriminant analyses

The box-plot collecting all leaf traits (Fig. 6.9) shows that *C. laeve* tends to be slightly larger than *C. acutum* subsp. *acutum* and *C. acutum* subsp. *sibiricum*, but there is a lot of overlap between the populations. While the box plots for some leaf traits like Total leaf length, petiole length, width length, maximum width length, leaf surface and leaf area (Fig.6.2-6.8) showed large variation at interquartile range and median that confirm the separation of three taxa under studied.

Canonical variate analysis showed a significant difference between taxa (*P*=0.001***), where the first discriminating axis of canonical variate analysis (CVA) separated *C. laeve* from two *C. acutum* subspecies expect few specimens from *C. acutum* subsp. *acutum* to indicate the relation between the two taxa with a total variation 83%. The second discriminating the axis isolated *C. acutum* subsp. *sibiricum* specimens at the top of biplot from relative *C. acutum* subsp. *acutum* to total variation of 67% (Fig. 7). By using Discriminant analysis, check the total variation between *C. acutum* subsp. *acutum* and *C. acutum* subsp. *sibiricum* (77%) then between *C. acutum* subsp. *acutum* and *C. laeve* to show 86% of the variation and significant value (*P*=0.002**) (Fig. 8).

4. Discussion

Cynanchum acutum is distributed in worldwide. It occurs in wet soils and nearby to freshwater, roadsides, wastelands, and gardens. It ranked as Least Concern (Akhani 2014).

The present study agrees with Rechinger, (1970) who stated that "The difference between two subspecies *C. acutum* is easy about extreme traits, but mainly problematic because the exploiting characteristics are not fixedly correlated with characteristics of leaf". Accordingly, the study going to use the modern method like Computerized geometric Morphometric analysis to a good distinction between them by selecting more viable characters to help the differentiation between taxa, depending mainly on quantitative measurement, test and visualize a digital image to identify the differences in form in extremely actual perfect and, statically powerful way (Viscosi and Cardini 2011; Cope *et al*. 2012; Mayer *et al.* 2014; López De Heredia *et al.* 2017 ; Klein & Svoboda 2017).

This close relationship between examined taxa appear at NJ topology and PCA biplot, Owing to the variables at the shape and size of leaves between individuals and different populations. However, the first cluster has individuals of *C. acutum* subsp. *acutum* and *C. acutum* subsp. *sibiricum* , which separated out to two sister groups, the first one collects all identified *C. acutum* subsp. *sibiricum* with the closeness of it from *C. acutum* subsp. *acutum*, that showed the slightly distinction between two subspecies with and approve that the main difference is the shape and base lobe of leaf show it in, display the leaf shape of hastate-cordate, the base of lobes to be auriculate or hastate, and the basal lobes distinctly divergent are the obvious characters for *C. acutum* subsp. *sibiricum* (Davis 1978; Rechinger 1970; Gilbert *et al.* 1995; Li *et al.* 1995; Boulos 2000).

The second sister group at cluster I has typically *C. acutum* subsp. *acutum*, which have a cordate leaf with cordate or broadly auriculate leaf base and the basal lobes scarcely divergent (Davis 1978; Boulos 2000). Whereas the second cluster II assembly two different species (*C. acutum* subsp. *acutum* and *C. laeve*) which contain typically cordate and broadly cordate leaves, maximum width

Table 3.1. Hierarchical sum of squares ANOVA of three examined taxa. SS = sum of squares, MS = mean sum of squares (i.e., SS divided by df) and df = degree of freedom.

	SS	MS	df	F value	Pr (>F)
Taxon	55762062	18587354	3	2626	<2e-16 ***
Residuals	424772	7080	60		
Population	55834218	4294940	13	609	<2e-16 ***
Residuals	352616	7052	50		

Signif. codes : 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’

Table 3.2. MANOVA test of three examined taxa. SS = sum of squares, MS = mean sum of squares (i.e., SS divided by df) and df = degree of freedom.

	Df	Pillais trace	Approx. F	num Df	den Df	Pr (>F)
Taxon	3	2.5215	4.2157	105	84	3.926e-11 ***
Residuals	60					
Population	13	8.1421	1.3408	455	364	0.001735 **
Residuals	50					

Signif. codes : 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

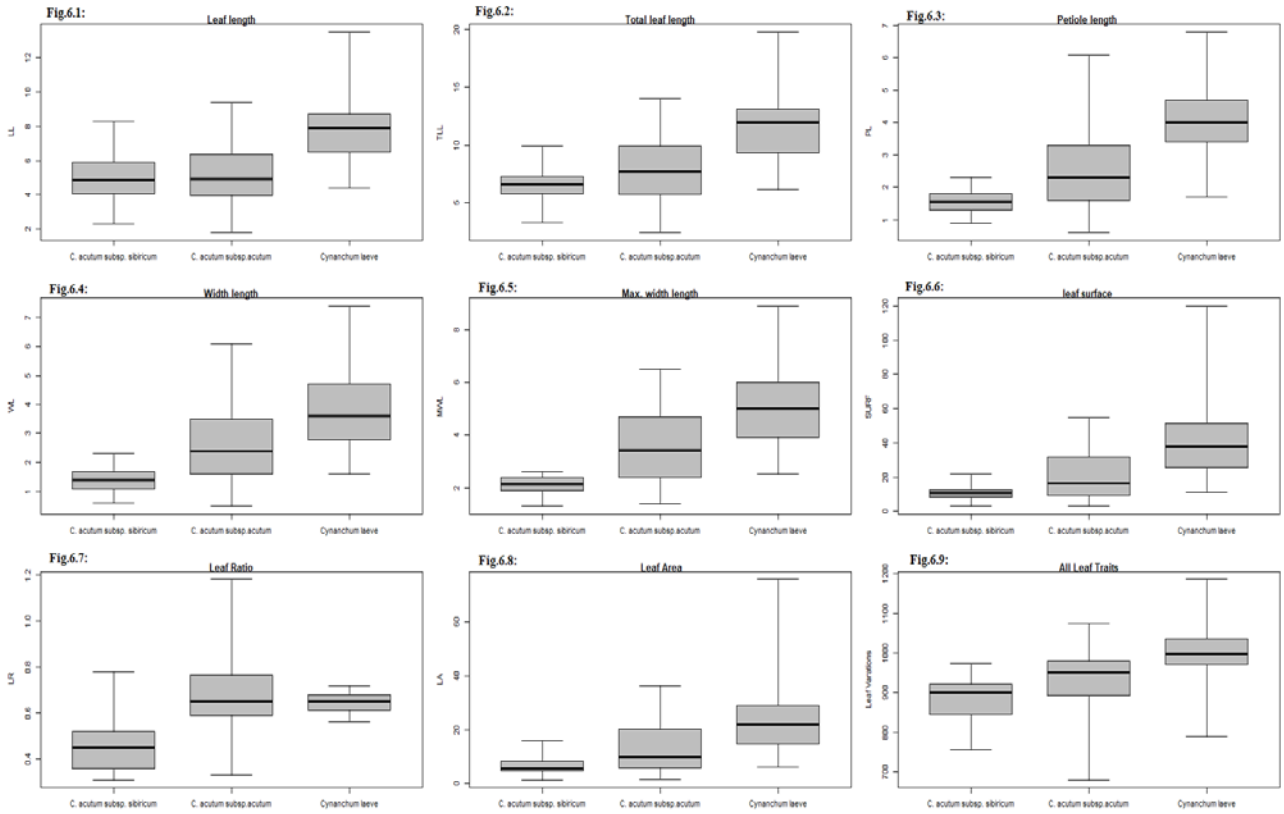


Fig.6. The box-plot of three examined taxa, depending on all leaf traits and selecting some of them showed that *C. laeve* tend to be slightly larger than *C. acutum* subsp. *acutum* and *C. acutum* subsp. *sibiricum*, with overlaps between the populations.

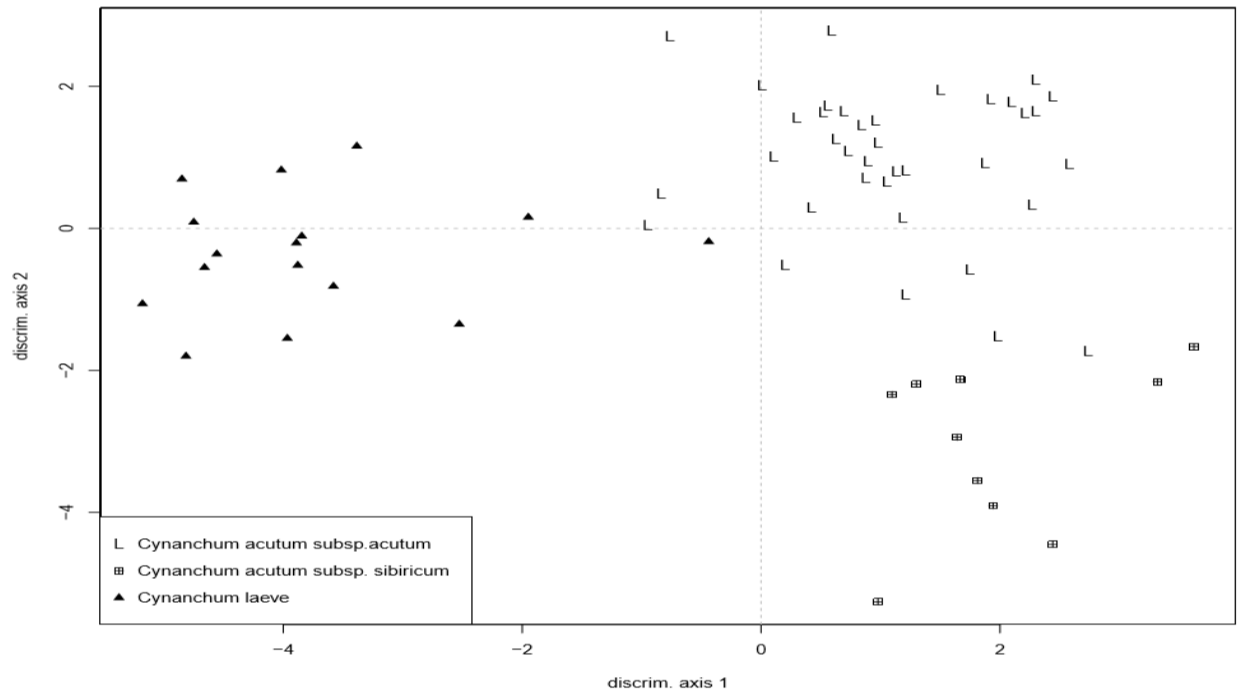


Fig.7. canonical variate analysis (CVA) for three taxa, which ranged in CCA1 and CCA2 as for C. *laeve* (-3.76 and -0.34), *C. acutum* subsp. *acutum* (1.096 and 0.97), *and C. acutum* subsp. *sibiricum* (1.98 and -3.05)

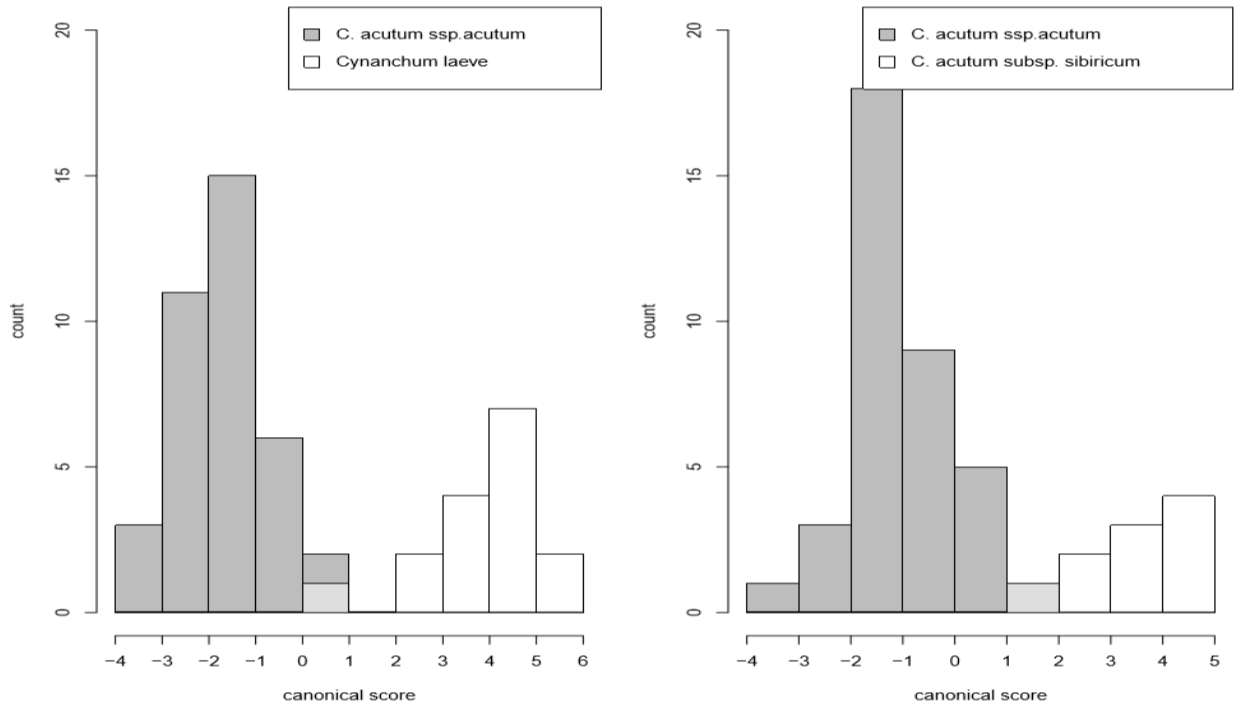


Fig.8. Discriminant analysis, in left histogram for *C. acutum* subsp. *acutum* and *C. laeve* and the right histogram *C. acutum* subsp. *acutum* and *C. acutum* subsp. *sibiricum*, the both point to the variance between taxa.

length ranged between (3-8 cm) and maximum petiole length ranged between (2.3-6 cm).

The main reason for chosen of *C. laeve* to be outgroup in the current study that is more resemblance species to C. *acutum* and also belong to different section according to Liede & Kunze (2002). The results displayed a relative relationship between the old world *Cynanchum* presented by C. *acutum* subsp. *acutum* and New World *Cynanchum* (*Cynanchum laeve*), was confirmed before by Liede & Tauber (2002) who used cpDNA spacers to analyze a lot of memberships of sections of *Cynanchum* and found that *C. laeve* subgen. *Mellichampia* is correctly placed under *Cynanchum* and formed a sister clade to each other. And they also suggested using the vegetative character were being good sign than floral characters as doing in this study.

While the multivariate analysis demonstrations the relationship and confusion between taxa the Box-plot, canonical variate analysis CVA and discriminant analyses (DA) confirmed separation of each three taxa with a few overlaps. The discriminant analyses revealed to the percent of medium width is the main trait that can distinguish the two subspecies with 39 % variation depending on Mw%.

That for the aim of this study about the presence of two subspecies at Egypt according to the Herbarium sheet kept at (CAI) has been confirmed by different methods either via classical taxonomy or by modern taxonomy (GMM). The current study agreed and recommended the using of Modern Geometric Morphometric Analysis, however, is very potential for applying, a relatively highly effective, accurate and viable tool to characterization and classification between closely related taxa (Viscosi and Cardini 2011; Vieira *et al.* (2014).; Lo Bianco and Mirabella 2018). In line with the finding of Viscosi and Cardini 2011 stated that "Taxonomists must be definitely keen on taking improvement for new quantitative methods for the description of taxa including semi-automated computerized tools".

5. Conclusion

The study aimed to revise *Cynanchum acutum* in Egypt and their relative subspecies by using classical taxonomy and modern geometric morphometric analysis. After extensive studies at the samples of the Herbarium sheet kept at (CAI) and study the description of *C. acutum* subspecies in different flora, the present study confirms of existence *C. acutum* subsp. *sibiricum* in Egypt. The morphometric analysis going to the same trend with classical taxonomy where NJ and PCA exhibited the relationship between taxa understudy to reveal the relation between two (*C. acutum* subsp. *acutum* and *C. acutum* subsp. *sibiricum*) and (*C. acutum* subsp. *acutum* and *Cynanchum laeve*). Finally, Boxplot, CVA and DA confirmed the separation of the taxa. This study clarifies the situation of *Cynanchum acutum* in Egypt and the concluded and confirmed the presence of two subspecies viz.: subsp. *acutum* and subsp. *sibiricum*, and recommended using the geometric modern analysis as a practical method of classification.

Acknowledgments

The author is greatly appreciative to the staff of Cairo University Herbarium (CAI) for facilities provided.

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Supplementary 1: The distribution of the monitored *Cynanchum acutum* subsp. *acutum* in Fayoum depression during summer 2017, collected by M. Sayed, M. Faker & F. Yousef (Fayium University Herbarium).

Site	Locality	Latitude	Longitude	date of collected
1	Madinet Senouris, Ezbet Mohammed Mahfouz, Senoures, Faiyum Governorate	29°26'08.3"N	30°53'03.4"E	25.8.2017
2	Qasr Rashwan,Tameyah, Faiyum Governorate	29°27'30.4"N	30°55'22.5"E	25.8.2017
3	Madinet Tameyah, Tameyah	29°29'02.2"N	30°56'28.1"E	25.8.2017
4	Ezbet El-Hariry, El-Rawda, Tameyah	2927'38.8"N	30°58'41.5"E	25.8.2017
5	Madinet Tameyah, Tameyah, Faiyum	29°29'25.5"N	30°58'41.9"E	25.8.2017
6	Kafr Al Maslat - Tamya,Fanous, Tameyah, Faiyum Govern	29°32'52.5"N	30°58'55.9"E	25.8.2017
7	Monshaat Doctor El-Gammal,Tameyah, Faiyum Governorate	29°30'32.1"N	31°03'21.3"E	25.8.2017
8	Forqos, Tameyah, Faiyum Govern	29°25'28.4"N	30°58'50.4"E	25.8.2017
9	Qasr Bayad,Ibsheway, Faiyum Governorate	29°21'30.2"N	30°44'12.1"E	12.9.2017
10	Kahk, Youssef El Seddik, Faiyum Governora	29°26'02.0"N	30°38'39.4"E	12.9.2017
11	An Nazlah, Youssef El Seddik, Faiyum Gove	29°17'59.8"N	30°38'14.4"E	12.9.2017
12	Al Hamouli,Youssef El Seddik, Faiyum Governorate	29°17'10"N	30°37'15.4"E	12.9.2017
13	Al Agameyin-Ibsheway way,Zaid, Ibsheway, Faiyum Governorate	29°20'57.9"N	30°41'26.0"E	12.9.2017
14	Madinat Al Fayoum - Tohbar way, Al Agameyin,Ibsheway, Faiyum Governorate	29°19'48.2"N	30°42'58.6"E	12.9.2017
15	Al Gaafra - Minya Al Hayt,Minya, ATSA, Faiyum Governorate,	29°13'32.8" N	30°45'29.5"E	16.9.2017
16	Al Gharq-Faiyum way,Izbat Ad Daw, Minya, ATSA	29°12'44.4" N	30°45'50.1"E	16.9.2017
17	Ezbet Ali Farag - Al Hadqa,Al Hadeqah, Al Fayoum, Faiyum Governorate	29°16 '2.9" N	30°50'42.5"E	16.9.2017
18	Hawaret Al Maqtaa,Al Fayoum, Faiyum Governorate	29°14 '50.7" N	30°54'29.5"E	16.9.2017
19	Madinat Al Fayoum - Kafr Mahzooz way,Matar Tares, Senoures, Faiyum Go	29°22 '46.1" N	30°54'17.1"E	16.9.2017
20	Behmo, Senoures, Faiyum Govern	29°22 '27.1" N	30°50'51.4"E	16.9.2017
21	Madinat Al Fayoum - Tersa way,Naqalifah	29°24 '39.8" N	30°49'31.9"E	16.9.2017
22	Senoures, Faiyum Governorate	29°17 '21.78" N	30°52'52.7"E	16.9.2017
23	Kofour an Nil,Al Fayoum, Faiyum Governorate	29°18' 46.63" N	30°53'23.14"E	20.9.2017
24	Al Edwah, Al Fayoum, Faiyum Gov	29°19 '58.7" N	30°55'52"E	20.9.2017
25	Al Eelam,Al Fayoum, Faiyum Governorate	29°19'38.2"N	30°52'07.5"E	20.9.2017
26	Zawyet Al Kerdaseya,Bani Saleh, Al Fayoum, Faiyum Gov	29°21'18.8"N	30°48'18.9"E	20.9.2017

Supplementary 2: Details about *Cynanchum* specimen used in present study.

code		Locality
Ca1 F	Nv	Fayoum, KomAushim, Faiyum, 21.9.1959; V. Täckholm; 6029(CAI).
Cs2 SW	O	Siwa Oasis, Meshanded 25Km West of Siwa, 26.10.1963; L. Boulos, 6023(CAI).
Ca3 SW	O	Siwa Oasis, Meshanded 25Km West of Siwa, 26.10.1963; Loutfy Boulos, 6024(CAI).
Ca4 Alx	M	Faculty of agriculture, farms, Alexandria; 28.8.1953; Loutfy Boulos, 6008(CAI).
Ca5 F	Nv	Fayoum, El-Faiyum, 28.5.1954, M. El Shafay, 6034(CAI).
Ca6 F	Nv	Fayoum, El-Faiyum, 28.5.1954, M. El Shafay, 6026(CAI).
Ca7 F	Nv	Fayoum, KomAushim, Faiyum, 29.9.1967; V. Täckholm - M. Imam - I. Elsayed- M. El Mahdi; 6036(CAI).
Ca8 F	Nv	Fayoum, KomAushim, Faiyum, 13.10.1967; V. Täckholm - M. Imam- N. El Hadidy; 6035(CAI).
Cs9 SW	O	Siwa Oasis, Siwa oasis/ Abu Sharour, 1.4.1968, Gun Pomeé- and Nabil El Hadidy;6021(CAI).
Ca10 F	Nv	Fayoum, KomAushim, Faiyum, 21.9.1959; Vivi Täckholm; 6032(CAI).
Ca11 B	Nv	Beheira, Beheira province, Rosetta, 16.7.1987; Alaa Amer, 11904(CAI).
Ca12 SW	O	Siwa Oasis, 27.12.1969 ; M. A. Zahran / L. Boulos, 6020(CAI).
Ca13 F	Nv	Fayoum, Kom Osheem, near Faiyum, Jan. 1953; Nabil El Hadidy 6025(CAI).
Ca14 F	Nv	Fayoum, Kom Osheem, near Faiyum, Jan. 1953; Nabil El Hadidy 6028(CAI).
Ca15 F	Nv	Fayoum, KomAushim, Faiyum, 15.7.1960; Loutfy Boulos 6027(CAI).
Ca16 GZ	Nv	Giza, Giza near Cairo, 19.10.1910; Ernst Hartmann 6016(CAI).
Ca17 ALX	M	Alex, Faculty of agriculture farm, Alexandria Univ., 28.8.1952; Nabil El Hadidy 6009(CAI).
Ca18 GZ	Nv	Giza, Orman Gardens, Giza, 6.10.1954; Soliman Sisi, 6011(CAI).
Ca19 GZ	Nv	Giza, Orman Gardens, Giza, 6.10.1954; Soliman Sisi, 6013(CAI).
Ca20 BO	O	Baharia Oasis, Baharia Oasis, Zabou, 11.9.1971; M. Imam, 5987(CAI).

Ca21 DK	Nv	Dakahlia, Dakahlia- Aga, 11.10.1929; Elhamy Greiss, 5968(CAI).
Ca22 DM	Nv	Demietta, Between Ras el Bar and Demietta, 13.9.1929; M.T. Hefnawy, 5980(CAI).
Ca23 ALX	M	Alex, Masraf El Gabaroti, El Aslah Mohatta, on the road Alexandria-Rosetta, 18.9.1970; Imam, Ibrahim, Mahdi 5986(CAI).
Ca24 DM	Nv	Demietta, Between Ras el Bar and Demietta, 13.9.1929, M.T. Hefnawy, 5978(CAI).
Ca25 BO	O	Baharia Oasis, Al-Zabw, 4.10.1978; Monier Abd El Ghani, 677(CAI).
Ca26 BO	O	Baharia Oasis,. Al-Harra, Ain Boulol, on walls & hedges, 24.10.1979; Monier Abd El Ghani , 2112(CAI).
Ca27 BO	O	Baharia Oasis, Al-Zabw, 18.7.1978; Monier Abd El Ghani, 490(CAI).
Ca28 BO	O	Baharia Oasis,. Al-Harra, Ain Boulol, on fences, 24.10.1979; Monier Abd El Ghani, 2112(CAI).
Ca29 BH	Nv	Beheira, 8-Mar-08, G. Maire, 5970(CAI).
Ca30 EN	ND	East ND, East of the Nile Delta/ El-Maneyl, 17.6.1980, A. Bakry, 1229(CAI).
Ca31 EN	ND	East ND, East of the Nile Delta/ El-Mullok, 25.4.1980, A. Bakry, 283(CAI).
Cs32 BO	O	Baharia Oasis, El-Helz, Tabliun, 15.5.1978; Monier Abd El Ghani, 320(CAI).
Cs33 WD	ED	Wadi Degla, Wadi Degla, Eastern Desert, 25.4.2006, M. Abdel Aleem; L1. (CAI).
Ca34 DAM	Nv	Damanhour, 15.8.1907, G. Maire; 5971(CAI).
Ca35 DAK	Nv	Dakahlia, the drain going parallel to the railways at Kilo 27 after Kaha, 21.7.1954; 5995(CAI).
Ca36 BH	Nv	Beheira, Boghaz, Rosetta, 30.10.1971; 5991(CAI).
Ca37 BO	O	Baharia Oasis,. Al-Harra, Ain Boulol, on walls & hedges, 24.10.1979; Monier Abd El Ghani , 2112(CAI).
Ca38 SHR	Nv	El-Sharqia, Ezbet EL Dleba, on gawsah drain:Hefna, 3.9.1953; Nabil El Hadidy 5994(CAI).
Ca39 EN	ND	East ND, East of the Nile Delta/ El-Maneyl, 12 km from Ismaila on the desert road, 17.6.1980, Leg. A. Bakry 1229(CAI).
Ca40 EN	ND	East ND, Cairo: Ain Shams University, 1977; A. Gazzar 5988(CAI).
Ca41 DAK	Nv	Dakahlia, Dakahlia- Aga, 11.10.1929; E. Greiss 6005(CAI).
Ca42 DAK	Nv	Dakahlia, Dakahlia- Aga, 11.10.1929; E. Greiss 6006(CAI).
Ca43 DAK	Nv	Dakahlia, Dakahlia- Aga, 11.10.1929; E. Greiss 6007(CAI).
Ca44 DEM	Nv	Demietta, Between Ras el Bar and Demietta, 13.9.1929; M.T. Hefnawy, 5983(CAI).
Ca45 DEM	Nv	Demietta, Between Ras el Bar and Demietta, 13.9.1929; M.T. Hefnawy, 6002(CAI).
Ca46 DEM	Nv	Demietta, Between Ras el Bar and Demietta, 13.9.1929; M.T. Hefnawy, 5972(CAI).
Ca47 DEM	Nv	Demietta, Between Ras el Bar and Demietta, 13.9.1929; M.T. Hefnawy 5976(CAI).
Cs 48 EN	ND	East ND, East of the Nile Delta/ El-Adlyah company for land reclamation, 14.9.1981; A. Bakry,. 3175(CAI).
Cs 49 SR	Nv	El-Sharqia, Ezbet EL Dleba, on gawsah drain: Hefna, 3.9.1954; Nabil El Hadidy 5993(CAI).
Ca50 BH	Nv	Beheira, Along the delta road to Edfina at sandioun, 29.9.1966; N. Hadidi M.Imam and V. Tackholm 5996(CAI).
Ca51 BH	Nv	Beheira, Along the delta road to Edfina at sandioun, 29.9.1966; N. Hadidi, M.Imam and V. Tackholm 5997(CAI).
Ca52 GZ	ND	Giza, At Parks and gardens, 10.10.1967; M. El-Mahdi 6045(CAI).
Ca53 GZ	ND	Giza, At Parks and gardens / Orman Gardens, Giza, 27.8.1963; M. El-Mahdi 6048(CAI).
Ca54 GZ	ND	Giza, At Parks and gardens, 10.10.1967; M. El-Mahdi 6050(CAI).
Ca55 OV	ND	Giza, Olive orchard, 16.11.2010; Bennoba s.n. (CAI).
Ca56 NT	ND	Beheira, Wadi El Natraun, in Olive orchard, 18.7.2009; A. B. Farhat.
Ca57 RY	ND	Fayoum,Wadi Wl. Rayan protected area herbarium,1.2.2000; L.Boulos (CAI).
Ca58 S		Damascus, Demaecue, 23.8.1959; S. Ghabbour. 6043(CAI).
Ca59 S		Damascus, Demaecue, 23.8.1959; S. Ghabbour. 6044(CAI).
Ca60 F	ND	Qasr Rashwan Tameyah, Fayoum, 29°27'30.4"N,30°55'22.5"E; 25.8.2017; M. Sayed, M. Faker & F. Yousef; CF1.
Ca61 F	ND	Madinet Tameyah, Fayoum, 29°29'02.2"N, 30°56'28.1"E;25.8.2018; M. Sayed, M. Faker & F. Yousef; CF2.
Ca62 F	ND	Ezbet El-Hariry, El-Rawda, Tameyah, Fayoum; 2927'38.8"N 30°58'41.5"E, 25.8.2019; M. Sayed, M. Faker & F. Yousef; CF3.
Ca63 F	ND	Madinet Tameyah, Fayoum; 29°29'25.5"N, 30°58'41.9"E; 25.8.2020; M. Sayed, M. Faker & F. Yousef; CF4.
Ca64 F	ND	Kafr Al Maslat – Tamya, Fayoum,29°32'52.5"N 30°58'55.9"E ;25.8.2021; M. Sayed, M. Faker & F. Yousef; CF5.
Ca65 F	ND	Monshaat Doctor El-Gammal, Fayoum, 29°30'32.1"N 31°03'21.3"E; 25.8.2022; M. Sayed, M. Faker & F. Yousef; CF6.
Ca66 F	ND	Forqos, Tameyah, Fayoum,29°25'28.4"N 30°58'50.4"E;25.8.2023; M. Sayed, M. Faker & F. Yousef; CF7.
Ca67 F	ND	Al Gharq-Faiyum way Izbat Ad Daw, Minya, Etsa, Fayoum; 29°12'44.4" N 30°45'50.1"E; 16.9.2017; M. Sayed, M. Faker & F. Yousef; CF8.
Ca68 F	ND	Madinat Al Fayoum - Tersa way –Naqalifah, Fayoum, 29°24 '39.8" N 30°49'31.9"E; 16.9.2018; M. Sayed, M. Faker & F. Yousef; CF9.
Ca69 F	ND	Al Eelam Al Fayoum, Fayoum, 29°19'38.2"N 30°52'07.5"E;20.9.2017; M. Sayed, M. Faker & F. Yousef; CF10.
Cl.70 FL	USA	Prince George Co., Illinois, USA; 10.8.1939; L. B. Smith; R. P. underlin; Barcode:13667.
Cl.71 FL	USA	Halifax Co., North Carolina, USA;19.7.1956; H. E. Ahles; R. P. underlin; Barcode:20265.
Cl.72 FL	USA	Jasper Co., Missouri, USA; 8.8.1920; E. J. Palmer; R. P. Wunderlin; Barcode:29684.
Cl.73 FL	USA	Fairfax Co., Virginia, USA; 7.8.1910; P. Dowell; R. P. Wunderlin; Barcode:29685.
Cl.74 FL	USA	Hamilton Co., Ohio, USA;7.8.1882; C. G. Lloyd; R. P. Wunderlin; Barcode:33754.
Cl.75 FL	USA	Sharkey Co., Mississippi, USA;11.7.1955; J. D. Ray, Jr.; R. P. Wunderlin; Barcode:41111,
Cl.76 FL	USA	Mercer Co., Kentuchy, USA;23.7.1955; M. E. Wharton; R. P. Wunderlin; Barcode:43698.
Cl.77 FL	USA	Union Co., Illinois, USA; 23.8.1958; R. F. Thorne; R. P. Wunderlin; Barcode:53192.
Cl.78 FL	USA	Independence Co., Arkansas, USA; 6.7.1968; R. D. Thomas; Barcode: 86801.
Cl.79 FL	USA	Macon Co., Tennessee, USA; 14.10.1968; K. E. Rogers; D. Drapalik; Barcode: 86937.
Cl.80 FL	USA	Macon Co., Tennessee, USA; 14.10.1968; K. E. Rogers; D. Drapalik; Barcode: 94278.
Cl.81 FL	USA	Cocke Co., Tennessee, USA; 29.7.1977; P. Genelle; Barcode: 95410.
Cl.82 FL	USA	Lincoln Co., Missouri, USA; 10.8.1978; W. G. D'Arcy; B. Summers; Barcode: 214841.
Cl.83 FL	USA	Westmoreland Co., Virginia, USA; 7.7.1990; T. R. Bradley; Barcode: 215064.
Cl.84 FL	USA	Champaign Co., Illinois, USA; 10.8.2008; S. R. Hill; S. R. Hill; Barcode: 277162.
Cl.85 FL	USA	Jackson Co., Illinois, USA; 23.7.1998; S. R. Hill; S. R. Hill; Barcode: 283635.