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Systematic implications of morphological traits variation and *rbcL* sequence polymorphism on inter-species relationships of the subtribe Plucheinae (tribe Inuleae-Asteraceae)

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The classification of species in the subtribe Plucheinae (Inuleae - Asteraceae) has been addressed by several authors using morphological characters and DNA sequences. However, neither of the published investigations afforded a convenient classification of the subtribe Plucheinae. Therefore, the present investigation aimed to evaluate the potential use of *rbcL* sequence, which has not been used before in phylogenetic studies of Plucheinae, taxa, either alone or in combination with morphological characters. In the current study, phylogenetic trees based on the morphological traits variation strongly support the differentiation of *Tessaria integrifolia, Cylindrocline commersonii, Geigeria alata, Sphaeranthus suaveolens*, and *Sphaeranthus indicus*, from a larger clade that encompasses all species of *Pluchea* and other species, such as *Dittrichia viscosa* and *Pulicaria dysenterica*, which served as outgroup species within the subtribe Inulinae in the current study. However, the *rbcL* polymorphism demonstrated the separation of *Dittrichia viscosa* and *Pulicaria dysenterica* from Plucheinae species. Furthermore, it revealed the paraphyly of *Pluchea* and *Laggera* and the incorporation of *Laggera alata* and *Tessaria integrifolia* into the *Pluchea* clades. The *rbcL* data confirmed the current taxonomic position of most of the Plucheinae species at the genus level. The analysis of the combined *rbcL* sequence polymorphism and variations in the morphological traits produced a better resolution of the examined species compared to the trees based on the analysis of the *rbcL* only. Heat maps illustrating the taxonomic relatedness of the studied species generally revealed results like those of the cluster trees and the PCA scatter diagrams. In conclusion, the *rbcL* sequences are helpful for the authentication and discrimination of Plucheinae species. However, the phylogenetic analysis of the *rbcL* barcode polymorphism revealed ambiguous taxonomic relationships among Plucheinae species and its heterogeneity of the taxonomic group.

Keywords: Systematics; *rbcL*; polymorphism; morphological traits; Plucheinae; Asteraceae

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INTRODUCTION

The Asteraceae is the largest plant family, including about 10% of the Earth's plant biodiversity (Mandel et al., 2019). The family is divided into 12 subfamilies and 43 tribes (Campos et al., 2016). The morphologically diverse tribe Inuleae (subfamily Asteroideae) consists of two subtribes, namely Inulinae and Plucheinae (Anderberg, 1991). Worldwide, the Inuleae–Plucheinae includes about 260 species in 31 genera (Nylinder and Anderberg, 2015). As pointed out by Merxmuller et al (1977), Inuleae was not altered substantially since Bentham (1873). However, Anderberg (1989) introduced the Inuleae sensu Merxmuellera et al. (1977) as a paraphyletic assemblage of three tribes: the Inuleae *sensu stricto*, the Gnaphalieae, and the Plucheeae, which together constitute the basal section of the hierarchy of the subfamily Asteroideae (Anderberg, 1989; Bremer, 1987; Eldenäs et al., 1999; Karis et al., 1992). The taxonomy and phylogeny of the tribe Plucheeae (Benth.) A. Anderb. were analyzed employing a computerized parsimony program (Anderberg, 1991). The results were presented in a consensus tree and cladogram showing four major monophyletic subgroups: The *Coleocoma* group (3 genera), The *Pterocaulon* group (3 genera), The *Laggera* group (6 genera) and The *Pluchea* group (12 genera). Anderberg (1991) described these four groups and supplied most genera with taxonomic notes, including comments on their taxonomic status. He concluded that genera such as *Blumea*, *Pluchea*, and *Epaltes* are unnatural assemblages.

Numerous combinations of traits such as habit, variation in capitulum shape and arrangement, flower disposition, and sex ratio in the capitula have been utilized to produce general descriptions and serve as delimiting criteria (Nylinder and Anderberg, 2015). In many cases, genera were delimited by technical characters, such as few pappus bristles, winged stems or combinations of features, like an abundance of female florets over the hermaphroditic ones in

association with filiform corolla (eg: Anderberg, 1991; Merxmuller et al., 1977). Some genera, such as *Sphaeranthus,* were distinguished by the absence of distinctive traits, such as lack of pappus bristles (Anderberg, 1991). On the other hand, receptacle, style, achene, and pappus traits were very helpful in classifying the examined species and, contributed more characters and clarified the phylogeny (Roque and Funk, 2013).

Based on analysis of sequence data from the chloroplast gene *ndhF*, Anderberg (2005) elucidated the delimitation between Inuleae s. str. and Plucheeae. The results reflected both tribes as a monophyletic group of two clades. The first hosted most Inuleae s.str. genera. Whereas the second clade included Plucheeae together with the remaining Inuleae s. str. genera, including *Iphionopsis*, *Pegolettia*, *Pechuel-loeschea*, *Ondetia*, *Geigeria, Calostephane*, and *Antiphiona*. Statistically, the first clade was strongly supported, while the second showed little support. Consequently, Anderberg (2005) considered Inuleae s. str. and Plucheeae as one tribe, where Inuleae included the two subtribes Inulinae and Plucheinae, corresponding to the clades having strong and weak support, respectively. Thus, compared with morphological criteria, the molecular data down-ranked Inuleae s. str. and Plucheeae into subtribes and modified the distribution of genera between them. At the nucleotide level, all Inulinae taxa possessed unique 3-bp CCT insertion in the *ndhF* gene that was missing in Plucheinae (Anderberg et al., 2005). This finding was supported by the floral micro-characters of Inuleae, which were studied by Anderberg (2012). Inuleae-Plucheinae share obtuse sweeping hairs reaching below the style bifurcation. Contrarily, Inuleae-Inulinae has acute sweeping hairs abaxially that do not reach below the stylar bifurcation. In addition, Inuleae-Plucheinae lacks the elongated oxalate crystals in cypsela epidermal cells that characterize Inuleae-Inulinae.

The classification of Anderberg (2005) reflected the paraphyly of the genera *Asteriscus*, *Chrysophthalmum, Inula, Pentanema*, and *Pulicaria* in Inuleae-Inulinae and *Laggera* and *Pluchea* in Inuleae-Plucheinae. Ten years later, Nylinder and Anderberg (2015) conducted a thorough investigation into the tribe Inuleae, with a particular focus on the Inuleae-Plucheinae using a specific set of chloroplast loci (*ndhF, trnL-F, trnH-psbA*) and two nuclear ones viz., external transcribed spacer (ETS) and internal transcribed spacer (ITS) sequences. In addition to *Laggera* and *Pluchea*, the authors recorded the paraphyly of the *Epaltes, Pterocaulon*, and *Nicolasia* genera. The last genus was absent in the study conducted by Anderberg (2005), while a single taxon represented the remaining two genera. Therefore, the potential use of new loci should be examined to establish the precise inter-specific and inter-generic relations in both subtribes.

In Egypt, the Asteraceae is represented by 98 genera and 234 species classified in the two subfamilies: Cichorioideae (Liguliflorae) and Asteroideae (Tubiliflorae) by Boulos (2002). The latter is exemplified by seven tribes: Anthemideae, Astereae, Calenduleae, Gnaphalieae, Heliantheae, Inuleae, and Senecioneae. Tribe Inuleae contains 13 Genera and 24 species within two subtribes: Inulinae (seven Genera & 18 Species) and Plucheinae (six Genera & six species).

The *rbcL* sequence is widely used in the discovery of new taxa (Shamso & Fouad, 2018), species conservation (Fouad et al., 2022a), biodiversity studies (Fouad et al., 2019b) and solving taxonomic challenges across large taxonomic groups (El-Sherif & Ibrahim, 2020). However, it was not used before for the establishment of phylogenetic studies of Inulae or its subtribes. Therefore, the present investigation aims to evaluate the systematic implications of the *rbcL* sequence polymorphism, either alone or in combination with variation in morphological traits to establish phylogenetic relationships in the subtribe Plucheinae. In addition, it aims to report a *rbcL*-based DNA barcoding for the six Pulchinae taxa growing in Egypt.

MATERIALS AND METHODS

Plant material

The current study revised 23 species representing nine genera of the subtribe Plucheinae, including six Egyptian species. In addition, the phylogenetic analysis used two species of subtribe Inulinae (*Dittrichia viscosa* and *Pulicaria dysenterica*) as outgroups (Supplementary Figure 1). The morphological description of the six Plucheinae species growing in Egypt (*Geigeria alata, Pluchea dioscoridis, Pseudoconyza viscosa, Pegolettia senegalensis, Sphaeranthus suaveolens,* and *Blumea bovei (syn. Doellia bovei*)) was based on herbarium specimens deposited at the Cairo University Herbarium (CAI) and Assiut University Herbarium (ASTU). Acronyms of herbaria were, according to Thiers (2023). The morphology of the remaining taxa was described from three-dimensional photos of

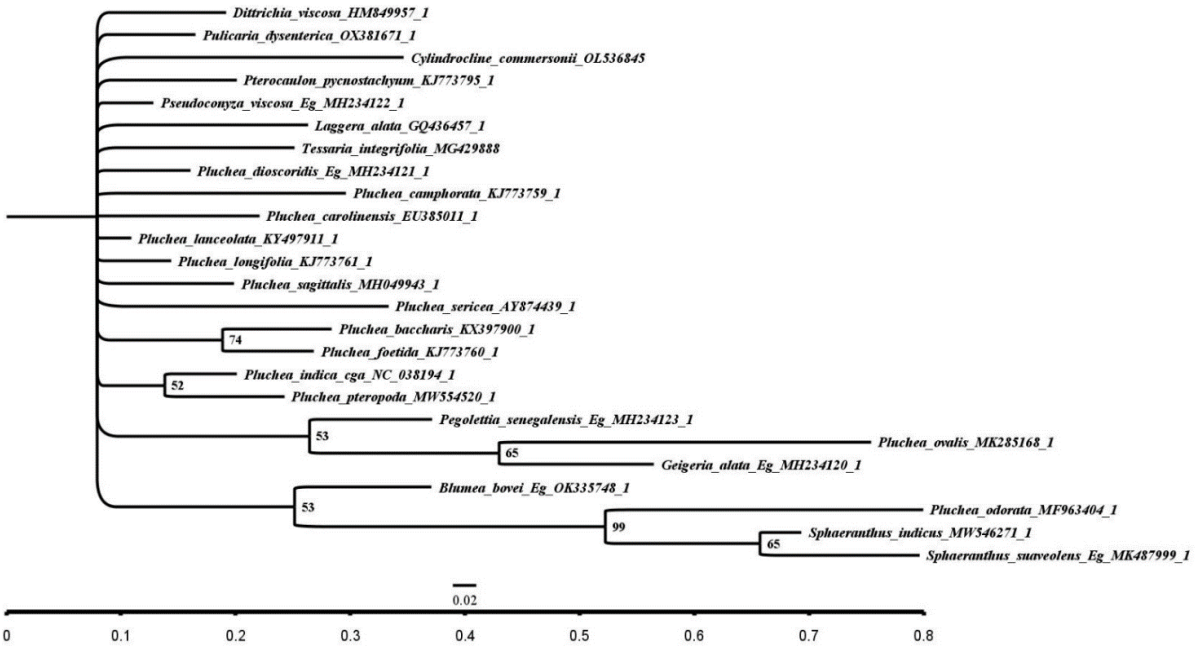

Figure 1. A Bayesian phylogenetic tree expressing the classification of Plucheinae species based on variation in the morphological features.

herbarium specimens and descriptions from the sources listed in Supplementary Figure 1. Pictures of the examined material were collected from the following websites: Plants of the World Online Portal - FTEA (https://powo.science.kew.org/); JSTOR Global Plants (JGP) (https://plants.jstor.org/); World Flora Online Data (https://www.worldfloraonline.org/); Flora of China (http://www.efloras.org/) and Flora of North America (http://floranorthamerica.org/Main_Page). The morphological description was based on thirty morphological characters listed in Supplementary Table 1.

Sequencing of *rbcL*

For the six Plucheinae species growing in Egypt, *the rbcL* sequence was obtained from DNA extracted from herbarium samples deposited at Cairo University Herbarium. Extraction of DNA was carried out using a Qiagen DNeasy kit (Valencia, California, USA) according to the manufacturer's protocol with some modifications for herbarium samples. Three samples (50 mg each) for each species from three herbarium specimens were ground to a fine powder in liquid nitrogen using a mortar and pestle. The AP1 buffer was fortified with dithiothreitol (Melford Laboratories, UK) and Proteinase K (Sigma) at 0.12 and 0.04 mg/ml, respectively (de Vere et al., 2012). The *rbc*L was amplified in a 50 µl reaction mixture containing 25 µl PCR Master Mix (Bioline), 20-50 ng genomic DNA, and 1 µl of each specific primer (5'-ATGTCACCACAAACAGAAAC-3' and 5'-TCGCATGTACCTGCAGTAGC-3'). The amplification protocol was 95°C for two min, followed by 34 cycles of 94°C for 1 min, 55°C for 30 sec, and 72°C for 1 min, then a final extension for 7 min at 72°C (CBOL Plant Working Group, 2009). PCR products were visualized on 1.5% (m/v) agarose gel under UV light. After purification employing the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), the amplified products were sequenced using Big-dye terminator chemistry in 3130xl Genetic Analyzer (Life Technologies, California, USA) following the manufacturer's protocol. Forward and reverse sequences repeated in at least three species samples were assembled using the Codon Code Aligner version 7.1.2 (Codon Code Cooperation, Dedham, USA). On the other hand, *rbcL* sequences of the remaining species were retrieved from the NBCI Gene Bank for material from the sources given in Supplementary Figure 1.

Phylogenetic and statistical analysis

The phylogenetic relationships among the examined species were expressed as cluster trees and PCA scatter diagrams constructed based on variation in morphological features coded as a multistate matrix (Supplementary Table 2) in addition to the four quantitative traits; leaf length, width, ratio, and area

(Supplementary Table 3). On the other hand, *rbcL* DNA sequence polymorphism was coded as 1 for the presence or 0 for the absence of nucleotides. The two data types were used independently and in combination to express the taxonomic relatedness of the examined species. A phylogenetic tree constructed using the Bayesian analysis represented the cladogenesis of species using the software MrBayes 3.2 (Ronquist et al., 2012). The best-fit substitution model (SYM+G) was selected based on the Akaike Information Criterion (AIC) as set by MrModel-test v.2.3 (Posada and Crandall, 1998). The Markov chain Monte Carlo (MCMC) process was conducted for three million generations while sampling one tree every 1000 generations with 16 chains. Stationary was recognized to be reached when "the average standard deviation of split frequencies" was less than 0.01; the first 25% of runs were ignored.

The thirty qualitative and four quantitative characters of morphological traits were analyzed using the R software (Vienna, Austria) (R Development Core Team, 2016). The four quantitative data were measured using the Image J program (version 1.51j8). The R-software was run utilizing the interface (Team, RStudio, 2015). To depict the degree of resemblance between the species, the "pheatmap" and "ggplot2" packages (Kassambara, 2020; Wickham & Wickham, 2016) were used. The color scale varies depending on how much the studied readings differ. According to Viscosi and Cardini (2011), red denotes substantial similarity between accessions, whereas blue denotes low similarity by using the "factoextra" and "ggplot2" packages in R for visualizing the distance matrices used in the PCA analysis. Kassambara and Mundt (2020) created the scatter diagram of the species produced.

RESULTS

Phylogeny of Plucheinae species based on variation in morphological traits

The Bayesian analysis of the 30 multistate morphological traits (Figure 1) failed to express reliable taxonomic relations between the examined species. It produced a polytomy tree with single branches for 14 species, including the *Dittrichia viscosa* and *Pulicaria dysenterica* of the subtribe Inulinae and seven *Pluchea* species, including *P. dioscoridis, P. camphorata, P. carolinensis, P. lanceolata, P. longifolia, P. sagittalis,* and *P. sericea*. It grouped the remaining eleven taxa into four clusters. At a low bootstrap value of 53, the first cluster from the bottom of the tree separated *Blumea bovei*, *Pluchea odorata,* and the two *Sphaeranthus* species (*S. suaveolens* and *S. indicus*). Internally, *Pluchea odorata* and *Sphaeranthus* species were grouped with a bootstrap value of 99, and then *Sphaeranthus* species were delimited at the bootstrap value of 65 from *P. odorata*. At the same bootstrap value of 53, the second clade delimited *Pegolettia senegalensis, Pluchea ovalis,* and *Geigeria alata; the latter two species were further delimited with 65 bootstrap supports.* The third cluster comprised *Pluchea pteropoda* and *P. indica* at bootstrap values of 52, while the fourth cluster comprised *Pluchea baccharis* and *P. foetida* at a bootstrap value of 74 (Figure 1).

The PCA scatter diagram constructed using the R software based on the variation in the 30 qualitative traits in addition to the four quantitative morphological traits generated a PCA heatmap for the 25 species (Figure 2). The figure shows total variation of 50.44%, including 18.7% at Dimension 1 (horizontal axis), and 13.1% at Dimension 2 (vertical axis). The PCA resolved the species into three groups; one includes *the majority of Pluchea species and is called the Pluchea group (P. ovalis, P. sericea, P. odorata, P. lanceolata, P. longifolia, P. dioscoridis*, *P. camphorata*, *P. carolinensis, P. foetida, P. baccharis) but also includes Tessaria integrifolia.* The *Sphaeranthus* species (*S. suaveolens* and *S. indicus*) are separated from all other taxa and called the *Sphaeranthus group.* The remaining *species of the subtribe* Plucheinae and *the two outgroup species*, *Dittrichia viscosa Pulicaria dysenterica, formed the third group called the Laggera group.*

The most contributing morphological characters to the separation of the examined species are listed in Table 1. For dimension 1, the highest positive correlation value was for pappus presence-absence (0.832) and disc florets surface (0.757). Meanwhile, dimension 2 recorded a more negligible correlation between leaf width (0.745) and leaf area (0.699). Leaf width, length, and area contributed the most to separating the *Pluchea* group, while pappus presence-absence, inflorescence type and base, and disc florets surface were the most important for separating *Sphaeranthus* species—separation of the *Laggera* group based on pappus color and row in addition to inflorescence number and plant habit.

Figure 2. Principal Component Analysis (PCA) of morphometric characters with the correlation between different variables and the first two components.

Phylogeny of Plucheinae species based on *rbcL* sequence polymorphism

The present investigation reports the first *rbcL* barcodes for *Geigeria alata, Pseudoconyza viscosa, Pegolettia senegalensis*, and *Blumea bovei* in GenBank, in addition to the Plucheinae species in Egypt, including the four species as well as *Pluchea dioscoridis* and *Sphaeranthus suaveolens*. The Bayesian analysis of *rbcL* sequence polymorphism separated the two out-group species, *Dittrichia viscosa* and *Pulicaria dysenterica* of the subtribe Inulinae from the studied Plucheinae *species* by a bootstrap value of 98 (Figure 3). In the subtribe Plucheinae, *Cylindrocline commersonii, Pterocaulon pycnostachyum, Blumea (Doellia) bovei,* and *Pegolettia senegalensis*, were isolated as separate branches. In addition, the two species, *Pseudoconyza viscosa* and *Geigeria alata*, were clustered together at a bootstrap value of 100, but separated from the other species. The main clade of the Plucheinae species had a bootstrap value of 73. It comprised a minor and a significant clade strongly supported with bootstrap values of 95 and 91, respectively. In addition, the two *Sphaeranthus* species and *Pluchea lanceolate* were recognized as separate branches. The minor clade comprised *Pluchea dioscoridis*, *P. pteropoda*, *P. ovalis*, and *P. indica*. The major clade included ten species, nine of which were clustered in a clade supported with a bootstrap value of 90. However, *Tessaria integrifolia* was associated with this clade. The nine species were resolved as a grid of *Pluchea sericea*, *P. longifolia*, *P. carolinensis, P. baccharis,* and *P. foetida* intermingled with two clades; the first was strongly supported with 98 bootstrap value and included *Pluchea odorata* and *P. camphorata* while the second included *P. sagittalis* and *Laggera alata* with a smaller bootstrap value of 84 (Figure 3).

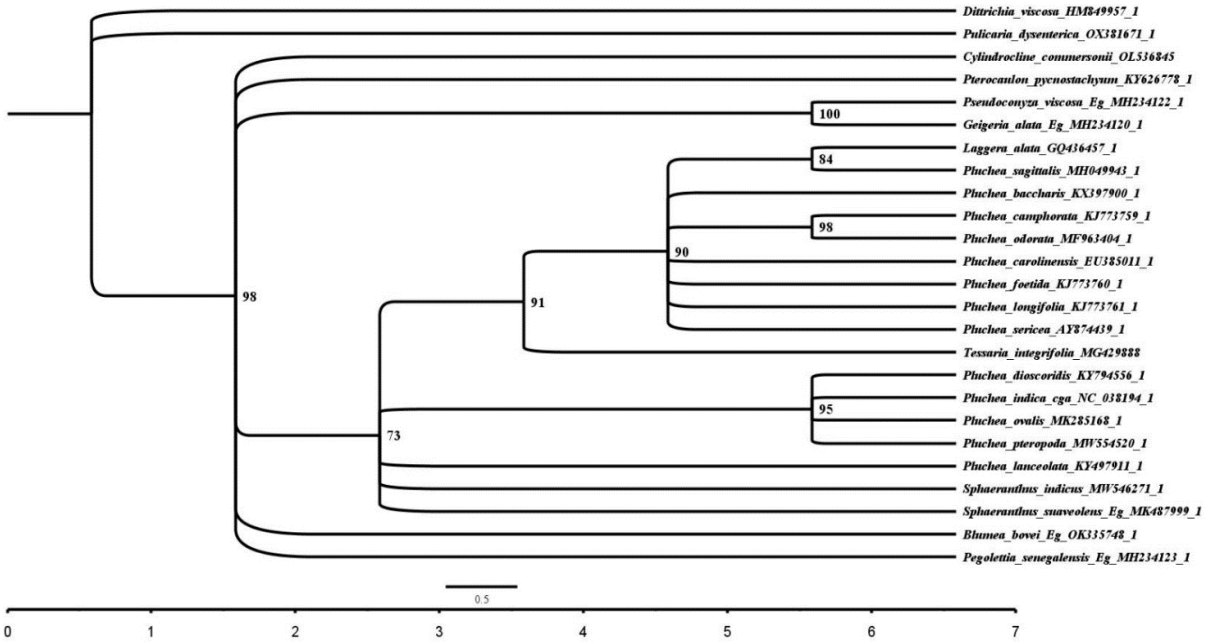

Figure 3. A phylogenetic tree based on the analysis of the *rbcL* sequence polymorphism in the examined species.

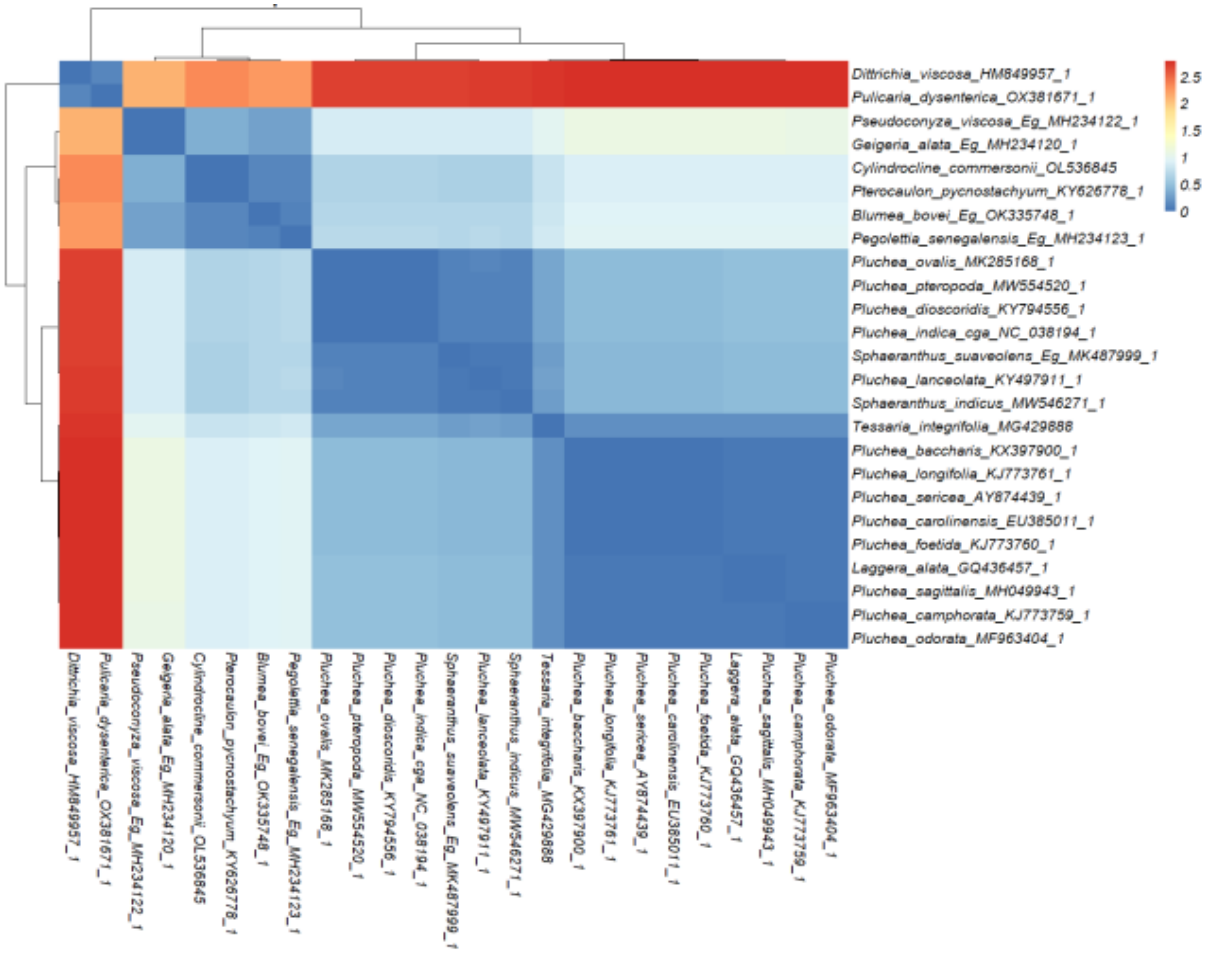

Figure 4. Heatmap showing the relationships among the species of Plucheinae, based on *rbcL*.

The heatmap built using the *rbc*L sequence data (Figure 4) also clearly shows the differentiation of the examined Plucheinae species from the two outgroup species of subtribe Inulinae (*Dittrichia viscosa* and *Pulicaria dysenterica*). It also reflected grouping patterns of the species corresponding to the significant groups produced by the Bayesian phylogenetic tree based on *rbcL* data analysis presented in Figure 3.

Phylogeny of Plucheinae species based on *rbcL* sequence and morphological traits

The analysis of the combined *rbcL* sequence polymorphism and variation among the described morphological traits produced a clear resolution of the examined species. The two outgroup species, *D. viscosa* and *P. dysenterica*, were isolated from the Plucheinae species by a bootstrap value of 99 (Figure 5). Within the Plucheinae clade, *Pegolettia senegalensis*, *Blumea bovei, Pterocaulon pycnostachyum,* and *Cylindrocline commersonii* were isolated as separate branches. The two species, *Pseudoconyza viscosa* and *Geigeria alata*, clustered together and separated with a 100-bootstrap support. The remaining Plucheinae taxa were grouped with 84% bootstrap support and internally divided into three clades in addition to *Pluchea lanceolate*, which was distinguished as a separate branch (Figure 5). The first clade was supported with a bootstrap value of 99% and included the two *Sphaeranthus* species (*S. suaveolens* and *S. indicus*). The other species were divided into two clades, one including four *Pluchea species. (P. dioscoridis, P. indica, P. pteropoda,* and *P. ovalis) and the other clade of ten species in which Tessaria integrifolia was* differentiated as a separate branch. *Laggera alata* and *Pluchea sagittalis* were also distinguished together at a substantial bootstrap value of 99. The remaining seven species that are all members of the genus *Pluchea* were clustered into two clusters: one of the four species, *P. longifolia*, *P. baccharis*, *P. sericea*, and *P. foetida* at a bootstrap value of 87, and the other of three species (*P. camphorata, P. odorata*, and *P. carolinensis*) with weak bootstrap support of 56. However, P. *camphorata* and *P. odorata* were separated as distinguished species with bootstrap solid support of 98 (Figure 5).

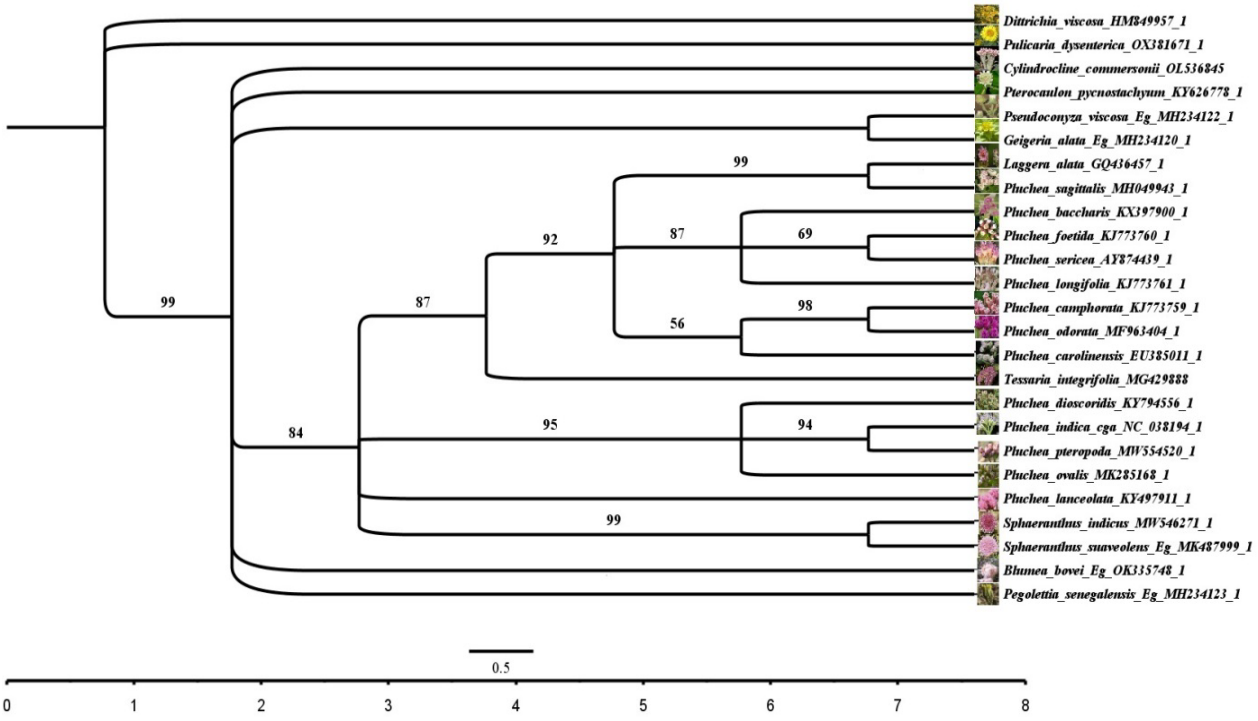

Figure 5. phylogenetic tree based on the analysis of the combined *rbcL* sequence polymorphism and morphological data in the examined species.

Table 1. PCA Eigenvalues and variance for the morphometric traits as variables and their correlation at dimension 1 and dimension 2 for the examined taxa.

Dimension 1		Dimension 2	
Variables	**correlation**	**Variables**	**correlation**
Pappus presence-absence	0.832	Leaf width	0.745
Disc florets surface	0.757	Leaf area	0.699
Inflorescences base	0.684	Leaf length	0.677
Inflorescences number	0.669	Inflorescences type	0.599
Growth form	0.649	Pappus color	-0.425
Type of plants	0.585	Disc florets color	-0.502
Phyllaries rows	0.496	Leaves Arrangement	-0.551
Inflorescences type	0.492	Pappus row	-0.565
Leaf width	-0.420		
Shape of inner phyllarie	-0.448		
Marginal florets color	-0.504		
Stem surface	-0.512		
Shape of outer phyllarie	-0.5812		
Stem wings	-0.6392		
Pappus color	-0.7155		
Eigenvalue	6.34	**Eigenvalue**	4.44
Variance	18.65	**Variance**	13.08
Cumulative variance	18.65	**Cumulative variance**	31.74

DISCUSSION

The morphometric analysis based on 34 qualitative and quantitative traits by R-software resolved the polytomy of Plucheinae and subtribe Inulinaea. Nonetheless, it did not clear the delimitation between the two subtribes. The polytomy tree based on morphological characters may be explained by the absence of ubiquitous unique morphological traits in the Inuleae, whose ample definition allowed the joining of some anomalous genera that cannot be included in other more obviously defined tribes (Merxmuller et al., 1977). Several studies indicated that the diagnostic characters used to delimit genera are not apomorphic. In several cases, genera were recognized by technical characters, including few pappus bristles, winged stems, or combinations of features like an abundance of female florets over the hermaphroditic ones and with filiform corolla (Anderberg, 1991; Merxmuller et al., 1977).

Therefore, many genera are small or monotypic. The poor delimitation between Inulinaea and Plucheinae may be understood by Anderberg (1989), who recognized the Inuleae sensu Merxmueller et al. (1977) to consist of three tribes: the Inuleae sensu stricto, the Gnaphalieae, and the Plucheeae. The author recorded *Pegolettia* and *Blumea* as belonging to the Inuleae clade hosting *Dittrichia viscosa* and *Pulicaria dysenterica*.

Pluchea is heterogeneous and not a monophyletic genus; it lacks sufficient diagnostic apomorphic traits. *Pluchea* species are usually defined by the absence of characters distinguishing other genera (King-Jones, 2001). Similarly, the scarcity of apomorphic traits in *Pluchea* species addressed in the present study resolved most of the belonged species as separate branches based on few diagnostic characters, for example, stem surface in *P. lanceolata* and stem wings as well as marginal florets shape in *P. sagittalis* (Supplementary Table 1). However, the clasping leaf base grouped *P. baccharis* and *P. foetida,* while stem sulcation grouped *P. indica* and *P. pteropoda*. On the other hand, the scabridulous leaf surface grouped *Pluchea ovalis with Geigeria alata and Pegolettia senegalensis. The disciform Inflorescences grouped Pluchea odorata with Sphaeranthus* species (*S. suaveolens* and *S. indicus*) and *Blumea bovei*; the *absence of pappus supported further resolution of Sphaeranthus* species. In this context, Anderberg (1991) recorded that some species of the polyphyletic genus *Blumea* could nest in the *Sphaeranthus clade of Plucheeae*.

Based on the morphological variations, the principal component analysis (PCA) scatter diagram confirmed the Bayesian cluster tree with a clear grouping of taxa. The most significant characteristics that are shared in the separation were pappus row and color, stem wing, shape of outer Phyllaries, stem surface, marginal florets color, shape of inner Phyllaries, and leaf width are positively correlated traits in the first dimension, while leaf width, length and area with

positive correlated at second dimension. The separation of the two *Sphaeranthus* species (*S. suaveolens* and *S. indicus*) in PCA analysis was based on pappus absence, subsessile inflorescence base, globose capitula disciform inflorescence type, and papillose disc florets surface. The *Geigeria* and *Laggera* are closer to each other, whereas the other 12 species, including two *Pluchea* species (*P. pteropoda and P. indica*) and the two outgroups, *Dittrichia viscosa, Pulicaria dysenterica* are differentiated depending on stem surface*, pappus* color and disc florets color. On the other hand, *Pegolettia senegalensis, Cylindrocline commersonii, Pterocaulon pycnostachyum, Laggera alata, and Blumea bovei are grouped* depending on the winged stem, shape of outer phyllaries, leaf width and length. However, ten *Pluchea* species and *Tessaria integrifolia* were located together in the PCA scatter diagram; the traits contributing to their grouping may be the plant type and inflorescence number (all are perennial, uniseriate pappus rows with numerous inflorescences.

The results above agree with the view of Nylinder and Anderberg (2015), who regarded the genera of Inuleae-Plucheinae as monotypic, polyphyletic, and strictly monophyletic. Many species in the Inuleae-Plucheinae have been considered morphologically distinct and complex and have therefore been recognized as monotypic genera like the pantropical genus *Pseudoconyza*, which appears as sister to *Laggera* in the Inuleae-Plucheinae whereas, several of the bigger genera of the Inuleae-Plucheinae are not monophyletic. The Bayesian analysis of *rbcL* sequences resolved the delimitation between the two subtribes Inulinae (*Dittrichia viscosa* and *Pulicaria dysenterica*) from taxa from subtribe Plucheinae. The delimitation between these subtribes was also recorded based on the plastid gene *ndhF* (Anderberg et al., 2005; Anderberg and Bengtson, 2022) or a set of nuclear (ETS, ITS) and chloroplast loci (*ndhF, trnL-F, trnH-psbA*) (Nylinder & Anderberg, 2015). The *rbcL* sequence polymorphism was employed successfully to delimit Astraceae from the remaining Asterales) (Nylinder and Anderberg, 2015) and to delimit subfamilies and tribes in Astraceae (Fu et al., 2016). Thus, our results may indicate using *rbcL* data to recognize intertribal relationships in Astraceae that were absent in available literature.

The Bayesian analysis of *rbcL* sequences showed that the Plucheinae species were represented by four distinct branches for *Cylindrocline commersonii*, *Pterocaulon pycnostachyum*, *Blumea* (*Doellia*) *bovei*, and *Pegolettia senegalensis*, as well as two clades. One clade included *Pseudoconyza viscosa* and *Geigeria alata*, while the other consisted of *Pluchea* species mixed with two *Sphaeranthus* species, *Tessaria integrifolia* and *Laggera alata*. These results agree with the comprehensive molecular study using nuclear (ETS, ITS) and chloroplast loci (*ndhF, trnL-F, trnH-psbA*) conducted by Nylinder and Anderberg (2015) who indicated that the genera *Pterocaulon*, *Pegolettia*, *Cylindrocline*, *Geigeria*, and *Blumea* (*Doellia*) were distinct from *Pluchea* taxa. Consistently with our findings, Nylinder and Anderberg (2015) also considered *Tessaria integrifolia* as part of the *Pluchea* clades. Robinson & Cuatrecases (1973) observed a substantial similarity between the genera but acknowledged the existence of two separate taxa. It has been suggested to classify *Tessaria* as a monotypic genus comprising only *T. intergrifolia*; Anderberg (1991) proposed that *Tessaria* and *Plucheae* are closely linked and could be seen as a sister group.

The large polyphyletic assemblage, including *Pluchea*, *Laggera*, and *Sphaeranthus* species, was established and can be explained in the light of Zdero et al. (1988), who presented the link between *Pluchea* and *Sphaeranthus based on* phytochemical components, which may explain the presence of *Sphaeranthus* in a clade with other *Plucheae* species. *Pluchea sagittalis* and *Laggera alata gathered on one cluster; both have significant winged stem character.* In contrast to King-Jones (2001), who believed that Old World and Australian species of *Pluchea* formed a homogeneous monophyletic group, the result of our study supports the paraphyletic nature of *Pluchea* and agrees with Anderberg (1991), who found that 14 *Pluchea*, species from the Old and New Worlds *were* heterogenous and paraphyletic. Our work included 13 species of *Pluchea*, eight from the New World (*P. sagittalis, P. foetida, P. baccharis, P. sericea, P. longifolia, P. camphorata, P. odorata, and P. carolinensis) and* five of the Old-World (*P. dioscoridis, P. indica*, *P. pteropoda, P. ovalis,* and *P. lanceolata).* Compared with the *rbcL*-based phylogenetic tree, trees based on the *rbcL* sequence and morphological traits variation enhanced resolution at the genus level. *The* absence of pappus supported the grouping of the *Sphaeranthus* species in a minor clade nested in the *Pluchea* clade.

CONCLUSION

In conclusion, our results confirm the treatment of subtribe Plucheineae as proposed by Anderberg

(1991) and generally agree with the proposals by Nylinder and Anderberg (2015) in demonstrating the separation of *Dittrichia viscosa* and *Pulicaria dysenterica* from the Plucheinae species. The results confirm that the *rbcL* sequences provided essential evidence for the authentication and discrimination of the six Plucheinae species in Egypt and indicated their phylogeny in the subtribe Plucheinae. The *rbcL* data also showed close relationships between *Tessaria integrifolia* and *Laggera alata* and the two species of *Sphaeranthus* (*S. suaveolens* and *S. indicus*) to *Pluchea species*. The results also support the destination of the six species: *Cylindrocline commersonii, Pterocaulon pycnostachyum, Pegolettia senegalensis, Pseudoconyza viscosa, Geigeria alata* and *Blumea bovei* from the species of *Pluchea*. The results reflected the advantage of *rbcL* sequence over morphological criteria to establish phylogenetic relationships at tribe and subtribe levels, corresponding to Inuleae and Plucheinae respectively.

AUTHORS' CONTRIBUTIONS

ASF, AB and AF. assessed the conceptualization of studied research point. ASF, AB, AF and FYE., conceived and designed the experimental methodology. ASF performed the experimental analysis. ASF, AB, AF and FYE, were responsible for data curation. ASF, AB, AF and FYE, prepared and wrote the original manuscript draft. ASF, AB, AF and FYE wrote, reviewed, and edited the approved manuscript. AB supervised. all procedures. All authors have read and approved the final manuscript.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interest.

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