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ASSESSMENT OF MORPHOLOGICAL CHARACTERISTICS AND MOLECULAR EVOLUTIONARY RELATIONSHIPS IN PARASITIC SPECIES OF THE LORANTHACEAE FAMILY

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Abstract

The Loranthaceae family is considered parasitic, and mistletoe negatively affects the growth and productivity of host trees. There are four genera and six species of Loranthaceae that grow naturally in Saudi Arabia. It grows on fruit trees and causes damage, and these species have not received sufficient morphological or molecular study. This work aims to evaluate the importance of morphological traits and the phylogenetic relationships between Loranthaceae species. Eleven morphological characteristics of leaves, flowers and fruit were investigated in the study samples. The discrimination power of these characteristics was evaluated using principal component analysis (PCA), hierarchical clustering analysis (HC)A, and analysis of variance (ANOVA). On the other hand, molecular experiments included extracting the DNA of the samples and amplification using different chloroplast regions cpDNA (rbcL, matK, and trnL–trnF), as well as nuclear regions nrDNA (ITS1 and ITS2). The results of DNA sequences of this study and available sequences of Loranthaceae species in GenBank were used to reconstruct the phylogenetic trees and investigate the genetic relationships. The morphological characteristics that were used in this study, especially "Lamina width, lamina area, and lamina pedicel length", were able to discriminate between Loranthaceae species and classify species within clusters based on their affiliation to subtribes Emelianthinae and Tapinanthinae. According to the current results, the maximum parsimony (MP) phylogenetic tree based on matK and rbcL genes has well-supported branch nodes compared to other genes, especially for tribe Lorantheae. Available morphological and molecular data in these studies are useful in determining the parasitic plants and will facilitate resistance efforts.

Key words: Morphology, Loranthaceae, Parasitic, Mistletoe, Molecular, Taxonomy, Phylogeny.

Introduction

Loranthaceae belong to the order Santalales, with more than 1000 species and 73 genera (Vidal-Russell & Nickrent, 2008; (Grímsson *et al*., 2018). The Loranthaceae family includes most of the mistletoe (parasitic) genera (Wilson & Calvin, 2006a). It is widespread in tropical and warm temperate climates (Barlow, 1997). Parasitic plants are classified as hemiparasites (photosynthetic), which are able to perform photosynthesis, or holoparasites (nonphotosynthetic), which cannot perform photosynthesis and are completely dependent on the host (Twyford, 2018). Mistletoe leads to reduced growth and productivity and eventually death of the host trees (Menezes *et al*., 2022).

The Loranthaceae family is divided into five tribes and eleven subtribes (Suárez *et al*., 2021). Loranthaceae has a complicated taxonomic issue related to overlapping delimitation features between species or differences among individuals of the same species, the majority of them serving as synonyms for mistletoes from other genera, in addition to the lack of adequate knowledge of the taxonomy of the family (Ibrahim & Ayodele, 2013; Nickrent & Vartak, 2021).

Four genera and six species of Loranthaceae grow naturally in the western, southwestern, and northern regions of Saudi Arabia: *Tapinanthus globiferus, Oncocalyx glabratus, Oncocalyx schimperi, Phragmanthera austroarabica, Plicosepalus curviflorus* and *Plicosepalus acacia* (Alqthanin, 2011). These species have not received sufficient morphological or molecular study.

Some studies have discussed the morphological characteristics of the species, but the importance of these characteristics from a taxonomic standpoint is not clear. In addition, there is a contradiction in their taxonomic ability. For example, Sivaramakrishna *et al*., (2021) screened morphological traits and mentioned that the morphology of vegetative and floral traits was helpful in defining *Dendrophthoe laljii* (Loranthaceae). Moreover, morphological features contributed to distinguishing the borderline species of mistletoe *Decaisnina tomentosa* (Loranthaceae) that was recorded on Samar Island, Philippines (Tandang *et al*., 2022). In contrast, Kazandjian (2011) studied the morphological characteristics of leaves and inflorescences and reported that these morphological features failed to discriminate between *S. dichotrianthus* and *S. phillyreoides* of Loranthaceae in Venezuela. Furthermore, (González & Pabón-Mora, 2018) note that it is completely impossible to distinguish mistletoes at the generic level due to similarities in seedling morphology among Loranthaceae.

In general, molecular phylogenetic techniques have been somewhat helpful in understanding the evolutionary history of the Loranthaceae family, and both chloroplast and nuclear areas have been used to assess the molecular variation in parasitic plant species. Phylogenetic and molecular data were used to reconstruct the Loranthaceae family's historical biogeography. Cabrera (2002) used the chloroplast gene matK to create a molecular phylogenetic tree of the 43 genera of the Loranthaceae family. He found that the clade that belonged to the subtribe Loranthinae contained numerous genera and was moderately supported. Wilson & Calvin (2006a) studied evolution within Loranthaceae using the nuclear gene ITS (internal transcribed segments) and chloroplast trnL–trnF region. In addation, (Nickrent *et al*., 2010; Vidal-Russell & Nickrent, 2008) studied Loranthaceae family based on molecular and morphological data, in addition to the analysed rdna gene

and rbcl, matk, and trnl-F genes, in addition chromosome numbers. The Loranthinae tribe distinguished by a chromosome number x = 9, While Psittacanthinae tribe has chromosome number x = 8. The tribes Elytrantheae and Nuytsieae characterized by a chromosome number x = 12 (Vidal-Russell & Nickrent, 2008).

The phylogenetic relationship of Loranthaceae species was investigated using 14 complete chloroplast genomes and included the clustered parasitic species *S. chingii* (Liu *et al*., 2021). The species that are native in Saudi Arabia were not included in these trees (Wilson & Calvin, 2006b; Liu *et al*., 2021). While thestudy by Al-Juhani *et al*., (2022) included comparative analyses and phylogenetic relationships among the chloroplast genomes of *Plicosepalus acaciae and Plicosepalus curviflorus* species only.

This work aims to evaluate the taxonomic importance of morphological traits of Loranthaceae species in Saudi Arabia. Assessing the phylogenetic relationships between Loranthaceae species native to Saudi Arabia and distributed around the world.

Material and Methods

Plant material collection and identification: Fresh leaf, flower, and fruit samples of Loranthaceae species native to Saudi Arabia were collected during field trips in 2021 and 2022 from six areas of Saudi Arabia. Samples were identified by experts at Sultan bin Abdulaziz Center for Research and Environmental Studies at King Khaled University, Abha. Voucher specimens for each species were prepared and deposited at the Herbaria of Biology Department, Faculty of Applied Science, Umm Al-Qura University, Mecca/KSA. This study also included herbarium samples of the Loranthaceae family collected from King Khaled University. For each study specimen, important information was noted, such as the sample number, name of the collector, location, latitude, longitude, and date of collection (Table 1). As well, some fresh leaves cutting and drying up in silica gel for molecular experiments.

Morphological methods

Screening morphological characteristics: For each sample, the examination focused on the quantitative characteristics, such as flowers, fruits, and leaves. Whether fresh or dry. All measurements were used in centimeters and millimeters based on the parts being measured. The study's morphological revision included eleven quantitative characteristics for each sample (Table 3), which were scaled by using manual methods (ruler) or ImageJ software (Schneider *et al*., 2012). These characteristics were examined in 5 replicates per species.

Statistical analysis

The importance of eleven quantitative morphological characteristics of leaves, flowers, and fruit has been evaluated (Table 3). The principal component analysis (PCA) (Sneath & Sokal, 1973) was preformed using morphological data in XLSTAT version 2023.1.1 Lumivero (2023).

One-way analysis of variance (ANOVA) and a box plot (Ashapkin *et al*., 2023) were applied in GraphPad Prism v. 9.5.1(95 permutations) to evaluate morphological characteristics. The statistics were calculated based on 95 permutations, and R squared the proportion of the variation was calculated (Sneath & Sokal, 1973; Glantz *et al*., 2016). The R squared value ranges from (0.1) to (1); for example, 0.9 shows a high correlation, whereas a value of 0.5 or less shows a low correlation.

The relationships between Loranthaceae species in KSA was Screened using cluster dendrogram, which was conducted in the 'factoextra' package Version 1.0.7 Kassambara & Mundt (2017) via RStudio software Version 2.0 (RStudio Development Core Team, 2020).

Molecular methods

DNA extraction: The DNA extract using CTAB method (Doyle & Doyle, 1987) of silica-dried leaves and herbarium leaves.

Primer selection: The chloroplast region cpDNA (rbcL, matK, and trnL–trnF) regions and nuclear region nrDNA (ITS1 and ITS2) were used in this study, as they have been used widely in phylogenetic reconstructions at the generic, tribal or sub-familial level (Paton *et al*., 2004; Li *et al*., 2016).

Table 1. The study samples List of Loranthaceae in Saudi Arabia, name, code, and locations.

No.	Scientific name	Locality	Collector	Longitude & Altitude	Voucher
1.	*Tapinanthus globiferus* (A. Rich.)	Wadi Alreem	Alqthanin, R	17.9805724°, 42.2380263°	T10011 (KKU)
2.	*Oncocalyx glabratus* (Engl.)	Thageef village		20.6197578°, 40.9233393°	G1001 (UQU)
3.	*Oncocalyx schimperi* (Hochst. ex A. Rich.)	Abha – Khamis mushat		18.185097°,42.818443°. 18,299187°,42.497780°.	S1002 (UQU)
4.	*Phragmanthera austroarabica* A.G. Mill. & J.A. Nyberg	Al-Taef, Gabel Ibrahium Wadi leiah	Althagafi, N.	20.402047°,41.138292°. 18.1913686°,42.8226008°. 18.0602509°,42.7076538°. 18.301805°,42.496372°	P1005 (UQU)
5.	*Plicosepalus curviflorus* (Benth. ex Oliv.) Tiegh.	Wadi leiah		21.218874°,40.557426°. 20.637140°,41.275528°. 18.1913686°,42.8226008°.	N C1008 (UQU)
6.	*Plicosepalus acaciae* (Zucc.)	Tabouk, Alola Alwajh		26.5737260°,36.3731150° 26.7392410°,37.1740530°	A1003 (UQU)

PCRs and sequencing: PCR reaction was performed following the methods mentioned by (Chen, 2010), total reaction volume is 25-µL contains 12.5 µL of PCR master mix, 8.5 µL of Nuclease-Free Molecular Grade Water, 1 µL of forward and reserved primers and 2 µL of the DNA strand. The 1.5% agarose gel and electrophoresis were used to check quality of PCR products. Sequencing was performed in Macrogen company (https://dna.macrogen.com/, Seoul, South Korea).

Bioinformatic analysis: Forwards and reverse sequences were assembled into contig sequences in Geneious Prime® 2023.1.1 (https://www.geneious.com). Sequence alignment accomplished in the Muscle algorithm window within MEGA11 programe (Tamura *et al*., 2021). Examples of tribes and subtribes sequences of Loranthaceae downloaded from the GenBank and used alongside with the sequences of Loranthaceae in presented in this study to created phylogenetic trees. Species from the families Santalaceae, Schoepfiaceae, Viscaceae, and Erythropalaceae were used as outgroups. The maximum parsimony (MP) method was used to build phylogenetic trees and 1000 bootstrap values, which was performed in the molecular evolutionary genetics analysis software Mega v. 11 (Tamura *et al*., 2021) version 11.0. The tree of each gene (ITS1 and ITS2, rbcL, trlF, mATK) was evaluated based on the bootstrap support value for each node and the ability of samples to form monophyletic groups.

Results

Morphological results: There was obvious variation in the PCA results of morphological characteristics of leaves, flowers, and fruits the Loranthaceae species. Which was reflected in the distribution of samples in different groups based on the results of the Principal Component (PCA), cluster Hierarchical clustering analysis (HC)A, and Analysis of Variance (ANOVA).

The Table 2 show Axis 1 & 2 PCA with high eigenvalues in PCA (54.623, and 23.172). Features that had the highest positive and negative loading in Axes 1 & 2 PCA are shown in Table (3), as well deduced from loading plot (Fig. 2) in appendix. The fruit Area (FA) and flower Pedicel length (FPL) characteristics had the highest positive and negative loading in Axes 1 (0.983 & -0.469). While the fruit Pedicel length (FEL) and style length (SL) had the highest positive and negative loading in Axes 2 (0.845 & -0.711).

In accordance with the PCA Axes 1 and 2 results, leaf and fruits characteristics that are important in discrimination and capable of separating the samples into groups according to genera and species are "Lamina Width, Lamina Area, Lamina Pedicel length, and Fruit Area, which is also evidenced by Figs. (1 & 2).

The relationships between Loranthaceae species in this study are presented in a cluster dendrogram based on the morphological characteristics of leaves, flowers, and fruits (Fig. 3). The Lorantheae species were divided into two main clusters.

The first cluster contained *Phragmanthera austroarabica* species and represented subtribe Emelianthinae. The second main cluster divided into two subclusters, first one included *Tapinanthus globiferus*, while the second subclusters included species *Plicosepalus curviflorus*, *Plicosepalus acacia*, *Oncocalyx schimperi*, and *Oncocalyx glabratus*. All species in the second main cluster are belongs to subtribe Tapinanthinae.

The results of ANOVA and boxplot presented in Table (4), shown that characteristics reflect highest contrast between species are Lamina Width, Lamina Area, and Lamina Pedicel length, (P value <0.0001 & R squared= 0.948, 0.903, and 0.905. *Phragmanthera austroarabica* (Pau) had the largest values recorded in the three previously mentioned traits (Fig. 4), with mean=4.867, 59.174, and 2.117, maximum= 5.500, 72.526, & 2.400, minimum value= 4, 39.164&1.500, standard deviation SD= 0.489, 10.07, and 0.308.

Lamina Length character (Table 4) also showed moderate level of variance, with (P value <0.0001 & R squared= 0.720). However, characteristics such as fruit width (FW), fruit length (FL), fruit Area (FA), fruit Pedicel length (FEL), petals length (PL), style length (SL), flower Pedicel length (FPL) were less importance according to ANOVA analysis (Table 4). These results on a large scale are consistent with the results obtained from the analysis of principle component PCA.

Table 2. Eigenvalues of principal component analysis based on morphological characteristics.

	F1	F2	F3	F4	F5
Eigenvalue	6.009	2.549	1.398	0.903	0.141
Variability (%)	**54.623**	**23.172**	12.713	8.213	1.278
Cumulative %	54.623	77.795	90.508	98.722	100.000

Bold text indicates to axes with high Eigenvalues values

Table 3. Characteristic loadings in PCA based on morphological characteristics.

Character	Code	F1	F2	F3	F4	F5
Lamina length	LL	0.831	-0.009	0.208	-0.514	-0.044
Lamina width	LW	0.943	-0.206	0.228	-0.007	-0.128
Lamina area	LA	0.940	-0.179	0.232	-0.161	0.066
Lamina Pedicel length	LPL	0.950	-0.162	0.211	-0.061	0.149
Fruit length	FL	0.691	0.436	-0.561	-0.036	0.127
Fruit width	FW	0.765	0.292	-0.143	0.542	-0.121
Fruit Area	FA	**0.983**	-0.122	-0.099	0.092	0.027
Fruit pedical length	FPL	0.407	**0.845**	-0.338	0.025	0.069
Petals length	PL	-0.294	0.800	-0.084	-0.503	-0.112
Style length	SL	-0.374	**-0.711**	-0.556	-0.183	0.109
Flower pedical length	FEL	**-0.469**	0.545	0.655	0.137	0.187

Bold text indicates to high positive and negative values in PCA axes 1& 2

Molecular and phylogenetic results

Amplification and sequencing results: The current results shown that 6 species of family Loranthaceae native in Saudi Arabia (represented by 10 samples) yielded 10 (100%) PCR products, and sequences100% for each ITS1and trnlF genes (Table 5). The rbcL genes successfully amplified PCR products by (100%), and 80% for sequencing, while, ITS2 gene successfully amplified PCR products (100%), and 70% in sequencing. However, matK gene amplified only 40% PCR products and 30% sequencing (Table 5). The gained sequences length was in the normal range of the expected length foe each gene. The average and range of sequences length in ITS1, TS2 genes were 270 (200–280), and 275 (250–285), respectively. While the mean and range of sequences in matK, rbcLand trnlF genes were 921 (850–1010), 570 (560–658), and 385 (300–400), respectively (Table 5).

Table 4. ANOVA results P value and R squared for macromorphological characteristics.

Characteristics	P value	R squared
Lamina length	<0.0001	0.720
Lamina width	<0.0001	0.948
Lamina area	<0.0001	0.903
Lamina pedicel length	<0.0001	0.905
Fruit length	0.0002	0.565
Fruit width	0.0003	0.563
Fruit Area	0.0041	0.446
Fruit pedical length	0.0003	0.557
Petals length	0.0025	0.443
Style length	0.2869	0.179
Flower pedical length	0.1887	0.211

Phylogenetic trees: The phylogenetic placement of the loranthaceae species of Saudi Arabia within the whole tribe and sub-tribes of loranthaceae was conducted using novel DNA sequences in the curent study and available sequences in GenBank. phylogenetic trees has been created of sequences ITS1, ITS2, matK; trnL-F, and rbcL.

The matrices of the DNA sequences of ITS1, ITS2, matK, trnL-F, and rbcL consisted of 93, 70, 47, and 60 Loranthaceae taxa including the important five tribes respectively; Elytrantheae, Psittacantheae, Gaiadendreae, Nuytsieae, Lorantheae, and seven sub-trib; Scurrulinae, Amyeminae, Dendrophthoinae, Tapinanthinae, Emelianthinae, Loranthinae, and Ileostylinae. The outgroups were sequnces represented of familes belongs to order order Santalales respectively; *Schoepfia schreberi* and *Schoepfia jasminodora* (Schoepfiaceae), *Erythropalum scandens* and *Maburea trinervis* (Erythropalaceae), *Osyris alba* and *Osyris quadripartite* (Santalaceae), *Viscum coloratum, Viscum album,* and *Viscum minimum* (Viscaceae).

The outcomes showed that family loranthaceae was well separated of the outgroups in Maximum parsimony MP matK tree, bootstrap supported value (BS= 100%), and MP rbcL tree (BS= 99%), similarly tribe Lorantheae represented a well-supported monophyletic clade, in matK tree (BS = 99%) composed of 38 taxa (Fig. 5). As well tree rbcL (BS= 82%) composed of 50 taxa (Fig. 6), tribe Lorantheae had well-supported clade in trlF tree too, 35 taxa (BS = 77%) (Fig. 7).

Subtribe Scurrulinae represented a highly monophyletic clade in matK and rbcL trees (BS = 96 %& 83%) composed of 28 taxa (Figs. 5 & 6). In an analogous way, subtribe Amyeminae had well supported monophyletic clade in trlF and ITS trees (BS = 95 %& 69%) composed of 9 taxa (Figs. 7 & 8).

On the contrary, the phylogenetic MP trees of the matK, trlF, rbcL, and ITS genes (Figs. 6 to 8), showed that subtribes Tapinanthinae and Emelianthinae are not monophyletic. Emelianthinae taxa represented a sister to Tapinanthinae clade (42 taxa). Similarly, subtribe Dendrophthoinae was polyphyletic in trlF and ITS trees composed of 15 taxa, (Figs. 7 & 8). We noticed, samples of genus Oncocalyx, are not form monophyletic group as well (Fig. 6).

Species native to Saudi Arabia of subtribe Emelianthinae (*P. austroarabica*) were conspicuously grouped with sequences of genus Phragmanthera (*P. usuiensis* & *P. austroarabica*) downloaded of NCBI. While species (*O. schimperi,* and *O. glabratus*) of subtribe Tapinanthinae of SA, were located in branch contains samples of genus Oncocalyx (*O. fischeri, O. schimperi, O. glabratus,* and *O. sulfureus*). As well *P. curvifloru,* and *P. acacia* samples collected of Saudi Arabia clearly grouped in same branch that includes sequences of *P. curviflorus,* and *P. sagittifolius* of NCBI. In the same way sequences of Tapinanthus globiferus native to Saudi Arabia were s grouped in same branch contain *T. globiferus* & *T. constrictiflorus* sequences.

Table 5. Evaluate amplification and sequencing of the nrDNA and cpDNA regions in the current study.

Gene	Nuclear regions		Chloroplast regions		
	ITS1	ITS2	matK	rbcL	trnlF
Samples number	10	10	10	10	10
PCR products number	10	10	4	10	10
Efficiency of PCR amplification (%)	100	100	40	100	100
Number of sequences	10	7	3	8	10
Success rate of sequencing (%)	100	70	30	80	100
Expected sequence length (bp)	278 - 294	364 - 398	631-1580	501-1000	372- 376
Study sequences length (Mean and range/bp)	270 (200–280)	275 (250–285)	921 (850-1010)	570 (560–658)	345 (300-450)

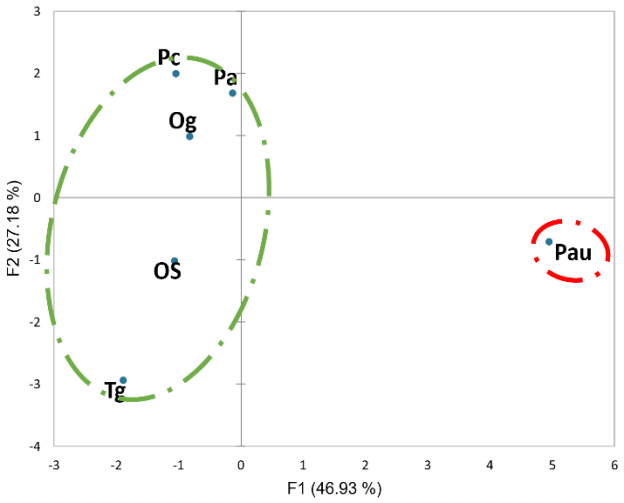

Fig. 1. Cluster loading for Loranthaceae species of the KSA, by the two axes principal components 1/2, based on observation of macromorphological characteristics of leaves, fruit and flower. The dotted circle indicates the subtribe. Tapinanthinae (green circle) and subtribe. Emelianthinae (red circle). *Tapinanthus globiferus* (Tg).*Oncocalyx glabratus* (Og). *Oncocalyx schimperi* (Os). *Phragmanthera austroarabica* (Pau). *Plicosepalus curviflorus* (Pc). *Plicosepalus acaciae* (Pa).

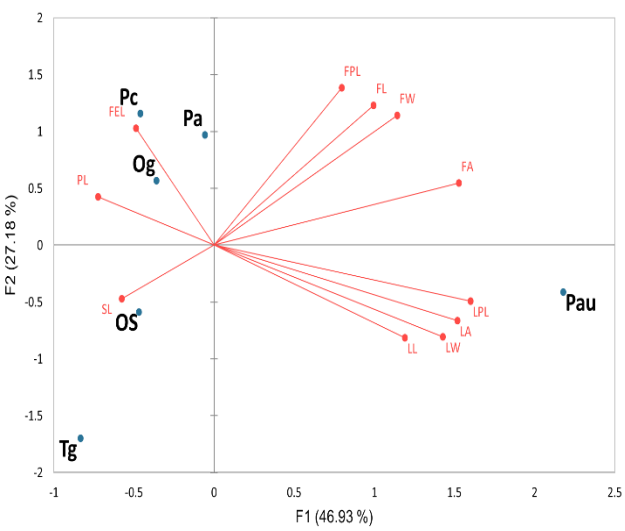

Fig. 2. Loading plots and cluster loading for Loranthaceae species of the KSA by the two axes principal components 1/2, based on observation of the macromorphological characteristics of leaves, fruit and flower. The characteristic code is available in Table 3.

Discussion

This study aims to evaluate importance of morphological traits of Loranthaceae species and the phylogenetic relationships between Loranthaceae species native to Saudi Arabia and those distributed around the world.

In accordance with the PCA and ANOVA analysis, the leaf and fruit characteristics that were used in the current study were capable of dividing samples into groups and discriminating between Loranthaceae species: lamina width, lamina area, and lamina Pedicel length. A cluster dendrogram based on used leaf and fruit characteristics showed distribution study samples within branches based on their affiliation to subtribes Tapinanthinae and subtribe Emelianthinae. This reflects the taxonomic status of the species under study, agrees with the literature (Vidal-Russell & Nickrent, 2008; Nickrent *et al*., 2010; Liu *et al*., 2018), and confirms the importance of these morphological traits as classification tools for differentiating the native species Loranthaceae. These results corroborate the findings of the previous work of (Sivaramakrishna *et al*., 2021; Tandang *et al*., 2022), which referred to the morphology of vegetative and floral traits in the family Loranthaceae and was helpful in defining the new species.

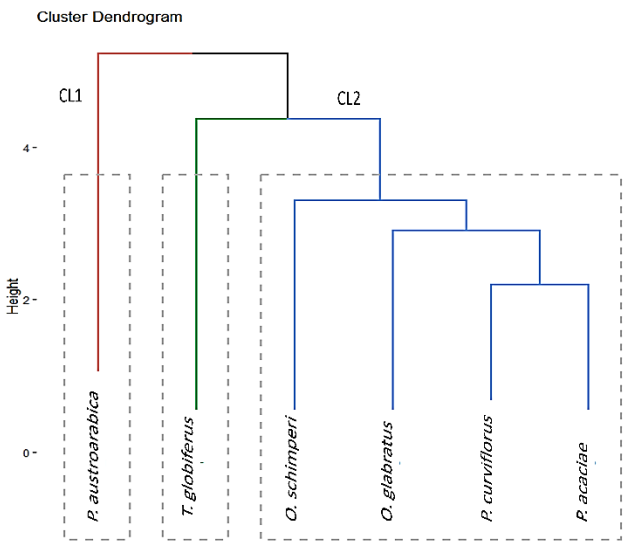

Fig. 3. Cluster dendrogram for Loranthaceae species local to the KSA based on observations of the morphological characteristics of leaves, fruit, and flower.

In the current study, we built a tree with ITS sequences and marK, trnL-F, in addition to rbcL sequences. The MB phylogenetic tree constructed based on sequences of matK and rbcL mostly has well-supported branches compared to other genes. (Der & Nickrent, 2008) reported that both rbcL and small subunit ribosomal DNA (SSU Rdna) sequences were beneficial in solve taxonomic issues between Santalaceae, and Viscaceae genera. On the other hand, (Hudson, 1990; Amico *et al*., 2007; Ortiz-Rodriguez *et al*., 2018) reported that coalescence occurs four times faster in maternally inherited chloroplast genes than in biparentally inherited nuclear genes. The topology of the current trees corresponds with those obtained in previous studies (Liu *et al*., 2018); some clades were monophyly and tribes such as Lorantheae, Amyeminae and Scurrulinae received strong support, while the other branches did not.

In the present study, we found some nodes with low support in phylogentic trees, which has been noted by many researchers, such as (Vidal-Russell & Nickrent, 2008; Liu *et al*., 2018). Despite continuous attempts to improve the trees matrix, for examples (Nickrent *et al*., 2010) used the molecular phylogeny tree, chromosome number and morphological data, and (Liu *et al*., 2018) built the phylogentic tree in Loranthaceae using an improved molecular data matrix (85% filled). This may be because of the physiological nature of the family, which loses many genes during its evolutionary history and its transformation into a parasitism (Liu *et al*., 2018).

On the other hand, we noticed a significant variation between examined morphological traits, which was useful in distinguishing between species at the level of tribe and subtribe, while this was not the case in some cases at the level of nodes in the genetic tree. Within Loranthaceae, Morphological characteristics may evolve faster and clearly more than evolution at the genome level in parasitic species (Vidal-Russell & Nickrent, 2008).

According to the present results subtribe Tapinanthinae and Emelianthinae are polyphyletic. The Tapinanthinae and Emelianthinae are native to Africa, they have the similar chromosome number (x = 9), and racemes inflorescences (Kuijt, 1981; Vidal-Russell & Nickrent, 2008; Nickrent *et al*., 2010), further studies should focus on these two subtribes to resolve the complex phylogenetic relationships between them. In our study, we found that the matK and rbcL regions, produced trees with well supported nodes.

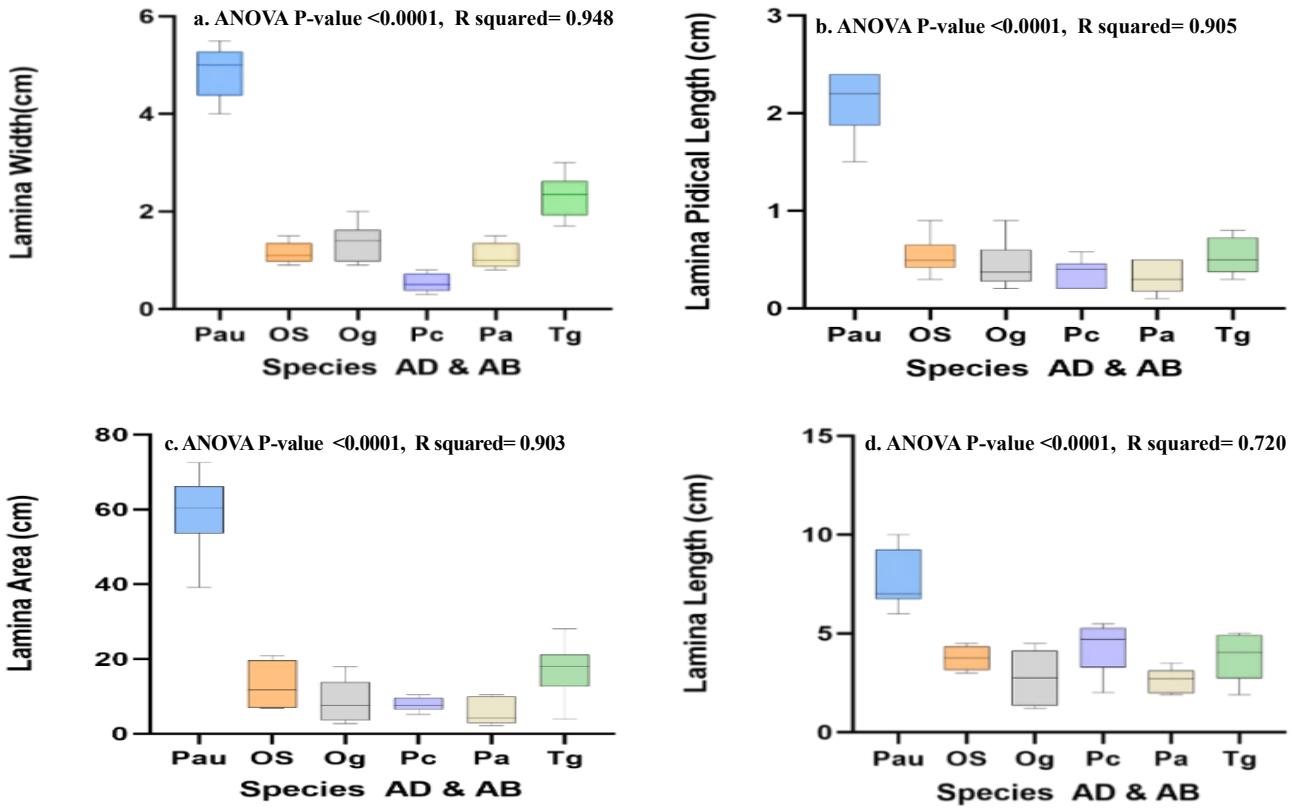

Fig. 4. Boxplots and ANOVA values for a.lamina width, b.lamina pedicel length, c.lamina area, and d.lamina length characteristics evaluated within Loranthaceae native to the KSA. *Tapinanthus globiferus* (Tg). *Oncocalyx glabratus* (Og). *Oncocalyx schimperi* (Os). *Phragmanthera austroarabica* (Pau). *Plicosepalus curviflorus* (Pc). *Plicosepalus acaciae* (Pa).

Conclusion

The current study aims to contribute to the identification, characterization, and understanding of the relationships among the Loranthaceae species native to Saudi Arabia. The leaf and fruit characteristics that were used in this study were able to discriminate between Loranthaceae species and classify species within clusters based on their affiliation with subtribes. We found that the MB phylogenetic tree based on matK and rbcL genes had well-supported branches compared to other genes. Although there have been continuous attempts to improve the molecular data of the phylogenetic tree in the Loranthaceae family, it still has unsupported nodes, which could be related to the physiological nature and gene loss in parasitic genera. Current findings confirmed that subtribe Tapinanthinae and Emelianthinae are polyphyletic. In contrast, we observed evolution and great variation in micromorphological characteristics compared to changes in molecular data. We recommend using morphological characteristics as taxonomical tools to identify Loranthaceae species.

We recommend that future work focus on two subtribes, Tapinanthinae and Emelianthinae, to resolve the complex phylogenetic relationships between them. Trying to analyse the largest possible number of species under these two subtribes, as well as a taxonomic review of the species belonging to these subtribes, consider the possibility of moving species between the two tribes to resolve the complicated taxonomic issues between them.

There is a need to detect effective DNA barcodes regions to resolve the taxonomic issues, that which could be applied as a low-cost tool for identifying complex relationships at lower levels, within and between subtribes. Therefore, we recommend focusing on these regions in future works.

Providing sequence data for these species and saving them in the GenBank NCBI database will be of great benefit in identifying and distinguishing between species belonging to this family. The molecular data that presented in the current results could be encourage biotechnology researchers to improve the host's resistance to parasitic plants.

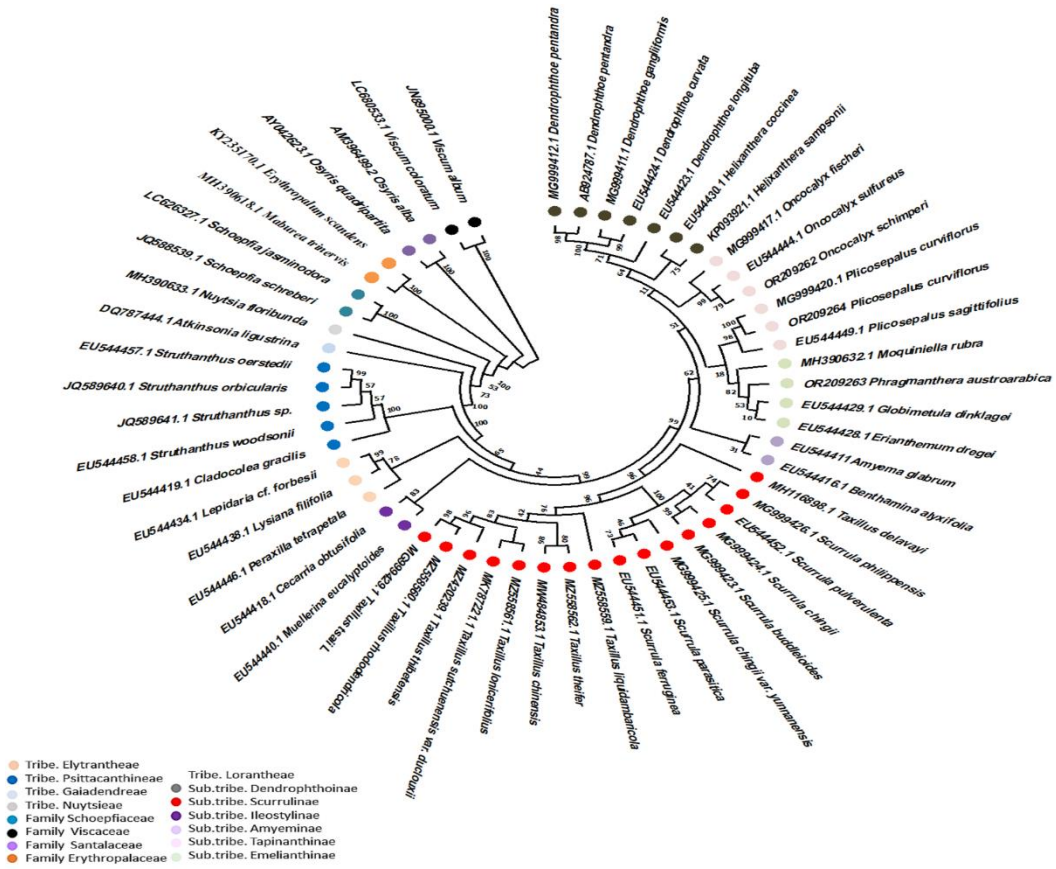

Fig. 5. Phylogenetic tree created of the matK sequences of local and worldwide species of the Loranthaceae family using maximum parsimony (MP). Bootstrap value shown in the branch nodes.

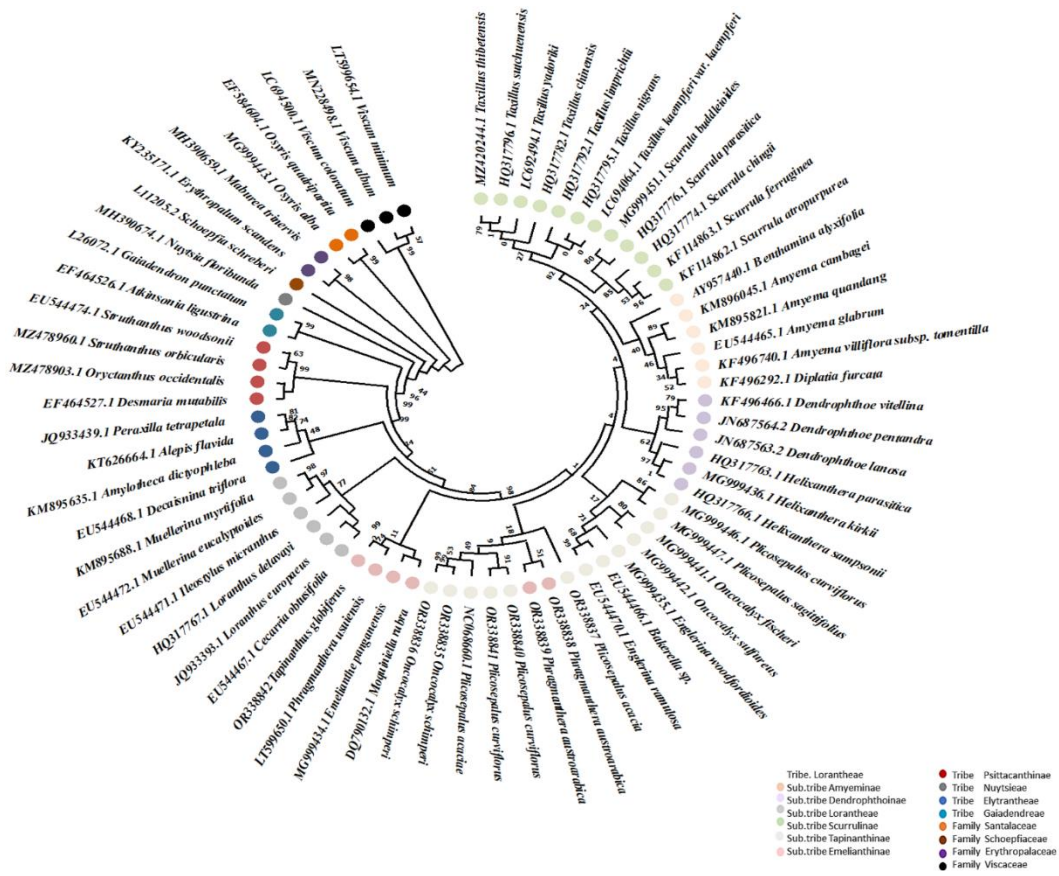

Fig. 6. Phylogenetic tree created of the rbcL sequences of local and worldwide species of the Loranthaceae family using maximum parsimony (MP). Bootstrap value shown in the branch nodes.

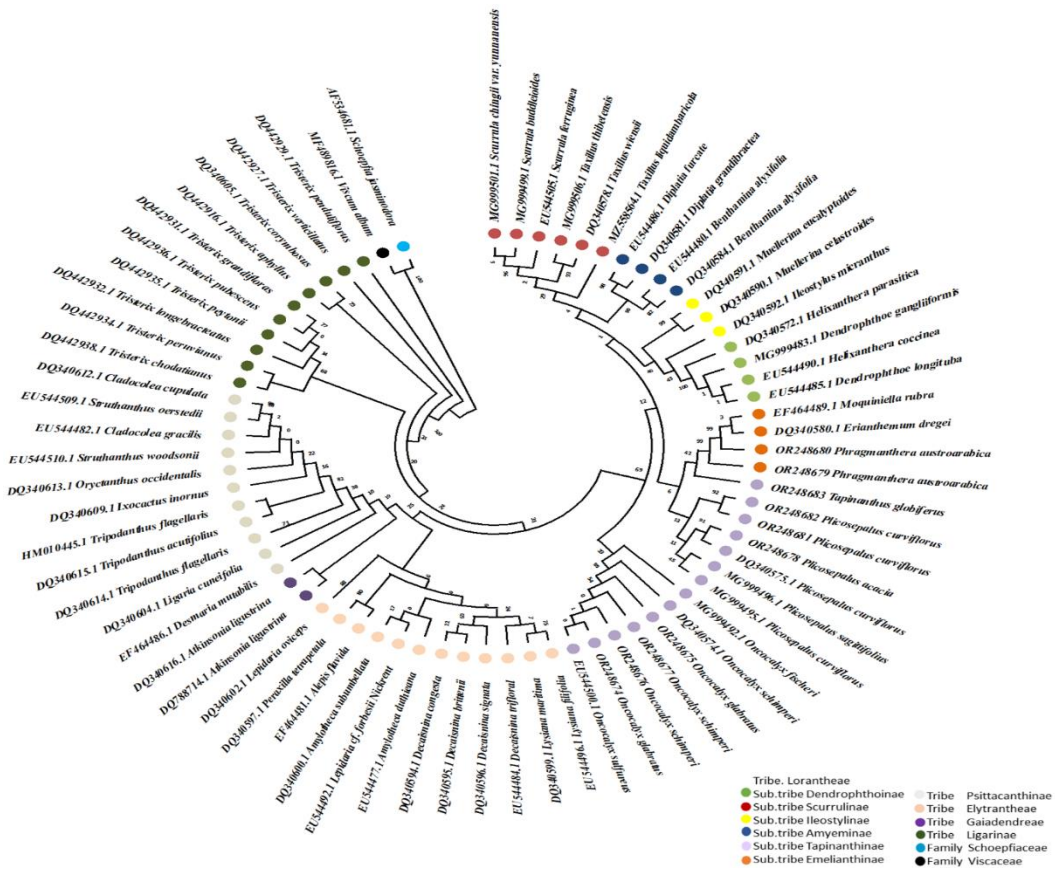

Fig. 7. Phylogenetic tree created of the trlF sequences of local and worldwide species of the Loranthaceae family using maximum parsimony (MP). Bootstrap value shown in the branch nodes.

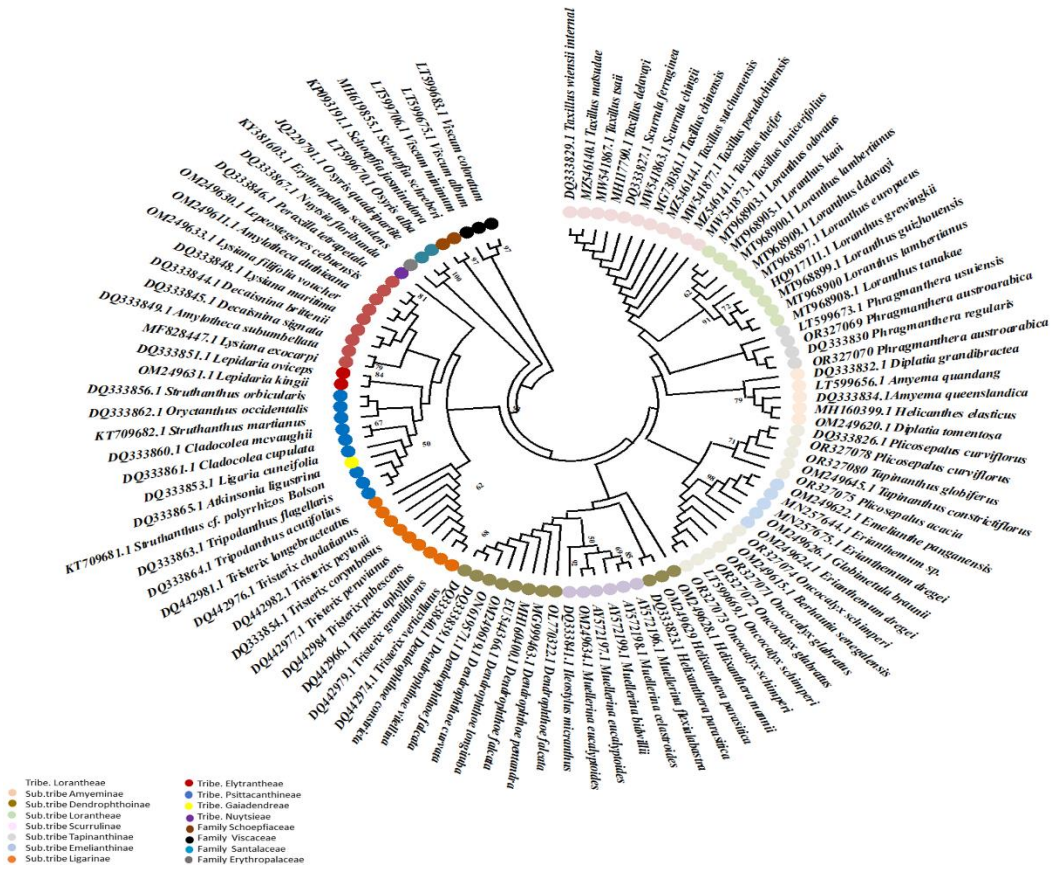

Fig. 8. Phylogenetic tree created of the ITS1+ITS2 sequences of local and worldwide species of the Loranthaceae family using maximum parsimony (MP). Bootstrap value shown in the branch nodes.

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