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First record of *Pluchea dioscoridis* (*Asteraceae*) in AL-Chibayish, Southern Iraq

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ABSTRACT

This study records the first confirmed occurrence of *Pluchea dioscoridis* (*Asteraceae*) in the southern wetlands of Iraq, thereby extending the known geographic distribution of the species into the Mesopotamian marshland system. The identification was established through an integrated framework combining morphological assessment with nuclear DNA regions (Internal transcribed spacer and external transcribed spacer), ensuring accurate taxonomic placement. The principal component analysis of morphological traits shows that the first two components (PC1 = 30.58% and PC2 = 20.87%) explain over half of the total variation and reveal three major clusters. Phylogenetic analyses further demonstrated its close affinities with related *Pluchea* taxa, providing new molecular evidence that enriches the understanding of intrageneric relationships within the group. Beyond its floristic significance, this discovery provides valuable baseline data for biodiversity monitoring in Iraq's marshes, a globally important yet understudied ecosystem. The occurrence and spread of *P. dioscoridis* in southern Iraq can be attributed to a combination of ecological conditions and human activities. This plant flourishes in damp environments such as river margins, irrigation ditches, and disturbed soils, all of which are widespread across the southern Iraqi plain. The records of this species highlight the importance of integrating classical botany with molecular systematics in exploring neglected habitats, while also underscoring the need for continued surveys to inform conservation and ecological restoration efforts in the region.

1. INTRODUCTION

Iraq encompasses a wide range of landscapes, stretching from the rugged mountains in the northeast to vast desert expanses in the west and the extensive marshes of the south. This geographical heterogeneity underpins the country's rich botanical diversity. Historically, plant research has been concentrated in the more accessible northern and central regions, favored by relatively stable climatic and socio-political conditions [1]. In contrast, the southern wetlands, most notably the Mesopotamian Marshes, have remained understudied, mainly due to past wars, environmental degradation, and the logistical challenges of conducting fieldwork in these areas [2,3]. Once among the most significant wetland ecosystems worldwide, the Mesopotamian Marshes represent a crucial ecological transition between aquatic and terrestrial zones, supporting highly specialized flora adapted to salinity and seasonal inundation [4,5]. Comprehensive floristic assessments in such overlooked habitats are vital for conservation planning and ecological monitoring, as they generate baseline records, clarify distributional ranges, and help detect biodiversity trends needed for management and restoration [6]. Since large-scale rehabilitation efforts began in the early 2000s, regular updates on plant diversity have become essential to evaluate the ecological recovery of Iraq's marshlands and to guide long-term conservation strategies [1]. Particular attention is warranted for taxonomically complex genera that thrive in dynamic or disturbed environments, such as *Pluchea Cass*. (*Asteraceae*), which includes approximately 80–100 species worldwide [7-11]. These plants, typically herbs or shrubs, are commonly found in wet, saline, or disturbed areas. Morphologically, *Pluchea* species are recognized by their dense pubescence, aromatic foliage, and discoid flower heads [12]. They are broadly distributed across tropical and subtropical regions, and are well documented in countries neighboring Iraq, including Iran, Kuwait, and Saudi Arabia [10,11]. Our findings expand the documented range of *Pluchea dioscoridis* to the Mesopotamian Marshes. This ecologically distinct and globally important wetland system has suffered for decades due to environmental and political crises [13]. Previous reports on this species have been limited to areas such as Northern Libya [7], Egypt's Nile Delta [14], and parts of the Arabian Peninsula [14], with an emphasis on ecological roles, morphology, or ethnobotanical uses. Unlike earlier studies that primarily relied on morphological

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traits for species delimitation, the present work combines nuclear DNA markers (internal transcribed spacer [ITS], external transcribed spacer [ETS]) with rigorous phylogenetic inference using maximum likelihood (ML), Maximum Parsimony, and Neighbor-Joining (NJ) approaches.

Beyond its ecological role, *P. dioscoridis* is well recognized for its medicinal potential. Extracts of the plant contain abundant flavonoids, terpenoids, and phenolic compounds, which contribute to antioxidant, anti-inflammatory, and antimicrobial activities [15,16]. Several studies further demonstrate anticancer effects, where bioactive metabolites exhibit cytotoxicity against a variety of tumor cell lines [17]. In traditional medicine across North Africa and the Middle East, *P. dioscoridis* is widely employed in the treatment of respiratory illnesses, fevers, wounds, and digestive problems. Many of these applications have been substantiated through modern pharmacological evaluations [18]. These properties highlight their promise for future phytochemical and therapeutic research.

P. dioscoridis (L.) DC. is a perennial shrubby herb indigenous to northeastern Africa and parts of the Arabian Peninsula, commonly found in moist, saline habitats [7]. It uses medicinal and aromatic leaves; its occurrence in Iraq had not been previously recorded through verified specimens or published surveys, making any new finding scientifically significant [16,19]. The present study aims to report the first confirmed record of *P. dioscoridis* in southern Iraq, based on a detailed morphological description and phylogenetic analysis.

2. MATERIALS AND METHODS

2.1. Study Area

The *P. dioscoridis* was first recorded in the Al-Chibayish region of the southern Mesopotamian marshes, situated within the Dhi Qar Governorate of Iraq [Figure 1]. The species was found growing along the margins of a seasonal water canal at an elevation of approximately 3 m above sea level [Table 1]. The habitat is characterized by semi-permanent marshland with fine alluvial soils, moderate salinity, and fluctuating water levels influenced by both rainfall and river inflow. This ecotonal zone supports a mixture of hygrophytic and halophytic vegetation. *P. dioscoridis* was observed in partial shade, typically growing as a scattered subshrub among dense assemblages of native marsh flora. The most associated species included *Typha domingensis*, *Phragmites australis, Juncus acutus*, *Tamarix nilotica*, and *Alhagi graecorum*, reflecting a transitional habitat between wetland and arid-steppe elements. The presence of *P. dioscoridis* in such a niche indicates its ecological tolerance to saline and waterlogged conditions, suggesting its potential role in the phytosociological composition of southern Iraq's wetland margins. The discovery of this species enriches the region's floristic diversity and highlights the importance of continued botanical exploration in the Mesopotamian wetland ecosystem [Figure 1].

2.2. Procedures

2.2.1. Specimen collection and identification

The study area lies within the Mesopotamian marshland ecosystem, characterized by semi-aquatic vegetation and seasonal water

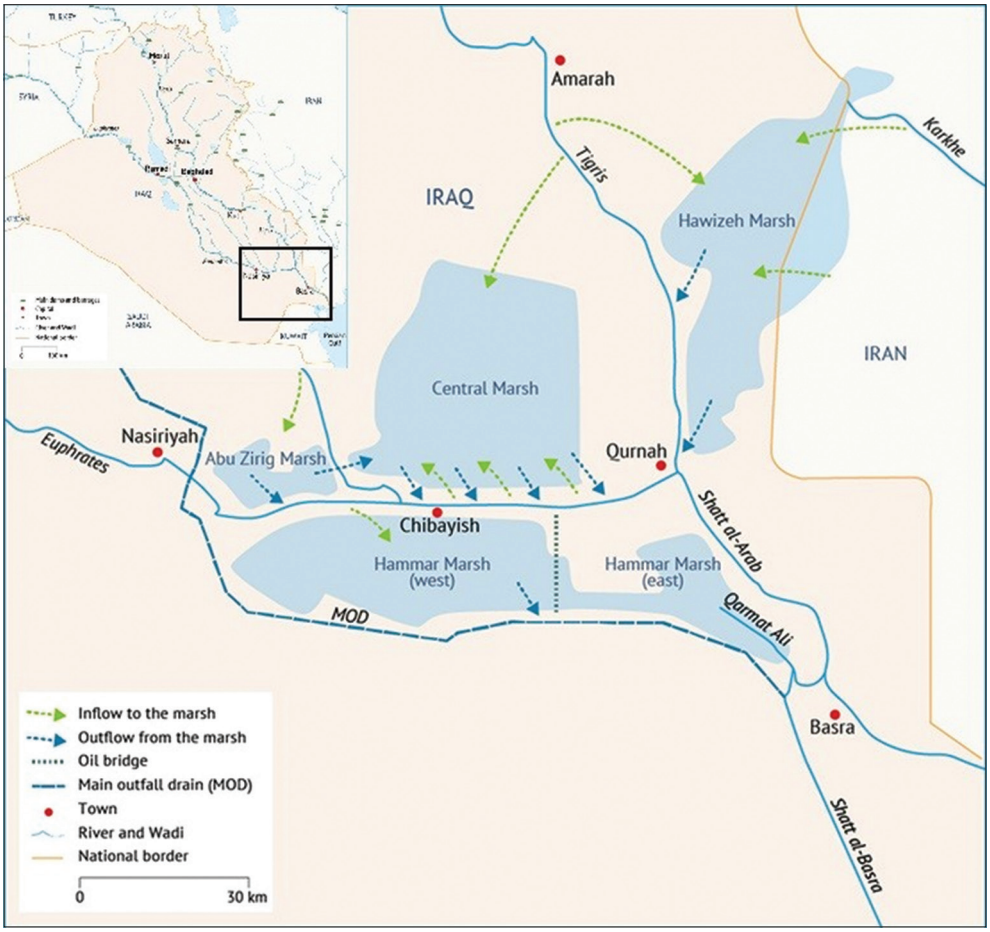

Figure 1: Map of the study area in Iraqi Marshlands Showing the Central, Hammar, and Hawizeh Marshes and Their Inflows and Outflows.

Table 1: Samples collations area of *Pluchea dioscoridis*, herbarium voucher numbers, localities.

S. No.	Collection locality	Location	Voucher	Altitude (m)	Collation name
1	Hammar Marsh, Al-Chibayish region, Dhi Qar	30°58′41″N, 47°05′12″E	MUPH 001	2.0	Haider M.J, Raad H. Alasadi
2	Hammar Marsh, Al-Chibayish region, Dhi Qar	30°58’44”N 47°05’09”E	MUPH 002	1.7	Haider M.J, Raad H. Alasadi
3	Hammar Marsh, Al-Chibayish region, Dhi Qar	30°58’42”N 47°05’06”E	MUPH 117	2.5	Haider M.J, Raad H. Alasadi
4	Hammar Marsh, Al-Chibayish region, Dhi Qar	30°58’48”N 47°05’11”E	MUPH 118	0.5	Haider M.J, Raad H. Alasadi
5	Hammar Marsh, Al-Chibayish region, Dhi Qar	30°58’47”N 47°05’06”E	MUPH 119	1.0	Haider M.J, Raad H. Alasadi
6	Hammar Marsh, Al-Chibayish region, Dhi Qar	30°58’46”N 47°05’05”E	MUPH 120	1.5	Haider M.J, Raad H. Alasadi
7	Hammar Marsh, Al-Chibayish region, Dhi Qar	30°58’45”N 47°05’13”E	MUPH 121	2.3	Haider M.J, Raad H. Alasadi
8	Central Marsh, Al-Chibayish region, Dhi Qar	30°58’42”N 47°04’56”E	MUPH 122	1.5	Haider M.J, Raad H. Alasadi
9	Central Marsh, Al-Chibayish region, Dhi Qar	30°58’51”N 47°04’52”E	MUPH 123	2.6	Haider M.J, Raad H. Alasadi
10	Hammar Marsh, Al-Chibayish region, Dhi Qar	30°58’33”N 47°05’08”E	MUPH 124	1.5	Haider M.J, Raad H. Alasadi
11	Central Marsh, Al-Chibayish region, Dhi Qar	30°58’41”N 47°04’51”E	MUPH 125	2.0	Haider M.J, Raad H. Alasadi
12	Central Marsh, Al-Chibayish region, Dhi Qar	30°58’33”N 47°04’56”E	MUPH 126	1.6	Haider M.J, Raad H. Alasadi
13	Central Marsh, Al-Chibayish region, Dhi Qar	30°58’42”N 47°04’40”E	MUPH 127	1.8	Haider M.J, Raad H. Alasadi
14	Abu Zirig Marsh, Al-Chibayish region, Dhi Qar	30°59’05”N 47°05’12”E	MUPH 128	3.0	Haider M.J, Raad H. Alasadi
15	Abu Zirig Marsh, Al-Chibayish region, Dhi Qar	30°59’05”N 47°04’36”E	MUPH 129	2.0	Haider M.J, Raad H. Alasadi
16	Abu Zirig Marsh, Al-Chibayish region, Dhi Qar	30°58’54”N 47°04’37”E	MUPH 130	2.0	Haider M.J, Raad H. Alasadi
17	Abu Zirig Marsh, Al-Chibayish region, Dhi Qar	30°58’37”N 47°04’27”E	MUPH 131	1.6	Haider M.J, Raad H. Alasadi
18	Abu Zirig Marsh, Al-Chibayish region, Dhi Qar	30°59’24”N 47°05’06”E	MUPH 132	1.0	Haider M.J, Raad H. Alasadi
19	Abu Zirig Marsh, Al-Chibayish region, Dhi Qar	30°59’09”N 47°04’57”E	MUPH 133	1.20	Haider M.J, Raad H. Alasadi
20	Abu Zirig Marsh, Al-Chibayish region, Dhi Qar	30°59’24”N 47°04’34”E	MUPH 134	2.0	Haider M.J, Raad H. Alasadi

fluctuations. A botanical survey was conducted in Oct 2024, in the Al-Chibayish marshes, Dhi Qar Governorate, located in the southern part of Iraq. The newly recorded plant species, *P. dioscoridis* (L.) DC. was found growing in a shallow, marshy habitat adjacent to a water canal, surrounded by halophytic and hydrophytic vegetation. Twenty voucher specimens were deposited at the Herbarium of Al-Muthanna University, College of Pharmacy, under the accession numbers MUPH001 to MUPH134 [Table 1]. Initial morphological identification was performed using diagnostic keys from Flora of Egypt [20] and Flora of Iraq [21]. Identification was confirmed through comparative analysis with authenticated specimens from digital herbaria (Global Biodiversity Information Facility, JSTOR Plants).

2.2.2. Morphometric analysis of morphological traits

Quantitative evaluation of morphological characters was carried out by coding 15 diagnostic traits (leaf length, leaf width, margin type, pubescence, bract series, inflorescence type, and achene features). Measurements were taken from 10 individuals whenever possible, and mean values were used for statistical comparisons. The dataset was subjected to principal component analysis (PCA) and Cluster Analysis (Unweighted pair group method with arithmetic mean based on Euclidean distances) using PAST v4.0 software. These analyses aimed to assess morphological divergence and to visualize the clustering pattern of *P. dioscoridis* relative to its congeners.

2.2.3. DNA extraction and molecular identification

To support morphological identification and assess phylogenetic placement, genomic DNA was extracted from silica-gel-dried leaf tissue using the modified cetyltrimethylammonium bromide (CTAB) method [22,23]. The modifications were applied especially when treating the old, succulent, not fresh, and over-dried succulent leaves. The modifications are: First, the amount of polyvinyl-pyrrolidone-40T was increased to 2% (w/v); the same amount of CTAB was used; second, the chloroform: Isoamyl alcohol (24:1) was added 3 times instead of two; and third, after adding the ice-cold isopropanol, the samples were kept in the freezer at −20°C for 2 days.

Two nuclear ribosomal regions, ITS and ETS, were amplified using universal primers ITS4/ITS5 and ETS-B/ETS-A [24,25], respectively. Polymerase Chain reactions were carried out in 25 Microliter volumes under optimized cycling conditions: initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, annealing temp at 52°C for 40 s for ITS, and 55°C for 1 min for ETS region. Initial extension 72°C for 1 min with a final extension at 72°C for 7 min [6,24]. All PCR reactions were performed with standard negative controls (no-template controls) to monitor potential contamination. No amplification was observed in any of the negative controls throughout the study. Amplified products were purified and sequenced bidirectionally using Sanger sequencing. Chromatograms were edited and assembled using BioEdit v7.2.6.

2.2.4. Molecular congruence analysis

The incongruence length difference (ILD) was tested to assess the congruence between ITS and ETS datasets using PAUP* v4.0. The test was conducted with 1000 replicates under the heuristic search option using tree-bisection/reconnection branch swapping and random addition sequences. $P > 0.05$ was considered evidence of no significant incongruence, thereby justifying the combination of datasets in a single phylogenetic analysis.

2.2.5. Phylogenetic analysis

The obtained ITS and ETS sequences were aligned using ClustalW in MEGA X [26]. Reference sequences from *Pluchea* and related taxa were downloaded from GenBank. Phylogenetic analyses were conducted using ML and NJ methods with 1000 bootstrap replicates [6]. The best-fitting nucleotide substitution model was determined using the Bayesian Information Criterion (BIC). *Ageratina adenophora* was used as an outgroup.

3. RESULTS

3.1. Morphological Description

P. dioscoridis is an erect, perennial, aromatic shrub, typically reaching heights of 1.0–2.5 m. The stems are robust, woody at the base, and densely branched in the upper parts, often displaying a reddish-green hue and covered with fine glandular hairs. Leaves are alternate, simple, and ovate to lanceolate in shape, measuring 5–15 cm in length and 2–6 cm in width. The leaf margins are coarsely serrate to subentire, and the lamina is gland-dotted, emitting a strong, aromatic scent when crushed [Figure 2] [27-30]. Petioles are short or absent, and the leaf surface is pubescent, particularly on the underside. Inflorescences are terminal and axillary corymbs or panicles composed of numerous small, discoid capitula. Each capitulum is subtended by involucre bracts arranged in 3–4 series, imbricate, ovate to lanceolate, and somewhat scarious. Florets are all tubular and bisexual; corollas are pink to purplish, with five small lobes. Others are syngenesious, and the style is bifid with sweeping hairs. Fruits are cypselae, narrowly oblong, ribbed, and crowned with a persistent pappus of numerous capillary bristles aiding in wind dispersal. Table 2 presents a comparative overview of morphological traits across ten *Pluchea* species. These species vary widely in habit, ranging from erect herbs to aromatic shrubs. Leaf morphology is diverse, with forms such as lanceolate, ovate, and sagittate, accompanied by differences in texture and margins. Inflorescence types include corymbose, axillary, and terminal clusters, with flower colors from pale pink to deep violet. Phyllaries and achenes also exhibit considerable variation in hairiness and color. Habitats range from wetlands and swamps to arid and sandy plains, reflecting the ecological adaptability and taxonomic diversity of the genus *Pluchea*. The PCA of morphological traits among ten *Pluchea* species (n = 100) shows that the first two components (PC1 = 30.58% and PC2 = 20.87%) explain over half of the total variation and reveal three major clusters. The solid ellipse encloses *Plantago lanceolata* and related taxa, which are clearly separated along PC2, while the dashed ellipse includes *Pluchea indica* and *Pluchea sericea*, distinguished mainly along PC1. The dotted ellipse groups *P. dioscoridis*, *Pluchea ovalis*, *Pluchea camphorata*, and allied species, display greater overlap and indicate shared or intermediate morphological features [Figure 3].

3.2. Synonyms

Baccharis dioscoridis L. (Cent. Pl. 1: 27, 1755), *Conyza odora* Forssk. (Fl. Egypt. Arab. 148, 1775), *Conyza dioscoridi* (L.) Desf. (Tabl. École Bot., ed. 2, 114, 1815), *Baccharis aegyptiaca* Forssk. ex-DC. (Prodr. 5: 450, 1836), and *P. dioscoridis* (L.) DC. var. glabra Oliv. and Hiern (Fl. Trop. Afr. 3: 329, 1877).

3.3. Phylogenetic Analysis

3.3.1. Nucleotide composition and substitution patterns

The nucleotide substitution rate analysis for ITS and ETS regions, based on the Kimura 2-parameter model, revealed clear differences in the relative frequencies of transitions and transversions across both markers. The substitution matrix for ITS showed a pronounced bias for transitions, particularly between G↔A and C↔T/U (rate = 16.20), while other substitutions occurred at significantly lower frequencies (rate = 4.39). Similarly, in the ETS region, the highest substitution rates were also observed for G↔A (15.63) and C↔T/U (15.63), confirming a strong transitional bias [Table 3]. These patterns are consistent with expected evolutionary trends in nuclear ribosomal regions, where transitions are favored over transversions due to biochemical stability and mutation mechanisms. The analysis was performed using MEGA software, with 18 and 20 nucleotide sequences included for the ITS and ETS datasets, respectively, resulting in maximum log likelihood values of −2194.642 (ITS) and -2320.317 (ETS). The equal base frequencies

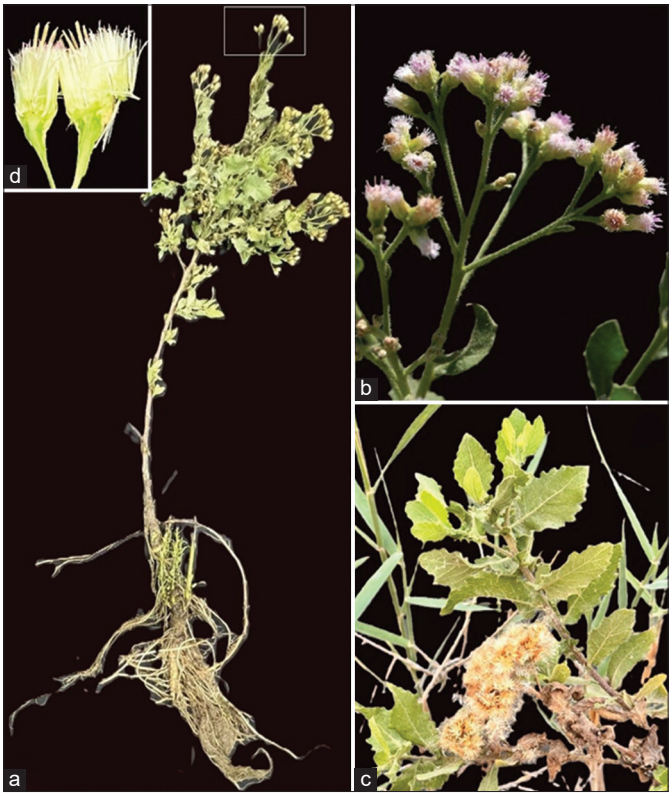

Figure 2: Morphological Features of *Pluchea dioscoridis* (L.) DC. (A) Whole plant showing root system and aerial parts. (B) Flowering branch with capitula bearing pinkish tubular florets. (C) Fruiting branch with mature capitula and pappus-bearing achenes. (D) Close-up view of individual flowers showing the flower structure.

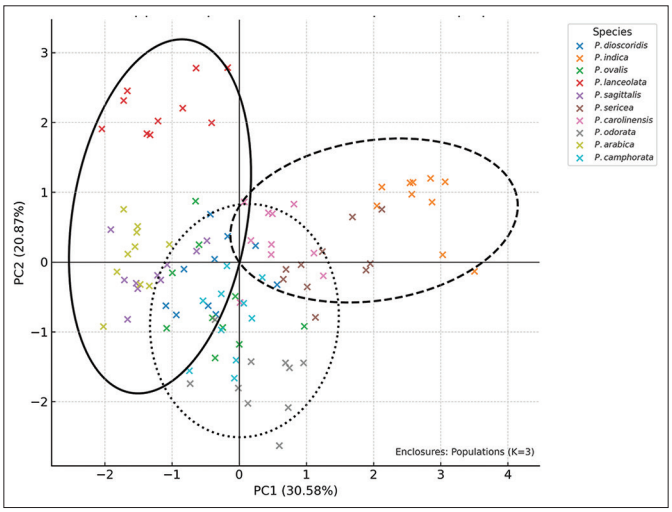

Figure 3: Principal component analysis of morphological traits among ten *Pluchea* species based on diagnostic characters.

Table 2: Morphological characters among 10 *Pluchea* species.

Morphological character	Species name									
	Pluchea dioscoridis	***Pluchea indica***	***Pluchea ovalis***	***Pluchea lanceolata***	***Pluchea sagittalis***	***Pluchea sericea***	***Pluchea carolinensis***	***Pluchea odorata***	***Pluchea arabica***	***Pluchea camphorata***
Habit	Aromatic subshrub, up to 1.5 m	Shrub or small tree, up to 3 m	Perennial herb, up to 1 m	Erect herb, 0.5–1.2 m	Herb, 0.3–1.0 m	Shrub, 1.5–3 m	Shrub, 1–2 m	Herb or small shrub	Perennial shrub, 0.6–1.5 m	Erect herbs, aromatic
Stem	Erect, branched, pubescent	Woody, thick, glabrous	Slender, hairy	Simple, glabrous	Soft, hairy	Woody, grayish	Thick, pubescent	Green, pubescent	Tomentose	Sticky, hairy
Leaves	Lanceolate, serrate, pubescent	Elliptic, glabrous	Ovate, toothed	Narrow-lanceolate, entire	Sagittate, soft pubescent	Linear-lanceolate, silvery	Broad, toothed	Serrated, aromatic	Oblong, gray green	Ovate, camphor-scented
Inflorescence	Corymbose, terminal	Dense terminal clusters	Compact terminal corymbs	Axillary heads	Loosely clustered	Dense axillary clusters	Large heads in terminal panicles	Corymbose, showy	Small corymbs	Terminal clusters
Flower color	Pink to purplish	Purple to red	Pale violet	Pale pink	Purple	Pink	Lilac to pink	Lavender	Pale pink	Violet
Phyllaries	Hairy, dark-tipped	Greenish, pubescent	Broad, hairy	Narrow, pubescent	Soft hairy, spreading	Silvery tomentose	Overlapping, pubescent	Green with purple margins	Densely hairy	Dark-tipped, hairy
Achenes	Ribbed, white pappus	Ribbed, reddish pappus	Ribbed, white pappus	Small, ribbed, white pappus	Slender, ribbed, white pappus	Silky pappus	Broad, hairy pappus	Ribbed, long pappus	Short, white pappus	Ribbed, white pappus
Habitat	Wetlands, canals	Coastal swamps	Disturbed plains	Sandy plains	Moist grasslands	Arid riverbanks	Tropical lowlands	Roadsides, wetlands	Semi-arid plains	Swampy ground

Table 3: Nucleotide substitution rates for ITS and ETS regions based on the Kimura 2-parameter model.

From\ To	ITS				ETS			
	A	T/U	C	G	A	T/U	C	G
A	-	4.39	4.39	16.20	-	4.68	4.68	15.63
T/U	4.39	-	16.20	4.39	4.68	-	15.63	4.68
C	4.39	16.20	-	4.39	4.68	15.63	-	4.68
G	16.20	4.39	4.39	-	15.63	4.68	4.68	-

ITS: Internal transcribed spacer, ETS: External transcribed spacer.

(A, T/U, C, G = 25.00%) ensured balanced substitution modeling. The ETS dataset contained 691 aligned positions, while the ITS dataset included 553 positions, excluding ambiguous sites and poorly covered positions. These findings reinforce the utility of ITS and ETS regions for phylogenetic reconstruction and evolutionary inference in plant systematics using ML-based models.

3.3.2. Phylogenetic analysis based on the ITS gene

The phylogenetic analysis of the *ITS* gene sequences, comprising 517 conserved sites, 199 variable sites, and 56 parsimony-informative characters, revealed a well-supported evolutionary relationship among the *Pluchea* species [Table 4], with a consistent clustering pattern across all three analytical methods: ML, maximum parsimony, and NJ. In all tree topologies, the target species *P. dioscoridis* with *P. dioscoridis* KF805091 (difference area) consistently clustered together with high bootstrap support (98%), indicating a strong genetic affinity and validating their conspecific status. These accessions formed a monophyletic group closely allied with *P. oval*, *P. indica*, and *P. sericea*, suggesting a close evolutionary relationship. Minor topological variations were observed between the methods, particularly in the placement of distant taxa. Still, the grouping of *P. dioscoridis* remained stable, reinforcing the robustness of ITS as a marker for species-level resolution within *Pluchea*. The outgroup (*Tithonia diversifolia*) successfully rooted the tree, further confirming the phylogenetic distinctiveness of *P. dioscoridis* within the genus [Figure 4].

3.3.3. Phylogenetic analysis based on the ETS gene

The phylogenetic reconstruction based on the ETS gene region (329 conserved sites, 221 variable sites, and 77 parsimony-informative sites) yielded consistent topologies across the ML, Maximum Parsimony (MP), and NJ analyses [Table 4]. In all three tree-building methods [Figure 5], *P. dioscoridis* clustered together with high bootstrap support, forming a strongly supported monophyletic clade along with *P. dioscoridis* LN606961. This clade was closely related to *P. indica* and *P. oval*, indicating a shared evolutionary lineage and potential taxonomic proximity. The ML tree provided greater resolution among internal nodes, with branch support consistently at or near 98–100%, confirming the reliability of this relationship. The MP and NJ trees reinforced the same topological structure, though with slight variation in branching order among closely related taxa. The congruence of topologies across methods and the strong support values confirm the distinct phylogenetic placement of *P. dioscoridis*, highlighting the robustness of the ETS region as a marker for species delimitation within the genus *Pluchea* [Figure 3].

3.3.4. Congruence of molecular datasets

The ILD test applied to the nuclear partitions (ITS, ETS, and the combined Nu regions) indicated significant incongruence among

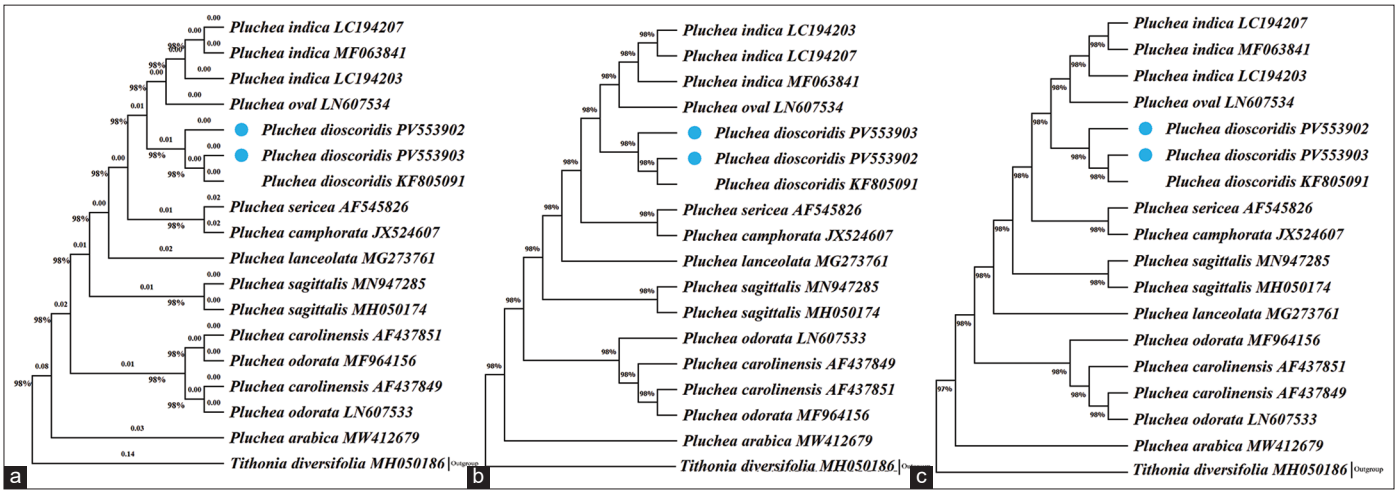

Figure 4: Phylogenetic relationships of *Pluchea* species based on ITS sequences using Maximum Likelihood (a), Maximum Parsimony (b), and Neighbor-Joining (c) Methods, Highlighting the Position of *Pluchea dioscoridis* (PV553902, PV553903).

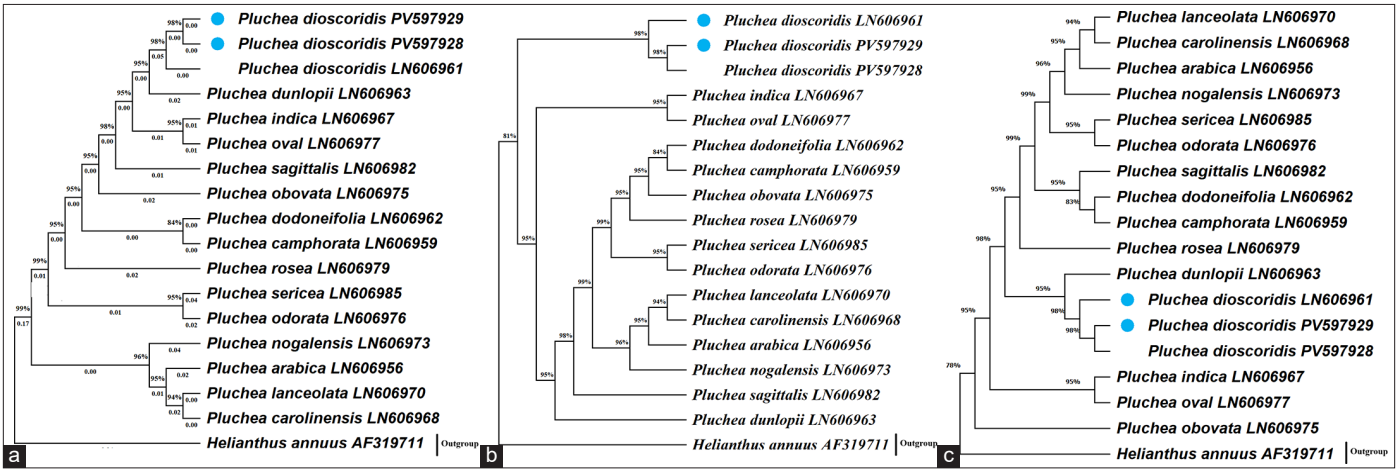

Figure 5: Phylogenetic relationships of *Pluchea* species based on ETS sequences using Maximum Likelihood (a), Maximum Parsimony (b), and Neighbor-Joining (c) Methods, Highlighting the Position of *Pluchea dioscoridis* (PV553902, PV553903).

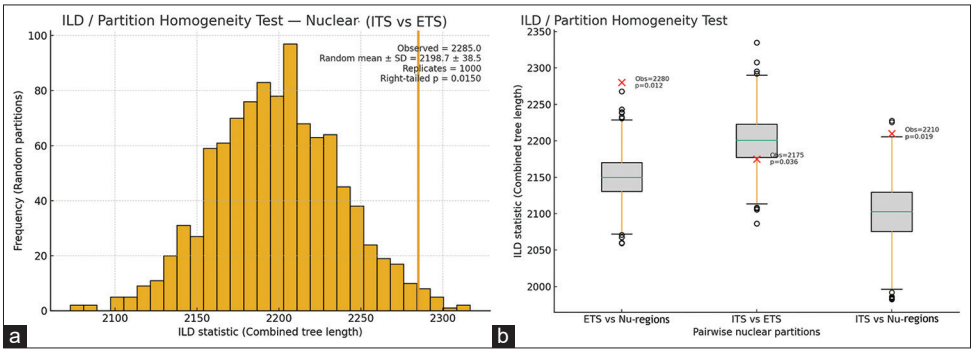

Figure 6: Incongruence Length Difference (ILD) or Partition Homogeneity Test for nuclear regions (ITS and ETS) of *Pluchea* species. (a) Histogram showing the frequency distribution of random partitions (n = 1000) with an observed ILD statistic (2285.0) exceeding the randomized mean (2198.7 ± 38.5; p = 0.015), indicating significant heterogeneity between ITS and ETS datasets. (b) Boxplot comparison of pairwise nuclear partitions (ETS vs Nu-regions, ITS vs ETS, and ITS vs Nu-regions) showing observed ILD values and corresponding p-values, confirming partial incongruence among the nuclear datasets.

all pairwise comparisons [Figure 6a]. The observed tree length values for ITS versus ETS (Obs = 2280, *P* = 0.012), ITS versus Nu-regions (Obs = 2175, *P* = 0.036), and ETS versus Nu-regions (Obs = 2210, *P* = 0.019), all exceeded the null distributions of randomized partitions, confirming that the nuclear datasets do not exhibit complete homogeneity [Figure 6b]. This result suggests

Table 4: Sequences comparison for ITS and ETS regions for 18 *Pluchea* taxa.

No	Scientific name	*ITS* gene					Scientific name	*ETS* gene				
		Maximum score	Total score	Query cover (%)	E-value	Accession		Maximum score	Total score	Query cover (%)	E-value	Accession
1	*Pluchea dioscoridis*	712	712	100	0	PV553902*	*Pluchea dioscoridis*	510	510	100	0	PV597928*
2	*Pluchea dioscoridis*	712	712	100	0	PV553903*	*Pluchea dioscoridis*	510	510	100	0	PV597929*
3	*Pluchea dioscoridis*	720	720	100	0	KF805091	*Pluchea dioscoridis*	540	540	100	0	LN606961
4	*Pluchea indica*	720	720	100	0	LC194203	*Pluchea indica*	530	530	100	0	LN606967
5	*Pluchea indica*	720	720	100	0	LC194207	*Pluchea dodoneifolia*	533	533	100	0	LN606962
6	*Pluchea indica*	720	720	100	0	MF063841	*Pluchea dunlopii*	540	540	100	0	LN606963
7	*Pluchea lanceolata*	720	720	100	0	MG273761	*Pluchea lanceolata*	540	540	100	0	LN606970
8	*Pluchea oval*	715	715	100	0	LN607534	*Pluchea oval*	510	510	100	0	LN606977
9	*Pluchea sagittalis*	713	713	100	0	MN947285	*Pluchea sagittalis*	510	510	100	0	LN606982
10	*Pluchea sagittalis*	713	713	100	0	MH050174	*Pluchea rosea*	540	540	100	0	LN606979
11	*Pluchea sericea*	713	713	100	0	AF545826	*Pluchea sericea*	540	540	100	0	LN606985
12	*Pluchea carolinensis*	713	713	100	0	AF437851	*Pluchea carolinensis*	540	540	100	0	LN606968
13	*Pluchea carolinensis*	713	713	100	0	AF437849	*Pluchea nogalensis*	540	540	100	0	LN606973
14	*Pluchea arabica*	713	713	100	0	MW412679	*Pluchea arabica*	540	540	100	0	LN606956
15	*Pluchea camphorata*	713	713	100	0	JX524607	*Pluchea camphorata*	540	540	100	0	LN606959
16	*Pluchea odorata*	713	713	100	0	LN607533	*Pluchea odorata*	540	540	100	0	LN606976
17	*Pluchea odorata*	713	713	100	0	MF964156	*Pluchea obovata*	540	540	100	0	LN606975
18	*Tithonia diversifolia*	718	718	100	0	MH050186	*Helianthus annuus*	500	500	100	0	AF319711

*Show the sequences data generated and recorded in NCBI by this study. ITS: Internal transcribed spacer, ETS: External transcribed spacer.

that although nuclear markers generally recover consistent clades of *Pluchea* spp., they retain distinct phylogenetic signals that may be attributed to incomplete lineage sorting, paralogy, or historical hybridization events. Therefore, while the combined dataset provides an overall framework for species relationships, the detected incongruence highlights the need for cautious interpretation and supports the inclusion of multiple independent loci to resolve complex evolutionary histories in *Pluchea*.

4. DISCUSSION

The phylogenetic and morphological evidence presented in this study confirms the presence of *P. dioscoridis* in the marshes of Al-Chibayish city in southern Iraq, expanding its known distribution into the Mesopotamian wetland system. The species exhibited distinctive morphological traits, described in regional floras [7,12].

Molecular analysis based on ITS and ETS regions further supported its taxonomic placement, revealing a clear phylogenetic distinction from closely related taxa. Across all analytical methods ML, maximum parsimony, and NJ *P. dioscoridis* formed a consistently resolved subclade, suggesting its relative topological position within the genus and supporting its identification as a distinct lineage [11,12]. The close clustering of *P. dioscoridis* with *P. ovalis* and *P. indica* reflects potential ancestral affinities or shared ecological adaptations to semi-saline environments, as all three species were associated with wetland or canal side habitats. These findings are consistent with previous studies highlighting the value of ITS and ETS markers for resolving intrageneric relationships in *Asteraceae* [31-34].

The occurrence and spread of *P. dioscoridis* in southern Iraq can be attributed to a combination of ecological conditions and human activities. This plant flourishes in damp environments such as river margins, irrigation ditches, and disturbed soils, all of which are widespread across the Mesopotamian plain. Factors similar to ongoing irrigation, changes in soil salinity, and land disturbance from farming and urban growth provide suitable habitats for its growth and expansion. Its strong adaptability, rapid vegetative development, and allelopathic effects further allow it to displace native species and establish dense populations along waterways. Comparable patterns of expansion have been observed in other parts of the Arabian Gulf and nearby Middle Eastern regions, highlighting the influence of altered hydrological regimes and human-facilitated dispersal in driving its localized expansion [35].

5. CONCLUSION

This investigation documents a verified record of *P. dioscoridis* within the southern marshes of Iraq, particularly in the Al-Chibayish district of Dhi Qar Governorate. The species was identified based on morphological traits, with confirmation provided by nuclear ribosomal ITS and ETS sequence analyses, which securely positioned it within a well-supported clade alongside its close relatives. Its occurrence in semi-saline wetlands that undergo seasonal flooding demonstrates notable ecological tolerance, extending the species' distribution into the Mesopotamian landscape. The addition of *P. dioscoridis* enriches the floristic inventory of Iraq, emphasizing the value of combining classical field botany with molecular phylogenetics when exploring understudied habitats. These results not only provide baseline data for conservation strategies but also open new directions for ecological and evolutionary research on wetland plant communities in the region.

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7. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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10. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

11. ETHICAL APPROVALS

Field collection of plant specimens was conducted in accordance with Iraqi environmental and academic research regulations, and necessary permissions were obtained from local authorities in Al-Chibayish, Dhi Qar Governorate. Herbarium specimens were deposited in the Herbarium of the College of Pharmacy, Al-Muthanna University, under voucher numbers MUPH001–MUPH134. Therefore, This study does not involve experiments on animals or human subjects.

12. DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article

13. PUBLISHER'S NOTE

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14. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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