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ORIGINAL ARTICLE

GENUS *RETAMA* RAF., 1838 (FABALES, FABACEAE): TAXONOMIC REVISION IN EGYPT SUPPORTED BY MOLECULAR FINGERPRINTING

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ABSTRACT

In Egypt, the taxonomic identity of the taxa under genus *Retama* Raf., 1838 (Fabaceae) is still unclear and its morphological resemblance precludes its identification. The current study aims to resolve the taxonomic identity of the species belonging to genus *Retama* in Egypt and clarify the morphological, molecular, and geographic distribution characterised each species. To achieve these goals, the fresh and herbarium *Retama* specimens were used for morphological investigations using 94 macro-morphological characters while the Inter Simple Sequence Repeats (ISSR) markers were used for the molecular identity. This revision revealed the identification of two distinct species namely: *Retama raetam* (Forsskk.) Webb. and *Retama monosperma* (L.) Boiss with five forms under *R. raetam* (Forms 2, 4, 6, 7, and 8). In addition to Form 5 from under *R. monosperma*; the morphological and molecular identities of forms showed variations in the meanwhile it's clustering were congruent. The geographic distribution of the *Retama* taxa in Egypt was elucidated. This study highlights the urgent need for *Retama* species conservation, due its vulnerability to climatic change.

Keywords**:** Flora of Egypt, *Retama monosperma*, *R. raetam*, Forms, ISSR, Vulnerable species.

INTRODUCTION

Globally, Fabaceae is among the most prominent families of Angiospermae, it includes more than 19500 species grouped into approximately 770 genera (Bruneau *et al.*, 2013; Abusaief and Boasoul, 2021). Economically, Fabaceae is in the second position to Poaceae (Mabberley, 1997; Yahara *et al.*, 2013). The Egyptian flora comprises a unique mixture of native African and Asiatic species with over 2079 species (Amer, 2008), where Fabaceae is represented by 212 species (Boulos, 1999).

Genus *Retama* Raf.m 1838 (Fabaceae), previously named *Lygos* Adanson (GBIF Secretariat, 2022); *Spartium* L. and *Retama* Boiss. (Lopez *et al.*, 1998; Benmiloud-Mahieddine *et al.*, 2011), distributed in the western Mediterranean region (Chiapella *et al.*, 2009). It comprises four closely related species included in the tribe Genisteae, namely *R. monosperma* (L.) Boiss., *R. sphaerocarpa* Boiss., *R. raetam* Webb and Berthel., and *R. dasycarpa* Coss. It also contains several subspecies and varieties distributed in North Africa and their diversification center is in the western Mediterranean basin, Canary Islands, and Southern Europe (Benmiloud-Mahieddine *et al.*, 2011). The morphological resemblance between these four species in this genus obstructs their taxonomical identification (Käss and Wink, 1997; Pardo *et al.*, 2004; León-González *et al.*, 2018).

Retama species can tolerate severe drought conditions in various ecosystems, including deserts, coastal dunes, and maquis (León-González *et al.*, 2018). In Egypt, *Retama* grows in the desert wadis, sandy and maritime plains (Boulos, 1999, 2009; Amer *et al.*, 2021). Economically, *Retama* species are important fodder for livestock and considered as alternative to the conventional forage species in the arid Mediterranean ecosystems (Barakat *et al.*, 2013). They are used for protection and stabilization against wind or water erosions, irradiation, and overheating (Maghrani *et al.*, 2005; Morsy *et al.*, 2015). Also, they are widely used for construction and ornamental purposes (Barakat *et al.*, 2013); and have several medicinal benefits for humans (Maghrani *et al.*, 2005; Awen *et al.*, 2011; Djeddi *et al.*, 2013; Muñoz Vallés *et al.*, 2013; Chouaibi *et al.*, 2019).

The taxonomic identity of the taxa grouped under genus *Retama* Raf. is still unresolved. Several reports have shown divergence such as the following: in Algeria, this genus is represented by *R. raetam* Webb, *R. spherocarpa* (L.) Boiss. and *R. monosperma* (L.) Boiss. subsp. *bovei* (Spach.) Maire (Quezel and Santa, 1962); in Europe, it represent by three species, according to Tutin *et al.* (1968) are *Lygos raetam* (Forssk.) Heywood (syn. *Retama raetam* (Forssk.) Webb & Berth.) subsp. *gussoniei* (Webb) Heywood, *L. spherocarpa* (L.) Heywood. (syn. *Retama spherocarpa* (L.) Boiss.) and *L. monosperma* (L.) Heywood. (syn. *Retama monosperma* (L.) Boiss.) or only *L. monosperma* (L.) Heywood. (syn. *R. monosperma* (L.) Boiss. (Polunin, 1969). In Palestine, Zohary (1972) reported the presence of *R. raetam* (Forssk.) Webb with two varieties, var. *raetam* (Forssk.) Webb and var. *sarcocarpa* Zoh. While, in Lebanon and Syria, this genus is represented by two species *R. raetam* (Forssk.) Webb. and *Retama duriaei* (Spach) Willd. (Mouterde, 1966), and only one species, *R. raetam* (Forssk.) Webb was seen in Libya (Jafri, 1980).

The literature data of the previous taxonomic treatment of genus *Retama* in Egypt increased its complexity. Where Täckholm (1974), reported it as *Lygos* Adans which represented by *Lygos raetam* (Forssk.) Heywood (syn. *Retama raetam* (Forssk.) Webb with two varieties, var. *sarcocarpa* (Zoh.) Täckh. et Boulos and var. *bovei* (Spach) Täckh. et Boulos, in addition to three unidentified anonymous varieties in Sinai. Later, Boulos (2009), reported the genus *Retama* Raf. inEgypt by two species, *R. raetam* (Forssk.) Webb & Berthel (syn. *Lygos raetam* (Forssk.) Heywood) and *Retama monosperma* (L.) Boiss. subsp. *bovei* (Spach) Maire (syn. *Spartium bovei* Spach. and *Retama bovei* (Spach) Webb). The complexity of this genus in Egypt also includes some taxa, such as var. *bovei*, which was treated as a variety under *Lygos raetam* (Täckholm, 1974) and a subspecies of *R. monosperma* by Boulos (2009).

Although the genus *Retama* is important in the Mediterranean region, several species have recently become vulnerable to climate change, mainly rainfall shortage and temperature increase resulting in the poleward shift of the xeric species. Among them, *R. raetam*, which recently showed distribution, density, and genetic decline; accordingly, it needs urgent conservation (Amer *et al.*, 2020; Elshayeb, 2020).

It is crucial to understand the genetic profile of *Retama* taxa (species/populations/taxa) in Egypt to promote future conservation strategies. Molecular markers are powerful tools for detecting polymorphism in related species as they are not influenced by developmental stages or environmental factors (Helm *et al.*, 2009; Karimi *et al.*, 2009). ISSR molecular markers are among the molecular tools that can distinguish closely related species, even at the interspecies level (Zietkiewicz *et al.*, 1994; Abdelhameed *et al.*, 2020; Amer *et al.*, 2021), and identify the diversity within and among populations (Mosaferi *et al.*, 2015; Minaeifar *et al.*, 2016; Abdelhameed *et al.*, 2020; Muraseva and Guseva, 2021).

The current study aims to resolve the following points concerning genus *Retama* in Egypt, naming the number of taxa representing this genus; clarifying the morphological, genetic and geographic features of these taxa.

MATERIALS AND METHODS

Plant materials: The fresh *Retama* specimens (63 from 21 populations) and their seeds were collected and examined from the current geographical range in Egypt between 2020–2022 (Tab. 1) and a distribution map was conducted using the geographic position system. The dry specimens (220 specimens) were kept in the Egyptian herbaria at Cairo University (CAI), National Research Center (CAIRC), Desert Research Institute (CAIH), and Agricultural Museum (CAIM) and the online virtual herbaria (Royal Botanic Garden Herbarium at Kew (K)), JSTOR Global Plants database and the Global Biodiversity Information Facility database were additionally studied. The acronyms for herbaria follow Thiers (2019). Fresh samples were preserved in formalin-aceto-alcohol (FAA: 50 mL ethyl alcohol, 10 mL formaldehyde, 5 mL glacial acetic acid, and 35 mL distilled water (Amer *et al.*, 2021).

Morphological investigation: Morphological investigations were conducted based on 94 macro-morphological characters including stem, leaves, flowers, inflorescences, fruits, and seeds of the fresh and herbarium *Retama* specimens (see Appendix 1). The identified *Retama* taxa were based on previous taxonomic treatments (Quezel and Santa, 1962; Tutin *et al.*, 1968; Polunin, 1969; Zohary, 1972; Täckholm, 1974; Jafri, 1980; Wickens, 1998; Boulos, 1999, 2009).

Molecular investigation: Genomic DNA was extracted from juvenile leaf samples according to Abdelhameed *et al.* (2020) and purified them using the Plant DNeasy Mini Kit (Qiagen, Santa Clarita, CA). Twelve ISSR primers were used and sequenced as listed in Table (3). The PCR mixture (25 μL) included 30 ng template DNA (this was applied to triple samples/taxa), 2.5 μL of 10X PCR buffer, 1.5 μL of 25-mM $MgCl_2$, 2.5 μL of the dNTPs mix, 30 pm of ISSR primer, and 1 U Taq DNA polymerase (Promega, WI, USA). DNA amplification was performed according to Amer *et al.* (2021) using a thermal cycler (Applied Biosystems, USA). The PCR cycle consisted of initial denaturation at 94 °C for 5 mins, 40 cycles of denaturation at 94 °C for 2 mins, annealing at 50 °C for 45 secs, extension at 72 °C for 2 mins, and final elongation for 7 mins at 72 °C. The PCR products were separated using 1.5 % agarose gel in 1x TBE buffer and visualized using ethidium bromide. The gels (3 per each studied taxa) were visualized under a UV-transilluminator, documented in Gel-Doc XR (Bio-Rad), and photographed. The amplicon sizes were determined using a 1 Kplus DNA ladder RTU (Genedirex).

Statistical analysis: The morphological data (expressed as mean ± standard error) were analyzed and plotted using Graph Pad Prism version 8.0.1. One-way analysis of variance was used for all statistical comparisons followed by Tukey's procedure for posthoc analysis. Values with $P < 0.05$ were considered significant. The radar plot was developed using the Excel program (Microsoft 365). The Jaccard's coefficient was applied using the SPSS program (version 20 for Windows) to estimate the similarity between the studied *Retama* taxa based on the macro-morphological characters. A dendrogram was generated by cluster analysis using the unweighted pair group method of the arithmetic averages (UPGMA) using Past software (version 3.26 for Windows). For the molecular part the generated/amplified bands were scored based on the presence (1) and absence (0) of bands using NTSYS-PC 2.21 software (Rohlf, 2009) and indirect gradient analysis was performed by Detrended Correspondence Analysis (DCA) using Past software (version 3.26 for Windows).

RESULTS

The current revision of genus *Retama* Raf. in Egypt revealed the presence of two distinct species, *Retama raetam* (Forssk.) Webb & Brantel and *Retama monosperma* (L.) Boiss. along with five forms of *Retama raetam* (Forssk.) Webb (Form 2, Form 4, Form 6, Form 7, and Form 8). In addition to Form 5 of *Retama monosperma* (L.) Boiss. The morphology of each form was interdisciplinary and shared the features of one of the two identified species (*Retama raetam* and *R. monosperma*) with distinct dissimilar characters rendering it consistent with the typical species; accordingly they were treated as forms. The differential morphological traits were tabulated in Appendix 1, details description will be followed:

Morphological features of *Retama raetam* (Forssk.) Webb & Berthel.
Hist. Nat. Iles Canaries 3(2; 2): 56 (1842)
Synonyms:
Genista raetam Forssk. in Fl. Aegypt.-Arab.: 214 (1775)
Lygos raetam (Forssk.) Heywood in Feddes Repert. 79: 53 (1968)
Spartium raetam (Forssk.) Spach in Ann. Sci. Nat., Bot., sér. 2, 19: 288 (1843)

Perennial, woody spartoid (branched from the base), "nebka-forming shrub = sand-mound forming shrub" up to 3 m in height. Stem green-grooved, branches ascending or spreading, young twigs richly branched, sparsely-densely covered with silky hair. Leaves 2–8 (-23) × 0.5–3.0 mm, simple, sericeous, deciduous, linear-oblong, entire margin and acute-obtuse apex, petiole 0.25 mm. Inflorescence: lax–dense raceme, with 1–7 flowers (up to 15 flowers in the traced Egyptian forms). Flower papilionate, 8–12 mm, pedicle 1–3 mm. Calyx 2–4 × 1.5–3 mm, green-purple, occasionally with purple spots, up to ⅓ flower length, calyx tube with 2-lipped: upper lip with 2 broader triangular teeth and the lower lip with 3 shorter teeth almost equal in length, caducous after anthesis. Corolla white occasionally with purplish tips and/or purple spots. Standard 8–12 × 5–7 mm, as long as the wings, elliptical with purple main vein, apex retuse, hairy on tips and main vein. Wings 8–12 × 1.5–2.5 mm, oblong with obtuse apex, hairy on tips and base, occasionally with spots. Keel 8–12 × 3–5 mm, oblong, apex acute, hairy on base, tips, and main vein. Stamens 10, monadelphous, connate all below into a closed tube, 6–10 mm, filaments one long and the rest random in length. Anthers 5 heart-shaped, apiculate, dorsal fixed, 0.25–0.5 mm, and 5 oval-shaped, terminal fixed, 0.5–1.0 mm. Ovary 3–6 × 1–2 mm, elliptical, attenuate on both sides. Style 5–7 mm. Stigma 0.25 × 0.25 mm, capitate. Fruit pod 10–15 × 3–7 mm green indehiscent, glabrous, elliptical attenuates gradually to a short erect peak 0.25–2.0 mm, 1-seeded. Fruit pericarp is fleshy, leathery smooth when dry. Seeds 4–6 × 3–4 mm, reniform to round, smooth, olive-green to brown (Pl. 1a-e). Flowering from January to April. Distribution mainly in the desert wadis, sandy and gravel plains in Egypt.

Morphological features of *Retama monosperma* (L.) Boiss

Voy, Bot. ESpagne 2: 144 (1840)

Synonyms:

Genista monosperma (L.) Lam. in Encycl. 2: 616 (1788)

Lygos monosperma (L.) Heywood in Feddes Repert. 79: 53 (1968)

Spartium gracile Salisb. in Prodr. Stirp. Chap. Allerton: 329 (1796), nom. superfl.

Spartium monospermum L. in Sp. Pl.: 708 (1753)

Perennial, woody spartoid, up to 3 m in height. Stems erect, ascending or spreading, green-grooved, young twigs sparsely-densely with silky hairs**.** Leaves simple, 2–7 × 0.5–1.25 mm, simple, sericeous, deciduous, linear to lyrate, entire margin and acute-obtuse apex, petiole 0.25–0.5 mm. Inflorescence lax raceme, with 1–7 flowers (up to 15 flowers in the traced Egyptian form). Flower papilionate, 7–10 mm, pedicle 0.25–1.5 mm. Calyx 2–4 × 1.5–2 mm, purple, up to ⅓ of the flower, 2-lipped: upper lip with 2 broader triangular teeth and the lower lip with 3 shorter teeth equal in length, caducous after anthesis. White corolla, standard 4–6 × 6–8 mm, shorter than the wings, orbicular with purple mid-vein, apex retuse, hairy on tips, and mid-vein. Wings 7–10 × 2–3 mm, oblong with obtuse apex, hairy along the main vein, tips, and base. Keel 7–10 × 4 mm, oblong, apex obtuse, hairy along mid-vein, tips, and base. Stamens 10, monadelphous, connate all below into a closed tube, 7–8 mm, filaments nearly equal in length. Anthers 5 heart-shaped, apiculate, dorsal fixed, 0.25–0.5 mm, and 5 oval-shaped, terminal fixed, 0.5–1 mm. Ovary 2–3 × 0.5–1 mm, ovate. Styles 4–5 mm. Stigma 0.25 × 0.25 mm, capitate. Fruit pod 10–15 × 7–10 mm, green indehiscent, glabrous, globoid abruptly short-apiculate to erect peak, 1–2 mm, 1 to 2 seeds. The pericarp is leathery smooth and wrinkled when dry**.** Seeds 5–8 × 4–6 mm, reniform to round, smooth, olive-green to brown (Pl. 1f-j). Flowering from January to June. Distributed along the Mediterranean coast from Sallum in the west to Rafah at the Palestinian authority borders.

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Identification key to the traced Egyptian *Retama* taxa:

1. Standard shorter than wings……………………………………………………… 2

– Standard as long as wings………………………………………………………… 3

2. Fruit elliptical, peak gradually apiculate, seed yellow to orange …….... *R. raetam* Form 2

– Fruit globose, peak abruptly apiculate, seed olive-green to brown……... *R. monosperma*

3. Fruit length to 1.5 cm, with one seed……………………………………… 4

– Fruit length exceeds 1.5 cm, with 1–2 seeds………………………………5

4. Standard elliptical, calyx green-purple…………………………… *R. raetam*

– Standard rhombic, calyx purple………………………………… *R. raetam* Form 4

5. Flower length not exceeding 1 cm, seed olive-green to brown ………………… 6

– Flower length up to 1.5 cm, seed yellow to orange ……………………………..7

6. Calyx up to ½ flower, standard elliptical……………………...... *R. raetam* Form 8

– Calyx up to ⅓ flower, standard orbicular……………………… *R. raetam* Form 7

7. Fruit globose, with inflorescence from 1 to 15 flowers…... …..…... *R. monosperma* Form 5

– Fruit elliptical, with inflorescence from 1 to 7 flowers.……………… *R. raetam* Form 6

Morphological traits distinguish the *Retama* Forms from the identified species

***R. raetam* Form 2:** This form represents the three typical forms (1, 2 and 3) with standard shorter than the wing and the keel; fruit elliptical, with a gradually apiculate peak. Yellow to orange seeds (Pl. 2a). Diagram 1 (a-f) shows that this form is significantly different from *R. raetam* in both leaf length and width, standard length and keel length (P = 0.0001), wing length (P = 0.0335).

***R. raetam* Form 4:** standard as long as the wing and the keel. Standard shape rhombic (Pl. 2b) keel mid-vein white. Fruit length to 1.5 cm with one seed. Diagram 1 (b & c), shows that this form is significantly different from *R. raetam* in leaf width (P = 0.0019) and flower length (P = 0.0001).

***R.monosperma* Form 5:** standard is as long as the wing and the keel. Flower length up to 1.5 cm, with inflorescence from 1–15 flowers (Pl. 2c). Fruit globose exceeds 1.5 cm in length with 1–2 yellow to orange seeds (Pl. 2a). Diagram 1 (a–f) shows the significant differences from *R. monosperma* in leaf length and width, flower, standard, wing and keel lengths (P < 0.0001).

***R. raetam* Form 6:** standard as long as the wing and the keel. Flower up to 1.5 cm (Pl. 2d). Inflorescence 1–7 flowers. Fruit elliptical exceeds 1.5 cm with 1–2 yellow to orange seeds. Diagram (1 a, b, d and f) shows that this form is significantly different from *R. raetam* in leaf length and width (P < 0.0001), as well in standard length and keel length (P = 0.0053, P = 0.0015; respectively).

***R. raetam* Form 7:** standard as long as the wing and the keel. Flower not exceeding 1 cm. Calyx up to ⅓ flower (Pl. 2e). Standard orbicular (Pl. 2f). Fruit exceeds 1.5 cm with 1–2 olive-green to brown seeds. Diagram 1 (a-e) shows that form 7 is significantly different when compared with *R. raetam* in leaf length and width, standard length ($P < 0.0001$), and wing length ($P = 0.0020$).

***R. raetam* Form 8:** standard as long as the wing and keel. Flower not exceeding 1 cm. Calyx up to ½ flower (Pl. 2g). Standard elliptical (Pl, 2h). Fruit exceeds 1.5 cm with 1–2 olive-green to brown seeds. Diagram 1 (a, b and f) shows that this form is significantly different from *R. raetam* in leaf length & width and keel length ($P < 0.0001$), as well as the standard length ($P = 0.0006$) and wing length ($P = 0.0001$) as shown in Diagram 1 (d and e).

Morphological similarity of the identified *Retama* species and forms: Diagram (2) shows the UPGMA dendrogram based on 94 macro-morphological characters. It demonstrates the grouping of the studied *Retama* taxa into two main clusters (I and II). Cluster I included *R. monosperma* and *R. monosperma* Form 5, and cluster II included *R. raetam* and five forms of *R. raetam* (Form 2, Form 4, Form 6, Form 7, and Form 8). While the Jaccard's coefficient (Tab, 2) was used to estimate the similarity between the studied *Retama* species and the forms based on the 94 macro-morphological characteristics. The similarity value between *R. raetam* and *R. monosperma* was 60.4 %. Evaluation of the similarity between *Retama* species and the identified forms denoted that the highest similarity (91.6 %) was between *R. raetam* and *R.raetam* Form 4. Also the lowest similarity was between *R. monosperma* and *R. raetam* Form 6 (49.4 %).

Diversity in morphological features of the identified *Retama* species and forms: There were notable differences among and between the morphological features of the studied *Retama* taxa such as leaves, flowers, standard, wing, and keel lengths (Diag. 1a-f). The comparable features of the standard (Diag. 3), verify that *R. monosperma* had the shortest standard length compared to *R. raetam* and the identified forms. While *R. monosperma* Form 5 had the widest standard among the rest of the forms, followed by *R. raetam* Form 4 and *R. monosperma* were nearly equal in standard width. While *R. raetam* Form 8 had the narrowest standard. The wings also showed diversity among the studied taxa. Diagram (4) shows that *R. monosperma* Form 5 had the longest wings, while *R. raetam* Form 8 had the shortest wing compared with *R. raetam*, *R. monosperma*, and other forms. Also, *R. monosperma* had the widest wing and *R. raetam* Form 7 had the narrowest wing, while *R. raetam*, *R. raetam* Form 2, *R. raetam* Form 6 and *R. raetam* Form 8 were equal in wing width.

The molecular identity of the identified *Retama* species and forms: The ISSR marker analysis using 12 primers produced a total of 152 bands, of which 85 bands are polymorphic and 37 are monomorphic (Tab. 3). The band sizes varied between 150 and 2500 bp. The highest matching primers were primers 10 and 14 with 20 bands/each, followed by primers 13 and 19. Further, the lowest number of bands (8 bands/each primer) was obtained with primers 6, 9, and 20. Primer 13 produced bands (13 bands) showing the highest polymorphism (81.3 %). Primer 19 produced more number of unique bands (7 bands) with *R. monosperma* and Forms (4, 6, and 8).

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Retama monosperma was distinguished by five unique bands at 900, 1400, 500, 1250 & 1400 bp with primers 9, 10, 14, and 19; respectively. While, *R. raetam* could be distinguished from *R. monosperma* by 2 unique bands at 450 bp (primer 9) and at 360 bp (primer 10). *R. raetam* Form 2 was distinguished by two unique bands with primers 10 and 20 at 180 and 220 bp, respectively, and an absent band with primer 5 at 200 bp. The two closely related forms, *R. raetam* Form 7 and *R. raetam* Form 8, could be distinguished using primer 14 (unique bands at 2500 and 490 bp, respectively). Primer 20 could distinguish between the *R. monosperma* Form 5 and *R. raetam* Form 6 (two bands at 400 and 450 bp were absent and one band at 150 bp was unique, respectively). Similarly, primers 5, 6, 10, 13, and 19 produced eight unique bands for *R. raetam* Form 4 (Tab. 3).

Molecular grouping of the identified *Retama* taxa based on ISSR data: The DCA plot (Diag. 5) based on the ISSR markers data revealed that the identified *Retama* species and forms could be grouped into two main clusters (I and II). Cluster I included *R. monosperma* and *R. monosperma* Form 5; while cluster II included *R. raetam* and its Forms 2,4,6,7 and 8. This grouping is congruent with the grouping derived from the macro-morphological characters (Diagram 2).

Geographic distribution of the identified taxa in Egypt: This revision revealed that *Retama raetam* was distributed in all the phytogeographic regions in Egypt, mainly in the desert wadis, sandy and gravel plains. Contrastingly, *R. monosperma* was distributed along the Mediterranean coast from Sallum in the west to Rafah at the Palestinian authority border & the Red Sea coast and close to the salt-affected littoral plains. The identified forms were basically from South Sinai with a westward extension to the northern part of the Eastern Desert (Map 1).

DISCUSSION

The confusion regarding the taxonomy of the *Retama* Raf. species is not only based on its generic characters (Lopez *et al.*, 1998) but also closely related to phenology (León-González *et al.*, 2018) and the presence of several varieties (Benmiloud-Mahieddine *et al.*, 2011). This confusion extends to the species description reported previously and the authors noticed misalignments in several points, which will be clarified in this part.

***Retama raetam* (Forssk.) Webb & Branthl:** The current revision of the *Retama* taxa in Egypt revealed the identification of two species and five forms. The first species was *R. raetam* (Forssk.) Webb, which was characterized by linear-oblong leaves, is consistent with previous reports (Zohary, 1972; Jafri, 1980). However, these reports mentioned larger leaf dimensions (5–20 × 3–8 mm) while the studied specimens showed smaller dimensions (2–8 (-23) × 0.5–3 mm). This reduction in leaf size might be due to the arid environment of the Egyptian desert (Barakat *et al.*, 2013; Amer *et al.*, 2021). While the specimens we examined exhibited lax–dense inflorescence with 1–7 flowers (Pl. 1e), previous studies only reported 1–5 (Zohary, 1972; Jafri, 1980; Boulos, 1999). These authors also reported longer flower length (15 mm) which did not exceed 12 mm in the current study (Pl. 1a). The calyx color has also been debatable among authors. For instance, reports have shown purple (Zohary, 1972; Jafri,

1980) and reddish calyx (Mouterde, 1966) while this work reported a greenish-purple calyx (Pl. 1a).

The standard elliptic, as long as wings (Pl. 1a) with standard length as reported previously (Mouterde, 1966; Zohary, 1972; Täckholm, 1974; Jafri, 1980; Boulos, 1999). While the shape varies, it was obovate-orbicular (Zohary, 1972; Jafri, 1980); rhombic-ovate (Webb, 1853); ovate-oblong (Tutin et al., 1968). The fruit length was 10–15 mm (Pl. 1c), while previous reports showed 20 mm (Tutin et al., 1968; Zohary, 1972; Jafri, 1980; Boulos, 1999). Authors have attributed the smaller fruit size to the aridity in Egypt (Amer et al., 2021). The observed one-seeded, indehiscent pods were consistent with Boulos (1999) and Täckholm (1974). Fruits were elliptical (Pl. 1b, c) consistent with Webb (1853) while relevant shapes were also mentioned as ovoid-oblong (Zohary, 1972; Jafri, 1980;), and ovoid (Mouterde, 1966); oblong-ovate -Webb and Berthelot, 1836) and obovate-ellipsoid (Tutin *et al*., 1968).

The fruit attenuated gradually to an erect peak (Pl.1b, c), allied with Zohary (1972) and Jafri (1980). In contrast, the oblique-peak shape described by Mouterde (1966) did not observe. Fruit pericarp is fleshy, leathery smooth when dry (Pl.1b, c), similar to Jafri (1980) and Zohary (1972). The same authors supported the observed seed color (olive-green to brown; Pl. 1d), as well as Boulos (1999). The seed was black (Webb, 1853; Täckholm, 1974). Chiapella *et al*. (2009) reported that Retama raetam (Forssk.) Webb subsp. raetam is a Saharo-Arabian taxon, distributed in North Africa, Syria, Israel, Lebanon, and the Arabian Peninsula.

***Retama monosperma* (L.) Boiss.:** The second species, was *R. monosperma* (L.) Boiss., the shrub height was up to 3 m, consistent with Tutin *et al.* (1968); Polunin (1969); Boulos (1999); it reached 4 m (Quezel and Santa, 1962) or up to 4.5 m (Muñoz Vallés *et al.*, 2013). The inflorescence was lax raceme with 1–7 flower/inflorescence (Pl. 1j), and it is consistent with Tutin *et al.* (1968) and Muñoz Vallés *et al.* (2013). While non-parallel data (10–26 flowers/inflorescence) was reported by the later. The flower length was 7–10 mm (Pl. 1f), similar to Tutin *et al.* (1968) and Muñoz Vallés *et al.* (2013). While previous studies showed larger flower, it reached 15 mm (Polunin, 1969) and 15–17 mm (Täckholm, 1974; Boulos, 1999). The flowers had purple-colored calyx (Pl. 1f), it appears similar to the reddish calyx reported by Muñoz Vallés *et al.* (2013).

The notable characteristics of *Retama monosperma* were: (1) The shorter standard compared to wings (Pl. 1f), which has been previously verified (Quezel and Santa, 1962; Täckholm, 1974; Boulos, 1999). The detected standard was orbicular while Tutin *et al.*, (1968) described it as rhombic-ovate. (2) The globoid fruit (Pl. 1g, h) was consistent with previous reports (Polunin, 1969; Täckholm, 1974; Muñoz Vallés, 2013). (3) The fruit was 1–2 seeded, abruptly attenuated to a short-apiculate peak (Pl. 1g) as shown by Muñoz Vallés *et al.* (2013) while the fruit pericarp was wrinkled (Pl. 1h), consistent with that shown by Barker-Webb and Berthelot (1836) in both *R. raetam* and *R. monosperma* fruits.

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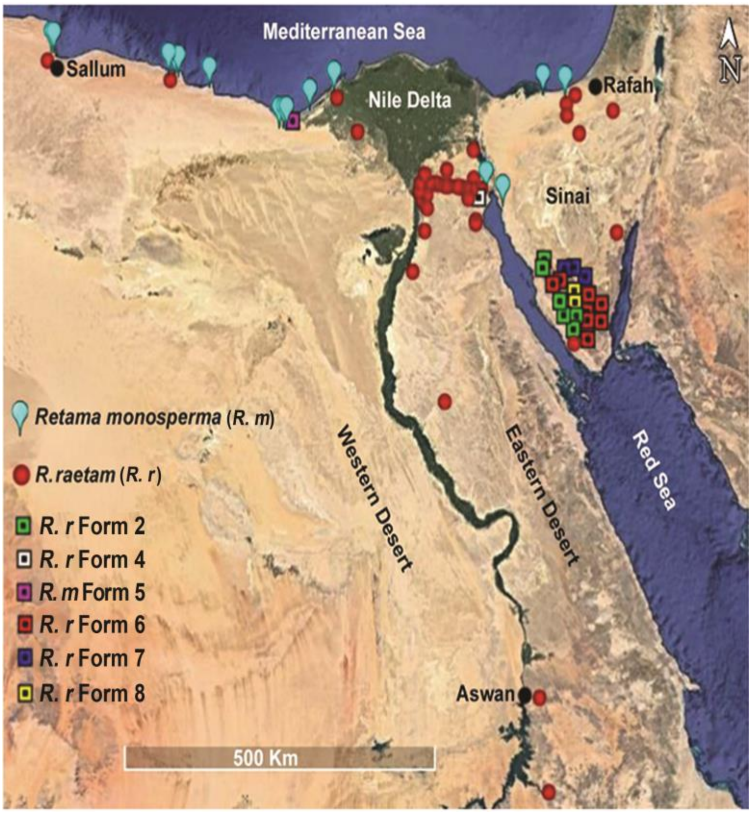
The identified species and Forms *vs.* the taxa reported by Täckholm (1974): Täckholm (1974) mentioned the presence of *Lygos raetam* (Forssk.) Heywood var. *sarcocarpa* (Zoh.) Täckh.et Boulos (syn. *R. raetam* subsp. *gussonei* (Webb) Greuter) in Egypt. Some of the identified forms (*R. raetam* Form 2, *R. raetam* Form 4, *R. raetam* Form 8) have characters similar to subsp. *gussonei*, such as diffusely deflexed-branching with silky-silvery indumentum (Zohary, 1972) and the fleshy fruit pericarp (Täckholm, 1974). The dehiscent ellipsoidal-ovoid pod distinguishes subsp. *gussonei* (Zohary, 1972), this inconsistent trait did not fit into any of the identified forms, where all forms possess indehiscent elliptical-globoid pods. *R. monosperma* Form 5 that have yellow seeds (Pl. 2a), and wrinkled fruit pericarp was similar in color to subsp. *gussonei* (Zohary, 1972; Täckholm, 1974). While, the presence of an indehiscent pod and the inflorescence with 15 flowers in *R. monosperma* Form 5 hampered the fitting it with subsp. *gussonei*. In addition, Chiapella *et al.* (2009) restricted the presence of subsp. *gussonei* to Italy on the Ionian coast of Calabria and Southern Sicily.

Täckholm (1974) was expected the presence of at least three nameless varieties in south Sinai. This revision identified four Forms (Form 2, Form 6, Form 7, and Form 8) from South Sinai, its seed length was 7–8 mm and the fruits were green, unlike three nameless varieties given by Täckholm (1974). She reported that the seeds ~ 6 mm and black to dirty-yellow fruit or seeds: ~ 3 mm with white hilum. Therefore, all the varieties expected by Täckholm (1974) were not consistent with the observations retrieved in the current work.

The identified Forms vs. *Retama monosperma* subsp. *bovei* recorded in Egypt by Boulos (1999): *Retama monosperma* (L.) Boiss. subsp. *bovei* (Spach) Maire (syn. *R. bovei* (Spach) Webb), found in Egypt, were characterized by flowers ranging from 15–17 mm, standard shorter than wings, pod obovoid and seeds reddish-brown or olive-green. The identified *R. monosperma* Form 5 has a globular pod exceeding 15 mm and a yellow-orange seed, unlike that of subsp. *bovei* (Boulos, 1999). Additionally, *R. monosperma* Form 5 lacking the pulvinus-branches base characterized the subsp. *bovei* (Webb, 1853). Moreover, subsp. *bovei* is endemic to Algeria and Morocco (Benmiloud-Mahieddine *et al.*, 2011).

The morphological and molecular similarity of the identified *Retama* taxa: The UPGMA dendrogram based on the macro-morphological characters grouped the *Retama* taxa into two main clusters (I & II). Cluster I included *R. monosperma* and *R. monosperma* Form 5, and cluster II included *R. raetam* and *R. raetam* Forms (2, 4, 6, 7, and 8; Diagram 2). The similarity value between *R. raetam* and *R. monosperma* was 60.4 % while the similarity values between the *R. raetam* & forms were between 64.8 % to 91.6 % (Tab. 2). The morphological similarities among the *Retama* taxa (species and forms) might be attributed to similarities in chromosome numbers. This postulation, based on the karyotype analysis of the *R. monosperma* (4 populations) and *R. raetam* (17 populations) from Algeria, showed that the chromosome number was the same (2n = 48) (Benmiloud-Mahieddine *et al.*, 2011).

The DCA plot based on ISSR markers (Diag. 5), showed congruent clustering whereas *R. monosperma* and its Form 5 and *R. raetam* as well as its forms were grouped in different clusters. The molecular grouping was consistent with the morphological grouping (Diag. 2) and might be attributed to the difference in the B chromosome, as reported by Chiapella *et al.* (2009) who revealed that the chromosome number in *Retama* taxa was 2n = 48 with up to 6 copies of the B chromosomes. For instance, *R. monosperma* from Portugal (2n = 48) and Spain (2n = 48 + 4B). *R. monosperma* var. *webbii* from different regions in Morocco were 2n = 48 + 4B and 48 + 6B; while *R. raetam* subsp. *raetam* from Algeria, Libya, Israel, and Algeria was 2n = 24, 48, 48 + 3B, and 48 + 3B, respectively. The subsp. *gussonei* populations from different regions in Italy had different numbers (2n = 48, 48 + 4B & 48 + 6B). Benmiloud-Mahieddine *et al.* (2011), clarify the significance of infraspecific variation in chromosome numbers when correlated with the geographic distribution of the studied *Retama* populations, and the identified forms might be newly adapted populations, consistent with Amer *et al.* (2021) who reported that a recent increase in aridity induced molecular variations in *R. raetam* populations and the pressure of grazing increased the use of these shrubs as fodder in these areas (Barakat *et al.*, 2013). Also, Stebbins (1971) claimed that polyploids are better adapted to extreme conditions than their diploid progenitors.



Map (1): Distribution pattern of *Retama* species and Forms in Egypt.

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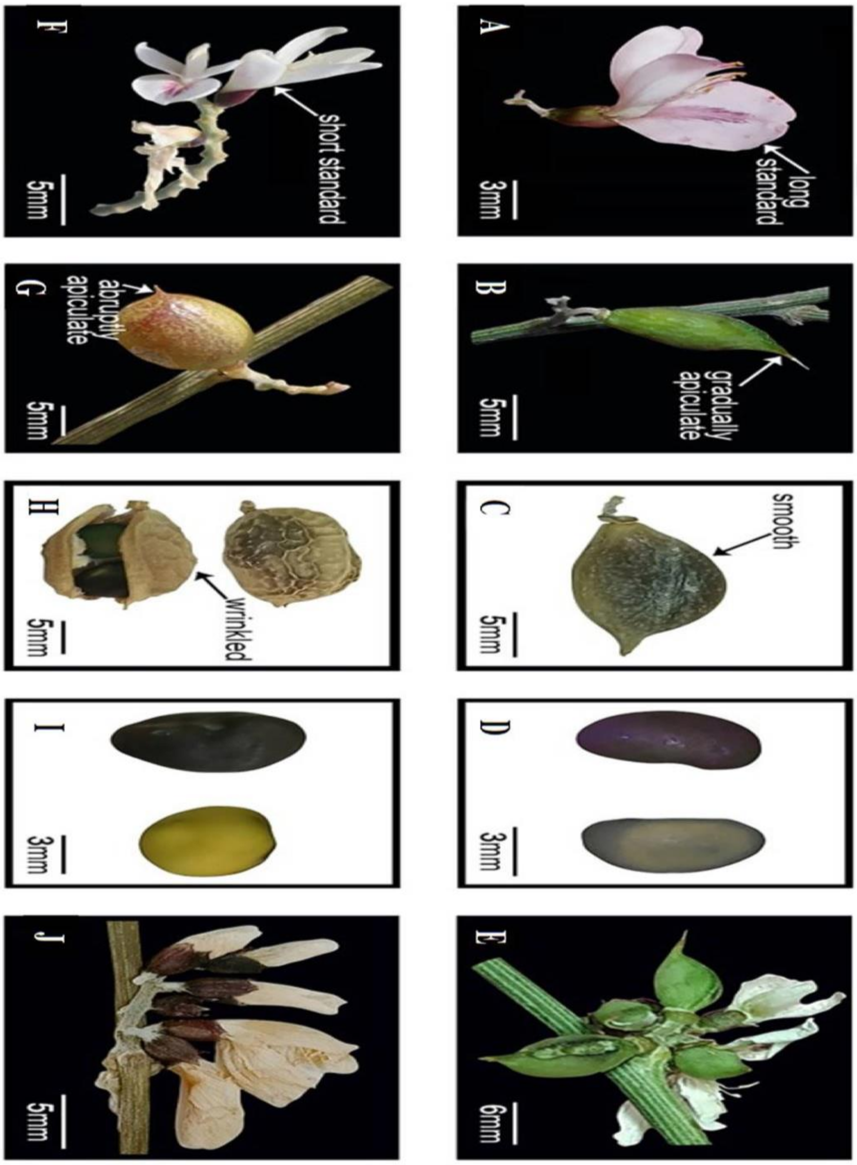

Plate (1): Characteristic morphological features of *Retama raetam* and *R. monosperma*; (A–E.) Represent *R. raetam*, (A) Flower showing standard as long as wings, (B) Elliptic fruit and gradually apiculate peak, (C) Dry one-seeded fruit with leathery smooth pericarp, D: Olive green and brown seeds, and E: Dense inflorescence; (F-J) Represent *R. monosperma*, (F) Flower showing standard shorter than wings, (G) Globose fruit and abruptly apiculate peak, (H) Dry two seeded fruit with wrinkled pericarp, (I) Olive green and brown seeds, and (J) Lax inflorescence.

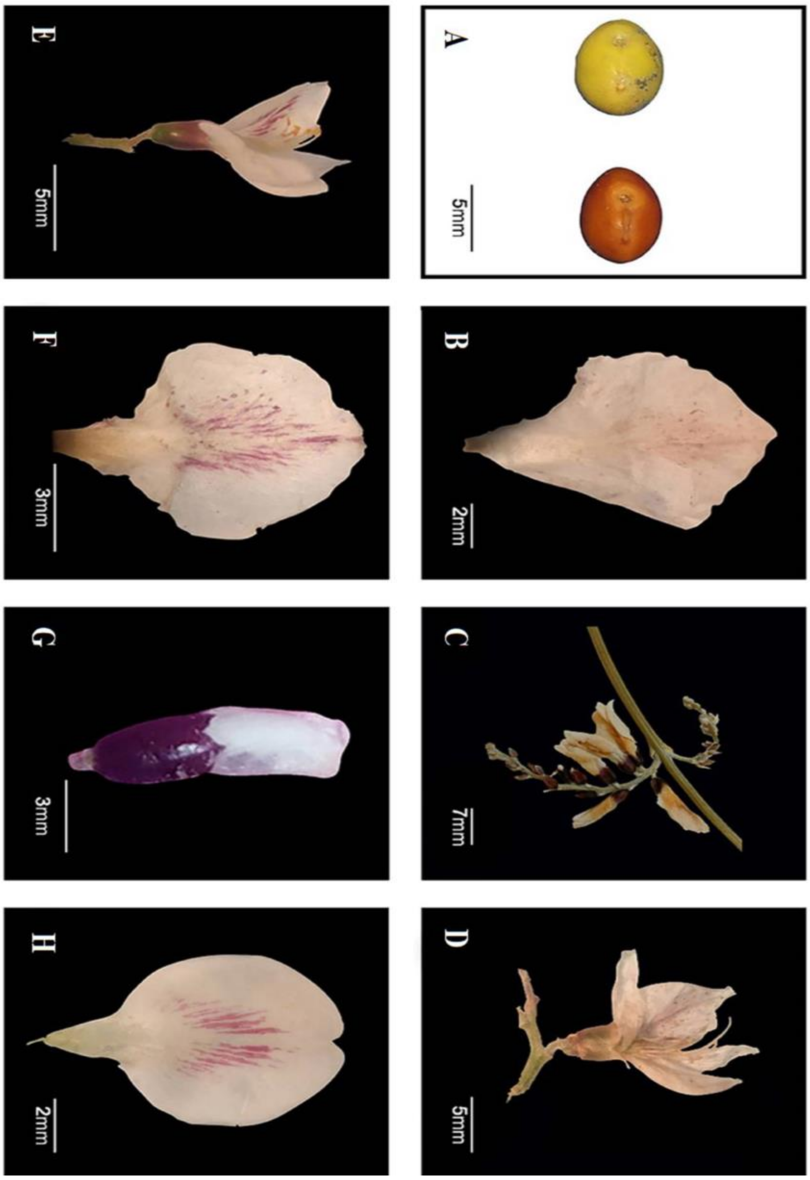

Plate (2): Characteristic morphological features of the identified forms; **(**A) Yellow and orange seed in *R. raetam* Form 2 and *R. monosperma* Form 5, (B**)** Rhombic standard in *R. raetam* Form 4, (C) Lax inflorescence with 15 flowered in *R. monosperma* Form 5, (D) Flower length up to 1.5 cm in *R. raetam* Form 6, (E) Calyx ⅓ flower length in *R. raetam* Form 7, **(**F) Orbicular standard in *R. raetam* Form 7, (G) Calyx ½ flower length in *R. raetam* Form 8, and (H**)** Elliptical standard *R. raetam* Form 8.

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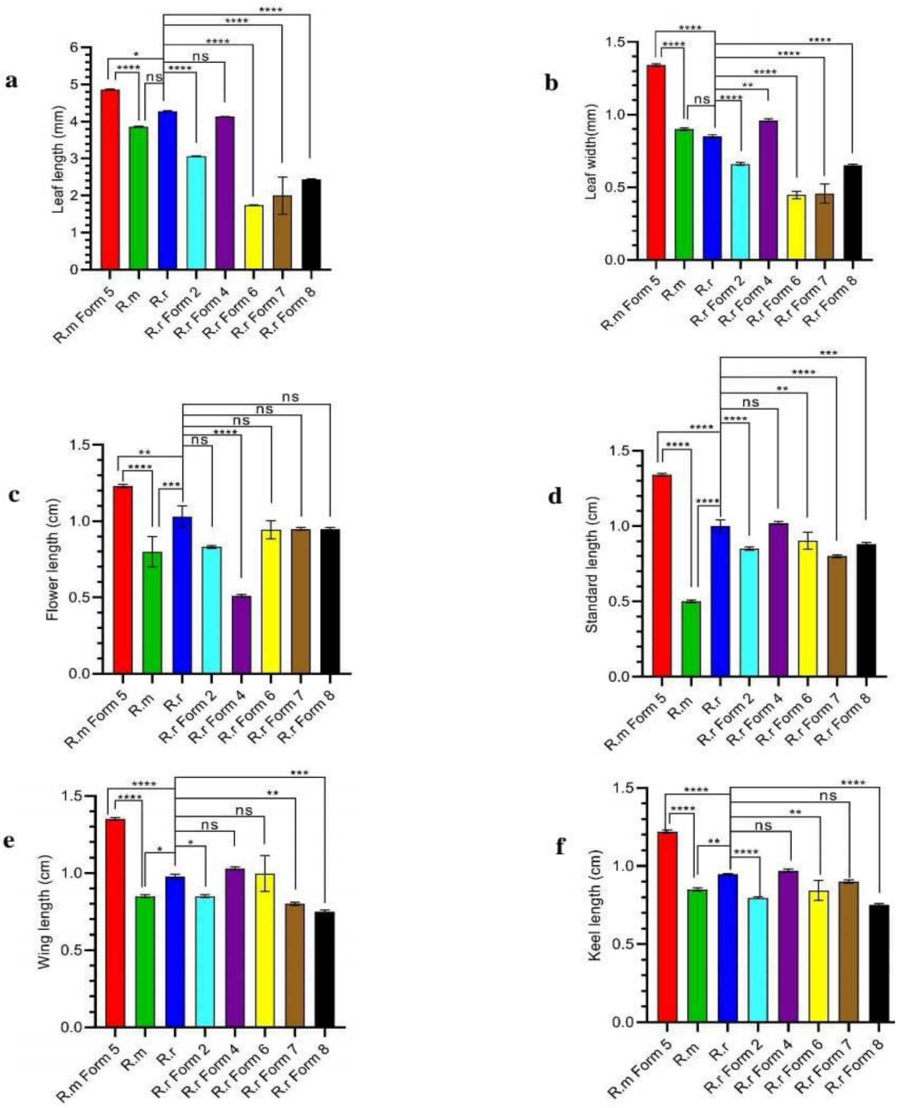

Diagram (1): Histograms of different morphological characters between *Retama raetam* (*Rr*), *Retama monosperma* (*Rm*), and the identified forms (*Rr* Forms 2, 4,6,7,8 & *Rm* Form 5). *: a Significant difference. (a) *: P = 0.0183; (b) **: P < 0.0019; (c) **: P = 0.0023 & ***: P < 0.0006; (d) **: P = 0.0053, ***: P = 0.0006; (e) *: P < 0.0335, **: P < 0.0020, ***: P < 0.0001; (f) **: P = 0.0015; ****: P < 0.0001 & ns: non-significant.

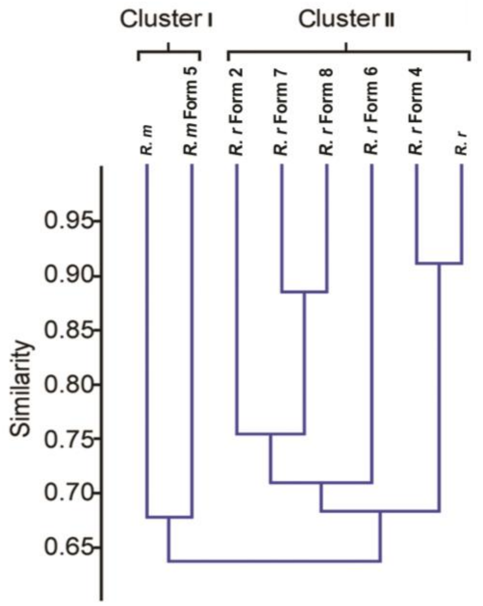

Diagram (2): UPGMA dendrogram of the identified *R. raetam* (*Rr*), *Retama monosperma* (*Rm*), and the identified forms (*Rr* Forms 2, 4,6,7,8 & *Rm* Form 5), based on macro-morphological characters using the Jaccard similarity matrix.

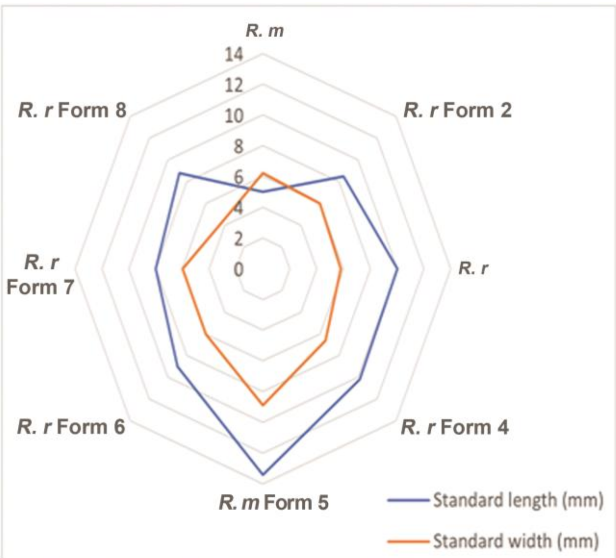

Diagram (3): Diversity in standard length and width of *R. raetam* (*Rr*), *Retama monosperma* (*Rm*), and the identified forms (*Rr* Forms 2, 4,6,7,8 & *Rm* Form 5), by Rader plot.

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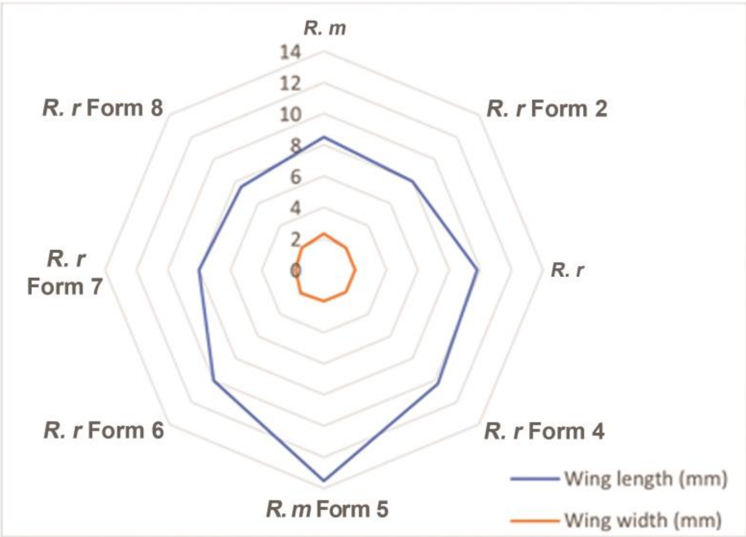

Diagram (4): Diversity in wing length and width of *R. raetam* (*Rr*), *Retama monosperma* (*Rm*), and the identified forms (*Rr* Forms 2, 4,6,7,8 & *Rm* Form 5), by Rader plot.

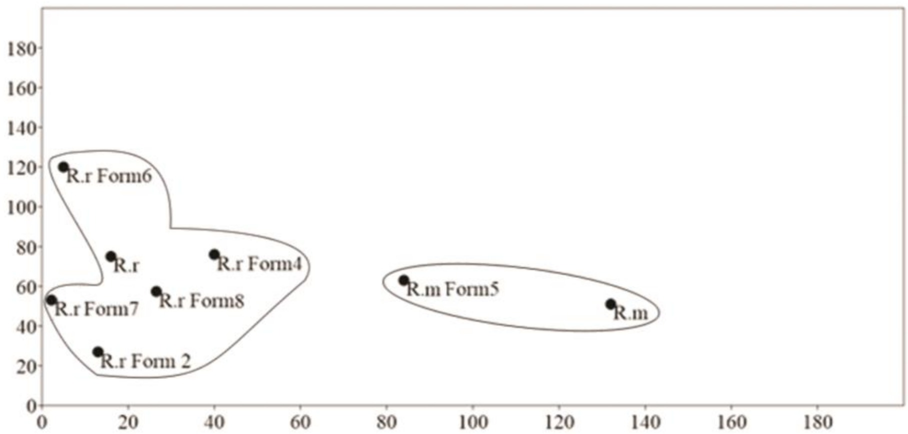

Diagram (5): Detrended correspondence analysis (DCA) based on the ISSR markers data of the studied taxa.

Table (1): Localities of the collected fresh populations (used for taxonomic and molecular investigations) and their GPS coordinates arranged from South to North.

Locality	Location	
	Latitude (N)	Longitude (E)
1. Wadi Nasb, South Sinai	28° 27’ 30.6”	34° 4’ 26.292”
2. Wadi Nasb, South Sinai	28° 28’ 4.008”	34° 5’ 58.308”
3. Wadi Nasb, South Sinai	28° 28’ 53.868”	34° 6’ 30.708”
4. Wadi Nasb, South Sinai	28° 29’ 13.632”	34° 6’ 55.98”
5. Wadi Nasb, South Sinai	28° 29’ 42.432”	34° 0’ 5.688”
6. Saint Catherine, South Sinai	28° 33’ 18.144”	33° 56’ 48.3”
7. Saint Catherine, South Sinai	28° 33’ 34.164”	33° 55’ 2.28”
8. Saint Catherine, South Sinai	28° 33’ 34.632”	33° 56’ 51.395”
9. Abo Sela, South Sinai	28° 35’ 21.264”	33° 55’ 44.939”
10. Abo Sela, South Sinai	28° 35’ 36.384”	33° 55’ 30.251”
11. El Sheikh Awad, South Sinai	28° 38’ 23.208”	33° 53’ 23.064”
12. El Sheikh Awad, South Sinai	28° 38’ 55.464”	33° 53’ 22.2”
13. El Sheikh Awad, South Sinai	28° 39’ 4.392”	33° 53’ 54.347”
14. Eltarfa, South Sinai	28° 41’ 40.164”	33° 56’ 35.16”
15. Eltarfa, South Sinai	28° 41’ 49.164”	33° 57’ 39.636”
16. Wadi Hagoul, Cairo-Suez Road	29° 51’ 04.4”	32° 16’ 06.8”
17. Wadi Hagoul, Cairo-Suez Road	29° 53’ 11.3”	32° 14’ 12”
18. Wadi Hagoul, Cairo-Suez Road	29° 58’ 08.3’	32° 08’ 13.1”
19. Western Mediterranean coast, Omyed	30° 49’ 41.7”	29° 12’ 15.4”
20. Western Mediterranean coast 24 km to Matrouh	30° 49’ 41.7”	29° 12’ 15.5”
22.Western Mediterranean coast, Marakia	30° 55’ 54”	29° 28’ 40.3”

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Table (2): Jaccard's similarity coefficient between the studied *Retama* species and forms based on macro-morphological characters.

Taxa	Jaccard's similarity coefficient							
	R. monosperma	*R. raetam* Form 2	*R. raetam*	*R. raetam* Form 4	*R. monosperma* Form 5	*R. raetam* Form 6	*R. raetam* Form 7	*R. raetam* Form 8
R. monosperma	100							
R. raetam Form 2	67.1	100						
R. raetam	60.4	75.3	100					
R. raetam Form 4	65.2	72.4	91.6	100				
R. monosperma Form 5	67.1	55.2	67.4	68.6	100			
R. raetam Form 6	49.4	65.9	71.8	76.8	67.9	100		
R. raetam Form 7	68.3	74.1	64.8	65.9	65.9	67.1	100	
R. raetam Form 8	70.2	71.8	71.4	72.2	64.0	71.1	86.1	100

Table (3): Polymorphism percentages and characteristic bands detected using ISSR marker analysis for the *Retama* species and forms; test was applied to triple for species and forms.

ISSR Primers	**Number of bands**			**Number of total amplified bands Unique bands between bracts**								**% Polymorphism**
	Monomorphic	**Polymorphic**	**Total & (Unique) bands**	***R. monosperma***	***R. raetam* Form 2**	***R. raetam***	***R. raetam* Form 4**	***R. monosperma* Form 5**	***R. raetam* Form 6**	***R. raetam* Form 7**	***R. raetam* Form 8**	
Primer 3 5\`-ACACACACACACACACYT-3\`	3	9	12 (0)	7 (0)	8 (0)	8 (0)	6 (0)	9 (0)	7 (0)	8 (0)	8 (0)	75 %
Primer 5 5\`-GTGTGTGTGTGTGTGTYG-3\`	1	7	11 (3)	4 (0)	3 (0)	4 (0)	8 (3)	7 (0)	8 (0)	8 (0)	7 (0)	63.64 %
Primer 6 5\`-CGCGATAGATAGATAGATA-3\`	2	4	8 (2)	3 (0)	3 (0)	4 (0)	8 (2)	5 (0)	5 (0)	5 (0)	5 (0)	50 %

Primer 9 5`-GATAGATAGATAGATAGC-3`	2	4	8 (2)	3 (1)	6 (0)	6 (1)	6 (0)	5 (0)	5 (0)	5 (0)	5 (0)	50 %
Primer 10 5`-GACAGACAGACAGACAAT-3`	2	12	20 (6)	9 (1)	8 (1)	9 (1)	11 (1)	6 (1)	10 (0)	11 (1)	3 (0)	60 %
Primer 11 5`-ACACACACACACACACYA-3`	3	8	13 (2)	9 (0)	9 (0)	6 (0)	4 (0)	7 (0)	5 (2)	10 (0)	10 (0)	61.5 %
Primer 13 5`-AGAGAGAGAGAGAGAGYT-3`	2	13	16 (1)	8 (0)	7 (0)	6 (0)	12 (1)	11 (0)	11 (0)	3 (0)	2 (0)	81.3 %
Primer 14 5`-CTCCTCCTCCTCCTCTT-3`	8	9	20 (3)	10 (1)	16 (0)	14 (0)	14 (0)	14 (0)	15 (0)	16 (1)	17 (1)	45 %
Primer 16 5`-TCTCTCTCTCTCTCTCA-3`	8	1	10 (1)	8 (0)	9 (0)	9 (0)	9 (0)	9 (0)	9 (0)	10 (1)	9 (0)	10 %
Primer 18 5`-HVHCACACACACACACAT-3`	3	8	12 (1)	5 (0)	4 (0)	3 (0)	5 (0)	10 (0)	11 (0)	10 (0)	11 (1)	66.7 %
Primer 19 5`-HVHTCCTCCTCCTCCTCC-3`	1	6	14 (7)	8 (2)	6 (0)	4 (0)	6 (1)	1 (0)	4 (1)	1 (0)	8 (3)	42.9 %
Primer 20 5`-HVHTGTGTGTGTGTGTGT-3`	2	4	8 (2)	5 (0)	5 (1)	4 (0)	4 (0)	2 (0)	5 (1)	6 (0)	6 (0)	50 %
Total	37	85	152 (30)	79 (5)	84 (2)	77 (2)	93 (8)	86 (1)	95 (4)	93 (3)	91 (5)	

CONCLUSIONS

The current revision was conducted to resolve the morphological diversity and addressed the nomenclature debates regarding the genus *Retama* Raf. in Egypt. We identified two distinct species, *R. raetam* (Forssk.) Webb and *R. monosperma* (L.) Boiss. along with five forms of *Retama raetam* (Forssk.) Webb & Berthel. (Form 2, Form 4, Form 6, Form 7, and Form 8). In addition to Form 5 of *Retama monosperma* (L.) Boiss. These forms showed significant differences in both morphological characteristics and genetic identity. Each of the identified forms showed inconsistent characteristics with its related species (*raetam* or *monosperma*). The results revealed that most of the forms were located in South Sinai, this may highlights the influence of geographic and climatic factors on taxa features and taxonomic identity. Further molecular investigations were highly recommended to resolve the taxonomic identity of the identified forms in genus *Retama*.

CONFLICT OF INTEREST STATEMENT

"The authors have no conflicts of interest to declare".

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مراجعة تصنيفية لجنس *Retama* Raf., 1838 في مصر مدعوما بالبصمة الجزيئية

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الخلاصة

في مصر، الهوية التصنيفية للأصنوفات تحت جنس *Retama* Raf., 1838 من العائلة البقولية (Fabaceae) لا يزال غير واضح وتشابهه الشكل الخارجي يجعل تحديد هويته أمرا صعبا. تهدف الدراسة الحالية إلى مراجعة هذا الجنس بمصر وذلك فيما يتعلق بالأنواع الموجودة وما يميزها من صفات شكلية ووراثية وجغرافية. وللوصول الى هذا الهدف، استخدمت عينات الجنس *Retama* الطرية والمعشبية. لقد أجرينا المراجعة المظهرية باستخدام 94 سمة شكلية ودرسنا الهوية الجزيئية باستخدام علامات تكرار التسلسل البسيط و كشفت هذه المراجعة عن تحديد نوعين متميزين هما *Retama raetam* (Forsskk.) Webb و Retama monosperma (L.) Boiss إلى جانب خمسة أشكال تحت اصنوفة *Retama raetam* (النموذج 2، النموذج 4، النموذج 6، النموذج 7، والنموذج 8). بالإضافة إلى النموذج 5 من تحت *R. monosperma*. وقد أظهرت الهوية المظهرية والجزيئية لهذه الأشكال اختلافات كبيرة في نفس الوقت كونت مجموعات إحصائية متطابقة. تم توضيح التوزيع الجغرافي للأصناف في مصر. وتسلط هذه الدراسة الضوء أيضًا على أن *Retama* هو من الأنواع المهمة، معرضة للتأثر سلبا بالتغير المناخي وتتطلب برامج صون عاجلة .