A systematic revision of *Capparaceae* and *Cleomaceae* in Egypt: an evaluation of the generic delimitations of *Capparis* and *Cleome* using ecological and genetic diversity

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**Abstract**

**Background:** The *Capparaceae* family is commonly recognized as a caper, while *Cleomaceae* represents one of small flowering family within the order Brassicales. Earlier, *Cleomaceae* was included in the family *Capparaceae*; then, it was moved to a distinct family after DNA evidence. Variation in habits and a bewildering array of floral and fruit forms contributed to making *Capparaceae* a “trash-basket” family in which many unrelated plants were placed. Indeed, family *Capparaceae* and *Cleomaceae* are in clear need of more detailed systematic revision.

**Results:** Here, in the present study, the morphological characteristics and the ecological distribution as well as the genetic diversity analysis among the twelve species of both *Capparaceae* and *Cleomaceae* have been determined. The genetic analysis has been checked using 15 ISSR, 30 SRAP, and 18 ISTR to assess the systematic knots between the two families. In order to detect the molecular phylogeny, a comparative analysis of the three markers was performed based on the exposure of discriminating capacity, efficiency, and phylogenetic heatmap. Our results indicated that there is a morphological and ecological variation between the two families. Moreover, the molecular analysis confirmed that ISTR followed by SRAP markers has superior discriminating capacity for describing the genetic diversity and is able to simultaneously distinguish many polymorphic markers per reaction. Indeed, both the PCA and HCA data have drawn a successful annotation relationship in *Capparacea* and *Cleome* species to evaluate whether the specific group sort individual or overlap groups.

**Conclusion:** The outcomes of the morphological and ecological characterization along with the genetic diversity indicated an insight solution thorny interspecies in *Cleome* and *Gynandropsis* genera as a distinct family (*Cleomaceae*) and the other genera (*Capparis, Cadaba, Boscia*, and *Maerua*) as *Capparaceae*. Finally, we recommended further studies to elucidate the systematic position of *Dipterygium glaucum*.

**Keywords:** *Capparaceae*, *Cleomaceae*, Systematic revision, Genetic diversity, Ecological distribution

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Background

The flora of Egypt is among the richest within the Arab countries and comprises very vital genetic resources of medicinal, fodder, and fiber plants. Currently, plant diversity is under threat as never before. In agriculture, the broad selection of a few developed varieties has reduced the genetic base of the most essential food crops, and it has added to the withdrawal of hundreds of landraces [1]. The complicated relationship between the crops, and it has added to the withdrawal of hundreds of species with a reduced genetic base of the most essential food crops [1].

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In the Egyptian flora, the set of Capparaceae plus Cleomaceae includes seven genera, twenty-two species, and four varieties with a wide range of ecological and geographical distribution [8]. They differ considerably in their life forms from trees (e.g., Boscia angustifolia) or shrubs (e.g., Capparis cartilaginea) to annual (e.g., Gynandropsis gynandra) or perennial herbs (e.g., Cleome amblyocarpa). The Egyptian taxa of Capparaceae refer to the xerophytic communities [9, 10], without Gynandropsis gynandra that is a common weed of the arable fields [11]. The taxonomic approach of the family in Egypt is interested only on seed morphology [12], leaf anatomy [13], and pollen morphology [14]. The systematic review of the natural species of Capparaceae (except Cleome) announced the unclear occurrence of Boscia angustifolia, while Capparis spinosa is described by three varieties, viz., deserti, spinosa, and inermis [15]. Split from Cleomaceae may be unreasonable since complications are met in selecting the genera. Accurate qualified information on fruit construction and gynoecium is obscure or non-present. Therefore, the taxonomic relationships between Capparaceae and Cleomaceae are still at discussion. Tackholm [6] distinguished among the two families concurring to gland formation, fruit type, and development of gynophores, whereas Zohary [16] included the exciting genus Cleome in the subfamily Cleomoideae of Capparaceae. The Capparaceae in Boulos [8], however, involved together Cleomaceae and Capparaceae. On the species level, Tackholm [6] identified eight species of Capparis, while Boulos [8] divided the genus as three species and four varieties.

The current investigation was aimed to provide a broad description of the morphological, ecological distribution attributes, and connected species along with three selected molecular markers of 12 representing species of Capparaceae and Cleomaceae. In detail, first, family Capparaceae...
is in clear necessity of more detailed morphological, ecological, and molecular study. Modulation inhabits and a confusing display of floral and fruit profiles provided to forming Capparaceae a “trash-basket” family in which several discrete plants were ordered. Second is to evaluate the discrimination capacity and the performance of the three marker system involving ISSR, SRAP, and ISTR and, finally, to infer the taxonomic questions and species borders treating the genetic diversity of Egyptian Capparaceae and Cleomaceae germplasm. To date, there has been no announcement concerning the performance and effectiveness of ISSR, SRAP, and ISTR markers in Egyptian Capparaceae and Cleomaceae species.

Methods
Plant materials
A total of twelve plant samples of Capparaceae and Cleomaceae were collected from Sinai Peninsula (three plants), Western desert (two plants), and Eastern desert (seven plants) (Fig. 1) (Table 1). Associate species were recorded, and identification was performed according to Boulos [8, 11]. Plant density and cover were assessed by the random plot and line intercept methods. A hierarchical dichotomous analysis (TWINSPAN) was used to classify plant species due to their density by PAST 3.14 [34].

DNA extraction
A fresh leaf of Capparaceae and Cleomaceae samples was used to extract the total genomic DNA according to the manufacturer’s protocol using plant DNA purification mini kit (Thermo Scientific GeneJET kit, K0791, USA). In each species, three to five replicate DNAs were utilized. However, the concentration and quality of the DNA samples were verified in a Quawell Q5000 UV-Vis spectrophotometer (V2.1.4, USA); then, the DNA was diluted to 50 ng/μl for use in ISSR, SRAP, and ISTR assay. Both the stock and diluted portions were kept at –20 °C.

![Fig. 1 Map showing the distributions and locations of 12 Capparaceae species within Western, Eastern desert, and Sinai Peninsula in Egypt](image-url)
INSTRUMENTATION AND METHODS

**ISSR analysis**

The ISSR-PCR amplification was prepared according to the earlier method demonstrated by Sankar and Moore [35]. The amplification reaction of ISSR analysis was done in a total volume of 25 μl on a Sure Cycler 8800 Thermal Cycler from Agilent Technologies. The reaction combination of 25 μl involved 3.5 μl of Green PCR buffer, 1 μM of each primer, 0.5 μM of dNTPs (10 mM each) (Thermo Fisher Scientific), 1 unit of taq DNA polymerase (5 U/μL) (Thermo Fisher Scientific), and 40 ng DNA template. The PCR program was as follows, denaturation (one cycle) in 94 °C for 2 min, followed by 40 cycles as follows: 94 °C for 30 s, 44 °C for 45 s, 72 °C for 1 min and 30 s, and finally one cycle extension at 72 °C for 10 min, and 4 °C (infinite). The amplified products were separated on 1.2% agarose gel by electrophoresis. A 100 bp DNA ladder (GeneRuler plus, Thermo Scientific, SM0321) was utilized as the molecular guideline to verify the competent ISSR markers. The gels were stained in ethidium bromide (0.5 μg/ml), and the amplicons were pictured below UV light using the Gel Doc XR system (Bio-rad, America).

**SRAP analysis**

The SRAP analysis was presented as illustrated by Li and Quiros [26]. SRAP primer combinations were tested using 30 various combinations which employed utilizing seven reverse and nine forward primers (Table 2). Every PCR reaction mix of 25 μl included 3.5 μl of green PCR buffer, 0.3 μM of each primer, 200 μM of dNTPs, 1 unit of taq DNA polymerase, 30 ng of genomic DNA, and deionized water up to 25 μl. PCR amplification program comprised 4 min of denaturing at 94 °C, five cycles of three steps: 1 min of denaturing at 94 °C, 1 min of annealing at 35 °C, and 1 min of elongation at 72 °C. In the next 35 cycles, the annealing temperature was increased to 50 °C, and for an extension, one cycle of 7 min at 72 °C. GeneRuler 50 bp Plus DNA ladder (Thermo Scientific, SM0371) was utilized as a molecular guideline to verify the accurate SRAP markers.

**ISTR analysis**

ISTR assessment was conducted following Aga and Brynælsson [36]. ISTR primer combinations were primarily examined using a total of 70 primer combinations from seven reverse and ten forward primers. Within all primers screened, only 24 ISTR combinations were picked for advanced analysis (Table 2). Each PCR included a reaction mix of 3.5 μl of green PCR buffer, 200 μM of dNTPs, 0.3 μM of each primer, 50 ng of genomic DNA, 1 unit of taq DNA polymerase, and finally deionized water up to 25 μl. PCR amplification performed involved of 1 cycle at 95 °C; 3 min; 40 cycles of 94 °C, 30 s; 45 °C, 30 s; 72 °C, 2 min; 1 cycle at 72 °C, 10 min; and 4 °C for infinitive. However, amplification products were separated and visualized subsequent the same procedure described for ISSR.

**Data analysis**

All clearly detectable ISSR, SRAP, and ISTR products were counted as band absence (0) and presence (1) using the Bio-Rad Gel Doc™ XR+ imaging analysis system with Image Lab™ (USA), and adjusted manually as necessary and collected onto a data matrix. However, the evaluations of the marker efficiency, level of polymorphism, discriminating capacity, and informativeness of the three marker profiles were calculated according to the indices of Powell et al. [37].

To measure the effectiveness of the three marker systems, polymorphic information content (PIC) was analyzed using the following formula of Roldán-Ruiz et al. [38] $PIC = 2fi(1 – fi)$, where $fi$ is the frequency of

| No. | Species                     | Latitude | Longitude | Altitude/m | Location          |
|-----|-----------------------------|----------|-----------|------------|-------------------|
| 1   | Capparis spinosa var. inermis Turra. | 31 19 21 | 27 04 20 4 | 85         | Western desert    |
| 2   | Capparis spinosa var. deserti Zahary. | 29 44 50 5 | 25 47 13 2 | 153        | Western desert    |
| 3   | Capparis spinosa var. Canescens Coss. | 29 54 05 6 | 33 42 56 1 | 402        | Sinai Peninsula   |
| 4   | Capparis cantilaginea Decne. | 28 42 48 1 | 33 39 45 7 | 696        | Sinai Peninsula   |
| 5   | Capparis egyptia Lam. | 29 04 58 2 | 33 05 55 2 | 23         | Sinai Peninsula   |
| 6   | Cleome chrysanthra Decne. | 23 25 20 7 | 34 52 30 5 | 291        | Eastern desert    |
| 7   | Cleome droserefolia Forssk. | 23 29 48 8 | 35 10 5 1 | 329        | Eastern desert    |
| 8   | Maerua crassifolia Forssk. | 22 55 18 8 | 36 21 15 8 | 22         | Eastern desert    |
| 9   | Cadaba farinosa Forssk. | 22 55 18 8 | 36 21 15 8 | 22         | Eastern desert    |
| 10  | Diptrygium glaucum Decne. | 23 24 17 7 | 35 29 52 1 | 21         | Eastern desert    |
| 11  | Capparis decidua (Forssk.) Edgew. | 22 55 18 8 | 36 21 15 8 | 22         | Eastern desert    |
| 12  | Cleome amblyocarpa Barratte & Murb. | 22 05 24 | 36 37 35 2 | 148        | Eastern desert    |
| Primer name | Sequence | Primer name | Sequence |
|-------------|----------|-------------|----------|
| HB1         | (CAA)5   | HB12        | (CAC)3 GC|
| HB2         | (CAG)5   | HB13        | (GAG)3 GC|
| HB4         | (GACA)4  | HB15        | (GTG)3 GC|
| HB8         | (GA)6 GG | 807         | (AG)8 T  |
| HB9         | (GT)6 GG | 814         | (CT)8 TG |
| HB10        | (GA)6 CC | 844A        | (CT)8 AC |
| HB11        | (GT)6 CC | 844B        | (CT)8 GC |
| 17899B      | (CA)6 GG |             |          |

| Marker type | Marker Name | Forward primer | Reverse primer | Marker Name | Forward primer | Reverse primer |
|-------------|-------------|----------------|----------------|-------------|----------------|----------------|
| SRAP        | Em 1R/DN 6 F | GACTGCGTACGAAATTAAT | /TGAGTCAAACGGGTTAA | Em 10 R/DN 8 F | GACTGCGTACGAAATCCAT | TGAGTCAAACGGGTGC |
|             | Em 1R/DN 7 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTAA | Em 10 R/DN 9 F | GACTGCGTACGAAATCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 8 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 10 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 9 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 11 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 10 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 12 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 11 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 13 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 12 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 14 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 6 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 15 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 7 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 16 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 8 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 17 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 9 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 18 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 10 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 19 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 11 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 20 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 12 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 21 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 6 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 22 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 7 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 23 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 8 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 24 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 9 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 25 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 10 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 26 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 11 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 27 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 12 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 28 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 6 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 29 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 7 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 30 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 8 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 31 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 9 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 32 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 10 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 33 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 11 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 34 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | Em 1R/DN 12 F | GACTGCGTACGAAATTAAT | TGGAGTCGAAACGGGTCA | Em 10 R/DN 35 F | GACTGCGTACGAAATTCAT | TGAGTCAAACGGGCTA |
|             | ISTR       | F1/B2        | AGGAGGTGAAATTCCCTTTAG | GGTATAATCAATTTTCATGGAATCAAGC | F3/B2 | GTGCAATGTCGATCTTTCATTTTCG | GGATATCATTAATCTGGAATACATCAGC |
|             | ISTR       | F1/B3        | AGGAGGTGAAATTCCCTTTAG | ATCCCTCAGTCTGACCAAT | F3/B3 | GTGCAATGTCGATCTTTCATTTTCG | ATCCCTCAGTCTGACCAAT |
|             | ISTR       | F3/B5        | GTGCAATGTCGACATTTTTTAG | CGTCTGAAGATCTGACCAAT | F3/B6 | GTGCAATGTCGATCTTTCATTTTCG | ATCCCTCAGTCTGACCAAT |
|             | ISTR       | F3/B8        | GTGCAATGTCGACATTTTTTAG | CGTCTGAAGATCTGACCAAT | F7/B3 | GTGCAATGTCGATCTTTCATTTTCG | ATCCCTCAGTCTGACCAAT |
|             | ISTR       | F3/B10       | GTGCAATGTCGACATTTTTTAG | CGTCTGAAGATCTGACCAAT | F7/B6 | GTGCAATGTCGATCTTTCATTTTCG | ATCCCTCAGTCTGACCAAT |
Table 2 List of ISSR, SRAP and ISTR primer combinations obtained from the current investigation (Continued)

| Combination | Forward Primer | Reverse Primer | Forward Primer | Reverse Primer |
|-------------|----------------|----------------|----------------|----------------|
| F5/B7       | ATATATGGACCTTAAGCAAGC | GGAATATCATTGATCCATAAG | F9/B2 | ATATGGACTTAAGCAAGCCA | GGATAATCCCTTAATGACAAGC |
| F5/B8       | ATATATGGACCTTAAGCAAGC | CCTCCTATTGATCCATAAG | F9/B3 | ATATGGACTTAAGCAAGCCA | ATCCATCTGATCCAAAT |
| F10/B6      | GGAATATCATTGATCCATAAG | ATATATGGACCTTAAGCAAGCCA | F9/B6 | ATATGGACTTAAGCAAGCCA | ATATATGGACCTTAAGCAAGCCA |
| F1/B8       | AGGAGTGAATACCCTTAG | CCTCCTATTGGAATGATAT | F9/B10 | ATATGGACTTAAGCAAGCCA | GACCCCTTTGAAAAACATG |
the amplified allele and 1 – \( f_i \) is the frequency of the null allele. While heterozygosity per locus was determined according to the formula: 

\[ H_e = 1 - p^2 - q^2, \]

where \( p^2 = f_i \). Meanwhile, the average heterozygosity per marker was evaluated based on 

\[ H_a = \frac{\sum (H_e/L)}{L}, \]

where \( L \) = total of detected bands. The multiplex ratio was calculated as 

\[ MR = \frac{L}{T}, \]

where \( T \) = the total number of primer combinations. The marker index (MI) was achieved by developing the average heterozygosity by the multiplex ratio: 

\[ MI = H_a \times MR. \]

To gain accurate perspectives on genetic diversity analyses among the Capparaceae and Cleomaceae germplasm, a graphic demonstration of principal coordinates analysis (PCA) and the heatmap cluster analysis (HCA) was provided an explanation to demonstrate the multidimensional genetic relationship and its split among species using ClustVis web tool for visualizing clustering of multivariate data [39].

Results

Morphological basis of Capparaceae taxonomy

In the present scenario, the main differences between Capparis and Cleome are growth habit and life span, as presented in Fig. 2. All individuals of Capparaceae are trees or shrubs, whereas species of Cleome are annual or perennial herbs. According to the type of fruits of the family, Cleomaceae were divided into Dipterygium (fruits one-seeded), Gynandropsis, and Cleome (fruits contains much seed), and then separated according to the number of stamens and presence or absence of androphore. On the other hand, family Capparaceae is classified into four species due to the presence of a stipule spine (Capparis) and the absence of a stipule (Maurea, Cadaba, and Boscia). Boscia species leave their group due to its fruit type (hard indehiscent). Recently, both Maurea and Cadaba are isolated according to the number of stamens and their contact with the androphore.

Distribution and habitat classification of Capparaceae and Cleomaceae species

According to vegetation density and cover, classification of the twelve targeted species and 29 associates are mainly divided according to species distribution, habitat, and locations to six vegetation groups. The first group comprises of Maerua crassifolia and Capparis deserti inhabiting the sandy formation of the southern wadis of the Eastern desert and Siwa oasis, respectively. Moreover, they are tending to form pure communities with high density and cover. The second group is formed of Cleome and Dipterygium, and they are collected from the main channel of Wadi Abirq, Shalatein area. The third group involves of Capparis spinosa var. canescens and C. aegyptia, and they are similar in habitat; both of them are hanging between the rock fissures at south Sinai. While the fourth group (Cadaba farinosa and Capparis decidua) is distributed on the sandy formation.
of red sea wadis, the fifth group is represented with *Capparis spinosa* var. *inermis*; this species is restricted to the maritime cliffs and rocky ridges at the western Mediterranean section. However, the last group is occupied with the biggest *Capparis* species (*Capparis cartilaginea*) (Fig. 3).

**Comparison of polymorphic levels and informativeness obtained with ISSR, SRAP, and ISTR markers**

In the present investigation, the levels of polymorphism of ISSR, SRAP, and ISTR markers and the index associating their informativeness are described in Table 3 and Fig. 4. All markers used pointed out to be helpful implements for the discovery of polymorphism and evaluating genetic diversity in *Capparaceae* and *Cleome* genotypes, but the level of sensitivity varied on the method applied. We primarily tested 20 of ISSR primers and 49 and 70 combinations of SRAP and ISTR primers among the twelve *Capparaceae* and *Cleome* species, respectively. Among all, only 15 ISSR, 30 SRAP, and 18 ISTR primers exhibited significant levels of polymorphism as shown in Table 3 and Fig. 4a–c. The total number of bands recorded for SRAP was almost high with 503 bands, followed by 337 and 252 bands for ISTR and ISSR markers, respectively. However, the total numbers of polymorphic bands (*p*) were ranged from 479 for SRAP, 333 for ISTR, to 239 for ISSR markers. On behalf of the total number of effective alleles (*Ne*), it was correlated significantly with the total number of bands (*L*) and the total numbers of polymorphic bands (*p*). Additionally, the average number of polymorphic bands/assay unit (*np/U*) was relatively high for ISTR being 18.5 with an intermediate value of 15.96 and 15.93 for SRAP and ISSR, respectively. Meanwhile, the PIC value for ISSR, SRAP, and ISTR marker system was almost parallel and relatively high being, 0.97, 0.98, and 0.99, respectively. Here, the present result showed that the ISTR marker was the most powerful marker in several detected parameters and PIC values. A comparative summary of the discriminating capacity of ISSR, SRAP, and ISTR markers are summarized in Table 3 and Fig. 5. On average, the three factors, assay efficiency index (*Ai*), effective multiples ratio (*E*), and marker index (*MI*), presented higher in ISTR marker, highlighting the notable characteristics of this marker compared to SRAP and ISSR (2.4×, 1.2× and 1.2× respectively). This certainly is due to the highest value of the assay efficiency index for the ISTR marker, inferring that ISTR has a higher discriminating capacity for counting the genetic diversity and can concurrently discover many polymorphic markers per
reaction. Although the variances in some of the diversity statistics, these outcomes reveal that ISTR following by SRAP markers can be applied to assess the level of polymorphism in Capparaceae and Cleome species.

Diversity analyses and phylogenetic heatmap

Here, we present a graphic demonstration of both PCA and HCA, utilizing variable information matrix as input, wherever numerous features of ISSR, SRAP, and ISTR marker data are assessed in several observations. The overall PCA plot data for the three marker profile as shown in Fig. 6 created four relatively clustered groups, with a total of 19.73% of the molecular variance (PC1—10.32%, PC2—9.41%). Cluster I compressed Capparis spinosa inermis, Capparis spinosa var. canescens, Capparis spinosa deserti, Capparis cartilaginea, and Capparis aegyptia with a closer relationship than other groups. Moreover, cluster II assembled Cleome drosersifolia, Cleome chrysantha, and Cleome amblyocarpa in a particular group, while Maerua crassifolia, Capparis decidua, and Dipterygium glaucum places jointly as cluster III. However, Cadaba farinosa was separated individually as out-group species.

To further determine the genetic diversity, HCA exhibits the abundance of the relationships between the twelve species of Capparaceae and Cleome. The distribution of hot points indicates significant variations between the major groups of the Capparaceae and Cleome species and able to cluster in a sub-clade. As a result, the HCA was constructed based on the three sets of ISSR, SRAP, and ISTR markers (Fig. 7). The results were similar to each other with a tiny difference in the placement of some species, where the ISTR-HCA tree was

| Marker efficiency                      | ISSR | SRAP | ISTR |
|----------------------------------------|------|------|------|
| Number of assay units (N)              | 15   | 30   | 18   |
| Total number of bands (L)              | 252  | 503  | 337  |
| Polymorphic bands (p)                  | 239  | 479  | 333  |
| Number of loci/assay unit (nu)         | 16.8 | 16.7 | 18.7 |
| Total number of effective alleles (Ne) | 912  | 2964 | 2687 |
| Average number of polymorphic bands/assay unit(np/U) | 15.93 | 15.96 | 18.5 |
| Polymorphic information content (PIC)  | 0.97 | 0.98 | 0.99 |
| Fraction of polymorphic loci (β)       | 0.94 | 0.95 | 0.98 |
| Assay efficiency index (Ai)            | 60.8 | 98.8 | 149.2|
| Effective multiples ratio (E)          | 15.9 | 15.9 | 18.5 |
| Marker index (MI)                      | 15.5 | 15.6 | 18.3 |
the most consistent with the morphological taxonomy data of Capparaceae. Overall, three confirmed clades were identified, which have the ability to clearly distinguish among the twelve species. In detail, the first clade assembled together Capparis decidua, Cadaba farinosa, and Maerua crassifolia in a particular monophyletic clade. However, the three Cleome species and Dipterygium glaucum were placed jointly in the second clade with a high proportion of close relationships. In the framework, the third clade formed two monophyletic
sub-clades, not based on their type but on their sampling origin, where the three species of *Capparis spinosa* are placed jointly with high portions within the first sub-clade, whereas the second sub-clade occupies *Capparis aegyptia* and *Capparis cartilaginea* with a close genetic relationship. Collectively, we found both PCA and HCA data have drawn a successful annotation relationship in *Capparaceae* and *Cleome* species to evaluate whether the specific group sort individual or overlap groups.

**Discussion**

The systematic approach and phylogenetic relationships of the Egyptian *Capparis* and *Cleome* species remain obscure, and unsolved dilemmas regarding their taxonomy and biology require further verifying and review [40]. Species identification in *Capparis* and *Cleome* is problematic because of the challenge of preserving the flowers [17] or maybe impossible when only vegetative parts are present, which is commonly the case during collection. Ettingshausen [41] made the primary comprehensive attempt to systematize the description of the vegetative leaf architecture together with his classification of venation patterns. Leaf architectural characters have demonstrated valuable taxonomic and systematic data both in fossil and living plants [42–44]. Leaf architecture and venation pattern were examined in numerous families of dicotyledons, among others, *Composite* [45], *Solanaceae* [46], *Bignoniaceae* [47], *Hamamelidaceae sensu lato* [48], *Leguminosae* [49], *Amaranthaceae* [50], *Ulmaceae* [51], *Fagaceae* [52], and in some monocots [53].

Various traditional taxonomic positions of *Capparis* and *Cleome* were derived from the study of both quantitative and qualitative macro-morphological characters which might become a broad margin of mistake. Therefore, additional knowledge about the genotype of plants is much required to resolve taxonomic problems in these genera [54]. Hence, evaluations of molecular statistics has had a serious effect on our perception of plant evolution and relations on all taxonomic levels, from the deep nodes dividing the key plant groups to species and populations [20]. Consequently, studying the levels of genetic diversity in natural populations is an essential precursor for the survey of plant species and will offer perceptions about the evolution of the species [55]. During the last two decades, there are little individual molecular studies involving *Capparis* and *Cleome* like the RAPD marker [54, 56] and therefore, the AFLP marker [57].

In the same context, the selection of the foremost appropriate marker system for a certain survey is not evident and principally depends on the aim of the research because the genetic structure of the species was varied [33]. The use of ISSR, SRAP, and ISTR marker is strongly recommended by several studies addressing the
effectiveness of such markers for investigating Capparaceae diversity. One of the challenges of the current research is the use of ecological and molecular markers to explore the genetic relationships among the Egyptian Capparis and Cleome species grown within the Egyptian desert. In the present investigation, the relatively high values of the effective number of alleles for ISSR, SRAP, and ISTR markers were used to provide indication of their discrimination capacity when study a huge number of plants. This trend is required for the germplasm bank’s certification when multiple species require to be correctly distinguished and classified [58]. In this revised, the effective number of alleles tracking the method: SRAP > ISTR > ISSR. This result suggests that the ISTR and SRAP is more useful evidence for Capparis and Cleome species classification and certification. It is well-known that the marker index (MI) may probably be a suitable value for marker effectiveness [58]. By this criterion, arithmetically 1.18 fold greater MI was estimated for ISTR against SRAP and ISSR, highlighting the unique character of the ISTR assay. This is definitely owing to the superior value of effective multiples ratio (EMR) and assay efficiency index (Ai) [59]. Many studies confirmed that the retrotransposons marker, e.g., ISTR had a superior discrimination capacity and have the flexibility to detect several polymorphic loci per individual reaction [60]. Recently, Du et al. [61] suggested that retrotransposons (RT) occupied 28.1 Mb of the genome sequence, accounting for 9.74% of the entire genome. These results indicated that ISTR had an abundant presence of Ty-1 Copia retrotransposons, which permit obtaining useful polymorphism among the tested genotypes of Capparaceae and Cleome germplasm. Indeed, our finding showed that RT-ISTR markers had numerous unique private loci that would allow diversity within the sub-species of Capparaceae and Cleome germplasm, which is in concurrence with earlier reports of this marker [31, 62].

In the Mediterranean area, five species are confirmed to be growing, e.g., Capparis aegyptia Lam. Boiss., Capparis spinosa L., Capparis orientalis Veil., Capparis sicula Veil., and Capparis ovata Desf [63, 64]. Mainly, in various floristic works, Capparis aegyptia is recognized from Northern Africa and also the Mideast [65]. In Flora Hellenica [66], it has been raised, Capparis aegyptia Lam., to a subspecies of Capparis spinosa, while Inocencio et al. [63] returned its status of individual species, while Özbek and Kara, [67] fully proposed that the two subspecies Capparis spinosa L. and Capparis ovata Desf. might be distinguished roughly. However, Inocencio et al. [63] confirm that Capparis aegyptia Lam. and Capparis ovata are very distinct from the opposite taxa but extremely tight to each other.

Recently, Al-Safadi et al. [64] recognized three Capparis species growing in Syria, C. sicula Duh, C. aegyptia Lam,
and C. spinosa L., and the results support this theory that C. aegyptia Lam. might be an independent species and not a varietal level of C. spinosa. Initially, Rivera et al. [68] discussed the origin of Capparis cartilaginea and indicated that this is often a widely accepted name. Following the phylogenetic analysis of Saifi et al. [69], the dendrogram was assembled together with Capparis cartilaginea, Capparis aegyptia Lam, and Capparis spinosa in a subgroup. In view of the previous revisions, our phylogenetic analysis clear evidence supporting the undisputed viewpoint that Capparis aegyptia and Capparis cartilaginea are very closely associated with Capparis spinosa in a sister clade and seem to be distinguished. These results might be applied in systematics and evolutionary biology studies within the Egyptian species of Capparis and Cleome to clarify the complex interactions among species, as demonstrated in previous studies [64, 70]. Indeed, we are able to tentatively imply forward this theory as Capparis decidua, Cadaba farinosa, Maerua crassifolia, and Dipterygium glaucum formed a particular monophyletic clade with Cleome species. Based on the above considerations, our results verified the conclusions of systematic and taxonomic analyses that were performed on the collected samples, which is in consensus with previous articles on Capparis species [54, 57]. Interestingly, we observed a thorny interspecies in Capparis decidua, Cadaba farinosa, Maerua crassifolia, and Dipterygium glaucum specimens and also the concerned during this study. Currently, the advent of molecular markers overcome the majority of the challenges related to utilizing morphological markers during which main phenotype-varying genes were applied as genetic markers [71].

Conclusion
In the present investigation, we emphasize that the outcomes of the morphological and ecological characterization as well as the genetic analysis based ISSR, SRAP, and SRAP can capture the taxonomy and systematics of the various subgroups recovered with a good performance in clarifying genetic diversity within and among populations in the Egyptian Capparis and Cleome species. Our findings show Cleome and Gynandropsis genera as an explicit family; therefore, an in-depth study like next-generation sequencing (NGS) technologies is now emerging as precision tools to assess the molecular systematics and evolution in Dipterygium glaucum (if belongs to Capparaceae or Cleomaceae or another family). Collectively our results not only help within the classification of species but may distinguish species limitations, flagging of modern species, and genus delimitation.

Abbreviations
ISSRs: Inter-simple sequence repeats; SRAP: Sequence-related amplified polymorphism; ORFs: Open reading frames; ISTR: Inverse sequence-tagged repeat; TWINSPLAN: A hierarchical dichotomous analysis; PIC: Polymorphic information content; MI: Marker index; PCA: Principal coordinates analysis; HCA: Heatmap cluster analysis

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Authors’ contributions
Both MSA and MAZ contributed equally to this paper. MSA formulated the idea, designed, and performed the ecological experiments, while MAZ carried out the practical work of genetic diversity experiments. MHA analyzed the data and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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