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Taxonomic evaluation of three Egyptian *Solanum* species based on morphology, DNA sequences, and chromosome analysis

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Abstract. We investigated the intra-specific relationships between three *Solanum* species in Egypt: *Solanum nigrum*, *Solanum villosum*, and *Solanum sinaicum*, in addition to the inter-specific relationships among the populations of the three studied species. These species' taxonomic difficulty is primarily due to its inconsistent infra-specific treatments in various floras. The morphological studies revealed distinguishing characteristics for the three investigated *Solanum* species mainly fruit and flower characteristics. The morphological differences allowed *S. villosum* to be divided into two subspecies: subsp. *villosum*, and subsp. *miniatum*. The genetic variation between the three *Solanum* species was clarified using AFLP technique. The ribosomal DNA ITS1-5.8S-ITS2 region of the three studied species was sequenced using the universal primers ITS4 and ITS5. The DNA sequences of *Solanum* species were counted. The karyotypes of the species under examination were established using the chromosome number and genome size acquired from mitotic chromosomal preparations.

Keywords: AFLP, karyotype, *Solanum*, *S. nigrum*, *S. sinaicum*, *S. villosum*.

INTRODUCTION

Solanaceae is one of the largest and most essential families of flowering plants. This family serves as a significant source for medicine, food, and spice (Afroz et al. 2020). It is extensively dispersed in both tropical and temperate regions; it has 100 genera and over 2500 species thriving in a variety of environments and ecologies and exhibits different morphologies (Nderitu et al. 2023). Solanaceae is represented in Egypt by eight genera including 30 species (Boulos 2002). *Solanum* L. is the family's largest genus (El-Shaboury et al. 2020). There are several species of *Solanum* that are used in traditional medicine, and some of these species are employed as sources of drugs in pharmacology (Jainu and Devi 2005). Due to its morphological plasticity and infraspecific genetic variation, *Solanum* possesses a taxonomic challenge (Jennifer and James 1997), also because of the 6,931 names that have been

published, many of which are connected to the cultivated and widely distributed species of the genus (Särkinen et al. 2018).

Solanum is represented in Egypt by 9 species (Boulos 2002). However, Täckholm (1974) recorded 10 species. Zohary (1966) recorded two subspecies of *Solanum villosum*: subsp. *villosum* and subsp. *puniceum*. Boulos (2002) recorded that *Solanum villosum* Mill. is represented in Egypt with two subspecies: subsp. *villosum* and subsp. *miniatum*. The name *Solanum sinaicum* is considered a synonym of *Solanum villosum* (Särkinen et al. 2018; powo.science.kew.org/) but the name is accepted by Khafagi et al. (2018) and (www.solanaceaesource.org).

Low taxonomic level phylogenetic relationships have been successfully solved using AFLP (Despres et al. 2003; Koopman 2005; Meudt and Clarke 2007). The use of AFLP offers numerous advantages. It generates data that is highly reproducible (Jones et al. 1997), and doesn't require a priori sequence knowledge. Previous attempts using AFLP markers have proved effective in resolving taxonomic issues and illuminating relationships among species of *Solanum* (Kardolus et al. 1998; Mace et al. 1999a, 1999b; Coulibaly et al. 2002; Jacoby et al. 2003; Dehmer and Hammer 2004; Olet 2004).

The objectives of this study are to investigate the morphological and genetic diversity found in the populations of the three selected *Solanum* species: *S. nigrum*, *S. villosum* and *S. sinaicum*, to solve the taxonomic problem of these species in Egypt due to the confused infraspecific groupings.

MATERIALS AND METHODS

Morphological study

This study was based on the examination of specimens deposited at Cairo University Herbarium (CAI), in addition to the authentic type specimens preserved in virtual herbaria that are accessible online (the JSTOR Global Plants database). Acronyms follow Index Herbariorum (http://sweetgum.nybg.org/ih/). In addition to the examination of fresh representative specimens of each of the three species collected between 2022–2024. These specimens belonging to 5 different localities from different phytogeographical regions of Egypt. The coordinates of these localities: 30°49'56" & 29°34'49"; 30°00'18" & 31°12'45"; 30°00'49" & 31°12'04"; 29°17'23"& 30°51'11"; and 28°33'38" & 33°58'20". All specimens were examined for morphological variations in all distribution localities. There were 44 different morphological features analyzed, including those for the stem, leaves, flower, and fruit. Scientific names for the species follow IPNI (http://www.ipni.org/). Vouchers Samples were placed in (CAI).

Data analysis

44 morphological characteristics were analyzed in order to establish a correlation between the samples of the three *Solanum* species. All samples had their characteristics measured and documented, and the software (R-4.3.1 for Windows) was used to analyze the data and create the heat map. A heatmap is a visual representation of numerical data where each value is represented as a square, with lighter squares representing smaller numerical values and darker squares representing larger values (Tiessen et al. 2017).

Cytogenetics study

Chromosomes number and Karyotype formulae

Chromosome preparations

Between 2022 and 2024, plant material was collected for cytogenetic analysis. A total of thirty specimens were sampled, comprising ten individuals from each of the three Solanum species. Seeds were extracted from the specimens and soaked in distilled water for 1 h prior to germination at room temperature. Root tips approximately 1 cm in length were excised and pretreated with 0.025% colchicine ($C_{22}H_{25}NO_6$) for 2 h at room temperature to arrest cells at metaphase, followed by rinsing with distilled water. The treated samples were subsequently fixed in a 3:1 (v/v) ethanol:glacial acetic acid solution.After thorough washing with distilled water, the root tips were hydrolyzed in 1 N HCl at 64 °C for 5 min. For slide preparation, the root tips were squashed in 45% acetic acid and stained with acetoorcein solution to visualize chromosomes. The procedure followed previously described protocols (Ibrahim et al., 2019; Elsayed et al., 2024).

Microscopic examination karyotyping, idiograming and signals imaging

Chromosomes examination was done via a vertical fluorescence microscope (Leica DM2500) equipped with a cooled monochrome digital camera (Leica DFC340FX). Twenty cells with clearly observed and well spread chromosomes were checked and photographed at 100X magnification under oil immersion. Chromosome counting and karyotype has performed via the automated Karyotype and FISH software processing (Leica

CW4000) system. Ideograms were constructed from complete chromosomes which showed the greatest possible banding pattern in at least ten different metaphase plates. (Ibrahim et al. 2019; Abdo et al. 2023).

Molecular study

DNA extraction

For the chosen samples of *Solanum nigrum*, *Solanum villosum* and *Solanum sinaicum*, genomic DNA was isolated from one gram of juvenile leaves using the CTAB (Cetyl-trimethyl ammonium bromide) extraction buffer approach as described by Doyle and Doyle (1990) and modified by Allen et al. (2006).

PCR reactions and data analysis

PCR amplification was carried according to Williams et al. (1990) with some modification. The reaction volume of 25 µl containing 12.5 µl Dream Taq Green PCR Master Mix (2X), 1 µl Forward primer, 1 µl Reverse primer, 2 µl Template DNA and completed to 25 µl with water (nuclease-free) was placed in a thin-walled PCR tube on ice, then gently vortex the samples and spin down. PCR reaction was performed using the recommended thermal cycling conditions: One cycle of initial denaturation at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 45 seconds followed by annealing at 57°C for 45 seconds, extension at 72°C for 60 seconds, and one cycle of final extension at 72°C for 10 minutes.

The reaction products were separated by electrophoresis on a 1.6% agarose gel in a 1x TBE buffer, run in the same buffer at 100 V for an hour, and then visualized by staining with 0.5 g/ml of ethidium bromide and being photographed under UV light.

To elucidate the genetic variation and construct the phylogeny of the studied *Solanum* species, the ribosomal DNA ITS1-5.8S-ITS2 region of the three species was sequenced using ABI377 DNA sequencer (ABI, USA). DNA was amplified using the universal primers ITS4 (5´-TCCTCCGCTTATTGATATGC-3´) and ITS5 (5´-GGAAGTAAAAGTCGTAACAAGG-3´), these primers described by White et al. (1990). Then, BLAST program was employed to look for sequence similarity in DNA databases. Multiple sequence alignment and the determination of genetic distances among the analyzed species were performed using MEGA5 software. Neighbour joining dendrogram was created to highlight the genetic links between the three species. The retrieved sequences were registered on the ncbi under accession numbers PP701899, PP707086, PP707087, and PP707088. The reference sequences from other countries of *Solanum nigrum* and *Solanum villosum* used to construct the heat map are available online in the Gene bank

RESULTS

Morphological diversity

Old herbarium specimens and 150 newly collected specimens of the three studied *Solanum* species were the subject of morphological studies and taxonomy revision based on 44 morphological traits, including plant height, leaf features, as well as inflorescence and fruit characters. The flower, fruit and leaf characters were the most distinctive characters between the species, and according to the variation in morphological characters we considered the two different forms of *Solanum villosum* as two different subspecies *Solanum villosum* subsp. *villosum* and *Solanum villosum* subsp. *miniatum* (Table 1, Figs 1-4).

***Solanum nigrum* L.,** Sp. Pl., ed. 1, 219 (1753).
Common name: Black nightshade

Annual erect herb; stem glabrous to pubescent, green, angular, woody, branched, up to 70 cm tall; leaves simple, alternate, petiolate, ovate or deltoid-rhomboid, entire or irregularly dentate, acute, base cuneate to truncate, 2-7 x 1.5-3.5 cm, petiole up to 2 cm, stipules absent; inflorescences unbranched cymes, number of flowers per inflorescence 5-10, peduncle length 1-1.5 cm; Flowers pentamerous, hermaphrodite; pedicel 3-10 mm; calyx lobes triangular with acute or rounded apex, green, (1.5-2.5 x 1 mm); corolla white with yellow midrib, oblong or ovate to lanceolate, 4-5 x 2-2.5 mm, acute; stamen filament 1.5-2 mm, anthers yellow, oblong, 1.5-2.5 x1 mm; ovary globose, 0.5-1 mm in diameter, style 2-3.5 mm long, stigma is small; fruit berry, globose, black, 6-7 mm in diameter, fruiting calyx lobes spreading to reflexed 2-3 x 2 mm; seeds ovoid, 1.5–2 mm in diameter; hairs non glandular unicellular papillose, or non-glandular basal cell with long narrow apical cell, or non-glandular bicellular uniseriate with hooked or obtuse apical cell.

Solanum villosum* subsp. *miniatum (Bernh. ex Willd.) J.M.Edmonds, Bot. J. Linn. Soc. 89: 166 (1984).
Common name: Woolly nightshade, Red-berried nightshade

Annual to perennial erect herb to small shrub; stem pubescent, green, angular, woody, branched, up to 70 cm tall; leaves simple, alternate, petiolate, broadly to narrowly ovate to elliptic or deltoid, entire or irregularly sinuate-dentate, acute to acuminate or obtuse, base

Table 1. Morphological variation among the studied *Solanum* species.

Character	*S. villosum* subsp. *miniatum*	*S. villosum* subsp. *villosum*	*S. nigrum*	*S. sinaicum*
Life cycle	Annual to perennial	Annual to perennial	Annual	perennial
Plant nature	Erect	Erect	Erect	Erect
Growth habit	herb to small shrub	herb to small shrub	herb	herb to small shrub
Plant surface	pubescent with multicellular glandualr hairs	pubescent	glabrous or with short hairs	sparsely pubescent
Stem length (cm)	up to 70	up to 70	up to 70	up to 60
Stem type	woody	woody	woody	woody
Stem shape	branched	branched	branched	branched
Stem outline	angular	angular	angular	angular
Stem color	green	green	green	green
Leaf structure	simple	simple	simple	simple
Leaf arrangement	alternate	alternate	alternate	alternate
Petiole length (cm)	up to 2	up to 2	up to 2	up to 3
Leaf shape	broadly to narrowly ovate to elliptic or deltoid	broadly to narrowly ovate to elliptic or deltoid	ovate or deltoid-rhomboid	oblong-rhombic to oblong-ovate
Leaf margin	entire or irregularly sinuate-dentate	entire or irregularly sinuate-dentate	entire or irregulary dentate	sinuate-dentate rarely entire
Leaf apex	acute to acuminate or obtuse	acute	acute	acute
Leaf base	cuneate to truncate or cordate	cuneate to truncate, cordate or hastate	cuneate to truncate	cuneate to truncate
Leaf color	green	green	green	green
Leaf length (cm)	2 to 6	2 to 8.5	2 to 7	2 to 6
Leaf width (cm)	1.5 to 5	1.5 to 6.5	1.5 to 3.5	1 to 3
stipules	absent	absent	absent	absent
number of lateral veins	4-7 per leaf	6-7 per leaf	6	6
Inflorescence type	unbranched cymes	unbranched cymes	unbranched cymes	unbranched cymes
number of flowers per inflorescence	2-5 flowers	3-6 flowers	5- 10 flowers	4-8 mostly 7
peduncle length (cm)	0.5-2.5	1-1.5	1-1.5	1.5-2.5
pedicel length	7-11 mm	9-12 mm	3-10 mm	8-11 mm
calyx lobes	rounded	rounded	triangular with acute or rounded apex	linear-oblong with acute or rounded apex
length	2.5 mm	2 mm	1.5-2.5 mm	1-2 mm
width	1 mm	1 mm	1 mm	1-2 mm
corolla color	white with black midrib	white with yellow midrib	white with yellow midrib	white with yellow midrib
corolla lobes length	8 mm	6-7 mm	4-5 mm	7-10 mm
corolla lobes width	4 mm	3 mm	2-2.5 mm	3-5 mm
filament length	2 mm	1.5 mm	1.5-2 mm	2 mm
anther length	2 .5 mm	3 mm	1.5-2.5 mm	2-3 mm
anther width	1 mm	1.5 mm	1 mm	1-1.5 mm
ovary shape	globose	globose	globose to ellipsoid	globose
ovary diameter	1 mm	1 mm	0.5-1 mm	1 mm
style	5 mm	5 mm	2-3.5 mm	5 mm
fruit type	berry	berry	berry	berry
fruit shape	globose	globose	globose	globose
fruit diameter	6-9 mm	6-9 mm	6-7 mm	7-9 mm
fruit color	bright red	orange	black	orange
friuting calyx legth	2-3 mm	3-4 mm	2-3 mm	4 mm

(Continued)

Table 1. (Continued).

Character	*S. villosum* subsp. *miniatum*	*S. villosum* subsp. *villosum*	*S. nigrum*	*S. sinaicum*
fruiting calyx width	1-2 mm	1.5-2 mm	2 mm	2 mm
hairs	non glandular bicellular uniseriate with hooked or obtuse apical cell	non glandular bicellular uniseriate with hooked or obtuse apical cell	non glandular unicellular papillose, or non-glandular basal cell with long narrow apical cell, or non-glandular bicellular uniseriate with hooked or obtuse apical cell	glandular, bicellular uniseriate stalk, with unicellular head, and glandular multicellular uniseriate stalk, unicellular head

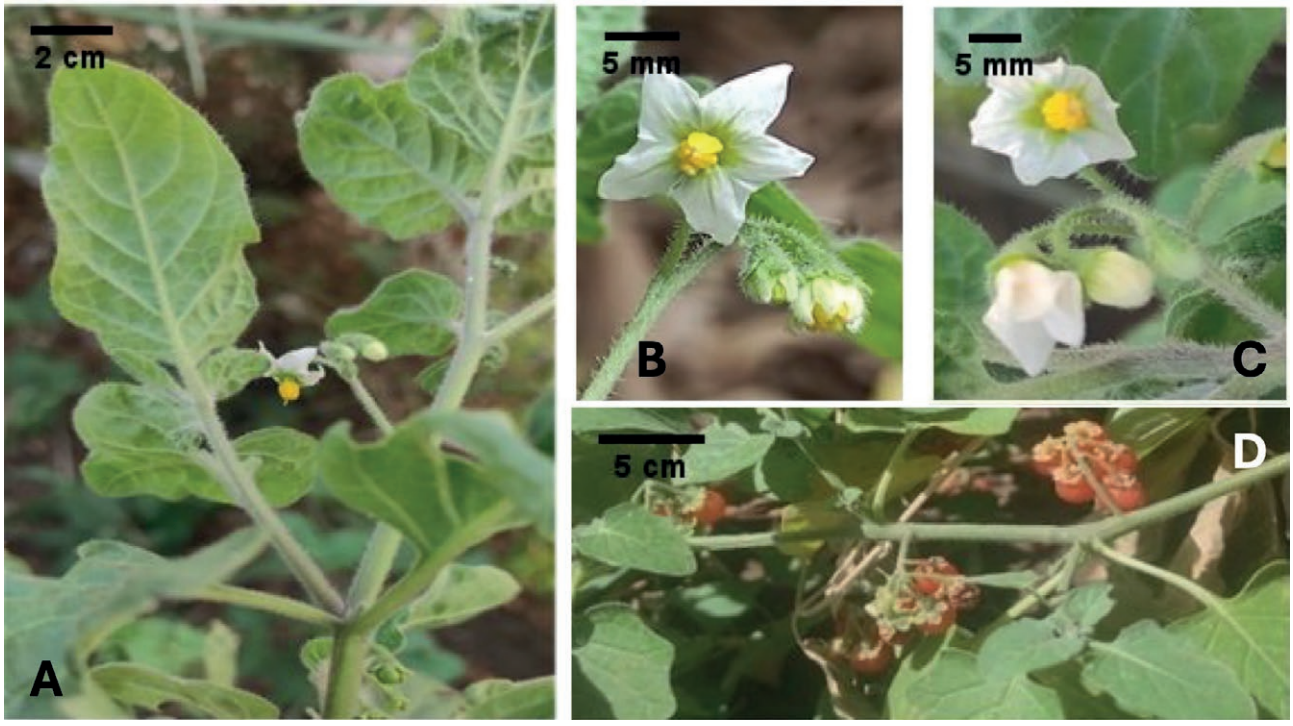

Figure 1. Specimen of *Solanum villosum* subsp. *villosum* showing the morphological features, (A) Leaf shape, (B), (C) flower shape, (D) fruit shape and color.

cuneate to truncate or cordate, 2-6 x 1.5-5 cm, petiole up to 2 cm, stipules absent; inflorescences unbranched cymes, number of flowers per inflorescence 2-5, peduncle length 0.5-2.5 cm; flowers pentamerous, hermaphrodite; pedicel 7-11 mm; calyx lobes rounded, green, (2.5 x 1 mm); corolla white with black midrib, oblong or ovate to lanceolate, 8 x 4 mm, acute; stamen filament 2 mm, anthers yellow, oblong, 2.5 x1 mm; ovary globose, 1 mm in diameter, style 5 mm long, stigma is small; fruit berry, globose, bright red, 6-9 mm in diameter, fruiting calyx lobes spreading to reflexed 2-3 x 1-2 mm; seeds 1.5–2.5 mm long, pale yellow; hairs non glandular bicellular uniseriate with hooked or obtuse apical cell.

Solanum villosum* subsp. *villosum Miller, Gard. Dict. 8th edn, no. 2 (1768)

It differs from subsp. *miniatum* in having acute leaves, base cuneate to truncate, cordate or hastate, 2-8.5 × 1.5-6.5; number of flowers per inflorescence 3-6 flowers, peduncle length 1-1.5 cm; pedicel 9-12 mm; calyx lobes 2 x 1 mm; corolla white with yellow midrib, 6-7 x 3 mm; stamen filament 1.5 mm, anthers 3 x1.5 mm; fruit orange, 3-4 x 1.5-2 mm.

Solanum sinaicum Boiss., Diagn. Pl. Orient. 11: 135 (1849)

Common name: Sinai Nightshade

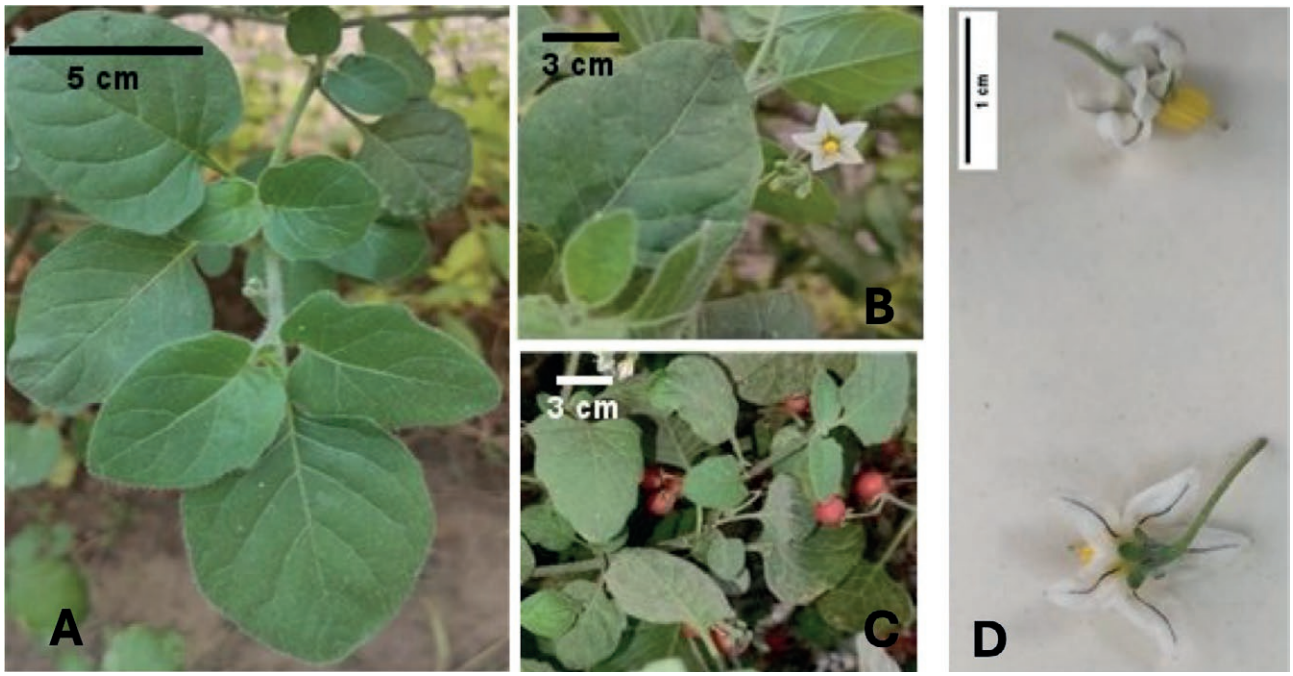

Figure 2. Specimen of *Solanum villosum* subsp. *miniatum* showing the morphological features, (A) Leaf shape, (B), (D) flower shape, (C) fruit shape and color.

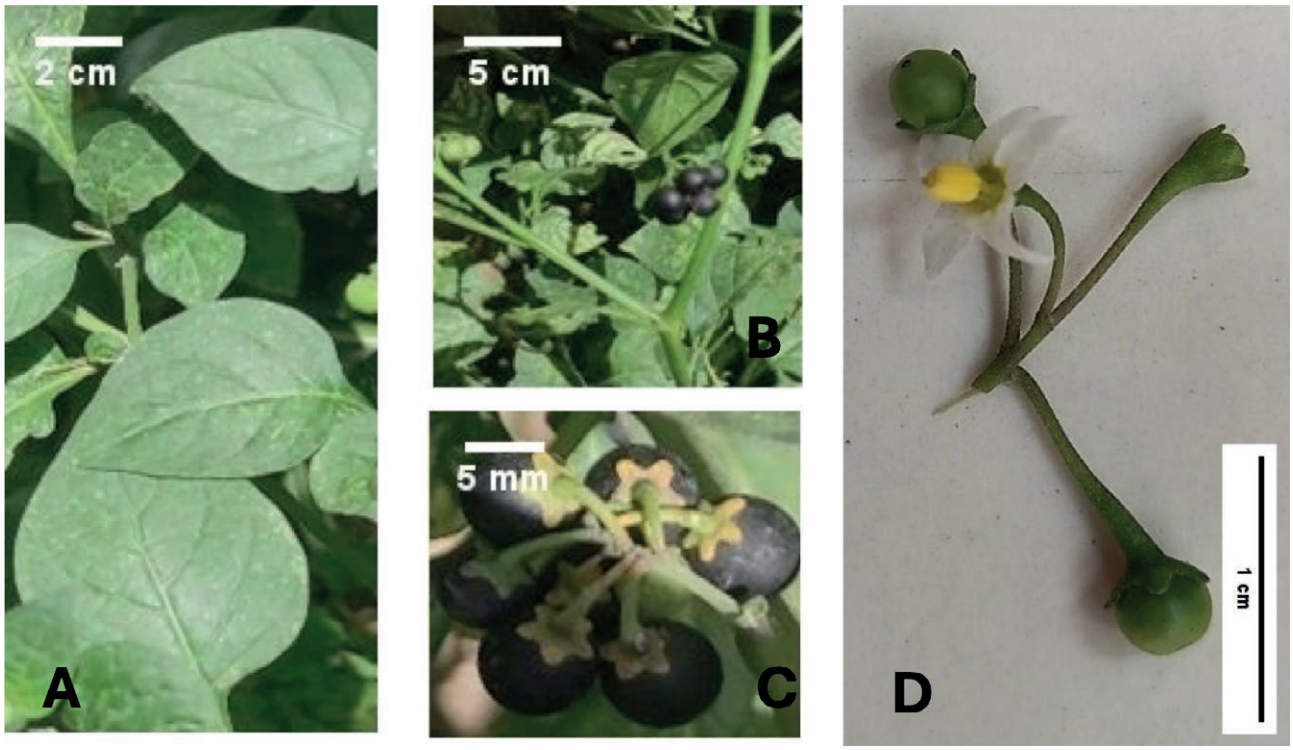

Figure 3. Specimen of *Solanum nigrum* showing the morphological features, (A) Leaf shape, (B), (C) fruit shape and color, (D) flower shape.

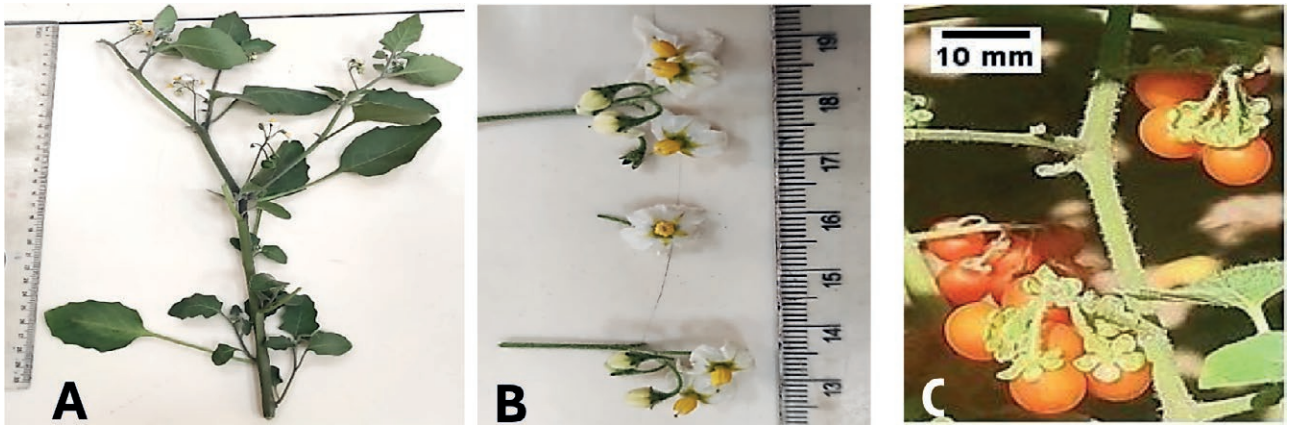

Figure 4. Specimen of *Solanum sinaicum* showing the morphological features, (A) Leaf shape, (B) Flower shape, (C) Fruit shape.

perennial erect herb to small shrub; stem sparsely pubescent, green, angular, woody, branched, up to 60 cm tall; leaves simple, alternate, petiolate, oblong-rhombic to oblong-ovate, sinuate-dentate rarely entire, acute, base cuneate to truncate, 2-6 x 1-3 cm, petiole up to 3 cm, stipules absent; inflorescences unbranched cymes, number of flowers per inflorescence 4-8 mostly 7, peduncle length 1.5-2.5 cm; flowers pentamerous, hermaphrodite; pedicel 8-11 mm; calyx lobes linear-oblong with acute or rounded apex, green, (1-2 x 1-2 mm); corolla white with yellow midrib, petals strongly recurved, 7-10 x 3-5 mm, acute; stamen filament 2 mm, anthers yellow, oblong, 2-3 x1-1.5 mm; ovary globose, 1 mm in diameter, style 5 mm long, stigma is small; fruit berry, globose, orange, 7-9 mm in diameter, fruiting calyx lobes spreading, 4 x 2 mm; seeds 1.5–2.5 mm long; hairs glandular, bicellular uniseriate stalk, with unicellular head, and glandular multicellular uniseriate stalk, unicellular head.

KEY TO STUDIED SPECIES OF THE GENUS *SOLANUM* IN EGYPT

1- Fruit berry black in color; calyx lobes triangular with acute or rounded apex ..... *S. nigrum*

- Fruit berry bright red or orange in color; calyx lobes rounded or linear-oblong 2

2- Fruit berry bright red in color; calyx lobes rounded; corolla white with black midrib ..... *S. villosum* subsp. *miniatum*

- Fruit berry orange in color; calyx lobes rounded or linear-oblong with acute or rounded apex; corolla white with yellow midrib 3

3- Leaves 2-8.5 × 1.5-6.5 cm, broadly to narrowly ovate to elliptic or deltoid; calyx lobes rounded; filament length 1.5 mm long ..... *S. villosum* subsp. *villosum*

- Leaves 2-6 × 1-3 cm, oblong-rhombic to oblong-ovate; calyx lobes linear-oblong with acute or rounded apex; filament length 2 mm long.. ..... *S. sinaicum*

Morphological correlation between the three Solanum species

Based on the 44 morphological characters that were investigated, the correlation between *S. nigrum*, *S. sinaicum*, *S. villosum* subsp. *miniatum*, and *S. villosum* subsp. *villosum* was constructed (Figure 5, Table 2). A clustering of the heat map identified two groups. *S. villosum* subsp. *miniatum* and *S. villosum* subsp. *villosum* together constituted the first group. *S. nigrum* and *S. sinaicum* were included in the second group. The heat map revealed that *S. nigrum* and *S. sinaicum* had the highest correlation (0.429), *S. villosum* subsp. *miniatum* and *S. villosum* subsp. *villosum* also have a high correlation of 0.415, followed by *S. sinaicum* and *S. villosum* subsp. *villosum* with correlation of 0.314**,** and there was a negative correlation (-0.127) between *S. villosum* subsp. *miniatum* and *S. nigrum*.

Cytogenetics study

Chromosomes number and Karyotype Formula

karyotype formula (Deanna et al., 2022), determined the fraction of m chromosomes for each of the three *Solanum* species (*S. nigrum*, *S. sinaicum* and two different subspecies of *S. villosum*) (Figure 6, Table 3) recorded highly variation. Chromosome counts from 10-20 well-scattered metaphase plates of each population of each species revealed interspecific differences in the diploid

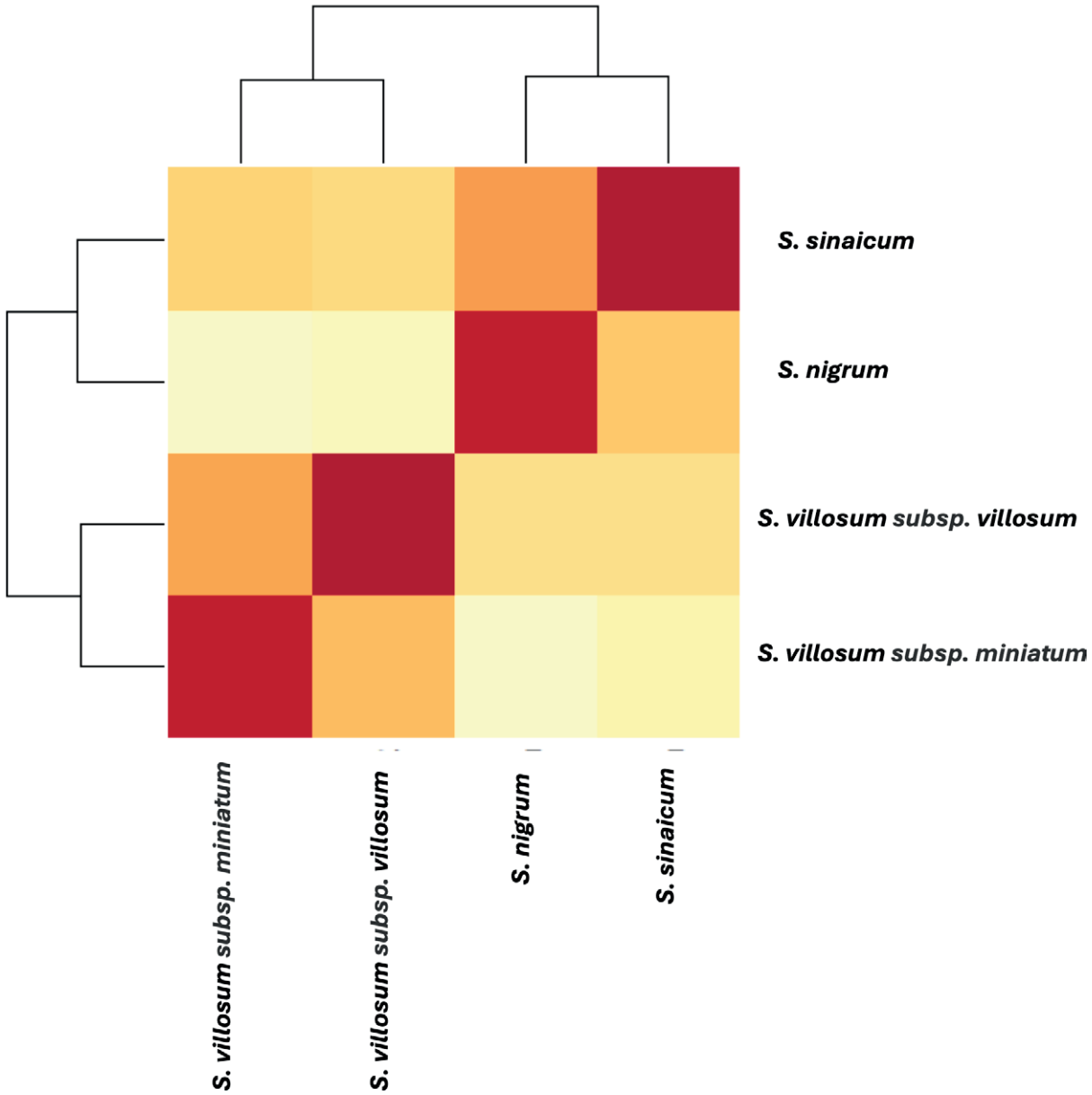

Figure 5. Heat map showing the correlation coefficients between morphological characters of the three studied *Solanum* species.

Table 2. Correlation coefficients between the three studied *Solanum* species, based on the investigated 44 morphological characters.

	S. villosum subsp. *miniatum*	*S. villosum* subsp. *villosum*	*S. nigrum*	*S. sinaicum*
S. villosum subsp. *miniatum*	1			
S. villosum subsp. *villosum*	0.415	1		
S. nigrum	-0.127	0.101	1	
S. sinaicum	0.217	0.314	0.429	1

chromosome number. *S. nigrum* showed 2n = 72 chromosomes, while *S. sinaicum* 2n= 24 chromosomes. The two different subspecies of *S. villosum* subsp. *villosum* and *S. villosum* subsp. *miniatum* showed 2n=24 and 2n= 48 chromosomes respectively. Accordingly, the x number of genome of the three *Solanum* species has been deter-

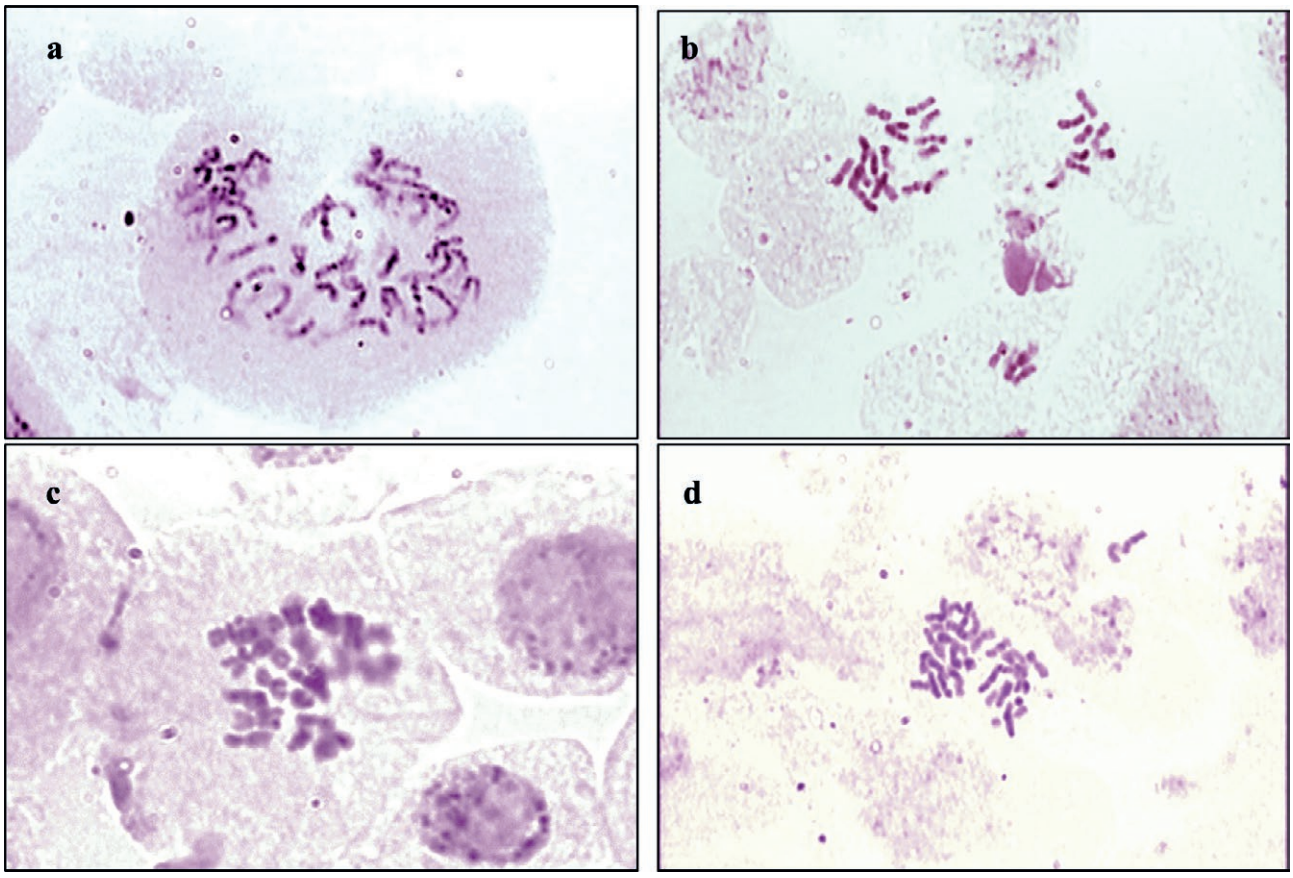

Figure 6. Photomicrographs showing well-spread mitotic metaphase of the three *Solanum* species, (a) *S. nigrum*, (b) *S. sinaicum*, (c) *S. villosum* subsp. *villosum*, (d) *S. villosum* subsp. *miniatum*.

Table 3. The chromosome number, genome size (x) and karyotype formula of the three *Solanum* species (*S. nigrum*, *S. sinaicum* and two different subspecies of *S. villosum*)

	Species	Chro. No. (2n)	Genome No. (x)	Karyotype Formula	Satellite chromosomes
1	*S. nigrum*	(2n=72)	6x	$M^{m}36+M^{sm}12+M^{ac}24$	-
2	*S. sinaicum*	(2n=24)	2x	$M^{m}12+M^{sm}4+M^{ac}8$	Chro. No. 5
3	*S. villosum* subsp. *villosum*	(2n=24)	2x	$M^{m}12+M^{sm}4+M^{ac}8$	Chro. No. 3
4	*S. villosum* subsp. *miniatum*	(2n=48)	4x	$M^{m}24+M^{sm}8+M^{ac}16$	Chro. No. 3 and 5

*M: medium, m: metacentric, sm: submetacentric, ac: acrocentric.

mined (Table 3). Both *S. nigrum* (6x) and *S. villosum* subsp. *miniatum* (4x) showed polyploidy, while *S. sinaicum* and *S. villosum* subsp. *villosum* showed diploid number (2x). (Figure 6, 7).

Molecular analysis

The PCR amplification products of the four investigated *Solanum* specimens produced four distinct, high-quality bands during gel electrophoresis. The DNA sequences ITS1-5.8S-ITS2 of 4 *Solanum* specimens that were the subject of molecular study have been registered on the National Center for Biotechnology Information (NCBI), and the following accession numbers PP701899, PP707086, PP707087, and PP707088 were given to these sequences.

Genetic correlation between the studied Solanum species

The phylogenetic dendrogram based on the ribosomal DNA ITS1-5.8S-ITS2 sequence of the three stud-

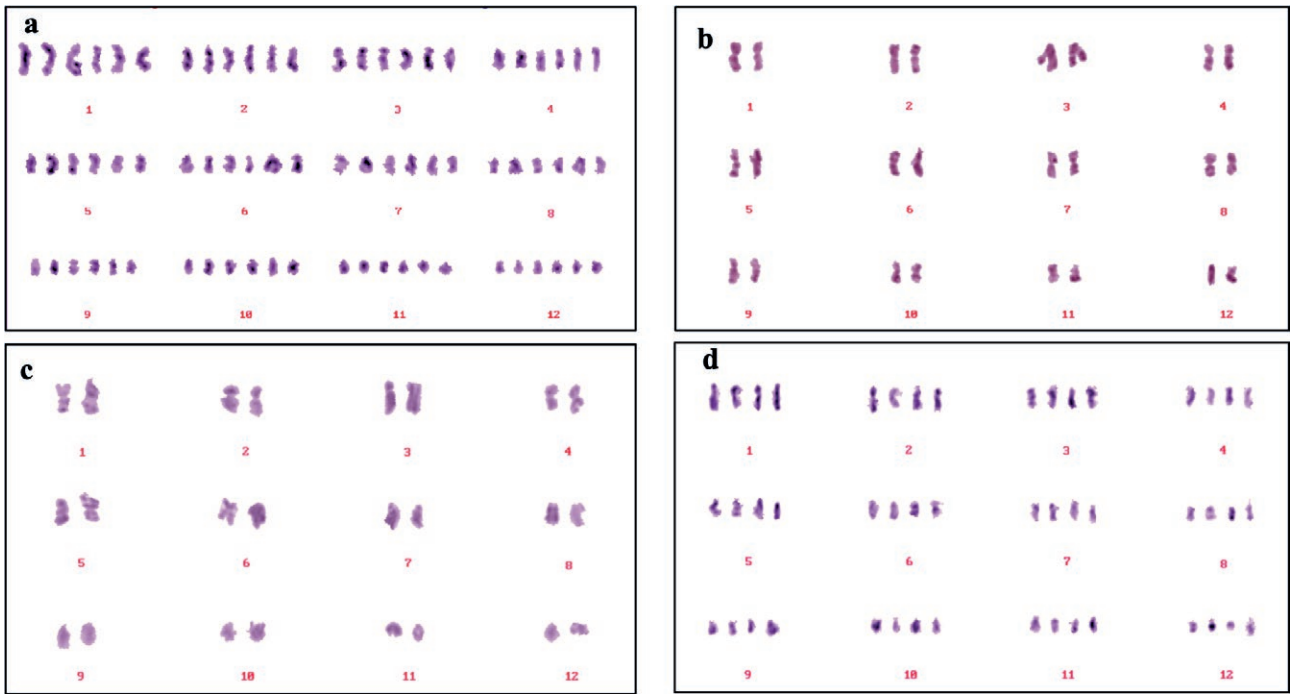

Figure 7. karyotypes of the three *Solanum* species, (a) *S. nigrum*, (b) *S. sinaicum*, (c) *S. villosum* subsp. *villosum*, (d) *S. villosum* subsp. *miniatum*.

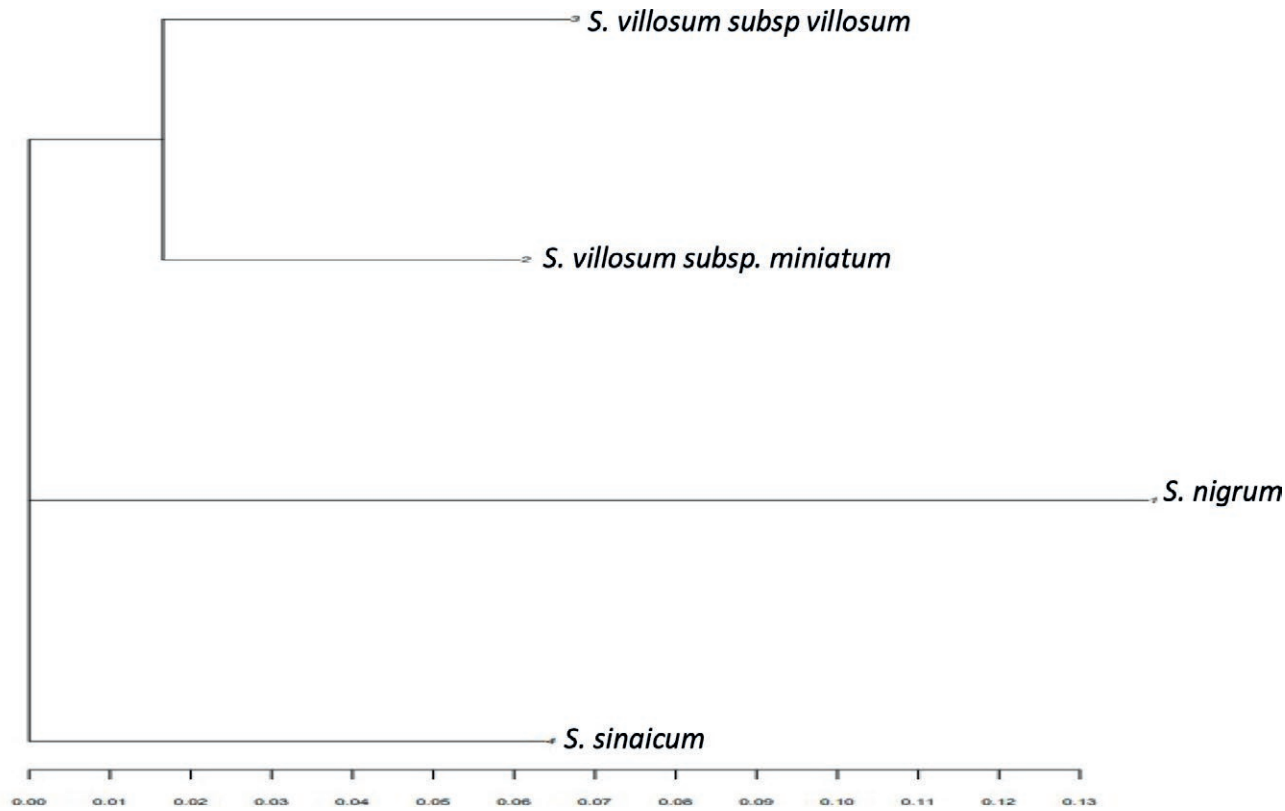

Figure 8. Phylogenetic dendrogram created by UPGMA using a combination of the ribosomal DNA ITS1-5.8S-ITS2 sequence data and values of the genetic dissimilarity distance between the investigated *Solanum* species.

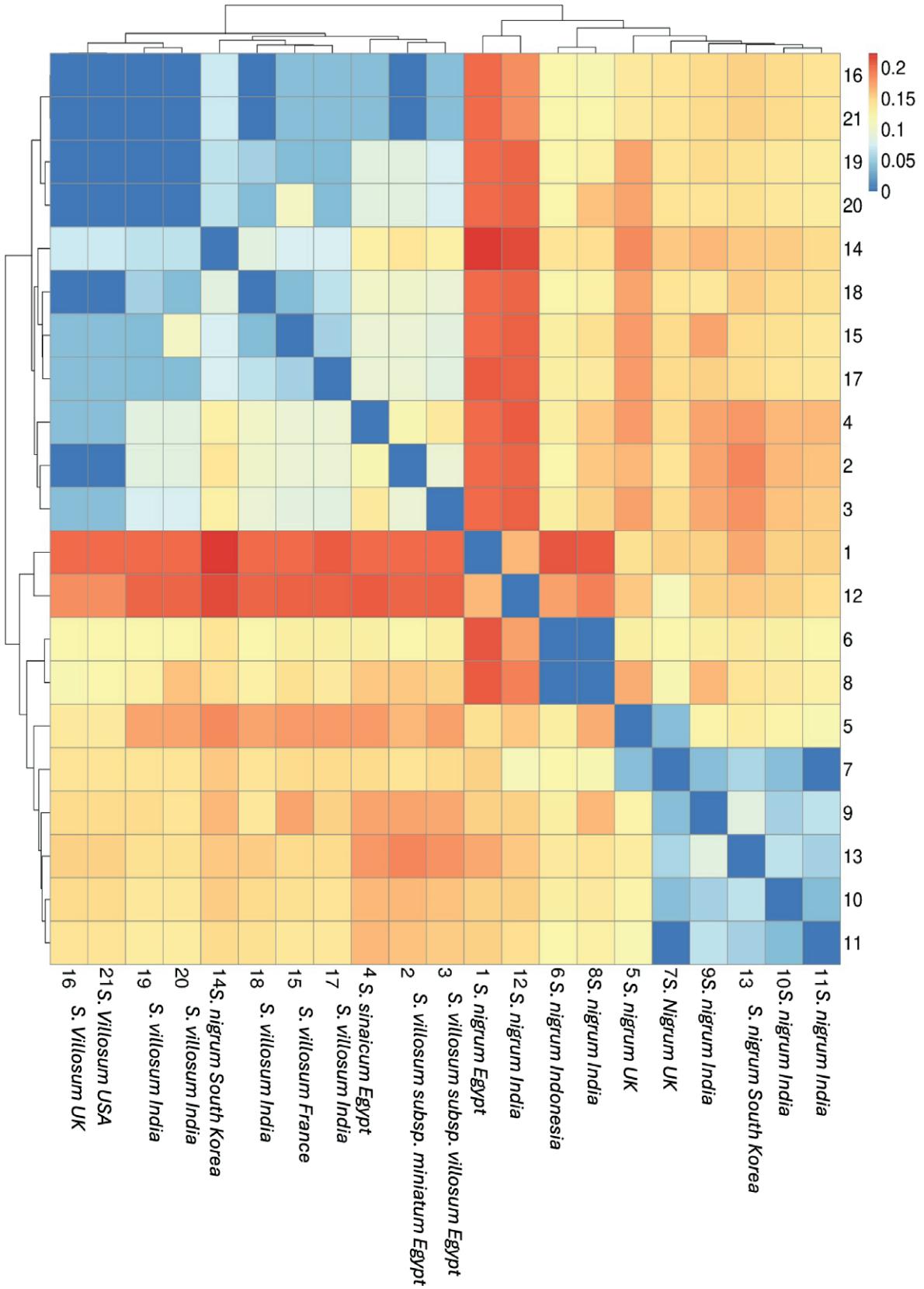

Figure 9. Heatmap of identity by state (IBS) distance matrix of 21 populations representing the three studied *Solanum* species worldwide.

ied species from Egypt (Figure 8), showed that these sequences generate three clusters. The first cluster included the two subspecies of *S. villosum* that showed close affinity with low divergence distance of 0.02. The second cluster included *S. nigrum* that showed high divergence distance from both *S. villosum* subspecies especially *S. villosum* subsp. *miniatum* and this result is consistent with the morphological correlations across these taxa. The third cluster included *S. sinaicum* which showed moderate genetic distance from *S. nigrum* and both *S. villosum* subspecies.

The genetic correlation between *S. nigrum* and *S. villosum* worldwide as shown in Figure 9 was indicated based on the constructed heat map using the ribosomal DNA ITS1-5.8S-ITS2 sequence and the genetic distance value between each two pairs, from the first sample to the last sample, is represented by each little square in the IBS distance matrix. The larger values of genetic distance between two specimens are closer to red, and smaller values are closer to blue. The heat map clustering separated two groups. The first group included specimens of *S. nigrum*, specimens 7 from UK and 11 from India have no genetic divergence, while specimens 9, 10 from India and specimen 13 from UK have very low genetic distance less 0.05, While other specimens of *S. nigrum* (Specimen 5 from UK, specimen 6 from Indonesia, specimens 6, 8 from India, specimen 1 from Egypt) have genetic distance from 0.05 to 0.2. *S. villosum* specimens comprised the second group, sp. 2 of *S. villosum* subsp. *miniatum*, sp. 3 of *S. villosum* subsp. *villosum*, and sp. 4 of *S. sinaicum* from Egypt comprised one subgroup with very low genetic distance less than 0.05, in addition the remaining specimens of *S. villosum* (sp. 17, 18, 19, 20 from India, sp. 15 from France, sp. 14 from South Korea, sp. 16 from UK, and sp. 21 from USA) also have low genetic distance less than 0.1.

DISCUSSION

Morphological identification might not be a trustworthy way to identify species in the Solanaceae because their members are cryptic and share phenotypic features that have been altered by genetic and environmental factors (Nderitu et al. 2023). However, Pojarkova (1997) and Yousaf et al. (2010) confirmed that an essential source for the classification of *Solanum* species was provided by morphological features. According to Knapp et al. 2019, *Solanum* L. possesses challenges for identification due to the lack of group-specific regional keys. There are 17 identified sections within the *Solanum* subgenus (Manoko 2007). About 50 global species make up the section *Solanum* (Child and Lester 2001), *the Solanum nigrum* complex is another name for the section *Solanum*. Since Linnaeus first described *S. nigrum* in 1753, the species in this section have undergone numerous reclassifications up till the present. The ploidy levels of the species, which range from diploid to hexaploid, the genetic variation between populations of the same species, and finally their naturally occurring inter-specific hybridization all contribute to the taxonomic complexity of this section (Edmonds and Chweya 1997).

Morphological studies of freshly collected species, in addition to the herbarium specimens of *S. nigrum*, *S. villosum*, and *S. sinaicum* based on 44 morphological characters (Table 1) showed that flower, fruit, and leaf characters were the most distinctive characters among the studied species. The examined *S. villosum* populations in Egypt exhibited inter-specific variations in morphology. These morphological differences allowed *S. villosum* to be divided into two subspecies: subsp. *villosum* has fruit with orange color, and white corolla with yellow midrib, while subsp. *miniatum* has fruit with a bright red color and white corolla with black midrib. These results agree with Boulos (2002). The morphological correlation of the three studied species as shown in Figure 5, Table 2 detected that *S. nigrum* and *S. sinaicum* had the highest correlation (0.429), followed by *S. villosum* subsp. *miniatum* and *S. villosum* subsp. *villosum* with a correlation of 0.415.

For each of the three *Solanum* species, we found the x number of genomes (Table 3). Polyploidy was demonstrated in *S. nigrum* (6x), This result agrees with Melo et al. (2011), Sultana and Alam (2007), and Särkinen et al. (2018), *S. sinaicum* and *S. villosum* subsp. *villosum* displayed diploid number (2x), however *S. villosum* subsp. *miniatum* displayed polyploidy (4x), this agrees with Sultana and Alam 2007 and Särkinen et al. 2018.

It is possible to determine the genetic diversity of individuals or populations by using morphological and molecular markers. Given that environmental factors and the stage of plant development determine morphological traits (El-Domyati et al. 2011). Previous research studies showed that ITS2 provides more accurate results on interspecific variation than intraspecific variation compared to RBCL (Duan et al. 2019). In this investigation, the ribosomal DNA ITS1-5.8S-ITS2 sequences of the studied species were used to examine the interspecific similarities showed the creation of three clusters (Figure 8), the two *S. villosum* subspecies that exhibited strong affinity and a low divergence distance of 0.02 were included in the first cluster. *S. nigrum* in the second cluster, had a high divergence distance from both *S. villosum* subspecies, particularly *S. villosum* subsp. *min-*

iatum. These findings were confirmed with the morphological correlation seen among these taxa Figure (5). *S. sinaicum* shows a moderate genetic divergence from *S. nigrum*, and both *S. villosum* subspecies were separated in the third cluster.

The heat map clustering based on the ribosomal DNA ITS1-5.8S-ITS2 sequence from worldwide specimens separated two groups (Figure 9). The first group included specimens of *S. nigrum* from Egypt and worldwide, the second group included specimens of *S. sinaicum*, *S. villosum* subsp. *miniatum*, and *S. villosum* subsp. *villosum* from Egypt in addition to worldwide specimens of *S. villosum*.

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