Plant Ecology and Evolution 155 (3): 394–403, 2022
https://doi.org/10.5091/plecevo.85790

RESEARCH ARTICLE

Species delimitation and phylogenetic relationships of *Silene villosa* s.l. (Caryophyllaceae, sect. *Silene* s.l.) using nrDNA ITS and cpDNA *rps16*

Farzaneh Jafari[1], Maryam Keshavarzi[1], Richard K. Rabeler[2]

1 Department of Plant Sciences, Faculty of Biological Sciences, Alzahra University, Tehran, Iran
2 University of Michigan Herbarium-EEB, Ann Arbor, Michigan, U.S.A

Corresponding author: Farzaneh Jafari (f.jafari@alzahra.ac.ir)

Academic editor: Federico Selvi ♦ **Received** 26 April 2022 ♦ **Accepted** 25 July 2022 ♦ **Published** 3 November 2022

Abstract

Background and aims – Species delimitation is a necessary investigation for widely distributed species. Examination of herbarium specimens and descriptions in local floras revealed that two forms of *Silene villosa* were recognized. Form B of *S. villosa* has been identified as a separate species named *S. wendelboi*. However, the latter species was not treated as a separate species in local floras. By using molecular tools, we investigated if these forms of *S. villosa* should be treated as two distinct species or be retained in *S. villosa*.
Material and methods – We created two datasets containing 84 and 46 accessions of nrDNA ITS and cpDNA *rps16* regions, respectively, which were extracted mainly from GenBank. Phylogenies were reconstructed using Maximum Likelihood and Bayesian analyses.
Key results – We propose that *S. villosa* and *S. wendelboi* are two separate species, morphologically and phylogenetically. *Silene wendelboi* was first recognized in SW Iran but we show that the species is distributed in the Persian Gulf and the E Mediterranean region as well. In most cases, *S. wendelboi* is erroneously identified as *S. villosa* in these regions. The distribution of *S. villosa* is much wider, also occurring in SW Asia and North Africa, Egypt, and Algeria. *Silene wendelboi* differs from *S. villosa* in calyx texture, calyx length, the ratio of calyx length to pedicel, the shape of the apex of the coronal scale, seed morphology, and molecular data, i.e. ITS and *rps16* sequences. Based on those two markers, *S. villosa* is closely related to *S. ayachica*, while *S. wendelboi* shows affinity to *S. arabica*.

Keywords

Mediterranean region, North Africa, Persian Gulf, *Silene villosa*, *S. wendelboi*

INTRODUCTION

Silene L. is the largest genus of the family Caryophyllaceae Juss., consisting of ca 870 spp. (Jafari et al. 2020) and often grows in dry habitats including rocky or gravelly places, sandy soils in steppes, chalky soil, and deserts, with little competition from other plants. According to a recent study (Jafari et al. 2020), *Silene* includes three subgenera and one section which is considered as incertae sedis, *S.* sect. *Atocion* Otth as circumscribed by Toprak et al. (2016).

Silene sect. *Silene* s.l., which is part of subg. *Silene*, consists of about 93 species. It is distributed in Europe, Asia, and Africa from the Mediterranean to Pakistan, some species extending to southern Africa (Jafari et al. 2020: appendix S6).

In the Flora of Iraq (Townsend et al. 2016), the annual species *S. villosa* Forssk. and *S. arabica* Boiss. are included in *S.* sect. *Bipartitae* (Boiss.) Melzh. The current infrageneric classification of *Silene* places the two species in *S.* sect. *Silene* s.l. (Jafari et al. 2020). The circumscription of section *Silene* was expanded to encompass species that previously were placed in *S.* sect. *Atocion*, *S.* sect. *Dipterospermae* (Rohrb.) Chowdhuri, *S.* sect. *Fruticulosae* (Willk.) Chowdhuri, *S.* sect. *Nicaeenses* (Rohrb.) Talavera, *S.* sect. *Rubellae* (Batt.) Oxelman & Greuter, *S.* sect.

Plant Ecology and Evolution is published by Meise Botanic Garden and Royal Botanical Society of Belgium.

Scorpioides (Rohrb.) Chowdhuri, *S.* sect. *Silene*, and *S.* sect. *Succulentae* (Boiss.) Chowdhuri (Chowdhuri 1957; Talavera 1988; Greuter 1995; Jafari et al. 2020: appendix S6).

Silene villosa was described from Egypt (Forsskål and Niebuhr 1775); the type specimen is preserved in the Copenhagen herbarium. The species is distributed from SW Asia to North Africa, Egypt, and Algeria. The diagnostic characters in the protologue "petalis bifidis, oblongis, basi sub germine in tubum connatis" are not helpful here. Furthermore, in the description, *S. villosa* was identified by opposite branches, long calyx, violet corolla, and exserted petals. Boissier (1867) described this species with more details, as follows: stems with numerous fleshy leaves, the calyx shorter than peduncles (pedicels) in the reflexed fruit, and petals white. Rohrbach (1868) presented a description similar to Boissier. He recognized the species with stem ramose, calyx shorter than the pedicel, and petals whitish. Zohary (1966) described *S. villosa* in the Flora Palaestina as follows: stem much-branched, leaves somewhat fleshy, pedicels shorter than or as long as or longer than calyx, calyx 10–25 mm, and reflexed in fruit, petals white, rarely pink. Melzheimer (1988) identified the species with these characteristics in the Flora Iranica: calyx shorter than the pedicel, the pedicel straight, bent backward in fruit, calyx at anthesis subcylindrical, fruiting ± clavate, (16–)18–22(–23) mm long, yellowish at the base, pale green above, petals white to pink. Chamberlain (1996) indicated that two forms, 'A' and 'B', of this species occur in Arabia. The forms were separated based on calyx length ((10–)12–18 vs 17–23(–25) mm), the position of the calyx (pendulous vs erect), and plant length (10–45 vs 7–18 cm). Townsend et al. (2016) described *S. villosa* with these characteristics: pedicels shorter than calyx, reflexed in fruit, calyx subcylindrical at anthesis, ± clavate in fruit, petals white to reddish, exserted from the calyx.

Silene wendelboi Assadi was reported as a new species for the southwestern part of Iran (Assadi 1977). It was distinguished by a globose habit, long and narrow calyx, and the sculpture of the seed surface. *Silene villosa* was recognized as a closely related species to *S. wendelboi* (Assadi 1977). However, Melzheimer (1988) mentioned in his note that *S. wendelboi* could be part of *S. arabica* and he did not accept it as a separate species. Although *S. wendelboi* was also reported in Iraq (Lazkov 2006), it has not been treated as a separate species in a recent flora of Iraq (Townsend et al. 2016). The specimen "110 km SSW of Basra, *Rechinger 8793*; B [B10 1107154]" that was reviewed by Lazkov (2006) and identified as *S. wendelboi* was listed as *S. villosa* by Rechinger (1964). A specimen from the same locality (110 km SSW of Basra; *Guest, Rawi & Rechinger, Natl. Herb. Iraq 17208*; K [K000609857]) was listed under *S. villosa* by Townsend et al. (2016).

We recognize the second form of *S. villosa* noted in the Flora of the Arabian Peninsula and Socotra (Chamberlain 1996) with a longer and erect calyx as *S. wendelboi*. We examined many herbarium specimens of these two forms of *S. villosa* s.l. from the Persian Gulf region, including a few specimens at E that were annotated by Chamberlain, and by using molecular tools we investigated if they could be treated as two distinct species or if they are the same.

MATERIAL AND METHODS

Taxonomic study

We examined material of the two species (*S. villosa* s.l. and *S. wendelboi*) in the following herbaria: IRAN, M, MIR, MSB, S, TUH, and TARI (abbreviations according to Thiers continuously updated). In addition, field trips were carried out during the period 2016–2019 in various regions of Iran. Plants were identified using the following references: Flora Aegyptiaco-Arabica (Forsskål and Niebuhr 1755), Flora Orientalis (Boissier 1867), Monographie der Gattung *Silene* (Rohrbach 1868), Flora Palaestina (Zohary 1966), Flora Iranica (Melzheimer 1988), Flora of the Arabian Peninsula and Socotra (Chamberlain 1996), and Flora of Iraq (Townsend et al. 2016). For studying the type specimen of *S. villosa*, we used online images at the virtual Copenhagen herbarium (http://www.daim.snm.ku.dk/search-in-types) and, for additional comparison of herbarium specimens, we used online images available from the Global Biodiversity Information Facility (GBIF) (https://www.gbif.org/), JSTOR (https://plants.jstor.org), and/or JACQ database (https://www.jacq.org/#database), as well as the herbarium websites of E, P, and US, and images provided by staff at B and K.

Phylogenetic study

We created two datasets containing 84 and 46 accessions of nrDNA ITS and cpDNA *rps16* regions, respectively, which were extracted mainly from GenBank (Supplementary file 1). Four accession numbers are newly sequenced. The datasets mainly consist of species that belong to *Silene* sect. *Silene* s.l. sensu Jafari et al. (2020). We tried to include all accessions belonging to this section in the datasets, with only some repetitions removed. Genomic DNA was extracted from herbarium material using the Sinaclon Plant DNA extraction kit (Tehran, Iran) according to the manufacturer's protocol.

Polymerase chain reaction (PCR) amplifications were performed in 25 µL reactions, containing 10 µl of deionized water, 12.5 µl of 2X Reddy® to use PCR Master Mix, 0.5 µl of each primer (10 pmol/µl), and 1 µl template DNA. Amplification of the ITS region was performed using the primers P17 and 26S-82R (Popp and Oxelman 2001). We used the primers rpsF and rpsR2R for amplification (Oxelman et al. 1997). Cycle sequencing was done using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, California, U.S.A.). DNA samples were sequenced with an ABI3130XL

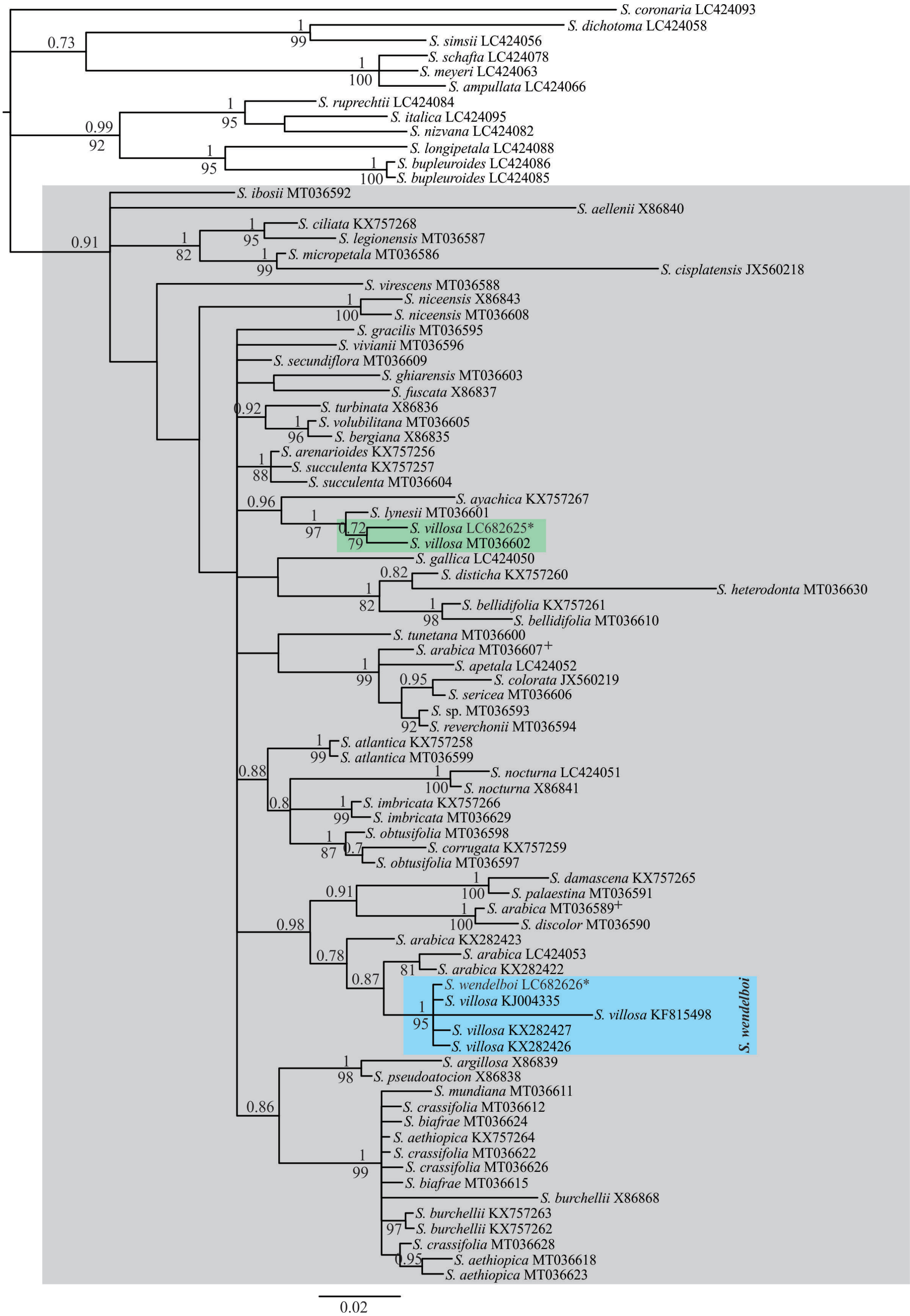

Figure 1. 50% majority-rule consensus tree obtained from the Bayesian inference analysis of the nrDNA ITS sequences in selected species of *Silene*. Posterior probabilities (PP) ≥ 0.70 are shown above the branches and bootstrap values (BS) ≥ 75 below. Gray box shows *S.* sect. *Silene* s.l. Accessions newly sequenced are indicated by an asterisk. Two accessions of *S. arabica* that we did not confirm are indicated by a plus sign.

DNA Analyser 16-well capillary sequencer (Applied Biosystems) performed by Niagene Noor (Tehran, Iran).

Sequence alignment was performed in MAFFT v.7 (Kuraku et al. 2013; Katoh et al. 2019) at the web service (http://mafft.cbrc.jp/alignment/server/). The default setting was applied for all options. The preliminary alignments were then corrected manually. PAUP* 4.0a169 (Swofford 2003) was used to select the best-fitted model of nucleotide substitution based on the corrected Akaike information criteria (AICc), and the General Time Reversible model with Gamma shaped rate variation (GTR+G) model was selected for both regions. Maximum Likelihood (ML) analyses were conducted in RAxML HPC v.8.2.12 (Stamatakis 2014) using the GTRGAMMA model with 1000 pseudo-replicates to evaluate bootstrap support for each node. Bayesian gene tree inference was performed using MrBayes v.3.2.7a (Ronquist et al. 2012) with 10 and 5 million generations for the nrDNA ITS and cpDNA *rps16* datasets. Four Metropolis-coupled chains were run with tree and parameter values saved every 1000th generation in two parallel runs. The first 25% of all trees were discarded as burn-in. Phylogenetic analyses were carried out on the CIPRES science gateway (Miller et al. 2010).

RESULTS

Molecular phylogeny

The phylogenies of both ITS and *rps16* are similar and compatible. The circumscription of *S.* sect. *Silene* sensu Jafari et al. (2020) is supported in both trees. The phylogenies of ITS and *rps16* show that *S. villosa* and *S. wendelboi* are placed in distinct clades. *Silene villosa* along with *S. lynesii* Norman and *S. ayachica* Humburt form a clade (Fig. 1, PP = 0.96; Fig. 2, PP = 1.00, BS = 87). *Silene lynesii* is the closest relative to *S. villosa* in the ITS phylogeny (Fig. 1, PP = 1.00, BS = 97) but since the *rps16* sequence of *S. lynesii* is not available, *S. ayachica* is most closely related to *S. villosa* in the *rps16* tree. In the ITS tree, *S. wendelboi* shows affinity with *S. arabica* (Fig. 1,

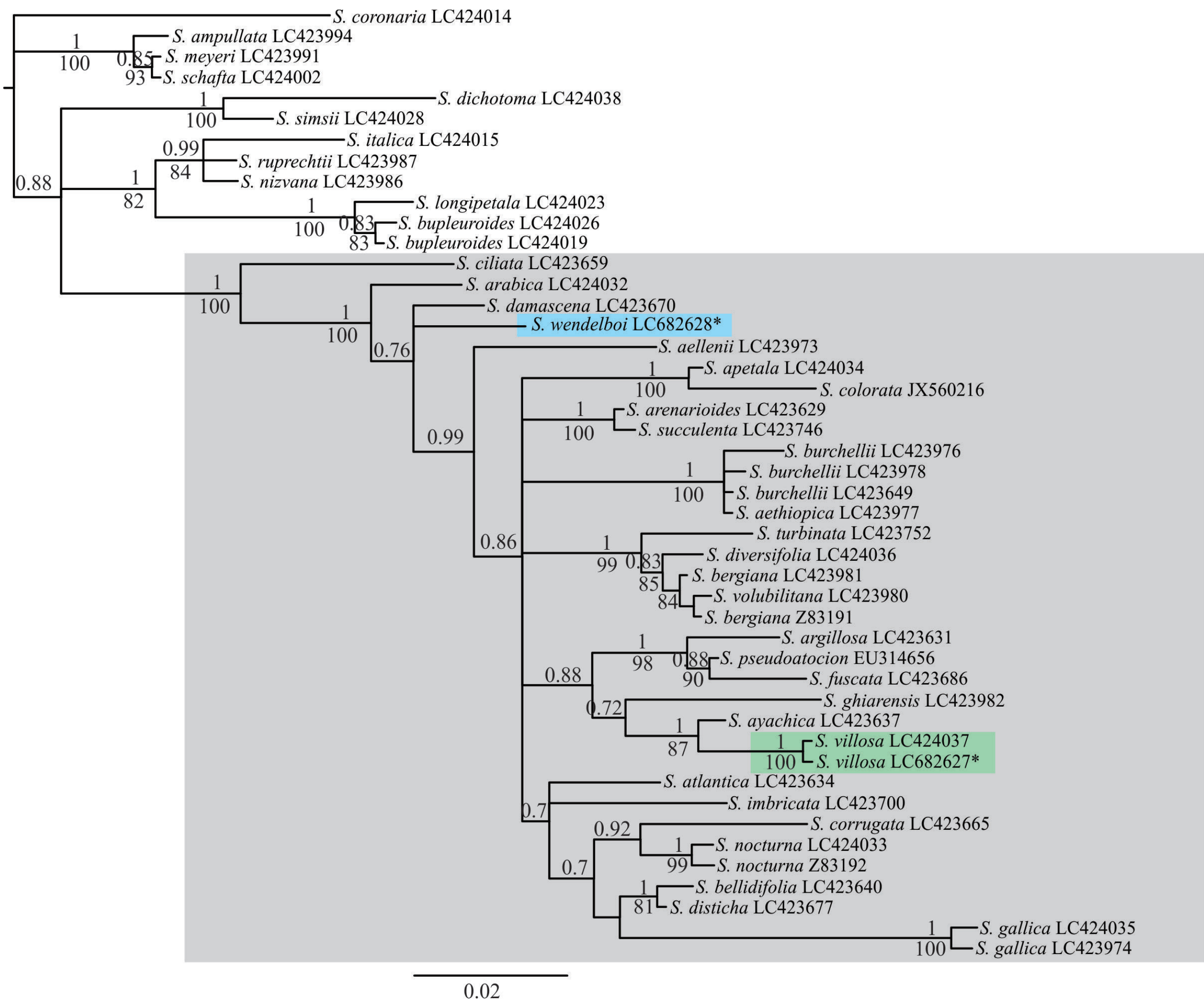

Figure 2. 50% majority-rule consensus tree obtained from the Bayesian inference analysis of the cpDNA *rps16* sequences in selected species of *Silene*. Posterior probabilities (PP) ≥ 0.70 are shown above the branches and bootstrap values (BS) ≥ 75 below. Gray box shows *S.* sect. *Silene* s.l. Accessions newly sequenced are indicated by an asterisk.

PP = 0.87). The *S. wendelboi-S. arabica* subclade is closely related to another subclade including one sample of *S. arabica*, *S. damascena* Boiss. & Gaill, *S. discolor* Sm., and *S. palaestina* Boiss. (Fig. 1, PP = 0.98). *Silene wendelboi* and its close relatives, i.e. *S. arabica* and *S. damascena*, are sisters to the rest of the *S.* sect. *Silene* s.l. in the *rps16* tree (Fig. 2, PP = 1, BS = 100).

Taxonomic treatment

Silene villosa Forssk., Fl. Aegypt-Arab.: 88. 1775.
(Forsskål and Niebuhr 1775)

Fig. 3D–E

S. villosa var. *stricte-refracta* Hausskn. & Bornm., Mitth. Thüring. Bot. Vereins 6: 49. 1894. (Bornmüller 1894) – Type: IRAN • Sinus Persicus australis, in apricis arenosis insulae Kischm; *J. Bornmüller Iter persico-turcicum 1892–93 96*; Jan. 1893; lectotype (**designated here**): JE [JE00013335], isolectotypes: B [B101156873, B101157369, B101157373, B101157374], E [E00987051], JE [JE00013336, JE00013337], PH [PH00024127], PI [PI042024]; probable isolectotype: KFTA [KFTA0001148].

Type. EGYPT • Prope pyramides Gizenses; Herb. P. Forsskål 550; lectotype: C [C10003076] (designated by Melzheimer 1988); probable isolectotype: Herb. P. Forsskål 560, C [C10003075].

Description. Annual herb, glandular and eglandular villous to pubescent. Stem 14.5–32 cm long, ascending. Leaves linear to oblanceolate, 19–43.5 × 2–9.5 mm. Inflorescence irregular cymose, mostly monochasial, rarely dichasial; pedicels 4–19 mm long; bracts herbaceous, linear to oblanceolate. Calyx cylindric, 12.5–18 mm long, pendulous in fruit. Petals white to pinkish, exserted, bifid. Coronal scales oblong, apex entire, obtuse. Anthophore 7–8.5 mm long. Capsule 7–9 mm long. Seed reniform, concave.

General distribution. Algeria, Egypt, Iran, Mauritania, Morocco, Palestine, Qatar, Saudi Arabia, Tunisia (Fig. 5).

Distribution in Iran. South-eastern Iran including Hormozgan, Kerman, Sistan and Baluchestan.

Additional material examined. ALGERIA • Bechar; 29°5'N, 2°38'W; 520 m; 1 Apr. 1980; *Podlech 33649*; MSB [MSB-113841] • Laghouat; 30°00'N, 2°32'E; 500 m; 26 Mar. 1981; *Podlech 35342*; MSB [MSB-113632].

EGYPT • Sinai Peninsula, ca 50 km ESE of Ismailiya near the road to Gifgata, sandy flats; 30°34'N, 32°44'E; 170 m; 3 May 1991; *Förther 4298*; MSB [MSB-113639] • Cairo: between Kirdasa and the Giza Pyramids; 10 Feb. 1926; *Cäckholm s.n.*; S.

IRAN • Hormozgan, 73 km after Kahir to Jak, near Tujak village; 78 m; 7 Mar. 2013; *Attar, Mirtadzadini &*

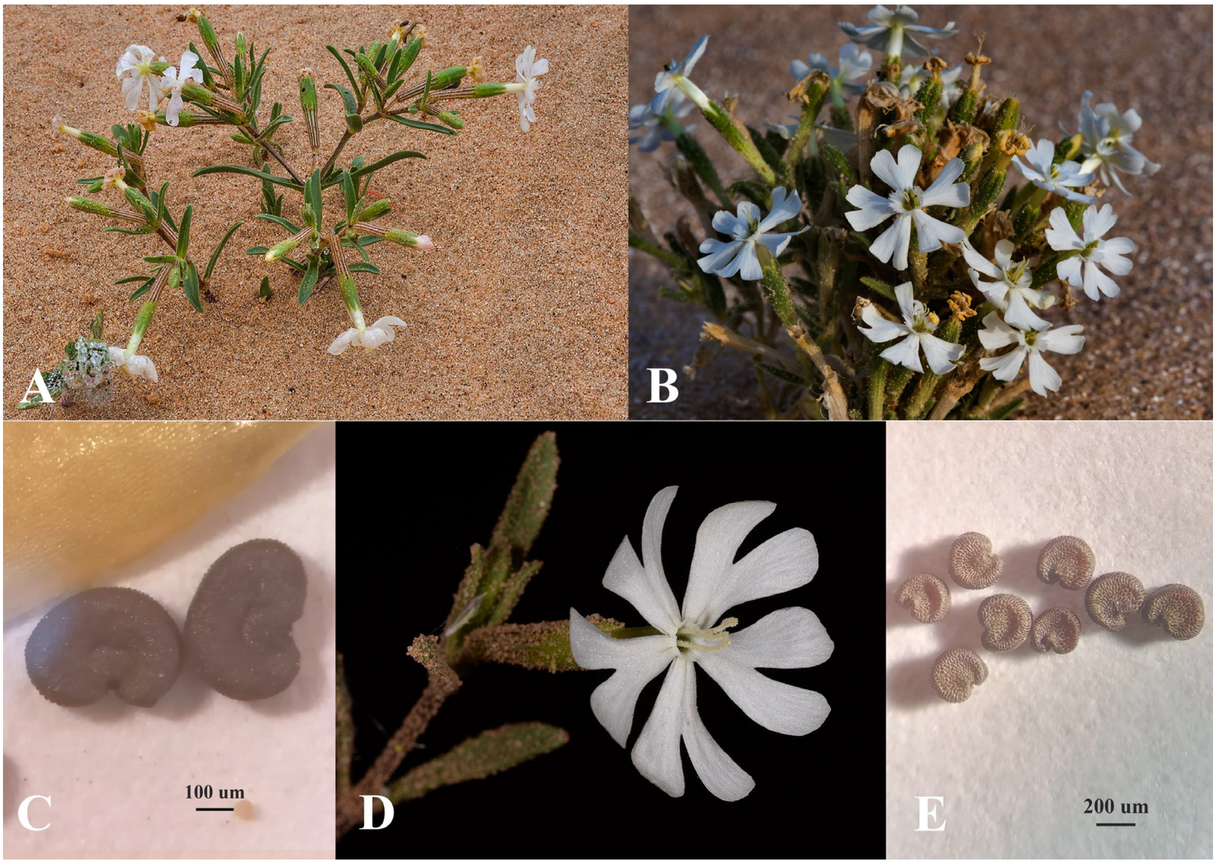

Figure 3. *Silene wendelboi* Assadi (A–C) and *Silene villosa* Forssk. (D–E). **A**. Habit and habitat. **B**. Flowers. **C**. Seeds (53480 TARI). **D**. Flower. **E**. Seeds (45936 TUH). Photos A and B by Alexey Sergeev (https://www.floraofqatar.com/); D by Ori Fragman-Sapir (https://powo.science.kew.org/); C and E by Farzaneh Jafari.

Table 1. Morphological comparison between *Silene arabica*, *S. villosa*, and *S. wendelboi*.

	S. arabica	*S. villosa*	*S. wendelboi*
Calyx texture	not herbaceous, more or less membranaceous	often herbaceous	membranaceous in the lower and herbaceous in the upper part
Calyx length (mm)	11–13.5	12.5–18	17–23
Pedicel length (mm)	2.5–7.5	4–19	(4–)5.5–16.5(–21)
Shape of coronal scale	oblong, apex dentate, acute	oblong, apex entire, obtuse	oblong, apex dentate, acute
Inflorescence	irregularly cymose, mostly monochasial	irregularly cymose, mostly monochasial, rarely dichasial	dichasial cyme

Rategar 45936; TUH • Kerman, SE Jiroft, E Anbarabad, NE Rudfarq village; 23 Feb. 2007; *Mirtadzadini 3853*; MIR • Kerman, Jiroft toward Kahnuj, before Anbarabad crossroad; 709 m; 28 Mar. 2011; *Mirtadzadini 3855*; MIR • Sistan and Baluchestan, Konarak to Jask, 11 km before Zarabad, near Tujak village, on a sandy hill; 78 m; 6 Mar. 2014; *Mirtadzadini, Attar & Rastgar 3854*; MIR • Sistan and Baluchestan, Iranshahr to Nikshahr, 12 km after Espake crossroad; 990 m; 3 Mar. 2014; *Mirtadzadini, Attar & Rastgar 3856*; MIR.

MOROCCO • d'Ouarzazate; 30°38'N, 6°10'W; 10 Apr. 1990; *Schuhwerk 90/903*; M • d'Ouarzazate; 30°57'N, 6°50'W; 12 Apr. 1990; *Podlech 49528*; MSB [MSB-113635].

PALESTINE • Negev, Revivim, sandy soil; 12 Apr. 1952; *D'Angelis s.n.*; M • Negev; 30°58'N, 34°22'E.; 200 m; 30 Mar. 1992; *Tielbörger s.n.*; M • Sinai, Rafa Bir El Meleha (Bi'r al Malalihah) at the coast; 23 Mar. 1938; *Cäckholm s.n.*; S.

Additional material examined (digital). ALGERIA • Aïn-Sefra (Sud de la prov. d'Oran); 7 May 1856; *Cosson s.n.*; JE [JE00015630], LY [LY0126056], MPU [MPU008778], US [US128945] • El-Goléa, in arenosis; 16 Mar. 1899; *Chevallier s.n.*; LY [LY0126054] • El Goléa, in cultis, ad ripas rivulon; 21 Mar. 1904; *Chevallier 88*; US [US1157498] • [Naâma], Aïn Sefra; 20 Apr. 1888; *Bonnet & Maury s.n.*; P [P05032549] • Sables et bords de l'Oued à Aïn Sefra, sud-ouest de la prov. d'Oran; 7 May 1856; *Kralik in Bourgeau, Pl. D'Algerie 229*; MPU [MPU008779, MPU008780, MPU008781], WAG [WAG.1562546] • El Abiod-Sidi-Cheikh, in arenis; May 1899; *Chevallier 170bis*; US [US550356] • Sud-Oranais, environs d'Aïn-Sefra; 1100 m; 11 May 1938; *Faure s.n.*; P [P01194665].

EGYPT • Km 24 Cairo–Suez road; 7 Apr. 1945; *Davis 10284*; E [E00987058] • Al Qāhirah, Sandhügel, 7 km südöstl. von Heluan; 19 Mar. 1885; *Volkens s.n.*; JE [JE00015632] • Bir Baadah el Messaid; 20 Mar. 1880; *Barbey 148*; LY [LY0686625] • [New Valley], Abydos; 26 Mar. 1881; *Letourneux s.n.*; P [P04914444] • Prope Kahiram, Gezisch; Apr. 1877; *Ball 248*; US [US292295].

MAURITANIA • Aguelt Oued Aouineght (Zemmour noir); 4 Mar. 1954; *Sougy 324*; P [P00799753].

MOROCCO • Ouarzazate, 7 km NE Ouarzazate nahe der Straße nach Skoura (P 32), sandiges oued; 30°56'59.999"N, 6°49'59.988"W; 1140 m; 12 Apr. 1990; *Podlech 49528*; P [P05075130].

SAUDI ARABIA • Near lip of the escarpment behind Police Post on the Taif-Jaddah road; 1920 m; 5 Feb. 1980; *Collenette 1742*; E [E01000686] • Red sand dunes, 12 km west of Zabirah Camp and 100 km north of Giba; 549 m; 24 Feb. 1985; *Collenette 5072*; E [E01000644].

TUNISIA • [Kebili], Bechilli (Nefzaoua merid.); 19 Mar. 1886; *Letourneux s.n.*; P [P04914526] • [Kebili], El Foouara in arenosis; 19 Apr. 1887; *Letourneux s.n.*; P [P04914522, P04914523] • [Sidi Bouzid], El houwara; 19 Apr. 1887; *Letourneux s.n.*; P [P04914525] • [Tozeur], Gouifla Tunisie; 9 May 1884; *Doumet-Adanson & Bonnet s.n.*; P [P04914527].

UNKNOWN • Herbier Mussat; 4 Oct. 1902; *s.col. s.n.*; LY [LY0126051].

Notes. Although Bornmüller (1894) was specifically describing new plants from Iran based on specimens that were included in the Iter persico-turcicum 1892–93 exsiccata set that he distributed, no single specimen is cited in the protologue of *S. villosa* var. *stricte-refracta*. We believe #96 is original material of this taxon and have selected one of the three sheets of #96 at JE to serve as the lectotype. The sheet at KFTA has a handwritten label with the same collection information except for the exsiccata number.

In Flora Iranica (Melzheimer 1988), five specimens were listed for *S. villosa* from Iran. The first author (FJ) examined three specimens (*Pabot 382*, *Pabot 392*, and *Iranshahr & Terme 14943-E*) (Fig. 4A, B) and identified them as *S. wendelboi*. Melzheimer (1988) recognized *Bornmüller 96* from Qeshm as *S. villosa* and we confirm the identification. However, we could not locate *Kunkel 16731* from Qeshm. It is noteworthy that *Rechinger 8787=8794* from Iraq, Basra, cited as the basis for the illustration of *S. villosa* in tab. 248 in Flora Iranica, should be *S. arabica* based on the characters shown in Table 1; *Rechinger 8794* was cited under *S. arabica* in the Flora of Lowland Iraq (Rechinger 1964). The specimen *Iranshahr & Terme 33683-E* was erroneously identified as *S. arabica* in Flora Iranica; it should be *S. villosa*.

The presence of *S. villosa* in Iraq has not been confirmed; we have not seen any specimens of *S. villosa* from Iraq. The description of *S. villosa* in Flora of Iraq (Townsend et al. 2016) includes characteristics of *S. wendelboi*, while parts 4–7 in figure 46, reproduced from the Flora of Pakistan (Ghazanfar and Nasir 1986), are similar to *S. villosa.* All specimens cited as *S. villosa* in Flora of Iraq (Townsend et al. 2016) are *S. wendelboi*.

Our phylogenetic analysis shows that *S. villosa* forms a clade with *S. ayachica* and *S. lynesii* in both the ITS and *rps16* tree.

Silene wendelboi Assadi, Iran. Journ. Bot. 1(2): 181–183. 1977. (Assadi 1977)

Figs 3A–C, 4A–B

Type. IRAN • Khuzestan, Albaji; 30 m; 28 Apr. 1971; *Gheisari 1252*; holotype: TARI.

Description. Annual herb, glandular and eglandular pubescent. Stem (4.5) 6–23 cm long, ascending. Leaves linear, 16–44 × 1–3.5 mm, sometimes internodes shorter than the leaves. Inflorescence more or less regular dichasial; pedicel (4–)5.5–16.5(–21) mm long; bracts herbaceous, linear. Calyx cylindric, membranaceous in the lower and herbaceous in the upper part, glandular-hairy, 17–23 mm long, more or less pendulous in fruit. Petals white, exserted, bifid. Coronal scales oblong, apex dentate, acute. Anthophore 8.5–12.5 mm long. Capsule 8–11 mm long. Seed reniform, compressed around the hilum.

General distribution. Bahrain, Cyprus, Iran, Iraq, Jordan, Kuwait, Lebanon, Qatar, Saudi Arabia, United Arab Emirates (Fig. 5).

Distribution in Iran. Khuzestan province, in the southwest of Iran.

Notes. *Silene wendelboi* was accepted as part of *S. arabica* in Flora Iranica by Melzheimer (1988), while the specimen from Khuzestan, *Pabot 392*, was listed in both *S. villosa* and *S. arabica*. The handwriting on the label of the herbarium sheet shows it was identified as *S. villosa*.

We analysed the ITS and *rps16* regions of a specimen from Basra, Iraq, from an area near Khuzestan province. It shows affinity with *S. arabica* in the ITS phylogeny (Fig. 1) and this subclade forms a clade with E Mediterranean species including *S. damascena*, *S. palaestina*, and *S. discolor*. The E Mediterranean species and *S. wendelboi* are sister to the rest of the *S.* sect. *Silene* s.l. in the *rps16* phylogeny (Fig. 2). In the ITS tree, the accessions KJ004335, KF815498, KX282426, and KX282427 from Saudi Arabia and Kuwait were erroneously identified and should be corrected to *S. wendelboi*.

Investigation of the herbarium specimens showed that the specimen from Basra (*Rechinger 8725* (M)), earlier cited as *S. villosa* (Rechinger 1964), belongs to *S. wendelboi*. We were able to examine the Rechinger specimen at B (*Rechinger 8793*) cited by Lazkov (2006) and the collection from the same locality (110 km SSW of Basra) cited by Townsend et al. (2016); as noted above, both specimens were indeed *S. wendleboi*.

Additional material examined. IRAN • Khuzestan, 14 km E aggeris Kharkheh (Karkheh); 15 Mar. 1959; *Pabot*

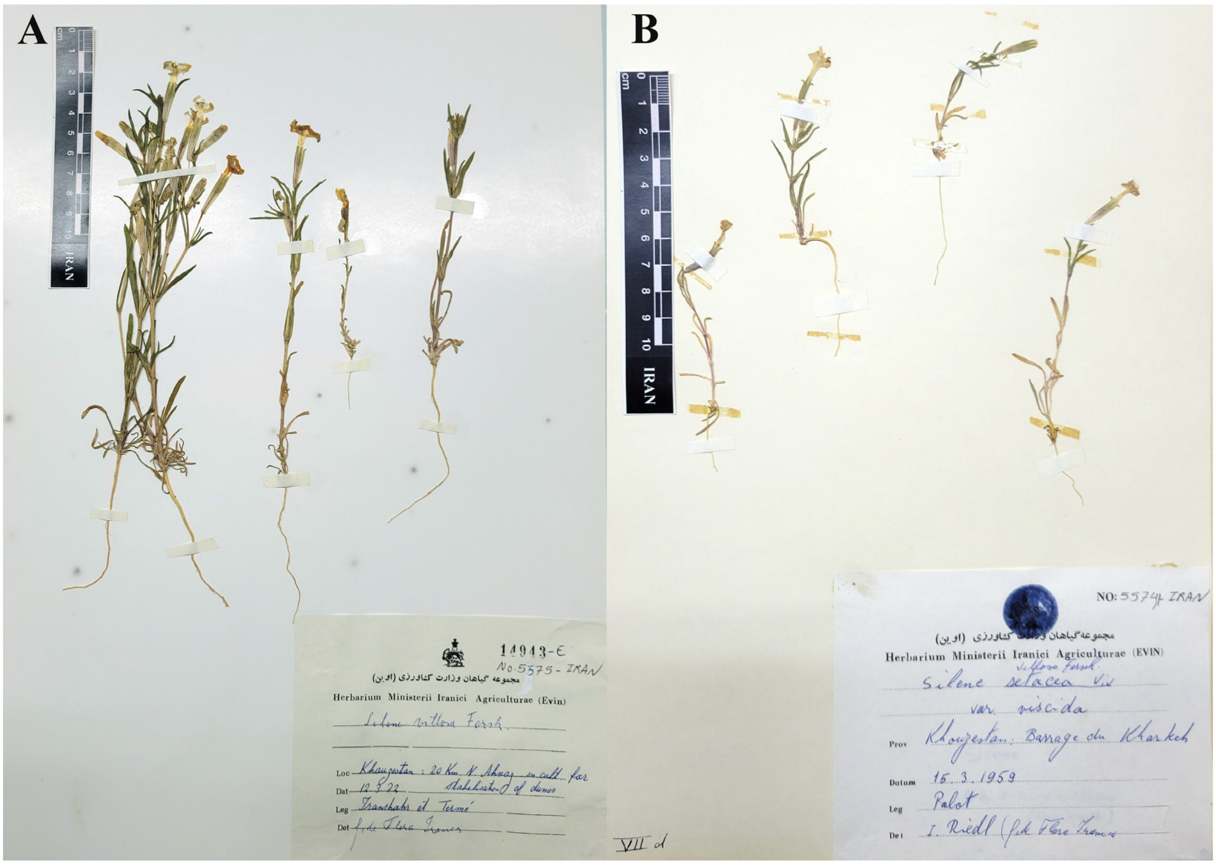

Figure 4. A. *Silene wendelboi* Assadi, *Iranshahr & Terme 14943-E*, IRAN. **B**. *Silene wendelboi* Assadi, *Pabot 382*, IRAN. Photos by Atiye Nejad Falatoury.

382; IRAN • ibid.; *Pabot 392*; IRAN • Khuzestan, 20 km N Ahvaz, in arenosis cultis; 12 Mar. 1972; *Iranshahr & Terme 14943-E*; IRAN • Khuzestan, NE Susangerd, Segure region, Farheh, sand dunes; 40 m; 12 Apr. 1985; *Mozaffarian 53480*; TARI • Khuzestan, NE Bostan around Mish-Dagh Mt.; 50–200 m; 16 Apr. 1985; *Mozaffarian 53727*; TARI • Khuzestan, Ahvaz, Karkheh; 110 m; *Jamzad, Naanaee & Salehi 79222*; TARI • Khuzestan, ca 14 km to Omidieh from Ahvaz-Omidieh road, sandy area; 40 m; 11 Apr. 2008; *Mozaffarian 93598*; TARI.

IRAQ • Basra, Jabal Sanam; ca 30°10'N, 47°30'E; 24 Mar. 1957; *Rechinger 8725*; M, E [E00987075, E00987076, E00987077].

JORDAN • Aqaba, Wadi Rum; *Emanuelsson 4371*; S [S14-22379].

QATAR • 5 km E of Umm Bab; 15 Apr. 1987; *El-Ghazaly & Nilsson 103*; S [S17-61907] • E of Umm Bab; 15 Apr. 1987; *El-Ghazaly & Nilsson s.n.*; S [S17-61908].

Additional material examined (digital). BAHRAIN • In sandy gullies along the western Imrock; 26–30 m; 17 Feb. 1986; *Alder 14*; E [E00046454].

IRAQ • Al Zarqa, 12 km SE of Samawa; 25 m; 30 Mar. 1957; *Alkas, Natl. Herb. Iraq 17672*; K [K000609855] • Basra, desertum meridionale (Southern Desert), ad confines territorii Kuweit, prope Chilawa, 110 km SSW Basra, in arenosis submobilibus; 170 m; 25 Mar. 1957; *Rechinger 8793*; B [B101107154] • Basra, nr. Chilwa, 110 km SSW of Basra; 170 m; 25 Mar. 1957; *Guest, Rawi & Rechinger, Natl. Herb. Iraq 17208*; K [K000609857] • Southern desert, Al-Ichrishi, 35 km E by N Busaiya; 115 m; 15 Apr. 1955 (1957?); *Guest & Rawi, Natl. Herb. Iraq 14186*; K [K000609859, K000609860] • Southern desert, Al Urmaigh, ca 30 km S of Tall Lahn; 75 m; 30 Mar. 1956; *Guest & Mahmoud, Natl. Herb. Iraq 15305*; K [K000609861] • ca 6 km W of Safwan (30 km S of Zubair); ca 10 m; 23 Mar. 1957; *Guest, Rawi & Rechinger, Natl. Herb Iraq 16946*; K [K000609858] • Jebel Samara; 23 Aug. 1919; *Watson & Sharples s.n.*; K [K000609856].

KUWAIT • 29°10'33"N, 47°43'39"E; 14 Feb. 2013; *Abdullah MTA274*; E [E00678805].

LEBANON • Qana, Neford; 27 Apr. 1982; *Dulici 2375*; ALF [ALF038623].

QATAR • Wadi Al Galaiel, toward the southern end of the Qatar Peninsula (Miocene); 2 Apr. 1977; *Boulos 11130*; E [E00648975].

SAUDI ARABIA • 20 km N of Manijah, Gulf Coast; 3 m; 30 Mar. 1987; *Collenette 6182*; E [E00540018] • 12 km west of Zabirah Camp and 100 km N of Gila; 550 m; 24 Feb. 1985; *Collenette 5073*; E [E00540036] • Dhahran, Ash Sharqiyah; 26°18'N, 50°08'E; 21 Feb. 1964; *Mandaville 52*; US [US2512948] • ibid.; 19 Mar. 1965; *Mandaville 366*; US [US2512826] • Hail; 22–23 Mar. 1981; *Chaudhary E-574*; E [E00648973] • Nafud, near Kharais; 8 Apr. 1980; *Chaudhary E 217*; E [E00648979] • Mada'in salih; 26°47'21.947"N, 37°56'44.988"E; 799 m; 18 Mar. 2018; *Bouchaud 6*; P [P00915699].

UNITED ARAB EMIRATES – **Abu Dhabi** • Al Markhaniyah, 2 km W of Tawwam Hospital at Al Ain; 190 m; 26 Mar. 1982; *Western 16*; E [E00648977] • Zibara, ca 30 km N of new Abu Dhabi airport on road to Dubai; 25 m; 14–16 Apr. 1982; *Western 41*; E [E00648978]. – **Dubai** • 45 km from Al Ayn to Dubai; 300 m; 15 Feb. 1980; *Edmondson E3005*; E [E00648972] • Dünen bei Al Awir; 100 m; 24 Mar. 1986; *Müller-Hohenstein 86292*; E [E00648971]. – **Umm al Qawain** • Tell Abrak, near Umm al Qawain; 10–50 m; 22 Feb. 1985; *Western 771*; E [E00046452].

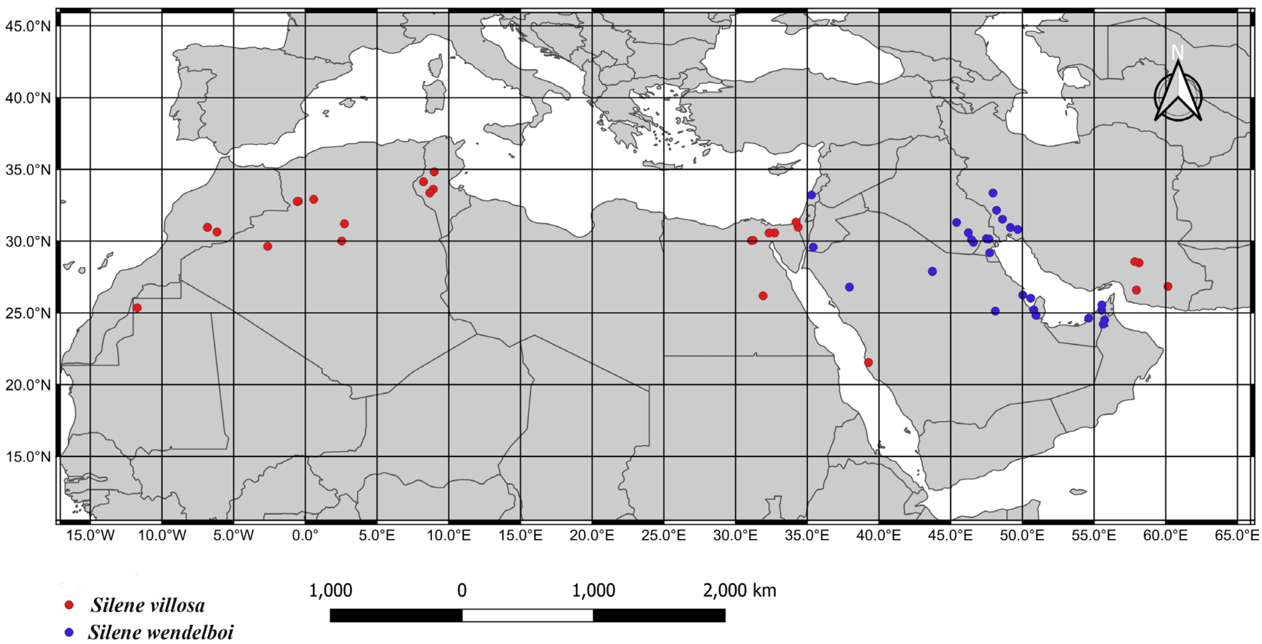

Figure 5. Distribution map of *Silene villosa* (red dots) and *S. wendelboi* (blue dots) based on specimens examined. The map was made with QGIS v.3.22.9.

Identification key

1. Stem branched, ramose at base; inflorescence monochasial; calyx 11–13.5 mm long, erect in fruit, petal limb cleft more than ½ length of limb, lobes linear .... ***S. arabica* Boiss.**
– Stem branched, ramose throughout; inflorescence monochasial or dichasial; calyx > 12 mm long, pendulous in fruit (sometimes erect); petal limbs cleft less than ½ length of limb, lobes oblong **2**
2. Inflorescence monochasial (rarely dichasial); calyx 12.5–18 mm, herbaceous throughout, pendulous in fruit; apex of coronal scale entire, obtuse .... ***S. villosa* Forssk.**
– Inflorescence dichasial; calyx 17–23 mm, non-herbaceous at the base, erect in fruit; apex of coronal scale dentate, acute .... ***S. wendelboi* Assadi**

DISCUSSION

Silene villosa and *S. wendelboi* are treated as two separate species in both the ITS and the *rps16* phylogenetic tree (Figs 1, 2). Calyx texture, calyx length, the ratio of calyx length to pedicel, shape of the apex of the coronal scale, seed morphology, and molecular data are reliable characters that confirm the distinction of these species. Herbarium investigations show that *S. wendelboi* was erroneously identified mainly as *S. villosa* or sometimes as *S. arabica* among collections from the Persian Gulf and E Mediterranean regions. The erroneous identification causes the description of *S. villosa* in local floras of the Persian Gulf and E Mediterranean regions (i.e. Zohary 1966; Melzheimer 1988; Chamberlain 1996; Townsend et al. 2016) to be mixed with *S. wendelboi*. Shahid and Rao (2014) erroneously identified *S. wendelboi* as *S. arabica* for the flora of the United Arab Emirates. Although the calyx length shows a very small degree of overlap, the characters coronal scales and seeds are diagnostic. The apex of the coronal scale is dentate and acute in *S. wendelboi* and seeds are recessed near the hilum, while the apex of the coronal scale is entire and obtuse and seeds are concave in *S. villosa*. Pedicels often are shorter than the calyx in *S. wendelboi*, while long pedicels and a pendent or reflexed calyx in fruit are characters indicated for *S. villosa* in most local floras (Zohary 1966; Melzheimer 1988; Chamberlain 1996; Townsend et al. 2016). Lazkov (2006) also indicated seed shape and colour as diagnostic characters for separating *S. wendelboi* and *S. villosa*.

In the African Plant Database (version 4.0.0), four additional varieties of *S. villosa* are listed. We refrain from treating these varieties since further morphological investigation and molecular data are needed. It seems that the seed shape and micromorphology of *S. villosa* and *S. wendelboi* were intermixed in both the Flora Iranica and the protologue of *S. wendelboi*; the seed image of *S. villosa* in Flora Iranica belongs to *S. wendelboi* and the seed illustration of *S. wendelboi* in the protologue shows the micromorphological characters of *S. villosa*. The seeds are reniform in both taxa, and compressed around the hilum in *S. wendelboi*, while concave in *S. villosa*.

Although similarities of morphological characters in *S. villosa* and *S. wendelboi* cause these taxa to have been considered as the same taxon, the phylogenetic trees show that *S. wendelboi* is distinct from *S. villosa* and that it is a close relative of *S. arabica* rather than of *S. villosa*.

Low-copy nuclear markers have sometimes been used due to the fast-evolving intron of low-copy nuclear genes when the variation within nuclear and chloroplast sequences was not sufficient to segregate closely related species (Sang 2002). However, here, the nrDNA ITS and cpDNA *rps16* are informative and support the distinction of *S. villosa* and *S. wendelboi*; therefore, low-copy nuclear genes were not applied here.

ACKNOWLEDGEMENTS

We greatly appreciate the curators at the herbaria IRAN, M, MIR, MSB, S, TARI, TUH for permission to study plant material used in this study. We are thankful to the staff at B and K for providing digitized images of *Silene wendelboi* in their collections. We are grateful to Alzahra University and the International Association for Plant Taxonomy (IAPT) for financial support to FJ. We are grateful to Atiye Nejad Falatoury and Somayyeh Kheiri for providing photos of herbarium specimens at the Iranian Research Institute of Plant Protection (IRAN) and literature. We thank Fatemeh Moein (University of Isfahan) for her assistance with the map (Fig. 5). We are also grateful to the two anonymous reviewers and the subject editor for their helpful comments on the earlier version of the manuscript.

REFERENCES

African Plant Database (version 4.0.0) Conservatoire et Jardin botaniques de la Ville de Genève and South African National Biodiversity Institute, Pretoria. http://africanplantdatabase.ch [accessed 01.08.2022]

Assadi M (1977) *Silene wendelboi* (Caryophyllaceae), a new species from SW Iran. Iranian Journal of Botany 1(2): 181–183.

Boissier E (1867) Flora Orientalis: sive, Enumeratio plantarum in Oriente a Graecia et Aegypto ad Indiae fines hucusque observatarum. Volume 1. Apud H. Georg, Basel & Genève, 1–1017. https://doi.org/10.5962/bhl.title.20323

Bornmüller J (1894) Ein beitrag zue Kenntniss der Küstenflora des Persischen Golfes. Mittheilungen des Thüringischen Botanischen Vereins 6: 48–77.

Chamberlain DF (1996) *Silene* L. In: Miller AG, Cope TA (Eds) Flora of the Arabian Peninsula and Socotra. Edinburgh University Press, Edinburgh, 209–222.

Chowdhuri PK (1957) Studies in the genus *Silene*. Notes from the Royal Botanic Garden, Edinburgh 22: 221-278.

Forsskål P, Niebuhr C (1775) Flora Aegyptiaco-Arabica: sive descriptiones plantarum. Ex officina Mölleri, Hauniæ (Copenhagen), 1–221.

Ghazanfar SA, Nasir, YN (1986) Caryophyllaceae. In: Ali SI (Ed.) Flora of Pakistan: 175. University of Karachi, Karachi.

Greuter W (1995) *Silene* (Caryophyllaceae) in Greece: a subgeneric and sectional classification. Taxon 44(4): 543–581. https://doi.org/10.2307/1223499

Jafari F, Zarre S, Gholipour A, Eggens F, Rabeler RK, Oxelman B (2020) A new taxonomic backbone for the infrageneric classification of the species-rich genus *Silene* (Caryophyllaceae). Taxon 69(2): 337–368. https://doi.org/10.1002/tax.12230

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160-1166. https://doi.org/10.1093/bib/bbx108

Kuraku S, Zmasek CM, Nishimura O, Katoh K (2013) aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. Nucleic Acids Research 41(W1): W22–W28. https://doi.org/10.1093/nar/gkt389

Lazkov GA (2006) *Silene wendelboi* Assadi (Caryophyllaceae), new to Iraq. Novosti Sistematiki Vysshikh Rastenii 38: 126.

Melzheimer V (1988) *Silene* L. In: Rechinger KH (Ed.) Flora des Iranischen Hochlandes und der umrahmenden Gebirge (Persien, Afghanistan, Teile von West-Pakistan, Nord-Iraq, Azerbaidjan, Turkmenistan): Caryophyllaceae II. Volume 163. Akademische Druck- und Verlagsanstalt, Graz, 341–508.

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, Louisiana, 14 Nov. 2010. Piscataway, IEEE, 45–52. https://doi.org/10.1109/GCE.2010.5676129

Oxelman B, Lidén M, Berglund D (1997) Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). Plant Systematics and Evolution 206: 393–410. https://doi.org/10.1007/BF00987959

Popp M, Oxelman B (2001) Inferring the history of the polyploid *Silene aegaea* (Caryophyllaceae) using plastid and homoeologous nuclear DNA sequences. Molecular Phylogeny and Evolution 20: 474–481. https://doi.org/10.1006/mpev.2001.0977

Rechinger KH (1964) Flora of Lowland Iraq. J. Cramer, Weinheim, 1–746.

Rohrbach P (1868) Monographie der Gattung *Silene*. W. Engelmann, Leipzig, 1–249. https://doi.org/10.5962/bhl.title.15462

Ronquist F, Teslenko M, Van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Sang T (2002) Utility of low-copy nuclear gene sequences in plant phylogenetics. Critical Reviews in Biochemistry and Molecular Biology 37(3): 121–147. https://doi.org/10.1080/10409230290771474

Shahid M, Rao NK (2014) New records of two species of Caryophyllaceae in the flora of the United Arab Emirates. Tribulus 22: 66–68.

Stamatakis A (2014) RAxML ver. 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Swofford DL (2003) PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Ver. 4.0a169. Sinauer Associates, Sunderland, Massachusetts. http://phylosolutions.com/paup-test/ [accessed 10.01.2022]

Talavera S (1988) Nombres y combinaciones nuevas en el género *Silene* L. (Caryophyllaceae). Anales del Jardín Botánico de Madrid 45: 361–362.

Thiers B (continuously updated) Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/ [accessed 24.02.2019]

Toprak Z, Pfeil BE, Jones G, Marcussen T, Ertekin AS, Oxelman B (2016) Species delimitation without prior knowledge: DISSECT reveals extensive cryptic speciation in the *Silene aegyptiaca* complex (Caryophyllaceae). Molecular Phylogenetics and Evolution 102: 1-8. https://doi.org/10.1016/j.ympev.2016.05.024

Townsend CC, Melzheimer V, Kandemir A, Ghazanfar SA, Haloob A (2016) Caryophyllaceae. In: Ghazanfar SA, Edmondson JR (Eds) Flora of Iraq. Volume 5(2). Ministry of Agriculture of the Republic of Iraq, Baghdad, 6–123.

Zohary M (1966) Flora Palaestina. Volume 1. Israel Academy of Sciences and Humanities, Jerusalem, 1–364.

SUPPLEMENTARY FILE

Supplementary file 1

Material used for phylogenetic analyses, with indication of taxon name, voucher information, and GenBank accession numbers.

Link: https://doi.org/10.5091/plecevo.85790.suppl1