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RALF HAND[1], GEORGIOS HADJIKYRIAKOU[2] & HOLGER ZETZSCHE[1]

Scaligeria alziarii (Apiaceae), a new sibling species of *S. napiformis* from Cyprus

Abstract

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Recent research has identified a new species of the genus *Scaligeria,* the rhizomatous perennial *S. alziarii,* endemic to Cyprus. Prior to the discovery of this species, the biennial *S. napiformis* was the only identified taxon of the genus in Cyprus. Molecular studies corroborate the placement of the new taxon in *Scaligeria* and its rank as a separate species.

Additional key words: *Umbelliferae,* taxonomy, molecular phylogenetics, ITS, *psbA-trnH*

Introduction

The genus *Scaligeria* DC. has been significantly altered in the last decades. The majority of species from SW and Middle Asia traditionally allocated to the genus have been transferred to other genera, primarily to *Elaeosticta* (Korovin 1928; Kljuykov & al. 1976; Pimenov & Kljuykov 1981; Kljuykov 1983; Pimenov & Kljuykov 1995). This classification is generally accepted today and has also been corroborated by molecular studies (see, e.g. Degtjareva & al. 2009). As a result, *Scaligeria* s.str. was determined to be a genus restricted to the E Mediterranean and comprising three taxa only: the widespread *S. napiformis* (Willd. ex Spreng.) Grande [Syn.: *S. cretica* (Mill.) Boiss.], known to occur from the southern Balkans and Libya to SW Asia; *S. moreana* Engstrand, an endemic of the Peloponnese and the island of Kefallinia; and *S. halophila* (Rech. f.) Rech. f., endemic to several islands of the southern Aegean Sea. The data on these species and especially the variability of *S. napiformis* have been summarised by Engstrand (1970), who also provided an identification key. There has been little substantial information added since that time, apart from the observation that *S. moreana* is perennial (Tan 2001), a fact that had already been observed in plants collected by Greuter and cultivated at the Botanic Garden Berlin-Dahlem in the 1980s (specimen *Schwerdtfeger 14405* at B).

In Cyprus, up to now, only the widespread *Scaligeria napiformis* had been documented (Meikle 1977). But in 1999, the second author noticed that the Cypriot populations seemed more variable than described in the literature. His initial studies led to the conclusion that these populations belong to an unnamed taxon within the polymorphic *S. napiformis*. After subsequent intensive studies both in the field and on herbarium collections, the distribution of the taxon was clarified. Moreover, plants from these enigmatic populations were successfully cultivated. Its subterranean parts and the life form proved to be so unusual that even the question was raised as to whether the plants really belonged to *Scaligeria* or instead to another related genus. Molecular studies were

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therefore conducted to clarify its phylogenetic position.

These results, in combination with other related research on the taxon, led to the decision to describe it as a species new to science.

Material and methods

Material — This paper is based (1) on intensive studies and field work in Cyprus, where both the new *Scaligeria alziarii* and *S. napiformis* were observed over the year. (2) The complete herbarium material of *Scaligeria* in Berlin (B) and the rich Cypriot collections in the private herbarium of the second author were analysed. Specimens studied for measurements are listed in the Appendix. (3) Plants of *S. alziarii* in greenhouse cultivation in the Botanic Garden Berlin-Dahlem were studied for several years.

Morphology, anatomy and caryology — Terminology and measurement follow, for the most part, Fröberg (2010), partly also Kljuykov & al. (2004). The mericarp anatomy was studied in thinly sliced dry fruits (moistened prior to study). Chromosome numbers were counted as described by Vogt & Aparicio (2000).

Taxon sampling for the molecular studies — Tissue sampling and preparation were carried out as recommended for higher plants in Gemeinholzer & al. (2010). Sequences of the closely related taxa *Scaligeria halophila,* S. *moreana* and *S. napiformis* were provided by Galina Degtjareva, while outgroup taxa were chosen and downloaded from GenBank/EMBL based on the phylogenetic hypothesis for *Bunium* and related taxa as published by Degtjareva & al. (2009). Collection data, including place of voucher deposit and GenBank/EMBL accession numbers are shown in the Appendix and are partly available online via GBIF and the DNA Bank Network (2009).

DNA preparation, amplification and sequencing — Dried leaf tissues were ground with 3 mm metal beads using a RETSCH MM301 shaking-mill at a mill frequency 30/s for 45s. Total genomic DNA was extracted from pulverised material by using the NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany) with RNase and lysis buffer PL1 following the manufacturer's instructions. DNA samples were stored at −20°C until further analysis was undertaken. The ITS and the *psbA-trnH* regions were amplified using the primers ITS-A and ITS-B (Blattner 1999), and psbAF (Sang & al. 1997) and trnH (Tate & Simpson 2003), respectively. PCR products were cleaned with MSB Spin PCRapace® (Invitek LLC; Berlin, Germany). PCR amplifications were performed using the following reaction mix: 1.5 mM MgCl2, 1X PeqLab Taq Buffer S (including MgCl2), 0.25 mM each dNTP, 0.8 pmol primer, 0.03 U/µl Taq polymerase (PeqLab, Erlangen Germany), betain at a final concentration of 1.0 M and 1.0 ng/µl DNA template. The PCR was conducted in a Mastercycler (Eppendorf, Hamburg, Germany) and the PCR regime for ITS was performed as given by Blattner (1999). The PCR program for the *psbA-trnH* region was: 35 cycles of denaturation (60 s at 97 °C), annealing (60 s at 51 °C), extension (45 s at 72 °C) and a final extension step (10 min at 72 °C). Quality and quantity of the purified amplicons were measured with a NanoDrop ND-1000 spectrophotometer (PeqLab Biotechnology LLC; Erlangen, Germany). The DNA cycle sequencing was conducted by Starseq® (GENterprise LLC; Mainz, Germany) for forward and reverse strands using amplification primers as sequencing primers.

Sequence alignment and phylogenetic analysis — Sequences were edited manually and improved using PhyDE-1 (Phylogenetic Data Editor) version 0.9971 (Müller & al. 2007). Inversions were re-inverted and coded as mutational event in the indel matrix as outlined in Löhne & Borsch (2005). The phylogenetic relationships were reconstructed based on aligned ITS and *psbA-trnH* datasets by Maximum Parsimony (MP) analysis using PAUP*(version 4.0b10; Swofford 2002) and Bayesian Inference applying the MrBayes software (version 3.2.1, Ronquist & Huelsenbeck 2003). MP analysis was performed with the heuristic search modus with equally weighted character states, random sequence addition, tree bisection-reconnection (TBR) branch swapping and with inital MAXTREES set at 1000. The consistency index (CI), retention index (RI) and rescaled consistency index (RCI) were calculated. Also, bootstrap support of branches was calculated with 1000 replicates. For the Bayesian analysis the GTR+I+G model was selected with MrModeltest 2.3 (Nylander 2004). The Metropolis-coupled Markov chain Monte Carlo algorithm was used to estimate posterior probabilities. Six Markov chains were run from a random starting tree for 200 000 generations and trees sampled every 100 generations. The first 10 000 trees were discarded as burn-in before convergence of the chains. The remaining trees were used to calculate posterior probabilities. TreeView (Page 1996) and Adobe Illustrator (Adobe Systems Inc., San Jose, California, USA) were used to visualise the phylogenetic tree calculated.

Results

Scaligeria alziarii Hand, Hadjik. & Zetzsche, **sp. nov.**
Holotype: Cyprus, Vasileiatis, 2 km south of Agios Amvrosios Keryneias, alt. c. 450 m (coordinates: long. 554140, lat. 3908870), *Pinus brutia* forest with low shrubs in the understorey, 5.5.2012, *Hadjikyriakou 7210* (B; isotypes: B, CYP, JE, MPU, STU, herb. Hadjikyriakou).

Diagnosis — *Scaligeria alziarii* is very similar to *S. napiformis* but differs primarily in the following ways: it is a perennial (not biennial) with long, branched, horizontal

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rhizomes that are difficult to uproot (not short, thickened vertical taproots which can easily be completely uprooted); its first leaves appearing in spring are 1–2-ternate-pinnate (not 2–3-ternate); its petals are 0.6–1.04 × 0.45–0.75 mm with an incision of (0.14–)0.24–0.4 mm length (not (1–) 1.2–1.68(–1.92) × 0.75–1.28(–1.92) mm, (0.32–)0.4–0.72(–0.96) mm incised).

Illustrations — Fig. 1A–C, 2–3 (for additional illustrations of *Scaligeria alziarii* and *S. napiformis* see Hand & al. 2011+).

Description — Erect, rhizomatous, polycarpic perennial. *Rhizome* cylindrical, elongated, ± horizontal, irregularly branched, up to 45 cm long, 3–22 mm wide, older parts warty, blackish brown, somewhat woody; *young roots* waxy yellow to yellowish brown, fleshy, terminally filiform for about 2–5 cm. *Stems* solitary, solid, bright green, sometimes with purplish tinge, especially lower parts often glaucous, glabrous, shallowly sulcate, 50–100 cm high, often branched in the upper third. *Leaves* glabrous, usually dark green above, whitish green and reticulate-veined below; *lamina* of basal and lower leaves



Fig. 1. A–C: *Scaligeria alziarii* – A: flowering shoots; Cyprus, Vasileiatis above Agios Amvrosios Keryneias, 3.5.2010; B: basal leaf; Cyprus, Vasileiatis above Agios Amvrosios Keryneias, 5.3.2011; C: subterranean parts; Cyprus, Vasileiatis above Agios Amvrosios Keryneias, 3.5.2010. – D: *S. napiformis,* subterranean parts; Cyprus, Malia Lemesos, 19.5.2010. – Photos by G. Hadjikyriakou.

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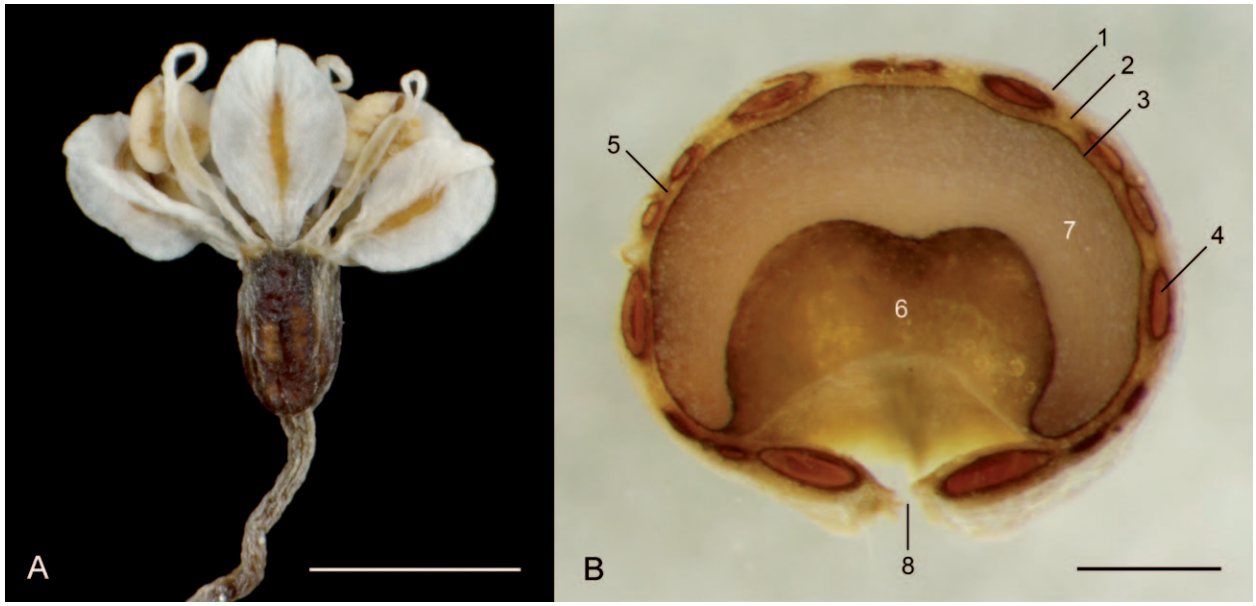


Fig. 2. *Scaligeria alziarii* – A: flower, from *Hand 4827;* B: cross section of mericarp, from *Hand CY-29;* 1 = exocarp, 2 = mesocarp, 3 = endocarp, 4 = oil duct, 5 = vascular bundle, 6 = funicle, 7 = endosperm, 8 = sclerenchyma (mostly collapsed). – Scale bars: A = 1 mm, B = 0.5 mm; photos by M. Lüchow.

narrowly to broadly deltoid in outline, (3)6–12(–23) × (5–)17–23 cm, 1–2-ternate-pinnate, usually withering at anthesis; ultimate segments of the basal and lower leaves ovate to rhomboid, usually cuneate at base, irregularly crenate-serrate to lobed, margins sometimes revolute, 8–34 × (5–)26–32 mm; upper cauline leaves (if present) continuously reduced to a lamina with linear lobes, uppermost leaves reduced to simple sheaths; *petioles* of the basal leaves partly subterranean, (4–)16–28 cm long, canaliculate above; *basal leaf-sheaths* narrow, 4–8(–21) mm long, with membranous margins and small membranous apical auricles. *Inflorescence* lax; *peduncles* 5–10(–12) cm long; *bracts* 0–2(–3), linear-subulate, caducous, 2–4 mm long, 0.2–0.5 mm wide; *umbels* lax, (6–)8–15(–20)-rayed; rays very slender, spreading to suberect, unequal, 1–4(–5) cm long at anthesis; *bractlets* (0–)1–5(–6), inconspicuous, linear-subulate; 1–2 × 0.2–0.5 mm. *Flowers* (Fig. 2A) 10–20 in each umbellule, about half of them hermaphrodite and the rest male; pedicels filiform, 1–4 mm long; calyx teeth short, rudimentary, often obsolete; petals subequal, oblong-obovate, white with a brownish or purplish median stripe,

Table 1. *Scaligeria alziarii,* selected characters compared to *S. napiformis.*

	Scaligeria alziarii	***Scaligeria napiformis***
Life form	perennial, polycarpic	biennial, monocarpic
Subterranean parts	elongated, cylindrical, creeping, ± horizontally orientated rhizome, almost always branched, very rarely simple, easily broken and generally not easily uprooted*	tuberous, fusiform, globose, sometimes forked root, ± vertically orientated, easily uprooted*
Length of thickened subterranean part [mm]	>230–410 (easily broken and sometimes probably much longer)	(15–)25–29(–133)
Lamina division of basal and lower leaves	usually 1–2-ternate but quite often 1–2-pinnate	2–3-ternate
Leaf sheath length [mm]	4–8(–21)	5–27(–32)
Number of bracts	0–2(–3)	0–1(–2)
Petal length [mm]	0.6–1.04	(1–)1.2–1.68(–1.92)
Petal width [mm]	0.45–0.75	0.75–1.28(–1.92)
Petal incision [mm]	(0.14–)0.24–0.4	(0.32–)0.4–0.72(–0.96)
Mericarp width [mm]	1.3–1.89	(0.88–)1.2–1.52(–1.84)

* both taxa are common and not threatened by moderate collecting

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0.6–1.04 × 0.45–0.75 mm, apex incurved-emarginate, incision (0.14–)0.24–0.4 mm; *filaments* 1–1.5 mm long, *anthers* oblong, 0.3–0.5 mm long, 0.3–0.4 mm wide, whitish yellow; *stylopodium* convex, later shortly conical, (0.26–)0.27–0.62(–0.64) mm long, *styles* 0.3–0.8 mm long and erect at anthesis, lengthening to (0.96–)1.12–1.28 mm in fruit and becoming sharply reflexed, *stigmas* capitate. *Fruit* broadly ovoid-subglobose, distinctly didymous, base distinctly cordate, separating readily at maturity; *mericarps* (1.45–)1.52–2.11(–2.2) × 1.3–1.89 mm, 0.8–1.13(–1.4) mm thick, blackish or dark brown when ripe, rugulose, glabrous; ridges very variable, from very obscure and nearly lacking to clearly visible; mericarp anatomy (Fig. 2B) similar to *S. napiformis*, with (14–)15(–16) oil ducts and conspicuous funicle, commissure (0.12–)0.2–0.32 mm wide; carpophore bipartite. – Flowering May to June.
Chromosome number: $2n = 20$ (see Fig. 3)

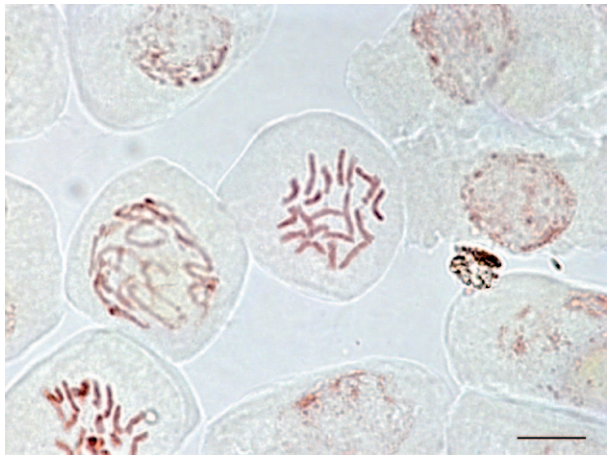


Fig. 3. *Scaligeria alziarii* – metaphase of root tip mitosis, $2n = 20$ (*Hand 5548 & Hadjikyriakou*); scale bar = 10 µm; photo by R. Hand & M. Lüchow.

Eponymy — The new species has been named in honour of Prof. Dr Gabriel Alziar (Cassagnes-Bégonhès/France), who was the Director of the Botanical Garden of Nice for 20 years, and a botanist and entomologist at the Muséum d'Histoire Naturelle de Nice for 15 years. He contributed greatly to the extant knowledge of the Cypriot natural history, especially the entomofauna. He also was involved in the organisation of the Optima Iter Mediterraneum IV which inspired botanical research on the Cypriot flora.

Distribution, ecology and conservation — *Scaligeria alziarii* occurs on the two mountain ranges of Cyprus, Troodos and Pentadactylos, as well as in the Akamas peninsula. Its altitudinal distribution ranges from 50 to 700 m a.s.l.

The new species is primarily associated with *Pinus brutia* forests, as, typically, its elongated rhizomes spread in quite deep forest soils, often encroaching into rock crevices. Large populations are found in semi-shaded areas, sometimes also in dense phrygana. In terms of substrata in its distribution area, *Scaligeria alziarii* does not appear to be very specialised. In the Pentadactylos range it concentrates on limestones of the Kyrenia terrane, mostly Triassic and Jurassic limestones and chalks. In the Troodos range it mostly occurs on igneous rocks, namely diabase, whereas the sites in the Akamas peninsula are characterised by Cretaceous serpentines. *S. alziarii* is widespread in the above-mentioned terrains in Cyprus where it is a common and characteristic plant of pine forests. Its preferred habitats are not currently under threat.

Phylogenetic positioning

The phylogenetic analysis was based on a combined ITS (ITS1 and ITS2) and *psbA-trnH* dataset of all known species of *Scaligeria* s.str., including the new taxon sequenced here, and selected taxa of subfamily *Apioideae* as outgroup (primarily from Degtjareva & al. 2009).

ITS and *psbA-trnH* data were combined since there was no contradiction between MP and Bayesian tree topologies derived from partial datasets. The combined dataset comprised 655 characters after alignment including gaps. The resulting phylogenetic MP tree shown in Fig. 4 had a length of 315 steps (CI = 0.77, RI = 0.66, RCI = 0.51). Clades with bootstrap support of 50 % or higher are shown. All *Scaligeria* taxa formed a strongly supported clade together with *Carum appuanum* (Viv.) Grande indicating a monophyly of that group. That clade shows some relationships to *Crithmum maritimum* and the representatives of the *Bunium*-I Clade, *Bunium intermedium* and *Galagania ferganensis,* in contrast to the suggestions of Degtjareva & al. (2009). However, the analysis revealed a strong affiliation among *S. halophila, S. napiformis* and *S. alziarii* and some of *S. moreana* with *Carum appuanum,* forming two to three separate sister groups within that clade. *S. napiformis* together with *S. halophila* appear separated from *S. alziarii* based mainly on ITS sequence differences.

Discussion

The two Greek endemics of *Scaligeria, S. moreana* and *S. halophila,* differ considerably from both the new taxon and *S. napiformis*. *S. moreana* has lanceolate leaves in outline and *S. halophila* has larger fruits, to name but the most obvious diagnostic features (for details see Engstrand 1970, Jahn & Schönfelder 1995, Tan 2001). Even though *S. moreana* has been shown to be perennial, its tuberous roots are somewhat thicker (diameter up to 2.5 cm) and much shorter (<10 cm; see Tan 2001, and cultivation of *Schwerdtfeger 14405* at B) than in *S. alziarii*. On the other hand, the latter is most similar to *S. napiformis* with regard to its above-ground parts. This may perhaps explain why it escaped the attention of botanists working on the island, although it is a quite

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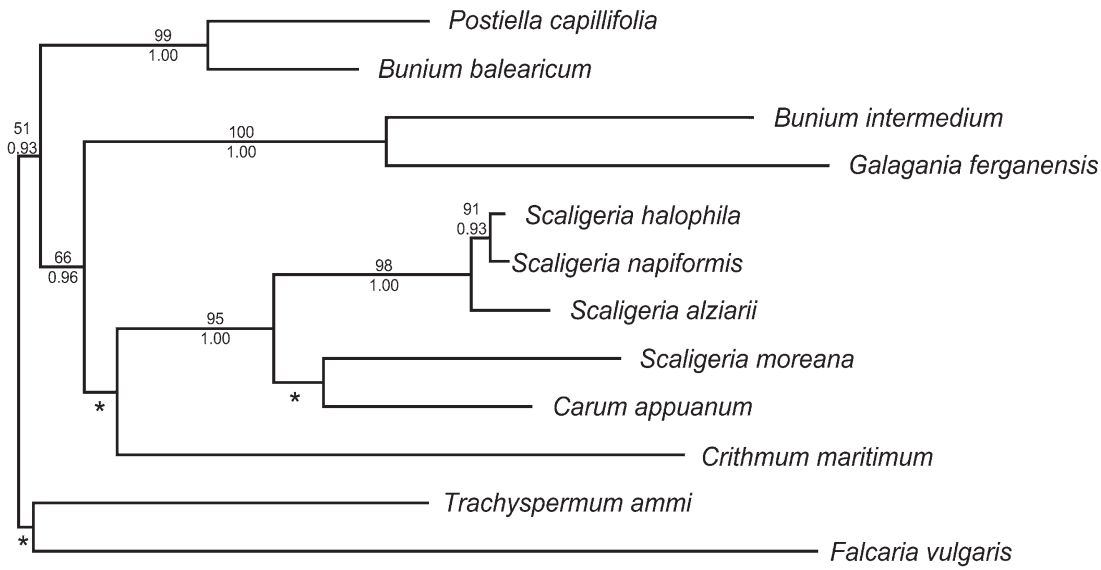


Fig. 4. Phylogram of one of the 354 equally most parsimonious trees of 12 taxa within subfam. *Apioideae* (selection compared with Degtjareva & al. 2009) including all known *Scaligeria* taxa, based on a combined sequence data set (ITS1, ITS2*, psbA-trnH*). Numbers above nodes indicate bootstrap support estimates greater than 50 % for the Maximum Parsimony analysis with 1000 replicates and numbers below Bayesian posterior probabilities, respectively. Asterisks (*) indicate nodes that collapse in the strict consensus tree.

common plant in several regions. Its subterranean organs seem to be unique for the genus and are diagnostic.

Autecology of *Scaligeria alziarii* and *S. napiformis* reveals considerable differences: *S. alziarii* is able to colonise larger areas by vegetative growth as it can penetrate rock crevices such that it may be impossible to uproot the entire subterranean system. In *S. napiformis* the taproot can easily be completely uprooted, which proved to be a very reliable identifying character in the field. Cultivation experiments showed that short rhizome fragments of *S. alziarii* plants (less than 10 cm) could produce rhizomes of about 1 m within less than a year. It appears that *S. alziarii* seeds germinate at very low rates, but this needs further corroboration. Engstrand (1970) observed that there was no successful germination in *S. moreana* at all. However, vegetative spread may play a key role in the dispersal of *S. alziarii,* as the rhizomes easily break, e.g. after landslides and torrential rains, resulting in new colonies along streams and similar places. *S. napiformis* seems to be strictly biennial (Engstrand 1970) or very rarely a short-lived perennial (3 years; pers. obs.). Consequently, dispersal by seeds is essential for this taxon. There seem to be no significant differences in the mericarp anatomy of *S. alziarii* and *S. napiformis* (see Kljuykov & al. 1976; Pimenov & Kljuykov 1995 in reference to only *S. napiformis*).

The chromosome number of *Scaligeria alziarii* is identical to known counts of *S. napiformis* and *S. halophila* (see summary by Engstrand 1970; for an exception see Pimenov & al. 1998). The number of *S. moreana* is not yet known.

As our phylogenetic results demonstrated, ITS and *psbA-trnH* sequences of all four *Scaligeria* species show unique characters supporting two to four different biological entities. *S. moreana* is certainly more distantly related, but whether it is a member of the genus *Scaligeria* requires further studies based on a complete sampling. *S. napiformis* and *S. alziarii* are not closest relatives but the former together with *S. halophila* are sister to the latter. Thus, if *S. halophila* is accepted to be a separate species, that status should be even more appropriate for *S. alziarii*. *S. halophila* "has possibly originated as an ecotype" of *napiformis* (see Engstrand 1970); our results do not contradict this.

Although *Scaligeria alziarii* and *S. napiformis* sometimes occur in close proximity in Cyprus, no signs of intermediacy between the two were found. It should be noted that we failed to confirm the occurrence of *S. napiformis* in the Pentadactylos mountain range. Obviously, all records for that area (see, e.g. Meikle 1977) seem to refer to *S. alziarii,* but this needs further mapping efforts.

All results speak in favour of treating *Scaligeria alziarii* as a clearly distinct sibling species of *S. napiformis*. *S. alziarii* is endemic to Cyprus and enhances the island's reputation as a hotspot of Mediterranean biodiversity and endemism.

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Appendix

1. Specimens seen

Scaligeria alziarii (complete list)
Cyprus (divisions sensu Meikle 1977): Division 1: Koudounomeni Akamas, slope with *Pinus brutia*, c. 250 m, 14.3.1999, *Hadjikyriakou 4144* (B, herb. GH); Fytorion Akamas, pine forest, c. 350 m, 15.6.1999, *Hadjikyriakou 4643* (B, herb. GH); Smigies – Koudounas Akamas, slope with open pine forest, c. 300 m, 15.6.1999, *Hadjikyriakou 4646* (B, herb. GH); Neo Chorio, Smigies, W Smigies picnic site, N of track, pine forest, 285 m, 14.6.2011, *Hand 5832* (B); ibid., 29.3.2012, *Hand 5944* (B). — Division 2: Argaki tou Gerakiou, crevices of vertical cliff, c. 600 m, 12.1.1999, *Hadjikyriakou 3924* (B, herb. GH); Mourmouros Pafos forest, roadside, c. 700 m, 22.1.1999, *Hadjikyriakou 3945* (B, herb. GH); ibid., c. 650 m, 19.6.1999, *Hadjikyriakou 4658* (B, herb. GH); Argaki tou Gerakiou at Frodichia Fleva valley, crevices of vertical cliff, c. 600 m, 4.4.1999, *Hadjikyriakou 4262* (B, herb. GH); ibid., 4.4.1999, *Hadjikyriakou 4266* (B, herb. GH); ibid., 29.5.1999, *Hadjikyriakou 4575* (B, herb. GH); ibid., 29.5.1999, *Hadjikyriakou 4576* (B, herb. GH); ibid., 6.6.2001, *Hadjikyriakou 5300 & T. Hadjikyriakou* (B, herb. GH); Frodichia Fleva valley, roadside, c. 600 m, 19.6.1999, *Hadjikyriakou 4657* (B, herb. GH); ibid., 10.7.1999, *Hadjikyriakou 4707* (B, herb. GH), ibid., 25.2.2012, *Hadjikyriakou 7206* (B, herb. GH); ibid., vertical cliff and slopes with N aspect in pine forest, c. 500 m, 10.7.1999, *Hadjikyriakou 4707* (herb. GH); Frodisia [same area but different label inscription], at the track S of the former village before turn-off to Stavros tis Psokas, bank of the track in *Pinus* forest, shaded rocky ground, c. 450 m, 5.5.2005, *Hand 4827 & Hadjikyriakou* (B); Agia valley, roadside, c. 650 m, 19.6.1999, *Hadjikyriakou 4661* (B, herb. GH). — Division 7: Mavri Skala Akanthou, rocky slope, c. 400 m, 14.5.2005, *Hadjikyriakou 6383 & Hand* (B, herb. GH); Kremmos tis Keryneias NW of Halevga, pine forest, c. 700 m, 21.5.2005, *Hadjikyriakou 6418* (B, herb. GH); ibid., at view point at ridge road c. 1300 m WNW of Armenomonastiro, pine forest, c. 700 m, 22.5.2009, *Hand 5548 & Hadjikyriakou* (B) and cult. until 26.05.2010, *Cubr 46994* (B), cult. until 30.6.2010, *Cubr 46994a* (B), ibid., 30.9.2010, *Hand S-246* (B-seedbank); above Chartzeia, pine forest, 450 m, 18.2.2012, *Hadjikyriakou 7205* (B, herb. GH); W of Halevga, roadside, c. 700 m, 21.5.2005, *Hadjikyriakou 6419* (B, herb. GH); Kremmos tou Fani above Agios Amvrosios Keryneias, pine forest, c. 550 m, 21.5.2005, *Hadjikyriakou 6421* (B, herb. GH); Vasileiatis above Agios Amvrosios Keryneias, pine forest, c. 450 m, 21.5.2005, *Hadjikyriakou 6422* (B, herb. GH); ibid., 6.2010, seeds only, *Hadjikyriakou* (= *Hand CY-29*, B-seedbank); ibid., 5.3.2011, *Hadjikyriakou 7184* (B, herb. GH); southern outskirts of Agios Amvrosios Keryneias, pine forest, 200 m, *Hadjikyriakou 7204* (B, herb. GH). — Division 8: Giouti, Eptakomi, slope with open forest and shrubs, 50 m, 4.5.2010, *Hadjikyriakou 7170* (B, herb. GH).

Scaligeria napiformis (collection numbers and dates of specimens and seeds from specimens at Berlin (B) and in the herbarium of G. Hadjikyriakou (herb. GH) used for comparative measurements)
Croatia: *Bornmüller* 8.5.1911 (B), *Poscharsky* 7.6.1904 (B).
Greece: *Böhling 1692, 5561, 7189* (B); 30.4.1926, 23.6.1926, *Bornmüller* (B); 7.5.1992, *Jagel* (B); 26.4.1987, *Jäth* (B); *Shay 377* (B); 8.6.1896, *Sintenis* (B); 17.6.1891, 28.6.1891, *Sintenis & Bornmüller* (B); cult., *Schwerdtfeger 16032* (B); *Willing 13010, 13950 I, 16131, 24083, 24237, 30245, 30657, 30941, 31974, 36618, 54980, 55785, 77457, 88850, 91211, 191823, 191919, 192573, 193447, 194211, 194988, 195230, 197189, 199039, 201651, 201749, 202011, 202096* (B). — Crete: 11.5. & 11.6.1982, *Risse* (B); 4.1980, *Shay 80-545, 82-940* (B). — Eastern Aegean Islands: 23.5.1978, *Engelhardt* (B), 26.5.1986, *Höhner & Potthoff* (B); *Raus 6002, 8815* (B).
Turkey: Anatolia: 11.5.1906, 29.5.1906, 2.–3.6.1906, *Bornmüller* (B); *Hennipman & al. 856* (B); 8.5.1979, 28.6.1979, 1.9.1979, *Kehl* (B); *Peşmen 445* (B); 6.1932, *Schwarz* (B); *Walter & Walter 411* (B).
Cyprus: 24.4.1991, *Alziar & al.* (B); *Hadjikyriakou 750, 2587, 3960, 3968, 4000, 4586, 4661a, 5299, 6294, 7203, 7207* (B, herb. GH); *Hand 2038, 4626, 4869, 5318, 5949* (B); *S-303, S-310, S-313, S-329* (B-seedbank).
Lebanon: 25.5.1853, *Blanche* (B); 6.6.1910, *Bornmüller & Bornmüller* (B).
Israel/Palestine: 3.5. & 12.5.1897, *Bornmüller* (B); 5.4.1989, *Danin & al.* (B).

2. EMBL/GenBank accessions used for sequence analysis in the present study

Sequence of data: Taxon [EMBL/GenBank accession number: ITS or ITS1/ITS2; *psbA-trnH*; herbarium*; collector(s) with collecting number*; origin*]. * = given only if sequences are from single voucher.

Bunium balearicum (Sennen) Mateo & S. López Udias [DQ435208/DQ435247; EU445720; G; *Porta & Rigo 679*; Spain]. ***B. intermedium*** Korovin [DQ435222/DQ435261; EU445733; MW; *Pimenov & al. 202*; Tadzhikistan]. — ***Carum appuanum*** Grande [AY840984/AY840985; EU445747]. — ***Crithmum maritimum*** L. [U30540/U30541; EU445751]. — ***Falcaria vulgaris*** Bernh. [AF077888; DQ457174]. — ***Galagania ferganensis*** (Korovin) M. Vassiljeva & Pimenov [DQ422819/DQ422838; EU445756; MW; *Pimenov & al. 227*; Tadzhikistan]. — ***Postiella capillifolia*** (Post ex Boiss.) Kljuykov, [DQ422829/DQ422848; EU445766; E; *Peşmen & Güner 1867*; Turkey]. — ***Scaligeria al-***

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ziarii [HE985079; HE985080; B; *Hand 5548 & Hadjikyriakou*; Cyprus]. ***S. halophila*** (Rech. f.) Rech. f., [HM229404; HM474851; LD; *Bentzer & Runemark 28236*; Greece], ***S. moreana*** Engstrand [HM229405; HM474852; LD; *Runemark & Snogerup 20727*; Greece]. ***S. napiformis*** (Willd. ex Spreng.) Grande [DQ422830/DQ422849; EU445768; MW; *Pimenov & Kljuykov T95-11*; Turkey]. — ***Trachyspermum ammi*** (L.) Sprague [U78380/U78440; EU445776].

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