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The rediscovery of *Stachys virgata* (Lamiaceae), a rare endemic of Peloponnisos, Greece: taxonomy, distribution, karyology and conservation

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Abstract

Stachys virgata, a rare Greek member of *S.* subsect. *Rectae*, was hitherto known from historical collections made in the north-eastern and southern parts of Peloponnisos, the last one dated in 1844. Its only recent record, on Poros Island in 1940, is not supported by a herbarium specimen. The species was rediscovered in 2005, 161 years after its last collection, and subsequently documented in 18 populations (1 of them now extinct) distributed along the eastern slopes of Mt. Parnonas and Mt. Madara in east Peloponnisos. We review the species' description and distribution based on both historical records and recent collections, select a specimen deposited in the Natural History Museum, Paris (P) as a lectotype, and count its chromosome number, 2n = 34, for the first time. We evaluate the species' taxonomic relationships and consider its threats and conservation status based on our own field work and several years of monitoring. *S. virgata* totals 355 mature plants and 12 out of its 18 populations comprise 20 plants or fewer. Based on the IUCN criteria, *S. virgata* falls under the Endangered (EN) category and specific conservation measures are proposed. Finally, we provide a key distinguishing *S. virgata* from the other Greek members of *S.* subsect. *Rectae*.

Key words: distribution, extinction, Stachydeae, threatened species, typification

Introduction

Stachys Linnaeus (1753: 580), the largest genus of Stachydeae (Lamiaceae subfamily Lamioideae), comprises approximately 300 species with a worldwide distribution (Salmaki *et al*. 2013, Tundis *et al*. 2014). *Stachys* is well represented in the Balkans, Turkey and the Irano-Turanian area, where 20 of the 23 currently recognized sections (excluding *Betonica* Linnaeus 1753: 573) are found (Salmaki *et al*. 2012). Greece hosts 38 species, 16 of which are endemics usually locally distributed; some of them, e.g. *S. cretica* Linnaeus (1753: 581), *S. recta* Linnaeus (1767: 82), *S. swainsonii* Bentham (1834: 535), are particularly polymorphic and further divided into distinct subspecies (Dimopoulos *et al*. 2013). Among the Greek narrow endemics, the least known is *S. virgata* Bory de Saint-Vincent & Chaubard (1832: 166), a local species undocumented for over 150 years. Similar plant species not found for many decades can be considered as presumably extinct until their rediscovery (Constantinidis & Vassiliades 1996, Pereira *et al*. 2002, Eker & Akan 2010, Simpson *et al*. 2013, Romero & Woodgyer 2014).

Stachys virgata, a tall and not easily overlooked plant endemic to Peloponnisos (south part of Greek mainland), was discovered in Argolida in the early 19th century, near the small town of Kranidi, as well as in the wider region of the Methana peninsula and Trizinia, where it was either reported or collected by the French botanists J.B.G.M. Bory de Saint-Vincent, L.A. Chaubard and J.M. Dèspréaux (Bory de Saint-Vincent & Chaubard 1832). *S. virgata* was probably first seen between 1828 and 1831; the exact year of its collection in Peloponnisos is rather vague. On most labels of the extant old collections (BR!, G!, P!, WU!) no date is provided and the locality is often discouragingly imprecise: 'Morée', an old name of Peloponnisos as a whole. When the species was described in 1832, it was also illustrated in plate XVII (Bory de Saint-Vincent & Chaubard 1832). Six years later, Chaubard & Bory de Saint-Vincent (1838: 37, Plate XVIII) reproduced the species' localities and illustration information. An early collection, apparently prior to 1835, was also made near Astros, by X. Landerer and J. Sartori (BR!). In 1844, the French amateur botanist S.R. Lenormand collected *S. virgata* in the ruins of an ancient temple or settlement in Cape Tenaron ('ruines

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de Taenarium'), in southernmost Peloponnisos (G-BOIS!). Nearly forty years later, Boissier (1879: 731) cited the already known localities of the species and also added Akrokorinthos, a rocky hill reaching 575 m a.s.l. close to the city of Korinthos as a new locality, where the plant was collected by F. Zuccarini, obviously prior to his death in 1833 (Kalheber 2006). At the start of the 20th century, Halácsy (1902: 525–526) did not add any new information regarding the species but noted, after citing its localities, "n.v." ("non vidi"), an indication that he neither collected nor observed *S. virgata* specimens. During World War II, Zaganiaris (1940) reported the species from Poros Island, lying east of the Methana peninsula, without an exact locality or any detailed comment. A large part of Zaganiaris' botanical specimens are not extant; some collections are located in the herbaria ATHU and TAU, yet they do not host this Poros specimen. If such a specimen ever existed it may have not survived to the Second World War. Without a proper documentation by Zaganiaris, the most recent *S. virgata* specimens apparently date back to 1844 and the species' historical localities are geographically restricted in the prefectures of Argolida (Kranidi, Methana, Trizinia and Poros), Arkadia (Astros), Korinthia (Akrokorinthos) and Lakonia (Cape Tenaron).

To our knowledge, there is no other published information regarding *Stachys virgata* during recent or historical years. The species has been considered extinct, at least in the Akrokorinthos area (Iatrou *et al*. 2007), due to the absence of any recent record, and was not found in the Methana peninsula (Kougioumoutzis *et al*. 2012). On the other hand, it is evident from the herbarium specimens' morphological evaluation that *S. virgata* is a distinct and easily recognisable taxon. Indeed, the clear taxonomic identity of *S. virgata* has never been questioned (Ball 1972, Bhattacharjee 1980, Greuter *et al*. 1986). Nevertheless, due to the paucity of the available material, *S. virgata* has always remained a little-known species requiring further investigation (Ball 1972).

Stachys virgata has been treated as Rare (R) in certain lists of threatened plant species (e.g. IUCN 1982, Walter & Gillett 1998), but this assessment is quite controversial, since it is based on insufficient information, incomplete documentation and lack of any recent gathering. Finally and rather surprisingly, *S. virgata* was not included in the revision of the endemic plants of Peloponnisos (Tan & Iatrou 2001).

The aim of the present study is to report on the rediscovery of *Stachys virgata.* New localities are added to its historical distribution, its morphology is defined in detail and its taxonomic position is reviewed. The species' name is typified and a lectotype is selected. Its chromosome number is given for the first time and its ecological preferences are briefly discussed. Its population structure is presented based on field work conducted during a 10-year period (2005 to 2014) and its conservation status is evaluated.

Materials and methods

Stachys virgata specimens were searched in the herbaria ACA, ATH, ATHU, B, BM, BR, G, K, LD, M, P, TAU, TUB, UPA, W and WU (acronyms follow Thiers 2015). The abbreviation G-BOIS refers to the E. Boissier collections, kept separate in G. Intensive field work was carried out during 2005–2014 in all the known *S. virgata* localities, historical and new. A detailed morphological study was undertaken based on plants from natural populations and collected vouchers. Two living plants of *S. virgata* were cultivated in Athens and used for karyological investigations. Root tips were collected and pretreated with an aqueous solution of 8-hydroxyquinoline (0.3g/l) for three hours at room temperature. Then, they were placed into a 3:1 mixture of absolute ethyl alcohol/glacial acetic acid at 4º C for two days, transferred to 70% ethyl alcohol and kept at -20 ºC for several months. Before being stained in Feulgen's reagent they were hydrolyzed for 10 minutes at 60º C. Stained roots were squashed over a glass slide in a drop of a 45% acetic acid solution and observed under the microscope. Metaphase plates of good quality were recorded and photographed. The term "ibidem" is used in the Appendix for populations found in the same Nomos and Eparchia (Greek administrative units).

Results

In order to rediscover *Stachys virgata*, we repeatedly visited the areas surrounding Cape Tenaron, Kranidi, southern Argolida, Arkadia, the Akrokorinthos hill, the lower hills mostly to the south of Akrokorinthos and the Methana peninsula; however, all visits proved unsuccessful. Finally, *S. virgata* was found on 30 May 2005 during an investigation of the eastern, lower slopes of Mt. Madara along the eastern coastal parts of Peloponnisos, ca. 5 km north-northwest of the Mitropoli village. The plants were in flower and immediately identified as *S. virgata*. More populations were found

on the same day in the area, all in new, hitherto unknown localities. These populations confirmed that the species is still extant and well-established, although very localized and always growing in small groups of individuals. Between 2005 and 2014, we discovered some additional populations, thus enabling voucher collection, threat evaluation and species' monitoring (see Appendix). Based on our voucher specimens kept in ATHU and field notes, we provide a revised description and illustrations of *Stachys virgata* (Figs. 1–2), as follows.

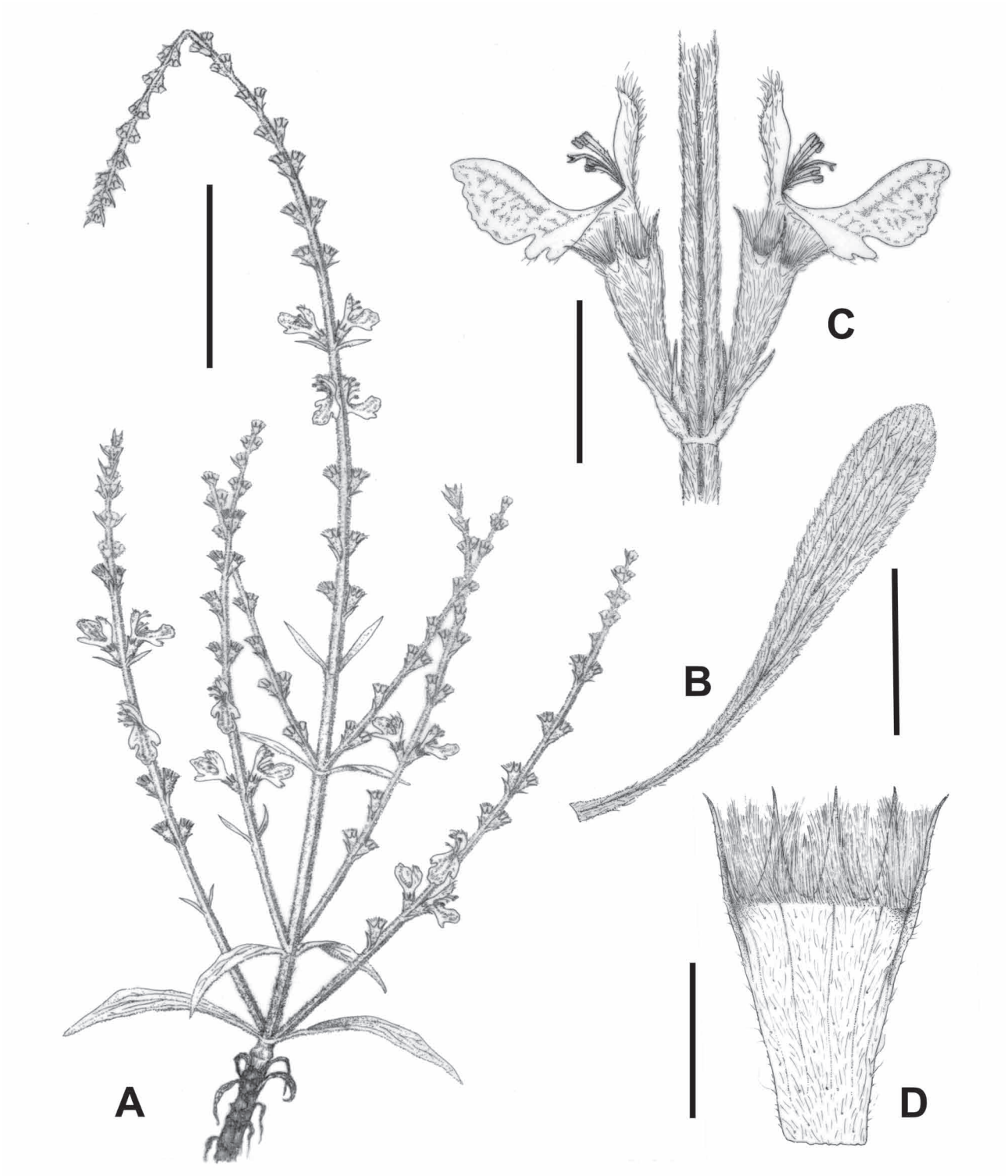

FIGURE 1. Drawings of *Stachys virgata* (from *Kalpoutzakis 1786* and *Constantinidis & Kalpoutzakis 11379*). A. Flowering shoot. B. Basal leaf. C. A verticillaster from middle part of inflorescence with two flowers. D. Inner part of an opened calyx. Scale bars: A = 10 cm, B = 2.5 cm, C = 10 mm, D = 5 mm. Illustrations by Z. Tziakou.

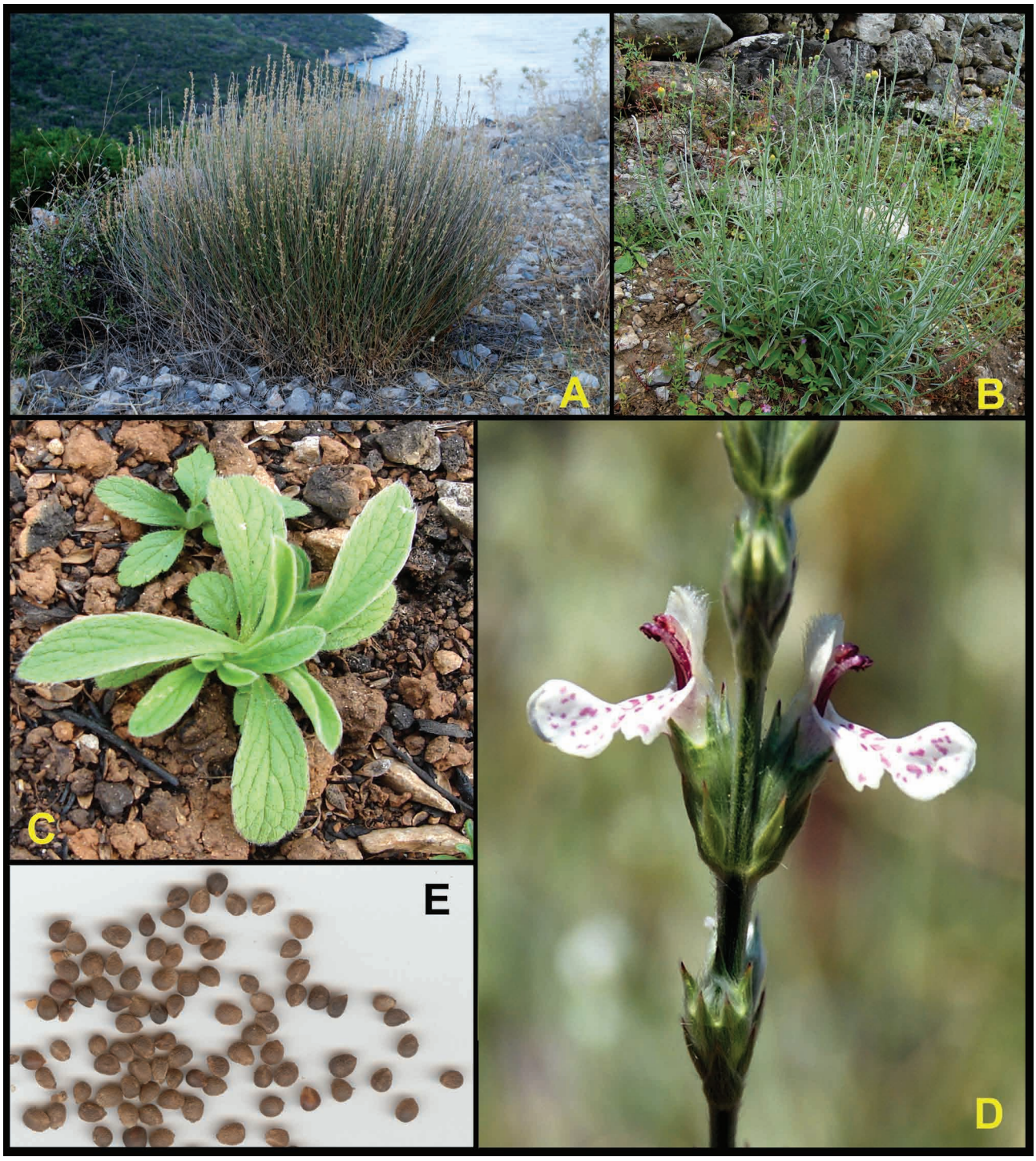

FIGURE 2. *Stachys virgata* in its natural habitat. **A.** A post-flowering plant from population no. 2, between Mitropolis and Kapsala (29.07.2013). **B.** A pre-flowering plant from population no. 9, near Poulithra village (28.04.2006). **C.** Seedlings in spring 2006, after a local fire in population no. 12, at Agrioachladia (28.04.2006). **D.** Part of an inflorescence in population no. 12, at Agrioachladia (22.06.2008). **E.** Ripe seeds. For population details see Appendix. All photographs by E. Kalpoutzakis.

Stachys virgata Bory de Saint-Vincent & Chaubard (1832: 166, plate XVII)

Type (lectotype, here designated):—GREECE. Peloponnisos: *Stachis* [sic] *virgata* Chaub. et Bory, Morée, s.d., *Chaubard, J.B.G.M. Bory de St-Vincent s.n.* (MNHN-P-P03683987!, isolectotype G!)

= *Stachys zuccarinii* Bentham (1834: 535). Type:—Not designated.

Notes on typification:—We traced a few historical specimens of *Stachys virgata* deposited in BR!, G!, P! and WU!. On the label of one G specimen we read '*Stachis* [sic] *virgata* Chaub. et Bory, Morée, Chaub.' in apparently the same handwriting as on the P lectotype label. Both specimens obviously comprise part of the same original gathering and it is therefore safe to consider the G specimen an isolectotype. The second specimen deposited in G-BOIS is a subsequent collection of 1844 by S.R. Lenormand. In WU, the *S. virgata* specimen (WU 0038150!) has the indication of an exact locality: 'Cranidi, Morée', followed by 'Chaubard' (collector?) in a handwriting different from that on P and G specimens. Since the town of Kranidi (Cranidi) is cited by Bory de Saint-Vincent & Chaubard (1832: 166) as a collection locality, WU 0038150 is obviously a syntype of *S. virgata*. We have not been able to trace F. Zuccarini's collection from Akrokorinthos. Bentham (1834: 535) indicated a specimen in the K.F.P. von Martius herbarium collected '*in Graecia ad Acrocorintham*' on which he based his *S. zuccarinii,* a later synonym of *S. virgata.* Von Martius' exsiccates are primarily housed in BR and M. No material was detected in M but a specimen does exist in BR!, apparently sent to von Martius by X. Landerer and J. Sartori in 1835. On its label we read: '*Astros, in montib. saxosis versus coenob. Sotyri*', which most probably corresponds to a locality ca. 4 km NW of Astros, near the convent of Transfiguration of Loukous. Both the toponym and the collectors do not match Bentham's (1834: 535) protologue and this specimen cannot serve as a lectotype for *S. zuccarinii*.

Description:—Perennials forming a persisting, partly subterranean, branched, suffruticose stock 2–5 mm wide and producing 4–25 (or more in large specimens) annual stems that die back in autumn. Flowering stems 40–90(–100) cm long, quadrangular, angles yellowish-green, sides slightly concave, with decussate branches at almost every node of main axis up to inflorescence; primary branches placed at an angle of 30–50º to main stem and further branched similarly one or two times; all parts of stem covered with simple, soft, predominately adpressed, whitish hairs. Lower leaves mostly withered at anthesis, 7–10 × 0.8–2.5 cm, elliptic to oblanceolate-elliptic, acute to subobtuse, attenuating at base into a 2.5–4 cm long petiole, rather remotely serrulate-serrate, tomentose, veins on lower surface evident; middle and upper leaves gradually smaller and narrower, often falcate, oblanceolate to ensiform, tomentose-sericeous especially on lower surface, petiole very short to absent; uppermost leaves 10–20 × 1.5–3 mm, entire. Inflorescence (5–)15–25 cm long, simple, rarely branched at base, sericeous particularly on upper parts; verticillasters (2–)5–18, clearly distant, those on lower parts placed at 3.0–5.5 cm intervals, those on upper parts at 0.7–1.6 cm intervals, 1–3(–6)-flowered but predominately 2-flowered. Bracts lanceolate, acute, 7–20 × 2–3.5 mm, tomentose-sericeous, 1/2 as long to somewhat longer (at lower parts of inflorescence) than calyx; bracteoles lanceolate, acuminate, 5–7 × 1–1.5 mm, sericeous, ca. 1/2 to 3/4 as long as calyx, ending into a ca. 0.5 mm yellowish to brownish mucro. Calyx on a ca. 2 mm pedicel, regular, campanulate to somewhat infundibular after anthesis, sericeous especially at lower part and along veins, 8–12 × 3–4 mm, with 5 main veins below each tooth and 2–4 secondary veins between each pair of main veins; tube 6.5–8 mm long, hairy inside, with a dense ring of ca. 2 mm long white hairs at mouth; teeth triangular, equal, 2.5–4.5 × ca. 1 mm at base, ending in a yellow-brownish 0.5–1 mm long mucro. Corolla bilabiate, white to cream or rarely ochre-yellow, 16–19 mm long; tube equalling to calyx, annulate inside, hairy on throat; upper lip straight, entire to shallowly emarginate, with long sericeous hairs on upper surface; lower lip 6–7 mm long, 3-lobed, with reddish-purple markings on upper surface, middle lobe largest, 4.5–5.5 × 9–10 mm at widest part, lateral lobes broadly triangular, obtuse to emarginate, ca. 2.5 × 3 mm at widest part. Stamens 4, epipetalous, ca. 6 mm long, exserted from corolla tube and shorter than upper lip; filaments with swollen hairs at lower half and with sparse sessile glands along their length; anthers dithecous, thecae divaricate. Style gynobasic, ca. 12 mm, terminal branches equal. Nutlets obovoid, 2–2.5 × 2 mm, brown to blackish, verruculose.

Distribution:—Endemic to eastern Peloponnisos (both southern and northern parts), Greece, and known from a few historical and some recent collections. The old records from the north-eastern (Akrokorinthos, Astros, Kranidi, Methana, Trizina) and southern (Cape Tenaron) parts of the region have not been confirmed for the last 171 years, but the species may still grow there in small populations. Extant populations were discovered in a relatively small stripe of eastern Peloponnisos, at the lower foothills of Mts Parnonas and Madara that face the Mirtoon Sea. The known distribution of *Stachys virgata* is presented in Fig. 3. Rather interestingly, the new and historical locations are quite distant from one another; yet, the former fall within a 100 km radius from the nearest historical collection site. This phenomenon has been observed in the presumed extinction and rediscovery of other species as well (Crowley 2011).

Habitat and ecology:—*Stachys virgata* was found growing in maquis clearings or openings, at an elevation of 4–660 m a.s.l. Maquis openings are found either in primary (e.g. in stony or rocky places, where scrub establishment may be difficult) or in secondary, man-made habitats (i.e. planted olive groves receiving little cultural care and replacing natural vegetation). In several cases, *S. virgata* was seen along dirt roads or paths, where the natural vegetation had been cleared. Likewise, road embankments and debris offer suitable habitats for *S. virgata*. The species cannot tolerate competition by tall and dense maquis vegetation, therefore, it is absent or rarely found within such formations. Human

interference (e.g. local fires occasionally used by land owners to clear slopes from natural vegetation) may provide new suitable niches and colonization opportunities to some *S. virgata* populations. In open places, roadsides and embankments, *Stachys virgata* occasionally grows together with *Asperula elonea* Iatroú & Georgiadis, a local endemic species, and other common species such as *Acer sempervirens* L., *Arbutus andrachne* L., *A. unedo* L., *Cytisus laniger* DC., *Erica manipuliflora* Salisb., *Fraxinus ornus* L., *Globularia alypum* L., *Hyparrhenia hirta* (L.) Stapf, *Hypericum empetrifolium* Willd. subsp. *empetrifolium, Olea europaea* L., *Phagnalon graecum* Boiss. & Heldr., *Phillyrea latifolia* L., *Quercus coccifera* L., *Teucrium flavum* L., *Thymbra capitata* (L.) Cav. and *Satureja thymbra* L. In maquis clearings, *S. virgata* grows together with *Bituminaria bituminosa* (L.) C.H.Stirt., *Cytisus laniger, Cistus salviifolius* L., *Erica manipuliflora, Globularia alypum, Pistacia lentiscus* L., *P. terebinthus* L., *Sarcopoterium spinosum* (L.) Spach and *Smilax aspera* L. *Stachys virgata* occurs singly or in small groups, on calcareous, schistose or other, non-calcareous substrates. Its populations are usually low in number of mature individuals, rarely exceeding 20 plants (Table 1).

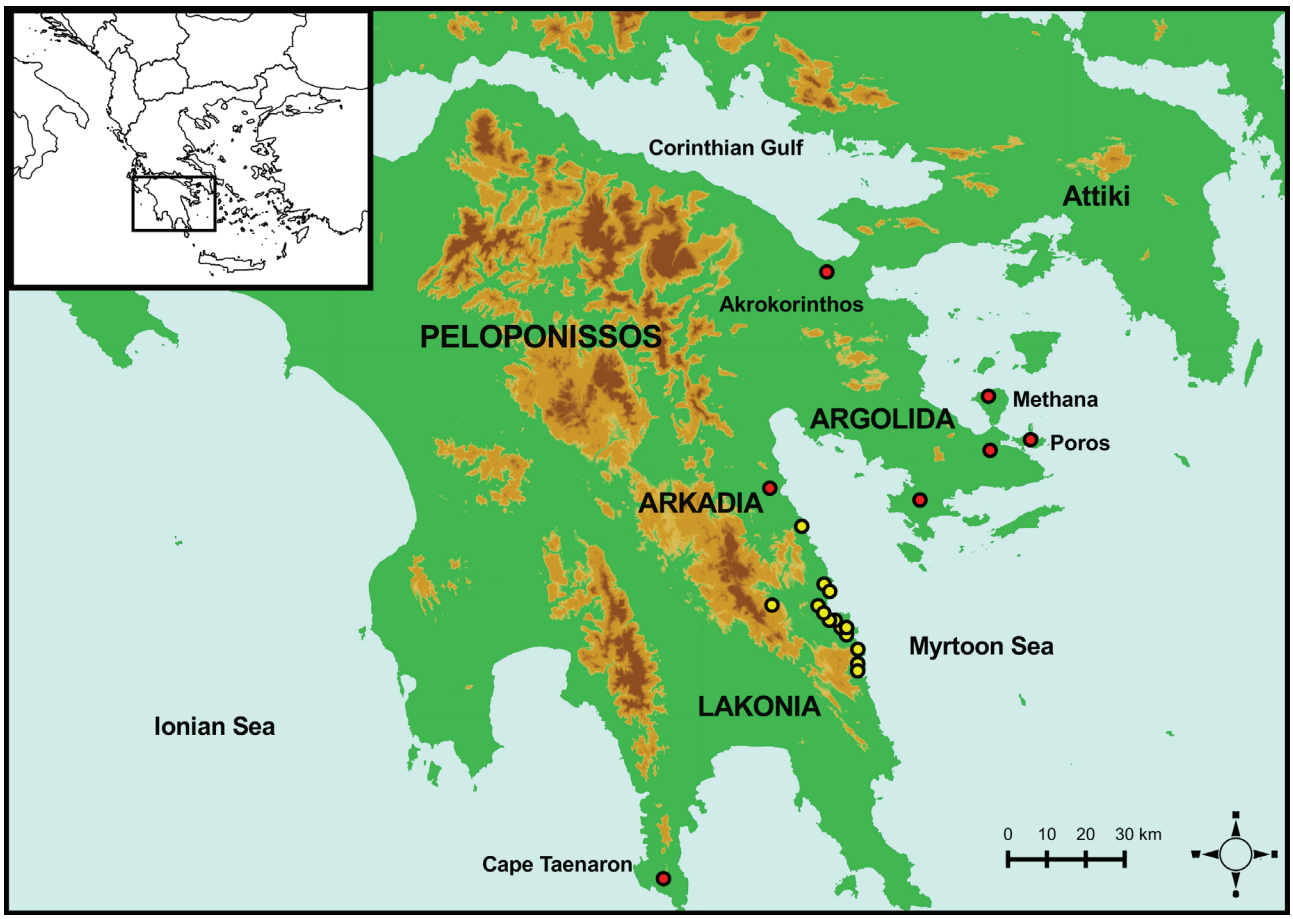

FIGURE 3. Total known distribution of *Stachys virgata*. Red circles indicate historical sites not confirmed the last 171 years. Yellow circles refer to the new populations discovered between 2005 and 2014.

Phenology:—*Stachys virgata* flowers from the end of May to the middle of July. The ripe seeds are released in August, perhaps also in early autumn. The annual, above-ground flowering stems die back in late autumn and new shoots appear in October and November, depending on rainfall.

Karyology:—Two plants originating from the area near Krioneri settlement (pop. no. 16) were cultivated for chromosome investigations. Both plants provided 2n = 34 (Fig. 4). The chromosomes are short, usually 1 µm or less, appearing blurry and without a clear centromere position in our metaphase plates. A distinct satellite was seen and probably some more exist in the complement, but they are smaller and faint.

Population structure, threats and conservation status:—*Stachys virgata* was first assessed as Rare (R), based solely on the historical information of old locations in Akrokorinthos, Kranidi, Trizina and Methana (IUCN 1982, Walter & Gillett 1998). These populations have not been confirmed in recent years, but 18 new populations were discovered in east Peloponnisos between 2005 and 2014 (Table 1). Population no. 10, consisting of 10 plants and located in private land, was destroyed between 2005 and 2011 following dramatic changes of its surrounding vegetation, extensive herbicide use and a building construction in its habitat. Twelve out of the 18 known populations harbour a very small number of

plants (20 mature individuals or less) and only 4 of the known populations exceed 30 individuals, with population no. 17 being the largest with 102 individuals. Most recent fieldwork estimated a total population of 355 mature plants. The species' extent of occurrence (EOO) does not exceed 100 km^2 and since *S. virgata* is a very local species, its area of occupancy (AOO) is much narrower, apparently less than 10 km^2. The main threats *Stachys virgata* is facing are mostly associated with land use changes and human interference and less with the development of thick maquis formations in the species' habitats. Population no. 12 was observed under retreat in both plant number and AOO, due to herbicide applications between 2011 and 2013. The uncontrolled herbicide use could affect 6 additional populations. Fires lit on purpose, usually aiming at controlling undesirable vegetation in olive groves and private land, also pose a serious threat to the species (particularly in pop. nos. 10, 11, 15). Changes or abandonment of traditional agriculture activities enable a thickening of maquis vegetation leaving thus no suitable niches for *S. virgata* in at least three localities. Road construction works may affect 9 populations. Finally, settlement construction activities and vegetation clearance pose less imminent threats to *Stachys virgata*, since they affect 4 populations. Mild human influence, on the other hand, such as the one traditionally expressed in many Peloponnesian villages (olive tree cultivation, avoidance of plugging and herbicides use, moderate grazing that controls the maquis development, maintaining dirt roads and paths) may have a positive impact on the *S. virgata* populations. Due to: i) the restricted EOO and AOO (less than 100 km^2 and 10 km^2, respectively), ii) the current existence of 355 mature individuals distributed in 18 small populations, iii) the apparent extinction of all historical populations, and iv) the high possibility that the extant species' localities and populations could be eradicated either as the result of human interference or by stochastic events, *Stachys virgata* is assigned to the Endangered (EN) IUCN (2001) category, following criteria B1ac(i,ii,iii,iv)+2ac(i,ii,iii,iv). Hence, *S. virgata* should be carefully monitored, cultivated ex situ in botanical gardens and special conservation measures be taken to safeguard at least the larger known populations. Further conservation suggestions for each separate population are described in Table 1.

TABLE 1. An annotated list on the existing *Stachys virgata* populations, their mature plant number and the conservation actions proposed. Abbreviations: Pop. no. = population number; NMP = number of mature plants; CAP = conservation actions proposed; Pr = private land, Pu = public land; Au = communication with local authorities, Co = communication with land owner, He = no herbicide use, Ma = maintenance of clearings in shrub formations, Ro = no changes of the dirt or paved roadsides, Tr = maintenance of traditional cultivation methods.

Pop. no.	NMP (2005–2010)	NMP (June/July 2011)	NMP (July 2013)	Comments	CAP
1	10	12	20	Pu	Ma, Ro
2	10	9	9	Pr, Pu	Ma, Ro
3	3	3	11	Pu	Ma, Ro
4	1	1	2	Pu	Ma, Ro
5	3	6	6	Pu	Ma, Ro
6	20	23	23	Pr	He, Ma, Tr
7	4	9	6	Pr	Ma, Ro
8	3	1	1	Pr	Co
9	7	9	20	Pr	Co, He, Tr
10	10	0	0	Pr	-
11	6	3	4	Pr	Co, He
12	51	86	54	Pr, Pu	Co, He, Ma
13	6	5	5	Pu	Au, Ro
14	12	25	33	Pr	Co, He, Tr
15	20	8	49	Pr	Co, He, Tr
16	1	1	1	Pu	Au, Ro
17	-	-	102	Pr	Co, Ma
18	-	-	9 (discovered in 2014)	Pu	Ma, Ro

Taxonomic position:—Bhattacharjee (1980) placed *Stachys virgata* in *S.* sect. *Olisia* Dumortier (1827: 44) subsect. *Rectae* Bhattacharjee (1980: 85), together with five additional Greek species: *S. angustifolia* Marschall von Bieberstein (1808: 52), *S. atherocalyx* Koch (1849: 691), *S. leucoglossa* Grisebach (1844: 140), *S. tetragona* Boiss. & Heldr. in Boissier (1879: 736) and *S. recta*. A sixth species, *S. iberica* Marschall von Bieberstein (1808: 51) was reported by Halácsy (1902: 525, Mt. Oeta, also known as Mt. Iti) and accepted by Bhattacharjee (1982: 244) as a member of the Greek flora, but its existence has never since been verified or documented. *Stachys virgata* produces long inflorescences predominately composed of 2-flowered verticillasters and resembles, in this respect, *S. angustifolia*

and *S. tetragona*. The former differs by its glabrescent stems, linear pinnatipartite middle and lower cauline leaves and longer pedicels (2–5 mm vs. 0–2 mm) on at least the lower verticillasters. The later differs by its shorter habit, glabrous or sparse patent hairy upper stem parts and calyx and lack of a dense tuft of protruding hairs on the calyx throat. Furthermore, *S. tetragona* is a chasmophyte with a particular preference for limestone rocks, cliffs and scree (Persson 1981), in contrast to the maquis and man-influenced habitats preferred by *S. virgata*. The rest of Greek *Stachys* subsect. *Rectae* members present denser verticillasters consisting of 3–16(–18) flowers and are presumably more distantly related to *S. virgata*. A key to all Greek species of *S.* subsect. *Rectae* (plus *S. iberica* subsp. *iberica*) is provided below.

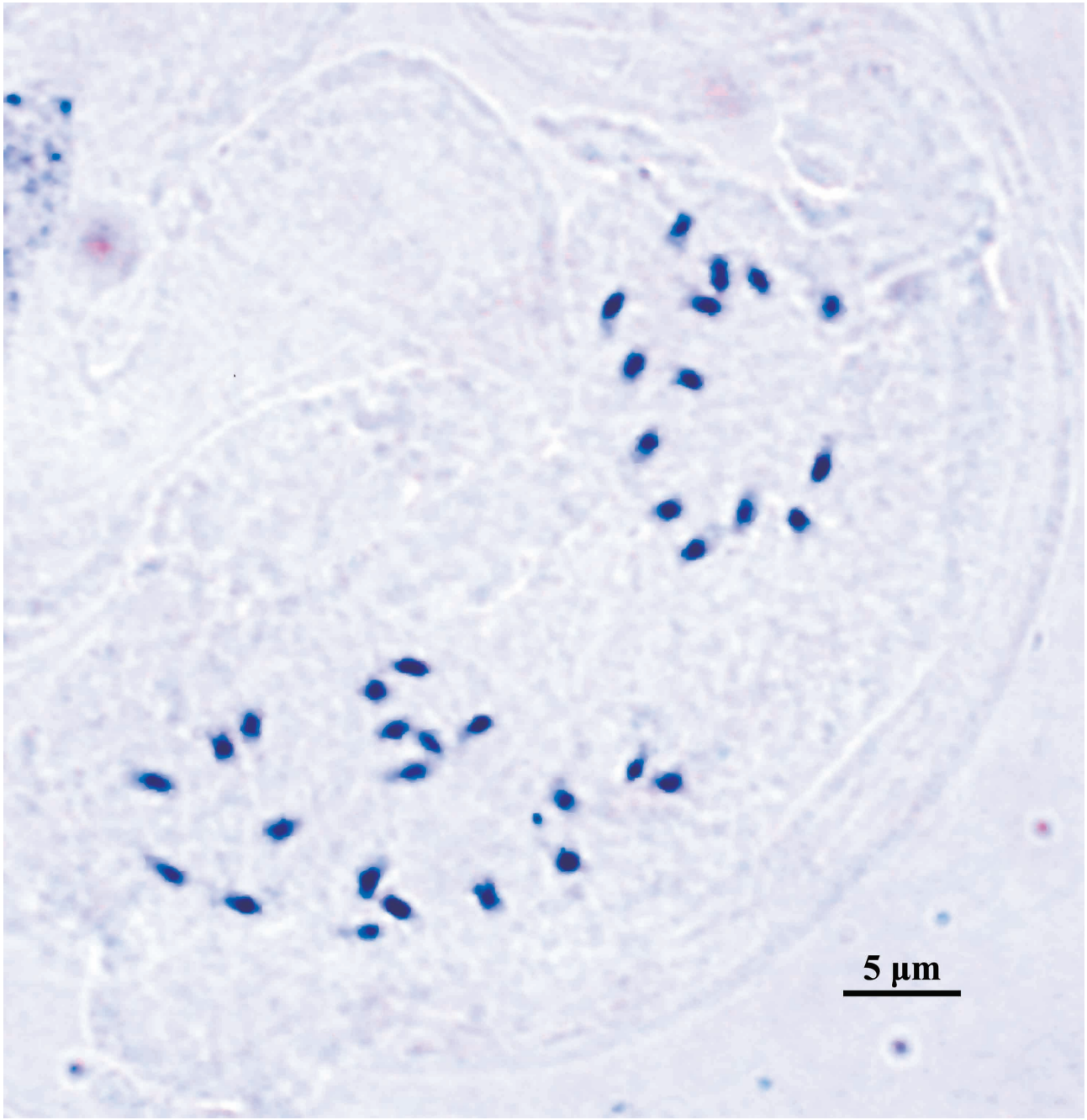

FIGURE 4. A metaphase plate of *Stachys virgata* with the diploid chromosome number of 2n = 34 on material near Krioneri settlement (population no. 16—see Appendix for details).

Key to Greek species of *Stachys* sect. *Olisia* subsect. *Rectae*

1. Verticillasters at middle parts of inflorescence 2-flowered; upper ones sometimes 1-flowered and lower ones rarely up to 5(–6)-flowered.....2

- Verticillasters at middle parts of inflorescence 3–16(–18)-flowered 4
2. Lower and middle leaves pinnatipartite, with 2–4 pairs of linear segments; pedicels (at least of lower verticillasters) 2–5 mm long ..... ***S. angustifolia***
- Lower and middle leaves entire, dentate to serrate; pedicels 0–2 mm long 3
3. Upper stem and calyx sericeous; throat of calyx with a dense tuft of long, protruding, white hairs ..... ***S. virgata***
- Upper stem and calyx glabrous or with sparse, patent hairs; throat of calyx with sparse short hairs ..... ***S. tetragona***
4. Corolla yellow, often with whitish or pink-purplish spots and markings 5
- Corolla white, cream, pink or purple, with or without markings 7
5. Calyx teeth linear-lanceolate to subulate, less than 1 mm wide at base, as long as or longer than tube ..... ***S. atherocalyx***
- Calyx teeth triangular-lanceolate, wider than 1 mm at base, shorter than tube 6
6. Most verticillasters 5–16-flowered, upper verticillasters crowded; stems slightly woody at base; corolla yellow ..... ***S. recta***
- Verticillasters 2–6-flowered, all distant; lower stems suffruticose and branched; corolla whitish to ochre-yellow ..... ***S. tetragona***
7. Stems sparsely to densely adpressed hairy; calyx with long adpressed hairs; corolla pink-purplish ..... ***S. iberica*** subsp. ***iberica***
- Stems glabrous to patent-hairy; calyx with sparse patent to erecto-patent hairs; corolla white to pale pink 8
8. Calyx teeth ca. 1/3 as long as tube, mucro absent or up to 1 mm; verticillasters up to 4 ..... ***S. tetragona***
- Calyx teeth approximately as long as tube, with a 1–3 mm long arista; verticillasters numerous ..... ***S. leucoglossa***

Conclusions

Stachys virgata is the last member in a list of rare plant rediscoveries in Greece (e.g. Yannitsaros *et al.* 1996, Constantinidis & Vassiliades 1996, Vassiliades 2003). With a total of 18 populations (1 recently extinct) and 355 mature individuals found within a 10-year span (Table 1), one may think that the species is now safe from vanishing. However, most populations are small in number: we detected only 4 localities comprising more than 30 individuals and the largest known population consists of 102 plants. Locations with 1–10 plants are common. This low number of individuals may be interpreted in two ways: they either represent very recently established, or define residual populations, indicating that the species' habitat is rather unstable and prone to drastic changes over time. Furthermore, most of the population size increase detected in 2013 (pop. nos. 1, 3, 9, 15, see Table 1) should not be attributed to actual population increase, viz. a successful local reproduction and spreading of youngsters. The additional plants found were of the same old age as the rest of the population and apparently were not noticed during earlier counts; they were detected when we repeatedly visited each location and spent adequate time to evaluate each separate population. In all cases, however, seedlings were rare. In an attempt to reinforce the natural populations, small scale *ex situ* propagation efforts were undertaken by seed collected either from small or large populations. Yet, seed germination proved largely unsuccessful and a considerable percentage of seeds appeared lacking an embryo or with an abortive embryo. The reason for this phenomenon is still unclear; if the species is cross-pollinated and self-sterile, the very small number of individuals could be responsible for ineffective fertilization and genetic corruption, despite the adequate numbers of available pollinators observed in the field. Another small-scale preservation experiment was conducted in pop. no. 16 that consists of only one plant. Cuttings were taken in late winter 2009 and rooted successfully. Six plants were then reintroduced around the only surviving plant of pop. no. 16 in autumn 2010, but none had survived when the area was revisited in July 2011. Failure of re-introducing the species in the wild is alarming, yet more experimentation is needed before final conclusions are drawn. Interestingly, the five largest *Stachys virgata* populations were found localized within private land (partly so in pop. no. 12). Communication with the landowners and providing a local-scale protection status would be an effective conservation measure in these cases. We informed most landowners on the species' rarity in their properties and will continue doing so with the rest of them in the future.

The chromosome number of 2n = 34 (x = 17) counted in *Stachys virgata* predominates in the group of *S. recta* and its relatives. Baltisberger & Lennherr (1984) counted 2n = 34 in 27 members of this group, belonging to 73 populations, the only exception being two Greek populations of *S. angustifolia*, which afforded 2n = 24. The same deviating chromosome number for *S. angustifolia* was also reported earlier by Koeva-Todorovska (1978) based on Bulgarian plants. *S. tetragona,* a local endemic species of Evvia and Northern Sporades Islands and a putatively relative of *S. virgata*, has 2n = 34 (Baltisberger & Lennherr 1984, Phitos 1988). Sharing the same chromosome number strengthens the taxonomic proximity of *S. virgata* to *S. tetragona* rather than with *S. angustifolia*. Still, phylogenetic relationships within members of *S.* sect. *Olisia* remain obscure and the section itself appears polyphyletic in a recent investigation by Salmaki *et al.* (2013).

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APPENDIX. Recent *Stachys virgata* specimens examined or observed (all in ATHU).

Stachys virgata:—GREECE. Nomos Arkadias, Eparchia Kinourias: foothills of Mt Parnonas, the entrance to the village of Poulithra along the road from Plaka, very close to the first house of the village, 37°07′N, 22°53′E, ca. 4–5 m, 12 June 2005, *Kalpoutzakis 1690* (pop. no. 8); *ibidem*: ca. 1.2 km SE of the village of Poulithra, along the road to the chapel of Agios Georgios, olive groves and remnants of maquis, stony calcareous places, 37°06′N, 22°54′E, ca. 60 m, 12 June 2005, *Kalpoutzakis 1691* (pop. no. 9); *ibidem*: locality known as Agios Georgios, ca. 4 km SE of Poulithra village, olive groves with terraces and remnants of destroyed maquis, stony calcareous places, 37°06′N, 22°55′E, ca. 150–220 m, 12 June 2005, *Kalpoutzakis 1692* (pop. no. 10); *ibidem*: 1.5 km SE of Poulithra village, partly destroyed scrub (maquis) and olive grove, limestone, 37º 06′ N, 22º 54′ E, ca. 60 m, 12 July 2005, *Kalpoutzakis obs*. (pop. no. 11); *ibidem*: east parts of Agrioachladia summit, ca. 2.5 km SE of Poulithra, an opening of the road, margins of maquis, 37°06′N, 22°54′E, ca. 275 m, 22 June 2008, *Kalpoutzakis 1980* (pop. no. 12); *ibidem*: ca. 1.4–1.7 km NNW of Fokianos settlement, maquis on stony slopes, abandoned fields in terraces, limestone, 37°05′N, 22°57′E, ca. 110–125 m, 25 June 2005, *Kalpoutzakis 1699 & 1700* (pop. no. 6); *ibidem*: ca. 2.3 km N of Fokianos settlement, above the gulf with fish-breeding constructions, maquis, limestone, 37°05′N, 22°57′E, ca. 150 m, 25 June 2005, *Kalpoutzakis 1701* (pop. no. 7); *ibidem*: ca. 7.2 km from the village of Pigadi towards the settlement of Fokianos, stony area in

openings of maquis, limestone, 37°05′N, 22°56′E, ca. 280 m, 25 June 2005, *Kalpoutzakis 1702* (pop. no. 4); *ibidem*: ca. 8.5 km from the village of Pigadi towards the settlement of Fokianos, stony area with rocks, maquis on limestone, 37°04′N, 22°57′E, ca. 205 m, 25 June 2005, *Kalpoutzakis 1703* (pop. no. 5); *ibidem*: ca. 4.2–4.5 km from Leonidio towards the village of Tsitalia, stony, calcareous slopes with maquis, limestone, 37°08′N, 22°52′E, ca. 220–230 m, 10 August 2005, *Kalpoutzakis 1744* (pop. no. 13); *ibidem*: ca. 0.3 km from the settlement of Krioneri towards Tiros, slopes with maquis at the right side of the road, stony places, together with *Pistacia lentiscus*, *Quercus coccifera*, *Acer sempervirens*, *Fraxinus ornus*, *Erica manipuliflora*, limestone, 37°18′N, 22°49′E, ca. 20 m, 12 September 2005, *Kalpoutzakis 1757* (pop. no. 16); *ibidem*: along the road from Tiros to Leonidion, ca. 0.3 km after the secondary road towards Pramateftis village, abandoned olive grove in destroyed scrub vegetation (maquis), stony slope, non-calcareous substrate, 37°11′N, 22°54′E, ca. 60–80 m, 25 May 2006, *Kalpoutzakis 1774* (pop. no. 14); *ibidem*: along the road from Tiros to Leonidion, ca. 1 km after the secondary road towards Pramateftis village, olive grove in clearings of maquis, non-calcareous substrate, 37°10′N, 22°54′E, ca. 100–150 m, 30 May 2006, *Kalpoutzakis 1786* (pop. no. 15); *ibidem*: ca. 0.8 km SE of Pramateftis village, slopes with maquis and olive groves, 37°11′N, 22°53′E, ca. 75–100 m, 28 August 2013, *Kalpoutzakis 4400* (pop. no. 17); *ibidem*: ca. 2.4 km from Elona convent towards Kosmas village, stony slope with maquis, 37°08′N, 22°44′E, ca. 657 m, 22 July 2014, *Kalpoutzakis 4546* (pop. no. 18). Nomos Lakonias, Eparchia Epidavrou-Limiras: 4.9–5.1 km NNE of Mitropolis village, on the way to Kapsala settlement, maquis, openings of maquis and roadsides, limestone, 36°59′N, 22°59′E, ca. 120–150 m, 30 May 2005, *Constantinidis & Kalpoutzakis 11377* (pop. no. 1); *ibidem*: ca. 8.0–8.2 km NNE of Mitropolis village, on the way to Kapsala settlement, maquis and roadsides, edges of olive groves, limestone, 37°01′N, 22°59′E, ca. 90–130 m, 30 May 2005, *Constantinidis & Kalpoutzakis 11379* (pop. no. 2); *ibidem*: 13.0–14.0 km NNE of Mitropolis village, on the way to Fokianos settlement, roadsides, limestone, 37°02′N, 22°59′E, ca. 210–230 m, 30 May 2005, *Constantinidis & Kalpoutzakis 11381* (pop. no. 3).