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Taxonomic study on the Greek endemic genus *Hymenonema* (Asteraceae: Cichorieae), using morphological and karyological traits

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Abstract: *Hymenonema* is a Greek endemic genus consisting of two species, *H. laconicum* and *H. graecum*, occurring in the lowlands of S Peloponnisos and on most of the C Aegean islands, respectively. Morphological investigation of 20 gatherings covering the entire distribution range revealed clear morphological differences between the two species, mainly in pappus, achenes, anther tube, ligules and basal leaf characters. A corresponding emended identification key to the species is given. Karyological investigation of 11 accessions included karyotypes, idiograms and karyological indices for both species. Six karyomorphological parameters were also statistically analysed. Populations with intermediate morphological characters between the two species are recorded for the first time and their relationship with the typical two species is discussed. The geographical distribution of the genus is mapped and doubtful locations are commented on. The cytotaxonomic data and the geographical distribution of the species support the characterization of *H. laconicum* and *H. graecum* as schizoendemics. The conservation status of both species is suggested as Vulnerable (VU) according to IUCN criteria.

Key words: Asteraceae, chromosome numbers, Cichorieae, Compositae, distribution, endemism, Greece, *Hymenonema*, karyotype analysis, plant morphology, taxonomy

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Introduction

Hymenonema Cass. is one of the seven endemic genera of Greece and the only one that consists of two species, while the rest are monotypic: *Horstrissea dolinicola* Greuter & al. (Apiaceae), *Jankaia heldreichii* (Boiss.) Boiss. (Gesneriaceae), *Lutzia cretica* (L.) Greuter & Burdet (Brassicaceae), *Petromarula pinnata* A. DC. (Campanulaceae), *Phitosia crocifolia* (Boiss. & Heldr.) Kamari & Greuter (Asteraceae) and *Thamnosciadium junceum* (Sm.) Hartvig (Apiaceae) (Phitos & Kamari 2009). *Leptoplax emarginata* (Boiss.) O. E. Schulz was

treated as a Greek endemic genus by Phitos & Kamari (2009), but was more recently included in *Bornmuellera* Hausskn. (Rešetnik & al. 2013).

Hymenonema laconicum Boiss. & Heldr. occurs in the lowlands of S Peloponnisos and *H. graecum* DC. on most of the C Aegean islands (Fig. 1). A record for *H. graecum* from NW Kriti (Crete) (Zaffran 1990: 331) has not recently been reconfirmed. The systematic classification of *Hymenonema* at the taxonomic level of family and tribe has not changed since the first description of the genus. It was classified by Cassini (1817) in the family Asteraceae and in the tribe Cichorieae (= Lactuceae).

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At subtribal rank, *Hymenonema* was placed by Stebbins (1953) in the *Cichoriinae* together with the genera *Arno-seris* Gaertn., *Catananche* L. and *Tolpis* Adans. Jeffrey (1966) included *Hymenonema* in the *Catananche* subgroup, whereas Bremer (1993, 1994) placed it in the subtribe *Catananchinae* with *Catananche* and *Rothmaleria* Font Quer. Recently, Kilian & al. (2009) and Tremetsberger & al. (2013), based on molecular phylogenetic evidence, included *Hymenonema* in subtribe *Scolyminae* along with *Catananche*, *Gundelia* L. and *Scolymus* L., while *Rothmaleria* was placed along with *Tolpis* in the *Cichoriinae*.

The combination of the homogamous capitula with 5-dentate, ligulate flowers and the presence of latex easily places *Hymenonema* among the members of *Cichorieae* (Kilian & al. 2009). The morphological features that distinguish *Hymenonema* from the other genera of the *Cichorieae* are mainly in the shape of the achenes and pappus. *Hymenonema* together with *Catananche* are the only genera with the combination of a pappus composed of large scales apically prolonged into bristles, and achenes that are densely appressed pilose. The presence of receptacular paleae in *Hymenonema*, a character that is considered cardinal for *Asteraceae* classification, is also observed in some genera such as *Crepis* L., *Hypochaeris* L., *Rothmaleria* and *Scolymus* (Bremer 1994; Kilian & al. 2009). For this shared character among *Hymenonema* and the above-mentioned genera, Bremer (1994) supported the hypothesis of plesiomorphy.

According to Kilian & al. (2009), the closest relative of *Hymenonema* is *Scolymus*. The two genera share several morphological features, namely: pinnatifid-pinnatisect leaves, involucre bracts in several gradually differing rows, yellow florets, pilose corolla tube, yellow echinolphate pollen grains, long style branches with long hairs, and scabrid-barbellate pappus bristles (Sell 1976a, 1976b; Bremer 1994).

Both *Hymenonema graecum* and *H. laconicum* are perennial, robust rosette herbs that usually grow on rocks, in stony places and on roadsides. The rosette leaves are pinnatifid-pinnatisect and hairy. The stems are unbranched or have few branches terminating in a capitulum. The involucre bracts are arranged in several imbricate rows and have a scarious margin. The achenes are obconic, pilose, unbeaked, with five ribs, and the pappus consists of linear-lanceolate scales (Sell 1976a; Bremer 1994). The width of the terminal segment of the basal leaves and the morphology of the receptacle and the pappus have played a major role in the distinction of the two species of *Hymenonema* (Sell 1976a).

Karyological data combined with morphology and geographical distribution were first used in the taxonomy of the *Cichorieae* by Stebbins (1953). The ancestral basic chromosome number of the tribe (and *Asteraceae* in general) has been assumed to be $x = 9$ (Stebbins & al. 1953; Wagenitz 1976; Tomb 1977; Tomb & al. 1978). According to Turner & al. (1961), the basic number is

$x = 5$ (or 4) as a result of an aneuploid reduction from the tetraploid level, which was suggested as an explanation for the frequent gaps in the series between $x = 4, 5$ and $x = 8, 9$. However, the numbers in *Cichorieae* known today do not exhibit such gaps and also $x = 9$ is the number present in most genera and subtribes. The hypothesis of $x = 9$ is assumed more parsimonious (Kilian & al. 2009).

The *Scolyminae* (*Catananche*, *Gundelia*, *Hymenonema* and *Scolymus*) have two basic chromosome numbers: $x = 9$ and $x = 10$. The genera *Catananche* and *Gundelia* share the basic chromosome number $x = 9$ and the species are usually diploid (*Catananche*: Stebbins & al. 1953; Reese 1957; Delay 1967, 1968; Löve & Kjellqvist 1974; Humphries & al. 1978; Natarajan 1978; Blanca López 1981; Molero & Montserrat Marti 1986; Mejías & Luque 1987; Verlaque & al. 1987; Galland 1988; Gemeinholzer & Faustmann 2005; Garcia & al. 2013; *Gundelia*: Waisel 1962; Brullo & Pavone 1978; Devesa 1980; Lack & al. 1980; Al-Taey & Hossain 1984; Ghaffari & Chariat-Panahi 1985; Nersesyan & Nazarova 1989; Nazarova & Gukasian 1990; Ghukasyan & Janjughazyan 2015). Only in *Catananche* has polyploidy been observed ($2n = 4x = 36$; Oberprieler & Vogt 1993). The other two genera of the subtribe, *Hymenonema* and *Scolymus*, share the basic chromosome number $x = 10$ (*Hymenonema*: Iatrou 1986; Tan & al. 2001; Liveri & al. 2014; Strid 2015; *Scolymus*: Murin & Sheikh 1971; Tomb & al. 1978; Kuzmanov & al. 1986, 1991; Oberprieler & Vogt 1994 and references therein; Gemeinholzer & Faustmann 2005).

Despite the great interest in the tribal and/or subtribal classification of *Asteraceae*, the Greek endemic genus *Hymenonema* has never been studied sufficiently. The morphological diversity of *H. graecum* observed during field work, the restricted distribution area and the inadequate data available for the genus led us to the present study. This is the first attempt to establish a broader framework on the phylogeny of the genus, in which molecular data will be included. Morphological characters play a major role in the preparation of classification systems, diagnostic keys, etc. (Sharma 2009), while karyological data significantly contribute to the understanding of evolutionary relationships (Peruzzi & Altinordu 2014). Thus, karyological and morphological features are used to create a taxonomic framework. The main goals of the present work are (1) to evaluate the taxonomic status of *Hymenonema* and (2) to determine the morphological and karyological diversity of the genus. In a follow-up study, the morphological and karyological data will be combined with molecular data to investigate phylogeny, speciation and biogeography of *Hymenonema*.

Material and methods

Plant material of *Hymenonema* was collected during field work in 2013 and 2014. Herbarium specimens of all col-

Table 1. The main morphological differences between *Hymenonema laconicum* and the typical and non-typical forms of *H. graecum*.

		<i>H. laconicum</i>	<i>H. graecum</i> (typical form)	<i>H. graecum</i> (non-typical form)
Stem	height	30–77 cm	14–67 cm	20–63 cm
Rosette leaves	shape	pinnatifid-pinnatisect	pinnatifid-pinnatisect with narrower segments	pinnatifid-pinnatisect with narrower segments
	width	24–62 mm	12–50(–69) mm	11–26 mm
	width of terminal segment	(9–)15–35 mm	3–15(–21) mm	5–12 mm
Cilia of receptacular pits	length	to 0.5 mm	to 0.5 mm	to 1 mm
Ligules	colour	orange-yellow usually with a purple spot at base	yellow	yellow
Anther tube, fertile portion	colour	dark purple	yellow	purple
Anther tube, apical appendage	colour	dark purple	yellow	yellow
	indumentum	dense	sparse	± sparse
Achenes	length	4.7–6 mm	3.5–5.4 mm	4–5 mm
	width	1.4–2 mm	0.8–1.8 mm	1.1–1.3 mm
	indumentum	densely appressed pilose	sparsely appressed pilose	medium appressed pilose
Pappus scales	colour	distal 1/3 dark purple	uniform (pale straw-coloured)	uniform (pale straw-coloured)
	relative length	unequal	equal	equal
	length	15–18.6 mm	10–14.5 mm	12.5–14 mm

lected populations are deposited at the Herbarium of the University of Patras (UPA). Additional *Hymenonema* material was studied from UPA and from digital images of the following herbaria: ATH, ATHU, B, GZU, K, LD, P, S, W and WU (herbarium codes according to Thiers 2017+).

We examined morphologically the two *Hymenonema* species from 20 localities, 14 for *H. graecum* from seven islands of the Kiklades (Anafi, Andros, Kithnos, Mikonos, Serifos, Siros and Tinos) and six for *H. laconicum* from S Peloponnisos (Mt Paronias and Mt Taigetos). The main morphological features measured were: stem height, width of rosette leaves and width of their terminal segment, length of cilia of receptacular pits, achene size, and pappus length (Table 1). Also, qualitative differences between the taxa were examined concerning shape of rosette leaves, ligule colour, anther tube (indumentum and colour) and its apical appendage, achene indumentum, and uniformity and colour of pappus.

Living plants from 11 different localities were cultivated in the experimental garden of the Botanical Institute, University of Patras, for karyological studies. These populations are indicated by an asterisk (*) in the specimen list (see Appendix).

The chromosome measurements were obtained from root-tip metaphases, using the squash technique (Östergren & Heneen 1962; Kamari 1976). Root tips were pre-treated for six hours in a mixture of 1:1 8-hydroxyquinoline (0.3 g/l):colchicine 0.2% w/v and followed

by fixation in Carnoy [3:1 (v/v) absolute ethanol:glacial acetic acid] for 24 hours at 0–4 °C. Afterwards, they were hydrolysed in 1N HCl for 12 minutes at 60 °C and placed in Feulgen's stain (Darlington & La Cour 1969) for about three hours. At least five metaphase plates of each population were examined and indices were calculated with Microsoft Excel (2007) and PAST (version 3.14, Hammer & al. 2001). Chromosome terminology follows Levan & al. (1964), Stebbins (1971) and Kamari (1976), taking into consideration comments and suggestions by Sybenga (1959), Bentzer & al. (1971) and Favarger (1978). For each taxon, the karyotype formula, maximum and minimum length of chromosomes, total chromosome length (TCL) and average chromosome length (ACL), along with their standard deviation (SD) are given. Moreover, r-index, R-length, centromeric index and arm difference ratio for the chromosome pairs of both taxa are estimated. The interchromosomal and intra-chromosomal asymmetry are given estimating the Coefficient of Variation of Chromosome Length (CV_{CL} ; Paszko 2006; Watanabe & al. 1999) and the Mean Centromeric Asymmetry (M_{CA} ; Peruzzi & Eroğlu 2013; Peruzzi & Altinordu 2014), respectively. Additionally, the Coefficient of Variation of Centromeric Index (CV_{CI}) measuring the centromere position heterogeneity is estimated following Paszko (2006) and Peruzzi & Altinordu (2014). A multivariate analysis (Principal Coordinate Analysis, PCoA) was made for six karyological parameters: $2n$, x , THL

(Total Haploid Length), CV_{CL} , CV_{CI} and M_{CA} (Peruzzi & Altinordu 2014; Samaropoulou & al. 2016).

Results

Hymenonema Cass. in Bull. Sci. Soc. Philom. Paris 1817: 34. 1817. – Type (designated by Pfeiffer 1875: 1707): *Catananche graeca* L. (= *H. graecum* (L.) DC.).

Description — Herbs perennial, rosette-forming. *Stems* solitary to few, branched, with glandular and longer, eglandular hairs. *Leaves* pinnatifid-pinnatisect with dense, appressed, rigid, glandular and longer, eglandular hairs. *Cauline leaves* resembling rosette leaves or bract-like. *Capitula* 1 to c. 20 per individual. *Involucral bracts* in several imbricate rows, greenish in middle with scarios margin. *Receptacle* paleate, pitted, with awned scales peripherally. *Receptacular paleae* membranous. *Ligules* bright yellow or orange-yellow, 5-dentate. *Achenes* obconic, 5-angled, appressed pilose. *Pappus* of up to 15 linear-lanceolate, awned scales.

Key to the species of *Hymenonema*

1. Ligules orange-yellow usually with a dark purple spot at base; anther tube dark purple throughout and densely hairy; achenes brown, densely hairy;

pappus of awned scales varying in length, shortest ones 2–10 mm long, longest ones 15–18.6 mm long

- **1. *H. laconicum***
 – Ligules yellow; anther tube yellow or sometimes purple, with yellow apical appendage and ± sparsely hairy; achenes light brown, ± sparsely hairy; pappus of awned scales, ± equal in length, 10–14.5 mm long
 **2. *H. graecum***

1. *Hymenonema laconicum* Boiss. & Heldr. in Boissier, Fl. Orient. 3: 715. 1875. – Lectotype (designated by Tan in Tan & al. 2001: 389): [Greece, Peloponnisos] “ad radices montis Malevo pr[o]pe Platanos”, *Orphanides* Flora Graeca Exsiccata no. 1152 (G-BOIS; isoelectotypes: P 02831038, WU 0077308). – Fig. 2.

– *Catananche graeca* sensu Bory & Chaub., Nouv. Fl. Pélop.: 55. 1838, non L.

Description — *Stem* 30–77 cm tall. *Rosette leaves* 10–25(–30) × 2.4–6.2 cm, pinnatifid with dentate, lobed segments; terminal segment (9–)15–35 mm wide, larger than lateral segments. *Capitula* (1–)5–15(–20) per individual. *Involucre* 14–25 × 15–26 mm at anthesis; *bracts* 28–54, in several imbricate rows, ovate to oblong, glabrous, with a distinct scarios margin 1–3 mm wide and an acute apex. *Receptacular pits* with unequal cilia, to 1 mm long. *Ligules* orange-yellow, usually with a purple spot at base; *tube* to 15 mm long; *limb* to 25 × 5 mm. *Anther* tube dark purple, to 8 mm long, densely hairy,

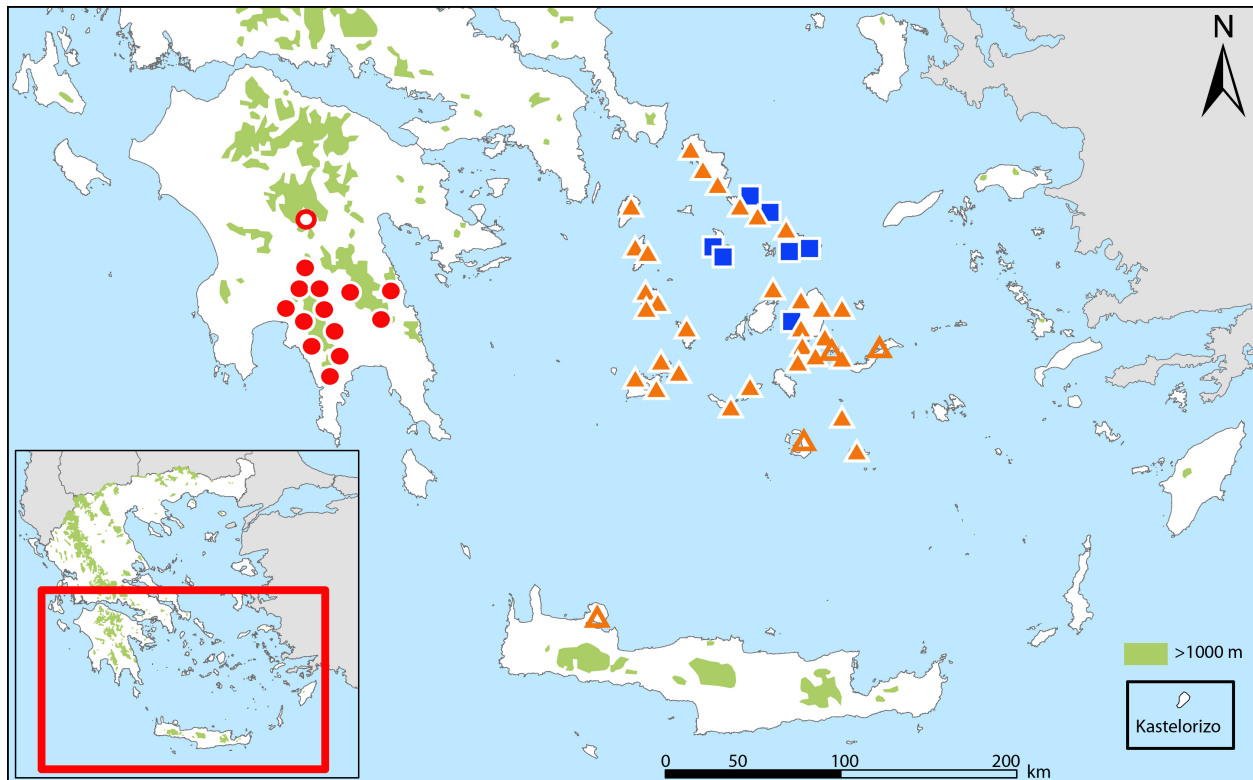


Fig. 1. Distribution map of the genus *Hymenonema* – *H. laconicum* (circles), typical form of *H. graecum* (triangles), non-typical form of *H. graecum* (squares). Solid symbols refer to specimens seen (see Appendix), open symbols refer to literature references.



Fig. 2. *Hymenonema laconicum* – A: individual; B: capitulum; C: involucre. – Photographs: A: village of Geraki, 7 Jun 2009, G. Kofinas; B: near town of Sparti, 21 May 2017, G. Kofinas; C: village of Krokees, 25 May 2014, E. Liveri (*Liveri & Kofinas 131*, UPA).

with triangular apical appendages of same colour. *Style* to 23 mm long. *Achenes* brown, 4.7–6 × 1.4–2 mm, 5-ribbed, punctate, densely hairy with rigid, appressed hairs. *Pap-*

pus with pale straw-coloured, awned scales, distal 1/3 dark purple; scales in 1 row, strongly varying in length, shortest ones 2–10 mm long, longest ones 15–18.6 mm long.

Table 2. Karyomorphometric data on *Hymenonema laconicum*. – l = length of long arm; s = length of short arm; SD = standard deviation; Sn = sum length of haploid chromosome set.

Chromosome pair	l (μm) (SD)	s (μm) (SD)	l+s (μm)	r-index l/s	centromeric index l/l+s	arm ratio l-s/l+s	relative length l+s/Sn(l+s)
1	1.97 (0.26)	1.70 (0.28)	3.67	1.18	0.54	0.076	0.049
2	1.89 (0.33)	1.56 (0.20)	3.45	1.21	0.55	0.090	0.058
3	1.72 (0.28)	1.53 (0.27)	3.25	1.13	0.53	0.058	0.055
4	1.77 (0.24)	1.36 (0.26)	3.13	1.32	0.57	0.131	0.053
5	1.92 (0.52)	1.28 (0.54)	3.06	1.73	0.63	0.252	0.051
6	1.62 (0.20)	1.14 (0.17)	2.99	1.20	0.55	0.089	0.050
7	1.73 (0.20)	1.37 (0.25)	2.83	1.19	0.54	0.083	0.048
8	1.55 (0.40)	1.13 (0.19)	2.69	1.38	0.57	0.145	0.045
9	1.35 (0.31)	1.16 (0.19)	2.84	1.16	0.54	0.071	0.042
10	1.42 (0.35)	0.79 (0.15)	2.21	1.82	0.64	0.280	0.037

Chromosome number — $2n = 2x = 20$.

Phenology — Flowering from May to July; fruiting from June to August.

Distribution — S Peloponnisos, in the lowlands surrounding Mt Parnonas, Mt Taigetos and (unconfirmed) Mt Menalo (Fig. 1).

Ecology — Dry slopes, abandoned terraces in *Quercus-Pistacia* scrub, roadsides, olive groves,

on limestone, at altitudes of (5–)20–800(–1300) m (Fig. 2A).

Karyology — Karyotype formula: $2n = 16m + 2sm + 2sm-SAT = 20$ chromosomes.

The karyotype of *Hymenonema laconicum* is diploid and symmetrical. It consists of 16 metacentric chromosomes, two submetacentric chromosomes, which are the fifth pair from largest, and two submetacentric, satellited chromosomes, which are the smallest pair (Fig. 5A, B). The size of chromosomes varies between 1.80–3.32 μm and the average chromosome length equals 2.61 μm . THL and TCL equal 26.06 μm and 52.11 μm , respectively. The interchromosomal asymmetry index (CV_{cl}) is estimated at 13.61 and the intrachromosomal (M_{CA}) at 11.15. The index related to centromere position heterogeneity (CV_{cl}) equals 11.05. The morphometric data of *H. laconicum* are given in Table 2.

All the material studied here was collected from the lowlands of Mt Parnonas and Mt Taigetos, and the exact locations are provided in the specimen list (see Appendix) indicated with an asterisk. The chromosome number $2n = 20$ has also been reported in material from Mt Parnonas and Mt Taigetos (Iatrou 1986; Tan & al. 2001) and from the Langada gorge in Mt Taigetos (Liveri & al. 2014).

Conservation status — No protection status is known until now; the species was only included in the directive

for threatened taxa according to the World Conservation Monitoring Centre (UNEP-WCMC 2013). However, *Hymenonema laconicum* is found in four protected sites of the NATURA 2000 network (Mt Parnonas: GR2520005, GR2520006; Mt Taigetos: GR2550006, GR2550009). For the protected area GR2520006 (Mt Parnonas) the presence of *H. laconicum* is characterized as very rare and for GR2550006 (Mt Taigetos) the population size was counted as 100–250 individuals by the NATURA 2000 network (standard data forms available at <http://natura2000.eea.europa.eu/Natura2000/SDF.aspx?site=GR2520006> and <http://natura2000.eea.europa.eu/Natura2000/SDF.aspx?site=GR2550006>, respectively). Based on our field observations most of the subpopulations of *H. laconicum* examined do not exceed 100 mature individuals each. The notably small number of mature individuals in each subpopulation lead us to assess *H. laconicum* as Vulnerable (VU) according to criteria C2a(i) of the IUCN (2016).

2. *Hymenonema graecum* (L.) DC., Prodr. 7: 116. 1838 \equiv *Catananche graeca* L., Sp. Pl. 2: 813. 1753 \equiv *Scorzonera elongata* Willd., Sp. Pl. 3: 1508. 1803, nom. illeg. (McNeill & al. 2012: Art. 52.1) \equiv *Hymenonema tournefortii* Cass. in Cuvier, Dict. Sci. Nat., ed. 2, 22: 316. 1821, nom. illeg. (Art. 52.1). – Lectotype (designated by Turland in Jarvis & Turland 1998: 356): [icon] “*Scorzonera Graeca saxatalis* [sic!] *et maritima, foliis varie laciniatis*” in Tournefort, Rel. Voy. Levant., ed. Paris, 1: t. fac-



Fig. 3. *Hymenonema graecum* – A: individuals of typical form; B: capitulum of typical form; C: involucre of typical form; D: capitulum of non-typical form; E: involucre of non-typical form. – Photographs: A, C: Kithnos island, 20 May 2007, G. Kamari (*Phitos & Kamari 27334*, herb. Phitos & Kamari); B, D: Tinos island, 18 Jul 2014, E. Liveri (*Liveri & Ketsilis-Rinis 121*, UPA); E: Siros island, 15 Jul 2013, E. Liveri (*Liveri & Ketsilis-Rinis 110*, UPA).

Table 3. Karyomorphometric data on typical form of *Hymenonema graecum*. – l = length of long arm; s = length of short arm; SD = standard deviation; Sn = sum length of haploid chromosome set.

Chromosome pair	l (µm) (SD)	s (µm) (SD)	l+s (µm)	r-index l/s	centromeric index l/l+s	arm ratio l-s/l+s	relative length l+s/Sn(l+s)
1	2.24 (0.83)	1.97 (0.74)	4.21	1.14	0.53	0.065	0.062
2	2.21 (0.33)	1.80 (0.15)	4.01	1.24	0.55	0.100	0.059
3	2.03 (0.31)	1.72 (0.18)	3.75	1.18	0.54	0.080	0.055
4	2.06 (0.27)	1.55 (0.24)	3.61	1.35	0.57	0.145	0.053
5	2.07 (0.40)	1.38 (0.25)	3.46	1.52	0.60	0.195	0.051
6	1.72 (0.24)	1.64 (0.24)	3.36	1.05	0.51	0.022	0.049
7	1.77 (0.18)	1.39 (0.24)	3.16	1.30	0.56	0.124	0.047
8	1.82 (0.39)	1.22 (0.19)	3.04	1.54	0.59	0.186	0.045
9	1.58 (0.27)	1.24 (0.24)	2.85	1.31	0.56	0.122	0.042
10	1.42 (0.16)	1.05 (0.17)	2.47	1.45	0.58	0.160	0.037

Table 4. Karyomorphometric data on non-typical form of *Hymenonema graecum*. – l = length of long arm; s = length of short arm; SD = standard deviation; Sn = sum length of haploid chromosome set.

Chromosome pair	l (µm) (SD)	s (µm) (SD)	l+s (µm)	r-index l/s	centromeric index l/l+s	arm ratio l-s/l+s	relative length l+s/Sn(l+s)
1	2.32 (0.52)	1.70 (0.36)	4.01	1.38	0.58	0.156	0.061
2	2.10 (0.37)	1.74 (0.39)	3.84	1.24	0.55	0.096	0.059
3	1.99 (0.48)	1.56 (0.28)	3.54	1.27	0.56	0.097	0.054
4	2.15 (0.59)	1.32 (0.24)	3.48	1.62	0.62	0.231	0.053
5	1.81 (0.35)	1.67 (0.44)	3.48	1.10	0.52	0.044	0.053
6	1.81 (0.36)	1.45 (0.26)	3.26	1.28	0.56	0.111	0.050
7	1.82 (0.38)	1.37 (0.25)	3.19	1.33	0.57	0.140	0.048
8	1.63 (0.37)	1.42 (0.27)	3.05	1.16	0.53	0.066	0.046
9	1.56 (0.37)	1.18 (0.27)	2.74	1.37	0.57	0.139	0.042
10	1.43 (0.20)	0.90 (0.24)	6.59	1.65	0.62	0.235	0.035

Table 5. Karyomorphometric indices of the two *Hymenonema* species: chromosome number (2n), karyotype formula, maximum (max l+s) and minimum (min l+s) chromosome length, total haploid chromosome length (THL), total (TCL) and average (ACL) chromosome length (SD = standard deviation), and karyotype asymmetry indices (M_{CA}, CV_{CL} and CV_{CI}).

	<i>Hymenonema laconicum</i>	<i>Hymenonema graecum</i>	
		typical form	non-typical form
2n	20	20	20
Karyotype formula	2n = 16m + 2sm + 2sm-SAT	2n = 18m + 2m-SAT	2n = 18m + 2m-SAT
Max l+s (µm)	3.32	4.27	4.11
Min l+s (µm)	1.80	2.21	2.31
THL (µm) (SD)	26.06 (4.51)	33.91 (4.08)	32.95 (6.44)
TCL (µm) (SD)	52.11 (9.01)	67.81 (8.16)	65.89 (12.88)
ACL (µm) (SD)	2.61 (0.45)	3.39 (0.41)	3.29 (0.64)
M _{CA} (SD)	11.15 (1.80)	11.86 (2.51)	13.27 (3.10)
CV _{CL} (SD)	13.61 (3.47)	16.25 (1.31)	15.63 (1.42)
CV _{CI} (SD)	11.05 (2.17)	11.27 (2.71)	10.37 (2.04)

ing p. 223. 1717. – Fig. 3. – “*Scorzonera graeca* Hort.” (Steudel, Nomencl. Bot., ed. 2, 2: 546. 1841), nom. inval. (Art. 36.1(c)).

Description — *Stem* 14–67 cm tall. *Rosette leaves* 3.2–25(–35.4) × 1.2–5(–6.9) cm, pinnatifid with dentate, lobed segments; terminal segment 3–15(–21) mm wide, larger than lateral segments. *Capitula* 1–5(–15) per individual. *Involucre* 15–26 × 10–24 mm at anthesis; *bracts* 24–50, in several imbricate rows, ovate to oblong, glabrous, with a distinct scarious margin 0.8–2.5 mm wide and an acute apex. *Receptacular pits* with unequal cilia to 0.5 mm long. *Ligules* yellow; *tube* to 11 mm long; *limb* to 18 × 4 mm. *Anther tube* yellow, to 6 mm long, sparsely hairy, with triangular apical appendages of same colour. *Style* to 14 mm long. *Achenes* light brown, 3.5–5.4 × 0.8–1.8 mm, 5-ribbed, punctate, ± sparsely hairy with rigid, appressed hairs. *Pappus* of pale straw-coloured, awned scales, in 1 row, ± equal in length, 10–14.5 mm long.

Chromosome number — 2n = 2x = 20.

Phenology — Flowering from May to July; fruiting from June to the beginning of September.

Distribution — Kiklades and (unconfirmed) NW Kriti (Fig. 1).

Ecology — Growing in garigue, phrygana, stony

places, cliffs, roadsides, residential areas, mostly on limestone, also on schistose and granitic substrate, margins of coastal saline ground, at altitudes of 0–450 m (Fig. 3A).

Morphological variation —

During the field work we observed that some individuals (Tinos) or even a whole population (Siros) of *Hymenonema graecum* (Fig. 1) have some morphological features resembling *H. laonicum*. This non-typical *H. graecum* has a purple anther tube as in *H. laonicum* but with a yellow apical appendage (Fig. 4E) and the achenes (Fig. 4F) are intermediate in indumentum between those of *H. graecum* and *H. laonicum*. With respect to the other characters, the non-typical plants largely match typical *H. graecum* (Table 1). This non-typical form of *H. graecum* has been observed on three islands: Mikonos, Siros and Tinos. On Mikonos, specimens (at LD) from two different localities were examined, and the plants belong to the non-typical form of *H. graecum*. On Siros, all the localities examined had plants belonging to the non-typical form of *H. graecum*. On Tinos, all the localities examined had both forms of *H. graecum*. On the islands of Anafi, Andros, Kithnos and Serifos, all the populations were of typical *H. graecum*.

Karyology — Karyotype formula: $2n = 18m + 2m\text{-SAT} = 20$ chromosomes.

All the populations of *Hymenonema graecum* are found to be diploid having a symmetrical karyotype, with 20 metacentric chromosomes. The smallest chromosome pair bears well-observed satellites (Fig. 5C, D). This satellited pair shows structural heterogeneity with one metacentric and one submetacentric homologue in material collected from Kithnos island. The chromosome size ranges from 2.21–4.27 μm . The average chromosome length is 3.39 μm , the total chromosome length is 67.81 μm and for

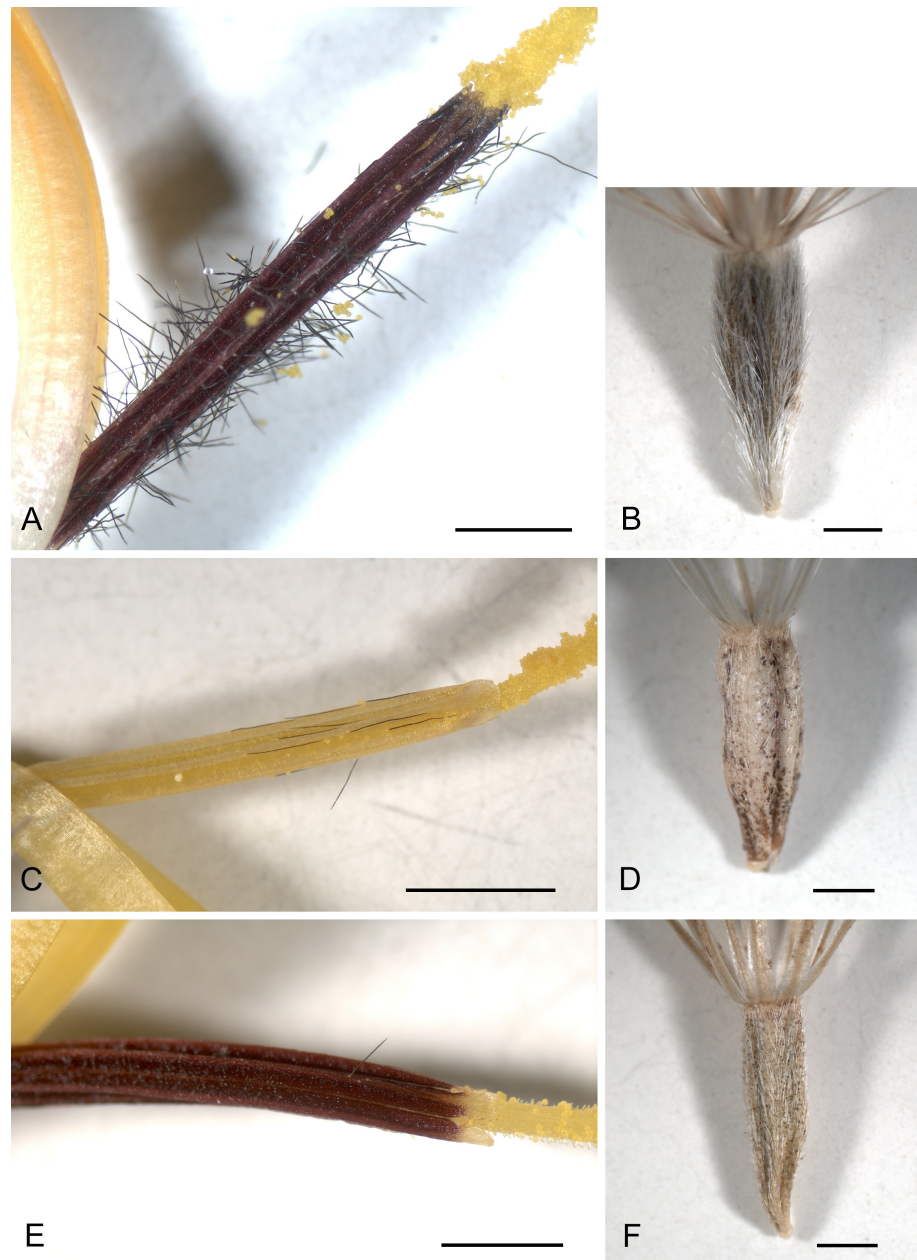


Fig. 4. Morphological differences of achene and anther tube of *Hymenonema*. – A, B: *H. laonicum* (Kyriakopoulos 1524, herb. Phitos & Kamari); C, D: typical form of *H. graecum* (Liveri & Ketsilis-Rinis 100, UPA); E, F: non-typical form of *H. graecum* (Liveri & Ketsilis-Rinis 107, UPA). – Scale bars = 1 mm.

the haploid series is 33.91 μm . The asymmetry indices, CV_{CL} and M_{CA} , equal 16.25 and 11.86, respectively. The coefficient of variation of centromeric index is estimated to 11.27. The morphometric data of the typical *H. graecum* are given in Table 3.

Individuals of the non-typical form of *Hymenonema graecum* were also examined karyologically, and the results show similar karyotype morphology to the typical form (Fig. 5E). The morphometric data from these populations were calculated separately in order to find possible variations (Table 4). The karyotype formula of non-typical *H. graecum* is: $2n = 18m + 2m\text{-SAT} = 20$ chromosomes (Fig. 5F). The chromosome size varies

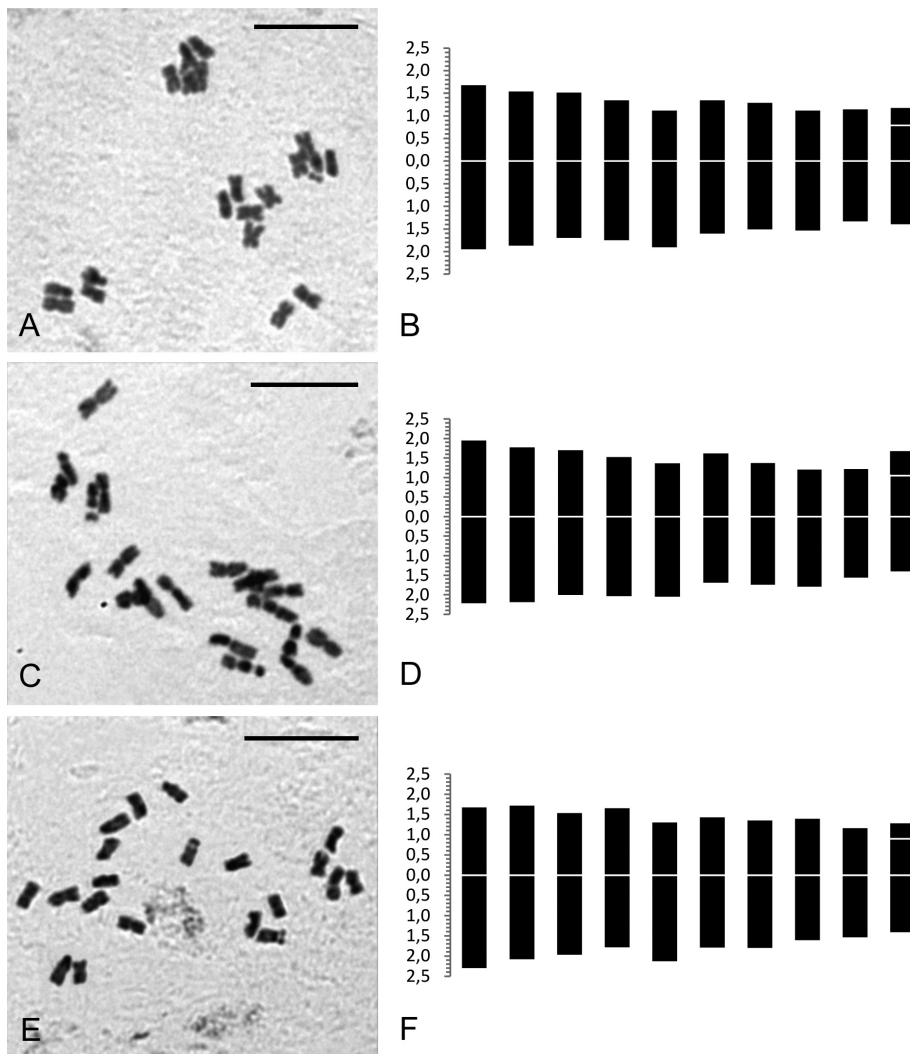


Fig. 5. Karyotype and idiogram of *Hymenonema*. – A, B: *H. laconicum* (Liveri & Ketsilis-Rinis 124, UPA); C, D: typical form of *H. graecum* (Liveri & Ketsilis-Rinis 100, UPA); E, F: non-typical form of *H. graecum* (Liveri & Ketsilis-Rinis 107, UPA). – Scale bars = 10 μ m.

from 2.314.11 μ m, while the average chromosome length is 3.29 μ m. THL and TCL equal 32.95 μ m and 65.89 μ m, respectively. M_{CA} is estimated at 13.27, CV_{CL} at 15.63 and CV_{CI} at 10.37. The morphometric data of the non-typical *H. graecum* are given in Table 4.

The chromosome number $2n = 20$, found here, is in accordance with previous references based on material from Kithnos (Liveri & al. 2014), Naxos and Schinoussa (Strid 2015). There is also one reference of the same chromosome number (Iatrou 1986), but the locality of the material is not mentioned.

The karyomorphometric indices of *Hymenonema laconicum* and *H. graecum* (typical and non-typical) are given in Table 5.

Conservation status — *Hymenonema graecum* is protected by Greek Presidential Decree 67/1981 (1981) on the protection of the native flora and wild fauna of Greece and was also included in the directive for threatened taxa according to the World Conservation Monitoring Cen-

tre (UNEP-WCMC 2013). Populations of *H. graecum* are found in five protected sites of the NATURA 2000 network (Anafi: GR422002; Iraklia, Schinoussa and nearby islands/islets: GR4220013; Naxos: GR4220014; Poliegos-Kimolos: GR422006; Santorini: GR4220003). The examined subpopulations of *H. graecum* do not exceed more than 100 mature individuals each. Only on Tinos island does the subpopulation occurring close to the villages of Arnados, Dio Choria and Monastiri comprise more than 100 mature individuals. The species distribution includes almost all the Kiklades islands and islets reaching an extent of occurrence a little more than 2500 km². However, in view of the severe fragmentation of its distribution area and the continuous decline of its habitats, we assess the species as Vulnerable (VU) according to criteria B1ab(iii); C2a(ii) of the IUCN (2016).

Discussion

For this study, the geographical distribution of *Hymenonema* (Fig. 1) is presented in detail (see Appendix). *Hymenonema graecum* is distributed at most of the islands and islets of the Kiklades, but there are also two references from Kriti and Turkey. The presence of *H. graecum* in NW Kriti was referred by Raulin (1869: 493) and Boissier (1875: 715) and it was later confirmed by Zaffran (1990: 331). Since then, several botanists (N. Turland, pers. com.) searched for the plant without success at the locality mentioned by Zaffran (“à la périphérie du terrain salé au fond de la baie de Souda”). It should be noted that this area has been occupied for military purposes since 1951–1952. Now, it is the location of three major military installations and so access is strictly restricted. Concerning the presence of *H. graecum* in Turkey, one specimen from Herb. Heldreich was mentioned by Boissier (1875: 715) collected from the region “Byzantium”. One additional reference from the *Flora of Turkey* (Matthews 1975: 626), from Istanbul, based on collections by Cadet de Fontaney also in Herb. Heldreich, is presumably wrong. The two references most likely refer to the

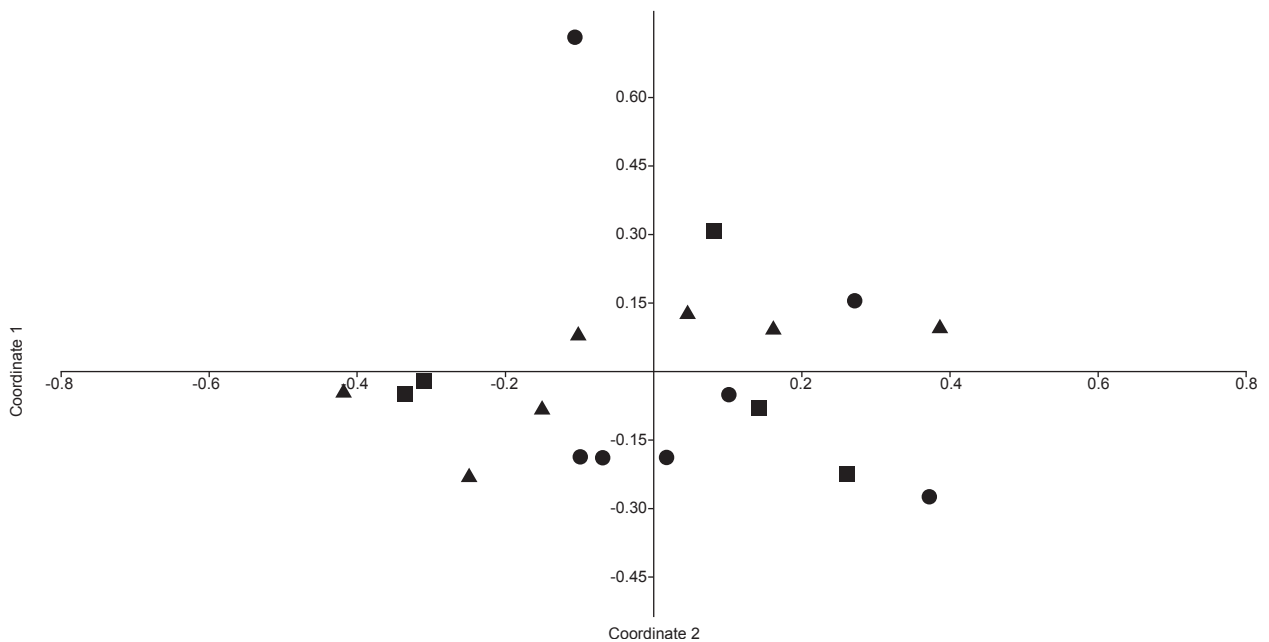


Fig. 6. PCoA analysis based on six quantitative karyological parameters of *Hymenonema* – *H. laconicum* (●), typical form of *H. graecum* (▲), non-typical form of *H. graecum* (■).

same specimen, which was probably a cultivated specimen. Therefore, in Euro+Med (2006+), the presence of *H. graecum* in Turkey as well as in Kriti is considered questionable.

There is also a reference of *Hymenonema laconicum* from Mt Menalo (Halácsy 1902: 173), with a herbarium specimen of Sartori, which, however, has not recently been confirmed.

The previous identification keys for the species of *Hymenonema* were based mainly on the width of the terminal segment of the basal leaves and the uniformity of the pappus (Sell 1976a). According to our results, the width of the terminal segment in contrast to the pappus structure is not a reliable diagnostic character. Moreover, new diagnostic features were observed: colour of ligules, colour and indumentum of anther tube, and indumentum of achenes (Fig. 4). It is noteworthy that the purple spot at the base of the ligules, which was mentioned for *H. graecum* in *Flora europaea* (Sell 1976a), is observed only on the ligules of *H. laconicum*. Additionally, Strid (2016) stated that the colour of the anther tube of *H. graecum* is orange-brown. We assume that the mentioned plants belong to the non-typical *H. graecum*. Also, the genus description by Sell (1976a) does not specify if the number of capitula (1–3) is per stem or per individual. However, we have counted in *H. laconicum* (1–)5–15(–20) and in *H. graecum* 1–5(–15) capitula per individual (Fig. 2A, 3A).

The main morphological differences between *Hymenonema laconicum* and *H. graecum*, presented in Table 1, support that they are two clearly separated species. Individuals or whole populations of *H. graecum* with intermediate morphological characters, characterized by purple anther tubes with a yellow appendage, are here reported for the first time. In this study, we define the

above-mentioned form of *H. graecum* as non-typical. The typical form with the yellow anther tube and other morphological differences (Table 1, Fig. 3, 4) agrees with the description by Candolle (1838: 116), which refers to “*capitula magna flava*”. The lectotype illustration (Tournefort 1717: t. facing p. 223), although it does not show the colour of the anther tube, resembles the form of *H. graecum* with the yellow anther tube.

A karyomorphological analysis of the genus *Hymenonema*, including populations from the most of its distribution area, is carried out for the first time. The karyotypes of *H. laconicum* and *H. graecum* show low intrachromosomal (M_{CA}) and interchromosomal (CV_{CL}) asymmetry, as was expected from the predominance of metacentric chromosomes and the similar chromosome size. The heterogeneity of the centromere position (CV_{CI}) is also low for both species. The above-mentioned indices are slightly higher for typical *H. graecum*. The karyological parameters concerning the chromosome length (THL, TCL, ACL) were also higher for *H. graecum*. The intrachromosomal asymmetry for non-typical *H. graecum* is even higher compared to the typical *H. graecum*, while the CV_{CI} is smaller than *H. laconicum* (Table 5). The karyological parameters about chromosome length (THL, TCL, ACL) for non-typical *H. graecum* are intermediate between the two species. Statistical analysis (PCoA; Fig. 6) of the six karyological parameters according to the method proposed by Peruzzi & Altinordu (2014) does not provide additional data to understand the relationships between these taxa. The accessions of the examined taxa overlap and no clear group is created.

Cytotaxonomic data have been used to explain the origin and evolutionary trends of endemics (Favarger & Contandriopoulos 1961; Favarger 1969; Favarger &

Siljak-Yakovlev 1986; Siljak-Yakovlev & Peruzzi 2012). Based on our karyological data, *Hymenonema* species are characterized as schizoendemics. *Hymenonema laconicum* and *H. graecum* share the same chromosome number, show similar morphological features and occur in different but close geographical areas. The evidence from the current study strongly supports the hypothesis of schizoendemism.

For *Hymenonema*, there is insufficient data to prove whether the differentiation of the two species started before or after the geographical isolation. However, the finding of the intermediate form of *H. graecum* suggests complex speciation events that occur in the Aegean archipelago.

The presence of intermediate plants between the two species may imply hybridization. Examples of hybridization between plant species have been studied extensively in the Aegean area, such as the *Crepis neglecta* L. complex (Kamari 1976). However, in this case the scenario of hybridization does not seem reasonable, since the non-typical *Hymenonema graecum* has not been found in the middle of the distribution areas of the two species, i.e. in the W Kiklades (Kimolos, Kithnos, Milos, Serifos and Sifnos). On the contrary, non-typical *H. graecum* occurs on Mikonos, Siros, Tinos (N Kiklades) and probably on Naxos (C Kiklades; Strid 2016). Finding the non-typical *H. graecum* is an interesting element in the evolutionary process of the genus, but still more populations from different islands need to be examined.

In conclusion, the karyological and morphological data provide a sufficient taxonomic framework for *Hymenonema*. The new findings of the current study contribute to a better understanding of the genus. The provided data combined with a molecular approach might elucidate the phylogenetic relationships between the species of *Hymenonema*, as well as with its closest genera.

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Appendix: List of material examined

Populations indicated by an asterisk (*) have been studied karyologically.

1. *Hymenonema laconicum*

GREECE: PELOPONNISOS: ARKADIA: NW slopes of Mt Parnon, 2–3 km E of village “Ajos Petros” along road to “Moni Malevis”, place called Zonanga, 900 m, dry, stony roadsides and rocky hillsides with *Quercus coccifera*, *Phlomis* and *Spartium* shrubs, limestone (ligules orange-yellow), 15 May 1970, *Stamatiadou* 8763 (ATH 14314); 1–2 km after crossing of road from Leonidion to Tsitalia village, 100 m, sandy-stony roadsides, dry fallow fields and phrygana (ligules orange-yellow with a purple spot at base), 18 May 1970, *Stamatiadou* 8915 (ATH 14313); c. 6.8 km from Leonidion towards Poulithra, close to Poulithra, 37°07'N, 22°53'E, 20–40 m, in roadsides and margins of abandoned fields (florets orange), 25 May 2002, *Constantinidis & Kalpoutzakis* 10112 (ATHU, UPA, herb. Phitos & Kamari); 3.1 km from small town of Leonidio towards Tsitalia village, 37°08'N, 22°53'E, 140–160 m, in stony slopes with abandoned olive groves, together with *Ceratonia siliqua*, *Pistacia lentiscus*, *Calicotome villosa*, *Cistus creticus* etc., 31 May 2004, *Kalpoutzakis* 1506 (ATHU, UPA, herb. Phitos & Kamari); **ibid.*, 37°08.90'N, 22°52.65'E, 113 m, 13 Jun 2014, *Kofinas & Dolianitis* 129 (UPA). — LAKONIA: in regione olearum ad radices Taygeti in herbis pr. Androuvasta, Jun 1844, *Heldreich s.n.* (K 000797232, P 02831032, P 02831034, P 02831035, P 02831043, P 02831044); in herbis reg. [radices] m. Taygeti Laconiae, Jun 1844, *Heldreich s.n.* (P 02831036, P 02831039); Taygetus, *Heldreich s.n.* (K 00797231); habit. in m. Malevo Laconiae, 3000', 24 Jun 1857, *Orphanides* 1152 (P 02831038, WU 0077308); Morea: in collib. pr. Golass, 700 m, Jul 1879, *Pichler s.n.* (P 02831001, P 02831037, P 02831041, P 02831045); Mani, Petria-Arna, 1 Jan 1950, *Goulimy* 6040 (ATH 51278); Krokeai, 13 May 1955, *Goulimy* 6041 (ATH 51280); inter Sparta et Skouras, Iter Graecum XI 1964, *Rechinger* 24754 (B 100209164); Mt Taigetos, NW of Arna, c. 25 km S of Sparti, 36°53'N, 22°24'E, 950 m, 21 May 1964, *Runemark & Snogerup* 20568 (LD 800100); prope pagum Krokeae, in argilosis, 36°53'N, 22°33'E, 9 May 1965, *Phitos* 1459 (UPA, herb. Phitos & Kamari); village “Pirgos Dirou”, 200 m, semi-cultivated fields and grassy roadsides, limestone (red soil), 15 May 1970, *Zoumpouli* 8609 (ATH 14315); inter oppidum Gythion et pagum Krokeae, ad viam, 36°49'N, 22°31'E, in argilosis, 11 May 1979, *Tzanoudakis & Iatrou* 5724 (UPA); prope pagum Krokeae, 36°53'N, 22°33'E, in argilosis, 22 Jun 1980, *Iatrou* 588i (UPA); 4 km from Siderokastron along road to Konakia, 400 m, (ligules orange yellow with brownish purple spot at base), 17 May 1991, *Tan & Strid* 31141 (ATH 42173); 1–2 km

from Siderokastron, along road to Skofianitika and Skamnitsa, 36°46'N, 22°25'E, 530 m, in *Quercus/Pistacia* scrub, (latex white, ligules orange-yellow with purplish black base stigmas bright lemon-yellow), 18 Jun 1991, *Tan & Vold* 9754 (UPA, herb. Phitos & Kamari); Eparchia Githiou, Siderokastron, 530 m, 28 Jul 1993, *Tan* 09754 (B 100185587); Ep. Lakedhemon: NE foothills of Mt Taijetos, Langadiotissa gorge SW of Parori, 37°03'30"N, 22°22'40"E, 350–500 m, on limestone substrate, 14 Jun 1995, *Kamari & al.* 1533 (UPA, herb. Phitos & Kamari); 3.9 km after village of Geraki towards village of Alepochori, stony slope at left side of road, 36°58'N, 22°43'E, 320–350 m, abandoned olive grove, calcareous and schistose substrate, with *Quercus coccifera*, *Phillyrea latifolia*, *Calicotome villosa*, *Coridothymus capitatus*, *Thymus laconicus*, 2 May 2005, *Kalpoutzakis* 1656 (ATHU); 13–14 km NNE of village of Mitropoli, along a secondary road, 37°02'N, 22°59'E, 80–100 m, roadsides, limestone, 30 May 2005, *Constantinidis & Kalpoutzakis* 11380 (ATHU); C Taigetos, Anavriti, 37°2.148'N, 22°23.864'E, 800 m, 10 Jun 2007, *Kyriakopoulos* 659 (UPA, herb. Phitos & Kamari); Geraki, road from village to Alepochori, 36°58'56"N, 22°43'46"E, 300–400 m, 27 May 2012, *Kofinas* 128 (UPA); on roadsides between villages Taigeti and Mistras, 600 m, 15 Jun 2012, *Kyriakopoulos & Kartsonas* 1073 (UPA, herb. Phitos & Kamari); *Lagkada gorge, climbing region, 37°04'59.09"N, 22°18'39.30"E, 800 m, 24 Jun 2013, *Kyriakopoulos* 1524 (UPA, herb. Phitos & Kamari); Geraki, road from village to Alepochori, 36°58'56"N, 22°43'46"E, 300–400 m, 24 May 2014, *Kofinas* 126 (UPA); *Lagkada gorge, climbing region, 37°04'59.09"N, 22°18'39.30"E, 800 m, 25 May 2014, *Liveri & Kofinas* 130 (UPA); Krokees village, 25 May 2014, *Liveri & Kofinas* 131 (UPA); entrance of Kaiaidas, 15 Jun 2014, *Kofinas* 132 (UPA); *Geraki, road from village to Alepochori, 36°58'56"N, 22°43'46"E, 300–400 m, 16 Jun 2014, *Liveri & Ketsilis-Rinis* 124 (UPA); Anavriti, 37°02.146'N, 22°24.049'E, 440 m, 5 Jul 2014, *Kyriakopoulos & Kofinas s.n.* (UPA). — MESSINIA: Ep. Kalamon: pr. Selitza ad radices m. Taygeti, 37°03'N, 22°07'E, 28 May 1894, *Heldreich* (LD 37837); *ibid.*, 15 May 1896, *Heldreich* 1355 (LD 37838, P 02831030, P 02831031); *ibid.*, 15 May 1896, *Heldreich* 1355 (K 000797230); Laconia boreo-occidentalis: in regione litorali ad radices m. Selitza prope Kalamata, 15 May–15 Jul 1896, *Zahn* 1355 (K 000797229, P 02831040, P 02831042, P 03763056, P 03763058, P 04310661); Ep. Kalamata: c. 2 km from Ano Amfia along road to Thouria, 37°05'45"N, 22°03'20"E, c. 150 m, olive groves and field margins, on sandstone, 14 Jun 1995, *Kamari & al.* 2591 (UPA, herb. Phitos & Kamari); 8 km SE of Kalamata, locally common on roadsides, along olive gr[oves] (this specimen taken above hotel Lida), 4 Apr 1996, *Emanuelsson* 1886 (S 10-12275); Rintomo, 680 m, 13 Jun 2009, *Kyriakopoulos s.n.* (photo!); S part of Mt Taigetos, peak of Zizali, in

area of Tsopania, 36°49'N, 22°24'E, 1000 m, 30 May 2013, *Kyriakopoulos & Kartsonas 1463* (UPA, herb. Phitos & Kamari); close to junction of provincial road Dirachiou-Thourias, 37°9.780'N, 22°11.488'E, 700 m, 1 Jun 2015, *Kyriakopoulos & Kofinas 2196* (UPA, herb. Phitos & Kamari).

2. *Hymenonema graecum*

GREECE: AEGEAN ISLANDS: KIKLADES: INS. ANAFI: Insula Anaphi, 3 Jun 1898, *Leonis 56* (P 02831024, P 02831048); Kalamos, 1 km E of monastery, 36°21'N, 25°51'E, 0–200 m, cliffs, garrigue, 8 May 1958, *Runemark & Snogerup 8132* (LD 1544455); *ibid.*, 8 May 1958, *Runemark & Snogerup 8134* (LD 1530335); Anafi, 0–1 km N of Chora, 36°21'N, 25°46'E, 200–300 m, gneiss-granite, 27 Apr 1995, *Runemark 50558* (1804865 LD); Anafi, substrate calcareous rocks, 36°21'36.0"N, 25°47'54.4"E, 320 m, 26 Apr 2011, *Kougioumoutzis 1172* (UPA); *ibid.*, 36°21'42.8"N, 25°47'58.9"E, 245 m, substrate gabbro-diorites, 26 Apr 2011, *Kougioumoutzis 1431* (UPA); *ibid.*, 36°21'20.7"N, 25°46'32.4"E, 210 m, substrate alluvial, 26 Apr 2011, *Kougioumoutzis 1499* (UPA); *ibid.*, 36°21'27.8"N, 25°46'23.4"E, 275 m, substrate granite, 28 May 2011, *Kougioumoutzis 1842* (UPA). — INS. ANDIKEROS: 36°51'N, 25°41'E, cliffs, 6 Jul 1958, *Runemark & Snogerup 12354* (LD 1555541). — INS. ANDROS: near sea 2 km SW of Zaganiari, 50–100 m, 16 Jun 1964, *Snogerup 21122* (LD 1532135); Oros Raxhi, 600–800 m, 17 May 1968, *Snogerup & Bothmer 32123* (LD 1532075); along road from Paleopolis to Stavropedha, place called “Kakia Melissa”, 150 m, stony roadsides and dry hillsides with phrygana and schistose rocks, (ligules orange-yellow), 13 Jun 1969, *Stamatiadou 6616* (ATH 14312); Batsi, terrace walls NE of village, 16 Apr 1990, *Snogerup & Snogerup 6666* (LD 1247052); Batsi, along roadsides NE of village, 37°51'09"N, 24°47'20"E, 20–25 m, phrygana, 14 Jul 2014, *Liveri & Ketsilis-Rinis 114* (UPA); *Batsi, residential area, Agios Filippos church, 37°51'09"N, 24°47'20"E, 45 m, 14 Jul 2014, *Liveri & Ketsilis-Rinis 115* (UPA). — INS. ANIDROS: insula Anhydros (Amorgopulos), 36°37'N, 25°41'E, in saxosis, substr. calc., 6 May 1934, *Rechinger & Rechinger 5260* (LD 1973900); SW part of island, 36°38'N, 25°41'E, limestone, garigue, 9 May 1958, *Runemark & Snogerup 8261* (LD 1530275). — INS. FOLEGANDROS: Adelfia Petra, E island, 36°37'N, 24°59'E, litoral limestone rocks, 23 May 1960, *Runemark & Nordenstam 14591* (LD 1555661). — INS. IRAKLIA: cycladum insula Heraklia, 36°51'N, 25°28'E, in lapidosis, 3 May 1934, *Rechinger & Rechinger 4892* (LD 1990997); S of harbour, 36°50'N, 25°29'E, 7 Jun 1957, *Runemark 4146* (LD 1544275); Ormos Pegadi, small valley W of bay, 36°50'N, 25°29'E, on sandy soil, 10 Apr 1958, *Runemark & Snogerup 5297* (LD 1530395); path SSW of abandoned settlement of Agios Athanasios at CW parts of island, 36°50'N, 25°26'E, 110–280 m, stony dry fields, path sides with sparse *Juniperus phoenicea*, *Cistus creticus*, *Coridothy-*

mus capitatus, mostly schists, (flowers yellow), 9 May 2013, *Constantinidis & Bazos 13190* (ATHU). — INS. KARDIOTISSA: 36°38'N, 25°01'E, garigue, 22 May 1960, *Runemark & Nordenstam 14528* (LD 1555601). — INS. KEA: in insula Cea, solo schistoso (“schiste cristallin”), 21–24 May 1898, *Svoronos s.n.* (P 02831021). — INS. KIMOLOS: Kimolos, *Kougioumoutzis s.n. (photo!)*. — INS. KITHNOS: Cycladum insula Cythnos, 17 May 1964, *Pinatzis 19069* (UPA, herb. Pinatzi); Merikha bay (W side), 20–40 m, garigue, 29 Jun 1966, *Strid 23332* (LD 1556201); in collibus supra pagi Merichas ad viam versus pagum Dryopida, c. 100 m, solo schistoso, 20 May 2007, *Phitos & Kamari 28005* (UPA, herb. Phitos & Kamari); in collibus ad meridiem pagi Merichas spectantibus, c. 50 m, in nanofruticetosis, solo schistoso, 20 May 2007, *Phitos & Kamari 27334* (UPA, herb. Phitos & Kamari); **ibid.*, 12 Jul 2013, *Liveri & Ketsilis-Rinis 100* (UPA). — INS. KOUFONISIA: Kopria, 36°59'N, 25°38'E, hard limestone cliffs, 5 Jun 1958, *Runemark & Snogerup 10279a* (LD 1554341). — INS. MAKARES: Prasonisi, 37°05'N, 25°42'E, 0–80 m, garigue, 26 May 1958, *Runemark & Snogerup 9662* (LD 1544515, LD 1555481). — INS. MIKONOS: island of Stapodia, 37°25'N, 25°34'E, 13 May 1968, *Runemark & Engstrand 35556* (LD 1530035); island of Ag. Georgios, 37°27'N, 25°18'E, 13 May 1968, *Runemark & Engstrand 35631* (LD 1529975); valley S of Mt Ag. Elias, 37°28'N, 25°20'E, 50–100 m, 13 May 1968, *Runemark & Engstrand 35355* (LD 1530155); S of Ag. Stefanos, 37°28'N, 25°20'E, 13 May 1968, *Runemark & Engstrand 35455* (LD 1530095); 2 km N of Ag. Stefano, 37°30'N, 25°19'E, 17 May 1968, *Runemark & Engstrand 36121* (LD 1541017). — INS. MILOS: en insula Melos, 1820, *Urville s.n.* (P 02831050); Milo, île d'archipel., *Urville s.n.* (P 02831011); coteaux de Melos, *Urville 22* (G 00498237); Erimomilos 29 Jun–2 Jul 1948, *Goulimy 6038* (ATH 51274); S of Akr. Roma, in a valley, 36°42'N, 24°32'E, 17 Jun 1967, *Runemark & Bentzer 29666* (LD 1556141); W and SW Chivadolimni, wet area, 36°44'N, 24°26'E, 5 May 2003, *Runemark 51574* (LD 1666654). — INS. NAXOS: Naxie, 1822, *Olivier s.n.* (G 00498239); Naxos, *Olivier & Bruguiere s.n.* (P 02831010, P 02831022); in Cycladum insula Naxos, 10 Jul 1897, *Leonis s.n.* (P 02831023); insula Naxos, 13 May 1898, *Leonis 122* (P 02831047, P 03763059); in cycladum insula Naxos, May 1898, *Leonis 4171* (LD 1989781, P 02831046, P 02831051, P 02831052, P 02831057); in monte Phanariotissa ad pagum Apiranthos, 37°08'N, 25°31'E, 600–800 m, in saxosis calc., 28 Jun 1932, *Rechinger 2237* (LD 1989973); Filoti-Apiranthos, 21–24 Jun 1954, *Goulimy 6037* (ATH 51273); Naxos, 37°08'N, 25°27'E, 13 May 1957, *Runemark 2637* (LD 1543915); E of Mytria, along a small stream, 37°08'N, 25°27'E, 100–150 m, limestone cliffs, garigue, 13 May 1957, *Runemark 2637* (LD 1544635); *ibid.*, 31 May 1957, *Runemark 3635* (LD 1544095); Metri N of Moni, 37°05'N, 25°30'E, 500–530 m, 20 May 1957, *Runemark 3174* (LD 1543975); Faneromeni, along a small stream, 37°09'N, 25°29'E, 50–120 m,

22 May 1957, *Runemark 3299* (LD 1544035); ENE of Skado, valley with a rill, 37°08'N, 25°33'E, 500 m, 1 Jun 1957, *Runemark 3736* (LD 1544155); SSE of Axapsis, along a small stream, 37°07'N, 25°26'E, 3 Jun 1957, *Runemark 3909* (LD 1544215); 2 km NNW of Ormos Liona, 37°09'N, 25°35'E, garigue near sea, 24 May 1958, *Runemark & Snogerup 9052* (LD 1530455); in valley 0–3 km N of Ormos Agiasou, 36°59'N, 25°26'E, 5–60 m, garigue, 3 Jun 1958, *Runemark & Snogerup 10134* (LD 1554281); Kalando, cultivated in Botanical Garden of University of Lund, 36°56'N, 25°28'E, 4 Jun 1958, *Runemark cult. 1212* (LD 1554161, LD 1554221); Psiliamos Ormos, 37°01'N, 25°34'E, 7 Jun 1958, *Runemark & Snogerup 10649* (LD 1555421, LD 1558004); NW outskirts of town of Naxos, 37°07'N, 25°24'E, 25 m, calcareous soils, 29 May 1982, *Franzén & Andersson 1009* (LD 1557944); Prokopios, 30 m, garrigue, 29 Sep 1989, *Jäth s.n.* (B 100209163); Ostküste, Moutsouna, Landzunge S des Hafens, 10 m, 16 May 2008, *Karl s.n.* (GZU 000272899). — INS. PAROS: Gaidouronisi, 37°09'N, 25°16'E, 30 m, 18 May 1967, *Runemark & Bentzer 29188* (LD 1556321). — INS. POLIYGOS: NW part, 36°47'N, 24°37'E, garigue-field, 19 Apr 1967, *Runemark & Bentzer 26301* (LD 1544395). — INS. SCHINOUSA: between principal village and harbour, 36°52'N, 25°31'E, 9 Jun 1957, *Runemark 4355* (LD 1544335); *ibid.*, cultivated in Botanical Garden of University of Lund, 9 Jun 1957, *Runemark cult. 124* (LD 1543795, LD 1543855); from village of Mersini towards N, on way to hill of Vardies, S and NW parts of hill, 36°52'N, 25°30'E, 10–120 m, phrygana with *Pistacia lentiscus*, *Coridothymus capitatus*, *Teucrium brevifolium*, and cultivated land, mostly schists, (flowers yellow), 10 May 2013, *Constantinidis & Bazos 13199* (ATHU). — INS. SERIFOPOULA: 37°15'N, 24°36'E, 27 Apr 1967, *Runemark & Bentzer 27771* (LD 1556021). — INS. SERIFOS: Koutalas, 28 Apr 1927, *Runemark & Bentzer 27978* (LD 1556081); rocks in Hora, (flowers bright yellow), 22 Jun 1961, *Gathorne-Hardy 75* (ATH 21294); just N of village of Serifos, 37°09'N, 24°30'E, 130–140 m, vertical rocks facing NW, 1 Jul 1966, *Strid 23354* (LD 1556501); 1–2 km SW of Livadion, 50–350 m, 26 Apr 1967, *Runemark & Bentzer 27457* (LD 1556561, LD 1556441); 2–2.5 km SW of Livadion, 37°08'N, 24°30'E, 250–350 m, 26 Apr 1967, *Runemark & Bentzer 27507* (LD 1555961); at strand and on rocks of Vagia-bay, 37°07'N, 24°27'E, 17 Jul 1982, *Tiniakou 285* (UPA); from Taxiarchis closter downwards Plati Gialos Bay, 37°11'N, 24°29'E, micaceous schist, 7 May 1983, *Tiniakou 478* (UPA); *ibid.*, 7 May 1983, *Tiniakou 494* (UPA); between villages of Panagia and Kentarchos, 37°10'N, 24°29'E, c. 300 m, micaceous schist, 7 May 1983, *Tiniakou 470* (UPA); Aspros Kavos, 37°07'N, 24°24'E, marbles, 9 May 1983, *Tiniakou 534* (UPA); Livadi, port, 37°08'N, 24°27'E, granitic rocks, 9 May 1983, *Tiniakou 545* (UPA); Koutalas, 400 m, 13 May 1990, *Malicky s.n.* (W); above Kato Ampeli bay, 37°07'N, 24°29'E, 200–300 m, granitic substrate, 11 May 1993, *Tiniakou & al. 2305* (UPA); at Petri as Mt, 1–2 km after Pano Chora, 37°09'N, 24°30'E, 300–500 m, granitic substrate, 11 May 1993, *Tiniakou & al. 2256* (UPA); Profitis Ilias, 28 Apr 1997, *Tiniakou 3454* (UPA); Koutalas, 28 Apr 1997, *Tiniakou 3654* (UPA); *ibid.*, 28 Apr 1997, *Tiniakou 3585* (UPA); S of Panagia, 37°11'N, 24°25'E, 400 m, 1 Jun 2008, *Runemark 51955* (LD 1804801); Aspros Kavos, marbles, 3 Aug 2014, *Tiniakou & Tiniakos 111* (UPA); Kalo Ampeli, 3 Aug 2014, *Tiniakou & Tiniakos 112* (UPA). — INS. SIFNOS: limestone hill S of Ormos Kondos, 36°54'N, 24°42'E, garigue, 10–100 m, 13 May 1958, *Runemark & Snogerup 8402* (LD 1530215); S of Kamares, 36°59'N, 24°40'E, 20 Jun 1967, *Runemark & Bentzer 29981* (LD 1555901). — INS. SIKINOS: E of Kastro, 36°42'N, 25°08'E, 200–350 m, N-exposed cliffs, 9 Apr 1967, *Runemark & Bentzer 24645* (LD 1544575); E promontory, 36°43'N, 25°11'E, S-exposed cliffs facing sea, 10 Apr 1967, *Runemark & Bentzer 24792* (LD 1555841); S-slope of highest mountain, 36°40'N, 25°06'E, 0–600 m, 10 Apr 1967, *Runemark & Bentzer 24941* (LD 1556261); NW parts of island, along path from ancient monument of Episkopi to Manali well, 36°40'N, 25°05'E, 260–310 m, edges of cultivated land, rocky slopes, stone walls and phrygana, siliceous schist (upper part) and limestone, (florets yellow-orange), 16 Apr 2014, *Constantinidis 13399* (ATHU). — INS. SIROS: Insula Syra, 1837, *Aucher-Éloy 3508* (G 00498240, P 02831012, P 02831014); legimus in ins. Syro, in collibus siccis et ad vias, 37°27'N, 24°55'E, 4 Jul 1889, *Heldreich 1055* (LD 1989909, P 02831052, P 02831054, P 02831055, P 02831056, P 02831058, P 04310662, P 04402753); insula Syros, 3–4 Jul 1889, *Heldreich* (P 02831027, P 02831028, P 02831049); Episkopion, 14 May 1921, *Ksenakis s.n.* (UPA, herb. Phitos & Kamari); Lotos-Kini, 14–16 Oct 1957, *Goulimy 6039* (ATH 51276); 1 km SE of Kini, 200 m, 20 Jun 1964, *Snogerup 21139* (LD 1555781); islet of Strongylo E of Didymi, 37°26'N, 24°59'E, 27 May 1968, *Snogerup & Bothmer 33426* (LD 1556381); island of Aspronisos, 37°24'N, 25°00'E, 50 m, hard limestone, 27 May 1968, *Snogerup & Bothmer 33443* (LD 1540897); islet of Ampelos E of Vari, 37°23'N, 24°57'E, 5 m, 27 May 1968, *Snogerup & Bothmer 33496* (LD 1532315); islet of Kommemo NE of Ermoupolis, 37°28'N, 24°57'E, 20 m, 28 May 1968, *Snogerup & Bothmer 33603* (LD 1532255); islet of Varvarousa, 37°28'N, 24°54'E, 25 m, limestone, 28 May 1968, *Snogerup & Bothmer 33655* (LD 1532195); *Kini, along roadsides from beach to village for a distance up to 1 km approximately, 37°26'N, 24°57'E, 13 Jul 2013, *Liveri & Ketsilis-Rinis 107* (UPA); *Agathopes, road towards beach, 37°23'N, 24°52'E, 0–10 m, on rocks very close to sea, 15 Jul 2013, *Liveri & Ketsilis-Rinis 110* (UPA); Megas Gialos, 37°22.75'N, 24°54.40'E, 25 m, 7 Aug 2014, *Ketsilis-Rinis 113* (UPA). — INS. TINOS: ad litore ins. Teni, 37°33'N, 25°06'E, 16 May 1851, *Sartori s.n.* (LD 1989717, P 02831025); Platia Ammos, 37°33'N, 25°08'E, 18 May 1968, *Runemark & Engstrand 36363*

(LD 1540957); c. 1 km W of Kionia, 16 Aug 1987, *Landström* 7346 (LD 1246992); *Monastiri, Arnados, Dio Choria, along roadsides connecting these three villages, 37°33'44"N to 37°33'47"N, 25°10'58"E to 25°11'29"E, 350–450 m, 17 Jul 2014, *Liveri & Ketsilis-*

Rinis 117 (UPA); *Kionia bay, 37°33'12.7"N, 25°08'10.5"E, 0–100 m, on rocks, phrygana, 18 Jul 2014, *Liveri & Ketsilis-Rinis* 121 (UPA); *Kolimpithra bay, 37°37'49.4"N, 25°08'44.5"E, 0–10 m, on rocks, phrygana, 19 Jul 2014, *Liveri & Ketsilis-Rinis* 123 (UPA).

Willdenowia

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