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

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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*), *Jankaea 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*).
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* sub-group, whereas Bremer (1993, 1994) placed it in the sub-tribe *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*, *Gundella* L. and *Scolymus* L., while *Rothmaleria* was placed along with *Tolpis* in the *Cichorieae*.

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* (Kilian & al. 2009). The morphological features of *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, involucral bracts in several gradually differing rows, yellow florets, pilose corolla tube, yellow echinolophate 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 involucral 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).


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-
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 Parnonas and Mt Taege-tos). 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 Leván & 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 (CVcL; Paszko 2006; Watanabe & al. 1999) and the Mean Centromeric Asymmetry (Mca; Peruzzi & Eroğlu 2013; Peruzzi & Altinordu 2014), respectively. Additionally, the Coefficient of Variation of Centromeric Index (CVcI) 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.

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

<table>
<thead>
<tr>
<th></th>
<th><em>H. laconicum</em></th>
<th><em>H. graecum</em> (typical form)</th>
<th><em>H. graecum</em> (non-typical form)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem height</td>
<td>30–77 cm</td>
<td>14–67 cm</td>
<td>20–63 cm</td>
</tr>
<tr>
<td>Rosette leaves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stem shape</td>
<td>pinnatifid-pinnatisect</td>
<td>pinnatifid-pinnatisect with narrower segments</td>
<td>pinnatifid-pinnatisect with narrower segments</td>
</tr>
<tr>
<td>width</td>
<td>24–62 mm</td>
<td>12–50(–69) mm</td>
<td>11–26 mm</td>
</tr>
<tr>
<td>width of terminal segment</td>
<td>(9–)15–35 mm</td>
<td>3–15(–21) mm</td>
<td>5–12 mm</td>
</tr>
<tr>
<td>Cilia of receptacular pits length</td>
<td>to 0.5 mm</td>
<td>to 0.5 mm</td>
<td>to 1 mm</td>
</tr>
<tr>
<td>Ligules colour</td>
<td>orange-yellow usually with a purple spot at base</td>
<td>yellow</td>
<td>yellow</td>
</tr>
<tr>
<td>Anther tube, fertile portion colour</td>
<td>dark purple</td>
<td>yellow</td>
<td>purple</td>
</tr>
<tr>
<td>Anther tube, apical appendage indumentum</td>
<td>dense</td>
<td>sparse</td>
<td>± sparse</td>
</tr>
<tr>
<td>Achenes length</td>
<td>4.7–6 mm</td>
<td>3.5–5.4 mm</td>
<td>4–5 mm</td>
</tr>
<tr>
<td>Pappus scales colour</td>
<td>distal ½ dark purple</td>
<td>uniform (pale straw-coloured)</td>
<td>uniform (pale straw-coloured)</td>
</tr>
<tr>
<td>relative length</td>
<td>unequal</td>
<td>equal</td>
<td>equal</td>
</tr>
<tr>
<td>length</td>
<td>15–18.6 mm</td>
<td>10–14.5 mm</td>
<td>12.5–14 mm</td>
</tr>
</tbody>
</table>
Liveri & al.: Taxonomic study on the Greek endemic genus *Hymenonema* (Total Haploid Length), CV<sub>Cl</sub>, CV<sub>CI</sub> and M<sub>CA</sub> (Peruzzi & Altnordu 2014; Samaropoulou & al. 2016).

**Results**


*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 scarious 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

   
   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 scarious margin 1–3 mm wide and an acute apex. Receptacular pappus 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.
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
dus with pale straw-coloured, awned scales, distal ½ 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.

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

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 (un confirmed) Mt Menalo (Fig. 1).

Ecology — Dry slopes, abandoned terraces in *Quercus-Pistacia* scrub, roadides, olive groves,
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 intrachromosomal asymmetry index \((CV_{cl})\) is estimated at 13.61 and the interchromosomal asymmetry index \((CV_{cl})\) 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](http://natura2000.eea.europa.eu/Natura2000) and [http://natura2000.eea.europa.eu/Natura2000/SDF.aspx?site=GR2550006](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).


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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).
**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. laconicum*. This non-typical *H. graecum* has a purple anther tube as in *H. laconicum* but with a yellow apical appendage (Fig. 4E) and the achenes (Fig. 4F) are intermediate in indumentum between those of *H. graecum* and *H. laconicum*. 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: $2n = 18m + 2m-SAT = 20$ 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 satellite 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 the haploid series is 33.91 μm. The asymmetry indices, $CV_{ct}$ and $M_{ct}$, 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: $2n = 18m + 2m-SAT = 20$ chromosomes (Fig. 5F). The chromosome size varies...
from 2.314.11 μm, while the average chromosome length is 3.29 μm. THL and TCL equal 32.95 μm and 65.89 μm, respectively. MCA is estimated at 13.27, CV CL at 15.63 and CVCI 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 Centre (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 sub-population 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
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<sub>CA</sub>) and interchromosomal (CV<sub>CL</sub>) asymmetry, as was expected from the predominance of metacentric chromosomes and the similar chromosome size. The heterogeneity of the centromere position (CV<sub>CI</sub>) 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<sub>CI</sub> 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 & Altinorlu (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 &

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**Fig. 6.** PCoA analysis based on six quantitative karyological parameters of *Hymenonema* – *H. laconicum* (●), non-typical form of *H. graecum* (■), typical form of *H. graecum* (▲).
Siljak-Yakovlev 1986; Siljak-Yakovlev & Peruzzi 2012). Based on our karyological data, *Hymenonema* species are characterized as schizoendemics. *Hymenonema laco-nicum* 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 nontypical *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 *Hyme-nonema*. 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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References


Appendix: List of material examined

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

1. Hymenonema laconicum

**Greece:** Peloponnisos: Arkadika: NW slopes of Mt Parnon, 2–3 km E of village “Ajios Petros” along road to “Moni Malevis”, place called Zonanga, 900 m, dry, stony roadsides and rocky hillsides with Quercus cocciifera, Phlomis and Spartium shrubs, limestone (ligules orange-yellow), 15 May 1970, Stamatiadou 8763 (ATH 14314); 1–2 km after crossing of road from Leonidio 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 Leonidio towards Pouliithra, close to Pouliithra, 37°07’N, 22°53’E, 20–40 m, in roadsides and margins of abandoned fields (florets orange), 25 May 2002, Constantinidis & Kalputzakis 10112 (ATHU, UPA, herb. Phitos & Kamari); c.6.8 km from Leonidion towards Poulithra, 37°07’N, 22°53’E, 20–40 m, roadsides, limestone, 30 May 2005, Constantinidis & Kalputzakis 11380 (ATHU); C Taigeto, 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, stony slope at left side of road, 36°58’N, 22°43’E, 300–320 m, abandoned olive grove, calcareous and schistose substrate, with Quercus cocciifera, Phillyrea latifolia, Calicotome villosa, Coriophyllum capitatus, Thymus laconicus, 2 May 2005, Kalputzakis 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 & Kalputzakis 11380 (ATHU); C Taigeto, 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, 25 May 2012, Kofinas 128 (UPA); on roadsides between villages Taigeti and Mistras, 600 m, 15 Jun 2012, Kyriakopoulos & Kartsounas 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, 25 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 Karia, 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 (K 00797230); Krokees village, 25 May 2014, Kyriakopoulos & Kofinas s.n. (UPA).
area of Tsopania, 36°49’N, 22°24’E, 1000 m, 30 May 2013, Kyriakopoulos & Kartsanos 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. Hymenomena graecum

GREECE: AEGEAN ISLANDS: KIKLADIES: INS. ANAFI: Insula Anaphi, 3 Jun 1898, Leonis 56 (P 02831024, P 02831048); Kalamos, 1 km E of monastery, 26°19’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, sub-strate 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, 210 m, substrate alluvial, 28 May 2011, Kougioumoutzis 1499 (UPA); ibid., 36°21’27.8”N, 25°46’23.4”E, 275 m, substrate granite, 26 Apr 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 Zaganari, 50–100 m, 16 Jun 1964, Snogerup 21122 (LD 1532135); Oros Rakhi, 600–800 m, 17 May 1968, Snogerup & Boothner 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 115 (UPA); Batsi, residential area, Agios Filippou church, 37°51’09”N, 24°47’20”E, 45 m, 14 Jul 2014, Liveri & Ketsilis-Rinis 115 (UPA). — INS. ANDROS: insula Anilidos: insula Anhydros (Amorgopulos), 36°37’N, 24°59’E, litoral area, Agios Filippos church, 37°51’09”N, 24°47’20”E, in saxosis calc., 28 Jun 1932, Stavrakakis 14591 (LD 1990997); S of harbour, 37°51’09”N, 24°59’E, 23 May 1960, Rechinger 2237 (LD 1556211); on sandy soil, 37°28’N, 25°41’E, 13 May 1898, Goulimy 6037 (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 & Bruguieres 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 Phanarotissa at 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 Mytira, 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); Faneromi, 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 Axapidias, 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); Psilamos 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, 30 May 1982, Runemark & Snogerup 102981 (LD 1554457); 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. 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, 24°40’E, 20 Jun 1967, Runemark & Bentzer 29981 (LD 1555901); NW 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. Sifnos: Ins. |Sul“ Ins. Kini, 37°28’N, 24°57’E, 200 m, 20 Jun 1964, Sifner & Snogerup 21139 (LD 1555781); islet of Strongylo E of Didymi, 37°26’N, 24°50’E, 27 May 1968, Sifner & Bothmer 33426 (LD 1556381); island of Aspronisos, 37°24’N, 25°00’E, 50 m, hard limestone, 27 May 1968, Sifner & Bothmer 33443 (LD 1540897); islet of Ampelos E of Vari, 37°23’N, 24°57’E, 5 m, 27 May 1968, Sifner & Bothmer 33496 (LD 1532315); islet of Kommeno NE of Ermonapolis, 37°28’N, 24°57’E, 20 m, 28 May 1968, Sifner & Bothmer 33603 (LD 1532255); islet of Varvaroussa, 37°28’N, 24°54’E, 25 m, limestone, 28 May 1968, Sifner & 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 110 (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 110 (UPA). — Ins. Tinos: at litore ins. |Teni, 37°33’N, 25°06’E, 16 May 1851, Sartori s.n. (LD 1989717, P 02831025); Platià Ammos, 37°33’N, 25°08’E, 18 May 1968, Runemark & Engstrand 36363

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(LD 1540957); c. 1 km W of Konia, 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).