

## Karyotaxonomy of *Myosotis alpestris* group

Chromozomové počty a taxonomické poznámky k zástupcům skupiny *Myosotis alpestris*

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Chromosome numbers of taxa belonging to the *Myosotis alpestris* group are provided and/or confirmed. A chromosome count is reported for the first time for *M. olympica*. A new ploidy level ( $2n = 24$ ) was revealed within *M. stenophylla* for which previously only tetraploid cytotypes are reported. *Myosotis stenophylla* is identified for the first time from Greece. Previous chromosome counts for *M. ambigens*, *M. alpestris*, *M. atlantica*, *M. corsicana*, *M. lithospermifolia*, and *M. suaveolens* are confirmed based on plants originating from karyologically poorly investigated parts of the distribution areas of this polyploid complex.

**Keywords:** distribution area, karyogeography, Morocco, *Myosotis alpestris* group, ploidy level, South Europe, taxonomy, Turkey

### Introduction

Representatives of the *Myosotis alpestris* group are arcto-alpine species with conspicuously scattered distribution areas. The population system of island differentiation is characteristic of this complex; suitable habitats are relatively small but scattered over a large region. Thus, a typical variation pattern develops as a result of limited gene flow, selection pressure of the environment and genetic drift. Along with morphological variation, extensive variability in ploidy level is found in this group. Several factors acting interactively must be invoked to explain the considerably complicated pattern of variability within the *M. alpestris* group. The most important ones are: polyploidy, ecological adaptation and geographical isolation.

In the *Myosotis alpestris* group, significant chromosome variation among species occurs. Karyological studies of this group have been published by several authors. Of particular interest are reports by Grau (1964), Luque (1992) and Štěpánková (1993a, 1993b, 1996). So far three groups of alpine forget-me-nots can be recognized based on their ploidy level: diploids ( $2n = 24$ ), tetraploids ( $2n = 48$ ) and hexaploids [ $2n = 72$  (70)]. It is quite clear that the process of polyploidization has greatly contributed to the evolution and speciation within this group. Along with conspicuous karyological variation and geographic discontinuities, the species of this group are well known for their interesting ecological disjunction. They grow on serpentine as well as non-serpentine sites both at alpine and lower altitudes. Hence the *M. alpestris* group might serve as a model for studies of the origin and consequences of geographic and ecological endemism, polyploid evolution and biogeographic history of the European mountain flora. However, the modes of diversification in the group are still unclear and no phylogenetic hypotheses are proposed. The results of karyological examination presented here are the first part of the project focussed on the biogeographic and evolutionary history of the complex, combining molecular phylogeny (data from restriction site

analysis of cpDNA and sequence data of ITS region of rDNA) with phylogenetic information from non-molecular data sets (morphology, karyology).

### Material and methods

Chromosome numbers were counted in samples from 21 populations collected in the field (Table 1) and cultivated in the experimental garden of the Institute of Botany at Průhonice. Counts were usually made on three different root tips from each of two to five individuals chosen from each population. Chromosome numbers were determined at the mitotic metaphase in the somatic cells of root tips. The material was pretreated for two hours with a saturated solution of bromnaphthalen and fixed in a freshly prepared 3 : 1 mixture of ethanol and acetic acid (Carnoy). The squash method and staining by lacto-propionic orceine were used. Voucher specimens are deposited in the herbarium of the Institute of Botany at Průhonice (PRA).

### Results and discussion

#### *Myosotis alpestris* F. W. Schmidt

A species distributed in almost all high mountain ranges in Europe and having broad ecological preferences with respect to the geological substrate. Its diagnostic set of morphological characters is: basal leaves with oblanceolate to obovate blade gradually narrowing into a petiol, hirsute with more or less patent hairs or glabrescent on lower surface, calyx narrowed at the base in fruit, with straight, short, appressed hairs not forming conspicuous white rim at the margin of calyx teeth, with few longer, patent, slightly curved hairs and sometimes with few hooked stiff hairs on the calyx tube, not extending up to the flower/fruit pedicel (Fig. 1a, b), pedicel usually as long as calyx in fruit.

*Myosotis alpestris* is well known for its ability to form cytotypes of different ploidy levels. However, this variation in ploidy level is not associated with particular morphological characters. According to previously reported chromosome data (e.g. Grau 1964, Štěpánková 1993b), diploid and tetraploid cytotypes occur sympatrically in the Pyrenees, Alps and Carpathians. Hexaploids are known only from the Alps in Germany and Austria (Grau 1964, Štěpánková 1993b). Tetraploid chromosome counts based on plants from the Velebit Mts (Croatia), Prokletije Mts (Montenegro and Albania), Mt Kajmakčalan (Greece), and Pindos Mts (Greece) are reported here for the first time.

During the revision of the *M. alpestris* group in Europe, a noteworthy discrepancy in taxonomic evaluation of sympatric diploid/polyploid cytotypes was noticed. While diploids, tetraploids, and hexaploids occupying areas of the Alps and the Carpathians are not usually classified at any taxonomic level (e.g. Grau 1964, Grau & Merxmüller 1972), diploids and tetraploids from the Pyrenees are usually treated as separate taxonomic units (Grau 1964, Grau & Merxmüller 1972, Blaise et al. 1992). While the tetraploids are attributed to *M. alpestris*, diploids are treated as *M. pyrenaica* (= *M. alpina* Lapeyer., nom. illeg.; Grau 1964, Grau & Merxmüller 1972), *M. corsicana* subsp. *pyrenaeorum* (Blaise et al. 1992), or *M. alpestris* (Luque 1992). Plants collected in the Pyrenees and included in this karyological analysis bear all of the diagnostic characters of *M. alpestris*, hence the diploid chromosome number of  $2n = 24$  reported here is attributed to *M. alpestris*.

Table 1. – List of localities and chromosome numbers of the populations of *Myosotis* studied.

Taxon	Voucher number	2n	Collection locality	Coordinates	Altitude (m a.s.l.)	Collectors
<i>M. atlantica</i>	2006/1	24	Morocco: Haute Atlas Mts, Oukaïmeden	31°13' N 07°51' W	2550	Štěpánková J., Kaplan Z., Kirschner J. 2002
<i>M. alpestris</i>	2005/1	24	Spain: Pyrénées-Orientales Mts, Puerta da la Bonaiqua, Mt Tuc de la Llanca	42°41' N 00°59' E	2483	Štěpánková J., 2005
<i>M. alpestris</i>	2005/2	48	Spain: Pyrénées-Orientales Mts, Parc National de Aigües Tortes, Port de Ratera	42°36' N 00°58' E	2380	Štěpánková J. 2005
<i>M. alpestris</i>	2005/3	48	France: Pyrénées-Orientales Mts, Mt Pic Carlit	42°34' N 01°54' E	2059	Štěpánková J. 2005
<i>M. alpestris</i>	2005/4	48	Montenegro: Prokletije Mts, Gusinje, Mt Volušnica	42°31' N 19°46' E	1750	Štěpánková J. 2005
<i>M. alpestris</i>	2005/5	48	Greece: Northern Pindos Mts, Metsovo, Kataras pass	39°40' N 21°21' E	1640	Štěpánková J. 2003
<i>M. alpestris</i>	2005/6	48	Greece: Nidže voras Mts, Mt Kajmakčalan.	40°54' N 21°49' E	1886	Štěpánková J. 2003
<i>M. alpestris</i>	2005/7	48	Croatia: Velebit Mts, Mt Crikvena	44°46' N 14°59' E	1620	Štěpánková J. 2005
<i>M. ambigens</i>	2005/8	24	Italy: Monte Baldo Mts, the crest Creta di Naole	45°38' N 10°47' E	1500	Štěpánková J., Kaplan Z. 2004
<i>M. corsicana</i>	2005/9	24	Corsica: Vizzavona, Mt Monte D'Oro	42°08' N 29°06' E	2203	Šafránek J. 2004
<i>M. lithospermifolia</i>	2005/10	24	Turkey: Esler Dagi Mts, Mt Honaz Dag	37°40' N 29°18' E	1950	Štěpánková J. 2004
<i>M. lithospermifolia</i>	2005/11	24	Turkey: Dedegöl Daglari Mts, Mt Dededöl Dagi	37°41' N 31°18' E	2036	Štěpánková J. 2004
<i>M. lithospermifolia</i>	2005/12	24	Turkey: Ak Daglar Mts: Mt Kofu Tepe	36°31' N 29°55' E	2108	Štěpánková J. 2004
<i>M. olympica</i>	2005/13	24	Turkey: Uludag Mts, Mt Uludag Tepe	40°05' N 29°11' E	2232	Štěpánková J. 2004
<i>M. stenophylla</i>	2005/14	24	Greece: Northern Pindos Mts, Mt Smolikas	40°06' N 20°54' E	2087	Štěpánková J. 2003
<i>M. stenophylla</i>	2005/15	24	Greece: Northern Pindos Mts, Mt Smolikas	40°05' N 20°55' E	2600	Štěpánková J. 2003
<i>M. suaveolens</i>	2005/16	24	Greece: Central Olympus Mts, Mt Skolio	40°05' N 22°22' E	2815	Štěpánková J. 2003
<i>M. suaveolens</i>	2005/17	24	Montenegro: Prokletije Mts, Gusinje, Mt Karanfili	42°30' N 19°47' E	1790	Štěpánková J. 2005
<i>M. suaveolens</i>	2005/18	24	Albania: Prokletije Mts, Mt Maja e Jezerce	42°27' N 19°49' E	2308	Štěpánková J. 2005
<i>M. suaveolens</i>	2005/19	24	Montenegro: Komovi Mts: Berane, Mt Kom Vasojevički	42°43' N 19°41' E	2100	Štěpánek J., Jr. 2005
<i>M. suaveolens</i>	2005/20	24	Montenegro: Moračke Planine Mts: Berane, Mt Moračka Kapa	42°50' N 19°15' E	1840	Štěpánková J. 2005

*Myosotis ambigens* (Bég.) Grau

A species considered to be restricted to the Apennine Mts (Italy) (Grau 1964, Grau & Merxmüller 1982). They are small mat-forming plants with small short petiolate basal leaves and narrowly oblanceolate blade hirsute with long patent hairs on both surfaces or sometimes glabrescent on the lower surface, gradually narrowing into a petiole, with conspicuously smaller linear to lanceolate hirsute cauline leaves (ca  $5 \times 15$  mm in the middle part of stem), obtuse at the apex, with fruiting calyx more or less narrowed at the base, densely covered with short hairs and with numerous long stiff appressed white hairs, forming conspicuous white rim at the margin of calyx teeth, calyx tube with no or very few hooked hairs, pedicel without hooked hairs (Fig. 1c).

During an excursion to the small mountain massif of Monte Baldo Mts (Italy, Veneto, near Lake Garda) small populations of plants with these characters were found. These plants were identified as *M. ambigens* on the basis of a detailed comparison with plants of *M. ambigens* from the Central Apennines (La Maiella Mts and Gran Sasso Mts). The chromosome number found agrees with that indicated by Grau (1964) and Blaise & Cartier (1982) for plants of *M. ambigens* coming from the Central Apennines.

*Myosotis atlantica* Vesterg.

This taxon is endemic to the high mountain systems of Morocco. It is distinct from the other species of this group as all its leaves are pilose with long soft nearly straight hairs on both surfaces, calyx rounded at the base in fruit, with white straight hairs forming conspicuous white rim at the margin of calyx teeth and with longer, patent, slightly curved hairs and hooked ones on the calyx tube extending halfway up the pedicel. Patent hooked hairs on the flower/fruit pedicels is a noteworthy morphological character, which occurs very rarely within the *M. alpestris* group (Fig. 2a). *Myosotis atlantica*, *M. corsicana* and *M. olympica* are the only representatives with this character.

The chromosome count presented here confirms the diploid level ( $2n = 24$ ) previously reported by Blaise (1970) and Galland (1988).

*Myosotis corsicana* (Fiori) Grau

A rare and local endemic of Corsica (France), *M. corsicana*, is an attractive perennial species occupying the highest parts of the central and northern mountains of the island. It differs from other representatives of the group *M. alpestris* by having strigose leaves with retrorse-appressed, short stiff hairs scattered on upper surface, glabrate or glabrescent lower surface, basal leaves long petiolate with blade broadly obovate or elliptical, more or less rounded at the base and suddenly constricted into a long petiole, cauline leaves coriaceous, broadly ovate, subacute at the apex, calyx rounded at the base in fruit, with straight, short, appressed hairs and long hairs on the calyx teeth forming conspicuous white rim, calyx tube with long patent or slightly decumbent hooked stiff hairs, extending up to the flower/fruit pedicel (Fig. 2b), pedicel not longer than calyx in fruit.

The chromosome count of  $2n = 24$  was observed in plants from Monte d'Oro. This number agrees with that previously reported by Countandriopoulos (1964).

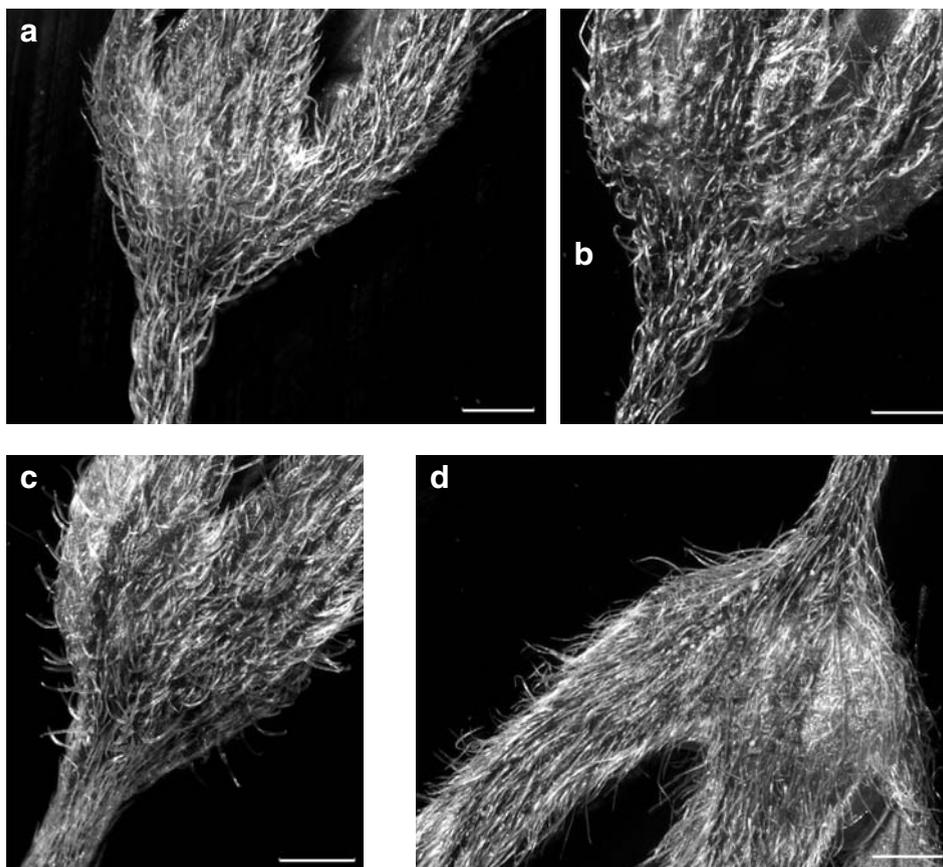


Fig. 1. – Hairs on the base of calyx: a – *M. alpestris* (diploid), Alps, Italy; b – *M. alpestris* (diploid), Pyrenees, Spain; c – *M. ambiguens*, Mt Gran Sasso, Italy; d – *M. stenophylla*, Czech Republic, Mohelno. Scale bar = 500  $\mu$ m.

*Myosotis lithospermifolia* (Willd.) Hornem.

The distribution area of this species includes Turkey, Iraq, Iran, Crimea and the Caucasus (Grau 1964, 1978). Its diagnostic characters are densely pilose leaves with long soft hairs on both surfaces, basal leaves indistinctly petiolate with oblanceolate to narrowly obovate blade, obtuse at apex, cauline leaves narrowly lanceolate, subacute to obtuse at apex, calyx narrowed at the base in fruit, equally densely hairy with straight, short, appressed hairs and long hairs on the calyx teeth not forming conspicuous white rim, calyx tube densely covered by long patent to deflexed hooked stiff hairs, extending halfway up the flower/fruit pedicel (Fig. 2c), fruit pedicels conspicuously longer than fruiting calyx.

The chromosome number of  $2n = 24$ , found in the three populations of *M. lithospermifolia*, is the first count for Turkey. This chromosome number agrees with the one previously established by Grau (1964) for plants from Iraq. Based on these reports, *M. lithospermifolia* seems to be a diploid with  $2n = 24$ .

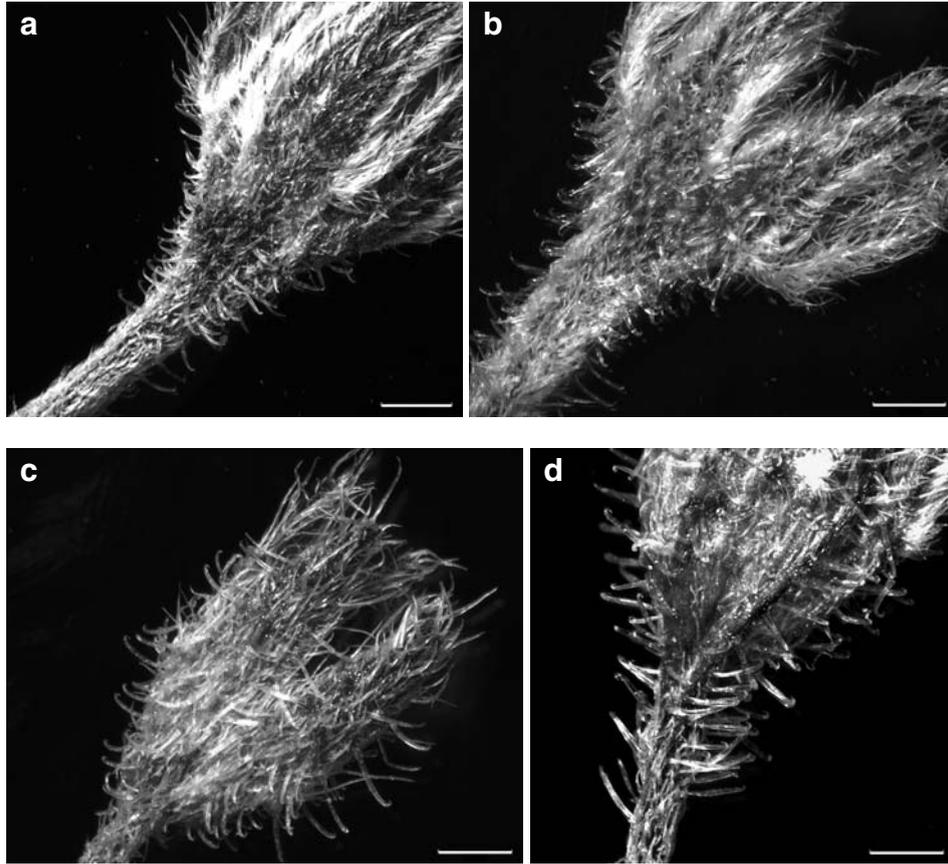


Fig. 2. – Hairs on the base of calyx: a – *Myosotis atlantica*, Haute Atlas Mts, Morocco; b – *M. corsicana*, Corsica; c – *M. lithospermifolia*, Mt Dedegöl Dagi, Turkey; d – *M. olympica*, Mt Uludag Tepe, Turkey. Scale bar = 500  $\mu$ m.

*Myosotis olympica* Boiss.

This species is described from the Turkey, Mt Olympi Bithyni (recently Mt Uludag Tepe) near Bursa and recorded from other high mountains of Turkey, predominantly the Pontic mountain systems (Grau 1978). The diagnostic characters of *M. olympica* include strigose leaves with short stiff hairs on the upper surface, glabrate or glabrescent lower surface, blade of basal leaves elliptical or obovate, subacute at apex, at base very gradually narrowing into a petiole, cauline leaves narrowly ovate to lanceolate, subacute at apex, fruiting calyx narrowed at base, with long, adpressed, straight hairs on calyx teeth forming distinct white rim at the margins and conspicuous white wad between teeth, calyx tube with long hooked hairs extending halway up the flower/fruit pedicel (Fig. 2d), pedicel usually shorter than calyx in fruit.

Karyological examinations of the *locus classicus* population revealed a diploid chromosome number  $2n = 24$ . As far as the author knows, this is the first report for this species.

*Myosotis stenophylla* Knaf

While most of the taxa belonging to the *Myosotis alpestris* group are considered to occur in alpine sites of high mountains systems, *M. stenophylla* is the exception; so far it is only recorded from lowland Central Europe. The distribution of this forget-me-not shows an interesting ecological disjunction. It grows on serpentine as well as non-serpentine sites at lower altitudes. During an excursion to the North Pindos Mts (Greece), plants of *M. stenophylla* were found by the author on serpentine slopes of Mt Smolikas. There are several populations in the upper parts of the mountain (ranging from 2080 to 2600 m a. s. l.). Plants from Mt Smolikas have all the important features of *M. stenophylla* growing on serpentine sites: small plants with stem highly branched, basal leaves with long slender petioles and elliptical to oblanceolate blades, sparsely hirsute or glabrescent upper surface, glabrate to glabrescent lower surface, cauline leaves narrowly lanceolate, sparsely hairy or glabrescent on both surfaces, corolla conspicuously glowingly blue, calyx narrowed at base in fruit, with straight, short, appressed hairs not forming conspicuous white rim at the margin of calyx teeth, with longer, more or less patent, hairs both on the teeth and calyx tube, calyx tube sometimes with a few short hooked hairs not extending up to the flower/fruit pedicel (Fig. 1d), pedicel as long as calyx in fruit or a little longer.

The chromosome count of  $2n = 24$  recorded for two populations from Mt Smolikas is a new diploid chromosome number for this species. Up to now, *Myosotis stenophylla* has been considered to be a tetraploid species (e.g. Štěpánková 1993b, 1996). However, a morphological comparison of tetraploid populations originating from lowland serpentine localities in Central Europe with diploid population from Mt Smolikas did not reveal any noteworthy difference. Hence, diploid populations from Mt Smolikas played very important role in the phylogeny of *M. stenophylla*.

*Myosotis. suaveolens* Waldst. et Kit. ex Willd.

Together with *M. alpestris*, *M. suaveolens* is the most common representative of the *M. alpestris* group in mountain areas of the Balkan Peninsula. Diagnostic set of characters is: leaves on both surfaces densely hirsute with more or less patent soft hairs, basal leaves with oblanceolate to obovate blade gradually narrowing into a short, sometimes indistinct petiole, cauline ones ovate, calyx narrowed at the base in fruit, densely covered by straight, short, appressed hairs not forming conspicuous white rim at the margin of calyx teeth, with longer, patent, slightly curved hairs and with many hooked stiff hairs on the calyx tube, not extending up to the pedicel, pedicel shorter than calyx in fruit.

Two ploidy levels, diploid and tetraploid, are recorded for this taxon. The chromosome number  $2n = 24$  is reported for *M. suaveolens* (Grau 1964, Greece, Akarnanika Mts, Boumistos; Štěpánková 1993a, Bulgarija, Rila Mts). The same count is recorded by Krogulevich (1978) for material from the East Sayan Mts (Vostočnyj Sajan). However, the taxon *Myosotis suaveolens* has been considered to be restricted to the Balkan Peninsula and its occurrence in the Sayan mountain range would require a taxonomic revision. Based on material from Northern Greece (Kozani), the only tetraploid chromosome number  $2n = 48$  is reported by Grau (1964). All the counts made by the present author confirm the diploid level ( $2n = 24$ ) for this species.

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## Souhrn

V práci jsou uvedeny výsledky karyologické analýzy druhů *Myosotis alpestris*, *M. ambigens*, *M. atlantica*, *M. corsicana*, *M. lithospermifolia*, *M. olympica*, *M. stenophylla* a *M. suaveolens*. Pro druh *M. olympica* byl poprvé stanoven chromozomový počet. Bylo zjištěno, že tento druh je diploidní s chromozomovým počtem  $2n = 24$ . Nově byl pro území Řecka zjištěn druh *M. stenophylla*. Zajímavá populace tohoto druhu známého jen z nižších poloh střední Evropy byla nalezena na hadcových svazích nejvyšší hory Severního Píndosu (severní Řecko) ve výšce od 2080 m až po vrchol (ca 2600 m). Populace je významná nejenom svojí izolovanou polohou a vysokou nadmořskou výškou lokality, ale zejména zjištěným chromozomovým počtem. Poprvé byl u tohoto druhu stanoven diploidní počet  $2n = 24$ . Z tohoto hlediska je řecká diploidní populace rostoucí na hadcovém substrátu významným článkem v evoluci druhu *M. stenophylla*.

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