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A new grass taxon in the *Phleum pratense* group (*Poaceae*) from the islands of Limnos and Lesvos (North Aegean, Greece)

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Abstract

Phleum pratense subsp. *boreoaegaeum* is described as a taxon new to science. It was found in wet-dry grassland in two localities on the island of Limnos (Lemnos) and one on the island of Lesvos (Lesbos) (North Aegean, Greece). Morphology, habitat and population size were noted, and the ploidy level was established using flow cytometry. Diagnostic features against the related members of the *P. pratense* group showed an intermediate position of the new taxon between the diploid *P. nodosum* and the hexaploid *P. pratense*. In terms of spikelet morphology, *P. pratense* subsp. *boreoaegaeum* is most similar to *P. pratense* s. str., while in terms of genome size, it corresponds to *P. nodosum*. When applying IUCN Red list criteria, the new taxon must be considered Vulnerable. Its locality and habitat on the islands are currently only partly protected. Apart from targeted conservation efforts and monitoring it is recommended that karyological and genetic studies be conducted on the new grass taxon to investigate its possible role in the origin and evolution of *P. pratense*.

Keywords

Aegean region, flow cytometry, grass, Greek flora, Mediterranean grassland, morphology, *Phleum*

Citation

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Introduction

The *Phleum pratense* group (*Poaceae*) comprises the hexaploid *P. pratense* L. ($2n = 42$), a common native Eurasian pasture grass (Timothy) which has been introduced as a fodder plant in temperate regions worldwide (Nordenskiöld 1945; Euro+Med 2006+; Stewart & al. 2009; Stewart & Ellison 2016; POWO 2025), and the diploid *P. nodosum* L. ($2n = 14$), widespread in Europe but in most areas much less common than *P. pratense* (Euro+Med 2006+; Dimopoulos & al. 2013; Tison & de Foucault 2014; Hand & Thieme 2025). In several other Floras, checklists and taxonomic works *P. nodosum* is known as *P. bertolonii* DC. (e.g., Doğan 1985, 1991; Conert 2000; Stace 2019; POWO 2025) or *P. hubbardii* Kováts (Kováts 1977; Kula 2003) or has been included in a variable *P. pratense* (Aedo 2020). Illustrations of diagnostic features of the two species can be found in, e.g., Foerster (1967), Conert (1983, 2000), Kula (2003) and Jauzein [2011; here *P. nodosum* is named *P. pratense* subsp. *serotinum* (Jord.) Berher].

Kováts (1980) noted that *P. pratense* and *P. nodosum* occurred in Hungary in different grassland habitats and at different elevations. Phytosociological studies revealed ecological differences between the two species in Central Europe. Oberdorfer (2001: 256) described the habitat of *P. nodosum* as warmer and drier than that of *P. pratense*.

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Voggesberger (1998: 286) added that *P. nodosum* occurs mostly in less productive habitats than *P. pratense*.

Experimental attempts to clarify the origin of *P. pratense* led to different assumptions as either autohexaploid (Nordenskiöld 1945, 1949; Wilton & Klebesadel 1973) or as allohexaploid (Kula 1989). As the karyotype of *P. pratense* turned out to consist of two different genomes, the latter assumption with different parental species is widely accepted (Kula 1989; Joachimiak & Kula 1993; Cai & Bullen 1994; Stewart & al. 2009; Rahimi & al. 2023). Four of the genomes are homologous and were most likely donated by *P. nodosum* (Cai & Bullen 1994; Joachimiak & Kula 1997), the others have been thought to be derived from the diploid *P. rhaeticum* (Humphries) Rauschert (referred to as *P. alpinum* L. by Cai & Bullen 1991, but the latter species is an allotetraploid thought to have originated from *P. rhaeticum*) and a second, undetermined parent. *Phleum rhaeticum* is a south-central and south-east European orophyte occurring among others in the Alps, Pyrenees and the Balkans (Zernig 2005; Tison & de Foucault 2014). Kula (2003), convinced that there are two diploid forms in the *P. pratense* group, distinguished *P. nodosum* (under the name *P. hubbardii*) and diploid *P. pratense*, the latter based on a collection from Coimbra, Portugal, which had been referred to *P. nodosum* by Nordenskiöld (1945). Tetraploid plants ($2n = 28$) in the *P. pratense* group are local or not found at all in areas where both species occur (Kožuharov & Petrova 1991; Perný & al. 2008), but in Italy tetraploid plants were found to be rather widely distributed (Cenci & al. 1984), though much rarer than hexaploid plants. In a review, Joachimiak and Kula (1997: 468) concluded that "the *P. pratense* karyotype contains four genomes of *P. nodosum* and two genomes of another form" which "could be the genomes of *P. rhaeticum*, or the genomes of a so far unidentified diploid taxon, closely related to *P. nodosum*".

In 2022 and subsequent years I observed three populations of a member of the *P. pratense* group on the islands of Limnos and Lesvos in the North Aegean which appeared distinct from both *P. pratense* and *P. nodosum*. Neither species is known to have been found on the Aegean islands in recent times (Strid 2016, 2024). In a contribution updating the flora of Limnos (Bergmeier 2022) the recorded plant has been named *P. pratense*. Along with an inventory of the flora and vegetation of the whole island of Limnos (Bergmeier & al. 2021; Bergmeier 2026), the sites where the *Phleum* populations were found have been thoroughly examined, specimens collected and flow cytometry applied. It turned out that indeed the populations show traits different from any of the two related taxa and that it is justified being described as a taxon new to science.

Methods

Specimens of the grass from the larger of the two sites on Limnos were collected in spring 2022, 2024 and 2025, when the plants were in flower or just after flowering. The two other occurrences in Limnos and Lesvos, respectively, were found in spring 2026. For flow cytometric studies (Galbraith & al. 2021; Loureiro & al. 2023), fresh leaf material of about 12 plants was collected on 26 April 2024, together with herbarium specimen *Bergmeier 24-156*, and preserved in silica gel. The relative genome size of the silica gel-dried leaf tissue of the sample was determined using a flow cytometer (Sysmex CyFlow Ploidy Analyser) at the Institute of Botany, University of Gießen, Germany, by Thomas Gregor, Schlitz, on June 3, 2024. The dried samples and an internal standard (*Pisum sativum*, 9,08 pg; Doležel & al. 1992) were chopped using a razor blade in a Petri dish with 1 ml of ice-cold Otto I buffer [0.1 m citric acid, 0.5% Tween 20; Otto (1990), Doležel & al. (2007)]. The suspension was filtered using Partec CellTrics® 30 µm (Partec, Germany). The isolated cell nuclei in the filtered suspension were stained with 1 ml Otto II buffer (0.4 m $Na_2HPO_4 \times 12H_2O$) and incubated for 10 minutes at room temperature. The AT-specific fluorochrome 4',6-diamidino-2-phenylindole (DAPI; 4 µg ml-1) was used as dye. The relative fluorescence was determined for 3000 particles. The relative genome size was determined from fluorescence histograms as the ratio of the mean values for the sample and standard (sample-standard fluorescence ratio; PSF).

Living material collected together with the holotype collection (*Bergmeier 24-195*) is currently cultivated at the Botanic Garden of the University of Göttingen (https://uni-goettingen.de/en/108651.html). For morphometric studies, I used herbarium specimens of *P. nodosum* from mainland Greece (*Bergmeier* 18-397, 24-412), the new taxon (*Bergmeier* 22-190, 24-156, 24-195 and 25-291) as well as *P. pratense* s. str. plants collected 2025 in Germany (*Bergmeier* 25-s.n.), as no Greek plants of that species were available.

Population size, area of occupancy, species composition and habitat characteristics were studied in spring 2024, 2025 and 2026 when the grass and most of the associated plants were best developed. Three representative plots, each of 20 m², were selected for vegetation sampling in the larger site on Limnos, and one in each of the smaller sites on Limnos and Lesvos, respectively. The plant community was classified and the species composition compared to similar habitats on the islands and by comparison with the European vegetation standard (Mucina & al. 2016; Bergmeier 2026). The population size was determined by counting the clonal clumps in representative plots and extrapolating. The extent of occurrence and area of occupancy were measured as polygon that includes all observed clumps.

Results and Discussion

Taxonomy

Phleum pratense subsp. *boreoaegaeum* Bergmeier, subsp. nov. (Figs 1–3)

Holotype: Greece, North Aegean, Island and munici-

Fig. 1. Holotype of *P. pratense* subsp. *boreoaegaeum*, Herbarium Göttingen (GOET075701).

pality of Limnos; 3 km E of Mýrina, plain between hills of Koukoúla and Kákavos, 39°52.50'N, 25°06.05'E, 132 m a.s.l., 2 May 2024, *Bergmeier 24-195* (GOET075701).

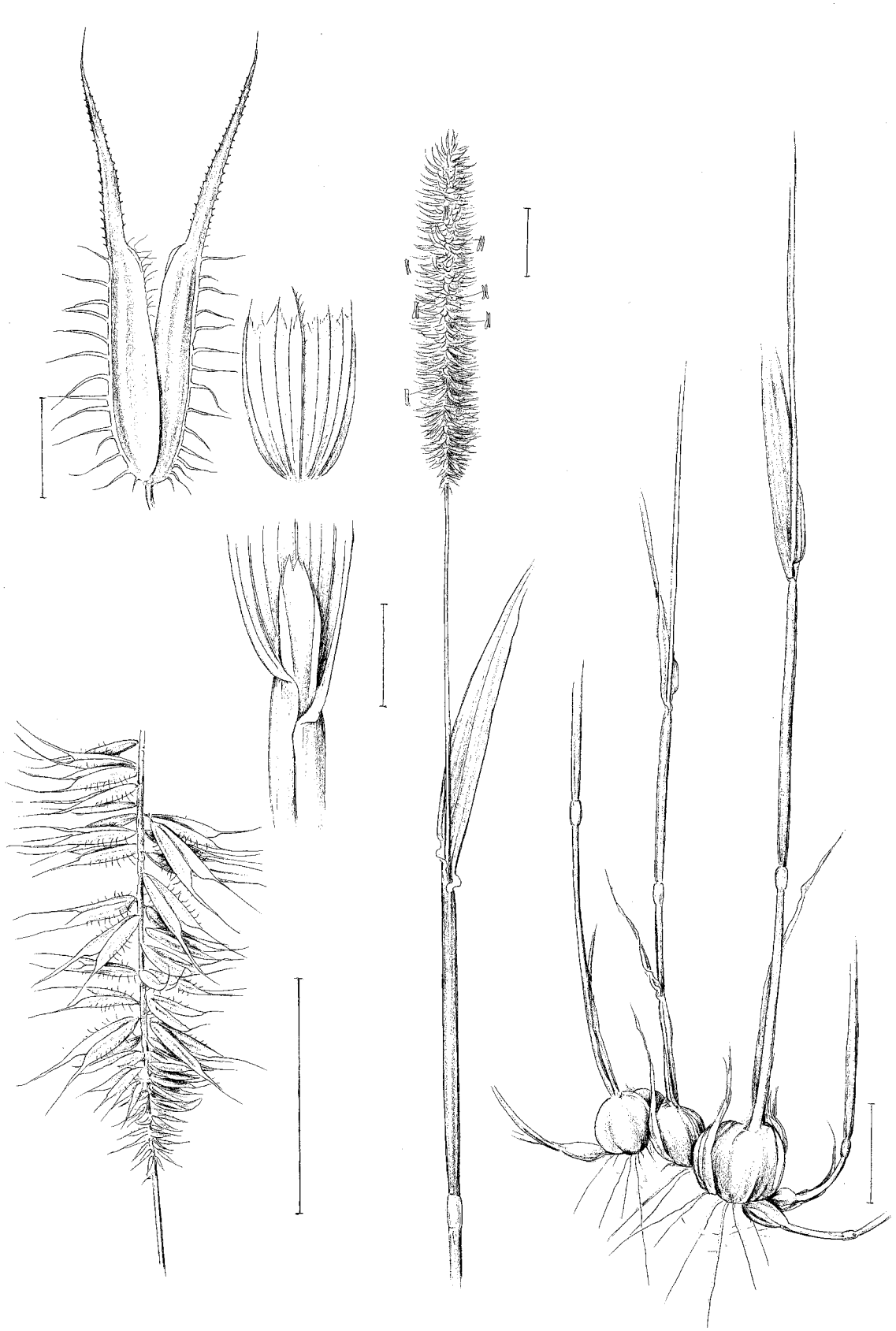
Diagnosis: Similar to typical *P. pratense* s. str. but differing in ploidy level (2x vs. 6x), shorter lemma (1.3 vs. 1.7-2.0 mm), usually fewer and somewhat shorter ciliae on keel, always bulbous stem base which is conspicuously set off, and somewhat smaller length-width panicle ratio (8-11 vs. >10). *P. pratense* subsp. *boreoaegaeum* differs from *P. nodosum* in its spikelet length (2.5-4.5 vs. 2.0-3.5 mm), longer glume awns (0.8-2.0 vs. 0.3-1.0 mm) and ratio of awn length against spikelet width (>1 vs. <1), and in ecology (seasonally wet grassland vs. moderately dry grassland).

Description (based on the holotype and the paratypes listed below as well as on living plants cultivated at the Botanic Garden, University of Göttingen, Germany): Laxly caespitose perennial; culms 35-50(-105) cm, erect or ascending, glabrous, with usually 4 nodes, abruptly set off from a conspicuously bulbose, almost globose base 1 × 1 cm, base of innovation shoots covered by chartaceous brownish leaf remains splitting longitudinally along nerves; leaf sheaths glabrous, ligule 2 mm, obtuse; leaf blades 8-17 cm long, 3.0-4.5 mm wide, usually flat, those of innovation shoots 1-2 mm wide; panicle cylindrical, branches adnate to main axis, i.e., not lobed, 2-12 cm long, 6-9 mm wide, green, not anthocyanin-pigmented, with vestigial spikelets at the basal 5 mm; spikelets oblong, 2.5(-4.5) mm long (incl. awns), 0.4-0.5 mm wide, truncate at apex, 1-flowered; glumes equal, oblong, 3-nerved, 2.5 mm long, with 3-12 stiff translucent 0.3-0.8 mm long hairs (ciliae) on keel, awn of glume 0.8-2.0 mm, apex of glumes and awns forming a vertical rectangle or subquadrate; lemmas ovate, membranous, 1.3 mm long, with up to 9 nerves ending in a minutely dentate margin; palea nearly as long as lemma; anthers 1.5-1.8 mm long; (immature) fruit up to 2 mm long. Table 1 shows traits of *P. pratense* subsp. *boreoaegaeum* in comparison to typical *P. pratense* and *P. nodosum*.

Table 1. Plant traits of *P. pratense* subsp. *boreoaegaeum* and the most closely related taxa. Measurements taken on plants from Greece (*P. nodosum*: *Bergmeier* 18-397, 24-412; P. pratense subsp. *boreoaegaeum*: 22-190, 24-156, 24-195, 25-291, 26-180, 26-214) and Germany (*P. pratense* s. str.; *Bergmeier 25-s.n.*), with additions from literature. Relevant diagnostic features in bold.

	***P. pratense* s. str.**	***P. pratense* subsp. *boreoaegaeum***	***P. nodosum***
Culm	80-120 cm	35-50(-105) cm	10-60(-100) cm
Culm leaves	20 cm × 5-6 mm	8-15 cm × 3-4.5 mm	4-12(-20) cm × 2-5 mm
Ligule	1.5-2.5 mm	2 mm	1-2 mm
Basal internodes	Often swollen but rarely bulbose, up to 7-10 mm wide, rather gradually transitioning into the culm	**Swelling bulb-like, up to 10 mm wide, abruptly set off**	Usually bulbosely swollen, but not abruptly set off
Panicle	3-14 cm × 6-8 mm	2-12 cm × 6-9 mm	2-8 cm × 4-6 mm
Panicle length-width ratio	Usually >10	**Usually 8-11**	Usually >10
Spikelet length (incl. awns)	3.5-4.5(-5.0) mm	2.5-4.5 mm	2.0-3.5 mm
Awn of glume	1-2 mm	0.8-2.0 mm	**0.3-1.0 mm**
Ciliae on glume keel	**10-20**	3-12	7-10
Cilia length	0.6-1.0 mm	0.3-0.8 mm	0.5 mm
Ratio awn length/ spikelet incl. awn length	0.30-0.45	0.30-0.45	**0.15-0.30**
Ratio awn length/ spikelet width	>1	>1	**<1**
Lemma	1.7-2.0 mm	ca. 1.3 mm	ca. 1.5 mm
Anthers	1.5-2 mm	1.5-1.8 mm	1-2 mm
Ploidy	6n	2n	2n

Fig. 2. *Phleum pratense* subsp. *boreoaegaeum*, with illustrations of spikelet with glumes, lemma, ligule (scale 1 mm), culm base with bulb-based tillers, panicle, mid-culm section with leaf (scale 1 cm). Drawings by Michael Schwerdtfeger 2026.



Phenology: *P. pratense* subsp. *boreoaegaeum* flowers in April-May.

Additional specimens (**paratypes**). Same locality as holotype, 6 May 2022, *Bergmeier 22-190* (Bergmeier 2022); 26 April 2024, *Bergmeier 24-156*; 12 May 2025, *Bergmeier 25-291*; Island of Limnos, 1.5 km SE of Paleó Pedhinó, 39°52.58'N, 25°12.04'E, 5 m a.s.l., 4 May 2026, *Bergmeier 26-180*; Island of Lesvos, Achladerí, 39°09.21'N, 26°17.01'E, 1 m a.s.l., 8 May 2026, *Bergmeier 26-214* (all Herb. Bergmeier).

Ploidy level: 2*n*. Two measurements were taken; they yielded a ratio of 0.27 and 0.30 of the value for *Pisum sativum* (9.09 pg), respectively, i.e., a DNA value of approximately 3 pg, which is close to absolute DNA values obtained from other diploid taxa of *Phleum*, such as *P. nodosum* with 3.01 pg and *P. phleoides* (L.) H. Karst. with 3.49 pg (Šmarda & al. 2019).

Taxonomic relations: The diploid P. *pratense* subsp. *boreoaegaeum* is morphologically very close both to the allohexaploid *P. pratense* and to the diploid *P. nodosum*.

Fig. 3. In situ photos of *P. pratense* subsp. *boreoaegaeum*, from top left to bottom right: panicle; *Phleum* meadow dominated by *Hordeum bulbosum*; detail of flowering panicle with anthers; bulbose culm base; detail of panicle with spikelets; meadow with clump of *Phleum*. (photos: U. Bergmeier & E. Bergmeier, 2024)

Subspecies rank is preferred for the new taxon, as there is overlap in most plant traits. The new subspecies and *P. nodosum* are equal in genome size. The important differences of *P. pratense* subsp. *boreoaegaeum* against *P. nodosum* (longer awns, larger awn length-spikelet width ratio) approach *P. pratense* s. str.

Phleum pratense subsp. *boreoaegaeum* is of considerable interest for studies on the origin of *P. pratense* s. str. According to current knowledge, *P. nodosum* donated four genomes (Kula 1989; Joachimiak & Kula 1997) but the origin of the other two has remained unclear (Rahini & al. 2023). The only diploid potential parental taxon discussed so far is *P. rhaeticum* (Cai & Bullen 1994; Tison and de Foucault 2014), although Kula (2003) compared *P. nodosum* (as *P. hubbardii*) with a diploid form of *P. pratense* from Portugal. With *P. pratense* subsp. *boreoaegaeum*, another potential donor candidate has emerged that could turn out to be the previously "unidentified diploid taxon, closely related to *P. nodosum*" (Joachimiak & Kula 1997) and thus provide further insight into the origin of *P. pratense* s. str.

Location and population size

Phleum pratense subsp. *boreoaegaeum* was found in two sites on the island of Limnos, the first about 3 km east of the municipality capital, Mýrina, in a grassland plain at the foot of Koukoúla hill just north of the dirt road towards Kákavos (with the rock shelter of Panagía Kakaviótissa), the second in a coastal meadow about 1.5 km southeast of the village of Paleó Pedhinó. A third site was discovered on the island of Lesvos, in a meadow in the coastal plain close to the settlement of Achladerí, municipality of Polichnítos. The first grassland site covers an area of almost 1.5 ha, more precisely 14,375 m². It consists of several connected meadows with some of the boundaries between the land parts still visible. The second site on Limnos and the site on Lesvos are smaller. They form parts of fenced coastal meadows where *Phleum* occurs at edges on about 250 m² and 150 m², respectively.

In the larger site, a total of approximately 400 clumps of *P. pratense* subsp. *boreoaegaeum* were estimated, each with (2-)6-12(-40) culms. About two thirds of the clumps were observed in the wetter parts of the meadow, a subarea of about 25% of the total extent of occurrence, characterized by abundant *Carex divisa* Huds., *Ranunculus marginatus* d'Urv. and *Oenanthe silaifolia* M.Bieb. In a slightly higher and dryer terrain dominated by *Hordeum bulbosum* L., the density of clumps of *P. pratense* subsp. *boreoaegaeum* was lower. In the smaller site on Limnos, 44 clumps were found, in the site near Achladerí on Lesvos only 7 clumps but here some clumps may have been overlooked in the tall-growing meadow.

Distribution and historical records

There is no other unequivocal record of *P. pratense* subsp. *boreoaegaeum* from the two islands or elsewhere. However, a record of *P. nodosum* by C. A. Candargy collected in the period between 1889 and 1894 on the island of Lesvos may refer to the new subspecies. Candargy was cited by Rechinger (1943: 800; under *P. pratense* var. *nodosum* (L.) Schreb.) and by Doğan (1985: 391; under *P. bertolonii*). Moreover, the latter author examined the specimen *Seçmen and Leblebici* 1640a from the Turkish Aegean island of "Bozca" (Bozcaada, or Tenedos in Greek; under *P. bertolonii*, collection date not known) (Doğan 1985). The distance between this island and Limnos is only about 60 km. Almost a century after Candargy, in 1986, Alfred Hansen and Henry Nielsen collected a *Phleum* on the island of Lesvos ("Delta area of the river Kalami, 8 km SE of Kalloni") (*Hansen* & *Nielsen* 1825, C; not included in Hansen & Nielsen 1993), which was interpreted as *P. pratense* by Strid (2016: 464; then queried by Strid 2024 who does not remember having seen the specimen). As the locality is close to the site near Achladerí, the record is likely to represent *P. pratense* subsp. *boreoaegaeum*. However, an attempt to examine the herbarium specimen failed despite a request having been made. My own records of plants in the *P. pratense* group on the Greek mainland, corroborated by herbarium specimens, refer to *P. nodosum*. They were found in moderately dry grassland habitats (e.g., Strid & al. 2017: 256 and *Bergmeier* 18-397, Prespa; *Bergmeier* 24-412, Peloponnese). Records of *P. nodosum* and *P. pratense* in Turkey-in-Europe and the western Anatolian mainland were cited and mapped by Doğan (1985: 651, under *P. bertolonii*). They should be re-examined.

Habitat and associated species

Phleum pratense subsp. *boreoaegaeum* grows on silty soil in seasonally wet grassland, the larger population in a plain at 130 m a.s.l. over sedimentary mudstone between volcanic hills, the two smaller populations in alluvial coastal plains. At the time of observation, in late April and early May, the soil was already dry. It was covered, in parts, by undecomposed grass litter. The meadows were dominated chiefly by perennial herbs and grasses, together with many species and considerable cover proportion of annual grasses and herbs, in particular of the genus *Trifolium*. The following species co-occurred with *P. pratense* subsp. *boreoaegaeum* in at least four of the five vegetation plots examined: *Carex divisa* Huds., *Gaudinia fragilis* (L.) P. Beauv., *Oenanthe silaifolia* M. Bieb., *Poa trivialis* subsp. *sylvicola* (Guss.) H. Lindb., *Ranunculus marginatus* d'Urv. and *Trifolium echinatum* M. Bieb. The following species were also common and occurred in three out of five plots:

Hordeum geniculatum All., *Hordeum bulbosum* L., *Trifolium lappaceum* L., *Trifolium petrisavii* Clementi and *Vicia parviflora* Cav. The following species prevailed locally—with more than 15 % cover—in one or more of the plots: *Carex divisa*, *Oenanthe silaifolia*, *Poa trivialis* subsp. *sylvicola*, and *Scirpoides holoschoenus* (L.) Soják. *Hordeum bulbosum*, abundant in places, and *Dittrichia viscosa* can be considered indicators of land-use abandonment. The historical land use is unknown but haymaking is most likely. At times, small-scale crop cultivation may have occurred in dry years. As the grasslands are surrounded by dry pastureland, the areas have likely been subject to occasional sheep grazing too. Among the rarer or uncommon plants of wet-dry meadows co-occurring with *P. pratense* subsp. *boreoaegaeum* the following seem noteworthy: *Anthoxanthum aristatum* Boiss., *Anthoxanthum ovatum* Lag., *Carex distans* L., *Crepis zacintha* (L.) Loisel., *Holcus annuus* C.A. Mey., *Juncus capitatus* Weigel, *Linum bienne* Mill., *Mentha pulegium* L., *Ornithogalum pyrenaicum* subsp. *sphaerocarpum* (A. Kern.) Hegi and *Ranunculus velutinus* Ten. The total number of vascular plant species associated with P. *pratense* subsp. *boreoaegaeum* was over 60. Species numbers in the three representative 20 m² plots were 15, 19, 26, 32 and 33, thus on average 25. The semi-wet grasslands might also be significant herpetological sites, as five individuals of snake (unfortunately unidentified) and, in a well, many individuals of a species of aquatic frog, most probably *Pelophylax bedriagae* Camerano, 1882 (Strachinis & Roussos 2016), were encountered on 12 May 2025. The vegetation was identified as belonging to the Mediterranean grass-rush meadow communities (Holoschoenetalia Br.-Bl. ex Tchou 1948), which are understudied in Greece and the East Mediterranean (Bergmeier 2026). The precise association where the vegetation belongs remains to be described on the basis of more, and more widely distributed, records.

Conservation

When following the IUCN Red list criteria (IUCN 2012) and the current guidelines (IUCN 2024), on the basis of quantitative criteria the category Vulnerable can be assigned to *P. pratense* subsp. *boreoaegaeum*. The new subspecies meets the criterion VU D1+2, as only three locations are currently known with less than 1000 mature individuals (clumps in the present instance), and the amount of suitable habitat (area of occupancy) is highly restricted. The species occurs in localised wet-dry grasslands, a rare habitat which is included in the Habitat type 6420 ("Mediterranean tall humid herb grasslands of the Molinio-Holoschoenion") of the EU Habitats Directive 92/43/EEC (European Commission, DG ENV 2013; Tsiripidis & al. 2025). Within the few years that I know the larger site on Limnos, I did not observe a decline in population size. However, the overall small population and the precarious habitat situation—which could be threatened in the medium term by litter accumulation, land abandonment and drought caused by climate change—necessitate regular monitoring. While the two smaller populations are located on the edge of the Natura 2000 sites GR4110006 and GR4110004, respectively, none of the two Natura 2000 sites of Limnos—GR4110006 and GR4110001—covers the area with the larger population of P. *pratense* subsp. *boreoaegaeum* (Tsiripidis & al. 2025). It is thus not protected under the EU Habitats Directive nor covered by any national conservation programme or area.

The conservation status of the meadows is not optimal as undecomposed grass litter has accumulated almost throughout the area, indicating abandonment. Although this does not appear to have had any impact on *P. pratense* subsp. *boreoaegaeum* yet, the accumulation of organic litter favours particularly competitive perennial plants such as *Hordeum bulbosum* and *Dittrichia viscosa*. It is thus likely to reduce the vitality, regeneration ability and population size of many less competitive plants, in particular leguminous annual herbs of *Trifolium* and *Vicia* which are of high ecosystem value for pollinators. It is recommended, therefore, to re-introduce traditional-intensive land-use practices, preferably haymaking once a year in early spring or mowing in summer and to remove the mown grass.

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