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Genetic, morphometric and microhabitat data reveal that papillose feather grasses in the Eastern Alps (*Stipa epilosa* auct.) do not constitute a separate taxonomic entity

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Abstract: Feather grasses (*Stipa*) are among the most typical constituents of steppe vegetation. Enigmatic *S. epilosa*, which was suggested to occur in various parts of the Alps, differs from common *S. eriocaulis* in the micromorphology of the cross section of its leaves. The rib flanks of the upper leaf surface lack the dense hairs typical of *S. eriocaulis*; instead, they are covered by short cone-shaped papillae. Populations of *Stipa* in the Vinschgau Valley in South Tyrol and the upper Inn Valley and Wipptal Valley in North Tyrol were studied using morphometric analysis of leaf cross sections, AFLP fingerprinting and microhabitat analyses. The following specific questions were addressed: (i) Is *S. epilosa* genetically divergent from *S. eriocaulis*? (ii) Are micromorphological differences between *S. eriocaulis* and *S. epilosa* consistent? (iii) Are there microhabitat differences between *S. eriocaulis* and *S. epilosa*, potentially reflecting adaptation of papillose and hairy individuals to different environmental conditions? First, this study revealed no genetic difference between *S. eriocaulis* and *S. epilosa* in the Eastern Alps. Second, there is a continuum in the variation in hair lengths from typical *S. epilosa* to typical *S. eriocaulis*, which makes it difficult to differentiate between two discrete groups. Finally, no microhabitat differences were recorded between papillose and hairy individuals. From a taxonomic point of view, our integrative approach combining genetic, morphometric and microhabitat data failed to support the hypothesis that *S. epilosa* is an independent taxon in the area studied.

Keywords: doubtful taxon, Eastern Alps, genetic drift, integrative taxonomy, micromorphology, *Stipa*

Introduction

Extrazonal steppes are isolated patches of steppe vegetation occurring separately from the climate-driven zonal steppes; they resemble islands of steppe in a sea of forest (Kirschner et al. 2022). They occur wherever edaphic factors along with a mildly continental climate prevent forest growth (Ellenberg & Leuschner 2010). Extrazonal steppes in major valleys in the interior parts of the Alps – inner-Alpine steppes – occur on southern slopes with shallow soils. They contribute significantly to regional biodiversity and, alongside harbouring a strong Alpine floristic element, share species with the zonal steppes (Pott 1996, Ellenberg & Leuschner 2010). In contrast to the classic hypothesis of postglacial colonization (Braun-Blanquet 1961), recent studies have shown that extrazonal steppe biota

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evolved independently from zonal steppe biota during the Pleistocene. Consequently, the inner-Alpine steppes harbour endemic genetic lineages and make a significant contribution to regional biodiversity (Kirschner et al. 2020).

Within the Alps, most major dry valleys (Durance Valley, Tarentaise, Maurienne, Susa Valley, Aosta Valley) are located in the Western Alps. In the Eastern Alps, by far the largest steppes occur in the Vinschgau Valley in the northern Italian province of Südtirol / Alto Adige / South Tyrol (Braun-Blanquet 1961), which is attributed to the low annual precipitation of ~530 mm in the municipality Schlanders in the middle Vinschgau (http://www.3pclim.eu), as they are in the rain shadow of the adjacent mountain ranges. To the north of the Eastern Alps' main divide, inner-Alpine steppes are highly localized; the most significant occurrences are in the upper Inn Valley in the Engadin / Engiadina (Switzerland) and Nordtirol / North Tyrol (Austria; Dobner 2007). Similar to the zonal steppes, the inner-Alpine steppes are under threat; in particular, land use changes (Lüth et al. 2011) and atmospheric nitrogen deposition (Willner et al. 2019) negatively affect this peculiar vegetation type.

One of the most typical constituents of both zonal and extrazonal steppe vegetation is the genus *Stipa* L., the feather grasses. It is one of the largest genera in the grass subfamily *Pooideae* (*Poaceae*), currently consisting of ~150, mostly perennial species distributed in xerophilic plant communities with low competition (Conert 1992) across Europe, Asia, and North Africa (Freitag 1985, Nobis et al. 2020). Feather grasses are xerophytes well adapted to arid environments and serve as bioindicators of extremely dry sites (Dobner 2007). Their altitudinal distribution ranges from the lowlands to the alpine belt and they grow in different edaphic conditions ranging from deep loess or sandy soils to rocky grasslands or rock crevices with very shallow soil (Rychnovská 1965, Úlehlová & Rychnovská 1967, Nobis et al. 2020). Feather grasses are predominantly cleistogamous, resulting in low levels of genetic diversity within populations and limited genetic exchange among populations of species with fragmented distributions (Durka et al. 2013). However, higher levels of genetic diversity are reported in regionally abundant species with large and well-connected populations (Liu et al. 2016).

The following species of feather grasses occur in the inner-Alpine steppes in North and South Tyrol. Taxonomically uncritical *S. capillata* L. from sect. *Leiostipa* Dumort. has a broad distribution in the Eurasian steppe belt, extending from the Iberian Peninsula to eastern Russia and China (Wagner et al. 2011). The taxonomically intricate *S. pennata* L. species group from sect. *Stipa* comprises *S. eriocaulis* Borbás, *S. pennata* s. str. (syn. *S. joannis* Čelak.) and *S. epilosa* Martinovský in this area; a report of *S. pulcherrima* K. Koch in North Tyrol proved to be erroneous (Moraldo 1986, Dobner 2007, Fischer et al. 2008). *Stipa eriocaulis* is a predominantly submediterranean species and is widely distributed from central Spain across the Alpine countries to central Europe; it is also relatively widespread in the Eastern Alps. Its northern and eastern limits are determined by the Danube (Martinovský 1967), but isolated occurrences are reported in the south-eastern part of the Czech Republic and western Slovakia (Danihelka et al. 2000), as well as in Poland (Nobis et al. 2017). In contrast, *S. pennata* s. str. is a predominantly eastern continental species distributed from the Rhine Valley to western Mongolia. Its north-south distribution ranges from southern Sweden to the mountains of Albania and from the southern limits of the birch forests in western Siberia to the steppes in the Altai Mountains (Martinovský 1970). In the Eastern Alps, this species is mostly restricted to the

Vinschgau Valley and adjacent areas. Finally, *S. epilosa* was suggested to occur in the area relatively recently (Moraldo 1986, Gutermann & Danihelka 2019). This species has a disjunct distribution in the Alps, the Balkan Peninsula and Anatolia (Moraldo 1986, Todorova & Tzonev 2010). In the Alps, the four species partly differ in their habitats, with *S. capillata* and *S. pennata* s. str. usually preferring deep soils and *S. eriocaulis* and *S. epilosa* rocky grasslands or rocky crevices (authors' field observations).

Stipa epilosa was described based on specimens collected in the Ak-Dagh mountains (Bornmüller: Plantae exsiccatae Anatoliae orientalis no 317) and in the Bulgar-Dagh mountains (Kotschy: Iter Cilicicum in Tauri Alpes s.n.) in eastern and south-eastern Anatolia, respectively (Martinovský 1967). Later, it was reported to occur on Mt Vitosha and Mt Lozenska in south-western Bulgaria, and near Štip in North Macedonia (Pedashenko et al. 2009, Todorova & Tzonev 2010). In the Alps, it was reported in the Western Alps (Ligurian Alps to Aosta Valley) as well as in the Vinschgau Valley, North Tyrol (Nauders) and the Engadine (Moraldo 1986, Gutermann & Danihelka 2019). It differs from *S. eriocaulis*, with which it shares the pattern of the ventral lemma indumentum, in the indumentum of the adaxial surface of the leaf blades. Specifically, the rib (costal) flanks of the upper (adaxial) leaf surface lack hairs in *S. epilosa*, but instead are covered with short cone-shaped papillae. In contrast, the rib flanks in *S. eriocaulis* are covered densely with 0.05–0.10 mm long hairs and the rib ridge is covered with conical setae (Martinovský 1967). Moraldo (1986) differentiates between Alpine endemic subsp. *montana* Moraldo with hairy leaf sheaths and the glabrous nominate subspecies distributed in the Vinschgau Valley and Anatolia; in later accounts this differentiation is rejected (Freitag 1985, Bartolucci et al. 2018). Finally, *S. pennata* s. str. differs clearly in the lemma indumentum from the two other species, and the tips of young leaves are usually hairy and brush-like (Martinovský 1970).

However, even though leaf indumentum has been commonly used to distinguish the species of sect. *Stipa*, it is recognized that transitional stages in hair length may occur. For instance, in populations of *S. pulcherrima*, individuals ("mutants") with glabrous rib flanks resembling *S. epilosa* are reported in the Bohemian Lower Mountains and referred to as *S. pulcherrima* f. *nudicostata* by Martinovský (1977). As a consequence, the taxonomy of *S. epilosa* is intricate; for example, the latest checklist of Italian vascular plants considers it "taxonomically doubtful" (Bartolucci et al. 2018).

Initial surveys (Nitz 2021 and unpublished field observations) report variation in the indumentum on the upper leaf surface in populations attributed to *S. eriocaulis* or *S. epilosa*; whereas in North Tyrol there is very limited within-locality variation, more (and continuous) variation was encountered in South Tyrol. The aim of the present study is to test the hypothesis that Eastern Alpine feather grass populations previously assigned to *S. epilosa* subsp. *montana* (Gutermann & Danihelka 2019) are an independent taxonomic entity. Four heterogeneous localities in the Vinschgau Valley and four fairly homogeneous localities in North Tyrol were studied using morphometric analysis of cross sections of leaves, AFLP fingerprinting and microhabitat analyses. The following questions were addressed: (i) Are Eastern Alpine populations of *S. epilosa* and *S. eriocaulis* genetically different? (ii) Are there consistent micromorphological differences between *S. eriocaulis* and *S. epilosa* in the Eastern Alps? (iii) Are there microhabitat differences between Eastern Alpine *S. eriocaulis* and *S. epilosa*, which potentially reflect adaptation of papillose and hairy individuals to different habitats?

Materials and methods

Field surveys and collection of plant material

Eight localities where feather grasses occur were studied (Fig. 1): four localities (1–4) in the Vinschgau Valley, where *S. epilosa* was thought to occur based on the inspection of available herbarium vouchers by T. Wilhalm (Museum of Nature South Tyrol, Bozen / Bolzano) as well as four localities in North Tyrol, where hair length was fairly uniform [either papillose (5, 6: *S. epilosa*) or long (7, 8: *S. eriocaulis*)]. The exact localities of the populations studied were (centroids of the coordinates of the sampled individuals): 1 – Tartscher Leiten, N46°40'50" E10°34'31", 1,212 m a.s.l.; 2 – close to the military cemetery at Spondinig, N46°37'58" E10°37'27", 954 m a.s.l.; 3 – below bus stop Putz near Allitz, N46°37'59" E10°41'30", 1,369 m a.s.l.; 4 – Schlanderser Leiten near Feuersteig, N46°37'54" E10°46'45", 814 m a.s.l.; 5 – Schöpfwarte, N46°53'48" E10°28'45", 1,430 m a.s.l.; 6 – opposite the fort Nauders, N46°54'48" E10°29'42", 1,245 m a.s.l.; 7 – Pfunds, along the main road, N46°58'19" E10°32'35", 1,000 m a.s.l.; 8 – Venn in the Vennbach Valley, N47°00'55" E11°31'47", 1,540 m a.s.l.

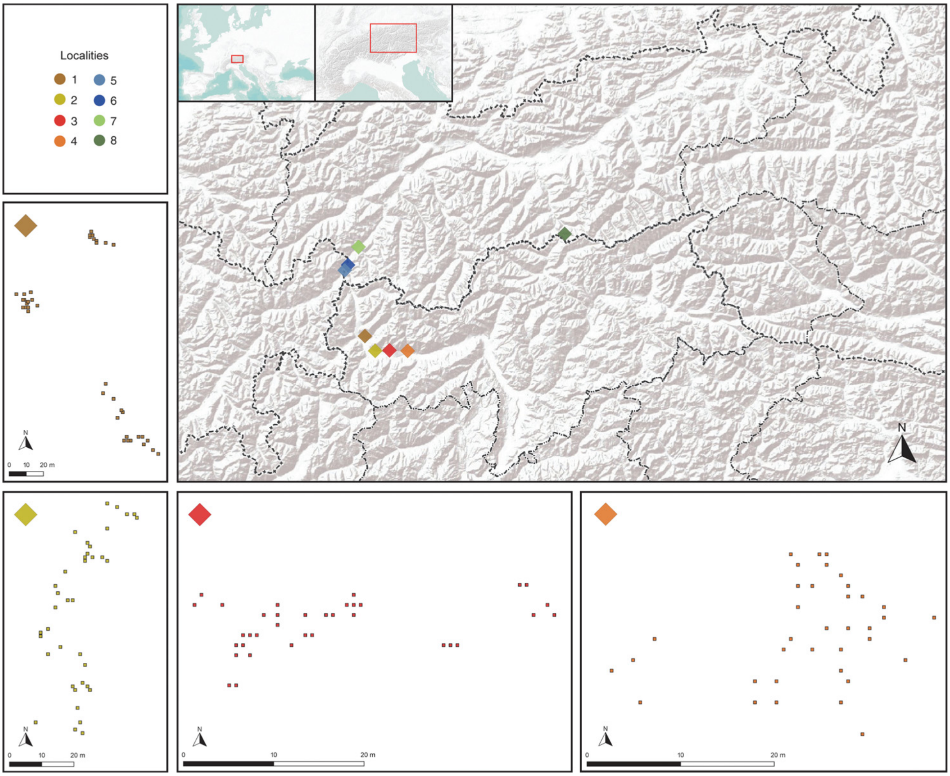


Fig. 1. Map of the area studied; the eight localities studied are in different colours; the scatter plots illustrate the spatial distribution of the investigated individuals at localities 1–4 in the Vinschgau Valley. The inserts show the location of the areas studied in Europe and the Alps.

Our study focused on the heteromorphic localities 1–4, where 40 *Stipa* individuals each were sampled for morphometric and AFLP analyses, accompanied by a microhabitat analysis (only 39 individuals were analysed at locality 3). At the homomorphic localities 5–8, 20 individuals (populations 5–6) or 10 individuals (populations 7–8) were sampled and no microhabitat analyses done. In addition, five individuals each of *S. pennata* s. str. were sampled at Allitz (N46°38'0" E10°41'37") and close to Vetzan (N46°37'37" E10°48'7") as an outgroup for the AFLP analyses. Plant material (i.e. leaves of vegetative shoots) for morphometric and AFLP analyses was dried and stored in silica gel.

Microhabitat analyses were carried out within a radius of 35 cm around each *Stipa* individual studied in order to characterize its microhabitat. The percentage covers of all vascular plant species, as well as rock, soil, litter and cryptogams (lichens and mosses) were estimated. Species were determined using Fischer et al. (2008) and Eggenberg & Möhl (2020). Coordinates, slope and exposition of the plots were recorded. An attempt was made to select 20 papillose and 20 long-haired individuals using a hand magnifying glass. In addition, the selection of individuals aimed to reflect the variability in the local vegetation.

Data were analysed mostly with R 4.2.3 (R Core Team 2024). The variability in percentage species cover data in the microhabitat analyses was projected into a reduced dimensionality space using a Detrended Correspondence Analysis (DCA). This approach is a detrended version of Correspondence Analysis, which result in a curved response even when based on non-linear relationships in the original data. Since species usually respond in a unimodal way to environmental factors, the DCA is the preferred approach for vegetation analyses (Legendre & Legendre 2012). The DCA was done in R package vegan (Oksanen et al. 2022) with the function decorana and visualized via R package ggplot2 (Wickham 2016); rare species were downweighted (iweigh = 1). The explanatory variables (slope, exposition, species richness, cryptogam cover, vascular plant cover, soil cover, rock cover and litter cover) were represented in the graph using vectors created using the function envfit and 999 permutations in vegan.

Morphometric analysis

A leaf cross section was made from each sampled individual at half the length of a leaf of a vegetative shoot. The ten longest hairs were measured in the furrow between the rib containing the main nerve and the next, fully developed rib. Measurements were done using a CH-2 Olympus microscope and a Periplan GF 10×M ocular (Leitz Wetzlar). The scale integrated in the ocular was calibrated with graph paper. A conversion factor of 7.81 µm/line was calculated for the scale. The data was checked for normality using the LeveneTest function in R package car (Fox et al. 2023). Due to the unequal variances between the localities, ANOVA was avoided and pairwise t-tests were conducted instead.

DNA extraction, AFLP fingerprinting and analysis of AFLP data

Similar amounts (~15 mg) of dried leaf tissue were used for DNA extraction following a CTAB protocol (Doyle & Doyle 1987) with some modifications (Schönswetter et al. 2009). AFLP profiles were generated following established protocols (Vos et al. 1995) with modifications described in Schönswetter et al. (2009). Three blank samples (DNA replaced by water) were included to test for systematic contamination, and reproducibility was tested by replicating 21 individuals (Bonin et al. 2004). The following primers were used in the second round of PCR ("selective PCR"): EcoRI (6-FAM)ATG / MseI-CTT, EcoRI (VIC)AAG / MseI-CTT and EcoRI (NED)ACC / MseI-CAG (6-FAM-labeled primer: Merck Sigma-Aldrich; NED- and VIC-labeled primers: Thermo Fisher Scientific, Applied Biosystems). Of each selective PCR product, 3.3 µL were purified by application to a Millipore MultiScreen-HV 96-Well Filter Plate (Merck Millipore) filled with a Sephadex G-50 fine (Merck Sigma-Aldrich, Cytiva) suspension (62.5 mL PCR grade water, 50 µL 100 × TE, 5 g Sephadex) in three steps of 200 µL each, packed at 750 g for 1, 1 and 5 min, respectively, and pre-wetted with 10 µL of PCR grade water. Then, 1 µL of the elution product was mixed with 10 µL Hi-Di Formamide (Thermo Fisher Scientific, Applied Biosystems) and 0.1 µL GeneScan 500 ROX dye Size Standard (Thermo Fisher Scientific, Applied Biosystems) and run on an automated capillary sequencer 3130xl Genetic Analyzer (Thermo Fisher Scientific, Applied Biosystems) at the Department of Botany and Biodiversity Research of the University of Vienna (Austria).

Due to the limited AFLP variation, the aim was to keep the amount of scoring errors to a minimum; binning and scoring of the fragments were therefore done manually. Raw data were collected and aligned with the internal size standard using ABI Prism Genescan analysis software 3.7.1 (Applied Biosystems). Subsequently, the GeneScan files were imported into Genographer 1.6.0 (version no longer available) for scoring of the fragments in the size range 70–500 bp. Each AFLP fragment was scored using the "thumbnail" option, which allows for comparison of the signal of each fragment in all the samples. The results of the scoring were exported as a presence/absence matrix. Monomorphic fragments were excluded. The error rate was calculated as the percentage of mismatches of the phenotypic comparisons of replicated samples.

A matrix of Jaccard distances among individuals was calculated in vegan (Oksanen et al. 2022) with the function vegdist. A Principal Coordinate Analysis (PCoA) with the function wcmdscale was done in vegan and illustrated via ggplot2. For visualization, a Neighbour Net and a Neighbour Joining (NJ) phylogenetic tree were produced. Bootstrap values were calculated based on 1,000 permutations with SplitsTree 4.17.1. The NJ tree was graphically edited using FigTree 1.4.4.

In order to investigate putative correlations of the genetic distances with geographic distances and differences in hair length, Mantel tests using the function mantel.randtest from the R package ade4 (Thioulouse et al. 2018) were done. The Mantel test aims at estimating correlation between two matrices (Mantel 1967). In addition, the correlation was tested by fitting a linear model for each pair of matrices using the function lm.

Results

Microhabitat analyses

The percentage cover of all species of vascular plants, as well as rock cover, soil cover, litter cover and cryptogam cover (lichens and mosses) are presented in Supplementary Table S1. The first two axes of the DCA explained 53.9% and 30.8% of the total variation (Fig. 2). Although there were big overlaps between localities 2–4, locality 1 was clearly separated from the other localities by its correlation with the explanatory variables exposition and soil cover. Localities 2 and 3 showed a strong tendency towards litter cover;

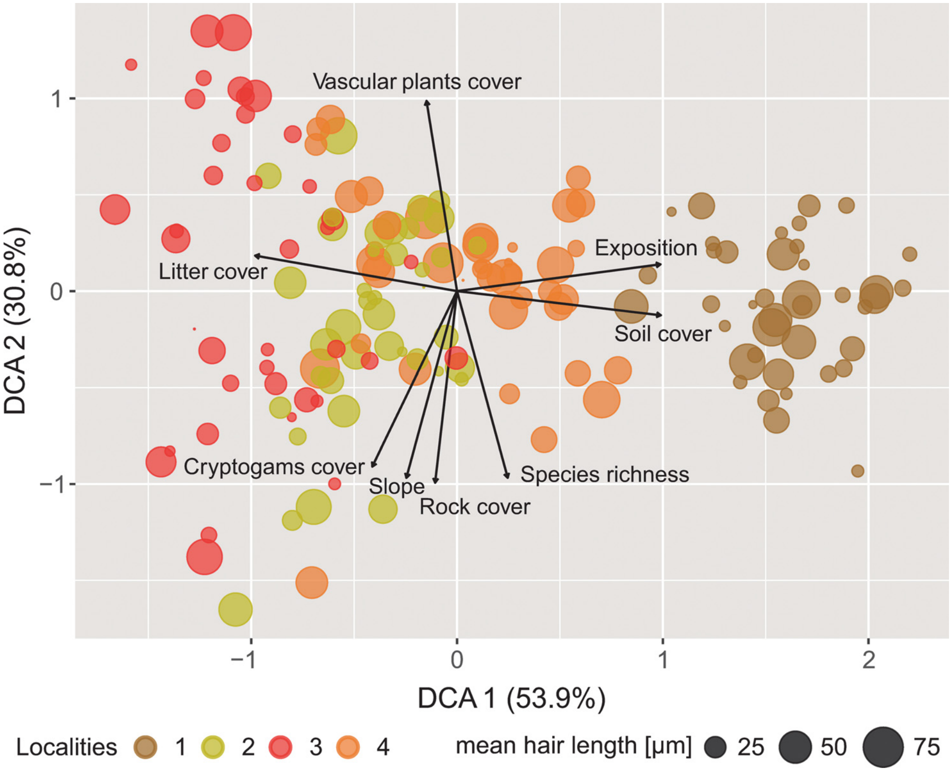
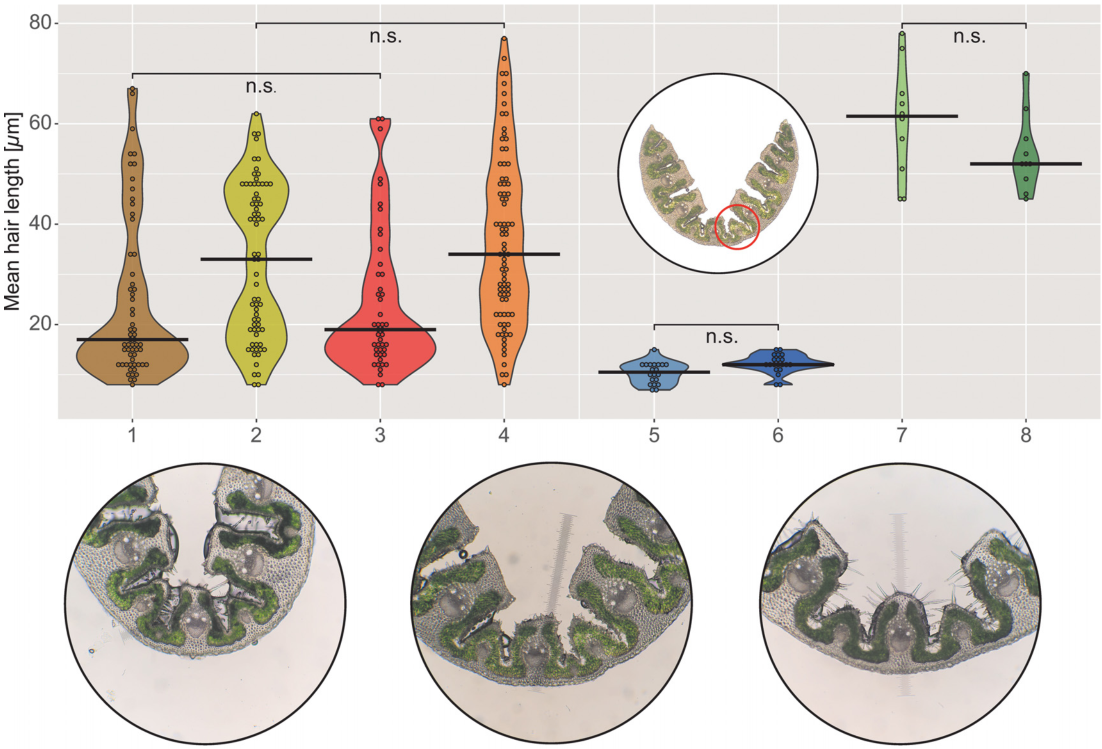


Fig. 2. Detrended correspondence analysis of microhabitat analyses centred on individuals of *Stipa eriocaulis* and "*S. epilosa*" occurring in four localities in the Vinschgau Valley. Different colours represent the localities and the diameter of the circle is the mean hair length. The arrows illustrate the relationships between the eight environmental descriptors projected in the same ordination space as the samples.

locality 4 exhibited the highest variation along axes 1 and 2. There was no clustering of individuals in the DCA according to hair length.

Hair length

The lengths of the 10 longest hairs measured in the furrow beside the main rib of the individuals studied at the eight localities generally showed large variation both within individuals and within populations (Supplementary Table S2). Nevertheless, differences among populations were clearly visible. The distribution of the mean hair length showed large variation at localities 1–4 and much less variation at localities 5–8 (Fig. 3; the data set is presented in Supplementary Table S2). At localities 1–4, individuals had a mean hair length of 8–77 µm, while at localities 5 and 6 the mean hair length did not exceed 15 µm and at localities 7 and 8 the mean hair length was above 45 µm. Pairwise t-tests showed significant differences between most pairs of localities (Fig. 3).



Population 1
Mean hair length: 37 μm

Population 6
Mean hair length: 8 μm

Population 7
Mean hair length: 68 μm

Fig. 3. Violin plots of the distribution of mean hair lengths of *Stipa eriocaulis* and "*S. epilosa*" sampled in eight localities in the Vinschgau Valley and North Tyrol; black bars are the medians. All comparisons using pairwise t-tests are significant except for the ones indicated (n.s. = not significant). Data points are means of the 10 longest hairs, measured in the portion of the cross section illustrated by a red line in the insert (magnification 100×) in the upper panel. The photographs (magnification 200×) depict typical cross sections bearing short, medium and long hairs. Pairwise t-tests indicate significances between all pairs except those marked (n.s. = not significant).

AFLPs

In total, 67 variable AFLP fragments were scored for 177 individuals, of which 11 belonged to the outgroup *S. pennata* s. str. as one individual from population 3 collected as *S. eriocaulis* / *S. epilosa* turned out to be *S. pennata* s. str. Mostly due to insufficiently high DNA concentrations, it was not possible to obtain reproducible fingerprints from 24 individuals relatively evenly distributed over the populations studied, which were excluded. The error rate based on 16 sample-replicate comparisons was 0.38%. The same fingerprint was recorded in up to 27 individuals.

In the NJ tree, samples of *S. eriocaulis* and *S. epilosa* (in the following "*S. epilosa*", based on the results of this study) together formed a well-supported lineage; the genotype retrieved from the geographically most distant locality 8 formed the sister group to all the other accessions (Fig. 4). The latter group was not supported and had no supported internal structure. The uniformly papillose individuals from localities 5 and 6 are clearly not

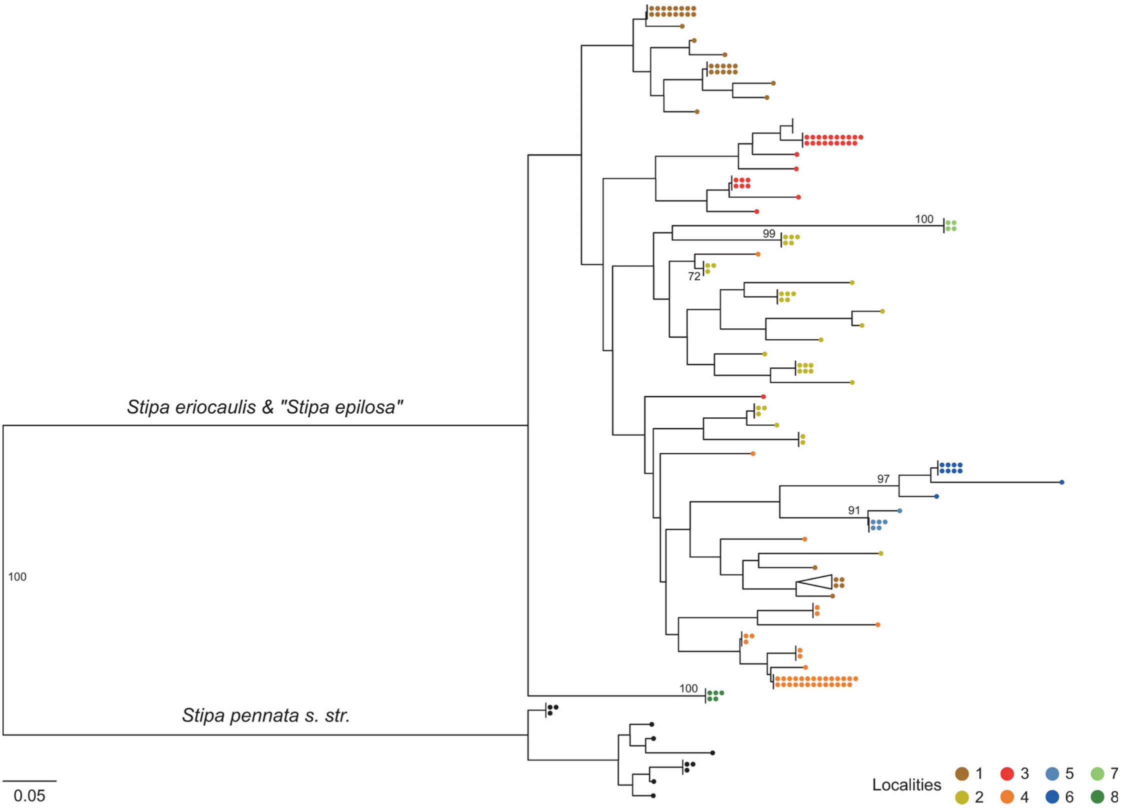


Fig. 4. Neighbour Joining tree of AFLP fingerprints for *Stipa eriocaulis* and "*S. epilosa*" individuals sampled at eight localities in the Vinschgau Valley and North Tyrol; the tree was rooted using *S. pennata* s. str. The number of individuals sharing the same genotype is indicated by coloured dots. Numbers at the tips of branches are locality identifiers, whereas numbers along the branches are bootstrap support values.

a different lineage as they are deeply nested within long-haired samples. The AFLP genotypes often clustered according to sampling localities, but there were several exceptions.

The first two axes of the PCoA explained 25.8% and 20.7% of the total variation (Fig. 5). The genotypes from localities 2 and 3 were separate whereas those from localities 1 and 2 strongly overlapped; genotypes from North Tyrol (localities 5–8) clustered with those from South Tyrol (1–4). There was no genetic clustering with respect to hair length. For instance, genotypes from locality 6 corresponding to papillose individuals tightly clustered with genotypes from locality 4 corresponding to long-haired individuals.

In the Neighbour Net (Fig. 6) individuals mostly clustered according to their provenance. At localities 5–8, a maximum of three different genotypes were recorded per locality, which were spread over the Neighbour Net. In some cases, individuals sharing the same genotype had strongly different hair lengths. Generally, there was no covariation between hair length and genetic structure. The Mantel tests revealed significant correlations between the genetic distance matrix on the one hand, and geographic distances and hair length differences on the other. The fitted linear models indicated significant positive

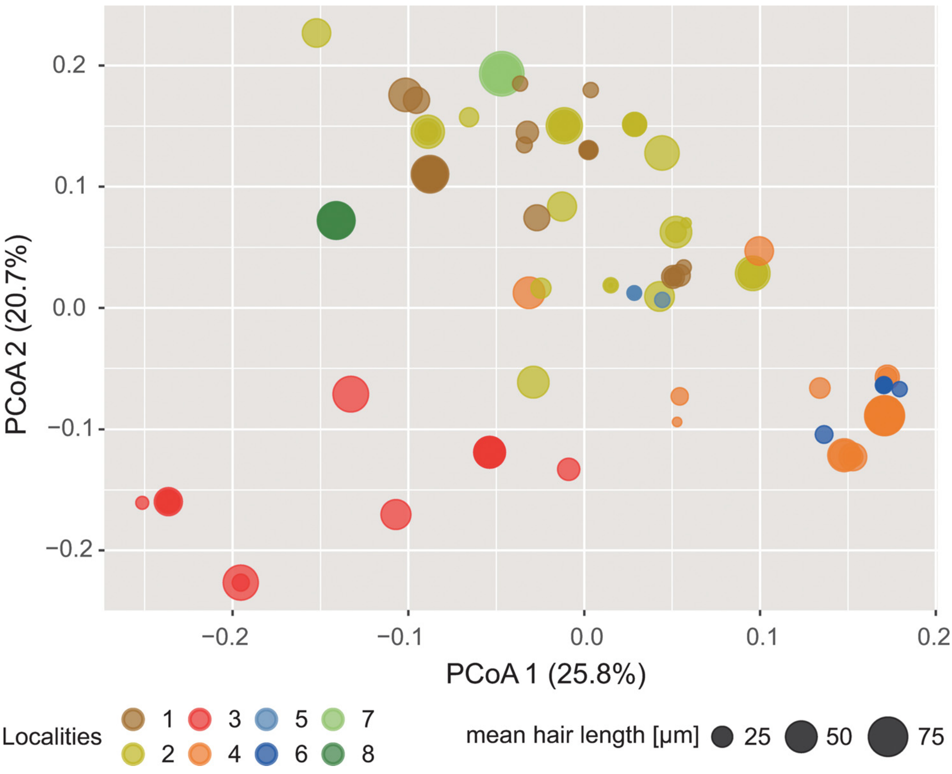


Fig. 5. Principal coordinate analysis of Jaccard distances among AFLP multilocus phenotypes derived from individuals of *Stipa eriocaulis* and "*S. epilosa*" sampled at eight localities in the Vinschgau Valley and in North Tyrol. Different colours represent the localities, the circle diameter the mean hair length of the respective individual.

relationships, although with a higher fit and slope for geographic distance ($R^2 = 0.174$, slope = 0.23) than for differences in hair lengths ($R^2 = 0.013$, slope = 0.00089 (Supplementary Figure S1).

Discussion

The central aim of this study was to test the hypothesis that Eastern Alpine feather grass from North and South Tyrol (Austria, Italy) with smooth or papillose upper leaf surfaces is an independent taxonomic entity, *S. epilosa*, which differs from sympatric *S. eriocaulis* with hairy upper leaf surfaces. Based on different sources of evidence, this hypothesis is rejected. First, there is no genetic difference between *S. eriocaulis* and Eastern Alpine "*S. epilosa*" (Figs 4–6). Second, at localities 1–4 in the Vinschgau Valley (Fig. 1) there is a continuum in hair length variation ranging from typical "*S. epilosa*" to typical *S. eriocaulis* (Fig. 3), which strongly indicates there is no morphological difference between these two groups. Finally, no microhabitat differences were recorded between the papillose and hairy individuals (Fig. 2).

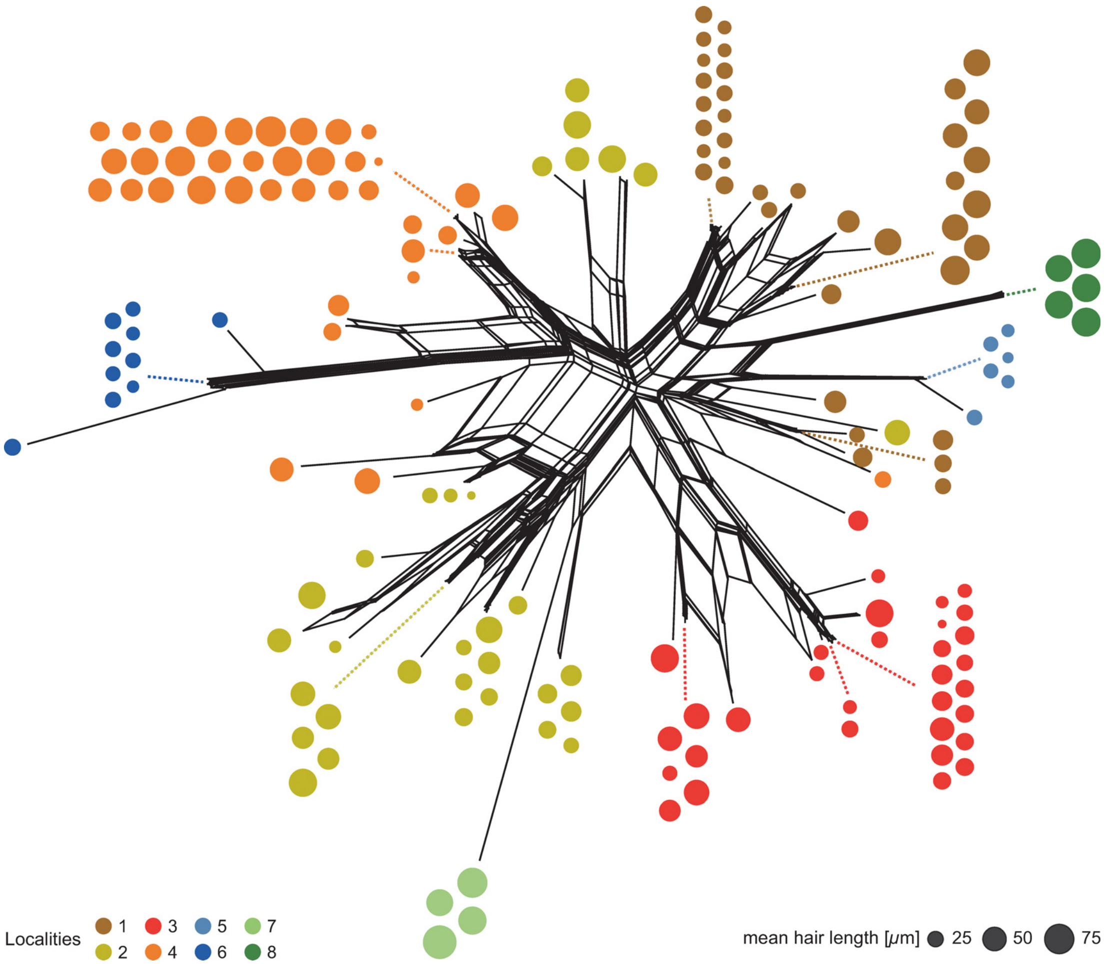


Fig. 6. Phylogenetic relationships among 159 individuals of *Stipa eriocaulis* and "*S. epilosa*" sampled at eight localities in the Vinschgau Valley and in North Tyrol depicted as a Neighbour Net based on uncorrected P distances. Different colours indicate the localities; the diameter of the circle diameter is the mean hair length of the individual.

In the Neighbour Joining tree based on AFLP data, the outgroup *S. pennata* s. str. is clearly different from the branch formed by *S. eriocaulis* and "*S. epilosa*" (Fig. 4). Within the latter, resolution was very limited and relationships largely mirrored geographical distance; for instance, the distant locality 8 situated near Brenner Pass (Fig. 1) is the sister of all other studied populations. Some localities harboured only a single or a few genotypes and all genotypes were restricted to a single locality. It is likely that this reflects the prevalence of cleistogamy in feather grasses (Durka et al. 2013), which was also reported in the populations investigated (E. Nitz, unpublished field observations). Conversely, the same genotype was detected in up to 27 individuals (Fig. 4). The number of variable AFLP fragments was 67; this is a low number compared to other studies (Hamasha et al. 2012, Durka et al. 2013). We suggest that the low variability is most likely caused by the low levels of outcrossing and thus intrinsic to the group studied. Thus, it is highly unlikely that the use of additional primer combinations would have qualitatively altered the results.

Most importantly, based on AFLP data *S. eriocaulis* and "*S. epilosa*" could not be separated. Both the PCoA (Fig. 5) and the Neighbour Net (Fig. 6) congruently indicate that there is no clustering reflecting hair length. Instead, papillose (localities 5, 6) and long-haired (localities 7, 8) individuals from North Tyrol are intermingled (PCoA: Fig. 5) or deeply nested (Neighbour Net: Fig. 6) among individuals from the morphologically heterogeneous localities 1–4 from South Tyrol, where individuals with papillae, intermediate and long hairs co-occur. This clearly indicates that the papillose individuals at localities 1–4 and the individuals in the uniform "*S. epilosa*" localities 5 and 6 have no common ancestry, rendering "*S. epilosa*" highly polyphyletic. Although the Mantel test indicated a correlation between genetic distance and differences in hair length, the linear model indicates a very poor fit (Supplementary Figure S1), which supports the conclusion that "*S. epilosa*" does not constitute a different entity in the area studied. Alternatively, the lack of genetic divergence between *S. eriocaulis* and "*S. epilosa*" could be due to introgression, as reported for Central-Asian *Stipa* species (Baiakhmetov et al. 2021). However, this hypothesis is clearly rejected by the results presented as the morphologically homogeneous populations 5–8 harbour only a very small fraction of the genetic diversity recorded in the variable populations 1–4 and their phylogenetic position is not correlated with the diagnostic morphological character (Fig. 6).

It is likely that the uniformity in indumentum in the North-Tyrolean populations is the result of a founder effect accompanying the colonization of North Tyrol from southern source populations in the course of postglacial range expansion from southern refugia, which were most likely situated along the southern margin of the Alps (Schönswetter et al. 2005). The founder effect predicts a reduction in diversity due to enhanced genetic drift in the newly founded populations, which can be observed as long branches in the Neighbour Net for the North-Tyrolean localities 5–8 (Fig. 6).

The finding that the hair length on the upper leaf surface does not follow species boundaries supports previous reports that in *Stipa* spontaneous mutations may lead to a switch between papillae and hairs. Specifically, it is suggested that (i) transitional stages in hair length may occur (Martinovský 1967) and that (ii) papillose individuals in otherwise hairy species (such as *S. pulcherrima*; Martinovský 1977) are "mutants" with no taxonomic value. Due to the prevalence of cleistogamy, individuals with aberrant character states can give rise to homogeneous, genetically depauperate populations (such as in the individual-poor localities 5 and 6 that consist of < 50 tussocks; Fig. 3), especially during phases of range expansion such as in the early Holocene. In contrast, rare allogamy may have resulted in a continuum in hair lengths, as observed in the individual-rich localities 1–4 (Fig. 3). There, the observed continuum makes the determination of feather grasses using the key in Fischer et al. (2008) difficult as most individuals are in the nonexistent hair length gap between "*S. epilosa*" (< 15 µm) and *S. eriocaulis* (> 40 µm). Only the uniformly long-haired or papillose plants from locations 7–8 and 5–6, respectively, can be unambiguously determined with this key.

As a dense leaf surface indumentum may reduce evapotranspiration (Monneveux & Belhassen 1996), we speculate that "*S. epilosa*" is restricted to less dry microhabitats than densely hairy *S. eriocaulis*. However, no support for this hypothesis was recorded.

Instead, the cover values of the accompanying plant species and the explanatory variables of the DCA (Fig. 2) did not show any microhabitat-related differences between papillose and long-haired individuals. In contrast, the differences observed between localities 2–4 and locality 1 in the DCA are likely site-related; locality 1 is grazed and the vegetation is relatively open, whereas at locality 3 scrub encroachment is advanced, with only a few open patches remaining. Localities 2 and 4 provided intermediate growing conditions. The approach based on small plots has been proven to be well-suited to characterize the target individuals' actual microsites; strong ecological differences between closely related, ploidy-differentiated species reported in previous studies employing the same approach support this view (*Senecio carniolicus* Willd. species group – Hülber et al. 2009, Sonnleitner et al. 2010, 2016; *Vaccinium uliginosum* L. and *V. gaultherioides* Bigelow – Silbernagl & Schönswetter 2019).

From an evolutionary point of view, our study of Alpine feather grasses provides an example of the effect of genetic drift on genes and character states. Indeed, a genetically diverse, morphologically polymorphic and individual-rich source population in South Tyrol has given rise to the genetically depauperate (Fig. 6), morphologically homogeneous (Fig. 3) and often individual-poor (E. Nitz & P. Schönswetter, field observations) North-Tyrolean satellite populations. Viewed differently, whereas the North-Tyrolean plants can be easily keyed out in two artificial groups, such an approach will fail for the polymorphic South-Tyrolean plants. From a taxonomic point of view, our integrative approach combining genetic, morphometric and microhabitat data failed to support the hypothesis that "*S. epilosa*" is an independent taxon in the area studied. Rather, papillose individuals with the lemma characteristics of *S. eriocaulis* should be included in the latter taxon. It is emphasized, however, that this regional study can neither clarify the identity of Western Alpine populations of "*S. epilosa*" nor provide an answer to the question, whether Anatolian and Balkan *S. epilosa* indeed deserves taxonomic recognition. Further studies of relevant populations in addition to a broad sampling of outgroup taxa are urgently needed to provide further insights into the evolution and taxonomy of feather grasses.

Supplementary materials

Figure S1. Scatterplots of genetic vs geographic distances, and genetic distances vs. hair length differences.

Table S1. Abiotic characteristics and estimated percentage cover of vascular plants included in the microhabitat analyses.

Table S2. Length of the 10 longest hairs of the individuals included in the morphometric analysis.

Supplementary materials are available at https://www.preslia.cz

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Genetická, morfometrická a mikrostanovištní data ukazují, že kavyly s papilnatými listy ve Východních Alpách (*Stipa epilosa* auct.) nepředstavují samostatný taxon

Kavyly (rod *Stipa*) patří k nejtypičtějším složkám stepní vegetace. Záhadná *S. epilosa*, která byla uváděna z různých částí Alp, se od běžné *S. eriocaulis* údajně liší mikromorfologickými znaky na průřezu listů; po stranách žeber na adaxiální straně čepele nejsou husté chlupy, které jsou typické pro *S. eriocaulis*, ale místo nich se tam vyskytují krátké kuželovité papily. S využitím morfometrické analýzy znaků na listech, AFLP fingerprintingu a analýzy mikrostanovišť jsme prozkoumali populace kavylů v údolí Vinschgau v Jižním Tyrolsku, v horní části údolí Inntal a v údolí Wipptal v Severním Tyrolsku. Položili jsme si otázky, (i) zda je *S. epilosa* geneticky odlišná od *S. eriocaulis*, (ii) zda jsou mikromorfologické rozdíly mezi *S. eriocaulis* a *S. epilosa* konzistentní a (iii) zda existují mezi *S. eriocaulis* a *S. epilosa* mikrostanovištní rozdíly, které potenciálně odrážejí adaptaci papilnatých a chlupatých jedinců na různé podmínky prostředí. Nejprve jsme prokázali, že mezi *S. eriocaulis* a *S. epilosa* ve Východních Alpách nejsou genetické rozdíly. Dále dokládáme, že existuje kontinuum variability délky chlupů od typické *S. epilosa* po typickou *S. eriocaulis*, což znemožňuje rozlišení dvou samostatných skupin. Závěrem bylo zjištěno, že mezi papilnatými a chlupatými jedinci nebyly pozorovány žádné rozdíly v mikrostanovištích. Z taxonomického hlediska náš přístup, kombinující genetická, morfometrická a mikrostanovištní data, nepotvrdil hypotézu, že *S. epilosa* je v naší studované oblasti samostatným taxonem.

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