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Bc. Eliška Krtilová

Genome duplication in *Stellaria* genus – the more, the merrier? Link between ploidy levels and sexual polymorphism.

Duplikace genomu v rodu *Stellaria* - čím více, tím lépe? Vztah mezi ploidní úrovní a sexuálními polymorfismy

Master thesis

Supervisor: **Mgr. Marek Slovák, Ph.D.**

Consultant: **doc. Clément Lafon Placette, Dr.**

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Declaration

I declare I have independently crafted the master thesis and have thoroughly documented all sources and literature referenced. Furthermore, no part of this work has been previously submitted for the award of another or the same academic degree.

In Prague, 18.4.2024 Bc. Eliška Krtilová

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Abstract

Polyploidization in angiosperms, a well-documented phenomenon, often correlates with a diversity of reproductive strategies, including sexual polymorphism, impacting their evolution and expression. This relationship is particularly intriguing in high polyploids, where the interaction can be complex yet largely enigmatic. We investigated the connection between high polyploidy and sexual polymorphism in *Stellaria palustris* (Caryophylaceae), a species complex with exceptionally high levels of polyploidy exceeding decaploids and reportedly exhibiting variable sexual expression. To broaden our analysis, we included its relatives, diploid-tetraploid *S. graminea* and diploid *S. longifolia*. Our study focused on Central and Northern Europe, utilizing various methodological approaches, including genome size estimation, chromosome counting, genetic analyses based on Sanger sequencing, and evaluating floral organ variability and sexual expression. Major hypotheses examined associations between ploidy level and latitude, as well as ploidy level and sexual polymorphisms. Chromosome counts of *S. palustris* ranged from 2x = 154 - 208, corresponding to 2n=12x, 14x, and 16x, while for *S. graminea* and *S. longifolia*, we confirmed their originally known ploidies. Although chromosome counts did not unambiguously reflect genome size, we found a weaker but significantly negative correlation of genome size with latitude, with Central European populations having slightly larger genome sizes, suggesting that marginal European populations may experience stressful conditions fostering genome size expansion and/or ploidy level increase. A similar trend was observed in flower organ size between these regions. Genetic analysis showed that all taxa forming their clusters, but with intermediate accessions, presented a blurred overall structure. *S. palustris* was closely related to *S. gramine,* while being more distant from *S. longifolia*. Notably, a unique clade related to *S. palustris* and *S. graminea* was identified, including tetraploid cytotypes (2n = 4x = 52) with different genome sizes from S. *graminea* and differing at least partially in morphology. We tentatively named this clade the 'Scandinavian entity,' exclusively collected in Northern Europe. Populations of *S. palustris* exhibited high sexual polymorphism, including female, hermaphroditic, and intermediate individuals with partially developed stamens, occurring at both population and individual levels. Despite varying ploidy levels, flower size dimorphism remained consistent, suggesting sexual selection as a driving force. No specific pattern in genome size correlated with latitude was identified. We propose that the intricate pattern of sexual expression in this high polyploid species is influenced by its high polyploidy, introducing complexity and dynamics into sex expression further influenced by intrinsic and extrinsic factors.

Content

1. Introduction

Throughout plant evolution, numerous processes have played pivotal roles in shaping the rich diversity of plant life observed today. These include hybridization (Stebbins, 1959; Soltis & Soltis, 2009; Goulet et al., 2017), the development of diverse sexual strategies (Bawa & Beach, 1981; Barrett, 1998; Barrett, 2002), or adaptation to various environmental conditions (An et al., 2019). Among these diversification and speciation mechanisms, however, one plays a pivotal role: whole-genome duplication, known as polyploidy (Otto & Whitton, 2000; Van de Peer et al., 2021; Heslop-Harrison et al., 2023). Although these evolutionary mechanisms have been extensively studied, still, much remains unknown about their occurrence and impact when two or more of these significant evolutionary mechanisms act simultaneously.

At the same time one of the most innovative trait in the evolution of flowering plants is the development of hermaphroditic flowers, capable of bearing both sexes (Charnov et al., 1976; Lloyd, 1982; Simpson, 2006). However, even in the early stages of angiosperm evolution, there was a divergence from this pattern, leading to the separation of individual sexes into distinct flowers (monoecy) and, notably, the emergence of plants with separate sexes (dioecy). This divergence paved the way for the evolution of various sexual polymorphisms, including gynodioecy - a phenomenon where morphologically and functionally hermaphroditic and female individuals coexist within the same species in a population (Darwin, 1977; Charlesworth & Charlesworth, 1978; Delph et al., 2007). This system is considered a transition from hermaphroditism to dioecy (Dufay et al., 2014; Godin & Demyanova, 2013; Käfer et al., 2017; Renner, 2014; Torices et al., 2011; Spigler & Ashman, 2012; Goldberg et al., 2017). On the contrary, the gynodioecious system demonstrates response to a range of intrinsic and extrinsic factors, enabling shifts between sexual morphs. These transitions occurring throughout the lifetimes of individuals and populations can markedly influence the overall genetic variability and fitness of populations, consequently affecting the evolution, fitness and survival of a given species (Aguirre et al., 2007; Caruso & Case, 2007; Ruffato et al., 2015).

As mentioned earlier, another fundamental driver that fuels diversification and speciation in angiosperms, is polyploidization (Otto & Whitton, 2000; Blanc & Wolfe, 2004; Soltis et al., 2009; Nieto Feliner et al., 2020). Polyploidy has played a significant role in the evolution and diversification of plants, and its involvement in sympatric speciation is well-documented (Otto & Whitton, 2000; Soltis & Soltis, 2009; Wood et al., 2009; Nieto Feliner et al., 2020). While the mechanistic pathways of polyploidization are generally well-studied, their interaction with other intrinsic and extrinsic evolutionary drivers is still not fully undrstood. In this regard, the evolutionary associations and consequences of sexual polymorphism and polyploidy represent a problem that warrants extensive exploration (Ashman et al., 2013; Glick et al., 2016). Indeed, this association is far from being a rule, and the evolutionary processes linking sexual polymorphism and polyploidy remain elusive.

Both polyploidy and sexual polymorphism can confer advantages to a given species, enhancing its ability to thrive in harsh environments or increase resistance to stress. While studies with models at diploid and tetraploid levels provide substantial support for this theory (reviewed in Ashman et al., 2013), the interaction between polyploidy and sexual polymorphism in systems with higher ploidy levels are quesitionable.

The focal species for my master's thesis is the perennial herb *Stellaria palustris* L., a member of the Caryophyllaceae family. This species is reported to be a high polyploid complex, with a reported chromosome number ranging between 130 and 188 (Peterson, 1936; Blackburn & Morton, 1957; Lövkvist & Hultgard, 1999; Stace, 2010), attributable to ploidies ten and higher (Peterson, 1936; Blackburn & Morton, 1957; Lövkvist & Hultgard, 1999; Stace,

2010; Šmarda et al., 2019). Existing data in the published literature highlights the presence of male sterile (female) flowers, suggesting a potential sexually polymorphic sexual system, particularly of the gynodioecious type. To enrich our comprehensive understanding of the associations between ploidy levels and sexual polymorphism, I have extended my analyses to include two closely related species with lower ploidies not previously reported in *S. palustris*. Consequently, I will undertake a comparative analysis involving *S. palustris, S. graminea*, and *S. longifolia.* All these species are considered to be related based on previous phylogenetic study (Sharples & Tripp, 2019). In the case of *S. graminea*, three ploidy levels (2n = 26, 39, 52) have been identified, with the triploid cytotype being a rare minor cytotype. Notably, both common cytotypes, 2x and 4x, have been shown to be gynodioecious (Kučera et al., 2021). On the other hand, *S. longifolia* is exclusively known for its diploid level of 2n = 2x = 26. This species is predominantly hermaphroditic, with occasional presumed gynodioecy (Kurtto, 2001). This study aims to investigate the model system *S. palustris*, focusing on fundamental biological characteristics including sexual expression variation. One of key goals of my thesis is to examine potential correlations between identified cytotypes and their geographic distribution and elucidate possible relationships between ploidy levels and sexual polymorphism manifestation (Ashman et al., 2013; Glick et al., 2016). To achieve this, I will conduct comprehensive analyses encompassing morphological, karyological, genetic, ecological, and sexual polymorphism to uncover the overall diversity of *S. palustris* and, to a certain extent, two related species within the study area. By investigating this intriguing model system, the study aims to fill knowledge gaps regarding the biology of this unique species, crucial for its legislative protection, particularly in Central Europe. Moreover, it seeks to enhance our understanding of highly polyploid plant species complexes' dynamics, behaviour, and evolutionary success.

1.1. Polyploidization – mechanism of its evolution

Polyploidization stands as a pivotal event that has played a vital role in the diversification of plants and facilitated sympatric speciation (Otto & Whitton, 2000; Blanc & Wolfe, 2004; Otto, 2007; Soltis et al., 2009; Nieto Feliner et al., 2020). During the process of polyploidization, the entire genome undergoes duplication, resulting in a multiplication of the chromosome set by more than two (Otto, 2007; Madlung, 2013). A polyploid can originate from the duplication of the genome of a single species, referred to as autopolyploidy (Vamosi & Dickinson, 2006; Parisod et al., 2010; Spoelhof et al., 2017). It arises through the fusion of unreduced gametes within a single species (Vamosi & Dickinson, 2006; Parisod et al., 2010; Spoelhof et al., 2017). This can occur when both gametes are unreduced or when one is reduced and the other is not, which might lead to the formation of odd ploidy-level cytotypes (Parisod et al., 2010). Alternatively, genome duplication may be directly associated with the hybridization of two species, known as allopolyploidy (Stebbins, 1971; Ramsey & Schemske, 1998; Soltis et al., 2009; Estep et al., 2014). A much rarer scenario occurs when hybridization takes place directly through the fusion of unreduced gametes from two distinct species (Ramsey & Schemske, 1998).

1.1.1. The impact of whole genome duplication on phenotype and mating strategies

Whole genome duplication profoundly affects various aspects of an organism's biology, encompassing morphology, physiology, and reproductive strategies. In terms of phenotype, WGD can shape morphological features, influencing size, shape, and structural characteristics (Levin, 1983; Flagel & Wendel, 2009; Hegarty et al., 2013; Ebadi et al., 2023) to increase DNA content, resulting in the expansion of plant organs (Sajjad et al., 2013; Sattler et al., 2016). This

phenomenon has been correlated with polyploid fitness advantages, enhancing their ability to establish and persist, ultimately contributing to speciation (Soltis & Visger, 2016; Heslop-Harrison, 2023). Corneillie et al. (2019) noted a correlation between ploidy level and cellular characteristics in the model plant species *Arabidopsis thaliana*. Specifically, they observed that as the ploidy level increased (ranging from 2x to 8x), there was a concurrent increase in cell size and a reduction in the number of cells per leaf blade. Furthermore, in specific members of the genus *Lilium*, tetraploids exhibited a larger leaf area than diploids (Zhang et al., 2022). Similarly, a study involving the common crop *Raphanus sativus* L., conducted by Pei et al. (2019), focused on diploids versus tetraploids. Remarkably, the gigas effect was evident in both vegetative and generative organs of synthetic tetraploids, which displayed increased height, larger leaves, and more prominent flowers. On the other hand, Becker et al. (2022) observed the Gigas effect in the *Oxalis* genus, where traits like pollen size, stomata size, and flower and leaf size showed no significant differences between diploids and polyploids cytotypes.

Morphological changes resulting from polyploidization can profoundly impact the overall functionality of a species and its mating strategies. Numerous studies have consistently demonstrated a general preference among pollinators for plants bearing larger flowers and more extensive flower displays (Klinkhamer et al., 1989; Andersson, 1991; Conner & Rush, 1996; Kennedy et al., 2006; Glaettli & Barrett, 2008). Moreover, comprehensive research conducted by Delgado et al. (2023) reveals that larger flowers tend to attract larger insects, while smaller flowers are more appealing to smaller insects. Consequently, in mixed-ploidy populations, it is reasonable to anticipate that groups of insects would exhibit a preference for plants with the same ploidy, potentially establishing a reproduction barrier between different cytotypes (Segraves & Thompson, 1999; Coyne & Orr 2004; Čertner et al., 2017; Palmqvist et al., 2021). The combined effects of an increased preference for larger flowers and establishing cytotype barriers can drive selection toward polyploids due to increased pollinator visitation (Kennedy et al., 2006; Oliveira et al., 2022).

However, the attractiveness of larger flowers to pollinators may be different, and the visitation rate of pollinators to flowers of varying sizes could decrease (Andersson, 1988; Klinkhamer & de Jong, 1990). Surprisingly, it's been observed that not only a single cytotype may not be preferred by insects, but pollinators might even show a preference for visitations between different cytotypes (Schmickl et al., 2024).

Polyploids frequently exhibit the capacity for self-pollination, a trait that may not have been present in their diploid ancestors (Okamoto et al., 2007). Self-pollination can confer advantages to newly formed polyploid cytotypes, facilitating their establishment and rapid expansion, particularly considering their immediate reproductive isolation from the diploid ancestor(s) following formation (Barringer, 2007). Despite the initial advantages, prolonged inbreeding in newly formed polyploid populations may result in inbreeding depression. Actually, however, cytotypes with higher ploidy levels may exhibit greater resistance to inbreeding than diploids. This resilience is attributed to the multiplicity of alleles in the genome, providing more effective mechanisms to mask deleterious mutations (Husband and Schemske, 1997; Osabe et al., 2012). However, the extent of selfing varies between autopolyploids and allopolyploids. While polyploids generally appear more inclined towards selfing, it is noteworthy that autopolyploids often demonstrate lower self-pollination rates when compared to their diploid counterparts (Husband et al., 2008). In autopolyploids, chromosome pairs arise from the same parental chromosome sets, known as homologous chromosomes, harbouring similar homologous genes. Consequently, during meiosis, they are prone to forming multivalents, where multiple chromosomes simultaneously pair, synapse, and recombine. This process leads to meiotic irregularities and challenges in fertility (Ramsey & Schemske, 2002; Briggs & Walters, 2016; Bohutínská et al., 2021). In contrast, allopolyploids typically form bivalents, as the two fused genomes differ (homeologous chromosomes). While

the pairing of homeologs in allopolyploids has the potential to create novel gene combinations and phenotypes, it can, on the other hand also disrupt the karyotype and lead to abnormal meiotic processes, thereby reducing fertility (Soares et al., 2021).

1.1.2. Associations between ecology and whole genome duplication: polyploids often exhibit wider ecological tolerance.

Polyploidization is frequently associated with stressful environmental conditions. Stress has been established as a triggering factor for the emergence of polyploids (Ramsey & Schemske, 1998). Consequently, polyploids are expected to demonstrate the ability to respond to stressful conditions, adapt effectively, and evolve stress resistance (Renny-Byfield & Wendel, 2014). This adaptation can lead to distinct ecological preferences between diploids and polyploids. However, it is crucial to note that the polyploidization mechanism significantly impacts the adaptation and physiological response of a particular polyploid cytotype. Physiological changes in polyploids may vary between auto- and allopolyploids. In allopolyploidy, physiological changes might be primarily influenced by hybridization rather than polyploidization (Rieseberg, 2001).

The general paradigm assumes that polyploids can occupy wider ecological niches and have a broader geographical distribution (Levin, 2002). Adapting to broader ecological niches aligns with the concept that genome duplication influences physiological and morphological structures, enhancing plasticity and providing more space for functional genetic variability (Levin, 1983; Otto & Whitton, 2000; Martin & Husband, 2009). This assumption is validated by studies demonstrating higher ecological tolerance in polyploid cytotypes than lower ploidy levels (e.g., Castro et al., 2023; del Pozo & Ramirez-Parra, 2015; Ramsey, 2011). For example, a study conducted by Castro et al. (2023) comparing water deficit tolerance in diploids versus well-established natural tetraploids and synthetically neotetraploids synthesized from diploids revealed significantly elevated traits in both types of tetraploids compared to diploids. These enhanced traits included belowground biomass, photosynthetic efficiency, and membrane stability. In a similar way, Manzaneda et al. (2012) explored the dependence of the distribution of diploids and tetraploids within the species *Brachypodium distachyon* (L.) P. Beauv. (*Poaceae*) on arid environments in the Iberian Peninsula. Cytotype frequency responded to aridity independent of geographic location, with distribution patterns associated with drought and annual precipitation. Under stressful drought-induced conditions, tetraploid cytotypes exhibited superior water management and coping mechanisms. However, this paradigm is not universally applicable, as the response of polyploids to various biotic and abiotic stresses can be variable and cannot be strictly generalized (Hannweg et al., 2016; Hias et al., 2017; see Van de Peer, 2021 for a review).

As already mentioned above, polyploidization often leads to expanding the range of the newly formed cytotype (Levin, 1983; Otto & Whitton, 2000; Levin, 2002; Martin & Husband, 2009; McIntyre, 2012). Compared to their diploid counterparts, distinct distribution patterns of polyploids were reported in the second half of the 20th century, suggesting environmentally induced shifts in distribution ranges, particularly in arctic and alpine flora (Johnson and Packer 1965). In a comprehensive meta-analytical study, Rice et al. (2019) confirmed this idea, showing that the frequency of polyploids increases with rising latitude and altitude. This trend is commonly explained by the superior adaptability and stress tolerance of polyploids. On the other hand, stressful conditions, such as low temperatures, can trigger the production of unreduced gametes. Results of experimental studies by Pecrix et al. (2011) and De Storme et al. (2012) indicated that temperature fluctuations critically impact meiosis, particularly in telophase II. The stress induced by temperature variations can facilitate the formation of unreduced gametes, contributing to the increased occurrence of polyploids. This phenomenon

is expected to be more prevalent in extreme conditions typical of higher latitudes and altitudes (e.g., Ramsey and Schemske, 1998; Otto Whitton, 2000; De Storme et al., 2012). In a recent review, Van de Peer (2021) mentions that polyploidization during evolution is often linked with significant climatic changes (Ramsey & Schemske, 1998), indicating that the formation of polyploids is often a result of some external stressful event.

Higher tolerance to environmental stressors is not linked just to tetraploids but can also be traced in higher ploidies and can be even more pronounced by an increase in chromosomal sets (Chandra & Dubey, 2010; Ramsey, 2011; Deng et al., 2012). For instance, Ramsey (2011) studied in the model species *Achillea borealis* the different behaviour and fitness of tetraploids and hexaploids in xeric dune habitats, which are naturally occupied only by hexaploids. His experiment revealed that wild hexaploids and the first generation of synthetic hexaploids have significantly higher fitness in dune habitats than tetraploids. Fixation of tetraploids on more mesic habitats and hexaploids to xeric habitats can indicate either different claims of the cytotype on the external conditions or the ability of higher cytotypes to adapt more quickly to more stressful conditions. Interesting findings emerged from the study by Prazak (2001), who subjected diploids, tetraploids, and hexaploids of wheat (*Triticum*) to salt stress. Notably, tetraploids exhibited the lowest salt tolerance, displaying reduced germination capacity and shorter shoots in high NaCl concentrations.

From a genomic perspective, polyploidization brings about significant alterations in genome structure. This transformation often results in a reduction in absolute genome size, particularly affecting non-coding DNA regions (Feldman et al., 1997; Ozkan et al., 2001). In larger genomes, disruptions during meiosis may arise due to challenges in identifying homologous genes amidst a high number of chromosomes (Zielinski & Scheid, 2012; Moore, 2013). Nevertheless, evolutionary mechanisms have evolved to enhance fertility in polyploids with unevenly paired chromosomes. This process frequently involves the loss of transposable elements (TEs) during genome reorganization, which contributes to increased genome stability (Parisod et al., 2009; Bourque et al., 2018). In contrast to diploid-tetraploid systems, the general rule of "higher is better" is not universally applicable in higher polyploid cytotypes. Mereďa et al. (2016) explored the ecological differentiation of tetraploid and octoploid cytotypes of *Jacobaea vulgaris*. Despite octoploids occupying slightly distinct ecological niches, the observed difference was not significant.

Specific chapter represent odd ploidy-level or aneuploid cytotypes, commonly regarded as evolutionary dead ends. Surprisingly, however, even triploids, known for problematic sexual reproduction during meiosis (Ramsey & Schemske, 2002; Pecinka et al., 2011), demonstrate higher stress resistance compared to diploids. Lourkisti et al. (2020), in their experiment exposing triploid citrus crops and their diploid parents to naturally lower temperatures, observed that triploids exhibited better adaptation to lower temperatures. This enhanced resilience was attributed to improved photosynthetic activity and chemical compounds in the leaves, contributing to more effective stress adaptation. In the end numerous studies have demonstrated that aneuploids not only have the capacity to survive but can also compete successfully with euploids (Kostoff, 1938; Bingham, 1968; Simonsen, 1975). Generally, higher polyploid species are more susceptible to the formation of aneuploids (Sears, 1953; Xiong et al., 2011), however the larger the genome, the lesser impact aneuploidy has on overall fitness and phenotype (Birchler, 2013), suggesting that aneuploidy may cease deleterious in highly polyploid organisms.

1.2. Sexual polymorphisms

Throughout the evolutionary trajectory of angiosperms, the original flower blueprint comprised male and female reproductive organs, making it hermaphroditic (cf. Sauquet et al. 2017). Although this hermaphroditic structure persists in many flowering plants, the early stages of evolution witnessed the emergence of sexual polymorphisms and deviations from hermaphroditism (Anger et al., 2017). This pivotal transition involved suppressing one sex within the flower, leading to the development of unisexual flowers. Two main types of unisexual flowers are recognized: monoecious and dioecious. Monoecious plants bear both male and female flowers on the same individual, while dioecious plants exhibit complete sexual separation, resulting in solely female or male plants. Estimates of dioecy prevalence in angiosperms vary, with around 5-6% of species displaying absolute sexual separation (Renner, 2014). Dioecy is widespread in approximately 38% of plant families, suggesting convergent evolution, with this trait emerging independently multiple times.

The evolutionary mechanism behind dioecy involves a gradual departure from hermaphroditism. Some plant species exhibit morphologically and functionally diverse transitional forms of sexual structures. In addition to dioecy, notable sexual polymorphisms include gynodioecy and androdioecy. These polymorphisms enhance genetic variability by having separate sexes while ensuring reproductive certainty under adverse conditions through hermaphroditic individuals.

1.2.1. Gynodioecy

Gynodioecy, a not uncommon sexual polymorphism, manifests as the coexistence of female and hermaphroditic plants within a species (Charlesworth & Charlesworth, 1978; Simpson, 2006; Delph et al., 2007; Singh, 2010). This sexual polymorphism involves morphologically and functionally distinct female and bisexual individuals within a single species (Darwin, 1877; Charlesworth & Charlesworth, 1978; Delph et al., 2007). In angiosperms, gynodioecy has independently evolved, in at least 97 families and over 1573 species (Godin, 2020), with the number of identified gynodioecious species continually rising, albeit still representing less than 1% of angiosperms (Caruso et al., 2016). Gynodioecy is notably prevalent in the Lamiaceae and Caryophyllaceae families, with the subfamily Silenoidea exhibiting an exceptionally high occurrence (Shykoff et al., 2003). In the Caryophyllaceae family, gynodioecious species constitute 10.3% of the total (Godin, 2020). A meta-analytic study by Caruso et al. (2016) exploring associations between gynodioecy and various biological and ecological factors revealed a higher prevalence of gynodioecy in perennial herbs of temperate climates. The evolution of females in gynodioecious systems relies on the male sterility of female plants. Male sterility in angiosperms refers to a phenomenon wherein stamen formation is impeded due to genetic predisposition, rendering them incapable of producing pollen in the anther or leading to incorrect anther development. The consequence is the absence of functional pollen capable of fertilization (Schnable & Wise, 1998). Phenotypic manifestations of male sterility can vary, with the most visually conspicuous sign being the absence of stamens or the preservation of rudimentary stamens or filaments without anthers. Thus, male sterility can be categorized into structural and functional types. Structural male sterility involves the absence of stamens, anthers, or their degeneration (Gottschalk & Kaul, 1974; Mishra et al., 2013). On the other hand, functional male sterile individuals produce viable pollen; however, factors such as anther opening disruption may hinder successful fertilization by such pollen (Mishra et al., 2013).

1.2.1.1. The genetic origin of gynodioecy

Male sterility in gynodioecy is contingent upon genetic information encoded in the nucleus and the cytoplasm. These genes, individually or in combination, exert control over the development of male reproductive organs, consequently influencing their fertilization capacity (Frank, 1989; Palmer et al., 1992; Schnable & Wise, 1998; Budar & Pelletier, 2001). The induction of male sterility can occur through two distinct genetic mechanisms. Firstly, nuclear-coded male sterility (NMS) involves specific genes located within the nuclear genome, where the recessive allele of the gene determines sterility (Kaul, 1988; Schultz 2002; Chang et al. 2016; Chen et al. 2019). Secondly, nuclear-cytoplasmic male sterility (CMS) is characterized by an intricate interplay between nuclear and cytoplasmic genes (Palmer et al., 1992; Charlesworth & Laporte, 1998; Schnable & Wise, 1998; Van Damme et al., 2004; Garraund et al., 2011; Touzet, 2012). This male sterility is more prevalent and has been identified in nearly 150 plant species (Laser & Lersten, 1972; Mackenzie et al., 1994; Levings & Vasil, 1995). The control of CMS involves genes residing in both the nucleus and the cytoplasm, with mitochondria housing genes responsible for male sterilization, while genes restoring male fertility are situated in the nucleus (Touzet, 2012). The emergence of CMS can occur through crossings involving either DNA damage caused by mutagens or the exchange of nuclear and cytoplasmic genes (Schnable & Wise, 1998). Notably, in both CMS and NMS male fertility can be restored (Schnable & Wise, 1998, Schultz 2002; Chang et al. 2016).

1.2.1.2. Impact of gynodioecy on phenotype of females and hermaphrodites

Sexual dimorphism is a prevalent phenomenon characterized by noticeable morphological differences between the sexes within a species (Barrett & Hough, 2013). Across various species, disparities in morphology between different sexes are frequently apparent (Yakimowski et al., 2011; Kučera et al., 2021). These distinctions can manifest in various ways, such as variations in flower size (Glaettli & Barrett, 2008; Yakimowski et al., 2011; Kučera et al. 2021) or floral display (Glaettli & Barrett, 2008). Numerous studies indicate that within gynodioecious systems, hermaphrodite and female individuals exhibit distinctions not only in sexual organs but also in perianth size (Delph, 1996; Shykoff et al., 2003; Ashman, 2006; Barrett & Hough, 2013; Dufay et al., 2014; Kamath et al., 2017; Kučera et al., 2021). A prevailing trend in gynodioecious systems is the smaller size of female flowers compared to hermaphroditic counterparts. This variation in flower size can be rationalized by the necessity of accommodating and safeguarding both functional stamens and pistils in hermaphroditic flowers, leading to a generally larger perianth.

Consequently, the perianth is smaller in females, given the absence of functional stamens. However, females typically exhibit a larger gynoecium than hermaphrodites, contributing to increased seed production (Delph, 1996; Varga, 2021). This phenomenon is often called female advantage (reviewed in Dufay and Billard 2012). An alternative explanation draws from the classic Bateman principle (Bateman, 1948), positing that reproductive success in males (or hermaphrodites in the case of gynodioecy) is limited by pollinators. In essence, plants generate more male gametes than female ones, resulting in effective selection for males (hermaphrodites). They have evolutionarily preferred plants with larger, more pollinator-attractive flowers. This principle, however, does not extend to females, exempting them from the need to invest energy in larger and more appealing flowers, subject to more lenient selection (Bateman, 1948; Paterno et al., 2020). The diminished size of the female perianth compared to hermaphrodites consequently entails a lower cost of formation and allows redirecting saved resources towards seed production (Delph, 1996; Miller & Venable, 2003). Also, the equilibrium can be set in the number of flowers per plant. Hermaphrodites tend to produce fewer flowers but larger ones. On the other hand, females tend to have a higher number of flowers but smaller sizes (Delph, 1996; Godin et al., 2021). Petals, contingent on sex, may not only diverge in size but also flower shape and symmetry (Kamath et al., 2017; Neustupa, 2020).

1.2.1.3. The associations between environment and sex expression

In general, under the influence of environmental stressors, the sex with lower reproductive demands gains an advantage. Environmental stressors can take various forms, with abiotic stressors including changes in temperature, flooding, drought, nutrient availability, altitude, humidity, or photoperiod (Dudash, 1990; Van Etten & Chang, 2009). Biotic stressors impacting plant development and reproduction encompass factors such as the activity of pollinators or the presence of herbivores and pathogens (Ashman, 2006).

In gynodioecious systems, females exemplify this trend, requiring less energy for seed production than hermaphrodites, which must produce both female and male gametes. Conversely, in dioecious populations, females face higher reproductive demands relative to males, as seed production is resource-intensive compared to the production of pollen grains (Varga & Soulsbury, 2020). Thus, the sex ratio in a sexually polymorphic population constantly fluctuates dynamically, depending on the environmental factors.

Contrastingly, in dioecious populations, the influence of these factors is notably diminished, posing challenges in understanding the determinants of female frequency. This suggests that dioecy represents a more stable system, exhibiting less dynamic responses than gynodioecy and sub-dioecy to environmental stressors affecting sex ratio variability. An alternative explanation could be that the influence of environmental factors on shifts in sexuality varies depending on the species (Varga & Soulsbury, 2020).

Among abiotic environmental stressors, temperature appears to exert the most significant influence on the ratio of hermaphrodites to females in sexually polymorphic systems. In the studied species *Plantago coronopus* L. (Plantaginaceae), partial male sterility was identified as the least stable condition, particularly sensitive to temperature fluctuations. In this species, the percentage of female flowers increased with rising temperatures, particularly in individuals exhibiting partial male sterility. Remarkably, while the proportion of female individuals remained constant, the prevalence of individuals with partial male sterility increased at the expense of hermaphrodites (Koelewijn and Damme 1996). While the impact of temperature is undoubtedly substantial, the response to temperature changes can vary across species. In several species, such as *Petunia* Juss. (Solanaceae) (Van Marrewijk 1969), *Daphne laureola* (Alonso and Herrera, 2001), or *Wurmbea biglandulosa* (Vaughton & Ramsey, 2005), similar trends as in *P. coronopus* mentioned above (Koelewijn & Damme 1996) were observed. However, other species, such as *Brassica napus* L. subsp. *napus* (Brassicaceae) (Burns et al. 1991), *Eritrichum aretioides* (Puterbaugh et al. 1997), *G. sylvaticum* (Asikainen and Murikainen 2003), or *Eichhornia paniculata* (Spreng.) Solms (Pontederiaceae) (Barrett & Harder, 1992) exhibited the opposite trend. These findings underscore the species-specific nature of temperature effects on sexual polymorphism in plants.

Another crucial abiotic factor influencing patterns of sexual expression in sexually polymorphic species is water availability. In gynodioecious species, females prefer drier and warmer environments (Caruso & Case, 2007; Ruffatto et al., 2015; Varga & Soulsbury, 2020). Caruso and Case (2007) propose that this preference could be attributed to the fact that, when resources are limited, females invest solely in seed production, which is less resource-intensive than the dual production of seeds and pollen. Vaughton and Ramsey (2005) suggest that the enhanced adaptation of females to drier locations may result from a decrease in the fitness of hermaphrodites under such conditions (cf. Alonso & Herrera, 2001).

Additionally, in *Wurmbea biglandulosa*, females exhibited higher seed fertility than hermaphrodites under dry conditions (Vaughton & Ramsey, 2005). Caruso and Case (2007) studied *Lobelia siphilitica* L. (Campanulaceae), revealing a distinct distribution pattern at different latitudes. Closer to the equator, where temperatures and drought are higher, populations are smaller but exhibit a higher proportion of females. This trend could be influenced by temperature or other factors, such as variable pollinator representation (Caruso

& Case, 2007). In contrast to these findings, in *Geranuim transversale*, females tend to be more commonly found in wetter sites (Abdusalam et al., 2017), aligning with a similar common trend observed in dioecious species (Dawson & Bliss, 1989; Xu et al., 2008; Miller & Compagnoni, 2022). However, despite these observations, Bailey et al. (2017) found no significant influence of temperature on the fitness of females or hermaphrodites. In addition to environmental factors, which undeniably play a significant role in shifts in sexuality, it is crucial to consider the species involved, as each species exhibits an individualized response to stressors (Koelewijn & Damme, 1996).

1.2.1.4. Equilibrium between females and hermaphrodites in the population

In a population where more than one sex exists, continuous selection occurs to balance the sexes in a ratio of 1:1 (Fisher, 1930). However, in a species where sex is dependent on environmental factors, maintaining a constant sex ratio may not be as straightforward. For females to persist within a population, they must possess advantageous traits that confer competitiveness against hermaphrodites. One such circumstance arises when inbreeding depression is high in a predominantly hermaphrodite population (Lloyd, 1975). In this scenario, the fitness of hermaphrodites diminishes, favouring females, as their capacity to enforce outcrossing enhances genetic variability (Charlesworth & Ganders, 1979). Another advantage for females may lie in resource allocation, directing resources toward increased seed production, resulting in seeds of superior quality and viability (Charlesworth & Charlesworth, 1978). Notably, the influence of pathogens, mainly sexually transmitted infectious diseases, significantly impacts sex representation in the population (Busch et al., 2004). As a general trend, females exhibit higher resistance to diseases and pests (Zuk & McKean, 1996; Zuk & Stoehr, 2002).

However, an increased proportion of females in gynodioecious populations can lead to pollen limitation. This phenomenon occurs when the representation of females is so high that hermaphrodite or male flowers cannot provide sufficient male gametes for fertilizing the eggs (McCauley & Taylor, 1997; Spigler & Ashman, 2012). A comparable study conducted by Wilson and Harder (2003) illustrates that when separate sexes are present, there is an increase in the variability of pollen income and seed dispersal. This phenomenon tends to favour hermaphrodites. Also, pollen limitation can favour hermaphrodites in the population by an overall decrease in female fitness due to the limitation of fertility (McCauley & Taylor, 1997). Even when the sex ratio is balanced and there is ample pollen, pollen availability can still be limited if pollinators are inactive or absent. In such cases, hermaphroditism becomes advantageous, as hermaphrodites can self-pollinate (Spigler & Ashman, 2012). However, hermaphrodite individuals face the threat of inbreeding, which can counteract their reproductive advantages. Repeated inbreeding results in inbreeding depression and reduced reproductive capacity (Charlesworth & Willis, 2009), ultimately influencing the shift of sexuality towards gynodioecy (Spigler & Ashman, 2012).

1.2.1.5. Environmentally induced lability in sexual expression

In constantly changing environmental conditions, plant species try to adapt to ensure further reproduction and maintain the population. Therefore, in response to changing conditions, changes in plant sexuality may occur, and entire populations may transition to a different sexual system due to changes in the reproductive organs. So-called sexual lability then refers to the constant fluctuation of the sexuality of a given population to ensure the highest possible reproduction and achieve the best possible adaptation to the surrounding environment (Charnov & Bull, 1976; Gabriel, 2005; Renner et al., 2007). Various factors can trigger changes in sexual expression, whereas the most common seem to be environmental conditions such as light and water availability or temperature (Horovitz & Galil, 1972; Koelewijn & Van Damme, 1996; Dang & Chinnapa, 2007; Friedman & Barrett, 2011). The transitions from a hermaphroditic individual to a female or male may not be instantaneous but may go through various intermediate forms. These forms can have both hermaphrodite and unisexual flowers or even

morphologically and functionally intermediate flowers with partial male or female fertility. Transitional forms in sexual expression encompass phenomena known as andromonoecy, gynomonoecy, and sub dioecy. Andromonoecy entails the presence of male and hermaphroditic flowers on a single plant (Simpson, 2006), while gynomonoecy involves the co-occurrence of female and hermaphroditic flowers on one plant (Koelewijn & Damme, 1996). Subdioecy refers to female, male, and hermaphroditic plants within one population (Spigler & Ashman, 2012). Sexual polymorphisms, especially their transitional forms, signify the flexibility of the sexual system.

1.3. Model species

1.3.1. Stellaria (Caryophyllaceae)

The genus *Stellaria* is recognized as one of the most species-rich in the family *Caryophyllaceae* (Singh, 2010), with the number of species varying depending on the taxonomical approach. Based on the current literature, the estimates of species number within the genus range between 115 and 200 (Singh, 2010; Mahdavi et al., 2012; Sharples, 2019). In a comprehensive phylogenetic study, Sharples (2019) conducted extensive RAD sequencing on the entire genus, confirming its paraphyletic nature. The study identified a "core group" comprising five major monophyletic groups: Labreae, Petiolares, Insignes, Nitentes, and Plettkeae (Sharples, 2019; Sharples & Tripp, 2019).

The genus *Stellaria* encompasses annual to perennial herbs characterized by fibrous, densely branched roots and slender rhizomes. The stems are square and glabrous, exhibiting a decumbent, ascending, or upright growth. Leaves are simple and arranged oppositely, predominantly lanceolate to linear, although variations such as ovoid, cordate, or elliptical shapes may occur. Leaf margins are generally entire, and the surface may be either hairy or glabrous. Bracts can either be membranous and whitish with a central stripe ranging from green to brown or green, resembling the upper stem leaves. The inflorescence is a dichasium. The flowers are small and typically white. The calyx consists of five (occasionally four) greenish sepals. True petals are absent, replaced by staminodial petals. The corolla comprises five (rarely four) petals, either fully divided or at least halfway, and may be entirely absent. The flower contains four to ten stamens. The uppermost ovary usually bears three, occasionally five, styles. The fruit is a capsule with six teeth, rarely an achene or berry.

1.3.1.1. Chromosomal diversity and polyploidization in the genus Stellaria

From a karyological perspective, the genus *Stellaria* exhibits considerable diversity in terms of basic chromosome numbers and ploidy levels. The base chromosome numbers are x = 11, 12, 13 (Jonsell et al. 2001; Kurtto 2001). Numerous species have been identified as polyploids, with tetraploid levels being prevalent. Tetraploids were observed in species such as *S. longipes* (Chinnappa et al., 2011), *S. borealis* (Kurtto 2001), *S. neglecta* (Kurtto 2001), *S. media* (Kurtto 2001), *S. graminea, S. ruderalis* (Lepší et al., 2019). Higher ploidy levels exceeding tetraploids were identified in only two species, namely *S. longipes* (Chinnappa & Morton, 1984; Antonova & Petrovsky, 1986) and *S. palustris*. The latter is recognized as a polyploid complex characterized by exceptionally high ploidies, extending up to decaploids and beyond. The presence of aneuploidy and odd ploidy levels is also quite common (Kurtto 2001).

1.3.1.2. Geographical distribution and ecology

Genus *Stellaria* exhibits a widespread distribution across both hemispheres, with a predominant presence in the Northern Hemisphere (Morton, 2005). It thrives in various climatic conditions, ranging from temperate to cold climates, and can be found in diverse ecosystems, from lowlands to alpine zones (Kurtto, 2001; Morton, 2005; Sharples & Tripp,

2019). Given its cosmopolitan distribution and extensive species diversity, *Stellaria* species occupy many habitats, from purely natural environments to those significantly influenced by human activities. Some species are commonly found in moist and shaded environments (e.g., *S. nemorum* L., *S. neglecta* Weihe, *S. palustris* Ehrh. ex Retz), while others prefer drier habitats (e.g., *S. pallida* (Dumort.) Piré, *S. holostea* L.). Additionally, various species exhibit preferences for specific soil conditions, such as high-nutrient soils (e.g., *S. palustris*), or thrive in ruderal habitats (e.g., *S. media (L.)* Vill., *S. hebecalyx* Fenzl) (Kurtto, 2001).

1.3.1.3. Sexual and asexual reproduction

In members of the genus, both vegetative and generative reproduction pathways are recognized. Vegetative reproduction commonly occurs through rhizomes, shoots, and rooting stems. Within sexual reproduction, species exhibiting both autogamous and allogamous tendencies (Kurtto, 2001; Morton, 2005; Dang & Chinnappa, 2007). In certain autogamous species, mechanisms preventing self-pollination have evolved, primarily proterandry, which is prevalent within the family (Bittrich, 1993; Kurtto, 2001). Proterandry was observed in various species, such as *S. holostea* L., *S. longipes* Goldie, and *S. longifolia* Muhl. Ex Willd. (Kurtto 2001). Alongside chasmogamous flowers, some species produce cleistogamous flowers, as seen in *S. pallida* (Dumort.) Piré and *S. media* (L.) Vill. from *S. media* aggregate (Richards, 1997; Kurtto, 2001; Weekley & Brothers, 2006).

1.3.1.4. Interspecific hybridisation

Hybridization between different species within the genus *Stellaria* has been documented, although the resulting offspring typically exhibit low fitness levels (Chinnappa, 1985; Kurtto, 2001). Natural hybridization occurrences have been reported (e.g. Kurtto 2001) andobserved, for instance in case of *S. longipes* and *S. borealis* Bigelow (Morton & Rabeler, 1989; Emery & Chinnappa, 1992). An experiment involving *S. longipes* crossed with five related species of the genus *Stellaria* (*S. calycantha* (Ledeb.) Bong., and *S. crispa* Cham. & Schltdl.) revealed successful seed production and the development of viable offspring only in the cross with *S. longifolia*. Conversely, crosses with other species resulted in the formation of poor-quality or deformed seeds that failed to germinate (Chinnappa, 1985).

1.3.1.5. Sexual polymorphisms in Stellaria genus

The presence of sexual polymorphisms, with gynodioecy being the most common, has been reported in the genus *Stellaria*. Male sterility has been observed in approximately 12.4% of species, including *S. graminea, S. palustris*, and *S. longipes* (Philipp, 1980; Kurtto, 2001; Dang & Chinnappa, 2006; Godin, 2020; Kučera et al., 2021). Gynodioecy has been explicitly proven and documented only in *S. longipes* and *S. graminea* (Philipp, 1980; Dang & Chinnappa, 2006; Kučera et al., 2021). Among the studied species, *S. longipes*, characterized by considerable phenotypic plasticity and widespread distribution, stands out as the most investigated gynodioecious species. In their study, Dang and Chinnappa (2006) conducted experiments with plants from diverse North American habitats, using a greenhouse and temperature chambers to simulate conditions for each season. The study revealed fluctuations in the degree of male sterility and fertility throughout the year, potentially influenced by temperature and environmental factors, as suggested by the authors. Philipp (1980) also studied *S. longipes*, conducting a cultivation experiment in Copenhagen. Plants were grown from seeds collected from female and hermaphroditic wild plants in Greenland. Within cultivated plants, transitional individuals with hermaphrodite and female flowers simultaneously were observed, indicating the sexual lability of this sexual polymorphism. The most significant differences between the sexes were found in the number of flowers, seeds in the fruit, and the overall seed production within the reproductive cycle.

Moreover, it was shown that the transitional form gradually changed during the

flowering period into a purely hermaphroditic one (Philipp, 1980). Kučera et al. (2021) conducted an extensive study on *S. graminea* in Central Europe, revealing that despite expectations, *S. graminea* is gynodioecious in both diploid and tetraploid levels. The gynodioecious sexual system observed in this study appears to be a significant driver of evolutionary processes, shaping the phenotype and the life and reproductive strategies within this species.

1.3.2. Studied model species

1.3.2.1. Stellaria palustris Ehrh. ex Retz

Stellaria palustris is a perennial herb with slender and creeping rhizomes, and its vegetative shoots are pale green but often appear glaucous. The stem is ascending or erect, , of a quadrangular shape, fragile and a smooth or rough texture. Leaves are linear-lanceolate or lanceolate, broadest at the base, with mostly glabrous margins. The bracts are membranous and whitish, often featuring a brownish or greenish stripe, and their edges are non-ciliated. The inflorescence is lax, with 2-13 flowers per inflorescence, although a low number of flowers or even a reduction to a single flower is standard. The corolla comprises five obovate petals, split almost to the base. The calyx is formed by five lanceolate sepals, which are glabrous, glossy, and possess a white membranous margin. Typically, sepals are shorter than the petals. The flowers are white and proterandric, usually containing ten stamens. The ovary is superior, usually featuring three styles. Flowers of *S. palustris* are either hermaphroditic or male-sterile (Kurtto, 2001). The fruit is an oblong yellowish-brown capsule, and the seeds are rugose and dark brown. *S. palustris* blooms from early summer to late summer. It is an entomogamous species, with flies and small dipterous insects being the most common pollinators, while small bugs or bees are less frequent visitors.

Stellaria palustris belongs to the Laureate group, forming a monophyletic branch alongside morphologically similar species such as *S. graminea* and *S. persica* (Sharples & Tripp, 2019). From a morphological point of view, *S. palustris* appears to be a highly variable species, particularly in northern regions. Six entities with distinct phenotypes (see Table 1) are typically recognized in Scandinavia. These entities are identified based on plant height, overall appearance, degree of branching, length of internodes and branches, leaf texture, size, shape and direction, density of inflorescence, and flower size. However, extensive phenotypic plasticity and intermediates between these entities complicate clear discrimination, making it challenging and necessitating a comprehensive biosystematic investigation.

The chromosome base number of *S. palustris* is x = 13. While there are not many recorded chromosome counts, the most frequently reported count is 2n = 10x = 130, corresponding to a decaploid ploidy level (Peterson, 1936; Blackburn & Morton, 1957; Lövkvist & Hultgard, 1999; Stace, 2010). Other recorded chromosome numbers include 2n = 174-176, 179, 180, 182, 188, which can be attributed to 2n = 14x = 182, including euploid and aneuploid chromosomes (Kurtto, 2001; Morton, 2005). Based on existing literature, it is reasonable to infer that the studied species harbour a diversity hotspot in Northern Europe (Kurrto, 2001; Morton, 2005). The only reported genome size estimate from Central Europe (2C = 6.44 pg) presumably corresponds to the 10x cytotype (Šmarda et al., 2019).

Hybridization of *S. palustris* is reported to be only with *S. graminea*. This species is native to Europe, Siberia, and Western and Central Asia. While it is spread, it is not native to North America, Australia, and New Zealand (Kurtto, 2001). It thrives in nutrient-rich moist to wet sites, often found in periodically flooded meadows, fens, lakes or rivers, and even by the sea, in ditches, and peat pits. Entities of *S. palustris* also exhibit differential distribution across the species' range.

Entity	Description						
	Height [cm]	Leaves	Middle internodes [mm]	Branching	Inflorescence	Flowers	Sex of the flowers
A	30-60	thin, usually the same lenght or longer than internodes	30-60	up to 10	Lax, long and flexous pedicels	Large	Hermaphroditic
B	similar to entity A, but smaller						
C	10-45	thick, usually shorter than internodes	40	few	Variable, denser than entity A	Large	Hermaphroditic
D	20-50	thick and stiff, same length or longer than internodes	35	up to 8	Variable, denser than entity A	Small	Hermaphroditic
E	30-60	thin, shorter than the internodes	30-60	0-5	Dense, 1-8 flowers	Small	Nearly always ± male-sterile
F	20-30	thick, shorter than the internodes	25-30	0-3	Dense, 1-3 flowers	Small	Hermaphroditic or ± male-sterile

Table 1: *Morphological classification of the six different morphological entities of S. palustris as recognized by Kurtto (2001).*

1.3.2.2. Stellaria graminea L.

Stellaria graminea is a perennial herb with slender, creeping rhizomes and procumbent to ascending vegetative shoots. The stem is slender, ascending, quadrangular, branched, and glabrous. The leaves are slender, linear-lanceolate to elliptic-lanceolate, single-veined, with a smooth margin. They are widest at the base, gradually taper upwards from the stem base, ciliated at the base, and otherwise smooth. The bracts in the inflorescence are membranous and whitish, often with a brownish or greenish stripe and ciliated edges, at least in the lower third. The inflorescence is lax, with a variable number of flowers, ranging from several to numerous. The calyx consists of five lanceolate leaves, usually smaller than the corolla, with mostly ciliated outer sepals. The corolla comprises five petals, split almost to the base, and the flowers are white and proterandric. Stamens are ten, and they may be developed and fertile; some of them are degenerate and sterile (Horne, 1914; Kurtto, 2001; Morton, 2005). Three styles emerge from the superior ovary. The fruit is an ovate-oblong capsule with rugose, dark, or reddish-brown seeds. The blooming period starts in May and continues until October (Kurtto, 2001; Morton, 2005; Kučera et al., 2012). It is an entomoganous species commonly pollinated by flies and small dipterous insects. It is a gynodioecious species with the presence of both hermaphroditic and female plants (Kučera et al., 2021).

From an evolutionary standpoint, *S. graminea* belongs to the Laureate group, forming a monophyletic branch alongside morphologically similar species such as *S. palustris* and *S. persica* (Sharples & Tripp, 2019).

Stellaria graminea is a species characterized by high morphological variability influenced by environmental factors. Factors such as soil moisture and incident radiation significantly shape the development and form of its vegetative organs, including roots, stems, and leaves (Kurtto, 2001).

S. graminea exhibits a basic chromosome number of x = 13. Presently, three distinct ploidy levels have been identified for this species: diploid (2n = 2x = 26), triploid (2n = 2x = 39), and tetraploid (2n = 2x = 52) (Gadella, 1977; Harmaja, 1992; Kurtto, 2001; Morton, 2005; Kučera et al., 2012; Kučera et al., 2021). While diploid and tetraploid forms are commonly encountered in Europe, triploid forms appear exceedingly rare (Gadella, 1977; Morton, 2005; Kučera et al. 2021). Additionally, infrequent aneuploid cytotypes have been detected in Scandinavia, featuring 2n = 40–44 (Harmaja, 1992; Keshavarzi & Bozchaloyi, 2014).

Interspecific hybridization is reported frequently, with presumed hybrids involving *S. borealis and S. fennica* (Murb). Perf., *S. longifolia, S. palustris*, and *S. uliginosa* Murray (Kurtto 2001; Kučera et al. 2012). However, experimental studies have indicated that the hybrid progeny often exhibits significantly reduced seed and pollen quality, frequently resulting in complete sterility (Chinnappa, 1985; Kurtto, 2001).

This species is native to Europe, Western and Central Asia, having been introduced and spread to North America and New Zealand (Kurtto, 2001; Morton, 2005; Kučera et al., 2012). It thrives in open, sunny habitats with moderate nutrient levels, preferring drier sandy to humid clay soils but not subject to long-term waterlogging. Commonly found in meadows, open grassalnds butfrequently occures also on roadsides, fields, and ruderal sites (Horne, 1914; Kurtto, 2001; Morton, 2005; Kučera et al., 2012)

1.3.2.3. Stellaria longifolia Muhl. ex Willd.

Stellaria longifolia is a perennial herb characterized by slender, short roots. The stem is crowded, ascending, quadrangular, branched, and rough with small papillae. Leaves are linear to oblong, widest at the base, single-veined, and primarily ciliated. Stems and leaves present yellowish to bright green hues. Bracts are scarious, with smooth lower ones featuring green midribs. The inflorescence is lax, with a variable number of flowers. Petals are obovate, split almost to the base. Sepals are ovate, obtuse to acute, smooth with scarious edges, usually of the same length or slightly shorter than the petals. The flowers are white and proterandric. Stamens typically number ten, and the styles are usually 3–4, rarely up to 6, with the ovary superior. The fruit is an ovoid capsule, light brown to brownish red, and the seeds are brown and glossy. *S. longifolia* blooms from May to July (Kaplan et al., 2019).

This entomogamous species is usually pollinated by small dipterous insects, and its flowers are primarily hermaphroditic. However, rare instances of female flowers have been reported (Kurtto, 2001), suggesting the presence of gynodioecy.

The chromosome base number of *S. longifolia* is x = 13, and the species is known only in the diploid ploidy level (2n = 2x = 26). Hybridization in *S. longifolia* is reported only with S. *graminea* and *S. borealis*.

It is widespread in the boreal and northern parts of Eurasia and Northern America, but it is rare in the temperate zone (Kurtto 2001). It often grows on wet meadows, wet spruce forests, lake shores, peat pits, and dry stony slopes (Kurtto, 2001).

2. Methods

2.1. Sampling design

The primary objective of this thesis was to focus on *S. palustris*. To provide context for the variability observed in *S. palustris* and potentially elucidate the evolutionary aspects of this species, we additionally collected and co-analyzed population samples of *S. graminea* (both diploid and tetraploid) and *S. longifolia* (diploid). These species were selected due to their co-occurrence in both Central and Northern Europe, allowing for comparative insights into closely related taxa with lower ploidy levels.

Throughout the 2021, 2022, and 2023 vegetation seasons, we conducted extensive sampling in Central Europe (Czech Republic, Slovakia) and Northern Europe (Latvia, Lithuania, Estonia, Finland, Norway, Sweden, and Denmark) (Figure 1). The collected specimens included 28 populations of *S. palustris* (243 individuals), 34 populations of *S. graminea* (236 individuals), and six populations of *S. longifolia* (31 individuals) (Table 2).

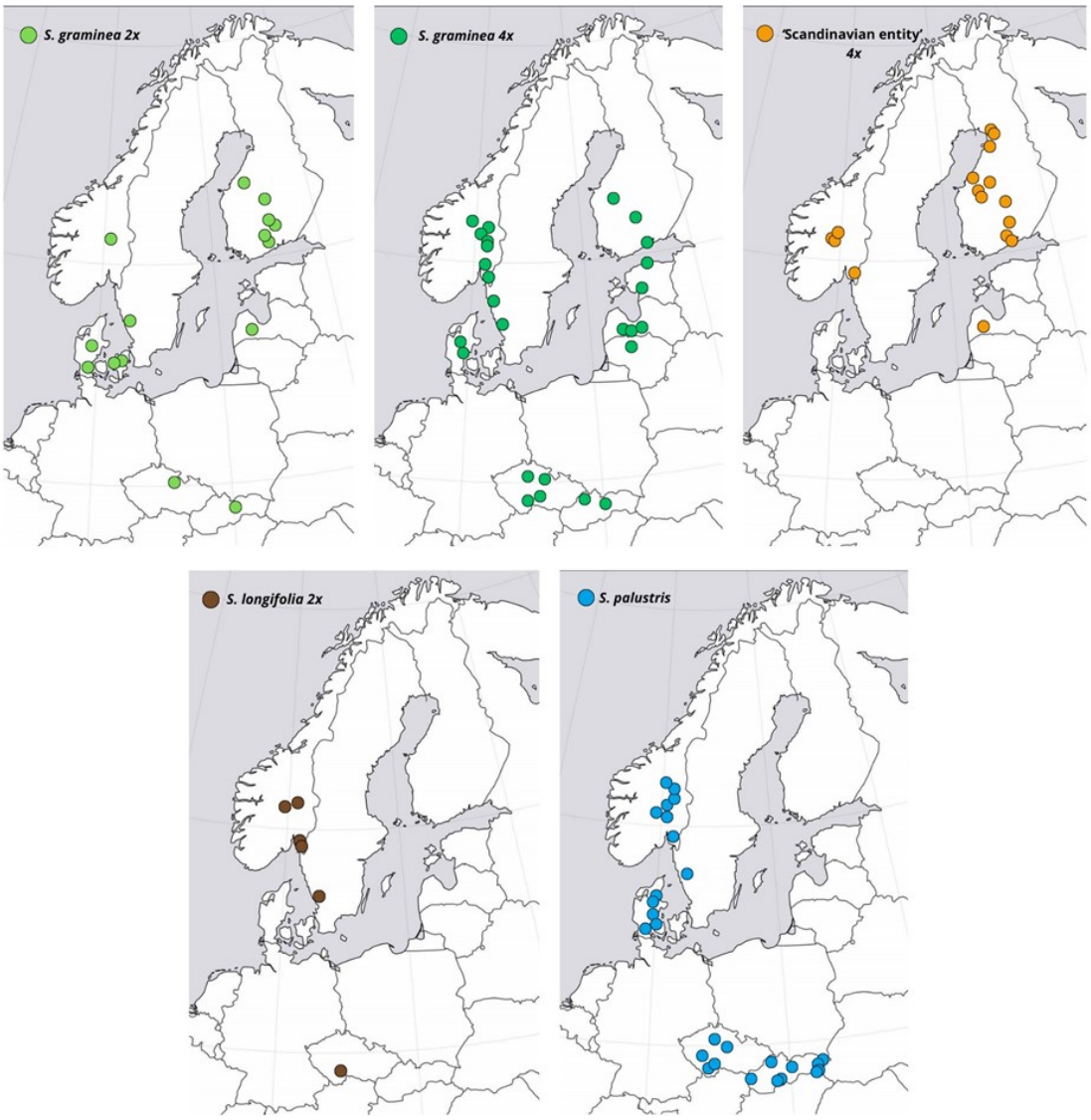


Figure 1: *Distribution maps of sampled populations of all collected species and cytotypes. Populations of the 'Scandinavian entity' were initially collected and attributed to the small-flowered S. palustris morphotype.*

Population	Country	Coordinates	Locality	S. palustris	S. graminea 2x	S. graminea 4x	Scandinavian entity'	S. longifolia
1 (15)	Slovakia	48.0925056N, 19.4199361E	Vrbovka, periodically flooded meadow.	42	x	x	x	x
2	Slovakia	48.5343269N, 16.9599011E	Abrod, Záhorská nížina, in the channel of the Malolevársky kanál.	8	x	x	x	x
3	Czechia	49.4327431N, 13.8221539E	Dolejší Pond near Tchořovice, waterlogged meadow.	7	x	x	x	x
4	Czechia	50.0876944N, 15.7121389E	Hrádek, Pardubice District, scattered in a dense thicket in a dried-up wetland.	3	2	2	x	x
5	Slovakia	48.8512200N, 20.0008200E	Pohorelá, along the Hron River, southwest of the village.	10	16	4	x	x
6	Czechia	48.9456492N, 14.7630581E	Hrachoviště, in the wetlands of Ruda Pond, north of the village.	4	x	3	x	x
7	Czechia	49.0382175N, 14.7930211E	Rožmberk Pond, wetlands, scattered among dense vegetation.	5	x	x	x	2
8	Czechia	49.1420717N, 15.1784325E	Budkov, a meadow along a dirt road, southwest of the village.	x	x	2	x	x
9	Slovakia	49.1811111N, 18.8144444E	Stráňavský Creek (Hýrov), Žilina.	18	x	12	x	x
10	Slovakia	48.4889959N, 22.0039482E	Poľany, Východný leleský canal, scattered in a dried-up channel.	9	x	x	x	x
11	Slovakia	48.3722022N, 21.8564992E	Strážne, an oxbow lake of Veľká Krčava.	2	x	x	x	x
12	Slovakia	48.7044347N, 22.0744436E	Iňačovce - a wet meadow by the Čierna Voda canal.	3	x	x	x	x
13	Slovakia	48.6670667N, 22.0360494E	Senné, wet meadows by the Čierna Voda canal, west of the village.	14	x	x	x	x
14	Slovakia	48.0728694N, 19.0396786E	Ipeľské Predmostie, the waterlogged meadow of Súdenina.	3	x	x	x	x
16	Finland	60.4547222N, 25.0616667E	Near the farm, in dense vegetation on the edge of the forest.	x	10	x	x	x
17	Finland	60.6818150N, 25.2125970E	Scattered in the forest south of Lake Kilpijärvi.	x	11	x	2	x
18	Finland	61.7000000N, 26.1000006E	The southern edge of Lake Vähä Saynätjärvi, in the waterlogged clearings.	x	18	x	x	x
19	Finland	61.8000000N, 25.7999994E	Edges of a mowed wet meadow at the forest's edge.	x	8	1	6	x
20	Finland	62.7348360N, 25.0309350E	The forest edge along a dirt road. In tall vegetation.	x	2	x	11	x
21	Finland	65.0792790N, 25.3875220E	Oulu, in the thickets on the edge of the Gulf of Bothnia on the outskirts of the city.	x	x	x	12	x
22	Finland	65.2266810N, 25.2860680E	The edge of the town of Martinniemi, near the beach on the shore of the Gulf of Bothnia.	x	x	x	9	x
23	Finland	64.8052778N, 24.5488889E	Siikajoki; The Baltic Sea coast, scattered around the rest area.	x	x	x	10	x
24	Finland	63.3434389N, 23.5306339E	A wet meadow near a farm, southwest of the village of Jokikylä.	x	3	x	10	x
25	Finland	63.0304622N, 23.8319481E	The roadside edge, in unmowed vegetation.	x	x	x	10	x
26	Finland	62.7869444N, 23.7383333E	North of the town of Virtala. In the ditch along the road.	x	x	x	11	x
27	Finland	62.7577778N, 23.7086111E	The north of the town of Virtala, in a meadow by the road.	x	x	1	9	x
28	Finland	60.1400000N, 24.9869444E	Helsinki - Suomenlinna - dry sunlit sites.	x	x	4	5	x
29	Estonia	59.3894444N, 24.6397222E	The channel of a ditch in a garden area on the outskirts of Tallinn. Waterlogged.	x	x	4	x	x
30	Estonia	58.3050614N, 24.5885822E	A rest area on the shore of the Baltic Sea, south of the city of Pärnu.	1	x	3	x	x
31	Latvia	56.6222222N, 23.2730556E	Dobeles; The bank of the town pond, in the park. Grasses, sedges, nettles.	x	x	12	x	x
32	Latvia	56.6394444N, 22.8608333E	Zebrus Pond; Meadow near the rest area, scattered, near the farm pond.	1	1	6	1	x
33	Latvia	56.6563889N, 23.7345278E	The riverbank, behind the nettle growth closer to the road (scattered in the grass between the road and the river, in the city).	x	x	4	x	x
34	Lithuania	55.8886111N, 23.2116667E	Overgrown area near the gas station; grasses, volunteer saplings; abundant.	x	x	11	x	x
35	Czechia	50.6031667N, 14.6242000E	From Stare Splavy camp to Provoding village, a wet meadow close to the railway.	3	x	2	x	x
NOR_1	Norway	58.2281042N, 11.9062669E	The village of Berg, around a parking lot in the meadow and in the thickets.	x	3	2	x	x
NOR_2	Norway	60.8897540N, 9.4144070E	In the ditch by the road at the edge of a dense forest.	x	x	x	12	x
NOR_3	Norway	61.8367500N, 9.2766667E	Around a ditch near the Otta River.	x	x	8	x	x
NOR_4	Norway	60.8897540N, 9.4144070E	A waterlogged forest and meadow, north of the town of Aurdal.	x	x	x	13	6
NOR_5	Norway	61.5184030N, 10.1573500E	Southeast of the town of Ringebu. A waterlogged area around a ditch.	10	x	4	x	x
NOR_6	Norway	61.4247970N, 10.2227380E	An overgrown, dried-up pond. Very abundant, with large clumps.	10	x	x	x	x
NOR_7	Norway	61.2170180N, 10.3680316E	Dried-up wetlands and waterlogged meadows. Southwest of the town of Hafjell.	x	2	3	x	3
NOR_8	Norway	60.9700260N, 10.6067860E	Clearings in a sparse forest around the Vismunda River. At the point where the river flows into Lake Mjøsa.	11	x	x	x	x
NOR_9	Norway	60.9131790N, 10.6649160E	A waterlogged edge of Lake Mjøsa.	7	x	3	x	x
NOR_10	Norway	60.8851220N, 10.6812560E	A maintained shore of Lake Mjøsa.	11	x	2	x	x

NOR_11	Norway	59.7183730N, 11.0560690E	A waterlogged edge of Lake Mjaer, southeast of the town of Ytre Enebakk.	4	x	3	x	x
NOR_12	Norway	59.4380922N, 10.8053854E	A dense waterlogged forest. Scattered in the brighter areas.	x	x	x	x	9
NOR_13a	Norway	59.1391615N, 11.4278909E	The forest edge on the northeastern outskirts of the city of Halden. A dry clearing.	x	x	x	x	10
NOR_13b	Norway	59.1395475N, 11.4312214E	Dry vegetation along a dirt road.	x	x	8	2	x
NOR_14	Sweden	57.8652400N, 12.0201400E	At the edge of a dense reed bed.	1	x	x	x	x
NOR_15	Sweden	56.7313600N, 12.8817800E	A dry meadow north of the city of Halmstad.	x	x	8	x	1
NOR_16	Denmark	55.5587230N, 12.2349010E	Along the Karlstrup Mosebaek stream.	x	10	x	x	x
NOR_17	Denmark	55.6005780N, 11.3750300E	A waterlogged, mowed meadow near Lake Torsø. North of the village of Jyderup.	x	11	x	x	x
NOR_18	Denmark	55.4288250N, 9.9524300E	The edge of a pond east of the town of Ejby. A waterlogged meadow.	11	x	x	x	x
NOR_19	Denmark	56.6956290N, 9.9970910E	A waterlogged bank of the Villestrup A River. East of the town of Oue.	10	x	x	x	x
NOR_20	Denmark	56.4495040N, 9.6777910E	A waterlogged meadow on the bank of the Nørea River.	10	x	x	x	x
NOR_21	Denmark	56.2154660N, 9.4943580E	The northwest of the town of Skaegkaer. A dried-up ditch.	x	5	6	x	x
NOR_22	Denmark	56.0531670N, 9.8332990E	Alken, north of the town. Wetlands, scattered in shady areas.	11	x	x	x	x
NOR_23	Denmark	55.8410350N, 9.5081010E	Tørring, southeast of the town. A waterlogged meadow.	x	x	10	x	x
NOR_24	Denmark	55.0767820N, 9.3604600E	Northeast of the town of Rødekro. A meadow with moisture-loving vegetation.	14	6	x	x	x
*'Scandinavian entity' - populations originally collected as S. palustris, later recognized as 'Scandinavian entity', see results later								

Table 2: *Details of population sites collected within the studied area*

Our sampling methodology aimed to collect ten individuals per population whenever feasible. However, material from all available individuals was carefully gathered in small populations (with fewer than ten individuals). For each plant collected, we obtained a living specimen for cultivation, plant material dried in silica gel for subsequent analyses (including flow cytometry - FCM and genetic analyses), herbarium specimens, and preserved flower parts for morphological analyses. Additionally, whenever possible, seeds from the plants were also gathered. To minimize potential biases in our final results, we took special care to address potential issues related to clonality. We collected individuals from individual plycormons that were spatially separated by a minimum of 20 meters, indicating there were no evident signs of direct interconnection between them. This approach enabled to minimize the possibility of including clones in our analyses.

The collected living plant cuttings were planted in coconut fibre pots in a mixture of peat and perlite (in a 1:2 ratio). Once they had rooted, each individual was transferred to a separate plastic pot, and we carefully monitored them to prevent long shoots from rooting in neighbouring pots. In the case of infestation by various insect pests, the plants underwent systematic spraying of insecticides. Subsequently, the cultivated plants were moved to the experimental greenhouse facilities of the Department of Botany, Charles University Prague.

2.2. Genetic analyses

Most published studies incorporating genetic data on the analyzed *Stellaria* species have primarily focused on whole-genus phylogenies. Consequently, they have typically included only a minimal number of accessions of the species analyzed herein, often limited to single representatives, and notably, they lack information regarding ploidy levels (e.g., Harbaugh et al., 2010; Sharples et al., 2019; Yao et al., 2021). To gain preliminary yet comprehensive insights into the genetic variability and interrelationships among the studied *Stellaria* species and their populations, we conducted genotyping on 72 individuals from 35 populations, whenever feasible, selecting 1-3 individuals from each collected population using classical Sanger sequencing techniques. Sequences from both coding and non-coding regions of nuclear ribosomal DNA (nrDNA) and chloroplast DNA (cpDNA) were employed.

2.2.1. DNA extraction

DNA from the analyzed samples was extracted using a DNA sorbitol extraction method. Approximately 0.5 g of dried material from silica gel was placed in Eppendorf tubes with two wolfram carbide beads and ground using a Retsch Mixer Mill 400. Each tube was then supplemented with 1300 µl of Extraction Buffer (EP), 5 µl of RNAseA, and PVP, thoroughly mixed, and allowed to incubate at room temperature for 20 minutes. Subsequently, the tubes were centrifuged at 7000 rpm for 5 minutes, and the supernatant was carefully discarded. To the resulting pellet, 300 µl of EP and 300 µl of Lysis Buffer (LP) were added, and the solution was mixed before transferring the tubes to a pre-heated thermomixer set at 65°C and 300 rpm for 15 minutes. Following this incubation, 600 µl of chloroform: isoamyl alcohol (24:1) was added and mixed, and the tubes were centrifuged at 9000 rpm for 10 minutes. The upper aqueous phase (600 µl) was pipetted into new labelled tubes and supplemented with 400 µl of ice-cold isopropanol. The tubes were stored at -20°C for 30 minutes, followed by centrifugation at 4°C for 15 minutes at 13000 rpm. After removing the supernatant, 700 µl of 80% ethanol was added to the tubes, mixed thoroughly for 2–5 minutes, and centrifuged again for 2 minutes at 13,000 rpm. The supernatant was carefully discarded, and the pellet was air-dried at room temperature before being transferred to a thermomixer and dried at 60°C for 10 minutes to eradicate residual ethanol. Finally, 45 µl of TE buffer was

added to the dried pellets, and the tubes were placed in the fridge overnight at 4 °C to allow the pellets to dissolve slowly.

2.2.2. DNA region amplification

Genetic variability was assessed using the nuclear ribosomal ITS1-5.8S-ITS2 region and two chloroplast DNA (cpDNA) regions. Initially, eight non-coding regions of cpDNA were screened for variability (psbJ-petA, trnK-trnH, trnF-f-trnL-c, trnH-psbA, rpoB-trnC, psbC-trnS, trnV-rbcL and K1F-K2R) (Taberlet et al. 1991; Steele et Vigalys 1994; Demesure et al. 1995; Dumolin-Lapegue et al. 1997; Manos et Steele 1997; Sang et al. 1997; Tate & Simpson 2003; Shaw et al. 2005, 2007). Following preliminary analysis, two regions demonstrating the highest variability and consistent amplification and sequencing success were selected for final analyses. These regions included the matK partial gene and flanking intergenic spacer K1F-K2R and the psbJ-petA intergenic spacer. Amplification of the ITS region was performed using primers ITS4 and ITS5 (White et al., 1990), while primers K1F and K2R were employed for matK amplification (Steele et Vilgalys, 1994; Manos et Steele, 1997). Primers psbJ and petA were used to amplify the psbJ-petA region (Shaw et al., 2007). Polymerase chain reactions were conducted in a total volume of 20 µl, comprising 14.2 µl of ddH2O, 1 µl of template DNA, 1 U MyTaq DNA polymerase (Bioline), 4 µl of 5× MyTaq buffer (Bioline), and 0.3 µl of 10 mM each forward and reverse primers. The PCR conditions were as follows: 1) ITS1-5.8S-ITS2 region: initial denaturation at 95°C for 60 s, followed by 35 cycles of denaturation at 95°C for 20 s, annealing at 53°C for 30 s, extension at 72°C for 45 s, and final extension at 72°C for 7 min, 2) matK region: initial denaturation at 95°C for 60 s, followed by 35 cycles of denaturation at 95°C for 15 s, annealing at 53°C for 30 s, extension at 72°C for 90 s, and final extension at 72°C for 7 min; 3) psbJ-petA region: initial denaturation at 95°C for 60 s, followed by 35 cycles of denaturation at 95°C for 15 s, annealing at 52°C for 25 s, extension at 72°C for 45 s, and final extension at 72°C for 7 min. PCR products were initially checked on a 1% TAE agarose gel for quality.

Magnetic Solid Phase Reversible Immobilization (SPRI) beads were employed to purify PCR products. To eliminate fragments shorter than 800 bp, the volume of PCR product was multiplied by 0,8. Thus, for the purification, 17 µl of PCR product and 13,6 µl (0,8 x 17) were mixed and left at a temperature room for 1 minute. After removing the clear supernatant, 200 µl of 85% ethanol was added to SPRI bands and incubated at room temperature for 30 seconds, and after removing ethanol, this step was repeated. The plate was removed from the magnetic stand, and 50 µl of ddH2O was added and mixed with SPRI beads. When the SPRI beads were resuspended, the mixture was incubated at room temperature for 1 minute. Then, the plate was placed back on the magnetic stand where the SPRI beads settled to the magnet. The clear eluate was transferred to the newly labelled stripe tubes.

DNA concentration was measured on a Nanodrop prior to the sequencing reaction. The mixture included 6 µl ddH2O, 1 µl primer (3,2 pmol), and 1 µl DNA (80 ng/µl). All three selected regions were sequenced only using the forward primers ITS5, K1F, and psbJ, respectively. PCR products were sequenced in BIOCEV, Faculty of Science, Charles University, where the sequencing proceeded.

2.2.3. Genetic data processing and analyses

Sequences were edited and aligned using Geneious Prime R24.0.3 (Biomatters Ltd., Auckland, New Zealand). Polymorphic sites were coded using IUPAC ambiguity codes (Cornish-Bowden, 1985). Due to limited informative indels, especially within the ingroup, these were treated as missing data. For phylogenetic analyses, diverse outgroup taxa from the genus *Stellaria*, available in GenBank, were employed.

Four data matrices were prepared:

Matrix_G_1 (145 individuals and 630 bp): This matrix comprised all available ITS sequences, comprising entire ITS1_5.8S-ITS2 region of *S. palustris, S. graminea*, and S. *longifolia*. It incorporated 69 sequences generated within this study, additional 15 sequences of *S. graminea* from the Carpathians (Slovakia, Ukraine, and Romania) generated within a yet unpublished study led by a supervisor. For population codes and locality details, see Kučera et al. (2021). Finally, 12 sequences of all three species available in GenBank (labelled in figures with GenBank accession numbers), including three accessions of *S. palustris*, six of *S. graminea*, and three of *S. longifolia,* were also co-analysed. In case of accessions from our unpublished study, the ploidy level was estimated in Kučera et al. (2021), while no ploidy level information was available for GenBank accessions.. Altogether, 96 ITS sequences belonging to *S. palustris, S. graminea*, and *S. longifolia* were analyzed. Additionally, the dataset was supplemented with 49 ITS sequences from 20 closely or more distantly related *Stellaria* taxa.

Matrix_G_2 (73 individuals and 1379 bp): This matrix was based on concatenated datasets of matK (alignment of 816 bp) and psbJ-petA (563 bp) regions. It comprised 72 sequences of cpDNA generated in this study for *S. palustris, S. graminea*, and *S. longifolia*, along with a single outgroup sequence available in GenBank for both cpDNA regions*, S. neglecta*.

Matrix_G_3 (69 individuals and 2006 bp): This matrix was based on concatenated datasets of ITS (alignment of 627 bp), matK (alignment of 816 bp), and psbJ-petA (563 bp) regions. It included 68 sequences generated in our study for *S. palustris, S. graminea*, and *S. longifolia*, along with a single outgroup sequence available in GenBank for all three amplified regions, *S. neglecta*.

Matrix_G_4 (71 individuals and 1345 bp): This dataset comprised concatenated sequences from the matK and psbJ-petA regions, encompassing 71 cpDNA sequences obtained from our study for *S. palustris, S. graminea*, and *S. longifolia*, without any outgroup taxa.

Phylogenetic relationships were analyzed using Bayesian inference (BI) conducted in MrBayes 3.2.7a (Ronquist & Huelsenbeck, 2003; Ronquist et al., 2012). Prior to phylogenetic analyses, the best-fit models of nucleotide substitutions were determined independently for each partition of the nucleotide data using jModelTest v.2.1.6 (Darriba et al., 2012) based on the Akaike information criterion (AIC; Akaike, 1974). The following models were inferred for specific regions: ITS1 and ITS - TPM1uf+G, ITS_5.8S - F81+G, matK – F81; psbJ-petA – TrNef. Indels present in the sequence alignments were treated as missing data. Bayesian analyses were conducted with four Markov chain Monte Carlo (MCMC) chains and two independent runs for 10 million generations, with a sampling frequency of every 1000th generation. The first 10% of trees were discarded as 'burn-in'. Analyses were run on the CIPRES Science Gateway (Miller et al., 2010). Phylogenetic trees wesamples,lized, and posterior probability (PP) values were appended using FigTree (v1.4.4). Posterior probability values of 0.95 and above were considered significant, while values below 0.95 were non-significant.

After conducting the initial network analysis, encompassing all samples including numerous and distant outgroup taxa (NN_1), we search for a finer structural resolution, particularly within our focal group. In the subsequent refinement, the vast majority of outgroup taxa were excluded, retaining only *S. calycantha* and *S. borealis*, which were found to cluster within the *S. longifolia* clade. Additionally, genebank sequences of *S. palustris*, *S. graminea*, and *S. longifolia* formed a separate clade with other related outgroup species,and accession SE_28_3, which seems to be hybrid of with *S. longifolia* lineage, were omitted (NN_2). To further refine the analysis, sequences of accessions of our three studied species located in the central part of the network were also removed, aiming to elucidate the structure and relationships among major lineages while minimizing the influence of evident hybrids.

For visualizing the relationships among cpDNA haplotypes, we utilised the parsimony method (Templeton et al., 1992) using TCS version 1.21 (Clement et al., 2000) with the cpDNA dataset (Matrix_B_G). A 90% connection limit for parsimonious connections was applied, with indels treated as missing data during the network construction process.

2.3. Karyological analyses

2.3.1. Flow cytometry

Absolute genome size content (AGS) and relative genome size content (RGS) were estimated using propidium iodide (PI-FCM) and 4,6-diamino-2-phenylindole (DAPI-FCM) flow cytometry, respectively. Overall, for absolute genome size we analyzed 54 individuals of *S. palustris* from 19 populations, 31 individuals of *S. graminea* from 17 populations, 6 individuals of *S. longifolia* from 5 populations and 20 individuals of ‚Scandinavian entity' from 11 populations. RGS was estimated for all analyzed samples across species, encompassing 633 individuals from 59 populations (see Table 1 in the appendix). Three individuals from each population were measured whenever feasible to estimate AGS. However, in some cases, living plants did not survive and could not be analyzed.

For RGS estimation using DAPI_FCM, leaves dried in silica gel were used and directly collected from field plants. The primary standard utilized was *Bellis perennis* (2C DNA = 3.38 pg; Schönswetter et al., 2007), as established in a previous Kučera et al. (2021) study. However, due to the overlap of the cytotype of presumed *S. palustris* moslty from Finland but also Norway and Lithuania with the primary standard in the peak position, an alternative standard, *Solanum pseudocapsicum* (2C DNA = 2.56 pg; Temsch et al., 2010), was employed for measurements of this cytotype. The dried plant material was cut with the standard in Petri dishes using a razor blade in 600 µl of Otto I buffer (0.1 M monohydrate citric acid, 0.5% Tween 20; Otto, 1990). The resulting solution was then pipetted through a filter into a tube, mixed with 700 µl of a pre-prepared dye mixture composed of 20-25 ml Otto II (solution of 0.4 M Na2HPO4 12H2O), 1 ml DAPI stock solution, and 50 µl β-mercaptoethanol. Measurements were conducted at a speed of 10-15 nuclei per second, up to 3000 nuclei. Relative genome size was calculated as the ratio between the sample positions and standard G0/ G1 peaks. The analyses were performed utilizing the Partec CyFlow ML equipped with a UV LED and fluorochrome as an excitation source at the Department of Botany, Faculty of Science, Charles University.

In the case of AGS, the procedure for sample preparation was the same as that for the measurement of relative genome size. The exception was the solution for analyses, which was composed of a mixture of 20-25 ml Otto II, 1 ml PI stock solution, 1 ml RNase, and 50 µl β-mercaptoethanol. Measurements were performed at a speed of 10-15 nuclei per second, up to a total of 5000 nuclei. Each individual was measured three times. The final genome size was calculated as an average of the three measurements, where the maximal difference between each measurement was not higher than 3%. *Bellis perennis* and *Solanum pseudocapsicum* were used as primary and secondary standards, respectively. Only histograms exhibiting symmetrical peaks and demonstrating a coefficient of variation (CV) of the sample G1 peak below 3% were taken into consideration. The AGS (2C value) estimation was computed as the ratio of the sample G0/G1 peak position to the standard G0/G1 peak position multiplied by the 2C DNA content of the standard (pgDNA; Doležel & Bartoš, 2005). Regarding the estimation of monoploid genome size, for all diploid and tetraploid cytotypes, monoploid genome size was calculated by dividing the AGS by the ploidy of the cytotype. The exception is *S. palustris*, where, due to the wide variation in genome sizes observed within

ploidy levels, monoploid genome size was determined exclusively in individuals with counted chromosomes.

Samples were measured on the Partec CyFlow SL equipped with a green solid-state laser (Cobolt Samba 532 nm, 100 mW) at the Department of Botany, Faculty of Science, Charles University. All analyses were evaluated using Partec FloMax software v. 2.4.

2.3.2. Statistical analyses

Differences between populations of *S. palustris* in Central and Northern Europe were assesed using parametric Mann-Whitney U test for both AGS and RGS.

The correlation between latitude and genome sizes in *S. palustris* was examined using Spearman's rank correlation coefficient. Variations in monoploid genome size among all cytotypes of studied taxa were evaluated using One-Way ANOVA. Variation in monoploid genome size within *S. palustris* was evaluated using Student's T-test.

2.3.3. Chromosome counting

The ploidy level inference, based on direct chromosome counting combined with DAPI flow cytometric analysis of diploid and tetraploid *S. graminea*, was previously conducted in a large-scale cytogeographic study in the Carpathians by the supervisory team. Therefore, a direct comparison of the DAPI measurements presented here with those previously published enabled unambiguous ploidy determination of the *S. graminea* accessions. Similarly, diploid *S. longifolia* was analyzed by Šmarda et al. (2019). To this end, direct chromosome counting was performed only for *S. palustris* and a cytotype from Northern Europe, morphologically resembling *S. palustris* but with smaller flowers. The RGS of this cytotype appears to be intermediate between diploid and tetraploid *S. graminea* and we named it with tentative name 'Scandinavian entity'. Specifically, 13 individuals from 9 populations of *S. palustris* and 4 individuals from 4 populations of the 'Scandinavian entity' were analyzed, with the kind collaboration of Magdalena Lučanová from the Department of Botany, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

Fixations of young root tips from cultivated plants followed the protocol outlined by Mandakova and Lysak (2016). Fresh root tips were placed into 1.5 ml Eppendorf tubes containing p-dichlorobenzene and left at room temperature for 3 hours. Subsequently, they were transferred to a freshly prepared fixative solution composed of ethanol and acetic acid in a 3:1 ratio (v/v) and stored overnight in a refrigerator set to approximately 4°C. The material was then stored at -20°C in the fixative until further use. The root tips underwent a series of washing steps: first, they were rinsed twice with distilled water for 5 minutes each time. Then, a citrate buffer was applied, and the roots were washed in an orbital shaker for two 5-minute cycles. Following this, the buffer was removed from the sample, and a mixture of pectolytic enzymes (containing pectolyase, cellulase, and cytohelicase) at a concentration of 0.3% was added, followed by an incubation period in an incubator set to 37°C for 120 minutes. The enzyme mixture was then replaced with fresh citrate buffer. The root tip meristems were examined under a stereomicroscope, the excess buffer was removed, and the sample was treated with 60% acetic acid for 1-2 minutes. The root meristem was disintegrated using dissecting needles, and the resulting meristematic suspension was covered with a cover slip. The slide was passed over a flame 2-3 times, and the material was gently squashed. The slides were then placed in a freezer at approximately -80°C, and after 10 minutes, the coverslips were separated from the slides using a razor blade. The samples were subsequently stained with 15 µl of Vectashield containing 4',6-diamidino-2-phenylindole (DAPI). After covering the preparations with new coverslips, they were fixed with nail polish. Chromosomes were

visualized using a Nikon Eclipse E600 microscope equipped with a Nikon DS-Qi1Mc camera, and images were captured using NIS-Elements AR software.

2.4. Morphological analysis

Morphological variation across different cytotypes, populations, and sexual expressions within the studied taxa was investigated using multivariate morphometrics. Consistent with a previous study on the closely related *S. graminea* (Kučera et al., 2021), eight floral characters were measured or scored. Stems and leaf-associated traits were considered irrelevant for morphometric assessment due to their susceptibility to ecologically driven phenotypic variability (cf. Kučera et al., 2021). To maintain the original character parameters of floral organs and ensure precise measurements and scoring, fresh flowers were dissected using a razor blade, and relevant parts were affixed to paper with translucent adhesive tape. Each paper sheet was then scanned with an appropriate scale, and traits were measured using the ImageJ program (Schneider et al. 2012). The resulting morphological dataset underwent analysis in RStudio (version R-4.1.1).

The morphometric analysis were performed on flowers from 370 individuals originating from 50 populations. Data matrices were constructed based on measurements from individuals or populations (represented by mean values of each character) as operational taxonomic units (OTUs) to assess differences between cytotypes or sexual morphs. *S. longifolia* was excluded from analyses due to the minimal number of flowering plants available. Additionally, characters associated with stamen morphology and functionality were scored as three separate binary traits: 1) all stamens well-developed, 2) all stamens vestigial, and 3) at least one stamen fertile and well-developed, but the rest are vestigial. These traits were scored as 1 for present and 0 for absent in all three cases.

Before the analyses, QQ plots were generated to visualize the normal data distribution, and Pearson's correlation coefficient was calculated. Due to a high correlation (>0.9) (Table 3), the character DPI was excluded from further analysis (Table 4). Subsequently, Pearson (parametric) or Spearman (non-parametric) correlation coefficients were computed depending on data type to identify highly correlated characters, which could potentially distort subsequent computations, particularly in discriminant analyses. The subsequent analyses included the following characters: maximum length of the petal (LP), maximum width of the petal (WP), maximum length of the sepal (LS), maximum width of the sepal (WS), maximum width of the membranous sepal margin (WMM), length from the base to the widest part of the sepal (LBS), maximum length of the ovary (LO), and maximum width of the ovary (WO).

	LP	WP	DPI	LST	LO	WO	LS	WS	WMM	LBS
LP	1	0.791	**0.978**	0.456	0.415	0.475	0.65	0.613	0.201	0.342
WP	0.791	1	0.777	0.428	0.418	0.493	0.629	0.687	0.273	0.393
DPI	**0.978**	0.777	1	0.425	0.386	0.448	0.608	0.58	0.197	0.324
LST	0.456	0.428	0.425	1	0.586	0.67	0.561	0.538	0.105	0.233
LO	0.415	0.418	0.386	0.586	1	0.835	0.521	0.527	0.115	0.279
WO	0.475	0.493	0.448	0.67	0.835	1	0.561	0.599	0.135	0.257
LS	0.65	0.629	0.608	0.561	0.521	0.561	1	0.754	0.423	0.483
WS	0.613	0.687	0.58	0.538	0.527	0.599	0.754	1	0.35	0.463
WMM	0.201	0.273	0.197	0.105	0.115	0.135	0.423	0.35	1	0.182
LBS	0.342	0.393	0.324	0.233	0.279	0.257	0.483	0.463	0.182	1

Table 3: *The values of the Pearson correlation coefficient for each pair of characters computed in the dataset. Characters with correlation above 0.9 are marked in red.*

	LP	WP	LST	LO	WO	LS	WS	WMM	LBS
LP	1	0.791	0.456	0.415	0.475	0.65	0.613	0.201	0.342
WP	0.791	1	0.428	0.418	0.493	0.629	0.687	0.273	0.393
LST	0.456	0.428	1	0.586	0.67	0.561	0.538	0.105	0.233
LO	0.415	0.418	0.586	1	0.835	0.521	0.527	0.115	0.279
WO	0.475	0.493	0.67	0.835	1	0.561	0.599	0.135	0.257
LS	0.65	0.629	0.561	0.521	0.561	1	0.754	0.423	0.483
WS	0.613	0.687	0.538	0.527	0.599	0.754	1	0.35	0.463
WMM	0.201	0.273	0.105	0.115	0.135	0.423	0.35	1	0.182
LBS	0.342	0.393	0.233	0.279	0.257	0.483	0.463	0.182	1

Table 4: *The values of the Pearson correlation coefficient for each pair of characters computed in the dataset after excluding character DPI.*

Finally, suites of canonical discriminant analysis (CDA) were employed to test for potential differences between cytotype or sexual expression morphs.

In analyses where cytotype was used to predefine class, the following groups were created: 1) diploid cytotype (represented by diploid *S. graminea*) – diploid1 hereafter; 2) tetraploid cytotype A (represented by tetraploid *S. graminea*) – tetraploid1 hereafter; 3) tetraploid cytotype B (represented by tetraploids of the 'Scandinavian entity' with genome size between diploid and tetraploid *S. graminea*) – tetraploid2 hereafter; 4) polyploid cytotype complex (including a heterogeneous mixture of all high polyploids belonging to *S. palustris*) – high_polyploid hereafter.

In analyses where sexual morph was used to predefine groups, the following groups were created: 1) hermaphrodite morph (all stamens well-developed), 2) female morph (all stamens vestigial), and 3) intermediate (transitional) morph (at least one stamen fertile and well-developed, but the rest are vestigial) (Figure 2).

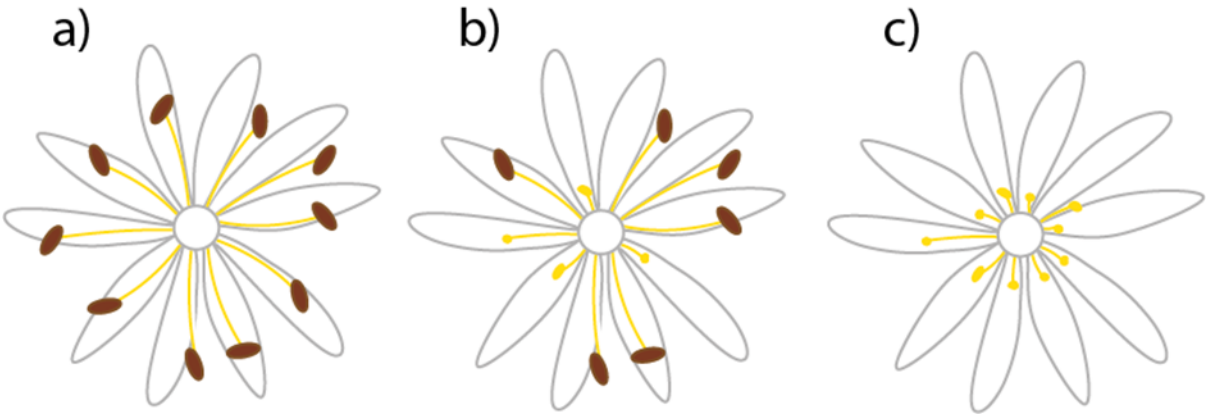


Figure 2: *Schematic illustration depicting various sexual morphs in Stellaria palustris:* ***a)*** *hermaphroditic morph with well-developed stamens* ***b)*** *intermediate morph with partly developed stamens* ***c)*** *female morph with undeveloped stamens*

To summarize morphological differences resulting from multivariate analyses, exploratory data analyses were performed and displayed as box plots, illustrating standard statistical parameters such as the median, the 10th, and the 25th percentiles (Figure 20).

CDA's based on cytotype as a criterion for class assignment

1. Matrix_M1 (370 individuals and 9 traits): this matrix includes the entire dataset with individuals as OTU's, encompassing diploid1, tetraploid1, tetraploid2, and high polyploid cytotypes. CDA1 was conducted to explore potential morphological differences between cytotypes at the individual level.

2. Matrix_M2 (50 populations and 9 traits): this matrix comprises the entire dataset with population mean values of each character as OTUs, including diploid1, tetraploid1, tetraploid2, and high polyploid cytotypes. Populations with measurements on fewer than three individuals were excluded. CDA2 aimed to investigate potential morphological differences between cytotypes at the population level.

3. Matrix_M3 (230 individuals and 9 traits): this matrix includes only hermaphrodites of diploid1, tetraploid1, tetraploid2, and high polyploid cytotypes as OTUs, excluding females and intermediates to eliminate the influence of sexual expression on morphological traits. CDA3 was performed to assess potential morphological differences between cytotypes at the individual level while mitigating the effects of sexual expression.

4. Matrix_M4 (30 populations and 9 traits): this matrix includes diploid1, tetraploid1, tetraploid2, and high polyploid cytotypes with population mean values of each character as OTUs. Females and intermediates were excluded to eliminate the influence of sexual expression on morphological traits. CDA4 aimed to explore potential morphological differences between cytotypes at the population level while controlling for sexual expression.

5. Matrix_M5 (84 individuals and 9 traits): this matrix includes only females of diploid1, tetraploid1 and high polyploid cytotypes as OTUs. CDA5 aimed to investigate whether the cytotypes differs morphologically even within females only. Tetraploid2 was excluded from the analysis because in the all collected individuals only four were scored as females.

6. Matrix_M6 (59 individuals and 9 traits): this matrix includes only intermediate individuals of diploid1, tetraploid1, tetraploid2 and high polyplois cytotypes as OTUs. CDA6 was conducted to investigate morphological differentiation between cytotypes of intermediate morphs.

7. Matrix_M7 (254 individuals and 9 traits): this matrix comprises individuals of diploid1, tetraploid1, and tetraploid2 cytotypes as OTUs, excluding the HPx cytotype. All sexual morphs were included. CDA7 was conducted to investigate potential morphological differences among lower ploidy cytotypes.

8. Matrix_M8 (29 populations and 9 traits): this matrix consists of populations of diploid1, tetraploid1, and tetraploid2 cytotypes as OTUs, excluding the HPx cytotype. All sexual morphs were included. CDA8 was performed to investigate the difference in morphological traits among lower ploidy cytotypes.

9. Matrix_M9 (174 individuals and 9 traits): this matrix includes only individuals of tetraploid cytotypes tetraploid1 and tetraploid2 as OTUs, encompassing all sexual morphs. CDA9 aimed to explore potential morphological differences among tetraploid cytotypes.

10. Matrix_M10 (116 individuals and 9 traits): this matrix includes individuals of high polyploid cytotype of Central and Northern Europe as OTUs. All morphs are included. CDA10 aimed to investigate morphological difference between individuals from Central and Northern Europe of high polyploid cytotype.

11. Matrix_M11 (45 individuals and 9 traits): This matrix includes only individuals representing high polyploid cytotype with well-developed stamens of Central and Northern Europe as OTUs. CDA11 aimed to investigate morphological distinction between individuals from Central Europe of high polyploid cytotype. Only hermaphrodite individuals with well-developed stamens were included to avoid biased results coming from different size of flowers woth different sex.

CDA's Based on sexual expression as a criterion for class assignment

The tetraploid cytotype belonging to the Scandinavian entity was not considered here due to significantly decreased variability in sexual expression.

12. Matrix_M12 (80 individuals and 9 traits): this matrix includes individuals representing all three sexual morphs of the 2x cytotype as OTUs. CDA12 aimed to assess potential morphological distinctions among females, hermaphrodites, and intermediate sexual morphs in diploid cytotypes.

13. Matrix_M13 (86 individuals and 9 traits): This this matrix includes individuals representing all three sexual morphs of the tetraploid1 cytotype as OTUs. CDA13 aimed to evaluate potential morphological distinctions among females, hermaphrodites, and intermediate sexual morphs in 4xA cytotype.

14. Matrix_M14 (116 individuals and 9 traits): This matrix includes individuals representing all three sexual morphs of the high polyploid cytotype as OTUs. CDA14 aimed to examine potential morphological distinctions among females, hermaphrodites, and intermediate sexual morphs in HPx cytotype.

15. Matrix_M15 (73 individuals and 9 traits): This matrix includes individuals representing only females and hermaphrodites of the diploid1 cytotype as OTUs. CDA15 aimed to explore potential morphological distinctions between females and hermaphrodites of 2x cytotype without including intermediate sexual morphs.

16. Matrix_M16 (76 individuals and 9 traits): This matrix includes individuals representing only females and hermaphrodites of the tetraploid1 cytotype as OTUs. CDA16 aimed to investigate potential morphological distinctions between females and hermaphrodites of 4xA cytotype without including intermediate sexual morphs.

17. Matrix_M17 (84 individuals and 9 traits): This matrix includes individuals representing only females and hermaphrodites of the high polyploid cytotype as OTUs. CDA17 aimed to assess potential morphological distinctions between females and hermaphrodites of HPx cytotype without including intermediate sexual morphs.

2.5. Sexual expression tests

We investigated the spatial distribution of sexual expression and its correlation with latitude in *S. palustris*. Our analysis involved 116 individuals of *S. palustris*. Species *S. graminea* and *S. longifolia* were not included in the correlation analysis with latitude. The decision to exclude *S. graminea* stemmed from our primary focus on *S. palustris*. While *S. graminea* is widespread and abundant throughout the studied region, we primarily collected populations of *S. graminea* for comparative purposes, gathering only 236 individuals from Central and Northern Europe. Such limited sampling could significantly bias the final inference regarding the frequency of sexual morphs within specific populations and regions. Similarly, *S. longifolia* was excluded from the analysis due to the collection of very few individuals exhibiting well-developed flowers (8), rendering any meaningful evaluation of such a small sample size untenable.

The assessment of sexual expression in *S. palustris* was further constrained to populations with more than three individuals scored for sexuality. Although this number is also low, unlike *S. longifolia* and especially *S. graminea*, this species often occurs in very small populations comprising only a few flowering plants.

Sexual expression was classified into three categories: 1) the proportion of females in the populations, 2) the proportion of intermediates in the populations, and 3) the overall proportion of sexually polymorphic morphs in the population, including summaries of females and intermediates.

To investigate the correlation between latitude and sexual expression, we calculated Spearman's or Pearson's correlation coefficient based on the normality of the data, as evaluated through QQ plots. Consequently, the correlation analysis was restricted to high polyploid cytotypes, as the 'Scandinavian entity' (tetraploid2) was exclusively detected in Northern Europe. To mitigate potential bias, two populations from Slovakia (population 1 comprising 42 individuals and population 14 comprising 3 individuals) consisting solely of female individuals were excluded from the analysis.

Difference of poportion of females, intermediates and hermaphrodites between Central and Northern Europe was evaluated using Mann-Whitney U test.

The following data matrices were compiled:

1. Matrix_S1: This dataset comprises 15 populations, totaling 116 individuals, of the high polyploid cytotype of *S. palustris*. These populations span both Northern Europe (8 populations, 46 individuals) and Central Europe (7 populations, 70 individuals).

2. Matrix_S2: This dataset includes 13 populations, encompassing 71 individuals of the high polyploid cytotype of *S. palustris*. These populations originate from both Northern Europe (8 populations, 46 individuals) and Central Europe (5 populations, 25 individuals). Two populations consisting solely of females were excluded from the analysis to avoid biased results.

3. Matrix_S3: This matrix contains 116 individuals (15 populations) of *S. palustris* from Northern and Central Europe. The dataset is divided into proportions of sexes from 70 individuals (7 populations) in Central Europe and 46 individuals (8 populations) in Northern Europe, with separate plots constructed for each region.

4. Matrix_S4: Matrix comprises 70 individuals from 7 populations of *S. palustris* from Central Europe to display intra-population variability of sexual expression.

5. Matrix_S5: Matrix includes 46 individuals from 8 populations of S. palustris from Northern Europe to show intra-population variability of sexual expression.

3. Results

3.1. Genetic analyses

3.1.1. Bayesian phylogenetic tree analyses

The Bayesian inference analysis of the ITS1_5.8S_ITS2 region based on Matrix_A_G (145 individuals and 630 bp) revealed that the majority of sequences from the studied species *S. palustris, S. graminea*, and *S. longifolia* clustered together in a single well-supported clade (PP = 0.98), with two major clades A and B observed (Figure 3). However, several genebank sequences from *S. palustris, S. graminea,* and *S. longifolia* were found also in clade A. This indicated that none of the studied taxa is monophyletic. Precisely, Clade A (PP = 1.00) comprised solely of genebank sequences, including two from *S. palustris* (KX158327 and JN589080) and one from *S. longifolia* (JN589146), along with nine related outgroup taxa. Clade B (PP = 1.00) consisted of two major subclades, B1 and B2 (PP = 0.98 and 1.00, respectively). Subclade B1 exhibited a shallow hierarchical structure with several statistically supported groupings (PP ranged from 0.51 to 0.87) and accessions in a basal polytomy. This subclade predominantly contained sequences generated within this study, which were grouped in an unsupported basal polytomy, with one grouping comprising *S. palustris* and another with *S. graminea* accessions (PP = 0.56) from Northern Europe, Central Europe (including samples from the Carpathians from previous investigations), and three accessions of *S. graminea* from genebanks. Interestingly, the accession of 'Scandinavian entity' SE_NOR_2_11 from Norway appeared intermingled among individuals of *S. palustris* in the basal polytomy. The third grouping comprised accessions of 'Scandinavian entity', appearing in a single statistically unsupported clade (PP = 0.82). Subclade B2 included all our samples of *S. longifolia* clustered together with genebank sequences, one *S. longifolia, S. calycantha*, two *S. borealis*, and one accession of the 'Scandinavian entity' (SE_28_3) in sister position. Regarding within-species variability, *S. graminea* showed apparent diversity across analyzed samples, while *S. palustris* and the “Scandinavian entity' were almost invariable.

A second BI analysis was conducted on Matrix_B_G (73 individuals and 1379 bp), including concatenated sequences of both cpDNA markers. The analysis revealed a well-supported clade of ingroup taxa but with minimal hierarchical structure restricted to a few statistically unsupported clades (PP ranged from 0.55 to 0.85; Figure 4). An exception was observed where haplotypes SE_24_10 of the 'Scandinavian entity' and SG32_1 of diploid *S. graminea* from Latvia clustered together in a strongly supported grouping (PP = 0.98). *S. palustris* and most of *S. longifolia* accessions formed their unsupported clades (PP = 0.58 and 0.85, respectively). However, three accession, belonging to *S. graminea* SG_NOR_9_9, and 'Scandinavian entity' (SE_NOR_2_8, SE_NOR_2_11) possessed more divergent haplotypes. Majority of samples of *S. graminea* and the 'Scandinavian entity' analyzed in this cpDNA analysis formed one unsupported clade (PP = 0.58).

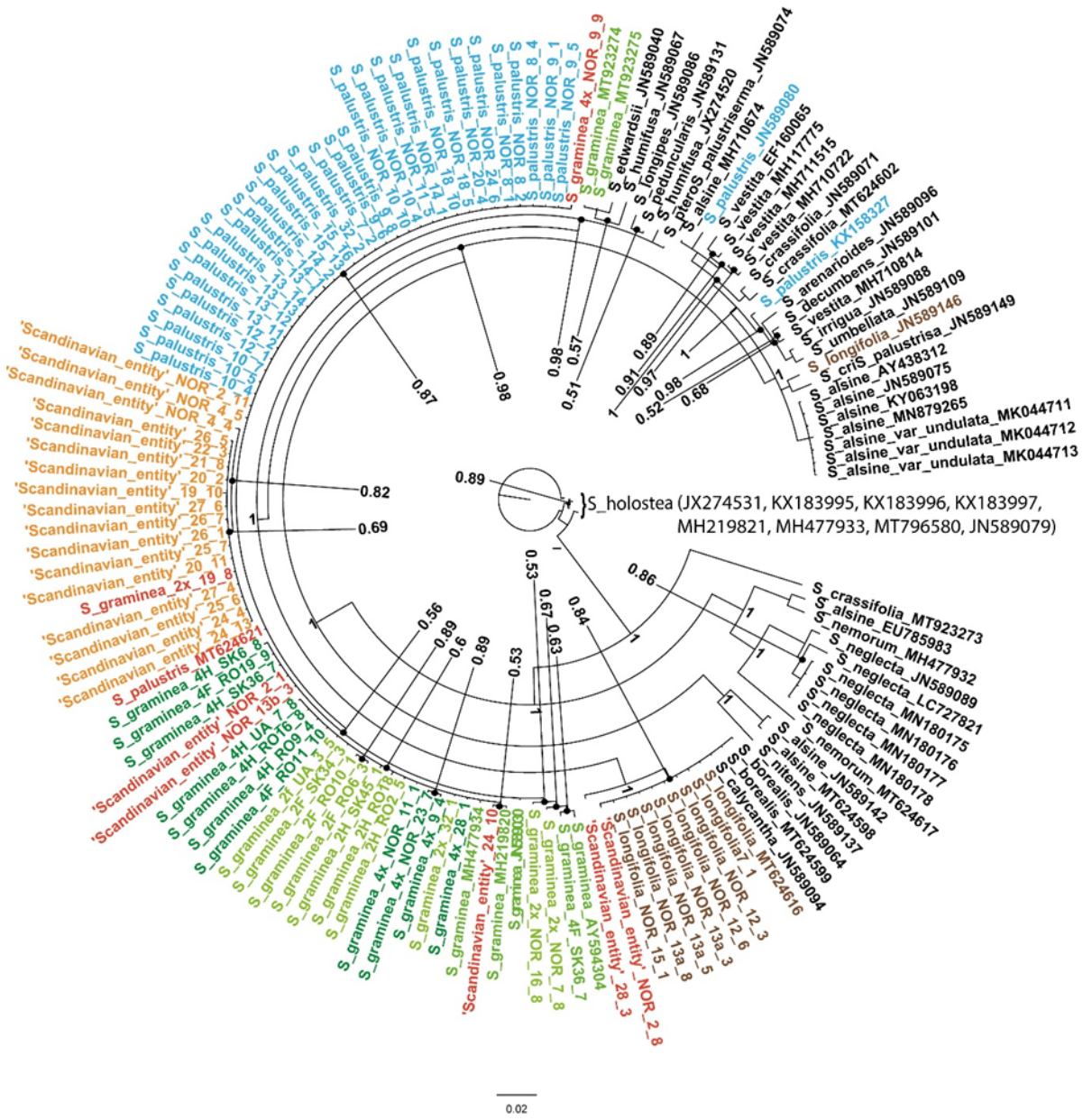


Figure 3: *Majority-rule consensus tree of Bayesian inference based on ITS1_5.8S_ITS_2 dataset (Matrix_A_G) of analysed Stellaria taxa. The posterior probability values of Bayesian inference are indicated by vertical bars. Colored labels indicate Stellaria species of interest. Sequences of S. graminea, including country codes SK (Slovakia), RO (Romania), and UA (Ukraine); ploidy levels (4 - tetraploid and 2 - diploid); and sexual morph designations (F - female and H - hermaphrodite), originate from the Carpathians and were generated within a yet unpublished study. Population codes and locality details follow those in Kučera et al. 2021.*

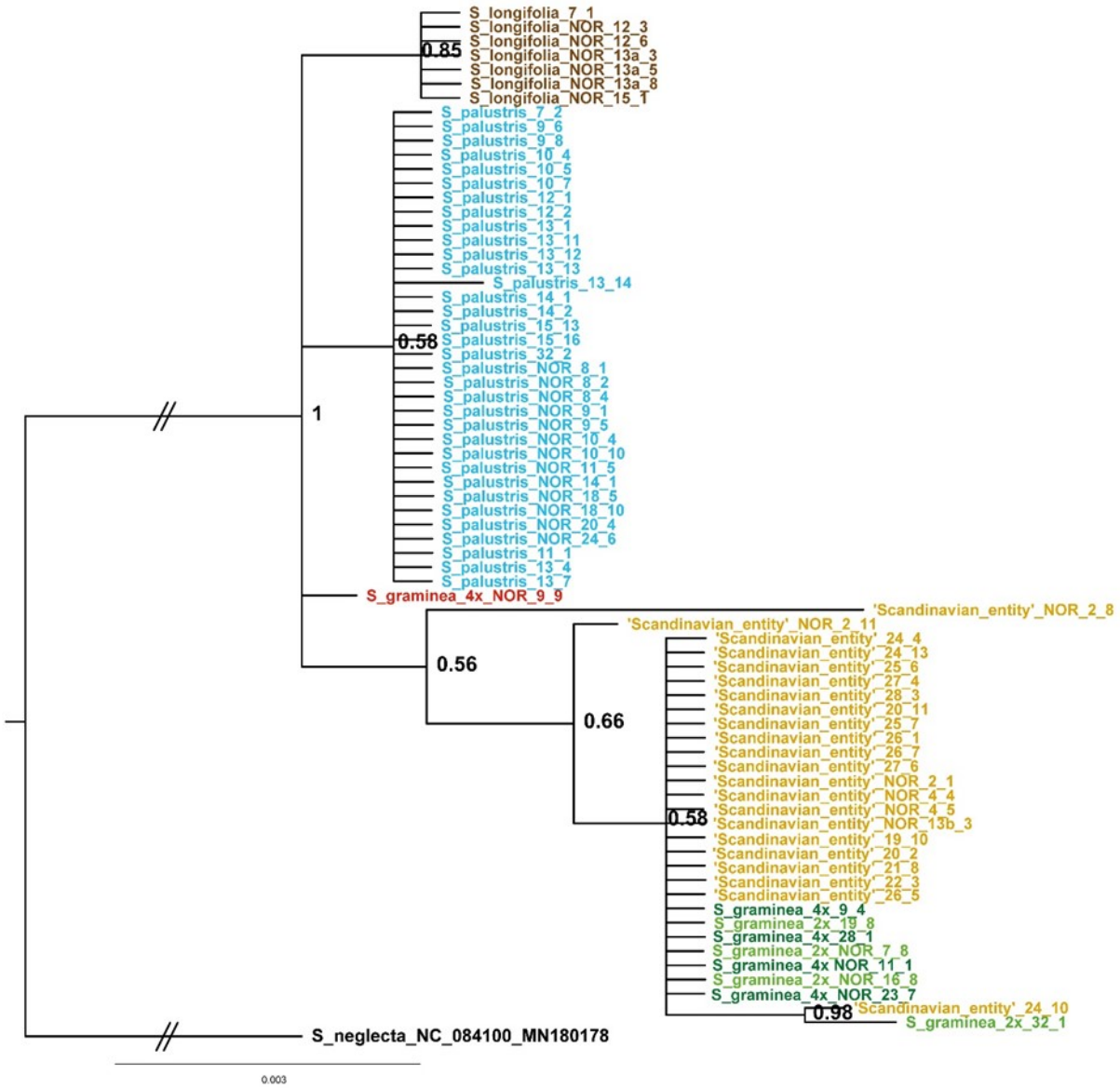


Figure 4: *Majority-rule consensus tree of Bayesian inference based on concatenated chloroplast dataset (psbJ-petA and K1F-K2R; Matrix_B_G) of analysed Stellaria taxa. The numbers above branches refer to posterior probability values of Bayesian inference. Coloured labels indicates Stellaria species of interest.*

Finally, BI inference based on Matrix_C_G (69 individuals and 2006 bp) of concatenated alignment of ITS and both cpDNA regions uncovered again shallow hierarchical structure with similar groupings a sin previous analysis. However, those of *S. palustris* and *S. longifolia* received significant statistical support (0.97 and 1.00, respectively, Figure 5). Accessions of *S. graminea* were shown to be the most heterogeneous, and those of the 'Scandinavian' entity' formed its own but unsupported grouping (PP = 0.69). Accessions of three 'Scandinavian entity' (SE_NOR_2_11, SE_NOR_4_5 and SE_28_3) appeared in outlying positions to groupings in which other accessions belonging to their taxa were clustered.

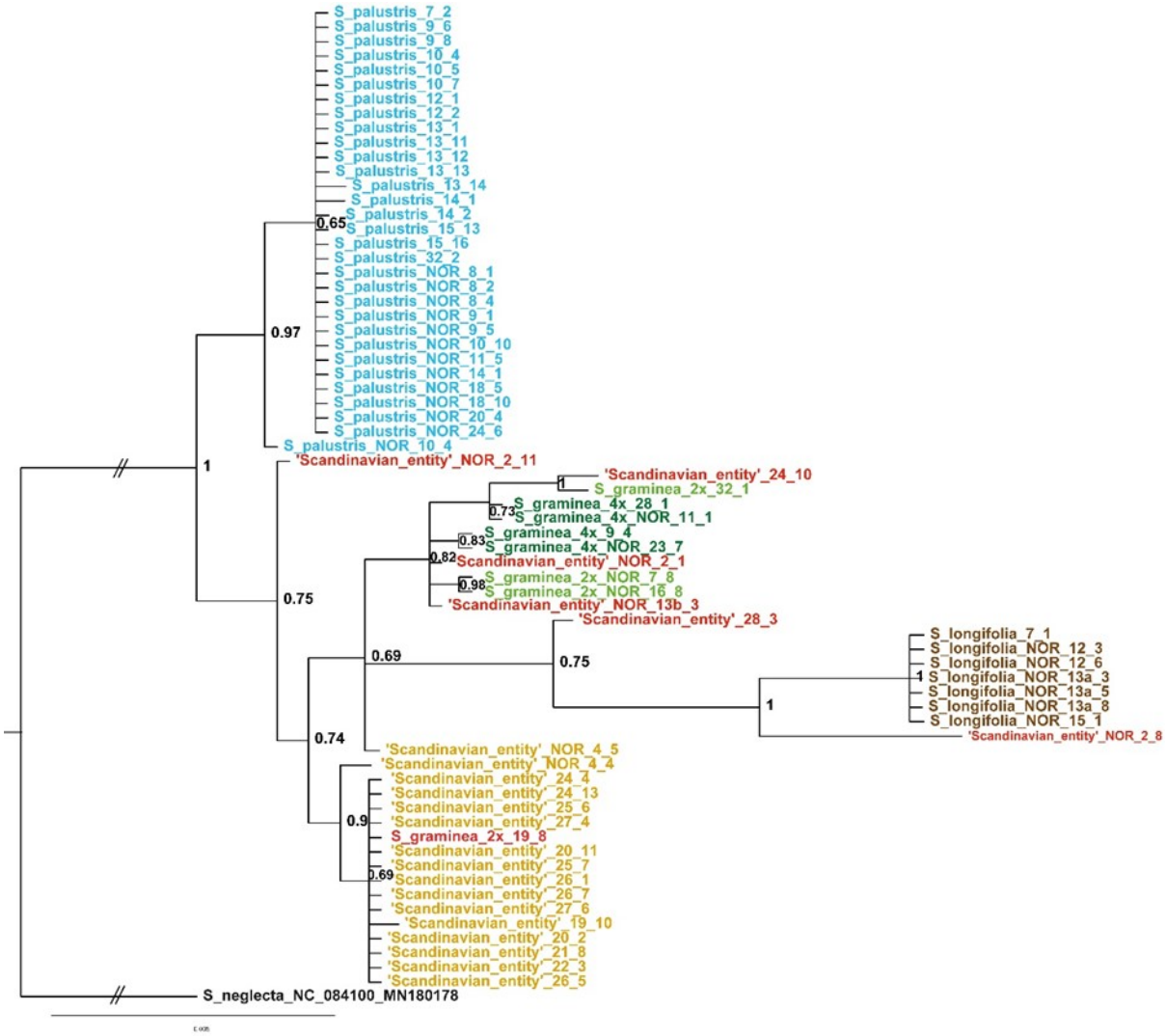


Figure 5: *Majority-rule consensus tree of Bayesian inference based on concatenated chloroplast (psbJ-petA and K1F-K2R) and ITS1_5.8S_ITS2 datasets (Matrix_C_G) of analysed Stellaria taxa. The numbers above branches refer to posterior probability values of Bayesian inference. Coloured labels indicates Stellaria species of interest.*

3.1.2. Phylogenetic network analyses

The first NeighborNet analysis (NN1) based on the entire ITS dataset (Matrix_A_G) uncovered a complex structure with significant vertical and horizontal splits across the network's backbone (Figure 6). Significant conflicts were apparent predominantelly among outgroup taxa. Pronounced conflicting signals were observed among clusters, including gene bank accessions of *S. palustris* (KX158327 and JN589080) and *S. longifolia* (JN589146), and a *S. graminea* (MT923274, MT923275) and accessions of related outgroup species. Notably, the accession SE_28_3 of the 'Scandinavian' entity' appeared intermediate between clusters containing *S. longifolia, S. calycantha,* and *S. borealis*, and clusters including accessions of *S. graminea*.

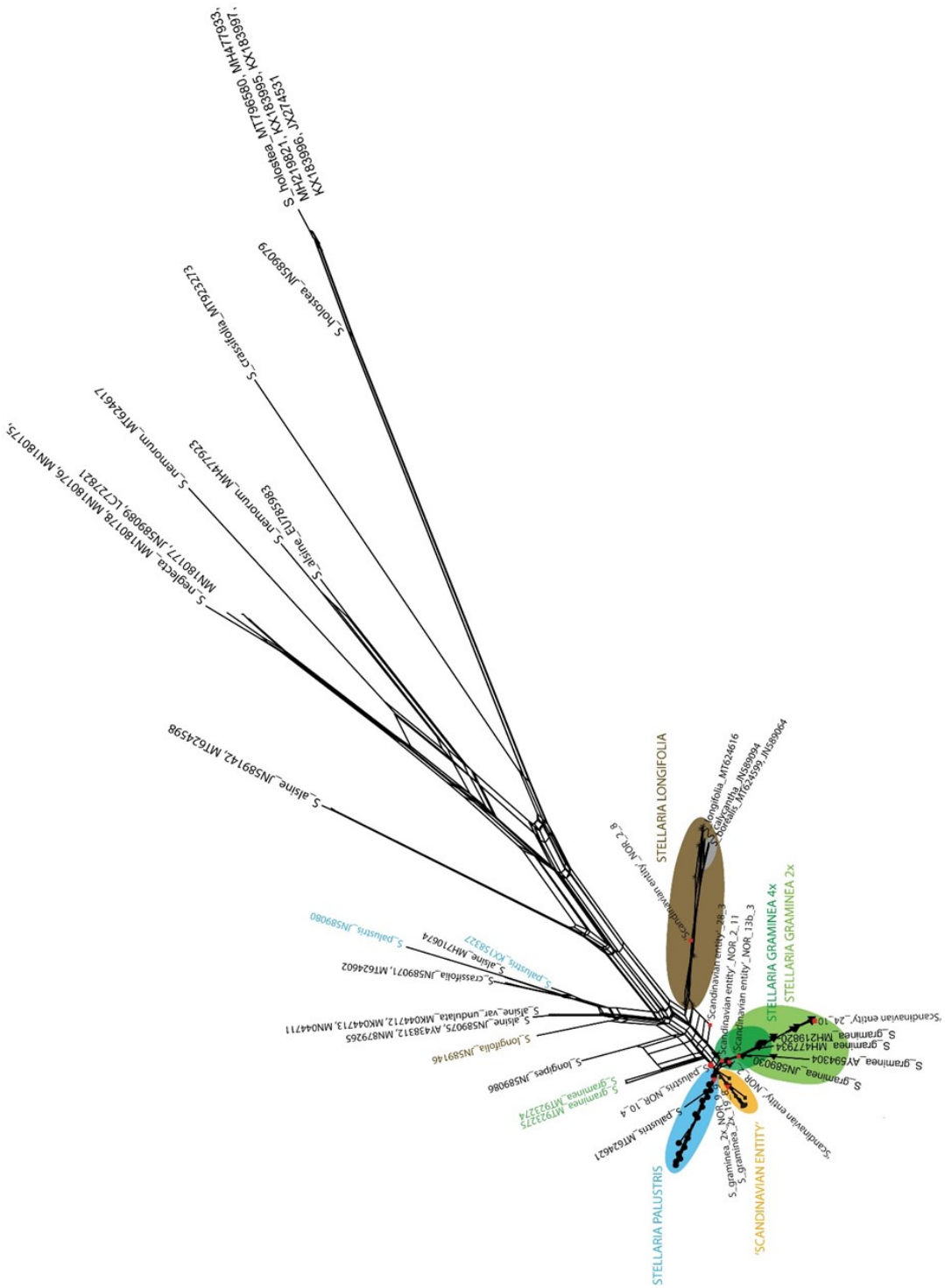


Figure 6: *Neighbour-Net diagram (NN1) and split support spectrum for the ITS dataset (Matrix_A_G). The Neighbour-Net diagram is based on uncorrected P-distances. Position of of studied individuals are marked by following symbols: S. palustris - circle, S. graminea - triangle, 'Scandinavian entity' - square, S. longifolia - star. Individuals placed out of their taxon specific cluster are in red. Colored labels indicate Stellaria species of interest. Sequences of S. graminea, including country codes SK (Slovakia), RO (Romania), and UA (Ukraine); ploidy levels (4 - tetraploid and 2 - diploid); and sexual morph designations (F - female and H - hermaphrodite), originate from the Carpathians and were generated within a yet unpublished study. Population codes and locality details follow those in Kučera et al. 2021.*

The second NN2 analysis (Figure 7), excluding most of the outgroup taxa, revealed a much clearer structure, including four, mostly taxon-specific lineages,: 1) *S. palustris*, including our individuals and accession MT624621 from the gene bank; 2) *S. graminea* and all our individuals and gene bank accessions AY594304, JN589030, MH477934, MH219820; 3) the 'Scandinavian' entity'; and 4) *S. longifolia*, including gene bank accessions MT624616 and *S. calycantha* (JN589094) and *S. borealis* (JN589064; MT624599). In all major lineages, accessions of given taxa appeared along the entire vertical splits of the network, indicating the presence of continuous intra-individual variability of polymorphic sites. In several cases, such accessions appeared in the central part of the network in conflicting positions indicated by horizontal splits (*S. graminea* - 4F_RO19_9; SG_NOR_9_9; SG_NOR_23_7; SG9_4; SG19_8; 'Scandinavian entity' -, SE_NOR_2_11, SE_NOR_4_5; SE27_6; SE19_10; SE26_7; SE20_2; SE22_3; SE21_8; SE_NOR_4_4; SE26_5; and *S. palustris* - SP_NOR_10_4; SP14_1).

After their exclusion (NN3), all but one major horizontal split between the 'Scandinavian entity' and *S. longifolia* lineages disappeared (Figure 8).

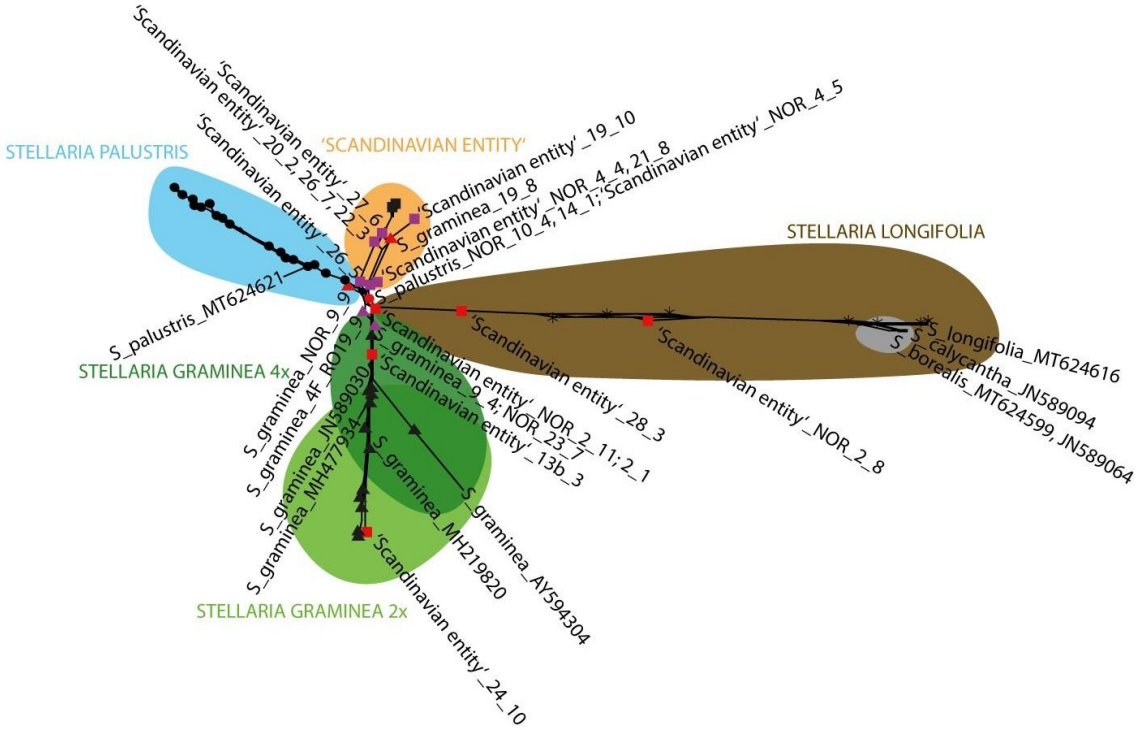


Figure 7: *Neighbour-Net diagram (NN2) and split support spectrum for the ITS dataset (Matrix_A_G). The Neighbour-Net diagram is based on uncorrected P-distances. Position of of studied individuals are marked by the following symbols: S. palustris - circle, S. graminea - triangle, 'Scandinavian entity' - square, S. longifolia - star. Individuals placed out of their taxon specific cluster are in red. Individuals in conflicting positions are in purple. Colored labels indicate Stellaria species of interest. Sequences of S. graminea, including country codes SK (Slovakia), RO (Romania), and UA (Ukraine); ploidy levels (4 - tetraploid and 2 - diploid); and sexual morph designations (F - female and H - hermaphrodite), originate from the Carpathians and were generated within a yet unpublished study. Population codes and locality details follow those in Kučera et al. 2021.*

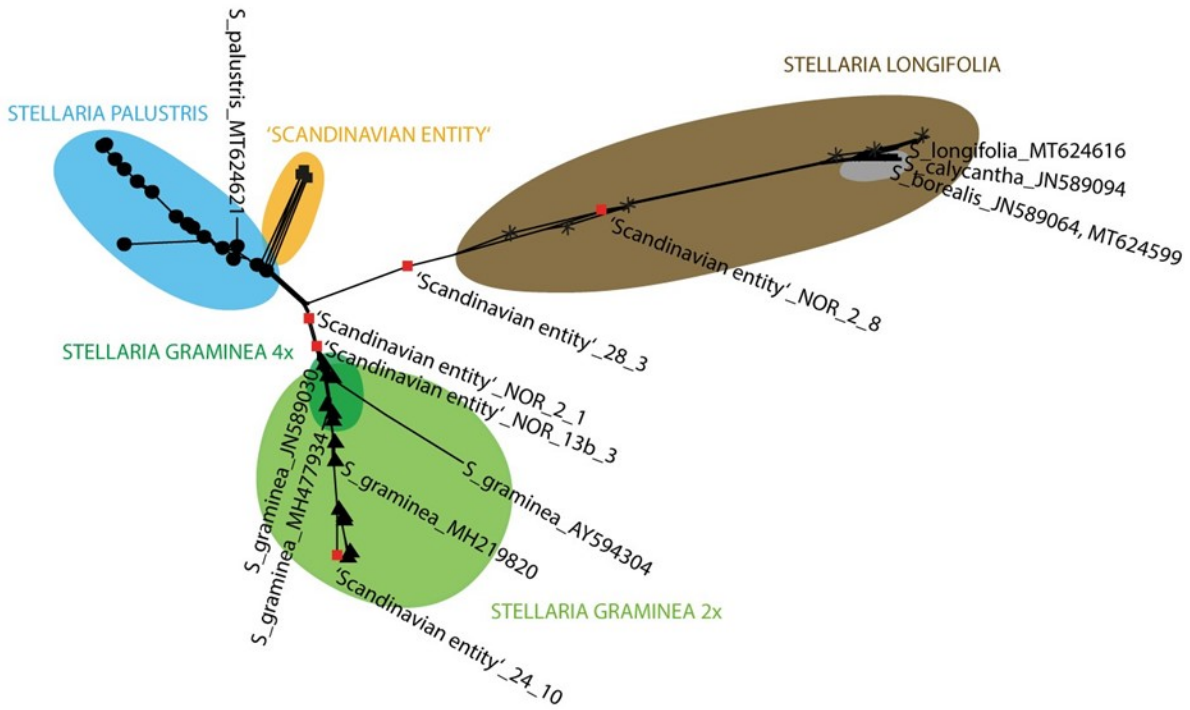


Figure 8: *Neighbour-Net diagram (NN3) and split support spectrum for the ITS dataset (Matrix_A_G). The Neighbour-Net diagram is based on uncorrected P-distances. The positions of the studied individuals are marked by following symbols: S. palustris - circle, S. graminea - triangle, 'Scandinavian entity' - square, S. longifolia - star. Individuals placed outside of their taxon specific cluster are in red. Colored labels indicate Stellaria species of interest.*

The parsimony-based haplotype network revealed low cpDNA diversity within the ingroup taxa, with only 8 haplotypes identified, primarily connected by one or two steps (Figure 9). An exception was observed with haplotype A found in 'Scandinavian entity', where nine mutation steps separated it from the closest haplotype B. The majority of *S. palustris* accessions posessed haplotype F.

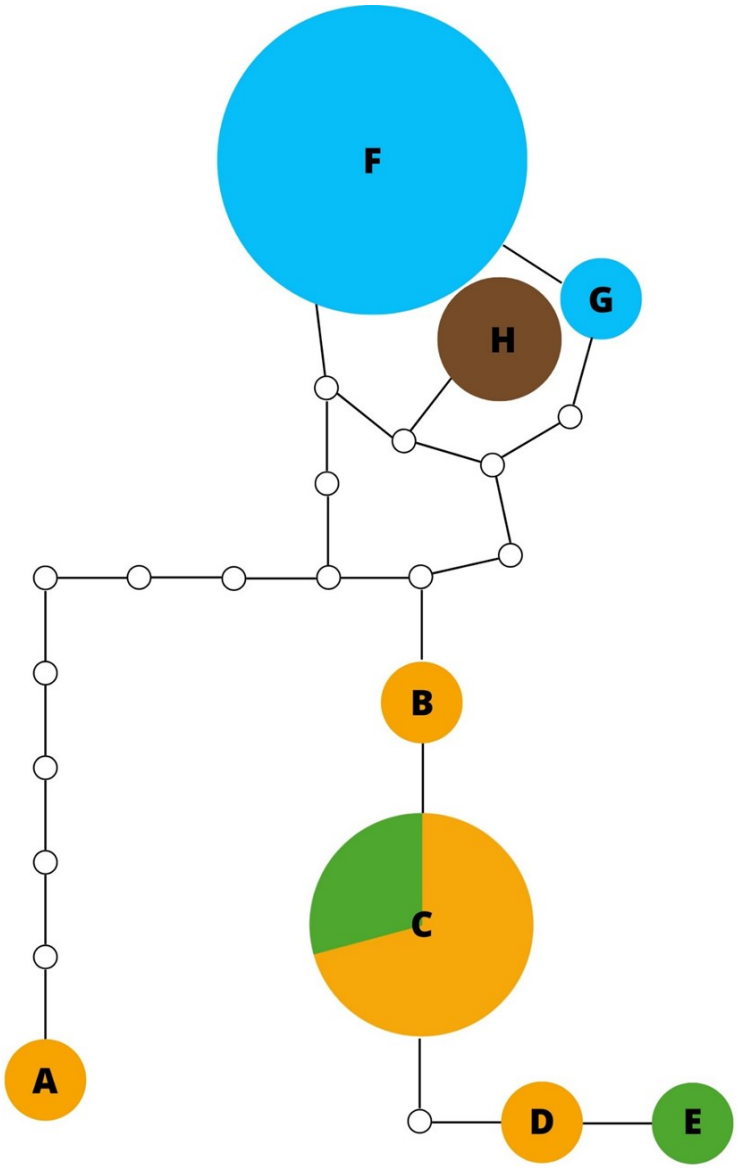


Figure 9: *Maximum parsimony network of the cpDNA haplotypes of studied Stellaria taxa. The symbol sizes are proportional to the haplotype frequencies, the lines represent mutational steps, and empty dots are unsampled haplotypes. Colour symbols indicate affiliation of accessions to the taxon (orange - 'Scandinavian entity', green - S. graminea, blue - S. palustris, brown - S. longifolia). Bold letters indicate haplotypes:* ***A)*** *'Scandinavian entity' (SE_NOR_2_8),* ***B)*** *'Scandinavian entity' (SE_NOR_2_1),* ***C)*** *'Scandinavian entity' and Stellaria graminea* ***D)*** *'Scandinavian entity' (SE_24_10),* ***E)*** *S. graminea (SG_32_1),* ***F)*** *S. palustris,* ***G)*** *S. palustris (SP_13_14), H) S. longifolia,*

3.2. Karyological analyses

3.2.1. Chromosome counting

Chromosome counts were conducted on 12 individuals of *S. palustris* from Central Europe and 4 individuals of the 'Scandinavian entity'. Only one chromosome count of *S. palustris* from Northern Europe has been reported. In *S. palustris*, we detected several cytotypes of exceedingly high ploidy levels (Figure 10).

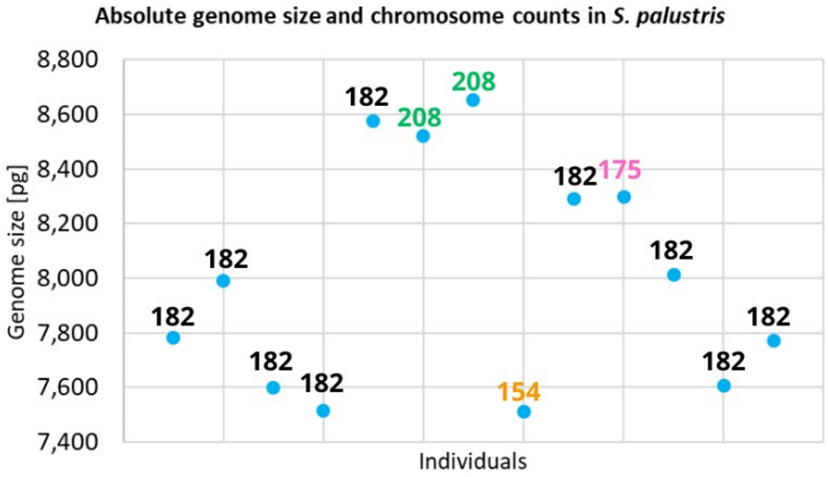


Figure 10: *Comparison of chromosome counts and absolute genome size values in S. palustris*

Specifically, the lowest ploidy level detected was in single individual (13_7) from Slovakia, with a chromosome number of 154, corresponding to aneuploid 2n = 12x = 154 (Figure 11b). Additionally, chromsome count for another individuals (13_14) from Slovakia estimated to be approximately 175 chromosomes, indicating aneuploid 2n = 13 - 14x = 175. Most counted individuals, specifically 1 from Czech Republic (3_7), 6 from Slovakia (1_10, 1_16, 2_6, 10_4, 13_12, 14_2) and 1 from Denmark (NOR_SP_19_3), possessed 182 chromosomes, indicating 14x ploidy (2n = 14x = 182) (Figure 11c). The highest chromosome count recorded was 208 chromosomes, corresponding to a ploidy level of 2n = 16x = 208 and was found in population 10 (individuals 10_6 and 10_9) from Slovakia (Figure 11d).

In the Scandinavian entity, all counted individuals were tetraploid, with a chromosome count of 2n = 4x = 52 (Figure 11a).

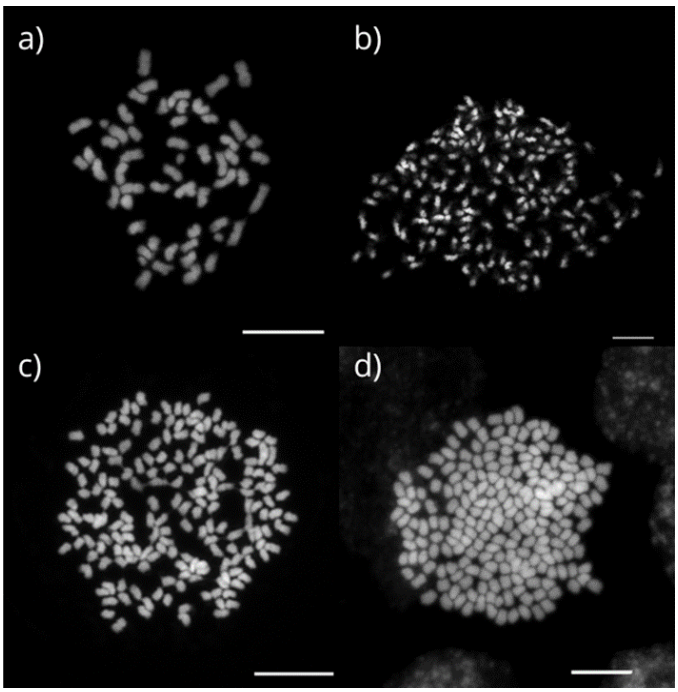


Figure 11. Microphotography of chromosomes of four detected ploidies. *A) 2n = 4x = 52, B) 2n = 12x = 154, C) 2n = 14x = 182, D) 2n = 16x = 208. The scale corresponds to 10 micrometers.*

3.2.2. Genome size estimation

The Spearman correlation coefficient analysis, conducted on 105 individuals, showed a robust positive correlation between RGS and AGS, with a correlation coefficient of 0.983 (Figure 12).

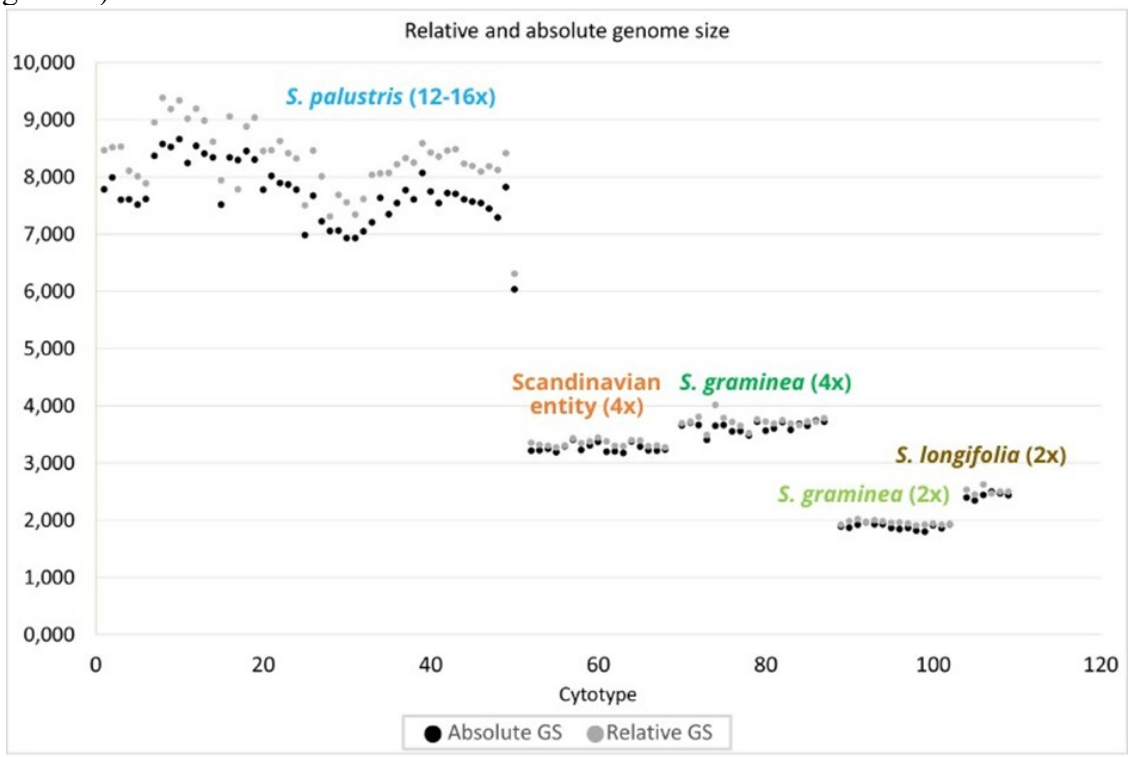


Figure 12: *Correlation between relative (a.u.) and absolute (pg) genome sizes of studied taxa and cytotypes.*

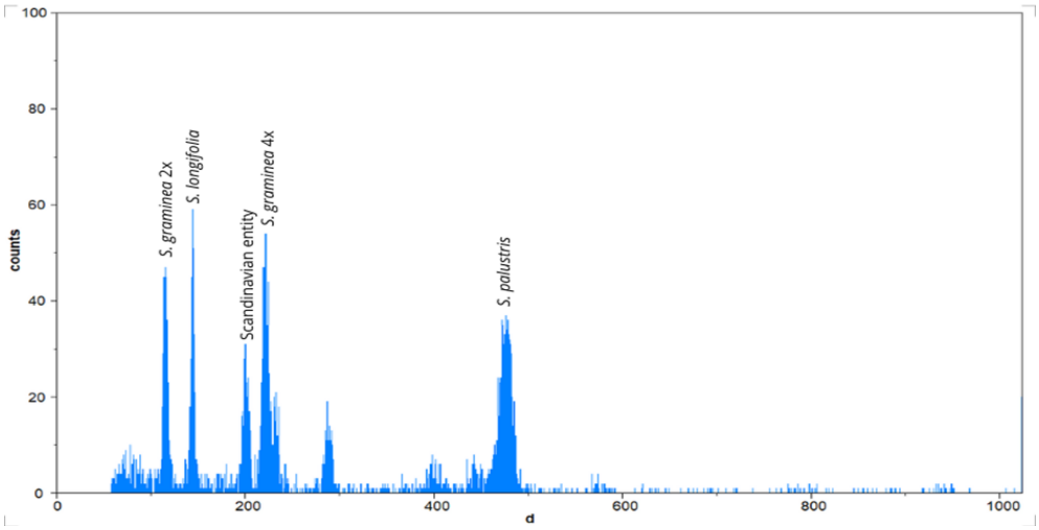


Figure 13: *Differences in reltive genome size among Stellaria taxa and cytotypes. The nuclei from all individuals were isolated, stained with DAPI and analysed simultaneously. The mean 2C-value estimated for the individuals were 1.84 pg (S. graminea 2x - NOR_SG_7_8), 2.4 pg (S. longifolia 2x - SL_7_1), 3.29 pg ('Scandinavian entity' 4x - 28_3), 3.66 pg (S. graminea 4x - 29_4) and 7.6 pg (S. palustris 3_2).*

RGS values (59 populations, 634 individuals; Figure 13) of analysed cytotypes ranged from 1.89 to 9.38 a.u. Specific RGS values for different taxa and cytotypes were as follows:

diploid *S. graminea*: 1.89 - 2.09 a.u. (mean = 1.97, σ = 0.04), tetraploid *S. graminea*: 3.35 - 4.02 a.u. (mean = 3.71, σ = 0.096), 'Scandinavian entity': 3.26 - 3.51 a.u. (mean = 3.34, σ = 0.06) with four individuals from population from Northern Europe (NOR_SE_2_5, NOR_SE_2_6, NOR_SE_2_8, NOR_SE_2_10) exceeding from this range, with RGS 5.09 - 5.22 a.u (mean = 5.13, σ = 0.05), *S. palustris*: 6.29 - 9.38 a.u. (mean = 8.24, σ = 0.53) and *S. longifolia*: 2.41 - 2.66 a.u. (mean = 2.49, σ = 0.07) . The comparison of relative genome size among all studied cytotypes is depicted in Figure 14.

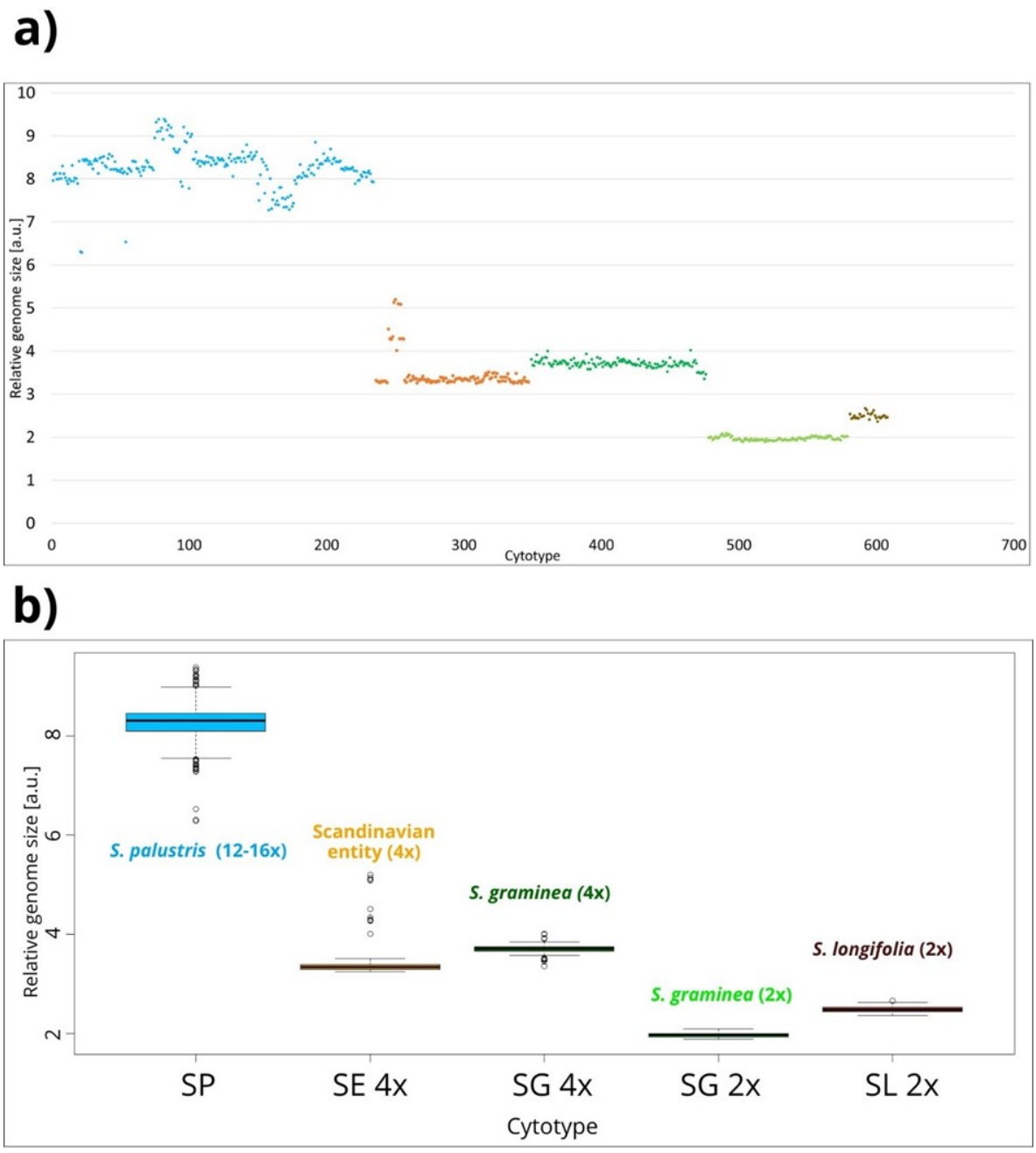


Figure 14: *Comparison of relative genome size ranges (a.u.) among studied taxa and cytotypes. a) relative genome size data displayed in scatter plot. b) variation in relative genome size data displayed by box-plot graphs. Rectangles define the 25th and 75th percentiles; bold horizontal lines show the median; whiskers are from the 10th to the 90th percentiles; empty dots show the extreme values.*

AGS values across analyzed taxa and cytotypes (112 individuals, 45 populations Figure 15) ranged from 2C = 1.80 to 8.65 pg. Specific AGS values for different taxa and cytotypes were as follows: diploid *S. graminea*: 2C = 1.80 - 1.97 pg (mean = 1.88 pg, σ = 0.05); tetraploid *S. graminea*: 2C = 3.40 - 3.74 pg (mean 3.63 pg, σ = 0.09); 'Scandinavian entity': 2C = 3.17 - 3.41 pg (mean 3.26 pg, σ = 0.07); *S. palustris*: 2C = 6.03 - 8.65 pg (mean 7.72 pg, σ = 0.53); *S. longifolia*: 2C = 2.34 - 2.50 pg (mean 2.43 pg, σ = 0.055).

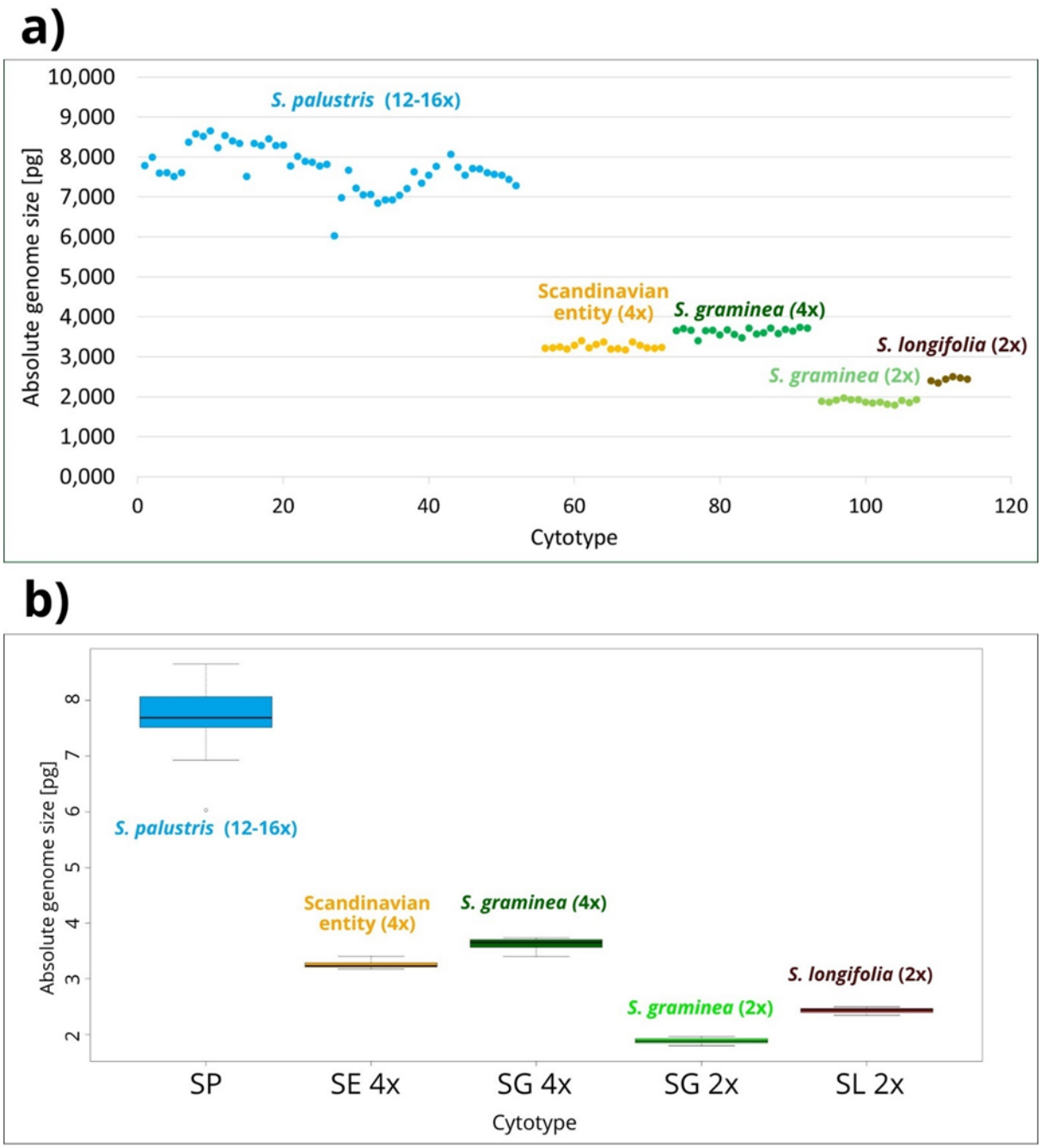


Figure 15: *Comparison of absolute genome size ranges (pg) among studied taxa and cytotypes. a) absolute genome size data displayed in scatter plot. b) variation in absolute genome size data displayed by box-plot graphs. Rectangles define the 25th and 75th percentiles; bold horizontal lines show the median; whiskers are from the 10th to the 90th percentiles; empty dots show the extreme values.*

Monoploid genome sizes was determined for all 113 individuals measured for AGS (Figure 16). For diploid *S. graminea* monoploid genome size ranged from *Cx* = 0.90 to 0.96 pg (mean = 0.94 pg, σ = 0.02), in tetraploid *S. graminea*, *Cx* = 0.85 to 0.934 pg (mean = 0.907 pg, σ = 0.02), in 'Scandinavian entity' *Cx* = 0.79 - 0.85 pg (mean = 0.81 pg, σ = 0.02), in *S. palustris* *Cx* = 0.53 - 0.626 (mean = 0.57 pg, σ = 0.03) and in for diploid *S. longifolia* *Cx* = 1.171 - 1.25 pg (mean = 1.22 pg, σ = 0.03).

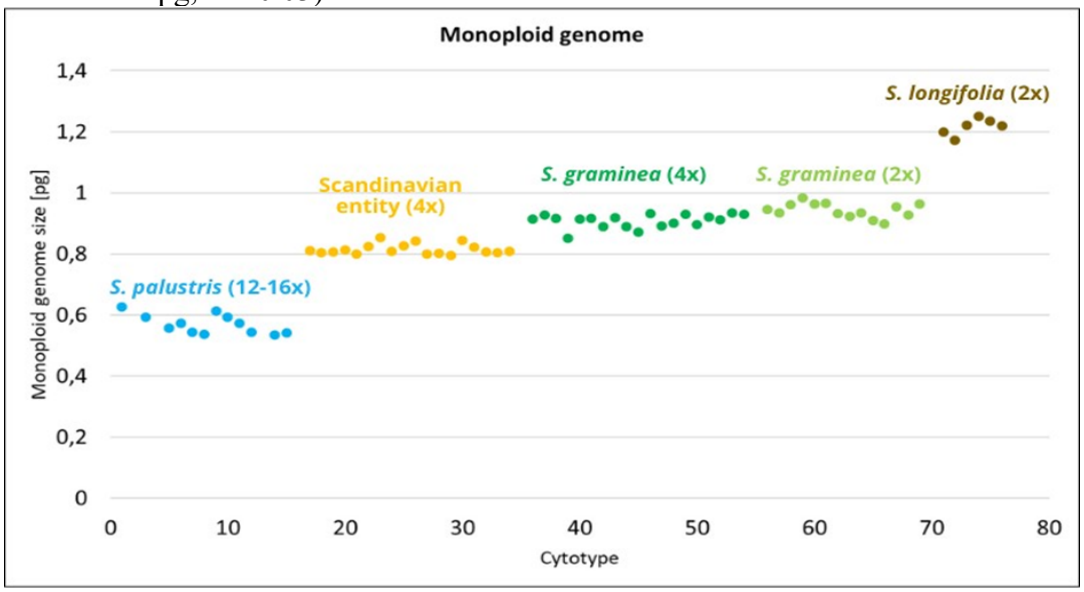


Figure 16: *Comparison of monoploid genome size (pg) among studied taxa and cytotypes.*

Difference in monoploid genome size between cytotypes was statistically significant (F(4)=8.66, p-value = < 0.001) as well as the difference in monoploid GS within *S. palustris* (t(11) = 62.4, p-value = < 0.001).
Overall variability in relative and absolute genome size across all cytotypes was illustrated using boxplots (Figure 17 and 18).

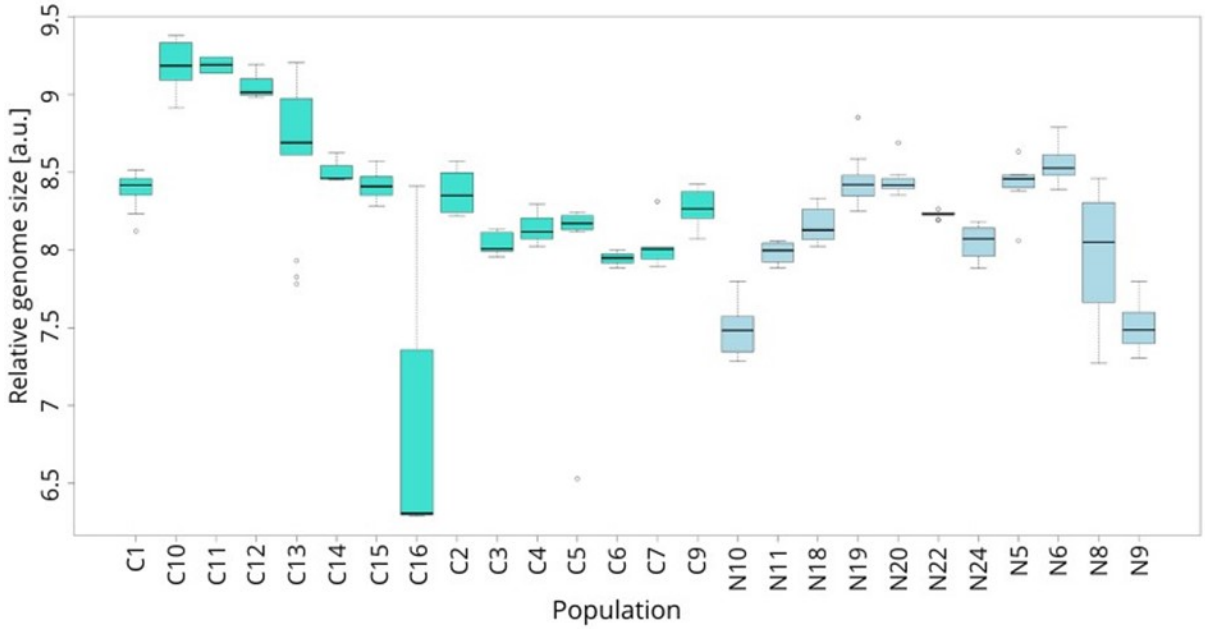


Figure 17: *Box-plot graphs depicting within population variability in relative genome size (a.u.) of S. palustris in Central (turquoise) and Northern (light blue) Europe. Rectangles define the 25th and 75th percentiles; bold horizontal lines show the median; whiskers are from the 10th to the 90th percentiles; empty dots show the extreme values.*

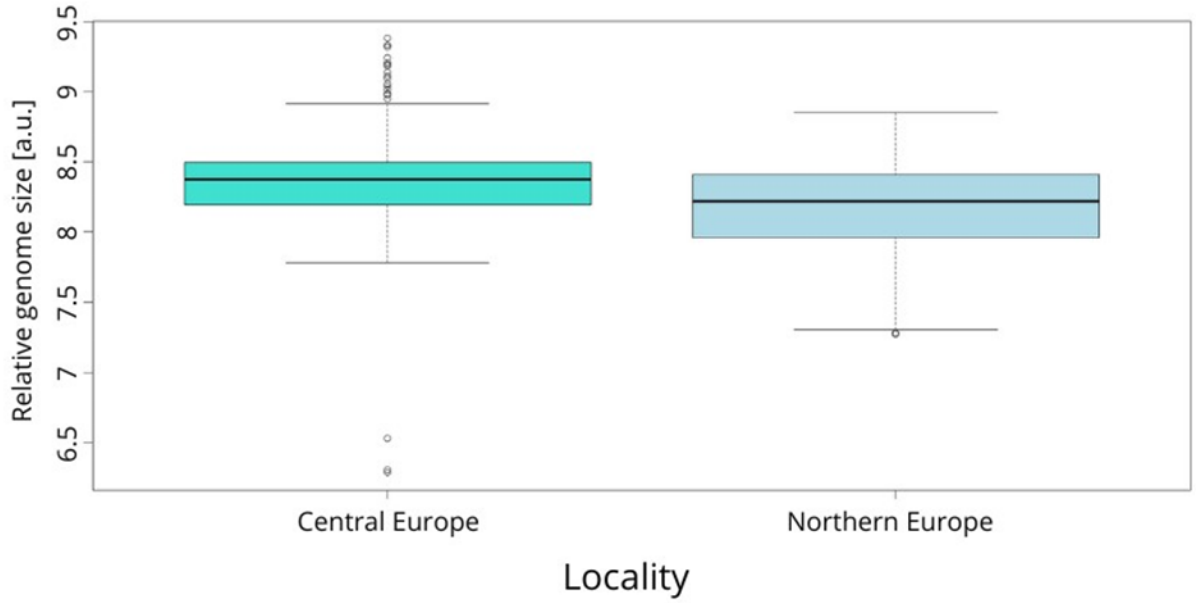


Figure 18: *Box-plot graphs depicting variation in relative genome size (a.u.) of S. palustris in Central (turquoise) and Northern (light blue) Europe. Rectangles define the 25th and 75th percentiles; bold horizontal lines show the median; whiskers are from the 10th to the 90th percentiles; empty dots show the extreme values.*

Even though Pearson's correlation coefficient is 0.671 and p-value 0.01204, this correlation is not observed consistently in *S. palustris* (Figure 10) and for chromosome number 2n = 14x = 182, the genome size varies by almost 1 pg.

3.2.3. The correlation between latitude and genome size in *Stellaria palustris*

The correlation analysis between latitude and both RGS and AGS revealed a statistically significant negative relationship, with correlation coefficients of -0.4023077 (p=0.04719) and -0.7069143 (p = 0.001449), respectively (Figure 19) indicating increase in genome size toward Central Europe.

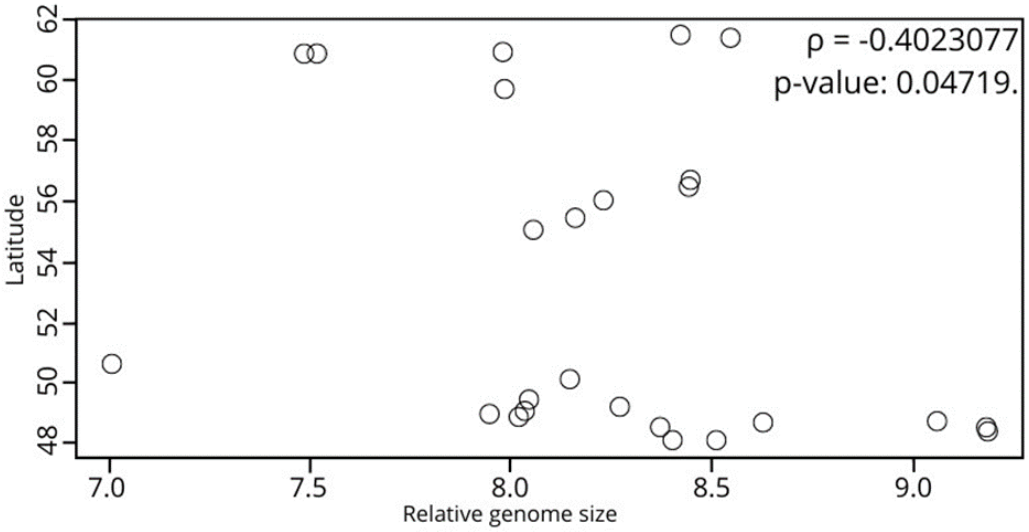


Figure 19: *Correlation plot between latitude and relative genome size (a.u.) in all analysed populations of S. palustris.*

3.3. Morphometric analysis

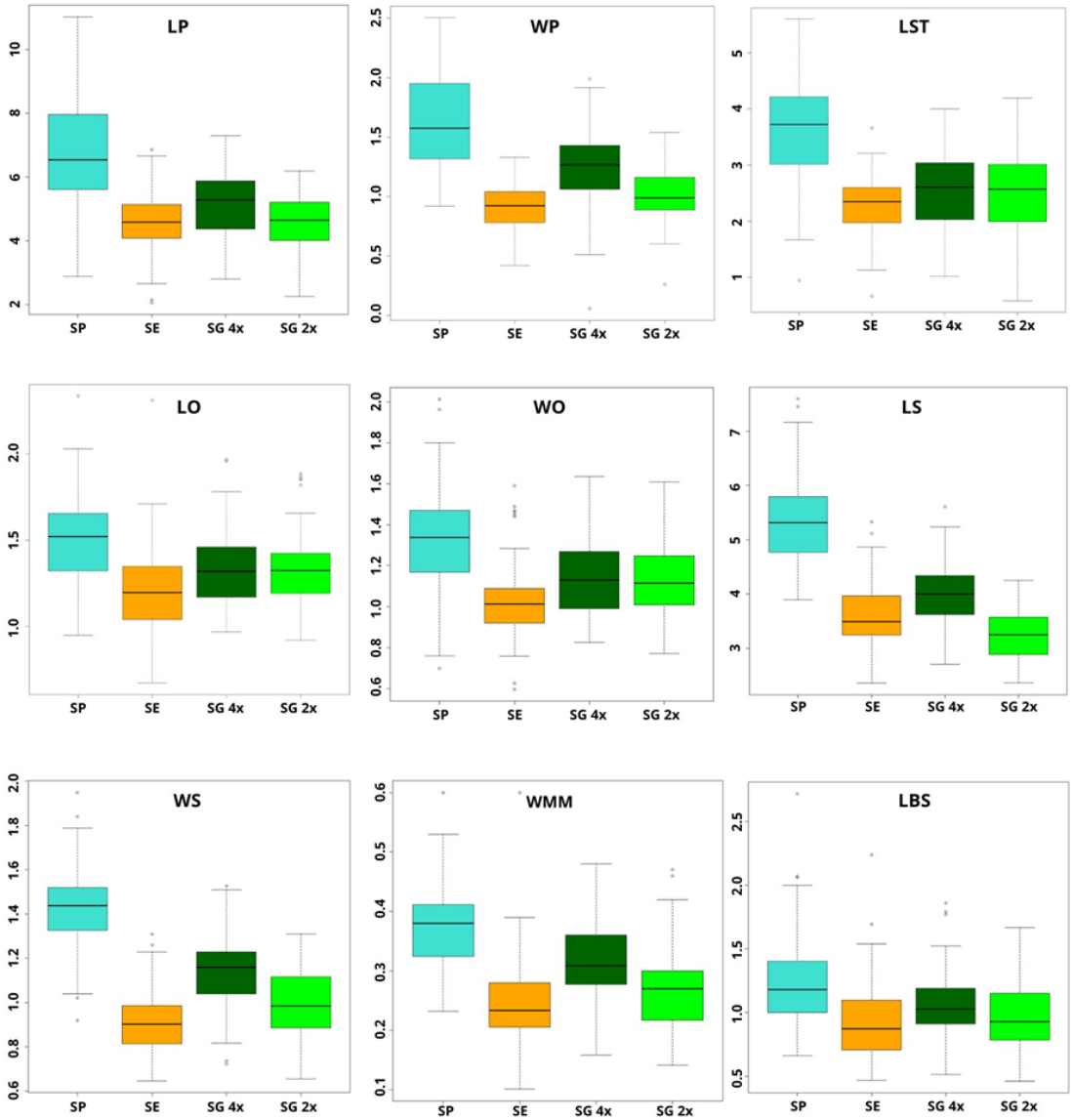


Figure 20: *Box-plot graphs depicting variation in selected morphological characters of cytotypes of studied Stellaria taxa. Rectangles define the 25th and 75th percentiles; bold horizontal lines show the median; whiskers are from the 10th to the 90th percentiles; empty dots show the extreme values. Taxon abbreviations are as followe: SP – S. palustris, SE - 'Scandinavian entity', CG 4x – S. graminea tetraploid cytotype, CG 2x – S. graminea diploid cytotype.*

In the first CDA1 conducted on Matrix_M1, encompassing individuals of all cytotypes, a single grouping was observed, with a noticeable tendency for the separation of the high polyploid cytotype (Figure 21a). However, diploid and tetraploid cytotypes were largely intermingled. The first axis explained 91.85% of the variability, with the most influential characters being the length of the petal, the length of the style, and the length of the sepal. The second axis accounted for only 6.6% of the variability.

CDA 2, based on population means of the same dataset (Matrix M2, Figure 21b), exhibited a more obvious separation of the high polyploid cytotype along the x-axis, with only one population (NOR_6) mixed with lower ploidy cytotypes. The first axis explained 88.93%

of the variability, with the most influential characters being the length of the sepal and the petal, consistent with the individual-level analysis.

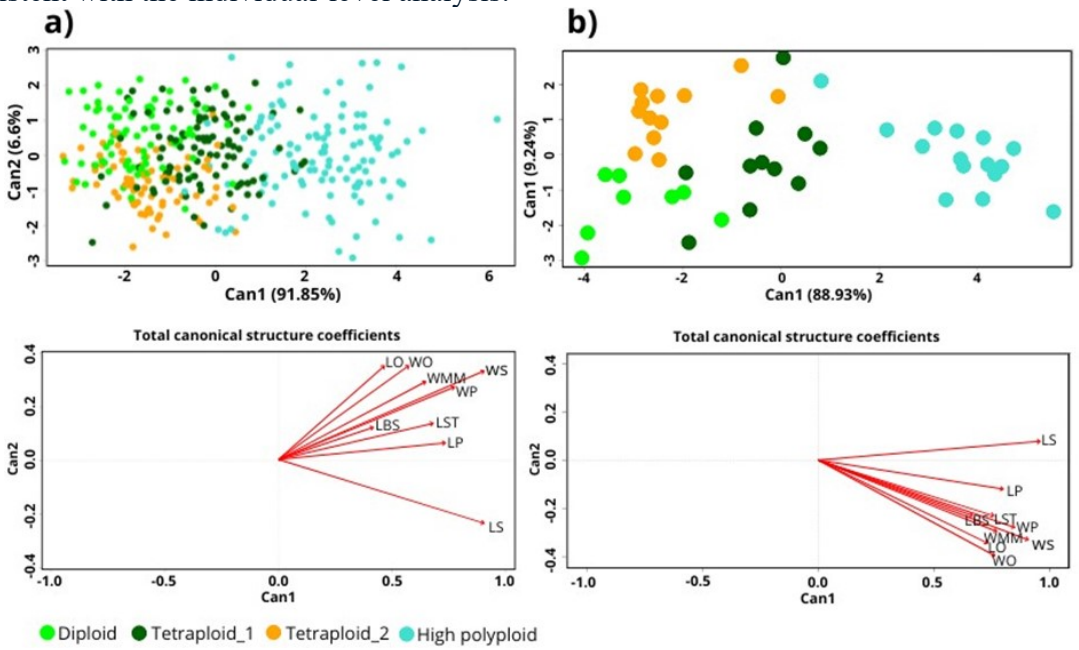


Figure 21: *Canonical discriminant analyses of cytotypes* ***a)*** *CDA 1 based on Matrix_M1 (370 individuals) with cytotypes as predefined groups including the whole dataset, performed on the individual level. The two axes explain 91.85% and 6.6% of variability, respectively.* ***b)*** *CDA 2 based on Matrix_M2 (50 populations) with cytotypes as predefined groups, conducted on the whole dataset, performed on the population level. The first two axes explain 88.93% and 9.24% of total variation. The contribution of characters is depicted in the plots below.*

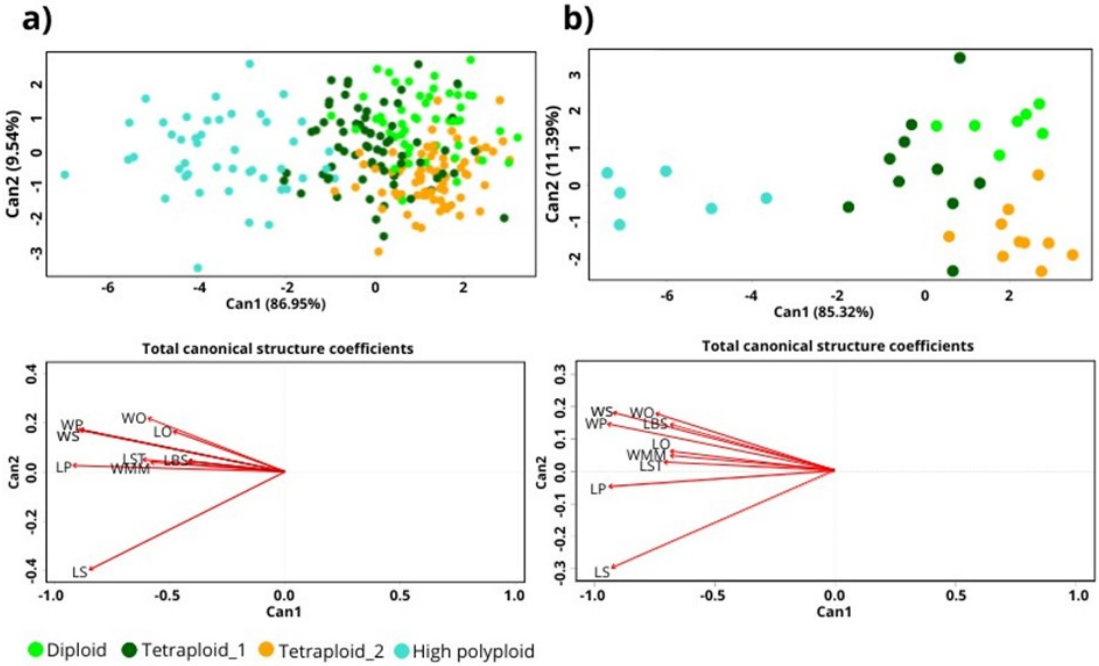


Figure 22: *Canonical discriminant analyses of cytotypes* ***a)*** *CDA 3 based on Matrix_M3 (230 individuals) with cytotypes as predefined groups including only hermaphroditic individuals, performed on the individual level. The two axes explain 86.95% and 9.54% of variability.* ***b)*** *CDA 4 based on Matrix_M4 (30 populations) with cytotypes as predefined groups, conducted only on hermaphrodites, performed on the population level. The first two axes explain 85.32% and 11.39% of variability. Contribution of characters is depicted in the plots below.*

Upon exclusion of female and intermediate individuals from both individual and population-level datasets (Matrices M3 and M4), CDA 3 and CDA 4 revealed a much clearer separation of high polyploid (Figures 22a and 22b), along the x-axis. In CDA 3, the first axis explained 86.95% of the variability, predominantly separating highly polyploid cytotypes from the rest. The characters contributing most to the separation were the length of the petal, the length of the style, and the maximum width of the membranous sepal margin. The second axis explained only 9.54% of the variability. In CDA4 the x-axis accounting for 85.32% of the variability, with the petal length being the most influential character. The second axis explained 11.39% of the variability. Additionally, populations of all lower ploidy cytotypes showed a clear trend of separation from one another.

CDA 5 (Matrix M5) including only females of all cytotypes revealed a tendency of separation of high polyploid from the rest (Figure 23a). The first axis explained 97.84% of variability with the most influential characters length of the style, length and width of the sepal and length and width of the petal. The second axis explained only 2.16% of the variability.

In CDA 6 (Matrix M6) including only intermediates individuals of all cytotypes (Figure 23b). High polyploid cytotype showed tendency to separation from other groups along the axis x, however with overlapped with females of tetraploid1. The first axis explained 82.5% of variability with most contributing character length and width of the sepal and width of the petal. The second axis explained only 11.8% of variability.

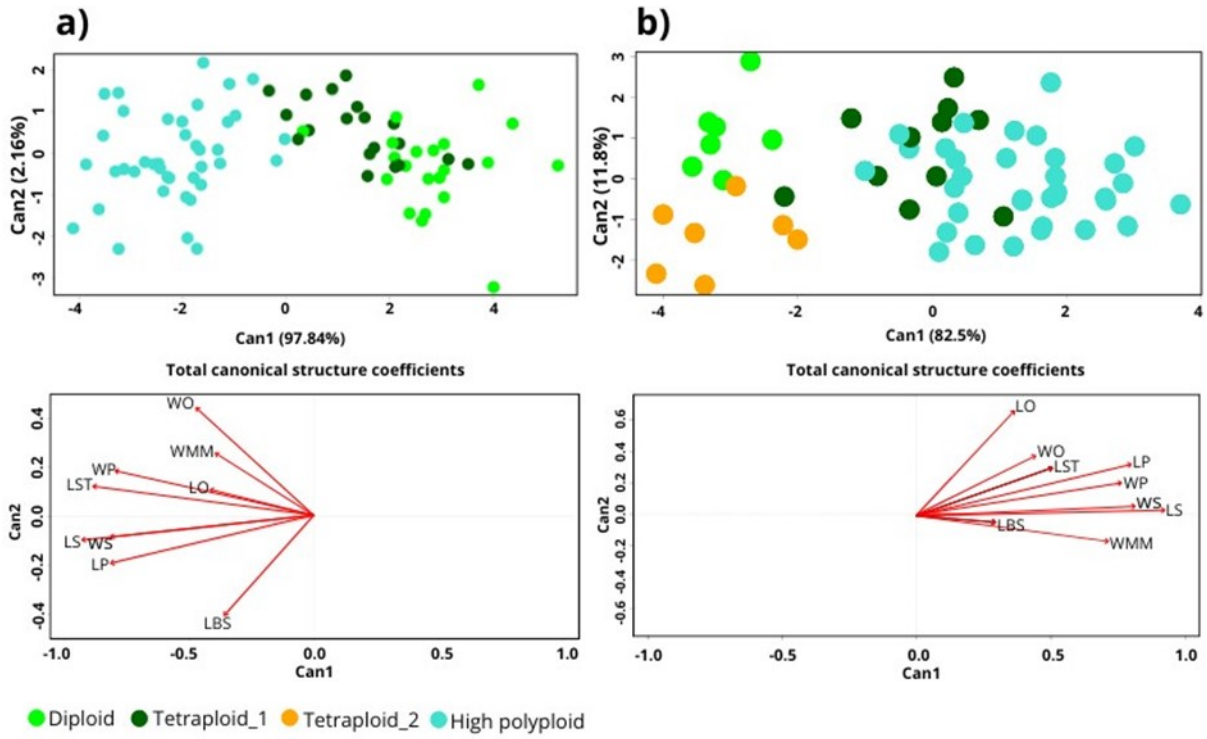


Figure 23: *Canonical discriminant analyses of cytotypes* ***a)*** *CDA 5 based on Matrix_M5 (84 individuals) with cytotypes diploid, tetraploid1 and high polyploid as predefined groups including only female individuals. Cytotype tetraploid2 was excluded due to the low number of individuals. The two axes explain 97.84% and 2.16% of variability.* ***b)*** *CDA 6 based on Matrix_M6 (59 individuals) with cytotypes as predefined groups, conducted only on intermediates. The first two axes explain 82.5% and 11.8% of variability, respectively. Contribution of characters to explained variability show plots below.*

CDA 7 (Matrix M7), including only diploid and tetraploid cytotypes, revealed a diffuse structuring with minor trends for separation along both axes (Figure 24a). The first axis explained 59.83% of the variability, tending to separate tetraploid 1 from the rest based on the length and width of the petal, maximum width of the membranous sepal margin, and width of the sepal. The second axis explained 40.17% of the variability.

CDA 8 (Matrix M8), including doploid and both tetraploid cytotypes, on the population level showed a more clear separation of the groups (Figure 24b). Groups were separated by both x-axis and y-axis explaining 67.38% and 32.62% of the variability, respectively. The tetraploid 1 cytotype was separated from rest mostly by the width of both petals and sepals. The diploid cytotype was separated from the other two groups mainly by the length of the sepal.

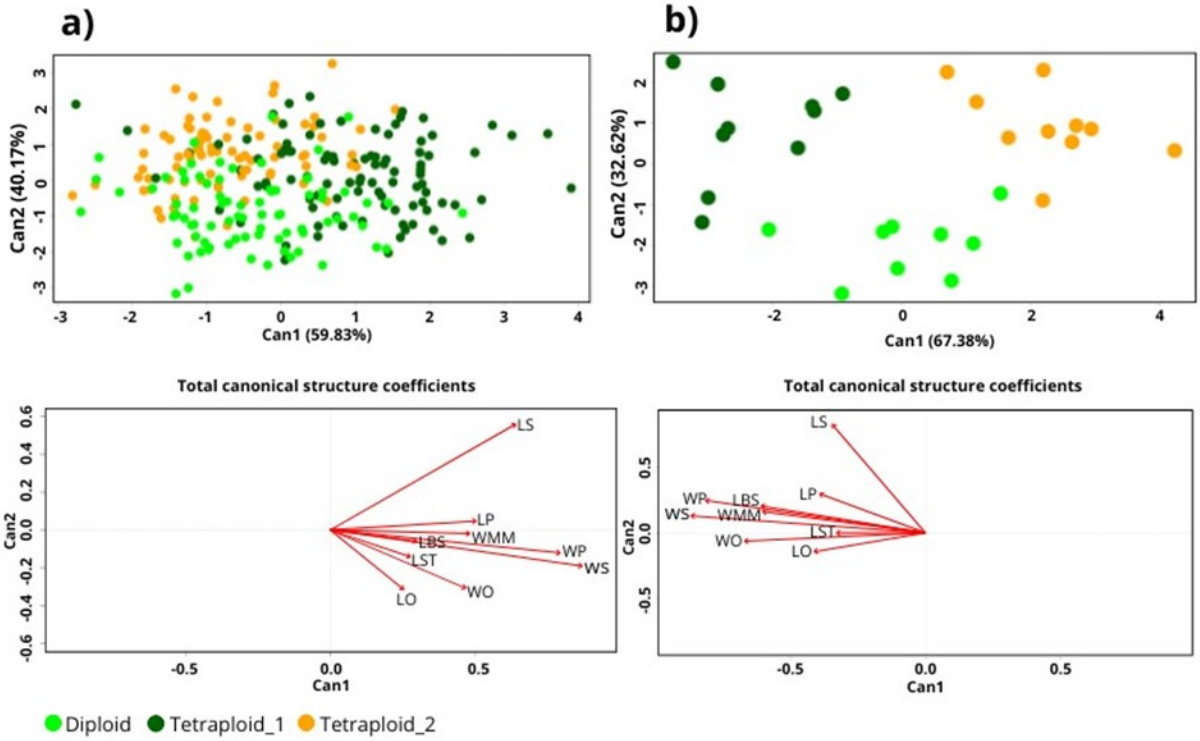


Figure 24: *Canonical discriminant analyses of cytotypes* ***a)*** *CDA 7 based on Matrix_M7 (254 individuals) with diploid and both tetraploid cytotypes as predefined groups, performed on individual level. The two axes explain 59.83% and 40.17% of variability.* ***b)*** *CDA 8 based on Matrix_M8 (29 populations) with diploid and both tetraploid cytotypes as predefined groups, conducted on the population level. The first two axes explain 67.38% and 32.62% of variability, respectively. Contribution of characters to explained variability show plots below.*

CDA 9 (Matrix M9) showed a relatively clear differentiation of both tetraploid cytotypes with only minor overlap (Figure 25). The most significant characteristics contributing to separation were the petal's width and the sepal's width.

CDA 10 (Matrix M10) showed almost clear separation between individuals of *S. palustris* from Central and Northern Europe with individuals from Central Europe being generally bigger in almost all characters, but mostly in length and width of the sepal and length of the style (Figure 26a). In CDA 11 (Matrix M11) including only hermaphrodites of *S. palustris*, differentiation between individuals from Central and Northern Europe was even more obvious, caused mainly by wider sepals and longer petal (Figure 26b).

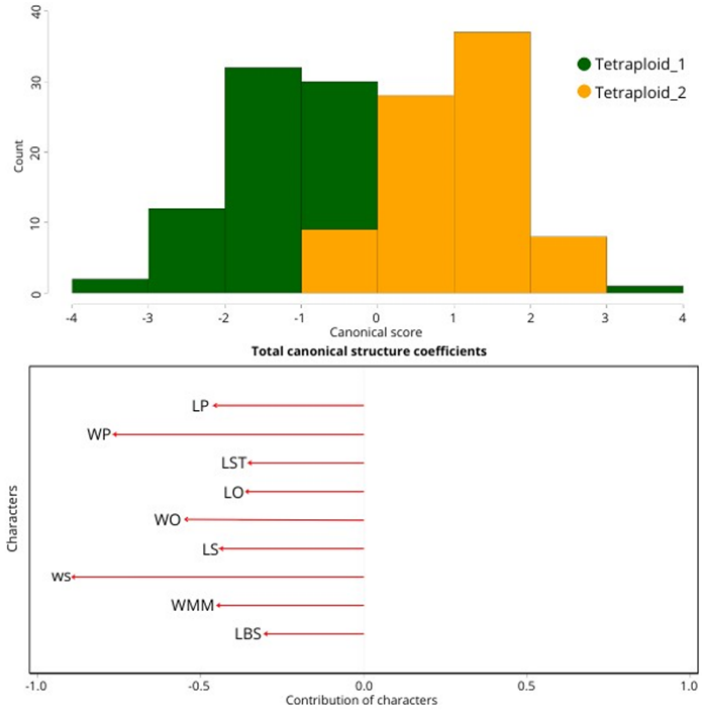


Figure 25: *Canonical discriminant analysis (CDA 9) of cytotypes based on Matrix_M9 (174 individuals) with two tetraploid cytotypes as predefined groups, performed on individual level. Contribution of characters is depicted on the plot below.*

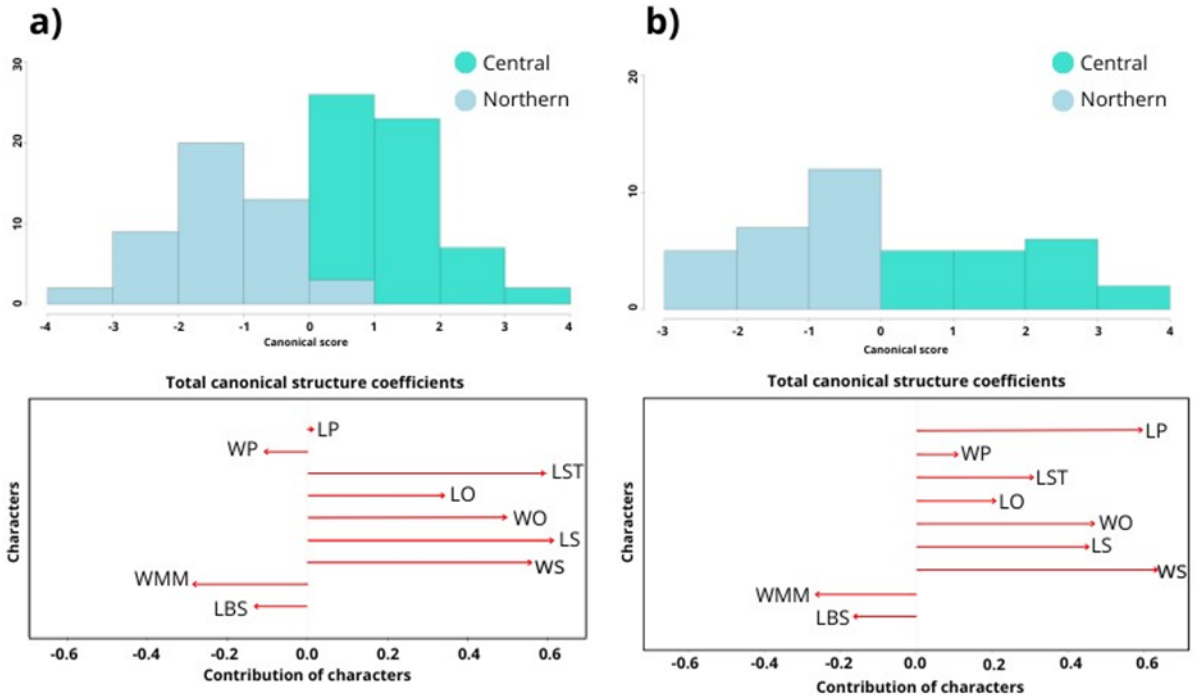


Figure 26: *Canonical discriminant analyses of cytotypes* ***a)*** *CDA 10 based on Matrix_M10 (116 individuals) with S. palustris from Central and Northern Europe as two predefined groups, performed on individual level including all sexual morphs.* ***b)*** *CDA 11 based on Matrix_M11 (45 individuals) with S. palustris from Central and Northern Europe as predefined groups, conducted on the individual level considering only hermaphrodites. Contribution of characters show the plots below.*

3.4. Evaluation of sexual polymorphisms

The variability in sexual expression analysed in the studied taxa is depicted in Figure 27a-f.

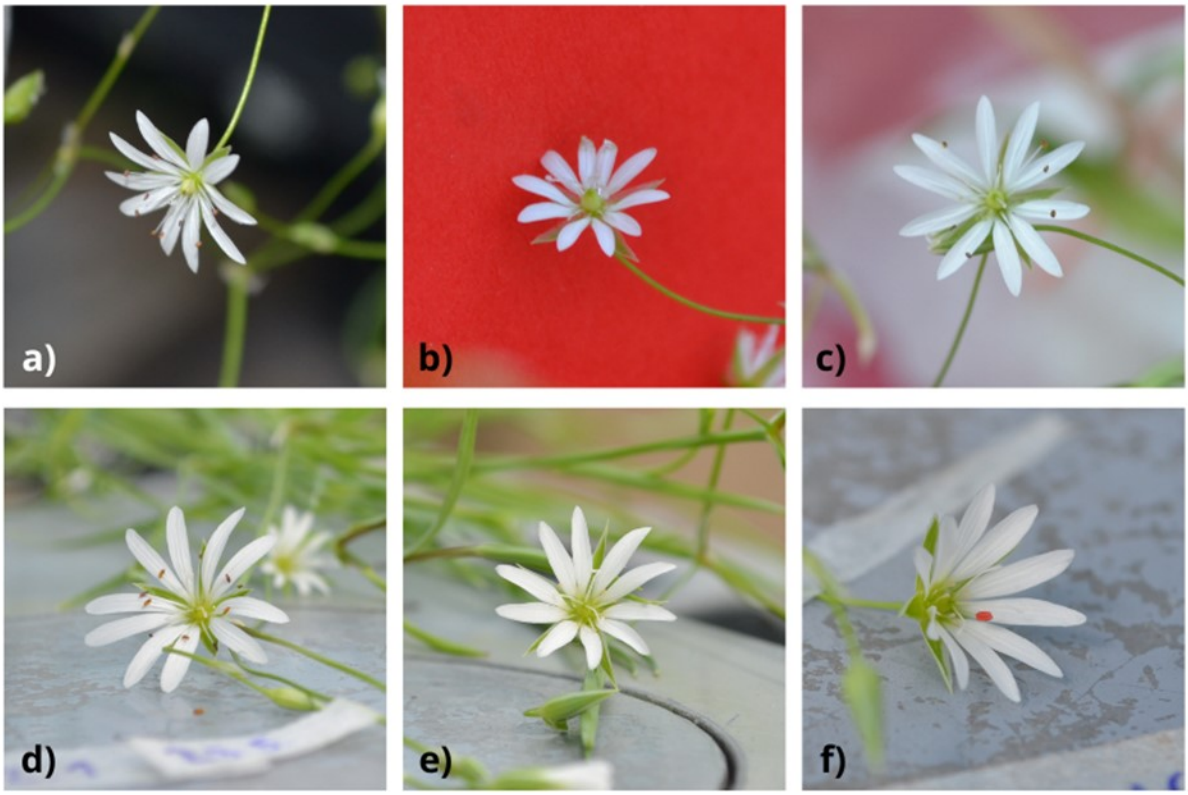


Figure 27: *Flower of sexual morphs in S. graminea and S. palustris.* ***a)*** *hermaphroditic morph of S. graminea,* ***b)*** *female morph of S. graminea,* ***c)*** *intermediate morph of S. graminea,* ***d)*** *hermaphroditic morph of S. palustris,* ***e)*** *female morph of S. palustris,* ***f)*** *intermediate morph of S. palustris.*

The CDA 12 (Matrix_M12) revealed a pattern where morphs were intermingled with only a subtle trend for separation (Figure 28a). The first axis explained 88.78% of the variability and mainly tend to separate groups with undeveloped and well-developed stamens. The character contributing most to the explained variability was the length and width of the petal. The second axis explained 11.22% of the variability, with the most influential character being the length of the style and the length and width of the ovary.

Similarly, CDA 13 (Matrix_M13) depicted a similar picture among the variability of sexual morphs in tetraploid cytotypes (Figure 28b). The first axis, which explained 84.37% of the variability, was most influenced by similar characters as in the tetraploid cytotype, such as the length and width of the petal, and the length from the base to the widest part of the sepal. The second axis, explaining 15.63% of the variability, was predominantly influenced by the maximum width of the membranous sepal margin.

Lastly, CDA 14 (Matrix_14) based on high polyploid *S. palustris* uncovered again continual variability among all three morphs. Along the first axis, which explained 76.65% of the variability, the groups with undeveloped and well-developed stamens were distinguished. The group with partly-developed stamens was mostly divided by the second axis, which explained 23.35% of the variability (Figure 28c).

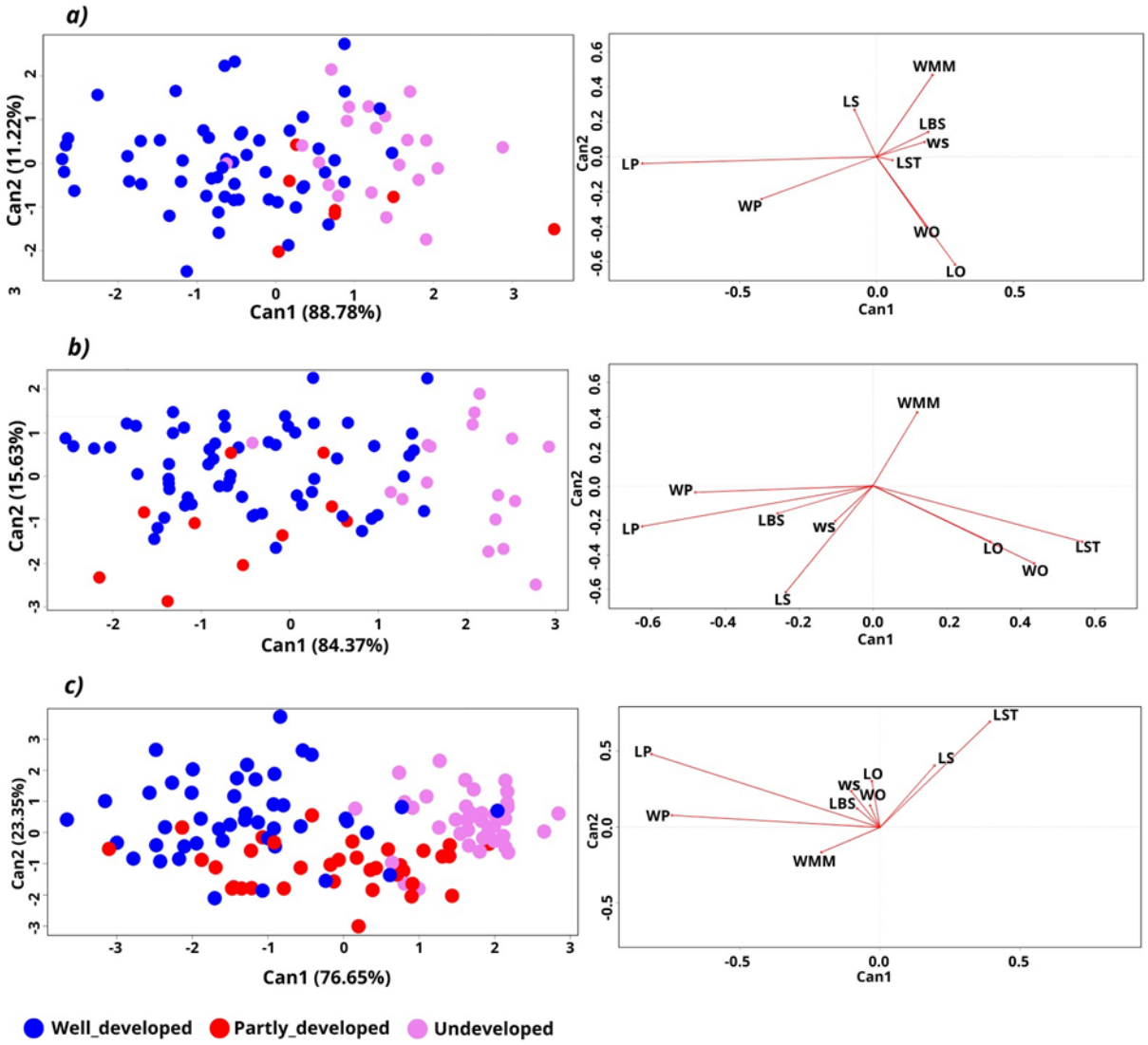


Figure 28: *Canonical discriminant analyses of sexual morphs* ***a)*** *CDA 12 based on Matrix_M12 (80 individuals) with morphs with well-developed, partly developed and undeveloped stamens of 2x cytotype as predefined groups, performed on individual level. The two axes explain 88.78% and 11.22% of variability.* ***b)*** *CDA 13 based on Matrix_M13 (86 individuals) with morphs with well-developed, partly developed and undeveloped stamens of tetraploid1 as predefined groups, performed on individual level. The two axes explain 84.37% and 15.63% of variability.* ***c)*** *CDA 14 based on Matrix_M14 (116 individuals) with morphs with well-developed, partly developed and undeveloped stamens of high polyploid as predefined groups, performed on individual level. The two axes explain 76.65% and 23.35% of variability. Contribution of characters is depicted in the plots on the right side.*

In summary, across all three cytotypes, the groups with well-developed and undeveloped stamens are primarily separated by the size of the petal, i.e., morphs with well-developed stamens have bigger petals compared to morphs with undeveloped stamens in all analyzed cytotypes. However, their separation is predominantly halted by morphs with partly-developed stamens.

The outcomes of CDA's based on datasets excluding intermediate individuals, CDA 15-17 (Matrices_M15-M17) revealed that the separation of the two major sexual morphs in all cytotypes was evident, with only minor overlap (Figure 29a-c). In all three cytotype groups,

morphs with well-developed stamens have bigger petals compared to morphs with undeveloped stamens. In the diploid cytotypes of *S. graminea* and high polyploid of *S. palustris*, the distinguishing trait contributing to the division of sex morph within these two groups is the length of the style, which is larger in females.

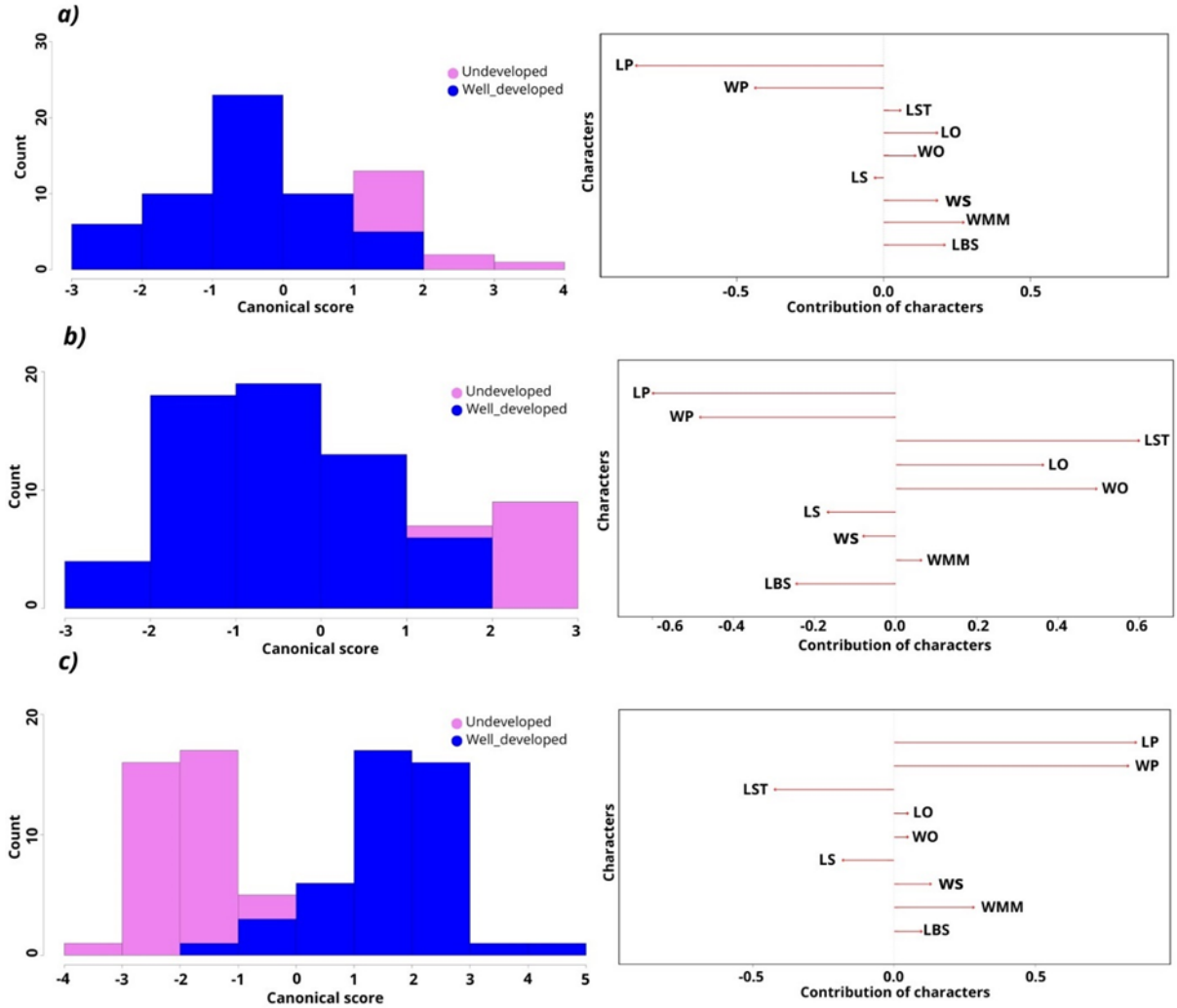


Figure 29: *Canonical discriminant analyses of sexual morphs* ***a)*** *CDA 15 based on Matrix_M15 (73 individuals) with morphs with well-developed and undeveloped stamens of 2x cytotype as predefined groups, performed on individual level.* ***b)*** *CDA 16 based on Matrix_M16 (76 individuals) with morphs with well-developed and undeveloped stamens of tetraploid1 as predefined groups, performed on individual level.* ***c)*** *CDA 17 based on Matrix_M17 (84 individuals) with morphs with well-developed and undeveloped stamens of high polyploid as predefined groups, performed on individual level. Contribution of characters is depicted in the plot on the right side.*

3.4.1. Correlation between latitude and sexual polymorphisms

QQ plots analysis revealed a non-normal distribution across all cytotypes and thus Spearman's correlation coefficient was utilized for analysis.

In the dataset including *S. palustris* (Matrix_S1), no significant correlations were identified between latitude and three sexual morphs (1- females, 2- intermediates and 3- females and intermediates together: -0.0795794, p = 0.778; 0.2073505, p = 0.4584; -0.1877359, p = 0.5204, respectively). Neither exclusion of two purely female populations (Matrix_S2) improved

results, and weak, non-significant correlation in all three cases left statistically insignificant (1- females, 2- intermediates and 3- females and intermediates together: 0.4832456, p = 0.09435; -0.08124811, p = 0.7919; 0.2049585, p = 0.5228; Figure 30).

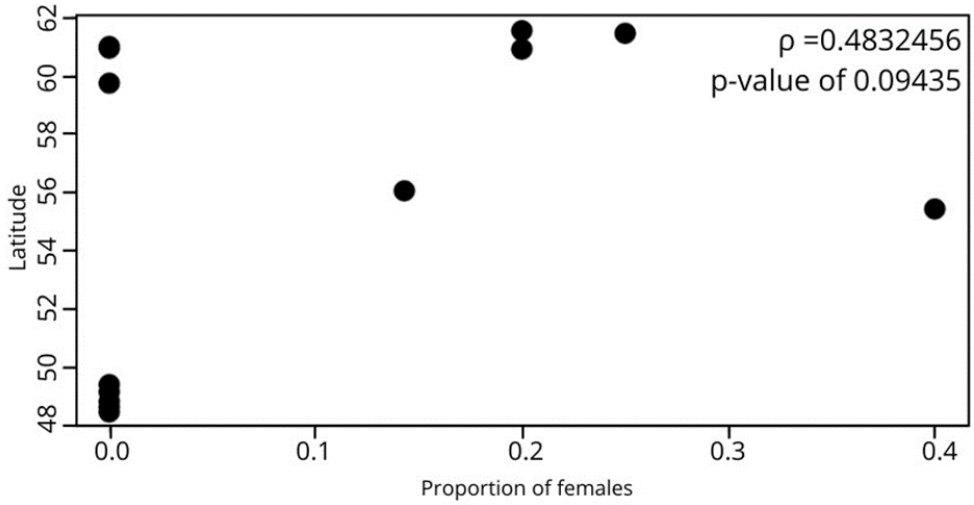


Figure 30: *Correlation plot between latitude and proportion of females in S. palustris based on Matrix_S2.*

The analysis of the overall proportion of sexual morphs within populations of *S. palustris* from Central and Northern Europe (Matrix_S3) revealed that in Central Europe, hermaphrodites constituted 46%, females 25%, and intermediates 29%, whereas populations from Northern Europe comprised 58% hermaphrodites, 12% females, and 30% intermediates (Figure 31). The overall proportion of sexes between Central and Northern populations of *S. palustris* did not differ significantly for any sexual morph (hermaphrodites p = 0.899, females p = 0.560, and intermediates p = 0.669). Intrapopulation variability of *S. palustris* in Central (Matrix_S4) and Northern Europe (Matrix_S5) showed more homogenous populations in Central Europe (Figure 32, table 4).

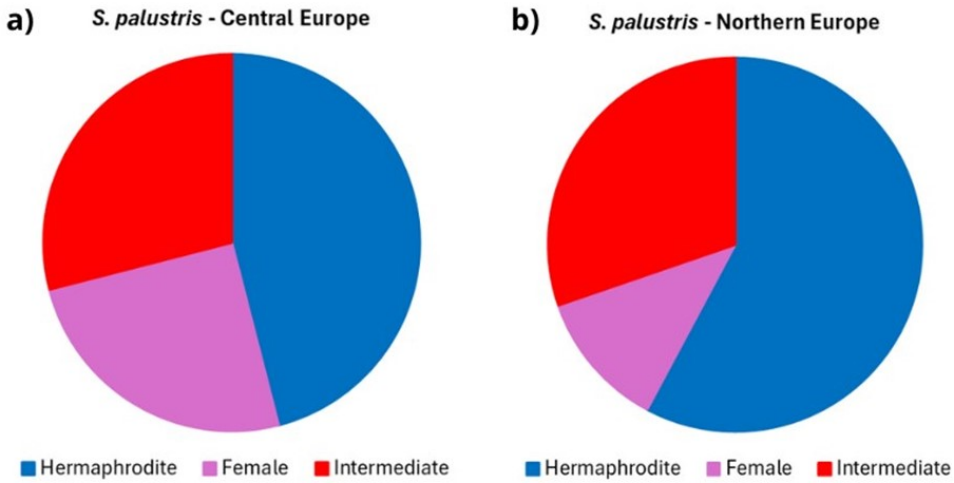
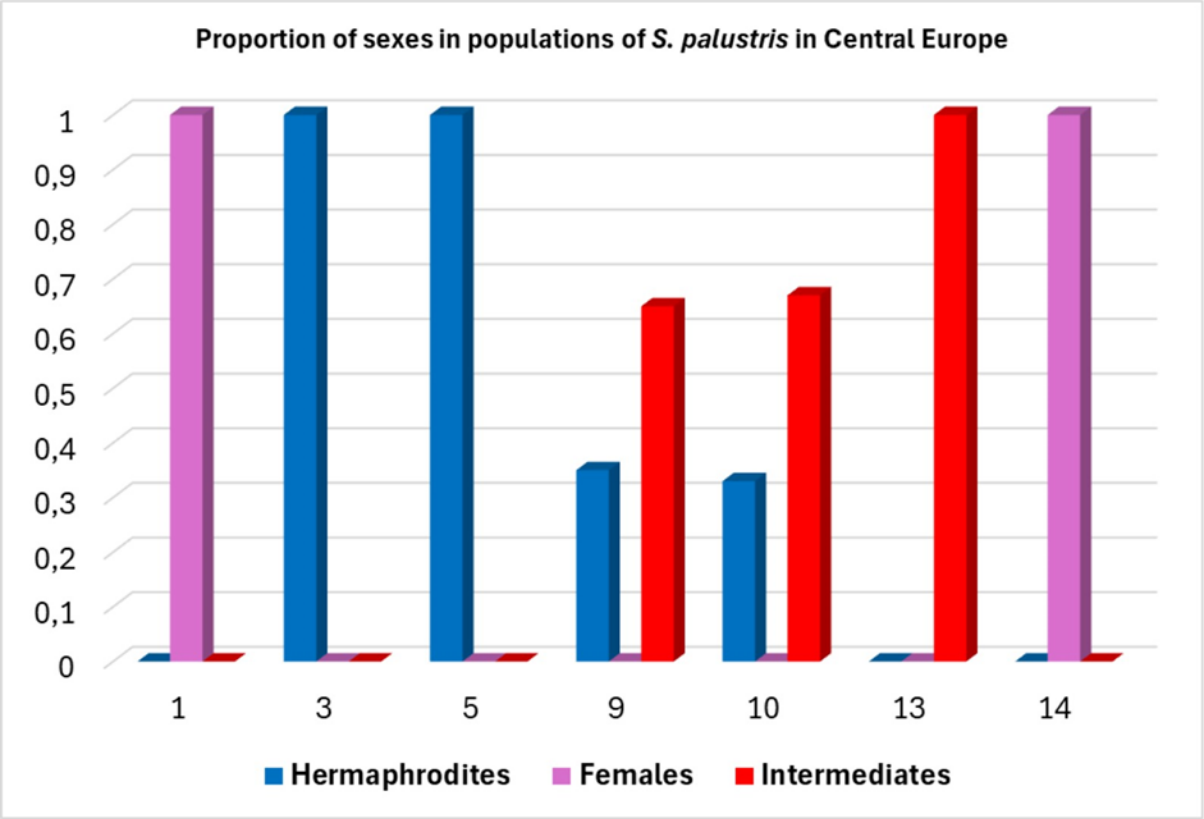


Figure 31: *Proportion of sexual morphs in the populations of S. palustris from Central and Northern Europe conducted on the Matrix_S3 (116 individuals, 15 populations). a) proportion of hermaphrodites (46%), females (25%) and intermediates (29%) from Central Europe (70 individuals, 7 populations), b) proportion of hermaphrodites (58%), females (12%) and intermediates (30%) from Northern Europe (46 individuals, 8 populations).*



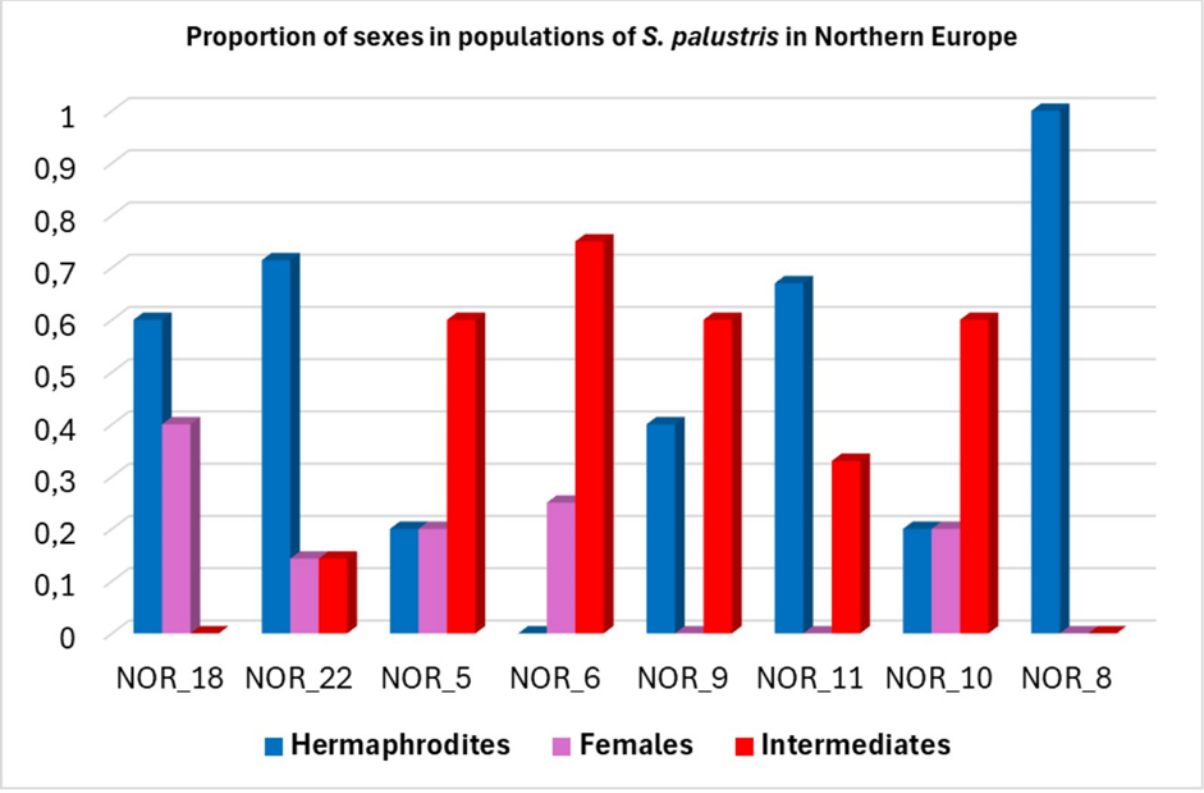


Figure 32: *Intrapopulation variability of sexual morphs in populations of S. palustris from Central (Matrix_S4, 70 individuals, 7 populations) and Northern (Matrix_S5, 46 individuals, 8 populations) Europe.*

a)

Population	Number of individuals	Hermaphrodites	Females	Intermediates
1	29	0	1	0
3	7	1	0	0
5	5	1	0	0
9	17	0.35	0	0.65
10	6	0.33	0	0.67
13	3	0	0	1
14	3	0	1	0

b)

Population	Number of individuals	Hermaphrodites	Females	Intermediates
NOR_18	5	0.6	0.4	0
NOR_22	7	0.71	0.14	0.14
NOR_5	5	0.2	0.2	0.6
NOR_6	8	0	0.25	0.75
NOR_9	5	0.4	0	0.6
NOR_11	3	0.67	0	0.33
NOR_10	5	0.2	0.2	0.6
NOR_8	8	1	0	0

Table 4: *Proportion of sexual morphs within scored populations from a) Central Europe and b) Northern Europe*

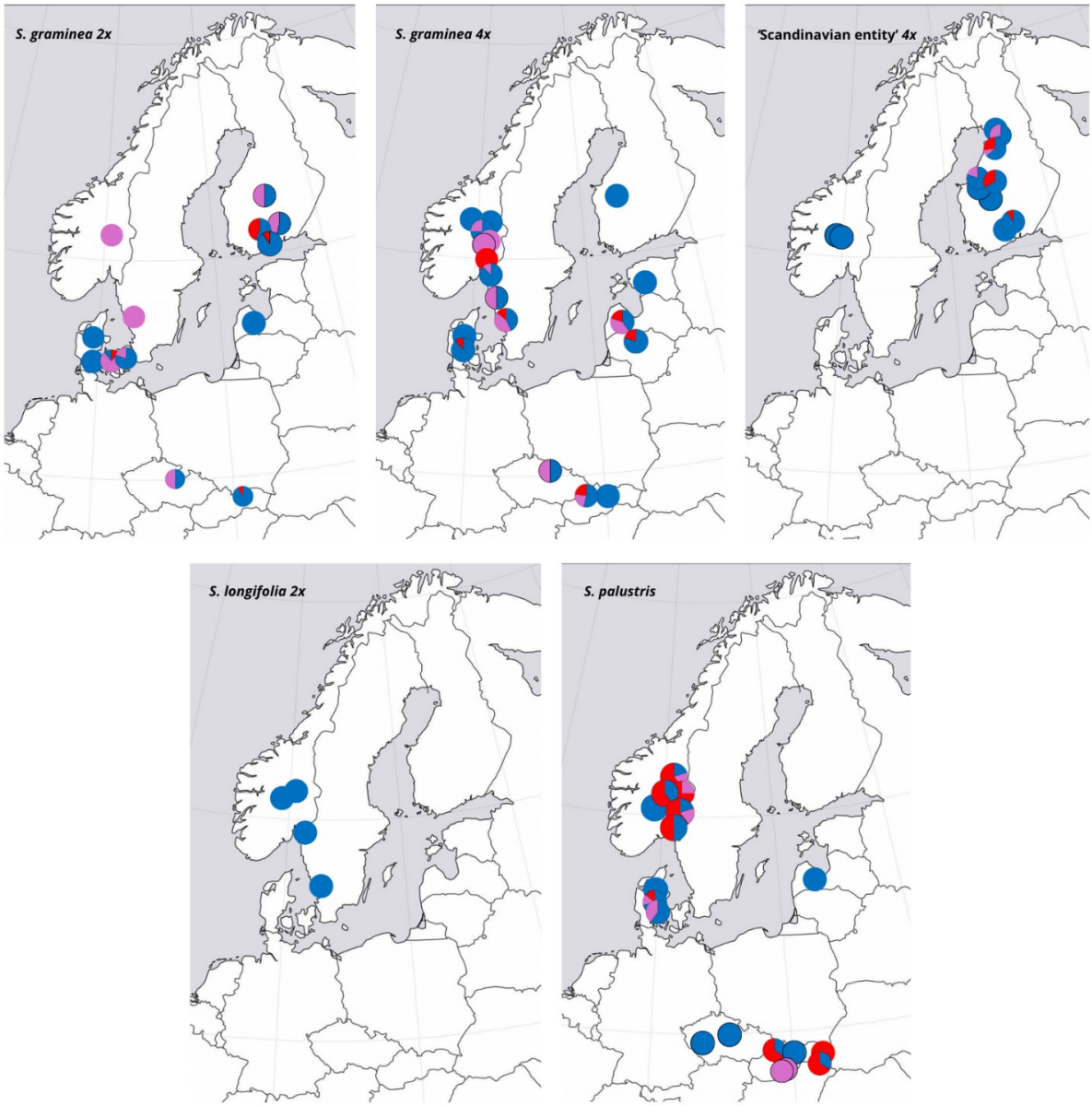


Figure 33: *Maps of geographic pattern of sexual expression in studied taxa and cytotypes at population level: hermaphrodites (blue), females (pink) and intermediates (red).*

4. Discussion

***Stellaria palustris* represents a highly polyploid complex with extensive karyological variability.**

Investigations into cytotype variability in plant species with high chromosome numbers, surpassing decaploid levels within species or even at the population level, have been undertaken only rarely, leaving high polyploids largely unexplored (but see D'Hont et al. 1998; Lagibo et al. 2005). Our study represents the first extensive karyological investigation of the understudied, high polyploid *S. palustris* to date. We have identified at least three major cytotypes with chromosome numbers ranging from 2n = 154 to 208, including three major even cytotypes attributable to 12x, 14x, and 16x ploidy levels (see Results, Figure 11). Additionally, two aneuploid cytotypes likely derived from the 14x cytotype were detected. The highest ploidy level, 2n=16x=208, was identified in this species as well as the entire genus for the first time (cf. Kurrto 2001; Morton 2005; Rice et al. 2015). Our findings align with previous literature reports predominantly conducted on plants from Northern Europe, which also exhibit lower chromosome counts, as found here, ranging from 130 to 188 chromosomes (Peterson, 1936; Blackburn & Morton, 1957; Lövkvist & Hultgard, 1999; Kurtto, 2001; Morton, 2005; Stace, 2010). Nevertheless, given our ongoing, yet unsuccessful, work with chromosome counting in individuals from Northern Europe and the absence of a clear correlation between genome size and chromosome numbers, there remains a possibility of detecting lower ploidies. However, we cannot rule out also the possibility that our team may have overlooked these cytotypes or that these high polyploid cytotypes evolved in more distant regions where intermediate cytotypes still exist and co-occur with their high polyploidy level derivatives. Indeed, the detection of lower ploidies could shed more light on the origin of the 12x, 14x, and 16x ploidies, which is especially enigmatic in the absence of intermediate cytotypes such as hexa-, hepta-, or octoploids. These intermediate cytotypes might offer a more parsimonious explanation for the emergence of these high polyploids, thus completing the evolutionary puzzle of this fascinating high polyploid species complex. Regardless of this, we provide compelling evidence that *S. palustris* constitutes an exceptionally high polyploid species complex with extensive within-species cytotype variability.

Furthermore, RGS and AGS analyses indicate significant cytotype variation within *S. palustris*, even at the population level (Figure 17 and 18). Precise cytotype identification and the revelation of the overall cytotype pattern of this species are challenging as already mentioned, making them unsuitable as indicators of ploidy level (cf. Štubňová et al. 2017). Consequently, chromosome number-based ploidy level inference cannot be used to confirm or reject our hypothesis regarding higher cytotype diversity positively correlated with latitude, suggesting higher ploidy levels in Scandinavia. Due to this phenomenon, we have to acknowledge that there might be multiple cryptic cytotypes in our dataset, which can only be detected using direct chromosome number counting. This is exemplified by the only published ploidy level inference from Central Europe to date, conducted by Šmarda et al. (2019), where the estimated genome size for the analyzed plant was 2C = 6.44 pg. This value falls within the AGS range of *S. palustris* analyzed here (6.03 pg to 8.65 pg); however, only two individuals from the Czech population (35_2_1 and 35_2_2) have similar AGS, and unfortunately, we have no chromosome counting for them. The authors attributed this AGS value to a decaploid ploidy level, although based on the mean monoploid genome size from our study (0.57 of ±15%), it rather indicates an 11x ploidy level. Considering the lack of correlation between genome size and chromosome number detected in our study, the true chromosome number and ploidy level may be lower or even higher due to the complex dynamics of genome evolution in such high

polyploids, including genome downsizing and/or recombination (Feldman et al. 1997; Ozkan et al. 2001; Wang et al. 2021).

Still, to test our hypotheses about the correlation between latitude and cytotypes, we could at least conduct correlation analyses using RGS data alone. Correlating RGS with latitude revealed a non-random pattern in genome size diversity within *S. palustris* populations across the studied regions. A statistically significant negative correlation (rho -0.4121739, p-value = 0.04643), suggests that genome size tends to be slightly higher in populations from Central Europe compared to those from Northern Europe. Surprisingly, this trend decreases towards the north, partially contradicting our hypothesis of higher karyological diversity in Northern Europe and deviating from the general paradigm proposed by Rice et al. (2019) suggesting that genome size increases with latitude (see also Adams & Wendel, 2005; Brochmann et al., 2004; Martin & Husband, 2009). However, it is essential to acknowledge that these results can be also affected by the limitations of our study, particularly the large unsampled areas between these two regions, especially in Poland and Germany. While it is unlikely that these regions would host entirely different karyological diversity with distinct cytotypes, data and information from these areas would be desirable for a more robust, final inference.

An intriguing question arises regarding the observed shift in RGS in Central European populations of *S. palustris*. Organisms with high chromosome numbers often undergo significant molecular changes, such as meiotic recombination (Pecinka et al. 2011) or loss of non-coding DNA regions (Feldman et al. 1997; Ozkan et al. 2001), leading to fluctuations in genome size within a given ploidy level. Molecular mechanisms underlying these rearrangements involve dynamic losses or gains in the abundance of transposable elements. Indeed, retrotransposons are considered significant drivers of genome expansion induced by genomic and environmental stress (Kumar and Bennetzen, 1999; Bui and Grandbastien, 2012). Such conditions are not uncommon in the marginal areas of distribution ranges. The center of diversity of this species, primarily inferred from morphology, abundance, and published chromosome numbers, is presumed to be located in Northern Europe (Kurtto 2001; Morton 2005). This region is characterized by cold and humid environments, to which this species is well adapted, thus most likely experiencing fewer challenging environmental stressors. Therefore, the observed increase in RGS of populations in Central Europe may be associated with adaptation to drier and warmer conditions in this region, which might be suboptimal or even challenging for this species. Moreover, changing environments with multiple stressors might stimulate the emergence of new polyploids with slightly different genome sizes compared to established populations, thereby broadening the range of genome sizes in Central Europe. Additionally, newly formed high polyploids are prone to meiotic problems and irregularities, which can lead to the production of aneuploid offspring (Ramsey & Schemske, 2002). Consequently, the formation of new cytotypes could increase the occurrence of aneuploids, contributing to increased genome size variability in Central Europe. The presence of aneuploids in *S. palustris* is confirmed in this study and has been documented in previous research as well (Peterson, 1936; Blackburn & Morton, 1957; Lövkvist & Hultgard, 1999; Kurtto, 2001; Morton, 2005; Stace, 2010; Rice et al. 2015). While it might be argued that aneuploidy could lead to reduced fertility and be non-competitive, thus quickly disappearing

from populations, studies have shown that aneuploids can persist and even compete with euploids (Kostoff, 1938; Bingham, 1968; Simonsen, 1975). Moreover, in highly polyploid organisms, aneuploidy may have a lesser impact on overall fitness and phenotype (Birchler, 2013), suggesting that it may be less deleterious. Finally, the maintenance of aneuploids within populations could be facilitated in *S. plalustris* by clonality (Van Drunen & Husband, 2019; Edgeloe et al., 2022).

Genetic data did not provide further insights into the within-species diversity of *S. palustris*. We observed no apparent differentiation or variations in genetics between populations in Central and Northern Europe (see Figures 3-8). On the other hand, morphometric analysis (Figure 26) showed morphological distinction of individuals from Central and Northern Europe with individuals from Central Europe being bigger on several characters. This finding might be linked with increased RGS detected in Central European populations and warrants further study.

The complex sexual system of *Stellaria palustris*: Gynodioecy and the frequent emergence of sexually intermediate forms

Our investigation confirmed the presence of sexual polymorphisms throughout the entire studied area of *S. palustris* (See Figure 33). However, our findings unveiled a far more intricate system of sexual expression than anticipated. In addition to dominant hermaphroditic individuals and purely female plants, we detected intermediate sexual morphs between both major sexes, a phenomenon already evidenced in other sexually polymorphic *Stellaria* species (Philipp 1980; Dang and Chinnappa 2007; Kučera et al. 2021). Additionally, several individuals demonstrated even varying sexual expressions among morphs within a single plant. We observed all possible combinations of morphs, including: a) hermaphrodites, females, and intermediates on one plant, but also b) females and intermediates coexisting on one plant, c) hermaphrodites and intermediates, and d) hermaphrodites and females. Similar extensive variability in sexual expression within a single individual was also identified in another sexually polymorphic species (*Hirschfeldia incana* (L.) Lagr.-Foss (Brassicaceae), Horovitz and Galil 1972; *Plantago coronopus* L.(Plantaginaceae), Koelewijn and Damme 1996; *Diospyros kaki* Thunb. (Ebenaceae) Akagi et al. 2016). We can conclude that *S. palustris* exhibits a gynodioecious reproductive system, yet the presence of diverse intermediate morphs suggests a highly labile and unstable sexual expression (Horovitz and Galil 1972; Koelewijn and Damme 1996).

This finding also crucially affects any analyses inferring from the frequency of sexual morphs at the population level, as well as at the overall pattern level. Specifically, at the population level across the studied area, the majority of populations exhibited sexual polymorphism, encompassing all three sex morphs (Figure 32). Thus we did not observe crucial difference in the sexual expression of *S. palustris* between Central and Northern Europe (Figure 31), which suggests no specific shifts in ecological niches in females compared to hermaphrodites, contrary to what has been shown in other species (e.g. *Salix arctica* Pall., (Salicaceae), Dawson and Bliss 1989; *Lobelia spicata* Lam. (Campanulaceae), Ruffatto et al. 2015; *Valeriana edulis* Nutt. ex Torr. & A.Gray, (Caprifoliaceae), Petry et al. 2016). This was

also evident in the lack of correlation between latitude and the presence of sexual polymorphisms, suggesting that this species may not be significantly influenced by changes in environmental conditions. However, this observation might be significantly blurred by the sexual instability of sexual expression in *S. palustris*. Thus, current data and the state of knowledge do not allow us to draw strong and final conclusions on analyses based on sexual morph frequency-based analyses.

In spite of this, at least two populations from southern Slovakia (1 and 14, Figure 33) deserve more additional attention, as both comprised solely female plants. In the case of population 14, this phenomenon could be attributed to its small size, as we only encountered 3 individuals due to untimely mowing of the protected wet meadow habitats during the species' flowering period in Slovakia. This occurrence recurred in both years of our fieldwork. Conversely, the second locality (population 1) experienced later mowing and a wetter environment, enabling the collection of most plants during both fieldwork seasons. Despite our thorough search efforts, hermaphrodite or intermediate individuals were not observed. One potential explanation for this unanticipated unisexual pattern, biased towards females, could be the clonality of this species (Kurtto 2001). The disappearance of hermaphrodites from the population may have led to an acute pollen availability. Thus, aside from sampling biases, the logical explanation is that females persisted in the locality after the disappearance of hermaphrodites through clonality, facilitated by the ecological conditions in the habitats. Notably, both sites experience spring and summer floods, during which vegetative fragments can easily disperse across flooded areas and establish new individuals. However, a more scientifically appealing explanation is supported by ongoing studies with plants from these populations (unpublished data). Upon transferring *S. palustris* plants to the greenhouse, female plants began to shift their sexuality during the subsequent flowering season. Several plants produced flowers with at least one well-developed stamen. This suggests a high degree of sexual system lability in *S. palustris*, potentially serving as a backup plan in cases of pollen insufficiency due to the lack of hermaphrodite individuals.

Despite the hight number of studies on gynodioecious species (e.g., Caruso & Case, 2007; Delph et al., 2007; Ruffatto et al., 2015; Varga and Soulsbury, 2020), including those within the *Stellaria* genus (Philipp, 1980; Dang and Chinnappa, 2007; Kučera et al., 2021), there remains a significant lack of information regarding intermediate flowers and plants (but see Horovitz and Galil 1972; Koelewijn and Damme 1996). Our own investigation unveiled an unexpectedly high prevalence of intermediates, particularly within *S. palustris*, but also within *S. graminea*. In the case of *S. palustris*, intermediates accounted for nearly one-third (29%) of all scored morphs in our study. At the population level, their frequency ranged from 0 to 100% across the entire area (Figure 33), with similar proportions observed when considering Central and Northern Europe separately (0-100%, average 33% and 0-75%, average 38%, respectively, see Figure 32 and Table 4). In contrast to our findings, a previous study examinated also the frequency of sexual morphs in *S. graminea* (Kučera et al., 2021) identified intermediate flowers in only 2% of examined individuals. However, considering that the identification of such morphs was not the primary focus of their study, it is plausible that these intermediates may not have received as much attention.

The high frequency and presence of intermediate flowers and/or plants is likely a phenomenon with a highly complex background, involving effect of genetic, epigenetic, and environmental factors (Janoušek et al. 1996; Koelewijn and Damme 1996). It might be linked to the mode of inheritance of sexual polymorphisms in this species. Assuming that male sterility is cytoplasmically encoded in this species, as already presumed for *S. graminea* (cf. Kučera et al., 2021), there exists the potential for the restoration of male fertility, which can occur either completely or incompletely during crosses (Palmer et al., 1992; Charlesworth & Laporte, 1998; Schnable & Wise, 1998; Van Damme et al., 2004; Garraund et al., 2011; Touzet, 2012). Such processes may be heightened in high polyploids due to the increased number of chromosomes and the associated challenges during mitotic division. However, this instability can also be presented in cases of purely nuclear inheritance (Schultz 2002; Chang et al. 2016; Chen et al. 2019), because in such high polyploids, the dynamics in the evolution of particular chromosomal sets and also given genes can be high, and even mutations in some portion of alleles do not necessarily completely block the function of such genes, resulting in such combined intermediate phenotypes (Schultz 2002).

Furthermore, numerous studies suggest that sex changes i.e., changes in sex within a single generation can be induced by environmental factors (Horovitz and Galil 1972; Zimmerman 1991; Koelewijn and Van Damme 1996). For instance, environmentally induced sex changes have been documented in the *Stellaria* genus, notably in *S. longipes* (Dang and Chinnappa 2007), where temperature was identified as a major influencing factor, whichplays role also in other species (e.g. Burns et al. 1991; Koelewijn and Damme 1996; Asikainen and Murikainen 2003; Vaughton & Ramsey, 2005).

In a study on *S. graminea*, Kučera et al. (2021) observed a trend of females occupying drier sites. While this may not directly demonstrate environmentally induced sex changes, it does imply that the species is responsive to environmental conditions, potentially affecting its sexual system. Such changes may be triggered, for example, by the absence of pollinators (Caruso and Case, 2007), variations in water regime (Freeman et al. 1984; Venkatasamy et al. 2007), ligth availability (Friedman and Barrett 2011, Berjano et al. 2014), or fitness of given individual (Nanami 2004; Blake-Mahmud and Struwe 2019).

The impact of sexual expression, especially its lability, on the morphology of sexual organs is intriguing. Hermaphroditic individuals exhibited larger perianth traits compared to females, consistent with a rather general trend observed in several studies of gynodioecious species (Delph, 1996; Shykoff et al., 2003; Ashman, 2006; Barrett & Hough, 2013; Dufay et al., 2014; Kamath et al., 2017; Kučera et al., 2021). The larger flowers in hermaphrodites may be explained by several hypotheses (Delph, 1996; Miller and Venable, 2003; Paterno et al., 2020; Varga 2021). One stems from the physiological need to accommodate reproductive organs of both sexes, compared to females which have only female organs, while anthers are dramatically reduced in size. Althought, females exhibited slightly larger styles compared to hermaphrodites, but this trait might be influenced by the time of collection of flowers as this species is considered to be proterandrous, and styles continuously enlarge from flower opening until maturing (Morbey and Ydenberg, 2001; Çetinbaş and Ünal 2014). Furthermore, this

sexual flower dimorphism may be driven by pollinator attraction, following Bateman's principle, which posits that male gamete-producing flowers (in this case hermaphrodites) are more limited by pollinators and therefore need to increase their attraction through larger perianth size (Bateman, 1948).

Although morphological traits differ among cytotypes, following the trend of increasing size with increasing ploidy level, sexual flower dimorphism remains consistent across cytotypes (see Figures 28 and 29). This suggests that ploidy has a minor impact, especially on perianth size, with sexual selection and pollinator attraction being the primary drivers of this phenomenon which was clear when cytotypes were analysed using either only hermaphrodites or only females (Figure 22 and 23a), but not in the intermediates (Figure 23b). In addition, intermediates further blurred this pattern, rendering it more diffuse and showing no obvious structuring. However, this indicates that intermediates were morphologically intermediate not only in terms of anther morphology and functionality but also in the size of other flower parts.

Insights into the diversity and evolution of the high polyploid cytotype complex of *S. palustris*: a genetic, karyological, and morphological perspective

Our study provides also the first more insight into the genetic, karyological, and morphological variability of the highly polyploid complex within the *Stellaria* genus, although it raises more questions than answers. Previous studies on *S. palustris* mostly involved single or a few accessions (Zhang et al., 2017; Greenberg & Donoghue, 2011; Sharples, 2019; Sharples & Tripp, 2019; Arabi et al., 2022). Phylogenetic trees inferred from ITS, cpDNA, and combined concatenated datasets (Figures 3, 4 and 5) suggest that the taxa and cytotypes collected within our study form a statistically strongly supported monophyletic group. However, the internal structure of the clade reveals a very shallow structure with shallow resolution, where species-specific clades were statistically unsupported and placed in a basal polytomy. Additionally, the cpDNA parsimony network reveals very low haplotype variability, with haplotypes separated mostly by very few mutation steps, except for an individual presumably belonging to 'Scandinavian entity' (SE_NOR_2_8), which appears distant from the rest of the haplotypes. This finding likely indicates a recent origin of this species group. Our results are at least partially congruent with the outcomes of studies based on RAD sequencing analyses, which showed a close relationship and monophyly of *S. graminea* and *S. palustris* (Sharples, 2019; Sharples & Tripp, 2019). However, this monophyly of *S. graminea* and *S. palustris* as a group, as well as each species separately, holds only until we analyze ITS sequences from the gene bank (Figure 3). In BI trees and neighbor-net analyses (Figure 3 and 6) based on all ITS data, all three species under study*, S. graminea, S. palustris*, and *S. longifolia*, appear to be non-monophyletic, as several accessions of each from the gene bank appear in a sister clade to that of individuals sequenced within this study, together with diverse *Stellaria* species. Specifically, in case of *S. palustris*, one accession originated from China (KX158327; Zhang et al., 2017), while the second accession (JN589080, Greenberg and Donoghue, 2011) likely originated from North America. Only the last sequence of *S. palustris* from GeneBank (MT624621; Arabi et al., 2022) collected from Finland belongs to the same clade as individuals from our study. Similarly, two sequences from GenBank belonging to *S.*

graminea (MT912275 and MT923274), both originating from China, appear in a statistically unsupported grouping with *S. edwardsii* and *S. humifusa* (Figure 3). Additionally, a sequence of *S. longifolia* (JN589146, Greenberg and Donoghue, 2011), also with an unknown origin, was found to be distant from the clade consisting of our accessions of *S. longifolia* and sequences of *S. borealis* and *S. calycantha*, which appear to be essentially identical and do not form their species-specific grouping. These findings strongly suggest that the taxonomy of these widespread species is far from ideal and underscores the necessity for a comprehensive taxonomic revision. This should involve extensive sampling across the major, if not the entire, distribution range of the studied species.

Our study also presents evidence for a likely new taxon, tentatively named the 'Scandinavian entity', which emerges from our collection of populations presumed to belong to the smaller flower morphotype of *S. palustris* (cf. Kurtto, 2001). This entity forms a very closely related but distinct genetic lineage to *S. palustris* and *S. graminea* in ITS and concatenated analyses (Figures 3 and 5). Karyological analyses, based on a combination of direct chromosome counting and genome size analyses, confirmed that this lineage possesses a tetraploid cytotype with 2n=4x=52, albeit with relative RGS and AGS lower than the ubiquitous tetraploid cytotype of *S. graminea* (Figures 14 and 15). Morphologically, it closely resembles both cytotypes of *S. graminea*, but it can be distinguished, at least at the population level, by width of petal and sepal and size of the ovary (Figure 24). Its morphology aligns closely with the taxon described in Flora Nordica (Kurtto, 2001), named *S. fennica*, which is closely related to *S. palustris* and is sometimes considered a variety thereof. Kurtto noted the difficulty in distinguishing this species from *S. palustris* (Kurtto, 2001), with the only reliable distinguishing trait potentially being small papillae on the stem and leaves, even though some varieties of *S. palustris* can be also papillose (Kurtto 2001). *S. fennica* typically occupies habitats similar to *S. palustris*, leading to potential confusion between the two species. Reported to be native to Russia, Finland, and Norway, our findings support its widespread presence in Finland and less frequent occurrence in Norway. However, its precise distribution range remains uncertain.

Complex signals from molecular, karyological, and morphological data do not help unambiguously distinguish between allo- or autopolyploid origin of the high polyploid cytotype of *S. palustris*.

One of the most intriguing questions concerns the origin of the high polyploid *S. palustris.* By combining morphological, genetic, and karyological traits, *S. palustris* can be clearly distinguished from *S. graminea, S. longifolia,* and the 'Scandinavian entity'. From a morphological perspective, it differs from congeners at the population and individual levels, predominantly by larger flower organs (Figures 21, 22 and 23). As *S. palustris* is exceptionally high polyploid, we presume that these differences most likely reflect polyploidization, particularly the well-established Gigas effect, typically presented in autopolyploidisation events (Pei et al., 2019; Becker et al., 2022). However, we cannot rule out the possibility of contributions from other species through allopolyploidization. Differentiating between these processes poses a challenge, given that *Stellaria* species, particularly from these group

including *S. graminea, S. palustris* tend to exhibit morphological similarities, with significant distinctions involving quantitative traits (Kurrto 2001; Morton 2005, Sharples & Tripp, 2019), that however offer limited discriminatory power to differentiate between auto- and allopolyploid origins. Furthermore, ITS sequences of *S. palustris* harbor a significant number of additive polymorphisms at numerous polymorphic positions, indicating that they possess multiple ribotype copies in their genome. We are convinced that they significantly contributed to the lack of statistical support for the given species-specific clades in the Bayesian inference phylogenies (Figures 3 and 5). This might be an indication of hybridization, and in this case, it is also linked with whole genome duplication, i.e., an allopolyploidization event. Most of individuals display varying levels of intra-individual ITS copy variability, suggesting diversity in the extent of concerted evolution towards specific parental copies. Such patterns can notably differ across individuals and populations in the studied distribution range of the species (cf. Fuertes and Nieto 2003; Nieto Feliner et al., 2004). This aligns with the position of specific individuals in the neighbor-net analysis, which reflects a continuous pattern of additive polymorphic sites across the studied individuals (Figure 6 and 7). However, numerous additive polymorphisms, also with varying levels of homogenization, were found in diploid and tetraploid cytotypes of *S. graminea, S. longifolia*, and the 'Scandinavian entity'. Thus, it remains to be distinguished whether ribotype copies forming such complex patterns in sequences on S. *palustris* accessions originated from different species or alternatively represent duplicated ancestral polymorphisms that were still enriched by the evolution of specific copies in the genome, deviating from the major ribotypes. Indeed, in such exceptionally high polyploids, as represent cytotypes of *S. palustris*, predicting and disentangling the dynamics of the evolution of multicopy ribosomal genes is exceedingly challenging.

The controversial position of specific individuals in genetic analyses blurs the overall genetic pattern among the analyzed species.

Several accessions deviate from their species-specific clades in NN and BI analyses of the ITS dataset, further complicating the overall relationship among taxa and cytotypes. One intriguing case involve two individuals sequenced for ITS, initially assigned to first to S. palustris, which were later uncovered to belong to the 'Scandinavian entity'. The first ot of them (NOR_SE_2_8) clustered with *S. longifolia*, while the second (NOR_SE_2_11) grouped with *S. palustris* accessions. This suggests their potential hybrid status, compounded by the high level of additive polymorphisms detected in ITS data. Nevertheless, accession NOR_SE_2_8, along with other unsequenced individuals from the same population (NOR_SE_2_5, NOR_SE_2_6, and NOR_SE_2_10), notably differs in its RGS significantly exceeds that of the 'Scandinavian entity' and *S. longifolia* but were also apparently lower than those of *S. palustris* (Figure 14). Based on RGS we could presumme that it is a tetraploid cytotype from *S. longifolia* (cf RGS values in Results section and Figure 14). However, no individual and cytotypes attributable *to S. longifolia* were found at the locality. The autopolyploid origin of this cytotype from diploid *S. longifolia* is further contradicted by findings in plastome phylogeny, where these two individuals possess unique haplotypes. Indeed, haplotypeof SE_NOR_2_8 ndiffers from its closest one (SE_NOR_2_11), by nine mutation steps (see Figure 9). This suggests this accession may belong to a completely different, unsampled taxon or within-species lineage. Unfortunately, flowering plants were unavailable, and those transplanted from the field died early, precluding chromosome counting, AGS measurement, examination of flower morphology and do another additional analyses.

Furthermore, we detected another instance (four individuals) which position strongly contradicts our assignment to specific taxa based on morphological and karyological data. Specifically individual SE_28_3 identified as a 'Scandinavian entity', which appeared at the branch's base encompassing *S. longifolia, S. borealis*, and *S. calycantha*. Additionally, two specimens of *S. graminea* exhibited notable deviations: the diploid individual SG_19_8 clustered within the Scandinavian entity branch, while the tetraploid individual SG_NOR_9_9, identified morhologically as *S. graminea*, fell within the branch associated with *S. palustris*. Their intermediate positions might be easily explainable by their hybrid origin between species and their cytotypes. However, each of these cases should be a case of heteroploid hybridization (diploid-tetraploid in the first two cases and tetraploid-high ploidy level in the last one, Table FCM). In such a case, however, it is logical to expect that they possess transitional and odd ploidy level cytotypes, which is not proven by RGS and AGS data. Additionally, in plastome phylogeny, at least SE_28_3 and SG_19_8 share a haplotype with other individuals of their species-specific clade, where they also belong morphologically (see Figure 9). Only the SG_NOR_9_9 possesses a unique haplotype that appears separated from species-specific clades. Thus, we can also return back to a scenario involving idiosyncratic lineage sorting of ancestral variability, which is at least in polyploid cytotypes inherited from parental diploids and has recently undergone extensive concerted evolution (Coyne & Orr, 2004; Feder et al., 2013). Distinguishing between these two processes presents a challenge, making it difficult to determine whether polymorphic positions solely result from one process or if both are involved.

5. Conclusion

This master's thesis presents original findings that contribute to understanding of high polyploid systems, which remain largely understudied despite being evolutionarily fascinating. High polyploids represent an interesting evolutionary phenomenon that might enable species to adapt to diverse intrinsic and extrinsic conditions. Despite facing numerous challenges associated with their extremely high number of chromosomes and issues with sexual reproduction, they seem to be not evolutionary dead ends. Through their ability to generate high cytotype variability, they may contribute to establishing and diversifying new lineages and potentially new taxa under selection pressures triggered by environmental factors.

Our findings challenge the traditional paradigm of increasing ploidy with latitude. While macroecological factors were shown to be crucial triggers contributing to the formation of patterns of genome size and ploidy levels, our findings suggest that in some species, their response to the environment is highly species-specific and may more accurately reflect the impact of microecological conditions, accompanied by population demographic aspects and some level of stochasticity. In the case of *S. palustris*, with a centre of diversity in Northern Europe, the warmer and drier conditions in Central Europe may be less favourable. This may be further exacerbated by a specific suboptimal condition typical for the distribution range margin, which, in synergy, acts as complex stressors potentially triggering genomic rearrangements or increases in chromosome number.

Our study reveals extraordinary and continual variability in sexual expression within this highly polyploid complex. The high presence of intermediate individuals in *S. palustris* indicates considerable sexual lability, driven by various internal and external factors, including stochastic processes. The results of our investigation, however, indicate a lack of ecological influence shaped by latitude on the performance of sexual polymorphism. We presume that one

plausible explanation of this diversity might rely on a high number of chromosomal sets per se, which influence the genetic basis and functionality of the sexual system.

Our study also sheds more light on the diversity of the genus Stellaria by identifying a new lineage, tentatively named the "Scandinavian entity." This lineage is characterized by at least partially unique genetic, karyological, and morphological variability compared to the analyzed congeners. This taxon requires further examination, potentially representing a newly undescribed entity or clarifying the existence of already described but forgotten taxa.

In conclusion, the complexity of this high polyploid system poses challenges for interpretation, and drawing straightforward conclusions based on current knowledge is difficult. Nevertheless, this study provides initial insights into one such high polyploid system, underscoring the need for further investigation to better understand the evolution and dynamics of high polyploidy in general.

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Appendix

Table 1: Relative genome size (a.u.) of all collected species and cytotypes

Relative genome sizes measured with secondary standard *Solanum pseudocapsicum* (2C DNA = 2.56 pg; Temsch et al., 2010) were recalculated using genome size value of primary standard *Bellis perennis* (2C DNA = 3,38 pg; Schönswetter et al., 2007). Table shows recalculated values.

Individual	**Species**	**Standard**	**Signal standard**	**Signal sample**	**CV standard**	**CV sample**	**Ratio**	**RGS**
1_1	S. palustris	*B. perennis*	186	487	1,40	2,55	2,62	8,45
1_2	S. palustris	*B. perennis*	191	498	1,94	2,77	2,61	8,41
1_3	S. palustris	*B. perennis*	188	493	2,09	2,99	2,62	8,46
1_4	S. palustris	*B. perennis*	188	491	2,01	3,05	2,61	8,42
1_5	S. palustris	*B. perennis*	190	491	2,24	2,95	2,58	8,34
1_6	S. palustris	*B. perennis*	189	492	2,75	1,35	2,60	8,40
1_7	S. palustris	*B. perennis*	188	486	2,71	2,47	2,59	8,34
1_8	S. palustris	*B. perennis*	190	485	2,35	2,28	2,55	8,23
1_9	S. palustris	*B. perennis*	208,06	543	2,28	2,46	2,61	8,42
1_10	S. palustris	*B. perennis*	191	501	1,33	2,68	2,62	8,46
1_11	S. palustris	*B. perennis*	197	514	1,29	2,48	2,61	8,42

1_12	S. palustris	*B. perennis*	197	496	1,44	2,97	2,52	8,12
1_13	S. palustris	*B. perennis*	199	523	1,75	2,21	2,63	8,48
1_14	S. palustris	*B. perennis*	200	527	1,8	2,41	2,64	8,50
1_15	S. palustris	*B. perennis*	200	519	1,48	3,01	2,60	8,37
1_16	S. palustris	*B. perennis*	200	528	1,59	2,57	2,64	8,52
1_17	S. palustris	*B. perennis*	203,59	527,66	1,57	2,72	2,59	8,36
1_18	S. palustris	*B. perennis*	202,39	527,99	2,32	2,34	2,61	8,42
1_19	S. palustris	*B. perennis*	201,62	518,30	1,73	2,03	2,57	8,29
1_20	S. palustris	*B. perennis*	199,96	521,31	2,75	2,42	2,61	8,41
1_21	S. palustris	*B. perennis*	199,49	518,00	1,74	2,98	2,60	8,38
1_22	S. palustris	*B. perennis*	203,70	508,19	1,52	1,53	2,49	8,05
1_23	S. palustris	*B. perennis*	199,53	516,70	2,09	2,83	2,59	8,35
1_24	S. palustris	*B. perennis*	193,68	503,68	2,32	3,02	2,60	8,39
1_25	S. palustris	*B. perennis*	197,18	521,01	2,73	2,98	2,64	8,52

1_26	S. palustris	*B. perennis*	199,63	518,90	2,74	2,91	2,60	8,38
1_27	S. palustris	*B. perennis*	198,94	523,88	2,37	2,34	2,63	8,49
1_28	S. palustris	*B. perennis*	199,27	513,79	3,00	2,13	2,58	8,32
1_29	S. palustris	*B. perennis*	196,88	517,82	1,78	3,01	2,63	8,48
1_30	S. palustris	*B. perennis*	197,95	517,09	2,29	1,73	2,61	8,43
1_31	S. palustris	*B. perennis*	203,82	527,28	1,43	2,03	2,59	8,35
1_32	S. palustris	*B. perennis*	202,10	523,72	1,78	2,02	2,59	8,36
1_33	S. palustris	*B. perennis*	198,69	522,67	2,06	2,98	2,63	8,49
1_34	S. palustris	*B. perennis*	201,73	527,53	1,94	2,17	2,62	8,44
1_35	S. palustris	*B. perennis*	202,03	528,61	2,35	2,70	2,62	8,44
1_36	S. palustris	*B. perennis*	195,81	514,25	1,69	2,92	2,63	8,47
1_37	S. palustris	*B. perennis*	202,96	530,17	2,03	2,46	2,61	8,43
1_38	S. palustris	*B. perennis*	196,11	503,57	2,15	1,68	2,57	8,28
1_39	S. palustris	*B. perennis*	196,26	507,83	2,22	2,40	2,59	8,35

1_40	S. palustris	*B. perennis*	198,13	510,29	1,83	2,54	2,58	8,31
1_41	S. palustris	*B. perennis*	200,33	526,82	1,69	2,22	2,63	8,48
1_42	S. palustris	*B. perennis*	199,84	531,04	1,34	2,81	2,66	8,57
2_1	S. palustris	*B. perennis*	190	489	2,23	2,62	2,57	8,30
2_2	S. palustris	*B. perennis*	199	510	1,48	2,84	2,56	8,27
2_3	S. palustris	*B. perennis*	192	504	1,55	2,42	2,63	8,47
2_4	S. palustris	*B. perennis*	181	481	1,32	2,62	2,66	8,57
2_5	S. palustris	*B. perennis*	199	507	1,47	2,62	2,55	8,22
2_6	S. palustris	*B. perennis*	199	526	1,71	2,33	2,64	8,53
2_7	S. palustris	*B. perennis*	199	507	2,44	2,35	2,55	8,22
2_8	S. palustris	*B. perennis*	199	518	1,58	3,15	2,60	8,40
3-1	*S. palustris*	*B. perennis*	180	444	1,89	2,35	2,47	7,96
3-2	*S. palustris*	*B. perennis*	191	480	1,53	2,51	2,51	8,11
3-3	*S. palustris*	*B. perennis*	190	479	1,39	2,55	2,52	8,13

3-4	*S. palustris*	*B. perennis*	193	478	1,71	3,1	2,48	7,99
3-5	*S. palustris*	*B. perennis*	196	486	1,43	2,42	2,48	8,00
3-6	*S. palustris*	*B. perennis*	191	481	1,9	3	2,52	8,12
3-7	*S. palustris*	*B. perennis*	193	479	1,43	1,94	2,48	8,01
SP 4-1	*S. palustris*	*B. perennis*	194	499	1,62	2,9	2,57	8,30
SP 4-2	*S. palustris*	*B. perennis*	184	463	2,02	1,75	2,52	8,12
SP 4-3	*S. palustris*	*B. perennis*	199	495	1,49	2,11	2,49	8,02
SG 4-1	*S. graminea*	*B. perennis*	203	125	1,69	2,68	0,62	1,99
SG 4-2	*S. graminea*	*B. perennis*	181	112	1,94	3,12	0,62	2,00
SG 4-3	*S. graminea*	*B. perennis*	201	237	1,27	2,72	1,18	3,80
SG 4-4	*S. graminea*	*B. perennis*	202	230	1,24	2,29	1,14	3,67
SP_5_1	S. palustris	*B. perennis*	184	469	1,63	1,75	2,55	8,22
SP_5_2	S. palustris	*B. perennis*	204	517	2,69	2,81	2,53	8,18
SP_5_3	S. palustris	*B. perennis*	198	503	2,89	2,55	2,54	8,19

SP_5_4	S. palustris	*B. perennis*	195	493	2,54	2,68	2,53	8,16
SP_5_5	S. palustris	*B. perennis*	194	495	1,86	1,38	2,55	8,23
SP_5_6	S. palustris	*B. perennis*	200	504	1,66	1,7	2,52	8,13
SP_5_7	S. palustris	*B. perennis*	203	514	3,05	2,29	2,53	8,17
SP_5_8	S. palustris	*B. perennis*	203	411	2,43	2,53	2,02	6,53
SP_5_9	S. palustris	*B. perennis*	203	511	2,43	2,54	2,52	8,12
SP_5_10	S. palustris	*B. perennis*	180	460	2,42	2,58	2,56	8,24
SG_5_1	S. gramine a	*B. perennis*	195,39	121,1	1,71	2,96	0,62	2,00
SG_5_2	S. gramine a	*B. perennis*	194,89	117,03	1,35		0,60	1,94
SG_5_3	S. gramine a	*B. perennis*	200,38	121,9	1,26	2,27	0,61	1,96
SG_5_4	S. gramine a	*B. perennis*	198,97	123,98	1,55	2,62	0,62	2,01
SG_5_5	S. gramine a	*B. perennis*	196,96	121,34	1,36	3,09	0,62	1,99
SG_5_6	S. gramine a	*B. perennis*	204,98	243,6	1,37	3,1	1,19	3,83
SG_5_7	S. gramine a	*B. perennis*	195,22	223,81	1,54	1,62	1,15	3,70

SG_5_8	S. graminea	*B. perennis*	198,08	120,96	1,32	1,89	0,61	1,97
SG_5_9	S. graminea	*B. perennis*	197,95	122,65	1,34	3,2	0,62	2,00
SG_5_10	S. graminea	*B. perennis*	199,92	126,97	1,29	3,02	0,64	2,05
SG_5_11	S. graminea	*B. perennis*	193,93	125	1,33	2,83	0,64	2,08
SG_5_12	S. graminea	*B. perennis*	195,56	123	1,05	2,45	0,63	2,03
SG_5_13	S. graminea	*B. perennis*	195,68	122,94	2,25	2,85	0,63	2,03
SG_5_14	S. graminea	*B. perennis*	196,11	127	1,17	3,01	0,65	2,09
SG_5_15	S. graminea	*B. perennis*	202,29	128	1,15	3	0,63	2,04
SG_5_16	S. graminea	*B. perennis*	191,26	122,65	1,26	2,68	0,64	2,07
SG_5_17	S. graminea	*B. perennis*	198,94	123	1,39	1,87	0,62	1,99
SG_5_18	S. graminea	*B. perennis*	191,92	229	1,21	2,45	1,19	3,85
SG_5_19	S. graminea	*B. perennis*	194,73	232,02	1,14	2,68	1,19	3,84
SG_5_20	S. graminea	*B. perennis*	192,91	121,18	1,15	1,89	0,63	2,03
6-2	*S. graminea*	*B. perennis*	206,71	240,33	1,85	3,02	1,16	3,75

6-3	*S. palustris*	*B. perennis*	197,27	486,19	1,65	1,90	2,46	7,95
6-4	*S. palustris*	*B. perennis*	207,86	508,14	1,47	1,84	2,44	7,89
6-5	*S. graminea*	*B. perennis*	195,89	237,53	1,56	2,99	1,21	3,91
6-6	*S. palustris*	*B. perennis*	200,84	497,98	1,87	2,19	2,48	8,00
6-7	*S. palustris*	*B. perennis*	211,34	520,64	1,46	2,06	2,46	7,95
7-1	*S. palustris*	*B. perennis*	196,00	505,13	1,86	2,54	2,58	8,31
SL 7-1	*S. longifolia*	*B. perennis*	190,21	149,28	2,03	2,57	0,78	2,53
SL 7-2	*S. longifolia*	*B. perennis*	190,17	143,15	2,87	2,93	0,75	2,43
SP 7-1	*S. palustris*	*B. perennis*	203,34	500,77	1,69	1,66	2,46	7,94
SP 7-2	*S. palustris*	*B. perennis*	204,76	508,90	1,63	1,96	2,49	8,02
SP 7-3	*S. palustris*	*B. perennis*	207,65	515,27	1,47	2,23	2,48	8,00
SP 7-4	*S. palustris*	*B. perennis*	197,62	483,46	1,53	1,91	2,45	7,89
8-1	*S. graminea*	*B. perennis*	184,97	214,23	1,43	2,00	1,16	3,74
8-2	*S.graminea*	*B. perennis*	176,13	208,01	2,37	2,47	1,18	3,81

9-1	S. gramine a	*B. perennis*	193,67	223,18	1,67	2,16	1,15	3,72
9-2	S. gramine a	*B. perennis*	180,03	205,3	1,52	2,45	1,14	3,68
9-3	S. gramine a	*B. perennis*	198,98	224,58	1,58	2,21	1,13	3,64
9-4	S. gramine a	*B. perennis*	199,75	229,07	1,76	2,22	1,15	3,70
9-5	S. palustri s	*B. perennis*	208,17	530,32	1,46	2,34	2,55	8,22
9-6	S. palustri s	*B. perennis*	200,64	508,44	1,64	2,45	2,53	8,17
9-7	S. palustri s	*B. perennis*	200,96	523,91	1,94	2,63	2,61	8,41
9-8	S. palustri s	*B. perennis*	200,8	523,21	2,86	2,97	2,61	8,41
9-9	S. palustri s	*B. perennis*	200,83	522,84	2,05	2,54	2,60	8,40
9-10	S. gramine a	*B. perennis*	203,23	237,65	1,25	2,3	1,17	3,77
9-11	S. gramine a	*B. perennis*	203,23	236,76	1,78	1,91	1,16	3,76
9-12	S. gramine a	*B. perennis*	197,69	226,34	2,81	2,34	1,14	3,69
9-13	S. palustri s	*B. perennis*	206,46	527,73	2,15	2,01	2,56	8,25
9-14	S. palustri s	*B. perennis*	206,45	526,89	1,97	3	2,55	8,23

9-15	S. palustris	*B. perennis*	206,23	521,48	1,45	2,31	2,53	8,16
9-16	S. graminea	*B. perennis*	208,49	239,96	2,66	2,97	1,15	3,71
9-17	S. graminea	*B. perennis*	204,01	236,84	2,7	2,81	1,16	3,74
9-19	S. graminea	*B. perennis*	204,54	235,17	2,13	2,57	1,15	3,71
9-20	S. palustris	*B. perennis*	202,96	507,99	1,99	2,9	2,50	8,07
9-21	S. graminea	*B. perennis*	203,77	238,55	1,46	2,78	1,17	3,78
9-22	S. palustris	*B. perennis*	203,71	522,92	2,4	2,78	2,57	8,28
9-23	S. graminea	*B. perennis*	211,57	247,23	1,76	2,98	1,17	3,77
9-24	S. palustris	*B. perennis*	210,83	535,82	1,26	1,81	2,54	8,20
9-25	S. palustris	*B. perennis*	212,74	545,42	1,71	2,29	2,56	8,27
9-26	S. palustris	*B. perennis*	203,62	531,82	1,31	2,73	2,61	8,43
9-27	S. palustris	*B. perennis*	204,71	531,53	1,65	2,61	2,60	8,38
9-28	S. palustris	*B. perennis*	205,91	528,22	1,86	2,00	2,57	8,28
9-29	S. palustris	*B. perennis*	206,35	529,08	1,50	1,79	2,56	8,27

9-30	S. palustris	*B. perennis*	199,10	509,79	1,45	2,25	2,56	8,26
9-31	S. palustris	*B. perennis*	208,39	530,00	1,37	2,71	2,54	8,20
10-1	S. palustris	*B. perennis*	190,09	527,44	1,97	2,98	2,77	8,95
10-2	S. palustris	*B. perennis*	196,73	568,34	2,21	2,95	2,89	9,32
10-3	S. palustris	*B. perennis*	193,97	546,82	2,47	2,80	2,82	9,09
10-4	S. palustris	*B. perennis*	202,81	589,73	1,78	2,73	2,91	9,38
10-5	S. palustris	*B. perennis*	198,70	561,24	1,66	2,09	2,82	9,11
10-6	S. palustris	*B. perennis*	195,99	558,00	1,66	2,18	2,85	9,18
10-7	S. palustris	*B. perennis*	201,44	556,75	2,14	2,72	2,76	8,92
10-8	S. palustris	*B. perennis*	201,07	584,71	1,39	1,85	2,91	9,38
10-9	S. palustris	*B. perennis*	199,28	576,61	2,67	3,05	2,89	9,33
11-1	S. palustris	*B. perennis*	209,30	592,91	1,65	3,00	2,83	9,14
11-2	S. palustris	*B. perennis*	196,15	561,88	1,64	2,43	2,86	9,24
12-1	S. palustris	*B. perennis*	195,62	546,52	2,00	2,73	2,79	9,01

12-2	S. palustris	*B. perennis*	196,62	560,32	2,86	2,46	2,85	9,19
12-3	S. palustris	*B. perennis*	196,91	548,42	2,74	2,10	2,79	8,98
13-1	S. palustris	*B. perennis*	197,51	532,30	1,42	2,34	2,70	8,69
13-2	S. palustris	*B. perennis*	198,91	532,78	1,56	1,99	2,68	8,64
13-3	S. palustris	*B. perennis*	196,50	524,56	1,79	2,36	2,67	8,61
13-4	S. palustris	*B. perennis*	196,43	525,28	1,08	1,67	2,67	8,63
13-5	S. palustris	*B. perennis*	198,63	534,69	1,73	1,74	2,69	8,68
13-6	S. palustris	*B. perennis*	197,56	485,86	1,66	2,96	2,46	7,93
13-7	S. palustris	*B. perennis*	197,35	478,91	1,68	1,73	2,43	7,83
13-8	S. palustris	*B. perennis*	198,49	566,43	1,53	2,29	2,85	9,21
13-9	S. palustris	*B. perennis*	198,01	544,96	1,86	2,44	2,75	8,88
13-10	S. palustris	*B. perennis*	197,23	541,26	2,03	2,63	2,74	8,85
13-11	S. palustris	*B. perennis*	204,44	573,82	2,43	2,35	2,81	9,05
13-12	S. palustris	*B. perennis*	199,96	482,34	1,85	1,85	2,41	7,78

13-13	S. palustris	*B. perennis*	203,43	565,96	2,52	2,95	2,78	8,97
13-14	S. palustris	*B. perennis*	203,37	569,72	1,39	1,74	2,80	9,04
14-1	S. palustris	*B. perennis*	197,04	516,24	2,08	2,56	2,62	8,45
14-2	S. palustris	*B. perennis*	198,00	519,27	1,67	2,63	2,62	8,46
14-3	S. palustris	*B. perennis*	197,77	528,83	1,31	1,91	2,67	8,63
16-1	*S. graminea*	*B. perennis*	200,99	122,03	1,83	2,18	0,61	1,96
16-2	*S. graminea*	*B. perennis*	201,01	121,17	2,05	2,23	0,60	1,94
16-3	*S. graminea*	*B. perennis*	202,27	120,5	1,72	2,77	0,60	1,92
16-4	*S. graminea*	*B. perennis*	199,14	120,01	2,99	2,43	0,60	1,94
16-5	*S. graminea*	*B. perennis*	201,93	122	1,79	1,67	0,60	1,95
16-6	*S. graminea*	*B. perennis*	200,48	119,29	2,61	3,1	0,60	1,92
16-7	*S. graminea*	*B. perennis*	200,65	120,02	2,28	3,06	0,60	1,93
16-8	*S. graminea*	*B. perennis*	195,52	117,4	1,47	2,33	0,60	1,94
16-9	*S. graminea*	*B. perennis*	196,48	117,71	2,14	2,77	0,60	1,93

16-10	*S. graminea*	*B. perennis*	203,34	125	1,37	2,87	0,61	1,98
17-1	*S. graminea*	*B. perennis*	196,10	117,94	1,39	2,04	0,60	1,94
17-2	*S. graminea*	*B. perennis*	200,58	121,13	2,20	2,88	0,60	1,95
17-3	*S. graminea*	*B. perennis*	199,20	118,73	1,94	2,78	0,60	1,92
17-4	*S. graminea*	*B. perennis*	195,54	119,25	2,12	2,13	0,61	1,97
17-5	*S. graminea*	*B. perennis*	206,25	127,14	1,83	3,05	0,62	1,99
17-6	*S. graminea*	*B. perennis*	198,19	120,47	2,12	2,40	0,61	1,96
17-7	*S. graminea*	*B. perennis*	197,67	119,74	2,10	2,43	0,61	1,95
17-8	*S. graminea*	*B. perennis*	205,72	125,27	1,91	2,96	0,61	1,96
17-9	*Scandinavian entity'*	*S. pseudocapsicum pseudocapsicum*	207,96	278,73	1,97	3,03	1,03	3,32
17-10	*S. graminea*	*B. perennis*	189,55	112,90	1,99	2,15	0,60	1,92
17-11	*S. graminea*	*B. perennis*	195,76	120,11	2,07	2,94	0,61	1,98
17-12	*Scandinavian entity'*	*S. pseudocapsicum*	191,88	253,48	1,11	1,08	1,01	3,27

17-13	*S. graminea*	*B. perennis*	192,70	118,30	2,75	3,05	0,61	1,98
18-1	*S. graminea*	*B. perennis*	194,92	120,63	1,84	2,21	0,62	2,00
18-2	*S. graminea*	*B. perennis*	194,89	119,70	1,65	2,87	0,61	1,98
18-3	*S. graminea*	*B. perennis*	196,95	124,33	2,13	2,56	0,63	2,04
18-4	*S. graminea*	*B. perennis*	191,90	119,97	1,68	2,54	0,63	2,02
18-5	*S. graminea*	*B. perennis*	203,81	126,53	1,93	2,90	0,62	2,00
18-6	*S. graminea*	*B. perennis*	198,06	124,88	2,98	2,10	0,63	2,03
18-7	*S. graminea*	*B. perennis*	205,18	127,72	2,21	1,98	0,62	2,01
18-8	*S. graminea*	*B. perennis*	192,44	117,76	1,87	2,14	0,61	1,97
18-9	*S. graminea*	*B. perennis*	193,63	121,03	2,47	2,00	0,63	2,02
18-10	*S. graminea*	*B. perennis*	194,31	120,09	2,15	2,93	0,62	1,99
18-11	*S. graminea*	*B. perennis*	206,84	127,00	1,92	2,12	0,61	1,98
18-12	*S. graminea*	*B. perennis*	196,57	121,32	2,03	2,32	0,62	1,99
18-13	*S. graminea*	*B. perennis*	208,28	126,92	1,89	2,20	0,61	1,97

18-14	*S. graminea*	*B. perennis*	199,51	125,52	1,92	2,90	0,63	2,03
18-15	*S. graminea*	*B. perennis*	196,12	212,57	1,40	3,00	1,08	3,50
18-16	*S. graminea*	*B. perennis*	192,41	118,85	2,19	3,02	0,62	1,99
18-17	*S. graminea*	*B. perennis*	195,08	122,67	2,17	3,08	0,63	2,03
18-18	*S. graminea*	*B. perennis*	206,17	128,83	1,39	2,96	0,62	2,02
19-1	S. graminea	*B. perennis*	196,35	120,4	1,5	2,3	0,61	1,98
19-2	*S. graminea*	*B. perennis*	203,49	123,4	1,21	2,43	0,61	1,96
19-3	*S. graminea*	*B. perennis*	199,62	120,18	1,54	3,07	0,60	1,94
19-4	*Scandinavian entity'*	*S. pseudocapsicum*	198,24	262,43	1,85	1,69	1,02	3,28
19-5	*Scandinavian entity'*	*S. pseudocapsicum*	198,88	272,08	1,52	2,99	1,05	3,39
19-6	*S. graminea*	*B. perennis*	207,54	124,89	2,71	2,48	0,60	1,94
19-7	*Scandinavian entity'*	*S. pseudocapsicum*	195,05	270,85	1,43	3,07	1,07	3,44
19-8	*S. graminea*	*B. perennis*	207,62	124,86	2,73	3,01	0,60	1,94
19-9	*S. graminea*	*B. perennis*	201,36	218,64	1,47	2,32	1,09	3,50

19-10	Scandinavian entity'	S. pseudocapsicum	202,64	267,75	1,63	1,53	1,01	3,27
19-11	S. graminea	*B. perennis*	199,32	121,8	1,79	2,36	0,61	1,97
19-12	S. graminea	*B. perennis*	203,05	122,72	1,86	2,28	0,60	1,95
19-13	Scandinavian entity'	*S. pseudocapsicum*	194,17	259,29	1,52	2,86	1,03	3,31
19-14	S. graminea	*B. perennis*	205,1	122,6	2,9	1,79	0,60	1,93
19-15	Scandinavian entity'	*S. pseudocapsicum*	199,85	268,21	1,17	2,84	1,03	3,33
20-1	Scandinavian entity'	*S. pseudocapsicum*	200,98	276,57	1,06	2,94	1,06	3,41
20-2	Scandinavian entity'	*S. pseudocapsicum*	196,78	266,21	1,07	2,1	1,04	3,35
20-3	S. graminea	*B. perennis*	192,56	120,85	2,9	3	0,63	2,02
20-4	Scandinavian entity'	*S. pseudocapsicum*	207,71	281,57	1,25	2,21	1,04	3,36
20-5	Scandinavian entity'	*S. pseudocapsicum*	206,25	271,59	1,13	2,12	1,01	3,26
20-6	Scandinavian entity'	*S. pseudocapsicum*	216,36	290,96	1,09	1,88	1,03	3,33
20-7	Scandinavian entity'	*S. pseudocapsicum*	205,01	274,51	1,07	1,73	1,03	3,32
20-8	Scandinavian entity'	*S. pseudocapsicum*	205,36	277,49	1,13	2,32	1,04	3,35

20-9	Scandinavian entity'	*S. pseudocapsicum*	199,99	275,27	1,07	2,01	1,06	3,41
20-10	Scandinavian entity'	*S. pseudocapsicum*	199,01	267,71	2,3	3,01	1,03	3,33
20-11	Scandinavian entity'	*S. pseudocapsicum*	210,07	282,58	1,98	2,93	1,03	3,33
20-12	S. graminea	*B. perennis*	198,43	123,08	1,04	1,54	0,63	2,02
20-13	Scandinavian entity'	*S. pseudocapsicum*	199,47	264,51	2,17	2,4	1,02	3,29
21-1	Scandinavian entity'	*S. pseudocapsicum*	195,11	262,86	2,71	3	1,03	3,34
21-2	Scandinavian entity'	*S. pseudocapsicum*	211,04	282,01	2,81	3,06	1,03	3,31
21-3	Scandinavian entity'	*S. pseudocapsicum*	201,46	265,77	2,32	2,48	1,01	3,27
21-4	Scandinavian entity'	*S. pseudocapsicum*	197,14	262,71	2	1,59	1,02	3,30
21-5	Scandinavian entity'	*S. pseudocapsicum*	198,46	262,97	2,05	1,86	1,02	3,28
21-6	Scandinavian entity'	*S. pseudocapsicum*	200,08	265,99	2,88	2,28	1,02	3,29
21-7	Scandinavian entity'	*S. pseudocapsicum*	197,17	260,73	2,51	2,76	1,02	3,28
21-8	Scandinavian entity'	*S. pseudocapsicum*	201,86	269,39	3,12	3,92	1,03	3,31
21-9	Scandinavian entity'	*S. pseudocapsicum*	190,35	253,37	2,23	2,47	1,02	3,30

21-10	Scandinavian entity'	*S. pseudocapsicum*	202,57	277,22	2,52	3,02	1,05	3,39
21-11	Scandinavian entity'	*S. pseudocapsicum*	193,01	263,25	3	2,06	1,05	3,38
21-12	Scandinavian entity'	*S. pseudocapsicum*	202,9	270,98	2,27	2,46	1,03	3,31
22-1	Scandinavian entity'	*S. pseudocapsicum*	191,48	260,49	1,95	1,28	1,04	3,37
22-2	Scandinavian entity'	*S. pseudocapsicum*	190,20	258,19	1,60	2,94	1,04	3,36
22-3	Scandinavian entity'	*S. pseudocapsicum*	201,46	265,77	2,24	2,32	1,01	3,27
22-4	Scandinavian entity'	*S. pseudocapsicum*	197,14	262,71	2,49	2,43	1,02	3,30
22-5	Scandinavian entity'	*S. pseudocapsicum*	198,46	262,97	2,05	1,86	1,02	3,28
22-6	Scandinavian entity'	*S. pseudocapsicum*	200,08	265,99	2,88	2,28	1,02	3,29
22-7	Scandinavian entity'	*S. pseudocapsicum*	198,72	275,01	2,28	2,31	1,06	3,43
22-8	Scandinavian entity'	*S. pseudocapsicum*	192,37	254,90	1,24	1,35	1,02	3,28
22-9	Scandinavian entity'	*S. pseudocapsicum*	199,35	269,13	3,02	2,98	1,04	3,34
23-1	Scandinavian entity'	*S. pseudocapsicum*	203,93	276,35	1,35	2,73	1,04	3,36
23-2	Scandinavian entity'	*S. pseudocapsicum*	207,44	280,99	1,72	2,97	1,04	3,36

23-3	Scandinavian entity'	*S. pseudocapsicum*	211,20	287,51	1,49	2,84	1,05	3,37
23-4	Scandinavian entity'	*S. pseudocapsicum*	213,11	287,69	1,22	2,72	1,04	3,34
23-5	Scandinavian entity'	*S. pseudocapsicum*	203,93	272,80	1,24	2,12	1,03	3,31
23-6	Scandinavian entity'	*S. pseudocapsicum*	208,58	281,44	1,32	2,16	1,04	3,34
23-7	Scandinavian entity'	*S. pseudocapsicum*	206,28	279,43	1,45	2,28	1,04	3,36
23-8	Scandinavian entity'	*S. pseudocapsicum*	213,34	287,67	1,10	2,12	1,04	3,34
23-9	Scandinavian entity'	*S. pseudocapsicum*	205,62	275,77	2,31	2,79	1,03	3,32
23-10	Scandinavian entity'	*S. pseudocapsicum*	203,93	272,63	1,68	2,67	1,03	3,31
24-1	Scandinavian entity'	*S. pseudocapsicum*	210,5	287,83	1,94	2,35	1,05	3,39
24-2	Scandinavian entity'	*S. pseudocapsicum*	198,34	271,45	2,01	2,98	1,05	3,39
24-3	Scandinavian entity'	*S. pseudocapsicum*	198,55	272,97	1,22	2,25	1,06	3,41
24-4	Scandinavian entity'	*S. pseudocapsicum*	201,69	271,95	1,22	2,15	1,04	3,34
24-5	S. graminea	*B. perennis*	198,18	122,80	2,00	2,75	0,62	2,00
24-6	Scandinavian entity'	*S. pseudocapsicum*	201,88	265,29	1,74	2,21	1,01	3,26

24-7	Scandinavian entity'	*S. pseudocapsicum*	201,37	274,31	1,57	2,3	1,05	3,38
24-8	Scandinavian entity'	*S. pseudocapsicum*	200,2	264,24	1,42	2,3	1,01	3,27
24-9	S. graminea	*B. perennis*	202,51	126,35	1,71	2,27	0,62	2,01
24-10	Scandinavian entity'	*S. pseudocapsicum*	197,64	263,4	1,15	2,56	1,02	3,30
24-11	S. graminea	*B. perennis*	202,33	213,83	1,48	1,86	0,62	2,01
24-12	Scandinavian entity'	*S. pseudocapsicum*	200,51	266,06	1,25	2,83	1,02	3,29
24-13	Scandinavian entity'	*S. pseudocapsicum*	201,9	272,06	1,12	2,52	1,03	3,34
25-1	Scandinavian entity'	*S. pseudocapsicum*	195,05	268,47	1,64	2,57	1,06	3,41
25-2	Scandinavian entity'	*S. pseudocapsicum*	207,88	292,06	0,94	1,85	1,08	3,48
25-3	Scandinavian entity'	*S. pseudocapsicum*	202,54	281,08	1,24	2,92	1,07	3,44
25-4	Scandinavian entity'	*S. pseudocapsicum*	200,97	284,89	1,38	2,48	1,09	3,51
25-5	Scandinavian entity'	*S. pseudocapsicum*	203,79	275,87	2,16	2,72	1,04	3,35
25-6	Scandinavian entity'	*S. pseudocapsicum*	205,17	279,56	1,2	2,53	1,05	3,38
25-7	Scandinavian entity'	*S. pseudocapsicum*	188,55	265,43	2,13	2,96	1,08	3,49

25-8	Scandinavian entity'	*S. pseudocapsicum*	201,21	283,41	1,38	2,33	1,08	3,49
25-9	Scandinavian entity'	*S. pseudocapsicum*	206,47	283,52	2,79	2,79	1,05	3,40
25-10	Scandinavian entity'	*S. pseudocapsicum*	205,65	288,91	2,57	2,73	1,08	3,48
26-1	Scandinavian entity'	*S. pseudocapsicum*	195,59	260,48	2,47	3,04	1,02	3,30
26-2	Scandinavian entity'	*S. pseudocapsicum*	198,56	263,59	2,37	2,13	1,02	3,29
26-3	Scandinavian entity'	*S. pseudocapsicum*	202,07	276,69	2,14	2,63	1,05	3,39
26-4	Scandinavian entity'	*S. pseudocapsicum*	194,60	259,59	1,75	2,53	1,02	3,31
26-5	Scandinavian entity'	*S. pseudocapsicum*	196,34	268,67	1,29	2,29	1,05	3,39
26-6	Scandinavian entity'	*S. pseudocapsicum*	196,07	261,06	1,20	2,59	1,02	3,30
26-7	Scandinavian entity'	*S. pseudocapsicum*	195,16	267,49	1,83	2,54	1,05	3,40
26-8	Scandinavian entity'	*S. pseudocapsicum*	208,83	292,45	2,37	2,01	1,08	3,47
26-9	Scandinavian entity'	*S. pseudocapsicum*	190,40	257,00	2,43	2,57	1,04	3,34
26-10	Scandinavian entity'	*S. pseudocapsicum*	192,14	254,89	2,68	2,45	1,02	3,29
26-11	Scandinavian entity'	*S. pseudocapsicum*	195,79	259,87	2,59	2,51	1,02	3,29

27-1	Scandinavian entity'	*S. pseudocapsicum*	200,11	262,64	2,27	2,68	1,01	3,25
27-2	Scandinavian entity'	*S. pseudocapsicum*	194,93	257,63	1,67	2,63	1,02	3,27
27-3	Scandinavian entity'	*S. pseudocapsicum*	198,47	260,96	1,94	2,03	1,01	3,26
27-4	Scandinavian entity'	*S. pseudocapsicum*	197,81	277,54	2,16	2,26	1,08	3,48
27-5	Scandinavian entity'	*S. pseudocapsicum*	203,77	267,8	1,34	1,63	1,01	3,26
27-6	Scandinavian entity'	*S. pseudocapsicum*	197,93	263,49	2,01	1,8	1,02	3,30
27-7	S. graminea	*B. perennis*	199,35	216,27	1,54	1,72	1,08	3,50
27-8	Scandinavian entity'	*S. pseudocapsicum*	201,33	267,99	1,47	2,74	1,02	3,30
27-9	Scandinavian entity'	*S. pseudocapsicum*	199,18	261,63	1,61	1,9	1,01	3,25
27-10	Scandinavian entity'	*S. pseudocapsicum*	200,7	268,67	1,36	2,78	1,03	3,32
28-1	S. graminea	*B. perennis*	196,07	212,03	1,42	2,02	1,08	3,49
28-2	S. graminea	*B. perennis*	197,09	212,94	1,68	1,58	1,08	3,49
28-3	Scandinavian entity'	*S. pseudocapsicum*	203,01	277,37	1,74	1,92	1,05	3,39
28-4	Scandinavian entity'	*S. pseudocapsicum*	200,1	265,76	2,13	2,09	1,02	3,29

28-5	S. graminea	*B. perennis*	197,04	214,44	1,34	2,12	1,09	3,51
28-6	S. graminea	*B. perennis*	194,1	201,84	0,02	3	1,04	3,35
28-7	Scandinavian entity'	*S. pseudocapsicum*	193,94	257,96	2,9	3,01	1,07	3,46
28-8	Scandinavian entity'	*S. pseudocapsicum*	197,72	262,77	1,59	1,66	1,02	3,29
28-9	Scandinavian entity'	*S. pseudocapsicum*	199,23	263,45	1,13	1,29	1,02	3,28
29-1	*S. graminea*	*B. perennis*	185,19	230,50	1,45	2,34	1,24	4,02
29-2	*S. graminea*	*B. perennis*	201,39	227,12	1,64	3,00	1,13	3,64
29-3	*S. graminea*	*B. perennis*	184,79	214,00	2,01	2,67	1,16	3,74
29-4	*S. graminea*	*B. perennis*	185,84	218,00	1,78	2,13	1,17	3,78
30-1	S. palustris	*B. perennis*	196,86	437,79	2,11	2,66	2,22	7,17
30-2	S. graminea	*B. perennis*	201,66	231,99	2,10	2,56	1,15	3,71
30-3	S. graminea	*B. perennis*	191,33	221,81	1,70	1,59	1,16	3,74
30-4	S. graminea	*B. perennis*	200,02	227,64	2,31	2,02	1,14	3,67
31-1	S. graminea	*B. perennis*	196,63	222,18	1,49	1,73	1,13	3,64

31-2	S. graminea	*B. perennis*	194,02	219,53	2,26	1,89	1,13	3,65
31-3	S. graminea	*B. perennis*	201,06	234,1	1,37	2,82	1,16	3,76
31-4	S. graminea	*B. perennis*	201,7	230,5	1,36	2,55	1,14	3,69
31-5	S. graminea	*B. perennis*	175,99	203,76	1,83	2,2	1,16	3,73
31-6	S. graminea	*B. perennis*	192,17	209,46	1,78	2,34	1,09	3,52
31-7	S. graminea	*B. perennis*	193,45	218,9	1,82	2,64	1,13	3,65
31-8	S. graminea	*B. perennis*	208,96	249,06	2,33	2,55	1,19	3,84
31-9	S. graminea	*B. perennis*	204,29	230,23	2,18	1,89	1,13	3,64
31-10	S. graminea	*B. perennis*	203,98	230,81	1,36	1,77	1,13	3,65
31-11	S. graminea	*B. perennis*	198,85	231,62	1,37	1,98	1,16	3,76
31-12	S. graminea	*B. perennis*	201,56	230,73	1,49	1,58	1,14	3,69
32-1	S. graminea	*B. perennis*	202,85	124,26	1,32	2,32	0,61	1,98
32-2	S. palustris	*B. perennis*	193,53	482,22	1,48	1,74	2,49	8,04
32-3	Scandinavian entity'	*S. pseudocapsicum*	200,39	265,79	1,22	1,64	1,02	3,29

32-4	S. graminea	*B. perennis*	200,16	227,08	1,69	1,82	1,13	3,66
32-5	S. graminea	*B. perennis*	204,86	231,86	1,42	3,02	1,13	3,65
32-6	S. graminea	*B. perennis*	201,38	232,01	2,38	2,54	1,15	3,72
32-7	S. graminea	*B. perennis*	198,82	231,91	1,89	2,29	1,17	3,76
32-8	S. graminea	*B. perennis*	201,9	234,02	1,83	2,83	1,16	3,74
32-9	S. graminea	*B. perennis*	205,7	233,61	1,28	2,49	1,14	3,66
33-1	S. graminea	*B. perennis*	191,7	220,94	2,27	2,48	1,15	3,72
33-2	S. graminea	*B. perennis*	197,13	226,18	1,24	2,03	1,15	3,70
33-3	S. graminea	*B. perennis*	207,95	239,67	1,43	1,99	1,15	3,72
33-4	S. graminea	*B. perennis*	198,94	225,97	1,97	2,07	1,14	3,66
34-1	*S. graminea*	*B. perennis*	200,46	229,57	1,85	2,19	1,15	3,69
34-2	*S. graminea*	*B. perennis*	199,4	223,56	1,57	1,15	1,12	3,62
34-3	*S. graminea*	*B. perennis*	198,5	225,15	1,98	1,98	1,13	3,66
34-4	*S. graminea*	*B. perennis*	201,06	232,24	1,4	2,77	1,16	3,73

34-5	*S. gramin ea*	*B. perennis*	205,07	231,66	2,14	2,34	1,13	3,64
34-6	*S. gramin ea*	*B. perennis*	204,95	229,21	1,38	2,38	1,12	3,61
34-7	*S. gramin ea*	*B. perennis*	197,82	221,01	2,12	2,88	1,12	3,60
34-8	*S. gramin ea*	*B. perennis*	200,5	230,01	2,25	2,92	1,15	3,70
34-9	*S. gramin ea*	*B. perennis*	199,54	223,54	1,78	2,65	1,12	3,61
34-10	*S. gramin ea*	*B. perennis*	196,83	225,24	2,87	2,65	1,14	3,69
34-11	*S. gramin ea*	*B. perennis*	198,31	223,57	1,53	2,45	1,13	3,64
35_1_1	S. gramine a	*B. perennis*	201,68	232,61	1,47	1,34	1,15	3,72
35_1_2	S. palustri s	*B. perennis*	204,85	534,19	1,11	1,12	2,61	8,41
35_1_3	S. gramine a	*B. perennis*	198,99	233,12	1,25	1,25	1,17	3,78
35_2_1	S palustri s	*B. perennis*	199,43	389,81	1,40	1,18	1,95	6,31
35_2_2	S palustri s	*B. perennis*	204,55	398,73	1,17	1,31	1,95	6,29
NOR-1-1	S. gramine a	B. perennis	198,83	118,68	2,17	2,98	0,60	1,93
NOR-1-2	S.grami nea	B. perennis	191,55	115,72	2,12	2,04	0,60	1,95
NOR-1-3	S.grami nea	B. perennis	197,95	118,46	2,47	2,97	0,60	1,93

NOR-1-4	S.graminea	B. perennis	198,21	229,35	2,45	2,47	1,16	3,73
NOR-1-5	S.graminea	B. perennis	197,84	226,04	2,31	2,37	1,14	3,69
NOR_2_1	Scandinavian entity	S. pseudocapsicum	208,66	291,92	1,41	2,67	1,07	3,47
NOR_2_2	Scandinavian entity	S. pseudocapsicum	198,8	264,26	3,03	2,89	1,02	3,29
NOR_2_3	Scandinavian entity	S. pseudocapsicum	196,55	260,3	1,28	2,58	1,02	3,28
NOR_2_4	Scandinavian entity	S. pseudocapsicum	203,75	273,89	1,28	2,04	1,03	3,33
NOR_2_5	Scandinavian entity	B. perennis	187,54	298,6	1,99	3,26	1,59	5,14
NOR_2_6	Scandinavian entity	B. perennis	195,65	315,22	1,33	4,16	1,61	5,20
NOR_2_7	Scandinavian entity	S. pseudocapsicum	193,09	263,39	1,13	2,52	1,05	3,38
NOR_2_8	Scandinavian entity	B. perennis	203,96	322,6	1,21	2,57	1,58	5,10
NOR_2_9	Scandinavian entity	S. pseudocapsicum	200,57	266,62	2,04	1,64	1,02	3,29
NOR_2_10	Scandinavian entity	B. perennis	205,62	324,3	1,46	2,87	1,58	5,09
NOR_2_11	Scandinavian entity	S. pseudocapsicum	210,24	279,64	1,48	1,62	1,02	3,30
NOR_2_12	Scandinavian entity	S. pseudocapsicum	207,56	275,45	1,48	1,77	1,02	3,29
NOR-3-1	S. gramine a	B. perennis	196,4	225,74	2,51	2,32	1,15	3,71

NOR-3-2	S. graminea	B. perennis	200,16	223,14	1,78	2,3	1,11	3,60
NOR-3-3	S. graminea	B. perennis	190,06	223,37	1,99	2,45	1,18	3,79
NOR-3-4	S. graminea	B. perennis	189,45	214,15	2,86	3	1,13	3,65
NOR-3-5	S. graminea	B. perennis	194,01	230,46	2,27	2,66	1,19	3,83
NOR-3-6	S. graminea	B. perennis	197,62	227,73	2,01	2,63	1,15	3,72
NOR-3-7	S. graminea	B. perennis	199,74	226,79	2,62	2,82	1,14	3,66
NOR-3-8	S. graminea	B. perennis	204,05	235,63	3,02	2,94	1,15	3,73
NOR_4_1	Scandinavian entity	S. pseudocapsicum	199,53	267,52	1,25	1,62	1,03	3,32
NOR_4_2	Scandinavian entity	S. pseudocapsicum	203,43	270,59	1,48	1,92	1,02	3,30
NOR_4_3	Scandinavian entity	S. pseudocapsicum	201,18	267,09	1,14	1,85	1,02	3,29
NOR_4_4	Scandinavian entity	S. pseudocapsicum	199,96	263,86	1,06	1,94	1,01	3,27
NOR_4_5	Scandinavian entity	S. pseudocapsicum	197,96	263,74	1,01	1,11	1,02	3,30
NOR_4_6	Scandinavian entity	S. pseudocapsicum	206,14	273,69	2,29	2,83	1,02	3,29
NOR_SL_4_6	Scandinavian entity	S. pseudocapsicum	209,03	279,08	1,32	1,51	1,03	3,31

NOR_4_7	Scandinavian entity	S. pseudocapsicum	206,06	274,14	2,84	2,73	1,02	3,30
NOR_SL_4_7	S. longifolia	B. perennis	195,24	147,15	2,01	1,85	0,75	2,43
NOR_4_8	S. longifolia	B. perennis	198,63	152,24	1,56	2,46	0,77	2,47
NOR_4_9	Scandinavian entity	S. pseudocapsicum	213,69	281,59	1,03	1,94	1,01	3,26
NOR_4_10	Scandinavian entity	S. pseudocapsicum	205,21	270,6	1,96	1,79	1,01	3,27
NOR_4_11	Scandinavian entity	S. pseudocapsicum	201,69	268,14	2,37	2,69	1,02	3,29
NOR_SL_4_11	S. longifolia	B. perennis	202,1	153,2	2,23	2,4	0,76	2,45
NOR_4_12	S. longifolia	B. perennis	201,94	156,25	2,14	2,73	0,77	2,50
NOR_4_13	Scandinavian entity	S. pseudocapsicum	203,23	270,31	1,67	1,92	1,02	3,30
NOR_4_14	Scandinavian entity	S. pseudocapsicum	201,51	268,21	1,93	2,03	1,02	3,30
NOR_4_15	S. longifolia	B. perennis	202,85	153,41	1,98	2,24	0,76	2,44
NOR_4_16	S. longifolia	B. perennis	200,46	152,09	2,09	2,14	0,76	2,45
NOR-5-1	S. graminea	B. perennis	195,84	220,11	1,78	2,5	1,12	3,63
NOR-5-2	S. palustris	B. perennis	197,97	523,32	2,47	2,35	2,64	8,53

NOR-5-3	S. palustris	B. perennis	206,57	538,44	2,41	3,05	2,61	8,41
NOR-5-4	S. gramine a	B. perennis	197,38	224,86	1,75	2,84	1,14	3,67
NOR-5-5	S. palustris	B. perennis	200,29	500,51	1,8	1,92	2,50	8,06
NOR-5-6	S. gramine a	B. perennis	196,19	226,83	2,38	2,9	1,16	3,73
NOR-5-7	S. palustris	B. perennis	198,5	520,36	2,21	2,36	2,62	8,46
NOR-5-8	S. palustris	B. perennis	199,34	517,92	2,19	2,34	2,60	8,38
NOR-5-9	S. palustris	B. perennis	196,05	515,63	2,6	2,13	2,63	8,48
NOR-5-10	S. gramine a	B. perennis	203,32	237,1	2,95	2,11	1,17	3,76
NOR-5-11	S. palustris	B. perennis	193,21	508,33	2,15	1,88	2,63	8,49
NOR-5-12	S. palustris	B. perennis	204,38	537,44	2,35	2,94	2,63	8,48
NOR-5-13	S. palustris	B. perennis	197,34	514,15	1,88	3,02	2,61	8,40
NOR-5-14	S. palustris	B. perennis	204,39	547,04	2,23	2,72	2,68	8,63
NOR-6-1	S. palustris	B. perennis	202,17	531,45	1,42	2,57	2,63	8,48
NOR-6-2	S. palustris	B. perennis	202,67	527,08	2,28	2,3	2,60	8,39

NOR-6-3	S. palustris	B. perennis	192,87	525,68	2,48	2,27	2,73	8,79
NOR-6-4	S. palustris	B. perennis	202,61	535,01	1,73	2,14	2,64	8,52
NOR-6-5	S. palustris	B. perennis	199,11	526,72	2,28	2,06	2,65	8,53
NOR-6-6	S. palustris	B. perennis	196,48	524,58	2,43	2,9	2,67	8,61
NOR-6-7	S. palustris	B. perennis	183,76	481,36	2,8	2,4	2,62	8,45
NOR-6-8	S. palustris	B. perennis	195,71	516,35	2,45	2,05	2,64	8,51
NOR-6-9	S. palustris	B. perennis	188,41	499,16	1,48	2,13	2,65	8,55
NOR-6-10	S. palustris	B. perennis	212,38	567,68	2,4	2,24	2,67	8,62
NOR-7-1	S. gramine a	B. perennis	189,7	218,22	1,89	1,87	1,15	3,71
NOR-7-2	S. gramine a	B. perennis	205,39	124,26	3,03	2,01	0,60	1,95
NOR-7-3	S. gramine a	B. perennis	196,09	225,56	1,36	1,73	1,15	3,71
NOR-7-4	S. longifol ia	B. perennis	202,74	152,87	2,22	1,69	0,75	2,43
NOR-7-5	S. longifol ia	B. perennis	189,67	149	1,83	1,27	0,79	2,53
NOR-7-6	S. gramine a	B. perennis	205,03	249,71	2,67	2,7	1,22	3,93

NOR-7-7	S. longifolia	B. perennis	198,99	152,57	2,02	2,73	0,77	2,47
NOR-7-8	S. graminea	B. perennis	209,41	127,26	2,98	2,43	0,61	1,96
NOR-8-1	S. palustris	B. perennis	203,54	497,51	1,5	2,42	2,44	7,88
NOR-8-2	S. palustris	B. perennis	195,64	454,92	2,29	2,31	2,33	7,50
NOR-8-3	S. palustris	B. perennis	198,48	497,86	1,55	2,25	2,51	8,09
NOR-8-4	S. palustris	B. perennis	190,19	498,7	2,56	2,59	2,62	8,46
NOR-8-5	S. palustris	B. perennis	193,47	483,05	2,33	2,89	2,50	8,05
NOR-8-6	S. palustris	B. perennis	197,5	513,29	2,98	2,99	2,60	8,38
NOR-8-7	S. palustris	B. perennis	198,17	470,89	2,34	2,49	2,38	7,67
NOR-8-8	S. palustris	B. perennis	196,21	505,08	2,68	2,47	2,57	8,30
NOR-8-9	S. palustris	B. perennis	197,09	502,23	2,44	2,34	2,55	8,22
NOR-8-10	S. palustris	B. perennis	214,61	484	1,62	2,42	2,26	7,28
NOR-8-11	S. palustris	B. perennis	199,7	495,7	2,86	3,01	2,48	8,01
NOR-9-1	S. palustris	B. perennis	198,82	450,25	1,53	2,97	2,26	7,31

NOR-9-2	S. graminea	B. perennis	198,33	219,88	1,34	2,37	1,11	3,58
NOR-9-3	S. palustris	B. perennis	192,17	447,65	2,22	2,16	2,33	7,51
NOR-9-4	S. graminea	B. perennis	201,44	232,56	1,6	1,66	1,15	3,72
NOR-9-5	S. palustris	B. perennis	197,71	471	3,05	2,3	2,38	7,68
NOR-9-6	S. palustris	B. perennis	200,53	460,4	2,23	2,97	2,30	7,41
NOR-9-7	S. palustris	B. perennis	201,91	468,56	1,25	2,96	2,32	7,49
NOR-9-8	S. palustris	B. perennis	198,39	454,54	1,38	1,79	2,29	7,39
NOR-9-9	S. graminea	B. perennis	198,81	220,74	1,11	1,73	1,11	3,58
NOR-9-10	S. palustris	B. perennis	192,09	448,98	1,32	2,96	2,34	7,54
NOR-10-1	S. palustris	B. perennis	194,23	469,55	1,42	2,63	2,42	7,80
NOR-10-2	S.palustris	B. perennis	196,28	458,27	2,06	2,33	2,33	7,53
NOR-10-3	S. palustris	B. perennis	193,4	452,78	1,3	1,93	2,34	7,55
NOR-10-4	S. palustris	B. perennis	194,42	442,2	2,04	2,09	2,27	7,34
NOR-10-5	S. palustris	B. perennis	192,78	450,09	1,34	2,11	2,33	7,53

NOR-10-6	S. palustris	B. perennis	188,06	428,24	1,81	2,4	2,28	7,35
NOR-10-7	S. palustris	B. perennis	198,36	465,71	1,43	1,82	2,35	7,57
NOR-10-8	S. palustris	B. perennis	199,67	450,85	2,02	2,08	2,26	7,28
NOR-10-9	S. gramine a	B. perennis	198,42	233,91	1,34	2,87	1,18	3,80
NOR-10-10	S. palustris	B. perennis	200,05	472,17	2,56	2,18	2,36	7,61
NOR-10-11	S. palustris	B. perennis	194,16	442,86	1,89	2,64	2,28	7,36
NOR-10-12	S. palustris	B. perennis	198,12	456,59	2,3	2,47	2,30	7,43
NOR-10-13	S. gramine a	B. perennis	201	231,05	1,78	1,56	1,15	3,71
NOR-11-1	S. gramine a	B. perennis	198,08	226,26	2,1	2,05	1,14	3,68
NOR-11-2	S. gramine a	B. perennis	186,3	209,73	2,12	2,73	1,13	3,63
NOR-11-3	S. palustris	B. perennis	194,85	481	1,98	1,9	2,47	7,96
NOR-11-4	S. palustris	B. perennis	199,1	486,78	1,95	2,78	2,44	7,89
NOR-11-5	S. palustris	B. perennis	186,68	464,75	1,75	2,55	2,49	8,03
NOR-11-6	S. palustris	B. perennis	176,99	442,05	1,86	2,89	2,50	8,06

NOR-11-7	S. graminea	B. perennis	189,89	226,55	1,03	2,32	1,19	3,85
NOR-12-1	S. longifolia	B. perennis	198,54	152,86	2,32	2,54	0,77	2,48
NOR-12-2	S. longifolia	B. perennis	209,75	162,3	1,88	2,26	0,77	2,50
NOR-12-3	S. longifolia	B. perennis	190	156,92	2,65	2,98	0,83	2,66
NOR-12-4	S. longifolia	B. perennis	193,66	157,76	2,22	2,35	0,81	2,63
NOR-12-5	S. longifolia	B. perennis	212,59	167,75	2,9	2,48	0,79	2,55
NOR-12-6	S. longifolia	B. perennis	192,34	143,5	1,69	2,58	0,75	2,41
NOR-12-7	S. longifolia	B. perennis	197,85	155	2,12	2,3	0,78	2,53
NOR-12-8	S. longifolia	B. perennis	190,39	151	2,52	2,83	0,79	2,56
NOR-12-9	S. longifolia	B. perennis	195,22	158,72	2,65	2,92	0,81	2,62
NOR-13a-1	S. longifolia	B. perennis	203,57	154,57	2,25	2,61	0,76	2,45
NOR-13a-2	S. longifolia	B. perennis	199,68	146,32	2,85	2,8	0,73	2,36
NOR-13a-3	S. longifolia	B. perennis	210,28	160,64	2,18	2,65	0,76	2,46
NOR-13a-4	S. longifolia	B. perennis	193,67	146,68	1,59	1,79	0,76	2,44

NOR-13a-5	S. longifolia	B. perennis	207,26	156,73	1,87	3,1	0,76	2,44
NOR-13a-6	S. longifolia	B. perennis	203,62	155,59	1,77	2,3	0,76	2,46
NOR-13a-7	S. longifolia	B. perennis	202,28	155,83	1,93	2,07	0,77	2,49
NOR-13a-8	S. longifolia	B. perennis	204,52	158,45	1,54	2,86	0,77	2,50
NOR-13a-9	S. longifolia	B. perennis	207,39	157,62	1,84	2,66	0,76	2,45
NOR-13a-10	S. longifolia	B. perennis	206,25	157,2	1,97	2,49	0,76	2,46
NOR-13b-1	Scandinavian entity	S. pseudocapsicum	197,07	277,62	2,05	1,65	1,08	3,49
NOR-13b-2	S.graminea	B. perennis	198,37	235,38	1,51	2,41	1,19	3,83
NOR-13b-3	Scandinavian entity	S. pseudocapsicum	207,8	292,76	1,95	1,83	1,08	3,49
NOR-13b-4	S. gramine a	B. perennis	202,92	228,36	1,99	2,12	1,13	3,63
NOR-13b-5	S. gramine a	B. perennis	206,41	240,17	2,51	2,98	1,16	3,75
NOR-13b-6	S. gramine a	B. perennis	205,9	234,82	1,79	2,58	1,14	3,68
NOR-13b-7	S. gramine a	B. perennis	204	235,37	2,98	2,2	1,15	3,72
NOR-13b-8	S. gramine a	B. perennis	208,07	239,35	1,72	2,29	1,15	3,71

NOR-13b-9	S. graminea	B. perennis	208	236,38	1,98	2,14	1,14	3,67
NOR-13b-10	S. graminea	B. perennis	209,89	236,22	2,43	2,23	1,13	3,63
NOR-14-1	S.palustris	B. perennis	194,49	477,09	2,98	2,1	2,45	7,91
NOR-15-1	S. longifolia	B. perennis	198,64	154	2,31	2,9	0,78	2,50
NOR-15-2	S. graminea	B. perennis	197,59	230,21	2,16	1,71	1,17	3,76
NOR-15-3	S. graminea	B. perennis	202,43	231,6	1,8	2,3	1,14	3,69
NOR-15-4	S. graminea	B. perennis	203,74	235,73	2,04	2,09	1,16	3,73
NOR-15-5	S. graminea	B. perennis	203,15	235,01	2,44	1,74	1,16	3,73
NOR-15-6	S. graminea	B. perennis	204,42	232,67	2,68	2,3	1,14	3,67
NOR-15-7	S. graminea	B. perennis	202	240,4	2,98	2,33	1,19	3,84
NOR-15-8	S. graminea	B. perennis	201,66	235	2,97	2,09	1,17	3,76
NOR-15-9	S. graminea	B. perennis	203,7	239,58	2,99	2,34	1,18	3,79
NOR-16-1	S. graminea	B. perennis	202,88	122,59	2,32	2,09	0,60	1,95
NOR-16-2	S. graminea	B. perennis	205,09	121	2,77	2,66	0,59	1,90

NOR-16-3	S. graminea	B. perennis	206,73	121	2,81	2,54	0,59	1,89
NOR-16-4	S. graminea	B. perennis	209,42	126	2,93	2,98	0,60	1,94
NOR-16-5	S. graminea	B. perennis	206,02	126	1,89	2,34	0,61	1,97
NOR-16-6	S. graminea	B. perennis	198,5	118,85	1,46	2,03	0,60	1,93
NOR-16-7	S. graminea	B. perennis	203,7	122,6	1,27	2,1	0,60	1,94
NOR-16-8	S. graminea	B. perennis	205,42	121,62	1,54	2,23	0,59	1,91
NOR-16-9	S. graminea	B. perennis	199,68	121,44	1,29	2,34	0,61	1,96
NOR-16-10	S. graminea	B. perennis	201,81	120,81	1,6	2,01	0,60	1,93
NOR-17-1	S. graminea	B. perennis	200,66	119,09	1,42	2,11	0,59	1,91
NOR-17-2	S. graminea	B. perennis	199,29	120,71	3,01	2,09	0,61	1,95
NOR-17-3	S. graminea	B. perennis	207,47	123,36	1,65	2,66	0,59	1,92
NOR-17-4	S. graminea	B. perennis	202,79	122,39	2,09	2,29	0,60	1,95
NOR-17-5	S. graminea	B. perennis	202,31	121	1,58	2,76	0,60	1,93
NOR-17-6	S. graminea	B. perennis	202,19	121,37	1,34	3,1	0,60	1,94

NOR-17-7	S. graminea	B. perennis	200,46	121,05	1,28	2,59	0,60	1,95
NOR-17-8	S. graminea	B. perennis	203,18	121,48	1,8	2,64	0,60	1,93
NOR-17-9	S. graminea	B. perennis	202,06	118,11	1,61	2,96	0,58	1,89
NOR-17-10	S. graminea	B. perennis	202,91	123	1,78	2,43	0,61	1,96
NOR-17-11	S. graminea	B. perennis	205,43	121,54	1,67	2,69	0,59	1,91
NOR_18_1	S. palustris	B. perennis	200,1	516,8	1,22	2,8	2,58	8,33
NOR_18_2	S. palustris	B. perennis	206,7	514,15	1,26	2,86	2,49	8,02
NOR_18_3	S. palustris	B. perennis	208,34	525,41	1,28	2,44	2,52	8,14
NOR_18_4	S. palustris	B. perennis	206,22	516,06	1,69	2,21	2,50	8,07
NOR_18_5	S. palustris	B. perennis	202,92	507,32	1,78	1,79	2,50	8,06
NOR_18_6	S. palustris	B. perennis	203,73	509,05	1,6	2,27	2,50	8,06
NOR_18_7	S. palustris	B. perennis	202,82	516,62	1,92	2,32	2,55	8,22
NOR_18_8	S. palustris	B. perennis	205,12	528,07	2,37	2,13	2,57	8,30
NOR_18_9	S. palustris	B. perennis	203,67	513,18	1,4	1,98	2,52	8,13

NOR_18_10	S. palustris	B. perennis	200,02	516,3	1,52	2,03	2,58	8,33
NOR_18_11	S. palustris	B. perennis	201,79	506,26	1,82	1,81	2,51	8,09
NOR-19-1	S. palustris	B. perennis	197,76	542,72	1,46	1,91	2,74	8,85
NOR-19-2	S. palustris	B. perennis	199,81	522,58	1,63	1,89	2,62	8,44
NOR-19-3	S. palustris	B. perennis	202,15	516,99	2,65	2,93	2,56	8,25
NOR-19-4	S. palustris	B. perennis	208,46	538,8	1,72	1,88	2,58	8,34
NOR-19-5	S. palustris	B. perennis	198,09	513,13	1,5	2,35	2,59	8,36
NOR-19-6	S. palustris	B. perennis	198,01	512,3	1,35	2,02	2,59	8,35
NOR-19-7	S. palustris	B. perennis	199,99	532,28	1,43	2,22	2,66	8,59
NOR-19-8	S. palustris	B. perennis	201,73	526,43	2,65	2,46	2,61	8,42
NOR-19-9	S. palustris	B. perennis	204,06	532,7	1,96	2,45	2,61	8,42
NOR-19-10	S. palustris	B. perennis	198,17	521,07	2,33	2,97	2,63	8,48
NOR-20-1	S. palustris	B. perennis	186,67	486,98	2,3	2,11	2,61	8,42
NOR-20-2	S. palustris	B. perennis	193,94	522,46	2,5	2,64	2,69	8,69

NOR-20-3	S. palustris	B. perennis	201,68	522,29	2,28	2,49	2,59	8,35
NOR-20-4	S. palustris	B. perennis	199,34	522,73	2,66	3,03	2,62	8,46
NOR-20-5	S. palustris	B. perennis	201,82	530,86	2,19	2,98	2,63	8,49
NOR-20-6	S. palustris	B. perennis	199,15	518,45	2,61	2,82	2,60	8,40
NOR-20-7	S. palustris	B. perennis	199,98	520,44	1,85	2,59	2,60	8,40
NOR-20-8	S. gramine a	B. perennis	205,58	239,11	1,61	2,41	1,16	3,75
NOR-20-9	S. palustris	B. perennis	203,23	528,48	2,43	2,19	2,60	8,39
NOR-20-10	S. palustris	B. perennis	204,98	534,75	1,7	2,66	2,61	8,42
NOR_21_1	S. gramine a	B. perennis	197,36	233,21	1,87	2,49	1,18	3,81
NOR_21_2	S. gramine a	B. perennis	198,64	118,7	2,15	2,65	0,60	1,93
NOR_21_3	S. gramine a	B. perennis	203,43	122,01	2,49	2,53	0,60	1,93
NOR_21_4	S. gramine a	B. perennis	206,19	241,49	3	2,95	1,17	3,78
NOR_21_5	S. gramine a	B. perennis	212,6	246,16	2,23	2,34	1,16	3,74
NOR_21_6	S. gramine a	B. perennis	203	234,36	3,01	2,38	1,15	3,72

NOR_21_7	S. graminea	B. perennis	208,23	124,72	2,09	2,03	0,60	1,93
NOR_21_8	S. graminea	B. perennis	207,03	123,14	1,95	2,09	0,59	1,92
NOR_21_9	S. graminea	B. perennis	201,14	119,93	1,65	2,4	0,60	1,92
NOR_21_10	S. graminea	B. perennis	194,41	227,37	1,98	2,48	1,17	3,77
NOR_21_11	S. graminea	B. perennis	200,87	233,31	2,21	2,75	1,16	3,75
NOR_22_1	S. palustris	B. perennis	197,78	504,64	2,53	2,29	2,55	8,23
NOR_22_2	S. palustris	B. perennis	199,3	508,43	1,1	1,2	2,55	8,23
NOR_22_3	S. palustris	B. perennis	197,31	501,42	1,09	1,06	2,54	8,20
NOR_22_4	S. palustris	B. perennis	195,71	501,44	1,18	1,28	2,56	8,27
NOR_22_5	S. palustris	B. perennis	195,41	498,39	1,37	1,24	2,55	8,23
NOR_22_6	S. palustris	B. perennis	201,18	504,43	1,65	1,83	2,51	8,09
NOR_22_7	S. palustris	B. perennis	194,33	496,37	2,13	1,87	2,55	8,24
NOR_22_8	S. palustris	B. perennis	195,2	498,23	1,7	1,27	2,55	8,23
NOR_22_9	S. palustris	B. perennis	195,73	499,35	1,33	1,41	2,55	8,23

NOR_22_10	S. palustris	B. perennis	194,91	499,32	1,13	1,2	2,56	8,26
NOR_22_11	S. palustris	B. perennis	194,89	494,88	1,31	1,19	2,54	8,19
NOR_23_1	S. graminea	B. perennis	205,25	236,36	1,85	2,67	1,15	3,71
NOR_23_2	S. graminea	B. perennis	198,85	226,77	2,77	2,49	1,14	3,68
NOR_23_3	S. graminea	B. perennis	205,95	236,13	1,55	2,18	1,15	3,70
NOR_23_4	S. graminea	B. perennis	207,01	235,32	2,24	2,1	1,14	3,67
NOR_23_5	S. graminea	B. perennis	203,05	231,98	1,62	1,47	1,14	3,69
NOR_23_6	S. graminea	B. perennis	197,84	233,6	2,8	2,52	1,18	3,81
NOR_23_7	S. graminea	B. perennis	200,36	227,36	2,31	2,17	1,13	3,66
NOR_23_8	S. graminea	B. perennis	193,23	223	1,19	2,34	1,15	3,72
NOR_23_9	S. graminea	B. perennis	212,8	245,61	2,42	2,97	1,15	3,72
NOR_23_10	S. graminea	B. perennis	197,65	230,85	2,43	2,01	1,17	3,77
NOR_SG_24_1	S. graminea	B. perennis	197,65	117,01	2,48	2,98	0,59	1,91
NOR_24_1	S. graminea	B. perennis	197,58	117,45	1,59	2,83	0,59	1,92

NOR_24_2	S.graminea	B. perennis	199,26	122,61	1,95	3	0,62	1,98
NOR_24_4	S. graminea	B. perennis	208,6	124,4	1,65	2,07	0,60	1,92
NOR_24_5	S. graminea	B. perennis	192,09	115,12	2,38	2,85	0,60	1,93
NOR-24-6	S. palustris	B. perennis	200,76	490,62	1,83	2,97	2,44	7,88
NOR-24-7	S. palustris	B. perennis	203	514,68	2,95	2,01	2,54	8,18
NOR-24-8	S. palustris	B. perennis	192,75	475,65	1,87	2,34	2,47	7,96
NOR-24-9	S. palustris	B. perennis	195,86	483,94	2,55	2,27	2,47	7,97
NOR-24-10	S. palustris	B. perennis	204,64	513,27	1,46	1,34	2,51	8,09
NOR-24-11	S. palustris	B. perennis	197,22	492,43	2,71	2,22	2,50	8,05
NOR-24-13	S. palustris	B. perennis	202,14	511,07	1,22	1,03	2,53	8,16
NOR-24-14	S. palustris	B. perennis	201,93	509,75	1,28	1,08	2,52	8,14
NOR-24-15	S. palustris	B. perennis	203	506,21	2,35	2,29	2,49	8,04
NOR-24-16	S. palustris	B. perennis	201,86	509,5	1,1	1,07	2,52	8,14
NOR-24-17	S. palustris	B. perennis	192,14	487,31	1,56	1,26	2,54	8,18

NOR-24-18	S. palustris	B. perennis	193,75	487,76	1,08	1,03	2,52	8,12
NOR-24-19	S. palustris	B. perennis	199,41	490,71	2,49	2,24	2,46	7,94
NOR-24-20	S. palustris	B. perennis	180,84	444,18	2,68	2,96	2,46	7,92

Table 2: Absolute genome size (pg) of all collected species and cytotypes

Population	Sample	Species	Standard	Signal standard	Signal sample	CV standard	CV sample	Ratio	GS	deviation	average GS
1	1_10	S. palustris	B. perennis	197,75	461,42	3,67	3,76	0,43	7,89	1,03	7,78
1	1_10	S. palustris	B. perennis	200,15	459,95	3,22	3,38	0,44	7,77		
1	1_10	S. palustris	B. perennis	205,47	467,65	3,7	3,42	0,44	7,69		
1	1_16	S. palustris	B. perennis	192,88	454,16	3,32	3,55	0,42	7,96	1,01	7,99
1	1_16	S. palustris	B. perennis	197,98	469,2	3,19	3,7	0,42	8,01		
1	1_16	S. palustris	B. perennis	198,23	469,07	3,94	3,44	0,42	8,00		
2	2_6	S. palustris	B. perennis	197,55	445,32	3,16	2,8	0,44	7,62	1,01	7,60
2	2_6	S. palustris	B. perennis	206,46	461,59	3,07	3,08	0,45	7,56		
2	2_6	S. palustris	B. perennis	201,73	454,8	2,93	2,55	0,44	7,62		
3	3_2	S. palustris	B. perennis	204,07	459,05	3,43	3,98	0,44	7,60	1,00	7,60
3	3_3	S. palustris	B. perennis	205,61	462,27	3,53	3,22	0,44	7,60		

3	3_4	S. palustris	B. perennis	202,43	455,39	3,19	3,54	0,44	7,60		
3	3_7	S. palustris	B. perennis	205,79	460,15	3,37	2,55	0,45	7,56	1,01	7,51
3	3_7	S. palustris	B. perennis	207,32	460,67	3,47	3,53	0,45	7,51		
3	3_7	S. palustris	B. perennis	204,72	452,58	3,5	3,1	0,45	7,47		
4	SG 4_3	S. graminea	B. perennis	209	223	3,2	3,1	0,94	3,61	1,02	3,66
4	SG 4_3	S. graminea	B. perennis	200,19	217,75	3,23	2,21	0,92	3,68		
4	SG 4_3	S. graminea	B. perennis	203,11	222,13	3,51	2,36	0,91	3,70		
6	6_1	S. palustris	B. perennis	199,92	449,45	3,89	3,91	0,44	7,60	1,02	7,61
6	6_1	S. palustris	B. perennis	201,36	448,84	3,09	3,98	0,45	7,53		
6	6_1	S. palustris	B. perennis	199,91	455,05	3,01	4,7	0,44	7,69		
6	6_4	S. palustris	B. perennis	220,75	500	3,19	2,98	0,44	7,66	1,02	7,61
6	6_4	S. palustris	B. perennis	218,16	495,47	3,43	2,04	0,44	7,68		
6	6_4	S. palustris	B. perennis	214,84	476,48	3,28	3,32	0,45	7,50		

7	SL_7_1	S. longifolia	B. perennis	200,57	140,34	3,19	3,9	1,43	2,37	1,02	2,40
7	SL_7_1	S. longifolia	B. perennis	196,4	140,4	3,4	3,79	1,40	2,42		
7	SL_7_1	S. longifolia	B. perennis	199,6	142,3	3,97	3,07	1,40	2,41		
10	10_1	S. palustris	B. perennis	215,55	532,42	2,66	3,4	0,40	8,35	1,02	8,37
10	10_1	S. palustris	B. perennis	213,83	525,09	3,18	3,25	0,41	8,30		
10	10_1	S. palustris	B. perennis	206,76	516,82	2,9	3,42	0,40	8,45		
10	10_4	S. palustris	B. perennis	198,55	513,19	1,69	3,28	0,39	8,74	1,02	8,67
10	10_4	S. palustris	B. perennis	199,54	507,83	2,66	3,02	0,39	8,60		
10	10_6	S. palustris	B. perennis	198,12	500,94	3,53	3,03	0,40	8,55	1,02	8,52
10	10_6	S. palustris	B. perennis	197,75	492,8	1,92	3,34	0,40	8,42		
10	10_6	S. palustris	B. perennis	205,74	522,98	2,3	3,4	0,39	8,59		
10	10_9	S. palustris	B. perennis	190,56	494,43	1,86	2,43	0,39	8,77	1,03	8,65

10	10_9	S. palustris	B. perennis	196,91	507,44	1,69	3,22	0,39	8,71		
10	10_9	S. palustris	B. perennis	198,98	499,39	2	3,2	0,40	8,48		
12	12_1	S. palustris	B. perennis	193,91	470,92	3,4	3,39	0,41	8,21	1,01	8,24
12	12_1	S. palustris	B. perennis	195,39	478,98	3,65	3,03	0,41	8,29		
12	12_1	S. palustris	B. perennis	204,73	497,97	3,49	3,84	0,41	8,22		
12	12_2	S. palustris	B. perennis	195,17	500,22	3,61	3,66	0,39	8,66	1,00	8,64
12	12_2	S. palustris	B. perennis	200,21	510,71	3,75	3,76	0,39	8,62		
12	12_3	S. palustris	B. perennis	204	508,17	2,28	2,96	0,40	8,42	1,01	8,41
12	12_3	S. palustris	B. perennis	207,86	518,84	3,32	3,33	0,40	8,44		
12	12_3	S. palustris	B. perennis	207,18	512,4	3,13	3,12	0,40	8,36		
13	13_03	S. palustris	B. perennis	206,07	512,8	3,55	3,87	0,40	8,41	1,02	8,34
13	13_03	S. palustris	B. perennis	207,79	511,48	2,65	2,86	0,41	8,32		
13	13_03	S. palustris	B. perennis	203,05	497,22	3,3	3,31	0,41	8,28		

13	13_06	S. palustris	B. perennis	201,36	449,33	3,24	2,68	0,45	7,54	1,02	7,51
13	13_06	S. palustris	B. perennis	201,04	450,26	3,41	2,66	0,45	7,57		
13	13_06	S. palustris	B. perennis	204,53	449,24	3,44	3,2	0,46	7,42		
13	13_11	S. palustris	B. perennis	207,33	510,5	3,29	3,54	0,41	8,32	1,01	8,34
13	13_11	S. palustris	B. perennis	201,17	500,55	3,19	3,22	0,40	8,41		
13	13_11	S. palustris	B. perennis	205,82	505	3,35	3,13	0,41	8,29		
13	13_12	S. palustris	B. perennis	202,37	498,32	1,98	3,1	0,41	8,32	1,01	8,29
13	13_12	S. palustris	B. perennis	201,38	494,12	3,07	2,89	0,41	8,29		
13	13_12	S. palustris	B. perennis	202,17	493,82	2,95	3,6	0,41	8,26		
13	13_9	S. palustris	B. perennis	198,66	489,09	3,21	3,88	0,41	8,32	1,03	8,45
13	13_9	S. palustris	B. perennis	204,31	519,78	3,74	3,55	0,39	8,60		
13	13_9	S. palustris	B. perennis	207,02	516,15	3,58	3,92	0,40	8,43		

13	13_14	S. palustris	B. perennis	207,21	512,02	3,2	3,3	0,40	8,35	1,01	8,30
13	13_14	S. palustris	B. perennis	207,33	507,6	2,7	2,34	0,41	8,28		
13	13_14	S. palustris	B. perennis	202,17	494,41	3,29	3,66	0,41	8,27		
14	14_1	S. palustris	B. perennis	199,52	464,49	3,33	3,58	0,43	7,87	1,02	7,78
14	14_1	S. palustris	B. perennis	202,42	462,01	2,85	2,51	0,44	7,71		
14	14_1	S. palustris	B. perennis	200,64	459,81	3,22	2,48	0,44	7,75		
14	14_2	S. palustris	B. perennis	199,95	475,43	3,05	2,61	0,42	8,04	1,01	8,01
14	14_2	S. palustris	B. perennis	200,7	474,06	3,54	2,91	0,42	7,98		
14	14_2	S. palustris	B. perennis	204,81	485,72	2,87	2,32	0,42	8,02		
14	14_3	S. palustris	B. perennis	201,52	475,64	3,22	2,89	0,42	7,98	1,02	7,89
14	14_3	S. palustris	B. perennis	193,82	451,47	3,03	3,76	0,43	7,87		
14	14_3	S. palustris	B. perennis	194,32	449,95	3,49	3,04	0,43	7,83		
1	1_20	S. palustris	B. perennis	197,62	460,88	3,26	2,94	0,43	7,88	1,02	7,87

1	1_20	S. palustris	B. perennis	200,9	470,61	3,2	2,8	0,43	7,92		
1	1_20	S. palustris	B. perennis	214,66	495,28	2,98	3,03	0,43	7,80		
1	1_28	S. palustris	B. perennis	202,63	470,69	2,92	2,19	0,43	7,85	1,02	7,95
1	1_28	S. palustris	B. perennis	203,13	478,15	2,8	3,8	0,42	7,96		
1	1_28	S. palustris	B. perennis	203,53	483,89	2,5	3,71	0,42	8,04		
1	1_27	S. palustris	B. perennis	200,24	464,58	2,17	3,53	0,43	7,84	1,01	7,77
1	1_27	S. palustris	B. perennis	211,01	482,98	2,87	2,9	0,44	7,74		
1	1_27	S. palustris	B. perennis	205,05	469,52	3,33	3,54	0,44	7,74		
17	17_10	S. graminea	B. perennis	205,37	115,05	3,39	3,44	1,79	1,89	1,01	1,89
17	17_10	S. graminea	B. perennis	199,16	110,72	2,47	2,86	1,80	1,88		
17	17_10	S. graminea	B. perennis	195,57	109,46	1,91	2,94	1,79	1,89		
17	17_13	S. graminea	B. perennis	201,72	109,9	2,85	2,34	1,84	1,84	1,03	1,87
17	17_13	S. graminea	B. perennis	202,62	112,11	2,7	3,64	1,81	1,87		

17	17_13	S. graminea	B. perennis	200,14	112,04	2,97	3,2	1,79	1,89		
18	18_4	S. graminea	B. perennis	215,66	122,11	2,26	2,24	1,77	1,91	1,01	1,92
18	18_4	S. graminea	B. perennis	198,04	113,19	2,12	2,27	1,75	1,93		
18	18_4	S. graminea	B. perennis	200,8	114	2,48	2,49	1,76	1,92		
18	18_8	S. graminea	B. perennis	201,47	118,05	2,48	3,25	1,71	1,98	1,01	1,97
18	18_8	S. graminea	B. perennis	205,4	118,86	2,46	3,07	1,73	1,96		
18	18_8	S. graminea	B. perennis	204,15	118,4	2,41	2,6	1,72	1,96		
19	19_4	Scandinavian entity	S. pseudocapsicum	195,83	249,67	2,93	3,2	0,78	3,25	1,01	3,24
19	19_4	Scandinavian entity	S. pseudocapsicum	199,6	253,21	2,46	2,22	0,79	3,23		
19	19_4	Scandinavian entity	S. pseudocapsicum	201,17	255,05	3,19	2,81	0,79	3,23		
20	20_2	Scandinavian entity	S. pseudocapsicum	204,28	258,7	3,37	3,53	0,79	3,23	1,01	3,21
20	20_2	Scandinavian entity	S. pseudocapsicum	198,08	249,25	2,94	3,63	0,79	3,21		
20	20_2	Scandinavian entity	S. pseudocapsicum	197,69	247,8	2,46	3,5	0,80	3,20		

20	20_7	Scandinavian entity	S. pseudocapsicum	196,65	249,13	3,78	3,62	0,79	3,23	1,01	3,22
20	20_7	Scandinavian entity	S. pseudocapsicum	198,95	251,71	3,57	3,39	0,79	3,23		
20	20_7	Scandinavian entity	S. pseudocapsicum	196,23	246,99	3,7	3,81	0,79	3,21		
21	21_9	Scandinavian entity	S. pseudocapsicum	184,54	234,13	2,27	3,23	0,79	3,24	1,00	3,24
21	21_9	Scandinavian entity	S. pseudocapsicum	203,26	258,84	2,77	3,13	0,79	3,25		
21	21_9	Scandinavian entity	S. pseudocapsicum	200,95	255,91	3,38	3,4	0,79	3,25		
22	22_3	Scandinavian entity	S. pseudocapsicum	194,19	244,19	3,16	3,52	0,80	3,21	1,01	3,19
22	22_3	Scandinavian entity	S. pseudocapsicum	202,73	252,54	2,97	3,21	0,80	3,18		
22	22_3	Scandinavian entity	S. pseudocapsicum	194,26	242,67	1,8	2,26	0,80	3,19		
22	22_4	Scandinavian entity	S. pseudocapsicum	197,4	254,72	2,79	2,8	0,77	3,29	1,00	3,29
22	22_4	Scandinavian entity	S. pseudocapsicum	195,43	251,43	3,1	3,14	0,78	3,28		
22	22_4	Scandinavian entity	S. pseudocapsicum	199,37	257,62	2,79	3,25	0,77	3,30		

22	22_7	Scandinavian entity	S. pseudocapsicum	195,25	258,48	2,24	2,92	0,76	3,38	1,02	3,40
22	22_7	Scandinavian entity	S. pseudocapsicum	195,53	260,84	3,34	3,4	0,75	3,40		
22	22_7	Scandinavian entity	S. pseudocapsicum	194,93	262,68	3,16	3,23	0,74	3,44		
23	23_4	Scandinavian entity	S. pseudocapsicum	204,45	259,45	3,26	3,55	0,79	3,24	1,01	3,23
23	23_4	Scandinavian entity	S. pseudocapsicum	201,74	256,28	2,69	3,16	0,79	3,24		
23	23_4	Scandinavian entity	S. pseudocapsicum	196,22	246,78	3,17	3,11	0,80	3,21		
24	24_5	S. graminea	B. perennis	198,32	111,83	2,6	3,1	1,77	1,91	1,02	1,92
24	24_5	S. graminea	B. perennis	200,88	115,48	3,46	3,5	1,74	1,94		
24	24_5	S. graminea	B. perennis	210,62	119,89	2,81	2,42	1,76	1,92		
24	24_7	Scandinavian entity	S. pseudocapsicum	204,71	265,83	3,63	3,71	0,77	3,31	1,03	3,30
24	24_7	Scandinavian entity	S. pseudocapsicum	205,25	261,75	3,56	3,29	0,78	3,25		
24	24_7	Scandinavian entity	S. pseudocapsicum	197,47	259,24	2,7	3,96	0,76	3,35		
25	25_3	Scandinavian entity	S. pseudocapsicum	201,26	265,99	1,68	2,81	0,76	3,37	1,00	3,37

25	25_3	Scandinavian entity	S. pseudocapsicum	204,49	269,44	2,08	3,26	0,76	3,36		
25	25_3	Scandinavian entity	S. pseudocapsicum	209,88	277,21	3,81	3,93	0,76	3,37		
25	25_6	Scandinavian entity	S. pseudocapsicum	193,87	240,5	3,63	3,96	0,81	3,16	1,01	3,19
25	25_6	Scandinavian entity	S. pseudocapsicum	197,57	248,42	2,88	3,69	0,80	3,21		
25	25_6	Scandinavian entity	S. pseudocapsicum	191,84	241,49	2,46	3,35	0,79	3,21		
26	26_1	Scandinavian entity	S. pseudocapsicum	195,61	243,32	2,66	3,01	0,80	3,17	1,02	3,20
26	26_1	Scandinavian entity	S. pseudocapsicum	193,73	243,33	2,62	2,99	0,80	3,20		
26	26_1	Scandinavian entity	S. pseudocapsicum	200,02	253,14	3,49	3,67	0,79	3,23		
26	26_2	Scandinavian entity	S. pseudocapsicum	200,69	253,09	2,12	3,53	0,79	3,22	1,03	3,17
26	26_2	Scandinavian entity	S. pseudocapsicum	221,54	275,03	2,89	2,92	0,81	3,17		
26	26_2	Scandinavian entity	S. pseudocapsicum	218,88	269,09	3,01	3,61	0,81	3,13		
26	26_3	Scandinavian entity	S. pseudocapsicum	209,23	277,85	3,55	3,11	0,75	3,39	1,02	3,37
26	26_3	Scandinavian entity	S. pseudocapsicum	216,7	282,88	3,6	2,83	0,77	3,33		

26	26_3	Scandinavian entity	S. pseudocapsicum	216,67	289,21	3,14	3,72	0,75	3,40		
28	28_2	S. graminea	S. pseudocapsicum	199,43	267,44	3,43	2,19	0,75	3,42	1,01	3,40
28	28_2	S. graminea	S. pseudocapsicum	202,11	267,11	2,66	2,17	0,76	3,37		
28	28_2	S. graminea	S. pseudocapsicum	204,49	273,38	3,37	2,53	0,75	3,41		
28	28_3	Scandinavian entity	S. pseudocapsicum	215,17	277,06	3,04	3,03	0,78	3,28	1,02	3,29
28	28_3	Scandinavian entity	S. pseudocapsicum	203,76	260,5	2,75	2,93	0,78	3,26		
28	28_3	Scandinavian entity	S. pseudocapsicum	199,81	259,69	3,01	3,22	0,77	3,31		
28	28_8	Scandinavian entity	S. pseudocapsicum	212,11	269,12	2,23	3,83	0,79	3,24	1,01	3,22
28	28_8	Scandinavian entity	S. pseudocapsicum	210,08	265,34	2,61	2,92	0,79	3,22		
28	28_8	Scandinavian entity	S. pseudocapsicum	211,05	265,01	3,74	3,88	0,80	3,20		
29	29_1	S. graminea	B. perennis	203,39	220,88	2,67	1,98	0,92	3,67	1,01	3,65
29	29_1	S. graminea	B. perennis	225,1	242,23	2,41	1,83	0,93	3,64		
29	29_1	S. graminea	B. perennis	212,62	228,77	2,59	2,22	0,93	3,64		

29	29_4	S. graminea	B. perennis	192,55	207,57	2,29	3,01	0,93	3,64	1,01	3,66
29	29_4	S. graminea	B. perennis	200,87	217,86	1,92	2,34	0,92	3,67		
29	29_4	S. graminea	B. perennis	202,77	220,73	2,12	2,96	0,92	3,68		
30	30_2	S. graminea	B. perennis	210,52	223,86	2,02	1,79	0,94	3,59	1,02	3,55
30	30_2	S. graminea	B. perennis	210,89	221,12	2,91	1,14	0,95	3,54		
30	30_2	S. graminea	B. perennis	209,2	217,52	2,54	1,24	0,96	3,51		
30	30_3	S. graminea	B. perennis	206,31	223,59	1,95	2,05	0,92	3,66	1,00	3,67
30	30_3	S. graminea	B. perennis	208,89	227,05	2,32	2,01	0,92	3,67		
30	30_3	S. graminea	B. perennis	202,61	219,98	2,12	2,31	0,92	3,67		
31	31_1	S. graminea	S. pseudocapsicum	204,15	287,41	2,57	3,77	0,71	3,59	1,02	3,56
31	31_1	S. graminea	S. pseudocapsicum	196,25	271,74	2,01	3,79	0,72	3,53		
31	31_1	S. graminea	S. pseudocapsicum	204,16	284,13	3,75	2,44	0,72	3,55		

31	31_6	S. graminea	S. pseudocapsicum	202,29	275,33	2,92	3,79	0,73	3,47	1,01	3,48
31	31_6	S. graminea	S. pseudocapsicum	205,34	281,11	2,42	3,34	0,73	3,49		
31	31_6	S. graminea	S. pseudocapsicum	205,43	279,3	3,62	3,26	0,74	3,47		
31	31_11	S. graminea	B. perennis	193,18	212,14	2,1	2,32	0,91	3,71	1,00	3,72
31	31_11	S. graminea	B. perennis	192,15	211,55	2,28	2,33	0,91	3,72		
31	31_11	S. graminea	B. perennis	205,41	226,26	2,17	2,55	0,91	3,72		
32	32_1	S. graminea	B. perennis	199,82	115,4	2,1	3,75	1,73	1,95	1,02	1,93
32	32_1	S. graminea	B. perennis	201,87	114,43	3,04	2,95	1,76	1,92		
32	32_1	S. graminea	B. perennis	196,41	111,27	2,5	2,74	1,77	1,91		
32	32_6	S. graminea	B. perennis	200	211	3,13	2,87	0,95	3,57	1,03	3,56
32	32_6	S. graminea	B. perennis	201	215	2,19	2,15	0,93	3,62		
32	32_6	S. graminea	B. perennis	220,93	229,59	3,08	1,17	0,96	3,51		
NOR_4	NOR_4_5	Scandinavian entity	S. pseudocapsicum	204,63	257,11	2,27	2,91	0,80	3,20	1,01	3,21

NOR_4	NOR_4_5	Scandinavian entity	S. pseudocapsicum	194,88	247,02	1,99	2,96	0,79	3,23		
NOR_4	NOR_4_5	Scandinavian entity	S. pseudocapsicum	193,07	242,74	2,29	3,65	0,80	3,21		
NOR_4	NOR_4_4	Scandinavian entity	S. pseudocapsicum	196,36	248,49	2,92	3,23	0,79	3,23	1,00	3,23
NOR_4	NOR_4_4	Scandinavian entity	S. pseudocapsicum	199,49	253,45	3,21	3,78	0,79	3,24		
NOR_4	NOR_4_4	Scandinavian entity	S. pseudocapsicum	199,4	252,86	2,99	3,12	0,79	3,23		
NOR_4	NOR_4_17	S. longifolia	B. perennis	190,7	133,74	3,26	5,16	1,43	2,37	1,02	2,34
NOR_4	NOR_4_17	S. longifolia	B. perennis	191,09	131,54	2,22	3,28	1,45	2,33		
NOR_4	NOR_4_17	S. longifolia	B. perennis	192,25	132,55	3,56	5,02	1,45	2,33		
NOR_7	NOR_7_2	S. graminea	B. perennis	208,17	114,66	2,95	3,59	1,82	1,86	1,01	1,86
NOR_7	NOR_7_2	S. graminea	B. perennis	195,03	106,88	2,15	2,42	1,82	1,85		
NOR_7	NOR_7_2	S. graminea	B. perennis	218,27	120,62	2,55	2,74	1,81	1,87		
NOR_7	NOR_7_8	S. graminea	B. perennis	207,14	112,72	3,58	3,31	1,84	1,84	1,00	1,84
NOR_7	NOR_7_8	S. graminea	B. perennis	203,09	110,77	3,75	2,86	1,83	1,84		

NOR_7	NOR_7_8	S. graminea	B. perennis	218,93	119,93	3,25	2,89	1,83	1,85		
NOR_8	NOR_8_2	S. palustris	B. perennis	198,89	400,91	2,63	3,02	0,50	6,81	1,01	6,84
NOR_8	NOR_8_2	S. palustris	B. perennis	193,95	394,05	3,35	2,08	0,49	6,87		
NOR_8	NOR_8_4	S. palustris	B. perennis	190,7	429,29	3,2	3,09	0,44	7,61	1,03	7,67
NOR_8	NOR_8_4	S. palustris	B. perennis	184,6	426,96	2,43	2,82	0,43	7,82		
NOR_8	NOR_8_4	S. palustris	B. perennis	201,06	451,48	2,36	3,04	0,45	7,59		
NOR_8	NOR_8_11	S. palustris	B. perennis	202,1	419,45	3,58	3,18	0,48	7,02	1,01	7,59
NOR_8	NOR_8_11	S. palustris	B. perennis	189,24	398,52	3,01	2,88	0,47	7,12		
NOR_9	NOR_9_1	S. palustris	B. perennis	195,24	423	2,83	3,76	0,46	7,32	1,03	7,21
NOR_9	NOR_9_1	S. palustris	B. perennis	199,06	418,2	3,65	3,17	0,48	7,10		
NOR_9	NOR_9_5	S. palustris	B. perennis	191,99	400,72	2,04	2,46	0,48	7,05	1,03	7,06
NOR_9	NOR_9_5	S. palustris	B. perennis	201,12	425,99	1,68	3,05	0,47	7,16		

NOR_9	NOR_9_5	S. palustris	B. perennis	194,55	401,22	2,37	3,27	0,48	6,97		
NOR_9	NOR_9_8	S. palustris	B. perennis	198,64	398,6	3,02	2,49	0,50	6,78	1,03	6,85
NOR_9	NOR_9_8	S. palustris	B. perennis	203,6	407,02	3,18	3,44	0,50	6,76		
NOR_9	NOR_9_8	S. palustris	B. perennis	203,92	422,33	2,55	3,28	0,48	7,00		
NOR_9	NOR_9_10	S. palustris	B. perennis	194,17	416,19	3,19	3,42	0,47	7,24	1,03	7,14
NOR_9	NOR_9_10	S. palustris	B. perennis	205,83	428,83	2,47	2,89	0,48	7,04		
NOR_10	NOR_10_3	S. palustris	B. perennis	199	416,71	3,2	3,66	0,48	7,08	1,03	6,93
NOR_10	NOR_10_3	S. palustris	B. perennis	198,74	401,81	2,94	2,98	0,49	6,83		
NOR_10	NOR_10_3	S. palustris	B. perennis	196,13	398,76	2,51	3,21	0,49	6,87		
NOR_10	NOR_10_4	S. palustris	B. perennis	209,4	428,88	3,31	3,46	0,49	6,92	1,01	6,93
NOR_10	NOR_10_4	S. palustris	B. perennis	200,89	414,61	3,8	2,85	0,48	6,98		
NOR_10	NOR_10_4	S. palustris	B. perennis	205,14	417,96	3,93	2,55	0,49	6,89		

NOR_10	NOR_10_10	S. palustris	B. perennis	193,88	406,11	2,86	2,92	0,48	7,08	1,01	7,04
NOR_10	NOR_10_10	S. palustris	B. perennis	207,02	433,38	3,25	3,33	0,48	7,08		
NOR_10	NOR_10_10	S. palustris	B. perennis	206,41	426,12	2,44	2,67	0,48	6,98		
NOR_11	NOR_11_1	S. graminea	S. pseudocapsicum	203,15	286,6	1,92	2,52	0,71	3,60	1,01	3,60
NOR_11	NOR_11_1	S. graminea	S. pseudocapsicum	197,56	280,44	3,61	2,79	0,70	3,62		
NOR_11	NOR_11_1	S. graminea	S. pseudocapsicum	202,81	285	2,86	2,24	0,71	3,58		
NOR_11	NOR_11_5	S. palustris	B. perennis	203,9	428,16	2,63	2,45	0,48	7,10	1,02	7,21
NOR_11	NOR_11_5	S. palustris	B. perennis	197,94	424,85	3,92	2,89	0,47	7,25		
NOR_11	NOR_11_5	S. palustris	B. perennis	202,86	435,91	3,85	3,88	0,47	7,26		
NOR_11	NOR_11_6	S. palustris	B. perennis	204,36	466,66	2,83	3,83	0,44	7,72	1,00	7,72
NOR_11	NOR_11_6	S. palustris	B. perennis	204,7	467,43	2,95	3,91	0,44	7,72		
NOR_12	NOR_12_9	S. longifolia	B. perennis	197,43	143,15	2,65	2,99	1,38	2,45	1,01	2,44
NOR_12	NOR_12_9	S. longifolia	B. perennis	205,16	148,79	3,28	3,14	1,38	2,45		

NOR_12	NOR_12_9	S. longifolia	B. perennis	205,4	147	2,59	3,7	1,40	2,42		
NOR_13a	NOR_13a_3	S. longifolia	B. perennis	194,93	142,31	3,09	3,9	1,37	2,47	1,03	2,50
NOR_13a	NOR_13a_3	S. longifolia	B. perennis	189,43	142,38	3,69	3,23	1,33	2,54		
NOR_13a	NOR_13a_3	S. longifolia	B. perennis	205,85	151,4	3,37	3,65	1,36	2,49		
NOR_13a	NOR_13a_8	S. longifolia	B. perennis	191,25	137,85	1,94	2,84	1,39	2,44	1,02	2,47
NOR_13a	NOR_13a_8	S. longifolia	B. perennis	200,17	147,18	2,56	2,54	1,36	2,49		
NOR_13a	NOR_13a_8	S. longifolia	B. perennis	201,62	148,21	2,85	3,97	1,36	2,48		
NOR_13b	NOR_13b_1	Scandinavian entity	S. pseudocapsicum	198,45	282,89	1,92	3,36	0,70	3,64	1,01	3,66
NOR_13b	NOR_13b_1	Scandinavian entity	S. pseudocapsicum	205,91	295,28	2	2,44	0,70	3,66		
NOR_13b	NOR_13b_1	Scandinavian entity	S. pseudocapsicum	210,94	303,86	2,13	2,29	0,69	3,67		
NOR_13b	NOR_13b_3	Scandinavian entity	S. pseudocapsicum	197,01	286,25	2,64	2,47	0,69	3,71	1,00	3,71
NOR_13b	NOR_13b_3	Scandinavian entity	S. pseudocapsicum	192,55	280,2	2,28	3,21	0,69	3,71		
NOR_13b	NOR_13b_3	Scandinavian entity	S. pseudocapsicum	194,03	281,49	1,98	3,05	0,69	3,70		

NOR_15	NOR_15_1	S. longifolia	B. perennis	198,89	144,36	1,86	1,96	1,38	2,45	1,01	2,44
NOR_15	NOR_15_1	S. longifolia	B. perennis	190,38	137,08	2,99	2,45	1,39	2,43		
NOR_15	NOR_15_1	S. longifolia	B. perennis	189,82	136	3,39	2,27	1,40	2,42		
NOR_16	NOR_16_7	S. graminea	B. perennis	175,41	96,74	2,97	4	1,81	1,86	1,02	1,86
NOR_16	NOR_16_7	S. graminea	B. perennis	167,44	93,17	2,2	2,57	1,80	1,88		
NOR_16	NOR_16_7	S. graminea	B. perennis	169,35	92,57	2,44	2,81	1,83	1,85		
NOR_16	NOR_16_8	S. graminea	B. perennis	175,68	93,71	1,77	3,09	1,87	1,80	1,02	1,81
NOR_16	NOR_16_8	S. graminea	B. perennis	181,84	98,65	2,5	3,33	1,84	1,83		
NOR_16	NOR_16_8	S. graminea	B. perennis	185,35	99,11	2,17	2,43	1,87	1,81		
NOR_17	NOR_17_3	S. graminea	B. perennis	189,7	99,42	2,64	3,14	1,91	1,77	1,03	1,80
NOR_17	NOR_17_3	S. graminea	B. perennis	199,23	104,99	2,63	2,53	1,90	1,78		
NOR_17	NOR_17_3	S. graminea	B. perennis	199,97	108,51	2,48	3,84	1,84	1,83		

NOR_17	NOR_17_6	S. graminea	B. perennis	213,26	118,37	2,39	2,52	1,80	1,88	1,01	1,88
NOR_17	NOR_17_6	S. graminea	B. perennis	209,7	117,1	2,79	2,89	1,79	1,89		
NOR_18	NOR_18_5	S. palustris	B. perennis	193,44	418,95	2,14	3,37	0,46	7,32	1,01	7,35
NOR_18	NOR_18_5	S. palustris	B. perennis	189,85	410,78	2,27	3,37	0,46	7,31		
NOR_18	NOR_18_5	S. palustris	B. perennis	203,69	446,37	2,78	3,34	0,46	7,41		
NOR_18	NOR_18_7	S. palustris	B. perennis	203,05	463,49	3,2	3,6	0,44	7,72	1,02	7,63
NOR_18	NOR_18_7	S. palustris	B. perennis	193,5	432,3	3,94	3,85	0,45	7,55		
NOR_18	NOR_18_10	S. palustris	B. perennis	197,53	455,37	1,69	3,38	0,43	7,79	1,01	7,77
NOR_18	NOR_18_10	S. palustris	B. perennis	195,8	446,94	1,6	3,65	0,44	7,72		
NOR_18	NOR_18_10	S. palustris	B. perennis	197,45	455	1,49	3,04	0,43	7,79		
NOR_19	NORS_19_3	S. palustris	B. perennis	172,28	382,75	3,77	3,95	0,45	7,51	1,03	7,60
NOR_19	NORS_19_3	S. palustris	B. perennis	196,61	441,35	2,54	3,32	0,45	7,59		
NOR_19	NORS_19_3	S. palustris	B. perennis	204,86	467,8	3,96	2,86	0,44	7,72		

NOR_19	NOR_19_7	S. palustris	B. perennis	197,56	472,02	1,94	3,21	0,42	8,08	1,00	8,06
NOR_19	NOR_19_7	S. palustris	B. perennis	198,18	472,32	2,18	3,76	0,42	8,06		
NOR_19	NOR_19_7	S. palustris	B. perennis	195,52	466,45	2,5	3,63	0,42	8,06		
NOR_19	NOR_19_9	S. palustris	B. perennis	184,4	428,65	3,13	3,68	0,43	7,86	1,00	7,86
NOR_19	NOR_19_9	S. palustris	B. perennis	207,36	482,7	2,32	3,05	0,43	7,87		
NOR_20	NOR_20_3	S. palustris	B. perennis	201,45	449,12	3,04	3,35	0,45	7,54	1,03	7,40
NOR_20	NOR_20_3	S. palustris	B. perennis	198,01	425,44	3,58	3,67	0,47	7,26		
NOR_20	NOR_20_4	S. palustris	B. perennis	193,37	446,91	2,89	3,61	0,43	7,81	1,02	7,71
NOR_20	NOR_20_4	S. palustris	B. perennis	204,71	462,11	1,81	3,62	0,44	7,63		
NOR_20	NOR_20_4	S. palustris	B. perennis	202,31	460,84	1,88	3,86	0,44	7,70		
NOR_20	NOR_20_5	S. palustris	B. perennis	194,35	439,35	1,75	2,87	0,44	7,64	1,01	7,70
NOR_20	NOR_20_5	S. palustris	B. perennis	195,96	448,02	1,55	2,8	0,44	7,73		

NOR_20	NOR_20_5	S. palustris	B. perennis	197,62	452,56	2,22	2,92	0,44	7,74		
NOR_21	NOR_21_8	S. graminea	B. perennis	195,06	107,39	2,3	3,35	1,82	1,86	1,01	1,85
NOR_21	NOR_21_8	S. graminea	B. perennis	195,98	106,53	2,18	2,53	1,84	1,84		
NOR_21	NOR_21_8	S. graminea	B. perennis	195,61	107,69	2,88	2,92	1,82	1,86		
NOR_21	NOR_21_11	S. graminea	B. perennis	204,94	224,83	2,47	2,06	0,91	3,71	1,00	3,71
NOR_21	NOR_21_11	S. graminea	B. perennis	213,2	234,98	2,66	2,07	0,91	3,73		
NOR_21	NOR_21_11	S. graminea	B. perennis	202,89	222,55	2,33	2,09	0,91	3,71		
NOR_22	NOR_22_9	S. palustris	B. perennis	206,06	465,73	3,97	3,73	0,44	7,64	1,03	7,60
NOR_22	NOR_22_9	S. palustris	B. perennis	209,32	463,82	3,73	3,68	0,45	7,49		
NOR_22	NOR_22_9	S. palustris	B. perennis	201,51	458,02	3,17	3,77	0,44	7,68		
NOR_22	NOR_22_11	S. palustris	B. perennis	199,59	452,62	3,43	2,71	0,44	7,66	1,02	7,57
NOR_22	NOR_22_11	S. palustris	B. perennis	200	445,9	2,76	3,51	0,45	7,54		
NOR_22	NOR_22_11	S. palustris	B. perennis	193,04	428,2	4,36	2,8	0,45	7,50		

NOR_23	NOR_23_5	S. graminea	S. pseudocapsicum	198,29	278,95	2,35	2,62	0,71	3,59	1,01	3,58
NOR_23	NOR_23_5	S. graminea	S. pseudocapsicum	193,58	269,88	2,53	3,75	0,72	3,56		
NOR_23	NOR_23_5	S. graminea	S. pseudocapsicum	200,63	282,33	3,07	2,71	0,71	3,59		
NOR_23	NOR_23_7	S. graminea	B. perennis	195,44	214,34	2,44	3,68	0,91	3,71	1,01	3,68
NOR_23	NOR_23_7	S. graminea	B. perennis	197,77	215,27	1,93	3,05	0,92	3,68		
NOR_23	NOR_23_7	S. graminea	B. perennis	200,48	217	2,77	2,87	0,92	3,66		
NOR_23	NOR_23_8	S. graminea	S. pseudocapsicum	193,59	275,09	2,42	2,21	0,70	3,62	1,01	3,65
NOR_23	NOR_23_8	S. graminea	S. pseudocapsicum	201,54	288,11	3,95	3,33	0,70	3,65		
NOR_23	NOR_23_8	S. graminea	S. pseudocapsicum	198,15	285,08	3,09	3,46	0,70	3,67		
NOR_24	NOR_24_5	S. graminea	B. perennis	197,4	109,78	2,32	3,37	1,80	1,88	1,01	1,87
NOR_24	NOR_24_5	S. graminea	B. perennis	194,68	107,67	1,78	3,08	1,81	1,87		
NOR_24	NOR_24_10	S. palustris	B. perennis	192,47	433,9	3,48	3,5	0,44	7,62	1,03	7,54

NOR_24	NOR_24_10	S. palustris	B. perennis	193,26	423,51	1,99	3,82	0,46	7,41		
NOR_24	NOR_24_10	S. palustris	B. perennis	188,95	424,65	1,99	3,66	0,44	7,60		
NOR_24	NOR_24_17	S. palustris	B. perennis	192,22	421,2	1,97	2,67	0,46	7,41	1,02	7,44
NOR_24	NOR_24_17	S. palustris	B. perennis	198,76	435,12	1,98	2,05	0,46	7,40		
NOR_24	NOR_24_17	S. palustris	B. perennis	199,1	443,22	3,38	2,36	0,45	7,52		
NOR_24	NOR_24_18	S. palustris	B. perennis	196,01	414	1,93	3,37	0,47	7,14	1,03	7,28
NOR_24	NOR_24_18	S. palustris	B. perennis	210,17	457,68	2,79	3,5	0,46	7,36		
NOR_24	NOR_24_18	S. palustris	B. perennis	204,05	443,84	1,95	3,6	0,46	7,35		
35	35_1_1	S. graminea	B. perennis	203,88	222,43	2,46	1,89	0,92	3,69	1,02	3,74
35	35_1_1	S. graminea	B. perennis	200,47	222,59	2,36	2,49	0,90	3,75		
35	35_1_1	S. graminea	B. perennis	201,33	224,46	2,67	2,9	0,90	3,77		
35	35_1_2	S. palustris	B. perennis	192,54	439,88	2,14	3,67	0,44	7,72	1,02	7,82
35	35_1_2	S. palustris	B. perennis	190,49	442,22	2,31	3,28	0,43	7,85		

35	35_1_2	S. palustris	B. perennis	183,47	427,94	2,6	3,33	0,43	7,88		
35	35_1_3	S. graminea	B. perennis	201,83	221,56	2,12	2,23	0,91	3,71	1,01	3,72
35	35_1_3	S. graminea	B. perennis	201,67	222,72	2,02	2,08	0,91	3,73		
35	35_1_3	S. graminea	B. perennis	204,74	224,5	2,1	2,21	0,91	3,71		
35	35_2_1	S. palustris	B. perennis	206,67	369,48	2,93	3,44	0,56	6,04	1,02	6,03
35	35_2_1	S. palustris	B. perennis	215,41	380,52	3,22	3,22	0,57	5,97		
35	35_2_1	S. palustris	B. perennis	195,27	351,23	3,1	3,32	0,56	6,08		