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REVIEW PAPER

Revisiting the *Pellia sensu lato* complex: an integrative review of taxonomic revision, phylogeny, and molecular biology

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Abstract

The genus *Pellia sensu lato*, a crucial early lineage in land plant phylogeny, has long presented significant taxonomic challenges. This is due to its high phenotypic plasticity and limited number of reliable morphological characters. This review synthesizes recent advancements in understanding this complex, focusing on taxonomic changes, cryptic speciation, phylogenetic studies, and molecular biology.

The advent of molecular methods has fundamentally changed our understanding of *Pellia sensu lato*, leading to a robust division of the traditional genus into two distinct, monophyletic entities: *Pellia* Hedw. (*sensu stricto*) and the newly recognized *Apopellia* Grolle. Phylogenetic analyses utilizing plastid, mitochondrial, and nuclear markers not only confirmed this reclassification but also revealed widespread cryptic speciation. Within *Pellia epiphylla*, two cryptic lineages (N and S) were identified, which were subsequently proven to be the parental species of the allopolyploid *Pellia borealis*. Similarly, the *Apopellia endiviifolia* complex exhibited the existence of cryptic sister species (aquatic form A and terrestrial form B), and *Apopellia alpicola* was segregated from a previous subclade. Although studies on cryptic speciation have primarily concentrated in Poland, the results strongly suggest a much larger, undiscovered diversity across the vast circumboreal range of this taxon.

Beyond taxonomy, *Pellia* has become a valuable model system in molecular biology, particularly in research on sex determination mechanisms (encompassing both monoicous and dioicous species) and microtranscriptomics. Shared conservative miRNA families present in both liverworts and green algae offer insights into the evolution of gene regulation in land plants. Analyses of complete organellar genomes (plastid and mitochondrial) have provided new “superbarcodes” for species identification and facilitated the study of RNA editing and heteroplasmy, highlighting their roles in the adaptation and diversification of liverworts.

Keywords

Pellia, *Apopellia*; liverworts; taxonomy; phylogeny; cryptic speciation; organellar genomes; RNA editing

1. Introduction

As a pivotal early branch in the phylogeny of land plants, liverworts offer unique insights into the evolutionary origins of terrestrial life on Earth. The genus *Pellia*, belonging to the family Pelliaceae, is a small yet widespread group of liverworts found in temperate and cool regions of the Northern Hemisphere (Schuster, 1992). Representatives of this genus are considered the most basal lineage of Jungermanniopsida

because of numerous plesiomorphic traits, such as a wedge-shaped apical cell, a thallus lacking a midrib, a spherical capsule, and a massive seta (Schuster, 1992). However, this apparent simplicity has proven profoundly deceptive, masking a far more complex evolutionary history (Konstantinova et al., 2023; Schuster, 1981, 1992; Schütz et al., 2016).

The core challenge in studying *Pellia* has always been its high degree of phenotypic plasticity, where environmental factors induce significant variation in form, coupled with

a low number of reliable morphological characters (Konstantinova et al., 2023; Schuster, 1981, 1992; Schütz et al., 2016; Zieliński, 1987). This combination has led to a long and often contentious taxonomic history, with boundaries between species frequently debated and redefined (Schuster, 1992). It has become increasingly clear that traditional morphological approaches are insufficient to resolve the true diversity and phylogenetic relationships within what was known as the *Pellia sensu lato* complex (Zieliński, 1987).

The advent of the molecular era has fundamentally reshaped our understanding of liverworts. The application of molecular phylogenetics has not only clarified species boundaries but has also revealed previously unimaginable levels of hidden diversity. The most significant outcome of this study is the division of traditional *Pellia sensu lato* into two distinct, well-supported genera: *Pellia* Hedw. (*sensu stricto*) and the newly recognized *Apopellia* Grolle. Furthermore, these studies have consistently uncovered widespread cryptic speciation, demonstrating that what was once considered a single species is often a complex of morphologically identical but genetically divergent lineages (Konstantinova et al., 2023; Schütz et al., 2016; Zieliński, 1987).

Beyond resolving these critical taxonomic puzzles, the *Pellia* complex is rapidly emerging as a valuable model system for addressing fundamental questions in molecular biology and evolution research. Its unique position in the plant tree of life makes it an ideal subject for comparative genomics, representing the broader understudied liverwort class, Jungermanniopsida. This is particularly important because Jungermanniopsida, with over 7,000 species (Söderström et al., 2016), is far more speciose than the well-studied Marchantiopsida, which comprises approximately 380 species, including the model organism *Marchantia polymorpha* (Bowman et al., 2017).

Scientific interest in the genus *Pellia* and order *Pelliales* has increased over the past few decades. An analysis of scientific publications featuring the keywords *Pellia*, *Pelliales*, or *Apopellia* reveals that research on this group was limited until the mid-20th century. Dynamic research development began in the 1970s, with notable intensification after 2000. The marked rise in studies over the last decade can be attributed to new systematic approaches, the use of molecular methods, and taxonomic revisions that led to the segregation of the genus, *Apopellia*. This trend suggests the growing importance of this plant group in research on the evolution, phylogeny, and biogeography of liverworts (Figure 1).

This review synthesizes these recent and transformative developments. We traced the journey of *Pellia* from its confusing classical taxonomy to its modern reclassification based on robust molecular evidence. We detail the phylogenetic studies that led to the recognition of *Apopellia*, explore the biological and systematic implications of cryptic speciation within the complex, and finally, summarize the burgeoning field of molecular biological studies that are positioning these humble liverworts at the forefront of evolutionary research.

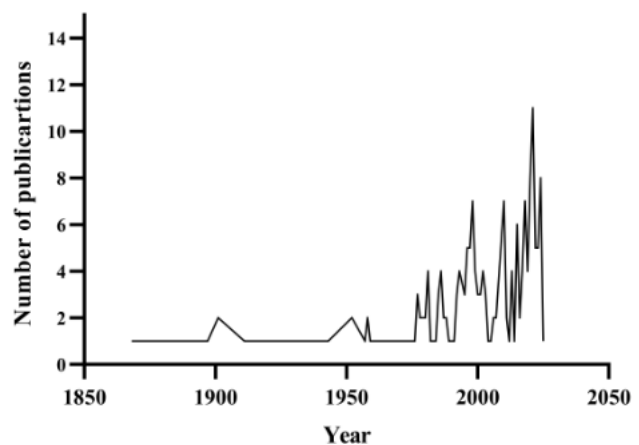


Figure 1 Bibliometric analysis of publication output concerning *Pellia*, *Apopellia*, and *Pelliales* (1850–2025). Data derived from Scopus database searches employing the query ‘pellia’ OR ‘apopellia’ OR ‘pelliales’. The temporal distribution demonstrates a pronounced upward trend commencing in the 1970s, with an accelerated publication frequency observed post-2000. The search was conducted on 05.04.2025.

2. Taxonomic history and classification

The earliest literary mentions of liverworts from the genus *Pellia* date back to 1616, when Colonna described them as *Lichen alter minor caule calceato*. The genus *Pellia* was formally established by Raddi in 1818, and the first critical taxonomic studies were conducted by Jack (1895). These foundational studies led to the distinction of three species: *P. epiphylla*, *P. neesiana*, and *P. endiviifolia*. Before 1876, three other described species were later synonymized: *Pellia fabroniana* Raddi and *Pellia calycina* (Tayl.) Nees (both now considered synonyms of *P. endiviifolia*), and *Pellia neesii* (Lindb.) Limpr. (a variant of *P. neesiana*). Currently, the genus *Pellia sensu lato* comprises eight species. Five species belong to the genus *Pellia* (*P. appalachiana*, *P. epiphylla*, *P. borealis*, *P. columbiana*, and *P. neesiana*), while three belong to the genus *Apopellia* (*A. alpicola*, *A. megaspora*, and *A. endiviifolia*) (Söderström et al., 2013; Stotler & Crandall-Stotler, 2017).

Morphological separation of taxa within *Pellia sensu lato* has always posed a significant challenge. The simple thaloid morphology of these liverworts, combined with their vast distribution across the Northern Hemisphere, complicates their identification. This high structural simplicity can mask genetic differences, limiting their phenetic expression and resulting in a lack of clear diagnostic characters required for classical taxonomic classification. Consequently, a high degree of genetic variability is often concealed by structural homogeneity and significant phenotypic plasticity (Schuster, 1981).

Research initiated by Schuster (1981), encompassing studies on geographic distribution, cytogenetics, and genetic variability, led to the identification of several new species within *Pellia sensu lato*. These studies also laid the groundwork for the subsequent elevation of *Apopellia* from a subgenus to distinct genus.

The subgenus *Apopellia* Grolle, established in 1983, initially contained only *Pellia endiviifolia*. In 2016, *Apopellia* was elevated to the rank of genus based on detailed morphological and molecular analyses by Schütz et al. (2016), which demonstrated that the family Pelliaceae comprises two distinct, monophyletic lineages. This study provides a more accurate classification, offering greater insight into the phylogenetic relationships, ecological preferences, and geographical distribution of Pelliaceae species. Morphological data corroborated the genetic results, confirming distinct diagnostic characteristics for both *Pellia* and *Apopellia* (Table 1). Biogeographical analysis has further revealed different distribution patterns, with *Pellia* species being widespread in temperate regions, whereas *Apopellia* species show more restricted distributions (Schütz et al., 2016).

The genus *Pellia* is distinguished by several key features: a hemidiscoidal apical cell; clustered two-celled slime hairs on both the ventral and dorsal surfaces of the thallus, as well as on the calyptra, pseudoperianth, and at the base of the archegonia; thickening bands in the thallus cell walls; and papilliform cells in the male tubercle aperture (Table 1, Table S1). The archegonia are grouped and arranged horizontally or ascending. In contrast, the genus *Apopellia* is characterized by clustered multicellular slime hairs on the ventral side of the thallus, a ciliate pseudoperianth, and vertically grouped archegonia (Table 1; Table S1) (Schütz et al., 2016).

2.1. Species of the genus *Pellia*

The genus *Pellia* (family Pelliaceae, order Peltiales) encompasses a small number of liverwort species characterized by simple thalloid gametophytes, specific anatomical features, and high phenotypic plasticity (Table 1). Representatives of this genus are found mainly in the temperate zone

of the Northern Hemisphere, inhabiting moist terrestrial habitats with variable microclimates, such as forests, meadows, and stream and river banks. However, their elusive diagnostic features make identification challenging.

Species-level identification within *Pellia* can be problematic because of their morphological homogeneity and the frequent absence of reproductive structures in herbarium specimens (Schuster, 1981; Konstantinova et al., 2023). Historically, *Pellia sensu lato* included a larger number of taxa; however, the advent of molecular and phylogenetic studies has led to a significant revision. Consequently, the separate genus *Apopellia* was established, and the current concept of *Pellia sensu stricto* includes five species: *P. epiphylla*, *P. borealis*, *P. neesiana*, *P. columbiana*, and *P. apalachiana* (Schütz et al., 2016).

This subchapter aims to present the currently recognized species of *Pellia*, with a focus on their morphological features, geographical distribution, and ecological preferences. Where relevant, systematic difficulties associated with the delimitation of individual taxa are also discussed.

Pellia epiphylla (L.) Corda

The first species of this genus to be described, *Pellia epiphylla*, was originally named *Jungermannia epiphylla* by Linnaeus in his 1753 work, *Species Plantarum*. The species was later reclassified in 1829 by August Carl Joseph Corda, who placed it in the genus *Pellia* and established the currently accepted binomial nomenclature.

This species is characterized by a simple thallus that can reach over 1 cm in width and several centimeters in length (Figure 2). The thallus is typically green but may develop red or purple tints. *Pellia epiphylla* is a monoecious species with antheridia scattered along the midrib and a single

Table 1 Selected characters of the genera *Pellia* and *Apopellia*. Based on Schuster (1981) and Schütz et al. (2016).

Character	<i>Apopellia</i> (Grolle) Nebel & D.Quandt	<i>Pellia</i> Raddi
Slime hairs	Pluricellular	Two-celled
Thickening bands	Lacking	Well developed
Male tubercles aperture	Cells inconspicuous	Cells papilliforme
Antheridia	Large	Large
Pseudoperianth present	Before fertilisation	Before fertilisation
Archegonia position	In cavities	In cavities
Archegonia number	Up to 10 per cavity	10–30 per cavity
Archegonia posture	Erect	Horizontal to ascending
Calyptra	Smooth, remains included or virtually so	With two-celled hairs; partially exserted at time of seta elongation
Apical cell	Wedge-shaped	Hemidiscoid
Perichaetium	Forms a complete ring, normally incised-lobulate and ± fimbriate, becoming tubular with fertilization	Short, horizontal, formed of a posterior flat ‘flap,’ lacking or very short anteriorly

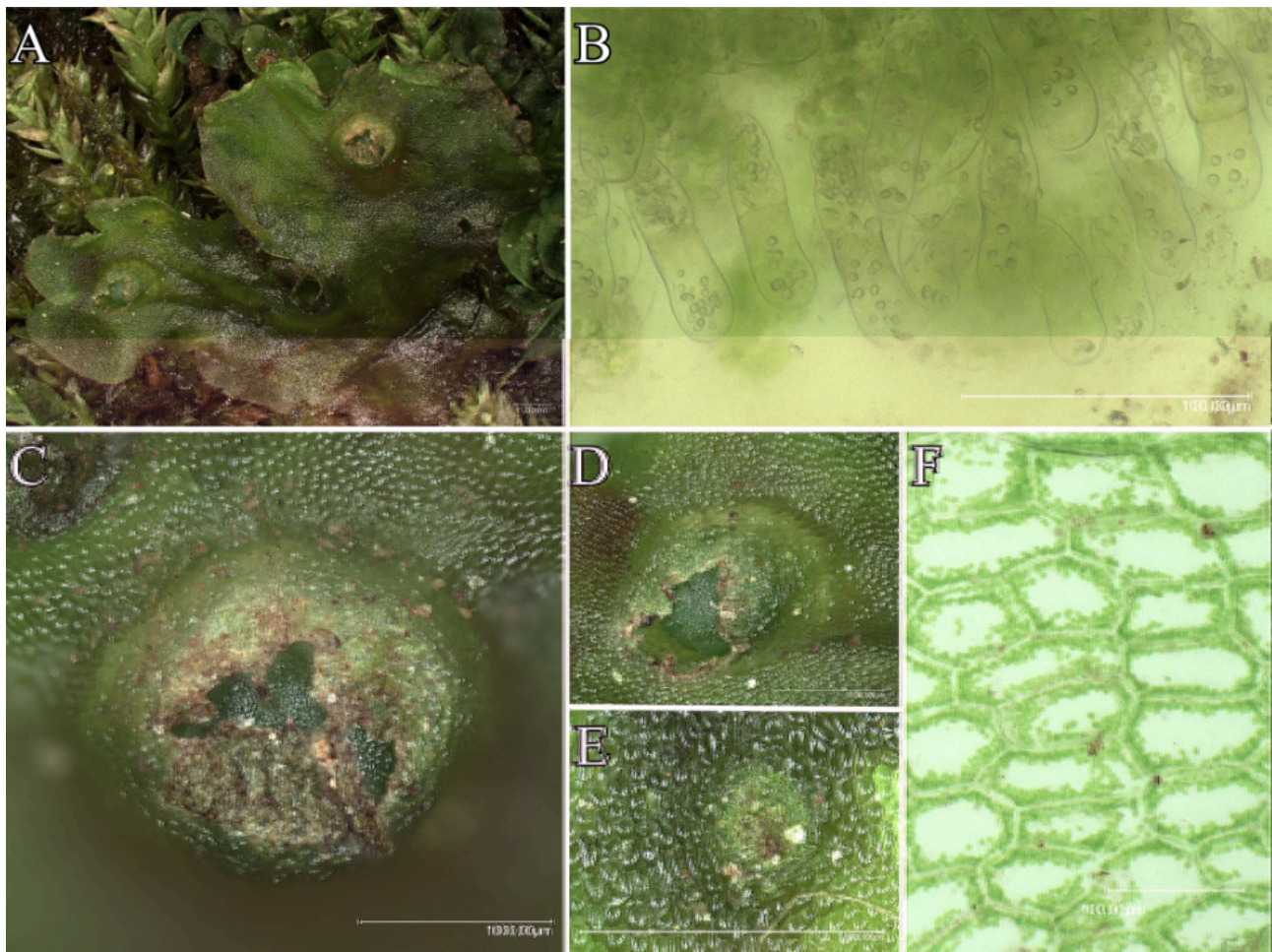


Figure 2 *Pellia epiphylla*: (A) General view of the thallus; (B) Slime hairs; (C, D) Archegonia on the thallus surface (close-up view); (E) Antheridium; (F) Thallus cells.

archegonium at the apex, surrounded by a vertical, flap-like involucre. Monoicousness and the structure of female sex organs are diagnostic characteristics that facilitate the taxonomic identification of this species during field observations. However, it is not uncommon to find large populations of *Pellia* that do not develop sexual organs, which makes it almost impossible to reliably identify them at the species level (Atherton et al., 2010).

Pellia epiphylla is one of the most widely distributed species in the genus, with a nearly cosmopolitan distribution (Figure 3). It is found in Europe, Asia, North America, North Africa, the Azores, Madeira, and Greenland (Schütz et al., 2016). This species thrives in soils with varying degrees of moisture and acidity, including nutrient-poor substrates. It is commonly found on clay and sand and often grows on thin soil layers over rocks. Its habitats include the banks of streams, lakes, and marshes, as well as eroded banks, shaded forests, and disturbed areas such as ditches and wet paths (Schütz et al., 2016).

Modern molecular studies using RAPD techniques have revealed the existence of two cryptic species within the *P. epiphylla* complex, designated as species N and S (Zieliński, 1984, 1987). The distribution of these cryptic species has thus far been described only in Poland, where they

exhibit an allopatric pattern: the N form is confirmed in the north, whereas the S form dominates in the south. Given the vast geographical range of *P. epiphylla*, further research is needed to determine the distribution of these cryptic lineages across the entire species range, as such data are currently unavailable.

Pellia neesiana (Gottsche) Limpr.

Pellia neesiana was first described by Gottsche in 1867 as a form of *Pellia epiphylla*. Although Limpricht (1877) elevated it to a separate species, its taxonomic status remained debatable until the early 20th century. Although Douin (1903) did not recognize *P. neesiana* as distinct, Frye and Clark (1937) later solidified its status as a separate species. In their study, they distinguished it based on key morphological features: a wide thallus (3–7 mm), dioicous sexual system, and an involucre forming a tube that is shorter on the side facing the thallus apex, with an exerted calyptra.

Pellia neesiana is characterized by a simple thallus, approximately 1 cm wide, which often develops red or purple pigmentation along the midrib. It can also be distinguished by its characteristic sharp odor. Despite their morphological similarity to *Pellia epiphylla*, the two species have different

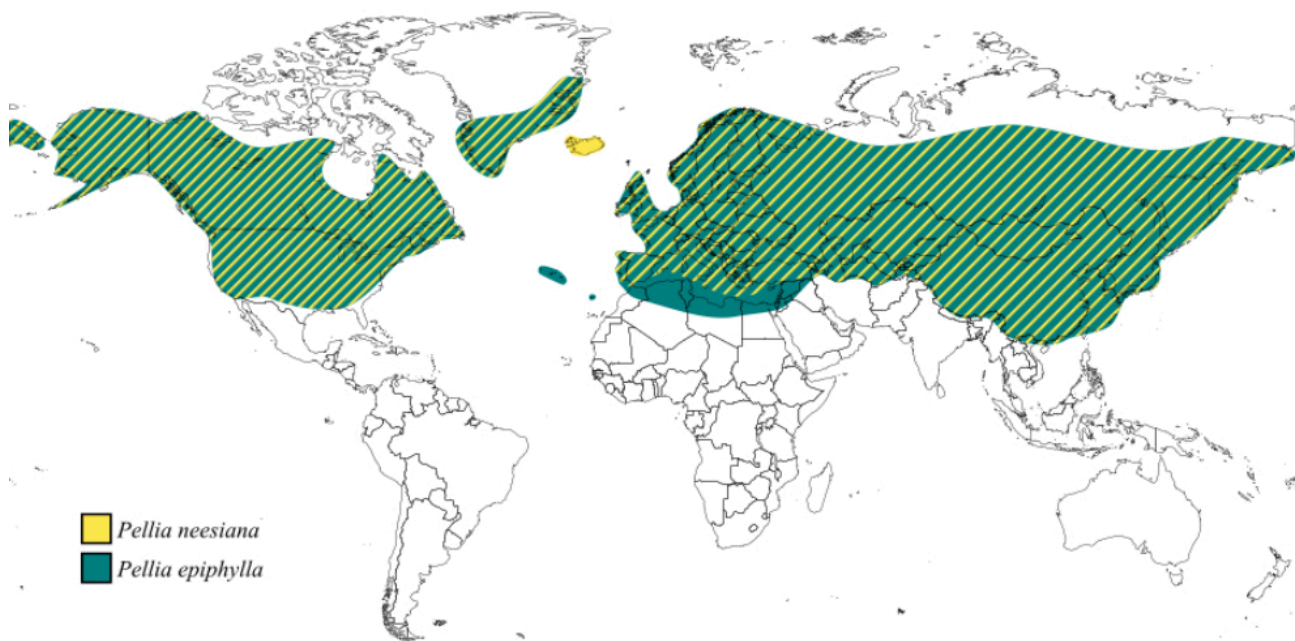


Figure 3 Distribution of *Pellia neesiana* and *Pellia epiphylla* based on the literature.

sexual systems. *Pellia neesiana* is dioicous, meaning that male and female reproductive structures develop on separate gametophytes. Consequently, field observations frequently reveal populations consisting of a single sex. The female involucre, which forms a tube that is shorter on its frontal side, is a key diagnostic feature (Atherton et al., 2010; Frye & Clark, 1937).

Pellia neesiana has a wide circumpolar distribution in Europe, Asia, North America, Greenland, and Iceland (Figure 3) (Schütz et al., 2016). It prefers moist to wet, nutrient-poor soils with a neutral to moderately acidic pH and clay or sandy-clay texture. This species tolerates seasonal flooding and is commonly found along streams, lakes, marshes, ditches, and wet paths, particularly within forests. It is occasionally found on gravelly forest tracks (Atherton et al., 2010; Schütz et al., 2016).

Pellia borealis Lorb.

Pellia borealis was first described by Lorbeer (1934) as a polyploid variant of *Pellia epiphylla* and immediately became the subject of extensive research on its origin, genetic structure, and taxonomic classification. Morphologically, it was distinguished from *P. epiphylla* by its larger gametophyte cells and higher average number of oil bodies. Additional diagnostic features include differences in the shapes of marginal thallus cells and epidermal capsule cells (Müller, 1954; Schuster, 1992). Comparative microstructural analyses have also revealed subtle differences in the distribution of papillose structures on the dorsal thallus surface and capsule surface cell patterns (Orzechowska et al., 2006).

The taxonomic status of *P. borealis* has long been debated because of its strong morphological similarity to *P. epiphylla*. Early cytological studies provided the first clues regarding its unique origin. Jachimsky (1935) discovered that

P. borealis possesses a unique chromosome set in which it was difficult to identify the homologous pairs typical of autopolyploids. Based on this observation, he hypothesized that *P. borealis* was an allopolyploid that originated from a hybridization event between *P. epiphylla* and another, then-unknown *Pellia* species. Although Newton (1986b, 1986a) later confirmed the chromosomal differences between *P. epiphylla* and *P. borealis*, she could not definitively resolve whether its origin was auto- or allopolyploid, only excluding *P. neesiana* as a potential parent.

A breakthrough in understanding the origin of *P. borealis* came with the discovery of cryptic species within *P. epiphylla*. Isozyme analyses demonstrated that all analyzed samples of *P. borealis* exhibited heterozygous phenotypes that combined enzymatic variants characteristic of the two cryptic species, *P. epiphylla* S and *P. epiphylla* N. This provided strong evidence that they were the parental species (Odrzykoski et al., 1996). Subsequent research using molecular techniques, including the analysis of *tRNA^{Gly}* (UAA) gene intron sequences, confirmed this hybridization-based origin. In *P. borealis*, one copy of the sequence matched that of *P. epiphylla* species S, while the other matched *P. epiphylla* species N, proving its allopolyploid nature.

Pellia borealis is found in Ireland, Great Britain, France, Belgium, Germany, Poland, northern Russia, Norway, Sweden, and Finland. Its habitat preferences closely resemble those of *P. epiphylla*, indicating a significant overlap between their ecological niches (Figure 4) (Schütz et al., 2016).

Pellia columbiana Krajina & Brayshaw

In the mid-20th century, Krajina and Brayshaw discovered a new liverwort species during field studies in British Columbia, which they named *Pellia columbiana*, and described it. This species is distinguished from other known *Pellia*

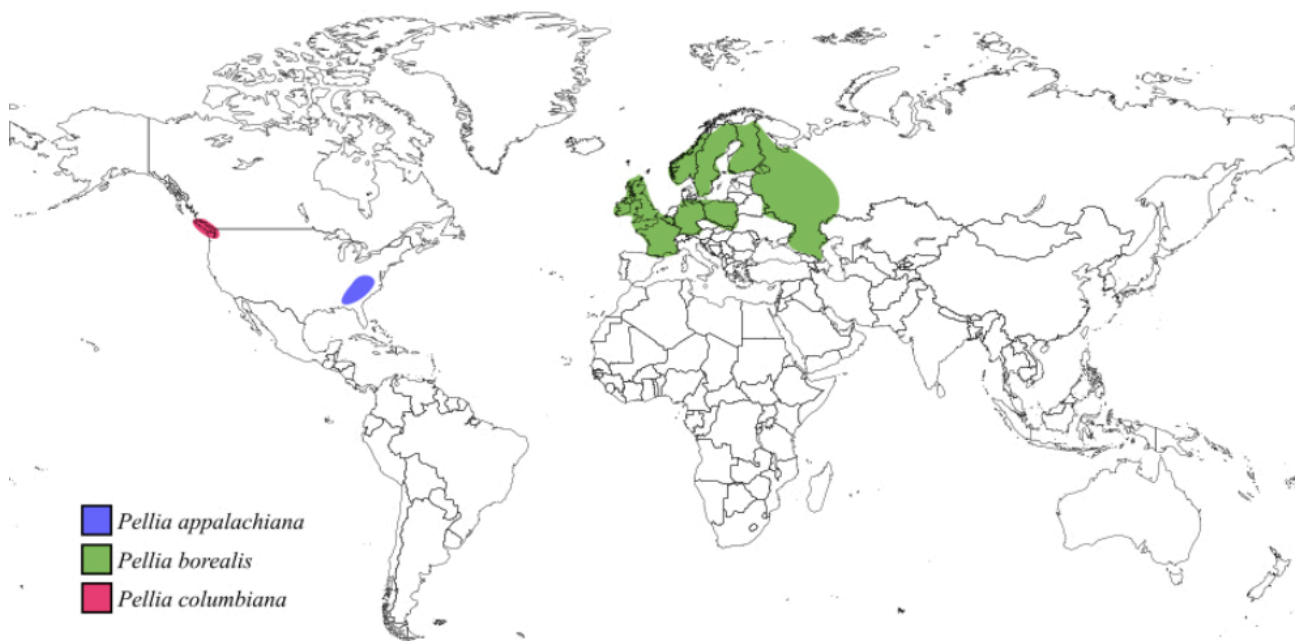


Figure 4 Distribution of *Pellia appalachiana*, *Pellia columbiana*, and *Pellia borealis* based on literature.

taxa by a unique combination of morphological features, including thalli lacking thickening bands, spores larger than those of other *Pellia* species, an exerted tubular calyptra, and unilayered inner walls of the sporangium with semiannular and annular thickenings. The taxonomic status of *Pellia columbiana* has been changing over the course of research on this species.

It was initially reclassified as a taxonomic synonym of *P. neesiana* by Prosauker (1963). Later, Schuster (1981) redefined it as a subspecies, *Pellia neesiana* subsp. *columbiana*; however, this name was not validly published because the basionym was not cited. However, following comprehensive molecular and morphological analyses by Schütz et al. (2016), recent taxonomic treatments have recognized *P. columbiana* as a distinct species based on its distinguishing characteristics (Stotler & Crandall-Stotler, 2017).

Pellia columbiana is distributed mainly along the Pacific Northwest coast of North America, with confirmed locations including Vancouver in British Columbia (Canada) and Grays Harbor in Washington (USA) (Figure 4). It typically grows in forested areas along ditches and streams and prefers moist environments at elevations of 40–100 m above sea level. This species occurs abundantly near water bodies with a neutral or slightly acidic pH but does not tolerate very low pH conditions (Schütz et al., 2016).

Pellia appalachiana R.M. Schust. ex L. Söderstr.,
A. Hagborg & von Konrat

Pellia appalachiana was first discovered in southern Appalachia (Schuster, 1991). It exhibits morphological characteristics that are intermediate between those of the two existing species, *Pellia neesiana* and *P. epiphylla*. Like *P. neesiana*, it is dioecious, but its perichaetium – a posterior

lamella that is absent anteriorly – resembles that of *P. epiphylla*. Schuster (1991) noted that *P. appalachiana* grew alongside both putative parent species, leading him to speculate that it might be a stabilized hybrid.

Reflecting this hypothesis, Schuster (1991, 1992) initially proposed the name *Pellia* X *appalachiana*. However, this designation was inconsistent with nomenclatural rules, leading to the later validation of the name *Pellia appalachiana* by Söderström et al. (2013). To date, there is no molecular evidence to support a hybrid origin for this species (Natcheva & Cronberg, 2004).

Pellia appalachiana is a thalloid liverwort that forms mats of overlapping thalli, sometimes arranged in rosettes. The thallus is up to 1 cm wide and several centimeters long, typically green, but capable of producing red pigments when exposed to direct sunlight. The midrib is not sharply demarcated and is “rather ill-defined” (Schuster, 1992). *Pellia appalachiana* is dioicous, with archegonia located on the dorsal surface of the thallus, protected by a posterior lobulate-incised involucre, antheridia sunken in dorsal pustules scattered or clustered along the thallus midline (Schuster, 1991).

Pellia appalachiana is an endemic species, with distribution limited to the Appalachian Mountains in the southeastern United States, including Alabama, Georgia, Kentucky, North Carolina, South Carolina, and Tennessee (Figure 4). It typically co-occurs with *P. neesiana* and *P. epiphylla*, suggesting that they share similar ecological preferences (Schütz et al., 2016).

2.2. Species of the genus *Apopellia*

Species currently classified within the genus *Apopellia* were initially categorized within the genus *Pellia*, which belongs

to the *Pelliaceae* family. This classification was primarily based on morphological characteristics, which were the fundamental criteria for distinguishing species in the 19th and early 20th centuries (Table 1). The subsequent elevation of *Apopellia* to genus rank was influenced by Schuster's research from 1981, which provided the foundation for molecular studies that demonstrated its phylogenetic distinctiveness (Schütz et al., 2016). These seemingly simple organisms represent interesting evolutionary challenges. *Pellia sensu lato* abounds in evolutionary paradoxes: many taxa in this group show structural simplicity (such as *Aneura pinguis*), which is often mistakenly interpreted as primitiveness, yet they have remarkably wide geographic distributions. Some species appear to have slowed down or ceased evolutionary changes, especially unisexual taxa that reproduce asexually (such as *Metzgeria leptoneura*); however, two factors complicate this matter. 1) Structural simplicity may limit the visible phenetic expression of genetic differences in the species. The genus *Pellia sensu lato* can be highlighted as a case in which genetic variation exists but is "masked" by structural uniformity, making species difficult to distinguish when they are sterile. 2) Dramatic climate change and continental fragmentation over the past 65 million years have caused population isolation. Consequently, this has led to the elimination of "old" taxa in most of their range and the fragmentation of once continuous populations into genetically isolated units. Geographical isolation could have led to increased genetic discontinuity and, consequently, the differentiation of new species (Schuster, 1981).

Data presented in a series of papers on the *Pellia endiviifolia* complex (Krzakowa, 1981; Mendelak, 1981; Newton, 1981; Schuster, 1981) indicate that these factors influenced

speciation within *Pellia sensu lato*; however, in this morphologically simple genus, genetic variation is "masked" by a high level of structural homogeneity (both within species and within the genus), combined with substantial phenetic plasticity. Based on these conclusions, Schuster indicated the distinctiveness of *Pellia endiviifolia* and *Pellia megaspora*, which provided the basis for the later differentiation of these species as a separate genus *Apopellia* – *Apopellia endiviifolia* and *Apopellia megaspora*. Currently, three species that were previously included in *Pellia* are classified under the genus *Apopellia*: *A. endiviifolia*, *A. megaspora*, and *A. alpicola*.

The aim of this chapter is to provide a synthetic overview of the current knowledge on species of the genus *Apopellia*, considering their diagnostic features, ecology, distribution, and systematic significance. Special attention is also given to issues related to taxonomic revisions.

Apopellia endiviifolia (Dicks.) Nebel & D. Quandt

Apopellia endiviifolia was first described by Dickson (1801) as *Jungermannia endiviifolia*. Over 180 years later, Grolle (1983) established the subgenus *Apopellia* to accommodate this species, emphasizing its unique morphological features that distinguish it from other taxa within *Pellia*. Subsequently, based on molecular and morphological evidence, *Apopellia* was elevated to the rank of genus, resulting in the current name *Apopellia endiviifolia* (Schütz et al., 2016).

This species has a green thallus up to 10 mm wide, which can develop red pigmentation when exposed to sunlight. A key diagnostic feature is the production of multiple apical proliferations (branches) in autumn, which can become

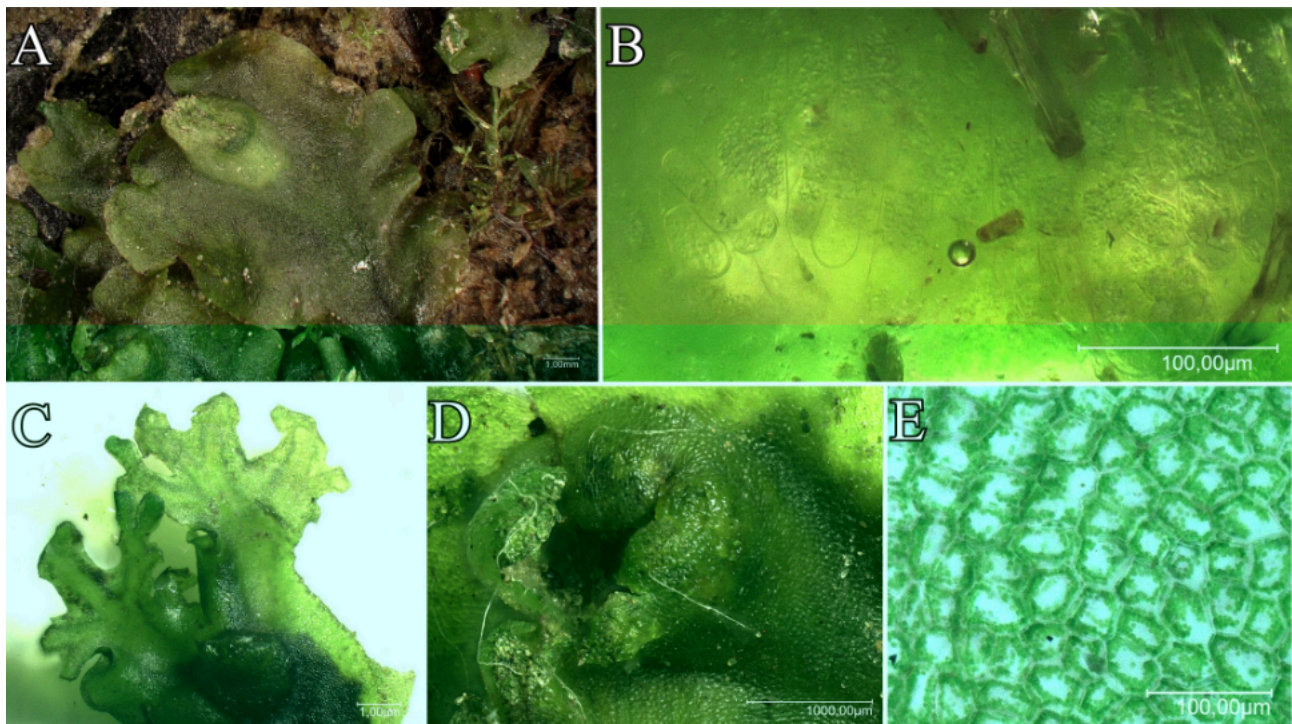


Figure 5 *Apopellia endiviifolia*: (A) General view of the thallus; (B) Slime hairs; (C) Apical proliferation; (D) Archegonium; (E) Thallus cells.

so prolific that they obscure the maternal thallus (Figure 5). As a dioicous species, its antheridia are surrounded by a vertical, toothed, tubular involucre (Atherton et al., 2010).

Apopellia endiviifolia is widely distributed in the temperate regions of the Northern Hemisphere (Figure 6). Its range extends from the lowlands of southern and western Europe to high mountains (2500 m in the Alps, 2300 m in the Caucasus) (Konstantinova et al., 2023). It is also found in the Mediterranean Basin, North Africa, southern Siberia, Central Asia, China, Korea, Japan, the Himalayas, and Oman, reaching as far north as 66°15' N in Scandinavia (Konstantinova et al., 2023).

Ecologically, *A. endiviifolia* prefers nutrient-rich, calcareous, and clayey substrate. It frequently colonizes disturbed and anthropogenically altered environments, such as concrete canal banks, masonry around springs and damp path edges. It is also common in natural habitats, including stream banks, springs, and marshes, and can be found in forested areas along ditches and gravel roads (Schuster, 1981; Schütz et al., 2016).

Apopellia megaspora (R.M. Schust.) Nebel & D. Quandt

The description of *Apopellia megaspora* emerged from Schuster's (1981) extensive research on the *Pellia endiviifolia* complex. Initial studies of populations from Europe, North America, and Japan suggested that American and European populations represent distinct evolutionary lineages.

This hypothesis was inspired by Schuster's long-term field observations, spanning over 15 years, of a liverwort population along the Green River in Massachusetts, USA. He noted that these eastern North American plants consistently lacked the autumnal proliferations and red pigmentation

characteristic of typical *Apopellia endiviifolia*. These observations prompted a collaborative, integrative study that led to the description of *Pellia megaspora* as a new species, supported by morphological, cytogenetic, and isoenzymatic evidence (Krzakowa, 1981; Mendelak, 1981; Newton, 1981; Schuster, 1981). *A. megaspora* is characterized by several key features: exceptionally large spores, lack of cell wall pigmentation, dioicous (unisexual) reproductive system, and a strongly incised and lacinate-dentate perichaetium mouth (Schuster, 1981).

Cytogenetic analyses provided further evidence of its distinctiveness. Although *A. megaspora* and *A. endiviifolia* share the same haploid chromosome number ($n = 9$), they differ significantly in karyotype symmetry and the presence of a sexually dimorphic ninth chromosome (m-chromosome) in *A. endiviifolia* (Newton, 1981). These findings were corroborated by isozyme analyses, which demonstrated clear biochemical differentiation (Krzakowa, 1981), and by detailed chromosome measurements that quantified the differences between the karyotypes of the two species (Mendelak, 1981).

Apopellia alpicola (R.M. Schust. ex L. Söderstr., A. Hagborg & von Konrat) Nebel & D. Quandt

Schuster (1992) first documented populations in the Canadian Cordillera at elevations of 2,134–2,896 m that exhibited morphological divergence from typical *Apopellia endiviifolia*. These high-elevation plants are characterized by their inability to form autumnal apical innovations, the presence of lower, frontally compressed juvenile gynoecea (similar to *Pellia neesiana*), and their exclusive occurrence in acidic regions. This montane taxon was initially described as *Pellia endiviifolia* subsp. *alpicola* Schust., a name that was later validated by Söderström et al. (2013). However,

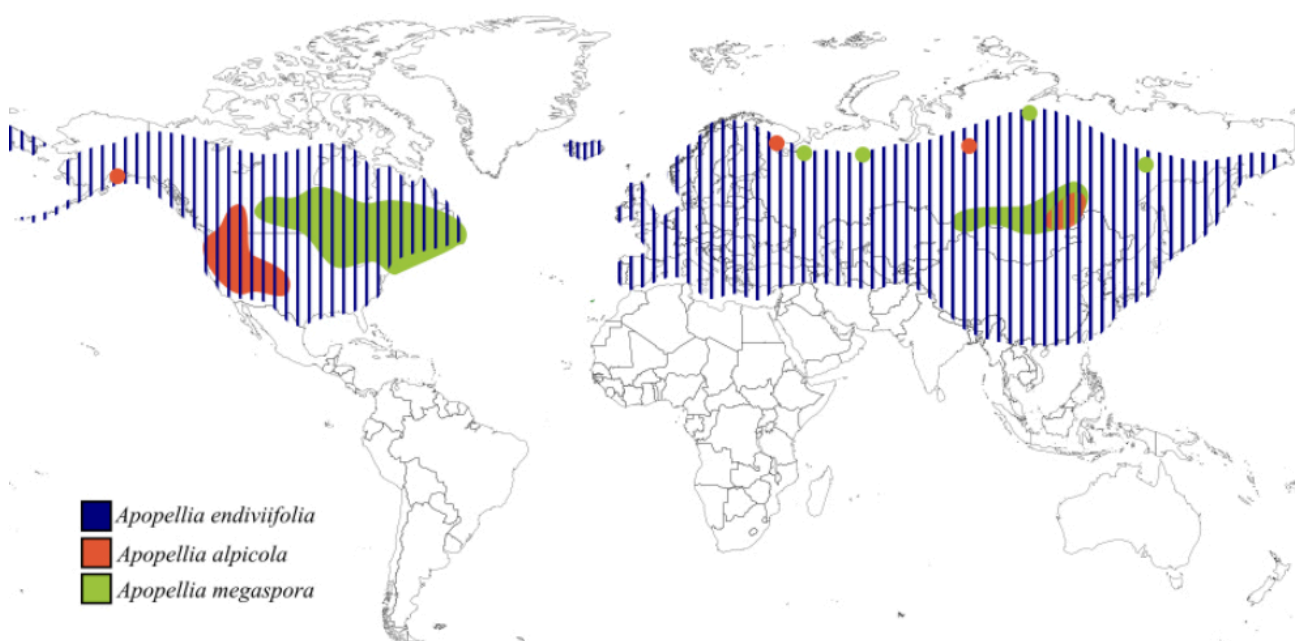


Figure 6 Distribution of *Apopellia endiviifolia*, *Apopellia alpicola*, and *Apopellia megaspora* based on literature.

because a type specimen deposit was not designated, the name was not considered to be validly published (Stotler & Crandall-Stotler, 2017). The taxon was finally accepted at the species rank, as *Apopellia alpicola*, based on a comprehensive molecular phylogenetic study (Schütz et al., 2016), and its distinctiveness has been confirmed by subsequent phylogenetic studies.

The principal diagnostic features of *Apopellia alpicola* include unisexual thalli with short, angled to erect, lacinate involucre, which separate this species from other species of the genus; lack of thickening of cell walls in the mid thallus; and three or more cells long slime hairs (Schütz et al., 2016).

Apopellia alpicola shows subarctic-montane and nearly circumpolar distribution (Figure 6). Initially recorded in western Canada, it has been confirmed at high elevations in California (~2682 m), New Mexico (>3048 m), and Alaska (600–900 m) (Konstantinova et al., 2023; Schuster, 1992). It is also found in southern Siberia and the Murmansk region, extending beyond the Arctic Circle in the north. *A. alpicola* is scattered throughout Eurasia and appears widespread in western North America but hasn't been found in eastern North America. (Konstantinova et al., 2023).

Apopellia alpicola develops in late snow areas, alpine meadows, and tundra vegetation in the far north. This species mainly occupies acidic environments and occurs primarily at elevations of 1800–2600 m above sea level in the alpine and subalpine regions. In the high Arctic latitudes, it occurs at lower elevations.

Apopellia alpicola has a subarctic montane and nearly circumpolar distribution. Initially recorded in western Canada, its presence has since been confirmed at high elevations in California (~2682 m), New Mexico (>3048 m), and Alaska (600–900 m) in North America (Schuster, 1992; Konstantinova et al., 2023). It is also found in southern Siberia and the Murmansk region, with its range extending beyond the Arctic Circle in the north. Although it is scattered throughout Eurasia and widespread in western North America, it has not been found in eastern North America (Konstantinova et al., 2023).

3. Taxonomic challenges and revisions in the shade of cryptic speciation

The identification of *Pellia sensu lato* species poses a major challenge, as distinguishing them based on their morphological features presents many problems. The great similarity of thalli, caused by their morphological simplicity, makes species identification based on macroscopic features impossible when specimens are sterile. Identification issues stem from the high phenotypic plasticity of *Pellia sensu lato*, combined with the rarity of sporophyte production in the wild. This leads to difficulties in distinguishing *Pellia* from other morphologically similar genera such as *Aneura*. Identifying sterile thalli of *Aneura* and *Pellia* species can be challenging and often leads to misidentifications, particularly because their aquatic forms inhabit similar environments as each other. While microscopic observation of slime hairs on the ventral side of the apical thallus is

considered the best method for distinguishing *Pellia sensu lato* species (Frahm, 2013), high phenotypic plasticity across wide geographical ranges and environmental variability introduce uncertainty. The significant number of misidentifications in herbarium specimens further highlights the difficulties in identifying *Pellia sensu lato* species (Konstantinova et al., 2023). Therefore, researchers have begun searching for alternative methods to enable the reliable identification of individual *Pellia sensu lato* species. The application of isoenzymatic markers is a response to this emerging need. Research on genetic variability has enabled the discovery and description of cryptic species of *P. epiphylla* and *A. endiviifolia*.

The first documented case of cryptic speciation within *Pellia* concerned *Pellia epiphylla*. Population studies conducted in Poland revealed the presence of two peroxidase phenotypes, which were initially considered to represent two distinct, previously unrecognized sibling species (Zieliński, 1984, 1987). These forms were characterized by allopatric distribution: the *P. epiphylla* N form dominated in the north of the country, whereas the *P. epiphylla* S form dominated in the south. The enzyme systems examined included peroxidases (PX), esterases (EST), aspartate aminotransferase (GOT), glutamate dehydrogenase (GDH), NADP-dependent malic dehydrogenase (ME), and acid phosphatases (AP). Subsequent genetic analyses showed significant differences between these two groups, with a genetic distance ($D_n = 0.552$) comparable to that typically found between species rather than populations of vascular plants (Zieliński, 1987). Zieliński (1987) proposed that these lines might represent separate biological species. This hypothesis laid the foundation for future genetic studies on cryptic speciation within *only the genus name (Pellia) should be in italics*. The presence of cryptic species in the *Pellia epiphylla* complex was confirmed through biochemical studies, which validated significant biochemical differences between these sister taxa. This strengthens the idea that geographical isolation and ecological differentiation are key factors driving cryptic speciation in *Pellia* (Prus-Głowacki et al., 1998).

With the increasing availability of molecular research methods, subsequent studies have consistently confirmed the existence of cryptic species within *P. epiphylla*. Molecular analyses of *Pellia* have utilized various genetic markers, enabling the differentiation of closely related cryptic species. Fiedorow and Szwejkowska-Kulińska (1998) were the first to employ intergenic tRNA sequences as phylogenetic markers for *Pellia* species, using PCR to amplify the nuclear tRNA^{Leu} sequences. Simultaneously, the presence of distinct cryptic species was confirmed by studies using the Random Amplified Polymorphic DNA (RAPD) technique, which demonstrated significant genetic divergence within the *P. epiphylla* complex (Pacak et al., 1998). Phylogenetic studies of the Polish *Pellia* population using tRNA^{Leu}_{CAA} intergenic sequences as molecular markers facilitated a more precise phylogenetic analysis of European *Pellia* species, including those from the *P. epiphylla* complex, thereby confirming the cryptic nature of *P. epiphylla* S and *P. epiphylla* N (Fiedorow et al., 2001). Szwejkowska-Kulińska et al. (2002)

introduced new chloroplast (cp) and mitochondrial (mt) tRNA molecular markers in their research: cp tRNA^{Leu}_{UAA}, cp tRNA^{Gly}_{UCC}, cp tRNA^{Lys}_{UUU}, mt tRNA^{Ser}_{GCU} and mt coxIII. Their results indicated that tRNA^{Leu} intron sequences are identical in the closely related species *P. borealis*, *P. epiphylla* S, and *P. epiphylla* N and therefore cannot serve as markers for their differentiation. However, the remaining sequences studied successfully allowed for the distinction of closely related cryptic species, thus serving as highly effective marker sequences for differentiating cryptic species of *P. epiphylla* (Szweykowska-Kulińska et al., 2002).

Similarly, cryptic species have also been found for *Apopellia endiviifolia*. Studies on the Polish population of *Pellia endiviifolia* (now classified as *Apopellia endiviifolia*) distinguished two groups based on peroxidase variation analysis, which were described as “sister species” (Zieliński, 1987). They were named *Pellia endiviifolia* species A (water form) and *Pellia endiviifolia* species B (terrestrial form). These species exhibit differences in their range of occurrence and environmental preferences (Szweykowski et al., 1995; Zieliński, 1987). *P. endiviifolia* sp. A occurred at sites near water courses, shallow water bodies, and mountain springs, growing around the water surface line and in periodically flooded areas. In contrast, *P. endiviifolia* sp. B occurred on moderately moist substrates (Szweykowski et al., 1995). Phylogenetic studies of the Polish *Pellia* population using tRNA^{Leu}_{CAA} molecular markers confirmed the occurrence of the cryptic species *P. endiviifolia* sp. A and sp. B, while also indicating the paraphyletic nature of these taxa (Fiedorow et al., 2001). Immunological analyses and studies of nuclear and chloroplast DNA have further confirmed their genetic distinctness, verifying the ecological and genetic separation of these forms (Polok et al., 2005; Sawicki et al., 2021).

Research on cryptic species within *Pellia sensu lato* has been conducted exclusively in Poland, which represents only a small fragment of the total distribution range (as discussed in the Taxonomic History and Classification section), which encompasses almost the entire Northern Hemisphere. Consequently, the distribution of cryptic species throughout their entire range remains unknown. A compelling parallel can be drawn from *Aneura*. In the case of *Aneura pinguis*, studies using DNA barcoding revealed that the nominally cosmopolitan *A. pinguis* was composed of 10 cryptic species, of which only five had been previously described (Bączkiewicz et al., 2017). A probable scenario is that *Pellia sensu lato* also conceals more cryptic species; however, it is essential to conduct analyses using material from a broader geographical range than has been previously studied. Such an analysis could reveal patterns indicating the habitat preferences of individual cryptic species. Habitat adaptation may be the main driving force of cryptic speciation within the taxon, as indicated by the aforementioned studies on *Aneura pinguis* (Bączkiewicz et al., 2017).

The description of cryptic species enabled the identification of the origin of the only described polyploid species in the genus *Pellia*: *Pellia borealis*. This species was identified as diploid ($n = 18$), with twice the number of chromosomes

as *P. epiphylla* ($n = 9$) (Newton, 1986b, 1977). Since its initial description, its distinction as a separate species has been debated. The taxonomic distinctness of *P. borealis* has been revealed in genetic studies, which have shown differences in peroxidase genotypes and phenolic compounds, suggesting that *P. borealis* is not merely a polyploid variant but a distinct taxon (Krzakowa & Szweykowski, 1977; Szweykowski & Krzakowa, 1977). Two main hypotheses have emerged regarding its polyploidy: autopolyploidy (chromosome duplication within one species) and allopolyploidy (hybridization between two species followed by polyploidization). Early skepticism toward the allopolyploidy hypothesis stemmed from the lack of evidence for a second parent species and the absence of known hybridization mechanisms in liverworts (Mendelak, 1972).

Chromosome staining techniques have demonstrated chromosomal differences between *Pellia epiphylla* and *Pellia borealis* (Newton, 1986b). *Pellia borealis* chromosomes exhibit unique structural arrangements and heterochromatin distribution. Cytogenetic studies subsequently excluded *P. neesiana* as a potential parent species in the hypothesized allopolyploidy scenario. The final verification of the allopolyploidy hypothesis was made possible by the discovery of cryptic species within the *Pellia epiphylla* complex. The cryptic species *P. epiphylla* S and *P. epiphylla* N were identified as the parental species of *P. borealis* (Odrzykoski et al., 1996). Isoenzymatic analyses have demonstrated that all polyploid samples of *P. borealis* from various geographic populations exhibit heterozygous phenotypes, combining enzymatic variants characteristic of the parental cryptic species (Odrzykoski et al., 1996). Furthermore, molecular analyses of tRNA intron sequences provided evidence that *P. epiphylla* N is the maternal organism from which *P. borealis* inherited exclusively the chloroplast and mitochondrial genomes through uniparental organelle inheritance (Pacak & Szweykowska-Kulińska, 2000).

To further investigate the polyploid genome of *Pellia borealis*, newer cytological and molecular techniques have been employed, such as the Random Amplified Polymorphic DNA (RAPD) technique (Pacak et al., 1998). Recent phylogenetic studies have supported the hypothesis of an allopolyploid origin, confirming its status as a distinct species. Analysis of tRNA^{Gly}_{UAA} gene intron sequences (Fiedorow et al., 2001) allowed for the differentiation of cryptic species through the insertion/deletion of three nucleotides (GCT). In *P. borealis*, one copy of the sequence matched *P. epiphylla* S, and the other copy matched *P. epiphylla* N. This confirmed that *P. borealis* originated through hybridization between these two cryptic species (Fiedorow et al., 2001). Subsequent studies using the RAPD-tRNA marker further confirmed that *Pellia borealis* is a hybrid between the two cryptic species, *P. epiphylla* S and *P. epiphylla* N (Pacak et al., 2002). Comparative karyotype analysis of *P. endiviifolia* and *P. borealis* using fluorescent differential staining, C-banding, silver staining, and FISH provided cytological confirmation of the allopolyploidy of *P. borealis* (Orzechowska et al., 2010).

Modern molecular methods have enabled the taxonomic validation of *Pellia* species using many complementary

approaches. The integration of isoenzymatic methods, cytogenetics, and classical botanical methods is crucial for the unambiguous acceptance of newly discovered species. This multifaceted approach is essential, as demonstrated by the case of *Apopellia megaspora* (formerly *Pellia megaspora*), which required years of observation to draw conclusions about its distinctiveness (Schuster, 1981). Cytogenetic analyses have provided detailed evidence for the distinctiveness of *A. megaspora* as a species. Although *P. megaspora* and *P. endiviifolia* have the same number of haploid chromosomes ($n = 9$), the ninth chromosome of *P. megaspora* does not show sexual dimorphism. Additionally, *P. megaspora* exhibits a more symmetrical karyotype with a predominance of acrocentric chromosomes, whereas *P. endiviifolia* shows greater chromosome asymmetry (Newton, 1981). These cytogenetic data were also confirmed through several methods: isoenzyme analysis (Krzakowa, 1981), which demonstrated biochemical differentiation; morphological analysis (Schuster, 1981), which documented structural differences; and detailed chromosome measurements (Mendelak, 1981), which quantified karyotypic differences. The distinction of *A. megaspora* as a separate species provided the basis for further research confirming the distinctiveness of *Apopellia* as a taxon.

4. Phylogenetic relationship

One of the first comprehensive phylogenetic studies of the genus *Pellia* was initiated by Schütz et al. (2016), who in her paper conducted a detailed phylogenetic analysis based on morphological and molecular markers. The foundation for this research stemmed from earlier analyses, including those by Schuster (1981), who repeatedly pointed out the difficulties associated with correctly identifying species, especially when they are sterile. In their study, the authors posed several key questions: What are the phylogenetic relationships within the genus *Pellia*? Are the species within this genus monophyletic? Is there any evidence of infraspecific and geographical differentiation? In their study, the authors used 61 samples from the Pelliaceae family, collected from across each area of occurrence (Schütz et al., 2016).

The Pelliaceae split into two maximally supported lineages: *Pellia* and *Apopellia* (new rank proposed in that study) (Schütz et al., 2016). Analysis of three plastid loci (*rpl16*, *rps4*, and *trnL-trnF*) showed maximum support for previous reports regarding the distinctiveness of these two genera. The level of genetic separation between these two groups was greater than that observed within either clade. The results of the genetic analysis were strongly supported by morphological data, which indicated that each clade was distinguished by distinct, unique morphological characters. Furthermore, the division into two clear evolutionary lineages reflects the ecological patterns of each group, as *Apopellia* prefers to grow on calcareous substrates, whereas *Pellia* is associated with more acidic environments (Schütz et al., 2016).

Within the genus *Pellia*, the first clade includes *P. epiphylla* and *P. borealis*, whereas the second is composed of *P. neesiana* and *P. appalachiana* (Schütz et al., 2016). These species exhibit close phylogenetic relationships within their clades.

Pellia borealis was classified as a variety of *P. epiphylla* because of the lack of sufficient genetic and morphological support for their distinctness. The results of this study indicate that *Pellia epiphylla* is a monophyletic taxon. The analysis showed that *Pellia neesiana* is a well-supported species and is sister to *Pellia appalachiana* (Schütz et al., 2016). The previous hypothesis regarding the origin of *P. appalachiana* as a hybrid between *P. epiphylla* and *P. neesiana* (Schuster, 1991) was not confirmed in this study. Hybridization between closely related species is possible; however, there is a high probability of overlooking this phenomenon because of the absence of diagnostic morphological characters (Schuster, 1992).

Within the genus *Apopellia*, two main clades were identified, corresponding to the species *A. endiviifolia* and *A. megaspora* (Schütz et al., 2016). The monophyletic *A. endiviifolia* was further differentiated into three subclades based on geographical origin: European (EU) and two Asian (A1 and A2). *A. megaspora* was divided into two subclades – eastern (E) and western (W) – a division which called its monophyly into question. Despite suggestions of paraphyly, the authors decided to maintain *A. megaspora* as a single species, arguing that a greater amount of genetic and morphological data were necessary to clearly differentiate these two clades (Schütz et al., 2016).

An integrative taxonomic study by Konstantinova et al. (2023) significantly supplemented and expanded our knowledge regarding the phylogenetic relationships of *Pellia sensu lato* and robustly supported the divergence of *Apopellia* as a distinct genus. Analyses were conducted based on two molecular markers: ITS1-2 nrDNA and *trnL-F* cpDNA. Sequencing of 36 new specimens from Alaska and Siberia, along with morphological analysis, provided new phylogenetic data and clarified the geographical distribution of the studied species. This work led to the description of the *Apopellia megaspora* W subclade as *Apopellia alpicola* (Konstantinova et al., 2023). According to this study, *Pellia neesiana* was divided into three subclades, which reflected the geographical distribution of the examined specimens (Konstantinova et al., 2023). This study also confirmed the occurrence of three subclades of *A. endiviifolia*.

The division into *Pellia* and *Apopellia*, initially revealed based on the analysis of nuclear and chloroplast genome regions (Konstantinova et al., 2023; Schütz et al., 2016), was subsequently confirmed by analyses of complete plastid and mitochondrial genomes (Paukszto et al., 2023). In these studies, 22 *Pellia* and *Apopellia* samples were subjected to barcoding analyses. Based on this work, the three subclades of *A. endiviifolia* – described as typical, aquatic, and spring forms – were recognized as monophyletic (Paukszto et al., 2023). Furthermore, the cryptic species *P. epiphylla* N (northern line) and *P. epiphylla* S (southern line) formed monophyletic, maximally supported groups that shared a common clade.

5. Reproductive biology and sex determination

In an evolutionary context, sex determination in the genus *Pellia sensu lato* is particularly interesting because it

encompasses both monoicous and dioicous species. In contrast to seed plants, dioicy is significantly more common in liverworts, with approximately two-thirds of all liverwort species being dioicous (Bell & Hemsley, 2000). A similar pattern is observed in the genus *Pellia sensu lato*, where the only monoicous species are *P. epiphylla* and *P. borealis*. The dioicous *Pellia* sl. species include: *A. endiviifolia*, *A. megaspora*, *A. alpicola*, *P. neesiana*, *P. appalachiana* and *P. columbiana*.

In bryophytes, dioicy is considered the primitive state, whereas monoicy is a secondary trait (Laenen et al., 2016; Longton & Schuster, 1983; Singh et al., 2023). For this reason, dioicous species can be viewed as evolutionarily older than forms that produce reproductive organs of both sexes on one individual. Within the genus *Pellia sensu lato*, heterogeneity in reproductive systems has been observed. In dioicous species, male and female thalli are phenotypically identical until the differentiation of sexual organs – antheridia and archegonia. The sexual organs of *Pellia sensu lato* are located on the dorsal surface of the costa of the main axis. Antheridia are colorless, immersed, and scattered in one to three irregular rows. The archegonia are solitary in cavities covered by a hemispherical tubercle with a small apical aperture (Paton, 1999).

Dioicous liverworts of the genus *Pellia sensu lato* encounter several obstacles that limit their reproductive success (Laenen et al., 2016). As in other bryophytes, sexual reproduction requires water, which means that increasing the distance between individuals of different sexes decreases the probability of fertilization. The distance of effective sexual reproduction in *Pellia sensu lato* is not well known, but it may not exceed 20 cm (Anand et al., 2013). The reproductive success of dioicous species may be further limited by two additional factors (Laenen et al., 2016): uneven sex distribution in populations, where one sex significantly predominates numerically (Bisang et al., 2014), and the tendency of males and females to colonize different habitats according to their distinct ecological requirements (sexually dimorphic niche partitioning) (Brzyski et al., 2014; Stark & McLetchie, 2006).

These obstacles result in rare sexual reproduction in dioicous bryophytes (Vanderpoorten & Goffinet, 2009). The sexual system has a decisive impact on reproductive success, with monoicous species characterized by a significantly higher frequency of successful fertilization and sporophyte formation than dioicous species (Longton, 1997). This difference is so pronounced that a significant proportion of dioicous moss and liverwort species have never been observed in the reproductive stage (Laenen et al., 2016). Among the liverworts of the genus *Pellia sensu lato*, vegetative reproduction clearly dominates, and the production of sexual organs occurs sporadically. For example, in *P. neesiana*, colonies consisting exclusively of thalli of one sex are frequently observed (Atherton et al., 2010). This pattern is consistent with the general tendency observed in dioicous species, where asexual reproduction constitutes an effective strategy for rapid population spread, whereas sporophyte production remains rare (Mishler & Newton, 1988).

The predominance of vegetative reproduction in *Pellia sensu lato* has been confirmed by observations of herbarium materials, where most specimens remain sterile, show only male characteristics, or contain unfertilized female structures (Schuster, 1981). A revision of *Apopellia endiviifolia* specimens by Konstantinova et al. (2023) demonstrated the scale of this phenomenon: among 100 examined specimens, only five had sporophytes, approximately 20 had pseudoperianths, and approximately 30 showed autumn proliferations. Furthermore, intensive vegetative reproduction was documented in populations of *Pellia borealis* (n = 18). Thalli growing in close proximity showed only one of two mutually exclusive peroxidase alleles, which is indicative of a clonal structure often extending over the entire colonies of *P. borealis* (Zieliński, 1986).

Although earlier studies indicated a strong correlation between dioicy and the production of specialized asexual reproductive structures (During, 2007; Hedderson & Longton, 1995; Longton & Schuster, 1983), contemporary phylogenetic analyses of mosses question this relationship (Crawford et al., 2009), pointing instead to other compensatory strategies. Dioicous species often achieve larger sizes than bisexual species and invest in intensive vegetative growth. This pattern, also known in angiosperms (De Jong, 2000), suggests that limitations in sexual reproduction are balanced by increased organism size and extended lifespan (Devos et al., 2011).

The occurrence of both monoicous and dioicous species within the genus *Pellia* creates broad possibilities for research on the mechanisms of sex determination, which has become the basis for intensive research on sex determination mechanisms in liverworts. The striking morphological similarity of these species, in contrast to their reproductive diversity, makes *Pellia* an exceptionally valuable model for understanding the evolution of sexuality in these plants.

Because liverworts are considered one of the oldest evolutionary lineages of currently living land plants, they can serve as a valuable model for studying the molecular foundations of mechanisms associated with sexual reproduction. However, knowledge of the molecular mechanisms of sex determination in liverworts remains modest. Currently, information on sex determination is available only for *A. endiviifolia* (Sierocka et al., 2011, 2020). Ultimately, these findings emphasize the need for further research to fully understand the complexity of the sex determination process and the development of monoecism in liverworts, using *Pellia sensu lato* as an example.

In the male gametophytes of *Apopellia endiviifolia*, Sierocka et al. (2011) identified four genes that were exclusively expressed: PenB_TUA1, PenB_Rab1/11, PenB_HMG-box, and PenB_MT. The expression of PenB_TUA1 and PenB_Rab1/11 was observed in male thalli, irrespective of whether antheridia were present, suggesting a role in the growth and development of general male gametophytes. In contrast, PenB_HMG-box and PenB_MT were expressed exclusively in male thalli producing antheridia and only under natural conditions. This indicates their involvement in reproductive organ development, which is influenced by environmental factors (Sierocka et al., 2011).

Using Representational Difference Analysis of cDNA (RDA-cDNA), Sierocka et al. (2014) identified three genes exclusively expressed in the female gametophytes of *A. endiviifolia*: PenB_CYSP, PenB_MT2, and PenB_MT3. These genes play roles in female gametophyte growth and differentiation, particularly in the formation of archegonia, the female reproductive organs. All three genes exhibited a tenfold higher expression in archegonial tissues than in vegetative tissues within female gametophytes grown in natural habitats (Sierocka et al., 2014).

A subsequent study by Sierocka et al. (2020) identified 87 genes with similar expression patterns during the reproductive phase of growth in *Apopellia endiviifolia* and *Marchantia polymorpha*. This suggests a common mechanism governing sex organ development in both species. Specifically, five female-specific and two male-specific Differentially Expressed Genes (DEGs) showed enriched expression in archegonia and antheridia, respectively. These DEGs included genes involved in various functions, such as ferric chelate reductase, potassium ion transporter, and chitinase class I (Sierocka et al., 2020).

6. Transcriptomic analyses

Liverworts, among other bryophytes, represent one of the most ancient lineages of land plants. This makes them a valuable group for comparative genomic studies, allowing for a better understanding of plant evolution. Microtranscriptome studies provide crucial information on evolutionary connections among bryophytes and between bryophytes and their algal ancestors. Analysis of the bryophyte microtranscriptome suggests that the basic molecular frameworks for microRNA (miRNA) biogenesis, degradation, and function were established in the common ancestor of all land plants (Pietrykowska et al., 2022). Understanding these mechanisms is crucial for comprehending how plants adapt to life on land and how they develop complex regulatory networks based on miRNAs, which currently control their development and response to environmental stresses. Research on conserved miRNAs within bryophytes has indicated that six miRNA families conserved in all bryophytes likely appeared before the common ancestor of all land plants (Pietrykowska et al., 2022). These conserved miRNAs generally target conserved mRNAs, suggesting that they play important roles in regulatory networks throughout plant evolution.

Some miRNAs were shared between the liverwort species *Marchantia polymorpha* and *Pellia endiviifolia*. Analysis of microtranscriptomic data from these two species showed that miRNA Pen-miR8163 (*P. endiviifolia*) shares a similar sequence with miRNA Mpo-miR11737a (*M. polymorpha*). Furthermore, *M. polymorpha* contains a short RNA sequence similar to Pen-miR8170 miRNA from *P. endiviifolia*, and Mpo-miR11889 (*M. polymorpha*) is homologous to Pen-miR8185 (*P. epiphylla*) (Pietrykowska et al., 2022). The presence of these three miRNAs, shared by *M. polymorpha* and *P. epiphylla*, is highly significant, as it may indicate their important role in liverwort physiology. Conversely, the limited number of shared species-specific

miRNAs supports the model of the frequent gain and loss of microRNA genes during evolution (Pietrykowska et al., 2022).

Research on *Apopellia endiviifolia* has shed new light on the evolutionary and functional aspects of miRNAs, challenging previous assumptions regarding their distribution and conservation in green algae and land plants. Earlier studies have suggested a lack of shared miRNA genes between these groups, as no evidence of overlap has been observed (Cuperus et al., 2011; Nozawa et al., 2012). Using Illumina-based next-generation sequencing, the identification and analysis of miRNAs in *Apopellia endiviifolia* enabled the discovery of hundreds of conserved plant miRNA families and the identification of new liverwort-specific miRNAs (Alaba et al., 2015). Analysis of the RNA degradome, which represents a collection of mRNA fragments cleaved by miRNAs, revealed that the target mRNA of only three miRNAs (miR160, miR166, and miR408) was conserved between liverworts and other land plants. This suggests that miRNA-mediated gene regulation has undergone significant diversification during the evolution of land plants.

Crucially, three miRNAs were identified in *Apopellia endiviifolia* with significant sequence similarity to miRNAs and small RNAs (sRNAs) found in the green alga *Chlamydomonas reinhardtii*. This discovery provides the first evidence for the existence of conservative miRNA species in different plant lineages and suggests a common evolutionary origin. The miRNAs in *Pellia* and *C. reinhardtii* were identified with a high degree of confidence, allowing for up to two mismatches in their sequences. One of these miRNAs (pen-miR1444) was previously described in algae, whereas the other two (pen-miR8183.2 and pen-miR8185) are novel miRNAs in *Pellia* that share homology with algal sRNAs. Experimental validation using northern hybridization confirmed their presence in *Pellia*, even when it was grown in controlled, contamination-free environments. These data strongly suggest that these miRNAs belong to a category common to liverworts and green algae, providing insight into the evolutionary relationship between land plants and their algal ancestors (Alaba et al., 2015). Broader studies of microtranscriptomes in other liverwort species are needed to enable a fuller understanding of the molecular mechanisms that facilitated the transition from aquatic to terrestrial ecosystems.

7. Organellar genomes, RNA editing and nuclear genome structure

Technological development and increased access to molecular biology methods have enabled the expansion of research on *Pellia sensu lato* to include the study of organellar genomes, plastid and mitochondrial. Thanks to these advances, it has become possible to sequence the complete organellar genomes of *Pellia sensu lato* representatives. The first complete plastid genome to be described was that of *Apopellia endiviifolia*, with a size of 120,546 bp (Grosche, 2012). The *A. endiviifolia* chloroplast genome is the largest known chloroplast genome of *Pellia sensu lato* (Table 2).

Table 2 Size of chloroplast and mitochondrial genomes (based on Paukszto et al., 2023).

Species	Chloroplast genome length (bp)	Mitochondrial genome length (bp)
<i>Apopellia endiviifolia</i> species A – water form	120,940–120,947	109,447–109,660
<i>Apopellia endiviifolia</i> species B – typical form	120,537–120,543	109,815–109,929
<i>Apopellia endiviifolia</i> species C – spring form	120,564–120,566	109,389–109,400
<i>Pellia borealis</i>	116,432–116,436	161,784–161,817
<i>Pellia epiphylla</i> species S	116,297–116,422	163,160–163,996
<i>Pellia epiphylla</i> species N	116,168–116,459	161,807–161,999
<i>Pellia neesiana</i>	115,878–115,879	158,690–158,710

In subsequent years, the chloroplast and mitochondrial genomes of *Pellia neesiana*, *Pellia borealis*, and cryptic species belonging to the *Apopellia endiviifolia* and *Pellia epiphylla* complexes were also described (Paukszto et al., 2023).

Among *Pellia sensu lato* species, distinct differences exist in mitochondrial genome sizes, ranging from the smallest mitogenome in *Apopellia endiviifolia* with a size of 109,389 bp to 163,996 bp in *Pellia epiphylla* (Table 2). Despite its relatively small size, the *A. endiviifolia* mitochondrial genome contains all protein-coding genes and the full intron set known from other Jungermanniopsida, including 41 protein-coding genes, three rRNAs, and 26 tRNAs (Sawicki, 2023). Flow cytometry analyses of genome sizes in species of the genus *Pellia sensu lato* have also revealed differences in their dimensions (Temsch et al., 2010), which may reflect differences in the amount of repetitive DNA and the accumulation of non-coding sequences.

Comparative organellar genomics can serve as a valuable tool for delimiting *Pellia* and *Apopellia* species, including cryptic species, using organellar genomes as ‘superbarcodes’. The identification of Molecular Diagnostic Characters (MDCs) in the mitogenome and plastome allowed for the successful identification of most evolutionary lineages of Pellidae, with the exception of the allopolyploid *P. borealis*. The mitogenome was the most effective for distinguishing species within the *P. epiphylla* complex, whereas the plastome was more effective for the *A. endiviifolia* complex (Paukszto et al., 2023).

The availability of complete organellar genome sequences enables their characterization and the study of post-transcriptional processes, particularly RNA editing. RNA editing is a post-transcriptional process that involves changes in single nucleotides in RNA sequences. This process is observable in the chloroplast genome, where it affects protein expression and the stabilization of secondary RNA structures (Huang et al., 2022). Among *Pellia sensu lato* species, RNA editing sites have been localized in the chloroplast genome of *Apopellia endiviifolia* and the mitochondrial genome of *Pellia epiphylla* (Grosche et al., 2012; Myszczyński et al., 2019).

In the chloroplast genome of *Pellia endiviifolia*, 54 RNA editing sites have been identified, all of which exclusively

involve C-to-U transitions (Grosche et al., 2012). These modifications lead to nonsynonymous codon changes that have functional significance. The exchange of amino acids in codons can restore or improve the functionality of encoded proteins. RNA editing is particularly important in cases which RNA editing restores the correct start codon (methionine), thereby enabling the translation of a functional protein (Grosche et al., 2012). However, not all chloroplast genes undergo RNA editing; for example, the *ndhB* gene, which encodes a subunit of the NADH dehydrogenase complex. This variation suggests that RNA editing patterns may differ between individual genes and in *Pellia* lineages. Unlike *M. polymorpha*, *Pellia* retained RNA editing capabilities, supporting the hypothesis that RNA editing was present in all early land plants and was subsequently lost in specific lineages (Grosche et al., 2012).

Analysis of mitochondrial genomes revealed that *Pellia epiphylla* is also a liverwort species rich in RNA editing sites. Comparative studies have demonstrated an evolutionary trend concerning the decreasing number of predicted editing sites along with diversification observed in the leafy liverworts group (Myszczyński et al., 2018; Ślipiko et al., 2017). This phenomenon may be related to structural changes in mitochondrial genes, such as the loss of introns. This trend is illustrated by comparing the early divergent *Haplomitrium hookeri*, in which 732 editing sites were predicted, with the simple thalloid *P. epiphylla*, in which 325 editing sites were predicted (Myszczyński et al., 2019).

Detailed analyses of plastidial genomes have revealed the occurrence of heteroplasmy in the mitochondrial genome of *Apopellia endiviifolia*, which has significant implications for understanding liverwort evolution (Sawicki et al., 2023). Intra-individual variability caused by this phenomenon may constitute a hidden source of material for natural selection, potentially increasing the adaptive plasticity of liverworts in response to variable environmental conditions. In the *A. endiviifolia* mitogenome, heteroplasmy manifested as a significant number of indels, up to 475 variants including those in coding regions, of which 30 potentially caused frameshift mutations (Sawicki et al., 2023). Notably, heteroplasmy has not been observed in the chloroplast genome of *A. endiviifolia* (Sawicki et al., 2023).

Recent advancements in long-read sequencing technologies have facilitated the expansion of genomic research on *Pellia* sensu lato at the nuclear level. A complete telomere-to-telomere (T2T) genome assembly was recently achieved for the haploid liverwort *Apopellia endiviifolia*, providing the first chromosome-scale nuclear genome reference for this lineage (Szablińska-Piernik et al., 2025). The assembled genome was 2,914,960,273 bp in size and organized into nine chromosomes, for which telomeric and centromeric regions were identified *in silico*. Genome annotation, supported by RNA-seq data, led to the identification of 34,615 genes, the majority of which were protein-coding. The analysis revealed substantial variation in gene density among the chromosomes, ranging from 5.52 to 9.78 genes per 500 kbp. The validated centromeres exhibited length variations ranging from 1.9 to 5 Mbp (Szablińska-Piernik et al., 2025). The availability of a complete nuclear genome complements earlier studies on the plastid and mitochondrial genomes of *Pellia* sensu lato, providing a broader framework for interpreting the evolutionary dynamics of this genus. Together, the complete organellar sequences and T2T nuclear assembly of *A. endiviifolia* contribute to a comprehensive understanding of genome organization in the early diverging lineages of land plants.

8. Ecological significance

Pellia species play an important role in various ecosystems. They are commonly found in damp, sheltered habitats such as streambanks, rivers, and ditches (Atherton et al., 2010; Schuster, 1992; Schütz et al., 2016). They also occur in wet woodlands, marshes, and wet rocks. *Pellia* species are particularly abundant in areas with acidic or neutral substrates and are often found growing in dense mats that cover extensive areas. These mats provide habitats for various invertebrates and contribute to nutrient cycling and soil stabilization. Furthermore, *Pellia* species are sensitive to environmental changes and can be used as indicators of water quality and habitat disturbance (Schuster, 1981).

Pellia endiviifolia, in particular, exhibits a preference for base-rich sites, growing near watercourses, springs, flushes, shaded tracks, woodland rides, fens, dune slacks, moist floors of chalk quarries, and dripping rock outcrops (Schuster, 1981; Schütz et al., 2016). This species' specific habitat preference further highlights the ecological diversity within the genus *Pellia*.

9. Future research directions

Despite the significant progress made in understanding the genus *Pellia*, several areas require further research. These include:

- Species delimitation and taxonomy: The exact number of species within *Pellia* remains uncertain, and further research is needed to clarify the boundaries between closely related species and to resolve cryptic species complexes.
- Phylogeny and biogeography: Although molecular studies have provided valuable insights into the phylogenetic

relationships within *Pellia*, further research is required to fully resolve the evolutionary history of the genus and understand the factors that have shaped its current distribution.

- Ecological interactions: The ecological roles of *Pellia* species are not fully understood, and further research is needed to investigate their interactions with other organisms and their responses to environmental change.
- Physiological adaptations: *Pellia* species exhibit a range of physiological adaptations that enable them to thrive in diverse habitats. Further research is needed to understand the mechanisms underlying these adaptations and their roles in the ecological success of this genus.

10. Conclusion

The genus *Pellia* represents a fascinating group of liverworts with a complex taxonomic history, diverse morphology, and important ecological role. Taxonomic changes, cryptic speciation, phylogenetic studies, and molecular research have significantly contributed to our understanding of the genus. Although significant progress has been made, several questions remain unanswered. Future research integrating molecular, morphological, and ecological approaches will be crucial to further unravel the mysteries of *Pellia* and gain a deeper appreciation of its contribution to the biodiversity of our planet.

11. Supplementary material

The following supplementary material is available for this article:

Table S1. Comparative morphological and reproductive characteristics of *Pellia* and *Apopellia* species. The table presents key diagnostic features, including sexual systems, chromosome numbers, cellular structures, reproductive organs, and spore characteristics. Data compiled from taxonomic and morphological studies (1966, 1981; Schütz et al., 2016). Unknown values indicate insufficient data in the literature.

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