Phylogeny, Species Delimitation and Taxonomy in *Polytrichum* sect. *Polytrichum* (Polytrichaceae Schwägr.; Bryophyta)

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“ There is an ancient conversation going on between mosses and rocks, poetry to be sure. About light and shadow and the drift of continents. This is what has been called the "dialect of moss on stone - an interface of immensity and minuteness, of past and present, softness and hardness, stillness and vibrancy, yin and yan.”

— *Robin Wall Kimmerer, Gathering Moss: A Natural and Cultural History of Mosses*

Declaration

I hereby declare that the work contained in this thesis is my own unless otherwise acknowledged and cited. This thesis has not in whole or in part been previously presented for any degree.

Isuru Udayanga Kariyawasam,

Edinburgh

31st March 2021

ABSTRACT

The Polytrichaceae Schwägr. is a relatively a small, distinct family of mosses (Phylum Bryophyta) usually recognised in its own order and class, Polytrichales and Polytrichopsida. The presence of the characteristic "polytrichoid peristome", lamellae on the adaxial side of the leaf, well developed conducting tissue and the occurrence of some robust species with heights of >40–60cm are some of the defining characters of the family. The type genus of Polytrichaceae, *Polytrichum* Hedw. comprises three sections viz. *Polytrichum* section *Polytrichum*, section *Aporotheca* and section *Juniperifolia.* The section *Polytrichum* is a well-circumscribed clade that includes eight species and all plants currently recognised within the species concept of *Polytrichum commune* Hedw., one of the most widespread and ecologically important moss species of northern temperate and boreal regions across Asia and North America and also known from southern temperate areas. Although some molecular taxonomic and monographic work has been done for certain genera within the family over the past three decades, a comprehensive integrated (molecular and morphological) taxonomic revision has not been done for *Polytrichum* sect. *Polytrichum,* which contains the type species of the genus, *P. commune* Hedw.

This study presents the first robust molecular phylogenetic framework for *Polytrichum* sect. *Polytrichum* using both Sanger and Next Generation Sequencing (NGS) approaches. For Sanger sequencing, a robust taxon sampling (114 ingroup accessions) was done to cover a substantial geographic distribution and morphological diversity. Six molecular markers were used, including two nuclear markers (ITS1 and ITS2) and four plastid markers (*rbcL*, *trnL-F*, *rpl16* and *trnG*). Phylogenetic relationships of the species were tested for the concatenated (3851 bp) matrix using both Maximum Likelihood (ML) and Bayesian inferences. The monophyly of five clades was strongly supported within *Polytrichum* sect. *Polytrichum* [Arctic and Subarctic *P. swartzii* and *P. jensenii* clades, a *P. commune sensu stricto* clade, and South American *P. angustifolium* and *P. brachymitrium* clades] while one poorly supported large clade was recovered including three morphologically and geographically distinct taxa, *P. perigoniale, P. subpilosum* and *P. ericoides*. Within this, *P. ericoides* and *P. subpilosum* were monophyletic, but ambiguously resolved in relation to elements within *P. perigoniale,* hence the clade is best viewed as a species complex. A haplotype network is presented from a representative subset of this clade using the ITS2 marker to illustrate and interpret the relationships of taxa and geographical populations.

To investigate the unresolved species relationships further, a target enrichment with *Physcomitrella* RNA baits was employed to sequence 809 low copy nuclear loci for 24 representative accessions of all extant taxa within the section. The data was assembled using different approaches (*de novo* and reference mapping) and with different software and settings to assess their impact on downstream phylogenetic analysis. All loci were concatenated and analysed under the ML phylogenetic framework and treated as a single partition. The final NGS phylogeny showed a similar phylogenetic pattern as was inferred using Sanger Sequencing. However, a striking difference between the NGS and Sanger phylogenies was that *P. ericoides* was separated as a well-supported monophyletic clade sister to the species complex comprising. *subpilosum*, *P. perigoniale* and *P. brachymitrium* in the NGS phylogeny. Moreover, *P. brachymitrium,* which was sister to the species complex with the Sanger data, was nested within it in the NGS results. Possible taxonomic and phylogenetic explanations are provided to address this issue.

This study presents the first worldwide monographic revision of *Polytrichum* sect. *Polytrichum* based on herbarium specimens, including all available type specimens. The unresolved nomenclatural issue of selecting a lectotype for the well-known moss taxon, *Polytrichum commune* Hedw., has been resolved. Interpreting the molecular phylogenetic results of the present study, it is now clearly revealed that *Polytrichum perigoniale* Michx. (earlier treated as *P. commune* var. *perigoniale*) is a distinct taxon at species rank which has been widely misunderstood, with many historic and recent collections from North America, Australasia, Africa and Southeast Asia erroneously named previously as *P. commune*. This taxonomic confusion is resolved by the molecular and morphological delimitations proposed in this study. Eight morphological species are confirmed from the study: *Polytrichum angustifolium* Mitt., *P. brachymitrium* Müll.Hal., *P. commune* Hedw., *P. ericoides* Hampe, *P. jensenii* I.Hagen, *P. perigoniale* Michx., *P. subpilosum* P.Beauv. and *P. swartzii* Hartm. New observations are reported and a taxonomic key to separate species is provided based on vegetative and reproductive characters. Typification, taxonomic descriptions, illustrations, geographic distributions, synonymy, ecological notes and new species records are provided under each species. All doubtful taxa are listed along with reasons for their exclusion. New combinations are also included under each taxon, with justifications provided.

The sporophytes of *P. ericoides* Hampe. are reported for the first time, described and illustrated. The taxon *Polytrichum commune* Hedw. in its revised circumscription is excluded

from Africa and China, while the geographic distribution of *P. brachymitrium* Müll.Hal. is expanded from Brazil to Venezuela, Argentina and Colombia. The Arctic and Subarctic taxon *P. swartzii* Hartm. is excluded from China, Taiwan and Japan.

This study provides the first phylogenetic study of Polytrichaceae using herbarium DNA to infer phylogenetic relationships using hybrid capture methods. Although the capture was variably successful it provided much target (nuclear) and off target (plastid) data for future research. This study will open avenues for inferring phylogenetic relationships of extant genera and species of the family Polytrichaceae through the design of genus- and species-specific DNA probes, to elucidate any reticulate evolutionary trajectories such as allopolyploidy and hybridisation within the group.

Lay Summary

Bryophytes mark a crucial step in land plant evolution. They are the first colonisers of the land which bear a long evolutionary history. Extant bryophytes include three morphological groups, namely liverworts (Marchantiophyta), mosses (Bryophyta in strict sense) and hornworts (Anthocerotophyta). Mosses in particular play a vital role in maintaining the ecological balance in many ecosystems including rain forests. Polytrichaceae is a common family of mosses in the northern boreal regions where the family contains enormous (by bryophyte standards) mosses such as the common bog moss *Polytrichum commune*, commonly known as the "Haircap Moss". Some allied taxa of *Polytrichum commune* can be seen in tropical, subtropical, arctic as well as subarctic areas. Over the last couple of centuries this group has been studied by botanists mainly with the light of morphological variations.

With the advancement of the modern molecular studies and DNA-based technologies, inferring the evolutionary relationships among the mosses have also accelerated. However, there are few studies which have done for this particular section of the genus *Polytrichum*. This is therefore the first study to revise the current understanding of this particular section of mosses (*Polytrichum* section *Polytrichum*) with the aid of DNA-based molecular markers.

The results have helped to eliminate some controversial nomenclatural and taxonomic issues botanists faced earlier in identifying morphologically similar taxa in various parts of the world, especially regarding application of the name "*Polytrichum commune"* in Africa and North America. Moreover, although the common moss *Polytrichum commune* was named by Johannes Hedwig, the "Father of Modern Bryology" in 1801, a specimen for authenticating the identity of this particular taxon (a lectotype) has never been formally designated. This study resolves the frequent centuries-old misapplication of the name *P. commune* in herbaria and taxonomic manuals and floras worldwide and provides a comparative understanding of morphologically allied taxa for field and herbarium botanists. The results showed both a correspondence and discrepancy between molecular and morphological data for recognizing species. The traditional morphological classification is congruent with five taxa studied within the group, while in another case molecular evolution outstripped morphological evolution by lumping three distinct morphological taxa into a "species complex".

This is the first detailed DNA-based study done for *Polytrichum* sect. *Polytrichum* with global scope, to identify and classify extant specimens both recent and old. This study also performed traditional Sanger Sequencing and modern Next-Generation Sequencing platforms and

assembled the most updated DNA sequence matrix of the moss family Polytrichaceae including all available taxa of the *Polytrichum* sect *Polytrichum*. This study also reveals some new species records of certain taxa in various geographical regions and the new discovery and description of the spore-bearing structures (sporophytes) of the Andean endemic taxon, *Polytrichum ericoides*.

Additionally, this study provides a lot of useful molecular data for future studies on mosses to infer phylogenetic relationships as well as for conservationists to use new data to support conservation of certain taxa and hopefully to enforce any necessary conservation measures.

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CHAPTER 01

INTRODUCTION

"A thinker sees his own actions as experiments and questions--as attempts to find out something. Success and failure are for him answers above all."

— Friedrich Nietzsche

1.1 General Introduction

Bryophytes have been an ecologically important group of plants established on land for over 400 million years (Mishler, 2001; Renzaglia & al., 2007; Vanderpoorten & Goffinet, 2009). They are unique and significantly different among the main groups of embryophytes (extant land plants) by two plesiomorphic traits; having a dominant haploid gametophyte in their life cycle (Frey & al., 2009; Vanderpoorten & Goffinet, 2009; Medina & al., 2018) and an unbranched matrotrophic sporophyte that remains dependent physically on the gametophyte. Although their vegetative body usually possesses a simple architecture compared to other extant plant lineages (Goffinet & Buck, 2012), bryophytes are the second largest group of land plants after angiosperms, with as many as 20,000 extant species (Crosby & al., 1999; Söderström & al., 2016) and can be found in almost every terrestrial habitat, as well as in many freshwater habitats (During & Van Tooren, 1987). Bryophytes comprise three morphologically distinct monosporangiate lineages including **liverworts** (phylum Marchantiophyta), **mosses** (phylum Bryophyta) and **hornworts** (phylum Anthocerotophyta).

Recent advances in the molecular phylogenetics and phylogenomics of land plants (or embryophytes) opens up an avenue to infer the relationships of bryophytes to the major lineages of vascular plants (tracheophytes). Kenrick & Crane (1997) introduced the two terms "monosporangiophytes" (for bryophytes, where the spore bearing structure contains a single sporangium) and "polysporangiophytes" (for the clade of non-bryophyte land plants). Bell & Hyvönen (2010 b) strongly supported these alternative terms citing two reasons. Firstly, water conducting cells have arisen independently in some monosporangiophyte (bryophyte) lineages (such as Polytrichopsida) as well as in polysporangiophytes (Ligrone & al., 2000; Carafa & al., 2005). These thick-walled water-conducting cells (hydroids) in mosses are analogous to the perforated water conducting xylem vessels and tracheary elements in polysporangiophytes,

with both types of cells undergoing cytoplasmic lysis (Ligrone & al., 2000). Hence the traditional terminology to introduce bryophytes as “non-vascular plants” and tracheophytes as “vascular plants” is debatable in terms of the architecture of the lignified water conducting cells. Hence, the term “non-vascular plants” indirectly provides an impression of bryophytes are a group of intrinsically “primitive” plants which are ancestral to the polysporangiophytes, irrespective of their enormous diversity which is only second to the diversity of flowering plants (angiosperms). Secondly, bryophytes possess a gametophyte-dominant lifecycle where the monosporangiate sporophyte is matrotrophic or gametophyte-dependent, whereas polysporangiophytes possess an independent, sporophyte-dominant life cycle stage in which multiple sporangia are produced on free-living sporophytes. Hence, this transition shows a distinct evolutionary innovation in the polysporangiophyte clade.

Previous pre-molecular (cladistic-based) and molecular phylogenetic studies postulated liverworts to comprise a monophyletic group sister to the rest of the land plants, with either mosses (Fig.1.1 F), hornworts (Fig. 1.1 E), or a moss−hornwort clade (Fig. 1.1 G) as the sister group to the polysporangiophytes (tracheophytes). Variants on these topologies have also been proposed, such as a liverwort−moss clade as the sister group to the remaining land plants (Fig 1.1 D) (e.g., Frey & al., 2009; Qui & al., 2006; Wolf & al., 2005). Moreover, based on recent phylogenomic studies (Wickett & al., 2014), two other variants of these topologies suggested that hornworts might be sister to all land plants (Fig.1.1 B), or that a monophyletic bryophytes is sister to the tracheophytes (Fig. 1.1 A).

However, these arguments have now been called into question and the respective phylogenetic positions of liverworts, mosses and hornwort is currently uncertain and debatable. A recent proposal by Cox & al. (2014) is that when compositional biases deriving from synonymous substitutions in protein-coding data are considered, bryophytes are best hypothesised to form a monophyletic group within the land plants, with mosses and liverworts (“Setaphyta”) comprising a monophyletic group sister to hornworts and bryophytes (mosses) sister to tracheophytes. The findings of Wickett & al. (2014) based on transcriptome data offer further tentative support for this hypothesis, while also suggesting that other topologies may be equally plausible.

Seven different alternative hypotheses of the relationships of bryophytes with land plants, postulated by different phylogenetic analyses during the recent past, are depicted in Figure 1.1. These alternative hypotheses have been used for the molecular dating analysis

(using a Bayesian relaxed molecular clock method) performed by Morris & al (2018) to determine a time scale for land plant evolution. From their study, Morris & al. (2018) supported the monophyly of land bryophytes and their sister relationship to land plants.

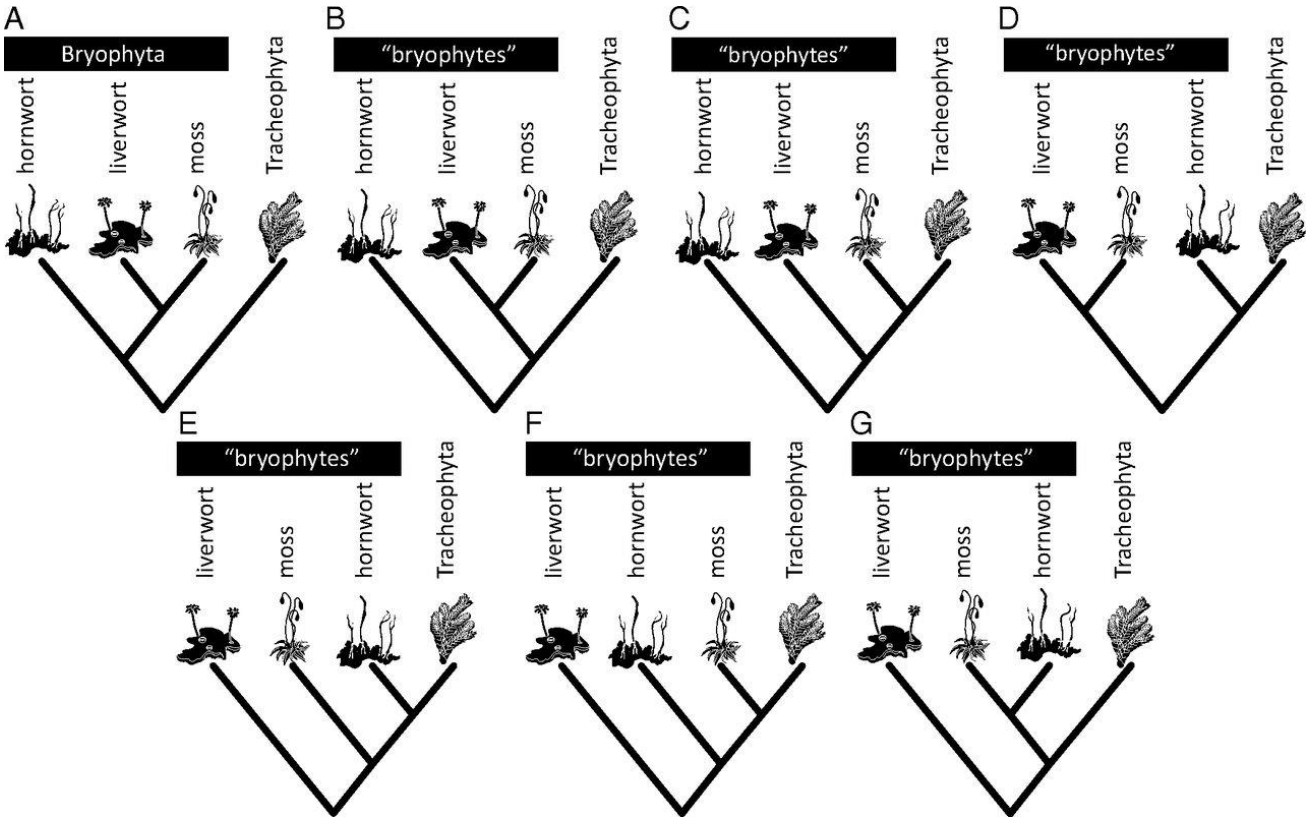


Figure 1.1: Seven alternative hypotheses postulated by various phylogenetic studies to show the evolutionary relationships of bryophytes (monosporangiophytes) and tracheophytes (polysporangiophytes): (A) Monophyletic bryophytes; (B) liverwort–moss sister clade to tracheophytes; (C) mosses, liverworts, and hornworts as successive sister lineages to tracheophytes; (D) a moss–liverwort sister clade to other embryophytes; (E) hornworts, mosses, and liverworts as successive sister lineages to tracheophytes; (F) mosses, hornworts, and liverworts as successive sister lineages to tracheophytes; and (G) a moss–hornwort sister clade to tracheophytes. [Figure and caption reproduced from Morris & al., 2018]

Although transcriptome-level datasets support both topologies A & B depicted in Figure 1.1 (Wickett & al., 2014), inferring the relationships among these early land plants is difficult due to sequence heterogeneity (Cox & al., 2014). However, the recent phylogenomic analyses of both nuclear (Finet & al., 2010; Cox & al., 2014) and chloroplast (Cox & al., 2014; Bell–D. & al., 2020) genome data of land plants (embryophytes / viridiplantae) have, controversially, supported the monophyly of both monosporangiate (mosses, liverworts, and hornworts) and polysporangiate (lycopods, ferns, and seed plants) lineages, with mosses and liverworts forming the "Setaphyta" clade. Sousa & al (2020) showed that phylogenetic relationships inferred from mitochondria are incongruent with these results and indicate the paraphyly of bryophytes with liverworts alone resolved as the earliest-branching lineage of land plants.

Among the three monophyletic, monosporangiophyte lineages (liverworts, mosses and hornworts), mosses (Phylum Bryophyta) are the most diverse group (Vanderpoorten and Goffinet, 2009). They occur in all major biomes of the world, from epiphytic communities in high elevation tropical rainforests to alpine montane areas. They can also occupy many relatively harsh xeric and semi-arid habitats periodically subjected to droughts (Vitt & al., 2014). Mosses exhibit a broad spectrum of strong adaptations in the form of their morphology, anatomy, physiology and genetic make-up in response to challenges in the terrestrial environment, such as desiccation tolerance (Proctor & al., 2007).

Unlike liverworts, mosses exhibit a great diversity in their sporophyte architecture. Sporophytes in mosses are relatively long-lived compared to those of liverworts and most species have a structurally complex specialised structure composed of one or more rings of "tooth-like" structures, which is collectively known as the peristome (Figure 1.5). This helps to regulate the liberation of spores over a long period of a time (moss capsules have synchronous production of spores) in response to the prevailing environmental conditions (Smith, 1974; Vanderpoorten & Goffinet, 2009; Bell & Hyvönen, 2010a). Even though peristomes are absent in the earliest-diverging moss lineages (Takakiopsida, Sphagnopsida, Andreaeopsida and Andreaeobryopsida), they characterise all other major groups of mosses, representing about 97% of species diversity in mosses (Bell & al., 2014)

1.2 Phylogeny & Extant Diversity in the Class Polytrichopsida

At present, there are a number of robust molecular phylogenetic and phylogenomic reconstructions of mosses available at the family rank and above (e. g. Cox & al., 2010; Liu & al., 2019). However, morphologically, the major lineages of mosses were defined by characters of the peristome in the past (Vitt, 1984). Within the current understanding of the phylogenetic reconstruction of mosses (Liu & al., 2019), the order Polytrichales comprises a monophyletic lineage of structurally complex mosses, with a well-developed water and food conducting system, complex multi-layered leaves with cuticle and photosynthetic lamellae (resembling the palisade layer of angiosperm leaves; a "pseudo-mesophyll") and underground rhizoidal mats with a perennating rhizome.

The class Polytrichopsida, informally referred to as the "hair-cap mosses" (due to the possession of a hairy calyptra which protects the sporangium; Figure 1.2), is a unique, phylogenetically isolated lineage within the acrocarpous mosses (i.e. those having the sporophyte produced at the apex of the stem or main axis), occurring outside of the major clade Bryopsida which harbours the vast majority of extant mosses. (Bell & Hyvönen, 2010b, Bell & al., 2021). The extant diversity of the class Polytrichopsida comprises 19 genera (Bell & al., 2021) with two known only from fossils, *Meantoinea alophosoides* Bippus, Stockey, G.W.Rothwell et Tomescu and *Eoplytrichum antiquum* Konopka, Herendeen, Smith Merrill et Crane. (Bippus, & al., 2017; Bippus & al., 2018). Most genera of Polytrichopsida generally contain a few species, however, *Pogonatum* is the largest genus within the class which comprises over 50 species (Hyvönen, 1989**)**. There are approximately 200 accepted species names found in the class Polytrichopsida, which included the largest extant mosses on the earth [*Dawsonia* R.Br., *Dendrologiotrichum* (Müll.Hal) Broth)] (Bell & Hyvönen, 2010b).

A vast majority of the Polytrichopsida are rather easily distinguished from other mosses even by non-bryologists by their distinctive robust habit. Figure 1.3 illustrates some members of Polytrichopsida exhibiting various forms of habits and statures.

Phylogenetic relationships and generic circumscriptions within the class Polytrichopsida are now fairly well understood by molecular phylogenetic analyses performed during the recent past and the relationships among the taxa were interpreted in terms of their morphology, ecology and phytogeography (e. g. Koskinen & Hyvönen, 2004; Bell & Hyvönen 2008, 2010a, 2010b, 2012; Bell & al., 2015; Hyvönen & al., 1998). These studies were largely

congruent with cladistic and/or morphological studies (e. g. Hyvönen 1989; Smith Merrill 1996; Smith 1971) performed during the pre-molecular era with only a few notable exceptions.

Figure 1.2: Habit of the common hair-cap moss *Polytrichum commune* Hedw. **(A)** showing the goldern coloured, hairy calyptrae covering the entire immature capsules (Photo: David Long); **(B)** showing the mature capsule with epiphram and peristome (Photo: Neil Bell).

Figure 1.3: Representatives of the Polytrichopsida showing overall morphology of different genera. (**A**) *Alophosia azorica* (Ren. & Card.) Card. showing the flattened sporophyte capsule; (**B**) *Dendroligotrichum* sp. showing the "tree-like" branched habit. (Photos: Neil Bell); (**C**) *Dawsonia superba* Grev., the largest extant moss with male inflorescences (perigonia) (Photo: Reiner Richter).

An updated phylogeny of the extant genera of the class Polytrichopsida (generated by Maximum Likelihood phylogenetic reconstruction based on previously published datasets; Bell & Hyvönen 2010a, 2010b, 2012; Bell & al., 2015, 2017) is depicted in Figure 1.4 (reproduced from Bell & al., 2021). This represents diversity across the class sampling molecular sequence data from four regions (the mitochondrial *nad5* gene, the 3′ half of the chloroplast *rbcL* gene, the chloroplast *rps4* gene and downstream intergenic spacer, and the chloroplast *trnL-trnF* region).

Based on molecular phylogenetic studies, it has been shown that *Alophosia* Card., *Bartramiopsis* Kindb., and *Lyellia* R.Br. make the earliest diverging lineages of the class Polytrichopsida (Figure 1.4). These are relict genera scattered and disjunctively distributed in the northern hemisphere (Bell & al., 2021). *Batramiopsis*, also monotypic, has a "northern Pacific Radiant" distribution (Smith Merrill, 2007). The distinct synapomorphy found in all these early lineages in Polytrichopsida, and also with the fossil *Meantoinea*, is the lack of a peristome. Instead, they possess a "stopper mechanism" at the capsule mouth composed of an enlarged apical section of the columella and often an expanded, flattened capsule rim (illustrated in Fig. 5A of Bell & Hyvönen, 2010b).

The position of Polytrichopsida within acrocarpous mosses is unique as their nematodontous peristome (i.e. a toothed structure made up of whole dead cells with very thickened walls) appears not to be homologous with the arthrodontous peristomes found in the majority of mosses (i.e. peristomes made up of only parts of cell wall remnants) nor with the nematodontous peristome of Tetraphidopsida (a class sister to Polytrichopsida which contains 4 large peristome teeth), although it is clearly homologous in its initial developmental stages (Bell & Hyvönen, 2008; 2010b). It is hypothesised that the nematodontous peristome of Polytrichopsida may be independently evolved from an ancestral type of spore-liberating apparatus which is completely devoid of peristome teeth (Bell & Hyvönen 2008; Bell & Hyvönen 2010a; Bell & al., 2015; Liu *et al*. 2019).

In Polytrichopsida, the nematodontous peristome has a very distinctive micro-architecture. This has been discussed in detail by Smith (1971, 1974) and Bell & Hyvönen (2010a), with variations being informative for sectional placements and generic circumscriptions in Polytrichopsida (see below). The peristome teeth in Polytrichopsida are developed from the U-shaped or deeply grooved amphithecial cells of the sporophytic capsule which are attached to the disc-like membranous structure called the "epiphragm" (Figure 1.5). The epiphragm is developed from the apex of the "columella", a sterile columnar structure in the central axis of the sporangium (capsule) that passes through the sporogenous tissue. Spores are released through the gaps between the peristome teeth and the epiphragm (in contrast to arthrodontous mosses, where spores are released due to the hygroscopic movements of the teeth).

Liu & al. (2019) hypothesised that the Oedipodiopsida, Polytrichopsida & Tetraphidopsida collectively form a monophyletic sister group to Bryopsida, the class containing the arthrodontous mosses (see Figure 1, Liu & al., 2019). The Oedipodiopsida lack a peristome and it is hypothesised that Oedipodiopsida may have lost the peristome secondarily (Liu & al., 2019). Moreover, within the "hypothesised monophyletic group" of Tetraphidopsida and Polytrichopsida, the genera *Alophosia*, *Bartramiopsis* and *Lyellia* lack peristomes and instead have a structure called a "stopper" which is homologous to the epiphragm found in most of the derived lineages of Polytrichopsida. The lack of a peristome in these lineages may be due either to secondary loss or to the independent evolution of the toothed peristome in the more derived Polytrichopsida (Bell & Hyvönen, 2008; Liu & al., 2019). Hence it is presumed that the "polytrichaceous" peristome has evolved by elongation and curvature in the innermost cell layers of the outer amphithecium to form tooth-like structures in Polytrichopsida (Smith, 1971; Bell & Hyvönen, 2010 a). However, Liu & al. (2019) state that transformational relationships between nematodontous & artrodontous peristomes still remain ambiguous due to uncertainty in homology.

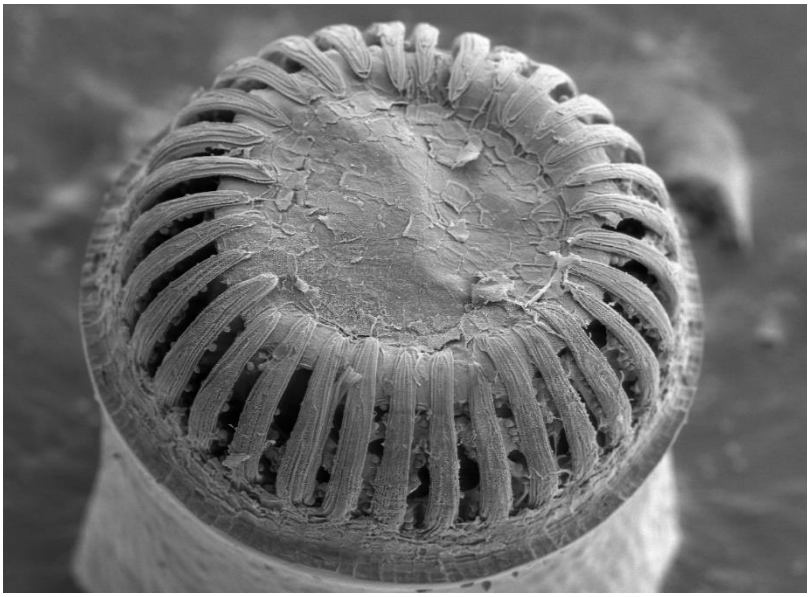


Figure 1.5: SEM photograph illustrating the peristome architecture (spore liberating apparatus) of *Atrichum undulatum* (Hedw.) P. Beauv. (Polytrichaceae); showing the peristome teeth and membranous structure called the epiphragm (Scale: 1cm=120µm; Photo: Neil Bell).

The genus *Dawsonia,* contains the largest extant mosses in the world (Figure 1.3 C). This extant species of this genus are predominantly found in tropical rain forest habitats in Australasia and South east Asia (Hyvönen, 2006). This genus is sister to all other genera of peristomate taxa in class Polytrichopsida (Figure 1.4). Members of this genus have a unique peristome structure which is composed of "thread-like" peristome teeth.

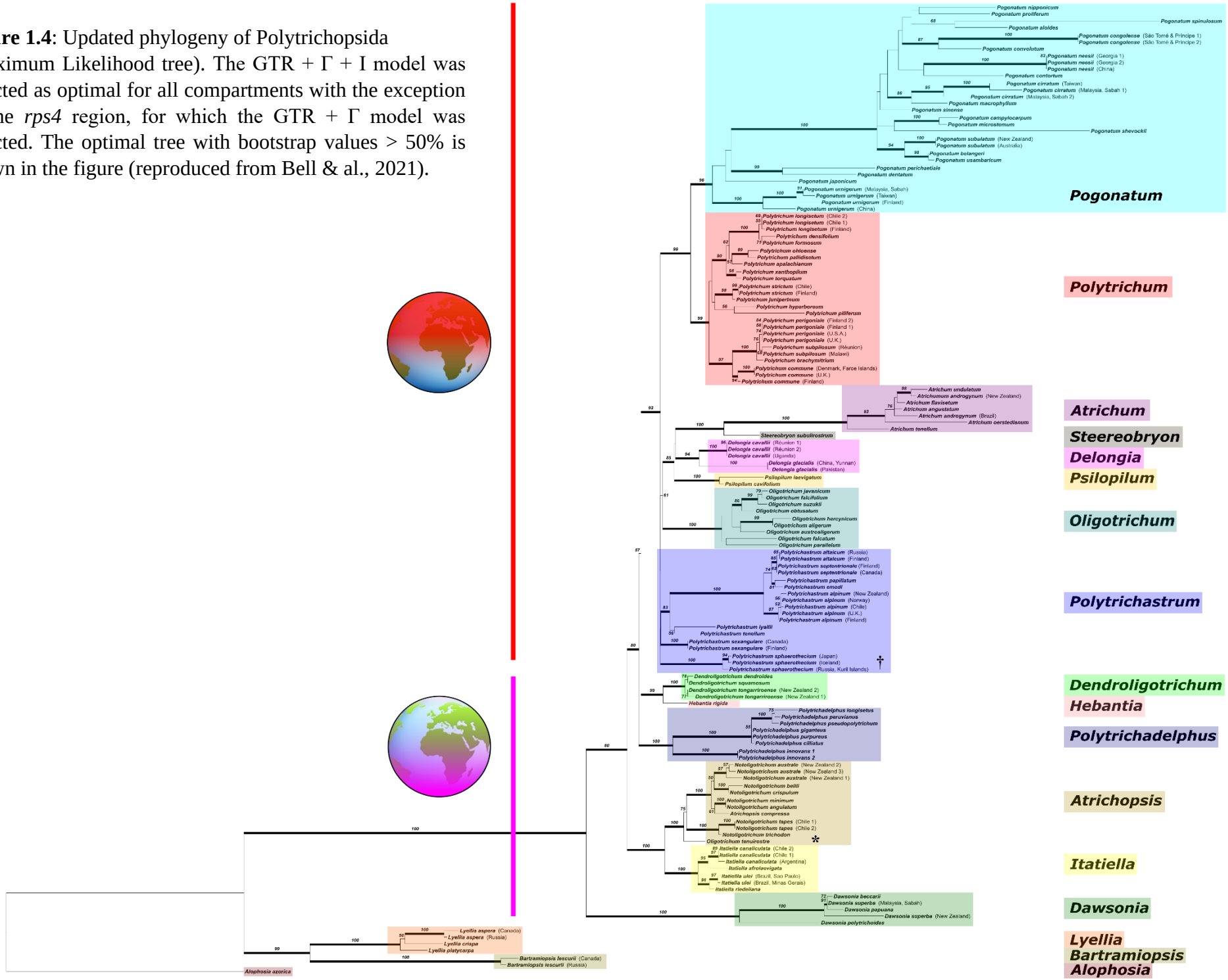
Dendroligotrichum (Müll.Hal) Broth. is a small genus, which is easily distinguished by its unique "tree-like" (dendroid) habit (Figure 1.3 B) and is composed of only three extant species found in New Zealand and Patagonia (Hyvönen, 2006).

Polytrichadelphus (Müll.Hal.) Mitt. is found on both sides of the Pacific, Australasia, South America, where the Andes is considered as the center of biodiversity of the genus (Hyvönen, 2006).

The large apical clade (Clade A) of the updated Polytrichopsida phylogeny (Figure 1.4) comprises eight genera (*Atrichum* P.Beauv., *Delongia* N.E. Bell, Kariyawasam, Hedd. & Hyvönen, *Oligotrichum* DC., *Pogonatum* P.Beauv., *Polytrichum, Psilopilum* Brid., *Steereobryon* G.L.Sm.) which cover the vast majority (~75%) of extant taxa within the class (Bell & al., 2021).

The genus *Oligotrichum* comprises 12 species and Sino-Himalaya is identified as the center of biodiversity of the genus by Kariyawasam & al. (2018). The genus *Delongia* is a new addition to the class Polytrichopsida recently described by Bell & al. (2015) and includes two disjunctively distributed species in Africa and Sino-Himalaya.

Figure 1.4: Updated phylogeny of Polytrichopsida (Maximum Likelihood tree). The GTR + Γ + I model was selected as optimal for all compartments with the exception of the *rps4* region, for which the GTR + Γ model was selected. The optimal tree with bootstrap values > 50% is shown in the figure (reproduced from Bell & al., 2021).



1.3 A Brief Taxonomic History of *Polytrichum* sect. *Polytrichum*

Mosses were never mentioned in the writings of Theophrastus (Hardy & Totelin, 2016). Although mosses were poorly defined, and their medicinal values were poorly discussed, they were first described in the 16th century herbals (Richardson, 1981). However, due to their larger plant body and fairly easily recognizable nature in the field, some of the "hair-cap mosses" (i.e. polytrichaceous mosses) were treated in 17th C. herbals (e.g. Sutherland 1689), most likely based on much older knowledge and usage, while *Polytrichum commune* was among the first mosses illustrated (Figure 1.6) in the era preceding Linnaeus (Dodoens 1578). Although *Polytrichum* was described and illustrated in *Historia Muscorum* (Dillenius, 1741), its authority was given in Hedwig's *Species Muscorum Frondosorum* (1801), which is the starting point of nomenclature for mosses (Drobnik & Stebel, 2014).

Figure 1.6: The Golden Maidenhair Moss ("Polytrichon" = *Polytrichum*) from Dodoens (1578). [Reproduced with the permission of the rare books collection, library of the Royal Botanic Garden Edinburgh]

Hedwig's classification of mosses (1801) was the first comprehensive approach to a natural classification system of mosses, as it was based on morphological characters (mainly peristome and inflorescence characters) and thus regarded as the starting point of the

nomenclature of mosses (Turland & al., 2018: Art. 13.1). Since then, the numbers of accepted taxa have increased with the accumulation of more detailed microscopic observations of the vegetative structure such as leaf anatomy. Hedwig (1801) included 17 species and one intraspecific taxon in the genus *Polytrichum* in his treatment of mosses (see Chapter 02). Since then, the species of the conglomerate genus *Polytrichum* Hedw. have subsequently been placed in separate genera to accommodate observed variation (Kariyawasam & al., 2021).

Lindberg (1868) divided the genus *Polytrichum sensu lato* into two subgenera, *Pterygon* and *Leiodon*, based on the peristome and epiphrgam characters. Fleischer (1923) presented ideas about phylogeny of Polytrichopsida in his extensive work on S.E. Asian mosses, and Brotherus (1925), in his general treatment of mosses, divided the group into two families, the Dawsoniaceae and Polytrichaceae. This taxonomic treatment provided the basis for further insights into peristome structure in Smith's taxonomic treatment of Polytrichaceae (1971) nearly a century later. Gary L. Smith laid the foundation stone for the modern taxonomy of the Polytrichopsida with numerous studies on the group, and particularly his "Conspectus of the Genera of Polytrichaceae" (Smith 1971). In his conspectus, Smith (1971) presented a schematic phyletic arrangement of the genera of the Polytrichaceae with a brief discussion, along with a list of phylogenetic trends, i.e., a table of characters and their assumed primitive and derived states. His revised generic classification is still largely in use today although concepts of some genera, e.g. *Pogonatum* P.Beauv., *Polytrichastrum* G.L.Sm. and *Polytrichum* Hedw., have subsequently been modified (e.g., Touw 1986; Hyvönen 1989; Bell & Hyvönen 2010b).

Prior to Smith's Conspectus of the Polytrichaceae (1971) the sole comprehensive taxonomic treatment for the family was Brotherus's monographic treatment presented in *Die naturlichen Pflanzenfamilien* (1904-1905). Brotherus divided *Polytrichum* into four sections with a clear distinction within the group between taxa chiefly based on capsule morphology (spherical capsules versus angled capsules). *Polytrichum* sect. *Alpina* I.Hagen encompassed many of the *Leiodon* spp. and included taxa with spherical capsules [Later Smith (1971, 1974) placed many of these into his new genus *Polytrichastrum*, while Bell & Hyvönen (2010a) placed some of these back into *Polytrichum* with the aid of molecular evidence and further morphological studies of peristome characters]. The section *Polytrichum* sect. *Communia* I.Hagen includes *Polytrichum commune* and allied taxa, whereas section *Polytrichum* sect. *Juniperina* I.Hagen includes most of the pterygodont taxa (i.e. those with a type of nematodontous peristome found in the Polytrichaceae that has longitudinal "wing-like" crests

or flanges on the inner surfaces of the teeth). This pterygodont peristome micro-architecture is shared by *P. commune* and its allied taxa in *P.* sect. *Polytrichum. Polytrichum sexangulare* Flörke ex Brid. [≡*Polytrichastrum sexangulare* (Flörke ex Brid.) G.L. Sm.]) was placed in its own monotypic section *Polytrichum* sect. *Sexangularia* I.Hagen. The taxonomic position of this species remains somewhat ambiguous (Bell & Hyvȍnen; 2010a), possibly reflecting its likely allopolyploid origin (Derda & Wyatt, 2000).

Lindberg's study of the pterygodont and leiodont peristome types provided the basis for Smith (1971, 1974) to distinguish *Polytrichum* (Hedw.) G.L.Sm. and *Polytrichastrum* G.L.Sm. Smith placed two main sections under the genus *Polytrichum*, i.e. *Polytrichum* sect. *Polytrichum* and *Polytrichum* sect. *Juniperifolia.* The well-developed apophysis and strictly 4-angled capsules were synapormorphies for both sections whereas the infolded leaves with pyriform apical leaf lamellar cells found in sect. *Juniperifolia* are distinctive characters distinguishing it from sect. *Polytrichum* (note however that *P. angustifolium* in sect. *Polytrichum* also has pyriform apical lamellar cells - the taxonomic position of this rather anomalous species has yet to be tested with molecular data-See Chapters 03 & 04). Smith (1971) in turn divided the genus *Polytrichastrum* into two sections, sect. *Polytrichastrum* and sect. *Aporotheca.* Rounded or slightly angled capsules found in sect. *Polytrichastrum* was used as a distinctive character to distinguish the section from members of section *Aporotheca*, which possessed 4–6 angled capsules. The first search-based cladistic analysis of Polytrichopsida was in the MSc thesis of Forrest (1995) and was based on 50 morphological characters. This was followed by an analysis by Hyvönen & al. (1998) that included, in addition to 39 morphological characters, nuclear and chloroplast gene sequences.

In 2010, Bell & Hyvȍnen published a comprehensive morphological and molecular study that revealed the taxonomic positions of *Polytrichum sensu lato* and *Polytrichastrum* and led to the rearrangement of species within the two genera. Members of *Polytrichastrum* sect. *Aporotheca* were placed under *Polytrichum* sect. *Aporotheca* after *Polytrichastrum sensu* Smith (1971) was shown to be a polyphyletic entity. *Polytrichum* was separated from *Polytrichastrum* based on capsule shape and the development of the peristome-epiphragm complex, strongly supported by Bayesian ancestral character state reconstruction (Bell & Hyvȍnen, 2010b). The pterygodont features such as vertical ridges on the insides of the peristome teeth, the presence of sacculi and the strong constriction on the apophysis are found in both *Poyltrichum* sect. *Polytrichum* and *Polytrichum* sect. *Juniperifolia*. [i.e., Smith's (1971) *Polytrichum sensu stricto*], but not in *Polytrichum* sect. *Aporotheca*. Hence, the members of

sect. *Aporotheca* do not possess all of the morphologically defining characters of *Polytrichum sensu* Smith (1971), although with the exception of *P. longisetum* they all have 4-angled capsules (albeit less sharply angled than in the other two sections of *Polytrichum*) and lack strongly elongated "epiphragm teeth" (members of *Polytrichastrum sensu stricto* all have terete or 5–6 angled capsules and all except *P. emodii* G.L.Sm. have elongated epiphragm teeth).

Polytrichum sect. *Polytrichum* is a well circumscribed clade that includes, amongst other taxa, all plants currently recognised within the species concept of *Polytrichum commune*, one of the most widespread and ecologically important moss species of northern temperate and boreal regions forests across Asia and North America and also known from southern temperate areas (Bell & Hyvönen, 2010a; Hyvönen & al.,1998, 2004). Within the monophyletic group of *Polytrichum* sect. *Polytrichum*, *P. perigoniale sensu lato* seems to be a paralyphyletic entity (see Chapter 03). Bijlsma & al., (2000) showed that their [1]*P. commune* (now *P. perigoniale* Michx.) and [2]*P. uliginosum* (now *P.* commune Hedw.) are two genetically distinct entities which were earlier treated as two varieties of *P. commune sensu lato*. This was not only supported by the molecular evidence (allozyme and RAPD) but also by ecological and morpho-anotomical studies. *Polytrichum perigoniale* (≡ *P. commune* var. *perigoniale*) is commonly found on exposed dry, sandy habitats whereas *P. commune* (≡ *P. commune* var. *commune*) is found almost exclusively in wet peat bogs, fens, wet heaths and the edges of pools etc. Moreover, *P. commune sensu lato* exhibits a consistent difference in morphology of the lamellar end-cells of the leaf lamellae. *P. commune* shows deep asymmetrically grooved apical end cells, the plants are comparatively taller, and leaves are arranged to form the characteristic "star-like" appearance with leaves distantly spaced on the stem. In contrast, *P. perigoniale* has much flattened or shallowly grooved apical end cells and plants are shorter than *P. commune* (Long, 1985; Smith-A. J. E, 2004). Later studies by Bell & Hyvönen (2010 a,b) showed that *P. commune* and *P. perigoniale* are phylogenetically distinct entities as well as being genetically distinguishable (see above). The true geographic distribution of *P. perigoniale* needs to be resolved by thorough herbarium study as well as in a phylogeographic context. It is now clear that *P. perigoniale* is paraphyletic as well as including morphologically cryptic or pseudocryptic species (Bell & Hyvonen, 2010a, 2010b; Bijlsma et al., 2000). One group of

[1] Bijlsma & al. (2000) have used the name "*P. commune* Hedw." for the taxon *P. perigoniale* Michx. is now confirmed as the correct name from the present study.

[2] Bijlsma & al. (2000) have used the name "*P. uliginosum* (Wallr.) Schriebl2 for the taxon P.commune Hedw. is now confirmed as the correct name from the present study.

entities in the section *P. commune* sect. *commune* comprises the earliest diverging lineages while another is derived within the clade, the two clearly separated by a grade of discrete tropical species (Bell & Hyvȍnen, 2010b). *Polytrichum commune* var. *uliginosum* Wallr. was raised to specific rank by Schriebl (1991) following his observation of stability in the described diagnostic features in artificial (*in vitro*) culture. Hill & al. (2006) accepted Schriebl's study and this was supported partially by Bijlsma & al. (2000), who described two distinct genetic entities within *P. commune sensu lato*. that they recognised as *Polytrichum uliginosum* and *Polytrichum commune*, with *Polytrichum commune* var. *perigoniale* (Michx.) Hampe treated under *Polytrichum commune* (see above).

The present study has confirmed that these two genetic entities exist, although this study has now shown with the integration of molecular phylogenetic and morphological characters, that *Polytrichum uliginosum* should be treated under *Polytrichum commune* while *Polytrichum perigoniale* Michx. is the correct name for the other entity. This is because Bijlsma & al. (2000) based their sampling of *Polytrichum commune* on Schriebl's concept of the species, with specimens corresponding to *Polytrichum commune* var. *perigoniale* (as can be seen in their Figure1C), while the concept of *Polytrichum uliginosum* they used presumably included plants corresponding morphologically to *Polytrichum commune* var. *commune*.

The present study has provided a solution for this long-lasting nomenclatural issue by selecting a lectotype for *P. commune* Hedw. (See Chapter 02; Kariyawasam & al., 2021) followed by the species circumscription of *P. perigoniale* to revise the geographic distribution within a phylogenetic context (See Chapters 03 & 04). The findings were also reported in the recent annotated checklist of bryophytes of Europe, Macaronesia and Cyprus (Hodgetts & al., 2020).

1.4 Anatomy & Physiology of Polytrichopsida

Some distinguishable features associated with the class Polytrichopsida are their larger size relative to other extant mosses, leaves with specialized basal sheaths (amplexicaul) and leaf blades (laminas) covered by special mesophyll-like photosynthetic structures known as "lamellae" (Figure 1.7 A & B), relatively larger sporophytes with rigid setae and capsules protected by hairy calyptrae (Smith, 1971). All these features can collectively be observed, for example, in "the common hair-cap moss", *Polytrichum commune* Hedw., often used as an exemplary moss in traditional botanical textbooks (Unwin, 1877).

Generally, bryophytes are [3]poikilohydric, and [4]ectohydric. However, many of the Polytrichopsida are relatively larger plants with well-developed water conducting cells (see above) and a "pseudo-mesophyll" (leaf lamina comprising lamellae) capable of supporting relatively high rates of photosynthesis in moist and well-illuminated environments (Smith, 1971; Proctor, 2005; Wang & al., 2017).

Recent studies (Marshall & Proctor, 2004; Proctor, 2005) have demonstrated that in contrast to other mosses, many species of Polyrichopsida with well-developed leaf lamellae exhibit higher levels of light saturation for photosynthesis. Proctor (2005) and Wang & al. (2017) also reported that Polytrichopsida species were distinct from other mosses with regard to their photosynthetic capacities, chlorophyll a and b concentrations and light requirements. It is obvious that the development of leaf lamellae of Polytrichopsida greatly increase the available surface area of leaves to capture sunlight and also for the uptake of atmospheric carbon dioxide, which is the limiting factor for photosynthesis in environments where water in adequate supply, such as the open mires where *Polytrichum commune* grows lavishly and most abundantly.

In certain species of Polytrichopsida, the apical (end) cell of each leaf lamellae is modified in such a way as to maximise capturing photons from sun light. They are presumed to act as "lenses" which maximize direction of the captured photons to the inner chlorophyllous layers and thus to maximize the rate of photosynthesis (Smith, 1971). In certain species, these lamellar end-cells develop varying amounts of epiculicular wax platelets collectively known as "papillae" (Figure 1.7 C) which provide a unique sculptural appearance in a transverse section of a leaf. These sculptured, papillose lamellar end-cells are also used as a distinct morphological character state to circumscribe certain taxa at the species level (e. g. Long, 1985; De Sloover, 1986; Peralta & Yano, 2010). The wax platelets cover the assimilating lamellae and allow maintenance of a high gaseous exchange rate even in wet environments (Robinson, 1971; Verdus, 1974; Proctor, 1979; Marschall & Proctor, 2004).

[3] Poikilohydry is the lack of ability to maintain and/or regulate water content to achieve homeostasis, i.e the water content of plant is determined by the surrounding atmosphere, becoming completely dry and dormant during the dry season after losing most of its water and resuming the metabolic activity and structure rapidly upon rehydration.

[4] Ectohydry in relation to mosses, water is conducted to the tissues externally.

The following figure (Figure 1.7) shows some Scanning Electron Microscope (SEM) photographs of leaf lamellae present in some species in the genus *Polytrichum*.

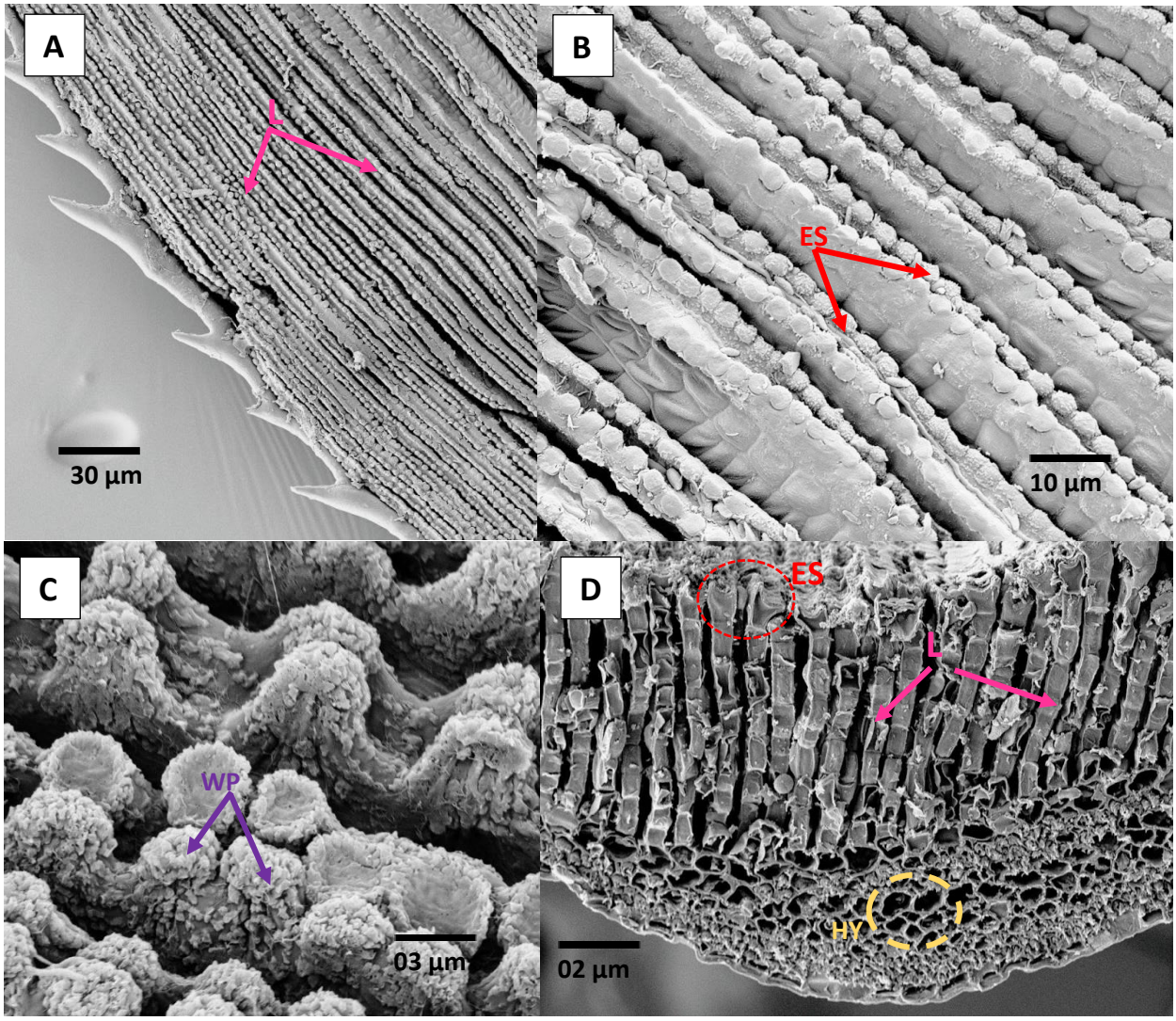


Figure 1.7: Scanning Electron Micrographs of *Polytrichum* spp. leaves illustrating the leafy anatomy of assimilatory tissue. (**A**) Adaxial leaf surface of *Polytrichum commune* - leaf lamellae (L) running longitudinally through the entire length of the leaf lamina; (**B**) A closeup view of the leaf lamellae of *P. commune* showing the compact arrangement and differentiated lamellar end-cells (ES); (**C**) Epiculticular wax platelets (WP) on the surface of lamellar end-cells of *Polytrichum subpilosum* ; (**D**) A transverse section of a vegetative leaf of P. commune showing the leaf lamellae (L), differentiated lamellar end-cells (ES) and the water-conducting cells (Hydroids) with larger cavities (HY) [Photos: A, B & D**:** *Long,41221(E)*, Photo C: *Hedderson, 16784 (E)*] -SEM photos by Isuru Kariyawasam].

Polytrichopsida possess a remarkably well-developed conducting tissue [which is not homologous with the vascular tissue in the polysporangiate lineage, (Carafa & al., 2005)] comprising water-conducting "hydroids" and food-conducting "leptoids", which presumably help certain species achieve their very robust stature (Smith, 1971). The increased uptake of atmospheric carbon dioxide due to the presence of leaf lamellae provides the high rate of photosynthesis compatible with the development of an efficient vascular system (the hydromel and leptome) to efficiently distribute the assimilates (water and sugars) within the plant body (Atala & Alfaro 2012; Wang & al., 2017). The hydroid-based vascular system in *Polytrichum*, which (unlike in any non-Polytrichaceous moss that has been studied so far) has long been known to be fully continuous from the "stem" (main axis) to the leaves (Goebel 1906), is also functionally capable of efficient water transport.

Recently Brodribb & al., (2020) demonstrated that *Polytrichum* hydroids are able to withstand the buckling forces associated with the transpiration pull and the negative water potentials exerted in vascular plants with the ascent of the sap from the soil through transpiration. This capability was previously thought to be restricted to the lignified vascular tissues of the tracheophytes. Furthermore, Brodribb & al., (2020) stated that the water transport efficiency of the *Polytrichum* "vascular system" and resistance to cavitation were compatible with maintaining a continuous water column in an endohydric plant. Moreover, *Polytrichum commune* is apparently able to regulate gaseous exchange in response to atmospheric conditions (i.e. to regulate transpiration rates to balance photosynthetic rates against desiccation), an ability that is presumed to be linked to closing off the inter-lamellar spaces of lamellar end-cells when dry, and changing the leaf orientation dramatically (Brodribb & al., 2020). This can be observed while rehydrating dried leaves of Polytrichaceae when sprinkling water, the leaf orientation changes from appressed to fully expanded state.

This study also demonstrated that, despite the suspected ability of lamellae to increase total atmospheric carbon dioxide diffusion rates, *Polytrichum* has a much less efficient exchange ratio of water for photosynthetic carbon dioxide than tracheophytes, likely linked to a comparatively high cellular resistance (boundary layer resistance) to carbon dioxide diffusion in its leaves. Thus, it requires moist air to support high rates of photosynthesis.

Connecting the above findings of Brodribb & al (2020) to the extant ecological diversity in Polytrichopsida, it is evident that these anatomical features associated with water relations in turn help members of Polytrichopsida to inhabit and thrive in a wide range of ecological

niches (Bell & Hyvönen, 2010b). This hypothesis fits with the wider range of habitats that very large Polytrichaceae members inhabit – humid tropical cloud forests (such as *Dawsonia*,) cool temperate rain forests (such as *Dendroligotrichum dendroides, Polytrichum formosum* etc.), and very wet open habitats such as mires in oceanic temperate areas (such as *Polytrichum commune* etc.), while some members are secondarily adapted to drier habitats (such as *Polytrichum perigoniale, Polytrichum brachymitrium, Polytrichum angustifolium* etc.).

1.5 Understanding the Species Concepts and Speciation in Species Delimitation Studies.

The basis for the current binomial nomenclature was established by Linnaeus in 1753, in his *Species Plantarum.* Even though Linnaeus's work was the starting point for liverwort nomenclature, the nomenclature of mosses began when Hedwig published his *Species Muscorum* in 1801. In the "typological species concept" taxa are determined and assigned to species level, based on observable morphological or phenetic characters (Simpson, 1951; Mayr, 1957; Bischler & Boisselier-Dubayle, 2000; Stuessy & al., 2014). In the "phylogenetic species concept", monophyly is the focal point and molecular sequence data is the basis for determining shared ancestry (Stuessy & al., 2014). Species concepts in bryophytes and species concepts used for delimiting the taxa in this study are discussed in both chapters 03 and 04.

"Speciation" is the process of origin of new and distinct species which is often linked to species concepts (Stuessy & al., 2014). However, de Querioz (2007a) argues that "a *species criterion* is some standard for judging whether a given entity qualifies as a member of the species category, that is, whether a particular entity should or should not be judged a species". There are four main evolutionary elements recognised in the speciation process: mutations, natural selection (Darwin, 1859), gene flow (migration) and genetic drift (Stuessy & al., 2014). Mutations are changes in nucleotide sequences which may result in evolutionary neutral or functionally silent genes (i. e. synonymous mutations) and thus have no effect on gene function. On the other hand, they could appear as nonsynonymous mutations which may change the protein sequences and are frequently subjected to natural selection (Page & Holmes, 1998). Natural Selection is a key evolutionary trajectory which alters the frequencies of genes because only the allelles (alternative forms of genes) that could confer a greater fitness are transmitted more frequently to the next generations, leading to significant changes in the allele frequency

in a given gene pool (de Querioz, 2007a; Stuessy, 2009). This will lead to better adaptation of organisms to their prevailing environments. Further to natural selection, genetic drift can be explained as a change in allele frequency of a population due to random sampling at each generation, which may lead certain alleles to disappear from the gene pool and a subsequent decline in the genetic variation in a population. Hence mutations, natural selection and genetic drift collectively lead isolated plant populations to gradually diverge from one another through continuous genetic change. However, if there is a migration of individuals or vegetative propagules transmitted between two populations, this will lead to gene flow, leading to a "homogenising effect" by increasing the proportion of alleles shared between those populations. Thus, the permanent cessation of gene flow between two populations by means of vicariance (geographic isolation) or reproductive isolation leads to speciation (de Querioz, 2007a; Stuessy, 2009; Stuessy & al., 2014).

1.6 Morphological and Molecular Data used in Species Delimitation Studies

In the process of speciation, one to many different character states are acquired and fixed by diverging lineages or populations to form "discrete entities" that we recognise as a "species". Species can be recognised using two major types of diagnostic characters, those from morphological and molecular origin.

"Homology" is a guiding principle of modern systematics. It is the name given to true similarity, as opposed to superficial similarity (or analogy, *sensu*, Scotland, 1992a). Homology is usually understood to be based on observations and estimates of similarity or correspondence and explained by a common ancestry.

Through the rapid flow of data utilisation over the past decades, all types of data bear on some aspects of phylogeny. Among those types, morphological data was one of earliest and most widely used types of data during the pre-molecular era of plant systematics.

1.6.1 Morphological Data

The great success of plant phylogenetics in the past decade was chiefly due to advancements in and comparative studies of genomics. In contrast, morphological data have received less interest. However, morphological data have usually been the primary source for

identifying species in the field and are traditionally used to delimit species boundaries, including in pre-molecular cladistic studies.

In alpha taxonomic studies such as classical revisions, monographs and floras, morphological characters are sought that can be easily employed and that are useful to circumscribe and key out species and higher taxa. Such features have also been used for morphological and phylogenetic analyses. Although morphological data have successfully been utilised for species delimitation in plant systematics, where species recognition is based on one or more qualitative (descriptive or non-measurable) or quantitative (continuous or discrete) morphological characters which are not overlapping with closely related taxa, some characters may not be equally valuable or useful, and some circumscriptions may be a complete mixture of non-homologous characters (Endress 2003; Duminil & Michele, 2009).

Problems associated with the use of morphological data are mainly due to intraspecific variation (i.e. the variation that exists between different individuals in a species due to genetic makeup and environmental variation) (Stuessy & al., 2014). Occurrence of cryptic and pseudo-cryptic species may also lead to erroneous results in classifications based solely on morphological data. Cryptic species are those morphologically very similar species due to convergence and/or genetic divergence without apparent morphological divergence and are thus grouped under the same species regardless of representing "separate functioning entities" (Duminil & Michele, 2009).

Moreover, "phenotypic plasticity", which is shown by plants to a greater extent than animals, is another problem encountered with morphological classification (Bachmann, 2001; Endress, 2003). Because of these major problems Page and Holmes (1998) stated that morphology is recognised as a "complex and non-neutral marker", even though its use is simple in practice. Therefore, species delimitation solely using morphological data may lead to inconsistent results as well as failure to understand the true evolutionary relationships among organisms (Endress, 2003).

1.6.2 Molecular Data

With the advancement of molecular techniques and availability of DNA sequence data, elucidating the phylogenetic relationships of organisms has accelerated over the quarter century (Stech & Quandt, 2010). Stech & Quandt (2010) mentioned that molecular characters used to infer phylogenetic relationships in plants are primarily obtained from three different sources: (i) DNA sequences of specific coding or non-coding regions from one of the three plant

genomes (plastid, mitochondrial, or nuclear markers) (ii) structural genomic characteristics (such as gene order, gain or loss of genes or non-coding regions such as spacers and/or introns) and (iii) genetic fingerprints such as microsatellites and/or variable number tandem repeats.

Due to the universal nature of DNA and the relative ease of obtaining numerous characters from different loci, the level of resolution provided by nucleotide data and the ability to distinguish divergence events even occurring at the population level favour DNA markers over morphological markers for delimiting species (Naciri & Linder, 2015).

Lacking a strong phylogenetic signal is one of the major drawbacks associated with data used in molecular systematics (Stuessy & al., 2014). Moreover, phylogenetic analysis of closely related species belonging to a recently diversified clade might be challenging if the analysis is based on a single or only a few loci. A single locus gives only one gene tree that may not reflect the true evolutionary history of a clade. Different loci may show incongruent phylogenetic patterns due to low rates of mutation over short evolutionary time periods since species divergence, retained ancestral polymorphism, hybridisation (found in plastid genes) and/or incomplete lineage sorting (Koopman & Baum, 2010).

In plant taxa which undergo hybridisation events, the use of plastid markers (generally uniparentally inherited) may fail to recognise the "true picture" of evolutionary history and produce conflicting or incongruent topologies with nuclear markers (biparentally inherited). This can lead to sharing of a chloroplast haplotype among a set of closely related taxa even when there is a stronger signal coming from a nuclear phylogeny (e.g. using nuclear ribosomal internal transcribed spacers, or ITS, sequences) (Stuessy & al., 2014). Nonetheless, ITS sequences have drawbacks related to paralogous copies (Buckler & al., 1997). However, ITS can still play a crucial role in investigating evolutionary relationships among species if analysed carefully, for example identifying pseudogenes and assessing orthology in the case of intra-individual polymorphism (Bailey & al., 2003).

The emerging high throughput DNA sequencing approaches offer a promising platform to greatly improve phylogenetic signal by the use of a large number of loci. The rapid revolution of next-generation sequencing accelerates the understanding of evolutionary processes in the plant kingdom and opens up novel research avenues for discovery of new taxa (Harrison and Kidner, 2011; Dodsworth, 2015).

Next-generation sequencing (NGS) covers a vast array of new techniques which enable sequencing of hundreds of independent nuclear loci, as well as loci from the plastid genome,

which together can provide many phylogenetically useful characters. In addition to generating a phylogeny using Sanger sequencing, the present study also incorporates a preliminary collaborative study on the potential of target enrichment (hybrid capture) for studying the phylogeny of *Polytrichum* sect. *Polytrichum* (see Chapter 03- Section B).

1.6.3 Integrating Morphological & Molecular Data in a Phylogenetic Context

Morphology, the science dealing with visible, observable characters, is the central discipline which should be firmly integrated with systematics. Systematics, on the other hand, struggles with convergences, and morphology must be linked in such a way as to form a system of mutual elucidation. Hence, any molecular systematic study should ideally be accompanied by a profound morphological study, with these studies particularly addressing homoplasies or convergences to overcome any conflicting results in species delimitation (Weber, 2003).

Hence, an idea put forward by Ruse (1998) is that the best way to characterise a species is to "make a pluralism", which is supported by the "Waltonian Concept" of Mann (1999), integrating the evidence from diverse areas such as anatomical, morphological, ecological and breeding characters to get a meaningful outcome. Hence, the main focus of this study is to amalgamate morphological and molecular data to define and reconcile species concepts within *Polytrichum* sect. *Polytrichum*.

1.7 Objectives of the Study.

May (2004) felicitously states that "*taxonomy provides the bricks and systematics the plan, with which the house of biological sciences is built*". This statement emphasizes that taxonomy deals with the recognition of taxa, whereas systematics deals with classifying them according to their phylogenetic relationships. Thus, the main objective of the taxonomic revision in this project will the application of sound molecular inference, integrated with morphological evidence, to circumscribe the extant species in *Polytrichum* sect. *Polytrichum* under the ICN (International Code of Nomenclature for Algae, Fungi and Plants; Turland & al., 2018).

Many previous taxonomic treatments of the Polytrichaceae have been restricted to relatively narrow geographic regions, thus covering only certain subsets of the group (Smith,

1971,1974; Crum & Anderson, 1981; Long, 1985; De Sloover, 1986; Smith Merril, 2007; Peralta & Yano, 2010; Aponte & Uribe, 2017). Even though *Polytrichum commune* Hedw. is one of the oldest names in bryology, it has historically most often been used in a general sense to encompass both of the two allied taxa *P. uliginosum* (= *P. commune* var. *commune*) and *P. commune* s.s (= *P. commune* var. *perigoniale*), while the relationship and circumscription of these groups has been uncertain. Hence, the necessity of producing a comprehensive alpha taxonomic revision of *Polytrichum* sect. *Polytrichum* became a priority to elucidate the taxonomic confusion within the group.

This taxonomic revision will be the first monographic study of *Polytrichum* sect. *Polytrichum* on a world-wide basis. Therefore, the main objectives of the present study were to:

1. Provide an overview of the taxonomic and nomenclatural history of Class Polytrichopsida with special emphasis on *Polytrichum* sect. *Polytrichum* (**Chapter 01**).
2. Find a lectotype for the taxon *Polytrichum commune* Hedw. in order to establish the correct identity of *P. commune* and its allied taxa. (**Chapter 02**).
3. Construct a phylogeny for *Polytrichum* sect. *Polytrichum* to circumscribe the extant species diversity using both Sanger and NGS approaches (**Chapter 03**).
4. Provide a detailed alpha-taxonomic revision for *Polytrichum* sect. *Polytrichum* on a global scale (**Chapter 04**)
5. Discuss the findings of the research and highlight suggestions for potential future work on Polytrichaceae (**Chapter 05**) In this study the following key topics are presented and discussed.

Chapter 02 addresses one of the most critical aims of the present study and has led to a major publication (Kariyawasam & al., 2021). This chapter provides a historical background to bryophyte nomenclature with special emphasis on Johannes Hedwig (1730-1799), “Father of Modern Bryology”, and his collections housed in the Geneva (G) herbarium. It describes a methodology to address a complex nomenclatural problem and to select a single lectotype from a mixture of a potential type material, ruling out other candidate specimens with the aid of historical taxonomic literature. This chapter also discusses the correct identity and diagnostic characters of the well-known moss taxon *Polytrichum commune* Hedw., based on the selected type material.

Chapter 03 comprises the large central core of this dissertation in providing a phylogeny for *Polytrichum* sect. *Polytrichum*. This has two parts. The ***main text*** constructs a detailed Sanger phylogeny using four plastid markers (namely, *rbcL*, *trnL-F*, *rpl16* and *trnG*), and two nuclear markers (ITS-1 and ITS-2) to infer relationships within the study group using DNA extracted from herbarium specimens. Using both Maximum Likelihood (ML) and Bayesian Inference (BI), the phylogeny generated by Sanger sequencing, which delimits five distinct phylogenetic species and a poorly supported apical clade (Clade A) representing a species complex comprising three morphologically distinct species within the monophyletic group. Possible explanations are given for the existence of this poorly supported apical clade with the aid of a haplotype network analysis of the nuclear ITS-2 compartment. This section provides the first detailed phylogenetic inference presented for *Polytrichum* sect. *Polytrichum* with thorough specimen sampling covering a broad geographic span of the extant taxa within the group. It confirms the identity of *Polytrichum perigoniale* Michx. as a separate entity/species in its own right, which is likely to be paraphyletic containing some undescribed pseudo-cryptic species. The ***appendix of this chapter*** presents a preliminary, collaborative NGS phylogenetic framework based on hybrid capture data, with much more limited taxon sampling but a much larger volume of multi-locus nuclear data, to compare the phylogenetic signal obtained with that derived from the Sanger sequencing data. Despite the very different numbers of markers and samples used in these approaches a similar tree topology was observed, demonstrating mutual corroboration of these methods for the phylogeny of *Polytrichum* sect. *Polytrichum.* It further discusses the utility of herbarium specimens in phylogenomic studies.

Chapter 04 provides a complete, up-to-date alpha taxonomic revision of *Polytrichum* sect. *Polytrichum* on a global scope. This is the first detailed taxonomic revision presented for the group. It includes the taxonomic circumscription of all extant species in *Polytrichum* sect. *Polytrichum* based on thorough herbarium study. In the light of the phylogenetic classification (Chapter 03), it addresses the current misapplication of species concepts and some nomenclatural issues encountered within the group. New discoveries (the sporophytes of *P. ericoides*), new species records, and the unearthing of some type material (e.g. a lectotype for *P. brachymitrium*) are also included in this chapter.

Chapter 05 synthesises the main findings and possible conclusions about the major taxonomic and phylogenetic advances of the present research as discussed in the two-central data-based chapters of the thesis. The chapter discusses whether Sanger sequence analysis is still relevant, and some possible improvements for future NGS phylogenomic studies. It also suggests useful future directions for improving the major taxonomic and phylogenetic approaches to research in the family Polytrichaceae.

1.8 Significance of the Present Study

The current research provides the first integrative alpha taxonomic revision for *Polytrichum* sect. *Polytrichum* by integrating both molecular and morphological data. The findings have contributed towards actual or potential publications.

Chapter 01 (This chapter) consolidates an updated review of the taxonomic history, phytogeography and current trends in anatomical and physiological studies of Polytrichosida in general.

Chapter 02 has already been published in the journal TAXON (Kariyawasam & al., 2021), addressing a century-old nomenclatural issue in bryology and establishing a lectotype for *Polytrichum commune* Hedw. It resolves many misapplications of names in herbaria worldwide and also helps field bryologists to identify *P. commune* and its allied taxon *P. perigoniale* in the field. The work also led to contribution as a co-author to the recent revised checklist of bryophytes of Europe, Macaronesia and Cyprus (Hodgetts & al., 2020), and to the latest review paper dealing with the Polytrichopsida (Bell & al., 2021), of which I am a co-author.

Chapters 03 & 04 provide updated species delimitations in a form of an alpha taxonomic revision. One of the major findings is circumscribing the two Arctic taxa *P. swartzii* and *P. jensenii* as distinct species in Holarctic region. These findings were used for the recent publication on the Moss Flora of Sweden (Lönnell, & al., 2019), in which I have been cited as a contributor. The phylogenetic studies in Chapter 3 should form the basis of at least one major publication in an international journal, with the taxonomic work leading to a further one, as well as likely to a number of smaller publications.

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CHAPTER 02

LECTOTYPIFICATION OF *POLYTRICHUM COMMUNE* HEDW.

"Fragments of the natural method must be sought with the greatest care. This is the first and last desideratum among botanists" — Carolus Linnaeus; *Philosophia Botanica*

GENERAL INTRODUCTION

The taxon *Polytrichum commune* Hedw. (Bryophyta; Polytrichaceae) was first described by Linnaeus (1753) and was validated by Hedwig (1801: 88) in his *Species Muscorum Frondosorum*, the starting point of moss nomenclature. It was previously described and illustrated by Dillenius (1747) and other contemporary botanists during the pre-Linnaean era as the "Square-headed great Goldilocks Moss" in Europe. However, even 200 years after its valid publication, *P. commune* remains to be typified. Moreover, recognising the "genuine" *P. commune* concept was a challenging, since it is one of the most widespread and ecologically important moss species of northern temperate and boreal regions. The name *P. commune* has often been used ambiguously for several morphologically cryptic and pseudocryptic taxa over the past centuries (see Chapters **03** & **04**). The "genuine" *P. commune* concept could only be formalised by assigning a lectotype from the original material; other nomenclatural issues for allied subspecific taxa were also addressed in the current study.

This lectotypification study was initiated in October 2017 with the aid of collaborators from Helsinki (Prof Jaakko Hyvönen), Geneva (Prof Michelle Price) and the Royal Botanic Garden, Edinburgh (Dr David Long and Dr Robert Mill) as a part of this PhD project.

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Unearthing a lectotype for *Polytrichum commune* Hedw. (Bryophyta, Polytrichaceae)

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2.1 Abstract

The name *Polytrichum commune*, validated in Hedwig's *Species muscorum frondosorum* of 1801, was based on earlier entities that can be traced back to the pre-Linnaean literature of the early 16th century. More than 200 years after its valid publication it remains to be typified. The single herbarium sheet for *P. commune* in the Hedwig-Schwägrichen Herbarium in G contains nine specimens from different continents that represent four different species (*P. commune, P. juniperinum, P. perigoniale, P. subpilosum*). After careful study of the origins and taxonomic affinities of the specimens on this sheet, a lectotype is designated.

Keywords: nomenclature, bryophyte systematics, *Polytrichum*, mosses.

2.2 Introduction

Polytrichum commune Hedw. (the "Common Haircap" moss), is one of the most ecologically important (Wilson & Provan, 2003; Bell & Hyvönen, 2010a,b) and widespread mosses globally (Osada, 1966; G.L. Smith, 1971; Crum, 1976; Crum & Anderson, 1981). Due to its large size and broad distribution, *P. commune* has been used as a model bryophyte species in ecological and physiological studies (Sarafis, 1971; Thomas & al., 1990; Bell & Hyvönen, 2010a; Biersma & al., 2017; Brodribb & al., 2020). It has been used for centuries as an important medicinal herb (one of the so-called Quinque Herbae Capillares, or Five Capillary Herbs), and as such has been included in many herbals and pharmaceutical dispensatories, including the list of plants cultivated at the forerunner of the modern Royal Botanic Garden Edinburgh (Sutherland, 1683) and the Edinburgh Pharmacopoea (Edinburgh Royal College of Physicians, 1699).

The genus *Polytrichum* Hedw. (Polytrichaceae) was one of the first mosses illustrated in the taxonomic literature of the pre-Linnaean era (Dodoens, 1578). *Polytrichum commune*, in particular, is one of the most commonly represented acrocarpous mosses in the herbaria of the early 16th century (Stech & al., 2018). However, more than 200 years after its valid publication in Hedwig (1801: 88), the name still lacks a designated type. Because of the long nomenclatural history of this taxon, crucial elements of the typification process have to include tracing the origins of the correct taxonomic concept, understanding how the concept of *P. commune* has developed over time, and outlining its taxonomic circumscription based on the validating description, as well as linking the name to a physical specimen.

Species muscorum frondosorum (hereafter abbreviated as SMF; Hedwig, 1801) is the adopted starting point for the nomenclature of mosses (apart from Sphagnaceae L.; see Dixon, 1933; Florschütz, 1960; Turland & al., 2018: Art. 13.1). After the adoption of SMF as the starting point of moss nomenclature, all names published in SMF that were not described as new to science within that work were subsequently ascribed to Hedwig (Florschütz, 1960; Geissler, 2000; Price, 2005). In most cases, original material suitable for lectotypification can be found in Hedwig's own herbarium (Hb. Hedwig-Schwägrichen) housed in G (Price, 2005). The Hedwig part of the Hedwig-Schwägrichen herbarium contains the original material that is essential for ensuring the correct application of many early moss names (Florschütz, 1960; Geissler, 2000; Price 2005), the importance of which has been explained by Geissler (2000) and Price (2005).

Hedwig (1801: 88) broadly defined *Polytrichum* Hedw. as follows: "*Peristomium simplex: denticuli breves, duplo plures, membranulam apicibus prehendentes. Flos masculus femineusque terminalis*", translated as: "a simple peristome with a doubled number of short teeth that possess a membrane at their apices. Male and female gametangia terminal". The genus *Polytrichum*, as treated by Hedwig (1801), comprised 17 species: *P. aloides* Hedw., *P. alpinum* Hedw., *P. commune*, *P. convolutum* Hedw., *P. dendroides* Hedw., *P. formosum* Hedw., *P. hercynicum* Hedw., *P. juniperinum* Hedw., *P. magellanicum* Hedw., *P. nanum* Hedw., *P. norwegicum* Hedw. (= *Polytrichastrum alpinum* (Hedw.) G.L.Sm.), *P. pensilvanicum* Hedw., *P. piliferum* Hedw., *P. pulverulentum* Hedw. (= *Pogonatum urnigerum* (Hedw.) P. Beauv.), *P. pumilum* Hedw., *P. undulatum* Hedw., and *P. urnigerum* Hedw., and one infraspecific taxon (*P. undulatum* var. *minus* Hedw.). Out of these 18 taxa *P. formosum*

and *P. pumilum* (= *Pogonatum nanum* (Hedw.) P.Beauv.) were newly described by Hedwig (1801). All the other names and polynomials used by Hedwig under the genus *Polytrichum* in SMF originated from earlier works, such as those of Vaillant (1727), Dillenius (1741), Linnaeus (1763), Hedwig, (1787) and Bridel (1798). Hedwig (1801) placed the species he enumerated into four main groups based on features of the apophysis of the capsule and the stems (simple or branched), as follows:

i. *Sporangio apophysi protuberante instructo, caule simplici* (*P. commune*, *P. juniperinum*, *P. piliferum*, *P. pulverulentum* and *P. formosum*)
ii. *Sporangio apophysi instructa, caule ramoso* (*P. alpinum*)
iii. *Sporangio absque apophysi protruberante, trunco simplici* (*P. convolutum*, *P. hercynicum*, *P. nanum*, *P. aloides*, *P. pensilvanicum*, *P. pumilum* and *P. undulatum*)
iv. *Sporangio absque apophysi, trunco ramoso* (*P. norwegicum*, *P. unigerum*, *P. magellanicum*, and *P. dendroides).*

Hedwig's broad concept of *Polytrichum* was gradually narrowed through subsequent studies by later bryologists, resulting in the recognition of six segregate genera: ***Atrichum* P.Beauv.** which includes *A. undulatum* (Hedw.) P.Beauv. and *P. undulatum* var. *minus* Hedw. (= *A. tenellum* (Röhl.) Bruch & Schimp.); ***Pogonatum* P.Beauv.** with *P. aloides* (Hedw.) P.Beauv., *P. convolutum* (Hedw.) P.Beauv., *P. nanum* (Hedw.) P.Beauv., *P. pensilvanicum* (Hedw.) P.Beauv. and *P. urnigerum* (Hedw.) P.Beauv.; ***Oligotrichum* Lam. & DC.** with *O. hercynicum* (Hedw.) Lam. & DC.; ***Polytrichadelphus* (Müll.Hal.) Mitt.**, with *P. magellanicus* (Hedw.) Mitt.; ***Dendroligotrichum* (Müll.Hal.) Broth.** with *D. dendroides* (Hedw.) Broth.; and ***Polytrichastrum* G.L.Sm.** with *P. alpinum* (Hedw.) G.L.Sm. Thus, out of the 18 taxa initially recognized under *Polytrichum* by Hedwig only four of his names now remain in this genus: *P. commune* (the type species), *P. formosum*, *P. juniperinum* and *P. piliferum*.

Hedwig's original concept of *P. commune* can be elucidated by exploring three major elements: (a) Hedwig's (1801) references to earlier treatments (b) his original description of *P. commune,* and (c) his specimens in the Hedwig-Schwägrichen herbarium in G.

a. Citations from earlier literature

Hedwig (1801) used the binomial "*Polytrichum commune*" from *Species plantarum* (Linnaeus, 1763), that also appeared in *Systema vegetabilium* (Linnaeus, 1784) as well as the slightly later work *Muscologia recentiorum* of Bridel (1798). Although moss names published before 1 January 1801 have no nomenclatural standing, it is useful to look at the earlier treatments of putative *Polytrichum* species to help understand Hedwig's taxonomic concept of the genus and its constituent species.

In the validating description of *Polytrichum commune*, Hedwig (1801: 88) cited the earlier polynomials of Plumier (1705), Barrelier (1714), Vaillant (1727), Micheli (1729) and Dillenius (1741), as well as the binomial "*Polytrichum commune*" of Linnaeus (1763, 1784), which was used by Bridel (1798). Linnaeus's (1763) concept of *P. commune* is clearly a broad one as he cited several earlier polynomials in synonymy. Moreover, Linnaeus (1763) stated that *P. commune* was common in Europe. To assist in understanding Linnaeus's concept of his species of *Polytrichum*, all specimens designated by himself as *P. commune* (mounted on sheets Herb. Linnaeus 1263.1 and 1263.2) housed in the herbarium of the Linnean Society of London (LINN), were studied by the first author (see the discussion below).

Dillenius's herbarium sheet, with several stems of *P. commune*, is housed in the herbarium of the University of Oxford (OXF [HM_420-001]). It is presumed that Linnaeus had studied material exchanged with Dillenius (Isoviita, 1970). Dillenius's polynomial "*Polytrichum quadrangulare vulgare, yuccae foliis serratis*" (= the Common 4-angled *Polytrichum*, with serrate leaves [like those] of *Yucca*) and proposed common name; "*The common bigger square-headed Bog Polytrichum, or great Goldilocks"*, both refer to the 4-angled form of the capsule, while 'great Goldilocks' alludes to the calyptra made up of golden hair-like fibres, these being prominent features of *Polytrichum* section *Polytrichum* to which *P. commune* belongs. These features are also found in *Polytrichum* section *Juniperifolia* Brid., however members of this latter section never have serrate leaves (Smith-A.J.E., 2004).

To establish the correct identity of the specimens of *P. commune* in the herbaria of Dillenius and Linnaeus, it was necessary to examine leaf sections to observe the anatomy of the leaf lamina, and particularly of the lamellar end-cells (or the apical cells of leaf lamellae), which are crucial for distinguishing many species of *Polytrichum* from each other. The deeply grooved or U-shaped lamellar end-cells of the lamellae are the most important anatomical

character of *P. commune* (Smith, 2004). The specimens on sheet HM_420-001 in the Dillenius herbarium (OXF), except for the smaller, fertile stem at the top left hand corner of the herbarium sheet and the specimens of the Linnaean herbarium (LINN 1263.1 – two sterile stems glued on a small sheet at the top left hand corner of the herbarium sheet & LINN 1263.2 – the first element comprising a single sterile stem at the left-hand side, out of five elements glued on the herbarium sheet) possess the deeply grooved lamellar end-cells characteristic of *P. commune*. This defining character of *P. commune* was not mentioned by Hedwig, Linnaeus or Dillenius as they did not include anatomical observations of leaf sections in their respective works. Characters, such as the prominent, transparent leaf sheath (amplexicaul), serrated leaf margins and tapering leaf apices, as well as the round ("disc-like") depressed apophysis of the four-angled capsule, were mentioned by Hedwig (1801) in his protologue, and these features were also observed in the specimens of Dillenius (OXF [HM_420-001] – both leaf and capsular characters) and Linnaeus (LINN 1263.1 and 1263.2 – leaf characters only).

The illustrations in the works cited in Hedwig's protologue also include details relevant to the current typification. Vaillant (1727: t. 23, fig. 8) depicted only sterile plants, whereas the other illustrations seen and studied by Hedwig, i.e., Plumier (1705: t. B, 2 figs. numbered 6), Barrelier (1714: fig. 251.III), Micheli (1729: t. 59, fig. 1) and Dillenius (1741: t. LIV, fig. 1) are of fertile plants but do not clearly illustrate a 4-angled capsule with a prominent constricted apophysis, an obvious and ubiquitous feature of *Polytrichum commune* and related species in *P.* sect. *Polytrichum* and sect. *Juniperifolia*. Hedwig himself (1782: tab. VII, fig. 37) had previously illustrated a capsule of *P. commune* (Fig. 2.1), showing the 4-angled capsule and the prominent, constricted apophysis, although he did not refer to his own illustration in what became the validating description of *P. commune*.

Figure 2.1: *Polytrichum commune* Hedw.: plate from Hedwig (1782, Tab. VII, Fig. 37) illustrating the strictly four-angled capsule with the attached operculum. (Reproduced with the permission of the rare books collection, library of the Royal Botanic Garden Edinburgh).

b. *Hedwig's original descriptions of* Polytrichum *and* Polytrichum commune

The validating description of *P. commune* (Hedwig, 1801:88) consists of a short diagnosis "*trunco simplici, foliis serrulatis acutis, sporangio quadrangulo*" followed by polynomials from Linnaeus (1763) and Bridel (1798) "*Polytrichum commune caule simplici, anthera parallelepipeda*", Dillenius (1741) "*Polytrichum quandrangulare vulgare Juccae foliis serratis*", Plumier (1705), Barrelier (1714) and Micheli (1729) "*Muscus capillaceus major, pediculo et capitulo crassioribus*" and Vaillant (1727) "*Muscus juniperifolious capitulo quadrangulo*". A description of this species was also elaborated under Hedwig's "observatio", in which *P. commune* was characterized by the rounded apophysis with a strong depression (constriction), the angled mature capsules and epiphragm. His earlier description of the number of peristome teeth from Hedwig (1782) was amended from '32' to 'numerous' [although the actual number of teeth found in the species is generally 64 (Smith, 1971; Smith, 2004; Bell & Hyvönen, 2010a].

Hedwig's (1801) original concept of *P. commune* was thus very broad and did not distinguish it from other related species, as presently delimited. Moreover, some features described were also included in the generic description of *Polytrichum*. In the protologue it was mentioned that

P. commune was common throughout Europe ("*vulgaris per totam Europam*") with no specific localities or collectors given.

c. The *Hedwig-Schwägrichen herbarium in G.*

Hedwig died on 7 February 1799 and his student Christian Friedrich Schwägrichen (1775–1853) took over editing SMF, bringing it to publication in 1801 (Florschütz, 1960; Price, 2005). At the time of his death Hedwig had finished the manuscript for *Phascum* Hedw., *Sphagnum* L., *Gymnostomum* Hedw., *Anictangium* Hedw. (= *Hedwigia* P.Beauv.), *Tetraphis* Hedw., *Andreaea* Hedw., *Octoblepharum* Hedw., *Splachnum* Hedw., *Cynontodium* Hedw.[rejected against *Distichium* Bruch & Schimp.] *Encalypta* Hedw., *Weissia* Hedw., *Grimmia* Hedw., *Pterigynandrum* Hedw., *Polytrichum*, *Didymodon* Hedw., *Trichostomum* Hedw. and *Barbula* Hedw. in part, i.e. up to p. 114 (Florschütz, 1960). Inevitably SMF included data that was added by Schwägrichen after Hedwig's death. This additional data was added in parenthesis into Hedwig's original text and suffixed with an "S" for 'Schwägrichen'. Schwägrichen took around one and a half to two years to finalise the SMF prior to its publication (Price 2005, Price & Ellis, 2011). Considering the number of genera and associated species descriptions that Schwägrichen completed, it is not surprising that some of Hedwig's original herbarium sheets were annotated entirely by Schwägrichen (Price & Ellis, 2011). Price & Ellis (2011) stated that the specimens on typical Hedwig herbarium sheets with labels that were annotated by Schwägrichen should not automatically be disregarded as potential type material for Hedwig's moss names for two reasons: firstly, based on the dates of each author's active contributions to compiling the SMF prior to its publication, and secondly, based on the information given by Schwägrichen himself on the magnitude of the work and the number of taxa that needed to be completed. Although Hedwig appears to have treated *Polytrichum* within SMF, none of the sheets of Hedwig's Polytrichaceae housed in G bear Hedwig's own handwriting; but were annotated by Schwägrichen, with specimens of multiple origins present on almost all of the sheets (see Price, 2005).

2.3 Materials and Methods

The single herbarium sheet for *Polytrichum commune* in the Hedwig-Schwägrichen herbarium (G barcode [G00040355]) contains nine different specimens (Figure 2: labelled by us A–I, from left to right). All nine stems were studied by the first and last authors while visiting G. A single leaf from each specimen was carefully removed and soaked in 70% ethanol and warmed in a

ca. 5% KOH solution before being soaked in warm water. Each leaf was carefully sectioned, and the sections were mounted in Hoyer's solution and retained as reference material along with the herbarium sheet in G. The first author prepared illustrations using a camera lucida attachment fixed to a Zeiss AX10 compound light microscope.

Material from all of the nine elements (specimens) needed to be carefully examined and their origins established in order to understand which belonged to the original material from which a lectotype could be selected. According to Schwägrichen's annotations, two elements (**Figure 2.2: A & G**) originated from outside Europe and one represented material that had been received from Linnaeus, approaching Bridel's '*P. appressum*.'

Element A, annotated by Schwägrichen on the sheet initially as "b" then corrected to "c" in the same handwriting and relating to "c. *Commers."* on the label, comprises a branched stem with three capsules. This collection had probably been gifted to Hedwig by the French collector Philibert Commerson (1727–1773). Commerson's voyages took place around 1770–71 mainly to Madagascar, Mauritius (*Isle de France)*, Réunion and the Straits of Magellan [where he collected plants in 1767 that Hedwig (1801) described as *P. magellanicum*; Cap, 1861]. After careful anatomical study, ***element A*** belongs to *P. subpilosum* P.Beauv., the most common and most variable species of *Polytrichum* found in Africa as well as in Madagascar, Mauritius and Réunion (De Sloover, 1986). Given the identification of the material, element A would not be a logical lectotype because it does not correspond to the current delimitation of *P. commune*.

Element B is a single stem with an intact dehisced capsule; ***element C*** is a single stem with a detached capsule; ***element D*** is a single fertile stem, however, the capsule itself is missing from the specimen. ***Elements F*** and ***H*** are single sterile stems and ***element I*** is a single stem with a complete but undehisced capsule. The exact provenance of each of these was not stated. All are presumed to be from Europe and could be parts of the same or different gatherings. Leaf cross sections of all six elements show deeply grooved, U-shaped lamellar end-cells, while the combination of this feature with a lack of papillose projections on lamellar end-cells, strictly serrate leaf margins, broad and excurrent costae, and longer inner perichaetial leaves with a comparatively larger leaf sheath and a white and hairy acumen collectively favours their identity with the modern concept of *P. commune* while excluding other superficially similar taxa with deeply grooved lamellar end-cells such as *P. jensenii* I.Hagen, *P. subpilosum* and *P. ericoides* Hampe (Long, 1985; De Sloover,1986; Smith 1976). Amongst them, ***B*** & ***I***

correspond most closely with the characters given by Hedwig in his brief diagnosis by virtue of including attached sporophytes. Of these two, ***I*** includes an undamaged capsule which shows the conspicuous constriction of the disc-like apophysis, the mucronate operculum and the four-angled capsule. Given its fertile status and the presence of the four-angled capsule (*sporangio quadrangulo*), ***element I*** is the most appropriate specimen to serve as a lectotype *P. commune.*

Element E comprises four short stems, one of which bears a very immature capsule covered by a calyptra. The plants have infolded leaf margins and leaf anatomical studies confirmed that the leaf lamellae possess ovate to pyriform end-cells with a distinct papillose knob, which confirms its identity with the modern delimitation of the taxon *P. juniperinum* (Smith-A.J.E, 2004). Since element ***E*** does not agree with the modern delimitation of the taxon it is not a logical choice to select as a lectotype for *P. commune*.

Element G is a short stem in the middle of the sheet; it was annotated by Schwägrichen as "b" and cited on the label as "b *Mhlbg. 251*". Hedwig received material from Rev. Gotthilf Heinrich Ernst Muhlenberg (1753–1815) from Lancaster in Pennsylvania, U.S.A. (see Price, 2005). This element belongs to *P. commune* var. *perigoniale* (Michx.) Hampe (= *P. perigoniale* Michx.), which is the most common member of the *P. commune* complex in North America (this, together with the application of the name *P. perigoniale* in North America, will be discussed in a future work; Kariyawasam & al., *in prep.*). The lamellar end-cells in this element from N. America are less deeply grooved or even flat-topped, ruling it out as belonging to *P. commune* in the strict sense. Moreover, the specimen is incomplete and sterile. Based on these observations and the North American origins of the material this element is excluded from potential original material for *P. commune*.

Typification

Polytrichum commune Hedw., *Sp. Musc. Frond*. 88. 1801.

Type citation: "Vulgaris per totam Europam".

Specimen label: "Polytrichum commune b. Mhlbg. 251. c. Commers. a. Linn ipso interum commune β interum polytr. appressum" (G[G00040355!].)

Lectotype *(designated here)*: [Europe?] *s.d., sin. coll., s.n.* (G, Hb–Hedwig-Schwägrichen [G00040355]), first specimen of the right-hand side (element I), see **Figure 2.3.**

Taxonomic Description Based on the Type Material

Stem erect, rigid, unbranched, often over 10 cm tall. ***Leaves*** densely aggregated, sharply divided into broad sheathing base and narrower, lamellate limb, when moist widely spreading often squarrose-recurved, when dry appressed to the stems at base with somewhat spreading tips above, linear-lanceolate, gradually narrowing from the base of the limb upwards to a sharp acumen, margin densely and sharply serrate from the limb base to the apex; number of lamellae 60–70, 4–6 cells high; lamellar end-cells broader than the others, deeply grooved or U-shaped. ***Perichaetial leaves*** morphologically distinct from the stem leaves, with a long sheathing base. ***Capsule*** 4-angled, shortly rectangular, 3.5–4.0 × 2–3 mm; apophysis very distinct, discoid, narrowly constricted above; exothecial cells composed of conical papillae with slit-like apertures; operculum with a short rostellate beak; peristome teeth ca. 0.4 mm high, obtuse, pale, basal membrane low, brownish; spores 7–9 µm. ***Seta*** 4(–5) – 7(–8) mm long. ***Calyptra*** golden-yellow, fibrillose and completely covering the capsule, 13–15 mm long.

Additional material of *P. commune* examined

Herb. Linn. [LINN 1263.1 (two top left-hand stems) & 1263.2 (first top-left hand stem out of five stems)]. *Polytrichum commune, sin. coll., s.n., s.d.*

Herb. Oxf. (OXF [HM_420-001]. *Polytrichum quadrangulare vulgare, yuccae foliis serratis (= P. commune), sin. coll., s.n., s.d.*

Figure 2.2: *Polytrichum commune* Hedw.: original Hedwig herbarium sheet from the Hedwig-Schwägrichen Herbarium collection in G (herbarium sheet G00040355) with the newly selected lectotype indicated [**Element I among nine (A–I) elements**].

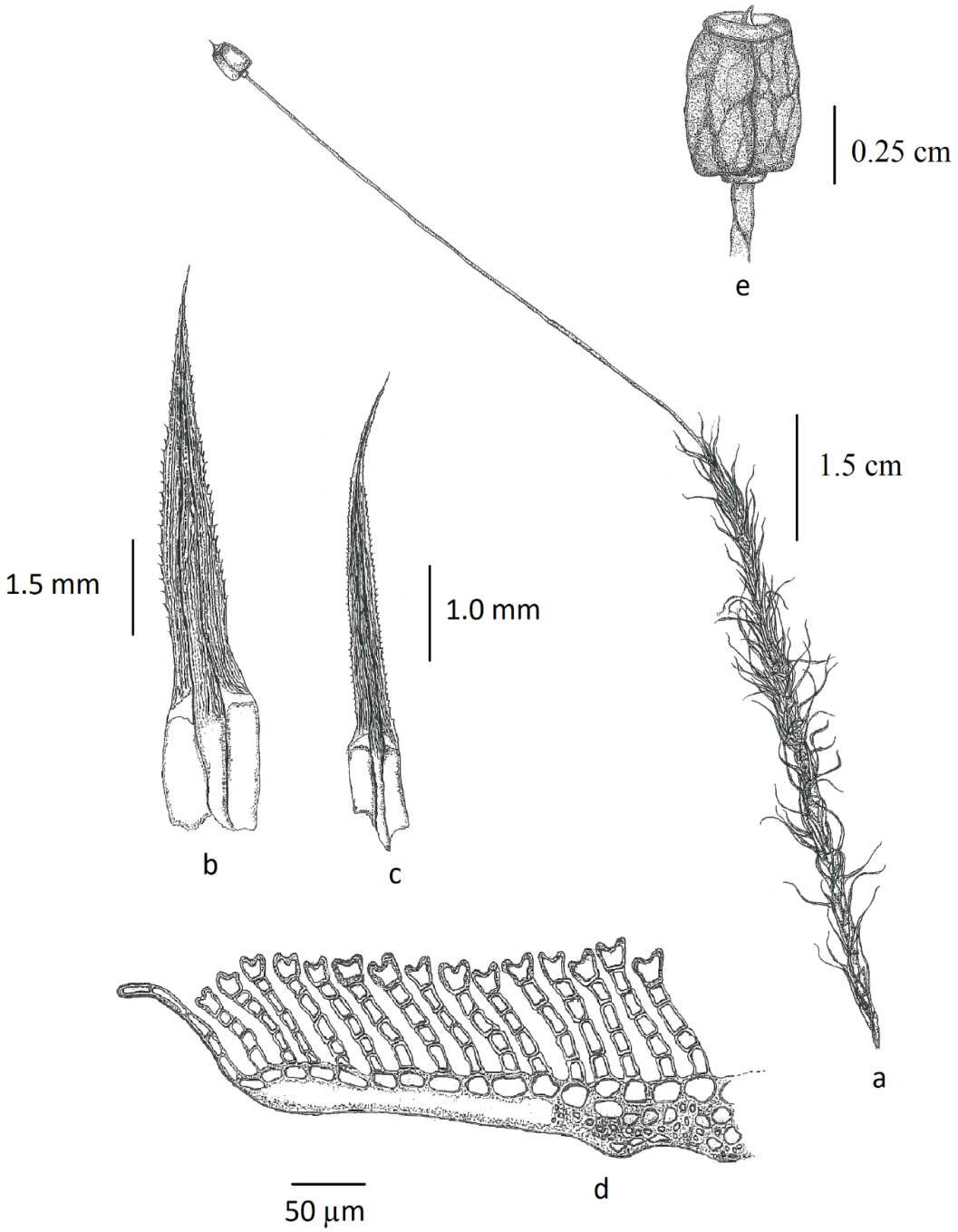

Figure 2.3: *Polytrichum commune* Hedw.: illustrations (a–e) of material on the original herbarium sheet in G (G00040355- drawn from the element I): (a) habit (non-hydrated), (b) adaxial side of a vegetative leaf (hydrated), (c) adaxial side of an inner perichaetial leaf (non-hydrated), (d) leaf cross-section illustrating the deeply-grooved or U-shaped lamellar end-cells, (e) strictly four-angled, undehisced capsule with a prominent apophysis [Drawn by Isuru Kariyawasam].

2.4 Discussion

Polytrichum commune, a widespread and fairly recognizable moss, is mostly distinguished by its large size, growing in tufts with 20–45 cm long wiry stems (Long, 1985; Smith, 1971; Smith 2004). Examination of the original material from G revealed that several different elements were present on the type sheet [G00040355], from different continents (Europe, North America and Africa) and representing different taxa (*P. commune, P. perigoniale* and *P. subpilosum*). The lectotype for *P. commune* selected here from amongst the material on the Hedwig herbarium sheet in G, corresponds most closely with the protologue and the current delimitation of this taxon. Although *P. commune* was not illustrated by Hedwig (1801), in his earlier publication (Hedwig, 1782) his illustration clearly shows the strictly four-angled capsule, that can also be seen in the lectotype selected. One of the anatomical features now recognized as diagnostic for this taxon, namely the end-cells of the leaf lamellae that are deeply grooved or U-shaped, was not mentioned in the early works of Linnaeus (1763), Dillenius (1741), Bridel (1798) or Hedwig (1782, 1801). However, this feature was observed in the lectotype, as well as in other specimens of *P. commune* in the collections of Linnaeus (LINN) and Dillenius (OXF). Detailed morphological features of the selected lectotype are illustrated in Figure 3.

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CHAPTER 03

MOLECULAR PHYLOGENY AND SPECIES DELIMITATION

"*We are survival machines – robot vehicles blindly programmed to preserve the selfish molecules known as genes. This is a truth which still fills me with astonishment*."

— Richard Dawkins, *The Selfish Gene*

3.1 Aims and Objectives of the Molecular Study

The present study integrates the current understanding of morphological concepts of extant taxa in *Polytrichum* sect. *Polytrichum* with a backbone molecular phylogeny. In this study, I have used the "phylogenetic species concept", considering the principle of "monophyly" [i.e. a monophyletic group consists of an ancestor and all of its descendants; commonly inferred from possession of shared derived character states] put forward by Willi Henning ([Henning, 1950] as cited in de Queiroz, 2007) to define species as clades, corroborated by consistent and definitive morphological character differences to differentiate and describe species. In a phylogenetic context, Coyne & Orr (2004) defined "a species" as the smallest (exclusive) monophyletic group of common ancestry.

Polytrichum sect. *Polytrichum* is a well circumscribed clade that includes, amongst other species, all plants currently recognised within the species concept of *Polytrichum commune* Hedw. *Polytrichum* sect. *Polytrichum* has not previously been studied or fully revised with the aid of molecular systematics on a global scale. However certain regional taxonomic revisions and phylogenetic work are available based on morphological characters (Smith, 1971; Long, 1985; De Sloover, 1986; Forrest, 1995; Hyvönen, 2006; Peralta & Yano, 2010; Aponte & Uribe, 2017). Moreover, only a few representative extant taxa within the section have been included in recent molecular phylogenetic studies (Hyvönen, 1998; Hyvönen & al., 2004, 2006; Bell & Hyvönen, 2008; 2010 a, b, 2012). Arctic and Sub-Arctic taxa such as *P. swartzii* Hartm. and *P. jensenii* I. Hagen and the South American taxon *P. angustifolium* Mitt. have never been included in molecular phylogenetic studies of Polytrichaceae.

Over the last few decades, based on different molecular systematic approaches, phylogenetic relationships among *Polytrichum* species have remained uncertain. Based on

allozyme and RAPD (Randomly Amplified Polymorphic DNA) analysis data, Bijlsma & al. (2000) showed that within the *P. commune sensu lato* complex there appear to be two genetically distinct species, which they treated as [5]*P. commune* (≡ *P. commune* var. *perigoniale*) and *P. uliginosum* (≡ *P. commune*. var. *commune*) [from this study-See Chapter 04, *P. commune* (≡ *P. commune* var. *perigoniale*) is confirmed as *P. perigoniale* Michx. and *P. uliginosum* (≡ *P. commune* var. *commune*) is confirmed as *P. commune* Hedw.], consistent with morphological and ecotype differentiation. This was corroborated by Bell & Hyvönen (2010a), who stated that "the common and widespread species *Polytrichum commune* is not monophyletic, the systematics of this important taxon will be the subject of further study…"

Bell & Hyvönen (2010a) showed that *P. commune sensu lato* is paraphyletic, with the South American species *P. ericoides* and *P. brachymitrium* apparently being more closely related to *P. commune sensu stricto* than the latter is to *P. uliginosum* (although their specimen of *P. ericoides* is here identified as *P. brachymitrium*, see below & Chapter 04). Within the *P. commune s.l* complex there appear to be a number of cryptic or pseudo-cryptic species (see the discussion below), as well as morphologically intermediate forms. Hence, this provides a stronger reason to perform a molecular taxonomic study, in order to circumscribe and delimit the species concepts in *Polytrichum*. sect *Polytrichum*.

Two molecular approaches are utilized:

1. A traditional Sanger Sequencing approach (see section 3.2: part A) was performed, with robust taxon sampling, sampling six molecular markers (including four plastid markers and two nuclear markers).

2. A Next Generation Sequencing (NGS) hybrid capture approach (see section 3.2: part B & Appendix of this chapter) was performed, with more limited taxon sampling (although still including all currently recognised taxa in the section) and a large quantity of molecular sequence data.

Liu & al. (2019) recently published a generic level moss phylogeny produced using an NGS target capture approach using organellar and nuclear exons. This only included a few taxa from the family Polytrichaceae. An extensive NGS approach following target enrichment (the

[5] Present study confirms the identities of two varieties of *P. commune* Hedw. used by Bijlsma & al. (2000) as follows: *Polytrichum commune* (≡ *P. commune* var. *perigoniale*) ≡ *Polytrichum perigoniale* Michx. and *P. uliginosum* (≡ *P. commune* var. *commune*) ≡ *P. commune* Hedw. *s.str*.

hybrid capture method) has never been performed to infer the phylogenetic relationships for this particular taxonomic group before. Hence, this is the first comprehensive phylogenetic study to delimit the existing "species" within *Polytrichum* sect. *Polytrichum* using an integration of both Sanger and Next Generation sequencing approaches.

The main objectives of the present study are:

- To perform a detailed molecular phylogenetic analysis of *Polytrichum* sect. *Polytrichum*, using Sanger sequence data for four plastid (*rbcL, trnL-trnF, rpl16* and *trnG*) and two nuclear (ITS1 and ITS2) markers.
- To perform a preliminary, collaborative phylogenetic study on *Polytrichum* sect. *Polytrichum* with the aid of hybrid capture (target-enrichment), using *Physcomitrella* RNA baits to sequence 809 low copy nuclear loci for 24 representative accessions of all extant taxa within the section.
- To test and infer the relationships among the species within *Polytrichum* sect. *Polytrichum* using Maximum Likelihood (ML) and Bayesian Inference (BI).
- To discuss the congruence between the extant molecular and morphological approaches and the evolutionary trajectories underlining the current species delimitations.

3.2 Materials and Methods

PART (A): Sanger Sequencing Approach

3.2.1 Taxon Sampling

Lecointre & al. (1993) considered the problem of the impact of species sampling on phylogenetic studies, as "contradictory trees of diverse reliability can be obtained from different species samples that have been chosen to infer the same evolutionary history". Hence, the selection of representative taxa to represent the extant broad picture of the phylogenetic problem is a crucial step in any kind of phylogenetic assessment. Lecointre & al. (1993) further discovered that the topology and robustness of trees obtained from a set of samples cannot be easily predicted from the topology and robustness obtained from the "whole defined universe" of taxa. Hence, a thorough taxon sampling which represents a broad spectrum of extant

morphological variation as well as the geographic distribution of the taxa will help to resolve this problem.

A node on a phylogram or a cladogram should be considered as "reliable", not only by the statistical support provided by the evolutionary model inferred in the phylogenetic analysis, but also fluctuations according to the representation of the taxa (species). Hence, the greater and denser the sampling, the better for phylogenetic accuracy (Zwickl & Hills, 2002; Heath & al., 2008).

Based on the above argument, representative accessions for each extant "morphological species" (as published in the literature) were carefully selected to cover the taxon's geographical distribution and the previously reported morphological variation. Moreover, certain herbarium specimens which have been overlooked for decades and which show certain morphological affinities with *P. commune sensu lato*, but are ambiguous with respect to some of the morphological and anatomical characters of the morphological species concepts described in *Polytrichum* sect. *Polytrichum,* were also included for the taxon sampling so that they could be placed in the molecular phylogeny.

This study is completely based on herbarium specimens and thus dried herbarium specimens were used to extract DNA. Each specimen was thoroughly examined under the dissecting microscope (Wild Heerbrugg M5) and all visible algae, fungi and soil debris were removed. Fully expanded leaves (selecting for the greenest coloured leaves in older specimens) from 1/3 of the apex with actively growing new shoots were used. For older herbarium specimens on loan from other herbaria and specimens with restrictions on destructive sampling, only a few detached leaves were taken from sub-pockets attached to the main pocket of each herbarium specimen, with the permission of the respective herbarium. Voucher information for all plant material used for the molecular analysis is given in Table 3.1.

3.2.2 Ingroup and Outgroup Taxa

(a) Ingroup Taxa

Initially 130 specimens were sampled in total, representing all currently recognised species of *Polytrichum* sect. *Polytrichum*. However, only 115 accessions (excluding the outgroup) were used to generate the final combined matrix (fifteen accessions which gave very low-quality degraded DNA resulting in a lot of missing data were excluded). Multiple accessions of each species were sampled from each widespread, variable and/or taxonomically

ambiguous taxon, such as *Polytrichum commune* Hedw. (≡ *P. uliginosum* (Wallr.) Schriebl.) and *P. perigoniale* Michx. (≡ *P. commune* var. *perigoniale* (Michx.) Hampe.). When selecting the exemplar accessions, morphological variation across the geographic regions was strongly considered, providing an opportunity to identify any unrecognised species-level diversity within the study group. With extensive sampling within the *Polytrichum commune* (*sensu lato*) (now confirmed as *P. perigoniale* from this study) and *Polytrichum uliginosum* (now confirmed as *P. commune* from this study) and *P. brachymitrium* Müll. groups, geographic representation was broader and tried to represent northern and southern temperate, arctic and sub-arctic and tropical diversity.

All ingroup sequences were newly generated for this study. Voucher information and accession numbers of all taxa used in this study are given in Table 3.1.

(b) Outgroup Taxa

Outgroup sequences downloaded from the nucleotide databases of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) are indicated in Table 3.1. Bell & Hyvönen (2010a) stated the disadvantages of selecting distantly related taxa within the family as outgroup taxa. They further mentioned that one major obstacle in previous phylogenetic studies (Bell & Hyvönen, 2008), when rooting the Polytrichopsida, was the difficulty of obtaining credible hypotheses of primary homology (alignment) for outgroup taxa. Bell & Hyvönen (2010a) considered that this is due to the phylogenetically isolated nature of the Polytrichopsida, which have considerable sequence-level divergence in non-coding regions. Therefore, to overcome alignment homology issues, *Polytrichum formosum* Hedw. and *Polytrichum piliferum* Hew., representing the two potential sister clades to *Polytrichum* sect. *Polytrichum* (*Polytrichum* sect. *Aporotheca* and sect. *Juniperifolia* respectively), were selected as outgroup taxa, and more distantly-related genera such as *Atrichum* and *Pogonatum* were avoided.

Table 3.1: Taxa sampled in the current molecular study with EDNA numbers and voucher details. [Herbarium acronyms follow the Index Herbariorum (www. nybg.org/science/ih)]

Taxon	Collector and Collection number	Country	Year of collection	Herbarium	EDNA number	Extraction ID
Polytrichum angustifolium Mitt.	D. F. Peralta, 5426	Brazil	2007	SP	EDNA18-0053091	IK 14
Polytrichum angustifolium Mitt.	D. F. Peralta, 16222	Brazil	2014	SP	EDNA18-0053092	IK 15
Polytrichum angustifolium Mitt.	D. F. Peralta, 15524	Brazil	2014	SP	EDNA19-0053313	IK 12
Polytrichum brachymitrium Müll. Hal.	H.S. Irwin, R.M. Harley & G.L. Smith, 32845	Brazil	1972	NY	EDNA19-0053155	IK 99
Polytrichum brachymitrium Müll. Hal.	D. M. Vital, 6285	Brazil	1976	NY	EDNA19-0053156	IK 100
Polytrichum brachymitrium Müll. Hal.	R. M. Harley, 19616 (with S. J. Mayo, R. M. Storr, T. S. Santos & R. S. Pinheiro)	Brazil	1977	NY	EDNA19-0053347	IK 116
Polytrichum brachymitrium Müll. Hal.	D. F. Peralta, 5398	Brazil	2009	SP	EDNA19-0053446	IK 135
Polytrichum brachymitrium Müll. Hal.	Carmo & D. F. Peralta, 822	Brazil	2013	SP	EDNA19-0053447	IK 136
Polytrichum brachymitrium Müll. Hal.	J. Hyvönen, 6230	Brazil	1997	H	EDNA19-0053150	IK 92
Polytrichum brachymitrium Müll. Hal.	O. Yano, 33830	Brazil	2014	SP	EDNA18-0053090	IK 13
Polytrichum brachymitrium Müll. Hal.	A. A. Speielmann, 7148	Brazil	2008	SP	EDNA19-0053450	IK 139
Polytrichum brachymitrium Müll. Hal.	D. F. Peralta, 3764	Brazil	2006	SP	EDNA19-0053448	IK 137
Polytrichum brachymitrium Müll. Hal.	D. F. Peralta, 3547	Brazil	2006	SP	EDNA19-0053449	IK 138
Polytrichum brachymitrium Müll. Hal.	S. P. Churchill & Julio C. Betancur B. 17033	Colombia	1990	MO	EDNA18-0053098	IK 24

Polytrichum brachymitrium Müll. Hal.	I. Sastre-de Jesús, 647	Venezuela	1984	NY	EDNA19-0053154	IK 98
Polytrichum brachymitrium Müll. Hal.	A. Schinini, 17696	Argentina	1979	NY	EDNA19-0053153	IK 97
Polytrichum commune Hedw.	D.H. Vitt, 13251	USA, Yukon	1975	UBC	EDNA19-0053148	IK 89
Polytrichum commune Hedw.	S. Talbot, 10-C-5	USA, Alaska	1986	NY	EDNA18-0053124	IK 52
Polytrichum commune Hedw.	O. Ivantov & D. Donskoff, 09-202	European Russia	2009	E	EDNA18-0053113	IK 40
Polytrichum commune Hedw.	W. B. Schofield, 115442	USA, Alaska	2000	NY	EDNA18-0053125	IK 53
Polytrichum commune Hedw.	K. L. Gray, 5269	USA, Idaho	2005	NY	EDNA18-0053135	IK 63
Polytrichum commune Hedw.	D.G. Long, 39518	Sweden	2010	E	EDNA18-0053083	IK 02
Polytrichum commune Hedw.	N. A. Brummit & S. McDonald, 172	Russia	2003	E	EDNA18-0053100	IK 26
Polytrichum commune Hedw.	T. Hallingback, 6394	Sweden	2012	E	EDNA18-0053110	IK 37
Polytrichum commune Hedw.	W.B. Schofield, 89416	Canada, Newfoundland	1987	UBC	EDNA19-0053147	IK 87
Polytrichum commune Hedw.	T. Hallingback,4062	Sweden	2010	E	EDNA18-0053108	IK 35
Polytrichum commune Hedw.	H. Crum & N.G. Miller ,284	USA, Michigan	1966	UBC	EDNA18-0053141	IK 70
Polytrichum commune Hedw.	W.B. Schofield, 121006	USA, Montana	2003	UBC	EDNA19-0053152	IK 96
Polytrichum commune Hedw.	I. U. Kariyawasam, IKAR, 145	Great Britain, Scotland	2016	E	EDNA19-0053168	IK 127
Polytrichum commune Hedw.	R. van der Valk 2006-10	Netherlands	2006	L [NL]	EDNA18-0053096	IK 22
Polytrichum commune Hedw.	W. R. Buck, 16232	Czechoslovakia (Czech Republic)	1988	NY	EDNA19-0053157	IK 101
Polytrichum commune Hedw.	L.E. de M. Filho, *s.n*	Brazil	1972	NY	EDNA19-0053170	IK 130
Polytrichum commune Hedw.	C. Schröck, 17016	Austria	2011	W	EDNA18-0053101	IK 27
Polytrichum commune Hedw.	T. Katagiri, 4271	Japan	2017	NICH	EDNA18-0053094	IK 18
Polytrichum commune Hedw.	D.G. Long, 44024	Great Britain, Scotland	2015	E	EDNA19-0053169	IK 129
Polytrichum commune Hedw.	D.G. Long, 41221	Latvia	2011	E	EDNA18-0053084	IK 03
Polytrichum ericoides Hampe	S. P. Churchill, 19311	Colombia	1988	MO	EDNA18-0053097	IK 23
Polytrichum ericoides Hampe	S. P. Churchill ,13325	Colombia	1985	MO	EDNA19-0053317	IK 28

Polytrichum jensenii I.Hagen	T. Hallingback, 45085	Norway	2007	E	EDNA18-0053111	IK 38
Polytrichum jensenii I.Hagen	D.G. Long, 42605	Norway	2013	E	EDNA18-0053082	IK 01
Polytrichum jensenii I.Hagen	M. Lewis, 457	USA, Alaska	1975	UBC	EDNA19-0053334	IK 95
Polytrichum perigoniale Michx.	Xu Shuming, Li Yuanmei, *s.n.*	China	1964	PE	EDNA19-0053320	IK 73
Polytrichum perigoniale Michx.	Yu Nigning, 02401	China	2008	PE	EDNA18-0053145	IK 77
Polytrichum perigoniale Michx.	X.-Y. Hu, H-0797	China	1987	PE	EDNA19-0053323	IK 78
Polytrichum perigoniale Michx.	G. Chien 33903	China	1983	UBC	EDNA18-0053095	IK 19
Polytrichum perigoniale Michx.	Y. Jia, 10093	China	2008	PE	EDNA18-0053144	IK 75
Polytrichum perigoniale Michx.	Matsumato & T. Katagiri, *s.n*.	Japan	2017	NICH	EDNA19-0053146	IK 79
Polytrichum perigoniale Michx.	N. Bell, 20.11.17.003	New Zealand	2017	E	EDNA18-0053104	IK 31
Polytrichum perigoniale Michx.	Allan J Fife, 6242	New Zealand	1984	NY	EDNA19-0054678	IK 153
Polytrichum perigoniale Michx.	J. A. Curnow, 2352	Tasmania	1988	NY	EDNA18-0053102	IK 29
Polytrichum perigoniale Michx.	W.B. Schofield, 50911	Australia	1972	UBC	EDNA19-0053324	IK 80
Polytrichum perigoniale Michx.	I. Williams, 3183/1	South Africa	1982	BOL	EDNA19-0053326	IK 82
Polytrichum perigoniale Michx.	W. R. Buck & H. Balsler, 654A	Kenya	1979	NY	EDNA19-0053159	IK 104
Polytrichum perigoniale Michx.	R.E. Magill, T. Pócs & C. LaFarge England, 9333	Tanzania	1990	GOET	EDNA19-0053325	IK 81
Polytrichum perigoniale Michx.	T. A. J. Hedderson, 17311	Madagascar	2009	BOL	EDNA19-0053311	IK 08
Polytrichum perigoniale Michx.	D.T. Holyoak. 07-493	Portugal	2007	E	EDNA18-0053107	IK 34
Polytrichum perigoniale Michx.	D.G. Long & D. Bell, 391444	Portugal	2010	E	EDNA18-0053099	IK 25
Polytrichum perigoniale Michx.	D.H. Vitt, 26350	Sweden	1990	PE	EDNA19-0053327	IK 83
Polytrichum perigoniale Michx.	R. Ochyra, 229	Poland	1978	UBC	EDNA19-0053329	IK 85
Polytrichum perigoniale Michx.	S. Lisowski,474	Germany, West Pomerania	1957	PE	EDNA19-0053330	IK 86
Polytrichum perigoniale Michx.	L. Kungu, *s.n.*	Great Britain, Ireland	2018	E	EDNA19-0053444	IK 133
Polytrichum perigoniale Michx.	D.T. Holyoak, 07-120	Great Britain, England	2007	E	EDNA18-0053114	IK 41
Polytrichum perigoniale Michx.	W. Darsalloch, *s.n.*	Great Britain, Scotland	1889	E	EDNA19-0053352	IK 132
Polytrichum perigoniale Michx.	Pirani, J.R. 5493	Brazil	2006	SP	EDNA19-0053451	IK 140
Polytrichum perigoniale Michx.	P. Majestyk, 2373	USA, Arkansas	2001	NY	EDNA18-0053121	IK 49
Polytrichum perigoniale Michx.	P. Majestyk, 2378	USA, Arkansas	2001	NY	EDNA18-0053122	IK 50
Polytrichum perigoniale Michx.	W. R. Buck, 43158	USA, Arkansas	2002	NY	EDNA18-0053123	IK 51

Polytrichum perigoniale Michx.	W. R. Buck, 15937	USA, Illinois	1988	NY	EDNA19-0054672	IK 147
Polytrichum perigoniale Michx.	W. R. Buck, 37577	USA, Oregon	2000	NY	EDNA18-0053128	IK 56
Polytrichum perigoniale Michx.	W. R. Buck, 46903	USA, Pennsylvania	2004	NY	EDNA18-0053131	IK 59
Polytrichum perigoniale Michx.	W. R. Buck, 49319	USA, Pennsylvania	2005	NY	EDNA18-0053132	IK 60
Polytrichum perigoniale Michx.	B. H. Allen, 27592	USA, Maine	2006	NY	EDNA18-0053137	IK 65
Polytrichum perigoniale Michx.	J. H. Thomas, 9801	USA, Massachusetts, Weston		UBC	EDNA18-0053140	IK 68
Polytrichum perigoniale Michx.	R .L. Wilbur & Webster, G. L, 2794	USA, Georgia	1950	UBC	EDNA19-0053333	IK 93
Polytrichum perigoniale Michx.	W. R. Buck, 51086	USA, Rhode Island	2006	NY	EDNA18-0053134	IK 62
Polytrichum perigoniale Michx.	W.R. Buck, 50398	USA, Ohio	2006	NY	EDNA18-0053127	IK 55
Polytrichum perigoniale Michx.	G.R.Smith,74-119	USA, Hawaii	1974	UBC	EDNA18-0053143	IK7 2
Polytrichum perigoniale Michx.	W. R. Buck, 44997	USA, Connecticut	2003	NY	EDNA18-0053138	IK 66
Polytrichum perigoniale Michx.	J.Y. Kekes, 964	USA, New York, Albany	2004	NY	EDNA18-0053130	IK 58
Polytrichum perigoniale Michx.	P.L. Redfearn, Jr. 9039	USA, Missouri	1961	UBC	EDNA19-0053319	IK 69
Polytrichum perigoniale Michx.	W. R. Buck, 51994	USA, New York, Clinton	2007	NY	EDNA18-0053133	IK 61
Polytrichum perigoniale Michx.	G.L.Smith, 68-2	USA, New York	1968	NY	EDNA19-0054669	IK 144
Polytrichum perigoniale Michx.	B. H. Allen, 24350	USA, New York, Putnam	2002	NY	EDNA18-0053126	IK 54
Polytrichum perigoniale Michx.	W. R. Buck, 37083	USA, West Virginia	2000	NY	EDNA18-0053129	IK 57
Polytrichum perigoniale Michx.	W. R. Buck, 31527	USA, South Carolina	1997	NY	EDNA19-0054673	IK 148

Polytrichum perigoniale Michx.	R. R. Ireland 15569	Canada, Ontario	1971	UBC	EDNA19-0053151	IK 94
Polytrichum subpilosum P.Beauv.	T.A.J. Hedderson, 19185-1	Réunion	2018	BOL	EDNA19-0054680	IK 155
Polytrichum subpilosum P.Beauv.	T.A.J. Hedderson,19208	Réunion	2018	BOL	EDNA19-0054679	IK 154
Polytrichum subpilosum P.Beauv.	T.A.J. Hedderson,18756	Réunion	2014	BOL	EDNA18-0053085	IK 04
Polytrichum subpilosum P.Beauv.	M. L. Jacobsz 5012	South Africa	1978	NY	EDNA19-0053158	IK 102
Polytrichum subpilosum P.Beauv.	W.R. Buck, 13504	South Africa	1986	NY	EDNA19-0054676	IK 151
Polytrichum subpilosum P.Beauv.	T.A.J. Hedderson, 17471	Malawi	2010	BOL	EDNA19-0053312	IK 09
Polytrichum subpilosum P.Beauv.	M. Koekemoer, 1782	Malawi	2000	NY	EDNA19-0053161	IK 120
Polytrichum subpilosum P.Beauv.	T.A.J. Hedderson, 18186	Rwanda	2012	BOL	EDNA18-0053087	IK 06
Polytrichum subpilosum P.Beauv.	M.J.E. Coode 4441	Mauritius	1974	NY	EDNA19-0053335	IK 103
Polytrichum subpilosum P.Beauv.	T.A.J. Hedderson & al. 20.5.2010/10 e	Mozambique	2010	BOL	EDNA18-0053088	IK 10
Polytrichum subpilosum P.Beauv.	T.A.J. Hedderson, 16784	Comores Archipelago, Grand Comore	2008	BOL	EDNA18-0053089	IK 11
Polytrichum swartzii Hartm.	J. Gangnon, 102.1	Québec	2015	QFA	EDNA18-0053116	IK 44
Polytrichum swartzii Hartm.	J. Gangon, D. Bastein & al., DB-2013-434	Québec	2013	QFA	EDNA18-0053117	IK 45
Polytrichum swartzii Hartm.	D. Bastien & al. DB-2013-488	Québec	2013	QFA	EDNA18-0053118	IK 46
Polytrichum swartzii Hartm.	D. Bastien & al. DB-2013-735	Québec	2013	QFA	EDNA18-0053119	IK 47
Polytrichum swartzii Hartm.	K. Hassel & T. Prestø, 691298	Greenland	2009	TRH-NTNU	EDNA18-0053106	IK 33
Polytrichum swartzii Hartm.	R. Fagerstén & M. Haapasaari s.n	Finland	1975	LD	EDNA18-0053120	IK 48
Polytrichum swartzii Hartm.	H. Roivainen s.n.	Finland	1961	UBC	EDNA19-0053328	IK 84
Polytrichum swartzii Hartm.	S. Heiømarsson *s.n.*	Iceland	2013	TRH-NTNU	EDNA18-0053093	IK 16
Polytrichum swartzii Hartm.	T. Hallingback, 4622-5	Sweden	2008	E	EDNA18-0053109	IK 36
Polytrichum swartzii Hartm.	T. Hallingback, 6271	Russia	2012	E	EDNA18-0053112	IK 39
Polytrichum swartzii Hartm.	D. H. Vitt, 7013	Canada	1972	NY	EDNA19-0054668	IK 143
Polytrichum swartzii Hartm.	W. B. Scofield & S. S. Talbot, 121386	USA, Alaska	2003	UBC	EDNA18-0053142	IK 71
Polytrichum swartzii Hartm.	W.C. Steere 16643	USA, Alaska	1963	UBC	EDNA19-0054667	IK 142
Polytrichum swartzii Hartm.	W. B. Scofield, 123648	USA, Wyoming	2005	UBC	EDNA19-0053149	IK 91

3.2.3 Selection of Molecular Markers

With the advancement of molecular technologies, deducing phylogenetic relationships using one or more molecular markers has expanded our current understanding of many bryophyte lineages. DNA markers have been employed extensively for species-level research in both bryophytes and tracheophytes. By allowing us to separate instances of homology and convergence in morphological characters, molecular phylogenies have greatly helped overcome the major constraints and controversies resulting from the classical morphology-based moss classifications, such as different taxonomic interpretations of the significance of gametophytic versus sporophytic traits and the presence of morphologically cryptic or pseudocryptic genera, families and orders that have frequently changed their circumscription through time (Carvalho-Silva & al., 2017; Huttunen & al., 2018; Bell & Ignatov, 2019).

There are three major genomes in plants from which to choose molecular markers for phylogenetic studies: mitochondrial (mtDNA), chloroplast (cpDNA) and nuclear DNA (nDNA). Both mitochondrial and chloroplast genomes are inherited through the maternal parent in mosses and many other land plants (Natcheva & Cronberg, 2007b). Mitochondrial genes have been utilised for resolving phylogenetic problems above the generic level (e.g. Cox & al., 2004) in bryophytes. The plastid genome shows moderate levels of variation, and is thus useful to delimit species and even for resolving phylogenetic patterns within species (Stech & Dohrmann, 2004; Budke & Goffinet, 2006; Bell & Ignatov, 2019).

Stech & Quandt (2010) provided a detailed overview of historical development of DNA markers in bryophyte systematics. In their publication (2010) they point out that a majority of DNA-based analyses in bryophyte systematics during the recent past have mostly used the "top five" molecular markers, namely *trnL-F*, *rbcL*, *rps4* (plastid markers) 18S and ITS (nuclear markers). They further mentioned some other useful markers newly employed during last two decades, including the plastid *atpB-rbcL* spacer, *trnG* intron, and *trnK-psbA-trnH* region, and the mitochondrial *nad5* group I (G1) intron. Many molecular phylogenetic studies on bryophytes have proven that the use of more than one molecular marker provides a better phylogenetic signal to understand the evolutionary relationships, to reconcile evolutionary trajectories of speciation and the evolution of morphological characters (Pedersen & Hedenäs, 2003; Grundman & al., 2005; Qui & al., 2006; Bell & Hyvönen, 2010 b; Bell & al., 2015).

During the last two decades bryologists have employed different DNA markers to explore the phylogenetic relationships among various taxa of Polytrichaceae, such as nuclear

18S (Hyvönen & al., 1998; Hyvönen & al., 2004; Bell & Hyvönen, 2008; Bell & Hyvönen, 2010 b; Bell & Hyvönen, 2012; Bell & al., 2015), the mitochondrial *nad5* coding region (Bell & Hyvönen, 2010b), the mitochondrial *nad5* coding region and intron (Bell & Hyvönen, 2010a; Bell & Hyvönen, 2012), and the plastid *rbcL, trnL-F* and *rps4* regions (Hyvönen & al., 1998; Hyvönen & al., 2004; Bell & Hyvönen, 2008; Bell & Hyvönen, 2010 a, 2010b; Bell & Hyvönen, 2012; Bell & al., 2015).

For the present study four plastid markers (*rbcL, trnL-trn*F, *trn*G and *rpl*16) and two nuclear markers (ITS1 and ITS2) were used. Nuclear internal transcribed spacer 1 & 2 (ITS1 and ITS2) sequences and plastid *trnG* and *rpl16* sequences are generated for the first time for *Polytrichum* sect. *Polytrichum*, while existing matrices for *rbcL* and *trnL*-F generated by Bell & al. (unpublished) were expanded by adding the newly generated sequences.

(a) *rbcL gene*

In many land plant lineages, the chloroplast protein coding ribulose bisphosphate carboxylase, or RUBSICO, enzyme gene (*rbcL)* is present in the Large Single Copy (LSC) region of the chloroplast genome between the *atp*B and *acc*D genes (Lee & al., 2007). The plastid *rbcL* gene is the most widely and extensively sequenced locus among all land plant lineages, including bryophytes (Stech & Quandt, 2010). Due to its conserved nature, and a low sequence variation at below family level, the popularity of using this gene has declined. Although, this gene has only been employed mostly at the family level and/or above family level circumscriptions (Shaw & al., 2003; Forrest & al., 2006; Bell & al., 2007), it is a designated DNA barcode marker for distinguishing species and is still very frequently used for that purpose. Although, due to the conserved nature of this gene, it is not recommended for species and intra-specific and/or population level analyses or barcoding approaches in mosses, it has been successfully used for species-level studies in liverworts (Forrest & al., 2005; Forrest & al., 2006; Newmaster & al., 2006). However, in combination with other genes, this gene will strengthen the phylogenetic signal, so currently it is used as a marker for a combined gene /multi-gene phylogenetic analyses.

Since many previous phylogenetic analyses of Polytrichaceae widely used the *rbcL* gene (see above) in order to generate a backbone phylogeny, I have sequenced the 5' part of the *rbcL* gene (consistent with the land plant DNA barcode marker) to expand the existing DNA sequence matrix of Polytrichaceae (Bell & al., unpublished) as well as to generate a better signal in the backbone of the combined cpDNA analysis.

(b) *trnL-trnF* region

The plastid *trnL-trnF* region contains sections of the plastid tRNA transferase coding gene *trnL*, in which an approximately 550 bp long intron is inserted, and of the tRNA transferase gene *trnF*, which is separated by an approximately 550bp intergenic spacer from *trnL* (Figure 3.1). Hence, the $trnL_{UAA}$-$trnF_{GAA}$ region includes both the *trnL* intron, the non - coding spacer sequence and also a small portion of the transferase gene (Quandt & Stech, 2004; Stech & Quandt, 2010).

Quandt & Stech (2004) reported that lengths of the *trnL-trnF* spacer region differ among the bryophyte lineages, hence this hyper-variable region is widely used to investigate intraspecific variations. Moreover, the *trnL-F* spacer contains a hairpin-associated inversion that overturns at the species or population levels, which may adversely affect the phylogenetic reconstructions if it hasn't been detected (Quandt & al., 2003; Borsch & Quandt, 2009).

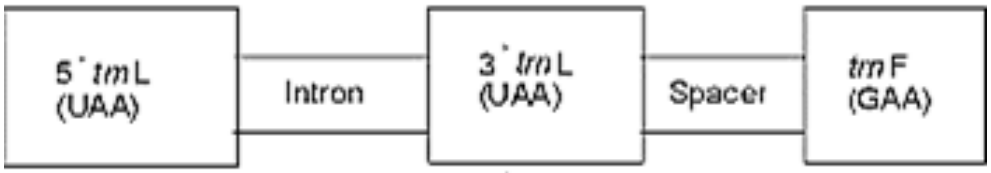


Figure 3.1: Structural organisation of the *trnL-F* region (not drawn to scale) of land plants. Coding regions are indicated in boxes (Reproduced from Yulita, 2013).

Previous phylogenetic studies on Polytrichaceae had extensively utilised the phylogenetic signal given by the *trnL-F* region (see above) to delimit the genera and species; the *trnL-F* region was therefore employed in the present study to infer the phylogenetic relationships among closely related taxa of *Polytrichum* sect. *Polytrichum*. The newly generated sequences of the *trnL-F* region have expanded the existing DNA sequence matrix of the Polytichaceae (Bell & al., unpublished).

(c) *trnG* UCC G2 intron

In bryophytes the *trnG* UCC gene is located in the LSC of the plastid genome, relatively close to the large *rpoC1-rpoC2* gene cluster, between *trnR*UCU and *ycf12* (Borsch & Quandt, 2009). The *trnG* UCC gene harbours a G2 intron, which is a non-coding region of phylogenetic interest in bryophytes. The primers developed by Pack & Szweykowska-Kulinska (2000) for

the phylogenetic studies of bryophytes (viz., trnGf and trnGr) are situated on the edges of the *trnG* exon and thus only amplify the *trnG* intron itself (Stech & Quandt, 2010).

The *trnG* intron has been utilised to infer the phylogenetic relationships in the family Bryaceae (Pedersen & Hedenäs, 2003) and the pleurocarpous order Hypnales (Hedenäs, 2009). In Bryaceae, Pedersen & Hedenäs (2003) showed that the *trnG* intron exhibits a higher level of variability compared to the *trnL-F* region.

Due to the ease of amplification and the potentially informative nature of the locus as described in the literature, the *trnG* intron was chosen as a further plastid marker for the molecular species delimitation. The present study is the first phylogenetic study in which sequences of the *trnG* intron has been utilised to infer the phylogenetic relationships of closely related species in the family Polytrichaceae.

(d) *rpl16* G2 intron

In almost all land plant lineages and charophytes (collectively known as "Streptophytes"), the *rpl16* gene (Figure 3.2) is flanked by *rpl14* and *rps3* in the LSC near the inverted repeat (IR) border of the chloroplast genome (Stech & Quandt, 2010). The chloroplast gene *rpl16*, encoding the ribosomal protein L16, is interrupted by an intron in many, but not all, land plants; for example, plant families Geraniaceae, Goodeniaceae and Plumbaginaceae lack the *rpl16* intron in their plastid genomes (Campagna & Downie, 1998).

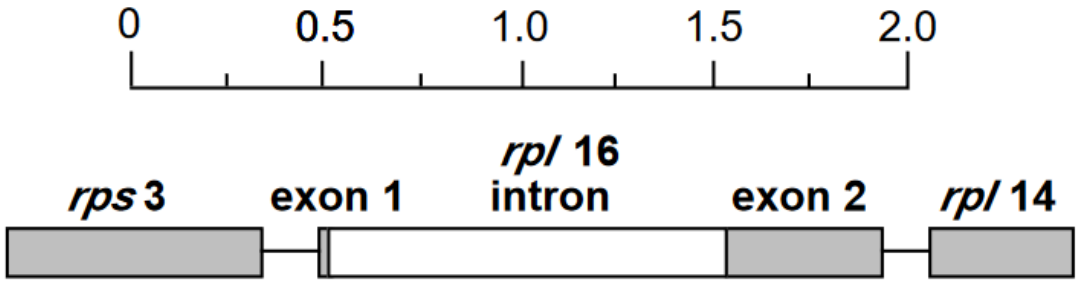


Figure 3.2: Structural organization of the *rpl16* gene and flanking DNA regions. Coding regions are indicated by shaded boxes; the *rpl16* intron (about 1.0 kb) is indicated by an open box. Scale units are in kilobase pairs (kb). (Reproduced from Campagna & Downie, 1998).

Stech & Quandt (2010) stated that, although many bryophyte systematic studies have employed the *rpl16* intron at the generic or higher taxonomic levels (Harris, 2008; Hutten & al., 2008; Olsson & al., 2009c), this plastid marker is becoming popular for phylogeographic and systematic analyses of closely related taxa such as those within the highly diverse order Hypnales (Pedersen & Hedenäs, 2003; Hedenäs, 2008; Olsson & al., 2009c). The present study is the first phylogenetic study in which sequences of the *rpl16* intron have been utilised to infer the phylogenetic relationships of closely related species in the family Polytrichaceae.

(e) Nuclear ITS

The most common nuclear region for species delimitation and intraspecific and/or population-level studies in many land plant lineages, including bryophytes (Vanderpoorten & al., 2006), is the internal transcribed spacer (ITS) of the ribosomal RNA repeat (nr DNA), this being more variable than any plastid region in most groups (Milyutina & Ignatov, 2015). The ITS cistron in many land plant lineages has the following architecture (Figure 3.3): The ITS transcription unit comprises a 5' external transcribed spacer (ETS), the gene coding for the SSU (Small Subunit) rRNA (18rRNA gene), the internal transcribed spacer 1 (ITS1), the 5.8S rRNA gene, the internal transcribed spacer 2 (ITS2), the gene coding for the LSU (Large Subunit) rRNA (26rRNA gene), as well as a short 3' external transcribed spacer (ETS), and is transcribed as a single cistron by the RNA Polymerase 1 enzyme.

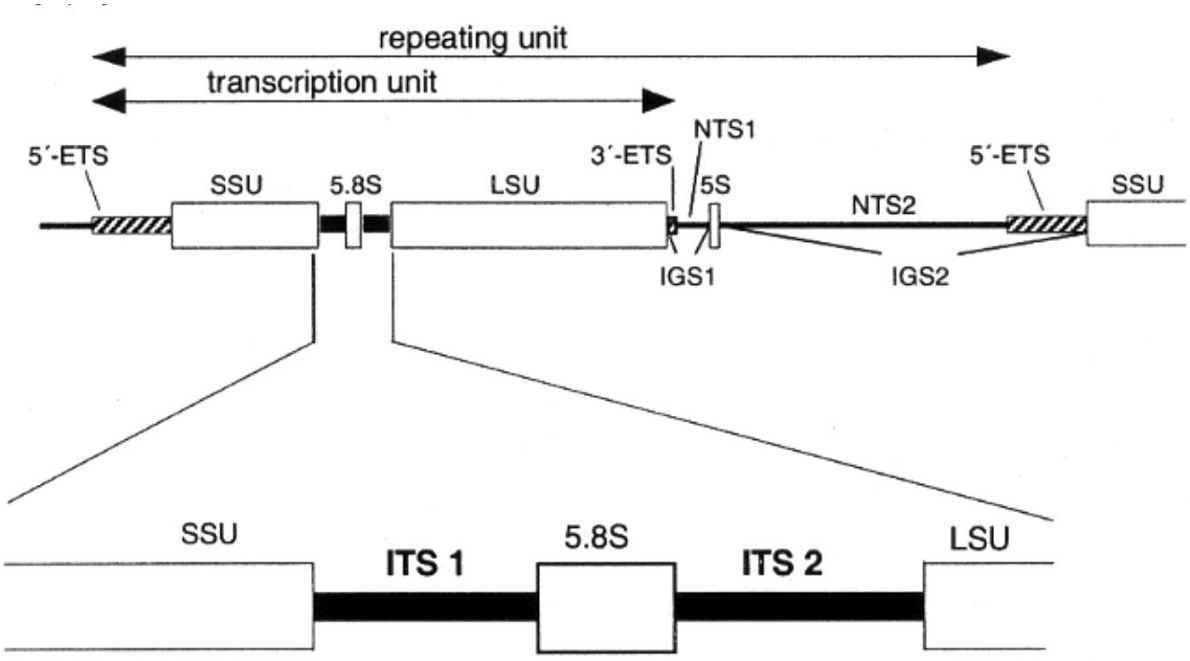


Figure 3.3: The diagrammatic representation of the ribosomal repeat unit and the transcription unit in bryophytes. The transcription unit comprises the external transcribed spacers (ETS), the gene coding for the SSU rRNA (18S rRNA gene), internal transcribed spacers 1 and 2 (ITS1 and 2), the 5.8S rRNA gene and the gene coding for the LSU rRNA (26S rRNA gene). In bryophytes, a 5S rDNA is incorporated in the ribosomal repeat unit [Reproduced from Quandt & Stech, 2003]

The secondary structure of ITS2 was found to be somewhat conserved, comprising a core structure with four associated hairpins, which are common for both plants and animals (Schultz & al., 2005). In general, ITS1 is more variable than ITS2, and its secondary structure has no definitive conserved core pattern like in ITS2. Hence the absence of a universal core structure underlies the great variability of ITS1 sequences in different taxa (Wang & al., 2006; Milyutina & Ignatov, 2015). In many acrocarpous moss lineages, many studies have shown that ITS1 is more variable, mainly due to several insertion and deletion (indel) events (Milyutina & al., 2010). This creates a lot of alignment issues, although for pleurocarpous moss groups ITS1 is reported to be easy to align without considerable indels (Huttunen & al., 2012).

Depending on the purpose of the study, ITS can be amplified either as separate compartments or as a single amplicon. The ITS1 and 2 loci have been used in molecular systematic studies of plants since the early 1990s onwards and are still regarded as widely used nuclear markers in plant molecular systematics (Baldwin, 19932; Baldwin & al., 1995). They

are generally easy to amplify and provide high levels of sequence variation to infer phylogenetic relationships as well as for population level studies (Quandt & Stech, 2003; Stech & Quandt, 2010). It has been shown that the amount of sequence variation seems to be largely lineage or taxon dependent in some bryophyte lineages, such as the common peat moss, *Sphagnum* spp. (Shaw, 2000; Shaw & Goffinet, 2000).

Although the 18S gene of the ITS nuclear ribosomal unit has been widely used to deduce generic level phylogenetic relationships in the Polytrichaceae (Hyvönen & al., 1998; Hyvönen & al., 2004; Bell & Hyvönen, 2008; Bell & Hyvönen, 2010 b; Bell & Hyvönen, 2012; Bell & al., 2015), the ITS1 and ITS2 spacers have not been employed before to infer phylogenetic relationships of closely related species of Polytrichaceae. Hence, the present study is the first attempt to sequence both the ITS1 and ITS2 regions for phylogenetic studies in Polytrichaceae.

3.2.4 DNA Extraction and Polymerase Chain Reaction (PCR)

(a) DNA Extraction

Shoots were selected individually for the genomic DNA extraction after thorough observation under the dissecting microscope (Wild Heerbrugg M5) to remove contaminants. For recent herbarium specimens containing robust plants, two or three shoot tips each containing 5-10 leaves plus a part of the shoot (excluding any perichaetia or perigonia) were used as the extraction material. Since many Polytrichopsida exhibit clear annual growth patterns from the shoot apex, shoot tips thus represent the most metabolically active parts of the plant as well as having less contact with soil and epiphytic algae and liverworts (Bell & Hyvönen 2010b). However, from the older herbarium material (several decades older, however carefully dried, ideally preserved, and curated) the greenest shoot tips were selected.

Approximately 10–20 mg plant material was used for each DNA extraction. Plant material was ground in 2 ml plastic Eppendorf tubes using a TissueLyser II Mill Grinder (Qiagen, Hombrechtikon, Switzerland) with flattened tungsten beads.

Genomic DNA was extracted using a modified CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle & Doyle, 1987). Depending on the age of the material, for older herbarium specimens, multiple extractions (2–5) were made from each accession from the same starting material (part of the same shoot), to allow for subsequent pooling to increase the DNA

yield. After precipitating DNA with ice-cold isopropanol, older specimens were frozen at −20°C for 3–5 days for a better precipitation of DNA. The DNA pellet was eluted in 50–100 µl TE buffer, and aliquots of the genomic DNA were electrophoresed in 2% agarose TBE gels at 80V for 40-60 minutes, stained with SYBRsafe™ (Invitrogen) and visualised under UV light on the Syngene G:BOX F3 Fluorescence Imaging System using GeneSys imaging software.

(b) Polymerase Chain Reaction (PCR)

For the *rbcL* locus, only the 5' half of the gene was amplified, using the primers NM34 (Forward) and M745R (Reverse) (Cox & al. 2000). For amplification of the *trnL-F* region, primers trnC (Forward) and trnF (Reverse) were used (Taberlet & al., 1991 in Cox & al., 2000). For amplification of the *trnG* intron, primers trnGf (Forward) and trnGr (Reverse) were used (Werner & al., 2009; Pack & Szweykowska-Kulinska, 2000 respectively for F and R primers). For amplification of the *rpl16* intron, primers F71(Forward) and *rpl16*R (Reverse) were used (Jordan & al., 1996; Olsson, 2009c respectively for F & R primers). The nuclear ITS1 and ITS2 loci were amplified in separate reactions due to problems with amplifying the complete ITS1-5.8S-ITS2 locus, especially in older herbarium specimens where the DNA was more fragmented. For the ITS1 region, ITS1_F (Forward) and ITS1_R (Reverse) primers were used (Sawicki & Szczecińska, 2011) for amplification. For the ITS2 region, ITS2SeqF (Forward) and ITS4 Bryo (White & al.,1990) primers were used. Primers used to amplify and sequence the genomic regions are summarised below (Table 3.2).

Table 3.2: Primers used to amplify and sequence the genomic regions.

Marker	Primer	Sequence 5'-3'	Direction	Reference
Plastid *rbcL*	NM34	GTTGTTGGATTTAAAGCTGGTGTT	Forward	Cox & al. (2000)
	M745R	CTTCACAWGTACCTGCRGTAGC	Reverse	Cox & al. (2000)
Plastid *trnL-F*	trnC	CGAAATCGGTAGACGCTACG	Forward	Taberlet & al. (1991)
	trnF	ATTTGAACTGGTGACACGAG	Reverse	Taberlet & al. (1991)
Plastid *trnG*	trnGf	GGCTAAGGGTTATAGTCGGC	Forward	Werner & al. (2009)
	trnGr	GCGGGTATAGTTTAGTGG	Reverse	Pack & Szweykowska-Kulinska (2000)
Plastid *rpl16*	F71	GCTATGCTTAGTGTGTGACTCGTTG	Forward	Jordan & al. (1996)
	*rpl16*R	GTAATCCAAGCTGGTTCAAGTGC	Reverse	Olsson (2009c)
Nuclear ITS1	ITS1_F	CAAGGTTTCCGTAGGTGAAC	Forward	Sawicki & Szczecińska (2011)
	ITS1_R	CAAGAGCCAAGATATCCG	Reverse	Sawicki & Szczecińska (2011)
Nuclear ITS2	ITS2Seq F	GCTGCGTTCTTCATCGATGC	Forward	White & al. (1990)
	ITS4 Bryo	TCCTCCGCTTATTGATATGC	Reverse	White & al. (1990)

The optimised PCR amplification conditions for each amplicon are as follows:

For the *rbcL* region, PCR mixtures had a total volume of 20 µl, and contained 2.5 mM of $MgCl_2$, 0.325 µM of each forward and reverse primer (Table 3.2), 0.2 mM of dNTP (Bioline, London, UK), 1x of PCR Reaction Buffer (Bioline, London, UK), 0.0375 units of Biotaq DNA polymerase (Bioline, London, UK), and 2.0 µl of unquantified template DNA. The thermocycling profile consisted of a 4 min initial denature at 94°C, then 40 cycles of denaturing at 94°C for 30 sec, annealing at 50°C for 40 sec, and extending at 72°C for 40 sec, with a final 5 min extension at 72°C followed by a 12°C holding stage.

For the *trnG* and *rpl16* regions, PCR mixtures had a total volume of 20 µl, and contained 1.5 mM of $MgCl_2$, 0.325 µM of each forward and reverse primer (Table 3.20.2 mM of dNTP (Bioline, London, UK), 1x of PCR Reaction Buffer (Bioline, London, UK), 1x of TBT-PAR (a trehalose-based PCR additive; Samarakoon & al., 2013), 0.075 units of Biotaq DNA polymerase (Bioline, London, UK), and 1.0 µl of unquantified template DNA. The thermocycling profile consisted of a 4 min initial denature at 94°C, then 35 cycles of denaturing at 94°C for 30 sec, annealing at 58°C for 60 sec, and extending at 68°C for 90 sec, with a final 4 min extension at 68°C followed by a 12°C holding stage.

For the *trnL-F* region, PCR mixtures had a total volume of 20 µl, and contained 2.5 mM of $MgCl_2$, 0.325 µM of each forward and reverse primer (Table 3.2), 0.2 mM of dNTP (Bioline, London, UK), 1x of PCR Reaction Buffer (Bioline, London, UK), 1x of CES (Combinatorial Enhancer Solution; Ralser & al., 2006), 0.075 units of Biotaq DNA polymerase (Bioline, London, UK), and 0.5 µl of unquantified template DNA. The thermocycling profile consisted of a 4 min initial denature at 94°C, then 35 cycles of denaturing at 94°C for 45 sec, annealing at 55°C for 60 sec, and extending at 72°C for 60 sec, with a final 10 min extension at 72°C followed by a 12°C holding stage.

For older herbarium specimens, to obtain more recalcitrant plastid DNA from the *rbcL, trnL-F, trnG,* and *rpl16* regions, the following "trouble shooting PCR protocol" was optimised: PCR mixtures had a total volume of 25 µl, and contained 2.0 mM of $MgCl_2$, 0.152 µM of each forward and reverse primer (Table 3.2), 0.2 mM of dNTP (Bioline, London, UK), 1x of PCR Reaction Buffer (Bioline, London, UK), 1x of BSA (Bovine Serum Albumin), 0.04 units of Biotaq DNA polymerase (Bioline, London, UK), and 2.0 µl of unquantified template DNA. The thermocycling profile consisted of a 5 min initial denature at 80°C, then 35 cycles of

denaturing at 94°C for 60 sec, annealing at 50°C for 60 sec, and extending at 65°C for 4 min, with a final 5 min extension at 65°C followed by a 12°C holding stage.

For the nuclear ITS1 and ITS2 regions, PCR mixtures had a total volume of 20 µl, and contained 2.5 mM of $MgCl_2$, 0.325 µM of each forward and reverse primer (Table 3.2), 0.2 mM of dNTP (Bioline, London, UK), 1x of PCR Reaction Buffer (Bioline, London, UK), 0.5x of TBT-PAR,), 0.0375 units of Biotaq DNA polymerase (Bioline, London, UK), and 1.0 µl of unquantified template DNA. The thermocycling profile consisted of a 4 min initial denature at 94°C, then 35 cycles (30 cycles for ITS1) of denaturing at 94°C for 60 sec, annealing at 58°C-60°C (59°C is the optimum temperature for the ITS1 locus to avoid the co-amplification of a shorter DNA strand) for 60 sec, and extending at 72°C for 90 sec, with a final 7 min extension at 72°C followed by a 12°C holding stage.

Blattner (2000) has also discussed the importance of amplifying ITS1 and ITS2 as two separate amplicons for herbarium material where DNA is severely degraded. Hence, to prevent the additional costs of trouble shooting and extra bench time for additional preparations, the ITS1 and ITS2 regions were amplified separately. A range of pilot PCR reactions were conducted to try to eliminate the co-amplification of an unwanted shorter DNA fragment along with the desired ITS1 fragment in certain older herbarium DNA extractions. Increasing the annealing temperatures in the range from 57°C-61°C and decreasing the number of PCR cycles from 35 to 30 helped to eliminate the co-amplification of this shorter DNA fragment. The annealing temperature to amplify ITS was optimised at 59°C and the number of PCR cycles optimised to 30.

Samarakoon & al. (2013) showed that the addition of a combination of PCR additives that they called ‘TBT-PAR’, containing trehalose and BSA, was effective in improving PCR amplification of DNA from plant specimens or from old and potentially degraded museum specimens with recalcitrant DNA. A second combined additive described by Ralser & al. (2006) as CES (Combinatorial Enhancer Solution) and modified at RBGE to contain betaine, BSA and DMSO was also used to improve PCR amplification processes for samples containing recalcitrant DNA.

(c) Gel Electrophoresis and Purification of PCR Products.

Samples of each PCR product were electrophoresed in 1% agarose TBE gels, with a 1Kb plus DNA ladder (Thermo Fisher Scientific, Waltham, USA), stained with SYBERSafe

™, and visualised under UV light by the Syngene G:BOX F3 Fluorescence Imaging System using GeneSys imaging software.

Samples which produced a clear single brighter band (under UV) which has the desired approximate length for each gene region were subsequently selected and their corresponding PCR products cleaned up using ExoSAP IT (USB Corporation, Ohio, USA). For this, a reaction mix of 5 µl of PCR product and 2 µl ExoSAP IT enzymes was incubated at 37°C for 15 min, followed by enzyme inactivation at 80 °C for a further 15 min and a 10°C holding stage.

3.2.5 DNA Sequencing and Sequence Alignment

(a) DNA Sequencing

For DNA sequencing, two separate reactions were performed for each sample (i.e. one for each forward and reverse primer, using the above [see 3.2.4 (b)] stated primers (Table 3.2) in all cases) to generate overlapping sequences for nucleotide confirmation (a bidirectional read of the DNA strand). For all six molecular markers, chain sequencing was performed for a total 10 µl sequencing reaction, using, 0.32 µl of primer (10 µM), 2 µl of 5x sequencing buffer (Applied Biosystems, Inc. Foster City, CA) and 0.5 µl BigDye™ mix [Terminator v3.1 Cycle Sequencing Kit, ThermoFisher Scientific, UK), 4.5 µl dH_2O and 0.8-1.0 µl template DNA (purified PCR product). The BigDye® Terminator protocol consisted of 25 cycles of denaturation at 95 °C for 30 sec, primer annealing at 50 °C for 20 sec and chain extension at 60 °C for 4 mins, followed by a 4 °C holding stage. Sequencing reactions were run on ABI capillary sequencers by the Genepool facility at the University of Edinburgh.

(b) Sequence Alignment

Electropherograms were assembled using Sequencher 5.4.1 (Gene Codes Corp., Ann Arbor, Michigan). Forward and reverse sequences were assembled into a single contig, ambiguities were checked, and consensus sequences edited manually to remove any primer sequences. These were exported from Sequencher in NEXUS or FASTA format. Sequences were aligned using MUSCLE (Multiple Sequence Comparison by Long-expectation, Edgar 2004) and optimised manually using PhyDE v.0.997 (Müller & al. 2010).

The total length for individual sequences after trimming obtained for *rbcL*, *trnL-F*, *trnG*, *rpl16*, ITS-1 and ITS-2 ranged between 574–633bp, 392–485bp, 669–713bp, 802–844bp, 672–698bp and 438–478bp respectively. Separate alignments were made for the *rbcL*, *trnL-F*, *trnG*, *rpl16*, ITS-1 and ITS-2 loci.

In order to maximise positional homology, areas of ambiguity and accessions with significant missing data were excluded when necessary. There was no alignment ambiguity in the protein-coding *rbcL* gene. Within the *trnL-F* region, Quandt & Stech (2004) previously identified hairpin-associated inversions that are variably present and known to be highly homoplasious; these were excluded from the alignment. The *trnG* locus was easily alignable, however, there is a 31 bp (between 624–654bp) repeat found in four populations of *P. brachymitrium* creating an insertion within the alignment. There was no alignment ambiguity in the *rpl16* locus. In the ITS -2 locus, a 40 bp insertion is found in all *P. commune s. str* accessions, with the rest of the locus mostly being easily alignable.

3.2.6. Phylogenetic Reconstruction

Many recent molecular phylogenetic studies on bryophytes have proven that the integration of more than one molecular region is desirable to deduce robust phylogenetic relationships among taxa and to reconcile and understand the evolutionary behaviour of morphological and molecular characters (Shaw & al., 2003; Forrest & al. 2006; Qui & al., 2007; Biersma, 2017). The data sets could be analysed as separate, individual data sets or as a combined dataset (simultaneous analysis) depending on the congruence of the data matrices (Cunningham, 1997; Buckley & Cunningham, 2002). The compatibility of datasets was compared from the individual trees generated from each locus by comparing the tree topologies as described by Farris & al., (1995). Moreover, two separate matrices for combined plastid and nuclear markers were prepared and topologies of trees generated from each combined matrix also checked for incongruence between markers, as inferred from potential occurrence of conflicting well-supported (bootstrap support greater than 50%) clades. Since there is no significant topological difference and no conflict in well supported clades in the trees generated separately from plastid and nuclear data sets, both matrices were combined to make a single data matrix.

The total amount of data in the combined data set ranged from 1176–3851bp per accession. Several gaps were inserted in the final combined data matrix, due to the insertions and deletions (indel events), and the final alignment had a length of 3851bp.

Separate preliminary assessments were performed using PAUP*4.0a169 (Swofford, 2002) for each separate DNA compartment and for the combined matrix to obtain the numbers of parsimony informative sites. Data is summarised in Table 3.3 below.

Table 3.3: A summary of total number of characters, constant characters and parsimony informative characters in each separate locus and the combined data matrix.

	rbcL	***trnL-F***	***trnG***	***rpl16***	**ITS-1**	**ITS-2**	**Combined**
Total number of characters	633	485	713	844	698	478	3851
Constant characters	607	467	693	803	501	391	3462
Parsimony informative characters	**15**	**09**	**17**	**23**	**127**	**65**	**256**

The combined data matrices were analysed using Maximum Likelihood (ML) and Bayesian Inference (BI) on unrooted and equally weighted characters.

3.2.6.1 Phylogenetic Reconstruction Using Model-Based Methods

Both Bayesian Inference (BI) and Maximum Likelihood (ML) methods are model-based methods employed for phylogenetic analyses.

(a) Maximum Likelihood (ML) Approach

The ML approach in phylogenetics is a statistical procedure which evaluates alternative trees and assigns a likelihood value to each of these trees. This approach tries to find the "best-fitted" phylogenetic tree from the tree space, that has the greatest likelihood (*L*) of generating the observed data, given a specified model of evolution. "A likelihood score" is assigned to each tree and the tree with the highest likelihood score is chosen as the best tree from the tree

space. Thus, this likelihood score is the optimality criterion for ML (Pagel, 1999; Hall, 2008; Stuessy & al., 2014).

Since the likelihood values are computed for each nucleotide position, and thus a product of many probabilities and are very small, they are usually presented on a logarithmic scale. The researchers select the tree with the largest log L value as the best ML tree. The ML method requires a model to assess the probability of changes in DNA sequences (such as point mutations and/or changes at the codon level). Hence, the likelihood of the evolutionary event depends on the model we employed in the analysis. Therefore, the requirement of a model to evaluate the probability of certain types of mutations has made ML a popular method of choice among systematists. However, the more the complex the model, the more computationally demanding the analysis will become (Hall; 2008; Stuessy & al., 2014).

All ML analyses were conducted under a maximum likelihood optimally criterion using RAxML v.7.4.2. (Stamatakis, 2006) with the raxmlGUI 2.0 front end (Edler & al., 2020). In all cases the "ML + thorough bootstrap" option within the raxmlGUI (RAxML option "-b" followed by an ML search) was used, with 100 runs and 10,000 replications. Separate analyses were conducted on the combined chloroplast data (*rbcL*, *trnL-F*, *trnG* and *rpl16*) and the nuclear data (ITS-1 and ITS-2) and for the concatenated data matrix. Gaps were treated as missing data in all analyses. All combined matrices were treated as a single partition and the General Time Reversible model of evolution with a gamma distribution of rate variation among sites and a proportion of invariant sites (GTR+ I+ G) was chosen as the model that best fits the data, according to the Akaike information (AIC) criteria as evaluated by MrModeltest v.2.4 (Nylander, 2004) using the Akaike information criterion (Akaike, 1974). RaxML produces a single most likely tree with bootstrap (BS) values attached to all nodes (Figures 3.4). For presentation of the results, TreeGraph 2.11.1 (Stöver & Müller, 2010) was used to collapse nodes in trees supported by BS values < 50%.

(b) Bayesian Inference (BI)

Bayesian Inference (BI) is a method that involves a statistical inference in which other evidence or observations can be used to calculate the probability that a hypothesis may be true. In phylogenetic studies the BI approach is somewhat similar to the ML approach in which there is a model of evolution and the method evaluates trees verses the molecular data. BI attempts

to maximize the probability of the tree given the data and model of evolution, and the *prior probability*. There are mainly three components in the BI approach. The first component is the prior probability, which represents the probability of a particular tree prior to the observations that have been made. Commonly, all trees are considered as equally probable trees (*a priori*; i.e. so-called "flat priors" are used). The second component is the *likelihood component*, which is proportional to the probability of the observations (i.e., a DNA or amino acid sequence alignment) given the hypothesis (i.e., the tree). This probability requires making a specific set of assumptions about the process of generating the observations. The third component is the *posterior probability* of a tree, which means the probability of the tree conditional on the observations (in other words, the probability of the hypothesis given the observations). It is generated by combining the prior and likelihood for each tree using Bayes's formula and thus prior probabilities estimated based on a model in its search for the best set of trees. The BI approach searches to maximize the probability of the tree considering the data and the model of evolution. In other words, the BI approach generates a posterior distribution for parameters, composed of a phylogenetic tree and a suitable model of evolution, based on the prior probability of those parameters plus the likelihood of the data, generated by a multiple sequence alignment.

The most popular computational programme that incorporates the BI approach is MrBayes (Huelsenbeck & Ronquist, 2007), which uses the Metropolis-coupled Monte Carlo Markov Chain (MCMC) method; a set of independent probabilistic searches that approximate the posterior probability distribution of trees and are also computationally efficient. Once the best set of trees which are consistent with the selected model and the data are generated, posterior probabilities of each clade can be obtained via summarizing the results in the form of a majority rule consensus tree (Huelsenbeck & Ronquist, 2007; Huelsenbeck & al., 2002; Hall 2008; Stuessy & al., 2014). While the ML approach calculates the probability of data given the desired model, the BI approach expresses the overall result as the probability of the model given the set of data. As the probability of the model, or the hypothesis including the tree topology, is more likely what the investigator expects, this straightforwardness is one of the main advantages of the BI approach.

For the BI model-based approach, six partitions were defined. Each locus was selected as a single compartment. MrModeltest v.2.4(Nylander, 2004) was used for initial estimation of the best-fitting substitution model for each compartment separately based on the AIC criterion (Akaike, 1974). For ML analyses the most complex model selected for any one partition was

applied independently to all partitions. For the BI analysis a single analysis was performed for the total combined data set using a heterogenous model using the MrModeltest. The optimal models for each compartment selected using the Akaike criterion within MrModeltest are listed in Table 3.4.

Table 3.4: Total number of characters, percentage (%) of parsimony informative characters for each region together with the optimal models selected for Bayesian analyses using the Akaike criterion within MrModeltest.

Region	Total number of characters	% of parsimony informative sites	Model
rbcL	633	2.37%	[6]HKY + I
trnL-F	485	1.86%	[7]GTR
trnG	713	2.38%	HKY + I
rpl16	844	2.73%	HKY + I
ITS-1	698	18.19%	[8]HKY + I + G
ITS-2	478	13.60%	[9]GTR + I + G

Bayesian phylogenetic analyses were performed using MrBayes v.3.2.7a (Ronquist & al., 2012) on unordered and equally weighted characters. In each analysis, three independent runs using the default prior settings, each with five MCMCP chains ("temp" parameter=0.1), were run simultaneously for 5×10^6 generations, with a sampling frequency of -1 with trees sampled every 1000 generations. Trace plots generated in Tracer v. 1.7.1 (Rambaut & al., 2018) were used to check for convergence of the runs (plateaus of all runs at comparable likelihoods), and the burn-in was determined by visualising the likelihood parameter against generation time using Tracer v.1.7.1. The first 50% of trees (including those from the burn-in phase) were discarded and a majority-rule consensus tree constructed using the remaining sample. Bayesian posterior probability (BPP) support values 95% or higher were regarded as significant (e. g. Forrest & al., 2006), although some authors discuss lower values (Nickrent & al., 2004).

[6] HKY +I :Hesegawa-Kishino-Yano substitution model with invariant sites
[7] GTR :General Time Reversible model
[8] HKY + I + G :Hesegawa-Kishino-Yano substitution model with a gamma distribution of rate variation among sites and a proportion of invariant sites
[9] GTR +I +G :General Time Reversible model of evolution with a gamma distribution of rate variation among sites and a proportion of invariant sites

3.2.6.2 Haplotype Network Analysis

A haplotype network is a diagrammatic representation depicting the relationships among the different haploid genotypes observed in each dataset. They are usually drawn unrooted, which is practically reasonable for within-species data, where the root location is often unknown. Each colour represents a distinct population or a geographic location. Each node represents a unique DNA sequence haplotype with the colour denoting the population (or populations) where it is found and the size of the circle accounting for its frequency. The lengths of the lines (in steps) connecting the haplotypes refer to the distance of relatedness, with each step usually representing one base pair change. This means that more distant nodes are more distantly related (more bp difference) haplotypes (Templeton & al., 1992; Paradis, 2018).

In order to explore and illustrate the genetic variation of the nuclear ITS-2 region among geographically separated and morphologically very distinctive populations of the three species *P. perigoniale*, *P. subpilosum* and *P. ericoides* [which together form a "species complex" with little phylogenetic resolution; See Figure 3.4 A], and *P. brachymitrium*, PopART v1.7 (http://www.leigh.net.nz/software.shtml) was employed to generate a 95% TCS haplotype network (statistical parsimony; Templeton & al., 1992). Sites with undefined states for some terminals (gaps) were excluded prior to haplotype analysis. The diagram was colour coded to illustrate the different geographic origins of each haplotype (Europe, China, Africa, America, Japan, Hawaii and Australia for *P. perigoniale* haplotypes, Africa for *P. subpilosum* haplotypes, Colombia for *P. ericoides* haplotypes and South America for *P. brachymitrium* haplotypes).

3.2.7 Results and Discussion on Phylogenetic Inference

Separate parsimony analyses of chloroplast data (*rbcL*, *trnL-F*, *trnG* and *rpl16*) and the nuclear data (ITS-1 and ITS-2) resulted in differently resolved (consensus) trees, due to different numbers of parsimony-informative characters (See Table 3.3). However, no supported incongruence between the different markers was observed, as no conflicting well-supported clades (greater than 50% BS support) were found by visual comparison of the respective tree topologies (See Appendix 1 of this chapter).

A single optimal phylogram was obtained under the ML criterion for the combined data set and is shown in Figure 3.4 A. Figure 3.4 B provides a cladogram to illustrate the nodes of the phylogram (Figure 3.4 A). Only branches with bootstrap support (BS) values >50% are annotated on both phylogram (Figure 3.4 A) and cladogram (Figure 3.4 B). Bayesian inference yielded a majority rule consensus tree with most of the branches strongly supported (not presented here). Both ML and BI analyses recovered very similar topologies for the final concatenated analyses. Hence a final ML tree is presented here (Figure 3.4 A) with Bootstrap (BS) and Posterior Probability (PP) values attached for each clade. Branches with PP values less than 0.5 are not annotated on the tree. Most clades were well-supported in both analyses, with the exception of the clade containing *P. perigoniale*, *P. subpilosum* and *P. ericoides* (see the discussion below) which is supported poorly with a posterior probability of 0.56 and a bootstrap value of 54%. However, within this poorly supported apical clade of the phylogram (Clade A in Figure 3.4 A), the monophyly of *P. ericoides* is supported by 100% BS and a 1.0 PP value.

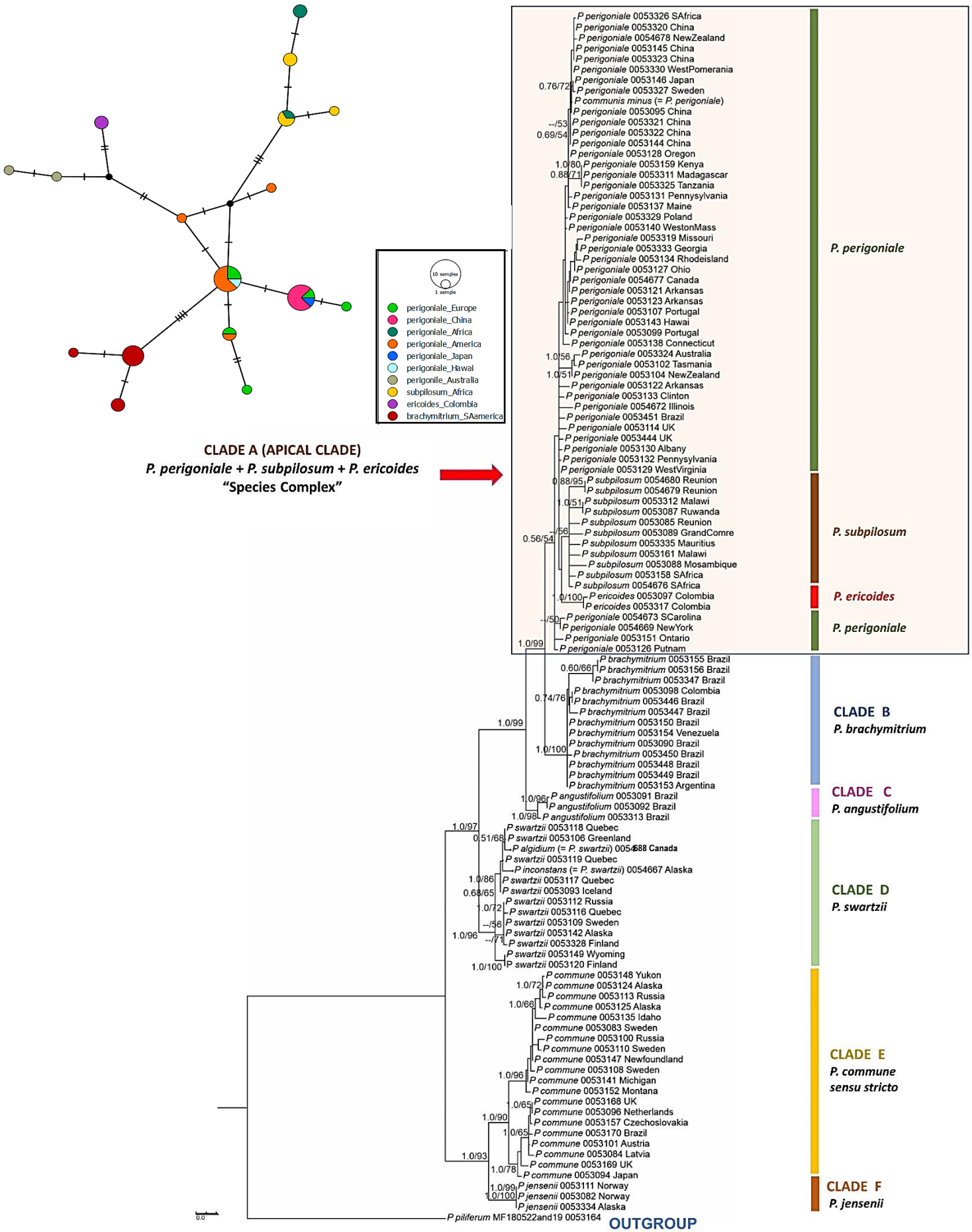


Figure 3.4 (A): Maximally likelihood (ML) topology resulting from the analysis of total combined dataset (*rbcL, trnL-F, trnG , rpl16,* ITS-1 & ITS-2) under the GTR +I +G model. Posterior probability (PP) values obtained from the Bayesian analysis are included. PP values < 0.5 and BS values < 50% are not included. A haplotype network for the ITS-2 region is shown for Clade A with colour codings for separate geographic regions.

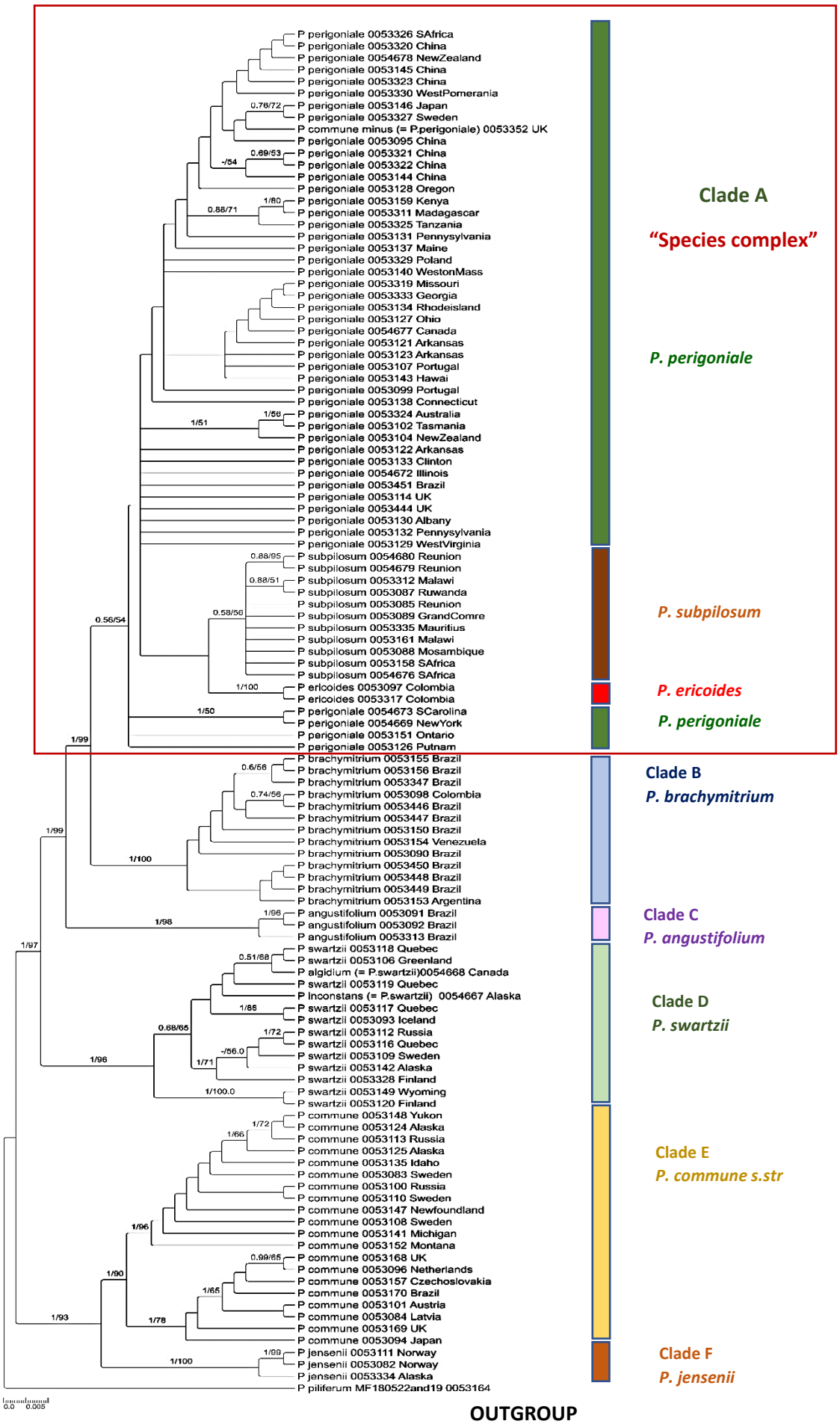


Figure 3.4 (B). A cladogram showing the ML topology resulting from the analysis of total combined dataset (*rbcL*, *trnL-F*, *trnG*, *rpl16*, ITS-1 & ITS-2) under the GTR +I +G model. Posterior probability (PP) values obtained from the Bayesian analysis are included. PP values < 0.5 and BS values < 50% are not included.

The present molecular data clearly resolves long-standing taxonomic and nomenclatural issues surrounding certain taxa and provides some well-supported evidence for phytogeographic structure within taxa. Most of the molecular determinations are corroborated by the extant delimitations of the taxa using morphological characters, however the phylogeny helps to resolve some long-standing debates in Polytrichaceae taxonomy.

The phylogram generated by the ML criterion with concatenation of nuclear and plastid data (Figure 3.4 A) exhibits the following overall phylogenetic patterns.

- **General Phylogenetic Topology**

Two major, well-supported clades were resolved the highest level (100% BS) within *Polytrichum* sect. *Polytrichum* - a clade containing *P. jensenii* and *P. commune s. str.* (1.00 PP, 93% BS), and a large clade with all the other species, including *P. brachymitrium, P. angustifolium, P. ericoides, P. subpilosum, P. perigoniale* and *P. swartzii*, supported with 1.00 PP and 97% BS. Within the latter, *P. swartzii* was supported as monophyletic (1.00 PP, 96% BS) and as sister (1.00 PP, 97% BS) to a well-supported clade comprising the other species (1.00 PP, 99% BS).

Within the *P. commune* + *P. jensenii* clade, *P. jensenii* is supported with 1.00 PP and 100% BS support, confirming its monophyly. Within this clade, *P. commune* is also monophyletic, with 1.00 PP and 90% BS support. Within the monophyletic clade of *P. commune* two strongly supported clades can be observed: a clade (1.00 PP, 78% BS) containing European, Brazilian and Japanese populations and another clade (1.00 BP, 96% BS) containing arctic and sub-arctic populations. However, relationships within these groups are not well supported.

Within the largest clade (comprising *P. angustifolium, P. brachymitrium, P. ericoides, P. subpilosum* and *P. perigoniale*), *P. angustifolium* is sister to the other taxa and forms a strongly supported (1.00 PP, 96% BS) monophyletic group containing the three accessions of that species from Brazil. *Polytrichum brachymitrium* is the next diverging clade (1.00 PP, 99% BS), within which the monophyly of some populations is moderately supported (0.6-0.7 PP; 60-70% BS). The remainder of this larger clade comprises a weakly supported (0.56 PP, 54% BS) apical clade (i.e., Clade A) containing one widespread species (*P. perigoniale*) and two geographically distinct species (the South American *P. ericoides* and the African *P. subpilosum*). Within this weakly supported "apical clade", *P. ericoides* is strongly statistically supported as monophyletic (1.00 PP,100% BS), occurring as sister to *P. subpilosum*, although

without support. The widespread species *P. perigoniale* appears paraphyletic with respect to *P. ericoides* and *P. subpilosum*, with population structure generally poorly supported. However, two geographically and morphologically distinctive groupings within *P. perigoniale* can be identified with moderate or strong support. An Australasian clade containing all Australian and New Zealand samples is supported with a strong Bayesian Inference (1.00 PP) but weak Bootstrap support value (56%), while a clade comprising three African accessions is strongly supported (0.88 PP and 77% BS) (see discussion for the Clade A below).

The sister clade, *P. swartzii*, comprises a monophyletic group which includes all samples of this arctic and sub-arctic species. Two populations from Finland and Wyoming, USA form a separate clade within *P. swartzii* with 100 % BS and 1.00 PP support. However, the phylogenetic relationships between two semi-aquatic forms, *P. algidum* and *P. inconstans* (now synonymised with *P. swartzii*), are not well-supported.

Each clade depicted in Figure 3.4 A is discussed in detail below.

- **CLADE A (*P. perigoniale* Michx., *P. subpilosum* P.Beauv. and *P. ericoides* Hampe)**

Within the monophyletic group of *Polytrichum* sect. *Polytrichum* an apical clade, which is poorly supported, comprises three species which are morphologically and geographically distinct. The vast majority of accessions included in this apical clade belong to the taxon *P. perigoniale* Michx. Almost all specimens labelled as *P. perigoniale* from North America used for the current study and depicted in the phylogram (Figure 3.4 A) were initially labelled and determined as *P. commune* by the collectors. For several decades, the concept of *P. commune* was erroneously applied in North America. Following the establishment of a lectotype for *P. commune* Hedw., the anatomical and morphological variation among and between these two taxa can now be much more clearly defined. Although *P. perigoniale* was earlier treated as a variety of *P. commune* (i.e., *P. commune* var. *perigoniale*; Smith, 2004), it has now been revised as a distinctive species in its own right (see Chapter 04). The morphological differences between *P. commune* and *P. perigoniale* are discussed below (see discussion under Clade E).

De Sloover (1986), in his taxonomic treatment of Polytrichaceae in Africa, erroneously applied the concept of *P. commune* to *P. perigoniale*. All accessions of *P. perigoniale* from Africa and Madagascar included in the phylogram (Figure 3.4 A) were initially determined as *P. commune*. However, the leaf anatomical characters in all of these specimens (leaf cross sections with flattened lamellar end-cells) corroborate the morphological species concept of *P. perigoniale*. Hence, in the taxonomic treatment in this study (Chapter 4), the taxonomy of the

African Polytrichaceae is revised by the exclusion of *P. commune* and the inclusion of *P. perigoniale*. Similarly, specimens identified as *P. swartzii* from China and Japan are also redetermined as *P. perigoniale* in this study. This revises the Polytrichaceae in China by excluding *P. swartzii* from the flora and redetermining *P. perigoniale* as a wide-spread taxon in the country.

Polytrichum subpilosum, which is morphologically distinct and endemic to Africa and Madagascar, seems to be derived from within the *P. perigoniale* clade. However, there is no relationship between *P. subpilosum* and *P. perigonaile* as *P. perigoniale* is paraphyletic. As De Sloover (1986) stated, *P. subpilosum* is a very distinctive plant which has a characteristic leaf anatomy exhibiting a "sinus-like" shallow furrow in the lamellar end-cells, bearing knob-like papillated thickenings on the top of each bifid portion of the cell. However, in the *rpl16* matrix, all *P. subpilosum* accessions used for the present study show a 28 bp deletion in the variable region.

P. ericoides is a geographically isolated, distinctive taxon which is an endemic to the tropical Andes (Peralta & Yano, 2010; Aponte & Uribe, 2017). Within this apical clade, *P. ericoides* accessions collected from Colombia were supported as forming a monophyletic group with 100 % BS and 1.00 PP support. *Polytrichum ericoides* is confined to high elevation Pàramo habitats in the Andes (Aponte & Uribe, 2017). This taxon is morphologically very distinctive with its shorter leaf blade (unique among the species in *Polytrichum* sect. *Polytrichum*), serrulate leaf margins and taller (10–12 cells high) lamellae on the adaxial leaf surface (Smith, 1975; Aponte & Uribe, 2017). *Polytrichum ericoides* appears in this phylogeny as a sister group to *P. subpilosum,* although there is no statistical support.

In order to explore and illustrate variation among geographically separated populations of *P. perigoniale, P. ericoides, P. subpilosum* and their possible sister group *P. brachymitrium*, a haplotype network analysis was performed (see 3.2.6.2) for the ITS-2 locus. The haplotype network diagram (Figure 3.5) is illustrated below.

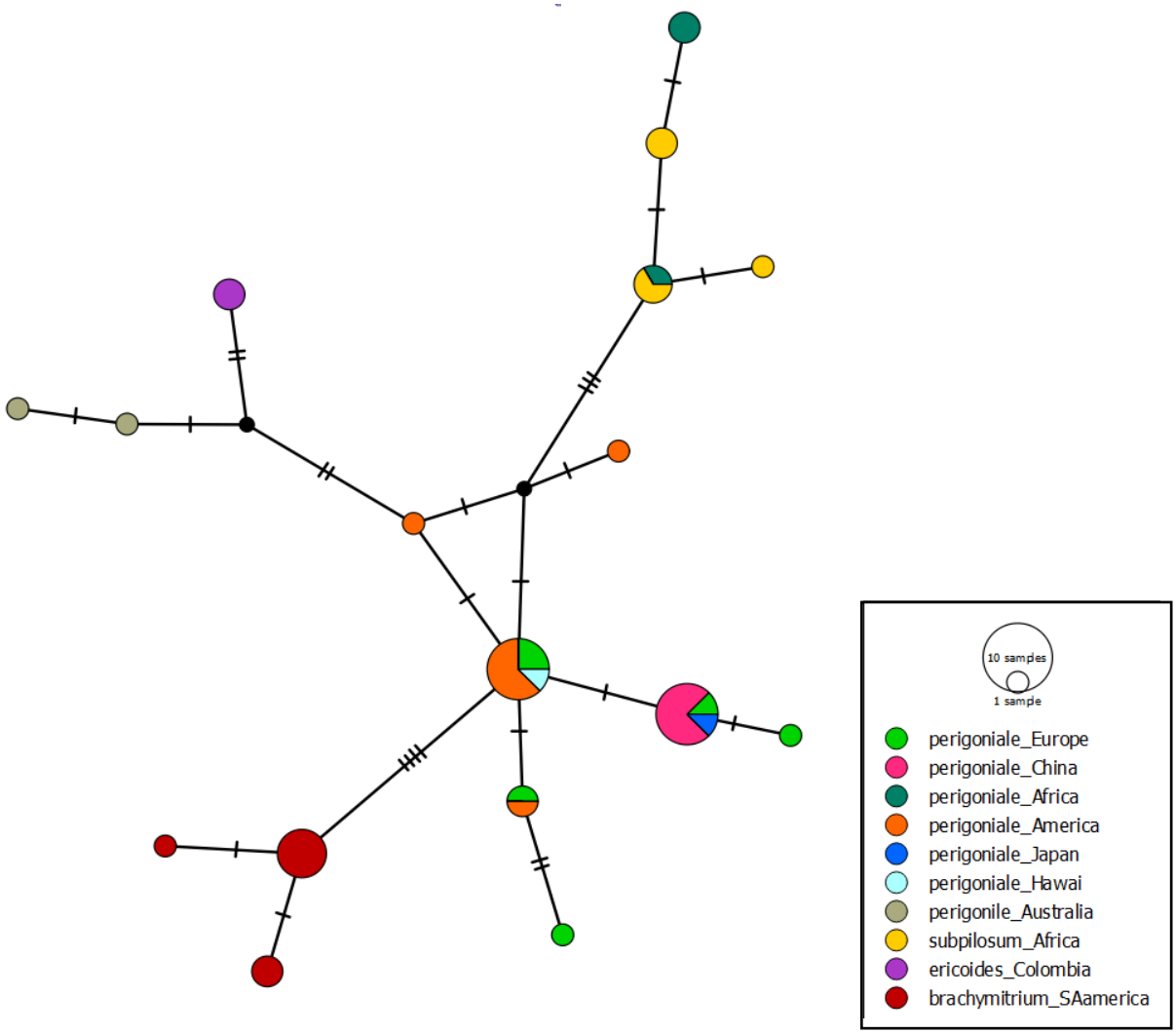


Figure 3.5: A haplotype network constructed for Clade A to illustrate the genetic differences within the ITS-2 locus of the species complex (*P. perigoniale* + *P. subpilosum* + *P. ericoides*) and *P. brachymitrium*.

The haplotype network diagram illustrates the genetic differences in the nuclear ITS-2 locus between accessions of *P. perigoniale*, *P. subpilosum*, *P. ericoides* and *P. brachymitrium* from different parts of the world. These include substitutions only (indels are not easily handled in TCS networks). Three haplotypes representing the South American *P. brachymitrium* are distinguished from a haplotype of European, North American and Hawaiian accessions of *P. perigoniale* by at least four substitutions. The African *P. subpilosum* and *P. perigoniale* samples are different and distinct from the European, North American and Hawaiian *P. perigoniale* accessions by at least three substitutions. The haplotypes containing the African *P. perigoniale* accessions are distinct from the *P. perigoniale* haplotypes elsewhere. One of the African *P. perigoniale* specimens is identical to some *P. subpilosum* specimens, and the haplotype representing the other African *P. perigoniale* accessions only differs by one

substitution from a *P. subpilosum* haplotype. This scenario would be compatible with regarding them as a single taxon with some degree of variation within it. Hence, the geographically isolated *P. subpilosum* is presumed to be a taxon which is derived from within *P. perigoniale* in Africa. It may have speciated relatively recently and is probably still in the process of speciation. Several haplotypes represent the European and North American (including Hawaii) specimens of *P. perigoniale*, with one common haplotype differing by a one bp substitution from a haplotype containing Southeast Asian (Chinese and Japanese) accessions as well as one from North America. The Australasian accessions of *P. perigoniale* are different from the European, North American and Hawaiian accessions by at least by two substitutions. The Colombian *P. ericoides* haplotypes are different from the Australasian *P. perigoniale* haplotypes by at least two substitutions.

It is now clear that *P. perigoniale* is paraphyletic, corroborating previous studies done by Bell & Hyvonen (2010a, 2010b) and Bijlsma & al. (2000). Hence, within this species complex *P. perigoniale* likely comprises an ancestral morphology from which *P. subpilosum* and *P. ericoides* are derived.

Moreover, based on the haplotype analysis (Figure 3.5) and the phylogenetic structure depicted in Figure 3.4 A, the paraphyletic *P. perigoniale* contains at least two well-supported clades containing potentially cryptic taxa (Figure 3.6). "***The Australasian P. perigoniale Clade***", which has strong Baysean support (1.00), contains three accessions from mainland Australia, Tasmania and New Zealand, while a well-supported (0.88 PP, 71% BS) clade containing three accessions from Kenya, Madagascar and Tanzania ("***The African P. perigoniale Clade***") is also distinguished as a morphologically distinct entity within the paraphyletic clade of *P. perigoniale*.

The accessions (accessions) in both of these clades have somewhat distinct leaf shapes (Figure 3.6). However, a thorough morphological study is required to definitively separate them morphologically from *P. perigoniale sensu lato*. Moreover, this would require more taxon sampling and more molecular markers to strengthen the phylogenetic signal and resolve the weak statistical support for evolutionary relationships within this apical clade. Therefore, in the present study these two groups are treated as potentially "pseudocryptic" species (Bickford & al., 2007) derived from within the paraphyletic *P. perigoniale* (Clade A), although it is too early to formally describe them. This observation is also congruent with the structure of the

nuclear ITS-2 haplotype network (Figure 3.5), which clearly shows the Australasian and African accessions of *P. perigoniale* as distinct.

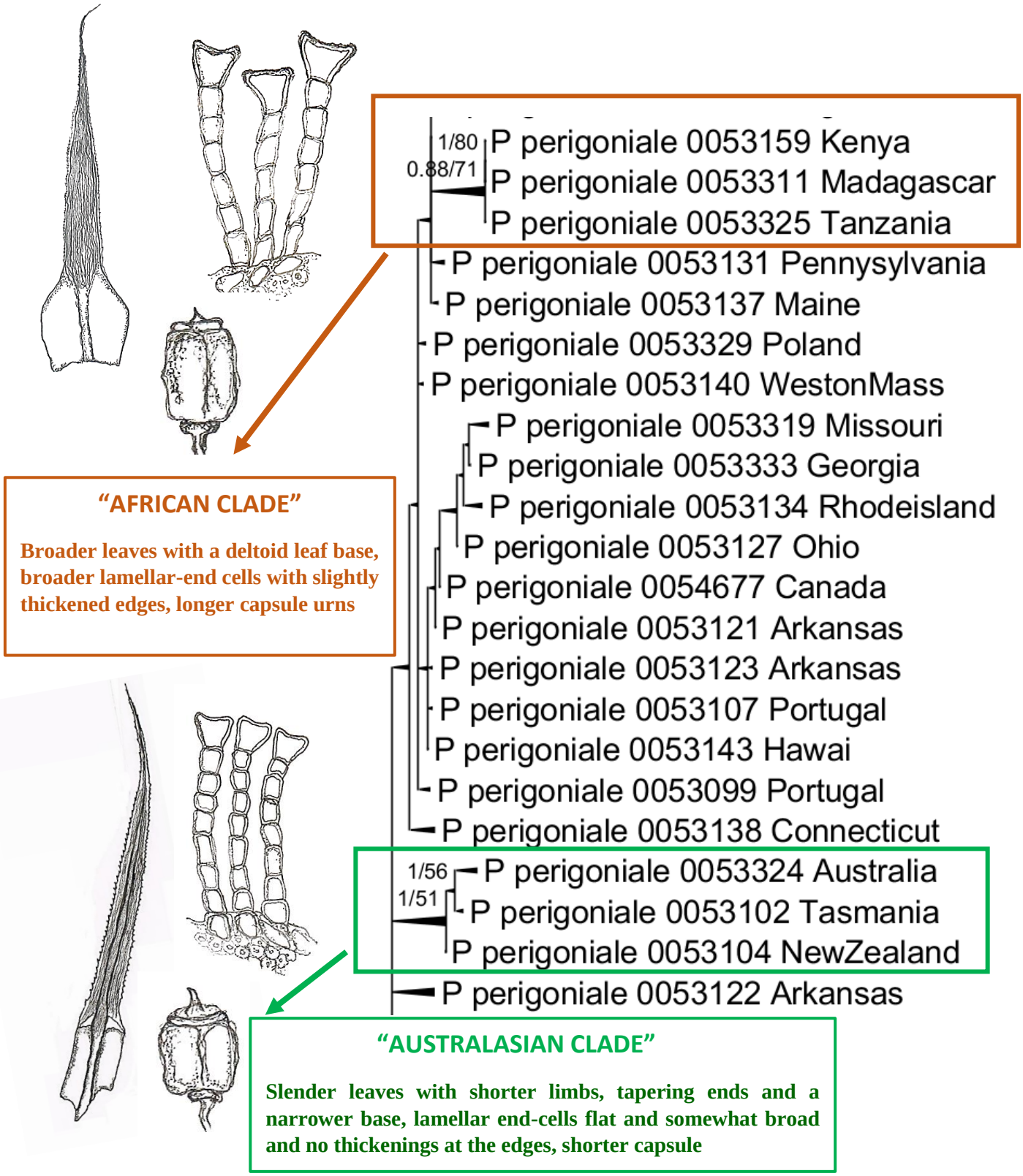


Figure 3.6: Phylogram showing a part of the apical clade (clade A) of the Maximum Likelihood tree and highlighting the pseudocryptic morphologies derived within the *P. perigoniale* Michx. clade in Africa and Australasia (all illustrations for groups are drawn to the same scale).

Although they can be separated morphologically, the characters that could be used to separate them have not yet been thoroughly investigated and were not noted in previous studies. They are within what Bickford & al. (2007) described “as a single nominal species” (*P. perigoniale*), because they are “at least superficially morphologically indistinguishable”. However, this is not due to the fundamental limitations of morphological methods, as taxonomists could recognise these as extreme variants or ecotypes of *P. perigoniale*. However, with the light of the phylogenetic analysis, these two groups are clearly distinguished as two well supported clades within the apical clade (clade A), which merits a future study with thorough taxon sampling.

The term “species complex” is a broad descriptor of organisms, where distinction into species is difficult due to overlapping morphological characteristics (based on the “typological species concept”; Mayr, 1957; Stussey, 2009) or weak phylogenetic signal (based on the “phylogenetic species concept”; Henning, 1950 as cited in de Queiroz, 2007). Cryptic species and species aggregates are merely specific types of species complexes. However, all these groupings are a part of informal taxonomy described for ease of communication and are not used in theoretical taxonomy. In theoretical taxonomy, there is no such thing as a "*species complex*" (see ICN, Turland & al., 2018).

Even though *P. perigoniale, P. subpilosum* and *P. ericoides* are distinctive morphological species, it is presumed that these taxa are just starting to diverge. Bell & al. (2015) have determined that the split between *P. commune* and *P. brachymitrium* (erroneously identified as *P. ericoides*) had happened ~15 million years ago. Since *Polytrichum* sect. *Polytrichum* represents quite a recent lineage, we can assume the species complex composed by these three species is at the early stages of its diversification.

A “species complex” may not always comprise monophyletic species. There is a possibility that it could arise through parallel events of interspecific hybridization. In other words, if two pairs of individuals of closely related species successfully interbreed, their respective offspring may not be exactly similar enough to be conveniently represented by one type specimen that comes from one of them. Once they have sufficient structural, genetic and biochemical similarity in addition to environmental range and reproductive compatibility, they may be seen either as distinct species or a 'species complex'. In other words, the term 'species complex' could be used to indicate existence of some reasonable or clearly observable level of heterogeneity among members of a species (Stussey & al., 2014).

In order to resolve the phylogenetic relationships within this species complex, a robust gene sampling (using a large number of nuclear markers) was undertaken and a phylogenetic reconstruction made for a selected subset of accessions of each species using a Next Generation Sequencing Approach (see Part B in this chapter).

- **CLADE B (*Polytrichum brachymitrium* Müll. Hall.)**

In the present phylogeny of *Polytrichum sect. Polytrichum, Polytrichum brachymitrium* represents a strongly supported clade (100 % BS and 1.00 PP), while there is also strong statistical support for the monophyly of a large apical clade that includes Clade A (the "species complex" of *P. perigoniale* + *P. subpilosum* + *P. ericoides*) together with *P. brachymitrium*, although not for the monophyly of Clade A to the exclusion of *P. brachymitrium*.

The variable region of the *trnG* intron shows a 30bp inverted repeat duplication in all accessions of *P. brachymitrium*. This is congruent with a single unique substitution characterising the species in the *trnL-F* matrix and a single unique bp insertion in the ITS-2 region.

The specimen identified as *P. ericoides* Hampe from Colombia (*Churchill & Betancur, 17033*, MO) used by Bell & Hyvönen (2010 a, 2015) was re-sequenced for all of the molecular markers used for the present study and is now redetermined as *P. brachymitrium*, this being corroborated by morphological study. This expands the current understanding of the geographical distribution of *P. brachymitrium* and further adds a new species to the flora of Colombia. The present study further expands the geographical range of *P. brachymitrium* by adding sequences from Argentina. This conflicts with the statement made by Peralta and Yano (2010) based on their morphological study of the Polytrichaceae in Brazil, in which they stated that *P. brachymitrium* is a species lacking in Brazil and found in Venezuela and Colombia. Rather, *P. brachymitrium* is a widely distributed taxon which is present in Brazil, Venezuela, Argentina and Colombia (see Chapter 04).

- **CLADE C (*P. angustifolium* Mitt.)**

Strongly supported monophyly of *P. angustifolium* within *Polytrichum* sect. *Polytrichum* is observed. Accessions of *P. angustifolium* have never been used in previous molecular studies of Polytrichaceae (Hyvönen, 1998; Hyvönen & al., 2004, 2006; Bell & Hyvönen, 2008; 2010 a, b, 2012). It is observable there is a 3 bp deletion (235-239 bp in the

matrix) found in the variable region of the ITS-2 matrix and two single bp changes in the spacer region of the r*pl16* matrix in all sampled accessions of the species. *Polytrichum angustifolium* is a very distinctive taxon which is regarded as endemic to Brazil. Sterile plants resemble *P. perigoniale* and the allied taxon *P. brachymitrium*; however, leaf anatomy delimits the species boundaries. Leaf transverse sections show pyriform lamellar end-cells with or without thickenings. This is the only taxon in the section which possesses pyriform lamellar-end cells (see Chapter 04), a common plesiomorphic character in *Polytrichum* sect. *Juniperifolia*. Hence, the present molecular study corroborates the current morphological delimitation of *P. angustifolium* and is compatible with its relatively early diverging position within the section.

- **CLADE D (*Polytrichum swartzii* Hartm.)**

Strong monophyly of *P. swartzii* is observed in both ML and BI analyses with a 1.00 PP and 96% BS support and it is strongly supported (1.00 PP and 97% BS) as a sister clade to the larger clade comprising clades A (apical clade), B (*P. brachymitrium* clade) and C (*P. angustifolium* clade). Although plastid data does not show much variation within the *P. swartzii* clade, the nuclear ITS-1 and ITS-2 data show some single base pair deletions and additions. Two specimens of *P. swartzii* from Wyoming, USA and Finland are strongly supported as monophyletic (1.00 PP, 100% BS) and are molecularly distinguishable from other populations of *P. swartzii* included for this study. This merits a population level study to observe the variation among the different populations of this widespread species.

Long (1985) synonymised two "semi-aquatic" taxa, *P. algidum* Hag. & C. Jens. and *P. inconstans* Hag., with *P. swartzii* based on morphological affinities. However, he distinguished these two taxa from *P. swartzii* using the degree of differentiation of the lamellar end-cell morphology. Single accessions of *P. algidum* (*La Frage, 74-112*, [BC]) and *P. inconstans* (Steree, *16643*, Alaska, NY) were included in the molecular study and both taxa found to be nested with the *P. swartzii* clade, although their relationships to other samples within *P. swartzii* are not strongly supported (68% BP and 0.56 PP). Specimens collected from China (*P. -C. Wu 21025*, [NICH, PE], *X.-Y, Hu, 797* [PE] labelled as *P. swartzii* (which were used to delimit the taxon *P. swartzii* in the Moss Flora of China, 2005)) were included in the present molecular study and they all grouped with *P. perigoniale*. This corroborates Hyvönen and Lai's (1991) suggestion that *P. swartzii* is a high-altitude species that should be excluded from the floras of Taiwan and South east Asia. The major confusion in the morphological determination of Chinese and Taiwanese specimens of *P. perigoniale* misidentified morphologically as *P.*

swartzii relates to the lamellar end-cell morphology (see Chapter 04). However, with careful observation *P. swartzii sensu stricto* can easily be distinguished by its broader, plate-like (flattish) lamellar end-cells without any thickenings (see Chapter 04), fragile plant form and leaves that always become blackish when old (this is a very distinctive, notable character which can even be observed in very old herbarium specimens of *P. swartzii*).

- **CLADE E (*Polytrichum commune* Hedw.)**

The long-standing nomenclatural issue of the identity of *P. commune* Hedw. was settled by selecting a lectotype (see Chapter 02). In order to establish the correct phylogenetic identity of *P. commune*, accessions were selected to cover a wide geographical range, including Southeast Asia (China, Japan), South Africa and Madagascar, Brazil, Arctic and Sub-arctic Europe (Russia, Sweden, Latvia, Austria etc.) and North America. The majority of the African (100%) and North American (90%) accessions labelled as *P. commune* on the specimen labels were determined as *P. perigoniale* (see the discussion above). Hence the taxon *P. commune s. str*. is excluded from Africa based on molecular and morphological determination. The *P. commune* concept was erroneously used in Africa for *P. perigoniale*, and a similar scenario was observed in North America. However, in their recent taxonomic revision of Polytrichaceae in Brazil, Peralta and Yano (2010) reported the presence of *P. commune* in Brazil and the present molecular study confirms its presence in South America. Although *P. commune* is presumed to have a European origin (Smith, 1971) and a predominantly northern distribution, this study corroborates its southern distribution as described by Peralta and Yano (2010). A specimen collected from Yukon, North America which was labelled as *P. commune* var. *yukonense* (*Vitt, 13251*, BC) was also included in this study based on the argument by Long (1985), as this taxon was widely treated as a separate species and merits further study to establish its identity. The present molecular results show that *P. commune* var. *yukonense* belongs with *P. commune s. str* with strong phylogenetic support and it is synonymised here. Two large sister clades within the *P. commune* clade are strongly supported with PP values of 1.00. However, it would be necessary to carry out a population level study with expanded taxon sampling to observe the variation among the populations in different geographic regions to conclusively identify any cryptic taxa within the group.

Schriebl (1990) described *P. uliginosum* (Wallr.) Schriebl based on *in vitro* and morphological studies, and a few specimens identified as *P. uliginosum* (e. g. *Hallingback,*

Sweden, 4062) were included for this study. All these accessions identified as *P. uliginosum* are now nested with the *P. commune* clade (Figure 3.4 A), with a strong PP (1.00) support. Hence, *P. uliginosum* (Wallr.) Schriebl is confirmed as a synonym of *P. commune* Hedw. *s. str* from this study (see Chapter 04). Van der Velde & Bijlsma (2000) also reported a high level of genetic relatedness of *P. commune* and *P. uliginosum.*

Although the plastid data does not exhibit much variation for the separation of *P. commune* and allied taxa, the nuclear ITS-2 matrix provides a lot of useful sites. *P. commune* and *P. jensenii* share a 14 bp insertion from 403-416 bp in the ITS-2 matrix, and another 8 bp insertion (371-379 bp) in ITS-2. Moreover, *P. commune* exhibited a few single bp deletions in the spacer region of ITS-2 (positions 160, 226 and 236).

Bijlsma & al. (2000) showed that *P. commune* (now *P. perigoniale*) and *P. uliginosum* (now *P. commune*) are two genetically distinct entities which were earlier treated as two varieties of *P. commune sensu lato* (*P. commune* ≡ *P. commune* var. *perigoniale* and *P. uliginosum* ≡ *P. commune* var. *commune*). This nomenclatural determination leads to a lot of nomenclatural issues and it has been erroneously used in identifying these two taxa, especially by non-bryologists in the field. However, Bijlsma & al., (2000) supported their idea not only by their molecular evidence (allozyme and RAPD) but also by ecological and morpho-anatomical studies. Later studies by Bell & Hyvönen (2010 a,b) showed that *P. commune* var. *commune* (now *P. perigoniale*) and *P. commune* var. *uliginosum* (now *P. commune)* are phylogenetically distinct entities.

Ecologically, *P. perigoniale* is commonly found in exposed, dry, sandy habitats whereas *P. commune* is found almost exclusively in wet peat bogs, fens, wet heaths and the edges of pools, etc. Moreover, *P. perigoniale* exhibits a consistent difference in the morphology of the lamellar end-cells of the leaf lamellae in transverse section. *Polytrichum commune* shows deep, asymmetrically grooved apical end cells, the plants are comparatively taller, and leaves are arranged to form the characteristic “star-like” appearance, being distantly spaced on the stem. In contrast, *P. perigoniale* has much flattened or shallowly grooved apical end cells and plants are shorter than those of *P. commune.* These characters are described in detail in Chapter 04.

- **CLADE F (*Polytrichum jensenii* I. Hagen)**

The *P. jensenii* clade comprises most of the Arctic taxa. This study is the first phylogenetic work to include *P. jensenii* accessions in analyses of Polytrichaceae. Long (1985)

treated this taxon as a variety of *P. commune*, *P. commune* var. *diminutum* (Hagen) Long, based on the morphological similarity of the leaf lamellar end-cell characters with *P. commune* (both taxa have deeply grooved, "U-shaped", lamellar end-cells). It is a morphologically distinctive taxon possessing entire leaf margins and a fragile texture, although sterile plants often get confused with the allied taxon *P. swartzii* Hartm. (see Chapter 04).

In his study Long (1985) mentioned that the relationships between *P. commune* var. *commune* (now *P. commune s. str*) and *P. commune* var. *diminutum* merit further study. The present study strongly supports the monophyly of the taxon and its identity as a distinct phylogenetic species, strongly supported (PP 1.00, 93% ML) as sister to *P. commune s. str*. The ITS-1 matrix provides useful molecular signals for distinguishing *P. jensenii* from *P. commune s. str*. In the nuclear ITS-1 matrix, *P. jensenii* shares many synapomorpihc insertions with *P. commune s.str* in that are lacking in other taxa within *Polytrichum* sect. *Polytrichum*. Both *P. commune* and *P. jensenii* share an insertion of 9–12 bp, from 400–412 bp in the ITS-1 matrix. Furthermore, there is a 15 bp insertion in *P. jensenii* in ITS-1 from 425–440 bp. Although plastid matrices do not provide strong evidence, nuclear sequences contain strong signal for the monophyly of *P. jensenii* as a distinct species.

Together with the morphological circumscription (entire leaf margins, leaf lamellar end-cells with knob-like papillae on the tops) these results now help to delimit *P. jensenii* as a distinctive taxon confined to Arctic and Subarctic areas.

3.2.8 Conclusions

The present molecular analysis is the first comprehensive phylogenetic reconstruction using the Sanger sequencing for *Polytrichum* sect. *Polytrichum*. Although a full ancestral character state reconstruction was not performed due to time constraints, the definitive morphological characters used to separate each taxon are discussed in Chapter 04. Based on the Sanger phylogenetic inference, the following conclusions are reached.

i. Samples from all of the accepted species in *Polytrichum* sect. *Polytrichum* were included in the molecular study, except for *P. elegans* Welw. & Duby (due to poor quality DNA extracted from the type specimen), with exemplar accessions to cover their geographical distributions to detect interspecific variations. Altogether 463 DNA sequences from six loci (86 sequences of ITS-1, 102 sequences of ITS-2, 66 sequences of *rbcL*, 74 sequences of

rpl16, 86 sequences of *trnG* and 49 sequences of *trnL-F*) were produced. All of these were newly sequenced for the study, thus considerably expanding existing Polytrichaceae data sampling.

ii. The molecular species delimitations resulting from Sanger sequencing of plastid and nuclear markers corroborate the current understanding of morphological species concepts within *Polytrichum* sect. *Polytrichum*. However, a robust taxon sampling with more accessions representing closely related populations may provide better resolution for weakly supported sister clades. Despite the weakly supported relationships within the species complex comprising the three geographically and morphologically distinct species *P. perigoniale*, *P. ericoides* and *P. subpilosum*, it can be hypothesised that these taxa are still in the process of speciation. Since there is no supported incongruence between plastid and nuclear datasets, it is suggested that it is unlikely that reticulate hybridisation processes are occurring among the closely related taxa.

iii. The distinct identity of *P. perigoniale* Michx. is established from this study. Based on this the nomenclatural misapplication of the name *P. commune* to North American accessions of *P. perigoniale* is identified. Similarly, the misapplication of *P. commune* Hedw. in Africa and of *P. swartzii* in China and Southeast Asia is highlighted by this molecular species delimitation. Existing synonymies based on morphological characters of the poorly studied taxa *P. commune* var. *minus* (≡ *P. perigoniale*), *P. commune* var. *yukonense* (≡ *P. commune*), *P. inconstans* (≡ *P. swartzii*), and *P. algidum* (≡ *P. swartzii*) were established and confirmed with the molecular species delimitation.

Appendix 1

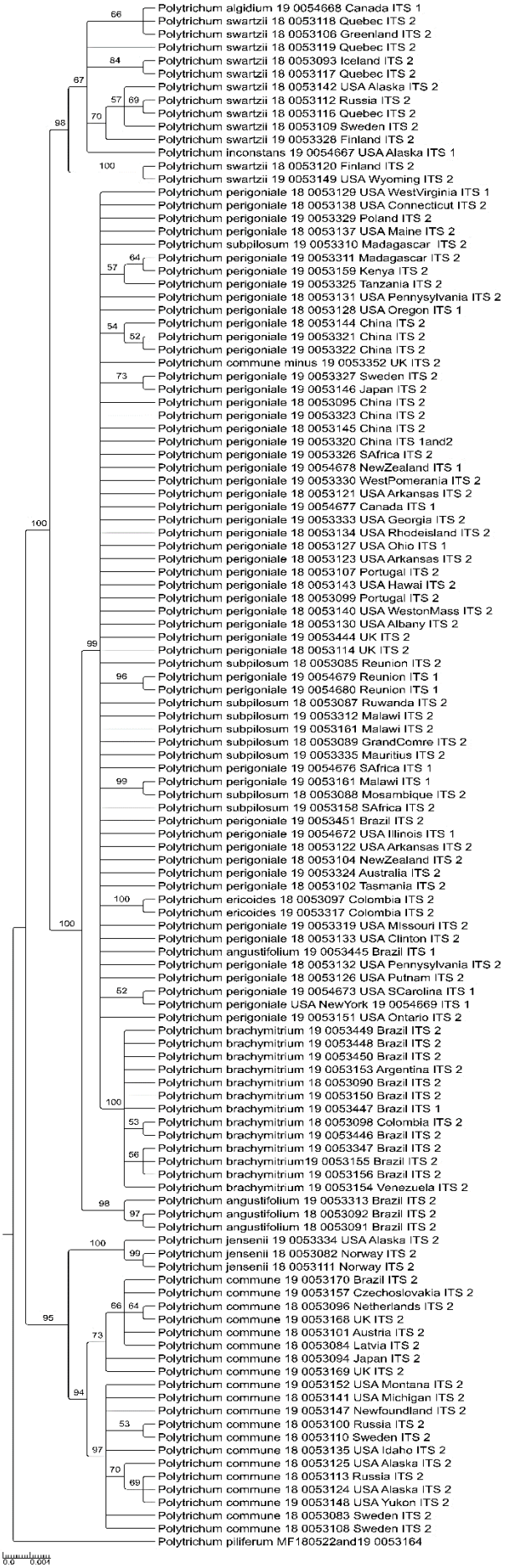


Figure A: A phylogram showing the ML topology resulting from the analysis of total combined dataset of nuclear ITS-1 & ITS-2 under the GTR +I +G model. BS values < 50% are not included.

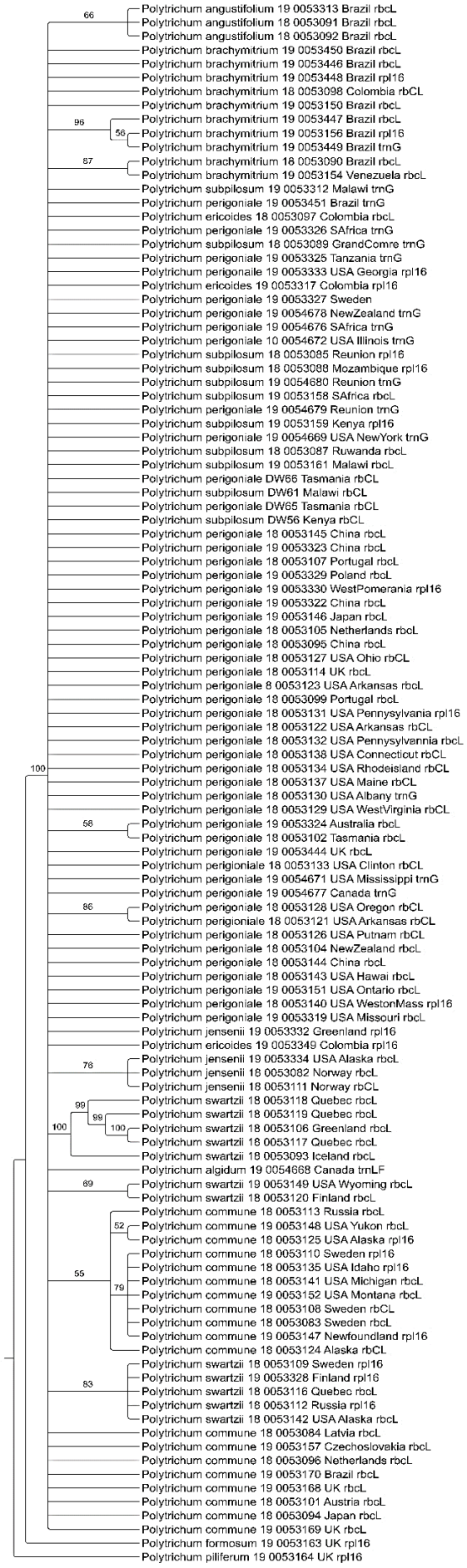


Figure B: A phylogram showing the ML topology resulting from the analysis of total combined dataset plastid markers (*rbcL, trnL-F, trnG, rpl16*) under the GTR +I +G model. BS values < 50% are not included.

Appendix 2

Next Generation Sequencing (NGS) Approach: A Collaborative Study:

Generating a NGS Phylogeny using Target Enrichment Approach.

- **A Brief Introduction to the NGS Project**

This appended section is largely based on a collaborative project to generate a preliminary NGS phylogeny for *Polytrichum* sect. *Polytrichum*, undertaken in parallel with the main Sanger sequencing project and carried out between October 2017 and January 2019.

Laboratory work (DNA extractions, library preparation, library pooling and target capture, etc.) was performed by Dr. Laura L. Forrest (RBGE) and me, selecting a representative subset of herbarium specimens (24 ingroup accessions and 3 outgroup taxa) covering all extant taxa within the section. Two methods of library preparation (DNA shearing by sonication and enzyme digestion) were used for each sample. Libraries were enriched with *Physcomitrella* RNA baits (received from Prof. Bernard Goffinet's Lab in Connecticut, USA) to sequence 809 low-copy nuclear loci. Bioinformatics processing was performed by Dr Flávia Pezzini (RBGE). The NGS data was assembled using different approaches (*de novo* and reference mapping) and with different software and settings to assess their impact on downstream phylogenetic analysis. All loci were concatenated, treated as a single partition and analysed under the ML phylogenetic framework. A final ML tree is presented below (Appendix 2, Figure C).

Not all technical and bioinformatics aspects of this collaborative NGS project are appended here, as some are not entirely my own work and thus do not form part of the main body of the thesis. Only the final ML tree generated from 809 low-copy nuclear loci for 27 accessions of *Polytrichum* sect. *Polytrichum* using the concatenated matrix approach is presented below (Appendix 2, Figure C), together with a discussion of its general phylogenetic structure, a comparison of the Sanger and NGS phylograms, and a general conclusion.

The final alignment built using the Nicholls & al. (2015) pipeline with 809 loci which posseses 226 informative sites and 16,962bp long. The gaps of the final alignment were improved using trimAl[V1.2] by removing columns with gaps in more than 50% of the samples. The phylogeny was inferred with 100 runs and 100,000 bootstrap replicates in RAxML v.7.4.2 as described in 3.2.61. The GTR+I+G model was selected treating the whole alignment as a single compartment.

- **General Phylogenetic Structure Generated from the NGS (Target Capture Approach) and a Comparison between NGS and Sanger Phylogenies**

The general phylogenetic structure of the phylogram generated for *Polytrichum* sect. *Polytrichum* using the NGS approach is similar to that obtained from the Sanger phylogeny (Figure 3.4 A), except for the relative positions of *P. ericoides* and *P. brachymitrium* (see below). The NGS phylogram consists of two major clades - a clade comprising *P. jensenii* and *P. commune*, and a larger clade comprising *P. angustifolium*, *P. ericoides*, *P. perigoniale*, *P. subpilosum* and *P. brachymitrium*, with a *P. swartzii* clade sister to the larger clade.

Although most intra-specific relationships are weakly supported (less than 50% BS), *P. commune*, *P. swartzii*, *P. angustifolium* and *P. ericoides* appear as distinctive clades with more than 80% bootstrap support. There is only a single accession of *P. jensenii* and its sister relationship with the *P. commune* clade is somewhat weakly supported (51%). However, this relationship was strongly supported in the Sanger phylogeny with 1.00 BP and 93% BS support.

Even though the intraspecific sequence variation and the phylogenetic relationships among major clades within *Polytrichum* set. *Polytrichum* has been resolved to a certain extent using the Sanger sequencing approach combining both nuclear and plastid markers (Figure 3.4 A), the aim of generating this NGS phylogeny (with a more limited number of representative taxa but a much larger number of nuclear loci) was to resolve relationships among three geographically isolated taxa (*P. perigoniale*, *P. subpilosum* and *P. ericiodes*), which comprise a poorly supported large apical clade (Clade A; Figure 3.4 A).

Although there is a limited number of accessions in the NGS study, the monophyly of *P. angustifolium* is confirmed with 100% BS support, *P. swartzii* is monophyletic with 95% BS support (the long branch of *P. swartzii* 0053119 is due to missing data), and *P. commune* s. str with 98% BS support.

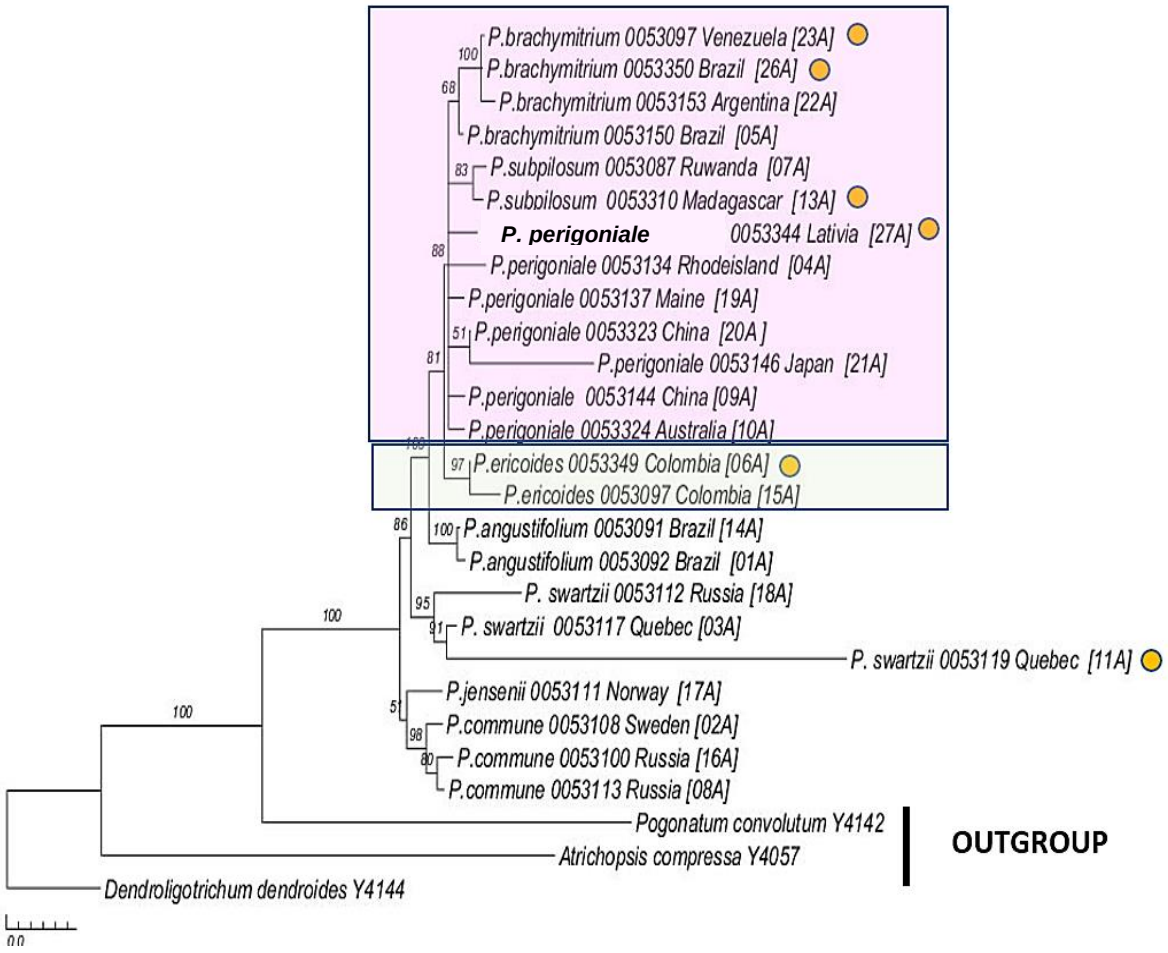


Figure C: Maximum likelihood phylogram derived from analysis of 809 low copy nuclear loci for 27 accessions of *Polytrichum* sect. *Polytrichum* using the concatenated matrix approach. Accessions which were not included in the Sanger sequencing and only used for the NGS study are marked with yellow circles.

The striking differences between the Sanger (Figure 3.4A) and NGS (Appendix 2, Figure C) topologies are the relative positions of two South American taxa *P. brachymitrium.* and *P. ericoides* in the corresponding phylograms. In the NGS phylogram (Appendix 2, Figure C), the monophyly of the *P. brachymitrium* clade is supported with 68% BS support and within this clade, there is a subclade of three specimens with 100% BS support. Along with *P. brachymitrium*, *P. perigoniale* and *P. subpilosum* comprise a strongly supported apical clade

(88% BP) where relationships among the three taxa are weakly supported and ambiguous. *Polytrichum ericoides* is well supported as sister to this clade (81% BS).

In contrast, the Sanger phylogeny circumscribes *P. brachymitrium* as a well-supported clade (100% BS and 0.99 PP) outside of a weakly-supported apical clade that here includes *P. ericoides*. *Polytrichum ericoides* forms a well-supported (100% BS and 1.00 PP) clade within the weakly supported apical clade. The relationships between *P. subpilosum* and *P. perigoniale* and between *P. ericoides* and *P. subpilosum* are weakly supported (56% BS) in the Sanger phylogeny.

In the NGS phylogeny, *P. subpilosum* is represented by two accessions and its monophyly supported with 88% BS support. However, there is no resolution to infer how it relates to *P. perigoniale* or *P. brachymitrium.*

In the above scenario of conflict between the NGS and Sanger phylogenies regarding the positions of *P. brachymitrium* and *P. ericoides*, it is obvious that the phylogenetic signal from many nuclear loci is greater than that of a single nuclear locus combined with a few plastid loci. The NGS (Appendix 2, Figure C) and combined Sanger (Figure 3.4 A) trees are not necessarily incompatible if strong support is regarded as necessary for incongruence. Thus, certain gene trees, whether plastid or nuclear, could help to resolve the picture found in the Sanger phylogeny. This could be achieved by running a constraint analysis to find a best-fitting Bayesian tree for the Sanger phylogeny which is congruent with the NGS tree.

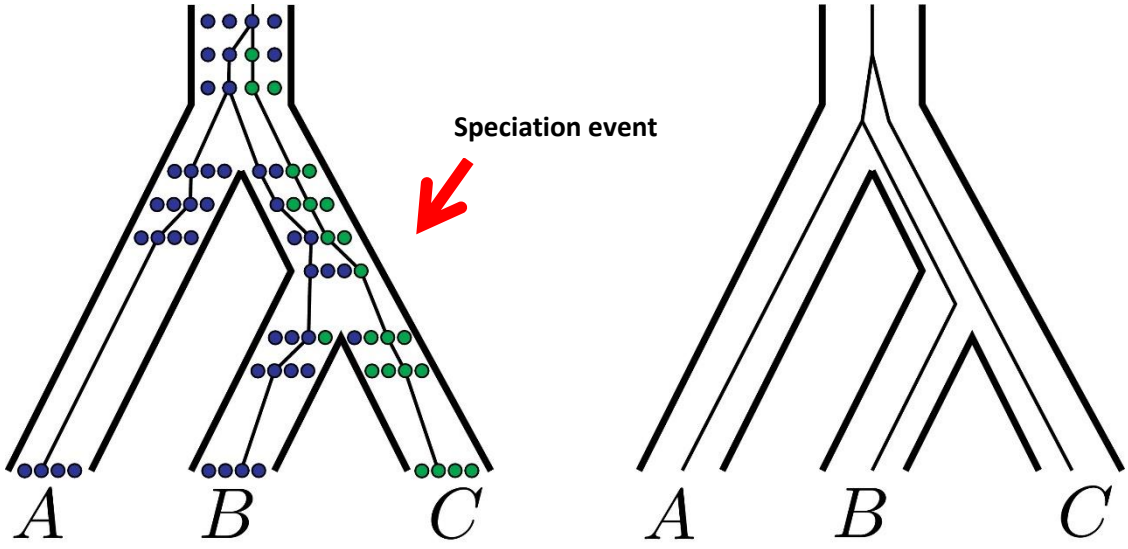
Moreover, in the Sanger phylogeny a conserved ITS signal and a mixed plastid signal can be explained by the presence of populations with mixed plastid genotypes due to incomplete lineage sorting (Naciri & Linder, 2015). As Naciri & Linder (2015) described in their paper, intra-population and intra-specific polymorphism may be observed due to incomplete lineage sorting or reticulate hybridisation events and these could cause non-monophyletic species affecting species delimitation. Potential reticulate hybridisation events happening in Polytrichaceae were observed by Bell & Hyvönen (2010b). These may collectively affect the overall phylogenetic resolution of a study group (Chan & al., 2017).

The present scenario, with mixed phylogenetic signal and problematic phylogenetic species delimitation in *Polytrichum* sect. *Polytrichum* might reasonably be explained by two of the three key evolutionary trajectories [i.e. (i). *Intergenomic Transfers*: transfer of chloroplast and mitochondrial genes to the nuclear genome, (ii) *Hybridization* and (iii) *Incomplete Lineage Sorting*] explained by Naciri & Linder (2015) in their publication:

i. **Hybridization** (i.e., interbreeding between individuals of different, closely related species). If the hybrids are viable and fertile, this leads to intraspecific transfer of genetic material which makes reticulate evolutionary relationships among closely related species (Bell & Hyvönen, 2010b).

However, there is no evidence from the ITS data suggestive of hybridisation having occurred, such as polymorphic sequences or polymorphic positions. Hence incongruence may be due to incomplete lineage sorting (See below).

ii. **Incomplete lineage sorting** (Figure D below), this refers to failure of gene copies to coalesce and homogenise, within the life span of a species (Chan & al., 2017).



(a) Population view (b) Reconciliation representation

Figure: D. A diagrammatic representation to illustrate incomplete lineage sorting: The impact of incomplete lineage sorting on simple populations of 4 haploid individuals. The originating population contains an allele (blue) for the considered gene. First, a mutation leads to a new green allele at this locus, then a first speciation takes place, rapidly followed by a second one. As the blue and green alleles still co-exist when the second speciation takes place, both alleles still have a chance to be fixed in the resulting daughter species *B* and *C*. For these species, the evolutionary history of this gene will thus be different from the species history due to incomplete lineage sorting (Photo and legend reproduced from Chan & al., 2017).

According to Chan & al. (2017), incomplete lineage sorting occurs when a genetically polymorphic trait predates a dichotomous speciation event which is inherited by one or both daughter species, which leads to share the alleles between populations or species which are not each other's closest relatives.

In the present study, the large apical clade has poor resolution and weak support values to infer phylogenetic relationships between *P. perigoniale, P. subpilosum, P. ericoides* and *P. brachymitrium*. Therefore, the species boundaries of these taxa are difficult to interpret in a phylogenetic context using nuclear ITS and a combination of plastid markers (*rbcL*, *trnL-F*, *rpl16*, and *trnG*).

- **General Conclusions**

1. This preliminary collaborative study provides an integrative approach to delimit the taxa of *Polytrichum sect. Polytrichum* using a robust Sanger phylogeny and a preliminary NGS phylogeny. Although a limited number of specimens were sampled for the NGS study (due to time limitations), enrichment was done with *Physcomitrella* RNA baits (universal baits used to investigate high level relationships between acrocarpous lineages of mosses), and the enrichment wasn't particularly successful (see appendix of this chapter), the phylogeny generated with 809 nuclear loci presented a more or less similar tree topology to that produced by the Sanger sequencing. This study could be improved in future by designing more *Polytrichum* or Polytrichaceae-specific baits sets to provide more data and more comprehensive taxon sampling from more populations representing a broader geographical range to help discriminate the weakly supported taxa in the present phylogeny. Further chloroplast data, which might be generated as an offshoot from a capture study or a from separate genome-skimming approach, could generate a more complete plastid phylogeny to facilitate detection of any reticulate hybridisation events happening within the group.
2. Despite the observed conflicting positions of *P. ericoides* and *P. brachymitrium*, and weakly supported apical clade with ambiguous relationships between populations of the paraphyletic entity *P. perigoniale* and *P. subpilosum* in both Sanger and NGS phylogenies, both support the existing morphological taxonomy of *Polytrichum* sect. *Polytrichum.* Furthermore, the lack of strong support in the Sanger phylogeny for the nodes that conflict with the NGS phylogeny mean that the two topologies need not be seen as fundamentally incompatible.

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CHAPTER 04

TAXONOMIC REVISION OF THE *POLYTRICHUM* SECT. *POLYTRICHUM*

"Taxonomy is described sometimes as a science and sometimes as an art, but really it's a battleground."
— Bill Bryson , *A Short History of Nearly Everything*

4.1 Introduction

From a historical perspective, de Candolle (1813), a Swiss botanist working in the herbarium at Geneva, Switzerland, first used the term "*taxonomy*" (as "*taxonomie*"), to explain the "the theory and practise of plant classification". The term "taxonomy" is now broadly used to encompass the methods, principles and rules of classification of any group of organisms (Simpson, G.G., 1961; Stace, 1980; Stuessy, 2009a; Stuessy & al., 2014). Since taxonomy has a long history, taxonomists must be able to find, archive and utilise the past concepts and sources of taxonomic literature of a desired group of plants, and to study, understand and incorporate novel discoveries and material from the present. This may provide a better and holistic understanding of the "taxonomy" of any desired group of plants. Since "taxonomy" can utilise data from a broad spectrum of disciplines such as morphology, anatomy, embryology, biochemistry, phenology, ecology and molecular biology, it provides an integrative platform for the study of organisms. Hence taxonomy shoulders major responsibilities in the process of identifying and describing organisms, which means it is directly and indirectly applicable as a tool in diverse fields of experimental biology.

From de Candolle to Darwin (1859), incorporating Darwin's "*theory of evolution*" by means of natural selection, the terms "taxonomy" and "systematics" were used synonymously in the past (Stuessy, 2009a; Stuessy & al., 2014). Simpson (2006) defined systematics as a "science that includes and encompasses traditional taxonomy, the description, identification, nomenclature and classification of organisms". However, there are some biologists who still equate the term taxonomy with systematics (Stace, 1980; Minelli, 1993; Singh, 2004). However, systematics is a broader science which also aims to interpret the processes of evolution, information which can then be used by other areas of biology (such as modern molecular biology); the pivotal role of systematics is to infer the phylogenetic relationships

among a desired group of organisms. The distinction and the interconnection between "*taxonomy*" and "*systematics*" is judiciously stated by May (2004): "taxonomy provides the bricks and systematics the plan, with which the house of the biological science is built". In a broad sense, taxonomy deals with naming and grouping organisms, while systematics classifies organisms based on their evolutionary relationships or phylogeny. Taxonomy without evolution is regarded as weak taxonomy (Hörandl, 2007). Hence systematics is a very positive stimulus for taxonomy as the best classifications incorporate a better understanding of the evolutionary trajectories which underpin evolutionary /phylogenetic processes (Stuessy & al., 2014).

Allkin (1988) states: "taxonomists subconsciously use their knowledge of the nature of taxonomic data and the rules governing taxonomic procedures to interpret and manage their data effectively". Taxon-based herbarium research can mainly be discussed under two headings, monographs and revisions (Radford, 1986; Jones & Luchsinger, 1987; Stace, 1989; Stuessy, 1990). The difference between these two processes is essentially one of scale and taxonomic focus (Radford, 1986). A monograph is a formal compilation of all available taxonomic information relating to a particular plant group (broader scope), whereas a revision is more limited and is largely confined to a formal compilation of morphological variation for a plant group, possibly from a particular geographical area (narrower scope).

Maxted (1992) proposed a definition for a taxonomic revision in his paper as follows: "A taxonomic revision is a novel analysis of the variation patterns within a particular taxon, considered in conjunction with information from the literature, which results in the generation of primary and secondary products. The primary product is a novel classification of the taxon and is complemented by a range of secondary products such as descriptions, keys, synonymised lists, taxon illustrations, critical notes etc."

Davis & Heywood (1963) proposed the first detailed, systematic attempt to break down the steps involved in the revision process. They listed the following nine empirical steps:

- Survey literature to delimit the taxon and clarify the associated taxonomic problems.
- Observe representative specimens and select characters which are discontinuous within the taxon.
- Group specimens on correlated characters.

- Identify diagnostic characters which distinguish specimen groups.
- Record the pattern of character variation both within and between specimen groups.
- Relate specimen groups to any geographical or ecological peculiarities; give taxonomic rank to each specimen group.
- Group taxa on the basis of similarities, so that natural series, sections, etc. build up into a natural classification of the whole taxon.
- Communicate the revision results in the appropriate stylised form of nomenclature, synonyms listed, keys, descriptions, scatter diagrams, maps, etc.
- Infer and discuss phylogenetic trends among taxa.

Although David & Heywood (1963) proposed these guidelines five decades before the present time, they are still invaluable and useful for taxonomists, especially beginners.

Hence, the main objective of a taxonomic revision is a circumscription of all known taxa in a desired study group, to account for and /or to inventorise all legitimate names ever applied within the group, to provide useful taxonomic descriptions of all the existing taxa, to provide the means of their identity (with the aid of taxonomic keys, illustrations, photographs, distribution maps) and to communicate this output to all potential users (field botanists, horticulturists, conservationists, policy makers, ecologists etc.).

According to Maxted (1992), "*a taxonomic revision paradigm*" contains three major components.

(a) ***Revision data***: herbarium specimens, live specimens, images, morphological data, non-morphological data, etc.,

(b) ***Revision process***: curatorial data, descriptive data, nomenclatural data and bibliographic data and,

(c) ***Revision products***:

- primary products – phenetic and/or phylogenetic classifications
- secondary products- character sets, illustrations, distribution maps, specimen citations, typifications, taxon ecogeography, popular field guides etc.

The schematic diagram of the taxonomic paradigm proposed by Maxted (1992) is reproduced below.

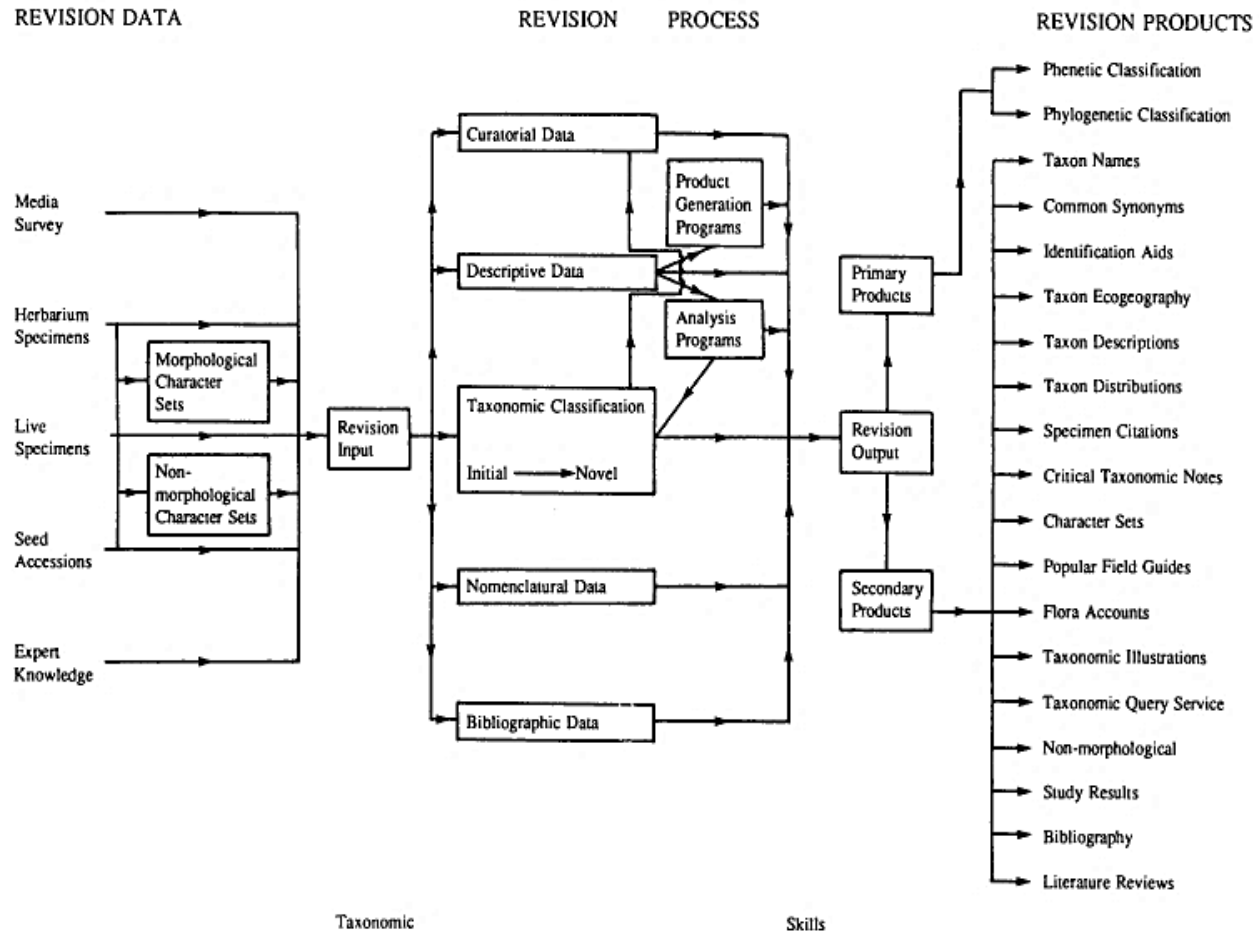

Figure 4.1: A schematic diagram of the taxonomic revision paradigm [Caption & figure reproduced from Maxted (1992)].

4.2 Objectives

Polytrichum sect. *Polytrichum* is a well-circumscribed clade that includes all taxa currently recognised within the broad species concept of *Polytrichum commune* Hedw., one of the most widespread and ecologically important moss species of northern temperate and boreal regions (Bell & Hyvönen, 2010 a, b). Although apparently abundant in mires and wet heaths across Asia and North America and also found in southern temperate areas, it is now clear that

Polytrichum sect. *Polytrichum* comprises a group of closely related taxa distributed in different geographic regions from the Arctic to Sub-arctic regions to the Tropics. There are a few available regional taxonomic studies for *Polytrichum* sect. *Polytrichum* [or the genus *Polytrichum* as a whole] (Frye, 1910; Nyholm, 1960; Husnot, 1967; Lawton, 1971; Howard, 1975; Crum & Anderson, 1981; Long, 1985; De Sloover, 1986; Greene, 1986; Noguchi, 1987; Forrest, 1995; Hyvönen, 2006; Peralta & Yano, 2010; Ivanova & al., 2015; Aponte & Uribe, 2017). However, a complete taxonomic revision to update the taxonomy of the extant taxa within *Polytrichum* sect. *Polytrichum* at a global scale has not been published, and the main objective of this revision is to revise the taxonomy in the light of a molecular phylogenetic study (Chapter 03).

Based on the molecular phylogenetic and phylogenomic work carried out in the present study (Chapter 03), *Polytrichum* sect. *Polytrichum* likely includes some morphologically cryptic and pseudocryptic species. One group of entities derived from within the clade includes all accessions of *P. perigoniale* together with a small number of distinct tropical and pseudocryptic species, this being clearly separated from a grade of earlier diverging lineages that includes *P. commune*. Hence, *Polytrichum* sect. *Polytrichum* is a moderately diverse group containing partially cryptic species, and some of these are geographically isolated. Moreover, there are a large number of accepted binomials applied over the past two centuries that have never been thoroughly studied and require nomenclatural revision. Therefore, earlier and somewhat conflicting taxonomic and nomenclatural treatments associated with the definition of P. commune and allied European taxa (Derda & Wyatt, 1990; Bijlsma & al., 2000; van der Velde & Bijlsma, 2000, 2001, 2003) require a thorough re-evaluation. As a starting point for this, it was imperative that the correct identity of the taxon *Polytrichum commune* Hedw. was established as a precursor to the present study by selecting a lectotype for *P. commune* from amongst Hedwig's own specimens (Chapter 02). Additionally, this will clarify the wide misapplication of the name *P. commune* in Southeast Asia (China, Japan and Taiwan), Africa and Madagascar, and to a great extent in North America.

Moreover, because *Polytrichum* is one of the oldest generic names in bryology and has never been completely revised, there are more than 100 poorly known species names that have been associated with *Polytrichum* sect. *Polytrichum*, in addition to the eight names that represent fairly well understood entities. Hence, a comprehensive taxonomic revision of *P.* sect. *Polytrichum* is considered a taxonomic priority and this alpha-taxonomic revision has

been undertaken to present the first monographic treatment of *Polytrichum* sect. *Polytrichum* on a global scale.

With an integration of molecular approaches (Sanger and Next Generation Sequencing approaches) for species discovery and phylogeny estimation and traditional morphological studies, this study provides a complete taxonomic revision of *Polytrichum* set. *Polytrichum* circumscribing species as traditional morphological taxa under the ICN (The International Code of Nomenclature for algae, fungi, and plants).

The main objectives of Chapter 04 are:

- To circumscribe all taxa in *Polytrichum* sect. *Polytrichum* (in the light of molecular phylogeny) and to resolve existing taxonomic and nomenclatural confusions within the study group.
- To evaluate the status of many unassigned synonyms and poorly understood taxa associated with the study group.
- To provide a taxonomic key to identify the species within *Polytrichum* sect. *Polytrichum*
- To provide complete taxonomic accounts for each species with taxonomic descriptions, revised and updated synonymy, typifications, illustrations (where necessary) and nomenclatural notes.
- To evaluate the ecology and revised biogeography of the accepted taxa with the aid of distribution maps.

4.3 Materials and Methods

4.3.1 Materials

(a) Literature Sources

As stated above, a thorough literature survey is a fundamental precursor to any taxonomic revision to understand the past and current taxonomic status of the study group and to know its existing classifications and groupings. Hence, such a survey was carried out to locate all published protologues (i.e. original descriptions) of all constituent taxa of *Polytrichum* sect. *Polytrichum.* A considerable amount of taxonomic literature including

regional floras, checklists, revisions, synopses and conspectuses published over the past couple of centuries was assembled to study and understand how these taxa were originally defined and to list the characters (morphological) used to delimit the taxa in earlier taxonomic treatments. This also helped to infer judgements of how application of these names has changed over time and possible justifications for the current understanding. A brief taxonomic history of the extant species is provided in 4.4.

(b) Herbarium Material

Herbaria are rightly regarded as "treasure chests" or "genomic treasure troves" by various scientists considering the crucial roles they can play in providing sources for molecular studies (Särkinen & al., 2012; Staats & al., 2013); they are vital repositories of specimens, a range of which can comprise the whole range of morphological variation within a taxon, as well as containing type specimens which are vital for correct application of the relevant names. In some cases, a type specimen may be the only recorded accession of a taxon.

Specimens and the associated data are also a vital resource for evaluating geographical distribution, ecological preferences, economic uses and conservation issues. Utilising herbarium specimens is therefore a fundamental step in any sort of monographic work and in revising the taxonomy of a given taxon or a group of taxa. Observation of original material including type specimens designated by the authors is a fundamental part of making taxonomic and nomenclatural decisions.

In bryophyte systematics, the most common preserved herbarium material is a dry herbarium specimen which is stored in a folded and labelled packet using conservation-grade paper, which may be glued onto a herbarium sheet, stored loosely in folders mounted on herbarium sheets, or filed as single packets placed vertically in drawers of a filing cabinet. Unlike vascular plants, bryophyte specimens are nowadays rarely glued directly onto sheets. The packets with appropriate protection and careful curation can be preserved indefinitely. Within individual specimen packets, smaller packets or paper capsules may hold smaller parts of the plants such as sporophytes, gametangia or fragments selected for dissection, microscopy or illustration. Where a field collection contains more than one taxon (as often happens), these can be separated in labelled mini-packets within a specimen packet, or re-curated as separate specimens with a new unique number (Bridson & al., 1992).

A vital part of any herbarium specimen is the collection or field number which is applied also to images and DNA samples for which the herbarium sample becomes a voucher for identification. Full specimen data are equally vital, nowadays including accurate georeferencing and information on habitat, substrate, elevation, etc. In the case of monographic studies, it is extremely valuable if the field collector has some knowledge of the group so that useful observations can be made and recorded in the field, such as sexual condition, occurrence of sporophytes, and information on associated species of plants.

Unlike higher plants (pteridophytes, gymnosperms and angiosperms) bryophytes are rarely kept as living collections in cultivation, often for practical horticultural reasons. Hence, almost all morphological and anatomical studies performed, and the data recorded for taxonomic revision, have primarily had to be obtained from dry herbarium specimens borrowed and assembled from different herbaria worldwide (Table 4.1). To study the distribution of each species all locality data was catalogued; this will open an avenue to re-find in future the sampled populations in the field (if they still survive); the data is also essential for documenting species distribution and future biogeographical modelling studies. This may also help in future to pinpoint poorly collected taxa in certain geographic regions, as well as to identify populations now presumed extinct in the wild.

Herbarium specimens of *Polytrichum* sect. *Polytrichum* (and any possibly allied taxa) were requested on loan from major international herbaria around the world. A list of herbaria and the number of specimens received from each is given below (Table 4.1). All specimens studied of each taxon are cited following individual species descriptions within this chapter.

Although a large number of specimens was observed to attempt to understand species concepts and how these differ across the geographic regions, and to attempt to identify misapplication of names in closely related taxa, at the initial stages of the project, only a selected range of representative specimens (representing a good geographic coverage) is cited under each taxon.

Table 4.1 Acronyms and names of herbaria and numbers of specimens of *Polytrichum* sect. *Polytrichum* received from each herbarium for the study.

Herbarium	No. of Specimens
BM - Natural History Museum, London, U.K.	**98**
BOL - Bolus Herbarium, University of Cape Town, Western Cape Province, South Africa	**35**
E - Royal Botanic Garden Edinburgh Herbarium, Scotland, U.K.	**163**
FH - Farlow Herbarium, Harvard University, Cambridge, Massachusetts, U.S.A.	**01**
GOET - Universität Göttingen, Göttingen, Germany.	**29**
H - Finnish Museum of Natural History (University of Helsinki), Helsinki, Finland	**19**
LD - Lund University Botanical Museum, Lund, Sweden	**43**
MNHN (PC)- Muséum national d'Histoire naturelle, Paris, France	**07**
MO - Missouri Botanical Garden Herbarium, St. Louis, Missouri, U.S.A.	**34**
NICH - Hattori Botanical Laboratory, Japan	**05**
NTNU-TRH - University Museum at Norwegian University of Science and Technology, Trondheim, Norway	**30**
NY - New York Botanical Garden, Bronx, New York, U.S.A.	**390**
PE- Chinese National Herbarium, (Chinese Academy of Sciences), Beijing, China	**117**
PH- Academy of Natural Sciences Philadelphia, U.S.A.	**01**
QFA - Herbier Louis- Marie Herbarium, University of Laval, Quebec, Canada	**27**
RB - Jardim Botânico do Rio de Janeiro, Brazil	**12**
S- Swedish Museum of Natural History (Naturhistoriska riksmuseet), Stockholm, Sweden	**14**
SP- Instituto de Botânica ,São Paulo, Brazil	**92**
SQB - Société québécoise de bryologie, St-Valérien-de-Rimouski, Québec, Canada.	**08**
UPS - Uppsala University, Uppsala, Sweden	**26**
L (NL) - Naturalis, Leiden, Nederland	**99**
UBC- University of British Columbia, Vancouver, British Columbia, Canada	**271**
W - Naturhistorisches Museum, Wien, Austria	**15**
Total Number of Specimens Studied	**1536**

4.3.2 Methods

(a) Herbarium Methods

The quality of the available herbarium material varied depending on the age of the specimen, the various drying methods used to dry the material, the quality and the condition of the specimen at the time of collection, the quality of the curatorial processes employed by different herbaria, and the quality of associated data. Specimens for comprehensive morphological and anatomical study were carefully selected after thorough observation of the morphological variation of each taxon across its geographical distribution. Five to ten representative specimens of each species from well-known geographic regions, and preferably from recent collections, were subjected to detailed anatomical and morphological studies. However, when the identity was uncertain, mostly leaf anatomical characters (under the compound light microscope) were studied irrespective of the age of the sample to establish the identity and each dissected leaf section was preserved as a permanent slide for future use [see 4.3.2 (c) microscopic methods]. Older and "valuable" herbarium specimens (including type specimens and specimens only known from single, older collections) were handled with utmost care to minimise potential damage and obeying the destructive sampling rules put forward by the corresponding herbaria with prior written permission.

Herbarium material was rehydrated by soaking in hot water [sometimes supplemented with a few drops of 10% KOH as described by Smith (1971)] for a variable period of time (15 mins-12 hrs), depending on the age and condition of the specimen (Kariyawasam & al., 2018; Kariyawasam & al., 2021). To study the leaf anatomy, first a few leaves were carefully removed from the stem using a pair of forceps and transferred to and soaked in 70% ethanol for 1-2 minutes, then warmed in a ca. 5% KOH solution for 10-15 minutes (or a longer time depending on the age of the material; this step helps the specimen to inflate nicely and to remove a substantial amount of secondary metabolites in older specimens), before being soaked in warm water (for 15 minutes to 12 hours).

(b) Field Methods

Although the present study is fully based on herbarium collections, field excursions were made where possible to observe the occurrence of certain taxa in their natural habitats in order to get an idea of their habit and ecology. Field excursions jointly with the BBS (British Bryological Society) to the Scottish Highlands and Wales, as well as visits to Quebec, Spain, Switzerland and Finland were helpful in studying natural habitats and growth habits of living

plants. Useful discussions with expert field bryologists in the field also helped, as did taking notes on the ecology of each taxon. Photographs were taken using a Nikon Coolpix P7700 digital camera. Field notes on the specimens, such as their height, the colour of mature leaves and sporophyte capsules and setae, habitat preferences and shared ecological niches were recorded. Colleagues from Brazil (*P. angustifolium* and *P. brachymitrium*), Colombia (*P. ericoides*) and South Africa provided the photographs of habits and the habitats of the South American and African taxa (*P. subpilosum*) to get an idea of the natural habitats of the plants.

A few specimens were collected from Scotland (*P. commune* and *P. perigoniale*) and Québec (*P. swartzii* and *P. commune*) to study the anatomy of leaves. All specimens collected were given their own unique identity numbers and georeferencing data were recorded using a Garmin 12XL Global Positioning System Navigator (GIS). A few stems were uprooted from a single population (if sporophytes were available, plants with sporophytes were selected and if the population contained both male and female plants, two representative stems from each sex were collected). The specimens collected in the field were blotted using tissues and air-dried in the field soon after collection and then inserted into temporary folders. The specimens were re-packeted, fully labelled and deep-frozen at -30 ^{O}C for five to seven days to prevent any insect or mould attacks (following the herbarium practices at E) before being used for the study.

(c) Microscopic Methods

Specimens were first observed under a Stereo dissecting microscope with a long arm stand (Lecia MZ8; 10445538 PLAN 1.0x). Most macro-morphological characters (e.g., leaves in wet and dry conditions, capsule shape and length : width ratio etc.) were observed and measurements were taken with the calibrated scale. Free-hand leaf sections were made with a razor blade. Leaf transverse sections were made from ordinary vegetative leaves, in the middle 1/3 of the leaf between the leaf apex and the base of the leaf blade (Figure 4.2). Lamellae were also scraped from the leaves to observe in profile. Median cells of the lamina were measured individually using a scale. Micro-morphological and anatomical characters such as the shape and thickenings of the lamellar end-cells in transverse sections of the leaves etc. were observed using a Zeiss Standard 20 compound microscope. Permanent mounts were prepared to observe the characters found in the leaf transverse sections and capsules (such as peristome teeth and peristome membrane). Measurements were taken from five to ten specimens with at least eight leaves observed per specimen. Leaf lengths were measured from the tip, down to the central longitudinal axis. Leaf lamina widths were measured at the broadest point, perpendicular to the

length measurement. Sections were mounted fixed in 5% Hoyer's solution and ringed with nail varnish to preserve them for future use.

(d) Scanning Electron Microscopic (SEM) Studies

Scanning Electron Microscopy (SEM) is a technique which is successfully used to study the ultrastructural details of organisms providing a three-dimensional representation and with a greater resolution than can be obtained using light microscopy (Heywood, 1971). SEM studies have been successfully used in bryology during the last five decades to resolve many phylogenetic and systematic questions (Mischler, 1985b.; Long, 1999; Long & al., 2000; Bell & al., 2015). Smith (1974) reported details of spore ornamentation in Polytrichaceae with the aid of SEM studies.

Scanning electron micrographs of spore ornamentations, the micro-anatomy of leaf transverse sections and the structure of stoma in the capsules of some selected taxa were studied using a Leo Supra 55VP scanning electron microscope at the Royal Botanic Garden Edinburgh. Dry leaves and capsules were transferred without treatment, directly from the herbarium specimens, to 12 mm carbon discs mounted on 12.5 mm aluminium pin stubs. Specimens were coated with gold palladium using an Emitech K 575x sputter coater to a thickness of approximately 12 nm at a rate of 25 mA for 1.3 minutes. SEM combined with the variable pressure technique was used to observe the spore ornamentations and the detailed structure of papillae on the leaf lamellar end-cells.

(e) Photographs and Illustrations

Photographs of permanent mounts (in 5% Hoyer's solution) prepared for whole leaves (both adaxial and abaxial sides), leaf transverse sections, capsules (epiphragm, peristome teeth, apophysis) were taken with an Axiocam MRc5 camera attached to a Zeiss Axiophot compound light microscope using the Axiovision 4.8 software.

A camera lucida is an optical device that performs an optical superimposition of the subject being viewed and allows the user to observe both the object under the microscope and the drawing surface simultaneously. This allows the user to duplicate and /or trace the key features of the object on the drawing surface, thus aiding in the accurate measurement and shape of the object (Hammond & Austin, 1987). Although this is an older technique, it is still

being successfully used in many taxonomic studies of bryophytes (Long, 2000; Bell & al., 2015; Kariyawasam & al., 2018; Kariyawasam & al., 2021), especially in monographic studies.

In the present study, all illustrations were made by the author using a camera lucida attached to a Zeiss Axioskop Standard 20 Compound Light microscope for micro-anatomical and micro-morphological characters. Drawings were then traced onto A3 tracing film and inked, then scanned using an Epson Expression 10000 XL scanner. The scanned photographs were edited using the Adobe Photoshop CC 2021 22.1.1.138 (64-bit) software and final plates made for the taxonomic revision.

(f) Mapping

Distribution maps were produced using the information available from the herbarium specimen labels. When coordinates were not available, geographic maps of the region, Google Earth and index gazetteers were used to obtain them. Longitude and latitude coordinates and approximate altitudes added by the author are in square brackets in the specimen lists provided under each species. Species distribution maps were generated using the ArcMap 10.1, ESRI, USA software. The base maps of countries and boundaries of provinces were downloaded from the Diva website (www.divagis.org/Data) and generated by the Diva GIS 7.5.0 software.

(f) Character Selection

All available literature was gathered to study the characters that have previously been used by the authors to circumscribe the taxa within the family Polytrichaceae and within the section *Polytrichum*. Then thorough observations were made for all representative specimens from each taxon prior to selection of the characters. In most cases type specimens were studied along with the protologues to check the correct application of names. Five to ten specimens from each taxon were studied and for each character five to ten measurements were taken. Both quantitative and qualitative characters were observed, measured and enumerated. Data were recorded in Excel spreadsheets. These observations were used in the taxonomic treatment below (see 4.5).

The genus *Polytrichum* has a remarkable structural complexity of both gametophytic and sporophytic generations and among these a few key morphological characters used to morphologically delimit the members of *Polytrichum* sect. *Polytrichum* were noted from relevant literature (Smith 1971, 1974; Long 1985; De Sloover, 1986; Forrest, 1995; Hyvonen

& al., 1998; Smith Merrill, 2007; Bell & Hyvönen, 2010 a, b; Peralta & Yano, 2010; Faubert, 2013; Bell & al., 2015; Bippus & al., 2018; Lönnell & al., 2019); these are summarised below.

- **Branching**: Most members of *Polytrichum* sect. *Polytrichum* are unbranched or sparingly branched by subfloral innovations from beneath the perigonium (Smith, 1971; Long, 1985; Forrest, 1995; Hyvonen & al., 1998). Only a few members (*P. angustifolium* Mitt. and *P. ericoides* Hampe) are occasionally branched (see the taxonomic treatment below).

- **Leaf characters**: As in all mosses, leaf characters in Polytrichaceae provide very useful taxonomic characters to delimit the taxa morphologically. Leaf shape, leaf margin, leaf apex, number and tallness of adaxial lamellae, and lamellar end-cell morphology in transverse section, have been widely used as taxonomic characters to delimit the members of *Polytrichum* sect. *Polytrichum* in the past (Smith, 1971, 1974; Long, 1985; Forrest, 1995; Hyvonen & al., 1998).

  Smith (2007) has mentioned that "the best analogy to the polytrichoid leaf is the grass leaf, with a clear distinction between a sheathing base and a divergent blade. Typically, a wedge-shaped group of transversely elongate, incrassate cells ('hinge tissue') is present at the shoulders or just above". Some taxonomically important leaf characters in *Polytrichum* sect. *Polytrichum* are depicted in Figure 4.2.

  In general, leaves of *Polytrichum* sect. *Polytrichum* have serrate leaf margins (Smith, 1971; Long 1985; Schofield, 1985). However, some Northern taxa such as *Polytrichum jensenii* I.Hagen possess entire or sub-entire leaves (Long, 1985; Smith Merrill, 2007; Faubert, 2013). Serrations of leaf margins are mostly composed of a large, single, terminal tooth cell or rarely some rudimentary tooth associated with the large terminal cell such as in some xeric forms of African *Polytrichum subpilosum* P.Beauv.(De Sloover, 1986). Leaf apices are mostly acute to acuminate in the members of sect. *Polytrichum* and certain taxa such as *Polytrichum perigoniale* Michx. possess a very long hyaline apical arista with spines on it. (Smith Merril, 2007).

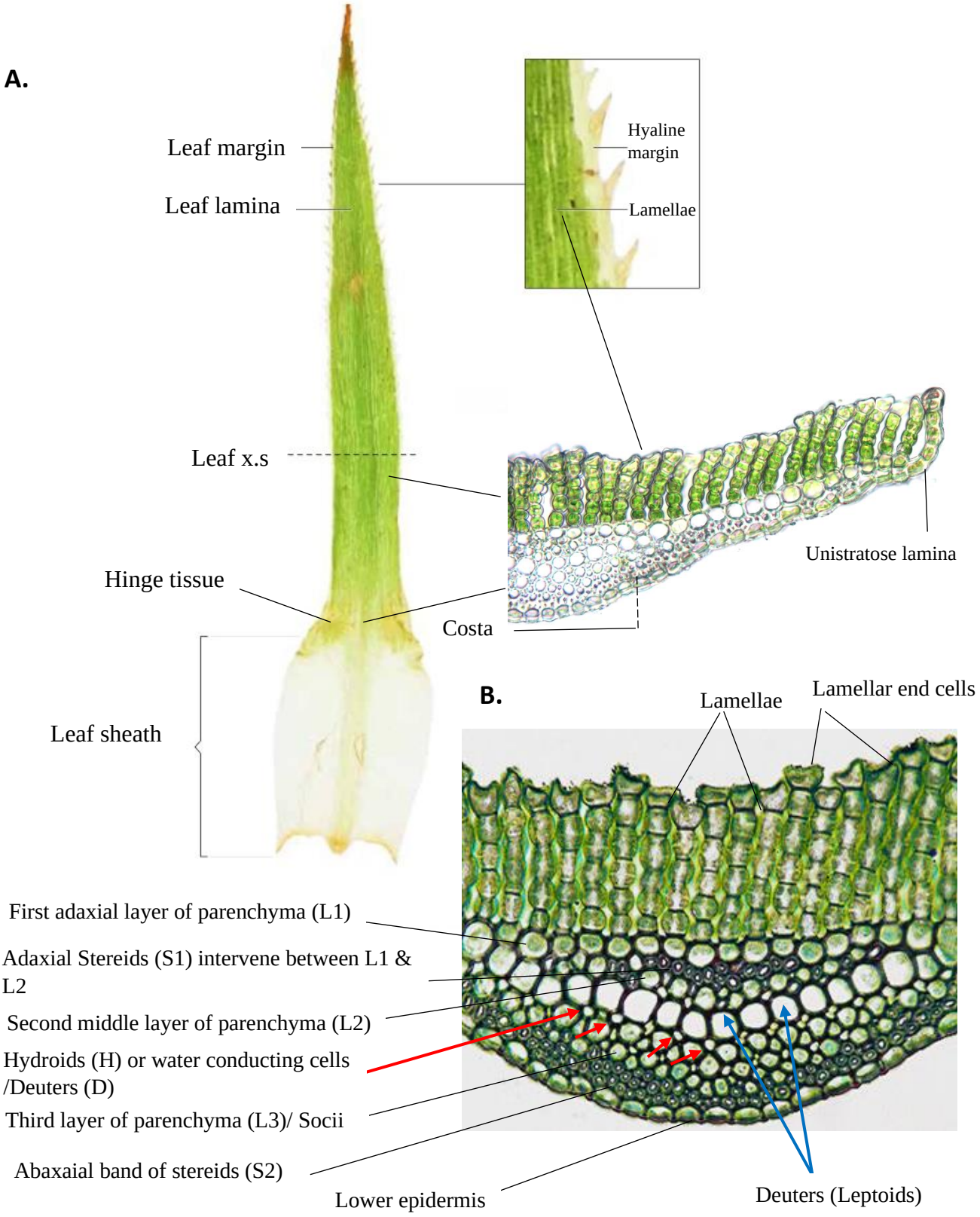

Figure 4.2: Leaf morphological and anatomical characters used in the present study **A:** General morphology and anatomy of a polytrichaceous leaf; **B.** Detailed micro-anatomy of a transverse-section of a *Polytrichum commune* leaf [adapted from Paolillo Jr. & Reighard, 1967; Smith, 1971 & Lönnell & al., 2019]

Leaf lamellae have a vital role in identifying the species of *Polytrichum* sect. *Polytrichum* microscopically with the aid of leaf transverse sections. Photosynthetic lamellae present on the adaxial surface providing a "pseudo-mesophyll" structure are a hallmark of the entire family. The physiological importance and occurrence of leaf lamellae in Polytrichaceae is extensively discussed in Chapter 01. Number of leaf lamellae, height of each lamella and the morphology of the lamellar end-cells in a leaf transverse section are taxonomically important characters to delimit the taxa in the section. Smith (1971) mentioned that the adaxial leaf lamellae of Polytrichaceae resulted evolutionarily (notably developmentally) by the division of ventral leaf lamina cells, and thus their establishment and subsequent elaboration was favoured because of photosynthetic efficiency. The adaxial photosynthetic lamellae of polytrichaceaous leaves dramatically increase the surface area available for the gaseous exchange and exchange of water vapour and efficient use of sun light and thereby enhance photosynthetic efficiency (Smith 1971; Proctor, 2005; Bell & Hyvönen, 2012; Kariyawasam & al., 2018).

In cross section a polytrichaceous leaf nerve or costa is dominated by an arc of larger cells (Figure 4.2 B) called "deuters" or "guide cells" (Smith, 1971). They are thin-walled vaculolated cells with a larger lumen. Alternating with deuters, to their adaxial side, there are smaller, inconspicuous cells called "central cells". Smith (1971) has mentioned that, "in *P. commune,* the deuters have been identified as leptoids, and the central cells as hydroids". There are three bands of parenchyma cells (also known as "socii") in the costa of the leaf which store lipid and starch reserves (Smith, 1971). They also integrate with leptoids and facilitate the conducting function. There are two bands of stereids (stereid cells) which possess thicker cell walls and provide additional mechanical strength to the leaves. They occur as two abaxial and adaxial bands in the costa and the borders (shoulders) of the leaves (Figure 4.2 B) (Paolillo Jr. & Reighard, 1967; Smith, 1971).

In almost all members of *Polytrichum* sect. *Polytrichum*, lamellae stand parallel to each other over the broad midrib (costa). As Lindberg (1868) described in his treatment of Polytrichaceae, the morphology of marginal cells of lamellae or the lamellar end-cells is often sufficient to distinguish between species of Polytrichaceae. This observation was confirmed by Conard (1956) and Smith (1971) in their taxonomic accounts. In *Polytrichum* sect. *Polytrichum* lamellar end-cells are mostly single, or occasionally geminate, and exhibit a wide range of shapes including pyriform, flat-topped, retuse or shallowly-grooved to deeply-grooved (or "U-shaped"). Some of them possess thickened-cell walls ornamented with epiculticular wax

flakes or papillae. (See Figure 1.7 C). A range of morphological forms of lamellar end-cells exhibited in *Polytrichum* sect. *Polytrichum* is depicted below in Figure 4.3.

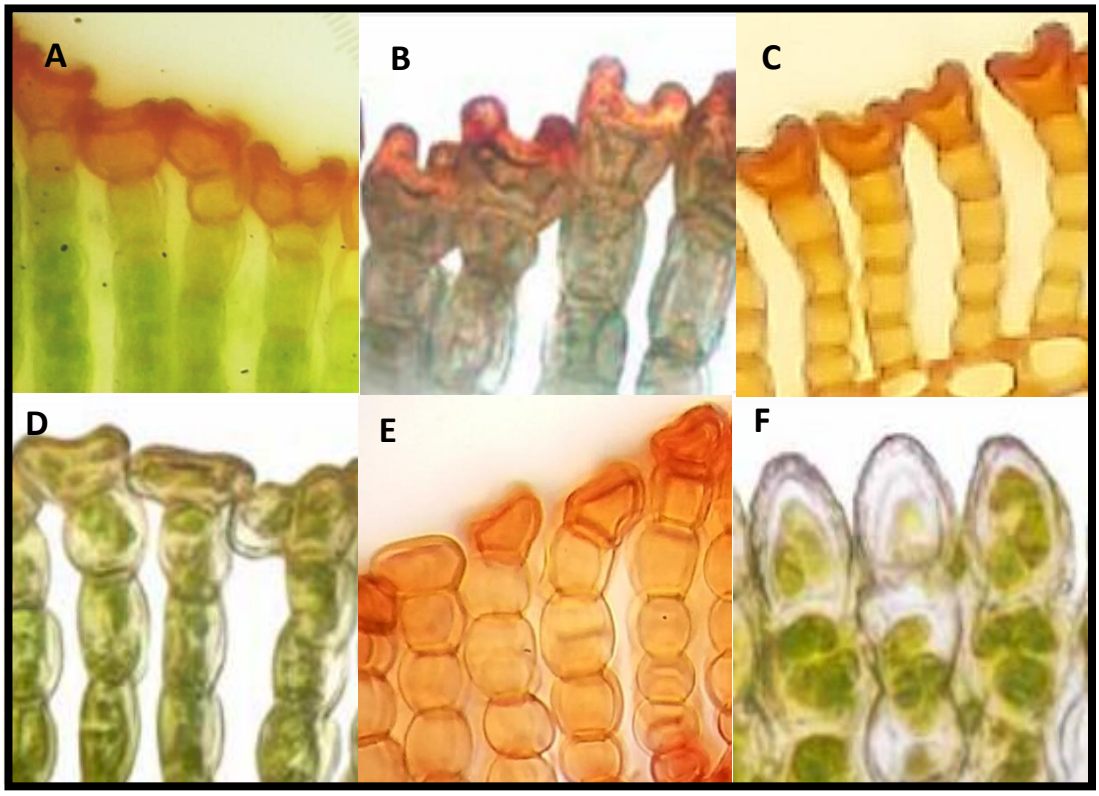

Figure 4.3: Morphological diversity found in the lamellar end-cells of the extant taxa of *Polytrichum* sect. *Polytrichum.*

***Group 1*: *Grooved lamellar end-cells*: A.** Shallowly or deeply grooved with geminate cells and thick papillated knobs at the edges (*P. subpilosum* type); **B**. Deeply-grooved lamellar end-cells with two heavily papillated knob-like projections at the edges (*P. jensenii* type); **C.** Deeply-grooved, U-shaped, lamellar end-cells with thickened walls (with or) without papillae (*P. commune s. str* and *P. ericoides* type)

***Group 2: Flat, asymmetric or pyriform lamellar end-cells*: D.** Flat-topped lamellar end-cells without papillae on top (*P. swartzii* type); **E.** A mixture of lamellar end-cells with dominant asymmetric, shallowly-grooved cells and pyriform cells (*P. brachymitrium* type and *P. perigoniale* to a lesser extent); **F.** Pyriform lamellar end-cells with or without papillae on the top (*P. angustifolium* type) [photos **B**, **D** & **F** are reproduced from Lönnell & al., 2019]

- **Capsular and spore characters:** The capsules of Polytrichaceae exhibit a wide variation in size and shape. They can be ovoid, cylindrical, bell-shaped, dorsiventrally flattened, radial, bilateral with sharp or rounded angles, with ridges or terete (Smith, 1971; Schofield, 1985). Almost all species of *Polytrichum* sect. *Polytrichum* possess strictly 4-angled (cubic) capsules (Smith, 1971; Long, 1985; Forrest, 1995; A.J.E. Smith, 2004; Smith Merrill, 2007). Exothecial cells of the capsules also exhibit some useful characters of pigmentation, and pits (apertures) (Hyvönen & al., 1998). Exothecial cells of the capsule in the members of *Polytrichum* sect. *Polytrichum* are bulging-mammillose, with a prominent "slit-like" pits which appear like tiny apertures (holes) on the centre of each exothecial cell (Smith, 1971; Bell & Hyvönen, 2010b).

  In *Polytrichum*, the peristome usually consists of 64 uniform teeth. Each tooth possesses a sharp keel on the inner surface, and a thin membranous structure called the "epiphragm" (see Figure 1.5) is firmly attached to the peristome. On the ventral surface of the epiphragm, a row of pendent projections alternates with the peristome teeth (Smith, 1974; Bell & Hyvönen, 2010b). The structure of the peristome and its importance in classifying the members of Polytrichaceae is discussed in Chapter 01 (section 1.3).

  Spores of the genus *Polytrichum* in general are rather small, [ca. 6(–8)–13µm in diameter], appearing smooth under the compound light microscope, however under the SEM spores show characteristic echinulate ornamentations. McClymont & Larson (1964) showed that the exine of *P. commune* forms "Christmas tree-like" ornamentations [see, McClymont & Larson (1964), Figure 8]. Smith (1974) employed SEM to distinguish the morphological characters of spores to identify the generic limits of Polytrichaceae. Smith (1974) reported and confirmed the findings of McClymont & Larson (1964) that the spores of the genus *Polytrichum* possesses "Christmas tree-like" ornamentations in the outer exine (perine) [see Smith, (1974), Figures 3& 4]. Ignatov & Smith Merrill (1995) also reported that spores of *Polytrichum* show "Christmas tree-like" ornamentations [see Ignatov and Smith Merril, (1995), Figures 2 & 3). However, while defining the generic limits employing the spore ornamentation morphology, Smith (1974) has described another type of spore ornamentation, i.e. "Cauliflower-like", a warty ornamentation type which is characteristic of the genus *Bartramiopsis*.

However, from the present study, a limited number of capsules (mature and immature and two capsules from each species) were used to study spore ornamentation using the SEM. In contrast to the previous studies (McClymont & Larson, 1964; Smith 1974; Ignatov & Smith Merrill, 1995) the African species *P. subpilosum* possesses "Christmas tree-like" ornamentations, whereas *P. perigoniale* exhibits "Cauliflower-like" spore ornamentations (Figure 4.4). However, due to time constraints a detailed morphological study of spore ornamentation could not be performed. A future study is recommended to study spore ornamentation of species across different geographic regions and at different maturity levels across the family Polytrichaceae to observe and to understand the ancestral states.

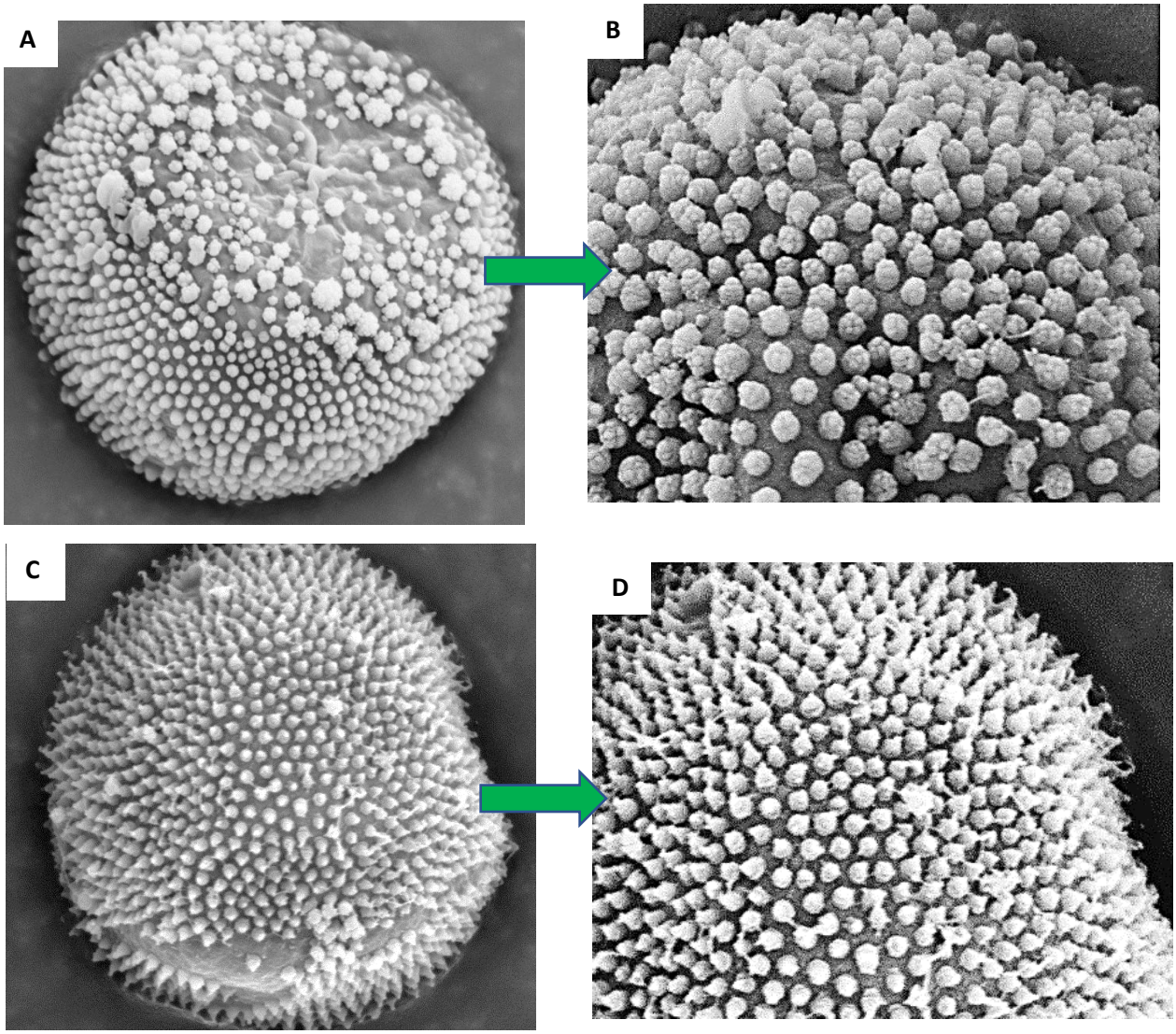

Figure 4.4: Spores of *Polytrichum* sect. *Polytrichum*: **A & B**: "Cauliflower-like" projections of *P. perigoniale* [*Kariyawasam 166* (E)]; **C & D**: "Christmas tree-like" ornamentations in *P. subpilosum* [*Hedderson 17471* (BOL)] (Scales A & C: 1cm=20 nm ; B & D 1cm=200 nm). SEM photographs by I.U. Kariyawasam

At the base of the capsule, all members of *Polytrichum* sect. *Polytrichum* possess a deep basal constriction. This strongly constricted area is called the "apophysis" (Schofield, 1985). There are a few to several stomata scattered in this area of the capsules of *Polytrichum*. In *Polytrichum* sect. *Polytrichum*, almost all members posses a large number of phaneropore stomata (i.e. superficial, fully exposed on the surface, with guard cells on the same level as the adjacent exothecial cells); these stomata (ca. 80–100) are present as a band on the apophysis (Figure 4.5).

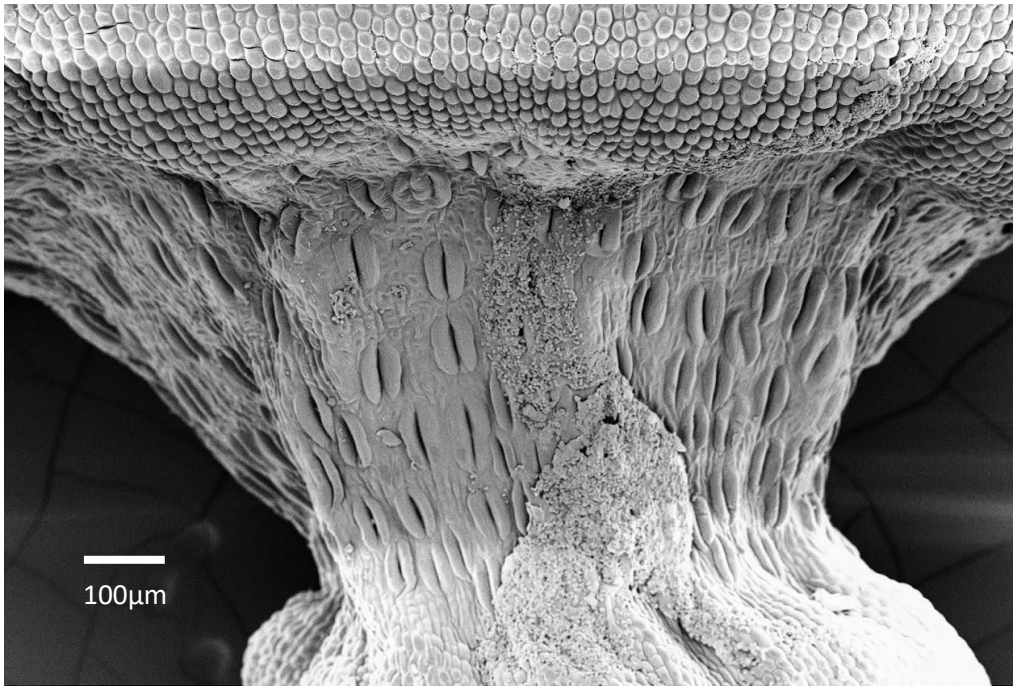

Figure 4.5: Apophysis and the distribution of stomata in the *Polytrichum subpilosum* capsule [*Hedderson 17471* (BOL)], SEM photograph by I. U. Kariyawasam

4.4 Taxonomic Treatment

4.4.1 The Genus *Polytrichum* Hedw.

***Polytrichum* Hedw.,** Sp. Musc. Frond, 88 (1801). Lectotype: *Polytrichum commune* Hedw. (designated here).

Plants medium-sized to robust, to ca. 45 cm in height, in loose or dense tufts, arising from a horizontal underground rhizome. ***Stems*** erect and seldom branched by innovations, loosely to densely leafy distally and bracteate or scaly proximally, rhizoidous or seldom woolly-tomentose. ***Leaves*** differentiated into sheath and limb (blade/lamina); sheath entire, hyaline-margined, composed of rectangular cells, often highly nitid (glossy); hinge tissue is well developed or sometimes not strongly developed; blade entire, sub-entire, serrate or serrulate, sometimes filmy, plane, erect or sometimes sharply inflexed, enclosing the adaxial lamellae; costa short-excurrent, scabrous or prolonged into a hyaline or reddish-brown toothed awn (especially in inner perichaetial leaves); lamellae numerous, mostly confined to the adaxial surface, only occurring as rudimentary spines on the abaxial surface along the costa; closely-spaced, occupying most of the blade including the costa, the end-cells of lamellae in transverse section distinctly differentiated, pyriform, flat-topped or retuse with a different levels of thickened walls and deposition of epicuticular flakes of wax or papillae. ***Sexual condition*** dioicous; male plants with conspicuous rosettes, formed by overlapping perigonial leaves (bracts), commonly forming innovations with successive male inflorescences (perigonia) per shoot, perigonial leaves oblong or deltoid with shorter lamina and broader sheath; perichaetial leaves typically possessing long-sheathing bases, with a weakly developed blade and sometimes inner perichaetial leaves are longer and more lanceolate than vegetative leaves with a very long acumen. ***Setae*** solitary, reddish-brown. ***Capsules*** strictly 4-angled or sometimes variably 4–5- or 5–6-angled; cubic or somewhat cylindric, sub-erect when young and becoming bent at the attachment of the seta and almost horizontal and nodding when shedding the spores, reddish to purplish-brown, glaucous when fresh; apophysis discoid, sharply delimited from the urn by a deep and narrow constriction or less pronounced, if prominent, stomata rather few or numerous and confined to this constricted area; exothecial cells smooth or protruding (bulging)-mammillose or papillose, often transversely-elongate, with a circular or slit-like pore in the outer wall; operculum umbonate and rostellate with a short beak; peristome teeth 64, but often somewhat fewer, pale, single, generally simple, usually with some teeth compound, not keeled at the back, with or without spurs inside, not deeply pigmented; epiphragm thin and

delicate, remaining attached to the peristome teeth or more rarely readily detached, usually without erect tooth-like projections opposite the peristome teeth or seldom with short pendent projections, with or without sacculi on the abaxial (dorsal) side alternating with peristome teeth, or with solid ridge-like circular "annulus" on the ventral edge of the epiphragm. ***Calyptrae*** fibrillose, with densely interwoven mat of golden-brown hairs, covering whole capsule or only upper portion of the capsule. ***Spores*** small, ca. 7–10 µm, smooth or echinulate with cauliflower-like or Christmas tree-like projections on the outer membrane.

The genus *Polytrichum* is subdivided into three main sections.

1. *Polytrichum* sect. *Aporotheca* (Limpr.) N.E.Bell & Hyvönen, Amer. J. Bot, 97(4): 577. 2010. ≡*Polytrichum* subgen. *Aporotheca* Limpr., Laubm. Deutschl. 2: 615. 1839. ≡*Polytrichastrum* sect. *Aporotheca* (Limpr.) G.L.Smith Merr., Bryologist, 95: 271. 1992. Lectotype (Smith, 1971): *Polytrichum formosum* Hedw.

2. *Polytrichum* sect. *Juniperifolia* Brid. Muscologiae Recentiorum Supplementum 1: 47. 1806. Lectotype: *Polytrichum juniperinum* Hedw. Sp. Musc. 89. 1801. = *Polytrichum* sect. *Juniperina* Brid., Musc. Recent. Suppl. 4: 194. 1819 (Type: *Polytrichum juniperinum* Hedw.) = *Polytrichum* sect. *Inflexifolia* Albr.Rohn., J. Bot., 72: 107, 1934, *nom. illeg*. (Type: *Polytrichum juniperinum* Hedw.)

3. *Polytrichum sect. Polytrichum*. Lectotype: *Polytrichum commune* Hedw.= *Polytrichum* sect. *Yuccifolia* Brid., Musc. Recent. Suppl, 1: 54. 1806 (Type: *Polytrichum commune* Hedw.) = *Polytrichum* sect. *Aloefolia* Brid., Musc. Recent. Suppl. 1: 81. 1806 (Type: *Polytrichum commune* Hedw.) = *Polytrichum* sect. *Adianta* Wallr., Fl. Crypt. Germ. 1: 198. 1831, *nom. illeg*. (Type: *Polytrichum commune* Hedw.) =*Polytrichum* sect. *Quadrangularia* Bruch & Schimp., Bryol. Eur. Fasc. 21/22: *Polytrichum* 11. 1844 (Type: *Polytrichum commune* Hedw.) = *Polytrichum* sect. *Porotheca* Limpr., Rab. Krypt.-Fl. 4(2): 623. 1893. (Type: *Polytrichum commune* Hedw.) = *Polytrichum* sect. *Communia* I.Hagen, Norske Vidensk. Selsk. Skr. 1913(1): 52. 1914 (Type: *Polytrichum commune* Hedw.) = *Polytrichum* sect. *Spinosifolia* Albr.Rohn., J. Bot. 72: 105. 1934, *nom illeg*. (Type: *Polytrichum commune* Hedw.)

4.4.2 Taxonomic Key to Sections of the Genus *Polytrichum*

A. Plants with broad, entire, sharply inflexed leaf margins that enclose the lamellae on the upper leaf surface; lamellar end-cells in transverse section always pyriform, with or without papillae on the top; capsules usually 4-angled without sharp edges, with a constricted apophysis at the base…….……....**1**. **Sect. *Juniperifolia***

+ Plants with narrow, entire, sub-entire, serrate-serrulate, and relatively erect leaf margins; lamellar end-cells in transverse section pyriform, retuse, deeply-grooved or flat-topped, with or without papillae on the top; capsules either strictly 4-angled with sharp edges or 4–5- or 5–6-angled, cubic or cylindric urns, with or without a prominent discoid apophysis at base of capsule…….……**B**.

B. Plants with narrow, entire, sub-entire or serrate to serrulate leaf margins; lamellar end-cells in transverse section pyriform, shallowly or deeply-grooved or flat-topped, with or without papillae on top; capsules strictly 4-angled with very sharp edges, urns cubic with a prominent discoid apophysis forming a strong constriction at base of capsule……**2**. **Sect. *Polytrichum***

+ Plants with narrow serrate to serrulate leaf margins, lamellar end-cells pyriform or flat-topped and never forming deeply-grooved end-cells; capsules 4–5- or 5–6-angled, cubic without sharp edges, apophysis not distinct and never forming a strong constriction at base of capsule…….……**3**. **Sect. *Aporotheca***

4.4.3 *Polytrichum* sect. *Polytrichum*

Plants medium to large (up to ca. 45 cm high), seldom branched. ***Stems*** erect and seldom branched by innovations, loosely to densely leafy distally and bracteate or scaly proximally, rhizoidous or seldom woolly-tomentose. ***Leaves*** differentiated into sheath and limb (blade/lamina); sheath entire, hyaline-margined, composed of rectangular cells, often highly nitid (glossy); hinge tissue well-developed or sometimes not strongly developed; blade lanceolate to oblanceolate; entire, sub-entire, serrate or serrulate, costa short-excurrent, scabrous or prolonged into a hyaline or reddish-brown toothed awn (especially in inner perichaetial leaves); lamellae numerous, mostly confined to the adaxial surface, only occurring as rudimentary spines on the abaxial surface along the costa; closely-spaced, occupying most of the blade including the costa, the end-cells of lamellae in a transverse section distinctly

differentiated, pyriform, flat-topped , shallowly to deeply-grooved (U-shaped) with different levels of thickened walls and deposition of epicuticular flakes of wax or papillae. ***Sexual condition*** dioicous; male plants with conspicuous rosettes, formed by overlapping perigonial leaves (bracts), commonly form innovations with successive male inflorescences (perigonia) per shoot, perigonial leaves oblong or deltoid with shorter lamina and broader sheath; perichaetial leaves typically possessing long-sheathing bases, with a weakly developed blade and sometimes inner perichaetial leaves longer and more lanceolate than vegetative leaves with a very long acumen. ***Setae*** solitary, reddish-brown. ***Capsules*** strictly 4-angled with very sharp edges, reddish to purplish-brown, glaucous in fresh capsules; apophysis discoid, sharply delimited from the urn by a distinct, deep and narrow constriction, stomata rather few or numerous and confined to this constriction; exothecial cells generally with protruding (bulging)-mammillose and papillose cells with a circular or slit-like pore in the outer wall of each cell; operculum umbonate and rostellate with a short beak; peristome teeth 64, but often somewhat fewer, pale yellow, single, generally simple, usually with some teeth compound, not keeled at the back, with or without spurs inside, not deeply pigmented; epiphragm thin and delicate, peristome usually seeming to attach to margin of upper epiphragm surface which often appears as a slightly raised rim, usually without erect tooth-like projections opposite the peristome teeth or seldom with short pendent projections; with or without sacculi on the abaxial (ventral) side alternating with peristome teeth. ***Calyptrae*** fibrillose, with densely interwoven mat of golden-brown hairs, covering the whole capsule or only the upper portion of the capsule. ***Spores*** small, ca. 7–10 µm, smooth or echinulate with cauliflower-like or Christmas tree-like projections on the outer membrane.

Distribution: Members of *Polytrichum* sect. *Polytrichum* are distributed throughout Europe, Arctic and Sub-arctic regions, Australasia, North America and Greenland, Central and South America, Sub-Saharan Africa and Madagascar, South-East Asia (confirmed from the herbarium material studied for the present study).

In the present study eight species have been recognised in *Polytrichum* sect. *Polytrichum*

A taxonomic key to species is given in 4.5.4 followed by a detailed taxonomic account for each species including a description, distribution, ecology, nomenclatural and taxonomic notes supplemented with illustrations, photographs and geographic distribution confirmed from the herbarium specimens used for the present study.

4.4.4 Taxonomic Key to Separate the Species of *Polytrichum* sect. *Polytrichum*

A. Plants 12–15(–18) cm high, with orange sheathing lamina; lamellar end-cells in transverse section pyriform, single or occasionally geminate……...**1**. ***P. angustifolium***

\+ Plants 2(–10)–15(–45) cm high, with hyaline or pale green sheathing lamina; lamellar end-cells flattish, slightly or deeply grooved in transverse section ……..……..**B.**

B. Lamina of vegetative leaves short (1.5–4.5mm); leaf margin serrulate; leaf with a mucronate, awnless apex……... **2**. ***P. ericoides***

\+ Lamina of vegetative leaves rather long (4.0–12 mm); leaf margin entire to serrate; leaf with an acute to acuminate apex and sometimes with a long acumen……..……...**C.**

C. Plants fragile with entire to finely and obscurely serrulate leaf margins; lamellar end - cells in transverse section grooved and possessing paired "knob-like" papillate projections……..…..…..…**3**. ***P. jensenii***

\+ Plants variable in stature, medium-sized to robust, with serrate to serrulate leaf margins; lamellar end-cells in transverse section flattish to deeply grooved and sometimes possessing thickened walls sculptured with papillae…… **D.**

D. Plants with serrate leaf margins; lamellar end-cells in transverse section either bifid or deeply grooved……..……**E.**

\+ Plants with serrate to serrulate margins; lamellar end-cells in transverse section mostly flattish to obliquely notched……..……....**F.**

E. Lamellar end-cells in leaf transverse section with a narrow "sinus", the two lobes terminated by papillae ("knob-like" projections) due to thickening of the upper cell wall, lamellar end-cells 12 × 10 (–12) µm……..…… **4**. ***P. subpilosum***

\+ Lamellar end-cells in leaf transverse section without a narrow "sinus-like" division, but deeply grooved, and with cells towards the base of the blade less deeply grooved; lamellar end-cells 12–12.5 × 17.5–20 µm.....**5**. ***P. commune s. str***

F. Plants 3–6 cm, soft and flexuose, becoming distinctly black when old; lamellar end-cells flattish, less broad, occasionally geminate and sometimes asymmetric; capsule with short, reddish-brown seta 2.5–5 cm, ……..……..…..……..**6**. ***P. swartzii***

\+ Plants 5–12 cm, stiff, not becoming distinctly black when old; lamellar end-cells flattish and somewhat broader; capsule with a brownish, long seta 6–12 cm …….**G.**

G. Leaf lamellar end-cells somewhat broad, flattened and occasionally asymmetric; leaf apex apiculate; leaf tooth cells very short, 30–33(–35) µm long; perichaetial leaves with a long acumen; capsules short…….....……...**7**. ***P. brachymitrium***

+ Leaf lamellar end-cells rather broad, never possessing thickened papillated projections, 11(–12)×10–14 µm in size; leaf tooth cells longer, ~75 µm long; perichaetial leaves with acuminate apex; capsules large…….……..……..….**8**.***P. perigoniale***

4.4.5 Descriptions and Taxonomic Accounts

1. *Polytrichum angustifolium* Mitt., J. Linn. Soc. Bot. 12: 622. 1869. Type citation: **[**Brazil] "Brasilia Tropica, Burchell, n. 3768." Type: Brazil, Brasilia Tropical, *s.d., Burchell 3768* (isotypes E00305525!, BM000919905!)

Syn.: *Polytrichum alticaule* Müll.Hal., Hedwigia 38(Beibl. 1): 59. 1899, *nom. inval*. (no description), ***syn. nov***.; citation of original material: [Brazil] *E. Ule, Bryotheca brasiliensis 226*; original material: Brazil, Santa Catarina, [Ouro Preto], [1180 m], [20.1715 S, 43. 3029 W], June 1890, *E.H.G. Ule 226* (E!, NY!, SP-not seen).

Syn.: *Polytrichum assimile* Hampe, Flora 64: 378. 1881. Type citation: [Brazil] "Prope Rio de Janeiro: Glaziou (11729)". Syntype: Brazil, Rio de Janeiro, *Glaziou 11729* (PC0709531!, PC0721235!, W140977- 04004649!)

Syn.: *Polytrichum brasiliense* Hampe, Vidensk. Meddel. Naturhist. Foren. Kjøbenhavn, ser. 3, 4: 53. 1872. Type citation: [Brazil] "Brasilia australis; Glaziou sub Nr. 5199." Lectotype (designated by Costa et al., 2016): Brazil, Rio de Janeiro, *Glaziou 5199* (BM000960645!); isolectotypes: BM!, B300267954!, PC0709516!, PC0721234! (fide Costa et al. 2016).

Figures 4.6, 4.7 & 4.8

Stem erect, variable in stature, medium to large, (3–)10-12(–18) cm. ***Leaves*** 5.5–7.5 × 0.5–1.0 mm, wide spreading, lanceolate, differentiated into sheath and lamina, leaf sheath hyaline, entire, lamina serrate to the apex and leaves crowded at the tip, costa prominent, percurrent and slightly spiny on the abaxial surface, lamellae present on both costa and lamina; in transverse section, 50–70 rows of lamellae of 6–8(–10) cells high, end-cells of the lamellae pyriform, smooth or with thick walls with papillae, 5(–7) –16(–20) µm wide; epidermal cells of the abaxial epidermis 6–12 × 5–15 µm; guide cells 3 (–4) –7.5(–10) × 5–12 µm; cells of the adaxial epidermis 2–6 × 5–12 µm; lamina cells 6–12 × 6–15 µm; sheath cells 60(–80) –120 × 5(–12) µm. ***Dioicous*** perigonia and perichaetia are terminal. ***Perichaetial leaves*** differentiated, lanceolate with a larger leaf sheath, 2–5(–6) × 1(–2) mm, acute apex, serrate margin, with spines on the abaxial surface. ***Pergonial leaves*** differentiated, oblanceolate with a larger leaf base, 6–7 × 0.5 –1(–2) mm, apex acute, margin serrate. ***Capsule*** 4-angled, cubic, 3.0–4.0 × 2–3 mm; apophysis distinct, discoid, narrowly constricted above; exothecial cells composed of conical papillae with slit-like apertures; operculum conic with a short rostellate beak; peristome teeth ca.0.3–0.45 mm high, obtuse, pale; basal membrane low, brownish; spores 6–8 µm. ***Seta***

4(–5) – 7(–8) mm long, reddish when young. ***Calyptra*** golden-yellow, fibrillose and completely covering the capsule, 13–15 mm long.

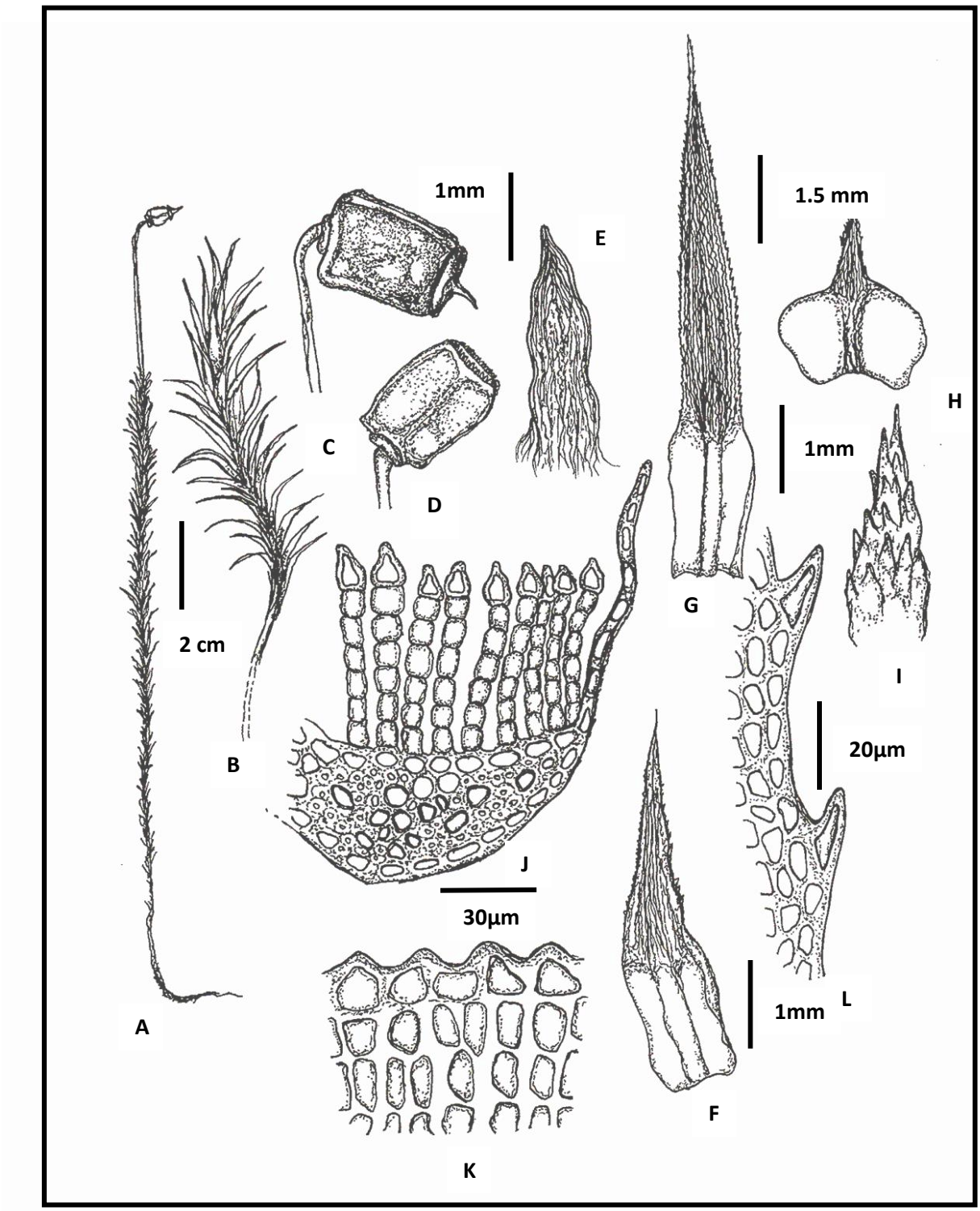

Figure 4.6: *Polytrichum angustifolium* **A**. Habit, dry; **B**. Habit, moist; **C**. Capsule - indehisced, with operculum; **D**. Capsule-dehisced; **E**. Calyptra; **F**. Vegetative leaf (moist); **G**. Inner perichaetial leaf (moist); **H**. Perigonial leaf (moist); **I**. Leaf apex with spines; **J**. Leaf transverse section showing pyriform lamellar end-cells; **K**. Leaf lamellae in side view; **L**. Leaf margin. (From the isotype E00305525, drawn by I.U. Kariyawasam)

Distribution: Southern Brazil – States of Espírito Santo, Minas Gerais, Paraná, Rio de Janeiro, Rio Grande do Sul, Santa Catarina and São Paulo (Based on herbarium specimens studied in the present study and Peralta & Yano (2010). Endemic to Brazil.

Although all specimens observed for the present study (from E, NY, SP, RB herbaria) originate only from Brazil, Smith (1971) has reported that this taxon is present in El Salvador, however no such specimen was observed in the present study.

Figure 4.7 shows the distribution of *P. angustifolium* in Brazil based on the herbarium records confirmed from the present study.

Figure 4.7: Distribution of *Polytrichum angustifolium* based on confirmed herbarium records

Ecology: On soil, gently open areas, along trails in the margins of humid montane forests at the Mata Atlântica Biome between ca. 500 (–700) – 2,200(–2350) m altitude.

Taxonomic & Nomenclatural Notes: *P. angustifolium* is characterised by its pyriform lamellar end-cells mostly having papillae on their surface. In a cross section of leaf lamellae from lamina to costa all possess pyriform end-cells without any intermediate forms (Figure 4.6). Gametophytes have rather thick and bright green leaves rather appressed to the stem (Figure 4.8). The plants can grow up to 16 cm high, sometimes occupying the whole height of a standard herbarium sheet.

Figure 4.8: *Polytrichum angustifolium* -sterile plants growing on a gravelly path along a ditch in Brazil. (Photo courtesy Denilson Peralta)

This taxon could be easily misidentified with sterile material of *Polytrichum formosum* Hedw. in Brazil. However, the shape of the strictly 4-angled capsule with a conspicuous apophysis and the height of the lamellae [always 6(–10) cells high] in a leaf cross section will help to diagnose the taxon. Peralta & Yano (2010) erroneously reported the taxon as possessing cylindric capsules; however, the urn of the capsule is cubic and strictly 4-angled, whereas *P. formosum* possesses 5–6-angled capsules with a less prominent apophysis.

Peralta & Yano (2010) stated that *Polytrichum brasiliense* Hampe is conspecific with *P. angustifolium* and placed the former in the synonymy of *P. angustifolium*, only citing syntypes. However, they have not selected a lectotype from the PC and BM specimens studied.

Costa & al. (2016) selected a lectotype for *Polytrichum brasiliense* Hampe with original material housed in BM, with sporophytes, serving as a lectotype.

P. angustifolium Schimp. (1897) is a later homonym and the original material belongs to *P. juniperinum* (Messner & Frye, 1947).

Additional Specimens Observed

BRAZIL, MINAS GERAIS: Caparaó Novo, Parque Nacional do Caparaó, along the road from park entrance to end of road (Tronqueira), dryish low forest, [~1600m], [20. 275 S, 41.50 W], 15-Sept-1984, *D.M. Vital & W.R. Buck 11518* (NY, SP); Caparaó Novo, Parque Nacional do Caparaó, along the road from park entrance to end of road (Tronqueira), dryish low forest, [~1600m], [20. 275 S, 41.50 W], 15-Sept-1984, *D.M. Vital & W.R. Buck 11600* (NY, SP); Caparaó Novo, Parque Nacional do Caparaó, along the road from park entrance to end of road (Tronqueira), dry, rocky hillsides with scattered shrubs and small trees, [~1970m], [20. 26 S, 41.44 W], 16-Sept-1984, *D. M. Vital & W.R. Buck 11721* (NY, SP); Parque Nacional do Itatiaia, along entry road near border of Rio de Janeiro, between Km 1.5 and Km 3, humid montane forest, [~1600m], [20. 275 S, 41.50 W], 04-July-1991, *D.M. Vital & W.R. Buck 19414* (NY, SP); Parque Nacional do Itatiaia, along entry road near border of Rio de Janeiro, between Km 1.5 and Km 3, humid montane forest, 04-July-1991, *D.M. Vital & W.R. Buck 19532* (NY); Catas Altas Reserva Particular do Patrimônio Natural "Parque Natural do Caraça", trilha até a capelinha, 1280 m, 20.0556 S, 43.2917 W, 30-May-2008, *D.F. Peralta, 6388* (SP, RB).

BRAZIL, PARANÁ: Morretes, Parque Estadual do Marumbi, trilha do rochedinho, cruza o rio Taquaral, Mata Atlântica com margem de riacho, em barranco, 1300 m, 22.2733S, 43.0017 W,21 -March -2017, *D. F. Peralta with O.S. Brito & F. Gonzatti 16222* (SP); Morretes, Rochedinho, Parque Estadual do Marumbi, terrícola, 532 m, 25.26132 S, 48.55191W, 14-Feb-2012, *R. Ristow with W.T. Ferreira & L.R. Granato 1872* (SP).

BRAZIL, RIO DE JANEIRO: Parque Nacional do Itatiaia, along entry road near border with Minas Gerais between km 9 and km 10, humid road sides, upper limits of continuous forest, 06- Jul-1991, *D. M. Vital & W.R. Buck 19764* (NY); Resende, Itatiáia National Park, South face of Mt. Itatiáia, on slope just below television relay tower area originally dense moist forest, then cleared but not burned, now a low open to dense shrubbery, leaf duff and humus, 30-Jul-1966, *G. Eiten & L. T. Eiten 7631* (NY); culta in horto in caminho velho do Botafogo, between Catate and Botafogo Bay, *s.d., W.J. Burchell 3768* (NY); vicinity of Rio de Janeiro, 26 Jul 1915 - 30 Jul 1915, *J.N. Rose & P.G. Russell 20436* (NY); Itatiaia, Parque Nacional do Itatiaia,

22- Oct-2005, mata atlântica, barranco úmido, vale dos lírios, 1100 m, 22.2946 S, 44.3348 W, *D.F. Peralta 2854*, with O. Yano, B.L. de Morretes & M. Kirizawa (SP); Itatiáia, Parque Nacional do Itatiaia, km 12, on banks, along road, [2250 m], [22.2946 S, 44.3348 W], 01-My-1977, *D.M. Vital, 7098* (SP); Itatiáia, Parque Nacional do Itatiaia, km 12, on banks, along road, [2350 m], [22.2946 S, 44.3348 W], 01-My-1977, *D.M. Vital 7103* (SP).

BRAZIL, RIO GRANDE DO SUL: Caxias do Sul - Criuva – Ilhéus, à beira da mata, 30-Oct-1988, *R.A. Wasum & al. 4740* (NY); Cambará do Sul- Fortaleza, nos barrancos, 25-Oct-1986, *R.A. Wasum & al, 2141* (NY); Cambará do Sul, estrada de acesso ao Parque Nacional de Aparados da Serra, barranco úmido, margem da Estrada, 1100 m, 29.0252 S, 50.0841W, 15-Dec-2005, *D.F. Peralta & P. Gonçalves 3314* (SP); Cambará do Sul, estrada de acesso ao Parque Nacional de Aparados da Serra, barranco úmido, margem da Estrada, 1100 m, 29.0252 S, 50.0841W, 15-Dec-2005, *D.F. Peralta & P. Gonçalves 3315* (SP); Cambará do Sul, estrada de acesso ao Parque Nacional de Aparados da Serra, barranco úmido, margem da Estrada, 1100 m, 29.0252 S, 50.0841W, 15-Dec-2005, *D.F. Peralta & P. Gonçalves 3322* (SP); Cambará do Sul, Parque Nacional Aparados da Serra, Canion Itaimbezinho, Mata Ombrófila Densa, em barranco, 23-July-2014, 1200m, 25.2611 S, 48.5514 W, *D.F. Peralta, with R. Wasum & J.M.R.P. Oliveira 10745* (SP).

BRAZIL, SANTA CATARINA: [Ouro Preto], [1180 m], [20.1715 S, 43. 3029 W], June 1890, *E.H.G. Ule 226* (NY, SP, E).

BRAZIL, SÃO PAULO: Pindamonhangaba, Serra da Mantiqueira, Pico do Itapeva, ca. 6 km SE of `Campos do Jordao, disturbed cloud forest with planted pines, 18-Oct-1994, *W.R. Buck 26427* (NY); Pindamonhangaba, along road, ca. 4 km S of the Pico de Itapeva, *on banks, 05-April-1977, D.M. Vital 7063* (NY); Parque Nacional Itatiaia, along road to Agulhas Negras, 18-Oct-1977, *L.R. Landrum 2145* (NY); Pindamonhangaba, Pico de Itapeva, associado a *Polytrichum brasiliense* Hampe. No limite do barranco a sombra de pequenos arbustos, crescendo junto com peteridófitos e liquens, [~560m], [22.5526 S, 45.2742 W], *04-March-1966, D.M. Vital 709* (SP).

2. *Polytrichum ericoides* Hampe, Ann. Sci. Nat., Bot. sér. 5, 4:350, 1865., non Hoffm. ex F.Hergt, Neues Bot. Taschenb. 1807: 214. 1807. Type citation: [Colombia] "Bogota, Boqueron, 2100 m., intermixtam leg. A. Lindig." Type specimen: Colombia, *s.d.*, *Lindig s.n.* (holotype BM 0000960749!)

Syn: *Polytrichadelphus ericoides* (Hampe) Mitt., Jour. Linn. Soc. Bot. 12: 611.1869.

Syn: *Polytrichum mutisii* G.L.Sm. *nom. nud*

This is an unpublished herbarium name which G.L. Smith applied to the type specimen of *P. ericoides* in 1975 before he published his paper on the identity of *P. ericoides* (1976).

Figures 4.9, 4.10, 4.11 & 4.12

Stem erect, rigid, occasionally branched, variable in stature, medium to large, (3–)10-12cm. ***Leaves*** 3.5–5.5 × 0.5–1.0 mm in the middle and 0.5–1.5 mm wide at the broadest; differentiated into sheath and lamina; leaf sheath hyaline-margined; lamina 1.5(–1.7)-3.5(–4.5) mm long, narrowly oblong with an obtuse, awnless apex; margin of lamina slightly incurved, serrulate to apex and crowded at stem apex; costa prominent, percurrent and spiny on the abaxial surface; lamellae present on both costa and lamina; in transverse section, 25(–30) –50 rows of lamellae of 6(–8) –12 cells high, end cells of the lamellae U-shaped (deeply grooved), smooth or with thick walls and occasionally papillose, 5(–7) –16(–20) µm wide; epidermal cells of the abaxial epidermis 5–15 × 4–15 µm; guide cells 3.5 (–4) –8.5(–10) × 6–15 µm; cells of the adaxial epidermis 3–8 × 6–15 µm; lamina cells 4.5–12 × 5–15 µm; sheath cells 60(–70) –120 × 5(–10) µm. ***Dioicous*** perigonia and perichaetia terminal. ***Perichaetial leaves*** differentiated, lanceolate to orbicular, 2–5(–6) × 1(–2) mm, acute apex, margin slightly serrate, spines present on abaxial surface, paraphyses hyaline to pale green, abundant, antheridia abundant, club-shaped, 1–4 µm long. ***Perigonial leaves*** differentiated, deltoid with a larger leaf base, 5–7 × 0.5 –1.5(–2.0) mm, apex acute, margin serrulate, paraphyses filiform, archegonia rare, when present, cylindrical with long necks**.** ***Capsule*** 4-angled, shortly rectangular, 3.0–4.0 × 2–3 mm; apophysis distinct, discoid, narrowly constricted above; exothecial cells composed of conical papillae with slit-like apertures; operculum with a short rostellate beak; peristome teeth ca.0.3–0.4 mm high, obtuse, pale, basal membrane low, brownish; spores 7–9 µm. ***Seta*** 4(–5) – 7(–8) mm long, reddish when young. ***Calyptra*** golden-yellow, fibrillose and completely covering the capsule, 12–15 mm long.

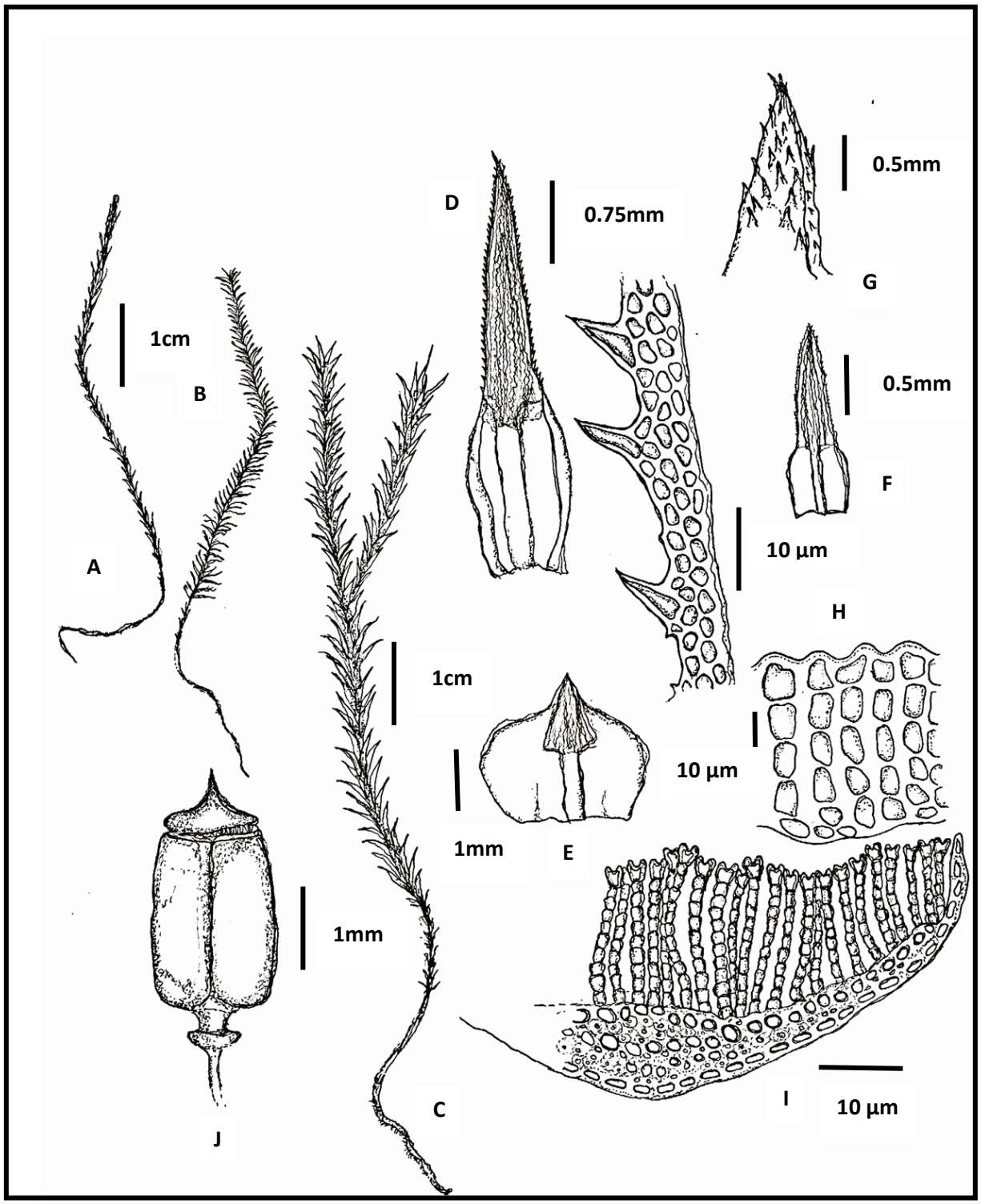

Figure 4.9: *Polytrichum ericoides* **A.** Habit -unbranched form, dry; **B**. Habit-unbranched form, moist; **C**. Habit-branched, taller form, moist; **D.** Vegetative leaf (moist); **E.** Perigonial leaf (moist); **F.** Perichaetial leaf (moist); **G.** Vegetative leaf apex with numerous spines; **H.** Side view of leaf lamellae; **I.** Leaf transverse section showing deeply-grooved lamellar end-cells; **J:** Undehisced young capsule with operculum attached. (From *Andrés Orejuela & Lily Castillo 3048* (E); drawn by I. U. Kariyawasam)

Distribution: *P. ericoides* is considered to be an endemic taxon in the tropical Andes. This taxon has been reported from Venezuela (1830–1920 m), Colombia: (500–3450 m), Ecuador (1500–1800 m) and Bolivia (3200 m) (Churchill & al., 2000).

Figure 4.10 shows the distribution of *P. ericoides* in Colombia based on the herbarium records confirmed from the present study.

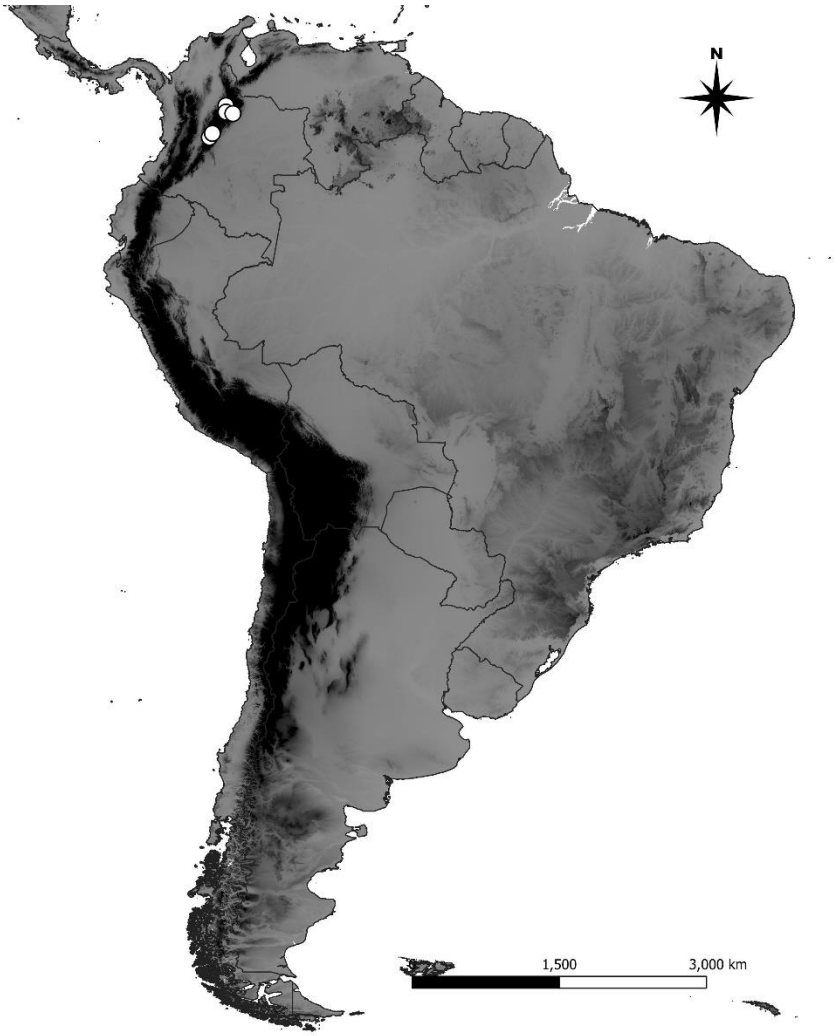

Figure 4.10: Distribution of *Polytrichum ericoides* based on confirmed herbarium records

Ecology: *P. ericoides* is a fairly common species on moorland usually associated with peat bogs (Aponte & Uribe, 2017). In the characteristic Páramo ecosystems in Colombia (Figure 4.11), it is confined to open, water-logged area and peat bogs. In Colombia it is located in the departments of Antioquia, Boyacá, Cundinamarca (from this study) and Meta (Aponte & Uribe, 2017) districts and regarded as a high-altitude species occur between 2700–3800 m altitudinal

range. Although it has also reported from Venezuela, Ecuador and Bolivia (Aponte & Uribe, 2017) no specimens were available for the present study.

Figure 4.11a: A Colombian Páramo ecosystems where some recent *P. ericoides* populations were collected for the present study in 2019 (Photo Courtesy Andrés Orejuela)

Taxonomic & Nomenclatural Notes: Although this is fairly a common species at the high altitudinal areas of Colombia, especially in the Northern Andean Páramo ecosystems (Figure 4.11), all the herbarium specimens available for the study received from the herbaria MO and NY lack sporophytes. Smith (1976) also described the taxon (as *Polytrichadelphus ericoides* (Hampe) Mitt.) without sporophytes.

In 2019, a PhD colleague Andrés Orejuela, who is working on Colombian plants in Páramo areas collected three specimens of *P. ericoides* from Cundinamarca, Municipality of Choachi, Bogotá-Choachi, Matarredenda Ecological Park, waterfall "La Abuela" (Figure 4.12A), where one of those was bearing sporophytes. Hence, the present study describes and illustrates [Figures 4.9 J & 4.11b (B)] the sporophytes of *P. ericoides* for the first time.

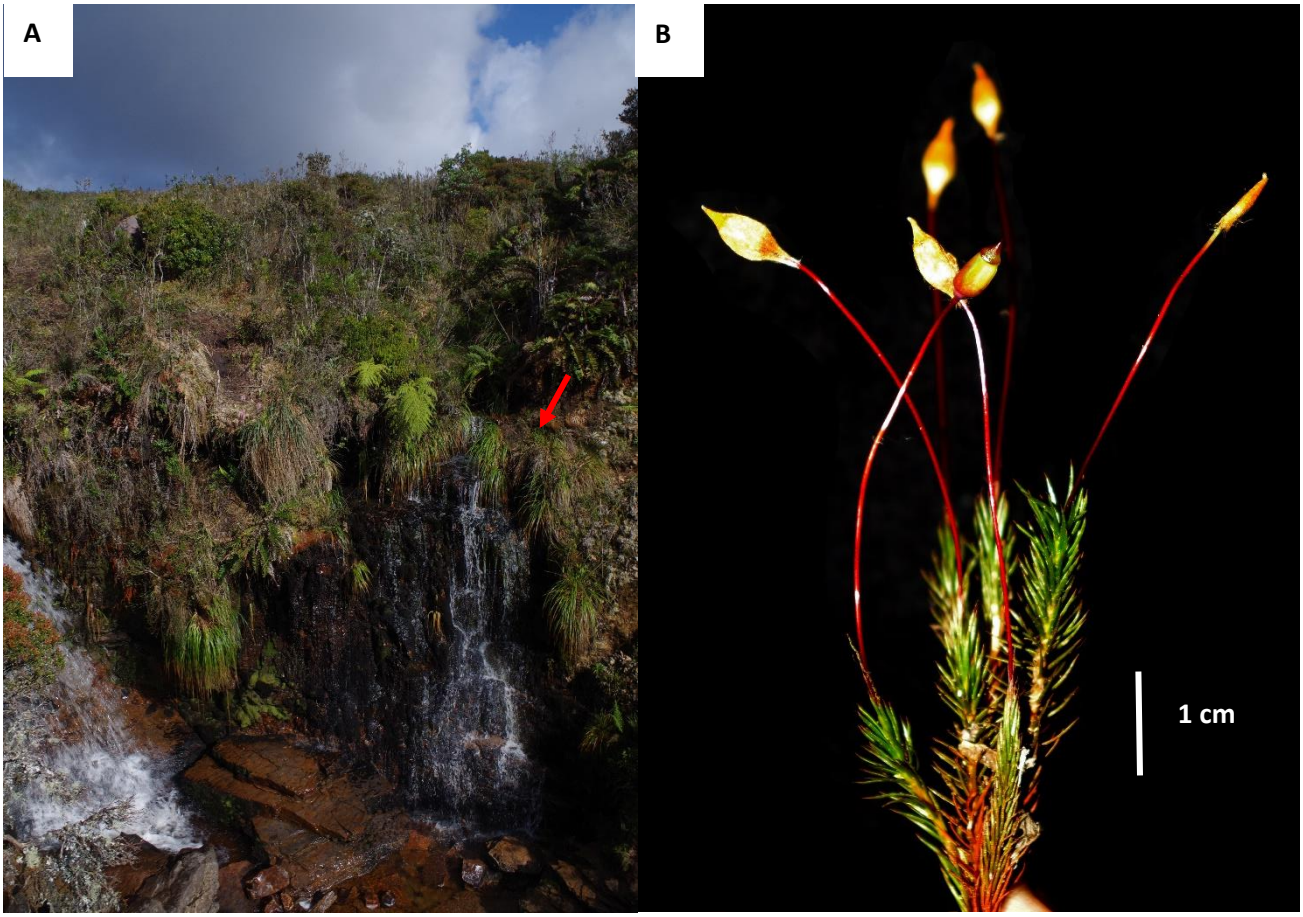

Figure 4.11b: (**A)**. The habitat where the sporophytes of *P. ericoides* collected (marked in a red arrow) near the"La Abuela" waterfall at Matarredenda Ecological Park, Bogotá-Choachi (Photo courtesy Andrés Orejuela); **(B)**. The newly discovered sporophytes of *P. ericoides*.

Smith (1976) stated that "*P. ericoides* Hampe of Colombia, [is] distinguished from *P. commune* by leaves with an obtuse, awnless apex and crowded marginal teeth and *P. angustifolium* Mitt. of Brazil, with the sharply-toothed leaf margins of *P. commune* Hedw. and the lamellae of *P. juniperinum*". This observation is seconded from this study as *P. ericoides* is the only taxon in *Polytrichum* sect. *Polytrichum* which has a short leaf lamina with strictly serrulate leaf margins. The leaf lamellae are very tall compared to those of *P. commune*, *P. angustifolium* and *P. brachymitrium* in South America, possessing lamellae 6(–8) –12 cells high.

Sterile plants of *P. ericoides* are very similar to *P. commune* in Colombia, however the short leaf lamina (or size of the limb relative to the costa and sheath) and shape of the leaf apex help to distinguish it from *P. commune*. Moreover, leaf transverse sections of *P. commune* Hedw. and *P. ericoides* Hampe could easily be confused as both taxa possess deeply grooved

lamellar end-cells. However, the lamellae are comparatively taller and more loosely aggregated in *P. ericoides* and the guide cells are comparatively smaller.

Additional Specimens Observed.

COLOMBIA, Antioquia, Urrano, [06°25' 00" N, 076°05'00" W], ~3500 m, 30-June-1985, *Churchill S.P., 13325* (MO, NY); Boyacá, Duitama, 3450 m, 05°59'00""N, 073°05'00""W, 3450 m, 23- July-1985, *Linda K. Albert de Escobar & J.I. Santa, 214* (MO); Boyacá, Soata, 3530 m, 05°53'00""N, 072°37'00""W, 16-Jun-1972, *Antoine M. Cleef, 4625* (MO); Distrito Capital, 3700 m, 04°17'00""N, 074°12'00""W, 16-Jul-1998, *Churchill S.P. 19311* (MO, NY); Cundinamarca, 3600 m, 04°31'00""N, 073°45'00""W, 3-Jan- 1969, *José Cuatrecasas, 26977* (NY); Cundinamarca, Municipality of Choachi, way Bogotá-Choachi, Matarredenda ecological Park, waterfall "La Abuela" 4° 32' 52.7" N; 73° 59' 46" W, 3316 m. 29-Aug- 2019, *Andrés Orejuela & Lily Castillo, 3048-3050* (E, JBB).

Additional notes:

Churchill, 17033, Colombia (MO, NY) This specimen was previously identified as *Polytrichum ericoides* Hampe. However, with careful observation of the leaf lamellar end-cells and other foliar characters this was morphologically identified as *P. brachymitrium* (see under *P. brachymitrium*). A duplicate specimen from NY was used to sequence this collection which had been misidentified as *P. ericoides* by Bell & Hyvönen (2010b) (See Chapter 3 Discussion & the foot note under *P. brachymitrium*).

3. *Polytrichum jensenii* I.Hagen, Meddel. Grønland 15(7): 444, 1898 – *Polytrichum commune* Hedw. var. *jensenii* (I. Hagen) Mönk ex Frye, in Grout, Moss Fl. N. America 1:125, 1937. Type citation: [Greenland] "ad Agpalisiorfik 70°49' lat., Grønlandiae boreali occidentalis, 5/7 1887 in itinere Ryderi detectum."; Type specimen: Northwest Greenland, Agpalisiorfik, 70°49' lat., 5-July-1887, *Ryder s.n.* (isotype C 197025!).

Syn*: Polytrichum fragilifolium* H.Lindb., Meddeland. Soc. Fauna Fl. Fenn. 24: 28, 200. 1901. Type citation: [Russia, Karelian Isthmus] "pa Karelska näset". Type specimen: [Russia] Isthmus Karelicus, bei Sakkola [Karelian Isthmus, near Sakkola], [60°24'38"N, 30°01'05"E], auf feuchten Sandboden [on damp sandy soil], 23-June-1897, *H. Lindberg s.n.* [isotype PC0132422!].

Syn*: Polytrichum commune* Hedw. var. *diminutum* (I.Hagen) D.G. Long, Meddel. Grønland 17: 40, 1985; *Polytrichum jensenii* I.Hagen var. *diminutum* I.Hagen, Kongel. Norske Vidensk. Selsk. Skr. (Trondheim), 1913(1): 56. 1914. Type citation: [Norway] "S.T. [Sør-Trøndelag] Opdal, pa vej til Snehætten: Kiær." Type specimen: Norway, Opdal, *Kiær s.n.*, [isotype S! - seen on a slide prepared by D.G. Long (E)].

Figures 4.12, 4.13, 4.14 & 4.15

Stem erect, medium to large, aggregated and forming extensive carpets or scattered stems, often soft and rather fragile or sometimes rigid, (2–)6–10(–12) cm, in lower part moderately to densely brownish tomentose. ***Leaves*** green, weakly flexuose, erect-spreading to widely-spreading when moist, closely appressed to the stem when dry, 6.0–7.5(–10.0) × 0.5–1.2 mm, caducous at the base of the lamina, lanceolate, differentiated into sheath and lamina, leaf sheath short, scarcely broadened, and weakly differentiated, margins usually entire or finely and obscurely serrate from the middle to the apex, teeth rather short and distant, costa abaxially smooth, ending in a short and very weakly toothed tip. Median cells of the sheath short and rectangular, 60–(–90) ×12–20(–25) µm. Median cells of leaf lamina quadrate, with slightly thickened cell-walls 9(–10)–15 µm broad. Lamellae crenulate in a profile, 20–30 rows, 5–(–10)–12 cells high, lamellar end-cells deeply-grooved), 8–12(–13) µm broad, thick-walled with paired "knob-like" papillated projections. ***Dioicous;*** perigonia and perichaetia terminal. ***Perichaetial leaves*** differentiated, lanceolate, with entire or very weakly dentate leaves, lamina subulate, apex acute. ***Perigonial leaves*** differentiated, lanceolate to oblanceolate with a larger leaf base, apex acute, margin slightly serrate and usually hidden by upper stem leaves. ***Capsule*** strictly 4-angled, cubic or shortly rectangular, 2.5–3.0(–3.5) mm; apophysis distinct, discoid,

narrowly constricted above; exothecial cells composed of conical papillae with slit-like apertures; operculum conic with a short rostellate beak; peristome teeth pale yellow, ca. 0.3–0.5 mm high, obtuse, spores 10–13 µm in diameter. ***Seta*** 1.5(–3) – 4.5(–5.2) mm long, brownish-red when young. ***Calyptra*** golden-yellow, fibrillose and completely covering the capsule, 10–12 mm long.

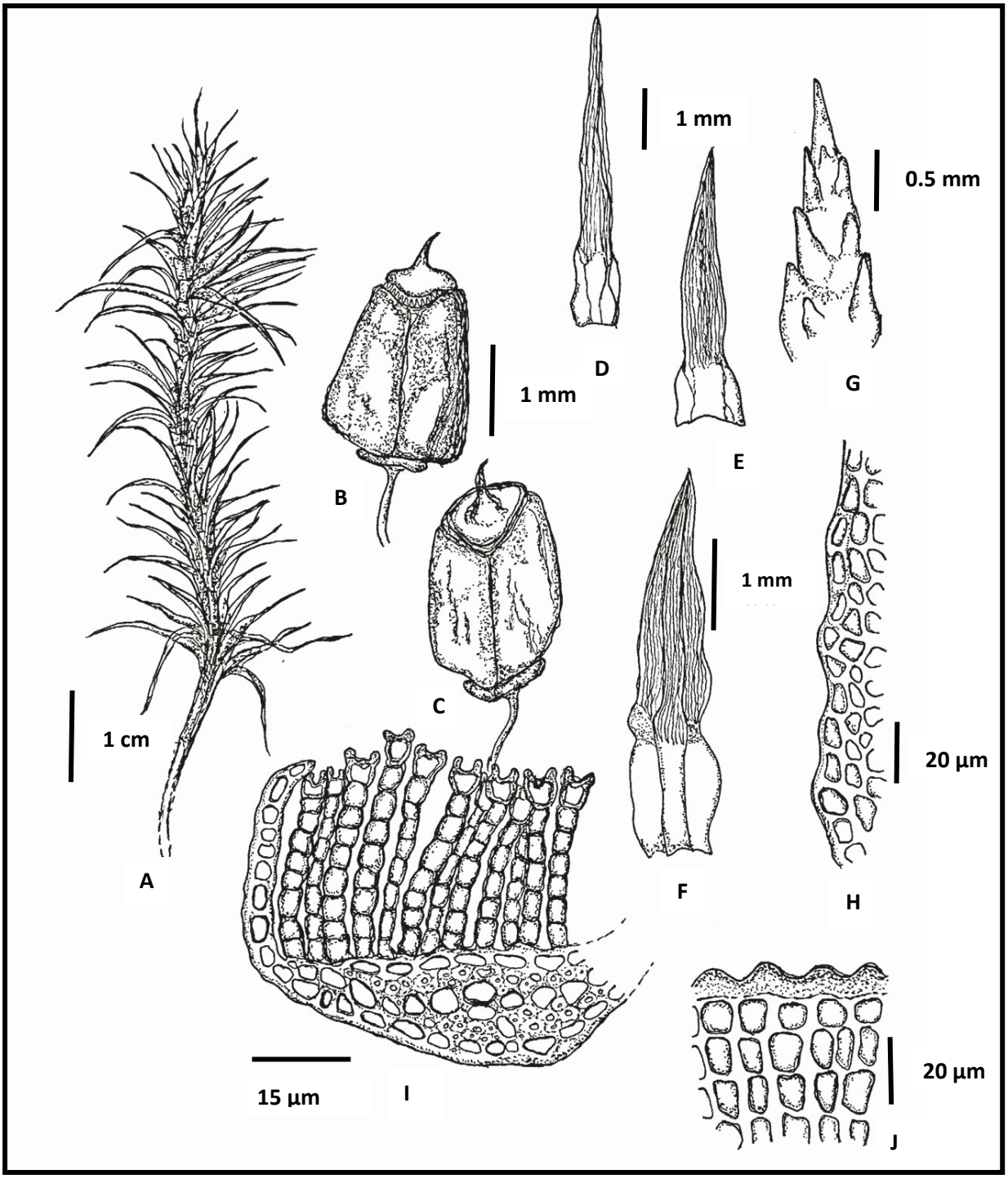

Figure 4.12: *Polytrichum jensenii* **A.** Habit-moist, showing the wide spreading leaves; **B &C**. Mature, undehisced, strictly 4-angled capsule; **D**. Perichaetial leaf (moist); **E & F**. Vegetative leaves with entire margins (moist); **G**. Leaf apex with spines; **H**. Entire leaf margin; **I.** Leaf transverse section showing deeply-grooved lamellar end-cells with "knob-like" projections; **J.** Lamellae side view (From *Long 42605* (E), drawn by I. U. Kariyawasam)

Distribution: In the Nordic countries, it is found in Sweden, Norway, Finland, Svalbard and Björnön. The total distribution is circumpolar, with an Arctic centre of distribution and including the northern parts of Europe, Arctic region of Russia and North America (Alaska, south to Labrador and northern Québec, with an outlying alpine station in the Rocky Mountains of Wyoming,) as well as Greenland (Frye, 1910; Long, 1985; Smith Merrill, 2007; Faubert, 2013; Lönnell & al., 2019; Hodgetts & al., 2020).

Figure 4.13 shows the distribution of *P. jensenii* in the Holarctic region based on the herbarium records confirmed from the present study.

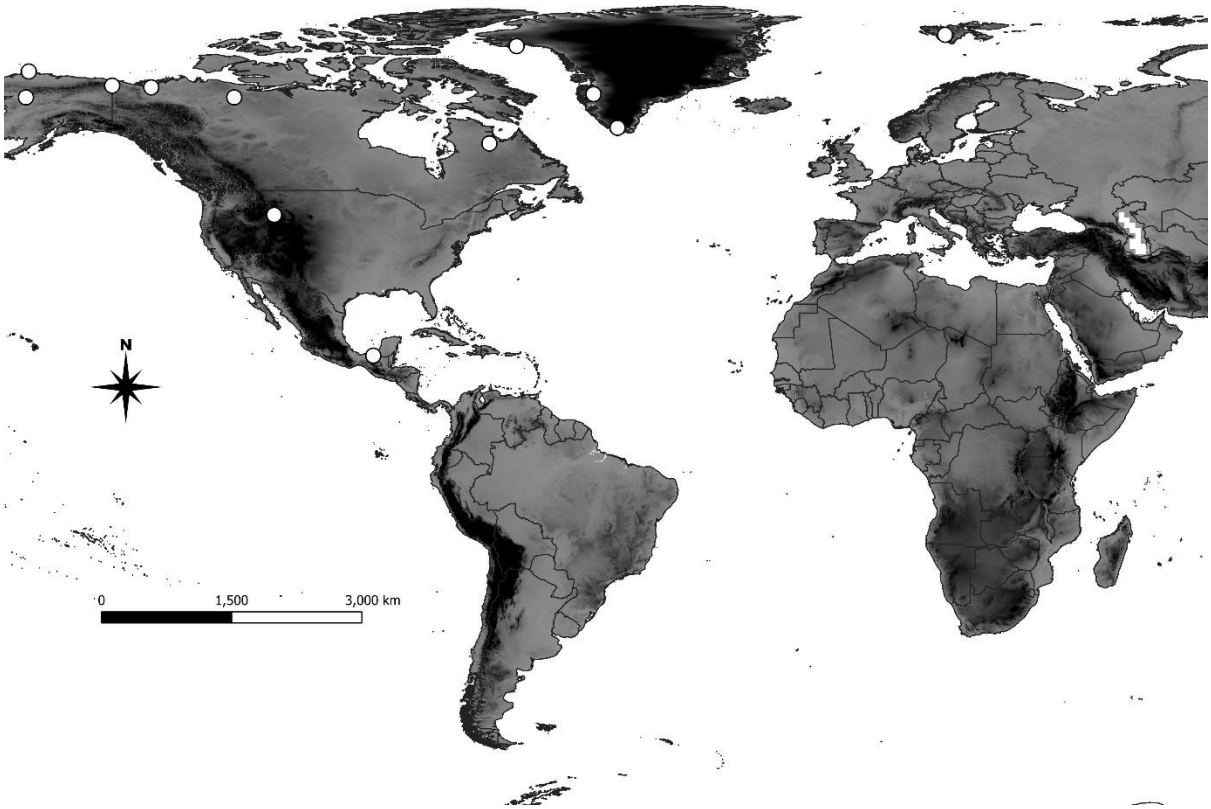

Figure 4.13. Distribution of *Polytrichum jensenii* based on confirmed herbarium records

Ecology: This is predominantly an arctic-alpine species. Long (1985) reported that "this taxon forms green carpets or more commonly as scattered stems amongst other bryophytes such as *Sphagnum* sp, *Calliergon samentosum* and *Aulacomnium palustre* in habitats subjected to regular or intermittent inundation, especially in *Sphagnum* bogs, sedge meadows, river and lake margins, also in hollows in open tundra and polygons, more rarely in wet *Picea* forest and muskeg swamps". *Polytrichum jensenii* often grows in environments that are occasionally flooded (Figure 4.14), such as banks by lakes and streams, ditches and bogs. In Arctic environments, it often grows on fairly thin peat soil with a mixture of mineral soil. It is predominantly found in low elevations to ~1500 m (Long 1985; Lönnell & al., 2019).

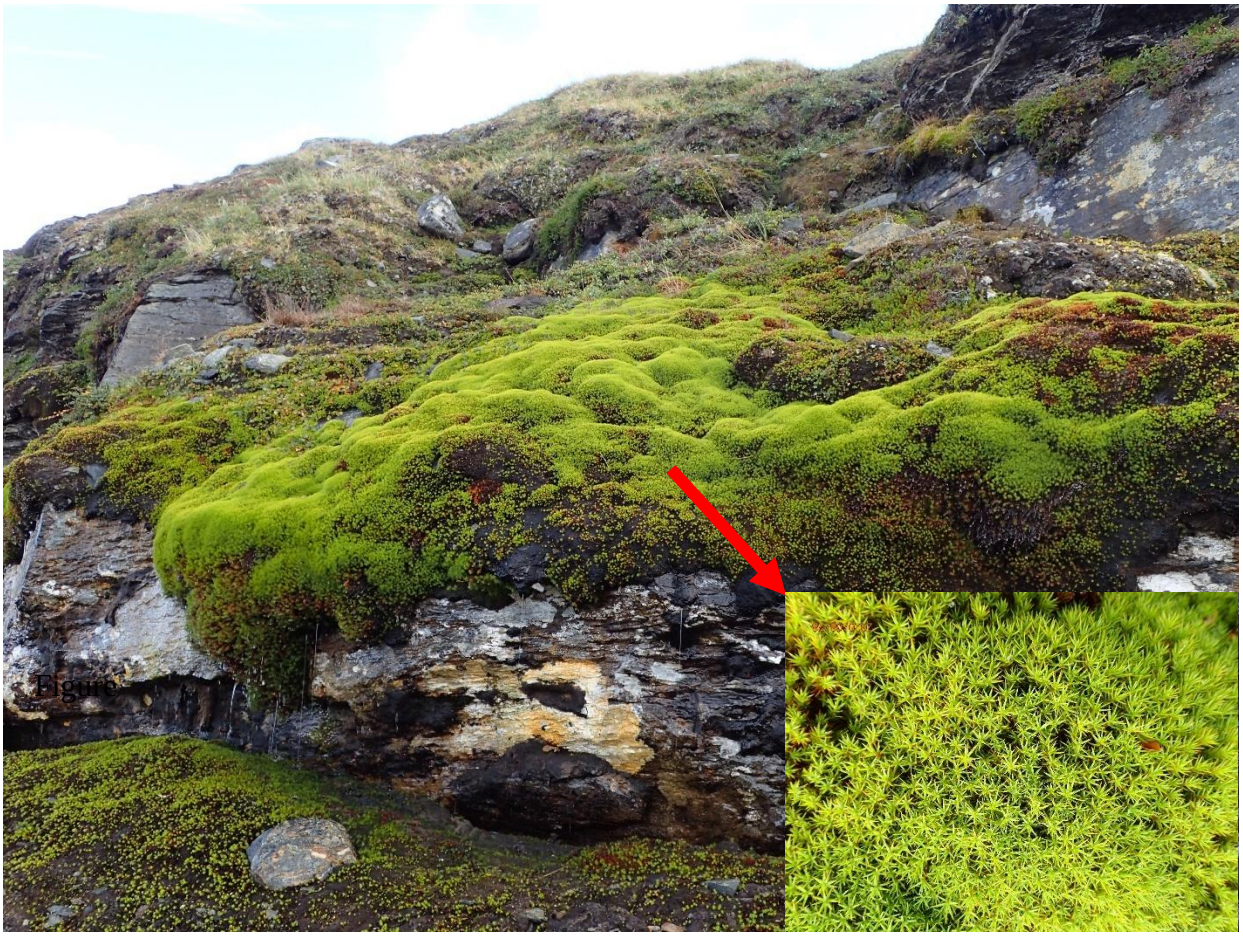

Figure 4.14: An extensive carpet of *P. jensenii* found at the wet rocky mountainous area in Northern Sweden (Photo courtesy Niklas Lönnell)

Taxonomic, Nomenclatural & Geographical Notes:

Long (1985) has treated this taxon as a variety of *P. commune* [i.e. *P. commune* var. *duminitum* (I.Hagen) D.G.Long.] from the arctic, based on its morphological affinity to the wide-spread taxon *P. commune* Hedw. However, in more recent taxonomic treatments (Smith Merrill, 2007; Faubert, 2013, Lönnell & al., 2019) *P. jensenii* is treated as a separate species. An examplar of *P.commune* var. *diminutum* collected and identified by Long [*Long 42605* (E)] from Norway was included in the molecular study along with *P. jensenii* examplars and confirms their identity. (See Chapter 03). Previous genetic evidence also corroborates with the molecular findings of the present study that *P. jensenii* is a distinct Arctic species (Derda & Wyatt, 2000).

Similarly, Long (1985) synonymised *Polytrichum yukonense* Card. & Thér. (*Polytrichum commune* var. *yukonense* (Card. & Thér.) Frye). under *P. jensenii* (≡ *P. commune* var. *jensenii*) in his taxonomic treatment. However, following recent designation of a lectotype for *Polytrichum commune* Hedw. (see Chapter 02) and using accessions identified as *P. yukonense* from the type locality [near Yukon river, Alaska; *Schofield 71150* (UBC)] and *P. commune* var. *yukonense* from Alaska [*Marko Lewis 457* (UBC)], the throrough morphological and molecular studies carried out have confirmed that *P. yukonense* (≡ *P. commune* var. *yukonense*) is now a synonym of *P.commune* Hedw. *s.str*. and is therefore excluded from the synonymy of *P. jensenii.*

Polytrichum jensenii is easily confused with *P. commune* and *P. swartzii*. This taxon can be readily identified in the field (as well as in the herbarium) from the brittle/fragile and flexuose nature of its leaves (Figure 4.15). Although the type specimen does not show the charactersic fragile leaves, the taxon *P. fragilifolium* which is now synonymised with this taxon (a very common form of *P. jensenii* especially in the Nordic countries) clearly exhibits this charactersitic feature. *P. jensenii* often grows in environments with varying levels of water, and possesses stiff leaves with entire or subentire leaves with weakly serrate leaf margins. This is a distinguishing fetaure of *P. jensenii* (≡ *P. fragilifolium*) between *P. commune* and *P. swartzii*.

This species can grow along with the common bog moss *Polytrichum commune* which can grow considerably larger, has leaves that are not fragile, leaf margins are clearly serrate and often bears a longer capsule. In a leaf transverese section, the lamellar end-cells in *P. jensenii* are always deeply grooved (note that the cross section should be made in the middle of the upper part of the leaf, as they may become flat-topped to slightly pyriform closer to the sheathing leaf base, exhibiting a developmental series of lamellar end-cells along the leaf

lamina), bearing the characteristic ”double knob-like” papillated thickenings (Figures 4.3B & 4.12 I), whereas in *P. commune*, the lamellar end-cells are usually deeply grooved, but never possess ”knob-like” thickenings on top of each half of the cell. In contrast, *P.swartzii* has flatter, never deeply-grooved lamellar end-cells and leaves that are often not as stiff and upright and which therefore give the plant a softer texture. Some specimens which have been called *P. jensenii* in the Nordic countries may probably be *P. swartzii* and some herbarium specimens labelled as *P. swartzii* turned out to be *P. jensenii* and *vice versa*. Future studies will hopefully clarify the range of morphological variation within this taxon with the aid of *evo-devo* studies as well as to correlate the occcurrence of such morphotypes of the taxon understand them and their distribution in various boreal environments.

This taxon is excluded from China as the specimens received from China (PE) identified as *P. commune* var. *jensenii* [e. g. Xu & Liu, 456, (PE)] are confirmed as *P. perigoniale* Michx. from this study.

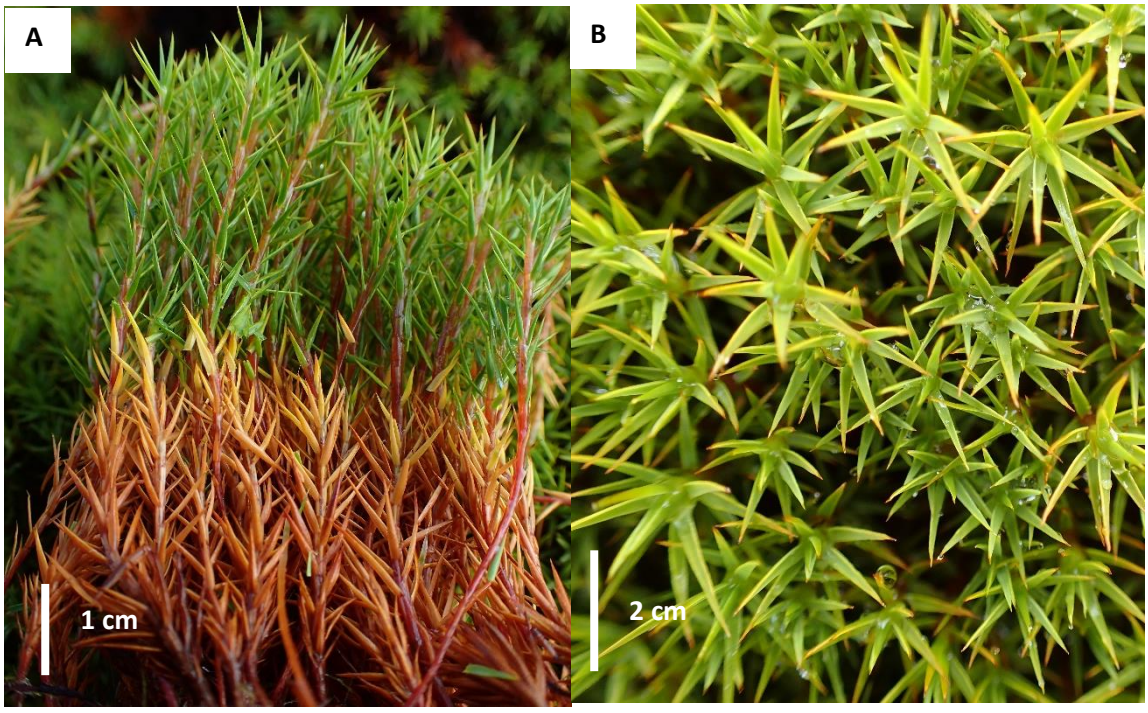

Figure 4.15: Sterile plants of *P. jensenii* **(A)** showing the characteristic stiff and fragile nature of leaves; **(B)** showing crowded plants with fully expanded leaves with entire leaf margins (Photo courtesy Niklas Lönnell)

Additional Specimens Observed

CANADA, **NORTHWEST TERRITORIES**, Northeast tip of Great Bear Lake, head of Hornby Bay, between harbour at extreme NE end of Hornby Bay and river coming from north, in wet sandy depression, on sandy point, [66.47 N, -118.09 W], 26-July-1948, *W.C. Steere 10488* (UBC, NY); Northwest Territories, Mackenzie, Mackenzie River delta, [68.35N, -133.67W], 1963, *W.C. Steere 63722* (NY)

CANADA, QUÉBEC: Nunavik, à environ 1.6 km à l'est du lac Narcy, 4.7 km à l'ouest du lac Napier et 7.3 km au sud du lac Imbault, sable humide riverain., 57.824, -70.2055, 24- Jul-2013, *D. F. Bastien with J. Gagnon, B. Tremblay DB2013-546* (NY); Ontario. Mt. Josephine, north shore of Lake Superior., 13 Aug 1901, *J. M. Holzinger, s.n.* (NY)

FINLAND: Lapponia Enontekiensis, Enontekiö, Lätäseno, Hirvasvuopio, in prato inundato, 09-Aug-1934, H. *Roivainen s.n*. (UPS); Pohjois-Pohjanmaa, Hailuoto, Marjaniemi, pond Hannuksen Rantalampi, on marshy, sandy NW shore of drained pond near seashore, *T. Ulivinen s.n.* (UBC); Pohjois-Pohjanmaa, Hailuoto, Marjaniemi, 27°E 72 17:385, on moist sandy half-open shore of drained pond Hannuksenlampi, Scattered. *+P. longisetum*, *P. commune*, 02-Aug-1977, *T. Ulvinen s.n.*(S).

GREENLAND: Crimson Cliffs, NW Greenland, 1940, *D.C. Nutt 47H-5083* (NY); Vestgrønland [West Greenland], small cove E of Nûpiluk, Kangerdluarssak Fjord, northwest across peninsula from Julianehaab, [60.77 N, 46.15W], 1962, *W.C. Steere 621132* (NY); NE Greenland, Liverpool Coast, Cape Lattershall, 1936, *R.A. Bartlett 1a* (NY); East of Sondre. Stromfjord airport, ca 7 km northwest of Keglen, [67 º05'N, 50º40'W], 29-Jul-1977, *G.S. Mogensen & G.R. Brassard 77-45* (UPS, NY); Melville Bay, Savigssivik [Meteorite Island], [76.1 N, 65.1 W], 20-Aug-1943, *M.P. Porsild s.n.* (UBC).

NORWAY: Svalbard, Nordenskiöld la District, Longyearbyen, Dammyra Strax, OSO om Longyearbyen, 78.2333°N, 15.5°E, 10-Aug-2007, *T. Hallingbäck 45085* (E); Svalbard, lower part of Bjorndalen, rocky valley slopes, by small stream in scree, c. 20 m, 78°13'46.9"N, 15°19'28.8"E , 26-Jul-2013, *D.G. Long 42605* (E).

SWEDEN: Lule Lappmark, Jokkmokk, ca. 87 m, Alep Stullo, N. branten, 13-Aug-2012, *T. Hallingbäck 6394* (E); Dalarna, Falun, Kvarnberget, in uliginosis, Sep-1913, *H. Möeller s.n.*

(UBC); Ångermanland, Högsjö sn., våt Ängsmark vid Havsstrand nära Vada, 25-Jul-1959, *E. Evans s.n*. (S); North Lapland, Katterjokk River, 07-Jul-1916, *E. Jaderholm s.n.* (S); North Sweden, in Nabwiesen und Sumpfen hochnordischer Lager mit salinem Einfluss, zwischen den Orten Lulea und Haparanda, 26-Jul-1977, *A.V. Hübschmann 1161* (UBC); Torne Lappmark, Jukkasjärvi, W of Lake Torneträsk, by Kärkejåkk, alt. 690 m, marshy ground, 03-Jul-1963, *A.C. Crundwell & E. Nyholm s.n*. (LD, S).

RUSSIA: Sankt-Petersburg, Isthmus Karelicus, par. Sakkola, in ripa arenosa humida fq, 29-Jun-1897, *H. Lindberg s.n.* (UPS); Sakhalin Island, Okhinskyi Region, unnamed lake campsite along a new road to the coast, next to the 15 km sign and jct. with main N/S Highway about 15–17 km east of Piltun Village in the transition zone between Taiga and Tundra, mixed in with a tuft of *Carex* spp. in part shade, 07-Aug-2003, *J.A. Harpel 32677* (UBC); Rossia Europea Arctica, Insula Kolgujev, Bugrino, tundra turfoso-tumulosa ad litus maris, in tumulis, 27-Jul-1935, *L.I. Savicz-Ljubitzkaja s.n*. (UBC).

USA, ALASKA: seepage meadow, Selawik Nature Wildlife Refuge, unnamed lake, lake shore, 66^{o} 28'19"N, -157^{o}11'43"W, 47 ft., 15-Jul-2003, *W.B. Schofield, S.S. Talbot 121386* (UBC, NY); North Slope, Point Barrow & vicinity, 71.33 N, -156.65W, 1951, *W.C. Steere 15003* (NY); North Slope, vicinity of Point Barrow, wet tundra SE of Laboratory, 18- Jun-1973 to 19-Jun-1973, *W. C. Steere 16 with H. Inoue & Z. Iwatsuki* (NY); North Slope, Schrader Lake, Ramanzof Mountains, Jul 1951, *R. Rausch, s.n*. (NY); North Slope, Romanzof Range, Okpilak River near Okpilak Lake, 04-Aug-1958, *S. Shushan B215 with J.W. Thomson, Jr.*, (NY); collected on upland tundra near Kochaskik Bay, Hooper Bay Quadrangle, 18N, 92W, 25 (SE quarter), Jul-1973, *M.T. Jackson 1275* (NY); Firth River Basin, near mouth of Mancha Creek, 68.67 N, -141.00 W, 1958, *A.J. Sharp MC58145* (NY); Nowitna National Wildlife Refuge, Ruby (C4) Quadrangle., 19-Aug-1986, *S.S. Talbot 10C5* (NY); North Slope, Brooks Range, Lake Schrader, 28-Jul-1960, *W.C. Steere 60á114 with O. Mårtensson & K. A. Holmen* (NY); vicinity of Isaac's Camp & Mt. Tomname, near Tomname Lagoon & Cape, N-shore of St. Lawrence Island, Bering Sea (about 10.0 miles W of "Lietnik" of many maps), Jul-1949, *W.C. Steere 13965* (NY).

USA, WYOMING: Yellowstone National Park, 24-Jul-1899, *A. Nelson & E. Nelson, s.n*., (NY); Beaver Lake, Yellowstone National Park [44.4280° N, 110.5885° W], 1899, *A. Nelson, s.n.* (NY).

4. *Polytrichum subpilosum* P.Beauv., Prodr. Aethéogam. 86, 1805. Type citation: [Réunion] "Cette plante de l'Ile de Bourbon, m'a été communiquée par M. de Jussieu." Type specimen: [Réunion] "Ile de Bourbon (Mr Adr. De Jussieu)" (E00238117!, isotype).

Syn: *Polytrichum afrorobustum* Besch. ex Renauld, Prodr. Fl. Bryol. Madagascar, 179. 1898. Type citation: "Madagascar: forêt de Vadivato, Calat, 6 août 1889, no. 1689 (Hb. Bescherelle)". Type specimen: "*Polytrichum afro-robustum* Besch. in litt. ad *Renauld, No. 1689*, Forêt de Vadivato, 6 août 1889, M. Calat" (BM000871214!, herb. Bescherelle, isotype).

Syn: *Polytrichum appressum* Brid., Muscol. Recent. Suppl. 1: 51. 1806. Type citation: [Réunion] "In Insulae Bourbonis Plaine des Chicots habitat. Bory de St. Vincent detexit et benigne communicavit." Type specimen: [Réunion] "*Polytrichum appressum* Bryol. Univ. Ile de Bourbon. Bory de St. Vincent. *Paris 1805*. Herb. Bridel." (B31066001!, lectotype selected here; *P. subpilosum* P.Beauv., det. J.L. de Sloover 1983).

Syn: *Polytrichum buchananii* Rehmann "*buchanani*", in Kindberg, Enum. Bryin. Exot., Suppl. 1: 94. 1889, *nom. inval.* [no description]. Original material: [South Africa] "*Polytrichum buchanani* Rehm. n. sp. Natal: in statione Umpolnuhe, leg. *Rev. Buchanan*, in *Rehmann*: Musci austroafricani cont. 580" (BM000871077!, BM000878414).

Syn: *Polytrichum calopogon* Besch., Ann. Sci. Nat., Bot., sér. 6, 10: 256. 1880. Type citation: [Réunion, Mauritius] "La Réunion: Bory (in herb. Cosson); Ile Maurice: Commerson". Type specimen: "Mousses de la Réunion. *Polytrichum calopogon* Besch., leg. *Richard*, hb. E. Bescherelle". (BM001087955!, lectotype selected here).

Syn: *Polytrichum calopogon* Besch. forma *majus* Besch., Ann. Sci. Nat., Bot., sér. 6, 10: 256. 1880. Type citation: [Réunion] "La Réunion: Richard, no. 570 (in herb. Mus. Par.)". Type specimen: [Réunion] "*Polytrichum calopogon* Besch., La Réunion: *Richard No. 570*, hb. E. Bescherelle" (BM001087960!, lectotype selected here).

Syn: *Polytrichum comorense* Müll.Hal. ex Besch., Ann. Sci. Nat., Bot., sér. 6, 10: 256. 1880. Type citation: [Comoro Islands] "Grand Comore: mai 1850, Boivin (herb. Mus. Par.)". Type specimen: [Comoro Islands] "Gd. Comore, Mai 1850, Voyage de M. *Boivin, s.n.* (PC0131174! lectotype selected here; ibid., PC0131175!, isolectotype); "*Polytrichum comorense* C.M., Iles Comores. Voyage de M. Boivin 1847–1852, herb E. Bescherelle" (BM000871202!, isolectotype).

Syn: *Polytrichum elatum* P.Beauv., Prodr. Aethéogam. 85. 1805. Type citation: [Réunion] "Cette espèce croit dans l'Ile de Bourbon." Type specimen: [Réunion] "*P. elatum* Beauv. Ile de Bourbon (Kunth). (E00428864!, isotype; *P. subpilosum* Besch. det. J.L. de Sloover 1983).

Syn: *Polytrichum elegans* Welw. & Duby, Mém. Soc. Phys. Genève 21: 217. 2. f. 1, 1872. Type citation: [Angola] "In dumetis rupestribus humidiusculus ad oram sylvae primitivae dicta *Mata de Pungo* suffruticibus parvis intertextum frequentem sed semper sterilem, in provincial Pungo Andongo inter 9 et 10° lat. austr., ad 3000 circa pedes altitudinis reperiit ce;. Welwitsch." Type specimen: [Angola] "Welwitsch Iter Angolense No. 50. Freq. in dumetosis rupestribus humidiusculus ad oram sylvae primitivae dicta "Mata de Pungo" suffruticibus parvis intertextum. Distr. Pungo Andonga (2400–3800 ped. elevat.) [Inter 9 et 10° Lat. austr.] Sine fructu ab Octob. 856, Mai 1857." (G00284088!, ex herb. Duby in herb. Boissier, lectotype, selected here). ***syn. nov***

Syn: *Polytrichum hoehnelii* Müll.Hal. "*höhneli*", Flora 73: 471. 1890. Type citation: [Tanzania] "Patria. Africa or. trop., monte Kilima-Ndscharo, sine loco speciali: L. Höhnel in Exped. Telckiana 1887." Type specimen: [Tanzania] "*Polytrichum höhneli* n. sp. Kilimandscharo*, Höhnel s.n.*. Herb. Bescherelle. (BM000871213!, lectotype selected here; *P. subpilosum* P.Beauv., det. J.L. de Sloover 1983).

Syn: *Polytrichum leioneuron* Besch., Ann. Sci. Nat., Bot., sér. 7, 2: 91. 1885. Type citation: [France, Department of Mayotte] "Mayotte, M'Sapéré, M'Bini, c. fr., M. Marie, no. 26. Se trouve également à Anjouan." Type specimen: [France, Department of Mayotte] "Mayotte, *Marie s.n.*, herb. Bescherelle" (BM000871196!, possible isotype).

Syn: *Polytrichum longissimum* Müll.Hal. ex Renauld, Prodr. Fl. Bryol. Madagascar 179. 1897 [1898]. Type citation: "Madagascar: Andrangoloaka, Hildebrandt; Analamainty, Campenon, 1890; Fianarantsoa, Dr Besson. La Réunion: Saint-Philippe, Rodriguez, 1889." Type specimens: [Madagascar] "Imerina, *Hildebrandt No. 3125* nov. sp. Herb. Bescherelle" (BM00871200!, syntype); "Ost Imerina, Andrangoloaka, *Hildebrandt 3125*" (BM001087959!, syntype).

Syn: *Polytrichim macropogon* Schimp., in Bescherelle, Ann. Sci. Nat., Bot., sér. 6, 10: 256. 1880, *nom. inval*. (herbarium name, cited as synonym of *P. calopogon* Besch.).

Syn: *Polytrichum madagassum* Hampe, Linnaea 38: 216. 1874. Type citation: [Madagascar] "Sub no. 11 leg. Borgen." Type specimen: [Madagascar] "1869 Madagascar, leg. *Borgen 11*, herb. Hampe" (BM000871204!, lectotype selected here).

Syn: *Polytrichum mahense* Besch., Ann. Sci. Nat., Bot., sér. 6, 10: 257. 1880. Type citation: [Republic of Seychelles] "Seychelles, sur les montagnes, à Mahé, 9 mars 1840, Pervillé (in herb. Mus. Par.)". Type specimen: [Republic of Seychelles] "Mahé, Seychellen, Pervillé No. 192" (BM001087953!, isotype).

Syn: *Polytrichum mauritianum* Müll. Hal. ex Besch., Ann. Sci. Nat., Bot., sér. 6, 10: 258. 1880. Type citation: [Mauritius] "Maurice: Commerson (hb. Mus. Par.); de Robillard (herb. Geheeb.)" Type specimens: [Mauritius] "In insula Mauritii, herb. *G. Robillard* 1876, *Polytrichum mauritianum* sp. nova. C. Müll. (BM001087966!, syntype); In insula Mauritii, herb. *G. Robillard* 1876, ex hb. Geheeb (BM001087963!, BM001087964!, syntypes); "Ile de France, *Commerson*, herb. Bescherelle" (BM001087965!, syntype).

Syn: *Polytrichum mildbraedii* Broth., Nat. Pfl. ed. 2, 11: 514 (1925), replacement name for *P. paludicola* Broth., Wiss. Ergebn. Deut. Zentr.-Afr. Exped., Bot. 2: 157. 1910, *nom. illeg*. non *Polytrichum paludicola* Cardot 1909. Type citation: [Rwanda] "Rugege-Wald: Waldmoor, ca. 1800 m.ü. M[Mildbraed] (männliche Pflanze im Aug. 1907 – n. 823)." Original material: [H-BR, not seen].

Syn: *Polytrichum pervillei* Besch., Ann. Sci. Nat., Bot., sér. 6, 10: 255. 1880. Type citation: [Madagascar] "N.-O. de Madagascar: Pervillé, no. 831, (in herb. Mus. Par.)." Type specimen: [Madagascar] "Madagascar, *Pervillé s.n.*, herb. Schimper" (BM000871198!, isotype); "N.O. Madagascar: *Pervillé s.n.*, herb. Bescherelle (BM000871199!, isotype).

Syn: *Polytrichum pervillei* Besch. var. *leptocaule* Besch., Ann. Sci. Nat., Bot., sér. 6, 10: 255. 1880. Type citation: "La Réunion: Lépervanche, 1877." Type specimens: "La Réunion, *Lepervanche s.n.*" (BM001087957!, isotype).

Syn: *Polytrichum preussii* Broth., Bot. Jahrb. Syst. 20: 193. 1894. Type citation: [Cameroon] "Kamerun: Gebirge, 3000 m (Preuss n. 837)." Type specimen: "Kamerunpik 3000 m, *Preuss 837*"(BM001087938! isotype); "Kamerun, *Dr Preuss 837"* (BM001087939! isotype); "Kamerunpik 3000 m, ii 1891, *Preuss 837"* (BM00871206! isotype).

Syn: *Polytrichum pun*gens Müll.Hal., Flora 71: 408. 1888. Type citation: [Tanzania] "Monte Kilimandscharo, ad finem sylvestrem superiorem inter 3000–4000 m." Type specimen:

[Tanzania] “Monte Kilimandscharo, ad finem sylvestrem superiorem inter 3000–4000 m, 1887, leg. Dr H. Meyer” (PC0131202!, lectotype selected here).

Syn: *Polytrichum purpurellum* Besch. ex Müll. Hal., Gen. Musc. Frond. 183. 1900, *nom. inval*., without description. Original citation: “von der Insel Bourbon”. Original material: [Réunion] “*Polytrichum purpurellum*, La Reunion, *Frappier s.n.*, 7/1880, herb. Bescherelle” (BM001087976!).

Syn: *Polytrichum purpurans* Besch., Ann. Sci. Nat., Bot., sér. 6, 10: 255. 1880. Type citation: “La Réunion: Frappier (in herb. Mus. Par.); sur la terre humide des plaines élevées, 1839, Lépervanche (hb. Thuret).” Type specimens: “La Reunion, *M. Frappier s.n.*, herb. Bescherelle (B001087967!, syntype); La Reunion, *Frappier s.n.*, herb. Bescherelle (B001087968!, syntype).

Syn: *Polytrichum radulifolium* Müll.Hal., Hedwigia 38: 62. 1899. Type citation: [South Africa] “Transvaal, Omtombi inter Delagoa-Bay et Lydenburg, Aug. 1884: Dr. Wilms. Hb. Jack mis. 1889.” Type specimen: [South Africa] “Bei Omtombi, zwischen Delagaobai und Lydenburg, Transvaal, August 1884, Dr. Wilms” (B300202766!, lectotype selected here).

Syn: *Polytrichum robustum* Müll. Hal. ex Cardot, Hist. Phys. Madagascar, Mousses 323. 1915, *nom. illeg*., [later homonym of *Polytrichum robustum* Lindb. 1868]; Original citation: Madagascar, “Zone supérieure des forèts: Andrangoloaka (Hildebrandt)”. Original material: [Madagascar] Imerina, *Hildebrandt 9126* (BM000871194!); Imerina: Andrangoloaka *Hildebrandt 3126* (BM001087974!), Andrangoloaka *Hildebrandt 3126* (BM000871193!), Central Madagascar, Imerino, Andrangoloaka, *Hildebrandt 2126* (BM001087975!).

Syn: *Polytrichum subappressum* Besch., Ann. Sci. Nat., Bot., sér. 6, 10: 252. 1880. Type citation: “La Réunion: herb. Montagne, sub *P. elato* P. Beauv.”. Type specimen: “*Polytr. subappressum*, La Réunion, ad. Delessert, ex herb. Montagne, herb. Bescherelle” (BM001087962!, isotype).

Syn: *Polytrichum subformosum* Besch., Ann. Sci. Nat., Bot., sér. 6, 10: 258. 1880. Type citation: “La Réunion: Bory Saint-Vincent (in herb. Mus. Par. No. 60^3); Lépervanche, 1875.” Type specimens (syntypes): “*Polytrichum subformosum* Besch., La Reunion, P. Lepervanche, hb. E. Bescherelle” (BM001087972!, syntype); “*Polytr. subformosum* E.Besch., La Reunion, Bory St. Vincent, herb. Bescherelle” (BM001087973!, syntype).

Syn: *Polytrichum transvaaliense* Müll.Hal., Hedwigia 38: 63. 1899. Type citation: [South Africa] "Transvaal, in minis auriferis prope Spitzkop, Aprili 1887, c. fr. Deoperculatis et junioribus." Type specimen: [South Africa] "an den Goldminen bei Spitzkop, Lydenburg, Transvaal, April 1887, Dr Wilms (B300203864!, lectotype selected here).

Syn: *Polytrichum trichodes* Müll.Hal., Hedwigia 38: 63. 1899. Type citation: [South Africa} "Natal, Inezanga: Dr. A. Rehmann." Type specimen: [South Africa] "Natal, Inezanga, Rehmann Musci austro-africani (1875-77) Nr. 277". (BM000871072!, isotype).

Figures 4.16, 4.17 & 4.18

Stem erect, rigid, occasionally branched, variable in stature, medium to large, (3–)10–12(–18) cm. ***Leaves*** 2.5–6.5 × 0.5–1.0 mm, appressed to the stem when dry and moderately to wide spreading when moist, leaves crowded at the tip, lanceolate, differentiated into sheath and lamina, leaf sheath hyaline, entire, lamina serrate to the apex, leaf margin sometimes presenting small teeth between the main teeth, costa prominent, excurrent and sometimes forming a pale white arista, leaf apex acuminate, forming a long acumen; lamellae present on both costa and lamina, crenulate in side view, in transverse section with 50–60 rows of lamellae of 4(–6)–8(–9) cells high, end-cells of the lamellae bifid, forming two lobes, divided by a sinus, thick-walled with single large papilla on the top of each lobe; sheath cells 60(–70)–100 × 5(–10) µm. ***Dioicous*;** perigonia and perichaetia terminal. ***Perichaetial leaves*** long, tapering to the end, differentiated, lanceolate with a larger leaf sheath, 2–5(–8) × 1(–2) mm, acute apex, serrate margin, with spines on the abaxial surface. ***Perigonial leaves*** differentiated, oblanceolate with a larger leaf base, 5–6.5 × 0.5–1(–1.8) mm, apex acute, margin serrate. ***Capsule*** 4-angled, cubic, 3.0–4.0 × 3–3.5 mm; apophysis distinct, discoid, narrowly constricted above; exothecial cells composed of conical papillae with slit-like apertures; operculum conic with a short rostellate beak; peristome teeth ca. 0.30–0.37 mm high, obtuse, pale; basal membrane low, brownish; spores 6–8 µm. ***Seta*** 4(–5)–7(–8) mm long, brownish-red when young. ***Calyptra*** golden-yellow, fibrillose and completely covering the capsule.

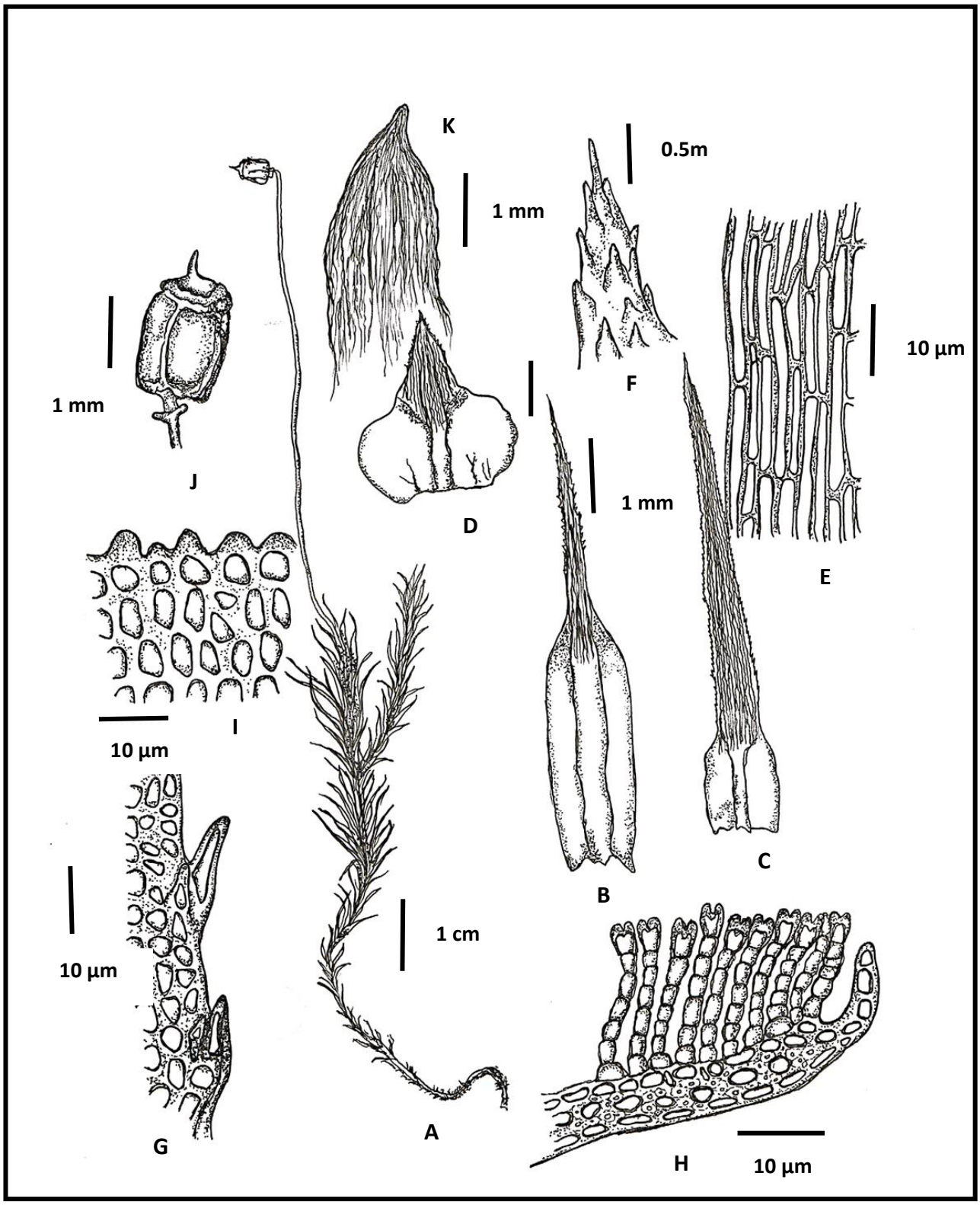

Figure 4.16: *Polytrichum subpilosum* **A.** Habit- branched female gametophyte with a sporophyte attached (dry); B.Perichaetial Leaf (moist); **C.** Vegetative leaf (moist); **D.** Perigonial leaf (moist); **E.** Median cells of the leaf sheath; **F.** Leaf apex with spines; **G.** Leaf margin with leaf teeth associated with small rudimentary teeth; **H.** Leaf transverse section showing bifid lamellar end cells with "papillated knobs"; **I.** Side view of lamellae; **J.** Undehisced mature capsule (dry); **K.** Calyptra (From *Hedderson 19304* (BOL), drawn by I.U. Kariyawasam

Distribution: This species is endemic to the African continent including Madagascar and Réunion, Sierra Leone, Cameroun, Zaïre, Lacs Edouard and Kivu, Uganda, Kenya, Rwanda, Burundi, Tanzania, Zambia, Malawi, Madagascar, La Réunion, Mauritius, South Africa (Transvaal, Natal) (De Sloover, 1986).

Figure 4.17 shows the distribution of *P. subpilosum* in the Africa based on the herbarium records confirmed from the present study.

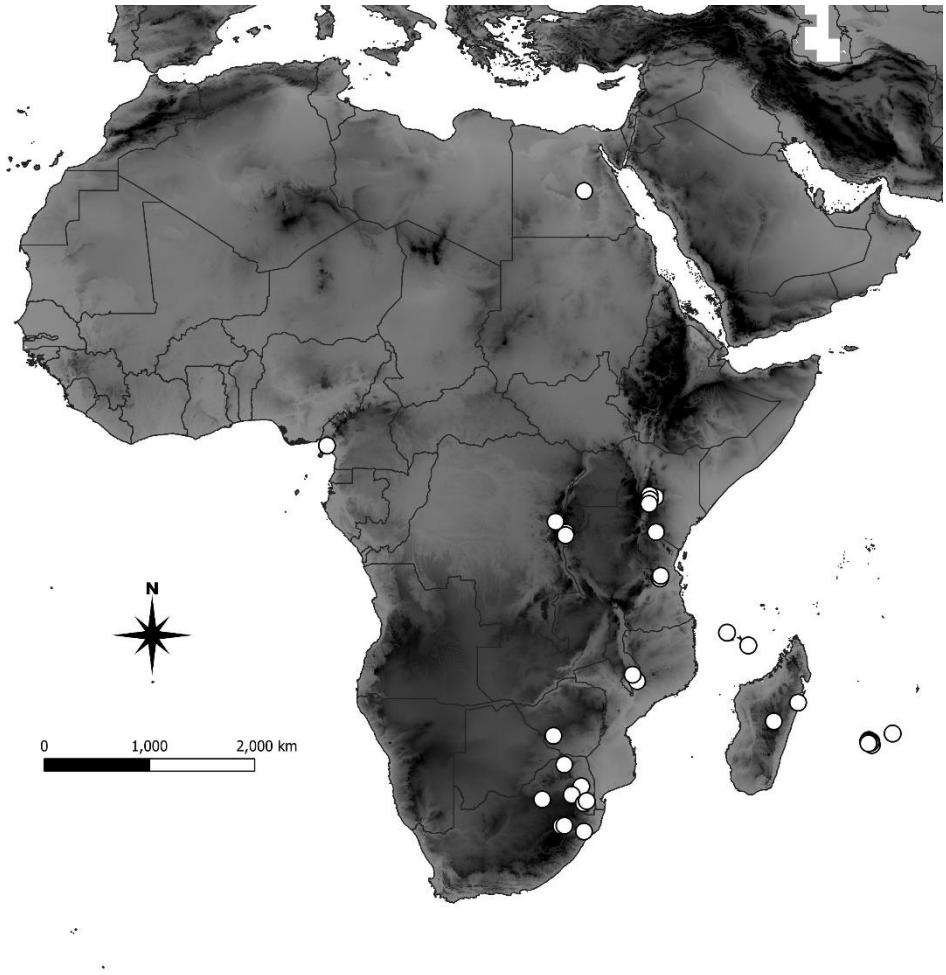

Figure 4.17: Distribution of *Polytrichum subpilosum* based on the confirmed herbarium records.

Ecology: *Polytrichum subpilosum* is regarded as the most frequent and most morphologically variable species of genus *Polytrichum* in Africa (Smith, 1971; Edwards 1976; De Sloover, 1986). *P. subpil*osum has the largest distribution in African montane mossy forests (see above) and also reported in Madagascar and Réunion.

In extreme cases plants are very short (~2–3cm) and sometimes very tall and robust (Figure 4.18), even growing up to 16–18 cm in height which is second only to *P. angustifolium* in Brazil.

Figure 4.18: A population of both male (pointed in red) and female plants of *P. subpilosum* representing both sporophytic and gametophytic generations [Photo courtesy John Brinda, accessed via TROPICOS (http://www.tropicos.org/Image/100740596)]

De Sloover (1986) also reported that this taxon is very robust and taller, often more than the tallest specimens of *Polytrichum commune s. str* of Europe. *P. subpilosum* is also a common species of Afromontane [2000(–3000) –3500m] regions, part of the ground floor

along with *Pogonatum simense* (Bruch & Schimp. ex Müll. Hal.) A. Jaeger. It is often found in wet moist locations in contrast to *P. perigoniale* Michx. [which De Sloover (1986) erroneously reported as *P. commune* in his treatment, see below] which is confined to more xeric environments in Africa. De Sloover (1986) further reported that this species could occur from 1700 to 4200 m altitude; down to 1500 m in Zambia, 900 m in Zimbabwe, 550 m in Comoros, 400 m in La Reunion, 700 m in Madagascar and 800 m in South Africa. In montane forests, in bamboo formations, more rarely with *Hagenia*, on moors and in ericaceous formations; rarely in afro-alpine formations dominated by *Senecio*.

Taxonomic & Nomenclatural Notes

Polytrichum subpilosum and *Polytrichum perigoniale* are the commonest species of *Polytrichum* sect. *Polytrichum* found in Africa. However, *Polytrichum perigoniale* was erroneously treated as *Polytrichum commune* in Africa by De Sloover (1986), as indicated by his anatomical and ecological notes. One of the main reasons for this was that no lectotypes had been designated for these species to clearly establish their identity, and to help to clarify differences between *P. commune* Hedw. and *P. perigoniale* Michx. (Present study has confirmed the identity of both *P. commune* and *P. perigoniale* by selecting lectotypes for each taxon). Another problem has been that during the last quarter century or more, some bryologists have treated *P. perigoniale* (*P. commune* var. *perigoniale*) as the true *P. commune* Hedw., and what had been traditionally known as *P. commune var. commune* became known as *P. commune var. uliginosum* Wallr..

The two striking anatomical differences to distinguish between *P. subpilosum* and *P. perigoniale* in Africa are in their leaf characters. A transverse section of a vegetative leaf of *P. subpilosum* shows characteristic lamellar end-cells. Lamellar end-cells are “bifid” forming a “sinus-like” structure dividing each end-cell into two lobes (Figure 4.16). This structure is remarkably different from the “deeply grooved” or “U-shaped” lamellar end-cells in *P. commune s. str* and the arctic taxon *P. jensenii*. Each lobe of the lamellar end-cell divided by the sinus possesses thick walls and is often sculptured by thin wax flakes or papillae. In side/lateral view, the lamellae exhibit a crenulate appearance due to this bifid nature. In contrary, in a leaf transverse section, leaf lamellar end-cells of *P. perigoniale* exhibit flattish or very shallowly grooved lamellar end-cells with or without thick walls and they never possess papillae. Moreover, the perichaetial leaves of *P. perigoniale* are much longer than the

perichaetial leaves of *P. subpilosum* and the leaf teeth of *P. subpilosum* show a lot of variation compared to those of *P. perigoniale*. In general, the leaf teeth of the vegetative leaves of *P. subpilosum* are somewhat shorter than those of *P. perigoniale*. However, shorter or extreme forms of *P. subpilosum* show shorter leaf teeth compared to robust forms and some rudimentary smaller teeth can be seen between the major teeth in certain forms.

These slight morphological and anatomical variations in different geographical regions in Africa may explain the broad spectrum of different names applied to this species (now mostly synonymised under the broad umbrella of *P. subpilosum*) in the late 19th century by various bryologists.

However, the molecular analysis (Chapter 03) in the present study could not provide strong statistical support for delimitation of *P. subpilosum* as a species. Molecular evidence shows that *P. subpilosum* is derived within the *P. perigoniale* clade in Africa, and that African *P. perigoniale* evidently comprises several pseudo-cryptic species. Despite the weakly supported relationships within the "species complex" of *P. perigoniale* and *P. subpilsoum* in Africa, it can be hypothesised that *P. subpilosum* and its allied pseudo-cryptic taxa are still in the process of speciation. In this taxonomic revision, notwithstanding the molecular evidence, *P. subpilosum* is treated as a distinct "morphological species". This concept may later lead to splitting of *P. subpilosum* into subspecific taxa based on further molecular work with more samples from different geographic regions in Africa.

Following nomenclatural novelties are added from the present study.

Nomenclatural Note 1: Lectotypification and new synonymy of *Polytrichum elegans* Welw. & Duby

De Sloover (1986) did not observe original material of this species and did not mention this taxon in his treatment of Polytrichaceae in Africa and only mentioned by O'Shea (2006) in his checklist without providing any synonomy. The type specimen of *P. elegans* housed in G [G00284088] was thoroughly examined for this study. This taxon is only known from its type specimen collected by Welwitsch from Angola. This type specimen has rather long, tapering leaves; however, the lamellar end-cell shape, and arrangement shows these to be similar to those within *P. subpilosum*. Since no type specimen has been designated for this taxon, the herbarium specimen of *P. elegans* housed in G, with the original annotations of Welwitsch and the illustration attached to it, is selected as a new lectotype for *Polytrichum elegans*.

Nomenclatural Note 2: Lectotypification of Carl Müller's African names now treated as synonyms of *Polytrichum subpilosum*

a. Carl Müller described *Polytrichum hoehnelii* Müll.Hal. collected by Höhnel and determined by Müller in 1887 from the expedition to Mount Kilimanjaro (~3000–4000 m) in Tanzania and described in 1890 in *Flora*. However, part of Höhnel's original material was housed in Émile Bescherelle's herbarium in Paris and later acquired by BM. Müller's complete bryophyte herbarium in Berlin (B) Germany, was tragically destroyed during WWII (Hiepko, 1987) resulting in almost total loss of the all-important type material (Merrill 1943). Part of Müller's original specimen housed in BM [BM000871213] was annotated as "part de type de *P. honhenelii* Müll.Hal" by De Sloover in 1983. Although he did not cite this as a lectotype anywhere in his text (De Sloover 1986) it is selected above as a new lectotype for *Polytrichum hoehnelii* Müll.Hal. After careful observation of the lamellar end-cell morphology and leaf characters this has been synonymised under *P. subpilosum.*

b. Similarly, in 1899 Müller described *Polytrichum pungens* Müll.Hal. from Mount Kilimanjaro in Tanzania collected by Dr H. Meyer. For this species part of the original material (PC0131202!), has been located and is designated above as a new lectotype and its synonymy with *P. subpilosum* confirmed.

c. *Polytrichum radulifolium* Müll.Hal. was also described by Müller in 1899. For this name original material from South Africa has been located in B (B300202766!) and is designated above as lectotype, and its synonymy with *P. subpilosum* confirmed.

d. *Polytrichum transvaaliense* Müll.Hal. was also described by Müller in 1899; original material from South Africa has been located in B (B300203864!) and is designated above as lectotype, and its synonymy with *P. subpilosum* confirmed.

Nomenclatural Note 3: *Polytrichum mildbraedii* Broth.; a proposed synonym for *P. subpilosum* from Brotherus' collection

Polytrichum mildbraedii Broth. is a replacement name for *P. paludicola* Broth., *nom. illeg*. non *Polytrichum paludicola* Cardot. Although the type specimen in H-BR wasn't observed, there were other accessions studied from the type locality (i.e. Rugege Forest, Rwanda) in the present study which confirmed their molecular identity with *P. subpilosum*.

Hence, *Polytrichum mildbraedii* Broth. is proposed as a new synonym for a *P. subpilosum* in this study.

Additional Specimens observed:

BURUNDI, MURAMWYA: Teza [-3.199998S +29.566712E], 2300 m, 23-Dec-1976, *M. Reekmans 5558* (GOET); Mont Manga-Mugongo [-3.443984S +29.546187E], 2350 m, 14-Sept-1974, *J.L. De Sloover 19.186* (GOET)

CAMEROON: Cameroon Mountains [+4.217438S +9.172863E], 2438 m, *s.d., G. Mann s.n.*, (NY); Camaroons, Aug 1875, *G. Thomson s.n.* (NY)

COMORES ARCHIPELAGO: GRAND COMORE. Kartala Forest; trail from La Convalescence to Kartala peak. Mist-belt forest grading into *Erica*-dominated vegetation on western slopes of Kartala volcano, [-11.756295S +43.335561E], ca. 1900 m, on soil along path, 26-May-2008, *T.A.J. Hedderson 16784* (BOL); Mayotte [-12.852794S +45.153272E], 1880, *E.A. Marie, s.n.* (NY).

KENYA: Mount Kenya, Naromoru Track and Teleki Valley [-0.170974S +37.212336E], 3048m, 21-Jul-1963, *M.D. Gwynne s.n.* (NY); below top of Vuria, Taita Hills, degraded forest, 2200 m, [-3.42S, 38.3W], 02-May-1975, *I. Friis 2821* (NY); below top of Vuria, Taita Hills, degraded forest, 2200 m, [-3.42S, 38.3W], 02-May-1975, *I. Friis, 2831* (NY); Nyeri. Central. North Nyeri Distr., not far from the Sirimon Track, Mount Kenya, [-0.007688S +36.716254E], on the ground at the roots of *Erica* bushes., 2800m, 04-Apr-1975, *C.C. Townsend 75/676* (NY); Nyeri. Central. South Nyeri District, Aberdare Mts., just past the East Gate into the Aberdares National Park, on a sandy trackside bank, 2800 m, 08-Apr-1975, *C.C. Townsend 75/869* (NY); Aberdare National Park, ca. 13 km W of the Rohuruini Gate, [-0.391555S +36.744558E], open forest of *Podocarpus* prominent with large patches of bamboo and open, heavily grazed, grassy areas. on vertical rocky roadside bank, 2800 m, 06-Mar-1974, *G. Davidse 7068A* (NY); Aberdares, S. Kinangop, Sasumwa Dam, [-0.762064S +36.681289E], on rocks and banks beneath Ericaceae/bamboo, 2591 m, 28-Apr-1970, *B.F. Mathew 6147* (NY)

MADAGASCAR: Fianarantsoa. near Chutes du Namorona, W of Ranomafana, 40 km NE by E of Fianarantsoa, along moist S-facing road and hillsides, on wet bank, [ca. 1200 m.], [-21.25S 47.42E], 05-Nov-1972, *M.R. Crosby with C.A. Crosby 7135A* (NY); Fianarantsoa. ca. 23 km S of Ambositra near 293 km S of Tananarive marker, 25 km S by W of Ambositra, [-20.77S, 47.27E], woods and road banks, ca.1700 m, 06-Nov-1972, *M.R. Crosby with C.A. Crosby 9333*

(NY); Tamatave, 9 km along road from village of Andonabe toward Foulpointe, 13 km WSW of Foulpointe, on clay bank, [-17.73S 49.4E], 28-Oct-1972, *M.R. Crosby with C.A. Crosby 5470* (NY); Tananarive: Massif de l'Ankaratra, Reserve Forestiere de l'Ankaratra, 20 km E of Ambatolampy [-19.33S 47.33E], trees at roadside. on ground, 2060 m, 24-Oct-1972, *M.R. Crosby with C.A. Crosby 5374* (NY).

MALAWI: MULANJE. Mount Mulanje, Chinzama -15.89791S, 35.65501E, closed-canopy *Widdringtonia whytei* forest in bouldery granite ravine, on humus under trees, 2100 m, 10-Jun-2010, *T.A.J. Hedderson 17471* (BOL); ZOMBA. Zomba Plateau, [-15.348943S, 35.273503E], locally common on moist rocky slopes, ca. 1500 m, 05-Jun-1946, *L.J. Brass 16453* (NY).

MAURITIUS: NW corner of Mare aux Vacoas, pine plantation, terrestrial. ca. 549m, [-20.37S 57.48E], 04- Dec-1972, *M.R. Crosby with C.A. Crosby 5671* (NY); near Riviere du Poste bridge, between Grand Bassin & Petrin, [-20.423206S 57.471934E], on bare, rotting, damp, nearly vertical basaltic rock, ca. 660 m, 12-Mar-1974, *M.J.E. Coode 4441* (NY); Mascarene Islands, Sine loco, 01-Oct-1966, *G. Erdtman E03* (S).

RÉUNION: Sentier de la roche Ecrite above St. Denis, [-20.980056S 55.432364E], growing with *Sphagnum* on paths and banks in forested area, ~1200m, 25-Nov-1973, *M.J.E. Coode 4193* (NY); Basse Vallee above St. Philippe, [-21.351597S 55.700165E], forest with high rainfall, 23-Nov-1973, *M.J.E. Coode 4673 b* (NY); Arrt. du Vent, along trail to La Roche Ecrite, from vicinity of forest station (gite) at NW corner of Plaine des Chicots down to parking area at end of road CF-1bis (near Mamode Camp), 8–12 km S of St. Denis, [-20.93S, 55.45E], on bank, 26-Nov-1972, *M.R. Crosby with C.A. Crosby 7448* (NY); Arrt, Sous le Vent, along beginning of trail, at end of road CF-8, to Grand Bernard, 22 km SSW of St. Denis, [-21.05S, 55.4E], in moist gully, ca. 2150m, 22-Nov-1972, *M.R. Crosby with C.A. Crosby, 8286* (NY); Vent. just SE of summit of La Roche Ecrite, 15 km S of St. Denis [-21.00 S, 55.47E], steep SE-facing slope. on rocks, ca. 2270 m, 25-Nov-1972, *M.R. Crosby with C.A. Crosby 7429* (NY); Vent, near km10 marker on road CD-53, 25 km SE of St. Denis [-21.05S, 55.65E], on concrete road embankment, ca. 500 m, 23-Nov-1972, *M.R. Crosby with C.A. Crosby 8806* (NY); Saint-Paul, vicinity of Pic Maïbo (Maïdo), 22 km SSW of St. Denis [-21.05S, 55.4E], on rock, ca. 2200 m, 22-Nov-1972, *M.R. Crosby with C. A. Crosby 8419* (NY); just below Naidho, E of St. Paul, apparently fairly early colonizer of lava and poor soil, 2134 m, 22-Nov-1968, *C. Barclay 1369* (NY); Sentier de la Roche Ecrite, [-20.980056S 55.432364E], growing on the ground, 1219 m, 25-Nov-1970, *C. Barclay 2069* (NY); Commune St. Louis, Forêt des

Makes, along trail to summit -21.1635S, 55.4127E, low vegetation dominated by *Erica*, *Stoebe*, *Acacia* and *Hypericum*, on soil banks, abundant, ca. 2350 m, 29-Oct- 2018, *T.A.J. Hedderson 19304* (BOL); COMMUNE SAINT BENOIT: Forêt de Bélouve. Sentier de L'Ecole Normale to Grand Mare, just at turning around Grand Mare. Wet cloud forest with Acacia heterophylla and some planted Cryptomeria, 1440 m, 21.06096ºS 55.55217ºE, 19-Oct-2018, *T.A.J. Hedderson 19185-1* (BOL); COMMUNE LES AVIRONS: Le Tévelave. Along forest route to Maido. Steep slopes and soil banks in Erica-dominated vegetation, 1060 m, -21.20104ºS 55. 35708ºE, 23-Oct-2018, *Terry A.J. Hedderson 19208* (BOL); au SW du Piton Mare-à-Boue, [-21.159041S 55.576599E], 1600 m, 16-Dec-1972, *J.L. De Sloover 17.272* (GOET); au pied du Piton de la Grande Montée, près des sources Reihlac, [-21.164300S 55.589739E], 1600 m, 16-Dec-1973, *J.L. De Sloover 17.244* (GOET); entre Guillaume et le Grand Bénard, point de vue du Piton de Maido, [-21.069080S 55.387176E], 2180 m, 20-Dec-1973, *J.L. De Sloover 17.499* (GOET); entre Guillaume et le Grand Bénard, point de vue du Piton de Maido, [-21.069080S 55.387176E], 2180 m, 20-Dec-1973, *J.L. De Sloover 17.485* (GOET); Plaines des Makes, [-21.209455S 55.411663E], 950 m, 17-Dec-1973, *J.L. De Sloover 17.359* (GOET); Cilaos, Sentir du Piton des Neiges, [-21.115107S 55.487857E], 1950 m, 22-Dec-1973, *J.L. De Sloover 17.650* (GOET).

RWANDA: NYUNGWE NATIONAL PARK: Kavamba, -02.481312S 29.111400E, boggy vegetation on thin soils, with extensive bryophyte hummocks and small shrubs, in hummocks, 1950 m, 4-Oct-2012, *T.A.J. Hedderson 18186* (BOL); Foret de Gishwati, [-01.820555S 29.368402E] along the route Gisenyi–Kibuye, 30 km à Gisenyi, 2050 m, 06-Feb-1972, *J.L. De Sloover 13.346* (GOET); Gisenyi, Vallée de la Sebeya, sous le poste minier de Gikungu, 2000 m, 30-Jul-1974, *J.L. De Sloover 18.704* (GOET); Massif des Virunga, Muhavura, versant E du volcan, [-01.387779S 29.591776E], 600 m, 20-Feb-1972, *J.L. De Sloover 13.603* (GOET); Cyangugu, Foret de Rugege, E du Mont Yahahi, 2450 m, 04-Mar-1972, *J.L. De Sloover 13.784* (GOET); Cyangugu, Foret de Rugege, 1900 m, 01-Sept-1974, *J.L. De Sloover 19.120* (GOET); Cyangugu, Foret de Rugege, km 91 de la route Butare – Cyangugu, [-2.483428S 28.897210E] 2400m, 14- Aug-1974, *J.L. De Sloover 18.904* (GOET).

SOUTH AFRICA: TRANSVAAL, Pelgrimsrus, God's Window, [-24.876570S 30.888980E], 2430DD, *Pinus* plantation on red lateritic soil along road embankment, semi shade, 05-Aug-1970, *B. De Winter 9297a* (NY); 13 km from Belfast to Dullstroom, [-25.604266S 30.068625E], 2530 CA, 1977, *C.H. Stirton 6721* (NY); upper slopes of Mount Lejuma, Soutpansberg, on Lajuma Ranch, [-23.023831S 29.434434E], 2329 AB, 1524 m, 1977, *R.E.*

Magill 3707 (NY); Houtbosberg, De Hoek, 2330 CC, 1420 m, 16-Nov-1977, *F. von Breitenbach 4* (NY); Nkandla forest, [-28.732116S 31.119115E], 2831 CA, on road embankment, 07-Oct-1972, *L. Smook 90 1*(NY); Lown Bush, Maritzburg, Jan-1916, *T.R. Sim 8640* (NY); Magaliesberg, Baviaanskranz Farm, in Tiger Kloof, [-25.992809S 27.554360E], 2527 CA., above stream on cliff with dense trees and shrubs, xeric grassland, 1600 m, 22-Dec-1976, *R.E. Magill 3027* (NY); KwaZulu-Natal, Knol, Hilton Road, 2030 CB., Feb-1917, *T.R. Sim 8639* (NY); E of Barberton, Fourieskraal area SE of Three Sisters, 2531 CB., patches of indigenous forest in base of main valley, 610 m, 09-Apr-1977, *E.G.H. Oliver 7151* (NY); FREE STATE, Platberg near Harrismith, [-28.262515S 29.182302E], 2829 AC, 21-Nov-1978, *M.L. Jacobsz 5012* (NY); Nelson's Kop, [-28.232948S 29.448611E], s.d., *H.A. Wager 11558 s.n.* (NY); Cape of Good Hope, *s.d., Miller, s.n.* (NY);

SWAZILAND: Hhohho, W of Mbabane, lower slopes of Mt. Kelley, 2631 AC,[-26.322260S 31.101613E], 1433 m, 1977, *R.E. Magill 3438* (NY); Hhohho NE of Mbabane, on hill with Mahunga Marker, 2631 AC, 1372 m, 1977, *R.E. Magill 3450* (NY); Mbabane, 2631 AC, 1128 m, 1977, *E.S. Kemp 1117* (NY); Hhohho NE of Mbabane, on hill with Mahunga Marker, 2631 AC, [-26.132139S 31.356846E], open grassland, 1372 m, 10-Mar-1977, *R.E. Magill 3461* (NY); Nord-Swasiland, nahe der Havelock-Bergwerke [= Havelock Mine, Bulembi, -25.954419S 31.136137E], Schieferfelsen, 20-Jul-1955, *B. J. Cholnoky 748* (NY).

TANZANIA: Moshi, Kilimanjaro Mountains, Marangu Route, Maundi Crater above Mandara Hut, -3.166605S, 37.266728E, 2780–2850 m, 15-Jun-1988, *T. Pócs, R. Ochyra & H. Bednarek-Ochyra, 88124/C* (GOET); Morogoro, South Uluguru Mountains, Nyandira, by the road to the village of Chenzema, -7.083346S, 37.550028E, 1450 m, 07-Jun-1988, *T. Pócs, R. Ochyra & H. Bednarek-Ochyra 88105/R* (GOET), S-Uluguru Mts., Lukwangule Plateau, [-7.128169S, 37.619363E], neutrophilous Cyperaceae bogs., 2400 m, 12-Nov-1972, *T. Pócs 6826/A* (NY); Morogoro. Uluguru Mountains, along "Landrover" road below Bondwa Peak, 9 km S by E of Morogoro, [-6.900011S, 37.670041E], 2150 m, on wood bark, 13-Dec-1972, *M.R. Crosby with C.A. Crosby & T. Pócs 13181* (NY).

DEMOCRATIC REPUBLIC OF CONGO (ZAIRE): KIVU, Kahuzi Massif, road Bukavu–Walikale, 2300 m, 22-Dec-1971, *J.L. De Sloover 12543* (GOET); Kahuzi Massif, road Bukavu–Walikale, 2180 m, 22-Dec-1971, *J.L. De Sloover 12558* (GOET); Kahuzi Massif, road to Kahuzi, [-2.284251S, 28.692422E], 2765 m, 25-Dec-1971, *J.L. De Sloover 12658* (GOET); Kahuzi Massif, Musisi, 2180 m, 22-Dec-1971, *J.L. De Sloover 12560* (GOET); Kahuzi Massif,

road Bukavu–Walikale, 2280 m, 22-Dec-1971, *J.L. De Sloover 12592* (GOET); road to Kahuzi, Mukaba, [-2.284251S, 28.692422E], 2250 m, 05-Jan-1972, *J.L. De Sloover 12.880* (GOET)

ZIMBABWE: Inyanga Distr., Rhodesia, summit of Inyangani Mt. [-18.295562S, 32.841996E], plateau along top of mountain, short, tussocky grasses, scattered rocky outcrops. common moss on rocks near top, 29-Apr-1967; *J.E. Rushworth 959* (NY); Rhodesia, Inyanga, on ground in wet hollow, [ca. 1829 m], Jul 1918, *E.A. Nobbs 1360* (NY); S. Rhodesia, Matopos, [-20.557553S, 28.511570E], 1524m, 1920, *T.R. Sim 8844* (NY).

5. *Polytrichum commune* Hedw., *Sp. Musc. Frond*. 88. 1801. Type citation: *"Vulgaris per totam Europam".* Type Specimen: *"Polytrichum commune b. Mhlbg. 251. c. Commers. a. Linn ipso interum commune β interum polytr. appressum"* [Europe?] *s.d.*, *s. coll*., *s.n*. (G00040355!, Hb–Hedwig-Schwägrichen, lectotype designated here, see Chapter 02).

= *Polytrichum yukonense* Cardot & Thér., Proc. Wash. Sci. 4: 329. 22 f. 1a. 1902 ≡ *Polytrichum commune* Hedw. var. *yukonense* (Cardot & Thér.) Frye, in Grout, Moss Fl. N. Amer. 1:125, 1937. Type citation: [USA, Alaska] 'Yukon River (W.H. Dall, in 1867)." Type specimen: [Alaska] "Yukon River, 1899, *W.H. Dall 1867"* (NY00465505!); *syn. nov.*

Figures 2.3, 4.19, 4.20 & 4.21

Stem erect, rigid, unbranched, often 2–30(–45) cm tall. ***Leaves*** densely aggregated, sharply divided into broad sheathing base and narrower, lamellate limb, when moist widely spreading, often squarrose-recurved, when dry appressed to the stems at base with somewhat spreading tips above, linear-lanceolate, gradually narrowing from the base of the limb upwards to a sharp acumen, margin densely and sharply serrate from the limb base to the apex; leaf sheath transparent and glistening when fresh, median cells of leaf bases linear-rectangular, 75–125 × 10–15 µm ; costa excurrent as short brown, entire or toothed point, in young leaves often present with long, slender, hyaline aristae; number of ventral lamellae 60–70, 4–6(–9) cells high; lamellar end-cells broader than the others, deeply grooved or U-shaped, in side view strongly crenate with a papilla-like thickenings and deep U-shaped groove between. ***Perichaetial leaves*** morphologically distinct from the stem leaves, with a long sheathing base. ***Perigonial leaves*** with broad, obovate sheathing bases and short limbs. ***Capsule*** 4-angled, shortly rectangular, 3.5–4.0 × 2–3 mm; apophysis very distinct, discoid, narrowly constricted above; exothecial cells composed of conical papillae with slit-like apertures; operculum with a short rostellate beak; peristome teeth ca. 0.4 mm high, obtuse, pale, basal membrane low, brownish; spores 7–9 µm. ***Seta*** 4(–5) – 7(–8) mm long, reddish-brown. ***Calyptra*** golden-yellow, fibrillose and completely covering the capsule, 13–15 mm long. Spores ca. 8 µm. with "Christmas tree-like" projections.

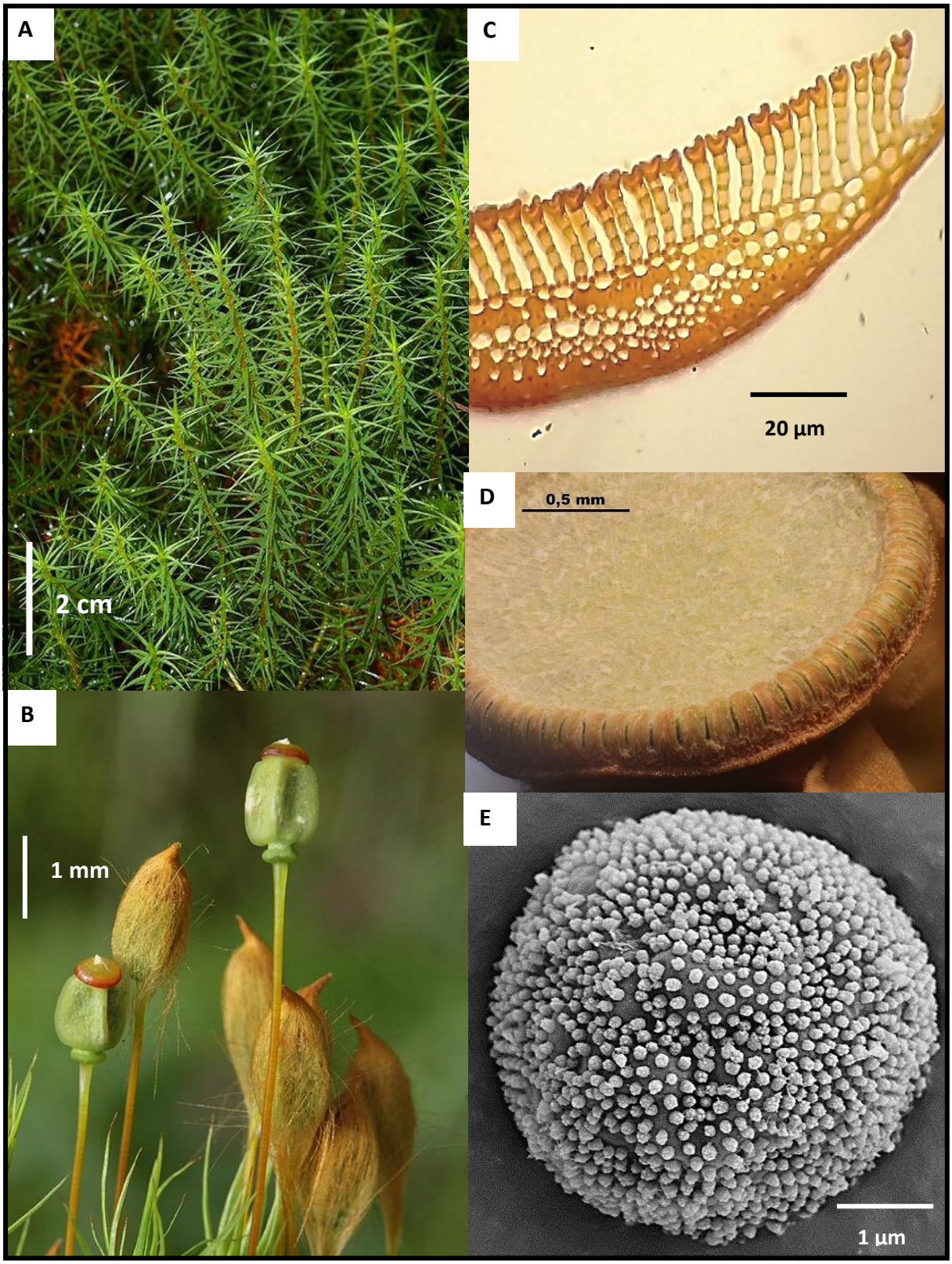

Figure 4.19: *Polytrichum commune* **A.** Habit (with characteristic "star-like" appearance; **B.** Capsules with golden, yellow, hairy calyptra completely covering the immature capsule, strictly four-angled capsules with the prominent constriction (apophysis) at the base of the capsule; **C**. A leaf transverse section showing the deeply-grooved (U-shaped) lamellar end-cells [A leaf cross-section taken from the historical collection of *P. commune* of Carl Linnaeus housed at LINN (LINN 1263.1)]; **D**. The membranous epiphragm with intact peristome teeth; **E**. A SEM photo of a spore of *P. commune* showing the "Christmas-tree like" projections on the exine [Photo courtesy **A, C & E**: *I. U. Kariyawasam*, **B & D** *Hermann Schachner* (accessed via https://commons.wikimedia.org)

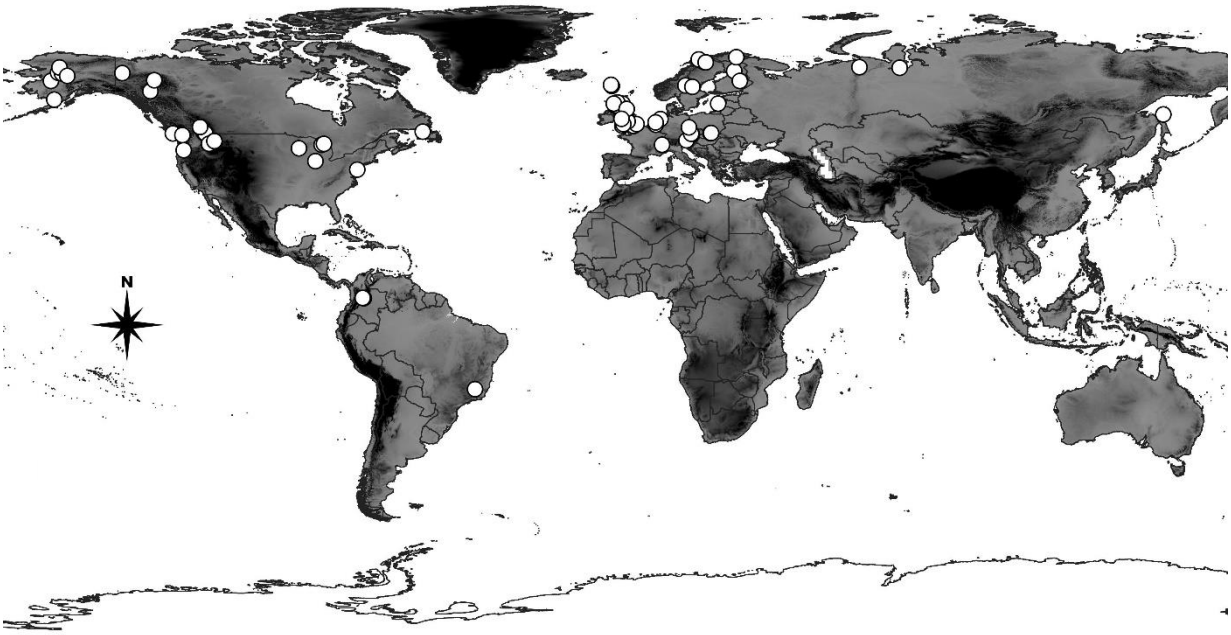

Figure 4. 20: Distribution of *Polytrichum commune* based on the confirmed herbarium records.

Ecology: *Polytrichum commune*, the Common Hair-cap Moss [which Dillenius (1741) called the "Great Goldilocks"], is characteristic of wet, boggy places. This plant is strikingly tall (up to ca. 45 cm) and is commonly found in a broad spectrum of damp, acidic habitats such as damp meadows, stream banks, lake margins, *Sphagnum* bogs and damp hollows in alpine tundra (Long, 1985; Atherton & al., 2010). Ignatov & Smith Merrill (1995) reported that, " in the boral zone, *P. commune* often dominates in forest and peatland communities. Avoiding xeric lowlands, *P. commune* is not rare in rather dry territories in high mountains, like that in Mongolia, where it is common throughout the country. […..] it often grows in conifer forests on litter or among moss carpets at middle elevations, forming pure mats in relatively wet places, and is also common on the edges of bogs among Sphagnum. On grazing meadows it occurs on ant hummocks. It occasionally grows on rock outcrops (on humus in crevices), and on rotten stumps and logs, and in the high mountains, it grows among dwarf *Betula* shrubs, and among big rocks in rock fields.

In South America it has been reported as a high-altitude species. In Colombia, it is also reported in the higher altitudes ranging from ca. 3300–4000 m (Aponte & Uribe, 2017) and commonly found in Páramos, near waterfalls, waterlogged areas of grasslands and on sandy soil rich with litter. In Brazil, this is commonly found on soil, along trails on banks at the margins of low, submontane and montane forests and high altitudes of the Amazonian basin, Mata Atlântica, Cerrado, Caatinga and Pampa Biomes, between ca. 600–2,000m altitudinal range (Peralta & Yano, 2010).

Atherton & al. (2010) report that " [….]this taxon is also frequent through western, lowland Britain in wet woodlands, bogs, ditches, by lake margins, on heaths etc. In the drier south-east it is perhaps most frequent in old gravel pits and sand pits by pools under willow and birch scrub". However, based on my field experience in Scotland this species can have scattered populations on drier gravelly paths with dominant populations of *P. perigoniale*. Since *P. perigoniale* is poorly documented in the Britain and elsewhere in the Europe it requires a proper revision of both taxa to study their geographic distribution.

In general, for non-bryologists, these two taxa (*P. commune* and *P. perigoniale*) can be easily identified in the field as follows: *P. commune,* which grows in wet, boggy places usually possesses longer stems and its leaves are widely spaced so that their shiny, whitish to pale yellow bases are noticeably exposed and they form the characteristic "star-like" appearance (Figure 4.19 A), whereas *P. perigoniale,* which grows on drier, gravelly paths, has usually shorter plants with more crowded leaves appressed to the stem (Figures 4.29 & 4.30), and possesses long perichaetial leaves.

Sarafis (1971) has summarised five major growth forms of *Polytrichum commune s.l* (including Australasian *P. perigoniale* in drier habitats). In his phenological account he has described *P. commune s.l.* as having five major types of growth patterns, **(i) *Short-loose sward***s: This form is found in highly insolated stations, on exposed clay soils and individuals with shorter internodes. Plants grow up to 2–3 cm high [This is probably the *P. perigoniale* form he has mentioned under *P. commune*]. **(ii) *Short-dense swards***: This form is quite common in *Sphagnum* bogs and on clay banks, it has been reported that plants form dense mats and thus the distance between plants are almost zero or negligible. The plants grow up to 10 mm high, **(iii) *Tall-dense swards***: This is the common most and easily recognisable growth form of *P. commune s. str*. Plants grow up to 30cm high. It has been reported that the distance between plants is between zero and 6 cm. Sarafis (1971) further mentioned that this growth

form is found on moist rich soils on forest floors. **(iv) *Cushion form*:** Sarafis (1971) reported that this form occurs mainly in "on bogs or along runnels and streams traversing moors and peatlands". He further states [..] "on the *Sphagnum* bogs, cushions occur free on the bog surface and later become contiguous as the forest margin is approached and finally grade over completely into the tall loose sward". Finally, Sarafis (1971) mentioned a fifth growth form; **(v) *in pools*:** This form of *P. commune* in pools fed by snow melts or in peat-cutting ditches. The first four types of growth patterns in *P. commune* are presented in Figure 4.21.

In his phenological account Sarafis (1971) further mentioned that *P. commune* exhibits four major ways of controlling water loss in dominant gametophytes namely (i) ***at community level***: by gregariousness and form of interface; (ii) ***at individual plant level***: by density of leaves, their size and height of the plant; (iii) ***at organ level***: movements of leaves and their inrolling and (iv) ***at molecular level:*** epicuticular wax on the surface. These have been extensively discussed in the last two decades in various physiological studies in bryophytes which chose *P. commune* as a model plant because of its robust vasculature in the stem and the characteristic leaf lamellae acting as pseudo-mesophyll (Proctor, 1979; Proctor, 2005; Proctor & al. 2007; Brodribb & al., 2020)

Figure 4.21: Some common growth forms of *P. commune* described by Safaris (1971): **(A).** Short-loose swards on exposed clay soil; **(B).** Short-dense swards; **(C).** Tall-dense swards in an open ditch; **(D).** Cushion form on a *Sphagnum* bog. [Photos courtesy: Photos A &B are accessed via https://commons.wikimedia.org, photos C & D were taken by I. U. Kariyawasam from Berwickshire (vc81), Scotland]

Taxonomic Notes & Nomenclatural Notes:

Nomenclatural Note 01: *Polytrichum yukonense* Cardot & Thér [≡ *Polytrichum commune* Hedw. var. *yukonense* (Cardot & Thér.)]

A lectotype has selected (Kariyawasam & al., 2021) for *P. commune* from this study (Chapter 02) which resolves a long-term misconception in identifying *P. commune* and *P.*

perigoniale in North America, Africa, Southeast Asia and elsewhere in Europe. *Polytrichum yukonense* Cardot & Thér [≡ *Polytrichum commune* Hedw. var. *yukonense* (Cardot & Thér.)] is newly synonymised from this study as a specimen identified as *Polytrichum commune* Hedw. var. *yukonense* collected near the type locality has been sequenced and its molecular study results in confirmation of its identity with *P. commune s. str*.

Nomenclatural Note 02: Status of *Polytrichum uliginosum* (Wallr.) Schreibl.

Scribel (1991) has described *Polytrichum uliginosum* (Wallr.) Schreibl, by raising the taxonomic status of *Polytrichum commune* Hedw. var. *uliginosum* Wallr., into a species based on his cultivation experiments. However, the morphological characters he listed under *P. uliginosum* based on his cultivation experiments are now fallen into the morphological circumscription of *P. commune* Hedw. after lectotypification of the taxon based on Hedwig's specimen. However, we could not find a type specimen for *P. uliginosum* or *P. commune* var. *uliginosum* to synonymise it under *P. commune s. str*. Hence, it has been treated under doubtful taxa in the present study (see below).

Taxonomic Note 01: **Morphological distinctions between *P. commune* & *P. perigoniale* and *P. commune* & *P. jensenii***.

Morphological distinctions of *P. commune s. str*. and morphologically related (allied) taxa in the *Polytrichum* sect. *Polytrichum* are discussed under *P. perigoniale* (Table 4.2) and under *P. jensenii* in this chapter. *P. jensenii* is strictly a Northern taxon, often reduced in size compared to *P. commune s. str*. The leaves often short and flexuose, easily breakable (somewhat brittle) and possess entire or subentire leaves with relatively short marginal teeth (if present). The lamellar end-cells are somewhat shallowly-grooved in *P. jensenii* compared to *P. commune*.

Taxonomic Note 02: Some useful morphological characters to distinguish *P. commune* from other related species with sterile material.

P. commune can easily be confused with *P. formosum* if not carefully observed in the field. Both species possess strictly four angled capsules, however *P. commune* (and all members of sect. *Polytrichum*) contains a very strongly constricted apophysis at the base of the capsule. In addition, in a fully developed leaf of *P. formosum* is a V-shaped (wedge-shaped) ridge formed by the abaxial surface of the costa whereas the abaxial surface of a *P. commune*

leaf is rounded. Sterile plants of *P. juniperinum* are sometimes confused with *P. commune* in the field; however, the distinctive red-brown leaf tip and strongly inrolled/ infolded leaf margins of *P. juniperinum* can easily distinguished it from *P. commune* in the field.

In Colombia, especially in Páramos, *P. commune* is usually found as mixed populations with *P. ericoides* and *P. juniperinum*. The leaves of *P. commune* is always erect in the field and leaves are distantly arranged and exhibits the characteristic "star-like" appearance, whereas in *P. ericoides* leaves are erect and slightly involute and the leaves are comparatively smaller and rather appressed to the stem. In leaf transverse section, lamellar end-cells of *P. commune* are deeply grooved (U-shaped) and cells towards the shoulders exhibit slightly pyriform shaped cells. Whereas in a leaf transverse section of *P. ericoides* all lamellar end-cells are predominantly U-shaped and never posses pyriform end-cells towards the shoulders and also the lamellae are comparatively taller (8 –12 cells) than the lamellae of *P. commune.*

Doubtful taxa

The following infraspecific taxa have not been lectotypified, and thus, until a lectotype is designated for each taxon, they cannot be accurately treated under the correct species in Sect. *Polytrichum.*

1. *Polytrichum commune* Hedw. var. *campestre* Wallr., Fl. Crypt. Germ. 1: 201. 1831. Type citation: [Europe] "in ericetis siccis eorumque novalibus Abiete consitis gregatim". Type specimen: not seen.

2. *Polytrichum commune* Hedw. var. *montanum* Wallr., Fl. Crypt. Germ. 1: 202. 1831. Type citation: [Europe] "in montium editiorum planitiebus apricis et udis." Type specimen: not seen.

3. *Polytrichum commune* Hedw. var. *uliginosum* Wallr., Fl. Crypt. Germ. 1: 202. 1831. ≡ *Polytrichum uliginosum* (Wallr.) Schreibl, Carinthia II 101: 485. 1991. Type citation: [Europe] "in caespitosis uliginosis, alnetis potissimum obumbrates sphagnetisque regionum subalpestrium nemusculi s. pineti instar horret." Type specimen: not seen.

4. *Polytrichum commune* Hedw. var. *fastigiatum* Wilson, Bryol. Brit. 212. 1855. Type citation: [Scotland, Stirlingshire] "on the drier parts of moors in mountainous districts. Near Airth, Scotland, Mr T. Lyle." Type specimen: not seen.

5. *Polytrichum commune* Hedw. var. *ehrenbergii* Lorentz, Abh. Königl. Akad. Wiss. Berlin 1867: 49. 14 f. 11–16, 15 f. 1–2. 1868. Type citation: "Syria, inter Sanim et Sachle, [1820–1826, *Ehrenberg s.n.*] Junio (ohne Nummer)." Type specimen: not seen.

6. *Polytrichum commune* Hedw. var. *pygmaeum* Lindb., Not. Sällsk. Fauna Fl. Fenn. Förh. 9: 118. 1868. Type citation: [Finland] "Locis spongiosis minusque humidis et in sphagnetis, inter *Sphagnum tenellum*, prope urbem Helsingfors Fenniae viget hæc varietas insignis." Type specimen: not seen.

7. *Polytrichum commune* Hedw. subsp. *cubicum* Lindb., Not. Sällsk. Fauna Fl. Fenn. Förh. 9: 106. 117. 1868. ≡ *Polytrichum commune* Hedw. var. *cubicum* (Lindb.) Habeeb, Kongel. Norske Vidensk. Selsk. Skr. (Trondheim) 1913(1): 60, 61. 1914. Type citation: [Europe] not specified. Type specimen: (Type: isosyntype E00007674 !).

Although the isosyntype has been seen this specimen needs a further investigation as it seems to be an extreme morphological form of *P. commune s.l.* with shorter leaves with distantly serrate and shallowly-grooved, lamellar end-cells. Hence this has been included as a doubtful taxon.

8. *Polytrichum commune* Hedw. var. *maximovickizii* Lindb., Contr. Fl. Crypt. As. 224. 1872. Type citation: [Japan] "Hab. Ad Nambu ins. Nippon, planta mascula, 1865 [*C. Maximowicz s.n.*]." Type specimen: not seen.

9. *Polytrichum commune* Hedw. var. *brevifolium* Geh., Flora 62: 477. 1879. Type citation: [Russia, W. Siberia] "Lepsa (15 Mai)" [15 May 1876, *F. Kurtz s.n.*]. Type specimen: not seen.

10. *Polytrichum commune* Hedw. var. *canadense* Kindb., Cat. Canad. Pl., Musci 156. 1892. Type citation: [Canada] "On damp earth at Rustico Bay and Royalty Junction, Prince Edward Island; woods, Mount Albert, Gaspé Co., Que.; McKay's woods, Ottawa, also at Belleville, Ont.; damp woods, Lake Nepigon; abundant on earth in the railway cuttings above the trestle at Albert Cañon Station, Selkirk Mountains, B.C., May 29th, 1890. (Macoun)." Type specimens (syntypes): not seen.

Excluded taxa

1. *Polytrichum commune* Hedw. var. *chalubinskii* Zmuda, Kosmos (Lvov) 37: 667. 1912. ≡ *Polytrichum commune* fo. *chalubinskii* (Zmuda) Podp., Consp. Musc. Eur. 68. 1954. Type specimen: [Poland] Tatry zacodnie: Dolina Chocholowska, las 22 vii 1912, A. Zmuda in Bryotheca Polonica 131 (PC0131420, isotype!).

   The isotype specimen has been studied online under high resolution; it belongs to *Polytrichum juniperinum* Hedw. with infolded leaf margins.

2. *Polytrichum commune* Hedw. var. *auranticum* (Hoppe ex Brid.) Wahlenb., Fl. Suec. 2: 737. 1826. ≡ *Polytrichum aurantiacum* Hoppe ex Brid., J. Bot. (Schrader) 1800(1): 286. 1801. Type citation: [Europe] "in uliginosis". Type specimen: not seen.

   = *Polytrichum longisetum* Sw. ex Brid. fide Crum & Anderson (1981).

3. *Polytrichum commune* Hedw. var. *attenuatum* (Menzies ex Brid.) Hook. & Taylor, Muscol. Brit. 26. 1818. ≡ *Polytrichum attenuatum* Menzies [Trans. Linn. Soc. London 4: 72, 1798] ex Brid., J. Bot. (Schrader) 1800(1): 286. 1801. Type citation: "ex ora occidentali Americae septentrionalis". Type specimen: [Canada or USA, Alaska] "Northwest coast of America A.M." [A. Menzies], Holotype E00011815!

   This holotype contains three stems (left) of *Polytrichum formosum* Hedw. and two stems of *Polytrichum ohioense* Renauld & Cardot.

Protologues and type citations could not be accessed for the following taxa during the study period

1. *Polytrichum commune* Hedw. var. *brevirostre* Papp, Ann. Sci. Univ. Jassy 14: 376. 1926. Type citation: ? Type specimen: not seen.

2. *Polytrichum commune* Hedw. var. *roemeri* Warnst., Krypt.-Fl. Brandenburg, Laubm. 1105. 1906. Type citation: ? Type specimen: not seen.

Additional Specimens Observed

AUSTRIA: Niederösterreich, Waldviertel, ca. 1 km nördlich Brand, Bummer Moos, Zerntralteil, ca. 526 m, 48.871944°N 15.020277°E [48. 87186 N, 15.02035 E], 01-Oct-2011, *C. Schröck 17016* (W); Salzburg, Pongau, Schladminger Tauern, Ortsgebiet von Obertauern, Hundsfeld, SE-Teil, ca. 1760–1800 m, 47.25301 N, 13.56364 E [47.253055°N 13.563611°E] 28-Jul-2005, C. *Schröck 14034* (W).

BRAZIL, MINAS GERAIS: Gandarela [-20.098294S -43.661584W], recobrindo a turfeira, em brandes extesnoes, grande comunidade lunuogenea, 17-Jul-1972, *L.E. de M. Filho s.n* (NY).

CANADA, BRITISH COLUMBIA: Blazed Creek Lake, headwater of Blazed Creek which drains into Summit Creek and into Creston floodplain, Selkirk Mts. [+49.184804N -116.931399W], 09-Aug-1977, *B.C. Tan 77-1455* (NY); Corn Creek marshy area unit, Creston Valley Wildlife Center, Creston, Hwy 3 [+49.126733N -116.637080W], 10-Aug-1977, *B.C. Tan 77-1465* (NY, UBC); Kennedy Lake, NE end, west central Vancouver Island [+49.128035N -125.427137W], 08-Aug-1980, *D. Gagnon 80-53-16* (NY); Mount Revelstoke National Park, Echo Lake Vicinity, 5 mi S of Revelstoke, by Akolkolex River, E of Upper Arrow Lake [+50.857352N -118.066093W], 01-Aug-1978, *B.C. Tan 78-1272* (NY).

CANADA, NEWFOUNDLAND: Gros Morne Natl Park, Gros Morne mountain; slope of gully, [+49.6 N -57.8W], 03-Aug-1987, *W.B. Schofield 89416* (UBC).

CANADA, YUKON TERITORY: Trout Lake, bank, drainage creek; [+60.553582N -131.497142W], 20-Jul-1965, *J. Lambert plot-95a* (NY, UBC); northern Ogilvie Mtns., Nahoni range, S-facing slope in saddle of limestone mtns., just below shale barren [65.448400N -139.093169W], 1158 m, 16-Jul-1975, *D.H. Vitt 13251* (UBC); Hess Mountains, Keele Peak area, at NE end of Keele Lake, on S facing slope [63.523101N -130.452849W], 07-Jul-1976, *D.H. Vitt 15900* (UBC).

COLOMBIA: Cundinamarca, Macizo de Bogota, vertiente oriental, Paramo de Chingaza, altos cerca de La Laguna [04.519888N, -73.750301W], 03-Jan-1969, *J. Cuatrecasas 26977* (UBC); Bogota, Region de Monserrate, El Granizo, [4.604996N -74.055339W]3100 m, 13-Apr-1980, *S. Zuluaga 181* (GOET).

CZECH REPUBLIC: Distr. Třeboň, Stará Hlína, zrašelinělý SV okraj rybníka Starý Vdovec, [49.034919N 14.832452E], 430 m, 08-Apr-2000, *J. Ernestová s.n.* (W); Distr. Děčin, [50.772384N 14.214255E], 15-Sept-1988, *W.R. Buck 16232* (NY).

DENMARK, FAROE ISLANDS: Sydero: Trangisvaag [Trongisvagur], [61.560490N -06.847022W], 12-May-1896, *C. Jensen s.n.* (UBC); Nolsoy, central part [61.980111N -06.646758W], 02-Jun-1985, *J. Lewinsky 4990* (UBC); Eysturoy, along Kviggjara w.Oyndarfjordur, [62.275891N -06.854663W], 21-Jun-1985, *J. Lewinsky 4457* (UBC).

FINLAND: Ostrobottnia Borealis, Ranua, Kuopasjarvi district n. of the church of Ranua, in a paludified shore forest near Paavonaho farm [66.019668N 26.472407E], 06-Aug-1943, *A.V. Auer s.n* (UBC); Feuchter nadelwald auf dem grossen Berg von Kevo bez. Utsjoki, Lappland [69.753563N 27.047638E], 19-Aug-1971, *A.V. Hübschmann 1114*? (UBC); Sb. Nilsia, Rahasmaki, Syvarinranta, Roninpuro, [63.323454N 28.003460E], 09-Sept-1975, *M. Haapasari, R. Fagersten 1034* (UBC).

GREAT BRITAIN, ENGLAND: North Devon (vc 4), Lynmouth, in woods near Watersmeet, bogs, [51.222896N -03.799560W], *s.d., C.D. Pigott s.n.* (E); Cheshire (vc 58), Oakmere Woods [53.228750N -02.638411W], on small floating raft of peat, SJ 573 685, 01-Jan-1965, *H.J.B. Birks s.n.* (E); Surrey (vc 17), Thursley Common [51.154011N -00.702704W], tufts in wet heath, *s.d., N. Wace s.n.* (E); South Essex (vc 18), Epping Forest, [51.644876N 00.050889E], 15- Apr-1947, *Chamberlain, P.J. 369* (E); Worcestershire (vc 37), Wyre Forest [52.377955N -02.364531W], *Sorbus* tree area, *s.d, J.M Holmes s.n.* (E); Perthshire, Ben Larenbro, kärr, 800 m.ö.h., 18-Jun-1950, *C.A. Torén s.n* (LD).

GREAT BRITAIN, SCOTLAND: Beinn na h Uamha, N. slopes, Morvern [+56.610772N -005.798577W], 10-Jul-1978, *W.B. Schofield 69347* (UBC); lower portion of Gleann Dubh, Morvern [+56.585359N -005.747142W], 06-Jul-1978, *W.B. Schofield 68954* (UBC); Westerness (vc 97), Ariundle Wood Nature Reserve, Strontian, Sunart ,[+56.717083N -005.547692W], 09-Jul-1978, *W.B. Schofield 69237* (UBC); Easterness (vc 96), Loch Morlich, [+57.160035N -003.719664W], 16-Jul-2000, *J. Davidson s.n.* (UBC); East Ross, (vc 106), near Loch Coireag nam Mang, Moruisg, open montane valley, at margin of acid flush, 57.510579N -05.140082W, *D.G. Long 42819* (E); West Ross (vc 105), Slattadale Forest, north of Slattadale, margin of conifer plantation, on side of track, 57.692493N -05.544921W, *D.G. Long 42808* (E); North Ebudes (vc 104), Skye, Camasunary, [57.194962N -06.114736W], marshy pasture, 23-Jun-1967, *H.J.B. Birks s.n.* (E); Selkirkshire (vc 79), above Over Phawhope, Ettrick,

55.358194N -03.290719W, conifer plantation, on slope by track, 410 m, 30-Aug-2015, *D. G. Long 44024* (E); Berwickshire (vc 81), near Spottiswoode Loch, 55.736803N -02.616609W, ca. 220 m, in ditch in *Betula* woodland, 25-Dec-2016, *I.U. Kariyawasam IKAR 145* (E); Berwickshire (vc 81), near Eastside, Spottiswoode, [55.741804N -02.622740W], ca. 225 m, in ditch by ride in conifer plantation, 25-Dec-2016, *I.U. Kariyawasam IKAR 146* (E).

GREAT BRITAIN, WALES: Glamorganshire (vc 41), Mynydd Maendy, near Ustrad Rhondda, on ground beneath roadside cliff, 51.643776N -03.533316W, 27-Jul-1966, *S.G. Harrison s.n.* (LD); Snowdon, Pen-y-Pass [53.080517N -04.020746W], on slopes with *Nardia* and *Galium*, 24-Jul-1964, *E. Nyholm 25/64* (LD); Snowdon, Pen-y-Pass, on moist, wet ground with *Nardus* and *Galium saxatilis*, 24-Jul-1964, *E. Nyholm 26/64* (LD).

JAPAN, HOKKAIDO: ca. 50 m, 28-Jun-2017, *T. Katagiri 4271* (NICH).

LATVIA: Ventspils District, Moricsala Island Nature Reserve, SW part, 57.191667N, 22.134167E, mixed mature ancient woodland, in hollow from upturned tree roots, 20 m, 5-Aug-2011, *D.G. Long 41221* (E).

NETHERLANDS: Groningen, Kloosterveen [52.997319N 06.518104E], 20-March 2006, *R. van der Valk 2006-10* (L [NL]); Friesland, 01-Sept-1965, *H.C. Wesseling s.n.* (L [NL]); Noord-Brabant [51.482583N 05.232269E], 22-Jul-1930, *D.E. Meijer s.n.* (L [NL]); Noord-Brabant, 27-Apr-1957, *A.J. Luitingh 2 59-4-6* (L [NL]); Utrecht, Heide bij de Bilt [52.110785N 05.202957E], 01-May- 1841 to 31-May-1841, *C.M. Sande Lacoste s.n.* (L [NL]).

RUSSIA: West Siberia, Yamal Peninsula, delta of Ob River, vicinity of Sjunyaj-Sale Settlement [66.896546N 71.252162E], 05-Aug-1966, *I.V. Czernyadjeva s.n.* (UBC); Sakhalin Island, Noglikskyi Region, along the Tym River about 20 km south of the town of Nogliki, in the mixed conifer forest around the campsite area [51.649313N 142.967983E], 02-Aug-2003, *J.A. Harpel 32567* (UBC); Sakhalin Island, Okhinskyi Region, Shmidta Peninsula, Tayezhnoye Lake, about 3-4 km west of the Forestry Camp, and about 70 km north of the town of Oxa [54.259838N 142.594297E], 10-Aug-2004, *J.A. Harpel 32786* (UBC); European Russia, ca. 100 m, [67.07N 60.36E], 27-Jul-2009, *O. Ivantov & D. Donskoff 09-202* (E).

SLOVAKIA Slovakia bor., montes Vsoke Tatry: ad lacum Velke Hincovo pleso in comvalle Mengusovska dolina, in terra humida, [49.176003N 20.060481E], 05-Sept-1973, *H. Zemanova 165* (UBC); Prešov Region, Biele Pleso, mountain heath, 1782 m, [49.22676°N 20.22186°E], 25-May-2016, *K. Hassel s.n.* (NTNU-TRH).

SWEDEN Norrbottens lan Lappland. lake Tornetrask area: SE of Laktatjakka along Laktatjakka stream, mostly about 2 km. w. of Laktatjakkastugan [68.419797N 18.361353E], 23-Aug-1980, *D.H. Vitt 26350* (UBC); Harjedalen, Linsell, S of Lofsdalen, S of Vastvallen [62.109516N 13.275296E], 25-Jun-2007, *L. Hedenas s.n*. (UBC); Vastergotland, Utvangstorp sn., zwischen Kryrkekvarn und Amurliden [58.030277N 13.873375E], 22-Aug-1929, *T.G. Halle s.n.* (UBC); Halsingland, Los, Lakes Takmyrtjarnarna, N of L. Malungen, [61.732798N 15.165477E], in a coniferous forest, 275–285 m, 23-Jun-2008, *L. Hedenas s.n*. (UBC); Lappland, Torneträsk Region, Abisko River near Abisko, 68.356944N 18.769722E, *Betula* woodland, on peaty side of footpath, 384 m, 16-Jul-2010, *D.G. Long 39518* (E).

SWITZERLAND: Valais, entre Finhaut et Gietroz, par la Ville de Geneve [46.062207N 06.953432E], 10-Jul-1951, *C.E.B. Bonner 794* (UBC).

USA, ALASKA Alaska Peninsula, Big Creek area; [+58.273971N -157.504683W], 15-Aug-1988, *S.S. Talbot 98-103-41* (UBC); Confluence Tagagawik & Selawik Rivers, Selawik Nat. Wildlife Refuge; [+66.615791N -160.012248W],18-Jul-2005, *S.S. Talbot 05-106-29* (UBC); Innoko NWR; [+63.333247N -158.416283W], 04-Sept-1985, *S.S. Talbot 1985-A8-12* (UBC); Koyukuk National Wildlife Refuge [+65.573806N -156.487369W], 01-Aug-1984, *S.S. Talbot 1984-L26-14* (UBC); Koyukuk National Wildlife Refuge, NE side of low mountain, 4.5 km west of Dulbatna Mountain [+65.362027N -155.406851W], 12-Aug-2014, *S. Noble 15270* (UBC); Angayucham, Mts Manneeluk [= Mauneluk] River [+66.980769N -156.109210W], 27-Jun-1974, *M. Lewis 457* (UBC); Nowitna National Wildlife Refuge [64.702312N -154.001256W], Ruby (C4) Quadrangle, 19-Aug-1986, *S.S. Talbot 10C5* (NY).

USA, DELAWARE: Kent County, 2 miles north of Route 6 on Route 9, [+39.331435N -75.523503W], 16-Nov-1969, *C.F. Reed 84567* (UBC).

USA, IDAHO: Idaho County, Eldorado Creek, [46.317279N -115.645359W], edge of fen east of Road 524, *Abies lasiocarpa*, *Thuja plicata*, *Picea engelmanni* forest. with *Ledum glandulosum*, *Carex angustata*, 02-Sep -2005, *K.L. Gray 5269* (NY).

USA, INDIANA: La Porte Co., Indiana Dunes National Lakeshore, Pinhook bog, near salt storage piles, along Highway 80/90, [+41.621348N -86.849309W], 08-Jun-1987, *Diana G. Horton 26319* (UBC).

USA, MICHIGAN: Mackinac County, Epoufette [46.055587N -85.167736W], on floor of rather dry *Thuja* swamp. 28-Jul-1966, *H. Crum & N.G. Miller 284* (UBC); Chippewa County,

0.2 mi from Garlinghouse Lake, 0.2 mi E of intersection of Thompson Rd and Bound Rd, on Thompson Rd. [46.293315N -84.545038W], associates *Ledum, Maianthemum, Myrica, Calopogon, Picea mariana, Pinus banksiana, Pinus strobus*, on top of hummock, 05-Jul-1991, *M. Bourell 4075* (UBC).

USA, MONTANA: on Mill Creek Road, ca. 3 mi. from Interstate 90 junction, Missoula, base of cliff, [47.045826N -114.179716W], 16-May-2003, *W.B. Schofield 121006* (UBC).

USA, OREGON: Crabtree Fen area. West Slope, Cascade Range, about 14.5 air miles E of Lacomb, T11S, R3E, Sec 16.; Co: Linn, [+44.584369N -122.480335W], 10-Aug-2001, *K. Merrifield 2021* (UBC).

USA, WASHINGTON: San Juan Co., Orcas Island, Moran State Park, along the trail to Cold Springs from Mt. Constitution, T37N R1W Sec. 21 [+48.672919N -122.846243W], 24-Aug-1991, *J.A. Harpel 5052* (UBC).

6. *Polytrichum swartzii* Hartm., Handb. Skand. Fl. ed. 5: 361. 1849. ≡ *Polytrichum commune* Hedw. var. *swartzii* (Hartm.) Nyholm, Ill. Moss Fl. Fennoscandia 2: 681, 1969; ≡ *Polytrichum commune* Hedw. subsp. *swartzii* (Hartm.) Hartm., Handb. Skand. Fl. (ed. 9), 2: 43, 1864). Type citation: "(Stockholm enl. ex. af Swartz. Gestr. Forsbacka bruk på homar i sjön nedom bryket – N. Kongsberg: Hj. Holmgr.)". Type specimen: Sweden, Stockholm, [Södermanland / Uppland], *s.d*., leg. *Swartz s.n.* (lectotype UPS B-855609!, selected by D. G. Long, 1985).

Syn*: Polytrichum algidum* I.Hagen & C.E.O.Jensen, Meddel. Grønland 15: 384, 1898. Type citation: [Greenland] "Habitat ad Scoresby-Sund, 1892 a N. Hartz lectum." Type specimen: Greenland, Scoresby Sund, June 1892, leg. Hartz *s.n*., (isotypes BM 001087919!, E 00007742!, W1904-0005929!).

Syn.: *Polytrichum commune* Hedw. var. *nigrescens* Warnst., Verh. Bot. Vereins Prov. Brandenburg 41: 65. 1899; ≡ *Polytrichum swartzii* Hartm. var. *nigrescens* (Warnst.) I.Hagen, Kongel. Norske Vidensk. Selsk. Skr. (Trondheim) 1913(1): 53. 1914. Type citation: [Germany] "Cladow auf der Havel bei Spandau, auf Sumpfwiesen (Prager 1897)". Type specimen: Germany, Brandenburg, Spandau: Cladow a/ H[avel], versandete Wiesen an der Havel, Juni 1897, E. Prager *s.n*., NY00913019 in herb. W.R. Uggla, lectotype, selected here.

Syn: *Polytrichum inconstans* I.Hagen, Nyt Mag. Naturvidensk 38: 339, 1901. Type citation: "Norvege et Islande". Syntype specimens: Norway, Iceland (isosyntype C!).

Figures 4.22, 4.23 & 4.24

Stem erect, variable in stature, medium to large, aggregated or scattered, often soft and flexuose or sometimes rigid, (2–)9–12(–14) cm, in lower part moderately to densely brownish tomentose. ***Leaves*** green, often becoming blackish when old, loosely imbricate to densely arranged, when dry erect-spreading and flexuose, upper leaves seldom appressed to the stem, when moist patent to widely spreading and somewhat recurved, 3.0–7.5(–8.0) × 0.5–1.0 mm, wide spreading, lanceolate to linear lanceolate, differentiated into sheath and lamina, leaf sheath hyaline, entire, rectangular, apex subulate, weakly channelled, margins entire (in semi-aquatic forms) to serrulate, costa prominent, excurrent as short brown entire to serrulate arista, abaxial surface smooth, slightly spiny near apex, lamellae present on both costa and lamina; in transverse section, 25–35(–40) rows of lamellae of 5–8(–10) cells high, end-cells of the lamellae single or geminate, flat, or very shallowly indented, oblique towards the margins of the lamina, broader than tall, walls not thickened with papillae, 12–16(–20) µm wide. Median cells of leaf base linear-rectangular, 75–110(–115) × 3 –5(–7) µm. ***Dioicous;*** perigonia and

perichaetia terminal. Median cells of leaf lamina quadrate, with slightly thickened cell-walls 9(–10)–15 µm broad. ***Perichaetial leaves*** differentiated, lanceolate, with a larger leaf sheath, 2–5(–7) × 1(–2) mm, lamina subulate, apex acute, margin serrate. ***Perigonial leaves*** differentiated, oblanceolate with a larger leaf base, 6–7 × 0.5 –1(–2) mm, apex acute, margin slightly serrate to serrulate. ***Capsule*** strictly 4-angled, cubic, 2.5–3.0 × 2.6–3.2 mm; apophysis distinct, discoid, narrowly constricted above; exothecial cells composed of conical papillae with slit-like apertures; operculum conic with a short rostellate beak; peristome teeth pale yellow, ca. 0.2–0.3 mm high, obtuse, pale; basal membrane low, 60–70(–75) µm, brownish; spores 12–15 µm. ***Seta*** 4(–5) – 7(–8) mm long, reddish-brownish reddish when young. ***Calyptra*** golden-yellow, fibrillose and completely covering the capsule, 13–15 mm long.

Distribution: *Polytrichum swartzii* Hartm. is a strictly arctic species distributed throughout the Holarctic region including Russia, Svalbard, Sweden, Lithuania, Finland, Iceland, Norway, Greenland, Canada, USA: Alaska, Wyoming, In Nunavut, it is known from Baffin and Devon islands (Frye, 1910; Long, 1985; Smith Merrill, 2007; Faubert, 2013; Lönnell & al., 2019; Jukoniene & al, 2019; Hodgetts & al., 2020).

Figure 4.22 shows the Holarctic distribution of *P. swartzii* in the world based on herbarium records confirmed from the present study.

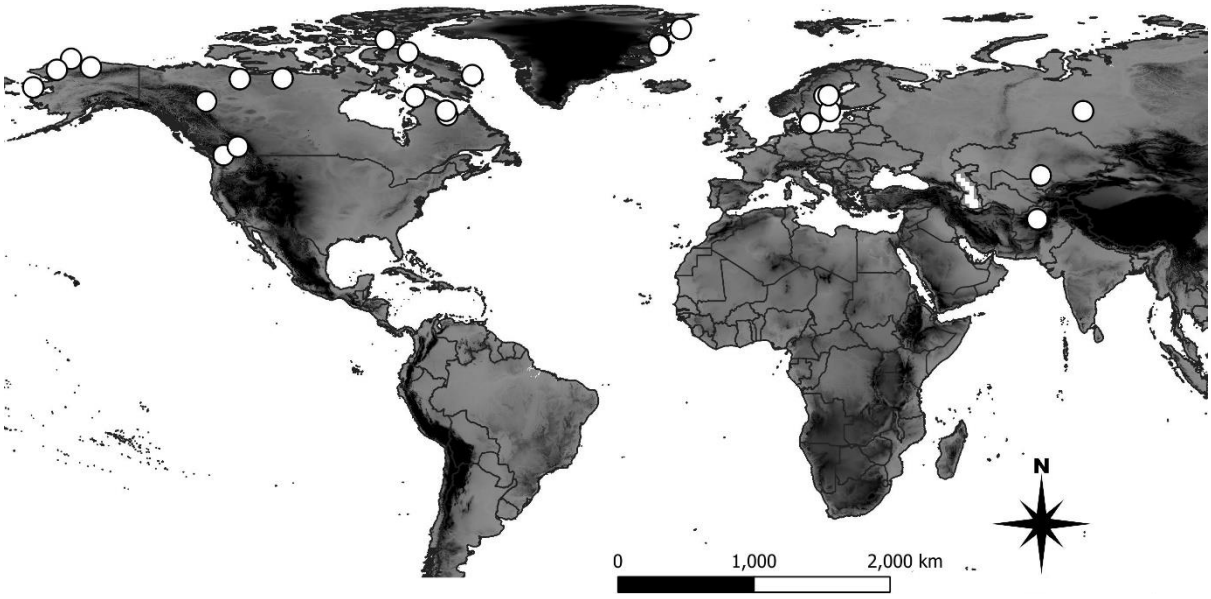

Figure 4.22: Distribution of *Polytrichum swartzii* based on the confirmed herbarium records.

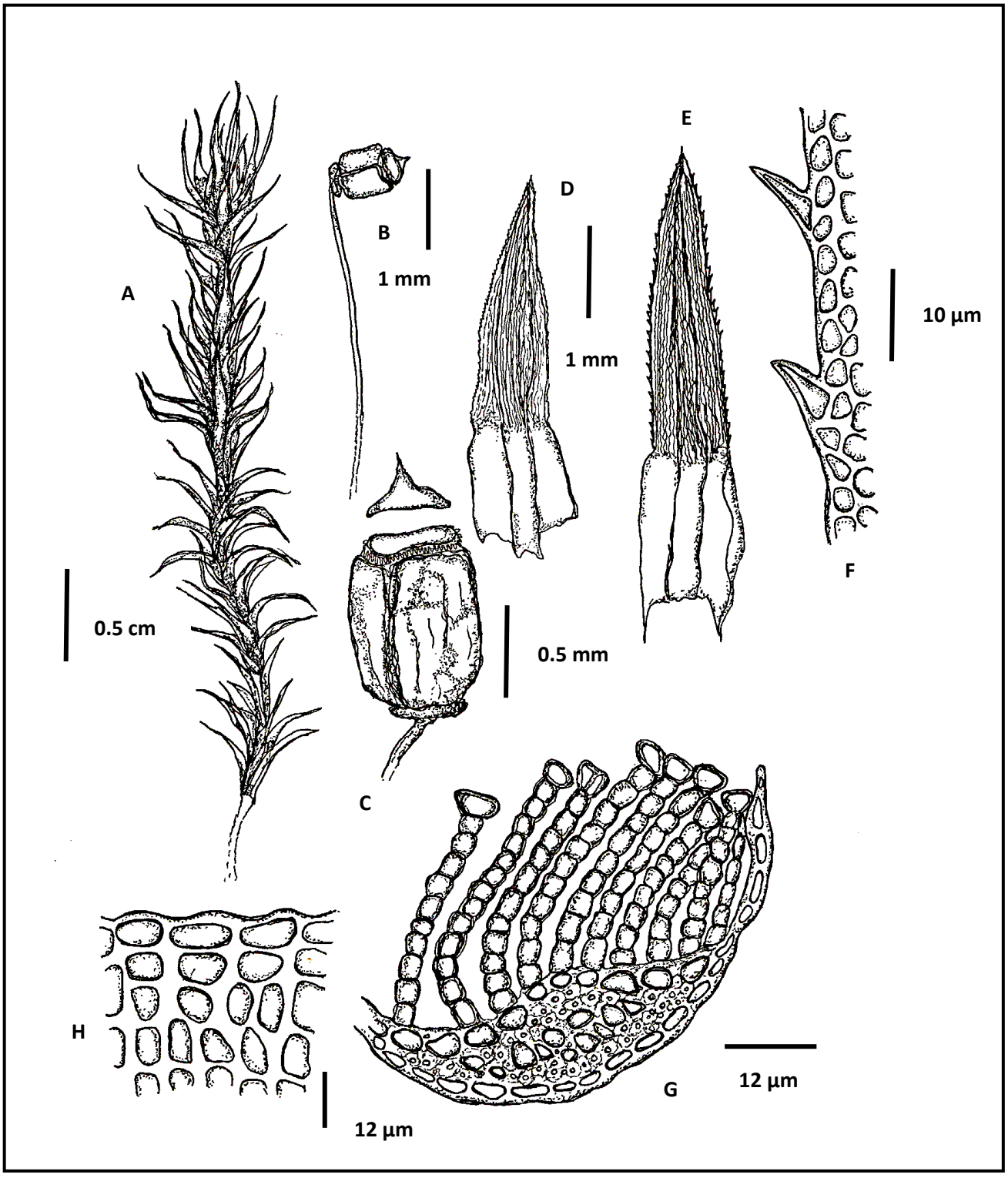

Figure 4.23: *Polytrichum swartzii* **A.** Habit-moist; **B.** Intact capsule & seta; **C.** Strictly 4-angled capsule with detached operculum; **D.** Vegetative leaf (moist); **E.** Perichaetial leaf (moist); **F.** Leaf margin; **G.** Leaf transverse section showing flat-topped (or plate-like) lamellar end-cells; **H.** Lamellae side view [From *Long 42554* (E), drawn by I. U. Kariyawasam]

Ecology: Long (1985) reported that "*P. swartzii* is occurring in very wet or regularly flooded situations". It is very common in habitats such as wet meadows, fens, near waterfalls, muskeg swamps, wet tundra and muddy and sandy lake shores. It grows from sea level to ca.150-200 m.

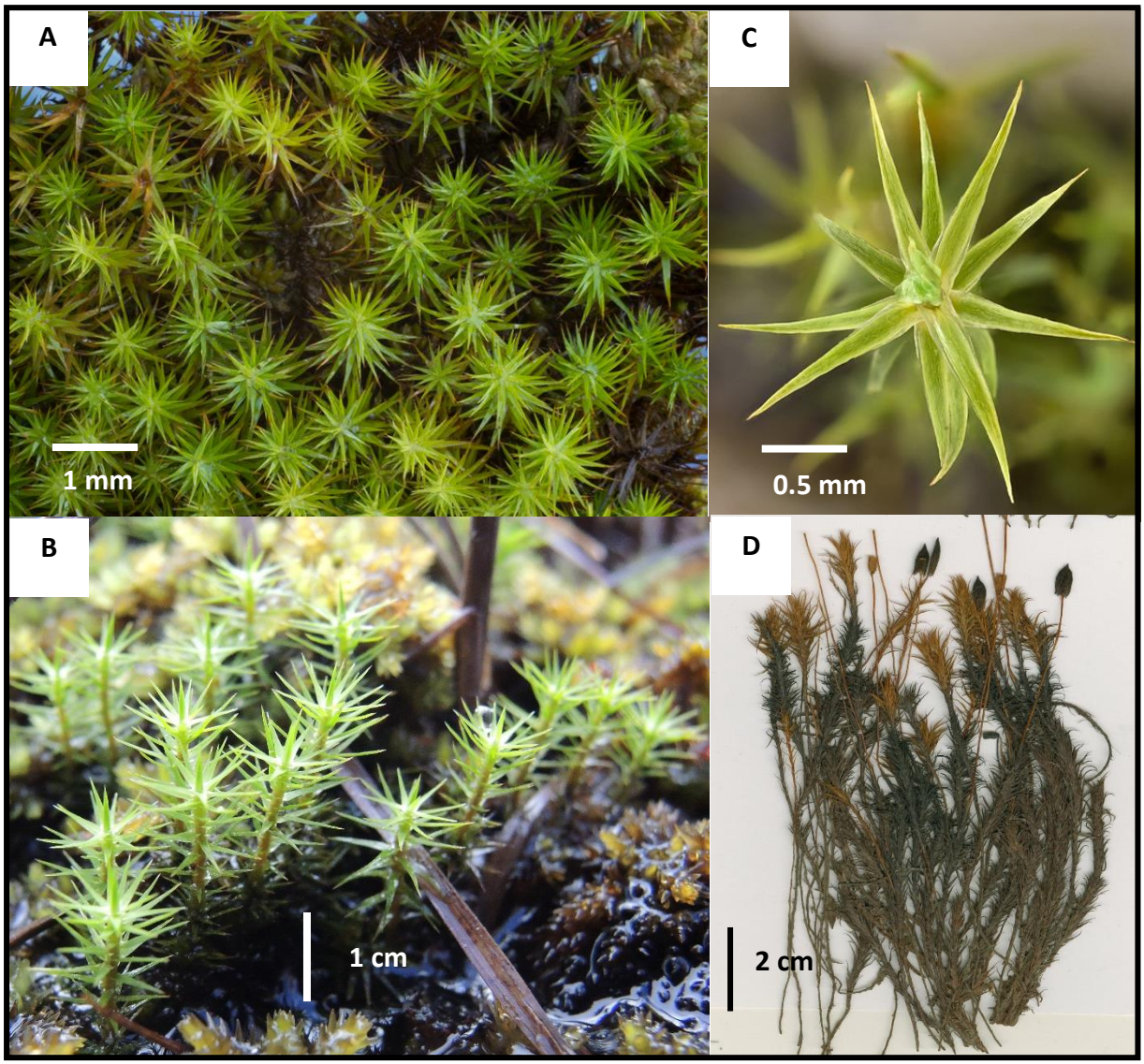

Figure 4.24: *P. swartzii* **(A)** Habit -densely aggregated plants; **(B)** Dwarf scattered plants on a forest floor; **(C)** Arrangement of leaves from the top showing slightly infolded leaf margins and leaf lamellae on the blade; **(D)** Characteristic blackish colour of the older parts of the plant in the herbarium specimens of *P. swartzii* [Photograph taken from the proposed lectotype specimen of *Polytrichum commune* var. *nigrescens* (NY00913019) which is now synonymised under *P. swartzii*. Photos A & C from Niklas Lönnell; photo B from Kristian Hassel (NTNU)]

Taxonomic & Geographical Notes:

Long (1985) treated *P. swartzii* at species rank and treated *Polytrichum algidum* I.Hagen & C.E.O.Jensen and *Polytrichum inconstans* I.Hagen as synonyms of *P. swartzii*. The present study has confirmed the correct placement of both taxa by Long (1985) under this taxon, as the accessions of each were molecularly proven to be *P. swartzii sesnu stricto* (See Chapter 03). It was not possible to borrow the syntypes of *P. inconstans* from Copenhagen, but a slide preparation made by Long (1985) of one of the syntypes confirms the synonymy. Although *P. algidum* and *P. inconstans* exhibit intermediate forms of phenotypic plasticity as to the degree leaf margins ranging from entire to serrate and differentiation of the lamellar end-cells, these taxa are nevertheless confirmed as belonging to *P. swartzii s. str*. *Polytrichum jensenii* I.Hagen is sometimes confused with *P. swartzii* in the field as well as in the herbarium when sterile. However, the soft texture, leaves often becoming blackish when old and the flat-topped lamellar end-cells of *P. swartzii* can easily be distinguished from the deeply-grooved end-cells with their "knob-like" papillar structures seen in transverse sections of leaves of *P. jensenii*.

Fry (1910) reported that *P. swartzii* had been found in England however, Crundwell (1957) concluded that these reports were misidentifications and excluded this taxon from Britain. Chuang (1973) reported *Polytrichum commune* var. *swartzii* (Hartm.) Mönk. from Taiwan. However, Hyvönen & Lai (1991) studied Chuang's specimen and confirmed that none of the specimens they studied from Taiwan including Chuang's (1973) specimen do not belong to *P. commune* var. *swartzii* and excluded it from Taiwan. This is corroborated by the present study as specimens observed from Taiwan [e. g. *Lin 238* (UBC)] identified as *P. commune* var. *swartzii* are now re-identified as *Polytrichum perigoniale* Michx.

Similarly, *P. swartzii* is excluded from mainland China by the present study. Specimens used for the *Moss Flora of China* by Wu & Wang (2005) are now confirmed to be *P. perigoniale* by both morphological and molecular determinations [*P.-C. Wu 21025* (NICH, PE) and *X.-Y. Hu 797* (PE)] from this study. Some resemblance in the shape of the lamellar end-cells between *P. perigoniale* and *P. swartzii* probably led to this misidentification. However, the type of *Polytrichum sinense* Card. & Thér. from Guizhou, synonymized under *P. swartzii* by Wu & Wang (2005), was not available for study and is excluded from the synonymy until it can be studied as it may also belong to *P. perigoniale.*

Also in East Asia, Osada (1966) reported *P. commune* var. *swartzii* from Japan. However, Noguchi (1987) has reported that "the plants are aquatic, and slender with leaves distant. I consider the material on which Osada's record is based, to be a form of the type variety". However, careful observation of this material, labelled as *P. commune* var. *swartzii* (NICH) confirms its identity as *P. perigoniale* and this is confirmed from the molecular study. Hence, *P. swartzii* must now also be excluded from Japan.

Nomenclatural note:

Selecting a lectotype for *Polytrichum commune* Hedw. var. *nigrescens* Warnst.

Polytrichum commune Hedw. var. *nigrescens* Warnst. is synonymised with *P. swartzii* Hartm. by Ignatov & Afonina (1992). A thorough morphological study was done to confirm this and the characteristic blackish colour of the material (when old) and the flat-topped lamellar end-cells and other leaf characters confirmed the decision of Ignatov & Afonia (1992) was correct. However, Warnstorf's herbarium was destroyed in Berlin during World War II (Merrill 1943; Hiepko, 1987) the duplicate in NY [NY00913019] is now selected to serve as a lectotype for *Polytrichum commune* Hedw. var. *nigrescens* Warnst. from this study.

Additional Specimens Observed.

CANADA, NUNAVUT. Baffin Island, Broughton Island, Cumberland Peninsula; Old Broughton settlement site, [+67.557861N, -63.975526W], 01–22-Jul-1974, *C. LaFarge 74112* (UBC, NY); Nunavut, Baffin Island, head of Maktak Fjord, Cumberland Peninsula, 26-Jul-1974–09-Aug-1974, *C. LaFarge B49462* (UBC, NY); Nunavut, Devon Island, Sparbo-Hardy lowland, on granite cliffs E of waterfalls on S side of lowland above small, protected cove meadow, [+75.742042N -83.971208W], 26-Jul-1972, *D.H. Vitt 7013* (UBC, NY); Nunavut, Bathurst Inlet, [+66.780976N -107.822254W], 01-Aug-1979, *W. Scotter 28641*(UBC).

CANADA, NORTHWEST TERRITORIES. Franklin, S-coast at mouth of Aktineq River, Eclipse Sound [+72.819032N -78.810489W], 25- Jul-1954, *W. Drury Jr. 2512c* (UBC); Great Bear Lake, on sandy point between harbour at extreme NE-end of Hornby Bay & river coming in from N [+66.654336N -117.686510W], 26-Jul-1948, *W.C. Steere 10477* (UBC, NY); Mackenzie, Lake Beechery, Back River, 03-Aug-1954, *M.E. Oldenburg 54594M* (UBC); Mackenzie, Nahanni National Park [+61.496460N -125.495493W], 01-Aug-1976, *S.S. Talbot*

T6175 (UBC, NY); Head of Hornby Bay, NE-tip of Great Bear Lake [+66.654336N -117.686510W], 27- Jul-1948; *W. C. Steere 10478* (NY).

CANADA, BRITISH COLUMBIA. Lake Lindeman [+49.113163N -121.451117W], 26-May-1898, *R.S. Williams 690* (UBC); Mt. Revelstoke National Forest, Balsam Lake Campground, [+51.040935N -118.148662W], 04-Sep-1962, *G.L.S. Merrill* with *S.K. Smith s.n.* (UBC, NY).

CANADA, QUÉBEC. Rimouski Co., Cap Caribou, Bic, 22-Jul-1907, *J.F. Collins 5117* (UBC); Québec, Nunavik, détroit d'Hudson, 61.1 km à l'est-sud-est du village nordiqued'Ivujivik, 58,5 km à l'ouest-nord-ouest du village nordique de Salluit, 12.4km au nord-ouest du lac Sirmiq, 21.3 km au sud du cap Pachot, Sols structurés avec Salix herbacea et bryophytes, pente 10%, expositionouest (250°), à la marge de dépressions remplies d'eau, entre blocsrocheux, sur sable fin, ~424m, +62 º 32’ 21.” N, -76 º 74’ 29.6” W, 25-Jul-2013, *J. Gangon 102.1* (QFA); Québec, Nunavik 9.9 km au sud-ouest du village d'Aupaluk, 1.9 km à l'ouest des collines Qingaujaup et 0.7 km au nord-nord-est du lac à la tête de la rivière Arsutaup. Fond de lac boueux peu profond et asséché. ~30m, +59º 15’ 21.6’’ N, -76 º 74’ 29.60’’, 25-Jul-2013, *D. Bastien* & al. *DB-2013-488* (QFA); Québec, Nunavik ~210m, Colline Siukkaq, environ 300 m au nord-est du rivage du lac Faujas et environ 3.6 km au nord-est du lac de la Pyroxénite, Affleurement rocheux basique, escarpé, +58º 20’ 45.52” N, -69 º 44’ 19.03”W, 25-Jul-2013, *Gangon, J., Bastein , D* & al., *DB-2013-434* (QFA); Québec, Nunavik,~155m, 9.6 km à l'ouest de la baie aux Baleines (bras ouest), 1,2 km au sud-ouest de l'exutoire du lac Monnet (dans la rivière Buron) et 4.6 km au nord-nordest du lac Chaperon, Alternance de landes tourbeuses et rocheuses basses, +59º 20’ 45.57’’ N, -70 º 01’ 15.92”W, 28-Jul-2013, *J. Gangon, D. Bastein* & al., *DB-2013-735* (QFA).

FINLAND: Lapponia enontekiensis, Kare-suvanto, Sakkara, in prato uliginoso ripensi ,08-Jul-1934, *H. Roivainen s.n.* (UBC); Lapponia enontekiensis, Enontekiö, NW-Lapponia Enontekiensis, Könkämäeno, Kouttamuotkanjärvi, In ripa paludosa, 06-Aug-1966, *H. Roivainen, s.n.* (UPS, L); Ostrobottnia ultima, Ranua, Kuopasjärvi district north of the church of Ranua, near Paavonaho farm, In a paludified shore forest, 06-Aug-1943, *A.V. Auer, s.n.*, (UPS); Nylandia, Helsinki, Helsingfors, 14-Aug-1868, *S.O. Lindberg, s.n.* (UPS); Nylandia, Helsinki, Helsingfors, 17-Aug-1868, *S.O. Lindberg, s.n.* (UPS); Le Markkina, Kuonnajoki, in prato interdum inundato, 20-Jul-1961, *H. Roivainen s.n.* (UBC); Pohjois-Savo, ~550 m, Grid 27ºE, 70.25W, 09-Sept-1975, *R. Fagerstén* & *M. Haapasaari s.n.* (LD); Suomi Varsinais-

Suomi, Mustfinn träsket, 20-May-1951, *H. Såltin s.n.* (H); Suomi, Pohjois-Savo, Järvikylä, Tiitunlampi, 25-Aug-1949, *O. Tiitinen s.n.* (H); Suomi, Koillismaa Sossonniemi, Piippusuo, 19-Jul-1949, *Y. Vasari s.n.*(H); Savonia borealis, Sprengel Jorois, Järvikylä, Auf dem sumpfigem Rande eines kleinen Sees, 03-Aug-1902, *S.O. Lindberg, s.n.* (UPS)

GREENLAND: East Greenland, Wollaston Foreland, Mt Zackenberg,[74.501013N -20.767106W.] 26-Jul-1950, *K.A. Holmen s.n.* (NY, TRH-NTNU); East Greenland. Wollaston Foreland, Mt Zackenberg, [74.501013N -20.767106W], 26-Jul-1947, *K.A. Holmen 99* (UBC, NY, TRH-NTNU); West Greenland, E of Sondre Stromfjord Airport, approx. 7.0 km NW of Keglen, 67º 05'N, 50º 40'W, 29-Jul-1977, *G.S. Mogensen 77-45* with *G. R. Brassard* (UBC, NY, TRH-NTNU); East Greenland, ~11 m, 74 º 27' 31.2" N, 20º 32' 15.2"W, 30-Aug-2009, *K. Hassel & T. Prestø 691298* (TNH-NTNU).

ICELAND: Melrakkaslétta, ~4m, 19-July-2013, *S. Heiømarsson s.n.* (TRH-NTNU); Natturrgripasafn Islands, South Iceland, Arnessysla, Skalhott, on mounds in a bog by a river side, alt. ~85m, 1961-Aug-24, *B. Johannsson s.n.* (UBC)

NORWAY: Svalbard, Adeventdalen, Adeventelva, 78°12'10.3"N, 15°45'38.4"E, 2 m, floodplain fen, in wet hollow, 23-Jul-2013, *D. G. Long 42554* (E); Oppland District, Dovrefjell National Park, Snøheim below Snøhetta, c. 1460 m, 09-Jul-1991, *D. G. Long 19938* (E); Hedmark, Lille Elvedal: Sumped Band ved en Skovvej ovenfor Byen, 08-July-1898, *C. Jensen, s.n.* (UPS); Torne lappmark, Jukkasjärvi, Torneträskområdet, 1 km SV stugan ("sjön"), 20-July-1945, *H. Persson, s.n.* (UPS)

RUSSIA: Murmansk Province, ~10–40m, 34.3166°N, 66.6750°W, 29-June-2012, *Hallingbäck 6271* (E); Irkutskaya oblast, NE-Sibiria, Jakutskaja ASSSR, regio ostiaria fluminis Lena, In Lariceto paludoso, 27-Aug-1901, *A.K. Cajander, s.n.* (UPS)

SWEDEN: Norrbotten Distr: Pajala Aereajoki, gla dolomitbrott, 500 m, V om Landsvbron, [44.427°N, 67.4290°W], 11-Aug-2008, *T. Hallingbäck 4622-5* (E); Uppland, Hölö, [59.27358000N,18.68530000W], 1943, *Sigge Hähnel, s.n.* (LD); Murmansk province District, Umba, mountain slope near outlet of Umba river in White Sea, [34.3166º N, 66.6750º W], 10–40 m, 29-Jun-2012, *T. Hallingbäck, 6271* (E); Ångermanland. Viksjö. N of Lake Abborrsjön.[62.768654N 17.420342W], 11-Sep-2013, *L. Hedenäs* with *G. Odelvik & K.*

Rönblom s.n. (NY); Ångermanland. [62.92239000 N 18.40417000 W],13-Jul-1921, H. Wilh. Arnell *s.n*. (LD); Ycksele Lappmark: Sorsele, in uliginosis,15-Jul-1925**,** *H. Möeller s.n*. (UBC); Torne lappmark, Jukkasjärvi, Torneträskområdet, Stordalen, området mot Torneträsk, 29-July-1945, *H. Persson & O. Mårtensson, s.n*. (UPS, LD); Dalsland, Öjersbyn, [59.09134000N 12.66851000 W],11-Jun-1918, *P. A. Larsson, s.n*. (LD); Skåne, Loshult, [56.47594000 N, 14.14257000 W], 19-Jul-1944, *S. Waldheim, s.n.* (LD)

USA, ALASKA: North Slope. In vicinity of Noluck Lake, northeastern slope of De Long Mountains, Brooks Range [+68.783333N -160.000000W], 10-Jul-1963, *W.C. Steere 63265* (UBC, NY); vicinity of Umiat, Colville River, on long steep ridge N of Umiat, above Umiat Lake [+69.366666N -152.133333W], 21, 22, 28-Jun-1973, *W. C. Steere 189 with H. Inoue & Z. Iwatsuki* (UBC); Meade River Camp [+69.366666N -152.133333W], 11-Jul-1951 to 15-Jul-1951, *W.C. Steere 15641* (UBC); Umiat. [+69.366666N -152.133333W], 01-Aug-1960, *W.C. Steere 60830* with *O. Mårtensson & K.A. Holmen* (UBC).

Shore of Glacial Lake, S-slope of Kigluaik Mountains, Seward Peninsula [+64.744759N -165.458431W], 17-Jul-1949, *W.C. Steere 13513* (UBC); ridge E of head of Snake River [+64.701738N, -165.408932W], 29-Aug-1949, *R.S. Sigafoos 49174* (UBC); Point Barrow & vicinity. [+71.333333N -156.700000W], Jun, Jul, Aug-1951, *W.C. Steere 15192* (UBC).

USA, WYOMING: Yellowstone National Park, Grizzly Lake Trailhead, 14-Jun-2005**,** *W.B. Schofield 123648* (UBC)

7. *Polytrichum brachymitrium* Müll. Hal., *Linnaea* 42: 468. 1879. Type citation: [Venezuela], 1855, A. Fendler, Musci Fendleriana Venezuelenses Nr. 71. Type specimen: Venezuela, [Valencia] Prope Colonia Tovar [-10.4056 S, -67.2894 W], 1854–5, *Fendler 71,* FH00220207!, (lectotype designated here); isolectotypes: BM000960640!, NY 00913008 !, NY00913009!, NY00913010!, PC0131169!, S170830!, S170831!

Syn.: *Polytrichum subcarinatum* Hampe, Vidensk. Meddel. Dansk Naturhist. Foren. Kjøbenhavn, ser. 3, 6: 150. 1874; replacement name for *Polytrichum subgracile* Hampe, Vidensk. Meddel. Dansk Naturhist. Foren. Kjøbenhavn, ser. 3, 4: 53. 1872, *nom illeg*. [non *P. subgracile* Hampe 1870]. Type citation: [Brazil] "Glaziou sub Nr. 5201."; lectotype, selected here: Brazil, Rio de Janeiro, *Glaziou 5201* (PC0709518!; isolectotype. PC0741423!), ***syn. nov***.

Syn.: *P. subremotifolium* Geh. & Hampe, Flora 64: 377. 1881. Type citation: [Brazil, São Paulo] "Prope Apiahy, Majo 1879: Puiggari; prope Faxina, sterile: Louis Puiggari." Type specimen: Brazil, São Paulo: prope Faxina, *Puiggari 634* (Syntypes H-BR!, BM000960744!); ***syn. nov***.

Figures 4.25, 4.26 & 4.27

Stem erect, rigid, unbranched, variable in stature, medium to large, (5–)10–12cm. ***Leaves*** more loosely imbricate as they are arranged on a rather more slightly compressed slender shoot, 5.5–7.5 × 0.5–1.0 mm, lanceolate, differentiated into sheath and lamina, leaf sheath hyaline, entire, sheathing base gradually acuminate into a narrowed, slightly longer blade, with serrate margin, with very short teeth, 30–33(–35) µm long, remote, patent, aculeiform, ending in a more or less elongate, serrate hair, leaf apex apiculate; costa prominent, percurrent and slightly spiny on the abaxial surface; leaf lamellae present on both costa and lamina; in transverse section with 50–60 rows of lamellae of 6–8(–10) cells high, end-cells of lamellae flat and broad, asymmetric and occasionally pyriform towards the shoulders of the lamina, never possessing papillae. ***Dioicous****;* perigonia and perichaetia terminal. ***Perigonial leaves*** differentiated, oblanceolate, with a larger leaf sheath, 2–5(–7) × 1(–2) mm, apex acute, margin serrate, lamina with spines on the abaxial surface. ***Perichaetial leaves*** with a long acumen, leaf apex apiculate; margin serrate. ***Capsule*** 4-angled, cubic, short, 2.0(–2.5)–3.0 × 1.5–2.7 mm; apophysis distinct, discoid, narrowly constricted above; exothecial cells bearing conical papillae with slit-like

apertures; operculum conic with a short and obliquely rostellate beak; peristome teeth obtuse, pale; basal membrane low, slightly brownish; spores 7–7.5 µm. ***Seta*** 4(–5) – 7(–8) mm long.

Distribution: Brazil, Venezuela, Colombia, Argentina.

Without citing any specimens Peralta & Yano (2010) stated that "*P. brachymitrium* Müll. Hal. is a species restricted to Venezuela and Colombia and lacking in Brazil", however the present study confirms this taxon is wide-spread in Brazil and also observed in Argentina, Venezuela and Colombia.

Figure 4.25 shows the distribution of *P. brachymitrium* in South America based on herbarium records confirmed from the present study.

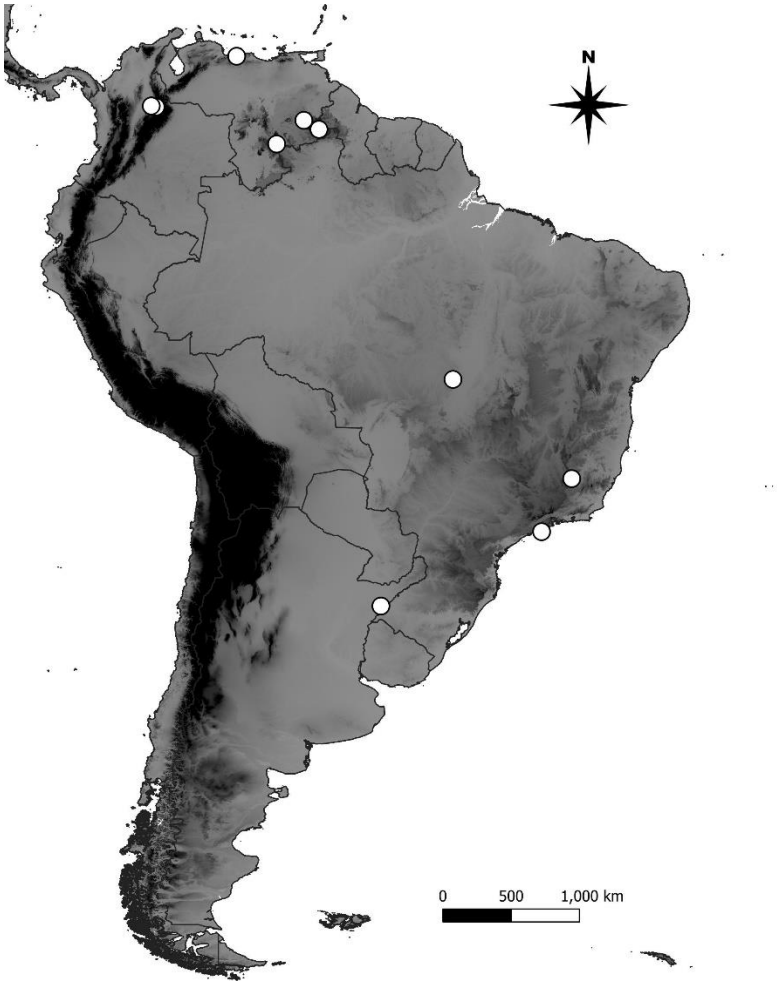

Figure 4.25: Distribution of *P. brachymitrium* based on the confirmed herbarium records.

Ecology: On sandy soil, exposed areas, creek boarders, damp places by rivers or waterfalls, ground of dry forests, between ca. 800–1200 m altitude.

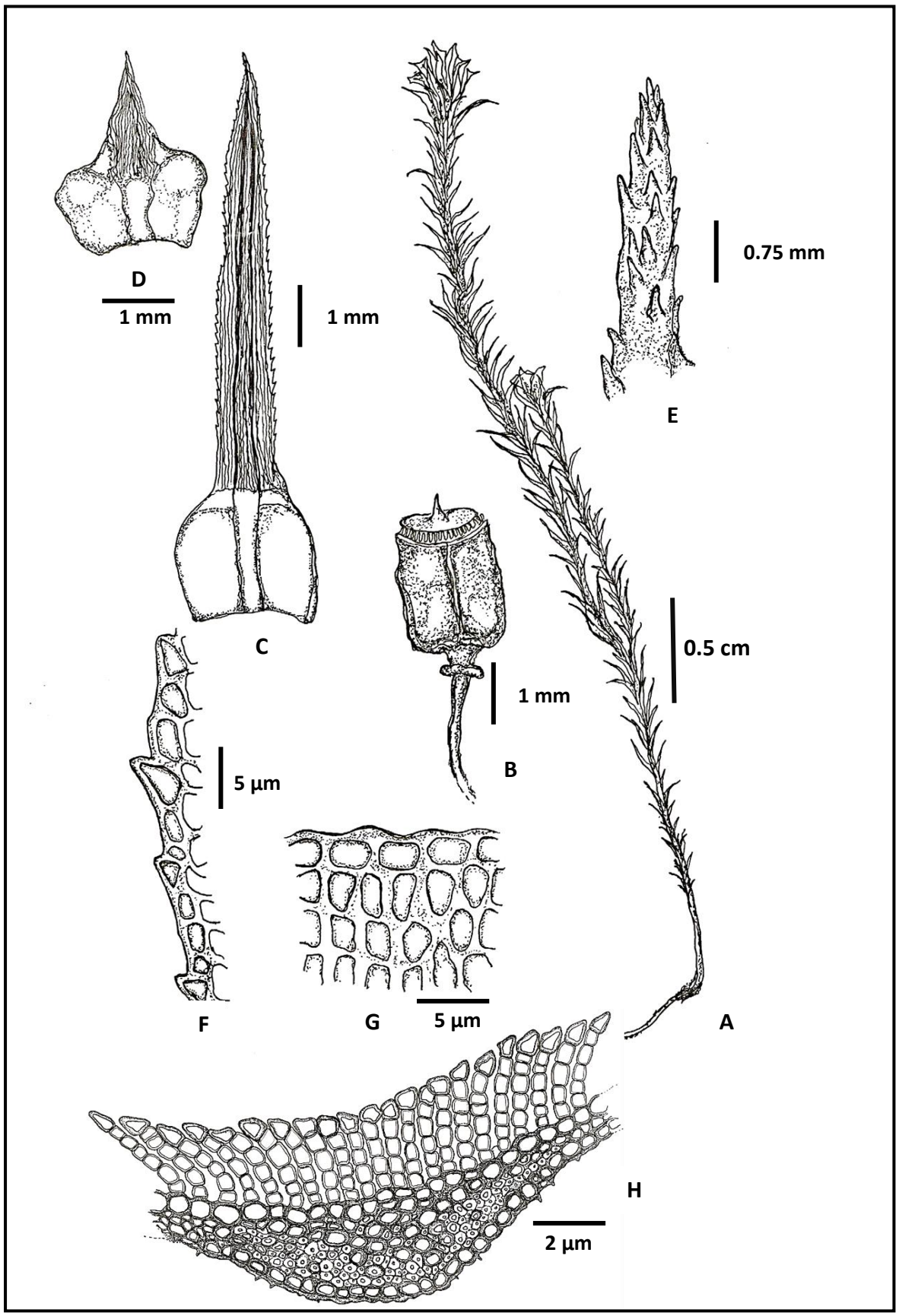

Figure 4.26: *Polytrichum brachymitrium* **A.** Habit- male plant (moist); **B**. Undehisced capsule; **C.** Vegetative Leaf (moist); **D.** Perigonial leaf (moist); **E**. Leaf apex with a larger number of shorter spines; **F.** Leaf margin with shorter teeth; **G.** Side view of leaf lamellae; H. Leaf transverse section showing pyriform to asymmetric shallowly-grooved lamellar- end cells (From the lectotype [FH00220207], drawn by I. U. Kariyawasam)

Taxonomic & Nomenclatural Notes:

Nomenclatural Note 1: Lectotypification of *Polytrichum brachymitrium* Müll. Hal.

Polytrichum brachymitrium Müll.Hal. was described by the German bryologist Carl Müller (1879) based on Augustus Fendler's collections in 1854–55 from Venezuela (Stieber & Lange, 1986; Todzia, 1989). Fendler visited the United States in March 1856, when William Starling Sullivant from Ohio purchased his moss and liverwort collections (Stieber & Lange, 1986). From 1856 onwards, A. Schrader worked as was a full-time illustrator for Sullivant and made illustrations of Fendler's Venezuelan mosses (Müller, 1879; Sayre, 1984). At Sullivant's request he made duplicate sets of these specimens to sell on his own, on condition of providing sketches in return for determinations. Some of these sets were sold to institutions in Germany (Correspondence 29th May 1873, to Sullivant's brother from Leo Lesquereux, Farlow Herbarium Archives Box 1, Folder 5). Sullivant kept one of Schrader's sets, along with the original illustrations, intending to use them to describe the new species himself. However, Sullivant died in 1873, and was not able to undertake this task. After his death his herbarium collections were transferred to Harvard University Herbarium in 1874 (Müller, 1879; Sayre, 1984).

Professor Asa Gray, at that time the Director of Harvard Herbarium, invited Karl Müller in Germany to study Fendler's Venezuelan mosses and he then sent them to Berlin (Müller, 1879). The results of his study, including Schrader's illustrations, were later published by Müller as "*Musci Fendleriani Venezuelensis*" in 1879. Müller must have kept original material of his new species in Berlin but the illustrations were returned to Harvard. Tragically, Müller's complete bryophyte herbarium was destroyed in Berlin during WWII (Hiepko, 1987) resulting in almost total loss of the all-important type material (Merrill 1943).

Thus, the original material of Müller's *P. brachymitrium* was lost and the name must be typified by a duplicate in another herbarium. *Polytrichum brachymitrium* is a poorly-understood species in Latin America and this name has been misapplied to plants of *P. commune* Hedw. (Peralta & Yano, 2010) showing a range of end-cell variation (U-shaped mixed with pyriform), as reported for *P. commune* by Crum & Anderson (1981). A duplicate of the *Fendler 71* material of *Polytrichum brachymitrium* is housed in the Farlow Herbarium of Harvard University (FH00220207!), to which is attached the original illustration by Schrader. This is therefore the most suitable specimen to select as a new lectotype for this

taxon. Duplicates of from the same collection housed at BM, NY, PC and S are treated as isolectotypes.

Nomenclatural Note 2: Hampe's two descriptions of *Polytrichum subgracile*

Hampe in 1870 and 1872 described two different species of *Polytrichum* with the same binomial, *Polytrichum subgracile*. The first was based on *Strebel* collection from Veracruz, Mexico, the second (an illegitimate homonym) based on *Glaziou 5201* from Brazil. Later, in 1874, he corrected his error by providing a replacement name *P. subcarinatum* Hampe for the later Brazilian taxon. Unfortunately, Costa & al. (2016), in lectotypifying moss names based on Glaziou's collections from Brazil, lectotypified the earlier 1870 Mexican name with the type of the illegitimate Brazilian homonym. Thus, the lectotype cannot stand as the selected specimen is not included in its protologue. Therefore, their selected lectotype for the 1870 binomial is now designated as a new lectotype for Hampe's replacement name *P. subcarinatum*.

In describing *Polytrichum subcarinatum*, Hampe treated it both as a replacement name for the later (illegitimate) *P. subgracile*, but also as a new species, citing another specimen, *Glaziou 7060* in his protologue. According to the ICN (Art. 6.12 and 6.13), therefore, this could be treated either as a replacement name or as a new species. Original specimens of *Glaziou 7060* are preserved in the Paris herbarium (PC) where, following study of leaf sections made by Denilson Peralta, this number has been shown to be a mixture of two species, *Polytrichum brachymitrium* and *P. commune*. Because it is not clear which element (if any) Hampe's description refers to, it is preferable to treat *P. subcarinatum* as a replacement name for the later *P. subgracile*, as proposed above. After careful observation of the capsule size and leaves of the newly-selected lectotype of *P. subcarinatum* (the later illegitimate *P. subgracile*) and comparison with the type specimen of *P. brachymitrium* (FH00220207) this is now synonymised (see above) under *P. brachymitrium*. The Mexican type of the earlier *P. subgracile* needs to be studied to ascertain its taxonomic identity. (*Polytrichum subgracile* Hampe, Bot. Zeitung (Berlin) 28: 51. 1870. Type citation: [Mexico, Veracruz] 'prope Veracrucem, leg. Strebel, ex herb W. Sonder').

Taxonomic Note: *Polytrichum brachymitrium*, *P. commune* and *P. perigoniale*

After careful examination of the newly designated lectotype material of *P. brachymitrium* (FH00220207!) there is a need to reconsider and slightly expand upon Müller's description of the specimen. Despite his failure to describe in detail the leaf lamellar-end cell morphology in cross-section (although the illustration by Schrader attached to the type specimen attempted to incorporate a cross section of the leaf, it does not demonstrate clearly the lamellar end-cell shape), his description of *Polytrichum brachymitrium* matches well with the designated lectotype. This material lacks only the male inflorescences; however, a specimen with numerous male inflorescences has been collected by *Killip & Smith 1522* from the Eastern Cordillera in Colombia in 1926 (NY 01835330!).

The name *Polytrichum subgracile* Hampe was synonymised recently with *P. commune* Hedw. by Costa & al. (2016). In their paper they have mentioned that "The type of *Polytrichum subgracile* was not studied during the revision of the family Polytrichaceae in Brazil (Peralta & Yano, 2010). This type is very important because the name was erroneously considered a synonym of *Pogonatum tortile* (Sw.) Brid. by Wijk & al. (1967). All of the specimens studied here belong to *P. commune*, a very common and wide-spread species in Brazil" based on the isotype of *P. subgracile* Hampe [PC0709518]. There are two problems with this statement: (a) they did not realise that *P. subgracile* had been described twice for two different taxa from Mexico and Brazil (as discussed above), and the specimen they cited was not that of the earlier name; and (b) *P. subgracile* was erroneously synonymised by them under *P. commune* due to their imprecise concept of *P. commune* in Brazil. Since a lectotype for *P. commune* has now been established from the present study (Kariyawasam & al., 2021) the characters defining *P. commune* have now been refined and clarified.

Peralta & Yano (2010) reported *P. commune* as present in Brazil; however, some of their determinations of specimens housed in PC were erroneously assigned to *P. brachymitrium*. In addition, *P. perigoniale* Michx. has now been confirmed as a third distinct entity in this taxonomic group in South America (see Chapter 03 in the present work) and Brazilian *P. perigoniale* (a new species record for Brazil, confirmed from the present molecular study) was erroneously assigned to *P. brachymitrium* by some workers in the past. Since *P. perigoniale* was earlier treated a synonym of, or as a variety of, *P. commune*, these past, inaccurate concepts of all three taxa have led to their confusion in literature reports, now resolved in Chapter 03.

Polytrichum brachymitrium and *P. perigoniale* are morphologically similar and easily confused if not carefully observed the lamellar end-cells in a leaf transverse section. The striking feature which distinguishes *P. brachymitrium* from *P. perigoniale* is, a transverse section of a leaf of P. *brachymitrium* shows flattened to asymmetric and pyriform end-cells (towards the leaf shoulder) [Figure 4.26], whereas in *P. perigoniale* all end-cells are flat-topped or very slightly grooved and never pyriform (see under *P. perigoniale*, Taxonomic Note1: Table 4.2). *P. brachymitrium* can be easily distinguished from the other species of *Polytrichum* sect. *Polytrichum* by the shorter, retuse leaf teeth and shorter capsule.

Study of the type of *P. subremotifolium* Geh. & Hampe indicates that it is a synonym of *P. brachymitrium*, not of *P. commune* as indicated by Peralta & Yano (2010).

Figure 4.27: Habit of *P. brachymitrium* from Brazil; Sterile plants showing comparatively shorter leaf lamina and distantly arranged leaves on the stem (Photo courtesy Denilson Peralta)

Additional Specimens Observed

ARGENTINA: Corrientes, San Martín. Tres Cerros, Pelon, 14-Nov-1979, 29°06'07"S, 56°56'20"W, *A. Schinini 17696* (NY).

BRAZIL, **BAHIA**. Serra das Almas, middle NE slopes of Pico das Almas, ca. 25 km WNW of Vila do Rio de Contas. in damp places by edge of river, 18 Mar. 1977, *R. M. Harley 19616 with S. J. Mayo, R. M. Storr, T. S. Santos & R. S. Pinheiro* (NY)

BRAZIL, **GOIÁS**, Chapada dos Veadeiros, on wet gravelly soil at roadside, gallery forest bordering riacho, with adjacent campo and cerrado, ca. 18 km N of Alto do Paraíso, elev. ca. 1250 m, *H.S. Irwin et al. 33068* (S, MO, NY); Serra dos Cristais, rooted in dense mats on porous rocks. Common. Creek border, ca. 5 km. S. of Cristalina, 17°S, 48°W, elev. 1175 m, 21-Mar- 1971, *H.S. Irwin, R.M. Harley & G.L. Smith 32845* (S, NY); territory of Roraima, vicinity of Auaris, high secondary forest, terrestrial, 04°06'N, 64°25'W, 3-Nov- 1965, *H.S. Irwin, R. Souza & R.R. dos Santos 9851* (NY, E).

BRAZIL, **MATO GROSSO**. Central Brazilian Plateau, 35 km north along road from the base camp; in bare damp ground formerly dry forest, recently burnt & cleaned, with *Pteridium aquilinum*, grasses etc., 12°49'S, 51°46'W, 23-Oct-1968, *R.M. Harley, R. Souza, R. De Castro & A. Ferreira 10783* (E).

BRAZIL, **MINAS GERAIS**. Serra do Espinhaço, moss growing along edges of road, sandy campo with outcrops, Serra do Cipo, ca. 120 km (ca. 145 km. N. of Belo Horizonte), elev. 1200m, 27-July-1974, *G.T. Prance, O. Fidalgo, B.W. Nelson & J.F. Ramos 21441* (NY); Serra do Espinhaço, on shaded tree branch, riacho margin, campo and gallery margin with outcrops, grey sandy soil, ca.12 km W. of Barão de Cocais, base of Serra da Caraça, elev. ca. 1500 m, 16-Dec-1968, *H.S. Irwin, H. Maxwell & D.C. Wasshausen 21825* (NY); Morro das Pedras, on sandy roadside embankment, campo on red clay, with very low generally sparse shrubs, ca. 25 km N.E. of Patrocínio, elev. 1050 m, 28-Jan-1971, *H.S. Irwin, R.M. Harley & E. Onishi 29339* (NY, E); on sandy roadside embankment, campo on red clay, with very low generally sparse shrubs, ca. 25 km NE of Patrocínio, [-19.948°S, -43.2486°W], 1050 m, 28-Jan-1970, H.S. *Irwin, E. Onishi, S.F. da Fonsêca, R. Souza, R. Reis dos Santos & J. Ramos 25549* (NY, E); Serra do Espinhaço, ca. 18 km E of Diamantina, forming dense masses in sandstone cervices,

wet with overflow from nearby brook, partial sun exposure, locally abundant, cerrado and brejo at creek margin, 900m, 18-Mar-70, *H.S. Irwin, S.F. da Fonsêca, R. Souza, R. Reis dos Santos & J. Ramos 27869*, (NY, E); Catas Altas, Sep-1997, *J. Hyvönen 6230* (H); Nova Limaon, bared soil, along road BR-135, 07-Feb-1976, *D. M. Vital 5517* (NY)

BRAZIL, **SÃO PAULO.** São Sebastião, cachoeira de Toque-Toque Pequeno, estrada Maresias-Barequecaba, no barranco arenoso, perto da cachoeira, 27-Jul-1983, *O. Yano, K.C. Porto & J.R. Pirani, 7931* (NY, SP).

COLOMBIA: Eastern Cordillera, Santander, Mesa de los Santos, [06°46'00"N, 073°05'00"W], rich soil, open meadow, 1500 m, 11–15 Dec. 1926, *E.P. Killip & A.C. Smith 15252* (NY); Antioquia, Municipio de Urrao, Páramo de Frontino, ca. 17 km directamente Norte de Urrao, ca. 06°25'00"N, 076°05'00"W, 500 m, 30-June-1985, *S.P. Churchill, I. Sastre de Jesús* & *M. Escobar A. 13325* (MO, NY); Caquetá, Musgo sobre suelo arenoso, Transecto Neiva-San Vicente del Caguán. Entre los ríos Pato y Miña Blanca. Bosque muy húmedo tropical, 500-575 m, 02°30'N 074°45'W, 02-Dec-1990*, S. P. Churchill & Julio C. Betancur B. 17033* (MO, NY).

VENEZUELA: Bolívar, Auyan-tepuí, Cumbre de la Parte sureste del Brazo Noroeste (division Occidental del Cerro), entre "Drizzly Camp" y "Río Lomita Camp", [05°09'00"S, 061°23'00"W], faldas secas de piedras "limonita" e ígneas con arbustos y árboles esparcidos, 05-May-1964, *J.A. Steyermark 93397* (NY); Bolívar. Urdaneta. Route 10, Quebrada Pacheco, along salto, big patch in savanna. boulders along stream, seepy sandy soil, 18-Dec-1984, *I. Sastre-de Jesús 647* (NY); Guyana, "Guisana angl."[“Guyana Angel” waterfall], [05.9701°N, 62.5362°W], Apr 1894, *R.H. Schomburgk s.n.* (NY).

8. *Polytrichum perigoniale* Michx. Fl. Bor.-Amer., 2: 293. 1803 (19 March); ≡ *Polytrichum yuccifolium* Ehrh. ex Funck var. *perigoniale* (Michx.) Mart., Fl. Crypt. Erlang., 83. 1817; ≡ *Polytrichum commune* Hedw. var. *perigoniale* (Michx.) Hampe, Linnaea 13: 44. 1839; ≡ *Polytrichum commune* Hedw. subsp. *perigoniale* (Michx.) Kindb., Eur. N. Amer. Bryin., 2: 163. 1897. Type citation: [U.S.A. and Europe] "In Carolina: etiam in Europa". Type Specimen: "*Polytrichum perigoniale*. a. Carolina Rich. [Richard]. b, Fontainbl. [Fontainebleau, France]. c. Fontainbl." (G00114590! lectotype, proposed in this study).

Syn: *Polytrichum glabrum* Schrad., J. Bot. (Schrader) 1801(1): 196. 1803 (April) [validating description for *P. glabrum* Brid. Musc. Recent. 1: 85. 1797]. Type citation: [La Réunion] "*P. glabrum* B. is eine neue Art. Von der Insel Bourbon"; Syn.: *Polytrichum glabrum* Brid. Musc. Recent. 1: 85. 1797; original citation: "In insula Bourbonis habitat. Illud nempe ea parte, quae Caffrorum planities dicitur in altissimus montibus, nec non in fossis aqua desiccates Augusto mense 1771 Commersonus invenit. Parisiis in Reipublicae herbario vidi. Duratio certe perennis.". Type specimen: La Réunion,1771, *Commerson s.n*. (PC, not seen).

Syn: *Polytrichum remotifolium* P.Beauv., Prodr. Aethéogam. 86. 1805. Type citation: [La Réunion] "Cette espèce de l'Ile de Bourbon, m'a été communiquée par M. de Jussieu." Type specimen*:* [La Réunion]" *P. remotifolium* ile de Bourbon (Beauv. herb.)" (E00428754!, herb. Arnott, isotype); ***syn. nov.***

Figures 4.28. 4.29, 4.30 & 4.31

Stems erect, medium to large, unbranched, often having a distinct "bushy" and densely tomentose appearance due to crowded leaves, (5–)6–14 cm, in lower part moderately to densely brownish tomentose. ***Leaves*** dark green, densely imbricate and crowded, erect-spreading when moist, appressed to the stem when dry, 7.5(–10.0) × 0.5–1.0 mm, lanceolate, differentiated into sheath and lamina, leaf sheath long, margins usually serrate up to the apex, marginal teeth rather long, costa broad covered by lamellae adaxially, abaxially spines towards the apex, costa excurrent to form a long hair-like structure. Median cells of the sheath short and rectangular, 70(–80) ×15–20 µm. Median cells of leaf lamina quadrate, with slightly thickened cell-walls 9(–10)–15 µm broad. Lamina bearing 60–70 rows of lamellae. Lamellae narrower and irregularly furrowed in profile, 8(–10)–12 cells high, lamellar end-cells shallowly-grooved,

irregularly furrowed or oblique, ca.10–14 μm broad, without papillated thickenings on the walls. ***Dioicous;*** perigonia and perichaetia terminal. ***Perichaetial leaves*** differentiated, lanceolate, with entire or very weakly dentate leaves or subentire, apex acute, longer than the vegetative leaves, distinctly ribbon-like, mostly hyaline, ending in a long, nearly smooth awn, irregularly twisted and curled when dry. ***Perigonial leaves*** differentiated, oblanceolate with a larger leaf base, apex acute, margins subentire or very weakly serrate and usually hidden by upper stem leaves. ***Capsule*** short, strictly 4-angled, cubic, 2.5–3.0 mm; apophysis distinct, discoid, narrowly constricted above; exothecial cells composed of conical papillae with slit-like apertures; operculum conic with a short rostellate beak; peristome teeth pale yellow, ca. 0.3–0.5 mm high, obtuse. ***Seta*** 5–9 mm long, reddish when young. ***Calyptra*** golden-yellow, fibrillose and completely covering the capsule, ca. 8–10 mm long.

Distribution: This taxon is a widespread species mostly in many parts of the drier regions of the Northern and Southern Hemispheres including Africa, North America, Europe, Australasia and Southeast Asia. From this study it is now confirmed as a new record for South America (Brazil).

Figure 4.28 shows the distribution of *P. perigoniale* based on herbarium records confirmed from the present study.

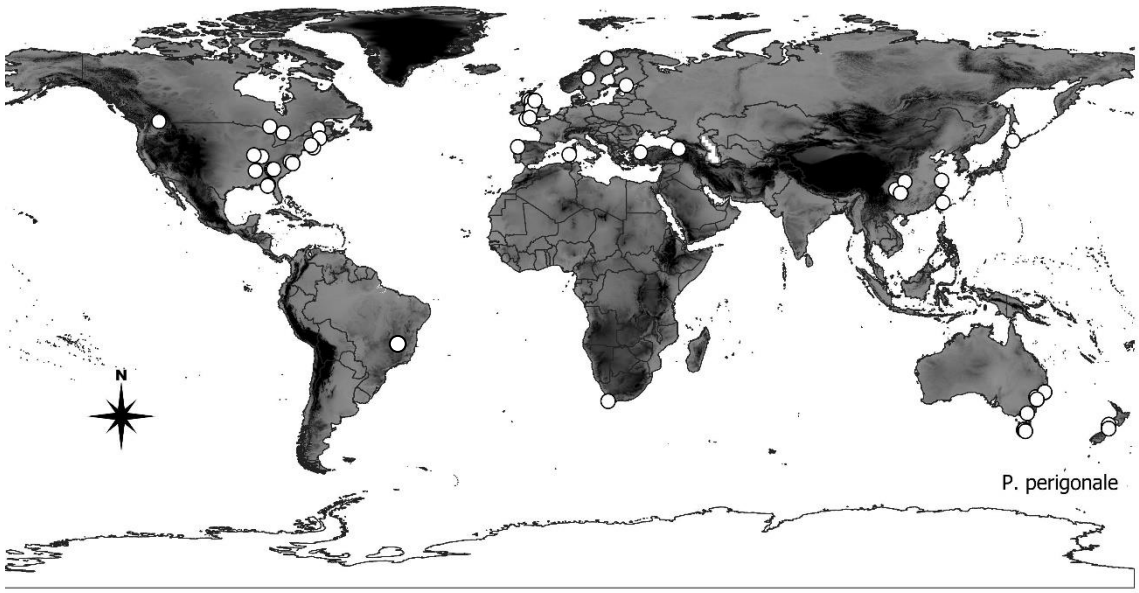

Figure 4.28: Distribution of *P. perigoniale* based on the confirmed herbarium records.

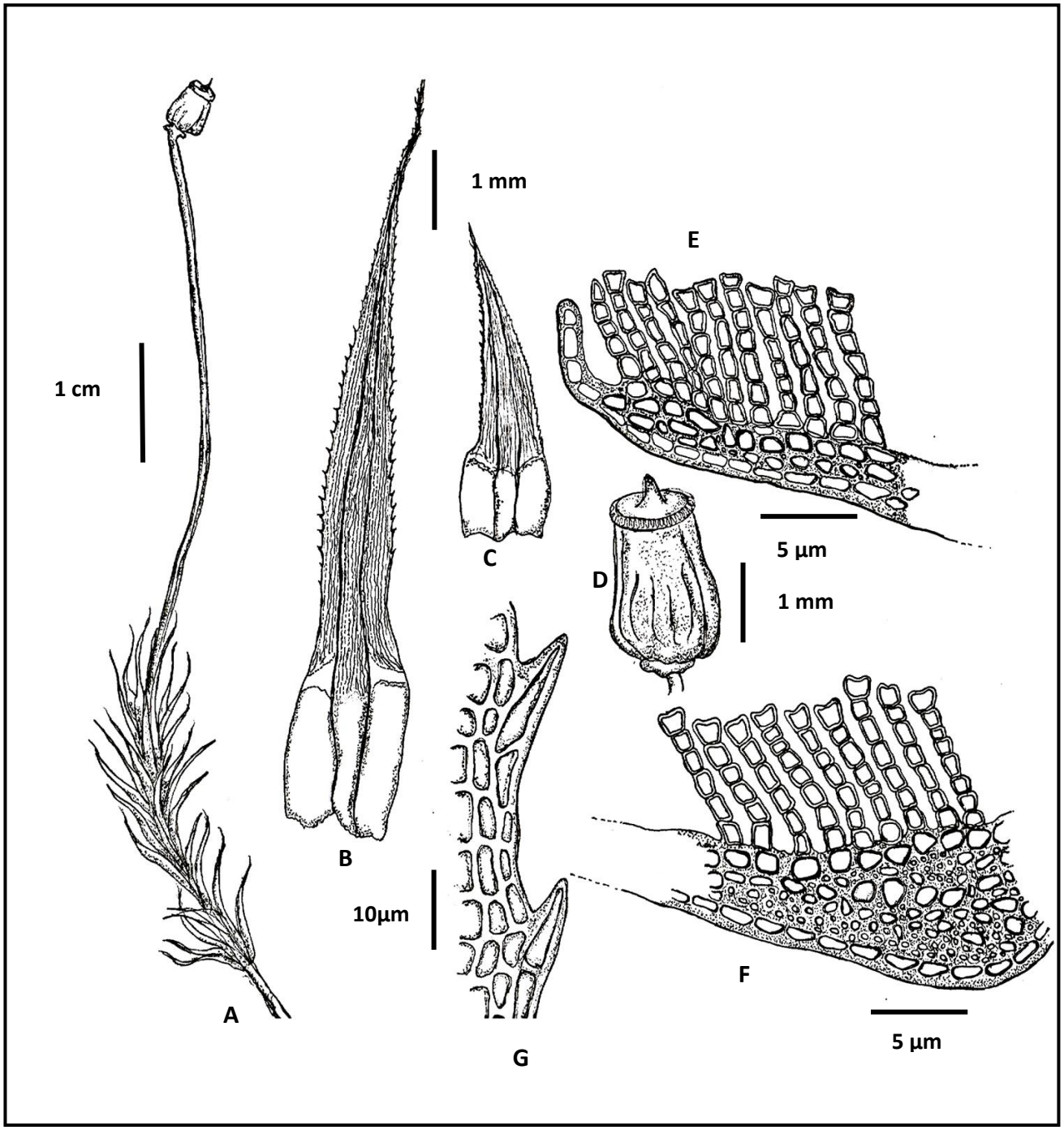

Figure 4.29: *Polytrichum perigoniale* **A.** Habit- female (moist); **B.** Perichaetial leaf (moist) showing the long dentate arista; **C**. Vegetative leaf (moist); **D.** Undehisced capsule with the operculum attached; **E & F.** Leaf transverse sections showing shallowly-grooved lamellar end-cells. (A, B, & C from *Buck 50398* (NY) & D, E & F from the proposed lectotype for *P. perigoniale* (G00114590), drawn by I. U. Kariyawasam)

Ecology: This species is predominantly found in drier and acidic habitats such as gravelly paths, heathlands, leached sand dunes, sand pits and gravel pits (Figure 4.30 C) in Britain and Ireland (Atherton & al., 2010). Moreover, Atherton & al. (2010) reported in "*Mosses and Liverworts of Britain and Ireland. A field Guide*" this species, as *P. commune* var. *perigoniale*, as being "particularly characteristic of shaly forestry tracks in western Britain and is almost ubiquitous in this habitat in mid-Wales (and doubtless elsewhere)."

However, Möller (1921) reported that it can grow on both acidic and calcareous soils in Scandinavia. Nyholm (1960) reported that this species grows in wet or moist habitats, besides lakes and rivers etc., in Scandinavia.

Smith Merrill (2007) reported that this taxon is commonly found in "humus and moist sandy soil, open woods and roadside banks; mainly in high elevations" in North America. Based on the herbarium work of the present study it seems to be the most widespread member of sect. *Polytrichum* in North America. In the past this taxon was erroneously identified as *P. commune* Hedw. in most North American collections.

De Sloover (1986) reported that *P. perigoniale* (as *P. commune* in his African taxonomic treatment) is found in Africa in much drier and xeric habitats including forests, grasslands, savanna and fynbos biomes. He (de Sloover, 1986) further reported that, "In South Africa it frequently grows on gravelly soils and steep banks of road cuts, and on soil over rock outcrops, and at the edge of marshes and also in gentle to steep slopes at 289–2865 m". In China (Wu & Wang, 2005), Taiwan (Hyvönen & Lai, 1991) and Japan (Iwatsuki, 2004; Suzuki, 2016) this taxon has been erroneously assigned to the arctic *P. swartzii* (*P. commune* var. *swartzii*) where it occurs in more exposed and drier habitats as mentioned above in other geographic regions.

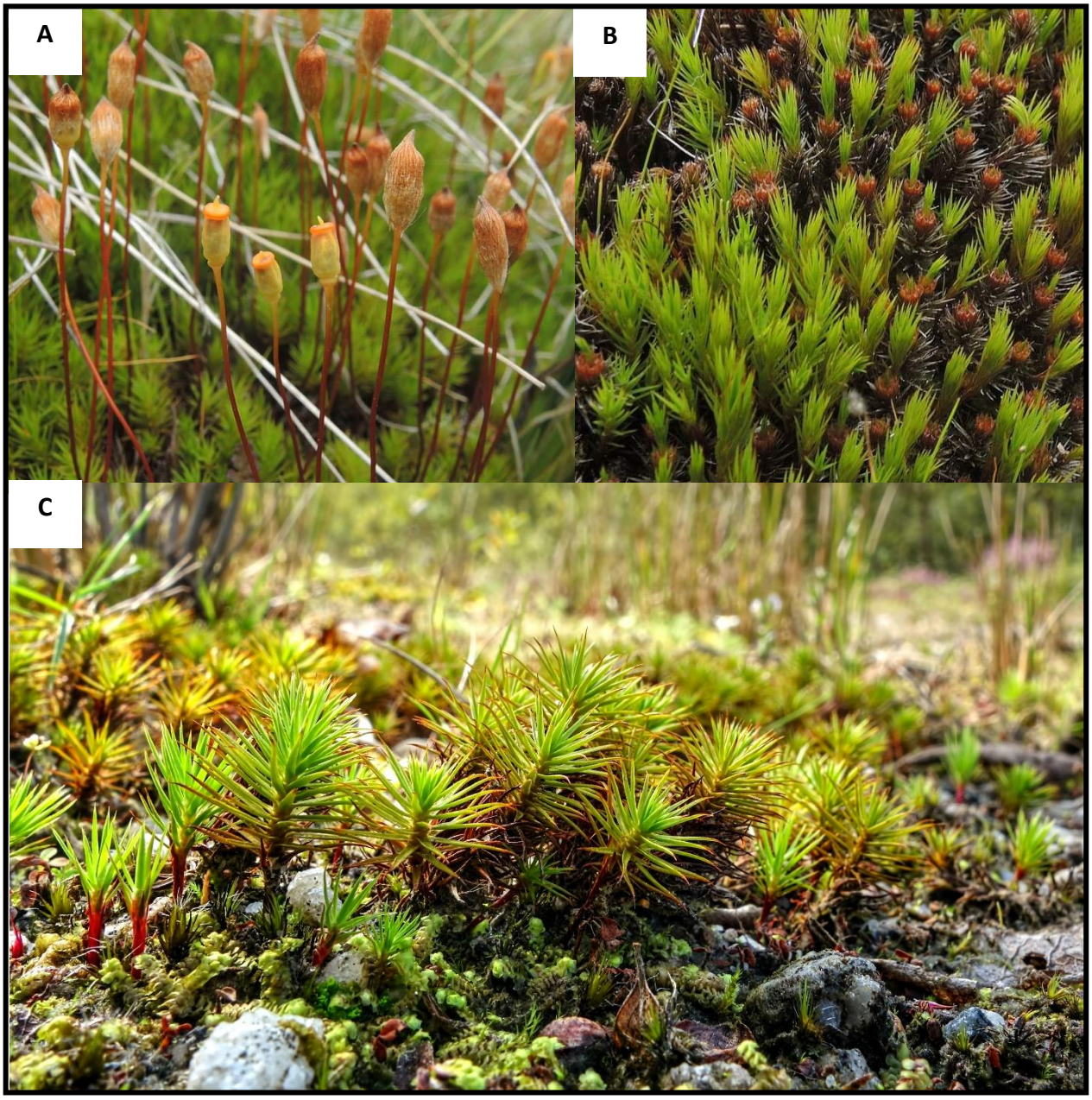

Figure 4.30: Habit of *P. perigoniale*: **(A)** Fertile female plants bearing strictly four-angled capsules; **(B)** Male plants with inflorescences (perigonia); **(C)** Short, scattered growth form in a much exposed gravel pit [Photo courtesy A & B by Michael Lüth and Photo C by Des Callaghan].

Taxonomic, Nomenclatural and Geographic Notes:

Taxonomic Note 1: **Distinguishing between *P. perigoniale* and *P. commune***

Molecular phylogenetic analysis in the present study (Chapter 03) has confirmed *Polytrichum perigoniale* Michx. to be a distinct taxon in its own right. Indeed, *P. perigoniale* in general has been an overlooked taxon worldwide. This is mainly due to its frequent confusion in the field by both bryologists and non-bryologists on account of its resemblance to the well-known and widespread *Polytrichum commune* Hedw., especially where they share drier habitats and similar ecological niches.

Based on the results of *in vitro* cultivation experiments, Schriebl (1991) suggested that *Polytrichum commune* var. *perigoniale* should be raised to specific rank but he did not present any convincing evidence in support of this. Bijlsma & van der Velde (2000) treated *Polytrichum perigoniale* as a distinct species with the aid of allozyme electrophoresis. In their study, they have included in this taxon the earlier *Polytrichum commune* var. *humile*. They applied the name "*Polytrichum commune sensu stricto*" for plants of the "*P. perigoniale* type" and the name "*P. uliginosum* (Wallr.) Schriebl" for plants inhabiting wet habitats. This creates confusion for bryologists as well as non-bryologists in applying the name "*Polytrichum commune*". However, selecting of lectotypes for both *P. commune s. str* (Chapter 02) and *P. perigoniale* (see below) has resolved this confusion.

Plant habit or the external appearance of the plant viewed from above (leaf orientation on the shoot), leaf characters such as leaf dentation, morphology of inner perichaetial leaves, microanatomical characters such as the nature of leaf lamellar end-cells in transverse section and size of the capsules are some distinguishing characters of these two taxa. Although the size and morphology of inner perichaetial leaves of *P. perigoniale* is a very useful character to distinguish it from *P. commune* in the field, it is only useful for fertile populations. Hence the most promising character to distinguish between *P. perigoniale* and *P. commune* is a study of leaf transverse sections to study the morphology of lamellar end-cells. Table 4.2 provides a summary to distinguish between *P. perigoniale* and *P. commune* from some notable morphological and anatomical characters.

Table 4.2: Some useful morphological and anatomical characters to separate *P. perigoniale* from *P. commune*

Character	***Polytrichum commune***	***Polytrichum perigoniale***
Habit	Shoots can grow up to ca.40–45 cm tall; starry appearance viewed from above.	Shoots comparatively short, can grow to ca.14 cm tall; forms rather dense tufts or tufts of tough leaves and crowded at the top.
Leaf dentation	Margins of the vegetative leaves very sharply toothed (serrulate).	Margins of vegetative leaves slightly dentate or serrate.
Perichaetial leaves	Inner perichaetial leaves or the perichaetial leaves surrounding the base of the seta strongly tapering and toothed above but never forming an irregularly twisted, ribbon-like, hyaline arista at the apex.	Inner perichaetial leaves surrounding the base of the seta are longer than the vegetative leaves and forming a irregularly twisted, ribbon-like, hyaline arista at the apex. Inner perichaetial leaves are even longer than those of *P. commune.*
Leaf transverse section	Lamellar end-cells in leaf transverse section showing deeply grooved or U-shaped lamellar end-cells with thick walls and sometimes ornamented with papillae.	Lamellar end-cells in a leaf transverse section irregularly furrowed, flattened or oblique without thick walls and papillae.
Capsule	Strictly 4-angled, cubic, somewhat larger capsule which is borne on a long (ca. 12–14 cm) long seta.	Strictly 4-angled, cubic and somewhat shorter than the capsule of *P. perigoniale* and borne on a comparatively shorter seta (ca. 8– 10 cm)

Taxonomic note 2: **Potential pseudocryptic taxa found in the "*P. perigoniale* complex"**

The molecular phylogenetic study conducted in the present study (Chapter 03) shows that the *P. perigoniale* clade is in fact a species complex with two or more pseudocryptic taxa (see Chapter 3, Figure 3.6). As mentioned above, *P. perigoniale* is widespread in drier habitats of Africa and Australasia. Accessions from Africa and Australasia show two different morphological forms with strong statistical support in the phylogeny. The African sub-clade comprising accessions from Tanzania, Kenya and Madagascar represents a morphological group containing leaves with a broader lamina and deltoid leaf base, broader lamellar end-cells with slightly thickened edges and longer capsule urns, whereas the Australasian sub-clade comprising the accessions from Australia, Tasmania and New Zealand represents a morphological group containing plants with slender leaves with shorter limbs, tapering ends and a narrower base, lamellar end-cells flat and somewhat broad and without thickenings at the edges and with somewhat shorter capsule urns. This implies that *P. perigoniale* requires broader attention with more taxon sampling from these geographic regions to study the cryptic and /or pseudo-cryptic speciation occurring within the taxon. These may turn out to be either extreme forms or varieties derived within *P. perigoniale* or potentially new species which have a close affinity to *P. perigoniale.*

Nomenclatural Note: **Selecting a lectotype for *Polytrichum perigoniale* Michx**.

The French botanist André Michaux described *Polytrichum perigoniale* in *Flora Boreali-Americana* (1803) from his collections from Carolina, USA, but he also included Europe in its distribution "In Carolina: etiam in Europa". In this protologue he noted that the taxon had an affinity to *P. commune major* (= *P. commune s.str*) and that it possessed serrate leaves and distinct perichaetial leaves. Clearly a lectotype is needed to resolve the application of the name, either from North America or Europe. Although most of Michaux's collections are housed in the Paris Herbarium (PC) (Sayre, 1976; Sorrie, 2004), no specimen of *P. perigoniale* from Michaux's herbarium has been located in PC to potentially select as lectotype.

However, Johannes Hedwig exchanged many specimens from other collectors (Chapter 02), including Michaux (Price, 2005) and, fortunately, an original herbarium sheet for this species is preserved in the Hedwig-Schwägrichen herbarium (G00114590). This is reproduced below as Fig. 4.31; it is annotated in Schwägrichen's hand as "*Polytrichum perigoniale* a. Carolina Rich. [Richard], b. Fontainebl. [Fontainebleau], c. Fontainebl.]". Specimens b and c clearly came from Fontainebleau south of Paris. The Carolina plants most

likely came to Hedwig from the French botanist L.C.M. Richard (1754-1852), who according to Sayre (1976), worked closely with Michaux on the bryophytes in *Flora Boreali-Americani*, and may even have contributed to or written the text. The Geneva sheet (G00114590) bears eight stems, each of which was annotated by Schwägrichen ‘a’, ‘b’ or ‘c’. Individual leaves from each of these stems have been dissected and studied anatomically, in order to identify each. As shown in Fig. 4, these individuals are newly labelled as eight ‘elements’ A to H, as follows: Schwägrichen a (Carolina, Richard) = elements C (sterile), D, E and F (all female plants with sporophyte); Schwägrichen b (Fontainebleau)= element G (female plant with sporophyte); Schwägrichen c (Fontainebleau) = elements A (sterile), B (male) and H (female with sporophyte and detached calyptra). Following detailed study, all the European plants (Elements A, B, G and H), have serrulate leaves with deeply-grooved, U-shaped lamellar end-cells, conforming to the current understanding of *Polytrichum commune* Hedw., as defined and lectotypified by Kariyawasam & al. (2021). The North American plants labelled ‘Carolina’ (Elements C, D, E and F) represent a single entity and may have come from a single collection. Study shows that they possess dentate leaves with narrowly-grooved lamellar end-cells and long perichaetial leaves (compared to elements A, B, G and H) and they agree with the current concept of *P. perigoniale* as well as with the protologue of Michaux (1803). Hence elements C, D, E & F are available for selection as a new lectotype for *P. perigoniale*, and one of the sporophyte-bearing plants, element E, has been selected as the most appropriate lectotype.

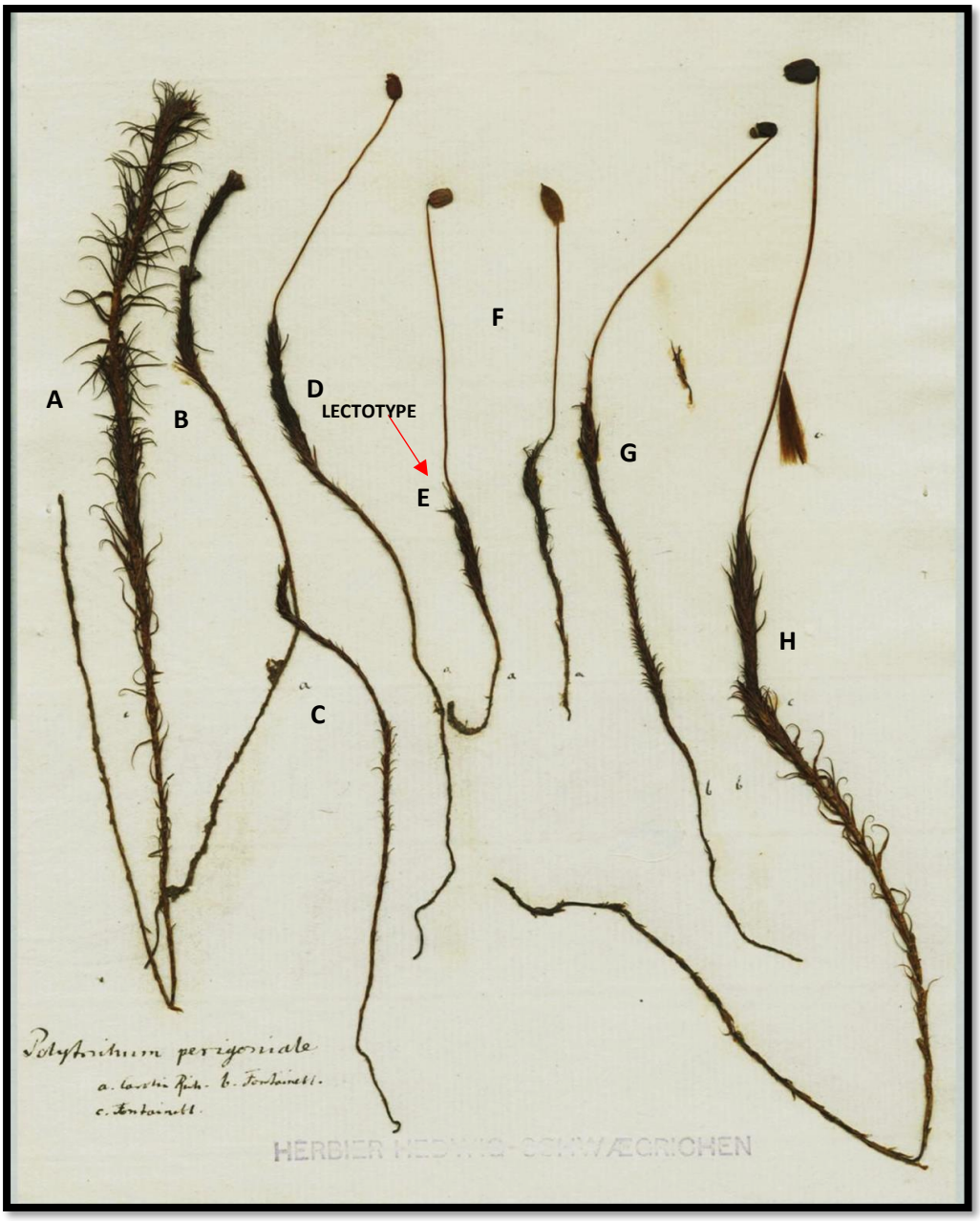

Figure 4.31: *Polytrichum perigoniale* Michx.: original Hedwig herbarium sheet from the Hedwig-Schwägrichen Herbarium in Geneva (G00114590), showing Schwägrichen's annotations a, b and c and the eight elements newly labelled A to H. The plants from Fontainebleau in France (A, B, G, H) are identified as *Polytrichum commune* Hedw., those from Carolina in the USA (C, D, E, F) are identified as *P. perigoniale* Michx., of which E is selected as a new lectotype.

Geographical Notes:

In the taxonomic treatment of Polytrichaceace in Brazil, Peralta & Yano (2010) did not report the occurrence of *P. perigoniale* in the country. However, in the present study, a specimen labelled as *P. brachymitrium* collected from Brazil (*Pirani 5493*; SP3888339) is confirmed as *P. perigoniale* in the molecular phylogeny (Chapter 03; Figure 3.6). Similarly, a few other specimens from Brazil (SP), initially identified as *P. brachymitrium,* are now confirmed as *P. perigoniale* from morphological study (see below). This greatly expands the known geographical distribution of *P. perigoniale* and also adds a new species to the Brazilian bryoflora. Since the poorly-studied South American taxon *P. brachymitrium* possesses a somewhat similar lamellar end-cell morphology (narrowly grooved, asymmetric, oblique end-cells) to that of *P. perigoniale,* they are easily confused microscopically.

In North America, *P. perigoniale* seems to be the most widespread species when compared to *P. commune s. str*. Almost all specimens used for the present molecular study from North America were previously labelled as "*Polytrichum commune*". This has necessitated revision of the phytogeography of *P. perigoniale* in North America. Based on the present study, the following states are added to those listed by Smith Merrill (2007) in *Moss Flora of North America*: Ga (Georgia), Rhode Island (RI), Arkansas (AR), Illinois (Ill), Connecticut (Conn), Pa (Pennysylvannia), Idaho (ID), Oregon (Ore) for *P. perigoniale* in North America. Crum & Anderson (1981) mentioned that *P. commune* var. *perigoniale* (now *P. perigoniale*) is common in the Atlantic coastal plain, as in the Pine Barrens of New Jersey (the type was from Carolina). Elsewhere, its distribution and ecology are poorly documented. Hence, extensive herbarium work is needed to re-determine the numerous specimens housed in North American herbaria currently labelled as *P. commune*.

In China, Taiwan and Japan, specimens identified as *P. commune* var. *swartzii* are now placed under *P. perigoniale*. Although *P. swartzii* is excluded from these countries, *P. perigoniale* is now added to the moss checklists of these countries, as confirmed morphologically and using molecular data in the present study.

In Australasia, *Polytrichum perigoniale* is poorly documented. In the Annotated Checklist of Mosses in Tasmania by Dalton & al. (1991), only *P. commune* was recorded. However, some specimens from Tasmania identified as *P. commune* (cited below in the specimen list) are confirmed as *P. perigoniale* from the present study. Similarly, Hyvönen (2006) has lumped *Polytrichum perigoniale* under *Polytrichum commune*. The illustration (Fig

15. E) of the leaf transverse section labelled *P. commune*, shows characteristic flattened or shallowly-grooved lamellar end-cells typical of *P. perigoniale*. However, Hyvönen (2006) has mentioned in his treatment as follows. "[…..] Two varieties (var. *commune* and var. *perigoniale*) have been distinguished in Australia, but their status is still in dispute, and they are not recognised here". From the present study, I presume *P. perigoniale* is the widespread taxon of *Polytrichum* sect. *Polytrichum* in Australasia and it was often erroneously identified as *P. commune s. str*, which is rare or completely absent in Australasia.

In conclusion, *P. perigoniale* now merits a detailed phylogeographic study with more intensive sampling in Africa, Australasia, South America and Southeast Asia to fully understand its diversity and speciation patterns.

Additional Specimens Observed

AUSTRALIA, NEW SOUTH WALES: Echo Point track to cascade, Blue Mts., Katoomba, [-33.719874S 150.322102E], damp trail margin, 10-Aug-1972, *W.B. Schofield 50911* (UBC); Leura Cascades trail, Blue Mountains, stream margin, 04-Aug-1983, *W.B. Schofield 81164* (UBC); Gannons Creek [-31.500034S 152.638288E], 11 km SW of Wauchope, cool temperate forest on edge of *Eucalyptus* forest, ca. 150 m, on ground in disturbed area, common, in scattered colonies, 28-Aug-1978, *H. Streimann 5946* (UBC); Megalong Valley Road, near top, Blue Mts., [-33.679349S 150.267721E], earth by streamlet, ca.150 m,19-Jun-1983, *W.B. Schofield 79639* (UBC);

AUSTRALIA, TASMANIA: ca. 74 km WNW from Hobart, N of Gordon River Road, ca. 15 km W from Maydena, State Forest SW of Mt. Field NP, Florentine road, ca. 1 km NW from The Gap (pass between Wherretts Lookout and Tim Shea), *Eucalyptus-Nothofagus cunninghamii* forest, roadside ditch, abundant on sand in partial shade, alt. ca. 550 m, 42°42´S, 146°28´E, 14-Jan-1993, *J. Hyvönen 6034* (H); ca. 74 km WNW from Hobart, N of Gordon River Road, ca. 15 km W from Maydena, State Forest SW of Mt. Field NP, common and adundant by trailside in partial shade in moorland dominated by *Gymnoschoenus sphaerocephalus* with *Restionaceae*, and shrubs *Banksia marginata* plus Myrtaceae and Ericaceae, alt. ca. 800-900 m, 42°42´S, 146°28´E, 14-Jan-1993, *J. Hyvönen 6044* (H); ca. 60 km SW from Hobart, ca. 15 km WSW from Geeveston, Hartz Mts. NP, minor road to Mt. Hartz, *Eucalyptus-Nothofagus cunninghamii* forest, very abundant on open mesic sand by roadside, alt. ca. 650 m, 43°12´S, 146°46´E, 17-Jan-1993, *J. Hyvönen 6054* (H); SW corner of Mount Field National Park and just W of it, trail from car park at E end of F8 East Road to

Growling Swallet, 530–570 m, *Eucalyptus regnans-Northofagus cunninghamii-Atherosperma moschatum* rainforest over limestone, -42.6879S, 146.496E, 04-Dec-2007, *W.R. Buck 52609* (NY); Southwest National Park, Wedge River Picnic Area, N of Gordon River Road (Hwy B61), 330 m, picnic area with shrubs and adjacent rainforest swamp, -42.8586S, 146.234E, 05-Dec-2007, *W.R. Buck 52736* (NY); Tasmania. Great Western Tiers, track to Westmoreland Falls, ca. 400 m, -41.33S, 146.17E, 28- Nov-1988, *J.A. Curnow 2352* (NY).

AUSTRALIA, VICTORIA: Dargo High Plains, Alpine National Park, 41 km NNW of Dargo, sub-alpine swampy grasslands. on moist ground amongst grasses, amongst *Empodisma minus*, 1620 m, -37.85S, 147.33 E, 17-Dec-1993, *H. Streimann 53175* (NY).

BRAZIL: Minas Gerais, Santana do Riacho [19°10′8″S 43°42′50″W], 21-Apr-2006, *Pirani, J.R. 5493* (SP)

CANADA, BRITISH COLUMBIA: Pass Creek [49.388589N -117.679178W], *s.d., J. Macoun 269* (NY, UBC).

CANADA, NEW BRUNSWICK: Carleton County, Mount Carleton Prov. Park, start of Sagamook Trail, at Nictau Lakeshore Rd., 290 m, on gravel, [+47.419281N -66.868998W], 09-Aug-1988, *V.N. Bristow with E. Haber & H.R. Hinds, s.n*. (NY); Albert County, Fundy National Park, along road connecting Point Wolfe Road & Highway 114, vicinity of Point Wolfe, on soil bank in clearing, [45.58N, -64.98W], 08-Jul-1968, *R.R. Ireland Jr. 11535* (NY,UBC); York County, 1.0-mile N of Lake George, clay bank along road, [45.85 N, -67.03W], 02-Jul-1974, *R.R. Ireland Jr. 16880* (NY); Kent County, 3 mi. W of Rexton [+46.637469N -64.948843W], 06-Aug-1970, *R.R. Ireland 14209* (NY,UBC).

CANADA, NOVA SCOTIA: Annapolis County, 5.0 miles E of Dalhousie West, Spruce-fir woods on humus over rock, [44.72N, -65.17W], 23-Jul-1974, *R.R. Ireland Jr. 17738* (NY); Shelburne Co., along Roseway River, 2.0 miles N of Upper Ohio, in woods, on ground, [+44.013412N, 65.441986W], 28-Jul-1968, *R.R. Ireland Jr. 12224* (NY, UBC); Halifax County, Halifax, in wet pastures, [44.63N -63.58W], 11-Jul-1899, *J. Macoun s.n*. (NY, UBC).

CANADA, ONTARIO: Shoals Prov. Park, 1 mi W, 32 mi W of Chapleau County [+47.862394N -83.839897W], 21-Aug-1971, *Robert R. Ireland 15569* (NY, UBC); Parry Sound Distr., ca. 4 km N of Restoule, Carleton twp., 205 m, bank along road, [+45.986798N -79.720426W], 26-Jul-1986, *R.R. Ireland 22129* (NY); Parry Sound District, ca. 8.0 km N of Dunchurch, Hagerman Township, in wet depression in woods, mixed deciduous woods,

[45.68N, -79.85W], 28-Jul-1987, *R.R. Ireland Jr. 23154* (NY, UBC); Haliburton Co., ca. 16.0 km SE of Tory Hill, Monmouth Township, sandy bank along road & adjacent coniferous-deciduous woods, on sandy soil along road, [44.92 N, -79.25W], 24-Jul-1987, *R.R. Ireland Jr. 22924* (NY).

CANADA, **PRINCE EDWARD ISLAND**: King's County, 2 mi. s. of Murray River [+45.986798N -62.600719W], 28-Jul-1970, *R.R. Ireland Jr.13845* (NY, UBC).

CHINA, GUIZHOU: Jiangkou County, Mt. Fanjing: Golden top, Jiu-long pool, ca. 2700m, on a bog, 22-Jul-1983, *G. Chien 33903* (UBC); Guizhou, Fanjingshan Nature Reserve. Wulin Mountains. Ju Long Chi (Nine Dragons Pools) below Jin Ding, 6000-7000 ft, [27.918931 N, 108.690449E], 05-Nov-1991, *M. Bourell 4644* (UBC).

CHINA, SHANGHAI: Sheshan, [31.695362N 121.195166E] 13-May-1964, *Xu Shuming, Li Yuanmei, s.n.,* (PE); behind the Peak of Sheshan Mountain, 13-May-1964, *Xu Shuming, Li Yuanmei, s.n.,* (PE); Sheshan ,07-May-1964, *Xu Shuming, Li Yuanmei, 456* (PE); Sheshan, 12-May-1964, *Xu Shuming, Li Yuanmei.481* (PE).

CHINA, SICHUAN: Chongqing City, Nanchuan County, Jinfo Mountain [29.019369N 107.175225E], under the ancient Buddha cave, under the tower head in the small basin, ca.1850 m, 29-Jul-1986, *P.-C. Wu 21025* (PE); Chongqing, Jinfo Mountain, Nanchuan County, below the ancient Buddha Cave (S entrance), ca. 2100 m, 20-Aug-1987, *X.-Y. Hu H-0808* (PE); Chongqing, Jinfo Mountain, Nanchuan County, below the ancient Buddha Cave (S entrance), ca. 2100m, 20-Aug-1987, *X.-Y. Hu H-0797* (PE); Chongqing, Dangyang Township, Guanyang Town, Wushan County [Woshanxian] [31.397143N 109.983753E], 11-May-2008, *Y. Jia 10093* (PE); Chongqing, Jinfo Mountain, Nanchuan County, below the ancient Buddha Cave (South entrance), ca. 2100, 20-Aug-1987, *X.-Y. Hu H-0799* (PE); Sichuan, Nanchuan County., Mt. Jinfu, ca. 1850m, on ground, 29-Jul-1986, *P.-C. Wu 21015* (UBC).

DENMARK: northern end of Jutland, Råbjerg Mile [57.648523N +10.401536E], between the dry zone of *P. piliferum* and the moist zone of *P. perigoniale* in dunes, 11-Jun-1968, *E. Nyholm, 10/68* (S); Northern end of Jutland, Råbjerg Mile, in depression in dunes, 11-Jun-1968, *E. Nyholm, 08/68* (S); Kjersgård Klit, Jylland [57.523904N +09.877607E] sanddyn, 16-Jun-1968, *G. Holmbrin s.n.* (S).

FINLAND: Nylandia (RT90), [60.2N, 24.9E], 0-Jul-1868, *S. O. Lindberg, s.n.* (LD)

GERMANY: West Pomerania, 12-Sept-1957, *S. Lisowski 474* (PE)

GREAT BRITAIN, ENGLAND: (vc 70) Cumberland, River Calder valley, path to Bomery Gill, near edge of path, [54.501996N -003.434536W], *s.d.*, *E.M. Kungu s.n.* (E); (vc 01)South of Georgia, West Cornwall, [50.162293N -005.528391W],15-April-1997, *D. T. Holyoak 97-213C* (E); (vc 02) East Cornwall, [50.504762N -004.539729W] ca. 225m, 10-Aug-2007, *D. T. Holyoak 07-120* (E)

GREAT BRITAIN, SCOTLAND: (vc 76) Renfrewshire, Cample Burn, Muirshiel Country Park, on thin soil over rocks, [55.821230N -004.715118W], 24-Apr-2015, *E.M. Kungu s.n.* (E); (vc 72) Dumfriesshire, Kello Water, Kelloholm, by path on N bank of river [55.373985N -003.994622W], by path, NS 737 107, 03-Jul-2016, *E.M. Kungu s.n.* (E); (vc 79) Yair Hill, Selkirk [55.581908N -002.904156W], 1886, *J. Noble s.n.* (BC); (vc 105) Bealach na Ba, Applecross Peninsula, walking path, North face, rock outcrop, under rocks, 57.418244N, -005.693964W (NG7828 4246), 24-July-2017, *I. U. Kariyawasam 166* (E); New Galloway, Mar-1889, *W. Darsalloch s.n*. (E)

GREAT BRITAIN, WALES: Snowdon, Pen-y-Pas [53.080678N -004.020850W], in *Sphagnum compactum*, 24-Jul-1964, *E. Nyholm 27/64* (S); Snowdon, Pen-y-Pass, On moist, wet ground with *Nardus* and *Galium saxatilis*, 24-Jul-1964, *E. Nyholm 26/64* (S).

JAPAN, **HOKKAIDO**: Prov. Kamikawa, around Kogen Spa, SE foot of Mt. Hakuun, Mt. Daisetsu [43.625795N 142.930896E], warm, moist, sloping soil, in open, 08-Jun-1971, *A.J. Sharp with E. Sharp and M. Haruki 10462a* (NY); Prov. Kamikawa, around Kogen Spa, SE foot of Mt. Hakuun, Mt. Daisetsu, on warm, moist, sloping soil, in open. 08-Jun-1971, *A.J. Sharp with E. Sharp and M. Haruki 10393* (NY).

JAPAN, KYUSHU: Yoshimuta, 03-Jul-2017, *Matsumoto & T. Katagiri s.n*. (NICH); Yakushima to Hananoego from Osugidani [30.327849N 130.493856E], earth, 1400 m, 02-Jun-1951, *Y. Kuwahara with Yukimobu 677* (NY).

JAPAN, HONSHU: Gifu-ken, Kamo-gun, Hichiso-cho, Iwai-dani, 250–350 m, on soil, 27-Oct-1992, *M. Mizutani 15746* (NY); Shiga. Mt. Hiei, Jogyo-do [35.073368N 135.833993E], *Cryptomeria* grove. On soil, 10-Oct-1975, *G. L. Smith J-1390* (NY).

KENYA: K 4, Sth./Nth, Nyeri, Aberdare National Park, [-0.451694S +37.262135E], alpine heath with *Erica arborea* and *Dendrosenecio* in protected places, in *Erica arborea* forest, 3000 m, 30-Nov-1979, *H. Balslev 654A* (NY).

MADAGASACAR: ANTANANARIVO PROV., Angavokely Forest, Pine and Eucalyptus plantation along track from station to indigenous forest, with extensive shaded granite cliffs among grasses on thin humus over rock, -18.9196 S, +47.7380E, 1500 m, 05-Dec-2009, *T.A.J. Hedderson 17341* (BOL); ANTALAHA PROV.: Marojejy National Park, montane mossy rain forest around upper rest camp, on open soil bank, 1320 m, -14.4381 S, +49.7444 E, 28-Nov-2009, *T.A.J. Hedderson 17311* (BOL).

NEWZEALAND: South Island, West Coast Region, Buller District. Track near Big Rimu Tree beside Umere Road, east of Karamea. On ground in mature forest. 41° 14' 55.32" S, 172° 11' 31.56" E, 20-Nov-2017, *N. Bell 20.11.17.003* (E); South Island. along Route 7, 0-4 mi W of Rahu Summit, subalpine bog and *Nothofagus* forest, 671 m, [-42.42S, 172.17E], 03-Dec-1972, *D. H. Vitt 8379* (NY); Roadside Ditch, *s.d., Renner 5150* (E)

POLAND: West Pomerania, Pobrzeze Koszalinskie, Slowinskie Nat. Park: ca 6 km W of village of Rabek & ca, 1 km from the Baltic shore (utm grid xa3, atpol grid ac43) [54.699856N 17.317407 E], 12-Nov-1957, *L. Piotrowska s.n.* (PE, UBC); Western Bieszczady mountains, raised bog "Wolosate" near Ustrzyki Gorne (distr. Krosno) [49.105904N 22.654912E], 06-Jul-1977, *R. Ochyra 98* (UBC); East Carpathians, Polish Eastern Beskids, western Bieszczady Mts., Pszezeliny [49.164525N 22.691065E], 27-Jun-1978, *R. Ochyra 299* (UBC).

PORTUGAL: MINHO PROV., Peneda-Gerês National Park, slopes near Portela de Leonte north of Gerês, ca. 958 m, open rocky hillside with *Pinus, Quercus* and *Erica arborea*; on open bank with *Erica umbellata* and *Calluna*, 41°46'35.4"N, 08°08'54.3"W, 07-Jun-2010, *D.G. Long & D. Bell 39144* (E); BARRAGEM PROV., Alto Alentejo 39.4692ºN 7.5576ºW , ca. 320 m, 23-Dec-2007, *D.T. Holyoak 07-493* (E); AZORES ISLAND, 1865, *F.D. Godman, Esqr., s.n.* (BC)

SOUTH AFRICA: LIMPOPO PROV., Louis Trichardt area, Blouberg, trail from Fransie SE Kraal to open valley below summit, -23.081677S 28.959444E, map sheet 2328BB, grassland with scattered *Widdringtonia* clumps on plateau, tufts in rock crevices, ca 1600 m, 09-Oct-2004, *T.A.J. Hedderson 15708* (BOL); CAPE PROV., Caledon Division [-34.219575S 19.415071E], 03-Feb-1982, *J. Williams, 3183/1* (BOL).

SWEDEN: Uppland, Sollentuna sn., Tunberget [59.449648N 17.947993E], 29-Apr-1928 *R. Florin s.n* (S); Vasterbotten, Lovanger, Uttersjon, [64.484274N 21.385245E], 1870, *L. Andersson s.n.* (S); Norrbottens Lan Lappland. Lake Tornetrask area. [68.251218N

18.892686E], ca. 1100 m, 23-Aug-1980, *Dale H.Vitt with D. G .Horton & N.G. Slack 26350* (PE); Härjedalen: Tännäs, Näsfjället [62.368323N 13.408614E], reg. alpina, 850-1060 m, 27-Jul-1928, *T. G. Halle, s.n.* (S)

TAIWAN: Taipei Hsien, Chihsing Shan, [25.166987N 121.560670E] on black soil of lake, 29-Mar-1974, *S. Lin 139* (UBC); Taichung Hsien, Toping Hsiang, the entrance to Chunghsueh Shan, 27-Feb-1978, *S. Lin 238* (UBC); Pa-yu Lake, co. [22.735710N +120.896404E], 28-Jul-1967, *C.C. Chuang 5079* (UBC); Bayu Lake, Tai-tung-hsien, [22.735710N +120.896404E], 21-Jan-1965, *S. Kurokawa 88(1801)* (UBC).

TANZANIA: Moshi, Mt. Kilimanjaro, ridge above Umbwe Stream Gorge, -3.123897S, 37.273145E, 2200 m, 01-Mar-1990, *R.E. Magill, T. Pócs & C. LaFarge England 9333* (GOET).

TURKEY: PROV. RIZE: about 3 km W of Ardesen, [41.179041N +40.940998E], +/- 5 m a.s.l., moist soil in hazel- and fruit garden, 26-Oct-1974, *T.-B. Engelmark & E. Nyholm 241a/74* (S); PROV. BURSA: N part of Uludag [40.115627N +29.249106E], +/- 1500 m a.s.l., sandy, moist soil on hillside, 09-Jul-1978, *E. Nyholm 578 (30), 78* (S).

USA, ARKANSAS: Van Buren County, Ozark National Forest, on Forest Service Road 1335, just uphill and W of Driver Lake, 245 m, *Pinus taeda* and *P. echinata* forest, 35.4667N, -92.7344W, 08-Nov-2002, *W.R. Buck 43158* (NY); Stone County, Cherokee Wildlife Management Area, N slope of Tater Hill, along County Rd. 23 (Signal Hill Rd) 2 mi SE of jct with Luber Cutoff Road, 6.1 mi SE of jct with County Rd. 21 (Hanover Rd), sandstone bluff. mixed hardwoods, 35.7628N, -92.0544W, 24-Oct-2001, *W.R. Buck 40345* (NY); Newton County, Boston Mountains, Ozark National Forest, 600 m, Alum Cove Recreation Area, hardwood forest and large sandstone outcrops along stream, 35.8697N, -93.2314W, 24-Apr-1988, *W.R. Buck 15810* (NY); Grant County, ca. 1.4 mi. E of Hwy 229 on Grant Co. 3., on soil, 27-May-2001, *P. Majestyk 2378* (NY); St. Francis County, mile marker 233 at rest area on I-40, bank of small ditch, 20-Jun-2001, *P. Majestyk 2394* (NY); Hempstead County, on co. rd. 16, Oak Grove Church, ca. 3 mi SW of Blevins, on soil, 33.8364 N, -93.6044W, 1996, *P. Majestyk 1963* (NY).

USA, CONNECTICUT: Litchfield County, Town of Canaan, Great Mountain Forest, Sam Yankee Woodloot, E of Canaan Mountain Road, mixed hardwood-conifer forest, 41.9536 N, -73.2764 W, 20-Sep-2003, *W.R. Buck 45001* (NY)

USA, FLORIDA: Leon County, Cascades [30.431939N -84.277870W], near stream, 06-Nov-1937, *R.O.S. Breen 212* (NY); Wakulla County, Apalachicola National Forest, Bradwell Bay Wilderness, along Apapalachicola Trail at Sopchoppy River, ca. 1/4 mi SW of jct. of Forest Serv. Rds. 348 and 329 [30.130265N -84.494914W], Hardwood scrub, 29-Nov-1988, *W. R. Buck 16479* (NY)

USA, GEORGIA: Coffee County, Broxton Rocks Ecological Preserve, 9 mi NE of Broxton, 3 mi S of Ocmulgee River, extensive sandstone outcrops with glades and bluffs in pine-oak forest, 31.73N, -82.75W, 16-17- Dec-1993, *W.R. Buck 24986* (NY).

USA, IDAHO: Idaho County, Eldorado Creek, edge of fen east of Road 524, 1103 m, *Abies lasiocarpa*, *Thuja plicata*, *Picea engelmannii* forest, with *Ledum glandulosum*, *Carex angustata*, 46.3096 N, -115.643W, 02-Sep-2005, *K.L. Gray 5269* (NY).

USA, ILLINOIS: Shelby County, Hidden Springs State Forest, ca. 14 mi W of Neoga, along Rocky Spring Nature Trail, Oak-hickory forest, [39.3186 N -88.69W], 28-Apr-1988, *W. R. Buck 15937* (NY)

USA, INDIANA: Cass County, 0.5 mi S of Lake Cicott, in woods, on soil, 26-Nov-1937, W. H. *Welch 14076* (NY); Lawrence County, along Back Creek, [38.875483N -86.304348W] dry cliff. Associated by *Tsuga canadensis*, 28-May-1933, *R. M. Kriebel 170* (NY)

USA, MAINE: Androscoggin County, Town of Durham; along S bank of Androscoggin River at Lisbon Falls, 44 m, on boulders along river just below falls, 43.9925, -70.0586, 08-Aug-2006, *B. H. Allen 27845* (NY); Aroostook County, Allagash Plantation. Along Saint John River 1.7 mi NW of Allagash Bridge on Rte. 161, [47.099185N -69.071555W] on ground in gravel pit. 23-Jun-2006, *B. H. Allen 27592* (NY); Kennebec County, Rome Town, Beaver Brook to Beaver Pond from Watson Pond road near Belgrade Lakes, 122 m, overturned stump, on bare soil, 44.5469 N, -69.9181W, 23-Aug-2003, *B. H. Allen 25806* (NY); Hancock County, near N. Sedgwick, [44.340877N -68.589507W] roadside on gravel, 05-Jun-1978, *Gillis Een, s.n.* (S).

USA, MASSACHUSETTS: Coonamesset River, 1 3/4 mi. NNW of East Falmouth, [41.596329N -70.568823W] under pines, edge of old field along road, 29-Jun-1946, *T.S. Githens 2568* (NY); Forest Street, Waltham, 05-Sept-1912, *A.B. Seymour s.n.* (S); Weston, Case Estate of the Arnold Arboretum, Wellesley Road, [42.332179N -71.311117W] common in oak-maple woods, 29-Oct-1961, *J.H. Thomas 9801*(UBC).

USA, MISSISSIPPI: Tishomingo County, Tishomingo State Park, above Swinging Bridge, Oak woods, exposed. Over sandstone, 100 - 200 m, [34.62 N -88.2W], 27-Sep-1992, *W. R. Buck 21926* (NY)

USA, MISSOURI: east-facing bluffs and wooded slopes with numerous dolomitic outcrops along Hunter Branch of Bryant Creek near Vera Cruz, 29R14W, T26N, Douglas County, [36.918118N -92.495501W], dolomitic ledge near summit of ridge, 02-Aug-1961, *P.L. Redfearn, Jr. 9039* (UBC).

USA, NEW YORK: Clinton County, Town of Mooers, Gadway Sandstone Pavement Barrens Preserve, SW of Cannon Corners Road, extensive Potsdam Sandstone pavements, *Pinus banksiana*-dominated forest, 44.9489 N, -73.7556W, 19-May-2007*, W.R. Buck 51194* (NY); Albany County, Town of Knox, Limestone Rise Preserve, along NY 146 ca. 5 mi W of Altamont, S of hwy, on soil, 42.7008N, -74.1403W, 16-Aug-2004, *J.Y. Kekes 964* (NY); Putnam Co., town of Southeast, Field Farmstead Preserve, N of Field Lane, 0.3 mi WSW of North Salen Road, 125 m, soil in trail, 30-Jun-2002, 41.3653 N, -73.6211W, *B.H. Allen 24350* (NY); [10]Herkimer Co. Little Falls. Moss Island in Mohawk River/Erie Canal, extensive gneiss outcrops. hardwood forest, 14-Jun-2011, *W.R. Buck 57829* (NY)

USA, NORTH CAROLINA: Macon County, Cliffside Lake, Highlands [35.078722N -83.235053W], *W.B. Schofield 9039* (UBC)

USA, OHIO: Adams County. Brush Creek Township, Spring Glen Natural Area, E side of Tulip Road (CR 59), E of dead end of Cline Road (Twp. Rd. 226), 1.8 mi S of Lynx, 215 m, wet calcareous seeps and dolomite exposures, *Juniperus* glades, 38.7603N, -83.4114W, 22-May-2006, *W.R. Buck 50398* (NY).

USA, OREGON: Wasco County, Mount Hood National Forest, Wapanita Pass near Mount Hood on US 26, Frog Lake Campground, Montane *Abies-Tsuga* forest, 1200 m, 45.225N, -121.7W, 10-Aug-2000*, W.R. Buck 37577* (NY).

USA, PENNSYLVANIA: Lancaster County, Martic Forge, Marticville. [39.905405N -76.327341W], Aug-1910, *J.F. Collins, s.n.* (NY); Pike County, Delaware Township, Delaware Water Gap National Recreation Area, Geo. W. Childs Recreation Area, 4 km NW of Dingmans

[10] This specimen has been used to sequence the complete transcriptome in the IKP project (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4306014/) however the specimen was misidentified as *P. commune* Hedw.

Ferry, along Dingmans Creek, 185–245 m, series of small waterfalls over acidic rock, along stream, Hemlock forest, 41.2378 N, -74.9147W, 23-Apr-2004, *W.R. Buck 46903* (NY).

USA, RHODE ISLAND: Washington County, border of towns of Exeter and South Kingstown, Marion Eppley Wildlife Sanctuary, ca. 1.5 mi NE of Usquepaug, along Queens River, *Chamaecyparis*-hardwood swamp, 41.5183 N, -71.5925 W, 16-Sep-2006, *W.R. Buck 50985* (NY); Providence Co., Town of Lincoln, Lime Rock Nature Preserve, ca. 1 mi SW of Lime Rock, N of Wilbur Road, mostly granitic outcrops around artificial pond, mixed hardwoods, 41.9219 N, -71.4675W, 17-Sep-2006, *W.R. Buck 51086* (NY).

USA, SOUTH CAROLINA: Greenville County, Table Rock Reservoir watershed, Buzzard Mountain summit to Slicking Creek, Gneissic-granite steep slopes; common in rock pockets, with *Selaginella tortipila*, [35.076191N -82.706210W], 609.6–853.4 m, 26-Jun-1992, *S.R. Hill* with *H. Douglass & S. Muzal 23584* (NY); Greenville County, Bald Rock, along US 276 ca. 3 mi N of SC 11 toward Caesars Head, Granitic flatrock, 650 m, [35.08N -82.62W], 14-Mar-1997, *W.R. Buck 31527* (NY).

USA, VIRGINIA: Chesterfield Co., Pocahontas State Park [37.372346N -77.571467W], *Quercus-Fagus-Carya* dominated forest on soil, 12-May-2002, *P. Majestyk 3388* (NY); Isle of Wight Co., Zuni Pine Barrens, now Blackwater Ecologic Preserve, 4.5 mi. SW of Zuni [36.820122 -76.852429W], 02-May-1989, *L.E. Anderson 25,342* (S).

USA, WEST VIRGINIA: Pocahontas County, Watoga State Park, Brooks Memorial Arboretum, trail along Two Mile Run, along Co. Rd. 27, 2.0 mi W of park office, shady, humid, mixed hardwood-conifer forest, 38.13 N, -80.15W, 15-May-2000, *W.R. Buck 37083* (NY).

4.5 Excluded and Doubtful Taxa

1. *Polytrichum juniperinum* Hedw. Sp. Musc. Frond. 89, pl. 18: f. 6–10. 1801.

Syn: *Polytrichum commune* var. *humile* Sw., Adnot. Bot. 141. 1829. Type citation: [Europe] "b. *humile*. *Polytrichum quadrangulare juniperifoliis brevioribus & rigidioribus*. Dill. l.c. [t. 54] f. 2. (b) totius Europae, vulgatissimum." Type specimen: OXF, herb. Dillenius, seen by G.L. Smith Merrill.

A.J.E. Smith (2004) in his key to three varieties of *Polytrichum commune* in Britain and Ireland, contrasted *P. perigoniale* (as *P. commune* var. *perigoniale*) against a poorly-known taxon *P. commune* var. *humile* as follows: "Similar to var. *perigoniale* but perichaetial leaves abruptly narrowed from sheathing base into short acuminate apex. Similar habitats to var. *perigoniale*. Rare; and not seen recently, extending from W. Cornwall and E. Sussex north to Angus and E. Inverness, Down. 20, H1. Central Europe, Morocco, Macaronesia". In an effort to try to find molecular support for varieties of *P. commune*, two accessions previously identified as *P. commune* var. *humile* Sw. and two of *P. commune* var. *minus* Brid. (see below) from Europe were included in the molecular phylogenetic study (Chapter 03); all were confirmed as *P. perigoniale*. However, the names nave been misapplied, and cannot be synonymised under *P. perigoniale*.

Hedwig (1801) gave the Dillenian polynomial *Polytrichum quadrangulare juniperifoliis brevioribus & rigidioribus* as a synonym of his *Polytrichum juniperinum* Hedw. and his figure would appear to confirm this. However, Swartz (1829) considered it to be a small form of *P. commune*. A. J. E. Smith (2004) accepted it in the British Isles as a variety of *Polytrichum commune*, without explanation, and it was given as a synonym of *P. commune* by Ignatov & Afonina (1992). The Dillenian specimen was checked by G.L. Smith Merrill in OXF in 1974 and has confirmed (in litt.) that it belongs to *P. juniperinum* rather than *P. commune*. Hence the above synonymy is accepted.

2. *Polytrichum commune* var. *minus* Brid. Bryol. Univ. 2: 150. 1827. Type citation: [USA, Alaska, Aleutian Islands] "In Unalaschka. Chamisso." Type specimen: not seen.

This taxon was given as a synonym of *P. commune* var. *humile* Sw. by Smith (2004) and given as a synonym of *P. commune* Hedw. by Tropicos (2021). However, it is a doubtful early name from North America and could belong to some other species. It was not treated by Long (1985) or Smith Merrill (2007). Study of Bridel's type is required.

The following names are illegitimate under the ICN and are excluded, as they are either later homonyms, just herbarium names or names without a valid description and a type specimen.

i. *Polytrichum armatum* Broth. f. minor Broth., Bot. Jahrb. 24: 253 (1897) *nom. inval*. incl. f. prior.

ii. *Polytrichum commune var. africanum* Müll.Hal., in Paris, Ind. Bryol.: 997 (1898) nom. nud.

iii. *Polytrichum flexicaule* Müll.Hal.. in Geheeb, Rev. Bryol. 5: 70 (1878) *hom. illeg*. non (Mitt.) Kindb., Enum. Bryin. Exot.: 72 (1888).

iv. *Polytrichum apuleja* Comm. ex Schwägr., *nom. nud*. in. syn = *Polytrichum glabrum*

v. *Polytrichum commune* var. *minus* Weiss ex De Not., *hom. illeg* (non Brid.) = *Polytrichum perigoniale* Michx.

vi. *Polytrichum dusenii* Müll. Hal. ex. Paris. *nom. nud*. Excluded. An undescribed herbarium name (*Dusén 346*) from Cameroon.

vii. *Polytrichum flaccidogracile* Müll. Hall. ex. Geh., *nom. nud*

viii. *Polytrichum lonchobasis* Müll. Hall. *nom. nud*. An undescribed name from Madagascar.

4.6 Discussion and Conclusions

The alpha taxonomic revision accepts eight species within *Polytrichum* sect. *Polytrichum*: *Polytrichum angustifolium* Mitt., *Polytrichum brachymitrium* Müll.Hal., *Polytrichum commune* Hedw., *Polytrichum ericoides* Hampe, *Polytrichum jensenii* I.Hagen, *Polytrichum perigoniale* Michx., *Polytrichum subpilosum* P.Beauv., *and Polytrichum swartzii* Hartm.. Lectotypes for *P. brachymitrium* and *P. commune* are designated here and a lectotype for *P. perigoniale* is proposed in this study (Chapter 02). Selection of a lectotype for *P. commune* to confirm the long debating taxonomic question of the identity of the taxon has been resolved (Kariyawasam & al., 2021). Similarly, proposing a lectotype for *P. perigoniale* clarifies the application of this name, and its identity as a species distinct from *P. commune* is supported both by the morphological study (Chapter 04) and by the molecular phylogenetic analyses (see Chapter 03). A much-updated synonymy for the African taxon *P. subpilosum* is

presented in this study with exclusion of erroneously synonymised taxa in the treatments of De Sloover (1986) and O'Shea (2006). Moreover, the uncertainty around the *Polytrichum commune* concept in North America, Africa, Australasia and Southeast Asia has been clarified by studying the morphology of a broad spectrum of herbarium specimens and by the molecular species delimitation (Chapter 03). It is now confirmed that the dominant and most common species in the above-mentioned regions is *P. perigoniale*, specimens of which had previously frequently been misidentified as *P. commune.* Selection of lectotypes for both these species has been crucial in clarifying their distinction. Because De Sloover (1986) misapplied the name *P. commune* in Africa to what is now known to be *P. perigoniale*, many species and some infraspecific taxa which were earlier ascribed for *P. commune* from Africa are now transferred to *P. perigoniale.*

The present taxonomic treatment provides an updated synopsis of accepted synonymy for the species in *Polytrichum* sect. *Polytrichum.* In addition, a broad spectrum of historical collections from the pre-Hedwigian era was examined and several unassigned names and their original material were studied to add to this up-to-date synonymy for each taxon. Useful nomenclatural and taxonomic notes are presented under each taxon. Sporophytes of *P. ericoides* have been found in Colombia and are described here. This taxon was known previously only from sterile specimens and sporophytes were never described, even in the recent taxonomic treatment of Polytrichaceae in Colombia by Aponte & Uribe (2017). Hence, the present study provides the first complete alpha taxonomic revision for *Polytrichum* sect. *Polytrichum* that is global in scope, as material of all formally accepted taxa within sect. *Polytrichum* has been observed (including both gametophytic and sporophytic generations) in this study. The species delimitations within the section were clearly and concisely defined and applied, and some comparative remarks regarding allied taxa are presented. Ranges of variation within and between species are discussed and the key morphological characters to distinguish between each species are documented and illustrated. A taxonomic key is presented to identify the species.

Many regional taxonomic treatments, recent and old, have been carried out by other workers within relatively narrow geographic ranges of the extant taxa of *Polytrichum* sect. *Polytrichum.* Therefore, a broad spectrum of herbarium specimens (ca. 1500) was borrowed for study from 23 different international herbaria, and as resources and time permitted, several herbarium visits were made to study and expand the geographic span of the study and also to observe the morphological variation for each species across geographic

regions. For the present taxonomic account, ca. 600 specimens, all individually identified, are listed in the text, including the type specimens, to generate distribution maps for each species to illustrate the current understanding of the geography of each. Hence, the present study provides several new geographic records for taxa such as *P. brachymitrium* and *P. perigoniale*. Figure 4.32 shows the global distribution of *Polytrichum* sect. *Polytrichum* based on the herbarium records used in the present study.

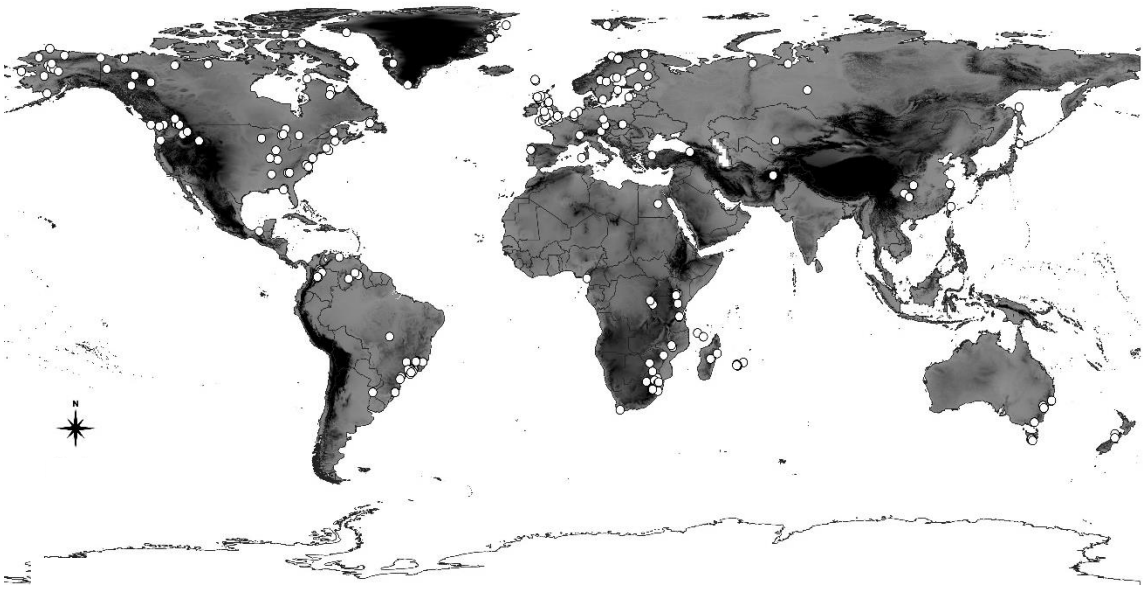

Figure 4.32: Distribution of *Polytrichum* sect. *Polytrichum* based on the confirmed herbarium records used for the present study.

However, due to time constraints, the full geographic span of *P. commune* is not presented due to the very large volume of specimens that would need to be examined in a very limited time period. However, its current geographic distribution within Europe and Macaronesia is provided in the recent publication of Hodgetts & al. (2020), where I have contributed and co-authored in compiling the distribution of *P. commune* within Europe by my observations based on the present study.

Certain geographic regions such as West Africa, Tasmania, Taiwan, Japan and the high altitude areas of the Sino-Himalaya, the Asian part of Turkey, etc. require more extensive sampling to fill geographical gaps in the knowledge of *P. commune* and its allies. The conclusion, therefore, is that some of these distributions must be considered as provisional, as in no way can they be taken as a true representation of genuine rarity or abundance of particular species, largely due to limited sampling by collectors. Members of *Polytrichum* sect. *Polytrichum* are found from the warm tropical to arctic and sub-arctic regions throughout the

Northern and Southern Hemispheres. Taxa such as *P. angustifolium, P. brachymitrium* and *P. ericoides* are confined to biodiversity-rich tropical South America, whereas *P. subpilosum* is restricted to only Africa, Madagascar and some adjacent African islands. *Polytrichum commune s.str* is widespread all over Europe and Arctic America and northern parts of East Asia. *Polytrichum perigoniale* is a widespread taxon in Europe, Australasia, Africa, South America, North America and South-east Asia.

Members of Polytrichaceae are presumed to be dispersed through spores by wind and occasionally by vegetative propagules. However, it is clear that there may be several factors involved in explaining both local endemism and the widespread nature of particular taxa in *Polytrichum* sect. *Polytrichum*; these cannot be fully understood only by studying distribution maps based solely on herbarium specimens studied. For example, there are several herbarium records of *P. commune* available in the Global Biodiversity Information Service [GBIF; https://www.gbif.org/], however, these may include a significant proportion of records based on specimens of *P. perigoniale* which have been misidentified as *P. commune* in North America, Europe, Africa, Australasia and elsewhere in South East Asia. There are also more records of *P. perigoniale* in Europe and North America than in Africa, where it is poorly documented, as Europe and N. America are more thoroughly studied. However, the present study has included a greater number of specimens world-wide than any other previous study of this taxonomic group and thus provides valuable insights into species delimitation with the benefit of molecular phylogeny (Chapter 03).

In the molecular species delimitation, *P. perigoniale* forms a "species complex" where two major pseudocryptic morphologies were observed (See Chapter 03), in Africa and Australasia. Further study is required to identify more accessions for these two pseudocryptic forms to perform a population level molecular study. It has now been demonstrated from the present study that *P. perigoniale* is the most common and widespread taxon in North America and Australasia; the misapplication of the northern *P. swartzii* and *P. jensenii* concepts in China is demonstrated and the species excluded from the country.

Branching and plant size in Section *Polytrichum* show phenotypic plasticity which varies depending on maturity. Hence, it is difficult to consider it as a useful taxonomic character; however, when the habitat of the plant is considered, growth form and height of the plant can be quite informative. For example, *P. jensenii* and *P. swartzii*, which can grow at high altitudes

(ca. 3500–4500 m), in habitats like snow beds and seepage below glaciers, exhibit dwarf (less than 4 cm) habits, whereas when growing on sandy lowland soils in Lapland they show vigorous plant growth up to 8–12 cm in height. Overall, plant habit is not a very useful character to define taxonomic identity, however it is a useful macroscopic character providing some clues to identify the taxa in the field. The texture and arrangement of leaves also provide some useful characters to identify the taxa in the field. For example, in *P. jensenii* the leaves are always flexuose and brittle, whereas those of *P. subpilosum* are somewhat thick and fleshy. The leaf orientation in both dry and wet conditions is a useful diagnostic character to identify some taxa. For, example the distant star-like arrangement of leaves is a characteristic diagnostic character of *P. commune* in the field. Moreover, the leaf arrangements and the morphology of inner perichaetial leaves help to distinguish between *P. commune* and *P. perigoniale*. Size and serration of leaf margins are also diagnostic characters to delimit some taxa in *Polytrichum* sect. *Polytrichum*. Of all the taxa, *P. ericoides* exhibits the smallest leaf size, *P. jensenii* shows entire leaf margins and *P. brachymitrium* exhibits the shortest leaf teeth among all taxa.

As Lindberg (1868) and many other authors explained, lamellar end-cell morphology has a vital role in identifying taxa within the Polytrichaceae and this particularly applies to *Polytrichum* sect. *Polytrichum*. However, it can sometimes seem not to be a discrete character where certain taxa exhibit a developmental series of different morphological forms of lamellar end-cells within a leaf transverse section. However, this is one of the most informative diagnostic characters in morphological species delimitation. Wax deposition and formation of papillae is another informative character in morphological species delimitation. Presence of papillated structures on the lamellar end-cells (e.g., the “knob-like” papillated projections in *P. jensenii*; see Figure 4.3) is also a useful diagnostic microscopic character to delimit species of the section.

The present study provides the solid groundwork for future taxonomic research in the family. Future collections will help to refine the knowledge of pseudocryptic and cryptic populations present in certain continents such as Australasia and Africa, as well as of some poorly sampled areas such as Asia, Africa and South and Central America, and may help to clarify gaps in ranges and the identities of some doubtful local endemics. Production of a dated phylogeny of the family should give a better understanding of its origin and historical dispersal, and potentially the routes of its migration.

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CHAPTER 05
GENERAL DISCUSSION AND CONCLUSIONS

"*The discussion itself is what most matters, the fact that we can reason together easily, with a blend of wit and seriousness, never descending into gossip or slander and always allowing room for alternative views."*

— Stephen Greenblatt, *The Swerve: How the World Became Modern*

5.1 Present species delimitation of *Polytrichum* sect. *Polytrichum*

The present study focussed on an integrative approach (molecular and morphological) to delimit the extant taxa of the *Polytrichum* sect. *Polytrichum* with three main objectives; firstly, to establish the correct identity of the well-known taxon *Polytrichum commune* Hedw. by selecting a lectotype, secondly, to delimit species using a molecular framework (using both Sanger and Next Generation Sequencing) and thirdly, to revise the taxonomy of all existing taxa of *Polytrichum* sect. *Polytrichum* using a monographic approach.

Polytrichum commune is frequently used as a "model" plant in diverse fields of biology, probably due to its large size and ubiquity in Europe and North America (although many plants identified as *P. commune* in North America may in fact be *P. perigoniale* Michx.; Kariyawasam & al., in prep.). The present study delimits the long-standing nomenclatural issues surrounding *P. commune* by selecting a lectotype from the Johannes Hedwig's herbarium (Chapter 02). Establishing the correct identity of *P. commune* leads to the correct understanding of the species concept of *P. commune*, which has been erroneously applied, particularly in North America, Africa and Southeast Asia (China and Japan). This morphological delimitation of taxa has been corroborated by molecular species delimitation derived from the present study.

An integrative approach combining morphological and molecular taxonomic delimitation was found to be most effective for establishing a stable classification for the study group. The two different molecular approaches employed (Sanger and NGS) to infer phylogenetic relationships have yielded a similar tree topology, which implies that a robust

phylogenetic classification can be used to delimit the taxa. Although the extant morphological (or typological) species concepts are largely congruent with the phylogenetic study, the Sanger phylogeny revealed a complex phylogenetic structure with weakly supported groupings inside a large apical clade that represents a species complex comprised of three distinct morphological species (i.e. *P. perigoniale*, *P. subpilosum* and *P. ericoides*). The phylogenetic relationships among these three species were weakly supported by the molecular markers used for the current study. However, the haplotype network constructed using the ITS-2 locus of a selected subset of taxa within this species complex was informative for the genetic relatedness of these taxa and some of the populations that comprise them (Chapter 03).

The phylogenetic framework resulting from the Next Generation hybrid capture sequencing approach resulted in a similar tree topology. Hence, with a large number of nuclear data also supporting a similar tree topology, these taxa are presumed to be still subject to reticulate evolutionary processes. Even though they are distinctive morphological species which are geographically isolated, they seem to be still subject to interspecific gene flow or incomplete lineage sorting. This may merit a phyogeographic study to generate a dated phylogeny to infer biogeographic and evolutionary process relevant to populations within the study group.

5.2 Future Work

The present study provides a robust phylogenetic framework for *Polytrichum* sect. *Polytrichum* based on DNA from herbarium specimens. It was inevitably challenging handling degraded DNA from older herbarium specimens, while optimising PCR protocols for this. However, the data generated from this study merits future work to study the behaviour of herbarium bryo-DNA amenable for different analytical techniques. Moreover, the NGS approach yielded a lot of useful data which could be used for future studies. Although the NGS hybrid capture in the present study wasn't as successful using the available *Physchromitrella* RNA baits as it might have been with more taxon-specific baits, the data generated will nonetheless provide a lot of useful information to perform further taxonomic, phytogeographic and /or ecological studies. These preliminary data may also help to develop *Polytrichum*-specific RNA baits for future work. This will potentially contribute towards inferring a time-calibrated phylogeny for the whole family Polytrichaceae, to elucidate the origins of the genera and species within the family.

Although there are certain homoplastic/apomorphic characters are shared by the members of *Polytrichum* sect. *Polytrichum* with the other two sections (sect. *Aporotheca* and sect. *Juniperifolia*), such as the lamellar end-cell morphology, it would be worth performing an ancestral character reconstruction to study the evolution of characters within the group. However, this wasn't performed in the present study due to certain homology problems in defining useful character states such as lamellar end-cell morphology. This is because, in certain species the morphology of lamellar end-cells in a transverse section of a leaf shows a developmental series (for example, in *Polytrichum brachymitrium*, the shape of lamellar end-cells progressing from pyriform to symmetric to completely flattened through a series of intermediates). Hence prior to perform a proper character state reconstruction, character stets need to be better understood, and perhaps with information from developmental studies that were beyond the scope of the present. study. However, it would be worth carrying out a future study with ancestral character state reconstructions for the whole family, to infer the evolution of characters such as presence and absence of leaf lamellae, lamellar end-cell morphology, presence and absence of stomata in the capsule, capsule morphology and peristome architecture, and to investigate correlations between these characters and ecology. This would contribute towards a more complete picture for the current circumscription of genera within the family Polytrichaceae.

With the aid of SEM studies, Smith (1974) stated that the spores of *P. commune* possess "Christmas tree-like" projections (see Smith, 1974, Figs 3 & 4). To infer the relationship between spore morphology and current species delimitations, I have initiated a pilot study on a subset of taxa (65 accessions) covering a wide geographic span and different developmental stages within *Polytrichum* sect. *Polytrichum*. However, due to time constraints, this is not yet complete and will provide material for a future study to investigate spore morphology in different clades to potentially corroborate molecular species delimitations.

The present study also suggests some important considerations for future taxonomic and nomenclatural work. The taxonomic position of *Polytrichum commune* var. *humile* Sw. and the taxonomic status of *Polytrichum elegans* Welw. & Duby. are yet to be investigated. Based on the morphological studies of *P. commune* var. *humile,* it seems to be an extreme ecological variant of *P. perigoniale* Michx., while *P. elegans,* which was only studied from the type specimen, also seems to be an extreme ecological variant of *P. subpilosum* collected from Angola. Although DNA was extracted from the type specimen using a few

leaves of the plant, due to the age of the material DNA yield was minute, and PCR protocols used to amplify DNA for ITS markers failed to amplify the locus. Hence no molecular data could be generated for this taxon. It is likely that field work to collect plants from the type locality will be required to infer its phylogenetic position within the family.

A complete genome sequence of *Polytrichum commune* Hedw. has recently been generated by colleagues in Helsinki. This will facilitate comparative genomic studies of the genus and allied taxa in the future. The 1KP consortium (https://db.cngb.org/onekp/). has already generated transcriptome data for *P. perigoniale* (The North American specimen used for this study was misidentified as *P. commune*, [Buck, *57829*, NY]), and a future study is merited to generate transcriptome data for *P. commune s. str*. This will provide useful data for comparative physiological studies of Polytichaceae with the aid of transcriptomic data. For example, the role of lamellar end-cells in modulating transpiration and water loss from the inter-lamellar spaces of "pseudo-mesophyll" would be a fascinating focus for physiological, developmental and comparative transcriptomic / genomic studies.

The Polytrichopsida would also be a fascinating group of mosses in which to further investigate the role of stomata in moss sporophytes and the variation in the expression of these, as recently studied by Renzaglia & al. (2020). Based on thorough sampling across several moss lineages, they concluded that stomata have been independently lost over 60 times, despite moss capsules having a complex architecture of internal spaces associated with stomata and with sporangial development and nourishment, the stomata themselves being fundamentally necessary for these processes. Variation in morphology, presence and absence of stomata, superficial and sunken stomata and their associated structures, complex peristome architectures (such as the epiphragm-peristome complex, presence and absence of annuli and sacculi) are highly evident in the Polytrichopsida (Smith, 1974; Bell & Hyvönen , 2010 a, b). In most derived two lineages such as, *Pogonatum* and *Atrichum*, stomata are completely absent, whereas in other lineages such as *Polytrichum* and *Delongia* have stomata associated with additional external structures in the apophysis as well as with internal spaces (Bell & al., 2015; Bell & al., 2021). Hence, studying the development of stomata across different lineages in Polytrichopsida with the aid of transcriptome data merits a future research project.

From the classical taxonomic point of view, it has been fascinating to unearth the nomenclatural history of *Polytrichum commune*. Since the name "*Polytrichum commune*" had been used in the pre-Linnean era (see Chapter 02), it would be interesting to study the

polynomial names historically applied to this taxon with the aid of pre-Linnean taxonomic literature as well as the specimens housed in 15th and 16th century herbaria. Moreover, a complete list of synonyms needs to be compiled and revised for *Polytrichum commune,* a potentially tedious nomenclatural research project.

Due to the large number of unassigned names available for the widely distributed taxa such as *P. commune,* it is worth to study different populations found indifferent geographic regions to infer any cryptic or pseudo-cryptic taxa using microsatellite markers or detecting Single Nucleotide Polymorphism (SNP) using high throughput platforms. Sampling at the population level to cover the entire geographic range of such species would be necessary. This would require more field sampling, and thorough morphological study and additional molecular work is recommended to study the populational level differences. However, due to the time constraint and limitations of sampling (which is not merely the genuine rarity of each taxon) a detailed study at populational level couldn't performed for the study group. However, this taxonomic study provides better insights for the fascinating group of Polytrichopsida addressing some long-lasting questions in the nomenclatural history and clearing a lot of confusions and misapplications in taxonomy and nomenclature and this will open a window for many intriguing questions remaining to answer in this fascinating group of mosses.

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