# Addendum

(1) Soon after submitting this thesis a more recent comprehensive classification by Crandall-Stotler *et al.* (2009)[1] was published. This recent publication does not undermine the information presented in this thesis. The purpose of including the comprehensive classification of Crandall-Stotler and Stotler (2000) was specifically to introduce some of the issues regarding the troublesome classification of this group of plants. Crandall-Stotler and Stotler (2000), Grolle and Long (2000) for Europe and Macaronesia and Schuster (2002) for Austral Hepaticae represent three previously widely used yet differing opinions regarding Lophoziaceae classification. They thus reflect a useful account of some of the motivation for initiating this project in the first place.

(2) Concurrently or soon after chapter 2 was published by de Roo *et al.* (2007)[2] more recent relevant papers were published. These include Heinrichs *et al.* (2007) already referred to in chapter 4, and notably Vilnet *et al.* (2008)[3] examining the phylogeny and systematics of the genus *Lophozia* s. str. The plethora of new information regarding taxa included in this thesis is encouraging and with each new publication we gain insight and a clearer understanding these fascinating little plants.



[1] Crandall-Stotler, B., Stotler, R.E., Long, D.G. 2009. Phylogeny and classification of the Marchantiophyta. *Edinburgh J. Bot.* 66: 155—198.

[2] de Roo, R.T., Hedderson, T. A., Söderström, L. 2007. Molecular insights into the phylogeny of the leafy liverwort family Lophoziaceae Cavers. Taxon. 56:301—314.

[3] Vilnet, A.A., Konstantonva, N.A., Troitsky, A.V. 2008. Phylogeny and systematics of the genus Lophozia s. str. (Dumort.) Dumort. (Hepaticae) and related taxa from nuclear ITS1-2 and chloroplast trnL-F sequences. *Molec. Phylogen. Evol.* 47: 403—418.

# Molecular systematics of the leafy liverwort family Lophoziaceae Cavers

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Thesis presented for the degree of

DOCTOR OF PHILOSOPHY

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# Abstract

Delimitation and classification of the large, cosmopolitan liverwort family Lophoziaceae is controversial. Many recent workers have included it in Jungermanniaceae, and even in its strictest sense, internal classification has varied widely among different treatments. Here an analysis of the variation in DNA sequences of the chloroplast *rps*4 gene and the *trn*G intron provides resolution of phylogenetic relationships in the leafy liverworts with emphasis on the various elements usually placed in Lophoziaceae. The following conclusions are drawn. Lophoziaceae is not closely related to Jungermanniaceae. Lophoziaceae, and perhaps also Cephaloziellaceae, should be included in Scapaniaceae unless many small families are recognized. *Delavayella* and *Blepharidophyllum* are excluded from Scapaniaceae. Jamesonielloideae is a family of its own (Jamesoniellaceae) sister to Adelanthaceae (or a subfamily of Adelanthaceae). The genus *Anastrophyllum* should be split into *Anastrophyllum* and *Sphenolobus*. *Lophozia* is polyphyletic and the genera *Isopaches* and *Schistochilopsis*, and perhaps *Obtusifolium*, should be recognized while *L. sudetica* could be transferred to *Barbilophozia*. *Barbilophozia* s. str. is monophyletic while *Orthocaulis* is polyphyletic with the four sampled species appearing in 3 different clades; their relationships are poorly resolved. *Lophozia silvicola* Buch is clearly separated from *L. ventricosa* and *Jamesoniella oenops* from *J. colorata* at species level. Further investigation within the clade comprising most *Anastrophyllum* species with the inclusion of additional sequences of the chloroplast rpoC1 and nuclear ITS regions was examined. The following conclusions are drawn. *Gymnocolea inflata* is possibly sister to the remaining taxa in the *Anastrophyllum* clade. *Anastrepta orcadensis* is possibly sister to *Isopaches*. Chandonanthoideae, *Sphenolobus*, *B. floerkei, B. attenuate*, *Anastrophyllum, B. atlantica* and *B. quadriloba* appear more closely related to each other than to *Isopaches, Gymnocolea* and *Barbilophozia* s. str. *Tetralophozia setiformis* is paraphyletic with *Plicanthus* and *Spenolobopsis* sister to *T. setiformis* (3) from Spain. *Sphenolobopsis* should be transferred to the Chandonanthoideae. *Barbilophozia atlantica* is the type for *Orthocaulis*; the genus should be re-instated for it and *Anastrophyllum cavifolium*. *Sphenolobus* is possibly sister to *Anastrophyllum*, *Orthocaulis, B. floerkei* and *B. attenuata*. *A. auritum* is paraphyletic with *A. auritum* (1) sister to *A. tubulosum*. Analysis of the divergence dates found that Jungermanniidae split from other liverworts and subsequently diversified after the mid-Permian (ca. 273 mya). The major leafy liverwort lineages mostly emerged by the end of the Cretaceous. Lineage-Through-Time (LTT) plots for liverworts were compared with those of other plant groups finding the correlation less clear for the diversification of liverworts following angiosperms as between angiosperms, ferns, lycopods and horsetails. A possible leafy liverwort radiation after the Cretaceous-Tertiary boundary was identified. Lastly, alternative changes to the classification under rank-based codes as well a phylogenetic classification was briefly explored.

# Contents

# Chapter 1
# General Introduction

### 1. 1. A Brief Introduction to the Liverworts

Liverworts, like other bryophytes, are small, herbaceous plants that are found in terrestrial ecosystems (Crandall-Stotler & Stotler 2000). Features of the life cycle, except for certain morphological differences, are essentially the same as for the hornworts and mosses (Schuster 1966). This life cycle is heteromorphic with a dominant gametophyte in the form of a free-living haploid gametophyte generation with a comparatively short-lived and nutritionally dependent diploid sporophyte (Schuster 1966).

The gametophytes produce sexual cells – the eggs and free-swimming spermatozoids – in archegonia and antheridia. Although fertilisation is thought generally to involve the existence of a continuous film of water between the sex organs (Schuster 1966), Cronberg *et al.* (2006) have shown recently that microarthropods can also mediate sperm transfer. The sex organs can occur on the same plants, in which case the gametophytes are monoecious, as opposed to dioecious. These sex cells undergo fusion resulting in a diploid sporophytic generation that is at least partially parasitic on the gametophyte and permanently epiphytic (Schuster 1966). The sporophyte undergoes all cell differentiation before the seta elongates to help effectively disperse spores. The sporangium has both fertile spores and sterile cells called elaters. Elaters assist in spore dispersal; as single celled tubular structures they are absorbent with spiral thickenings that twist and contort when drying. At maturity the sporangium wall usually splits longitudinally into valves and spores are released. On germination the spore develops a rudimentary and ephemeral protonema that usually gives rise to a single gametophyte (Paton 1999). The sporophyte stage is often bypassed by asexual and vegetative reproduction of the gametophyte.

The morphological divergences of liverworts in this life cycle from other bryophytes are highlighted by Crandall-Stotler & Stotler (2000). Unlike with either mosses or liverworts, the sporophyte matures completely within the confines of the gametophytic tissue. The sporophyte also has no differentiated meristematic zone and lacks a columella and stomata. Gametophytes normally grow prostrate on their substrates and are of three fundamental types: a leafy shoot system (Jungermanniopsida:

Jungermanniidae), a simple thallus (Jungermanniopsida: Metzgeriidae), and a complex thallus with air chambers (Marchantiopsida).

### 1. 1. 1. Characteristic features of liverworts and leafy liverworts

The diagnostic features of the liverworts (Schuster 1966) include: i) the presence of oil bodies and many small, spherical to ellipsoidal, chloroplasts in the gametophyte cells, ii) Sex organs in the form of externally developing antheridia and flask shaped archegonia, and iii) Sporophytes with capsules typically spherical to ovoid, usually with 4 valves, and usually including spiral elaters. Crandall-Stotler & Stotler (2000) note that while gametophyte architectures are very heterogeneous, the sporophytes are rather homogeneous in their organization. Sporophytes develop entirely within the gametophytic tissue, this tissue derived from the archegonium (a true calyptra), the female gametophore (a solid perigynium), or a combination of the two (a shoot calyptra). Additionally, there are usually other structures, associated with the female 'inflorescences', that may enlarge after fertilization to surround the sporophyte, such as perianths, pseudoperianths, scales, and paraphyllina (Crandall-Stotler & Stotler 2000). While variation among liverwort groups is found in embryology, in all of them the sporophyte is determinate and, except in the Ricciales, is differentiated into a foot that forms a placental zone with the gametophyte, a seta that is made up of thin-walled parenchymatous cells, and a capsule (Crandall-Stotler & Stotler 2000). Liverworts are also unique in having lunularic acid, a stilbene derivative (Damsholt 2002).

Some diagnostic features of the leafy liverworts *sensu stricto* (Jungermanniales), as highlighted by Schuster (1966), include i) the gametophytes developing from a tetrahedral apical cell in which three rows of segments are cut off and all of which develop leaves, ii) the leaves of the third row are often small or may be reduced to stalked slime papillae, iii) in their earliest stages of development, leaves have two lobes, however mature leaves often have two to several lobes or may be secondarily entire, iv) cells usually develop marked collenchymatous thickenings, v) archegonia formation involves the apical cell, resulting in the sporophyte being terminal on the plant, and vi) spores are numerous and usually small (6-25 µm).

### 1. 1. 2. Overview of liverwort classification

The time at which liverworts appear to first receive recognition begins by denotations to the Greek term λειχηυ in the writings of Theophrastus (c. 372-287 BC) and Aristotle

(384-322 BC) that are thought to apply to *Marchantia polymorpha* (Schuster 1966). Later Dioscorides (c. AD 40-90) attributed certain medical properties to the same group of plants, suggesting that the herb, while not of use internally to treat the liver, may however be applied externally for the purpose of restoring the natural colour of those afflicted by liver disorder (Brunfels 1531, quoted in Schuster 1966 p:120). It is thought that the distinct morphology of large, conspicuous, thallose forms like *Marchantia* suggested the lobes of the liver, resulting in ascribed medicinal properties, the Latin name Hepaticae and the common name liverworts (Schuster 1966). The nomenclature starts with Linnaeus (1753) in his Species Plantarum where 25 described leafy taxa were all placed in the genus *Jungermannia*. Hedwig, in 1784, first separated the liverworts from mosses and named them Musci Hepatici; De Jussieu in 1789 simplified this name to Hepaticae (Schuster 1966). Around 1820 this genus was divided into 22 more genera independently by contemporaries: in 1818 by Raddi, in 1821 by Gray and in 1822 by Dumortier. Almost simultaneously, this resulted in some confusion which extended into the twentieth century - for instance three different generic names for the same taxon (Schuster 1966, Crandall-Stotler & Stotler 2000). The first world-wide treatment of liverworts was Synopsis Hepaticarum of Gottsche, Lindenberg and Nees (1844-47). Von Nägeli (1845) described the apical cell and its mode of segmentation and following this Leitgeb (1874-1881) provided detailed anatomical descriptions of many hepatic taxa and importantly showed how the formation of archegonia in leafy liverworts terminates further apical cell segmentation ("akrogyne") whereas in simple thalloid hepatics it does not ("anakrogyne"). These findings quickly were incorporated into classifications like that of Schiffner (1893-1895) where categories Jungermanniales anakrogynae (simple thalloids) and Jungermanniales akrogynae (leafy liverworts) were established (Crandall-Stotler & Stotler 2000). Evans (1939) provided the formal names Jungermannineae and Metzgerineae (Crandall-Stotler & Stotler 2000).

With cladistic methods providing an objective way to reconstruct phylogeny, the idea that liverworts comprise a single natural unit has recently been questioned. Through a cladistic analysis of morphological characters, Kenrick & Crane (1997) proposed liverworts to comprise the phylum Marchantiophyta based on evidence of shared sporophyte characters, comparable in rank to mosses and hornworts. Capesius & Bopp (1997) in an analysis of 18S rRNA gene sequences found that liverworts do not form a natural unit, but found them to be polyphyletic. However, numerous molecular studies including Hedderson *et al.* (1996, 1998) using the nuclear 18S rRNA sequence, Lewis *et*

*al.* (1997) using the chloroplast *rbc*L sequence, Duff & Nickrent (1999) using the mitochondrial 19S rDNA sequences and Groth-Malonek & Knoop (2005) using the mitochondrial nad5 gene sequence all resolve a single liverwort clade.

Table 1.1 provides an outline of the classification of the Marchantiophyta *sensu* Crandall-Stotler & Stotler (2000) with focus on the Jungermanniidae, the leafy liverworts. The major distinction of liverworts into the complex thalloid Marchantiopsida versus the Jungermanniopsida, which are further distinguished into the simple thalloid (metzgeriid) and leafy species (jungermanniid) is generally accepted but the usefulness of taxonomic ranks below that of class is questionable (Crandall-Stotler *et al.* 2005; Davis 2004; Forrest & Crandall-Stotler 2004; Forrest & Crandall-Stotler 2005). This is explored in more detail in chapter two. The liverworts contain up to 8,000 species which have been grouped into 380 genera, 78 families, 26 suborders and 14 orders, respectively, in the comprehensive classification of Crandall-Stotler & Stotler (2000)[1], until recently utilised in the NCBI database.



[1] See Addendum (1)

**Table 1. 1.** Classification of the Marchantiophyta focusing on orders of the Jungermanniidae and suborders of the Jungermanniales sensu Crandall-Stotler and Stotler (2000)

PHYLUM: Marchantiophyta
- CLASS: Marchantiopsida Stotler & Stotl.-Crand.
- CLASS: Jungermanniopsida Stotler & Stotl.-Crand.
  - SUBCLASS: Metzgeriidae Barthol.-Began.
  - SUBCLASS: **Jungermanniidae** Engl. Emend. Stotler & Stotl.-Crand.
    - ORDER: Lepicoleales Stotler & Crand.-Stot., ordo nov.
    - ORDER: **Jungermanniales** H. Klinggr. Emend. Stotler & Stotl.-Crand.
      - SUBORDER: Herbertineae R. M. Schust.
      - SUBORDER: Balantiopsidineae R. M. Schust.
      - SUBORDER: Lophocoleineae Schljakov
      - SUBORDER: Lepidoziinae R. M. Schust.
      - SUBORDER: Cephaloziineae Schljakov
      - SUBORDER: Antheliineae R. M. Schust.
      - SUBORDER: Brevianthineae J. J. Engel & R. M. Schust.
      - SUBORDER: **Jungermanniinae** R. M. Schust. ex. Stotl. & Stotl.-Crand.
    - ORDER: Porellales (R. M. Schust.) Schljakov emend. Stotler & Stotl.-Crand.
    - ORDER: Radulales (R. M. Schust.) Stotler & Stotl.-Crand.
    - ORDER: Pleuroziales (R. M. Schust.) Schljakov



## 1. 2. Lophoziaceae Cavers

Currently identified diversity of the Lophoziaceae (sensu Grolle & Long 2000) shows the family to contain ca. 268 species grouped into 85 subgenera, 29 genera and 6 subfamilies (Söderström unpublished). The Lophoziaceae have mostly lateral branching (Damsholt 2002) and succubous leaves (Paton 1999) with 2-4 lobes (Schuster 2002a). Lophoziaceae are mostly terrestrial plants, ranging from tiny to large (shoots mostly 0.5-5 mm broad) (Schuster 2002a) and found on a variety of substrates (Damsholt 2002; Schuster 2002a). It is clear when examining the various descriptions of Lophoziaceae that it appears to be quite a mixed assortment of elements and it is easy to see how broad versus narrow familial circumscriptions have resulted.

Table 1.2 illustrates the different classifications of the Lophoziaceae by Grolle & Long (2000), Schuster (2002a) and Crandall-Stotler & Stotler (2000). It is clear that individual researchers have adopted radically different circumscriptions, and in particular the distinctness of Lophoziaceae from, and their relationship to, Jungermanniaceae has been debated. As with many other treatments, the most complete recent classification of hepatics (Crandall-Stotler & Stotler 2000) includes Lophoziaceae as a subfamily of the latter. Similarly, Schuster (2002a) does not distinguish the two at family level because of perceived exceptions to any diagnostic characters that can be identified for each group.

On the other hand, Grolle & Long (2000) recognise European Lophoziaceae (including two subfamilies, Lophozioideae and Jamesonielloideae) as distinct from Jungermanniaceae. The placement of subfamilies Chandonanthoideae, Gottschelioideae and Syzygielloideae is also uncertain, but they are often affiliated with Lophoziaceae (Schuster 2002a; Inoue 1966).

This is a very large group of plants, globally distributed, with a very questionable taxonomy. Clearly the systematics of the Lophoziaceae requires a detailed examination. The Lophoziaceae are explored in this thesis with the application of a molecular systematic approach. The main groups recognised within Lophoziaceae, here treated at subfamilial level as in Grolle & Long (2000), are described below.

**Table 1. 2**. Classification of Lophoziaceae/ Jungermanniaceae

Sensu Grolle and Long (2000) for Europe and Macaronesia:

Class Marchantiopsida (=Hepaticae s.str.)
- Subclass Marchantiidae
- Subclass Jungermanniidae
  - Order Metzgeriales
  - Order Calobryales
  - Order Jungermanniales
    - Suborder Jubulineaeae (=Porellineae)
    - Suborder Jungermanniineae
      - Family Jungermanniaceae
      - Family Mesoptychiaceae
      - Family Gymnomitriaceae
      - Family Plagiochilaceae
      - Family Geocalycaceae
      - Family Scapaniaceae
      - Family Adelanthaceae
      - Family Cephaloziellaceae
      - Family Cephaloziaceae
      - Family Antheliaceae
      - Family Lepidoziaceae
      - Family Calypogeiaceae
      - Family Pseudolepicoleaceae
      - Family Trichocoleaceae
      - Family Ptilidiaceae
      - Family Lepicoleaceae
      - Family Herbertaceae
      - Family Radulaceae
      - Family Pleuroziaceae
      - Family **Lophoziaceae**
        - Subfamily Lophozioideae
          - *Tetralophozia*
          - *Barbilophozia*
          - *Anastrepta*
          - *Lophozia*
          - *Leiocolea*
          - *Gymnocolea*
          - *Sphenolobopsis*
          - *Anastrophyllum*
          - *Tritomaria*
        - Subfamily Jamesonielloideae
          - *Jamesoniella*

Sensu Schuster (2002) for Austral Hepaticae:

Class Hepaticae
- Subclass Marchantiidae
- Subclass Jungermanniidae
  - Order Treubiales
  - Order Metzgeriales
  - Order Calobryales
  - Order Jungermanniales
    - Suborder Lepicoleineae
    - Suborder Herbertineae
    - Suborder Antheliineae
    - Suborder Lepidoziineae
    - Suborder Cephaloziineae
    - Suborder Geocalycineae
    - Suborder Brevianthineae
    - Suborder Perssoniellineae
    - Suborder Balantiopsidineae
    - Suborder Ptilidiineae
    - Suborder Lepidolaenineae
    - Suborder Porellineae
    - Suborder Radulineae
    - Suborder Pleuroziineae
    - Suborder Jungermanniineae
      - Family Scapaniaceae
      - Family Delavayellaceae
      - Family Blepharidophyllaceae
      - Family Gymnomitriaceae
      - Family Stephaniellaceae
      - Family Jungermanniaceae
        - Subfamily Chandonanthoideae
          - *Chandonanthus*
          - *Plicanthus*
          - *Tetralophozia*
        - Subfamily **Lophozioideae**
          - *Lophozia*
          - *Gymnocoleopsis*
          - *Tritomaria*
          - *Anastrophyllum*
          - *Andrewsianthus*
          - *Sphenolobopsis*
          - *Roivainenia*
          - *Pseudocephaloziella*
        - Subfamily Jamesonielloideae
          - *Anomacaulis*
          - *Cryptochila*
          - *Cuspidatula*
          - *Denotarisia*
          - *Jamesoniella*
          - *Pisanoa*
          - *Vanaea*
          - *Nothostrepta*
        - Subfamily Jungermannioideae
        - Subfamily Gottschelioideae
        - Subfamily Scaphophylloideae
        - Subfamily Notoscyphoideae
        - Subfamily Eremonotoideae

Sensu Crandall-Stotler & Stotler (2000):

Phylum Marchantiophyta
- Class Marchantiopsida
- Class Jungermanniopsida
  - Subclass Metzgeriidae
  - Subclass Jungermanniidae
    - Order Lepicoleales
    - Order Porellales
    - Order Radulales
    - Order Pleuroziales
    - Order Jungermanniales
      - Suborder Herbertineae
      - Suborder Balantiopsidineae
      - Suborder Lophocoleineae
      - Suborder Lepidoziineae
      - Suborder Cephaloziineae
      - Suborder Antheliineae
      - Suborder Brevianthineae
      - Suborder Jungermanniineae
        - Family Mesoptychiaceae
        - Family Gymnomitriaceae
        - Family Scapaniaceae
        - Family Jungermanniaceae (**inc. Family Lophoziaceae**)
          - *Anastrepta*
          - *Anastrophyllum*
          - *Andrewsianthus*
          - *Anomacaulis*
          - *Barbilophozia*
          - *Bragginsiella*
          - *Cephalolobus*
          - *Chandonanthus*
          - *Cryptochila*
          - *Cryptocolea*
          - *Cryptocoleopsis*
          - *Denotarisia*
          - *Diplocolea*
          - *Girhildiella*
          - *Gottschelia*
          - *Gymnocolea*
          - *Gymnocoleopsis*
          - *Hattoria*
          - *Horikawaella*
          - *Jamesoniella*
          - *Jungermannia*
          - *Lophonardia*
          - *Lophozia*
          - *Mylia*
          - *Nardia*
          - *Nothostrepta*
          - *Notoscyphus*
          - *Pisanoa*
          - *Protosyzygiella*
          - *Pseudocephaloziella*
          - *Rhodoplagiochila*
          - *Roivainenia*
          - *Scaphophyllum*
          - *Sphenolobopsis*
          - *Syzygiella*
          - *Tetralophozia*
          - *Tritomaria*
          - *Vanaea*

### 1. 2. 1. Lophozioideae

The Lophozioideae *s. str.* has up to ca. 214 species in 15 genera (Söderström unpublished). The type of Lophozioideae, the genus *Lophozia* (Dumort.) Dumort. is itself described by Schuster (2002a) as a large, complex, difficult and almost cosmopolitan genus with up to ca. 64 species (Söderström unpublished). The species are so variable that the genus is difficult to define (Paton 1999). It is usually recognised by elimination of other genera and some combination of the following characters: leaves predominantly bilobed, obliquely inserted to almost transverse antically; underleaves absent or very inconstant; gemmae mostly stellate or angular and often pigmented; branches terminal and lateral intercalary; perianths free, terete and distally plicate; stem medulla in some species dorsiventrally differentiated; stems 8+ cells high in t.s.; antical cortical cells mostly 16-30 μm wide or wider in some species (Paton 1999). Figure 1.1 shows *L. ventricosa* (Dicks.) Dumort., the type of *Lophozia*, to illustrate some of these features. *Lophozia* species are mostly found in areas of cool to cold climates, with the highest numbers of species found in the boreal-arctic regions; in the Tropics and warm temperate regions it is usually montane or alpine (Schuster 2002a). Taxa mostly occur on organic soils, some on mineral soils, decaying wood and on moist rocks or spreading from soil in cliff crevices to rock faces in alpine areas (Schuster 2002a).



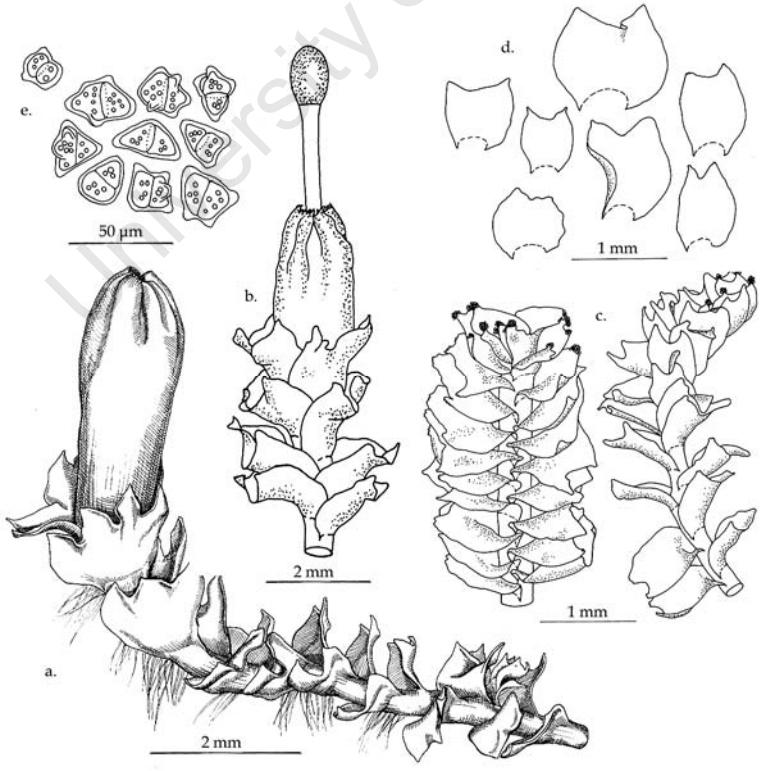


**Fig. 1. 1.** *Lophozia ventricosa*: a. Female gametophyte with perianths in lateral view

(modified from Damsholt 2002: Pl. 24). b. Female gametophyte with sporophyte emerging from perianth (modified from Paton 1999: Fig. 77). c. Gemmiferous shoots with gemmae in dorsal view (left) and lateral view (right) (modified from Paton 1999: Fig. 78). d. Bilobed leaves (modified from Paton 1999: Fig. 77). e. Angular gemmae (modified from Paton 1999: Fig. 78).

Another large genus in Lophozioideae is *Anastrophyllum* with up to ca. 65 species (Söderström unpublished). *Anastrophyllum* is described by Schuster (2002a) as a difficult genus, and internal classification is contentious. It is distinguished from other Lophozioideae by transverse or arcuate antical leaf insertion so that the leaves at the antical part are folded up over the postical part with underleaves usually absent or obsolete (Paton 1999). Figure 1.2 shows *A. donnianum* (Hook.) Steph., the type of *Anastrophyllum*, and *A. minutum* (Schreb.) R.M. Schust. to illustrate some of these features. *Anastrophyllum* species are very widely distributed, however the majority of taxa are found in montane to alpine parts of the tropics (Schuster 2002a). Taxa occur on damp to moist rock walls, decaying wood, occasionally on the bark of trees, on soil between rocks and in crevices on cliff walls in alpine areas, but not on mineral soil (Schuster 2002a).

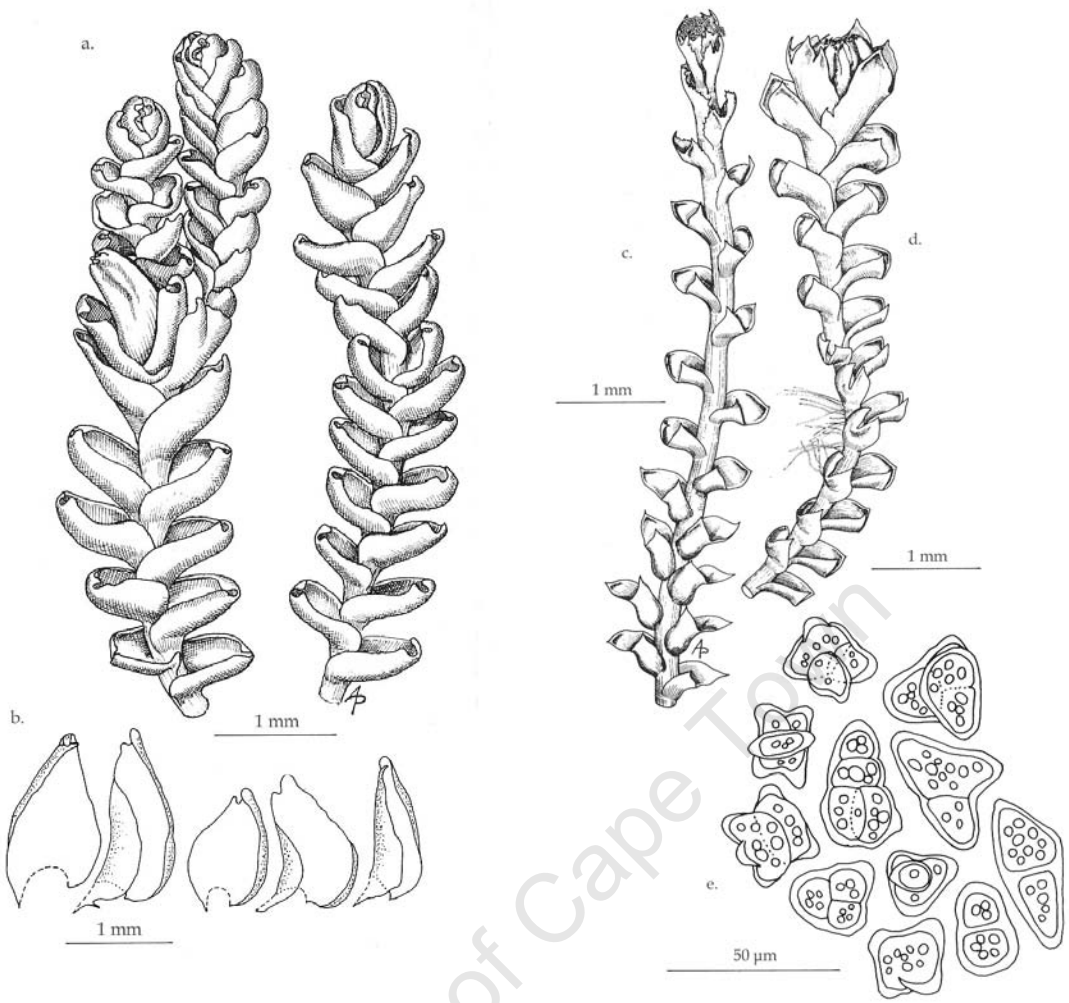




**Fig. 1. 2.** *Anastrophyllum donnianum*: a. Gametophyte shoot in dorsal view (modified from Damsholt 2002: Pl. 55). b. Leaves in apical view (modified from Paton 1999: Fig. 109). *A. minutum*: c. Male gametophyte gemmiferous shoot in dorsal view (modified from Damsholt 2002: Pl. 60). d. Female gametophyte shoot with young perianth in dorsal view (modified from Damsholt 2002: Pl. 60). e. Angular gemmae (modified from Paton 1999: Fig. 106)

### 1. 2. 2. Jamesonielloideae

Jamesonielloideae has up to ca. 33 species and 8 genera (Söderström unpublished). Schuster (2002a) regards Jamesonielloideae as showing characters of both Lophozioideae and Jungermannioideae. In general plants are seen to have a firmer gametophyte structure including leaf cells with large to coarse trigones. The leaves have expanded ventral bases obscuring the stem, and the gametophytes are also always unisexual, lacking asexual reproduction (Schuster 2002a). The type of the Jamesonielloideae, the genus *Jamesoniella* (Spruce) Carrington, has ca. 20 species (Söderström unpublished). Figure 1.3 shows *J. autumnalis* (DC.) Steph. to illustrate some of these features. Jamesonielloideae occur mostly in more exposed localities with longer periods of desiccation than the Jungermannioideae (Schuster 2002a).

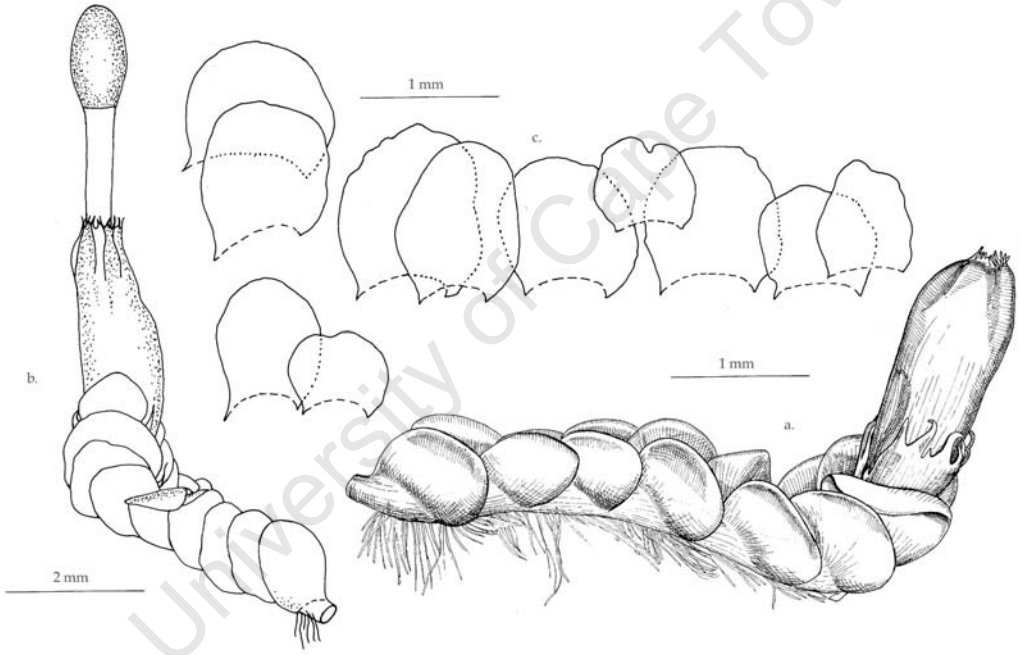




**Fig. 1. 3.** *Jamesoniella autumnalis*. a. Female gametophyte with perianth in lateral view (modified from Damsholt 2002: Pl. 67). b. Female gametophyte with sporophyte emerging from perianth (modified from Paton 1999: Fig. 115). c. Leaves (modified from Paton 1999: Fig. 115).

### 1. 2. 3. Jungermannioideae

Jungermannioideae has up to ca. 188 species and 11 genera (Söderström unpublished). The Jungermannioideae according to Schuster (2002a) show close affinities to the Lophozioideae and Jamesonielloideae, specifically sharing distinction in symmetry of sterile and gynoecial regions, the gynoecia, lack of underleaves and conspicuous bracteoles. However, Jungermannioideae tend to have an *Isotachis*-type perigynium, a feature that lacks in Lophozioideae and Jamesonielloideae (Schuster 2002a). Jungermannioideae also lacks asexual reproduction as is found in the Jamesonielloideae, unlike in the Lophozioideae. The type of the Jungermannioideae, the genus *Jungermannia* L. has up to ca. 159 species (Söderström unpublished). Figure 1.4 shows *J. atrovirens*, the type of *Jungermannia*, to illustrate some of these features.



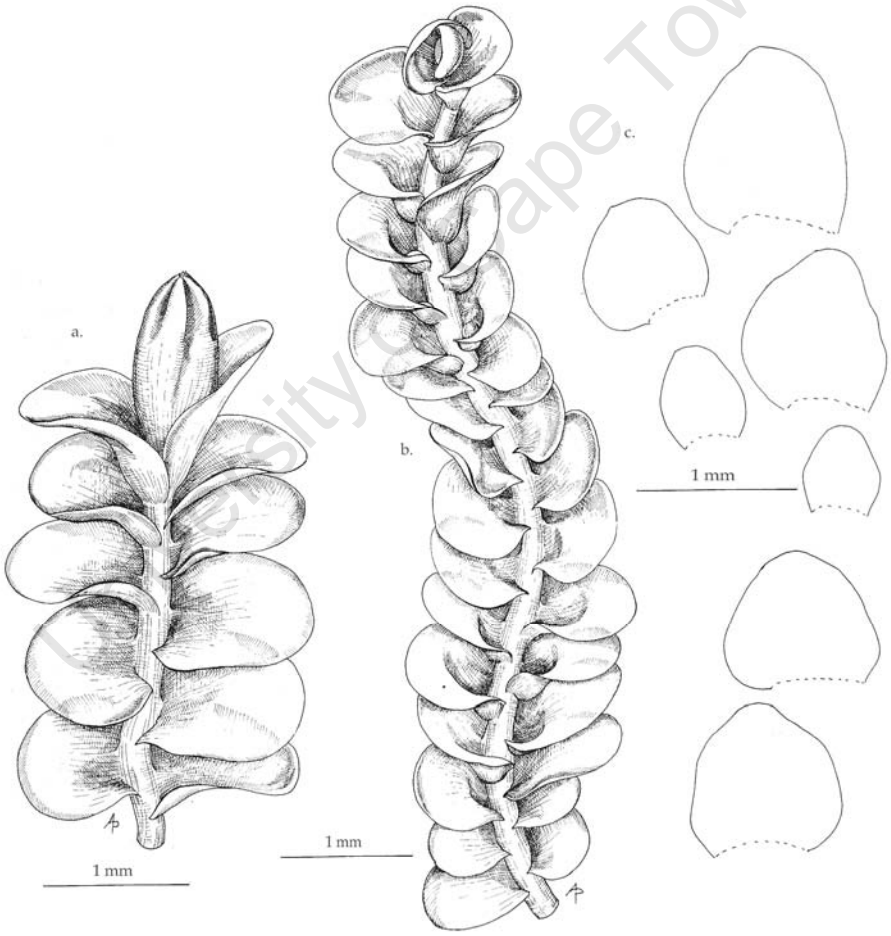


**Fig. 1. 4.** *Jungermannia atrovirens*. a. Female gametophyte with perianth in dorsal view (modified from Damsholt 2002: Pl. 70). b. Male gametophyte with androecia in dorsal view (modified from Damsholt 2002: Pl. 70). c. Leaves (modified from Damsholt 2002: Pl. 70).

### 1. 2. 4. Chandonanthoideae, Gottschelioideae and Syzygielloideae

Chandonanthoideae has ca. 11 species in 3 genera (Söderström unpublished). The Chandonanthoideae appear closely allied to the Lophozioideae and differ in having thick capsule walls with a distinctive anatomy, deeply plicate perianths and xeromorphic structure with rigid stems (Schuster 2002a).

Gottschelioideae is monogeneric with ca. 3 species (Söderström unpublished). According to Schuster (2002a), *Gottschelia* Grolle appears to be allied to Jamesonielloideae although the similarities are superficial. Species of *Gottschelia* produce angular or angular-stellate gemmae as seen in Lophozioideae (Schuster 2002a) and a similarity has also been noted by Grolle between *Gottschelia* and *Anastrophyllum* although the sporophyte is different and *Gottschelia* has interlocking merophytes dorsally (Schuster 2002a).

Syzygielloideae is also monogeneric, unless including *Protosyzygiella* (Váňa pers. comm.) with up to ca. 25 species (Söderström unpublished). Inoue (1966) suggested that *Syzygiella* is part of the Lophoziaceae, and specifically affiliated it with Jamesonielloideae. However, Schuster (2002a) notes that the genus has perfectly opposite leaves (Jamesonielloideae has alternate leaves) and places it as a subfamily in Plagiochilaceae.

**1. 3. A molecular systematic approach**

Systematics is the science of the organismal diversity that exists on earth today and its evolutionary history and entails the discovery, description and interpretation of this diversity (Judd *et al.* 2002). This thesis uses a molecular phylogenetic approach towards systematics wherein the phylogeny provides information on the sequence of evolutionary events for the taxa studied.

> "The characters which naturalists consider as showing true affinity between any two or more species, are those which have been inherited from a common parent…" Darwin (1859: 391).

The first step in inferring a phylogeny involves the identification of characters and states for the relevant taxa. Some of the advantages of a molecular approach include: i) the ability to generate data on a large number of characters relatively easily; ii) the relatively neutral character of nucleotide-level changes since sequences of basic universal genes change more easily than their function; iii) character states are unambiguously defined; and iv) relatively fewer problems in making homology assertions. The identification of shared derived characters (synapomorphies) is the key to identifying groups composed of an ancestor and all its descendants (monophyletic groups).

Molecular systematic approaches use genetic markers as characters and character states. A range of molecular approaches is available including various PCR-based techniques, restriction site analysis, analysis of DNA arrangements and gene and intron loss, and DNA sequencing (Soltis & Soltis 1998). This study utilises comparative DNA sequencing. To be appropriate, target DNA regions should be of a suitable length and level of variation to provide an adequate number of phylogenetically informative nucleotide positions (Olmstead & Palmer 1994). The regions targeted should provide phylogenetic information at a level of resolution spanning the spectrum of the taxonomic level being examined. For example the chloroplast region *rbc*L, which is typically characterised by a slower rate of evolution than the tRNA-Gly (*trn*G) gene intron would thus be utilised at relatively higher taxonomic levels – usually family and above (Soltis & Soltis 1998). The rate of evolution for any specific region varies among different taxa; this is related to a number of factors including generation time, extinction, episodic changes of rates of sequence divergence, and lineage-specific rate variation (Soltis & Soltis 1998). A pilot study of a few regions thought to be potentially suitable for a

phylogenetic analysis is usually required. Previous studies of related taxa help in choosing gene regions and actual sequences can be accessed from GenBank (http://www.ncbi.nlm.nih.gov). Regions initially identified as potentially providing suitable phylogenetic information for this study were the chloroplast *rps*4 region (Newton et. al. 2000, Capesius & Bloecher unpublished) and the tRNA-Gly (*trn*G) gene intron (Pacak & Szweykowska-Kulińska 2003).

Homology is a term used for similarity due to common descent and can be considered synonymous with synapomorphy (Doyle & Davis 1998). Entire regions and genes must be orthologous, that is, one must be able to distinguish genes related by gene duplication within a genome from genes that are homologous by organismal phylogeny (Olmstead & Palmer 1994). This is more an issue with the analysis of some nuclear genes, whereas chloroplast genes used in this thesis evolve as single copy genes and questionable homology is less of a problem (Olmstead & Palmer 1994).

Once specific regions are sequenced these must be aligned. This is essential for a correct establishment of character homology hypotheses, often called primary homology assessment (De Pinna 1991). This is often recognised as the most difficult part of using sequence data as there is no good, or at least logically justifiable analytic solution (Judd *et al.* 2002). For instance methods that select among alignments using parsimony-based tree lengths (such as POY or MALIGN) arrange the data to be consistent with a minimum-evolution model (Simmons 2004). Since I am not trying to reinforce earlier hypotheses about relationships by favouring congruence with other characters (Simmons 2004), alignments are instead done manually “by eye”.

### 1. 3. 1. Parsimony

Parsimony analysis is the ordering of synapomorphies into a nested hierarchy by choosing the arrangement of taxa in a way that accounts for the greatest number of characters in the simplest way (Kitching et. al. 1998). In terms of phylogenetically informative information, a short sequence with high substitution rates will not necessarily be comparable to a long sequence with a low substitution rate (Olmstead & Palmer 1994). For each nucleotide position, the distribution of character states is a set of hypotheses of homology that may disagree with homologies implied by other characters (Doyle & Davis 1998). Some of the apparent homologies are revealed as characters that do not suggest the same groupings as the reconstructed tree or trees of the parsimony analysis; these are explained as homoplasies (Doyle & Davis 1998). Homoplasies are the products of parallel evolution (convergences and reversals).

Parsimony relies on the fact that only some of the data represent homoplasy. Thus, the chance of substitution along a branch of a tree must be relatively low for parsimony to succeed (Olmstead & Palmer 1994). Reversals or convergence at any specific site are undetectable "by eye" and are only revealed by the actual phylogenetic analyses. With high rates of mutation or long evolutionary times between speciation events or sampling, parallelisms and reversals increase because of random changes that result in the actual amount of evolutionary change being underrepresented by observed differences (Judd *et al.* 2002). A common effect of this phenomenon is known as long-branch attraction. This is possible in a situation of high mutation rates occurring in a small included subset of taxa in an analysis. Some of these mutations might by chance make the sequences of divergent taxa look more similar than they are in reality (Felsenstein 1978; Hendy & Penny 1989; see also Bergsten 2005). This problem can, to a great extent, be circumvented by using appropriate models of substitution in reconstructing phylogeny. This is done in the application of Likelihood and Bayesian methods.

### 1. 3. 2. Likelihood

With a higher degree of understanding of how DNA evolves comes the realisation that biases may exist in the rates of substitution or character change as in, for instance, the frequently observed bias in transition/transversion rates, base frequencies not all 0.25, and substitution rates varying from site to site (Lewis 1998). So instead of simply ordering synapomorphies into a nested hierarchy by choosing the arrangement of taxa that accounts for the greatest number of characters in the simplest way, maximum likelihood is model-based, allowing for biases inherent in DNA mutation. Empirical information from the sequences themselves is incorporated into a model of how the selected piece of DNA behaves. Given the model, trees are evaluated just as with parsimony. Instead of choosing the shortest tree, we prefer the tree that has the highest probability of giving rise to the sequences that we observe in the organisms sampled. The approach is problematic, though, in that insertions and deletions are excluded from the analysis since there is no inherent model for these types of characters, and evaluating trees is computationally consuming on large data sets.

### 1. 3. 3. Bayesian inference

Bayesian inference of phylogeny uses the same models of evolution as many other methods of analysis (Heulsenbeck *et al.* 2001). It is based on a quantity called the posterior probability of a tree where Bayes's theorem combines the prior probability of a phylogeny (Pr [Tree]) with the likelihood (Pr [Data | Tree]) divided by the prior probability of the data (Pr [Data]) to give the posterior probability distribution on trees given the data (Pr [Tree | Data]) and is interpreted as the probability that the tree is correct (Heulsenbeck *et al.* 2001) given a particular model. Instead of searching for the optimal tree, one samples from the set of all possible trees (theoretically), weighted by their posterior probabilities.

The posterior probability requires summation over all possible trees and integration over all possible combinations of branch length and substitution model parameter values which is impossible to do analytically for large data sets. However the numerical method of Markov Chain Monte Carlo allows the posterior probability of a tree to be approximated (Heulsenbeck *et al.* 2001). To do this we use a Markov chain that has as its state space the parameters of the statistical model and a stationary distribution with the posterior probability distribution of the parameters. A new tree is proposed by stochastically perturbing a current tree and this is either accepted or rejected: if accepted the new tree is subject to further perturbation (Heulsenbeck *et al.* 2001). For a Markov chain, the proportion of times that any tree is visited is an approximation of the posterior probability of that tree (Heulsenbeck *et al.* 2001). Sometimes chains fail to converge to the stationary distribution, and to avoid this Metropolis-coupled Markov chain Monte Carlo algorithms are employed (Heulsenbeck *et al.* 2001), which run a number of chains simultaneously. The actual sampling is then taken by swapping between an optimal running chain (called a heated chain) and those less so. To further avoid this problem one can run multiple Metropolis-coupled Markov chain Monte Carlo analyses and combine the resulting samples.

Once a sample is available, features that are common among the trees can be discerned; for example, the sample can be used to construct a consensus tree with the posterior probability of the individual clades indicated on the tree. This is roughly equivalent to performing a maximum likelihood analysis with bootstrap resampling, only much faster (Heulsenbeck *et al.* 2001).

### 1. 4. Aims of the thesis

One must remember that a tree is simply a hypothesis or model of the evolutionary history. When reconstructing a phylogeny using specific genes, one is estimating the gene tree and making inferences about species phylogenies (Moritz & Hillis 1996). There are many processes leading to gene trees having different histories from each other and/or being different to species phylogenies for example introgression, lineage sorting, reticulation and/or concerted evolution among alleles, and mistaken orthology (Wendel & Doyle 1998). Hence, it is important to apply multiple data sets to a common group of taxa so to understand the evolutionary processes underlying them; however, when occurring, these processes will often confuse species phylogeny reconstruction. Where only chloroplast data is utilised it is important to realise the limitation imposed by this fact on the results.

This thesis investigates the phylogenetic relationships and evolutionary history of the Lophoziaceae. The main aims are to i) establish a defensible delimitation of the Lophoziaceae using a molecular approach; ii) gain a clearer understanding of relationships within and among its often poorly delimited genera; iii) hypothesise a time line for the diversification of the group; and iv) attempt a reclassification that reflects phylogenetic relationships.

# Chapter 2

# Molecular insights into the phylogeny of the leafy liverwort family Lophoziaceae Cavers.[4]

## 2. 1. Introduction

The Lophoziaceae are a large (ca. 280 species), globally distributed family of leafy liverworts including species ranging from narrow endemics to those that are very widespread, rare species to very abundant ones, species with very frequent sexual reproduction to those unknown to produce spores, and species with and without asexual reproduction. Most taxa occur in cool to cold areas and in the tropics mostly in montane or alpine regions. In some environments (e.g. cooler and cold portions of the Northern Hemisphere, and in humus-rich acid habitats in montane tropical and temperate rain forests) the group is ecologically significant.

Classification of the Lophoziaceae is controversial at all levels, including views on the very existence of the family. Individual researchers have adopted radically different circumscriptions, and in particular its distinctness from, and relationship to, Jungermanniaceae has been debated. As with many other treatments, the most complete recent classification of hepatics (Crandall-Stotler & Stotler, 2000) includes Lophoziaceae as a subfamily of the latter. Similarly, Schuster (2002a) does not distinguish the two at family level because of perceived exceptions to any diagnostic characters that can be identified for each group. On the other hand, Grolle & Long (2000) recognise European Lophoziaceae (including two subfamilies, Lophozioideae and Jamesonielloideae) as distinct from Jungermanniaceae.

The delimitation of subfamilies and genera has also been problematic. Schuster (2002a), who subsumed the family within Jungermanniaceae, writes: "The problem of how to circumscribe subfamilies is also illuminated by the fact that most generalisations used to separate groups … are exactly that, generalisations; almost all are *transgressed* by one or



[4] This chapter has been published : de Roo, R.T., Hedderson, T. A., Söderström, L. 2007. Molecular insights into the phylogeny of the leafy liverwort family Lophoziaceae Cavers. Taxon. 56:301—314.

The data gathering and analytical phases of the study were performed by de Roo, who also produced the first draft of the paper, Hedderson and Söderström contributed in the form of ideas and comments before and during the writing of this paper.

more exceptions". He notes that generalisations derived from examination of Holarctic taxa alone may be deceptive and that in Austral areas a very different mix of characters may be seen (Schuster, 2002a). He does suggest that subfamilies Lophozioideae Cavers and Jamesonielloideae Inoue have closest affinity to each other, concordant with their treatment as subfamilies of Lophoziaceae by Grolle & Long (2000).

The same problems exist at generic level; Schuster (2002a) notes that when the criteria used for subdividing Holarctic members of the large and complex genus *Lophozia* are used for taxa from elsewhere, they largely fail for recognising segregate genera in this broader context. This is seen in the use of leaf-lobe number to separate *Barbilophozia* and *Orthocaulis*, a character that is not consistent when including the richer southern hemisphere flora rather than just Eurasian representatives (Schuster, 2002a).

Whilst molecular data have been used to great effect in improving our understanding of evolutionary relationships in many groups of plants and animals including bryophytes (e.g. Hedderson *et al.*, 1996, 1998, 2004; Capesius & Stech, 1997; Cox & Hedderson, 1999; Stech, 1999; Newton *et al.*, 2000), only a few recent studies have addressed higher-level relationships in hepatics (e.g. Davis, 2004; He-Nygrén *et al.*, 2004; Heinrichs *et al.*, 2005; He-Nygrén *et al.*, 2006). With respect to the Lophoziaceae, several recent studies (e.g. Davis, 2004; Yatsentyuk *et al.*, 2004; Heinrichs *et al.*, 2005; He-Nygren *et al.*, 2006; Hentschel *et al.*, 2006) [2] have shown that the family Jungermanniaceae s. lat. is polyphyletic and that current family and subfamily delimitations are largely artificial. In addition Schill *et al.* (2004) demonstrated that the large family Scapaniaceae is nested within Lophoziaceae (see also Yatsentyuk *et al.*, 2004; Heinrichs *et al.*, 2005; He-Nygrén *et al.*, 2006) and Heinrichs *et al.* (2005) formally include Lophoziaceae in Scapaniaceae.

In this paper we provide an analysis of chloroplast DNA variation in leafy liverworts, with particular emphasis on the relationships of Lophoziaceae. Our main objectives are to 1) test the monophyly of Lophoziaceae and the main taxonomic groupings within it and 2) evaluate the relationship between Jungermanniaceae and Lophoziaceae.



[2] See Addendum (2)

## 2. 2. Materials and Methods

The 190 exemplars included in the analyses are listed in Table 1. The nomenclature for families and higher taxonomic levels follows Crandall-Stotler & Stotler (2000) except for Jungermanniaceae and, by definition, also Lophoziaceae where we follow Grolle & Long (2002). Taxa were chosen to represent a wide range of Jungermanniidae with emphasis on the Lophoziaceae and taxa placed near it in most classifications. The sampling attempts as far as possible to represent the major lineages of the Jungermanniidae, at least as these are currently understood. In addition, we included *Riccardia, Metzgeria*, *Symphyogyna*, *Haplomitrium* and *Pellia* (Metzgeriidae) as outgroups. Most sequences were generated in the course of this work (35 *rps*4 sequences were taken from GenBank); see Table 1 for voucher information and GenBank accession numbers. The sequence for *Plicanthus* sp. (taxon 59 in Table 1) is labelled as *Chandonanthus* sp. in GenBank. However, it most probably belongs to *Plicanthus*, since "true" *Chandonanthus* occurs only in New Zealand. This specimen is from China and therefore almost certainly represents the segregate genus *Plicanthus* (Schuster, 2002b), either *P. hirtellus* or *P. birmensis*.

Total genomic DNA was extracted from herbarium specimens by the method of Edwards *et al.* (1991). Two chloroplast regions were sampled, the protein coding *rps*4 (Nadot *et al.*, 1994) and the tRNA-Gly (*trn*G) gene intron (Pacak & Szweykowska-Kulińska, 2003). Primers *rps*5 and *trna*S (Nadot *et al.*, 1995) were used to amplify the *rps*4 gene whilst primers A and B (Pacak & Szweykowska-Kulińska, 2003) were used to amplify the tRNA-Gly (*trn*G) gene intron. PCR amplification employed 30 cycles of one minute at 94ºC (denaturing), one minute at 52ºC (annealing), and two minutes at 72ºC (extension), preceded by an initial melting step at 94ºC and followed by a final extension period of seven minutes at 72ºC. Fragments were cleaned with the GFX™ (Amersham Biosciences) PCR DNA and gel band purification kit. Amplification primers, used in conjunction with the ABI Prism™ Dye Terminator Cycle Sequence kit (Version 3.1), were also used as sequencing primers, and sequencing products were resolved on an ABI Prism 3100 genetic analyser.

Sequences were assembled and checked for inaccurate base calling using SeqMan II (Laser Gene System Software, DNAStar, Inc). Assembled sequences were aligned manually using MegAlign (Laser Gene System Software, DNAStar, Inc). The non-coding sequence at the 3' end of the amplified segment of *rps*4 was excluded, and *Trn*G

intron positions that are difficult to align over the wide range of taxa sampled were also excluded from the current analysis.

**Parsimony analysis. –-** Topologies were evaluated under the parsimony criterion using PAUP 4.0b8a (Swafford, 1998). A heuristic search was conducted with 40,000 replicates of random taxon addition using TBR branch swapping; only one tree was saved for each replicate. All characters were given equal weight and states were unordered. All most parsimonious trees (MPTs) were saved, to a maximum of 10, 000. Nodal support was evaluated by the jackknife as implemented in PAUP 4.0b8a, using 1000 resampling replicates. For each replicate 10 replicates of random taxon addition using TBR branch swapping were implemented, with a maximum of one tree saved at each replicate. Each replicate had 33.67% of characters deleted, and the "emulate Jac" resampling option was implemented.

**Bayesian Inference. –-** Bayesian phylogenetic analyses were conducted using MrBayes v3.0B4 (Huelsenbeck & Ronquist, 2003). We assume a uniform (uninformative) prior for the tree topology, branch lengths, and parameters of the substitution model. We feel this approach is justified given the lack of previous information on the group and the attendant taxonomic uncertainty.

A mixed-model approach (Huelsenbeck & Ronquist, 2003) was used for the substitution process. Hierarchical likelihood-ratio tests, implemented in Modeltest 3.6 (Posada & Crandall, 1998), indicated that each of the data partitions was best fit by the GTR+I+Γ model. This model incorporates separate time-reversible estimates of each possible substitution type, an estimate of the proportion of sites fixed at invariance, and an estimate of the shape of the gamma distribution to which variable sites are fitted. Parameter values were estimated independently across the two partitions. We used two independent runs of the Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) approach for sampling parameter values in proportion to their posterior probability. Each analysis used four chains, three heated and one unheated, run for $5 \times 10^6$ generations. Model parameters, including trees, were sampled every 500 generations. Plots of the likelihoods of each sample were used to ascertain the number of generations for stationarity to be reached in order to obtain the posterior probability tree set. Trees from the burn-in were excluded.

## 2. 3. Results

Of the 647 *rps*4 nucleotide positions included in the alignment, 452 (70%) exhibited variation and 389 (60%) were parsimony-informative across the range of taxa included in our analysis. Of the 828 included characters for the trnG intron, 492 (59%) exhibited variation and 388 (47%) were parsimony-informative. In the total DNA matrix there was, therefore, a total of 1475 characters of which 944 (64%) were variable and 777 (53%) potentially parsimony-informative.

Under the parsimony criterion, 10,000 trees were retained (L=4926, CI=0.3218, RCI=0.2242). Not all relationships are well-supported (Fig. 2.1); areas of disagreement are found among accessions of the same species, within clades of closely related species and among some of the deeper nodes of the phylogeny. Within the "core" Lophoziaceae clade (Fig. 2.1b), a number of strongly supported clades of affiliated species are revealed, but relationships among these clades are for the most part poorly resolved. Overall, relationships are better resolved and more strongly supported under Bayesian inference (Fig. 2.2); the MCMCMC search required 400,000 and 750,000 generations respectively for each analysis to reach stationarity; and the combined 2,300 trees obtained during these burn-in periods were discarded. The first analysis had a 95 % credible set containing 8,741 trees (of 9,201 trees), the second analysis had a 95 % credible set containing 8,076 trees (of 8,501 trees). These formed a combined total of 16,817 sampling points for the posterior probability tree set. The median and 95% credible intervals of the model parameters are shown in Table 2.2.

**Table 2. 2.** Parameter values for the first Bayesian analysis.

| | 95% Cred. Interval | | |
|---|---|---|---|
| Parameter | Lower | Upper | Median |
| TL{all} | 11.969000 | 15.319000 | 13.335000 |
| r(G<->T){trnG} | 1.000000 | 1.000000 | 1.000000 |
| r(C<->T){trnG} | 2.432825 | 4.556134 | 3.337974 |
| r(C<->G){trnG} | 0.675148 | 1.515353 | 1.012487 |
| r(A<->T){trnG} | 0.235286 | 0.465749 | 0.329869 |
| r(A<->G){trnG} | 3.039681 | 4.716709 | 3.766826 |
| r(A<->C){trnG} | 0.619415 | 1.256443 | 0.886786 |
| r(G<->T){rps4} | 1.000000 | 1.000000 | 1.000000 |
| r(C<->T){rps4} | 6.260180 | 10.475860 | 7.985856 |
| r(C<->G){rps4} | 1.309878 | 2.604897 | 1.836572 |
| r(A<->T){rps4} | 0.205861 | 0.410085 | 0.295015 |
| r(A<->G){rps4} | 7.460351 | 11.572561 | 9.248623 |
| r(A<->C){rps4} | 1.624953 | 2.895411 | 2.167063 |
| pi(A){trnG} | 0.356964 | 0.409225 | 0.383303 |
| pi(C){trnG} | 0.126317 | 0.162729 | 0.143550 |
| pi(G){trnG} | 0.094024 | 0.125552 | 0.108344 |
| pi(T){trnG} | 0.339705 | 0.391168 | 0.363824 |
| pi(A){rps4} | 0.326683 | 0.381278 | 0.353291 |
| pi(C){rps4} | 0.124268 | 0.156549 | 0.140382 |
| pi(G){rps4} | 0.164841 | 0.199547 | 0.181862 |
| pi(T){rps4} | 0.298162 | 0.350048 | 0.324145 |
| alpha{trnG} | 0.534336 | 0.694894 | 0.611168 |
| alpha{rps4} | 0.489224 | 0.587253 | 0.535510 |
| m{trnG} | 1.142932 | 1.336475 | 1.240365 |
| m{rps4} | 0.569395 | 0.817082 | 0.692392 |

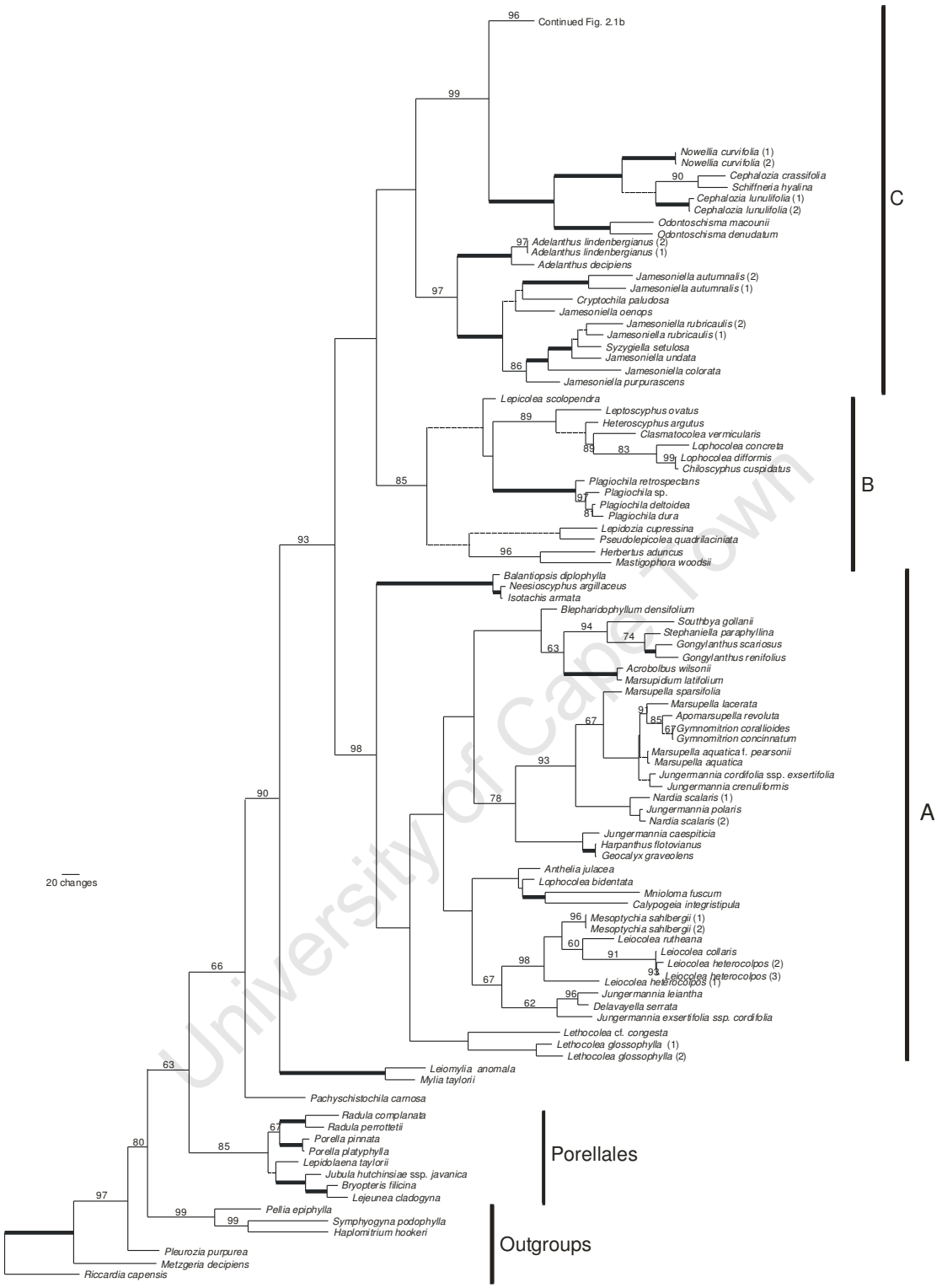




**Fig. 2. 1.** One of 10, 000 parsimonious trees from unweighted analysis with jackknife support (>60%) indicated for individual nodes. Stippled branches collapse in the strict consensus. Because of its size, the tree has been split into two parts. Numbers after taxa correspond to numbers in Table 2. 1.

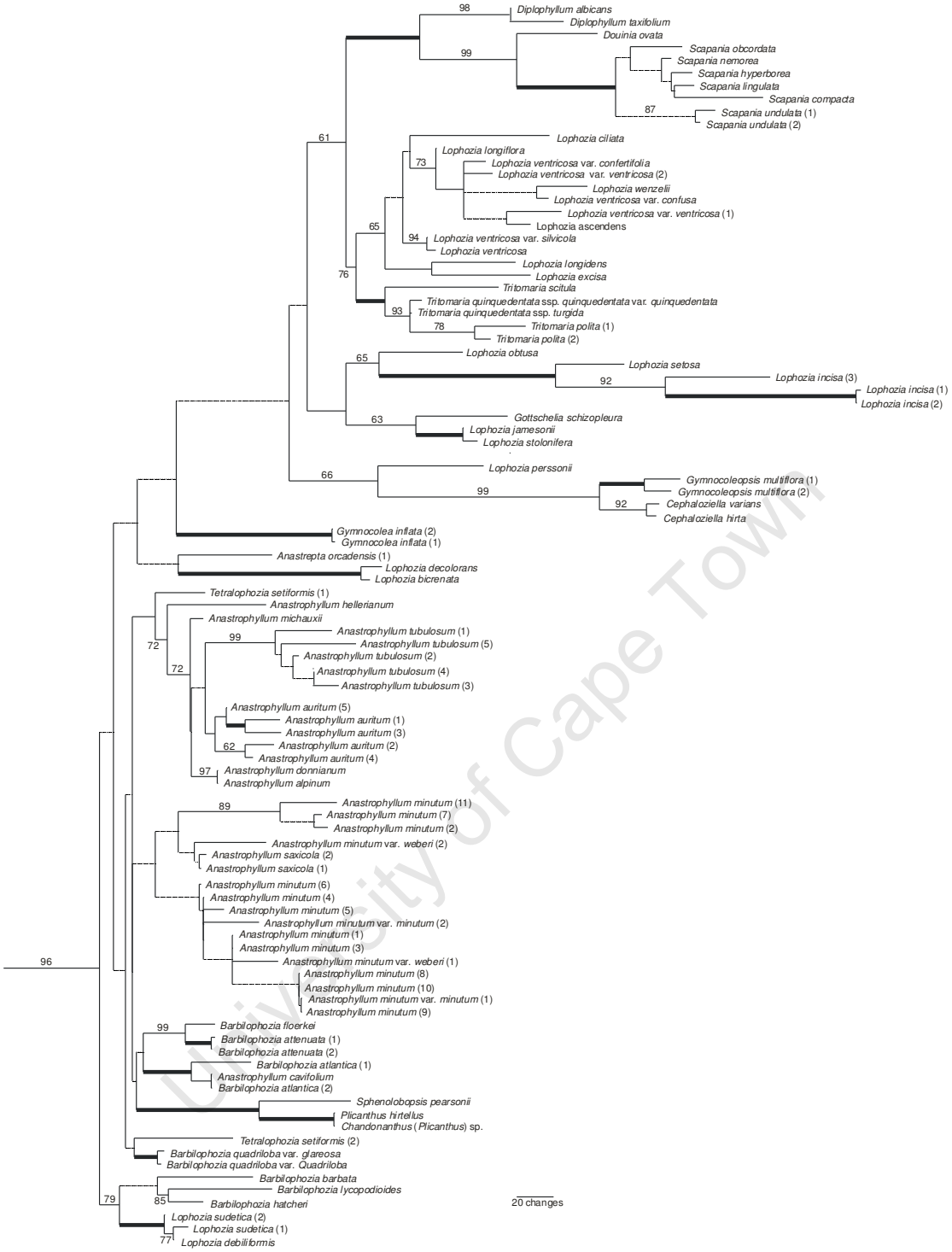
Diplophyllum albicans
Diplophyllum taxifolium
Douinia ovata
Scapania obcordata
Scapania nemorea
Scapania hyperborea
Scapania lingulata
Scapania compacta
Scapania undulata (1)
Scapania undulata (2)
Lophozia ciliata
Lophozia longiflora
Lophozia ventricosa var. confertifolia
Lophozia ventricosa var. ventricosa (2)
Lophozia wenzelii
Lophozia ventricosa var. confusa
Lophozia ventricosa var. ventricosa (1)
Lophozia ascendens
Lophozia ventricosa var. silvicola
Lophozia ventricosa
Lophozia longidens
Lophozia excisa
Tritomaria scitula
Tritomaria quinquedentata ssp. quinquedentata var. quinquedentata
Tritomaria quinquedentata ssp. turgida
Tritomaria polita (1)
Tritomaria polita (2)
Lophozia obtusa
Lophozia setosa
Lophozia incisa (3)
Lophozia incisa (1)
Lophozia incisa (2)
Gottschelia schizopleura
Lophozia jamesonii
Lophozia stolonifera
Lophozia perssonii
Gymnocoleopsis multiflora (1)
Gymnocoleopsis multiflora (2)
Cephaloziella varians
Cephaloziella hirta
Gymnocolea inflata (2)
Gymnocolea inflata (1)
Anastrepta orcadensis (1)
Lophozia decolorans
Lophozia bicrenata
Tetralophozia setiformis (1)
Anastrophyllum hellerianum
Anastrophyllum michauxii
Anastrophyllum tubulosum (1)
Anastrophyllum tubulosum (5)
Anastrophyllum tubulosum (2)
Anastrophyllum tubulosum (4)
Anastrophyllum tubulosum (3)
Anastrophyllum auritum (5)
Anastrophyllum auritum (1)
Anastrophyllum auritum (3)
Anastrophyllum auritum (2)
Anastrophyllum auritum (4)
Anastrophyllum donnianum
Anastrophyllum alpinum
Anastrophyllum minutum (11)
Anastrophyllum minutum (7)
Anastrophyllum minutum (2)
Anastrophyllum minutum var. weberi (2)
Anastrophyllum saxicola (2)
Anastrophyllum saxicola (1)
Anastrophyllum minutum (6)
Anastrophyllum minutum (4)
Anastrophyllum minutum (5)
Anastrophyllum minutum var. minutum (2)
Anastrophyllum minutum (1)
Anastrophyllum minutum (3)
Anastrophyllum minutum var. weberi (1)
Anastrophyllum minutum (8)
Anastrophyllum minutum (10)
Anastrophyllum minutum var. minutum (1)
Anastrophyllum minutum (9)
Barbilophozia floerkei
Barbilophozia attenuata (1)
Barbilophozia attenuata (2)
Barbilophozia atlantica (1)
Anastrophyllum cavifolium
Barbilophozia atlantica (2)
Sphenolobopsis pearsonii
Plicanthus hirtellus
Chandonanthus (Plicanthus) sp.
Tetralophozia setiformis (2)
Barbilophozia quadriloba var. glareosa
Barbilophozia quadriloba var. Quadriloba
Barbilophozia barbata
Barbilophozia lycopodioides
Barbilophozia hatcheri
Lophozia sudetica (2)
Lophozia sudetica (1)
Lophozia debiliformis
20 changes

**Fig. 2. 1.** cont.


**Fig. 2. 2.** A majority-rule consensus of the trees (excluding burn-in) for the first analysis (generated by MrBayes). The posterior probabilities from the combined 95% credible tree set are indicated for individual nodes. Again, the tree has been split into two parts. Numbers after taxa correspond to numbers in Table 2. 1.

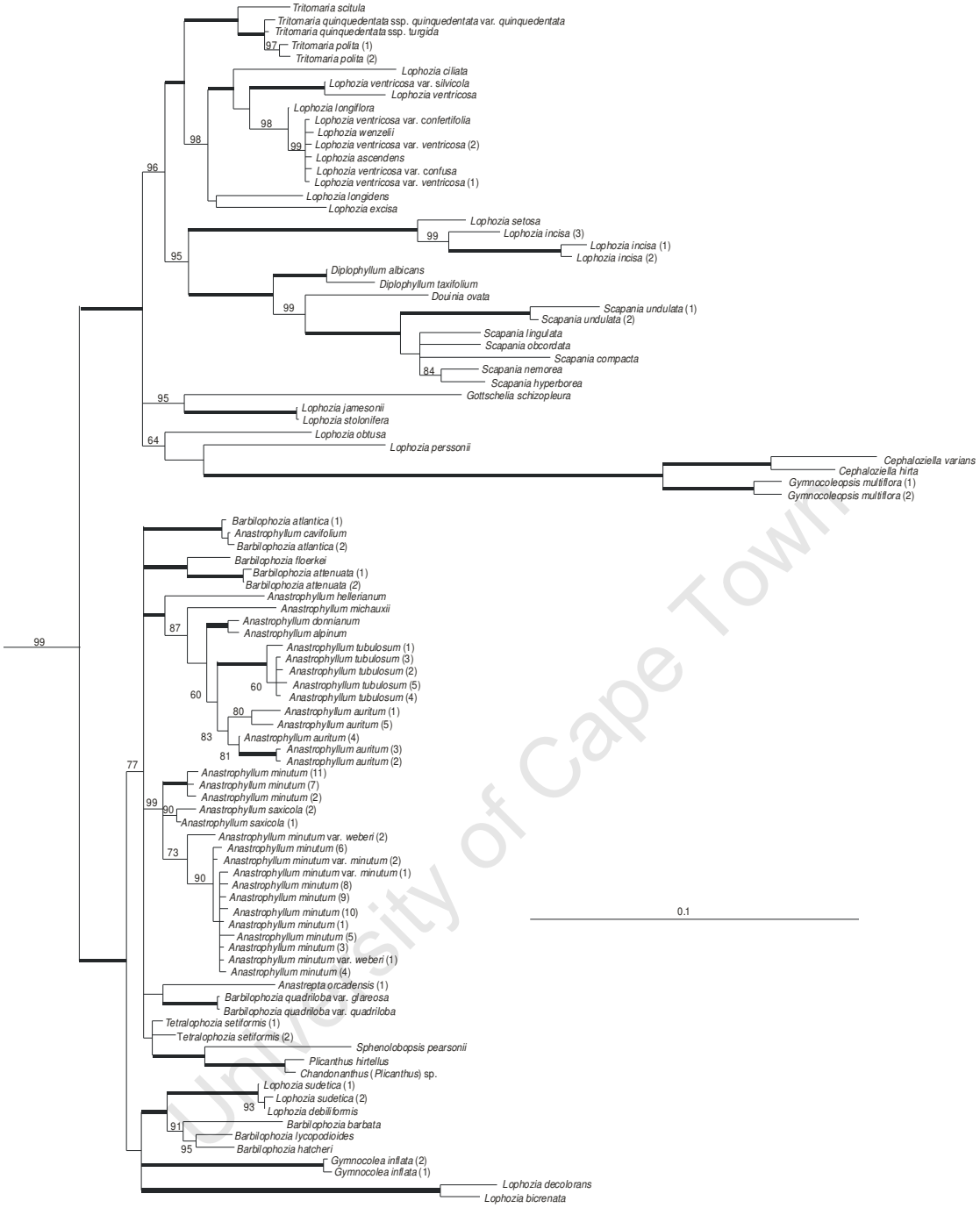


**Fig. 2. 2.** cont.

The Jungermanniidae form two clades that, together, are resolved as a poorly supported monophyletic group (jackknife percentage (JK) = 63, posterior probability (PP) = 66). The first clade (Porellales Clade; JK = 85, PP = 99) includes elements of Porellales, Radulales and Lepidolaenaceae (*Lepidolaena taylorii*). The second clade (JK = 66, PP = 96) includes Schistochilaceae (*Pachyschistochila carnosa*) as sister to a large clade (JK = 90, PP = 100) of the remaining leafy taxa within which the majority of taxa fall into one of three well-supported, high-level clades. The first of these (Clade A; JK = 98, PP = 100) includes elements of Jungermanniaceae s. str., Gymnomitriaceae, Geocalycaceae

(*Lophocolea bidentata*), Balantiopsaceae, Acrobolbaceae, Antheliaceae, Arnelliaceae, Scapaniaceae (*Blepharidophyllum* and *Delavayella*), Mesoptychiaceae, and one genus of Lophoziaceae (*Leiocolea*). It is noteworthy that *Stephaniella* is nested within a strongly supported (JK = 94, PP = 100) Arnelliaceae. The second (Clade B; JK = 85, PP = 90) includes Pseudolepicoleaceae, Plagiochilaceae, Geocalycaceae (*Clasmatocolea*, *Heteroscyphus*, *Lophocolea* and *Leptoscyphus*), Lepidoziaceae (*Lepidozia cupressina*), Lepicoleaceae(*Lepicolea scolopendra*), Herbertaceae (*Herbertus aduncus* ) and Mastigophoraceae (*Mastigophora woodsii*). The final group (Clade C; unsupported in the parsimony tree; PP = 100) encompasses all Lophoziaceae except *Leiocolea*, a core group of Scapaniaceae, Cephaloziaceae, Adelanthaceae and Cephaloziellaceae. Ambiguous in its position with respect to Clades A, B and C is a well-supported group (JK = 100, PP = 100) made up of *Mylia taylorii* and the segregate genus *Leiomylia anomala* (Jungermanniaceae). In the parsimony analysis this is placed sister to the rest whereas in the Bayesian analysis it is sister to Clade A alone (PP = 97). Relationships among clades A, B and C are ambiguous; in the parsimony analysis Clade A is sister to Clades B and C, but this relationship is unsupported by the jackknife and the Bayesian analysis.

Within Clade C, a strongly supported grouping (JK = 97, PP = 100) of *Adelanthus* (Adelanthaceae) sister to a strongly supported (JK = 100, PP = 100) Jamesonielloideae (*Jamesoniella*, *Cryptochila* and *Syzygiella*) is sister to a strongly supported group (JK = 99, PP = 100) comprising the rest of the taxa. The remaining Lophoziaceae, Scapaniaceae and *Cephaloziella* (Fig. 2.1b) form a well supported group (JK = 96, PP = 99) to which a strongly supported (JK = 100, PP = 100) clade of *Cephalozia*, *Nowellia, Schiffneria* and *Odontoschisma* is sister. Within the former group, two strongly-supported clades are resolved. The first (PP = 100) includes most elements of the Lophoziaceae (*Barbilophozia, Anastrophyllum, Anastrepta, Tetralophozia*, *Sphenolobopsis, Plicanthus*, *Lophozia and Gymnocolea*) and is hereafter denoted the *Anastrophyllum* clade, whilst the second (PP = 100), including Scapaniaceae (*Diplophyllum*, *Scapania* and *Douinia*), some Lophoziaceae (*Lophozia*, *Gymnocoleopsis, Gottschelia* and *Tritomaria*) and Cephaloziellaceae (*Cephaloziella*) is hereafter denoted the *Scapania* clade.

Within the *Anastrophyllum* clade a number of well-supported groupings emerge, but the relationships among these are not resolved. One of these is a strongly supported group

(JK = 100, PP = 100) with the mono-generic *Sphenolobopsis pearsonii* sister to *Plicanthus*. *Barbilophozia hatcheri*, *B. barbata* and *B. lycopodioides* and a strongly supported group (JK = 100, PP = 100) of *Lophozia sudetica* and *L. debiliformis* together form a well-supported group (JK = 79, PP = 100).

In the *Scapania* clade, again a number of well-supported groupings emerge. *Gymnocoleopsis multiflora* is strongly supported (JK = 99, PP = 100) as sister to a strongly supported group (JK = 100, PP = 100) comprising Cephaloziellaceae (*Cephaloziella*). The Scapaniaceae (*Diplophyllum*, *Douinia* and *Scapania*) are strongly supported (JK = 100, PP = 100) as monophyletic. Another well supported group (JK = 76, PP = 100) includes a strongly supported (JK = 100, PP = 100) grouping of sampled *Tritomaria* taxa placed sister to a well supported group (JK = 65, PP = 98) of *Lophozia* taxa including *L. ciliata*, *L. longiflora*, *L. wenzelii*, *L. ascendens*, *L. longidens*, *L. excisa* and the *L. ventricosa* species complex. *Lophozia incisa* and *L. setosa* form a strongly supported group (JK = 100, PP = 100).

With respect to the taxa of interest to this study, a few differences emerge in comparisons between the Bayesian and Parsimony results. For example the *Barbilophozia hatcheri* - *Lophozia sudetica* grouping falls within the *Anastrophyllum* clade in Bayesian analysis, but is placed as sister to the other Lophoziaceae, Scapaniaceae and *Cephaloziella* in the Parsimony tree. Similarly, the *Lophozia incisa* group is found supported (PP = 95) as sister to the Scapaniaceae in the Bayesian analysis, whereas it is found sister to the group of *Gottschelia schizopleura*, *Lophozia jamesonii* and *L. stolonifera* in the parsimony tree. Within Clade A, *Leiocolea spp.* and *Mesoptychia sahlbergii* form a strongly supported group (JK = 98, PP = 100). However while *Leiocolea* itself is resolved as monophyletic, albeit without support, in the Bayesian analysis, *Mesoptychia* is nested within this genus under the Parsimony criterion. None of these are strong conflicts in the sense that where the two differ the parsimony results are usually poorly supported.

## 2. 4. Discussion

TrnG and *rps*4 provide considerable resolution of phylogenetic relationships in the leafy liverworts. In almost all instances the posterior probabilities recovered were higher than the jackknife levels of support; this can be explained through a) differences in the concepts of support implicit in the different statistical methods and b) better performance of an explicit modelling approach. Overall the Bayesian analyses yield better resolution, especially in the core *Lophozia* clade. There is no strong conflict between the two sets of results, and where the two differ the parsimony results are usually poorly supported. Therefore much of the discussion centres on the Bayesian results.

The relationships recovered here correspond well to results of previous studies. The Porellales-Radulales Clade identified here corresponds to the Leafy I clade of Davis (2004), the Porellales–Lepidolaenineae clade of He-Nygrén *et al.* (2004), the Porellales-Radulales clade (Ahonen, 2004) and the Porellales clade of Heinrichs *et al.* (2005). The second clade, comprising the remaining leafy liverworts, corresponds to the Leafy II clade of Davis (2004), the Perssoniellineae–Herbertineae clade of He-Nygrén *et al.* (2004) and the Jungermanniales clade of Heinrichs *et al.* (2005).

*Mylia* forms a deep branch in both Bayesian and parsimony analyses, being either sister to all remaining leafy liverworts or to Clade A. This supports the recent resurrection by Engel & Braggins (2005) of the family Myliaceae and suborder Myliinae to accommodate the genus (see also Hentschel *et al.*, 2006). Their argument was based on the occurrence in *M. taylorii* of unique cell wall characters, namely the presence of perforations over the middle lamella, and the leaf surface having irregular plates or strips of smooth wall material separated by fibril-filled grids. In the same study, these authors erected the genus *Leiomylia,* retained in Jungermanniaceae, to accommodate *M. anomala*, which lacks these cell wall features. However, in our analyses the two *Mylia* species form a very strongly supported grouping, and we would argue that separate generic status is unwarranted. *Trabacellula tumidula* Fulford, usually allocated to the Cepohaloziaceae, was also found to possess these cell wall characters and was placed back by Engel and Braggins in its own family Trabacellulaceae, in the new suborder Myliineae (Engel & Braggins, 2005). Inclusion of this taxon in molecular analyses would shed further light on the evolution of what appears to be a unique set of cell wall characteristics and allow

determination of whether the absence of these in *Mylia anomala* represents a secondary reversal or the plesiomorphic condition.

The remaining Jungermanniaceae *s. lat.* (sensu Crandall-Stotler & Stotler, 2000; and others) are clearly polyphyletic. Of the Jungermanniaceae s. str. included in this study, all, with the exception of *Mylia,* all are found nested within Clade A. However, even in the strict sense the family is not monophyletic with the bulk of the sampled Gymnomitriaceae (ex. *Stephaniella*) located in the same clade with *Nardia* and some of the *Jungermannia* spp. *Jungermannia* itself is also polyphyletic, with *J. polaris* (nested within *Nardia*), *J. caespiticia*, *J. cordifolia* and *J. crenuliformis* placed in a clade containing Gymnomitriaceae and Geocalycaceae whilst *J. exsertifolia* and *J. leiantha* are in a group with *Leiocolea*, Mesoptychiaceae, *Delavayella* and Calypogeiaceae. *Stephaniella,* a genus hitherto placed in Gymnomitriaceae or in a family of its own, is found nested as part of Arnelliaceae. Schuster (2002a) comments that oil-body criteria suggest that *Stephaniella* does not belong in the Gymnomitriaceae. The genus is quite unique in its nearly chlorophyll-free leaves and elaborated paraphyllia (Schuster, 2002a). The placement of *Lophocolea bidentata* is odd, and may be based on a misidentification or contamination. The sequence was retrieved from GenBank and verification of identity is beyond the scope of this study.

The majority of sampled Lophoziaceae (s. str.) fall into a well-supported group that is not particularly closely related to Jungermanniaceae. However, *Leiocolea,* usually classified as Lophoziaceae, does not belong to this clade but is nested (with *Mesoptychia*) in a mixed group of Jungermanniaceae, *Delavayella* (Scapaniaceae) and Calypogeiaceae. Schuster (1969) refers to *Leiocolea* as a closely allied group of species, and suggested that the retention of the taxon as a part of *Lophozia* s. lat. results in a "sharply circumscribed and very isolated sub-generic group in *Lophozia*". However, he retained *Leiocolea* as a part of *Lophozia* s. lat. because characters used by others to segregate the genus recur sporadically in taxa of *Lophozia*. Schuster (1969) also comments that the perianth of *Leiocolea* is extremely characteristic, being tubular and terete and quickly constricted near the apex into a small narrow beak. Supporting the position of *Mesoptychia* as sister to *Leiocolea*, the immature perianth of *Mesoptychia* is described as beaked, and there is a strong resemblance in leaf form between *L. rutheana* and *Mesoptychia* with both showing a very oblique line of leaf insertion (Schuster, 1969).

Our results (cf. also Yatsentyuk *et al.*, 2004) therefore suggest the inclusion of *Leiocolea* in Mesoptychiaceae.

The subfamilies Jamesonielloideae and Adelanthaceae form a group, with the sampled Jamesonielloideae monophyletic. Inoue (1966) placed the subfamily in Lophoziaceae, whereas Schuster (2002a) regards it as showing both Lophozioideae and Jungermannioideae characters, thus placing all three groups in an expanded Jungermanniaceae. Whilst He-Nygren *et al.* (2006) recently elevated Jamesonielloideae to the rank of family, (Jamesoniellaceae), an alternative is to transfer the subfamily to Adelanthaceae, as suggested by Hentschel *et al.* (2006). Morphologically, the Adelanthaceae have geotropic rhizoidous axes which are sometimes present in Jamesonielloideae, both have ventral to lateral intercalary and, when present, Frullania-type terminal branching, both share reduced/ephemeral (absent in some Adelanthaceae) underleaves and both lack a perigynium (Schuster, 2002a).

*Jamesoniella* itself is not monophyletic because *Syzygiella* and *Cryptochila* are nested within it. Schuster (2002a) regards *Syzygiella* as belonging to a subfamily of Plagiochilaceae because of perfectly opposite leaves (Jamesonielloideae have alternate leaves). Inoue (1966) saw *Syzygiella* as being strongly related to Lophoziaceae by having pluriplicate perianths with the mouth contracted, well-developed bracts and bracteoles that are united at least at the base, a distinct tendency toward reddish or purple pigmentation, and distinct mycorrhizae among the cells of the stem. He placed *Syzygiella* as part of Jamesonielloideae because of small, vestigial (or often totally absent) underleaves, perianths having plicae in the upper half, postical-intercalary branching and a total absence of vegetative propagulae. Based on *rbc*L sequences, Groth & Heinrichs (2005) also concluded that relationships of *Syzygiella* are with the Lophoziaceae rather than Plagiochilaceae. Schuster (2002a) notes that criteria used to distinguish *Cryptochila* Subg. *Acinaria* Grolle (including *C. paludosa*), are all features that recur in *Jamesoniella* and that *Acinaria* could be assigned to *Jamesoniella*. It is also clear that *Jamesoniella colorata* as defined by Grolle (1971) includes more than one species since *J. oenops* (synonymised with *J. colorata* by Grolle, 1971) is well separated from *J. colorata*.

Cephaloziaceae is a large family; with only 4 of 15 genera sampled (Crandall-Stotler & Stotler, 2000). The sampled species form a strongly supported clade sister to the main clade containing Lophoziaceae, Scapaniaceae and the sampled Cephaloziellaceae taxa.

The genus *Cephalozia* itself appears paraphyletic since *Schiffneria hyalina* is sister to *Cephalozia crassifolia*.

The majority of Scapaniaceae are nested within Lophoziaceae as reported previously (Schill *et al.*, 2004; Yatsentyuk *et al.*, 2004; Heinrichs *et al.*, 2005; He-Nygren *et al.*, 2006), but *Blepharidophyllum* and *Delavayella* do not belong with the rest of the family. Schuster (1961, 1974, 1984, 1999) has already noted that these are not members of Scapaniaceae (for *Delavayella* see also Schill *et al.*, 2004) remarking that a whole ensemble of differences, including rhizoid dispersion, type of asexual reproduction, leaf symmetry, leaf lobing and shoot apex orientation, separate Scapaniaceae, *Blepharidophyllum* and *Delavayella*, and that these three should go into autonomous families, placing the latter two in Blepharidophyllaceae and Delavayellaceae respectively (Schuster, 1999). Schuster (1974) proposed that Scapaniaceae s. str. is closely related to the "less derivative" Lophoziaceae, and noted that most taxa of the two families share the following features: i) bilobed leaves with the dorsal lobe tending to be smaller than the ventral, ii) succubous ventral and almost transverse dorsal insertion of the leaves, iii) normally exclusively lateral branching, iv) gemmae produced freely in branched fascicles and of similar form, v) terminal perianths, vi) androecia relatively unmodified and intercalary, vii) paraphyllia often found with the male bracts and ix) a multistratose capsule wall.

The main elements of Lophoziaceae fall into two main clades. There is strong support for a clade including mainly *Anastrophyllum* and *Barbilophozia* (the "*Anastrophyllum*" clade). Also included in this clade are *Tetralophozia setiformis, Plicanthus, Sphenolobopsis pearsonii*, *Anastrepta orcadensis*, *Gymnocolea inflata* and a few *Lophozia* species (*L. bicrenata*, *L. decolorans, L. sudetica* and *L. debiliformis*). This group needs more sampling but it is clear that none of the sampled genera with more than one representative is monophyletic. *Anastrophyllum minutum* and *A. saxicola* form a strongly supported clade which corresponds to the previously recognised genus *Sphenolobus*. The remaining *Anastrophyllum* species except *A. cavifolium* form a separate clade to which this genus name can be applied. *A. cavifolium* seems to be a form of *Barbilophozia*, possibly conspecific with *B. atlantica*. With the present sampling the monophyly of the genus *Anastrophyllum* cannot be rejected. It is also notable that *A. minutum* specimens form two strongly supported clades that form a trichotomy with *A. saxicola*. Three specimens of *A. minutum* from the Southern Hemisphere (Venezuela and

South Africa) and one from Norway form a group that is clearly separated from the rest. The species may therefore not be monophyletic and possibly cryptic speciation occurs in this widespread taxon. Alternatively the apparent paraphyly could be due to gene-level coalescent processes. This interesting species complex requires a wider and more detailed study to explore these possibilities.

*Barbilophozia* s. str. is monophyletic while the group of taxa sometimes placed in the genus or subgenus *Orthocaulis* (Buch) Schust. is polyphyletic. *Lophozia sudetica* (incl. *L. debiliformis*) is sister to *Barbilophozia* and may be transferred to the latter. The two sampled representatives of subgen. *Isopaches*, *Lophozia decolorans* and *L. bicrenata,* form a well-supported group. The two remaining members of this subgenus, *L. alboviridis* R.M.Schust. and *L. pumicicola* Berggr. have not been sampled, but our results indicate that *Isopaches* should be recognised at generic level.

The second clade of "Lophoziaceae" (the "*Scapania*" clade) is strongly supported but also needs more sampling. Included in this clade are also *Gottschelia*, *Gymnocoleopsis* and *Tritomaria*. Relationships in general are better resolved in this clade than in the "*Anastrophyllum*" clade, but the "backbone" relationships of this group are still unclear.

Our results support the inclusion of Lophoziaceae by Heinrichs *et al.* (2005) and perhaps also Cephaloziellaceae (type species not sampled) in Scapaniaceae unless many small families are erected. Affinities of *Cephaloziella* have sometimes been assumed to be with the Lophozioids as Douin's genus name "*Lophoziella*" testifies (Schuster, 1971, 2002a). Schuster (1971) notes that reduced members of Cephaloziellaceae and of the Lophoziaceae both may have very reduced setae, and that additional plicae occur in the perianth of most *Cephaloziella* and *Cylindrocolea* species, lending the impression that this group is allied to the Lophoziaceae.

*Cephaloziella varians* and *C. hirta* (Cephaloziellaceae) form a clade sister to *Gymnocoleopsis*. Taxa currently in *Gymnocoleopsis* have previously been assigned to both *Gymnocolea* and *Lophozia* (Schuster, 2002a). However, *Gymnocoleopsis* is not closely associated with *Gymnocolea,* which is found in the "*Anastrophyllum*" clade. The two differ mainly in that *Gymnocoleopsis* is autoecious, while *Gymnocolea* is dioecious (Schuster, 2002a). *Gymnocoleopsis* also differs from *Lophozia* in the seta being consistently 8 + 4 seriate, a condition also found in some Cephaloziellaceae (Schuster,

1971), the branches are uniformly lateral-intercalary and the capsule walls are bistratose, also the case for most Cephaloziellaceae (Schuster, 1971), with both strata almost equal in height (Schuster, 2002a).

Another strongly supported branch is the Scapaniaceae when *Delavayella* and *Blepharidophyllum* are excluded. The sampled *Scapania* species are monophyletic and sister to *Douinia* and a monophyletic *Diplophyllum*. Clearly, the suggestion by Potemkin (1999) to segregate *Diplophyllum* and *Douinia* (into Diplophyllaceae) as "a group of different origins resulting in a different morphology" is not supported.

The strong support for *Tritomaria* and its sister relationship with a clade comprising species from *Lophozia* (mainly subgen. *Lophozia*) is supported morphologically. In both groups, ventral sectors of the stem medulla becoming brown and strongly mycorrhizal, ventral merophytes are narrow and consequently unable to produce underleaves, the gynoecial bracteole is lacking (or reduced), perianths are plicate, and branching is of the *Frullania* and *Radula*-type as well as lateral-intercalary (Schuster, 2002a). *Tritomaria* itself has morphological support by the possession of trilobed leaves, and by having the cuticle of the leaves and stem almost always verruculose to finely ridged (Schuster, 1969).

*Lophozia* s. lat. as delimited by most recent authors is polyphyletic and several of the previously segregated genera should be re-instated. Thus, for example, one of the strongly supported clades includes species sometimes placed in *Schistochilopsis* (*L. setosa* and *L. incisa*) and that genus should probably be recognised although the type species, *S. cornuta* (Steph.) Konstantinova, has not been sampled. It is worth noting that *Lophozia ventricosa* var. *silvicola* is well separated from var. *ventricosa* and should be re-instated as a species. On the other hand, *L. jamesonii* and *L. stolonifera* are suggested by J. Váňa (pers. comm.) as possibly conspecific; Schuster (2002a) notes that except for *L. jamesonii* being gemma-free, the two are virtually identical and our results support such a treatment. The generic position of the species is unclear as is the position of *L. perssonii* and *L. obtusa*. The recognition of *Obtusifolium* S. Arn. for the last species could be advocated, but as the clades around it are poorly resolved, more sampling of *Lophozia* taxa is needed.

These suggestions, as well as many relating to higher-level (i.e. supra-familial) relationships within the Jungermanniales as a whole, need to be addressed in the interests of a stable classification that truly reflects common ancestry. However, alterations need to be made in the context of much wider species sampling. Furthermore, the addition of molecular and anatomical characters would contribute greatly to better understanding the evolution of the diversity in these groups. In addition, hypotheses advanced here need to be tested by data from additional DNA regions. This study has utilised plastid loci; however the plastid genome is inherited as a unit and usually uniparentally, so there is a danger that our current understanding of phylogeny in reality encompasses only the chloroplast history. It is important therefore that future studies include information from morphology, anatomy and other genomes, especially nuclear loci, to facilitate a better understanding of liverwort evolutionary history.

## 2. 5. Conclusions

The current classification of leafy hepatics is highly inconsistent with phylogeny as revealed by chloroplast markers. We highlight the following conclusions from our analyses:

1. Lophoziaceae, and perhaps also Cephaloziellaceae, should be placed in Scapaniaceae unless many smaller families are recognized. They are not closely related to Jungermanniaceae.

2. *Delavayella* and *Blepharidophyllum* should be excluded from Scapaniaceae.

3. Jamesonielloideae is a family of its own (Jamesoniellaceae) sister to Adelanthaceae, or should be included in Adelanthaceae.

4. The genus *Anastrophyllum* should be split into *Anastrophyllum* and *Sphenolobus*.

5. *Lophozia* is polyphyletic and the genera *Isopaches* and *Schistochilopsis*, and perhaps *Obtusifolium*, should be recognized while *L. sudetica* could be transferred to *Barbilophozia*. However, the generic position of many *Lophozia* species is still unclear.

6. *Barbilophozia s. str*. is monophyletic, while *Orthocaulis* is polyphyletic with the four sampled species appearing in 3 different clades. However, their relationships to other taxa are poorly resolved.

7. *Lophozia silvicola* Buch is clearly separated from *L. ventricosa* at species level.

8. *Jamesoniella oenops* is clearly separated from *J. colorata* at species level.

9. The generic status of *Leiomylia* is unwarranted.

# Chapter 3

# The *Anastrophyllum* Clade: phylogenetic evidence from multiple data sets

## 3. 1. Introduction

Our previous analyses of sequence variation at the chloroplast loci *rps*4 and *trn*G intron clarified phylogenetic relationships considerably among leafy liverworts, particularly the various groups assigned to Lophoziaceae (Chapter 2: de Roo *et al.* 2007). One of the key findings was a strongly supported "core" clade comprising Scapaniaceae-Cephaloziellaceae and most of the sampled genera of Lophoziaceae *s. str.* (Lophozioideae). Under the Bayesian criterion, two, very well supported, main clades were resolved within this large clade – one denoted the *Scapania* clade comprising Scapaniaceae (*Diplophyllum*, *Scapania* and *Douinia*), some Lophoziaceae (*Lophozia*, *Gymnocoleopsis, Gottschelia* and *Tritomaria*) and Cephaloziellaceae (*Cephaloziella*), and the other, denoted the *Anastrophyllum* clade, comprising most elements of the Lophoziaceae (*Barbilophozia, Anastrophyllum, Anastrepta, Tetralophozia*, *Sphenolobopsis, Plicanthus*, *Lophozia and Gymnocolea*). This *Anastrophyllum* clade was not supported in the parsimony analysis.



The taxa placed in the *Anastrophyllum* clade are all 2-4-lobed and if gemmae are produced these are red or brown. In chapter two it was found that within the *Anastrophyllum* clade a number of well-supported groupings emerge, but the relationships among these were not resolved. This clade is of particular interest due to problems in delimitation of subfamilies and genera of the Lophoziaceae as discussed in chapter two. Whilst the rps4 and trnG Intron data resolved *Anastrophyllum*, *Sphenolobus*, *Isopaches*, *Schistochilopsis*, *Obtusifolium* and *Barbilophozia* lineages within the *Anastrophyllum* clade, relationships among these were not recovered (de Roo *et al.* 2007).

Resolution of phylogenetic relationships depends greatly on the number of characters available for analysis. Thus, the ability to recover robust phylogenies often is improved by using more data from multiple sources (Kluge 1989; Baker & De Salle 1997; Sanderson & Shaffer 2002). In this chapter, therefore, I undertake a more detailed

analysis of the *Anastrophyllum* clade. Further characters are available from the previously sampled chloroplast regions because of the ability to reliably align length variable regions among this reduced set of more closely related taxa. Furthermore, an additional chloroplast region is sampled for representatives of this group, generated as part of the DNA bar-coding project (Chase *et al.* 2007), the rpoC1 intron sequences (Liston & Wheeler 1994).

In addition, variation in the internal transcribed spacer (ITS) region of the nuclear-encoded 18S–26S rRNA cistron is evaluated. The ITS region has been used extensively in plant molecular phylogenies (see for example Kropf *et al.* 2002; Holderegger & Abbot 2003; Vargas 2003; Olsen *et al.* 2004) and has been shown to have high levels of variation at and below the species level in bryophytes (e.g. Hartmann *et al.* 2006; Hedderson & Nowell 2006).

Despite the perceived benefits of using multiple data sets, the possibility always exists that different partitions of the data (e.g. chloroplast versus nuclear genomes, different nuclear genes) may have different histories (Kluge 1989; De Queiroz 1993; Farris *et al.* 1994; Huelsenbeck *et al.* 1996). This problem is likely to be especially acute for more closely related entities, because coalescent events may not correspond with species boundaries. Usually due to incomplete lineage sorting, the result is a failure of gene lineages to form reciprocally monophyletic groups corresponding to species boundaries (e.g. Hudson & Coyne 2002; Rosenberg 2003; Hedderson & Nowell 2006). The problem of incongruence when combining data for analyses can therefore be very complicated (see for example Huelsenbeck *et al.* 1996, Farris *et al.* 1994). In most instances, it is advisable initially to analyse different data sets separately if there is a possibility they might have different histories. All chloroplast regions will have the same history so any differences would be due to factors like inadequate modelling of evolutionary process, such as sampling effects. Data sets after separate analyses are combined to give the best estimate of phylogeny by maximising the chance of convergence to the correct phylogenetic tree (Huelsenbeck *et al.* 1996).

The main objectives of this chapter are to i) further test the monophyly of the *Anastrophyllum* clade, given the observed differences between parsimony and Bayesian approaches observed in chapter two, ii) attempt to further resolve relationships among the

major clades previously recovered for this group, iii) further delimit the genera of the *Anastrophyllum* clade.

### 3. 2. Materials and methods

The exemplars used in this chapter are listed in the appendix. The sampling attempts as far as possible to represent the *Anastrophyllum* clade including multiple exemplars for included taxa where possible. The DNA was extracted, amplified in the same way as described in chapter 2. The additional chloroplast region rpoC1 was sampled, generated as part of the DNA bar-coding project (Chase *et al.* 2007). One nuclear region, the Internal Transcribed Spacer of nuclear ribosomal DNA (ITS) (Baldwin 1992) was sampled. Primers needed to be designed to avoid amplification of mycorhizal symbionts. This was done using available liverwort sequences of ITS, 18S and the 25S regions from GenBank as templates. The primers designed were: "3'18sF" (TGA ATG GTC CGG TGA AGT T) and "5'25sR" (TGC AGA GGA CGC TTC TCC A). Chloroplast and nuclear data sets are analysed separately and any "hard" incongruency is determined. The datasets are subsequently analysed in a combined analysis. Taxa sampled for only one DNA region are excluded.

Sequences were assembled and checked for inaccurate base calling using SeqMan II (Laser Gene System Software, DNAStar, Inc). Assembled sequences were aligned manually using MegAlign (Laser Gene System Software, DNAStar, Inc). The non-coding sequence at the 3' end of the amplified segment of *rps*4 was for the most part included. Where difficult to align over the range of taxa sampled, several *trn*G intron and ITS positions were excluded from the current analysis. In chapter 2 it was shown that the Bayesian analyses yield better resolution, especially seen in the "core" *Lophoziaceae* clade. There was no strong conflict between the Bayesian and Parsimony results, and where the two differ the parsimony results were usually poorly supported. For this reason only Bayesian inference is used to reconstruct the phylogenies.

Bayesian phylogenetic analyses were conducted using MrBayes v3.1.2 (Huelsenbeck & Ronquist 2003). Phylogenies for each DNA data set (nuclear and chloroplast) were obtained in separate analyses. The chloroplast data set comprising *rps*4 (partitioned into coding and non-coding regions), the *trn*G intron and RpoC1 was used for a mixed model approach (Ronquist & Huelsenbeck 2003) whereby the substitution process fit by the GTR+I+Γ model. This model incorporates separate time-reversible estimates of each

possible substitution type, an estimate of the proportion of sites fixed at invariance, and an estimate of the shape of the gamma distribution to which variable sites are fitted. Two independent runs of the Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) approach were used for sampling parameter values in proportion to their posterior probability. Each analysis used four chains, three heated and one unheated, run for 2 x $10^6$ generations. The combined region phylogeny again utilised a mixed-model approach for the substitution process. Each of the data partitions was fitted by the GTR+I+Γ model. Parameter values were estimated independently across the five partitions. The analysis used four chains, three heated and one unheated, run for 6.5 x $10^6$ generations.

For all analyses, model parameters, including trees, were sampled every 250 generations. Plots of the likelihoods of each sample were used to ascertain the number of generations for stationarity to be reached in order to obtain the posterior probability tree set. Trees from the burn-in were excluded.

### 3. 3. Results

#### 3. 3. 1. Chloroplast analysis

The chloroplast analysis included 61 exemplars, with 59 belonging to the *Anastrophyllum* clade and 2 outgroup taxa *Tritomaria quinquedentata* ssp. *quinquedentata* and *Lophozia longidens*. The coding portion of the *rps*4 region included 612 characters with 449 (73%) unique site patterns; the non-coding partition included 393 characters with 207 (52%) unique site patterns. The *trn*G intron included 792 characters with 480 (61%) unique site patterns. RpoC1 included 528 characters with 87 (16%) unique site patterns. The MCMCMC search required around 500,000 generations for each analysis to reach stationarity; and the combined 4,000 trees obtained during these burn-in periods were discarded. Each run produced 8,000 samples of which 6,000 samples were included. The tree is shown in Fig. 3.1.

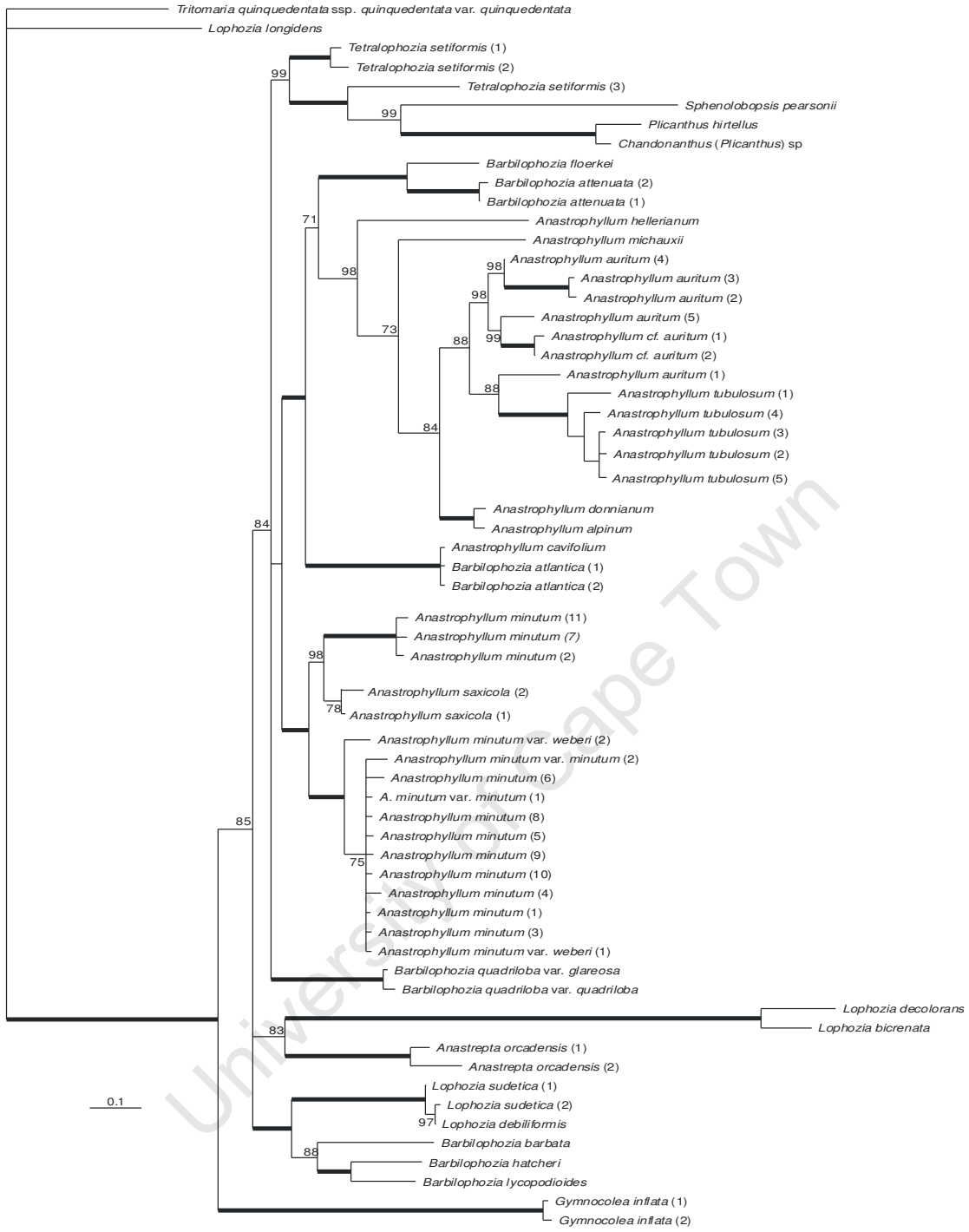




**Fig. 3. 1.** A majority-rule consensus of the chloroplast trees (excluding burn-in) generated by MrBayes. The posterior probabilities from the combined 95% credible tree set are indicated for individual nodes. Thick bars indicate PP=100.

The *Anastrophyllum* clade includes *Anastrophyllum*, *Barbilophozia*, *Tetralophozia*, *Sphenolobopsis*, *Plicanthus*, *Anastrepta, Gymnocolea* and some *Lophozia* sp. A number of well-supported groupings are evident. *Gymnocolea inflata* is sister to a poorly supported (PP = 85) clade comprising the remaining taxa. Within this group taxa are resolved into three clades, relationships among these three are unresolved: 1) *Barbilophozia hatcheri*, *B. barbata* and *B. lycopodioides* and a strongly supported group (PP = 100) of *Lophozia sudetica* and *L. debiliformis* which together form a strongly supported group (PP = 100); 2) a poorly supported (PP = 83) group of *Anastrepta orcadensis* sister to the long-branched well supported (PP = 100) group of *Lophozia decolorans* and *Lophozia bicrenata*; 3) a large, well supported group (PP = 98) of *Tetralophozia, B. floerkei, B. attenuata*, *Anastrophyllum*, *Sphenolobopsis*, *Plicanthus*, *B. atlantica* and *B. quadriloba*. Within this large group are three unresolved clades: 1) a strongly supported group (PP = 100) of the mono-generic *Sphenolobopsis pearsonii* and *Plicanthus* which is sister to *Tetralophozia setiformis* (3) from Spain suggesting *Tetralophozia setiformis* to be paraphyletic; 2) *Barbilophozia quadriloba*; and 3) a large unsupported clade of *Anastrophyllum* and some *Barbilophozia* species.

A strongly supported (PP = 100) group of *Anastrophyllum minutum* and *A. saxicola* within which *A. saxicola* is well supported (PP = 98) as sister to a strongly supported group (PP = 100) of *A. minutum* (2, 7 & 11) from Norway, Venezuela and South Africa. The remaining *A. minutum* taxa form a strongly supported (PP = 100) group. This *A. minutum-saxicola* group is sister to a strongly supported (PP = 100) group of the remaining *Anastrophyllum* species. *A. cavifolium* is strongly supported (PP = 100) as sister to *B. atlantica* and this group is sister to the poorly supported (PP = 71) group with *B. floerkei* strongly supported (PP = 100) as sister to *B. attenuata* and a well supported (PP = 98) "core" *Anastrophyllym* clade including *A. hellerianum, A. auritum, A. michauxii, A. cf. auritum, A. tubulosum, A. donnianum* and *A. alpinum*. Within the "core" *Anastrophyllum* clade, *A. donnianum* strongly supported (PP = 100) as sister to *A. alpinum* and *A. auritum* (1) is poorly supported (PP = 88) as sister to *A. tubulosum* suggesting a paraphyletic species.

### 3. 3. 2. ITS analysis

Included in the ITS analysis were 34 taxa, with 32 belonging to the *Anastrophyllum* clade and 2 outgroup taxa *Tritomaria quinquedentata* ssp. *quinquedentata* var. *quinquedentata* and *Lophozia longidens*. ITS included 1075 characters with 507 (47%) unique site patterns. The MCMCMC search required 25,000 generations for each analysis to reach stationarity; and the combined 200 trees obtained during these burn-in periods were discarded. Each run produced 8,000 samples of which 7,900 samples were included. The tree is shown in Fig. 3.2.

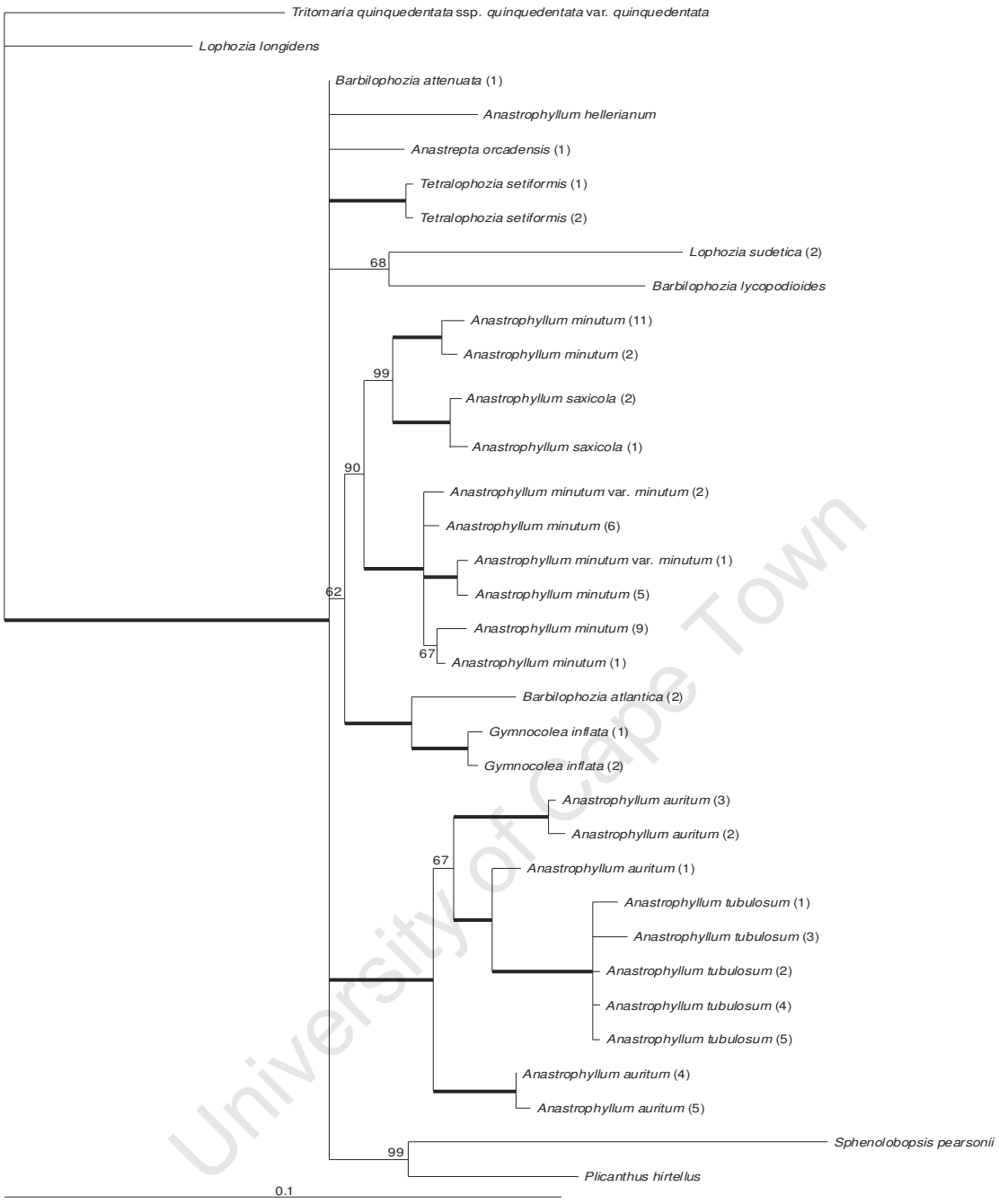




**Fig. 3. 2.** A majority-rule consensus of the ITS trees (excluding burn-in) generated by MrBayes. The posterior probabilities from the combined 95% credible tree set are indicated for individual nodes. Thick bars indicate PP=100.

Relationships within the *Anastrophyllum* clade are mostly poorly resolved as seen by the large polytomy. Some incongruence with the chloroplast data is apparent, specifically the strongly supported (PP = 100) sister relationship of *Barbilophozia atlantica* (2) with *Gymnocolea inflata*; these taxa very poorly supported (PP = 62) as sister to the *Anastrophyllum minutum-saxicola* clade. For this reason *Barbilophozia atlantica* (2) is excluded from the combined analysis. Some well supported groups do emerge in this analysis: *Sphenolobopsis* is strongly supported (PP = 99) as sister to *Plicanthus* and *A. auritum* and *A. tubulosum* form a strongly supported group (PP = 100) with *A. tubulosum* again nested within *A. auritum* with strong support (PP = 100) for this relationship.

### 3. 3. 3. Combined analysis

Included in the combined analysis were 53 accessions, with 51 belonging to the *Anastrophyllum* clade and 2 outgroup taxa *Tritomaria quinquedentata* ssp. *quinquedentata* var. *quinquedentata* and *Lophozia longidens*. The MCMCMC search required 1,500,000 generations for each analysis to reach stationarity; and the combined 12,000 trees obtained during these burn-in periods were discarded. Each run produced 26,000 samples of which 20,000 samples were included. The tree is shown in Fig. 3.3.

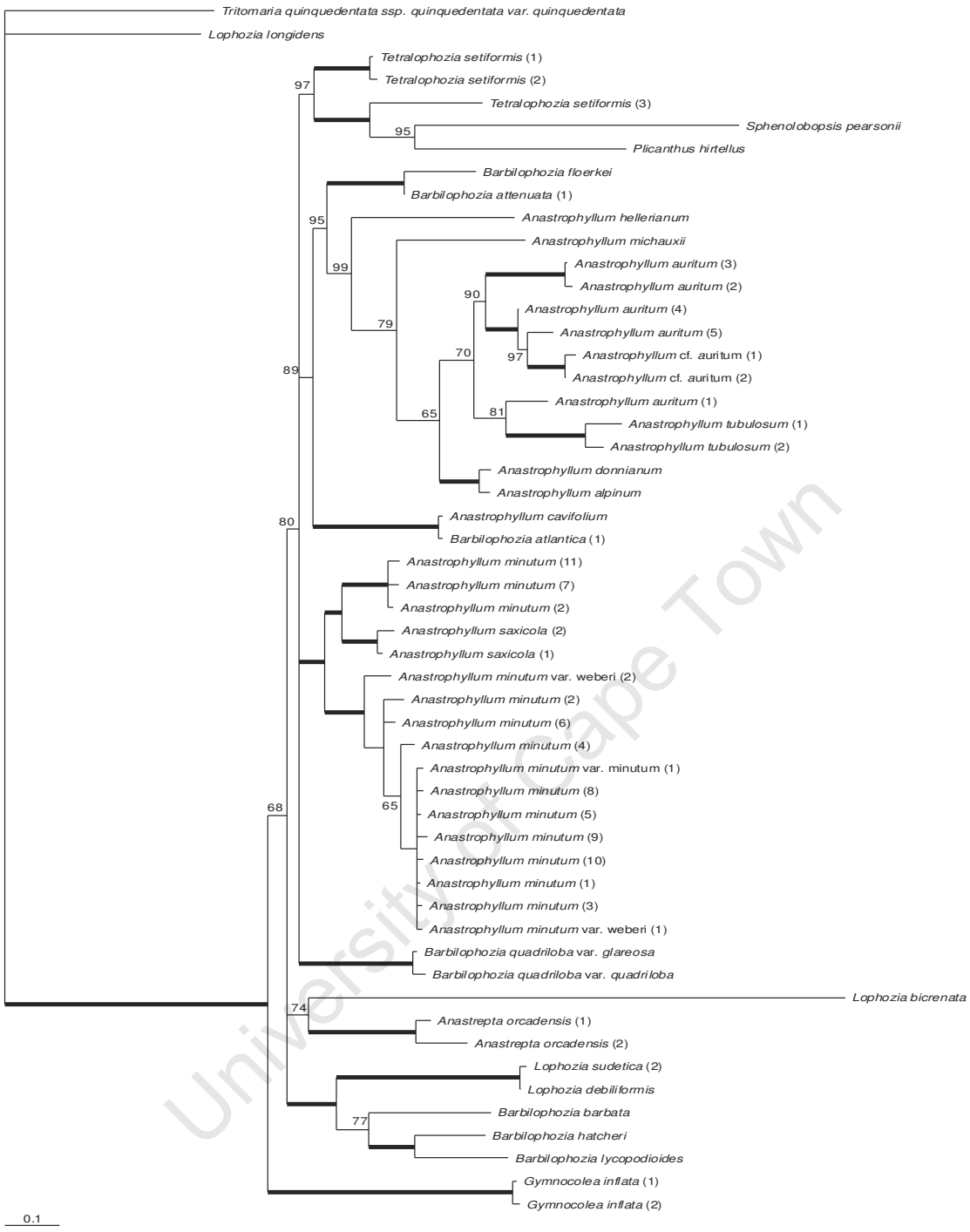




**Fig. 3. 3.** A majority-rule consensus of the combined analysis trees (excluding burn-in) generated by MrBayes. The posterior probabilities from the combined 95% credible tree set are indicated for individual nodes. Thick bars indicate PP=100.

The results of the combined analysis are much the same as in the chloroplast analysis, noticeable changes include a drop in resolution for certain relationships for example the "core" *Anastrophyllym* clade including *A. hellerianum, A. auritum, A. michauxii, A. cf. auritum, A. tubulosum, A. donnianum* and *A. alpinum* together with *A. cavifolium* and *Barbilophozia atlantica* form a poorly (PP = 89) supported group that is not resolved as sister to the *A. minutum-saxicola* group (although this relationship was not shown with any support in the chloroplast analysis). Similarly support for the relationships within the "core" *Anastrophyllym* clade is decreased for some groups and support for *Gymnocolea inflata* as sister to the remaining taxa has less support (PP = 68). An increase in resolution appears in some instances with the addition of the ITS data for example in the *A. minutum-saxicola* group there is more support for the group of *A. minutum* and *A. saxicola* within which *A. saxicola* is now strongly supported (PP = 100) as sister to the group of *A. minutum* (2, 7 & 11) from Norway, Venezuela and South Africa. Resolution for the remaining *A. minutum* taxa is increased, although lacking in support.

### 3. 4. Discussion

Overall, although mostly poorly supported, there is more resolution in the combined analysis of the *Anastrophyllum* clade compared to the two chloroplast regions in chapter two. The group in both the chloroplast and nuclear analyses are resolved strongly supported as monophyletic. Having analysed the different data sets separately, it is clear that congruence was found between the data sets, with the exception of *B. atlantica* (2). Having excluded this sample from the combined analysis it is found that *Gymnocolea inflata* is retained sister to the remaining taxa although larger group is poorly supported. In chapter two, *G. inflata* was unresolved with two sampled representatives of subgen. *Isopaches*, *Lophozia decolorans* and *L. bicrenata* and the group including *B. hatcheri*. More sampling of the ca. 7 species of *Gymnocolea* is probably required to properly establish its position within the *Anastrophyllum* clade.

The group of *Barbilophozia hatcheri*, *B. lycopodioides* and *B. barbata* and a group of *Lophozia sudetica* and *L. debiliformis* together form the *Barbilophozia hatcheri* - *Lophozia sudetica* grouping which is strongly supported in the combined analysis. *B. hatcheri* and *B. lycopodioides* both have cilia made up of much longer cells than those of other *Barbilophozia* species (Paton 1999); this is not shared with *B. barbata* and supports that *B. hatcheri* and *B. lycopodioides* are more closely related as seen in the phylogeny. Schuster (1969) notes that *B. hatcheri* is likely to be confused with both *B. barbata* and *B. lycopodioides*, *B. hatcheri* being smaller than the other two while *B. barbata* is intermediate in size. Juveniles of *L. debiliformis* and *L. sudetica* resemble each other and share red-brown gemmae but *L. debiliformis* differs in having concolorous gemmiparous leaf apices and gemmae (Damsholt 2002). Paton (1999) notes that *B. barbata* sometimes resembles *L. sudetica* when its leaves are 3-4 lobed, however, *L. sudetica* differs in its leaf cells being smaller, gemmae often present, cell walls are sometimes red and the base of rhizoids are purplish red, some morphological support for the fact that *L. sudetica* is affiliated with this group as seen in the molecular phylogeny. Given this sampling *Barbilophozia* s. str. is monophyletic and should be recognised, *Lophozia sudetica* and *L. debiliformis* being sister to *Barbilophozia* may be transferred to the latter as suggested in chapter two.

*Anastrepta orcadensis* from being ambiguously placed in the phylogeny in chapter two with *Barbilophozia quadriloba* is here found poorly supported as sister to Isopaches. The two sampled representatives of subgen. Isopaches, *Lophozia decolorans* and *L. bicrenata,*

still form a strongly supported group in the chloroplast analysis and as discussed in chapter two, *Isopaches* should be recognised.

The large group of *Tetralophozia, B. floerkei, B. attenuata*, *Anastrophyllum*, *Spenolobopsis*, *Plicanthus*, *Anastrepta*, *B. atlantica* and *B. quadriloba* was present in the analysis from chapter two, is retained in the chloroplast and combined analyses, albeit again with weak support. Within this group *Tetralophozia setiformis* forms a well-supported group with *Plicanthus* and *Spenolobopsis*, with the latter sister to *T. setiformis* (3) from Spain possibly rendering *T. setiformis* or at least the genus paraphyletic. It is likely that *T. setiformis* (3) is misidentified and is probably *T. filiformis*, reports of arctic-alpine species of *T. setiformis* from Spain are erroneous (Váňa pers. comm.). *Sphenolobopsis* is nested within these representatives of the Chandonanthoideae, and placed with strong support by both the ITS and chloroplast data as sister to *Plicanthus*. *Sphenolobopsis* has variously been associated with a wide range of genera (Schuster 2002a). The plants resemble *A. minutum* in miniature, but differ in that the cells of the capsule wall have a type of ornamentation that is described (Schuster 2002a) as stalked, remote, few and coarse, with sharply defined "nodular" thickenings extending to the tangential walls. In addition the gynoecia have the bracteole free from the bracts, and the seta is much reduced. *Plicanthus* has a massive seta and the bracteole is more or less connate with the bracts (Schuster 2002a). However taxa of the subfamily Chandonanthoideae while having more capsule wall layers (4-8 layered) than *Sphenolobopsis* (2 layered), do have capsule walls with a distinct anatomy wherein some of the cell walls have strong "nodular" thickenings (Schuster 2002a). This similarity may be taken as supporting the relationships indicated by molecular evidence and *Sphenolobopsis* should be transferred to the Chandonanthoideae.

In chapter two *Anastrophyllum minutum* and *A. saxicola* formed a strongly supported clade suggesting the genus *Sphenolobus* should be re-instated for these. In these analyses this group is maintained in the combined analysis, with strong support, *A. minutum* (2, 7 & 11) from Venezuela, South Africa and Norway are grouped with *A. saxicola* resulting in *A. minutum* being paraphyletic. Damsholt (2002) mentions that *A. minutum* has traditionally been placed near *A. saxicola*; *A. minutum* has narrower shoots and obliquely to transversely inserted leaves, whereas *A. saxicola* has arcuately inserted leaves. Schuster (1969) notes that *A. minutum* is a highly polymorphic and widely distributed taxon.

It was mentioned in the introduction to this chapter that incongruence is likely to be especially acute for more closely related entities, because coalescent events may not correspond with species boundaries. Possibly due to incomplete lineage sorting, resulting in a failure of gene lineages to form reciprocally monophyletic groups corresponding to species boundaries (e.g. Hudson & Coyne 2002; Rosenberg 2003; Hedderson & Nowell 2006), this phenomenon is possibly the cause of the paraphyly seen with *A. minutum*. A strict take on the pattern found in the results would suggest that *A. saxicola* is a synonym of *A. minutum*. This would be undesirable from the perspective of morphology since the two are very different.

Shaw (2001) notes that in general bryophytes are morphologically simple and that genetic differentiation among intercontinentally disjunct populations might not always be reflected in morphological traits. The phylogeny suggests a possibility of cryptic speciation for the currently delimited *A. minutum*. Szweykowski & Krzakowa (1979) first suggested cryptic speciation in liverworts for *Conocephalum conicum* finding two different allozyme types in allopatric populations of the species, since then there have been many other suggested cryptic species of liverworts (e.g. Szweykowski *et al.* 1995; Fiedorow *et al.* 2001; Shaw 2001; Wachowiak *et al.* 2007). Schuster (2002a) notes a wide geographic distribution of *A. minutum*: North America, Europe and Asia into the high arctic and extending into South America, southern Africa and New Guinea. According to Shaw (2001) this is not such an unusual situation in bryophytes. He notes that the "majority of boreal bryophytes have a more or less continuous circumpolar distribution across the northern parts of North America, Europe and Asia" (Shaw 2001). He also notes that "some circumboreal species also have isolated populations on high mountains in tropical zones" for example *Hylocomium spendens* (Hedw.) Schimp. and *Plagiobryum zieri* (Hedw.) Lindb. (Shaw 2001).

Interestingly, the *A. minutum* (2, 7 & 11) exemplars that are grouped with *A. saxicola* are from Venezuela, South Africa and Norway, whereas the remaining *A. minutum* exemplars were all sampled from Europe (Norway, Svalbard, Sweden and Spain) suggesting the two lineages to be sympatric. Possibly this is a result of more recent dispersal from older disjunct lineages. Clearly more sampling and a detailed morphological study of the species complex is required, specifically a phylogenetic approach based on coalescence models to distinguishing current and past population processes (Templeton 1998) or

specifically using phylogeographic analyses of gene trees to test species status and processes (Templeton 2001). *Sphenolobus* overall needs closer examination and more sampling is required, including more populations of *A. minutum* and *A. saxicola*. Possibly also taxa such as *A. austroamericanum* Váňa., *A. intermedium* Schust, *A. ambiguum* Schust. should be examined.

Unresolved in chapter two, the results here suggest that *Sphenolobus* is sister to the remaining *Anastrophyllum* species including some *Barbilophozia* species. As discussed in chapter two, *A. cavifolium* seems to be conspecific with *B. atlantica*. *B. atlantica* is the type for *Orthocaulis* and the genus should be re-instated for this group. *B. floerkei* and *B. attenuata* also form a group, again strongly supported in these analyses, and unresolved in chapter two is now sister to the "core" *Anastrophyllum* clade. A name for this group is lacking, possibly it could be included within *Anastrophyllum.*

The "core" *Anastrophyllym* clade is strongly supported in these analyses. As mentioned in chapter two the genus name should be retained for these taxa. *A. hellerianum* is sister to the rest, though still weakly supported as in chapter two. *A. hellerianum* is different from other *Anastrophyllum* in this clade, having only unicellular angular gemmae (Paton 1999) and erect gemmiparous shoots (Damsholt 2002). *A. cf. auritum* from South Africa, as expected, groups with most of the *A. auritum* accessions from Venezuela, however, *A. auritum* appears paraphyletic with *A. auritum* (1) sister to *A. tubulosum*. However, it is likely that *A. tubulosum*, also present in the *A. auritum* (1) voucher (Váňa pers. comm.) was used for the analysis.

### 3. 5. Conclusion

Congruence was found between the data sets, with the exception of the placement of a single specimen of *B. atlantica* (2). This sample was excluded and the combined analysis revealed much the same results as the chloroplast data. With additional taxa and data some further insight is gained from these analyses. Significant groups revealed in this chapter are highlighted:

1. *Gymnocolea inflata* is possibly sister to the remaining taxa in the *Anastrophyllum* clade.
2. *Anastrepta orcadensis* is possibly sister to *Isopaches*.
3. Chandonanthoideae*, Sphenolobus*, *B. floerkei, B. attenuate*, *Anastrophyllum, B. atlantica* and *B. quadriloba* appear more closely related to each other than to *Isopaches, Gymnocolea* and *Barbilophozia* s. str.
4. *Tetralophozia* is paraphyletic with *Plicanthus* and *Spenolobopsis* sister to *T. setiformis* (3) (cf. *T. filiformis*) from Spain.
5. *Sphenolobopsis* should be transferred to the Chandonanthoideae.
6. *Barbilophozia atlantica* is the type for *Orthocaulis*; the genus should be re-instated for it and *Anastrophyllum cavifolium*.
7. *Sphenolobus* is possibly sister to *Anastrophyllum*, *Orthocaulis, B. floerkei* and *B. attenuata*.

# Chapter 4

# Establishing the timeline of diversification - molecular estimates of divergence times

## 4. 1. Introduction

Molecular data offer the possibility of investigating the timeframe of evolutionary events; they essentially put a timescale on the history of life (Bromham & Penny 2003). All such methods use molecular phylogenies to convert measures of the genetic distance between sequences into estimates of the time at which the lineages diverged. Welch & Bromham (2005) summarise the technique: "The genetic distance estimates require topology (…) and branch lengths (…). To convert these into measures of time, the methods also require one or more externally derived dates, usually based on fossil or biogeographical evidence (…)." Using various methods the calibration dates are extrapolated to the rest of the tree. The dating of divergence is useful for many evolutionary investigations such as co-speciation and historical biogeographical analysis.

Molecular data have been used to great effect in improving our understanding of evolutionary relationships in many groups of plants and animals including liverworts (e.g. Davis, 2004; He-Nygrén *et al.*, 2004; Yatsentyuk *et al.*, 2004; Heinrichs *et al.*, 2005; He-Nygrén *et al.*, 2006; Hentschel *et al.*, 2006; de Roo *et al.* 2007). However there are many theories on the timing of, and factors affecting, diversification of the leafy liverworts – often with little supporting data. For instance Frey & Stech (2005), in their morpho-molecular classification of the liverworts, suggest that the main diversifications of the Jungermanniidae were in co-evolution with those of the angiosperms and the establishment of tropical rainforest ecosystems, with major evolutionary events confined to Gondwanaland; little to no evidence was offered in support of these theories. For other groups of plants molecular approaches have been well utilised to link various plant diversification events. Schneider *et al.* (2004) used molecular approaches to estimate divergence times for ferns and angiosperms and found that polypod ferns (more than 80% of living fern species) "diversified in the shadow of angiosperms". A similar pattern is found with lycopods (Wikström & Kenrick 2001), horsetails (Des Marais *et al.* 2003) and with pleurocarpous mosses (Newton *et al.* 2007). It would be of interest to see when

main diversification of the Lophoziaceae, a widespread and ecologically diverse group, occurred and whether this corresponds to any obvious paleo-ecological changes.

Schuster (1966) notes that because of a greater delicacy compared to other plants there is a lower percentage of fossilization of liverworts, leading to a more fragmentary fossil record than is available for most groups. The oldest fossil evidence for liverworts comes from ultrastructure of lower Silurian (424-439 mya) spores of *Dyadosora* suggested to have an affinity with Sphaerocarpales (Taylor 1995). Recently Graham *et al.* (2004), in a study using experimental degradation of liverworts as modern analogues to compare remains with Cambrian-to-Devonian microfossils, have suggested that marchantioid liverworts were present from at least the Silurian (409-439 mya) based on their interpretation of Nematophytales fossils. However, the oldest unequivocal fossil evidence for liverworts is often taken to be *Pallaviciniites devonicus* (Heuber) Schust. (Hueber 1961; Schuster 1966) consisting of a branching thallus with nonseptate rhizoids from the Upper Devonian, specifically the Frasnian 367-377 mya (Kenrick & Crane 1997).

There is some evidence for leafy liverwort lineages in the Mesozoic. *Jungermaniites keuperianus* (De Gasparis) Oostendorp, thought to be the earliest known member of the Jungermanniidae (Frey & Stech 2005), is present as far back as the Upper Triassic (200-228 mya). The earliest taxon assigned to the Jungermanniales is *Jungermannites gracilis* (T.Halle) Oostendorp (Frey & Stech 2005), known from the Middle Jurassic (161-175 mya). It has been suggested that the majority of modern leafy liverwort groups did not make an appearance much before the Paleogene (65 mya); specimens from before 65 mya that resemble extant leafy liverworts are mostly assigned to the genus *Jungermannites* whilst those younger than 65 mya are mostly assigned to extant genera (Stewart & Rothwell 1993). Leafy liverwort fossils, representing a wide range of taxa are well known from Baltic, Bitterfeld and Dominican amber deposits. According to Weitschat (1997), Baltic and Bitterfeld amber are the same age, Bitterfeld amber being a Late Oligocene redeposit of Baltic amber from the Eocene, found to be from over 50 mya (Ritzkowski 1997). Dominican amber from the Miocene is suggested to be from between 20 and 40mya (Gradstein 1993). *Scapania hoffeinsiana* (Grolle & Schmidt 2001) and *Lophozia kutscheri* (Grolle & Meister 2004) represent Lophoziaceae fossils from the Eocene.

With exceptions including of the study by Heinrichs *et al.* (2007) (see also Hartmann *et al*. 2006; Heinrichs *et al*. 2006; Wilson *et al.* 2007); molecular dating has not been often utilized on liverwort taxa. This is probably due to the relatively recent emergence of molecular studies involving liverworts, coupled with the poor fossil record available for calibration. Heinrichs *et al.* (2007) used penalized likelihood on two data sets (using *rbc*L, *psb*A and *rps*4). The first comprised representatives of all major clades of land plants, with the objective of obtaining a fixed calibration point for their second data set comprising representatives of the main lineages of Jungermanniopsida. As acknowledged by these authors their estimates incorporate a number of problems, most notably that the lack of a robust hypothesis of relationships among the four main lineages of land plants hinders divergence time estimates for stem lineages. Results from their first data set suggest a Late Ordovician origin of Marchantiophyta (around 454.4 ± 0.6 Ma), and a split of Metzgeriidae and Jungermanniidae in the Late Carboniferous (308.7 ± 7.8 Ma). From their second data set, they concluded that many extant genera of Jungermanniidae originated in the Cretaceous or Early Tertiary, thus suggesting a similar pattern as that found with ferns (Schneider *et al.* 2004).

In this chapter, I explore whether similar results emerge using the *rps*4 molecular phylogeny using a "relaxed phylogenetics" approach with calibration nodes situated within the study group.

### 4. 1. 1 Genetic distance measures and time

As a concept, molecular dating was first proposed by Zuckerkandl and Pauling (1965) who postulated that the degree of difference between a pair of protein sequences is approximately proportional to the time elapsed since their divergence from a common ancestor (Magallón 2004). Molecular substitution was proposed to behave approximately as a stochastic Poisson-distributed process (Zuckerkandl and Pauling 1965) which implies that the rate of molecular substitution does not occur with exact precision, but rather with probabilistic regularity (Magallón 2004).

Earlier applications of the principle thus based estimates of divergence on the assumption that genetic change accumulated steadily over time, i.e. that divergences constituted a "molecular clock". Under the molecular clock the genetic distance between sequences, corrected for saturated sites, is proportional to the elapsed time since their divergence (Magallón 2004). This is now known to be a special case where the total distance between root and all tips are constant; the tree is then termed ultrametric (Magallón 2004).

Under the assumption of a molecular clock, divergence times and rates can be estimated by linear regression. Here the molecular distance between each member of a sister pair and their most recent common ancestor is one-half of the distance between the two sequences. Because the underlying Poisson process introduces greater variance in molecular distance as time increases, a weighted linear regression, in which the scatter of data points around the regression line provides a confidence interval around estimated ages, is more appropriate (Magallón 2004). The Mean Path Length method proposed by Britton *et al.* (2002) estimates the rate and divergence times based on the mean branch length between a node and each of its terminals. Commonly used are Maximum Likelihood clock optimizations whereby divergence times are estimated by optimising a single constant rate of substitution that best fits the entire phylogeny (Langley & Fitch 1974). Here one starts with a phylogeny and a constrained constant rate of substitution is optimized through maximum likelihood to estimate branch lengths and ages of nodes.

However in many, if not most, cases rates of evolution are heterogeneous among branches of a phylogenetic tree such that the correlation between distance and elapsed time is disrupted and the clock is not a good model for the process of molecular evolution (Wu & Li 1985, Britten 1986, Takahata 1987, Sanderson 1997, Thorne *et al.* 1998, Gaut

1998). This is caused by the common phenomenon of overdispersion in the substitution process i.e. the variance to mean ratio is greater than one (Ohta & Kimura 1971; Langley & Fitch 1973, 1974). The fact that rates of substitution have also been shown to vary across lineages (Gillespie 1991) also renders problematic the direct application of the clock method.

Methodological factors causing measured rates of substitution to vary across lineages include taxonomic sampling, data type, incorrect phylogenetic hypothesis and incorrect temporal calibration (Magallón 2004). Biological factors causing variation among estimates include differences in generation time, metabolic rate, mutation rate, and the effect of population size on the rate of fixation of mutations (Rutschmann 2006). Thorne *et al.* (1998) comment that while, not surprisingly, rates of evolution differ between lineages as divergent as mammals and viruses, it does seem that there is a correlation of evolutionary rates among closely related lineages.

If the divergence among factors that influence evolutionary rates were better understood, dating of evolutionary events from comparisons of homologous sequences could be performed even without an assumption that the rates of evolution for different lineages are exactly equal. Similarly, inferences made about evolutionary processes would be more accurate. Much more work remains to be undertaken on the identification and characterization of factors affecting evolutionary rates of lineages, but it is clear that the divergence of many of these factors will be lineage-specific rather than gene-specific (Thorne *et al.* 1998). This is why reliance upon a molecular clock is likely to be unwarranted even for the analysis of data sets with sequences from many different loci (Thorne *et al.* 1998). It is important to note that ignoring deviation from the clock and undetected rate variation leads to potentially significantly incorrect estimations of divergence times (Bromham *et al.* 2000).

Several methods are available for testing whether sequence evolution departs from clock-like behaviour. For example, Felsenstein's (1988) likelihood ratio test compares the likelihood score of trees with branch lengths constrained to clock-like behaviour, to those in which branch lengths are unconstrained. Other tests are the Wu & Li (1985) relative rates test and Tajima's (1993) test. However these are all known to lack power for shorter sequences – the power of any relative-rate test increases with the amount of divergence in the sample (Cutler 2000) – and detect only a relatively low proportion of

cases of rate variation for the types of sequence that are typically used in molecular clock studies (Bromham *et al.* 2000). Contrary to common expectation, sequences that "pass" a relative rates test cannot always be considered sufficiently clock-like for the purposes of date estimation (Bromham *et al.* 2000). So one should, instead of relying on the results of these tests, rather accept an *a priori* expectation that rates vary across the tree.

There are numerous methods to accommodate rate heterogeneity. Some such as the linearized trees method (Takezaki *et al.* 1995) correct for observed rate heterogeneity by removing branches. Similarly one can exclude data partitions that do not meet the clock assumption (Kato *et al.* 2003). These methods are not universally recommended (Cutler 2000; Welch & Bromham 2005) since, as discussed above for instance, tests for detecting deviation from clock like behaviour lack power and any rate variation that remains undetected can result in consistently biased date estimates (Welch & Bromham 2005). In addition the exclusion approach is practical only if rate variation is the exception rather than the rule; otherwise, a large proportion of the sequences should have to be excluded (Welch & Bromham 2005).

Another way to accommodate rate heterogeneity is by using several rate classes as employed in the ML-based local molecular clock method (Hasegawa & Kishino 1989, Yoder & Yang 2000). This is feasible when the number of rates assigned is small, as these can be jointly estimated with the divergence times as is done with a single fixed rate. However, when the number of rates is large, then the rates and dates become unidentifiable with an infinite number of rate and date combinations that are equally probable (Welch & Bromham 2005).

One can also incorporate rate heterogeneity by estimating branch lengths without assuming rate constancy for any part of the tree, also known as "relaxed clock" methods (Welch & Bromham 2005). The most common way is to base models on the concept of temporal autocorrelation in rates (Magallón 2004). With temporal rate autocorrelation the speed with which a rate can change from an ancestral lineage to a descendant lineage is limited. Reasons why temporal autocorrelation might exist include descendant lineages inheriting the rates of their ancestor, with subsequent independent evolution of new rates (Takahata 1987, Gillespie 1991; Sanderson 1997), or descendant lineages inheriting from their ancestor traits that regulate rates of evolution, such as habit, life-form, metabolic rate, generation time, and/or descendant lineages being subject to similar

environmental conditions (Thorne *et al.* 1998). Thus temporal autocorrelation becomes an explicit *a priori* criterion to guide inference of among-lineage rate change in these methods (Magallón 2004).

These methods of dating either base analyses on trees with branch lengths, hence do not incorporate branch length errors or parameters of the substitution model into the dating analyses (e.g. Nonparametric Rate Smoothing (Sanderson 1997) or Penalized Likelihood (Sanderson 2002)), or they base analyses on tree topologies to asses rates and divergence times and estimate the branch lengths themselves, e.g. the increasingly popular (Rutschmann 2006) Bayesian implementation of rate autocorrelation (Thorne *et al.* 1998, Kishino *et al.* 2001, Thorn & Kishino 2002), which uses a fully probabilistic and highly parameterised model to account for changes in evolutionary rate over time with an MCMC procedure to derive the posterior distribution of rates and times (Rutschmann 2006).

Huelsenbeck *et al.* (2000), instead of using rate smoothing or autocorrelation in rates, utilise a Poisson-distributed process that introduces changes in the rate of substitution in different places in a phylogeny that are imposed on the primary Poisson distribution process of molecular substitution. This they call the compound Poisson process of rate change (Huelsenbeck *et al.* 2000). Instead of assuming that substitution rates change only at nodes, the Compound Poisson process method allows for rate variation at any point in the tree (Heulsenbeck *et al.* 2000). Like rate smoothing, the model of rate change penalizes large rate changes (Welch & Bromham 2005). However, the model also enables the number of rate changes to vary during the estimation, with departures from the expected number of changes penalized (Welch & Bromham 2005). Unfortunately programs utilising this method are still inaccessible.

In another method, Cutler (2000) relaxes the clock based on the fact that evolution is "overdispersed". Instead of assuming that different rates of molecular evolution characterize the different lineages, the process of molecular evolution is seen as identical in all lineages, with differences due to a high variance (substitutions might tend to cluster in time). He uses a model employing a Gaussian distribution, and the rate variation is implicit in the way likelihood values are assigned to the branch lengths. The method again resembles rate smoothing in that departures from rate constancy are penalized during the estimation (Welch & Bromham 2005). However, it does not assume that

bursts of substitutions are most likely to occur on closely related lineages or, alternatively, that rapidly evolving lineages are most likely to give rise to other rapidly evolving lineages. Rather, the method penalizes departures from the overall mean rate of the tree; regardless of the smoothness with which the changes take place (Welch & Bromham 2005). Again programs utilising this method are inaccessible.

Drummond *et al*. (2006) describe a method that allows for the implementation of a variable rate or a ‘‘relaxed phylogenetics’’ approach using Bayesian inference and MCMC procedures to derive a posterior distribution of rates and times. In this method rates are unconstrained and no assumption about substitution rate autocorrelation is made; instead rates are assumed to be drawn from a statistical distribution that can be estimated from the calibration points (Pulquério & Nichols 2006). The method is implemented in BEAST (Drummond & Rambaut 2007), software that does not require a starting tree topology, importantly allowing phylogenetic uncertainty to be included in the estimations (Rutschmann 2006). The analysis thus estimates the divergence times, the topology of the tree and the rates, all as part of the same calculation (Pulquério & Nichols 2006), effectively accounting for uncertainty in evolutionary rates and calibration times (Drummond et al. 2006).

### 4. 1. 2. Calibration

Once rate heterogeneity has been accommodated, genetic distance estimates must be converted into measures of time. This requires one or more externally derived calibration dates. The way in which age estimates are incorporated differs between methods of dating. These differences include single *vs.* multiple point calibrations and fixed ages to age bounds on nodes. The fossil record is the most commonly used source of non-molecular information regarding the ages of clades (Magallón 2004). Clade ages are equated with the age of the oldest known fossil that can be assigned to that clade (Heads 2005).

Fossils need to be identified and dated, either through stratigraphic correlations or radiometric dating (Magallón 2004). Thus fossil dates are themselves subject to various sources of error including the uncertainty in identifying fossilized taxa, a lack of preservation of the earliest fossils representing a lineage, and uncertainties around the geological dating of those fossils (Lee 1999). It is perhaps not surprising that that calibration is one of the most problematic issues in molecular dating analyses (Conti *et al.* 2004).

The fossil record documents only the first appearance of morphologies in stratigraphic sequences (Foote *et al.* 1999). The age of a lineage is thus dated by the first appearance of defining synapomorphies (hence taxonomic group) in the fossil record, which of course can only mark the minimum age of that taxon (see figure 4.1) and will always underestimate the actual divergence time (Shields 2004, Heads 2005). Magallón (2004) suggests that for a synapomorphy to appear in the fossil record, it was probably abundantly produced and a fossil's first appearances most likely document the time when that structure became abundant rather that its time of origin. Magallón (2004) further suggests that the best fossils are decay-resistant structures with unambiguous synapomorphies documenting clade membership that, as a result, provide a high probability of a small time lapse between becoming abundant and first fossil occurrence (Magallón 2004). This is a problem in terms of leafy liverworts due to a general lack of decay-resistant structures (Schuster 1966), as well as limited available unambiguous synapomorphies.

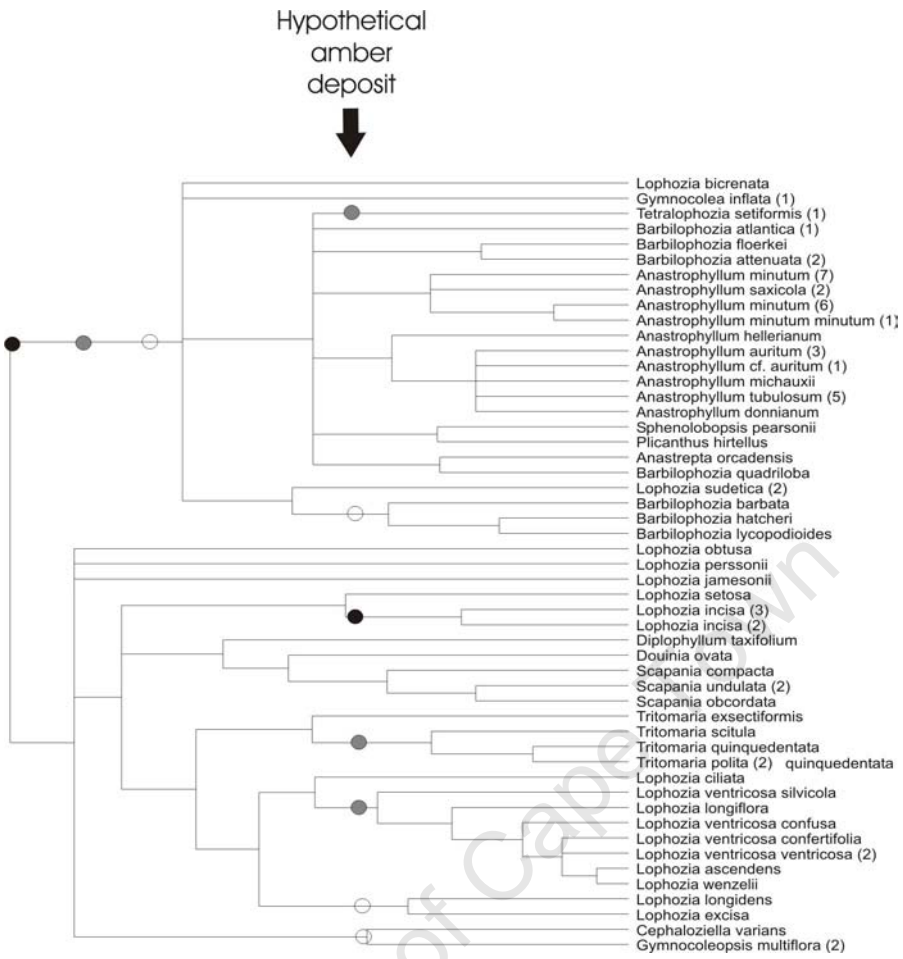




**Figure 4.1.** A hypothetical phylogeny of the Lophoziaceae-Scapaniaceae-Cephaloziellaceae clade. Ten calibration points are available for use, with the solid circles indicating examples of "good" calibration points, the clear of "poor" calibration points and the grey in-between. This shows how fossils indicate the minimum age of taxa (Modified from Hedges & Kumar 2004). A hypothetical amber deposit suggests the same calibration age for seven points in the tree, only one of which can be considered a "good" calibration point.

Multiple calibration points are desirable in order to minimise errors associated with single calibration points (Lee 1999). It has been argued (Wang *et al.* 1999, Doolittle *et al.* 1996, Cooper & Penny 1997, Bromham *et al.* 1998) that the desirability of multiple calibration points not withstanding, their use might introduce error because some might be inaccurate. Lee (1999), however, suggested that the risk of inaccurate calibration must be greater if only a single calibration point is used, unless the reliability of calibration points can be rigorously assessed. Near *et al.* (2005) proposed "Fossil Cross-Validation" as a method for assessing the accuracy of specific fossil calibration points. Using the impact of different individual calibrations on overall estimation identifies fossils that have an exceptionally large error effect and that may warrant further examination (Near & Sanderson 2004).

Calibration nodes should also be situated within the study group (Shaul & Graur 2002, Linder *et al.* 2005). Linder *et al.* (2005) found a linear relationship between the degree to which node ages are underestimated and their distance from the calibration point using Nonparametric Rate Smoothing or Bayesian analysis. In other words increased distance from dated node might increase error. Because of this, dated nodes should be in the proximity of fixed nodes; this is thought to reduce the error that might accumulate over longer time spans (Linder *et al.* 2005).

### 4. 1. 3. Objectives

This chapter explores divergence dates of the major lineages of leafy liverworts, with special focus on the Lophoziaceae. The primary objective is to examine possible radiations. Specifically, objectives are to i) establish when the major lineages in the Lophoziaceae arose, ii) test whether the main diversifications of the Jungermanniidae were in 'co-evolution' with the evolution of angiosperms and the establishment of tropical rainforest ecosystems, and iii) establish possible paleo-ecological correlates with observed radiations.

I first examine candidate calibration points in the leafy liverwort fossil record. Based on these the ''relaxed phylogenetics'' approach described by Drummond et al. (2006) as implemented in BEAST (Drummond & Rambaut 2007) and implied Lineage-Through-Time (LTT) plots for liverworts are compared with angiosperms and ferns.

## 4. 2. Methods

Literature searches (Table 4.1) were used to identify leafy liverwort fossils that would be good candidates for calibration points. Taxa from Baltic and Bitterfeld amber (>50 mya) include the following: *Plagiochila groehnii* (Grolle & Heinrichs 2003), *Scapania hoffeinsiana* (Grolle & Schmidt 2001), *Mastigolejeunea contorta* (Grolle & Meister 2004), *Radula sphaerocarpoides* (Grolle 1980), *Calypogeia stenzeliana* (Grolle 1985), *Metacalypogeia sp*. (Grolle 1999) and *Porella subgrandiloba* (Grolle & So 2004). The Dominican amber deposit (15-20 mya) (IturraldeVinent & MacPhee 1996) provides *Bryopteris sp.* (Grolle 1984). The use of these as calibration points has been to assign an age of at least 50 mya to the Plagiochilaceae, Scapaniaceae, Lejeuneaceae, *Radula*, *Calypogeia*, and an age of at least 20 mya to *Bryopteris*. Since it is difficult to place some taxa into immediate extant groups due to peculiarities, a conservative placement (in the context of minimum age for a particular group) was preferred, assigned based on stem-crown group distinction to nodes with Bayesian support of ≥0.95. Based on data from Heinrichs *et al*. (2007) who analysed a much broader range of taxa including liverworts, mosses, hornworts and tracheophytes, an additional calibration is used for the divergence of Metzgeriidae and Jungermanniidae between 158 mya and 316.5 mya. The minimum age is derived from the fossil *Cheiorrhiza brittae* from the Talynjan Formation of Bureja Basin in Amur (Heinrichs *et al*. 2007). The maximum age derived from a penalized likelihood approach to representatives of all major clades of land plants (Heinrichs *et al*. 2007). The exemplars used in this chapter are listed in the appendix.

**Table 4.1.** Potential calibration points for dating diversification of liverworts.

| Fossil Species | Taxon constrained | Fossil Matrix | Age | Reference |
|---|---|---|---|---|
| *Plagiochila groehnii* | Plagiochilaceae | Baltic amber | <50 mya | Grolle & Heinrichs (2003) |
| *Scapania hoffeinsiana* | Scapaniaceae | Bitterfeld amber | <50 mya | Grolle & Schmidt (2001) |
| *Mastigolejeunea contorta* | Lejeuneaceae | Baltic amber | <50 mya | Grolle & Meister (2004) |
| *Radula sphaerocarpoides* | *Radula* | Baltic amber | <50 mya | Grolle (1980) |
| *Calypogeia stenzeliana* | *Calypogeia* | Bitterfeld amber | <50 mya | Grolle (1985), Grolle (1999) |
| *Metacalypogeia Bryopteris* | *Bryopteris* | Baltic amber Dominican amber | <20 mya | Grolle (1984) |
| | Jungermanniidae | | < 158 mya >316.5 mya | Heinrichs *et al.* 2007 |

The BEAST manual, 'A rough guide to BEAST 1.4' (Drummond et al. 2007) was used as a guide to implementing the software. Sequence data for *rps*4 was first converted to the required XML format using the software BEAUti (Bayesian Evolutionary Analysis Utility) v1.4.7 (Drummond & Rambaut 2007). For calibration constraints a number of monophyletic taxon subsets within the sequence data were set up: *Brypoteris* (*Bryoperis filicina* and *Lejeunea cladogyna*); Jungermanniidae (all taxa except *Pellia epiphylla* and *Symphyogyna podophylla*); Lejuneaceae (*Bryopteris filicina*, *Jubula hutchinsiae* and *Lejeunea cladogyna*); Plagiochilaceae (*Chiloscyphus cuspidatus*, *Clasmatocolea vermicularis, Heteroscyphus argutus*, *Leptoscyphus ovatus*, *Lophocolea concreta*, *Plagiochila deltoidea*, *Plagiochila dura* and *Plagiochila retrospectans*); *Radula* (*Porella pinnata*, *Porella platyphylla*, *Radula complanata* and *Radula perrottetii*); Scapaniaceae (*Diplophyllum taxifolium*, *Douinia ovata*, *Lophozia ascendens*, *Lophozia ciliata*, *Lophozia excisa*, *Lophozia incisa* (2), *Lophozia incisa* (3), *Lophozia longidens*, *Lophozia longiflora*, *Lophozia setosa*, *Lophozia ventricosa* var. *confertifolia*, *Lophozia ventricosa* var. *confusa*, *Lophozia ventricosa* var. *silvicola*, *Lophozia ventricosa* var. *ventricosa* (2), *Lophozia wenzelii*, *Scapania compacta*, *Scapania obcordata*, *Scapania undulata* (2), *Tritomaria exsectiformis*, *Tritomaria polita* (2), *Tritomaria quinquedentata* ssp. *quinquedentata* var. *quinquedentata* and *Tritomaria scitula*) and *Calypogeia* (*Calypogeia integristipula* and *Mnioloma fuscum*). The dating was executed in BEAST v1.4.7 (Drummond & Rambaut 2007). A Yule tree prior was set with the time to most recent common ancestor ($t_{MRCA}$) for each taxon subset set to a uniform Distribution Model set

with priors for the constrained taxa as follows: *Brypoteris* (lower 20: upper 316.5 mya); Jungermanniidae (lower 158 and upper 316.5 mya); Lejuneaceae, Plagiochilaceae, *Radula*, Scapaniaceae and *Calypogeia* (lower: 50 and upper: 316.5 mya). The DNA substitution model was: GTR with estimated base frequencies and gamma site heterogeneity model with four categories. The molecular clock model was set to the uncorrelated, relaxed, lognormal clock. MCMC options were set for a chain length of 10 x $10^6$, with parameters sampled every 1000 generations. The burn-in phase was set to 1 x $10^{6.}$

The MCMC output of BEAST was visualised using the software TRACER v1.4(Rambaut & Drummond 2007). Information from the resultant sample of trees produced by BEAST was summarized onto a single "target" tree by the TreeAnnotator v1.4.7 (Drummond & Rambaut 2007) software. Using the "Maximum clade credibility" option, the node height and rate statistics are summarized on the tree with node heights rescaled to reflect the posterior mean node heights for the clades contained in the target tree.

The mean node height estimates were used to calculate a proportional LTT plot for all liverwort lineages included in the above analyses as well as for the Lophoziaceae-Scapaniaceae-Cephaloziellaceae lineages. For comparison this was also done using data (the estimated mean dates for individual nodes) from Heinrichs *et al.* (2007) and the LTT plots for polypod ferns and angiosperms from Schneider *et al.* (2004).

### 4. 3. Results

Table 4.2 shows the summary statistics for the analysis as summarised by Tracer v1.4 including the mean value and the Effective Sample Size (ESS) for each parameter. The ESS is an estimate of how many effectively independent samples from the marginal posterior distribution the MCMC is equivalent to. It is recommended that the ESS be of a value greater than 200 accurately represent the posterior distribution (Drummond et al. 2007). Figure 4.2 shows the *rps*4 tree from the analysis with associated node ages. Also represented are bars of 95 % confidence limits for those clades with a posterior probability of >0.5. Figure 4.3 shows the *rps*4 tree from the analysis with 95 % confidence limit values for those clades with a posterior probability values of >0.5.

**Table 4.2.** Summary statistics for the analysis

| Statistic | Mean | ESS |
|---|---|---|
| posterior | -1.256E4 | 1034.941 |
| prior | -596.851 | 893.155 |
| likelihood | -1.196E4 | 1471.338 |
| meanRate | 8.433E-4 | 949.898 |
| treeModel.rootHeight | 312.491 | 654.121 |
| tmrca(Brypoteris) | 46.894 | 218.913 |
| tmrca(Jungermanniidae) | 273.259 | 892.017 |
| tmrca(Lejuneaceae) | 114.299 | 289.02 |
| tmrca(Plagiochila) | 87.724 | 425.652 |
| tmrca(Radula) | 179.009 | 358.304 |
| tmrca(Scapaniaceae) | 62.858 | 383.313 |
| tmrca(Calypogeia) | 62.353 | 646.486 |
| yule.birthRate | 1.459E-2 | 1246.57 |
| gtr.ac | 0.225 | 2712.577 |
| gtr.ag | 1.046 | 1227.413 |
| gtr.at | 2.545E-2 | 3107.462 |
| gtr.cg | 0.259 | 3386.057 |
| gtr.gt | 0.127 | 2181.935 |
| siteModel.alpha | 0.439 | 6294.821 |
| ucld.mean | 8.23E-4 | 959.39 |
| ucld.stdev | 0.547 | 305.737 |
| coefficientOfVariation | 0.554 | 305.473 |
| covariance | 7.41E-2 | 1155.623 |
| treeLikelihood | -1.196E4 | 1471.338 |
| speciation | -566.747 | 894.308 |

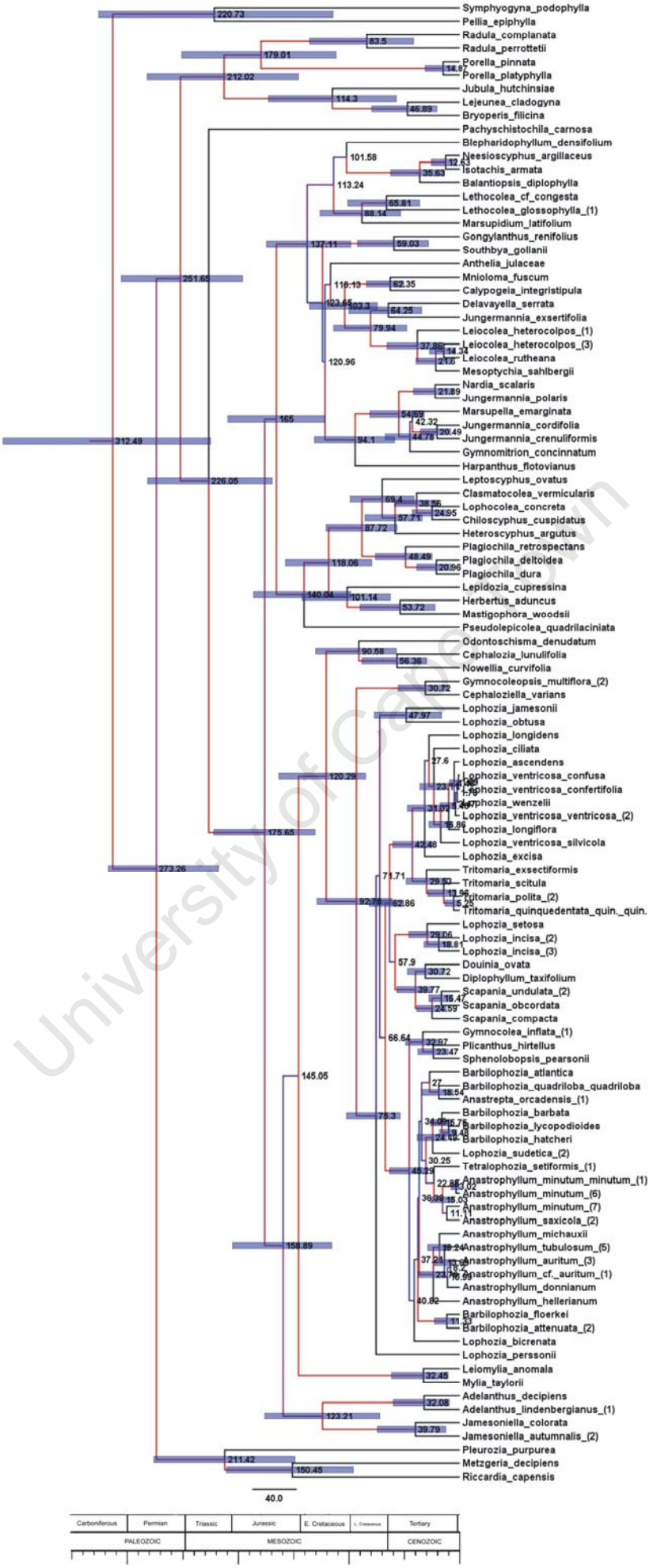
Symphyogyna_podophylla
Pellia_epiphylla
Radula_complanata
Radula_perrottetii
Porella_pinnata
Porella_platyphylla
Jubula_hutchinsiae
Lejeunea_cladogyna
Bryoperis_filicina
Pachyschistochila_carnosa
Blepharidophyllum_densifolium
Neesioscyphus_argillaceus
Isotachis_armata
Balantiopsis_diplophylla
Lethocolea_cf_congesta
Lethocolea_glossophylla_(1)
Marsupidium_latifolium
Gongylanthus_renifolius
Southbya_gollanii
Anthelia_julaceae
Mnioloma_fuscum
Calypogeia_integristipula
Delavayella_serrata
Jungermannia_exsertifolia
Leiocolea_heterocolpos_(1)
Leiocolea_heterocolpos_(3)
Leiocolea_rutheana
Mesoptychia_sahlbergii
Nardia_scalaris
Jungermannia_polaris
Marsupella_emarginata
Jungermannia_cordifolia
Jungermannia_crenuliformis
Gymnomitrion_concinnatum
Harpanthus_flotovianus
Leptoscyphus_ovatus
Clasmatocolea_vermicularis
Lophocolea_concreta
Chiloscyphus_cuspidatus
Heteroscyphus_argutus
Plagiochila_retrospectans
Plagiochila_deltoidea
Plagiochila_dura
Lepidozia_cupressina
Herbertus_aduncus
Mastigophora_woodsii
Pseudolepicolea_quadrilaciniata
Odontoschisma_denudatum
Cephalozia_lunulifolia
Nowellia_curvifolia
Gymnocoleopsis_multiflora_(2)
Cephaloziella_varians
Lophozia_jamesonii
Lophozia_obtusa
Lophozia_longidens
Lophozia_ciliata
Lophozia_ascendens
Lophozia_ventricosa_confusa
Lophozia_ventricosa_confertifolia
Lophozia_wenzelii
Lophozia_ventricosa_ventricosa_(2)
Lophozia_longiflora
Lophozia_ventricosa_silvicola
Lophozia_excisa
Tritomaria_exsectiformis
Tritomaria_scitula
Tritomaria_polita_(2)
Tritomaria_quinquedentata_quin._quin.
Lophozia_setosa
Lophozia_incisa_(2)
Lophozia_incisa_(3)
Douinia_ovata
Diplophyllum_taxifolium
Scapania_undulata_(2)
Scapania_obcordata
Scapania_compacta
Gymnocolea_inflata_(1)
Plicanthus_hirtellus
Sphenolobopsis_pearsonii
Barbilophozia_atlantica
Barbilophozia_quadriloba_quadriloba
Anastrepta_orcadensis_(1)
Barbilophozia_barbata
Barbilophozia_lycopodioides
Barbilophozia_hatcheri
Lophozia_sudetica_(2)
Tetralophozia_setiformis_(1)
Anastrophyllum_minutum_minutum_(1)
Anastrophyllum_minutum_(6)
Anastrophyllum_minutum_(7)
Anastrophyllum_saxicola_(2)
Anastrophyllum_michauxii
Anastrophyllum_tubulosum_(5)
Anastrophyllum_auritum_(3)
Anastrophyllum_cf._auritum_(1)
Anastrophyllum_donnianum
Anastrophyllum_hellerianum
Barbilophozia_floerkei
Barbilophozia_attenuata_(2)
Lophozia_bicrenata
Lophozia_perssonii
Leiomylia_anomala
Mylia_taylorii
Adelanthus_decipiens
Adelanthus_lindenbergianus_(1)
Jamesoniella_colorata
Jamesoniella_autumnalis_(2)
Pleurozia_purpurea
Metzgeria_decipiens
Riccardia_capensis
220.73
83.5
179.01
14.87
212.02
114.3
46.89
101.58
12.63
35.63
113.24
65.81
88.14
137.11
59.03
251.65
116.13
62.35
123.65
103.3
64.25
79.94
37.88
14.34
21.6
120.96
21.89
165
54.09
42.32
20.49
312.49
94.1
44.79
226.05
69.4
38.56
24.95
57.71
87.72
48.49
118.06
20.96
140.04
101.14
53.72
90.58
56.35
30.72
47.97
27.6
120.29
23.74
1.79
31.02
6.86
175.65
42.48
273.26
71.71
29.59
13.56
5.26
92.78
62.86
29.06
18.81
57.9
30.72
39.77
15.47
24.59
66.64
32.97
23.47
145.05
27
18.54
75.3
34.09
15.75
24.43
30.25
40.29
22.85
13.02
38.39
15.03
11.1
158.89
18.24
37.28
23.78
40.82
11.35
32.45
32.08
123.21
39.79
211.42
150.45
40.0
Carboniferous
Permian
Triassic
Jurassic
E. Cretaceous
L. Cretaceous
Tertiary
PALEOZOIC
MESOZOIC
CENOZOIC
300 mya
200 mya
100 mya
0 mya

**Figure 4.2.**

**Figure 4.2.** (Page 80) The *rps*4 relaxed clock tree. Branches are coloured according to posterior probability (red-high to blue-low), and labelled with node ages and bars of 95% confidence limits for nodes with greater than a 0.5 posterior probability.

The divergence between the Metzgeriidae and Jungermanniidae is estimated as 273.26 mya (95% CI 217.16 – 316.49 mya). The divergence between the Jamesonielloideae-Adelanthaceae clade and the Cephaloziaceae-Lophoziaceae-Scapaniaceae-Cephaloziellaceae clade is 158.89 mya (114.9 – 204.62 mya). Cephaloziaceae and the Lophoziaceae-Scapaniaceae-Cephaloziellaceae clades are estimated to have split at 120.29 mya (84.74 – 162.8 mya), whilst the divergence between the *Anastrophyllum* clade and the *Scapania* clade is poorly supported at 66.64 mya. The divergence between the *L. incisa*, *L. setosa* and Scapaniaceae clade from the 'core' *Lophozia* and *Tritomaria* clade is 62.86 mya (50 – 81.31 mya). The "core" *Lophozia* clade is estimated to have diverged from the *Tritomaria* clade at 42.48 mya (24.84 – 60.24 mya).

Figure 4.4 shows Lineage Through Time (LTT) plots for liverworts based on this study and that of Heinrichs *et al.* (2007) as well as for angiosperms and ferns from Schneider *et al.* (2004). This LTT plot shows the number of lineages present at intervals of 10 myr as a percentage of the terminal taxa (as seen in Schneider *et al.* 2004; Newton *et al*. 2007). Only the plot showing the strict age constraints, fixing the crown group at 132 mya for the angiosperms (Schneider *et al.* 2004), is shown in this plot. This is because these ages have been suggested (Newton *et al.* 2007) to be more closely congruent with the fossil history of the angiosperms (see Crane *et al*. 1995; Wing & Boucher 1998; Friis *et al.* 1999) and more closely reflect results of other molecular analyses (see Wikstrom *et al.* 2001; Soltis *et al*. 2002; Magallon and Sanderson 2005; Bell *et al.* 2005).

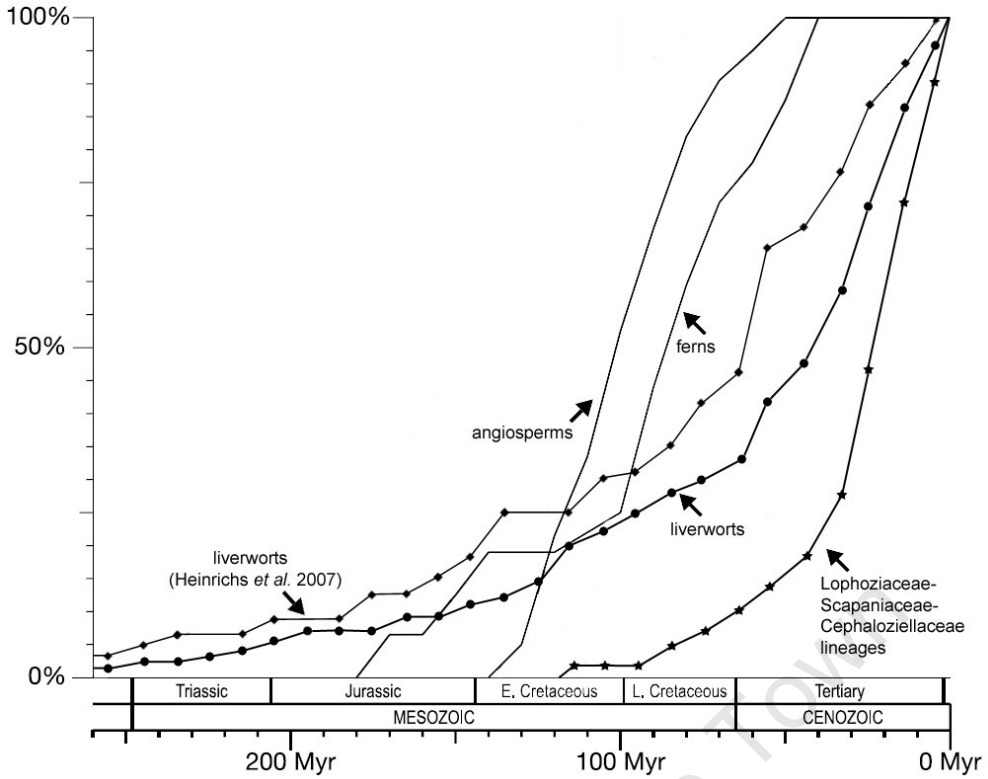


**Figure 4.4.** Lineage-through-time plots modified from Schneider *et al.* (2004) for angiosperms and ferns (Schneider *et al.* 2004) and liverworts. Angiosperm plot is from a strict age constraints analysis that fixed the crown group at 132 myr (Schneider *et al.* 2004). For the liverwort plots, lines with dots indicate the lineages from this study and lines with squares indicate the lineages from Heinrichs *et al.* (2007). Additionally the lines with stars indicate Lophoziaceae-Scapaniaceae-Cephaloziellaceae lineages.

Note that a sharp rise in the proportion of angiosperm lineages present is followed by a sharp rise in fern lineages (Schneider *et al.* 2004). The diversification of all liverwort lineages for this data shows much the same pattern, but with smoother diversification rates, as seen in the Heinrichs *et al.* (2007) study. The diversification of liverwort lineages shows a similar pattern to the fern lineages prior to the onset of angiosperm diversification, but unlike the ferns there is no evidence for an increase in diversification rates after the origin of flowering plants. The plots for all liverworts and for Lophoziaceae-Scapaniaceae-Cephaloziellaceae lineages examined in this study show a strong increase in the rate of diversification after the Cretaceous-Tertiary boundary; the latter shows strong increase in the mid-tertiary while the overall liverwort plot suggests an increase after the Cretaceous-Tertiary boundary. For the liverwort lineages there appears to be a decrease in rate of diversification with a noticeable absence of any flattening out of the LTT curve as seen for the other lineages.

**4. 4. Discussion**

The general absence of decay-resistant structures in leafy liverworts leads to an extremely slender fossil record, and most available samples are associated with amber. The amber from Baltic, Bitterfeld and Dominican deposits with leafy liverwort inclusions essentially represent very slender snapshots of liverwort history in time and space. Baltic and Bitterfeld amber being equated to the same age (Weitschat 1997) further limits the potential to place age constraints on specific nodes in dating divergence analyses. While the taxa from Baltic and Bitterfeld amber provide useful constraints on node ages (i.e. these nodes must be at least 50 mya) placing upper limit constraints on these nodes is essentially guesswork. However, these nodes are not older than 316.5 mya and this was used as an upper limit constraint, since a constraint for the divergence of Metzgeriidae and Jungermanniidae was set between 158 mya and 316.5 mya. Since the age of a lineage is dated by the first appearance of a taxonomic group in the fossil record, which of course can only mark the minimum age of that taxon, this problem is exacerbated when these calibrations are drawn essentially from only two deposits or snapshots from the past.

Another major source of error in fossil-based divergence dates is the uncertainty in identifying fossilized taxa. This is especially a problem when dealing with taxa traditionally associated with the Lophoziaceae. An example is the *Lophozia kutscheri* fossil (Grolle & Meister 2004) from the Bitterfeld deposit in Central Germany, which could have been used in this analysis to constrain the *Anastrophyllum* clade. Grolle & Meister (2004) draw attention to similarities of *L. kutscheri* to the extant *Barbilophozia hatcheri*. However, the nearly transverse insertion of the leaves in the dorsal half distinguishes it from *B. hatcheri* and suggests a vague affinity with *Anastrophyllum* (Grolle & Meister 2004). Further complicating this is a superficial similarity to certain species of *Acrobolbus* (Grolle & Meister 2004). The solution in this study might have been to assign a constraint to the *Anastrophyllum* clade which includes both *Anastrophyllum* and *B. hatcheri*. However due to the poor support for this part of the tree, this constraint was excluded. Conti *et al.*'s (2004) suggestion that calibration is one of the most problematic issues in molecular dating analyses is perhaps particularly apt for liverworts.

Many authors (Wang *et al.* 1999, Doolittle *et al.* 1996, Cooper & Penny 1997, Bromham *et al.* 1998) suggest that multiple calibration points introduce error because of some

calibration points being inaccurate. However, as noted by Lee (1999), the risk of inaccurate calibration is greater if only single calibration points are used unless the reliability of calibration points can be rigorously assessed. Near *et al.*'s (2005) fossil cross-validation method appears useful for assessing the validity of specific calibration points where calibrations are used to fix node ages; for example, when using Penalised Likelihood (Sanderson 2002). This procedure relies on the assumption that the majority of calibration points are accurate in the context of individual calibration point impact on the overall estimation and associated error effects. The method used here, however, does not require a cross validation procedure since it is inherent in this method that multiple calibration constraints be utilised. This is because rates are assumed to be drawn from a statistical distribution that can be estimated from the calibration, not assuming significant rate autocorrelation among branches. This in particular is a motivation for using the method of Drummond *et al*. (2006). Additionally constraints in BEAST analyses allow for the specification of upper and lower bounds on nodes, rather than specifying a specific point in time as a calibration point. Given the lack of fossil evidence older than the Baltic and Bitterfeld deposits useful for the taxa in this analysis, there is no justification in attaching more specific upper bounds on these nodes (i.e. >50 mya, <316.5 mya).

Some nodes have large error estimates associated with their dates: possibly with more evidence in the form of fossil taxa, importantly from deposits other than those used in this study, these age estimates could be refined. The data presented here suggests that the split of Jungermanniidae from other liverworts, and its subsequent diversification occurred after the mid-Permian around 273 mya. The major leafy liverwort groups seem to have mostly emerged by the end of the Cretaceous.

Overall it appears that rates of diversification have been fairly smooth. The LTT plot for all liverworts in this study, reflect this, showing a smooth, almost exponential increase in the rate of lineage formation through the Late Cretaceous and into the Tertiary. Looking at both the data from this study and that from Heinrichs *et al.* (2007) there seems to be a slight increase in the rate of diversification around the Late Cretaceous and into the Tertiary. A similar pattern is found for Pleurocarpous mosses (Newton *et al*. 2007), and might be explained by sampling density of species for specific clades (Newton *et al*. 2007). Because of this, the LTT plot for the better-sampled Lophoziaceae-Scapaniaceae-Cephaloziellaceae clade is included. This plot shows a similar pattern of a smooth,

exponential increase in the rate of lineage formation, although as expected for this later lineage (appearing in the early Cretaceous) diversification happens later than with the liverworts as a whole.

A couple of things should be noted from this figure when examining the plots of ferns, angiosperms and liverworts in conjunction with each other. Polypod ferns diversified in the Cretaceous, after angiosperms, perhaps as an ecological response to the diversification of angiosperms as suggested by Schneider *et al.* (2004). Liverworts show large increases in percentage representation of lineages in the Cretaceous, well after both angiosperms and ferns. The lineages from Heinrichs *et al.* (2007) show an earlier increase in percentage when compared to those from this study. The observed differences in these results are probably due to differences in sampling (see Nee *et al*. 1994; Pybus & Harvey 2000; Shaw *et al.* 2003), specifically differences in the depth of the liverwort phylogeny sampling. For instance Heinrichs *et al.* (2007) included representatives of the main lineages of Metzgeriidae, Pelliidae and Jungermanniidae; this study includes major lineages of Jungermanniidae, with special focus on the Lophoziaceae in other words a greater representation of recent lineages. Possibly also differences could be as a result of different dating methods (the use of Penalised Likelihood in the Heinrichs *et al.* study).

The data from Heinrichs *et al.* (2007) shows a similar pattern between the fern and liverwort lineages prior to the diversification of the angiosperms and less after, with ferns showing a sharp increase in diversification rate after about 100 Mya. If ferns did respond to the diversification of angiosperms then this at least suggests less of a direct impact of the diversification of angiosperms on liverworts as on ferns, lycopods (Wikström & Kenrick 2001) and horsetails (Des Marais *et al.* 2003). A reason for this could be that liverworts are found in a wider range of habitats when compared to ferns. For instance most taxa of the Lophoziaceae occur in cool to cold areas and in the tropics – mostly in montane or alpine regions. This might result in less dependence of liverworts on the specific habitats provided for by angiosperms prior to the Tertiary.

The increase in the rate of diversification of liverworts after the Cretaceous-Tertiary boundary, suggests radiation after the major global extinction associated with this episode. This suggests that liverworts responded to changes after this boundary, again not simply a response to the evolution of angiosperms. This is contrary to the idea (Frey

& Stech 2005) that the main diversifications of the Jungermanniidae were in 'co-evolution' with angiosperms. Liverworts in general might have responded less to the evolution of angiosperms and more to various unidentified abiotic events such as climate change and geological activity. These are possibilities suggested by Schneider *et al.* (2004) for polypods and by Newton *et al.* (2007) for pleurocarpous mosses.

However, global temperatures were high in the Cretaceous, and it has been suggested that angiosperms initially diversifying and spread during this period (115-120 mya), possibly only gaining dominance at high-middle paleolatitudes by the Upper Cretaceous (93-70 mya) (Stewart & Rothwell 1993). Behrensmeyer (1992) suggests that the fossil record shows that complex angiosperm forests with a rich diversity of habitats appeared late in the process of diversification of angiosperms. In North America angiosperms in the Late Cretaceous accounted for no more than 12 % of cover (Wing *et al.* 1993) with dominance likely to be restricted to disturbed and riparian habitats (Behrensmeyer 1992; Wing *et al.* 1993). Rather, the kind of forest habitat with a wide diversity of habitats characteristic of extant angiosperm forests did not appear until the end of the Cretaceous and early Palaeogene (Lupia *et al.* 2000), although probably earlier in the lower paleolatitudes (Lupia *et al.* 2000; Morley 2000). Thus the suggestion that the main diversifications of the Jungermanniidae were in 'co-evolution' with the evolution of angiosperms *and* the establishment of tropical rainforest ecosystems (Frey & Stech 2005) is thus still compatible with these results.

When examining the Lophoziaceae-Scapaniaceae-Cephaloziellaceae lineages in the LTT plot, it appears that these lineages were not significantly affected by events following the Cretaceous-Tertiary boundary. Many of the taxa sampled in this group depend less on complex, multilayered forest habitats (unlike many of the epiphyte and epiphyll taxa) and are often found in non-forest habitats in cool to cold areas and in the tropics mostly in montane or alpine regions (often forming important components of the ground level flora). Thus in the mid-tertiary and later in the Pleistocene, events such as cooling climates in the late Eocene with increasingly pronounced seasonal changes may have promoted diversification of these taxa. Supporting this is the suggestion that rates of diversification do not seem to decrease or flatten out as with angiosperms and ferns. The liverwort curves do not flatten out in the LTT plot as with the other lineages, recent events in the Pleistocene such as major glaciations possibly favour diversification of leafy liverworts.

## 4. 5. Conclusion

Acknowledging the uncertainty in identifying fossilized taxa, and incorporating uncertainty associated with lack of preservation of the earliest fossils used for calibration constraints, results in large confidence limits on the divergence dates estimated in this study. The latter uncertainty is exacerbated in leafy liverworts, since they lack decay-resistant structures resulting in "snapshots" of available fossil material in amber deposits.

The data suggests a split of Jungermanniidae from other liverworts, and its subsequent diversification occurred after the mid-Permian around 273 mya. The major leafy liverwort lineages mostly emerge by the end of the Cretaceous. Results are compatible with the theory that the main diversifications of the Jungermanniidae were in 'co-evolution' with the development of angiosperm habitats such as the establishment of tropical rainforests in the Cretaceous period. At the same time, the results suggest less correlation of the diversification of liverworts following angiosperms as between angiosperms, ferns, lycopods and horsetails. A reason for this could be that liverworts are found in a wider range of habitats when compared to ferns, lycopods and horsetails. An increase in the rate of diversification of liverworts after the Cretaceous-Tertiary boundary suggests a radiation after this event.

The Lophoziaceae-Scapaniaceae-Cephaloziellaceae lineages appear to not be significantly affected by events following the Cretaceous-Tertiary boundary. Possibly due to less dependence on complex, multilayered forest habitats; many taxa favour non-forest habitats in cool to cold areas. Increased rate of diversification of these taxa in the mid-tertiary is possibly related to cooling climates with increasingly pronounced seasonal changes. Recent events in the Pleistocene such as major glaciations appear to favour diversification of leafy liverworts.

# Chapter 5

# Classification in the context of phylogeny

## 5. 1. Introduction

The naming of biological groups has a long history. The *nomen specificum legitimum* did not begin with Linnaeus (1707-1778); rather the starting point of modern nomenclature is seen with his consistent use of the binomial by linking a *nomen triviale* to the generic name (McNeill 2000). The Linnaean hierarchical system has been the basis of classification ever since. Relationships among entities are also ordered into various hierarchical ranks such that each rank is nested within the higher rank: species within genera within families within orders etc.

The assertion that evolution is the single most powerful and general process underlying biological diversity summarises most arguments as to why formal taxonomic names should be used solely to represent monophyletic groups (Mishler 1999). This idea was widely catalyzed by Hennig (1966) with the idea of grouping by synapomorphy, thought to result in the best way to construct maximally efficient and predictive diagnoses of taxa in a hierarchical classification (Schuh 2003). Thus the most effective and natural classification systems are those grouping taxa according to the processes that generated them in the first place (Mishler 1999). Woodger (1952) pointed out that evolution is not something that can be grafted onto the Linnaean system of classification, suggesting that: "The taxonomic system and the evolutionary phylogenetic scheme are different things doing different jobs and that confusion will result from identifying or mixing them". Others have also pointed out that the Linnaean system is incompatible with a phylogenetic system (e.g. Griffiths 1976; Cronquist 1987; de Queiroz & Gauthier 1992; Brummitt 2002).

To illustrate, often there are no fully resolved phylogenetic trees to be used and one can usually simply superimpose Linnaean ranks onto a tree (dé Queiroz & Gauthier 1992). This works because both cladistic and ranked hierarchies are nested (dé Queiroz & Gauthier 1992), illustrated in Figure 5.1 (a). Problems with this arise when relationships are better understood or if nodes collapse, for example as illustrated in figure 5.1 (b). One begins with a tidy ranked classification of a family with two genera and four species.

With more data the previously recognised genus (C, D) is no longer a monophyletic group. This creates an unranked node between family and genus; one must create a rank for it and in this case it could become a sub-family. In reality this could lead to multiple sub-ranks (dé Queiroz & Gauthier 1992), as well as redundancy in asymmetric trees where certain ranks are used in one part and not needed in another part of a particular tree.

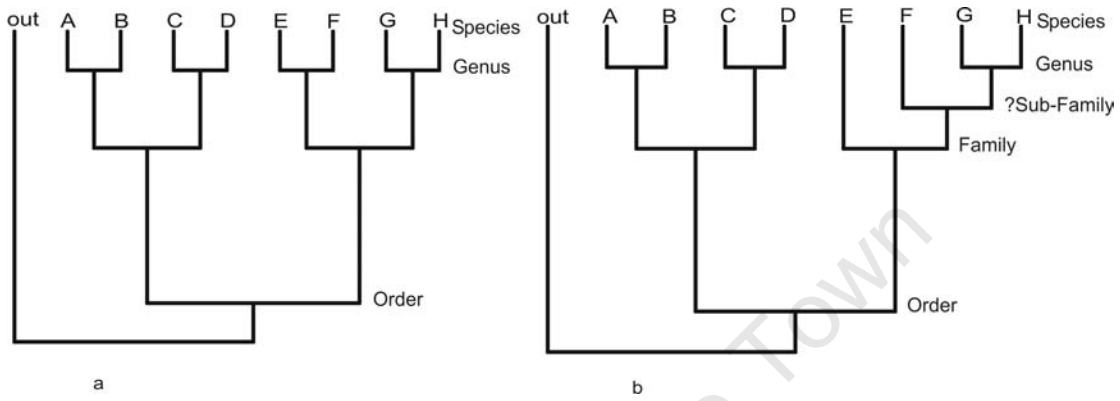


**Figure 5. 1.** (a) Both cladistic and ranked hierarchies are nested, often Linnaean ranks can be superimposed onto a tree. (b) Problems arise when relationships are better understood or if nodes collapse creating unranked nodes (in this case a sub-family) and redundancy (dé Queiroz & Gauthier 1992).



Brummitt (2002) argues that dividing up an evolutionary tree into mutually exclusive families, genera and species that are all monophyletic is a logical impossibility. He illustrates that if one classifies all the products of evolution in the Linnaean system, every taxon recognised inherently makes another taxon paraphyletic; if one were to classify using this system without paraphyletic taxa the whole classification collapses into the original genus or species (Brummitt 2002). Hence the hierarchical nature of phylogenies is incompatible with the ranked hierarchical nature of Linnaean classification (Brummitt 2002). Largely these problems in Brummitt's arguments give way if one relaxes the monophyly criterion for species.

Most importantly, groups given the same rank are not necessarily comparable in nature (dé Queiroz & Gauthier 1992). For example they are not comparable in age, size, amount of divergence or even the amount of diversity they contain (Mishler 1999). While many people are aware of this fundamental fact, many more are not, often taking number of taxa of a particular rank as a measure of biodiversity (Mishler 1999). Thus ranks become

a formality creating hindrances to those utilising the systems (Mishler 1999). Worse than this, in the wrong hands they often lead to incorrect comparisons and bad science (Mishler 1999).

Based on this perceived incompatibility of Linnaean nomenclature with representation of phylogenetic (monophyletic) groups, a suggested alternative is a phylogenetic nomenclature called the PhyloCode (Cantino & de Queiroz 2006). The PhyloCode provides rules to name the various parts or clades of the tree of life as uninomials. This name is tied to a specific clade definition with no significance to ranks and hence familiar endings such as "–aceae" for family (Forey 2002). Not all clades need to be named; those that are should be done so on the basis of evidence for monophyly (Mishler 1999). Langer (2001), examining the differences between Linnaean and phylogenetic nomenclature, notes that "Phylogenetic nomenclature (does) not attempt to merge the Linnaean nomenclature with phylogeny. Instead, it represents a completely independent system, which can be used not only instead of traditional taxonomy, but also alongside it" (Langer 2001). According to Pickett (2005), the concomitant use of the PhyloCode and current codes may soon be a reality.

The PhyloCode is still criticised by many (e.g. Benton 2000; Nixon & Carpenter 2000; Forey 2001; Carpenter 2003; Keller *et al.* 2003; Kojima 2003; Nixon 2003). Some criticise the premise that there should be concordance between phylogeny and nomenclature (e.g. Forey 2002; Nixon 2003; Keller *et al.* 2003), many question the stability offered (e.g. Benton 2000; Nixon & Carpenter 2000; Forey 2001; Carpenter 2003), and yet others criticise the communicability of the code (e.g. Kojima 2003; Nixon 2003). Since the Linnaean system of nomenclature has been used for over 250 years (Schuh 2003) it is understandable that the change of taxon names referencing categorical ranks to terms of common ancestry will be contentious. Pavlinov (2004) comments on the 'new phylogenetics' trend: "['New phylogenetics'] rejects some basical principles of classical phylogenetic (originally Linnean) taxonomy such as recognitions of fixed taxonomic ranks designated by respective terms and definition of taxic names not by the diagnostic characters but by reference to the ancestor. The latter makes the PhyloCode overburdened ideologically and the "newest" systematics self-controversial, as concept of ancestor has been acknowledged non-operational from the vary beginning of cladistics." This opinion seems excessive; so this chapter explores an application of the PhyloCode.

Under the PhyloCode there are three main ways to define taxa (see figure 5.2) using: node based, stem (or branch) based and apomorphy based definitions (de Queiroz & Gauthier 1992). However, the PhyloCode (Cantino & de Queiroz 2006) lists five examples of phylogenetic definitions. Schuh (2003) suggested that most of the literature is organised around the stem (or branch) based definitions, taking the form of "the clade consisting of A and all organisms or species that share a more recent common ancestor with A than with Z" (Cantino & de Queiroz 2006). A node based definition can take the form of "the clade stemming from the most recent common ancestor of A and B" (Cantino & de Queiroz 2006) while an apomorphy based definition can take the form of "the clade stemming from the first organism or species to possess apomorphy M as inherited by A" (Cantino & de Queiroz 2006).

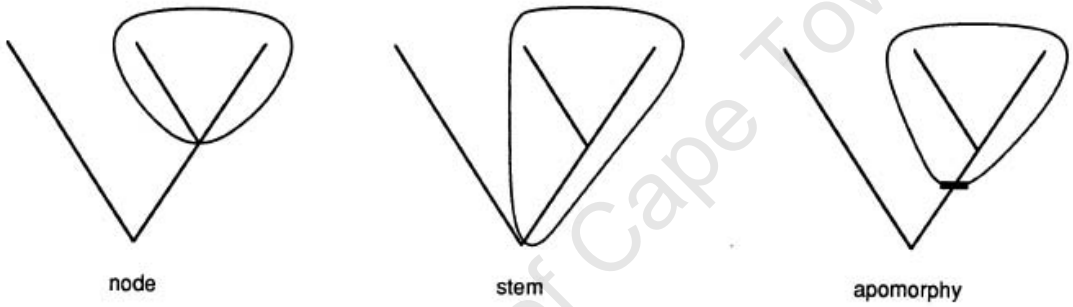


**Fig. 5. 2.** Node based, stem (branch) based and apomorphy based taxon definitions (from: de Queiroz & Gauthier 1992)



Acknowledging the recommendation of The International Code of Phylogenetic Nomenclature (ICPN) (Cantino & de Queiroz 2006) that the formal conversion of pre-existing names should only be done with thorough knowledge of the group concerned, in this chapter I explore an implementation of a phylogenetic classification of the Lophoziaceae-Scapaniaceae-Cephaloziellaceae clade. The main objectives are to i) Erect a phylogenetic classification and ii) contextualise this classification in the context of issues that would arise in applying a Linnaean classification whilst simultaneously attempting to recognise only monophyletic groups.

## 5. 2. Methods

The Bayesian phylogeny from the analysis in chapter two is used to label current classifications of taxa and groups within and around the Lophoziaceae-Scapaniaceae-Cephaloziellaceae clade. Existing classifications for families and higher taxonomic levels will again follow Crandall-Stotler & Stotler (2000) except for Jungermanniaceae and, by definition, also Lophoziaceae where I follow Grolle & Long (2002). Given the phylogeny, alternative changes to the classification under rank-based codes are explored, and an implementation of a phylogenetic classification is presented. To define taxa a stem (or branch) based definition is utilised. Following the ICPN's recommendation, the letter "R" bracketed will be used to designate names governed by the rank based codes and the letter "P" to designate names governed by the PhyloCode. For example, the name Lophoziaceae can be distinguished as Family Lophoziaceae[R] versus Clade *Lophoziaceae*[P]. Names designated "…" are my suggested names for clades or ranked groups where appropriate names are not yet published.

## 5. 3. Results and Proposals for alternative taxonomic treatments

Figure 5.3 illustrates the classification, for families and higher taxonomic levels following Crandall-Stotler & Stotler (2000) and for Jungermanniaceae and Lophoziaceae following Grolle & Long (2002), labelling groups of the sampled taxa. Given the phylogeny the family Lophoziaceae[R] and genera *Lophozia*[R], *Barbilophozia*[R] and *Anastrophyllum*[R] are clearly polyphyletic.

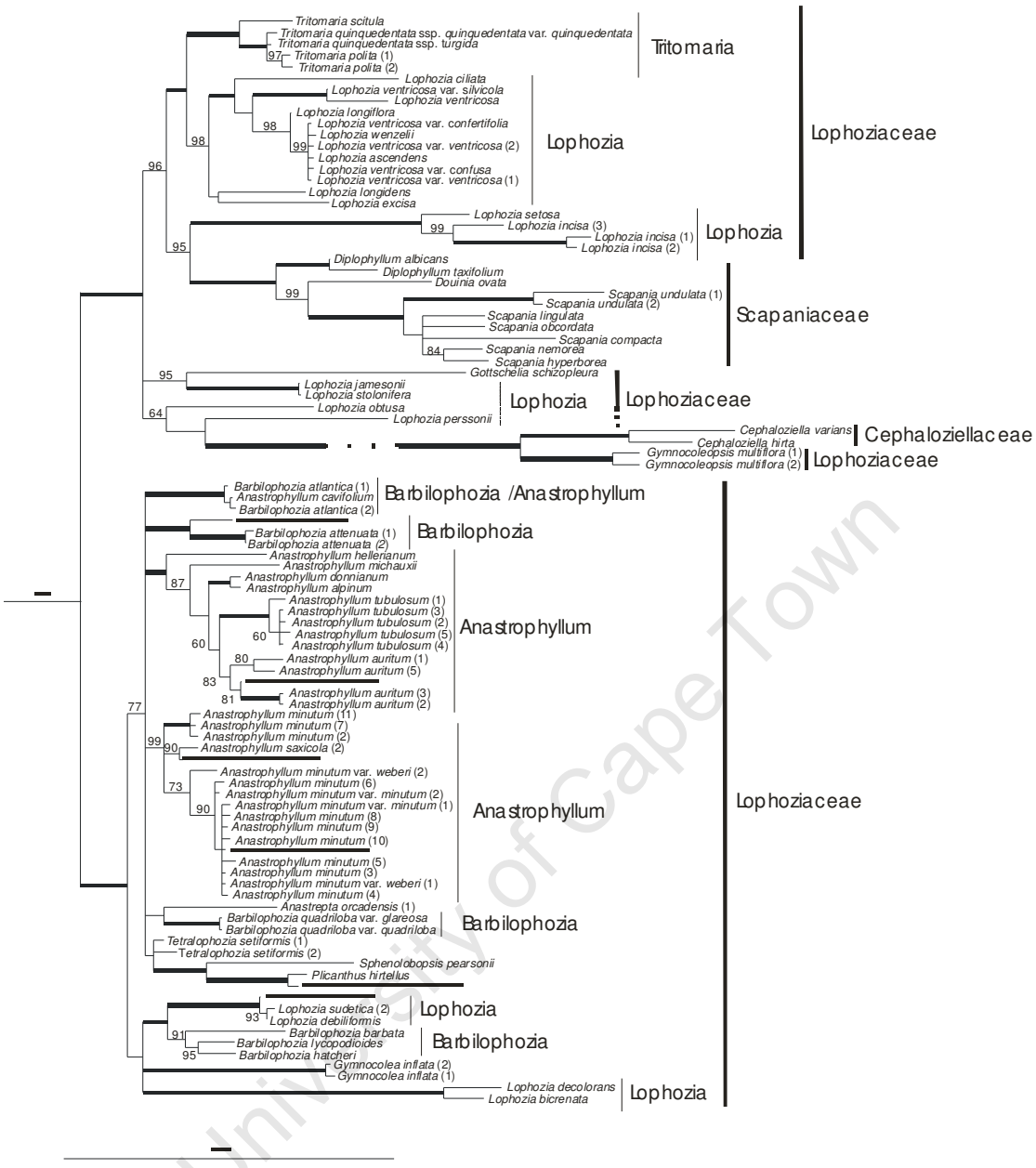




**Figure 5. 3.** An illustration of an example of the 'traditional' classification following Grolle & Long (2002) of taxa and groups within and around the Lophoziaceae-Scapaniaceae-Cephaloziellaceae clade as currently classified.

Figure 5.4 illustrates a revision of the current classification, similar to that suggested by Heinrichs *et al.* (2005), wherein Scapaniaceae is expanded and Lophoziaceae and Cephaloziellaceae are relegated to synonymy. The problems of polyphyletic genera can be solved by spitting *Lophozia*[R] into six genera (*Lophozia*[R], *Schistochilopsis*[R], *Hypolophozia*[R], *Obtusifolium*[R], a new genus for *L. perssonii,* and *Isopaches*[R]), *Anastrophyllum*[R] into two (*Anastrophyllum*[R] and *Sphenolobopsis*[R]), *Barbilophozia*[R] into four (*Orthocaulis*[R], a new genus for *B. floerkei* and *B. attenuata*, a new genus for *B. quadriloba,* and *Barbilophozia*[R]).

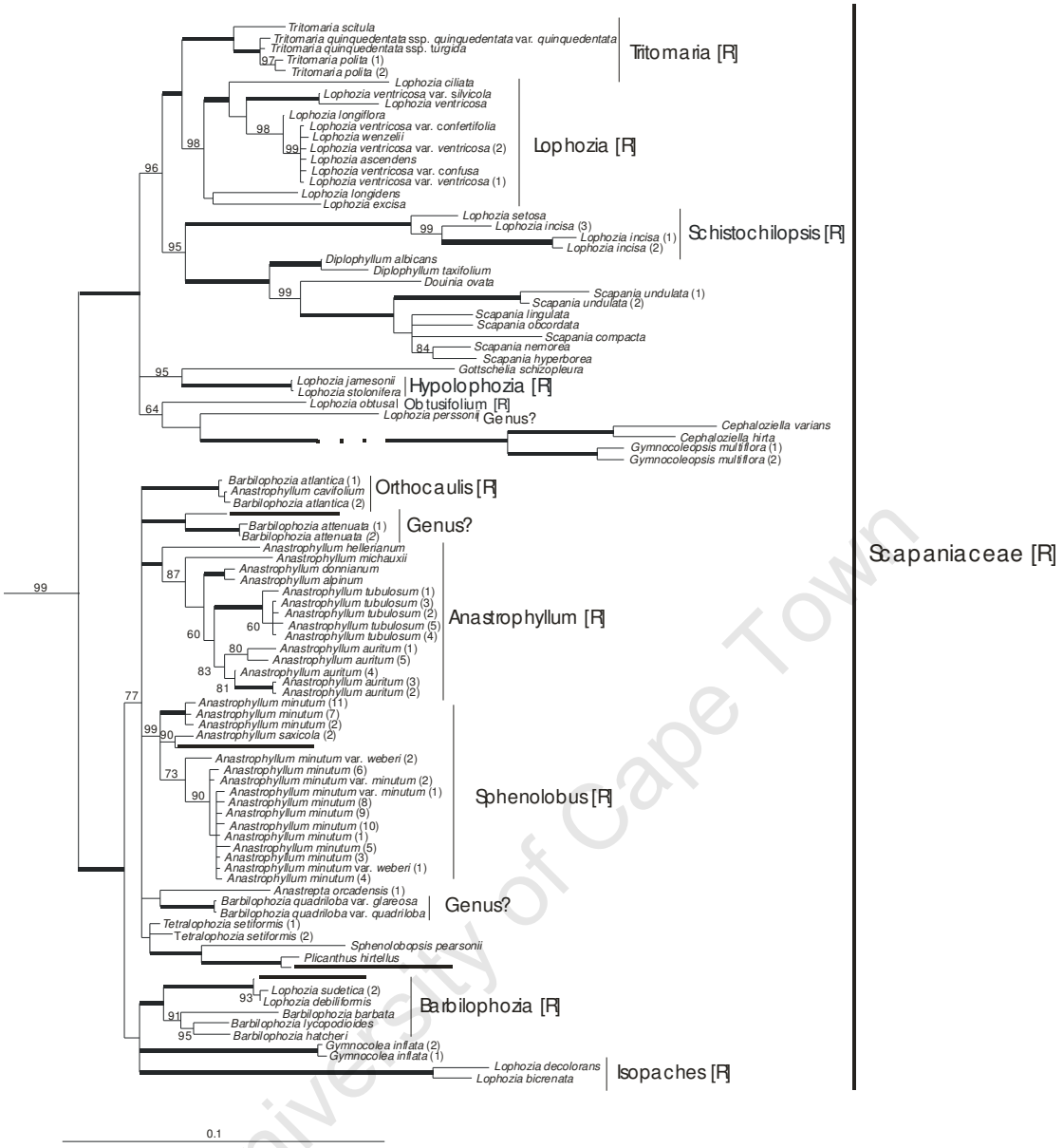


**Figure 5. 4.** A potential new "inclusive" classification of the Lophoziaceae-Scapaniaceae-Cephaloziellaceae clade, similar to that suggested by Heinrichs *et al.* (2005), wherein one family Scapaniaceae is recognised of which Lophoziaceae and Cephaloziellaceae become synonyms.

Figure 5.5 shows an alternative revision, wherein several smaller families are recognised with the same genera as in figure 5.4. Lophoziaceae[R] comprises *Lophozia*[R] and *Tritomaria*[R]. Scapaniaceae[R] comprises taxa currently recognised (except *Blepharidophyllum* and *Delavayella* are excluded). Cephaloziellaceae[R] includes *Gymnocoleopsis*[R]. At least three new families would need to be erected: 1) a family for *Schistochilopsis*[R], e.g. "Schistochilopsaceae"[R], 2) a family or families including *Gottschelia*[R] and *Hypolophozia*[R], 3) *Obtusifolium*[R] and *L. perssonii*, either form new families or are put in Cephaloziaceae and 4) a family for the "*Anastrophyllum* clade", e.g. "Anastrophyllaceae"[R].

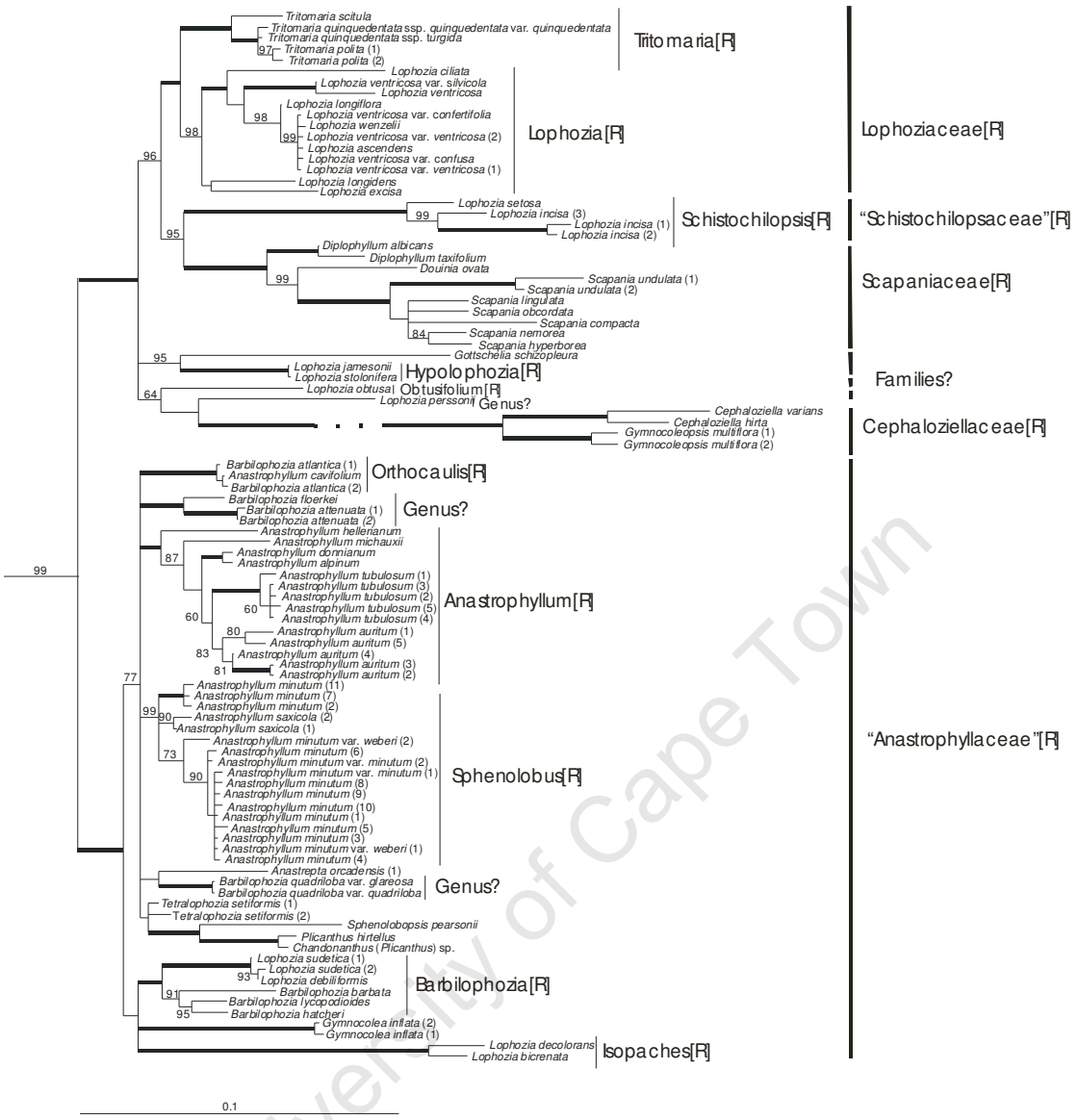


**Figure 5. 5.** An illustration of an alternative revision of taxa and groups within and around the Lophoziaceae-Scapaniaceae-Cephaloziellaceae clade, wherein many smaller families are recognised with the same genera as in figure 6.2.

Figures 5.6 and 5.7 show an implementation of a phylogenetic classification. *Lophoziaceae*[P] includes *Tritomaria*[P] and *Lophozia*[P]; "*Scapochilopsis*"[P] (name a concatenation of Scapania and Schistochilopsis) is a clade comprising *Schistochilopsis*[P] and *Scapaniaceae*[P]; *Chephaloziellaceae*[P] includes Cephaloziellaceae[R] and *Gymnocoleopsis*[R]. *Lophoziaceae*[P], "*Scapochilopsis*"[P], *Chephaloziellaceae*[P] as well as *Gottschelia*[P], *Hypolophozia*[P], *Obtusifolium*[P] and "*Personii*"[P] are all part of clade "*Ryabonapsis*"[P] (name a concatenation of rya (a Swedish shag rug) and *bona fide*). The "*Anastrophyllum* clade" is called "*Anastropsis*"[P] (a name derived from Anastrophyllum) and comprises *Orthocaulis*[P], a clade called "*Floerkuata*"[P] including *B. floerkei* and *B. attenuata*, *Anastrophyllum*[P], *Sphenolobus*[P], *Anastrepta*[P], *Quadriloba*[P], *Chandonanthoideae*[P], *Barbilophozia*[P], *Gymnocolea*[P] and *Isopaches*[P]. Both "*Anastropsis*"[P] and "*Ryabonapsis*"[P] form the clade "*Scapocephaphyllum*"[P] (name a concatenation of *Scapania*, Cephaloziellaceae and *Anastrophyllum*). Table 5.1 is a rank free treatment of the Lophoziaceae and related taxa based on the cladogram in figure 5.6.

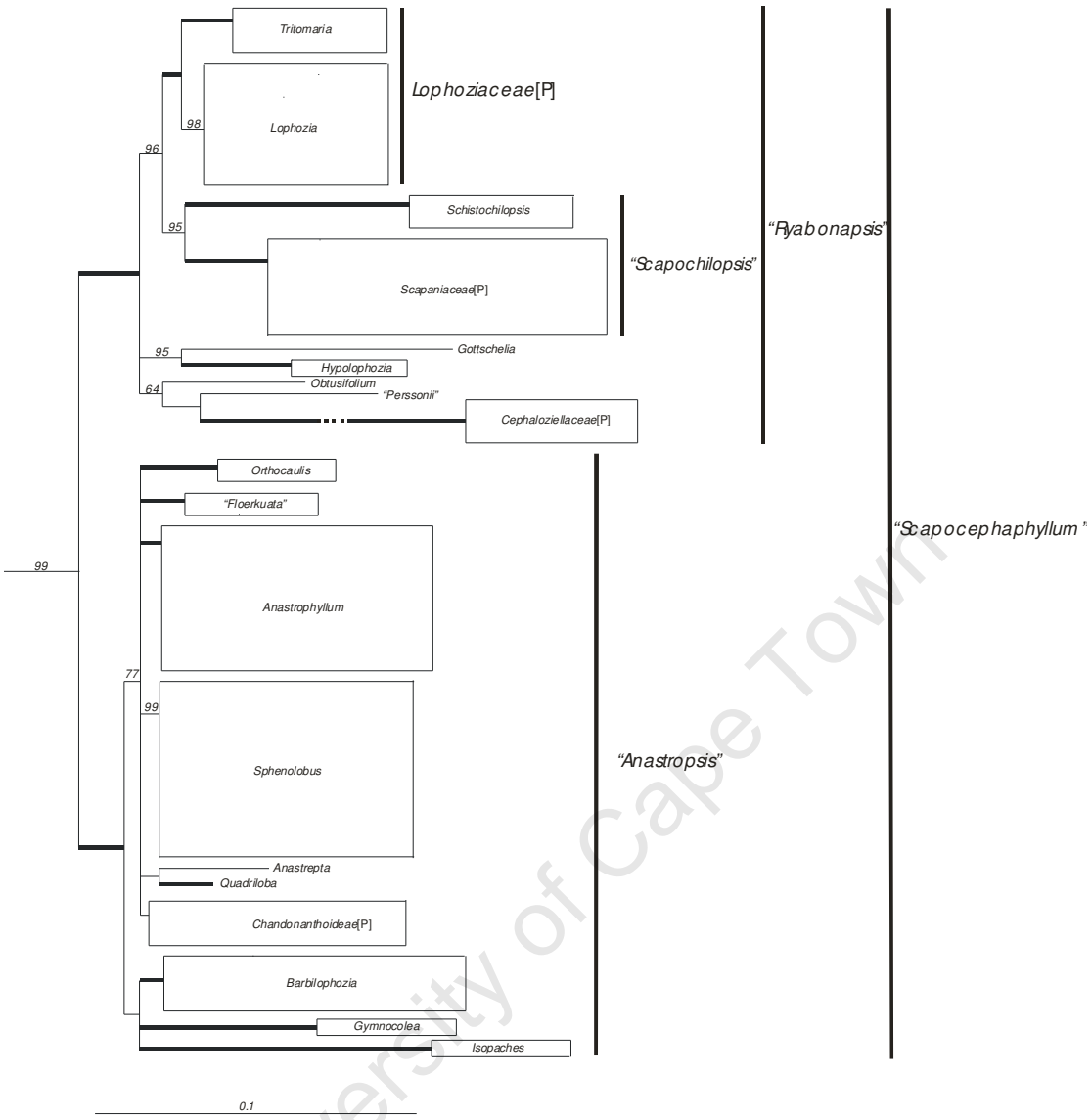


**Figure 5. 6.** A proposed phylogenetic classification of taxa and groups within and around the Lophoziaceae-Scapaniaceae-Cephaloziellaceae clade.

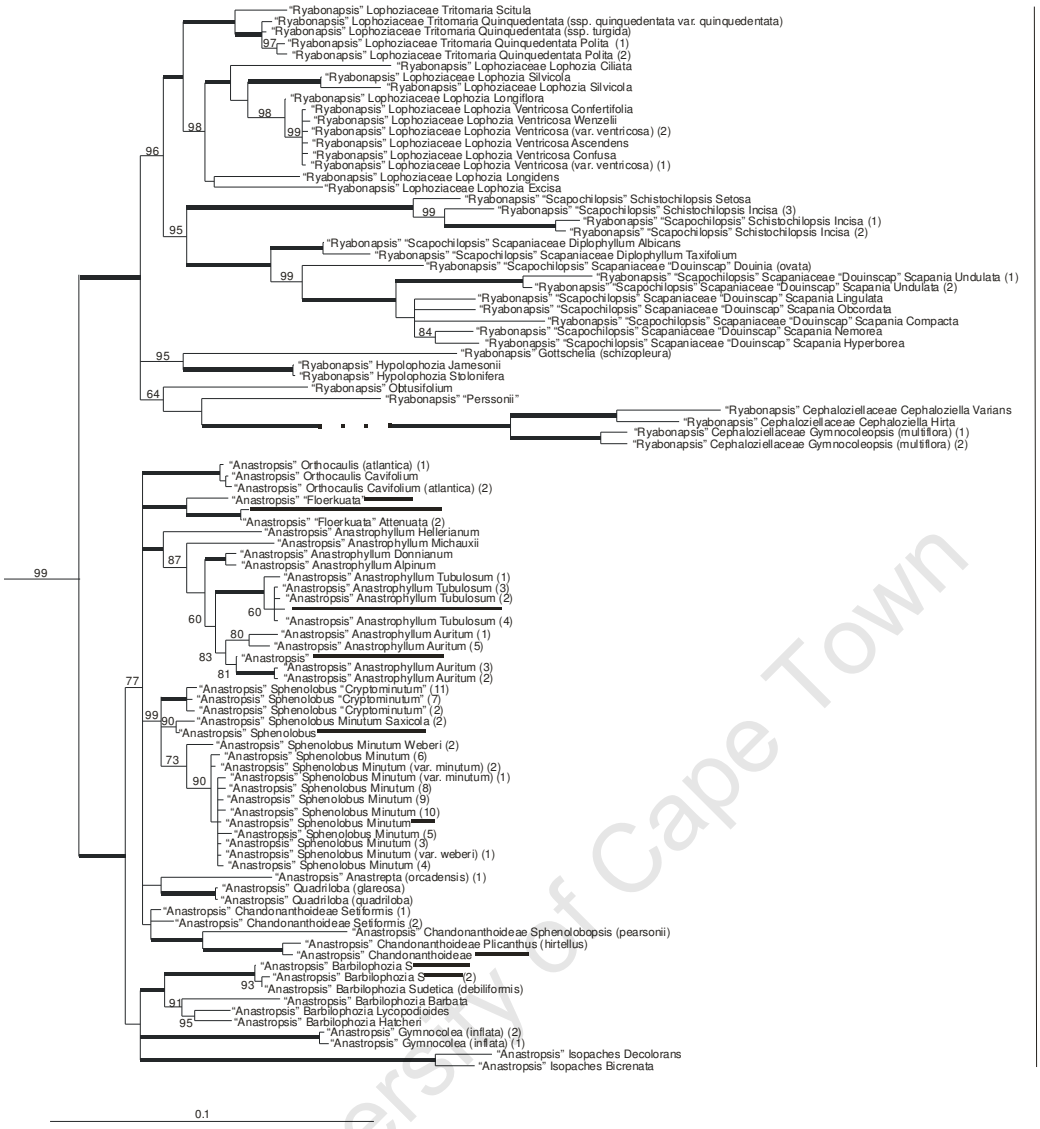


**Figure 5. 7.** A more detailed illustration of the phylogenetic classification of taxa and groups within and around the Lophoziaceae-Scapaniaceae-Cephaloziellaceae clade.

**Table 5.1**

A rank free treatment of the Lophoziaceae and related taxa based on the cladogram in figure 5.6. Terminal clades are included within higher-level taxa, but definitions for those are not specifically given here.

**Scapocephaphyllum**
Nomen cladi novum
Stem based definition
Internal specifier: *Tritomaria scitula* (Taylor) Jørg.
Internal specifier: *Lophozia* (Isopaches) *bicrenata* (Schmidel ex Hoffm.)
External specifier: *Cephalozia lunulifolia* (Dumort.) Dumort.
Included terminal clades

**Ryabonapsis**
Nomen cladi novum
Stem based definition
Internal specifier: *Gymnocoleopsis multiflora* (Steph.) R.M.Schust.
Internal specifier: *Tritomaria scitula* (Taylor) Jørg.
External specifier: *Barbilophozia atlantica* (Kaal.) Müll.Frib.
Included terminal clades
Perssonii (*Lophozia perssonii* H.Buch et S.W.Arn.); Gottschelia (*Gottschelia schizopleura* (Spr.) Grolle); Obtusifolium (*Lophozia obtusa* (Lindb.) A.Evans)

**Lophoziaceae**
Nomen cladi conversum, Lophoziaceae Cavers
Stem based definition
Internal specifier: *Tritomaria scitula* (Taylor) Jørg.
Internal specifier: *Lophozia excisa* (Dicks.) Dumort.
External specifier: *Lophozia setosa* (Mitt.) Steph.
Included terminal clades

**Tritomaria**
Nomen cladi conversum, *Tritomaria* Schiffn. ex Loeske
Stem based definition
Internal specifier: *Tritomaria scitula* (Taylor) Jørg.
Internal specifier: *Tritomaria quinquedentata* (Huds.) H.Buch
External specifier: *Lophozia excisa* (Dicks.) Dumort.
Included terminal clades
Scitula (*Tritomaria scitula* (Taylor) Jørg.); Quinquedentata (*Tritomaria quinquedentata* (Huds.) H.Buch)

**Lophozia**
Nomen cladi conversum, *Lophozia* (Dumort.) Domort.
Stem based definition
Internal specifier: *Lophozia wenzelii* (Nees) Steph.
Internal specifier: *Lophozia excisa* (Dicks.) Dumort.
External specifier: *Tritomaria scitula* (Taylor) Jørg.
Included terminal clades

Ciliata (Lophozia ciliata Damsh. *et al.*); Silvicola (Lophozia ventricosa var. silvicola (H.Buch) E.W. Jones); Longiflora (*Lophozia longiflora* (Nees) Schiffn.); Longidens (*Lophozia longidens* (Lindb.) Macoun); Excisa (*Lophozia excisa* (Dicks.) Dumort.)

**Ventricosa**
Nomen cladi conversum, *ventricosa* (Dicks.) Dumort.
Stem based definition
Internal specifier: *Lophozia ventricosa* var. *confusa* R.M.Schust.
Internal specifier: *Lophozia ventricosa* var. *confertifolia* (Schiffn.) Husn.
External specifier: *Lophozia longiflora* (Nees) Schiffn.
Included terminal clades
Confertifolia (*Lophozia ventricosa* var. *confertifolia* (Schiffn.) Husn.); Wenzelii (*Lophozia wenzelii* (Nees) Steph.); Ascendens (*Lophozia ascendens* (Warnst.) R.M.Schust.); Confusa (*Lophozia ventricosa* var. *confusa* R.M.Schust.)

**Scapochilopsis**
Nomen cladi novum
Stem based definition
Internal specifier: *Lophozia setosa* (Mitt.) Steph.
Internal specifier: *Scapania hyperborea* Jørg.
External specifier: *Lophozia excisa* (Dicks.) Dumort.
Included terminal clades

**Schistochilopsis**
Nomen cladi conversum, *Schistochilopsis* Kitagawa
Stem based definition
Internal specifier: *Lophozia incisa* (Schrad.) Dumort.
Internal specifier: *Lophozia setosa* (Mitt.) Steph.
External specifier: *Scapania hyperborea* Jørg.
Included terminal clades
Incisa (*Lophozia incisa* (Schrad.) Dumort.); Setosa (*Lophozia setosa* (Mitt.) Steph.)

**Scapaniaceae**
Nomen cladi conversum, Scapaniaceae Mig.
Stem based definition
Internal specifier: *Diplophyllum albicans* (L.) Dumort.
Internal specifier: *Scapania hyperborea* Jørg.
External specifier: *Lophozia incisa* (Schrad.) Dumort.

**Diplophyllum**
Nomen cladi conversum, *Diplophyllum* (Dumort. Emend. Lindb.) Dumort.
Internal specifier: *Diplophyllum albicans* (L.) Dumort.
Internal specifier: *Diplophyllum taxifolium* (Wahlenb.) Dumort.
External specifier: *Douinia ovata* (Dicks.) H.Buch
Included terminal clades
Albicans (*Diplophyllum albicans* (L.) Dumort.); Taxifolium (*Diplophyllum taxifolium* (Wahlenb.) Dumort.)

**Douinscap**
Nomen cladi novum

Internal specifier: *Douinia ovata* (Dicks.) H.Buch
Internal specifier: *Scapania hyperborea* Jørg.
External specifier: *Diplophyllum taxifolium* (Wahlenb.) Dumort.
Included terminal clades
Douinia (*Douinia ovata* (Dicks.) H.Buch)

**Scapania**
Nomen cladi conversum, Scapania (Dumort.) Dumort.
Internal specifier: *Scapania undulata* (L.) Dumort.
Internal specifier: *Scapania hyperborea* Jørg.
External specifier: *Douinia ovata* (Dicks.) H.Buch
Included terminal clades
Undulata (*Scapania undulata* (L.) Dumort.); Lingulata (*Scapania lingulata* H.Buch); Obcordata (*Scapania obcordata* (Berggr.) S.W.Arnell); Compacta (*Scapania compacta* (Roth) Dumort.); Nemorea (*Scapania nemorea* (L.) Grolle); Hyperborea (*Scapania hyperborea* Jørg.)

**Hypolophozia**
Nomen cladi conversum, *Hypolophozia* Schust.
Stem based definition
Internal specifier: *Lophozia jamesonii* (Mont.) R.M. Schust.
Internal specifier: *Lophozia stolonifera* R.M.Schust.
External specifier: *Gottschelia schizopleura* (Spr.) Grolle
Included terminal clades
Jamesonii (*Lophozia jamesonii* (Mont.) R.M. Schust.); Stolonifera (*Lophozia stolonifera* R.M.Schust.)

**Cephaloziellaceae**
Nomen cladi conversum, Cephaloziellaceae Mig.
Stem based definition
Internal specifier: *Gymnocoleopsis multiflora* (Steph.) R.M.Schust.
Internal specifier: *Cephaloziella varians* (Gottsche) Steph.
External specifier: *Lophozia perssonii* H.Buch et S.W.Arn.
Included terminal clades
Gymnocoleopsis (*Gymnocoleopsis multiflora* (Steph.) R.M.Schust.)

**Cephaloziella**
Nomen cladi conversum, Cephaloziella (Spruce) Schiffn.
Stem based definition
Internal specifier: *Cephaloziella hirta* (Steph.) R.M. Schust.
Internal specifier: *Cephaloziella varians* (Gottsche) Steph.
External specifier: *Gymnocoleopsis multiflora* (Steph.) R.M.Schust.
Included terminal clades
Hirta (*Cephaloziella hirta* (Steph.) R.M. Schust.); Varians (*Cephaloziella varians* (Gottsche) Steph.)

**Anastropsis**
Nomen cladi novum
Stem based definition
Internal specifier: *Barbilophozia atlantica* (Kaal.) Müll.Frib.
Internal specifier: *Lophozia bicrenata* (Schmidel ex Hoffm.)
External specifier: *Gymnocoleopsis multiflora* (Steph.) R.M.Schust.

Included terminal clades
Anastrepta (Anastrepta orcadensis (Hook.) Schiffn.); Quadriloba (*Barbilophozia quadriloba* (Lindb.) Loeske); Gymnocolea (*Gymnocolea inflata* (Huds.) Dumort.)

**Orthocaulis**
Nomen cladi conversum, *Orthocaulis* (Buch) Schust.
Stem based definition
Internal specifier: *Barbilophozia atlantica* (Kaal.) Müll.Frib.
Internal specifier: *Anastrophyllum cavifolium* (H.Buch et S.W.Arnell) Lammes
External specifier: *Barbilophozia floerkei* (F.Weber et D.Mohr) Loeske
Included terminal clades
Cavifolium (*Anastrophyllum cavifolium* (H.Buch et S.W.Arnell) Lammes)

**Floerkuata**
Nomen cladi novum
Stem based definition
Internal specifier: *Barbilophozia attenuata* (Mart.) Loeske
Internal specifier: *Barbilophozia floerkei* (F.Weber et D.Mohr) Loeske
External specifier: *Barbilophozia atlantica* (Kaal.) Müll.Frib.
Included terminal clades
Attenuata (*Barbilophozia attenuata* (Mart.) Loeske); Floerkei (*Barbilophozia floerkei* (F.Weber et D.Mohr) Loeske)

**Anastrophyllum**
Nomen cladi conversum, *Anastrophyllum* (Spruce) Steph.
Stem based definition
Internal specifier: *Anastrophyllum hellerianum* (Nees ex Lindenb.) R.M.Schust.
Internal specifier: *Anastrophyllum auritum* (Lehm.) Steph.
External specifier: *Anastrophyllum minutum* (Schreb.) R.M.Schust.
Included terminal clades
Hellerianum (*Anastrophyllum hellerianum* (Nees ex Lindenb.) R.M.Schust.); Michauxii (*Anastrophyllum michauxii* (F. Weber) H. Buch); Donnianum (*Anastrophyllum donnianum* (Hook.) Steph.); Alpinum (*Anastrophyllum alpinum* Steph.); Tubulosum (*Anastrophyllum tubulosum* (Nees) Grolle); Auritum (*Anastrophyllum auritum* (Lehm.) Steph.)

**Sphenolobus**
Nomen cladi conversum, *Sphenolobus* (Lindb.) R.M. Schust
Stem based definition
Internal specifier: *Anastrophyllum minutum* (Schreb.) R.M.Schust. (L. Söderström et. al. 2004/316)
Internal specifier: *Anastrophyllum minutum* (Schreb.) R.M.Schust. (L. Söderström et. al. 2004/135)
External specifier:
Included terminal clades
Saxicola (*Anastrophyllum saxicola* (Schrad.) R.M.Schust.)

**Cryptominutum**
Nomen cladi novum
Stem based definition
Internal specifier: *Anastrophyllum minutum* (Schreb.) R.M.Schust. (L. Söderström et. al. 2004/135)

Internal specifier: *Anastrophyllum minutum* (Schreb.) R.M.Schust. (T. Hedderson 15437)
External specifier: *Anastrophyllum minutum* (Schreb.) R.M.Schust. (L. Söderström et. al. 2004/316)

**Minutum**
Nomen cladi conversum, *minutum* (Schreb.) R.M.Schust.
Stem based definition
Internal specifier: *Anastrophyllum minutum* (Schreb.) R.M.Schust. (L. Söderström et. al. 2004/316)
Internal specifier: *A. minutum* var. *weberi* (Mart.) Kartt. (L. Söderström et. al. 2004/204)
External specifier: *Anastrophyllum minutum* (Schreb.) R.M.Schust. (L. Söderström et. al. 2004/135)

**Chandonanthoideae**
Nomen cladi conversum, Chandonanthoideae Inoue
Stem based definition
Internal specifier: *Tetralophozia setiformis* (Ehrh.) Schljakov
Internal specifier: *Plicanthus hirtellus* (Weber) R.M.Schust
External specifier: *Barbilophozia quadriloba* (Lindb.) Loeske
Included terminal clades
Setiformis (*Tetralophozia setiformis* (Ehrh.) Schljakov); Sphenolobopsis (*Sphenolobopsis pearsonii* (Spruce) R.M.Schust.); Plicanthus (*Plicanthus hirtellus* (Weber) R.M.Schust)

**Barbilophozia**
Nomen cladi conversum, *Barbilophozia* Loeske
Stem based definition
Internal specifier: *Lophozia sudetica* (Nees ex Huebener) Grolle
Internal specifier: *Barbilophozia hatcheri* (A.Evans) Loeske
External specifier: *Gymnocolea inflata* (Huds.) Dumort
Included terminal clades
Sudetica (*Lophozia sudetica* (Nees ex Huebener) Grolle); Barbata (*Barbilophozia barbata* (Schmidel ex Schreb.) Loeske); Lycopodioides (*Barbilophozia lycopodioides* (Wallr.) Loeske); Hatcheri (*Barbilophozia hatcheri* (A.Evans) Loeske)

**Isopaches**
Nomen cladi conversum, *Isopaches* H.Buch
Stem based definition
Internal specifier: *Lophozia decolorans* (Limpr.) Steph.
Internal specifier: *Lophozia bicrenata* (Schmidel ex Hoffm.) Dumort.
External specifier: *Gymnocolea inflata* (Huds.) Dumort
Included terminal clades
Decolorans (*Lophozia decolorans* (Limpr.) Steph.); Bicrenata (*Lophozia bicrenata* (Schmidel ex Hoffm.) Dumort.)

**5. 4. Discussion**

The first example of a revised rank-based classification for the group has already been suggested by Heinrichs *et al.* (2005), wherein only one family is recognised for the Lophoziaceae-Scapaniaceae-Cephaloziellaceae clade: Scapaniaceae[R], based on the priority of publication (Scapaniaceae Mig. Krypt.-Fl. Deutschl. 1:479; Gera. 1904). The second example of a rank based classification shows how the clade could be classified into several smaller families. In the first classification, Scapaniaceae[R] includes a sampling of 23 revised genera, a large and formidable family even given the sampling in this study. The second requires at least three new families to be erected, "Anastrophyllaceae"[R] would be suggested for the "*Anastrophyllum* clade" since *Anastrophyllum* was the first named genus within this clade (*Anastrophyllum* (Spruce) Steph. Hedwigia 32: 139. 1893).

The main difference between these two classifications is that in the first, more ranked groupings (e.g. Subfamily or Tribe) are required to convey further information regarding relationships of genera within the large Scapaniaceae[R]. This clearly would be necessary since this large family would embrace a very divergent range of morphologies; for example *Scapania* is very different from *Lophozia*. This I feel is cumbersome and as more resolution is gained with further studies of the phylogeny in this group, this is likely to lead to a proliferation of categories (de Queiroz & Gauthier 1992). It is clear that in these rank based classifications, differences between 'generic' and 'familial' concepts amount to little more than personal judgement. With the aim of producing a workable classification, these judgements are likely to be highly subjective especially given a particular sampling of taxa. One reason for not recognising many smaller families could be the resulting elevation of taxa such as *Lophozia obtusa* to being monotypic or monogeneric families. It seems often the case that monotypic families are only recognised when they are separated from others by wide gaps, but large families are often separated by very small gaps; there is no logic in this.

The phylogenetic classification bypasses these issues by removing the ranked categories and simply names clades. In this classification the clade *Lophoziaceae*[P] can be defined in a branch-based definition (as utilised in table 5.1) as the most recent common ancestor of *Lophozia*[P] and all extant organisms or taxa that share a more recent common ancestor with *Lophozia*[P] than with "*Scapochilopsis*"[P]. In a node-based definition the *Lophoziaceae*[P] is the clade stemming from the most recent common ancestor of

*Lophozia*[P] and *Tritomaria*[P]. It is important to note that not all clades need to be named. For instance the clade stemming from the most recent common ancestor of *Lophoziaceae*[P] and "*Scapochilopsis*"[P] is not named in this classification; the clade does not cease to exist, it is still part of the clade "*Ryabonapsis*"[P] and can be given a name without causing a shift in ranked categories or a proliferation of categories in order to accommodate it. Likewise the clade *Chandonanthoideae*[P] is the clade stemming from the most recent common ancestor of *Sphenolobopsis*[P], *Plicanthus*[P] and *Setiformis*[P]. Since support for this clade is weak, should it turn out that *Setiformis*[P] shares a more recent common ancestor with *Anastrepta*[P] than with *Sphenolobus*[P] and *Plicanthus*[P] it can be transferred to a new clade with *Anastrepta*[P] in the classification with little effect on *Chandonanthoideae*[P] other than its definition changing to be the clade stemming from the most recent common ancestor of *Sphenolobopsis*[P] and *Plicanthus*[P]. In reality, based on the weak support in this phylogeny for the clade *Chandonanthoideae*[P], it would not necessarily be formally named in the literature, removing the possibility that such a change would have to be performed; in the meantime it can be referred to as the "*Chandonanthoideae*"[P] until evidence suggests it to be legitimate or otherwise.

## 5. 5. Conclusion

By removing the ranks in classification systems and adopting the PhyloCode, biologists can work with a system of classification that is capable of representing and compatible with phylogenetic groups. This is essential given that evolution is the single most powerful and general process underlying biological diversity.

The PhyloCode system has the potential to be more stable with less input of personal judgement, and prevents proliferation of categories. It has been shown here to take away some difficult judgement choices in the naming of groups, moving away from a subjective towards more objective system of classification. Where support is weak for specific clades, these need not be formally named, yet they can still be referred to within the context of other established clades until further data becomes available. This chapter has made it clear to me that Pavlinov (2004) in his comment on the 'new phylogenetics' trend requires a refreshed grasp of phylogenetics to see that in practice, even when applied to the disorderly classification often associated with leafy liverworts, the PhyloCode is quite operational.

# Chapter 6
# General Discussion

## 6. 1. Summary of results

This thesis uses DNA sequence variation to investigate the phylogenetic relationships and evolutionary history of the large and systematically troublesome leafy liverwort family Lophoziaceae Cavers. The numerous fascinating insights gained in the course of this investigation highlight the utility of molecular data in revealing evolutionary pattern. The main aims were to i) establish a phylogenetically-defensible delimitation of the Lophoziaceae; ii) gain a clearer understanding of relationships within and among its often poorly delimited genera; iii) hypothesise a time line for the diversification of the group; and iv) attempt a reclassification that reflects phylogenetic relationships. In this chapter the main findings of this thesis are summarised, their broader relevance discussed, and an agenda for future research is explored.

### 6. 1. 1. A defensible delimitation of the Lophoziaceae

Analysis of DNA sequence data showed that the current classification of leafy hepatics is highly inconsistent with phylogeny as revealed by chloroplast markers. This will scarcely surprise anyone working with leafy liverworts, and several studies appearing at the beginning of this research project pointed to the problem (e.g. Davis, 2004; Yatsentyuk *et al.*, 2004; Heinrichs *et al.*, 2005; He-Nygren *et al.*, 2006; Hentschel *et al.*, 2006).

Extensive sampling of taxa representing a wide range of Jungermanniidae, with emphasis on the Lophoziaceae and taxa placed near it in most classifications, provide stark evidence that current family and subfamily delimitations are largely artificial. A key finding is that Lophoziaceae is in fact not closely related to Jungermanniaceae, where it is often placed, and should rather be included in Scapaniaceae unless many smaller families are recognized. If treated the same, Cephaloziellaceae, although poorly sampled should likewise be placed in Scapaniaceae. Jamesonielloideae is found either as a family of its own (Jamesoniellaceae) sister to Adelanthaceae, or should be included in Adelanthaceae.

The artificial delimitations are not limited to family level, and many genera were also found to be misplaced. For example, *Delavayella* and *Blepharidophyllum* should be excluded from Scapaniaceae, *Leiocolea* should be placed in Mesoptychiaceae, and the generic status of *Leiomylia* is unwarranted. At lower levels, it appears that the genus *Anastrophyllum* should be split into *Anastrophyllum* and *Sphenolobus*. *Lophozia* is polyphyletic and the genera *Isopaches* and *Schistochilopsis*, and perhaps *Obtusifolium*, should be recognized. *Barbilophozia s. str*. is found to be is monophyletic, while *Orthocaulis* is polyphyletic with the four sampled species appearing in 3 different clades.

Unsurprisingly, at species level changes were also highlighted. It was found that *L. sudetica* could be transferred to *Barbilophozia*. *Lophozia silvicola* Buch is separated from *L. ventricosa* at species level. And *Jamesoniella oenops* is separated from *J. colorata* at species level.

Limitations in sampling that I feel require particular attention include sampling of the following genera sometimes placed in Lophoziaceae: *Andrewsianthus*, *Cephalolobus*, *Gerhildiella*, *Hattoria*, *Pseudocephaloziella*, *Roivainenia*, *Nothostrepta*, as well as sampling more of the c. 130 species of *Lophozia* and more *Anastrophyllum* species. These are likely to be closely affiliated with groups explored in this research and it is important that future molecular studies attempt to include them for a clearer picture of leafy liverwort systematics. The use of chloroplast markers also limited the study; the plastid genome is inherited as a unit and usually uniparentally, so there is the danger that the phylogeny presented here in reality encompasses only the chloroplast history. This limitation was somewhat addressed in chapter 3 although only for the *Anastrophyllum* clade. It can be noted that whilst lower level relationships established using chloroplast markers might be very different with those identified using a nuclear marker, higher level ones shouldn't be so affected. This is mostly a function of population-level processes like lineage sorting and coalescence. Important in establishing a “bigger picture” phylogeny, future studies should also include information from morphology and anatomy to facilitate a better understanding of liverwort evolutionary history.

**6. 1. 2. A clearer understanding of relationships within the *Anastrophyllum* clade**

Within the clade comprising most sampled Lophoziaceae, two well supported clades emerged - here referred to as the *Scapania* clade and the *Anastrophyllum* clade. The former clade comprises the Scapaniaceae, some Lophoziaceae taxa and

Cephaloziellaceae and the latter clade comprises most elements of the Lophoziaceae. The second part of this research focused on the *Anastrophyllum* clade, attempting to further test its monophyly and to further resolve relationships within this large, diverse group. A slightly clearer picture of the phylogenetic status of various genera was gained using additional markers including nuclear data and additional taxa.

The combined nuclear and chloroplast analysis revealed much the same results as the chloroplast data alone. Congruence was found between the data sets with the exception of *B. atlantica* which was excluded from the combined analysis. Additionally, the results show that *Gymnocolea inflata* is possibly sister to the remaining taxa in the *Anastrophyllum* clade. *Anastrepta orcadensis* is possibly sister to *Isopaches*. Chandonanthoideae*, Sphenolobus*, *B. floerkei, B. attenuate*, *Anastrophyllum, B. atlantica* and *B. quadriloba* appear more closely related to each other than to *Isopaches, Gymnocolea* and *Barbilophozia* s. str. *Tetralophozia setiformis* is paraphyletic with *Plicanthus* and *Spenolobopsis* sister to one of the *T. setiformis* exemplars. *Sphenolobopsis* should perhaps be transferred to the Chandonanthoideae. Since *Barbilophozia atlantica* is the type for *Orthocaulis*; the genus needs to be re-instated for it and *Anastrophyllum cavifolium*. *Sphenolobus* is possibly sister to *Anastrophyllum*, *Orthocaulis*, *B. floerkei* and *B. attenuata*. And *A. auritum* is paraphyletic with an *A. auritum* exemplar sister to *A. tubulosum*.

Overall the increased resolution in this combined analysis of the *Anastrophyllum* clade compared to the two chloroplast regions in chapter two is mostly poorly supported. Again, further sampling of suitable markers as well as missing taxa mentioned earlier might improve this. However, as one approaches more closely related entities, essentially reaching the "species" level in the phylogeny, the fact that coalescent events may not correspond with species boundaries due to incomplete lineage sorting becomes more of an issue (see Knowles & Carstens 2007). Additional sampling of taxa at these levels will probably not clarify relationships to the degree hoped for in this kind of study I noted above. In the context of examining the paraphyly seen with *Anastrophyllum minutum*, a detailed morphological study of the species complex is required - specifically in the context of a phylogenetic approach based on coalescence models to distinguishing current and past population processes or using phylogeographic analyses of gene trees to test species status and processes. A denser sampling of *A. minutum* would be required for such a study, particularly including exemplars from all of its wide geographical

distribution (North America, Europe and Asia into the high arctic and extending into South America, southern Africa and New Guinea). One could better estimate species phylogeny from gene-tree probabilities (Carstens & Knowles 2007; Knowles & Carstens 2007) in this context of incomplete lineage sorting.

### 6. 1. 3. A time line for diversification

A "relaxed phylogenetics" approach described by Drummond et al. (2006) with calibration nodes situated within the study group for the *rps*4 data was used to explore divergence dates of the major leafy liverwort lineages. This investigated i) when the major lineages in the Lophoziaceae arose, ii) testing whether the main diversifications of the Jungermanniidae were in 'co-evolution' with the evolution of angiosperms and the establishment of tropical rainforest ecosystems, and iii) establishing possible paleo-ecological correlates with observed radiations.

The results suggest that Jungermanniidae split from other liverworts and subsequently diversified after the mid-Permian (ca. 273 mya). The major leafy liverwort lineages mostly emerged by the end of the Cretaceous. Lineage-Through-Time (LTT) plots for liverworts were compared with those of angiosperms and ferns. The plots does not reject the notion that Jungermanniidae diversified in 'co-evolution' with the development of angiosperm habitats such as the establishment of tropical rainforests in the Cretaceous period. However, the correlation is less clear for the diversification of liverworts following angiosperms as between angiosperms, ferns, lycopods and horsetails possibly because liverworts are found in a wider range of habitats when compared to these taxa. In addition a possible leafy liverwort radiation after the Cretaceous-Tertiary boundary was identified.

At the same time, Lophoziaceae-Scapaniaceae-Cephaloziellaceae lineages appear to have not been significantly affected by events following the Cretaceous-Tertiary boundary. A reason suggested for this is that these taxa in general depend less on complex, multilayered forest habitats with many taxa favouring non-forest habitats in cool to cold areas. An increased rate of diversification of these taxa in the mid-Tertiary is possibly related to cooling climates with increasingly pronounced seasonal changes. In particular recent events in the Pleistocene such as major glaciations appear to have favoured the diversification of leafy liverworts.

An extremely slender fossil record as well as uncertainty in identifying fossilized taxa makes calibration hard for liverworts. Further studies should include denser sampling in conjunction with a detailed morphological study of taxa in and around those used for calibration. This should ensure that constraints assigned for particular nodes are as realistic as possible, thereby reducing some of the more extreme estimation error. Also useful would be an analysis of existing data for angiosperms and ferns using the "relaxed phylogenetics" approach described by Drummond et al. (2006) to compare results to liverworts.

### 6. 1. 4. A reclassification that reflects phylogenetic relationships

Essentially the culmination of this research is an attempt at a reclassification that is in concordance with phylogenetic relationships. The ordering of taxa into various hierarchical *ranks* such that each rank is nested within the higher rank: species within genera within families within orders etc, is suggested to be inadequate. Currently the most utilised alternative to traditional classification schemes is the PhyloCode, which essentially defines taxon names not by diagnostic characters but by reference to the ancestor without the fixing of taxonomic ranks.

Alternative changes to the classification under rank-based codes as well implementing a phylogenetic classification was briefly explored. Examining alternative treatments, it became clear that in rank based classifications, differences between 'generic' and 'familial' concepts amount to little more than personal judgement. The phylogenetic classification bypassed these issues by removing the ranked categories and in a simple manner, without extravagance or embellishment, allows for the naming of clades. It seemed evident that in this application, the PhyloCode system has the potential to be more stable with less input of personal judgement, and will prevent a proliferation of categories. Where support is weak for specific clades, I took the stance that these need not be formally named, yet they can still be referred to within the context of other established clades until further data becomes available.

The ability to understand the diversity of taxa is crucial in biodiversity conservation as well as in understanding responses to environmental changes such as global warming. It is hoped that this classification will help in future systematic studies and ensure a realistic viewpoint of the Lophoziaceae-Scapaniaceae-Cephaloziellaceae clade, especially in the context of conservation and global climate change.

## 6. 2. Agenda for further research

### 6. 2. 1. The morphological gap

A major research component lacking in this thesis is an in depth study of the morphology of leafy liverworts taxa. To do this justice, firstly the construction of a comprehensive morphological character matrix is required: this is very time consuming. Initially I started doing this by simply using taxonomic treatments in the literature. However, I found this to be grossly insufficient as a source of data, since these treatments have a main purpose of identifying diagnostic characters. This either resulted in incomplete coding or a lack of detail required in the primary homology assessment process. Rather, I suggest that characters need to be examined from individual exemplars and coded very carefully (unfortunately a study beyond the time scale of this thesis). The overall purpose would be to examine the evolution of morphological characters in Lophoziaceae in the context of a total evidence phylogeny.

Particularly insightful would be exploration of characters that are often used to differentiate different groups of liverworts. For instance Crandall-Stotler *et al.* (2005), in a paper examining evolutionary trends in simple thalloid liverworts, show considerable homoplasy in their morphological data set. Reconstructing morphological character evolution using a combined analysis topology they found homoplasy even in characters that are traditionally considered diagnostic of hierarchical relationships, such as apical cell geometry, calyptra type and capsule wall thickness (Crandall-Stotler *et al.* 2005). Schuster (2002) discusses the issue of circumscribing higher level taxa in the leafy liverworts, stating that for the most part taxonomists rely on generalisations to separate groups, most of which are transgressed by exceptions.

In addition to identifying homoplasy, Crandall-Stotler *et al.* (2005) use ancestral state reconstructions to show that many established hypotheses of character evolution in liverworts are incorrect. For example, it is often suggested that the ancestral liverwort prototype was not an erect, radially symmetric plant (Crandall-Stotler *et al.* 2005); more likely it was a prostrate, bilaterally symmetric plant with the diagnostic features of a simple thalloid liverwort (Crandall-Stotler *et al.* 2005). Similar study needs to be undertaken with the clades identified in this thesis. This should be secondary, however, to a deeper level of understanding that is required of the supposed homoplasticity of morphological characters in leafy liverworts.

An immediately useful spin-off of this kind of data would be the ability to pick characters more sensibly to diagnose specific clades identified from the molecular analyses. Additionally this data would be very useful in tying clades to synapomorphies and as a result enable a more realistic tying down of dates for calibrations in future dating analyses. A further possibility includes testing for "adaptiveness" by looking at speciation rates in clades characterised by particular states as opposed to their sister taxa.

### 6. 2. 2. Cephaloziaceae and Cephaloziellaceae

The Cephaloziaceae and Cephaloziellaceae are two groups that both show reductions morphologically. Cephaloziaceae includes many taxa - the few sampled in this study forming a strongly supported clade sister to the main clade containing Lophoziaceae, Scapaniaceae and the sampled Cephaloziellaceae. It would be interesting to see how well these groups are supported together with additional exemplars. Similarly Cephaloziellaceae needs further sampling specifically when deciding on issues such as inclusion in Scapaniaceae, a currently well supported family.

Reduced taxa are interesting and these taxa are good candidates for investigating the associated biology of these liverworts. Especially significant is the question of what selects for these reductions in size. Reduced epiphyllous taxa such as the Lejuneaceae could be used for comparison with these predominantly terrestrial plants growing on a variety of substrates. Diversification rates and potential radiations in this context could also be explored.

### 6. 2. 3. More on dating and diversification

By examining individual clades, their sizes and rates of diversification, one could determine whether there are any departures from the clade size 'expectations' of random trees or, more interestingly, apparent departures from clock-like behaviour. Using this one could start to account for possible differences between clades to account for possible departures in clade size and diversification rate associated with taxa. For instance, one could examine the ecological contexts of clades associated with habitats like bogs and taiga in the Northern Hemisphere. Other drivers for differences in diversification rates could be explored including morphology, reproductive characters and life history. For example, one could examine the effects of morphological reduction (e.g. Cephaloziaceae

and Cephaloziellaceae), the reliance on asexual reproduction or even ecological nice specificity on diversification rate.

In the broader context it would be interesting to explore this because the levels of diversity in certain clades could be linked with specific characteristics in specific habitats. In this sense one could explore diversification rates of broader biogeographical patterns for instance north versus south splits. Using this example, on a very broad scale certain habitats might be inherently more common in the north versus south which could result in different diversification patterns within the same lineages or affect diversification in taxa sharing the same traits (e.g. branching, morphological reductions and rhizoids) located in different regions. A generalised global approach however would need to be augmented with specific habitat parameters, examining niche coverage by specific lineages. This would essentially enable one to get to grips with perceived morphological plasticity in the context of evolutionary history and habitat. This would also enable one to relate this perceived plasticity to its effect on diversification rates in specific lineages.

This chapter has highlighted many findings regarding the phylogenetic relationships and evolutionary history of the Lophoziaceae. A large, morphologically complex group, the variation over various spatial scales and the morphology reveals fascinating opportunities to further investigate and explore these organisms.

# References

Ahonen, I. 2004. Molecular phylogeny of the liverwort order Porellales (Marchantiophyta, Jungermanniopsida). *Syst. Bot. Missouri Bot. Gard*. 98: 168—188.

Baker, R.H., De Salle, R. 1997. Multiple sources of character information and the phylogeny of Hawaiian Drosophilids. *Syst. Biol*. 46: 654—673.

Baldwin, B.G. 1992. Phylogenetic utility of the Internal Transcribed Spacers of nuclear ribosomal DNA in plants: An example from the Compositae. *Mol. Phylogenet. Evol.* 1: 3—16.

Behrensmeyer, A.K. 1992. *Terrestrial Ecosystems Through Time: Evolutionary Paleoecology of Terrestrial Plants and Animals*. University of Chicago Press. Chicago

Bell, C.D., Soltis, D.E., & Soltis, P.S. 2005. The age of the angiosperms: A molecular timescale without a clock. *Evolution*. 59: 1245—1258.

Benton, M. J. 2000. Stems, nodes, crown clades, and rank-free lists: is Linnaeus dead? *Biological Reviews*. 75: 633—648.

Bergsten, J. 2005. A review of long-branch attraction. *Cladistics*. 21: 163—193.

Britten, R.J. 1986. Rates of DNA sequence evolution differ between taxonomic groups. *Science*. 231: 1393—1398.

Britton T., Oxelman, B., Vinnersten, A. & Bremer, K. 2002. Phylogenetic dating with confidence intervals using mean path lengths. *Mol. Phylogenet. Evol*. 24: 58—65.

Bromham, L., Rambaut, A., Fortey, R., Cooper, A. & Penny, D. 1998. Testing the Cambrian explosion hypothesis by using a molecular dating technique. *Proc. Natl. Acad. Sci. USA*. 95: 12386—12389.

Bromham, L. & Penny, D. 2003. The modern molecular clock. *Nat. Rev. Genet*. 4: 216—224.

Bromham, L., Penny, D., Rambaut, A. & Hendy, M.D. 2000. The power of relative rates tests depends on the data. *J. Mol. Evol.* 50: 296—301.

Brummitt, R.K. 2002. How to chop up a tree. *Taxon*. 51: 31—41.

Cantino, P.D. & de Queiroz, K. 2006. *International Code of Phylogenetic Nomenclature.* Version 3a. URL: www.ohiou.edu/phylocode (accessed 06/2007).

Capesius, I. & Blocher, R. unpublished. A molecular approach to bryophyte systematics. Botanisches Institut, Universitaet Heidelberg, Im Neuenheimer Feld 360, 69120 Heidelberg.

Capesius, I. & Bopp, M. 1997. New classification of liverworts based on molecular and morphological data. *Plant Syst. Evol*. 207: 87—97.

Capesius, I. & Stech, M. 1997. Molecular relationships within mosses based on 18S rRNA sequences. *Nova Hedwigia* 64: 525—533.

Carstens, B.C. & Knowles, L.L. 2007. Estimating Species Phylogeny from Gene-Tree Probabilities Despite Incomplete Lineage Sorting: An Example from Melanoplus Grasshoppers. *Syst. Biol.* 56: 400—411.

Carpenter, J. M. 2003. Critique of pure folly. *Bot. Rev.* 69: 79—92.

Chase, M.W., Cowan, R.S., Hollingsworth, P.M., van den Berg, C., Madrinan, S., Petersen, G., Seberg, O., Jorgsensen, T., Cameron, K.M., Carine, M., Pedersen, N., Hedderson, T.A.J., Conrad, F., Salazar, G.A., Richardson, J.E., Hollingsworth, M.L., Barraclough, T.G., Kelly, L., Wilkinson, M. 2007. A proposal for a standardised protocol to barcode all land plants. *Taxon*. 56: 295—299.

Conti, E., Rutschmann, F., Eriksson, T., Sytsma, K.J. & Baum, D.A. 2004. Callibration of molecular clocks and the biogeographic history of Crypteroniaceae: a reply to Moyle. *Evolution*. 58: 1874—1876.

Cooper, A. & Penny, D. 1997. Mass survival of birds across the Cretaceous–Tertiary boundary: Molecular evidence. *Science.* 275: 1109—1113.

Cox, C. J. & Hedderson, T.A.J. 1999. Phylogenetic relationships among the ciliate arthrodontous mosses: evidence from chloroplast and nuclear DNA sequences. *Pl. Syst. Evol.* 215: 119—139.

Crandall-Stotler, B. & Stotler, R.E. 2000. Morphology and classification of the Marchantiophyta. Pp. 21—70. In: Shaw, A.J. & Goffinet, B. (Eds.). *Bryophyte Biology*. Cambridge University Press. Cambridge.

Crandall-Stotler, B.J., Forrest, L.L. & Stotler, R.E. 2005. Evolutionary trends in the simple thalloid liverworts (Marchantiophyta, Jungermanniopsida subclass Metzgeriidae). *Taxon*. 54: 299—316.

Crane, P.R., Friis, E.M. & Pedersen, K.R. 1995. The origin and early diversification of angiosperms. *Nature*. 374: 27—33.

Cronberg, N., Natcheva, R. & Hedlund, K. 2006. Microarthropods mediate sperm transfer in mosses. *Science.* 313: 1255.

Cronquist, A. 1987. A botanical critique of cladism. *Bot. Rev*. 53: 1—52.

Cutler, D.J. 2000. Estimating divergence times in the presence of an overdispersed molecular clock. *Mol. Biol. Evol*. 17: 1647—1660.

Davis, C.E. 2004. A molecular phylogeny of leafy liverworts (Jungermanniidae: Marchantiophyta). *Syst. Bot. Missouri Bot. Gard.* 98: 61—86.

Darwin, C.R. 1859. *On the Origin of Species*. John Murray. London.

Damsholt, K. 2002. *Illustrated Flora of Nordic Liverworts and Hornworts.* Nordic Bryological Society. Lund.

de Jussieu, A.L. 1789. *Genera Plantarum Secundum Ordines Naturales Disposita*. Paris.

de Pinna, M.C.C. 1991. Concepts and tests of homology in the cladistic paradigm. *Cladistics.* 7: 367—394.

de Queiroz, K. & Gauthier, J. 1992. Phylogenetic Taxonomy. *Annu. Rev. Ecol. Syst.* 23: 449—480.

de Queiroz, K. 1993. For Consensus (Sometimes). *Syst. Biol*. 42: 368—372.

de Roo, R.T., Hedderson, T.A. & Söderström, L. 2007. Molecular insights into the phylogeny of the leafy liverwort family Lophoziaceae Cavers. *Taxon*. 56: 301—314.

Des Marais, D.L., Smith, A.R., Britton, D.M., & Pryer, K.M. 2003. Phylogenetic relationships and evolution of extant horsetails, *Equisetum*, based on chloroplast DNA sequence data (*rbcL* and *trnL-F*). *Int. J. Plant Sci.* 164: 737—751.

Doolittle, R.F., Feng, D.F., Tsang, S., Chao, G. & Little, E. 1996. Determining divergence times of the major kingdoms of living organisms with a protein clock. *Science.* 271: 470—477.

Doyle, J.J. & Davis, J.I. 1998. Homology in Molecular Phylogenetics: A Parsimony Perspective. In: Soltis, D.E., Soltis, P.S. & Doyle, J.J. (Eds.) *Molecular Systematics of Plants II: DNA Sequencing*. Kluwer Academic Publishers. Massachusetts.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. 2006. Relaxed Phylogenetics and Dating with Confidence. *PLoS Biol*. 4: 699—710.

Drummond, A.J., Ho, S.Y.W., Rawlence, N., & Rambaut, A. 2007. A Rough Guide to BEAST 1.4. URL: http://beast-mcmc.googlecode.com/files/BEAST14_Manual_6July2007.pdf

Duff, R.J., Nickrent, D.L. 1999. Phylogenetic relationships of land plants using mitochondrial small-subunit rDNA sequences. *Am. J. Bot.* 86: 372—386.

Dumortier, B.C. 1922. *Commentationes Botanicae*. Tournay.

Edwards, K., Johnstone, C. & Thompson, C. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Res.* 19: 1349.

Engel, J.J. & Braggins, J.E. 2005. Are Mylia and Trabacellula (Hepaticae) related? Unsuspected links revealed by cell wall morphology, with the transfer of *Mylia anomala* to a new genus (*Leiomylia* J.J. Engel & Braggins) of Jungermanniaceae. *Taxon.* 54: 665—680.

Evans, A.W. 1939. The classification of the Hepaticae. *Bot. Rev.* 5: 49—96.

Farris, J.S., Kallersjo, M., Kluge, A.G. & Bult, C. 1994. Testing significance of incongruence. *Cladistics*. 10: 315—319.

Felsenstein, J. 1978. Cases in which parsimony or compatability methods wil be positively misleading. *Syst. Zool*. 27: 401—410.

Fiedorow, P., Odrzykoski, I., Szweykowski, J., Szweykowska-Kulińska, Z. 2001. Phylogeny of the European species of the genus *Pellia* (Hepaticae; Metzgeriales) based on the molecular data from nuclear tRNALeu (CAA) intergenic sequences. *Gene*. 262: 309—315.

Foote, M., Hunter, J.P., Janis, C.M. & Sepkoski Jr, J.J. 1999. Evolutionary and preservational constraints on origins of biologic groups: divergence times of eutherian mammals. *Science.* 283: 1310—1314.

Forey, P.L. 2001. The PhyloCode: description and commentary. *Bull. Zool. Nomencl.* 58: 81—96.

Forey, P.L. 2002. PhyloCode - pain, no gain. *Taxon*. 51: 43—54.

Forrest, L. & Crandall-Stotler, B. 2004. A phylogeny of the simple thalloid liverworts (Jungermanniopsida, subclass Metzgeriales) as inferred from the chloroplast genome. In: Goffinet, B., Hollowell, V., Magill, R. (Eds.), Molecular Systematics of Bryophytes. *Monogr. Syst. Bot. Missouri Bot. Gard.* 98: 119—140.

Forrest, L. & Crandall-Stotler, B. 2005. Progress towards a robust phylogeny for the liverworts, with particular focus on the simple thalloids. *J. Hattori Bot. Lab.* 97: 127—159.

Frey, W. & Stech, M. 2005. A morpho-molecular classification of the liverworts (Hepaticophytina, Bryophyta). *Nova Hedwigia.* 81: 55—78.

Friis, E.M., Pederson, K.R., & Crane, P.R. 1999. Early angiosperm diversification: The diversity of pollen associated with angiosperm reproductive structures in Early Cretaceous floras from Portugal. *Ann. Miss. Bot. Gard.* 86: 259—296.

Gaut, B.S. 1998. Molecular clocks and nucleotide substitution rates in higher plants. *Evol. Biol.* 30: 93—120.

Gillespie, J.H. 1991. *The Causes of Molecular Evolution*. Oxford University Press.

Gottsche, C.M., Lindenberg, J.B. & Nees, C.G. 1844-47. *Synopsis Hepaticarum*. Meissner, Hamburg.

Gradstein, S.R. 1993. New Fossil Hepaticae Preserved in Amber of the Dominican-Republic. *Nova Hedwigia*. 57: 353—374.

Graham, L.E., Wilcox, L.W., Cook, M.E. & Gensel, P.G. 2004. Resistant tissues of modern marchantioid liverworts resemble enigmatic Early Paleozoic microfossils. *Proc. Natl. Acad. Sci. USA.* 101: 11025—11029.

Gray, S.F. 1821. *A Natural Arrangement of British Plants*. Vol. 1. London.

Griffiths, G.C.D. 1976. The future of Linnaean nomenclature. *Syst. Zool*. 25: 168—173.

Grolle, R. 1968. Lebermoose aus Neuguinea. 7.Vierte Fundliste. *J. Hattori Bot. Lab*. 31: 1—12.

Grolle, R. 1971. Jamesoniella und Verwandte. *Feddes Repert*. 82: 1—100.

Grolle, R. 1980. Lebermoose im Bernstein 2. *Feddes Repert*. 91: 401—407.

Grolle, R. 1984. *Bryopteris* und *Cyclolejeunea* fossil in Dominikanischem Bernstein. *J. Hattori Bot. Lab.* 56: 271—280.

Grolle, R. 1985. Zwei weitere Lebermoose in Bernstein aus Bitterfeld (DDR). *Feddes Repert*. 96:41—46.

Grolle, R. 1999. *Metacalypogeia* (Calypogeiaceae, Hepaticae) new to Europe as Baltic amber fossil. *Bryobrothera*. 5: 87—91.

Grolle, R. & Long, D.G. 2000. An annotated check-list of the Hepaticae and Anthocerotae of Europe and Macaronesia. *J. Bryol*. 22: 103—140.

Grolle, R. & Heinrichs, J. 2003. Eocene *Plagiochila groehnii* sp. nov. – the first representative of Plagiochilaceae in Baltic amber. *Cryptog. Bryol*. 24: 289—293.

Grolle, R. & Meister, K. 2004. *Lophozia kutscheri*, a new hepatic (Jungermanniales) in Bitterfeld amber from central Germany. *Bryologist.* 107: 79—81.

Grolle, R. & Schmidt, A. 2001. A fossil *Scapania* (Hepaticae) with perianth and Capsule in Bitterfeld Amber (Eocene) from Germany. *Bryologist.* 104: 362—366.

Groth, H. & Heinrichs, J. 2005. Maximum likelihood analyses of chloroplast gene rbcL sequences indicate relationships of *Syzygiella* (Jungermanniopsida) with Lophoziaceae rather than Plagiochilaceae. *Cryptogam. Bryol*. 26: 49—57.

Groth-Malonek, M., Knoop, V. 2005. Bryophytes and other basal land plants: the mitochondrial perspective. *Taxon*. 54: 293—297.

Hartmann, F.A. Wilson, R., Gradstein, S.R., Schneider, H., Heinrichs, J. 2006. Testing hypotheses on species delimitations and disjunctions in the liverwort Bryopteris (Jungermanniopsida: Lejeuneaceae*). Int. J. Pl. Sci*. 167: 1205—1214.

Hasegawa, M. & Kishino, H. 1989. Confidence limits on the maximum-likelihood estimate of the homonid tree from mitochondrial-DNA sequences. *Evolution*. 43: 672—677.

Hendy, M.D. & Penny, D. 1989. A framework for the quantitative study of evolutionary trees. *Syst. Zool*. 38: 297—309.

Heads, M. 2005. Dating nodes on molecular phylogenies: a critique of molecular biogeography. *Cladistics.* 21: 62—78.

Hedderson, T.A., Chapman, R.L. & Rootes, W.L. 1996. Phylogenetic relationships of bryophytes inferred from nuclear-encoded rRNA gene sequences. *Plant Syst. Evol.* 200: 213—224.

Hedderson, T.A., Chapman, R.L. & Cox, C.J. 1998. *Bryophytes and the Origins and Diversification of Land Plants: New Evidence from Molecules*. Pp. 65—77. In: Bates, J.W., Ashton, N.W. & Duckett, J.G. (Eds.). *Bryology for the twenty-first century*. Leeds: Maney.

Hedderson, T.A., Murray, D.J., Cox, C.J. & Nowell, T.L. 2004. Phylogenetic relationships of haplolepideous mosses (Dicranidae) inferred from rps4 gene sequences. *Syst. Bot*. 29: 29—41.

Hedderson, T.A., Nowell, T.L. 2006. Phylogeography of Homalothecium sericeum (Hedw.) Br. Eur.; toward a reconstruction of glacial survival and postglacial migration. *J. Bryol*. 28: 283—292.

Hedges, S.B. & Kumar, S. 2004. Precision of molecular time estimates. *Trends Genet.* 20: 242—247.

Hedwig, J. 1784. *Theoria Generationis et Fructificationis Plantarum Cryptogamicarum*. Petropoli.

Heinrichs, J., Gradstein, S.R., Wilson, R. & Schneider, H. 2005. Towards a natural classification of liverworts (Marchantiophyta) based on the chloroplast gene rbcL. *Cryptogam. Bryol*. 26: 131—150.

Heinrichs, J. Hentschel, J., Wilson, R., Feldberg, K. & Schneider, H. 2007. Evolution of leafy liverworts (Jungermanniidae, Marchantiophyta): estimating divergence times from chloroplast DNA sequences using penalized likelihood with integrated fossil evidence. *Taxon*. 56: 31—44.

Heinrichs, J., Lindner, M., Groth, H., Hentshel, J., Feldberg, K., Renker, C., Engel, J.J., von Konrat, M., Long, D.G., Schneider, H. 2006. Goodbye or welcome Gondwana? – insights into the phylogenetic biogeography of the leafy liverwort *Plagiochila* with a description of *Proskauera*, gen. Nov. (Plagiochilaceae, Jungermanniales) *Pl. Syst. Evol.* 258: 227—250.

Hennig, W. 1966. *Phylogenetic Systematics*. Davis, D.D. & Zangerl, R. trans. University of Illinois Press. Urbana.

Hentsche1, J., Wilson, R., Burghardt, M., Zündorf, H.-J., Schneider, H. & Heinrichs, J. 2006. Reinstatement of Lophocoleaceae (Jungermanniopsida) based on chloroplast gene rbcL data: exploring the importance of female involucres for the systematics of Jungermanniales. *Pl. Syst. Evol*. 258: 211—226.

He-Nygrén, X., Ahonen, I., Juslén, A., Glenny, D. & Piippo, S. 2004. Phylogeny of liverworts beyond a leaf and a thallus. Molecular Systematics of Bryophytes. *Syst. Bot. Missouri Bot. Gard.* 98: 87—118.

He-Nygrén, X., Juslén, A., Ahonen, I., Glenny, D. & Piippo, S. 2006. Illuminating the evolutionary history of liverworts (Marchantiophyta) - towards a natural classification. *Cladistics*. 22: 1—31.

Holdregger, R., Abbot, R.J. 2003. Phylogeography of the arctic-alpine Saxifraga oppositifolia (Saxifragaceae) and some related taxa based on cpDNA and ITS sequence variation. *Am. J. Bot.* 90: 931—936.

Hudson, R.R. & Coyne, J.A. 2002. Mathematical consequences of the genealogical species concept. *Evolution*. 56: 1557—1565.

Hueber, F.M. 1961, *Hepaticites devonicus*, a new fossil liverwort from the Devonian of New York. *Ann. Miss. Bot. Gard.* 48: 125—132.

Huelsenbeck, J.P., Bull, J.J. & Cunningham, C.W. 1996. Combining data in phylogenetic analysis. *Trends Ecol. Evol.* 11: 152—158.

Huelsenbeck, J.P. Larget, B. & Swofford, D. 2000. A compound Poisson process for relaxing the molecular clock. *Genetics.* 154: 1879—1892.

Huelsenbeck, J.P. & Ronquist, F. 2003. *MrBayes*. Version 3.0B. URL: http://morphbank.ebc.uu.se/mrbayes3/info.php.

Huelsenbeck, J.P., Ronquist, F., Nielsen, R. & Bollback, J.P. 2001. Bayesian Inference of Phylogeny and Its Impact on Evolutionary Biology. *Science.* 294: 2310—2314.

Inoue, H. 1966. A monograph of the genus Syzygiella Spruce. *J. Hattori Bot. Lab*. 29: 171—213.

IturraldeVinent, M.A., MacPhee, R.D.E. 1996. Age and paleogeographical origin of Dominican amber. *Science.* 273: 1850—1852.

Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F. & Donoghue, M.J. 2002. *Plant Systematics: A Phylogenetic Approach*. Second Edition. Sunderland: Sinauer Associates, Inc.

Kato, Y., Aioi, K., Omori, Y., Takahata, N. & Satta, Y. 2003. Phylogenetic analyses of Zostera species based on rbcL and matK nucleotide sequences: Implications for the origin and diversification of seagrasses in Japanese waters. *Genes Genet. Syst*. 78: 329—342.

Keller, R.A., Boyd, R.N. & Wheeler, Q.D. 2003. The illogical basis of Phylogenetic Nomenclature. *Bot. Rev*. 69: 93—110

Kenrick, P. & Crane, P.R. (1997). *The Origin and Early Diversification of Land Plants. A Cladistic Study*. Smithstonian Institution Press. Washington.

Kitching, I.J., Forey, P.L., Humphries, C.J. & Williams, D.M. 1998. *Cladistics: The Theory and Practice of Parsimony Analysis* (Second Edition). Oxford University Press. New York.

Kishino, H., Thorne, J.L. & Bruno, W.J. 2001. Performance of a divergence time estimation method under a probabilistic model of rate evolution. *Mol. Biol. Evol.* 18: 352—361.

Kluge, A.G., 1989. A concern for evidence and a phylogenetic hypothesis of relationships among Epicrates (Boidae, Serpentes). *Syst. Zool*. 38: 7—25.

Knowles, L.L. & Carstens, B.C. 2007. Delimiting Species without Monophyletic Gene Trees. *Syst. Biol*. 56: 887—895.

Kojima, J.L. 2003. Apomorphy-based definition also pinpoints a node, and the PhyloCode names prevent effective communication. *Bot. Rev.* 69: 44—58.

Kropf, M., Kadereit, J.W. & Comes, H.P. 2002. Late Quaternary distributional stasis of the submediterranean mountain plant *Anthyllis montana* L. (Fabaceae) inferred from ITS sequences and amplified fragment length polymorphism markers. *Mol. Ecol*. 11: 447—463.

Langer, M.C. 2001. Linnaeus and the PhyloCode: where are the differences? *Taxon*. 504: 1091—1096.

Langley, C.L. & Fitch, W.M. 1974. An examination of the constancy of the rate of molecular evolution. *J. Mol. Evol.* 3:161—177.

Lee, M.S.Y. 1999. Molecular clock calibrations and metazoan divergence dates. *J. Mol. Evol*. 49: 385—391.

Leitgeb, H. (1874-1881). *Untersuchungen über die Lebermoose*. I-VI. Jena.

Lewis, P.O. 1998. Maximum Likelihood as an Alternative to Parsimony for Inferring Phylogeny Using Nucleotide Sequence Data. In: Soltis, D.E., Soltis, P.S., Doyle, J.J. (Eds.). *Molecular Systematics of Pants II: DNA Sequencing.* Kluwer Academic Publishers. Massachusetts.

Lewis, L.A., Mishler, B.D. & Vilgalys, R. 1997. Phylogenetic relationships of the liverworts (Hepaticae), a basal embryophyte lineage, inferred from nucleotide sequence data of the chloroplast gene rbcL. *Mol. Phylogenet. Evol*. 7: 377—393.

Linder, H.P., Hardy, C.R. & Rutschmann, F. 2005. Taxon sampling effects in molecular clock dating: An example from the African Restionaceae. *Mol. Phylogenet. Evol.* 35: 569—582.

Linnaeus, C. 1753. *Species Plantarum* 2 vol. Holmiae.

Liston, A., Wheeler, J.A. 1994. The phylogenetic position of the genus Astragalus (Fabaceae) – evidence from the chloroplast genes rpoC1 and rpoC2. *Biochem. Syst. Ecol.*22: 377—388.

Lupia, R., Crane, P.R. & Lidgard, S. 2000. Angiosperm diversification and Cretaceous environmental change. In: Culver, S.J. & Rawson, P.F. (Eds.). *Biotic Responses to Global Change: the Last 145 Million Years*. Cambridge University Press. Cambridge.

Magallón, S.A. 2004. Dating lineages: molecular and paleontological approaches to the temporal framework of clades. *Int. J. Plant Sci*. 165: 7—21.

Magallón, S.A. & Sanderson, M. 2005. Angiosperm divergence times: The effect of genes, codon positions and time constraints. *Evolution.* 59: 1653—1670.

McNeill, J. 2000. Naming the groups: developing a stable and efficient nomenclature. *Taxon*. 49: 705—720.

Mishler, B.D. 1999. Getting Rid of Species. Pp: 307—315. In: Wilson, R.A. (Ed.). *Species: New Interdisciplinary Essays*. MIT Press, Cambridge, Massachusetts.

Moritz, C. & Hillis, D.M. 1996. Molecular Systematics: Context and Controversies. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.). *Molecular Systematics Second Edition*. Sinauer Associates. Massachusetts.

Morley, R.J. 2000. *Origin and Evolution of Tropical Rainforests*. John Wiley & Sons. Chichester.

Nadot, S., Bajon, R. & Lejeune, B. 1994. The chloroplast gene rps4, as a tool for the study of Poaceae phylogeny. *Pl. Syst. Evol*. 191: 27—38.

Nadot, S., Bittar, G., Carter, L., Lacroix, R. & Lejeune, B. 1995. A phylogenetic analysis of monocotyledons based on the chloroplast gene rps4, using parsimony and a new numerical phenetics method. *Mol. Phylogenet. Evol*. 4: 257—282.

Near, T.J., Meylan, P.A. & Shaffer, H.B. 2005. Assessing concordance of fossil calibration points in molecular clock studies: an example using turtles. *Am. Nat.* 165: 137—146.

Near, T.J. & Sanderson, M.J. 2004. Assessing the quality of molecular divergence time estimates by fossil calibrations and fossil-based model selection. *Philos. Trans. R. Soc. Lond. Ser. B-Biol. Sci.* 359: 1477—1483.

Nee, S., Holmes, E.C. May, R.M. & Harvey, P.H. 1994. Extinction rates can be estimated from molecular phylogenies. *Proc. R. Soc. B.* 344: 77—82.

Newton, AE., Cox, C.J., Duckett, J.D., Wheeler, J.A., Goffinet, B., Hedderson, T.A.J. & Mishler, B.D. 2000. Evolution of the major moss lineages: Phylogenetic analyses based on multiple gene sequences and morphology. *Bryologist*. 103: 187—211.

Newton, A.E., Wikström, N., Bell, N., Lowe Forrest & L., Ignatov, M.S. 2007. Dating the Diversification of the Pleurocarpous Mosses. In: Newton, A.E. & Tangney, R.S. (Eds.). *Pleurocarpous Mosses: Systematics and Evolution*. The Systematic Association Special Volume Series 71. CRS Press. Boca Raton.

Nixon, K.C. & Carpenter, J.M. 2000. On the other 'phylogenetic systematics'. *Cladistics*. 16: 298—318.

Nixon, K.C. 2003. The phylocode and rankles nomenclature: Fatally flawed. *Cladistics*. 19: 158—159.

Olmstead, R.G. & Palmer, J.D. 1994. Chloroplast DNA systematics: a review of methods and data analysis. *Am. J. Bot*. 81: 1205—1224.

Olsen, J.L., Stam, W.T., Coyer, J.A., Reusch, T.B.H., Billingham, M., Boström, C., Calvert, E., Christie, H., Granger, S., La Lumière, R., Milchakova, N., Oudot-Le Sec, M., Procaccini, G., Sanjabi, B., Serraõ, E., Veldsink, J., Widdicombe, S., Wylie-Echeverria, S. 2004. North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina* L. *Mol. Ecol.*. 13: 1923—1941.

Pacak A. & Szweykowska-Kulińska, Z. 2003. Organellar inheritance in liverworts: an example of *Pellia borealis*. *J. Molec. Evol.* 56: 11—17.

Paton, J.A. 1999. *The Liverwort Flora of the British Isles*. Harley Books. Colchester.

Pavlinov, I. Y. 2004. Foundations of the new phylogenetics. *Zhurnal Obshchei Biologii.* 65: 334—366.

Pickett, K. M. 2005. The new and improved PhyloCode, now with types, ranks, and even polyphyly: a conference report from the First International Phylogenetic Nomenclature Meeting. *Cladistics*. 21: 79—82.

Posada, D. & Crandall, K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics*. 14: 817—818.

Potemkin, A. D. 1999. Circumscription of the family Scapaniaceae, with segregation of the new family Diplophyllaceae (Hepaticae). *Ann. Bot. Fenn*. 36: 271—283.

Pulquério, M.J.F. & Nichols, R.A. 2006. Dates from the molecular clock: how wrong can we be? *Trends Ecol. Evol*. 22: 180—184.

Pybus, O.G. & Harvey, P.H. 2000. Testing macro-evolutionary models using incomplete molecular phylogenies. *Proc. R. Soc. B.* 267: 2267—2272.

Raddi, G. 1818. Jungermanniografia Etrusca. Reprinted in 1820. *Mem. Soc. Ital. Sci. Moderna*. Vol. 18.

Rambaut, A. & Bromham, L. 1998. Estimating divergence dates from molecular sequences. *Mol. Biol. Evol.* 15: 442—448.

Rambaut, A., Drummond, A.J. 2007. Tracer v1.4. URL: http://beast.bio.ed.ac.uk/Tracer

Ritzkowski, S. 1997. K-Ar – Altersbestimmung der bernsteinführenden Sedimente des Samlandes (Pälaogen, Bezirk Kalingrad). *Metalla*. 66: 19—23.

Rosenberg, N.A. 2003. The shapes of neutral gene genealogies in two species: probabilities of monophyly, paraphyly and polyphyly in a coalescent model. *Evolution.* 57: 1465—1477.

Rutschmann F. 2005. *Bayesian molecular dating using PAML/multidivtime*. A step-by-step manual. University of Zurich, Switzerland. URL: http://www.plant.ch

Rutschmann, F. 2006. Molecular dating of phylogenetic trees: A brief review of current methods that estimate divergence times. *Diversity Distrib.* 12: 35—48.

Sanderson, M.J. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. *Mol. Biol. Evol.* 14: 1218—1231.

Sanderson, M.J. (2002). Estimating absolute rates of molecular evolution and divergence times: A penalized likelihood approach. *Mol. Biol. Evol.* 19: 101—109.

Sanderson, M.J. (2004). r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. *Bioinformatics*. 19: 301—302.

Sanderson, M.J & Shaffer, H.B. 2002. Troubleshooting molecular phylogenetic analyses. *Annu. Rev. Ecol. Syst*. 33: 49—72.

Sanderson, M.J., Thorne, J.L., Wikstrom, N. & Bremer, K. 2004. Molecular evidence on plant divergence times. *Am. J. Bot*. 91: 1656—1665.

Schiffner, V. 1893-1895. Hepaticae. In: Engler, H.G.A. & Prantl, K. (Eds.). *Nat. Pflanzenfam*. 1: 3—141.

Schill, D.B., Long, D.G., Moeller M. & Squirrell, J. 2004. Phylogenetic relationships between Lophoziaceae and Scapaniaceae based on chloroplast sequences. *Syst. Bot. Missouri Bot. Gard.* 98: 141—149.

Schuh, R.T. 2003. The Linnaean system and its 250-year persistence. *Bot. Rev*. 69: 59—78.

Schuster, R.M. 1961. Studies on Hepaticae. III.-VI. *Bryologist*. 64: 198—208.

Schneider, H., Schuettpelz, E., Pryer, K.M., Cranfill, R., Magallón, S. & Lupia, R. 2004. Ferns diversified in the shadow of angiosperms. *Nature*. 428: 553—557.

Schuster, R.M. 1966. *The Hepaticae and Anthocerotae of North America. Volume 1*. Columbia University Press. New York and London.

Schuster, R.M. 1969. *The Hepaticae and Anthocerotae of North America – east of the hundredth meridian. Vol. 2*. Columbia University Press. New York.

Schuster, R.M. 1971. Studies on Cephaloziellaceae. *Nova Hedwigia*. 22: 121—165.

Schuster, R.M. 1974. *The Hepaticae and Anthocerotae of North America – east of the hundredth meridian. Vol. 3*. Koeltz Scientific Books. Koenigstein.

Schuster, R.M. 1984. Morphology, phylogeny and classification of the Hepaticae. Pp. 892—1070. In: Schuster R. M. (Ed.). *New Manual of Bryology, Vol. 2*. The Hattori Botanical Laboratory, Nichinan.

Schuster, R.M. 1999. Studies on Jungermanniidae. IV. On Scapaniaceae, Blepharidophyllaceae and Delavayellaceae. *J. Bryol.* 21: 123—132.

Schuster, R.M. 2002a. Austral Hepaticae: Part II. *Nova Hedwigia Beih*. 119: 1—606.

Schuster, R.M. 2002b. Revisionary studies of the Chandonanthoideae (Jungermanniales, Jungermanniaceae). *Nova Hedwigia.* 74: 465—496.

Shaul, S. & Graur, D. 2002. Playing chicken (*Gallus gallus*): methodological inconsistencies of molecular divergence date estimates due to secondary calibration points. *Gene*. 300: 59—61.

Shaw, A.J. 2001. Biogeographic patterns and cryptic speciation in bryophytes. *J. Biogeogr*. 28: 253—261.

Shaw, A.J. Cox, C.J., Goffinet, B., Buck, W.R. & Boles, S.B. 2003. Phylogenetic evidence of a rapid radiation of pleurocarpous mosses (Bryophyta). *Evolution*. 57: 2226—2241.

Shields, R. 2004. Pushing the envelope on molecular dating. *Trends Genet*. 20: 221—222.

Simmons, M.P. 2004. Independence of alignment and tree search. *Mol. Phylogenet. Evol.* 31: 784—879.

Söderström, L. unpublished. A current classification of Hepaticophyta (2003).

Soltis, D.E. & Soltis, P.S. 1998. Choosing an Approach and an Appropriate Gene for Phylogenetic Analysis. In: Soltis, D.E., Soltis, P.S., Doyle, J.J. (Eds.). *Molecular Systematics of Pants II: DNA Sequencing*. Kluwer Academic Publishers. Massachusetts.

Soltis, P.S.. & Soltis, D.E., Savolainen, V., Crane, P.R. & Barraclough, T.G. 2002. Rate heterogeneity among lineages of tracheophytes: Integration of molecular and fossil data and evidence from molecular living fossils. *Proc. Natl. Acad. Sci. USA*. 99: 4430—4435.

Stech, M. 1999. A reclassification of the Dicranaceae (Bryopsida) based on non-coding cpDNA sequence data. *J. Hattori Bot. Lab.* 86: 137—159.

Stevenson, D. 2004. Nomenclatural use and abuse: comments on the PhyloCode. *Cladistics*. 20: 606.

Stewart, W.N. & Rothwell, G.W. 1993. *Paleobotany and the Evolution of Plants*. Second Edition. Cambridge University Press.

Swofford, D.L. 1998. *PAUP*. Phylogenetic Analysis Using Parsimony (and Other Methods).* Version 4. Sunderland: Sinauer.

Szweykowski, J. & Krzakowa, M. 1979. Variation of four enzyme systems in Polish populations of *Conocephalum conicum* (L.) Dum. (Hepaticae, Marchantiales). *Bull. Acad. Pol. Sci. Biol.* 27: 37—41.

Szweykowski, J., Zieliński, R., Odrzykoski, I., Buczkowska, K. 1995. Geographic distribution of *Pellia* ssp. (Hepaticaea, Metzgeriales) in Poland based on electrophoretic identification. *Acta Soc. Bot. Pol.* 64: 59—70.

Takahata, N. 1987. On the overdispersed molecular clock. *Genetics*. 116: 169—179.

Takezaki, N., Rzhetsky, A. & Nei, M. 1995. Phylogenetic test of the molecular clock and linearized trees. *Mol. Biol. Evol*. 12: 823—833.

Taylor, W.A. 1995. Spores in earliest land plants. *Nature.* 373: 391—392.

Templeton, A.R. 1998. Nested clade analyses of phylgeographic data: testing hypotheses about gene flow and population history. *Mol. Ecol*. 7: 381—397.

Templeton, A.R. 2001. Using phylogeographic analyses of gene trees to test species status and processes. *Mol. Ecol.* 10: 779—791.

Thorne, J.L. & Kishino, H. 2002. Divergence time and evolutionary rate estimation with multilocus data. *Syst. Biol*. 51: 689—702.

Thorne, J.L., Kishino, H. & Painter, I.S. 1998. Estimating the rate of evolution of the rate of molecular evolution. *Mol. Biol. Evol*. 15: 1647—1657.

Vargas, P. 2003. Molecular evidence for multiple diversification patterns of alpine plants in Mediterranean Europe. *Taxon*. 52: 463—476.

Von Nägeli, C.W. 1845. Wachsthumsgeschichte der Laub- und Lebermoose. 1-4. *Zeit. Wiss. Bot*. 2: 138—150, 3: 150—166.

Wachowiak, W., Baczkiewicz, A., Chudzinska, E. & Buczkowska, K. 2007. Cryptic speciation in liverworts – a case study in the Aneura pinguis complex. *Bot. J. Linnean Soc.* 155: 273—282.

Weitschat, W. 1997. Bitterfelder Bernstein – ein eozäner Bernstein auf miozäner Lagerstätte. *Metalla*. 66: 71—84.

Welch, J.J. & Bromham, L. 2005. Molecular dating when rates vary. *Trends Ecol. Evol*. 20: 320—327.

Wikström, N., Savolainen, V. & Chase, M.W. 2001. Evolution of angiosperms: Calibrating the family tree. *Proc. R. Soc. B.* 268: 2211—2220.

Wikström, N. & Kenrick, P. 2001. Evolution of Lycopodiaceae (Lycopsida): Estimating divergence times from rbcL gene sequences by use of nonparametric rate smoothing. *Mol. Phylogenet. Evol*. 19: 177—186.

Wilson, R., Heinrichs, J., Hentschel, J., Gradstein, S.R., Schneider, H. 2007. Steady diversification of derived liverworts under Tertiary climatic fluctuations. *Biol. Lett.* 3: 566—569.

Wing, S.L., Boucher, L.D. 1998. Ecological aspects of the Cretaceous flowering plant radiation. *Annu. Rev. Earth Planet. Sci.* 26: 379—421.

Wing, S.L., Hickey, L.J. & Swisher, C.C. 1993. Implications of an exceptional fossil flora for Late Cretaceous vegetation. *Nature.* 363: 342—344.

Wendle, J.F. & Doyle, J.J. 1998. Phylogenetic Incongruence: Window into Genome History and Molecular Evolution. In: Soltis, D.E., Soltis, P.S., Doyle, J.J. (Eds.). *Molecular Systematics of Pants II: DNA Sequencing*. Kluwer Academic Publishers. Massachusetts.

Woodger, J.H. 1952. From biology to mathematics. *Brit. J. Phil. Sci*. 3: 1—21.

Wu, C.I. & Li, W.H. 1985. Evidence for higher rates of nucleotide substitutions in rodents than in man. *Proc. Natl. Acad. Sci. U.S.A.* 82: 1741—1745.

Yang, Z. 1997. PAML: a program package for phylogenetic analysis by maximum likelihood. *CABIOS*. 13: 555—556. URL: http://abacus.gene.ucl.ac.uk/software/paml.html.

Yatsentyuk, S.P., Konstantinova, N.A., Ignatov, M.S., Hyvönen, J. & Troitsky, A.V. 2004. On phylogeny of Lophoziaceae and related families (Hepaticae, Jungermanniales), based on trnL-trnF intron-spacer sequences of chloroplast DNA. *Syst. Bot. Missouri Bot. Gard*. 98: 243—260.

Yoder, A.D. & Yang, Z.H. 2000. Estimation of primate speciation dates using local molecular clocks. *Mol. Biol. Evol*. 17: 1081—1090.

Zuckerkandl E. & Pauling, L. 1965. Evolutionary divergence and convergence in proteins. In: Bryson, V., Vogel, H. (Eds.). *Evolving genes and proteins*. pp. 97—166. Academic Press, New York.

# Appendix

*Table 2. 1. Taxa and DNA regions used for this study. All specimens are placed in BOL except where stated. Further information on the specimens can be obtained from the author.*

Acrobolbus wilsonii Nees *UNITED KINGDOM 2001 D. Long & Rothero 29767(E) AM398297 (*rps4*);* Adelanthus decipiens *(Hook.) Mitt. SOUTH AFRICA 2004 L. Söderström 2004/177 AM398307 (*rps4*);* A. lindenbergianus *(Lehm.) Mitt., 1, ARGENTINA 2003 D. Long 31828(E) AM397738 (*trnG*) AM398292 (*rps4*); 2,* AY608042 *(*rps4*);* Anastrepta orcadensis *(Hook.) Schiffn., 1, NORWAY 2003 L. Söderström 2003/017 AM397771 (*trnG*) AM398339 (*rps4*); 2, SPAIN 2004 M. Infante et. al. no accession (*trnG*) no accession (RC24);* Anastrophyllum alpinum *Steph. NEPAL 2001 D. Long 30460(E) AM397754 (*trnG*) AM398320 (*rps4*) no accession (RC24);* A. auritum *(Lehm.) Steph., 1, VENEZUELA 2004 L. Söderström et. al. 2004/028 AM398240 (*rps4*) no accession (ITS) no accession (RC24); 2, VENEZUELA 2004 L. Söderström et. al. 2004/029 AM397703 (*trnG*) AM398243 (*rps4*) no accession (RC24); 3, VENEZUELA 2004 L. Söderström et. al. 2004/065 AM397699 (*trnG*) AM398238 (*rps4*) no accession (ITS) no accession (RC24); 4, VENEZUELA 2004 L. Söderström et. al. 2004/111 AM397714 (*trnG*) AM398255 (*rps4*) no accession (ITS) no accession (RC24); 5, VENEZUELA 2004 L. Söderström et. al. 2004/110 AM397701 (*trnG*) AM398241 (*rps4*) no accession (ITS) no accession (RC24);* Anastrophyllum cf. auritum*, 1, SOUTH AFRICA 2005 R. de Roo 17d (*rps4*,* trn*G); 2, SOUTH AFRICA 2005 R. de Roo 17e (*rps4*,* trn*G);* A. cavifolium *(H.Buch et S.W.Arnell) Lammes NORWAY 2004 A. Séneca & L. Söderström 2004/233 AM397742 (*trnG*) AM398301 (*rps4*) no accession (RC24);* A. donnianum *(Hook.) Steph. UNITED KINGDOM 2000 D. Long 30045(E) AM398319 (*rps4*) no accession (RC24);* A. hellerianum *(Nees ex Lindenb.) R.M.Schust. SWEDEN 2003 L. Söderström & P. Manyanga 2003/081 AM397798 (*trnG*) AM398364 (*rps4*);* A. michauxii *(F. Weber) H. Buch* AY507433 *(*rps4*);* A. minutum *(Schreb.) R.M.Schust., 1, NORWAY 2004 A. Séneca & L. Söderström 2004/224 AM397762 (*trnG*) AM398330 (*rps4*) no accession (ITS) no accession (RC24); 2, NORWAY 2004 L. Söderström et. al. 2004/217 AM398326 (*rps4*) no accession (ITS) no accession (RC24); 3, NORWAY 2004 L. Söderström et. al. 2004/271 AM397764 (*trnG*) AM398331 (*rps4*); 4, NORWAY 2004 L. Söderström et. al. 2004/316 AM397774 (*trnG*) no accession (RC24); 5, NORWAY 2004 L. Söderström et. al. 2004/444 AM397763 (*trnG*) no accession (ITS) no accession (RC24); 6, NORWAY 2004 L. Söderström et. al. 2004/205 AM398284 (*rps4*) no accession (ITS) no accession (RC24); 7, SOUTH AFRICA 2003 T. Hedderson 15437 AM397723 (*trnG*) AM398268 (*rps4*) no accession (RC24); 8, SPAIN 2004 M. Infante et. al. s.n. AM398290 (*rps4*) no accession (RC24); 9, SVALBARD 2004 L. Söderström et. al. 2004/327 AM397761 (*trnG*) AM398327 (*rps4*) no accession (ITS) no accession (RC24); 10, SVALBARD 2004 L. Söderström et. al. 2004/387 AM398329 (*rps4*) no accession (RC24); 11, VENEZUELA 2004 L. Söderström et. al. 2004/135 AM397712 (*trnG*) AM398253 (*rps4*) no accession (ITS) no accession (RC24);* A. minutum *(Schreb.) R.M.Schust. var.* minutum*, 1, NORWAY 2004 L. Söderström et. al. 2004/202 AM398278 (*rps4*) no accession (ITS) no accession (RC24); 2, SWEDEN 2003 L. Söderström & P. Manyanga 2003/054 AM397780 (*trnG*) AM398348 (*rps4*) no accession (ITS) no accession (RC24);* A. minutum *(Schreb.) R.M.Schust. var.* weberi *(Mart.) Kartt., 1, NORWAY 2003 L. Söderström 2003/013 AM397765 (*trnG*) AM398332 (*rps4*); 2, NORWAY 2004 L. Söderström et. al. 2004/204 AM398279 (*rps4*) no accession (RC24);* A. saxicola *(Schrad.) R.M.Schust., 1, FINLAND 2003 L. Söderström & P. Manyanga 2003/099 AM397794 (*trnG*) AM398360 (*rps4*) no accession (ITS) no accession (RC24); 2, NORWAY 2004 L. Söderström et. al. 2004/196 AM398285 (*rps4*) no accession (ITS) no accession (RC24);* A. tubulosum *(Nees) Grolle, 1, VENEZUELA 2004 L. Söderström et. al. 2004/030 AM397697 (*trnG*) AM398237 (*rps4*) no accession (ITS) no accession (RC24); 2, VENEZUELA 2004 L. Söderström et. al. 2004/064 AM397713 (*trnG*) AM398254 (*rps4*) no accession (ITS) no accession (RC24); 3, VENEZUELA 2004 L. Söderström et. al. 2004/066 AM397702 (*trnG*) AM398242 (*rps4*) no accession (RC24); 4, VENEZUELA 2004 L. Söderström et. al. 2004/078 AM398257 (*rps4*) no accession (ITS) no accession (RC24); 5, VENEZUELA 2004 L. Söderström et. al. 2004/120 AM397720 (*trnG*) AM398266 (*rps4*) no accession (ITS) no accession (RC24);* Anthelia julacea *(L.) Dumort.* AY608044 *(*rps4*);* Apomarsupella revoluta *(Nees) R.M.Schust. ICELAND 2004 A. Séneca & L. Söderström 2004/464 AM397759 (*trnG*);* Balantiopsis diplophylla *(Hook. f. & Taylor) Mitt.* AY608047 *(*rps4*);* Barbilophozia atlantica *(Kaal.) Müll.Frib.; 1, NORWAY 2003 L. Söderström 2003/052 AM397781 (*trnG*) AM398349 (*rps4*) no accession (RC24); 2, SWEDEN 2003 L. Söderström & P. Manyanga 2003/057 AM397782 (*trnG*) AM398350 (*rps4*) no accession (RC24);* B. attenuata *(Mart.) Loeske, 1, NORWAY 2003 L. Söderström 2003/020 AM397777 (*trnG*) AM398344 (*rps4*) no accession (RC24); 2, NORWAY 2004 L. Söderström et. al. 2004/208 AM398282 (*rps4*);* B. barbata *(Schmidel ex Schreb.) Loeske SWEDEN 1990 T. Hedderson 8856 AM398313 (*rps4*);* B. floerkei *(F.Weber et D.Mohr) Loeske ICELAND 2004 A. Séneca & L. Söderström 2004/457 AM397753 (*trnG*) AM398318 (*rps4*) no accession (RC24);* B. hatcheri *(A.Evans)*

*Loeske NORWAY 2003 L. Söderström 2003/001 AM397770 (*trnG*) AM398338 (*rps4*);* B. lycopodioides *(Wallr.) Loeske NORWAY 2003 L. Söderström 2003/019 AM397766 (*trnG*) AM398333 (*rps4*) no accession (ITS) no accession (RC24);* B. quadriloba *(Lindb.) Loeske var.* glareosa *(Jørg.) Lammes SVALBARD 2004 L. Söderström et. al. 2004/408 AM397758 (*trnG*) AM398324 (*rps4*) no accession (RC24);* B. quadriloba *(Lindb.) Loeske var.* quadriloba *SWEDEN 2003 L. Söderström & P. Manyanga 2003/061 AM397808 (*trnG*) AM398375 (*rps4*) no accession (RC24);* Blepharidophyllum densifolium *(Hook.) Ångstr. ARGENTINA 2003 D. Long 31696 AM398306 (*rps4*);* Bryopteris filicina *(Sw.) Nees* AY608051 *(*rps4*);* Calypogeia integristipula *Steph. FINLAND 2003 L. Söderström & P. Manyanga 2003/090 AM397795 (*trnG*) AM398361 (*rps4*);* Cephalozia crassifolia *(Lindenb. et Gottsche) Fulford VENEZUELA 2004 L. Söderström et. al. 2004/060 AM397746 (*trnG*) AM398309 (*rps4*);* C. lunulifolia *(Dumort.) Dumort., 1, SVALBARD 2004 L. Söderström et. al. 2004/415 AM397748 (*trnG*) AM398311 (*rps4*); 2, SVALBARD 2004 L. Söderström et. al. 2004/424 AM397750 (*trnG*) AM398315 (*rps4*);* Cephaloziella hirta *(Steph.) R.M. Schust.* AY608054 *(*rps4*);* C. varians *(Gottsche) Steph. SVALBARD 2004 L. Söderström et. al. 2004/365 AM397747 (*trnG*) AM398310 (*rps4*);* Chandonanthus *(*Plicanthus*)* sp. AY462347 *(*rps4*);* Chiloscyphus cuspidatus *(Nees) J. J. Engel & R. M. Schust.* AY462348 *(*rps4*);* Clasmatocolea vermicularis *(Lehm.) Grolle VENEZUELA 2004 L. Söderström et. al. 2004/116 AM397706 (*trnG*) AM398246 (*rps4*);* Cryptochila paludosa *(Steph.) Grolle SOUTH AFRICA 2003 T. Hedderson 15333 AM397729 (*trnG*) AM398275 (*rps4*);* Delavayella serrata *Steph. NEPAL 2001 D. Long 30522 AM398305 (*rps4*);* Diplophyllum albicans *(L.) Dumort. NORWAY 2004 K. Hassel s.n. AM397726 (*trnG*) AM398272 (*rps4*);* D. taxifolium *(Wahlenb.) Dumort. FINLAND 2003 L. Söderström & P. Manyanga 2003/098 AM397788 (*trnG*) AM398354 (*rps4*);* Douinia ovata *(Dicks.) H.Buch NORWAY 1996 T. Hedderson 11792 AM397786 (*trnG*) AM398353 (*rps4*);* Geocalyx graveolens *(Schrad.) Nees SWEDEN 2003 L. Söderström & P. Manyanga 2003/072 AM398367 (*rps4*);* Gongylanthus renifolius *(Mitt.) Steph. SOUTH AFRICA 2004 R. de Roo s.n. AM397717 (*trnG*) AM398261 (*rps4*);* G. scariosus *(Lehm.) Steph. SOUTH AFRICA 2003 T. Hedderson 15409 AM397711 (*trnG*) AM398252 (*rps4*);* Gottschelia schizopleura *(Spr.) Grolle REUNION 2004 T. Hedderson 15883 AM397736 (*trnG*);* Gymnocolea inflata *(Huds.) Dumort., 1, NORWAY 2004 K. Hassel s.n. AM397725 (*trnG*) AM398271 (*rps4*) no accession (ITS); 2, NORWAY 2004 A. Séneca & L. Söderström 2004/223 AM397755 (*trnG*) AM398321 (*rps4*) no accession (ITS);* Gymnocoleopsis multiflora *(Steph.) R.M.Schust., 1, VENEZUELA 2004 L. Söderström et. al. 2004/035 AM397710 (*trnG*) AM398251 (*rps4*); 2, VENEZUELA 2004 L. Söderström et. al. 2004/091 AM397700 (*trnG*) AM398239 (*rps4*);* Gymnomitrion concinnatum *(Lightf.) Corda* AY608065 *(*rps4*);* G. corallioides *Nees NORWAY 2004 L. Söderström et. al. 2004/270 AM397743 (*trnG*) AM398302 (*rps4*);* Haplomitrium hookeri *(Sm.) Nees* AJ251064 *(*rps4*);* Harpanthus flotovianus *(Nees) Nees FINLAND 2004 L. Söderström & P. Manyanga 2003/095 AM397791 (*trnG*) AM398357 (*rps4*);* Herbertus aduncus *(Dicks.) Gray IRELAND 2004 D. Long 33458 (E) AM397737 (*trnG*) AM398291 (*rps4*);* Heteroscyphus argutus *(Reinw. & Nees) Schiffn.* AY462355 *(*rps4*);* Isotachis armata *(Nees) Gottsche* AY462358 *(*rps4*);* Jamesoniella autumnalis *(DC.) Steph., 1,* AJ251066 *(*rps4*); 2, SWEDEN 2003 H. Weibull s.n. AM397730 (*trnG*) AM398276 (*rps4*);* J. colorata *(Lehm.) Spruce ex Schiffn. SOUTH AFRICA 2003 R. de Roo s.n. AM397749 (*trnG*) AM398314 (*rps4*) no accession (ITS);* J. oenops *Lindenb. & Gottsche SOUTH AFRICA 2003 T. Hedderson s.n. AM398233 (*rps4*);* J. purpurascens *Steph. SOUTH AFRICA 2003 P. Manyanga 89 AM397693 (*trnG*) AM398232 (*rps4*) no accession (ITS);* J. rubricaulis *(Nees) Grolle, 1, VENEZUELA 2004 L. Söderström et. al. 2004/023 AM397707 (*trnG*) AM398247 (*rps4*) no accession (ITS); 2, VENEZUELA 2004 L. Söderström et. al. 2004/093 AM397709 (*trnG*) AM398250 (*rps4*) no accession (ITS);* J. undata *(Mont.) Steph. VENEZUELA 2004 L. Söderström et. al. 2004/123 AM397708 (*trnG*) AM398249 (*rps4*);* Jubula hutchinsiae *ssp.* javanica *(Hook.) Dumort. (Steph.) Verd* AY688794 *(*rps4*);* Jungermannia caespiticia *Lindenb. NORWAY 2004 L. Söderström et. al. 2004/451 AM398288 (*rps4*);* J. cordifolia *subsp.* exsertifolia *(Steph.) Amak.* AY608077 *(*rps4*);* J. crenuliformis *Austin* AY608078 *(*rps4*);* J. exsertifolia *ssp.* cordifolia *(Dum.)Vaňa UNITED KINGDOM 1990 T. Hedderson 8819 AM397802 (*trnG*) AM398369 (*rps4*);* J. leiantha *Grolle* AY507451 *(*rps4*);* Jungermannia polaris *Lindb. SVALBARD 2004 L. Söderström et. al. 2004/403 AM398308 (*rps4*);* Leiocolea collaris *(Nees) Schljakov SWEDEN 2003 L. Söderström 2003/064 AM398377 (*rps4*);* L. heterocolpos *(Thed. ex Hartm.) H.Buch, 1, NORWAY 2003 L. Söderström 2003/014 AM397769 (*trnG*) AM398337 (*rps4*); 2, NORWAY 2003 L. Söderström 2003/015 AM397767 (*trnG*) AM398334 (*rps4*); 3, NORWAY 2003 L. Söderström 2003/021 AM397776 (*trnG*) AM398343 (*rps4*);* L. rutheana *(Limpr.) Müll.Frib. NORWAY 2003 L. Söderström & P. Manyanga 2003/022 AM397778 (*trnG*) AM398345 (*rps4*);* Leiomylia anomala *(Hook.) J.J. Engel & Braggins NORWAY 2004 K. Hassel s.n. AM398269 (*rps4*);* Lejeunea cladogyna *A. Evans* AY608079 *(*rps4*);* Lepicolea scolopendra *(Hook.) Dumort. ex Trevis.* AY462365 *(*rps4*);* Lepidolaena taylorii *(Gottsche) Trevis.* AY462368 *(*rps4*);* Lepidozia cupressina *(Sw.) Lindenb. SOUTH AFRICA 2004 R. de Roo s.n. AM397719 (*trnG*) AM398265 (*rps4*);* Leptoscyphus ovatus *(Spruce) Grolle VENEZUELA 2004 L. Söderström et. al. 2004/127 AM398248 (*rps4*);* Lethocolea *cf.* congesta *(Lehm.) S. Arnell SOUTH AFRICA 2003 T. Hedderson 15301 AM397721 (*trnG*) AM398267 (*rps4*);* L. glossophylla *(Spruce) Grolle, 1, VENEZUELA 2004 L. Söderström et. al. 2004/047 AM397704 (*trnG*) AM398244 (*rps4*); 2, VENEZUELA 2004 L. Söderström et. al. 2004/131 AM397705 (*trnG*) AM398245 (*rps4*);*

Lophocolea bidentata *(L.) Dumort.* AY608085 *(*rps4*);* L. concreta *Mont. SOUTH AFRICA 2004 R. de Roo s.n. AM398262 (*rps4*);* L. difformis *Nees SOUTH AFRICA 2000 T. Hedderson 13427 AM397784 (*trnG*) AM398352 (*rps4*);* Lophozia ascendens *(Warnst.) R.M.Schust. SWEDEN 2003 L. Söderström & P. Manyanga 2003/077 AM397796 (*trnG*) no accession (ITS);* L. bicrenata *(Schmidel ex Hoffm.) Dumort. FINLAND 2003 L. Söderström & P. Manyanga 2003/100 AM397789 (*trnG*) AM398355 (*rps4*);* L. ciliata *Damsh. & al. SWEDEN 2003 L. Söderström & P. Manyanga 2003/084 AM397797 (*trnG*) AM398363 (*rps4*) no accession (ITS);* L. debiliformis *R.M.Schust. & Damsh. NORWAY 2003 K. Hassel s.n. AM397806 (*trnG*) AM398373 (*rps4*);* L. decolorans *(Limpr.) Steph. INDIA D. Long 22566(E) AM398300 (*rps4*);* L. excisa *(Dicks.) Dumort. NORWAY 2003 L. Söderström & P. Manyanga 2003/030 AM397804 (*trnG*) AM398371 (*rps4*) no accession (ITS);* L. incisa *(Schrad.) Dumort., 1, NORWAY 1996 T. Hedderson 11802 AM397785 (*trnG*); 2, SWEDEN 2003 L. Söderström & P. Manyanga 2003/079 AM397800 (*trnG*) AM398366 (*rps4*); 3, VENEZUELA 2004 L. Söderström et. al. 2004/129 AM397694 (*trnG*) AM398234 (*rps4*);* L. jamesonii *(Mont.) R.M. Schust. VENEZUELA 2004 L. Söderström et. al. 2004/043 AM397696 (*trnG*) AM398236 (*rps4*) no accession (ITS);* L. longidens *(Lindb.) Macoun NORWAY 2003 L. Söderström 2003/016 AM397772 (*trnG*) AM398340 (*rps4*) no accession (ITS);* L. longiflora *(Nees) Schiffn. SWEDEN 2003 L. Söderström & P. Manyanga 2003/082 AM397799 (*trnG*) AM398365 (*rps4*);* L. obtusa *(Lindb.) A.Evans FINLAND 2003 L. Söderström 2003/094 AM397793 (*trnG*) AM398359 (*rps4*);* L. perssonii *H.Buch et S.W.Arn. NORWAY 2003 L. Söderström & P. Manyanga 2003/036 AM397807 (*trnG*) AM398374 (*rps4*) no accession (ITS);* L. setosa *(Mitt.) Steph. BHUTAN D. Long 28644 (E) AM397752 (*trnG*) AM398317 (*rps4*);* L. stolonifera *R.M.Schust. VENEZUELA 2004 L. Söderström et. al. 2004/130 AM397756 (*trnG*) AM398322 (*rps4*) no accession (ITS);* L. sudetica *(Nees ex Huebener) Grolle, 1, FINLAND 2003 L. Söderström & P. Manyanga 2003/096 AM397792 (*trnG*) AM398358 (*rps4*); 2, NORWAY 2003 L. Söderström & P. Manyanga 2003/049 AM397783 (*trnG*) AM398351 (*rps4*);* L. ventricosa *(Dicks.) Dumort.* AY462369 *(*rps4*);* L. ventricosa *(Dicks.) Dumort. var.* confertifolia *(Schiffn.) Husn. FRANCE 2004 J. Vaňa s.n. AM397734 (*trnG*) AM398287 (*rps4*) no accession (ITS);* L. ventricosa *var.* confusa *R.M.Schust. CANADA 1986 T. Hedderson 5008 AM397801 (*trnG*) AM398368 (*rps4*);* L. ventricosa *(Dicks.) Dumort. var.* silvicola *(H.Buch) E.W. Jones NORWAY 2003 L. Söderström 2003/018 AM398336 (*rps4*) no accession (ITS);* L. ventricosa *(Dicks.) Dumort. var.* ventricosa, *1, SWEDEN 2003 L. Söderström & P. Manyanga 2003/048 AM397805 (*trnG*) AM398372 (*rps4*) no accession (ITS); 2, SWEDEN 2003 L. Söderström & P. Manyanga 2003/070 AM397790 (*trnG*) AM398356 (*rps4*) no accession (ITS);* L. wenzelii *(Nees) Steph. NORWAY 2003 L. Söderström & P. Manyanga 2003/024 AM398346 (*rps4*) no accession (ITS);* Marsupella aquatica *(Lindenb.) Schiffn.* AY608087 *(*rps4*);* M. aquatica *f.* pearsonii *(Schiffn.) Schljakov NORWAY 2004 L. Söderström et. al. 2004/198 AM398281 (*rps4*);* M. lacerata *(Steph.) Váňa SOUTH AFRICA 2001 T. Hedderson 13613 AM397728 (*trnG*) AM398274 (*rps4*);* M. sparsifolia *(Lindb.) Dumort. SOUTH AFRICA 2003 T. Hedderson 15338 AM397722 (*trnG*);* Marsupidium latifolium *R.M. Schust.* AY608088 *(*rps4*);* Mastigophora woodsii *(Hook.) Nees UNITED KINGDOM Rothero 11005(E) AM397741 (*trnG*) AM398298 (*rps4*);* Mesoptychia sahlbergii *(Lindb.) A.Evans, 1, EAST SIBERIA Ignatov s.n. AM398323 (*rps4*); 2, EAST SIBERIA Ignatov AM397757 (*trnG*) AM398328 (*rps4*);* Metzgeria decipiens *(C.Massal.) Schiffn. SOUTH AFRICA 2004 R. de Roo s.n. AM398259 (*rps4*);* Mnioloma fuscum *(Lehm.) R.M.Schust. SOUTH AFRICA 2004 R. de Roo s.n. AM397718 (*trnG*) AM398263 (*rps4*);* Mylia taylorii *(Hook.) Gray NORWAY 2004 K. Hassel s.n. AM397724 (*trnG*) AM398270 (*rps4*);* Nardia scalaris *Gray, 1,* AY608092 *(*rps4*); 2, NORWAY 2004 K. Hassel s.n. AM397727 (*trnG*) AM398273 (*rps4*);* Neesioscyphus argillaceus *(Nees) Grolle VENEZUELA 2004 L. Söderström et. al. 2004/022 AM397695 (*trnG*) AM398235 (*rps4*);* Nowellia curvifolia *(Dicks.) Mitt., 1,* AY608094 *(*rps4*); 2, UNITED KINGDOM 2001 D. Long 29513(E) AM398293 (*rps4*);* Odontoschisma denudatum *(Mart.) Dumort. IRELAND 2001 D. Long 29937(E) AM397760 (*trnG*) AM398325 (*rps4*);* O. macounii *(Austin) Underw. SVALBARD 2004 L. Söderström et. al. 2004/431 AM397744 (*trnG*) AM398303 (*rps4*) no accession (ITS);* Pachyschistochila carnosa *(Mitt.) R.M.Schust. & Engel ARGENTINA 2003 D. Long 31755(E) AM398296 (*rps4*);* Pellia epiphylla *(Gottsche) Limpr.* AY330479 *(*rps4*);* Plagiochila deltoidea *Lindenb.* AY547699 *(*rps4*);* P. dura *De Notaris* AY547700 *(*rps4*);* P. retrospectans *(Nees ex Spreng.) Lindenb.* AY547721 *(*rps4*);* P. *sp. SOUTH AFRICA 2003 R. de Roo 12k AM397716 (*trnG*) AM398260 (*rps4*);* Pleurozia purpurea *Lindb.* AY608100 *(*rps4*);* Plicanthus hirtellus *(Weber) R.M.Schust NEPAL 2001 D. Long 30335 AM397745 (*trnG*) AM398304 (*rps4*) no accession (ITS) no accession (RC24);* Porella pinnata *L.* AY608101 *(*rps4*);* P. platyphylla *(L.) Pfeiff.* AY462387 *(*rps4*);* Pseudolepicolea quadrilaciniata *(Sull.) Fulf. & J.Taylor ARGENTINA 2003 D. Long 31658(E) AM398299 (*rps4*);* Radula complanata *(L.) Dumort UNITED KINGDOM 2001 D. Long 29904(E) AM397740 (*trnG*) AM398295 (*rps4*);* R. perrottetii *Gottsche ex Steph.* AY608105 *(*rps4*);* Riccardia capensis *S.W.Arnell SOUTH AFRICA 2003 R. de Roo s.n. AM398264 (*rps4*);* Scapania compacta *(Roth) Dumort. NORWAY 2002 L. Söderström 2002/160 AM398312 (*rps4*);* S. hyperborea *Jørg. NORWAY 2004 L. Söderström et. al. 2004/191 AM397732 (*trnG*) AM398283 (*rps4*);* S. lingulata *H.Buch NORWAY 2004 L. Söderström et. al. 2004/199 AM398280 (*rps4*);* S. nemorea *(L.) Grolle NORWAY 2004 L. Söderström et. al. 2004/200 AM397731 (*trnG*);* S. obcordata *(Berggr.) S.W.Arnell SVALBARD 2004 L. Söderström et. al. 2004/379 AM397735 (*trnG*) AM398289 (*rps4*);* S. undulata *(L.)*

*Dumort., 1, CANADA 1985 T. Hedderson 3432 AM397787 (*trnG*); 2, NORWAY 2004 L. Söderström et. al. 2004/201 AM397733 (*trnG*) AM398286 (*rps4*);* Schiffneria hyalina *Steph.* AY462393 *(*rps4*);* Southbya gollanii *Steph. NEPAL 2001 D. Long 30537(E) AM397739 (*trnG*) AM398294 (*rps4*);* Sphenolobopsis pearsonii *(Spruce) R.M.Schust. UNITED KINGDOM 2004 D. Long 33507(E) AM397751 (*trnG*) AM398316 (*rps4*) no accession (ITS) no accession (RC24);* Stephaniella paraphyllina *J.B.Jack VENEZUELA 2004 L. Söderström et. al. 2004/030b AM397698 (*trnG*);* Symphyogyna podophylla *(Thunb.) Mont. et Nees SOUTH AFRICA 2004 R. de Roo s.n. AM398258 (*rps4*);* Syzygiella setulosa *Steph. VENEZUELA 2004 L. Söderström et. al. 2004/073 AM397715 (*trnG*) AM398256 (*rps4*);* Tetralophozia setiformis *(Ehrh.) Schljakov, 1, NORWAY 2004 L. Söderström et. al. 2004/195 AM398277 (*rps4*) no accession (RC24); 2, SWEDEN 2003 L. Söderström & P. Manyanga 2003/056 AM397803 (*trnG*) AM398370 (*rps4*) no accession (ITS) no accession (RC24); 3, SPAIN 2004 M. Infante et. al. 11/06/2004 no accession (*trnG*) no accession (RC24);* Tritomaria polita *(Nees) Jørg., 1, NORWAY 2003 L. Söderström & P. Manyanga 2003/037 AM397775 (*trnG*) AM398342 (*rps4*) no accession (ITS); 2, SWEDEN 2003 L. Söderström 2003/063 AM398376 (*rps4*) no accession (ITS);* T. quinquedentata *(Huds.) H.Buch ssp.* quinquedentata *var.* quinquedentata *NORWAY 2003 L. Söderström 2003/002 AM397768 (*trnG*) AM398335 (*rps4*) no accession (ITS);* T. quinquedentata *(Huds.) H.Buch ssp.* turgida *(Lindb.) Damsh. NORWAY 2003 L. Söderström 2003/012 AM397773 (*trnG*) AM398341 (*rps4*);* T. scitula *(Taylor) Jørg. NORWAY 2003 L. Söderström & P. Manyanga 2003/028 AM397779 (*trnG*) AM398347 (*rps4*)*