Pleurocarpous Mosses in Space and Time:

Biogeography and Evolution of the Hookeriales

by

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology in the Graduate School of Duke University

2012
ABSTRACT

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Abstract

Morphological characters from the gametophyte and sporophyte generations have been used in land plants to infer relationships and construct classifications, but sporophytes provide the vast majority of data for the systematics of vascular plants. In bryophytes both generations are well developed and characters from both are commonly used to classify these organisms. However, because morphological traits of gametophytes and sporophytes can have different genetic bases and experience different selective pressures, taxonomic emphasis on one generation or the other may yield incongruent classifications. The moss order Hookeriales has a controversial taxonomic history because previous classifications have focused almost exclusively on either gametophytes or sporophytes. The Hookeriales provide a model for comparing morphological evolution in gametophytes and sporophytes, and its impact on alternative classification systems. Sometimes, placement of certain groups within Hookeriales remains challenging even at the molecular level. That is the case of the genus *Calyptrochaeta*. We study diversification dynamics in this genus to elucidate possible mechanisms obscuring its placement and we address biogeographic questions using the Tropical Conservatism scenario as our null hypothesis. Furthermore, to better understand biogeographic patterns in the Southern Hemisphere, infraspecific molecular patterns are compared in two species of the genus *Calyptrochaeta* (i.e., *C. apiculata* and *C.*
asplenioides) and vicariance and recent long distance dispersal are tested to explain the disjunct distributions observed in these species.

In this study we reconstruct relationships among pleurocarpous mosses in or associated to the Hookeriales, in Calyptrochaeta, and within Calyptrochaeta. Six molecular markers are explored in total from all three genome compartments to reconstruct the evolution of morphological characters and habitat preferences in our phylogenies. Divergence times are estimated in a Bayesian framework using a relaxed molecular clock, and diversification rates are calculated on the chronograms resulting from these estimations.

As a result, we found that the Hookeriales, as currently circumscribed, are monophyletic and that both sporophyte and gametophyte characters are labile. We documented parallel changes and reversals in traits from both generations. We show that diversification rates in Calyptrochaeta have changed through its history. Also, though we lack support to clearly reject the tropical conservatism hypothesis, our data point to a more complex scenario where both temperate and tropical species can be ancient and give rise to one another, since shifts between tropical and temperate regions seem to be possible in any direction. Finally, we have show that recent long distance dispersal best explains the distribution of both C. apiculata and C. asplenioides in the Southern Hemisphere.
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Gracias.
1. Pleurocarpous Mosses in Space and Time

1.1 Foreword

This dissertation is divided into an introductory chapter (1), three data chapters (2-4), and a general conclusions chapter (5). A general introduction to these three data chapters follows.

1.2 An Introduction

Like all land plants, bryophytes have a life cycle with alternation of generations. However, unlike most land plants, in bryophytes both generations are macroscopic and, most notably, the dominant generation is the haploid gametophyte. Haploid gametophytes can disperse clonally via specialized propagules or via plant fragments, although the main role of this generation is assimilation and growth. At gametophyte maturity, egg and sperm are produced in gametangia (i.e., antheridia and archegonia) and after fertilization a new sporophyte is produced. Diploid sporophytes, which are attached to the maternal haploid gametophyte, produce meiotic spores and facilitate dispersal (Goffinet et al. 2008). Since these alternating generations probably have uncoupled evolutionary trajectories, some very interesting questions on differential selective pressures can be addressed in these organisms. Moreover, since bryophytes diverged from the rest of the embryophytes before vascular plants evolved, they offer a unique perspective on the transition to land by plants (Shaw et al. 2011).
Within bryophytes, the ability to produce sporophytes on specialized lateral branches is a synapomorphy that characterizes pleurocarpous mosses and is, supposedly, a key innovation that has allowed this clade to greatly diversify (Bell et al. 2007). These pleurocarpous mosses comprise about half of all known mosses and include the moss orders Hookeriales M. Fleisch., Hypnales (M. Fleisch.) W.R. Buck & Vitt, Hypnodendrales N.E. Bell, A.E. Newton & D. Quandt, and Ptychomniales W.R. Buck, C.J. Cox, A.J. Shaw & Goffinet. The order Hypnodendrales is sister to the “homocostate pleurocarps”, informal node-based name referring to the orders Ptychomniales, Hookeriales and Hypnales (the latter two are also known as crown pleurocarps), whereas the order Ptychomniales is sister to the crown pleurocarps (Bell et al. 2007). Within the crown pleurocarps clade, the Hookeriales includes ca. 650 species in seven families, these being: Daltoniaceae Schimp., Hookeriaceae Schimp., Hypopterygiaceae Mitt., Leucomiaceae Broth., Pilotrichaceae Kindb., Saulomataceae W.R. Buck, C.J. Cox, A.J. Shaw & Goffinet, and Schimperobryaceae W.R. Buck, C.J. Cox, A.J. Shaw & Goffinet (Buck et al. 2005).

Although Hookeriales is a clade in most phylogenetic reconstructions (i.e., Bell et al. 2007, Buck et al. 2005), Newton et al. (2007) inferred an alternative topology that suggested that Hypnales was paraphyletic with respect to Hookeriales (i.e., Rutenbergia Geh. & Hampe ex Besch. and Trachyloma Brid., traditionally included in the Hypnales, were nested within the Hookeriales), and that Hypopterygiaceae was outside
Hookeriales and sister to both this order and the Hypnales. Though this disagreement in the classification of pleurocarpous mosses has only recently been noted, this group of mosses has a long history of conflict where contradictory classifications replace each other through time (Buck 2007), probably as a combination of two factors: a poor understanding of their evolution, on one hand, and rampant homoplasy of morphological characters, on the other. This homoplasy can result from parallel changes to derived states (convergence) or from reversals to ancestral states. Therefore, determining the existence and frequency or reversals is key to understanding morphological evolution in this (or any other) group of organisms (Collin & Miglietta 2008), which is what we will do in chapter 2. Also, the order Hookeriales has been around for at least 140 Myr (Newton et al. 2007) and contains the highest phylogenetic diversity among all pleurocarpous orders (Shaw et al. 2003b). Understanding its evolution can give us insights on how biodiversity is generated and maintained through time and across space.

support is lacking from some critical nodes in the backbone of this group, and the position of *Achrophyllum*, *Calyptrochaeta*, *Crossomitrium*, and *Hookeria*, with respect to other Daltoniaceae and other families in the Hookeriales, is uncertain.

In particular, attempts to solve the relative position of *Achrophyllum* and *Calyptrochaeta* have returned inconclusive results, despite increased taxon and molecular sampling (Ho 2010). *Achrophyllum* is invariably reconstructed in a clade of its own, often sister to all other Daltoniaceae (with the exception of *Calyptrochaeta*), though support for this relationship is never high. *Calyptrochaeta* is inferred to be a clade that is either sister to all other Daltoniaceae, or sister to a clade of the families Hookeriaceae, Leucomiaceae, Pilotrichaceae and Schimperobryaceae. In order to get insights into possible causes behind this conflict we will piece together the timing and diversification dynamics in *Calyptrochaeta*.

The genus *Calyptrochaeta*, containing ca. 30 species (Crosby et al. 2000), is found primarily in the Southern Hemisphere, with most of its diversity concentrated in the tropics. We can use this broadly distributed genus to test broad biogeographic questions pertaining to biodiversity patterns in the tropics versus temperate regions. In particular, the tropical conservatism (TC) hypothesis, which states that tropical climates have been environmentally stable for a longer time than temperate ones, and that, if niches are conserved, transitions to temperate climates are rare, will be used as a null in chapter 3.
Under a TC scenario we expect tropical regions to concentrate more species and sporadic temperate clades to be younger than, and nested within, tropical ones.

As in many land plants (Raven and Axelrod 1974), transoceanic distributions can also be found in bryophytes (Tan and Pócs 2000); though, unlike in angiosperms, these transoceanic distributions are often infraspecific, rather than interspecific (Schuster 1983). *Calyptrochaeta* is no exception and species in this genus have broad distributions, some encompassing multiple continents. These broad disjunct distributions, in combination with the ancient estimated age for bryophytes (Wellman et al. 2003), recent evidence of a much slower molecular substitution rate in bryophytes than in vascular plants or green algae (Stenøien 2008), and some morphological stasis, have led to a general perception of bryophytes as “unmoving, unchanging sphinxes of the past” (Frahm 2008). Nonetheless, not only can vicariance account for these broad disjunct distributions, but also more recent long distance dispersal (Muñoz et al. 2004).

Divergence time estimation can used to test alternative biogeographic hypothesis (Renner 2005). Specifically, in chapter 4 we will compare the distribution of, seemingly, Gondwanan *C. apiculata* and *C. asplenoides*.

*Calyptrochaeta asplenioides* (Brid.) Crosby is a widespread African endemic found in tropical and subtropical moist forests in South Africa, E Africa and Indian Ocean islands, which displays a wide range of morphological variation that appears to be related to its geographic distribution. *Calyptrochaeta apiculata* (Hook. f. & Wilson) Vitt
exhibits a Holantarctic distribution and is found in S America, Australasia and various sub-Antarctic islands, and also has a number of varieties, as a reflection of the spectrum of morphologies this taxon encompasses. We address if these disjunct distributions of *C. apiculata* and *C. asplenoides* result in phylogeographic structure, if the morphological variation observed in these species corresponds to the plausible phylogeographic structure, and if these disjunct distributions can be explained by vicariance or if instead they are the result of recent long distance dispersal.

To summarize, in this dissertation we use the Hookeriales (with a particular focus on the genus *Calyptrochaeta*) to address general questions in evolutionary biology. Homoplasy and irreversibility, both in morphological characters and habitat preferences, are addressed broadly in the order Hookeriales (chapter 2) and, more narrowly, in the genus *Calyptrochaeta* (chapter 3). Diversification rate shifts through time, in view of paleoclimatic events, are estimated in *Calyptrochaeta*, and character state reconstructions of habitat preferences through time are used to test the tropical conservatism hypothesis (chapter 3). The role of long-distance dispersal in shaping observed distribution patterns is explored in Holantarctic *C. apiculata* and African *C. asplenoides* (chapter 4). In all, we expect to offer insights into how evolutionary processes may have shaped bryophytes, with their unique life cycles, and hopefully shed some light on selective pressures that may have acted upon plants early on their colonization of land.
2. Phylogenetic Analyses of Morphological Evolution in the Gametophyte and Sporophyte Generations of the Moss order Hookeriales


2.1 Introduction

Bryophytes (liverworts, mosses, hornworts), like all land plants, exhibit a life cycle with alternation of sporophytic and gametophytic generations. However, in contrast to other groups, in bryophytes both generations are well developed and the haploid gametophyte is dominant. The unbranched diploid sporophytes are attached to the maternal gametophytes and are, at least partially, nutritionally dependent on them (Goffinet et al., 2008). With few exceptions, the gametophyte is leafy and long-lived. The sporophyte, in contrast, is short-lived, and consists of a foot that attaches it to the maternal gametophyte, and a sporangium (capsule) that is generally subtended by a more or less elongate seta (Goffinet et al., 2008). The two alternating generations in bryophytes have different morphologies and functions. Gametophytes are assimilative and at maturity produce archegonia and antheridia, with egg and sperm, respectively. Sporophytes produce meiotic spores and facilitate their dispersal. The two generations experience different selection pressures and the evolutionary trajectories of sporophytes and gametophytes can be uncoupled. Classifications that emphasize morphological characteristics of one generation or the other may, as a consequence, be substantially divergent (Buck, 1980; Dixon, 1932; Miller, 1979; Rohrer, 1988). Although both
generations have been utilized for inferring relationships and constructing classifications in other groups of land plants (Atkinson, 1973; Stuessy, 2009; Wagner and Beitel, 1992), bryophyte taxonomy is uniquely characterized by having access to relatively well-developed sporophytes and gametophytes that provide potentially informative morphologies.

Incongruence between classifications emphasizing gametophyte versus sporophyte characters is well exemplified by the moss order Hookeriales (Buck, 1980, 1991; Miller, 1979). The Hookeriales belong to the so-called pleurocarpous mosses (core pleurocarps sensu Bell et al., 2007), where the sexual structures and consequently the sporophytes are produced on specialized, short, lateral branches. Comprising about 5,300 to 6,600 species; i.e., about half of all known mosses (Crosby et al., 2000; Shaw et al., 2003b), this well-supported monophyletic group contains the orders Hookeriales, Hypnales, Hypnodendrales, and Ptychomniales (Bell et al., 2007). Although the phylogenetic branching order among early diverging pleurocarps (Hypnodendrales first, Ptychomniales second) is fairly well established (Bell et al., 2007), resolving relationships within the crown groups (Hypnales and Hookeriales) remains challenging because both orders are characterized by extensive homoplasy in morphological traits (Buck, 2007; Hedenäs, 2007; Huttunen et al., 2004; Olsson et al., 2009a; Quandt et al., 2009).
As currently circumscribed (Goffinet et al., 2008), the Hookeriales include about 650 species, predominantly distributed in humid forests in the tropics and the South Temperate Zone. The sporophytic capsule of the Hookeriales opens via a lid or operculum as in other “true mosses” (Bryopsida), permitting release of haploid meiospores. Lining the mouth of the capsule are the outer and inner rows of teeth known as exostome and endostome, respectively (collectively, the peristome). Although the peristome teeth are often highly ornamented and able to perform hygroscopic movements, details of their function are mainly speculative. It is generally agreed that the peristome participates in the regulation of spore discharge (Mueller and Neumann, 1988), though only one experiment on the matter can be found in the literature (Ingold, 1959). It is thus not surprising that we have no understanding on how variations in peristome morphology affect fitness in bryophytes.

The significance of sporophytes, and especially that of peristomes, for moss classification was emphasized in a series of studies by Philibert (for review see Taylor, 1962). Subsequently, Fleischer (1904-1923; 1920) presented a first systematic arrangement of mosses that applied Philibert’s principles. Although Fleischer argued that major moss lineages above the ordinal rank are best characterized by differences in peristome structure, at lower ranks gametophytic characters were considered to have more importance. His philosophy and classification scheme was followed by Brotherus (1925) with little modification and has, until recently, been the widely accepted standard.
Dixon (1932) agreed that sporophytic similarities are synapomorphic for major groups, and that, within these groups, taxa can be very diverse in gametophyte morphology. It was assumed that peristomial phenotypes are not as much influenced by the external environment as are gametophyte characters, either because peristomes are protected within the operculum (lid of the capsule) during development (Allen et al., 1985; Fleisher, 1904-1923, 1920) or because sporophytes are significantly shorter-lived than gametophytes (Buck, 1980). These views, together with Philibert’s observations on peristome structure, bolstered for many decades the idea that the sporophyte generation should be emphasized in higher-level classifications of moss diversity.

Miller (1971), stimulated by Bessey’s (1915) dicta for flowering plants, proposed an alternative system based on 23 generalized “principles for moss systematics” that considered both gametophytes and sporophytes. Miller recognized nine families in the Hookeriales and intuitively postulated directions of morphological evolution. However, Crosby (1974), being an ardent follower of the philosophy that major groups of mosses (i.e., families) are best defined by differences in peristome structure, applied Philibert’s principles of sporophyte and peristome conservatism to the family level classification of Hookeriales. As a result, hookerialean genera were divided into two families, separated strictly by outer exostome surface ornamentation (Crosby, 1974). Exostomes with the outer surfaces cross-striate at the base are termed “hookeriaceous” peristomes, whereas those completely papillose are termed “daltoniaceous”. It was on this basis that Crosby
distinguished the Hookeriaceae and Daltoniaceae. In this scheme, several pairs of genera
with indistinguishable gametophytes, but different exostome ornamentation (e.g.,
*Lepidopilum* and *Lepidopilidium*), were arranged in different families.

Buck (1987; 1988) reversed the traditional philosophy and reassessed familial
delimitation and relationships within the Hookeriales with emphasis on gametophytic
characters. He distinguished five families and discussed inferences about interfamilial
relationships based on differences in gametophyte structure. Whittemore and Allen
(1989) revisited Buck’s (1987; 1988) system, but focused on similarities of both
generations, rather than differences, and lumped Buck’s proposed five families into two
main families, Daltoniaceae and Hookeriaceae. They were conservative about inferring a
relationship for the highly reduced *Ephemeropsis*, which is epiphytic and has persistent
protonemata rather than “normal” leafy gametophytes, and tentatively retained it in a
family of its own. Later, Hedenäs (1996) coded 75 morphological characters from both
generations and analyzed the data applying cladistic methods. This analysis resolved
three clades within the Hookeriales, none of them with strong bootstrap support.

Buck et al. (2005) conducted a molecular analysis based on four genes, two from
the plastid genome (*trnf*LF and *rps4*), one from the mitochondrial genome (*nad5*), and the
large ribosomal RNA subunit gene from the nucleus. Their analyses included 89 taxa
traditionally classified in the Hookeriales, with representatives from the other orders of
pleurocarpous mosses as outgroups. Evolutionary transitions in 13 morphological traits
used previously to diagnose families within the Hookeriales were reconstructed on the molecular phylogeny. Their work led to a re-arrangement of the order into seven families. Phylogenetic relationships suggested substantial homoplasy in peristome morphology.

Hedenäs (2001), Huttunen et al. (2004), and Olsson et al. (2009b) showed that peristome reduction, at least in some epiphytic mosses, is correlated with habitat shifts, and thus contradicted the traditional view that sporophytic characters are not under selection. In addition, at least two or three distinct types of daltoniaceous peristomes have been identified, suggesting that daltoniaceous peristomes have evolved multiple times from hookeriaceous peristomes (Buck, 1987; Tan and Robinson, 1990; Whittemore and Allen, 1989). Also, a series of studies on peristome development have helped clarify when and how during ontogenesis the major types of moss peristomes differ from one another (Edwards, 1979, 1984; Goffinet et al., 1999; Shaw and Anderson, 1988; Shaw et al., 1987; Shaw et al., 1989a; Shaw et al., 1989b). Buck (1991) and Hedenäs (1998; 1999) have already pointed out that various peristomial structures may have evolved in parallel or convergently in many unrelated taxa in response to similar habitat conditions.

The controversial taxonomic history and disagreement about whether gametophyte or sporophyte characters are better indicators of phylogenetic relationships make the Hookeriales a good group in which to test this long-standing issue in
systematic bryology. Moreover, it is particularly important to corroborate reversals in morphological characters and to determine the frequency of such reversals (Collin and Miglietta, 2008). The current chapter seeks to identify synapomorphic morphological characters for clades within the Hookeriales and focuses on the question of whether most homoplasy represents parallel changes to derived states, versus reversals to seemingly ancestral conditions. As such, this chapter addresses the general question of reversibility in morphological evolution.

Specifically, this chapter is undertaken to (1) ascertain the monophyly of Hookeriales sensu Buck et al. (2005), (2) resolve relationships among families and genera within the Hookeriales clade, (3) trace the evolution of morphological characters utilizing ancestral state reconstruction, and (4) identify the occurrence and frequency of reversals in morphological characters.

2.2 Materials and Methods

2.2.1 Taxon Sampling and Molecular Protocols

DNA was sampled, when necessary, from 122 species representing 71 genera. Preexisting DNA templates generated for previous studies (e.g., Buck et al., 2005; Olsson et al., 2009a; Pedersen et al., 2006; Shaw et al., 2008; Yu et al., 2010) were also used. The outgroup is composed of five exemplars from the pleurocarpous orders Hypnodendrales and Ptychomniales. The ingroup consists of 95 species from 46 genera (out of 52) in the Hookeriales and 22 species (11 of which have been associated with the...
Hookeriales) currently classified in the Hypnales. Whenever possible, type species for
genera were sampled. Other than the type species, sampling efforts were made to
include a few additional exemplars from each genus, especially in large genera such as
*Callicostella, Cyclodictyon,* and *Distichophyllum,* to better represent their morphological
and geographical diversity.

Nucleotide sequences were obtained for five DNA regions, namely (1) the plastid
*trn*LF region, including the *trn*LUAA group I intron and the *trn*LF intergenic spacer (IGS)
(hereafter *trn*LF), (2) the plastid *rps4* gene, including the *trn*S–*rps4* IGS, (3) the
mitochondrial *nad5* group I intron, (4) the nuclear ribosomal ITS1–5.8–ITS2 region
(hereafter, ITS), and (5) the large ribosomal RNA subunit (hereafter, 26S). Appendix A
provides voucher information and GenBank accession numbers. The identifications of
all new samples were reconfirmed in this study.

Total genomic DNA was extracted as in Shaw (2000). DNA templates were
amplified by polymerase chain reaction (PCR) employing standard protocols and
amplification primers as described in Shaw et al. (2003a) and Olsson et al. (2009a).
Cleaned PCR products were sequenced by Macrogen Inc., South Korea
(www.macrogen.com), or in the DNA Sequencing Facility at the Institute for Genome
Sciences & Policy, Duke University (http://www.genome.duke.edu/cores/sequencing/).
2.2.2 DNA Sequence Editing and Alignment

For each sample and sequenced DNA region, forward (5'–3') and reverse (3'–5') sequences were assembled and checked for inaccurate base calling using Sequencher v4.1 (GeneCodes Corporation 2000) or PhyDE® 0.995 (Müller et al., 2008). Consensus sequences were aligned manually in PhyDE® 0.995 following alignment rules described in Kelchner (2000), trying to minimize substitutions and indels. The approach combines event based and similarity criteria to produce a hypothesis about the homology of the characters (Morrison, 2006; Simmons, 2004; Simmons and Freudenstein, 2003). Simple sequence repeats were isolated based on strict motif recognition as advocated by Kelchner (2000) and Quandt & Stech (2005). The matrix was visually inspected for hairpin-associated inversions. Detected inversions were positionally separated in the alignment. Apart from previously reported inversions in the trnLF IGS (Quandt and Stech, 2004) and in the trnL intron (Quandt and Stech, 2005), three additional inversions were detected (Appendix A). Following Quandt et al. (2003), inversions were not scored for the phylogenetic analyses. Nevertheless, in order to maximize information within detected inversions; i.e., substitutions that occurred prior the inversion event, a second alignment file was generated with the inversions included as reversed and complemented sequences for the phylogenetic analyses (Borsch and Quandt, 2009; Sotiaux et al., 2009). Regions of ambiguous alignment in the data matrix were defined as outlined in Olsson et al. (2009a) and excluded from phylogenetic analyses (Table 1).
Table 1: Hotspots (Hs) and inversions (Iv). Genes in the merged data matrix follow, rps4: 1–880, trnLF: 881–1753, nad5: 1754–3154, ITS: 3155–6894, and 26S: 6895–7958

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2.2.3 Phylogenetic Analyses

Both parsimony (MP) and Bayesian (BI) analyses were performed on the individual loci as well as the concatenated matrix. Maximum Likelihood (ML) analyses, and 1,000 ML bootstrap replicates were performed in GARLI v0.96 beta (Zwickl, 2006)
on the concatenated dataset. Analyses of the single locus data sets revealed no significant conflicts (i.e., \( \geq 70\% \) bootstrap support or \( \geq 0.95 \) posterior probability) among the different regions (data not shown). Command files for using the parsimony ratchet (Nixon, 1999) were generated using the program PRAP2 (Müller, 2007) applying default settings, and executed in PAUP* v4.0b (Swofford, 2002), and 10,000 bootstrap replicates were performed using heuristic search algorithms under parsimony. Bayesian analyses were performed with MrBayes v3.1.2, applying the GTR+\( \Gamma \)+I model for the sequence data. For each partition, the best-fit substitution model was selected according to the AIC and calculated with the aid of MrModeltest v2.2 (Nylander, 2004) and PAUP* v4.0b (Swofford, 2002). To allow for possibly deviating substitution matrices for the different genomes the data set was divided into the following sequence data partitions: partition 1, plastid (\( rps4 + trnLF \)); partition 2, mitochondrial (\( nad5 \)); and partition 3, nuclear (ITS1 \& 2 + 26S). Model parameters for each partition were sampled independently. The a priori probabilities supplied were those specified in the default settings of the program. Posterior probability (PP) distributions of trees were created using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method. Ten runs with four chains (3\( \times \)106 generations each) were run simultaneously. Chains were sampled every 1,000 generations. The program Tracer v1.5 (Rambaut and Drummond, 2009) was used to estimate the burnin and to examine the log likelihoods, ensuring that the runs were in the stationary phase and that adequate effective sample sizes (ESS) were attained.
Calculations of the consensus tree and of the posterior probability of clades were performed based on the trees sampled after the chains converged. Consensus topologies and support values from the different methodological approaches were compiled and drawn using TreeGraph 2.0.42-187 beta (http://treegraph.bioinfweb.info/).

2.2.4 Ancestral State Reconstructions and Correlation of Morphologies and Habitat Types

Six selected morphological characters (four gametophytic and two sporophytic) and three characters describing habitat occupation (presence versus absence from a particular substrate) were coded (Table 2). Each taxon was scored for the following morphological characters: differentiated leaf border (character 1: absent=0, present=1); leaf nerve or costa (character 2: absent=0, present and single=1, present and double=2); cell length/width (L/W) ratio in the middle of the leaf lamina (character 3: short=0, L/W≤3:1; long=1, L/W>3:1; though this character is continuous, pleurocarpous mosses are regularly divided into clear short an long-celled taxa); ornamentation of the outer surface of the exostome teeth at their bases (character 4: smooth, papillose or weakly striolate=0, conspicuously striate=1); furrow at the central divisural line of the exostome teeth (character 5: absent=0, present=1); and calyptra (character 6: cucullate=0, mitrate=1). Crosby (1974) and Buck (1988) used these gametophytic and sporophytic characters in their respective family level classifications of the Hookeriales, which emphasized either sporophytes (the former) or gametophytes (the latter).
Table 2: Character matrix. 1) Limbidium, leaf margin (0: absent; 1: present). 2) Costa, leaf nerve (0: absent; 1: single; 2: double). 3) Cell L/W, length/width ratio of cells in the middle of the leaf lamina (0: 1-3:1; 1: >3:1). 4) Ex. ornam., ornamentation of the outer surface of the exostome teeth at their bases (0: smooth, papillose or weakly striate; 1: conspicuously striate). 5) Furrow, which can be found in the outer surface, at the central divisural line, of the exostome teeth (0: not furrowed; 1: furrowed). 6) Calyptra dehiscence (0: cucullate; 1: mitrate). 7) ST (strict terricolous), mosses found on bare ground and/or decaying matter (0: absent; 1: present). 8) EL+TB (epilithic), mosses on rocks (shaded or exposed, wet or dry) or at the base of trees or shrubs (0: absent; 1: present). 9) SEP (strict epiphytes), mosses on tree trunks, branches, and leaves (0: absent; 1: present). (?) Unknown. (-) Does not apply.

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In order to address possible correlations between habitat shifts and morphological evolution in the Hookeriales, the occurrences of these taxa in three habitat categories were coded according to their presence or absence from these habitats (Table 2). When plants were found on bare ground or decaying matter (decaying logs inclusive) they were coded as present in terricolous habitats (character 7). When restricted to rocks and bases of trees or shrubs they were coded as present in epilithic habitats (character 8). When they grow exclusively on tree trunks and branches, or on leaves, they were coded as present in epiphytic habitats (character 9). Terricolous habitats, as here defined, represent loose, temporal substrates. Epilithic habitats, on the
other hand, are compact, long-lasting surfaces. Epiphytic habitats, as listed above, are rather unique and factors such as water shortage, nutrient availability, levels of irradiation (Zotz and Bader, 2009; Zotz and Hietz, 2001), pool them together and single them out from other habitats. Morphological and habitat character states for each taxon were taken from literature as well as collection labels.

Character state reconstruction analyses were performed in a Bayesian framework with BayesTraits (http://www.evolution.reading.ac.uk/BayesTraits.html). Reconstructions of these characters were mapped onto 500 trees sampled, after burnin, from the cloud of most optimal trees generated in MrBayes. All reconstructions were performed for a selection of 39 nodes defining important, and mostly well-supported (i.e., PP≥ 0.90), clades in the Hookeriales (Figs. 2-7). The Multistate Markov model was implemented to estimate the probabilities of character state change on each branch. For all characters except leaf nerve, the instantaneous forward (q_{01}) and backward (q_{10}) rates between two states were computed. Leaf nerve had three possible character states (0, absent; 1, single; 2, double) so three instantaneous forward (q_{01}, q_{02}, q_{12}) and three backward (q_{10}, q_{20}, q_{21}) rates had to be calculated for this character. The space of rate parameter values was visited with an MCMC chain (10,050,000 iterations, 50,000 burnin) and, for each repetition, a tree was sampled (every 10,000 generations) and the new state accepted or rejected. The rate at which these parameters change had to be set at the beginning of each run (ratedev), and its value confined the acceptance rate of the
proposed change between 20 and 40 percent. Not all trees selected contain the 39 nodes we aimed to reconstruct. For this reason reconstructions were performed via a MRCA (most recent common ancestor) approach so that, when the node of interest did not exist, the minimal node that contained all terminal taxa present in the clade defined by our node of interest (plus one or more extra taxa) was reconstructed instead.

Six evolutionary models describing trait evolution were compared for each trait (Barker et al., 2007): transitions and reversals both possible at different rates \( q_{01} \neq q_{10} \); transitions and reversals at the same rate \( q_{01} = q_{10} \); no transitions possible \( q_{01} = 0 \); no reversals possible \( q_{10} = 0 \); transitions possible but infrequent \( 0 < q_{01} < 1 \) and \( 0 < q_{10} < 100 \); and reversals possible but infrequent \( 0 < q_{01} < 100 \) and \( 0 < q_{10} < 1 \). The model where \( q_{01} = 0 \) presents a scenario in which a state when present at the root, is subsequently lost in different lineages and cannot be regained (Barker and Pagel, 2005). When \( 0 < q_{01} < 1 \), the state can be regained with low probability. The opposite situation occurs when \( q_{10} = 0 \); that is, once a certain state is gained for a trait, it cannot be lost. If \( 0 < q_{10} < 1 \), loses can occur on rare occasions. We used Bayes Factors (BFs) to determine which model best fit our data for each trait (Kass and Raftery, 1995). Harmonic mean estimators (HME) were used to calculate BFs. Thus, given \( 2(H_{1} - H_{0}) \), values between 0 and 2 were considered not significant, between 2 and 6 as significant, 6 to 10 as strongly significant, and greater than 10 as very strongly significant. The best-fit model in each case (Fig. 1) was then used to map each character.
Figure 1: Model selection using Bayes Factors for the reconstructed characters, them being: C1) differentiated leaf border or limbidium; C2) leaf nerve or costa; C3) cell length/width ratio in the middle of the leaf lamina; C4) ornamentation of the outer surface of the exostome teeth at their bases (ex. ornam.); C5) furrow at the central divisural line of the exostome teeth; C6) calyptra; C7) ST (strict terricolous).
mosses found on bare ground and/or decaying matter; C8) EL+TB (epilithic + tree bases), mosses on rocks or at the base of trees and/or shrubs; and C9) SEP (strict epiphytes), mosses on tree trunks, branches, and leaves. BF values between 0 and 2 were considered not significant, between 2 and 6 as significant, 6 to 10 as strongly significant, and greater than 10 as very strongly significant. Evolutionary models describing trait evolution for each trait compared: transitions and reversals both possible at different rates ($q_{01} \neq q_{10}$); transitions and transversals at the same rate ($q_{01} = q_{10}$); no transitions possible ($q_{01} = 0$); no reversals possible ($q_{10} = 0$); transitions possible but rare ($0 < q_{01} < 1$); and reversals possible but rare ($0 < q_{10} < 1$).

Pair-wise correlations among morphological characters and correlations of these morphological characters with habitat occupation where tested, again in a Bayesian framework, with Discrete in BayesTraits. The character, leave nerve, was recoded as absent (0) versus present (1) in order to test any possible correlation of this character with others because Discrete only allows correlation tests between binary traits.

Two models were compared using Bayes Factors (Kass and Raftery, 1995). In one the two traits evolve independently. Trait A has instantaneous rates $q_{01}$ and $q_{10}$ and trait B has instantaneous rates $q_{23}$ and $q_{32}$. In the second model the evolution of one trait depends on the state of the other, and dual transitions/reversals are not allowed. In this second model, given traits A and B at state 0, possible changes include either trait B transitioning to 1 while trait A stays at 0 ($q_{12}$) or trait A transitioning to 1 while B stays at 0 ($q_{13}$). Also in this model, given traits A and B at state 1, possible changes include either trait B reverting to state 0 while trait A stays at state 1 ($q_{42}$) or trait A reverting to state 0 while trait B stays at 1 ($q_{34}$). It is also possible, if correlated evolution is found, to test whether changes in one trait are contingent on the state of the other trait (comparing a
dependent model without restrictions to a dependent model where \( q_{12}=q_{34} \) and if there is directionality in this change (comparing a dependent model without restrictions to a dependent model where \( q_{12}=q_{13} \)), provided that that contingency exists (that is, only if the dependent model where \( q_{12}=q_{34} \) can be refuted). Best-fit model tests were evaluated using BF s (Table 3) comparing marginal log-likelihoods of these different models.

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(-) Does not apply.
2.3 Results

2.3.1 Sequence Amplification

We obtained plastid *rps4* and *trnLF* for all 123 accessions (except for *trnLF* for *Actinodontium adscendens*). Thirty-seven (30%) and 39 sequences (32%), respectively, were newly generated for this study. For mitochondrial *nad5* the final data matrix includes 115 sequences, 31 (25%) of which were newly generated. For nuclear ITS, the matrix included 105 sequences, 78 of them (63%) new, and for 26S, 109 sequences, 47 (38%) new. In total, the combined matrix included 574 sequences and missing data represented 7% of the matrix. The combined dataset included 6627 nucleotides, excluding 1502 indel characters. The highest number of informative characters came from the nuclear genome (644), followed by the plastid (407) and the mitochondrial (285).

2.3.2 Phylogenetic Analyses

Among supported clades, all data partitions converged to nearly identical topologies (data not shown) with no significant disagreement (plastid DNA topology differs but, since it lacks support, it does not conflict with other phylogenetic
reconstructions). Two main clades were resolved corresponding to the Hypnales and Hookeriales, each well supported as monophyletic.

In the Hypnales clade (Fig. 2), the genera *Rutenbergia* and *Trachyloma* are resolved as sister to the core Hypnales, although their early diverging position is not supported in most exploratory runs (data not shown). Sampling within the Hypnales was not extensive because our study focuses on the Hookeriales. Nevertheless, there were taxa included for which an ordinal placement has been debated. In that regard, a well-supported clade (i.e., “Symphyodontaceae”) consisting of *Chaetomitriopsis*, *Chaetomitrium*, *Dimorphocladon*, *Glossadelphus*, *Phyllodon* and *Symphyodon*, is resolved within the Hypnales; most of these taxa have previously been included in the Hookeriales.

Monophyly of the order Hookeriales (Fig. 2, node 1) sensu Buck et al. (2005) is corroborated with strong support from all analytical approaches (with the exception of chloroplast-only runs, where deep nodes are not supported). Within the Hookeriales clade, the monophyletic Hypopterygiaceae (Fig. 2, node 2) is resolved as sister to other families with strong support. Relationships in the Hypopterygiaceae clade do not differ from those reconstructed in Shaw et al. (2008) for all strongly supported clades. In the clade sister to the Hypopterygiaceae (Fig. 2, node 6; i.e., remaining Hookeriales), *Ancistrodes genuflexa* and *Sauloma tenella* form a well-supported clade (Fig. 2, node 7; i.e., Saulomataceae) sister to the remaining taxa (Fig. 3, node 8).
Figure 2: Phylogram inferred in PAUP* v4.0b (concatenated dataset) with details on the Outgroup (Hypnodendrales, Ptychoniales, and Hypnales), and the hookerialean families Hypopterygiaceae and Saulomataceae. The thickness of subtending branches is proportional to number of analysis supporting any given clade. Above branches from left to right ML bootstrap support (BS) and
heterogeneous Bayesian model posterior probability (PP). Below branches from left to right MP BB and homogeneous Bayesian model PP. (*) Maximum support (i.e., 100 BS and 1.00 PP). (-) BS < 80% and PP < 0.80. Branch length (BL) units: substitutions per site per year (see scale). Numbers in grey indicate nodes used in character state reconstruction.

Figure 3: ML Phylogram inferred in PAUP* v4.0b (concatenated dataset) with details on the hookerianal families Daltoniaceae, Schimperobryaceae, Hookeriaceae,
The genus *Achrophyllum* is resolved in a clade with most of the Daltoniaceae (Fig. 3, node 9; hereafter Daltoniaceae I), though support is lacking from this node in all reconstructions. However, *Calyptrochaeta* (Fig. 3, node 21), which is generally included in the Daltoniaceae, is reconstructed as sister to *Schimperobryum, Hookeria*, and *Crossomitrium*, three monogeneric clades, and the Leucomiaceae (clade composed of three genera) plus Pilotrichaceae (Fig. 3, nodes 20 and 22). This relationship is, however, not supported in any of our trees.

Within Daltoniaceae I, deep nodes resolve a series of clades mostly composed of monotypic or small genera such as *Adelothecium, Beeveria, Benitotania*, and *Ephemeropsis*. A clade (Fig. 3, node 12) that includes the large and polyphyletic genus *Distichophyllum*, and also the genera *Daltonia* and *Leskeodon*, which are not monophyletic, follows suit. Clades resolved within *Distichophyllum* and *Leskeodon* correspond to geographical regions rather than to groups united by morphology (see Appendix A for distributions).

Within the Pilotrichaceae (Fig. 4, node 28), two well-supported subfamilies traditionally recognized in the family, namely the monogeneric Pilotrichoideae (Fig. 4, node 29) and the Hypnelloideae (Fig. 4, node 30), are resolved as reciprocally monophyletic.
Figure 4: ML Phylogram inferred in PAUP* v4.0b (concatenated dataset) with details on the hookerilian family Pilotrichaceae. The thickness of subtending branches is proportional to number of analysis supporting any given clade. Above branches from left to right ML BS and heterogeneous Bayesian model PP. Below branches from left to right MP BB and homogeneous Bayesian model PP. (*) Maximum support (i.e., 100 BS and 1.00 PP). (-) BS < 80% and PP < 0.80. BL units:
substitutions per site per year. Numbers in grey indicate nodes used in character state reconstruction.

Resolution within the Hypnelloideae is for the most part poor and most genera in the subfamily appear to be polyphyletic as currently circumscribed; e.g., Callicostella, Lepidopilidium, Lepidopilum, Stenodictyon, Thamniopsis, Trachyxiphium. Based on present sampling, Actinodontium (Hypnelloide, Fig. 4, clade within node 38), Cyclodictyon (Hypnelloideae, Fig. 4, clade within node 39), Diploneuron (Hypnelloideae, Fig. 4, node 36), Pilotrichidium (Hypnelloideae, Fig. 4, node 32), and Pilotrichum (Pilotrichoideae, Fig. 4, node 29) appear to be monophyletic. Some monotypic genera (e.g., Callicostellopsis, Hemiragis, Philophyllum, Stenodesmus) are nested within larger genera.

2.3.3 Ancestral State Reconstructions and Morphological and Habitat Type Correlations

For the presence/absence of a leaf border or limbidium (character 1), two models were substantially better than all other models (Fig. 1), i.e., a model where reversals are not possible (q_{10}=0) and a model where reversals are possible with a very low probability. The former, with one less parameter, was chosen (Sullivan and Joyce, 2005). Differentiated leaf borders are reconstructed as absent for the earliest node including all Hookeriales (Fig. 5, node 1), and gained multiple times without any reversals.
Figure 5: Reduced cladogram with morphological character state reconstruction results for characters 1 to 3. C1) differentiated leaf border, 0: absent, 1: present. C2) Leaf nerve or costa, 0: absent, 1: present and single, 2: present and double. C3) Cell length/width ratio, 0: short (≤3:1), 1: long (> 3:1). (0) in white, (1) in black, and
(2) in grey. Reconstructed nodes are numbered. The thickness of subtending branches is proportional to BS and PP values.

A model where losses and gains occur with equal probability ($q_{01}=q_{10}$) is optimal (BF>2) for the presence/absence (and number) of leaf nerves (Fig. 1). The leaf nerve (character 2) is reconstructed as single for node 1 (Fig. 5). It was subsequently lost in Hookeria (Fig. 5, node 25), and regained as double in Pilotrichaceae (node 28). For leaf lamina cell length/width ratio (character 3), a model where transitions are not allowed ($q_{01}=0$) best fits the data (Fig. 1). Cells with a length/width ratio (Fig. 5) greater than 3:1 represent the ancestral condition in Hookeriales (node 1). State changes from long to short cells are inferred for the families Hypopterygiaceae (nodes 2–5) and Daltoniaceae (nodes 9–19), and for the genera *Calyptrochaeta* (node 21), *Hookeria* (node 25), *Pilotrichum* (node 29), *Diploneuron* (node 36), *Callicostella* (node 37), and *Cyclodictyon* (node 39).

The best-fit model (Fig. 1) for character 4 (outer exostome ornamentation) allowed for transitions and reversals at the same rate ($q_{01}=q_{10}$). The ancestral peristome state for the Hookeriales (Fig. 6, node 1) is striate. Transitions to a papillose exostome cannot be refuted for the genera *Daltonia* (Fig. 6, node 19), *Pilotrichum* (Fig. 6, node 29), and *Lepidopilum* (node 34). Similarly, regains of striate ornamentation cannot be rejected for *Distichophyllum* sensu stricto (Fig. 6, node 18), and several clades in the Pilotrichaceae (Fig. 6, nodes 30, 35).
Figure 6: Reduced cladogram showing morphological character state reconstruction results for characters 4 to 6. C4) Exostome ornamentation, 0: smooth, papillose or weakly striolate, 1: conspicuously striate. C5) Exostome furrow, 0: absent, 1: present. C6) Calyptra, 0: cucullate, 1: mitrate. (0) in white, and (1) in black.
Reconstructed nodes are numbered. The thickness of subtending branches is proportional to BS and PP values.

The presence of a median furrow on the outer surfaces of the exostome teeth (character 5) was reconstructed as the ancestral character state in Hookeriales (node 1) under a best-fit model (Fig. 1) that allows for frequent loses of this furrow and very rare regains (0<q<1). Hypopterygiaceae (Fig. 6, nodes 2, 3, 4, 5) lack this furrow, as do Daltonia (Fig. 6, node 19), Hookeria (Fig. 6, node 25), and Pilotrichum (node 29). Since ancestry could not be inferred for nodes 22, 23, and 24 (the position of Hookeria, node 24, is not supported in any of our final reconstructions), regains of this furrow cannot be refuted in subsequent nodes (Fig 6).

Calyptreae (character 6) in Hookeriales can be either cucullate or mitrate. Ancestral state reconstruction under a model allowing for transitions and reversals at different rates (q≠q) suggests likely states for many nodes but does not resolve the state for node 1, which is the most recent common ancestor for all extant Hookeriales.

Both Daltoniaceae I (Fig. 6, nodes 9 to 19) and Pilotrichaceae (Fig. 6, nodes 28 to 39) have mitrate calyptrae, whereas Hypopterygiaceae (Fig. 6, nodes 1 to 5) and Leucomiaceae (Fig. 6, node 27) ancestrally have cucullate calyptrae.

Few species are restricted to rocks or tree bases so taxa scored as present in saxicolous habitats were not necessarily absent from terricolous or epiphyllous substrates.
Figure 7: Reduced cladogram showing character state reconstruction results for characters 7 to 9. C7) Terricolous habitat, 0: absent, 1: present. C8) Saxicolous habitat, 0: absent, 1: present. C9) Epiphytic habitat, 0: absent, 1: present. (0) in white, and (1) in black. Reconstructed nodes are numbered. The thickness of subtending branches is proportional to BS and PP values.
The best-fit model (Fig. 1) for taxa that grow in both terricolous (character 7) and saxicolous (character 8) habitats was where transitions and reversals were possible at different rates ($q_{01} \neq q_{10}$). However, ancestral states at few nodes were reconstructed with significant probabilities (Fig. 7). The only genera that ancestrally grew on terricolous substrates are *Hookeria* (node 25) and *Diploneuron* (node 36). The only ancestor that was reliably absent from epilithic substrates is the MRCA of the genus *Hookeria* (node 25). Epiphytism best fits a model with both transitions and reversals possible at the same rate ($q_{01} = q_{10}$). All nodes were reconstructed as ancestrally absent from trees, except for nodes 11, 15, 22, and 38, which could not be reconstructed with confidence.

Pairs of traits that showed correlations (Table 3) were leaf limbidium (character 1) and leaf cell length ratio (character 3), exostome divisural line (character 5) and exostome ornamentation (character 4), and epiphytism (character 9) and exostome divisural line (character 5). The state of any of these characters was not contingent on the state of the character they were correlated to ($q_{12} = q_{34}$ could not be rejected). Since contingency did not exist, directionality could not be assessed.

### 2.4 Discussion

#### 2.4.1 Monophyly of the Order Hookeriales

The Hookeriales are currently circumscribed (see Goffinet et al., 2008) to include seven families, the Daltoniaceae, Hookeriaceae, Hypopterygiaceae, Leucomiaceae, Pilotrichaceae, Saulomataceae, and Schimperobryaceae. However, Newton et al. (2007;
2009) raised reasonable doubts about the validity of this circumscription. They suggested that the Hypnales are paraphyletic relative to the Hookeriales, since in their phylogenetic analyses the latter include the genera *Rutenbergia* and *Trachyloma*. Moreover, they reconstruct the Hypopterygiaceae (as summarized in Goffinet et al., 2008) as a clade outside the Hookeriales and Hypnales. Nevertheless, results from our study corroborate the monophyly of the order Hookeriales with the inclusion of Hypopterygiaceae, supporting the findings of Buck et al. (2005). Furthermore, the genera *Rutenbergia* and *Trachyloma* are resolved within the Hypnales clade with maximum posterior probability, though bootstrap support was only moderate (74% ML and 64% MP). Newton et al. (2009) based their inferences exclusively on sequences from the plastid genome, specifically the *trn*LF and rps4 genes, whereas our analyses include information from the nuclear and mitochondrial genomes as well. Indeed, our plastid data alone do not resolve relationships of the Ptychomniaceae, Hypnales, Hypopterygiaceae, and the rest of Hookeriales with strong support. Much of our sequence data supporting this topology resolved by our combined multilocus data matrix (Fig. 2) comes from the nuclear genome.

Within the Hypnales, the well-supported clade consisting of *Chaetomitriopsis*, *Chaetomitrium*, *Dimorphoclados*, *Glossadelphs*, *Phyllodon* and *Symphyodon* is noteworthy because *Glossadelphs* and *Phyllodon* have never previously been associated with the Symphyodontaceae, and also because all taxa in this clade were once considered allied
to the Hookeriales. If these genera are included in the Symphyodontaceae, morphological circumscription of the family summarized in Goffinet et al. (2008) appears to remain unchanged. Nevertheless, more taxon sampling within the Hypnales, especially members of Hypnaceae, is necessary for a better delimitation of the Symphyodontaceae.

2.4.2 Familial and Generic Relationships in Hookeriales

The relationships of familial clades reconstructed in our analyses are in agreement with those of Buck et al. (2005). In addition, clades lacking support coincide with results from their analyses, although the topologies retrieved within these clades are slightly different. In Buck et al. (2005), *Calyptrochaeta* is placed in a clade together with all other Daltoniaceae. In our phylogeny, *Calyptrochaeta* is sister to the Schimperobryaceae, Hookeriaceae, Leucomiaceae and Pilotrichaceae; and *Achrophyllum* is sister to the core Daltoniaceae. However, in both cases, the critical nodes (here nodes 9 and 20, Fig. 4) lack support. Morphology of *Calyptrochaeta* is ambiguous and does not help infer relationships. Species in this genus have leaf borders. Early diverging core Daltoniaceae and *Achrophyllum* lack leaf borders, but so do *Schimperobryum*, *Crossomitrium*, *Hookeria*, the Leucomiaceae and most Pilotrichaceae. Then, *Calyptrochaeta* and the Daltoniaceae (including *Achrophyllum*) have short cells (L/W ≤ 3:1, see description of character 3 in section 2.2.4), but other clades (e.g., *Schimperobryum*,
Crossomitrium, Hookeria) around these conflicting nodes have long cells (L/W > 3:1).

Further work is required to resolve the placement of the genus Calyptrochaeta.

Although the Hookeriaceae, Leucomiaceae and Pilotrichaceae form a clade (Fig. 4, node 24) with moderate support, the position of Hookeria (node 25) with respect to Crossomitrium (node 23) and the Leucomiaceae plus Pilotrichaceae (node 26) remains uncertain. Node 23 is fully supported in all Bayesian runs but not so in the MP and ML analyses; node 24 lacks support in all analyses. In fact, in our parsimony analyses, the positions of Hookeria and Crossomitrium are exchanged if compared to all other reconstructions. The segregation of Crossomitrium into its own family, which was hinted at by Buck et al. (2005), is not justified by our results. As Buck et al. (2005) did, we tentatively retain the genus in the more traditionally defined, and probably paraphyletic, Hookeriaceae. Additional data are needed to resolve this issue.

Relationships within the Hypopterygiaceae are similar to those reconstructed by Shaw et al. (2008). The only divergence from their topology lies in the position of Lopidium concinnum, which in this study is sister to all other Hypopterygiaceae except for Cyathophorum bulbosum. Shaw et al. (2008) resolved L. concinnum as sister to a clade consisting of the genera Dendrohypopterygium and Hypopterygium, a topology that we observe in our plastid tree without support. Although these topologies seem incongruent, support at the critical nodes is weak in both analyses. Sauloma and Ancistrodes are the only two Saulomataceae genera included in molecular phylogenetic
analyses to-date. The position of the Chilean endemic, *Vesiculariopsis spirifolium*,
hypothesized to belong in this family (Buck et al., 2005), remains to be tested.

The Daltoniaceae form a strongly supported monophyletic group, with the exception of the genera *Achrophyllum* and *Calyptrochaeta*. Deep nodes within this clade correspond to small genera with one to two species. Larger genera such as *Daltonia*, *Distichophyllum*, and *Leskeodon* are clearly not monophyletic. The phylogenetic position, in the Daltoniaceae, of the monotypic genera *Leskeodontopsis* and *Metadistichophyllum* remains uncertain. Relationships within *Daltonia* are further discussed in Yu et al. (2010), and a more thorough phylogeny of the polyphyletic *Distichophyllum* can be found in Ho (2010).

Although the family Leucomiaceae is well supported, relationships among the three genera in this family are not fully supported in all reconstructions. The double-costate Pilotrichaceae includes large genera that are not monophyletic as currently circumscribed. Current taxon sampling within the genera *Cyclodictyon* and *Actinodontium*, including their types, indicates that these genera are monophyletic. Species of *Callicostella* sampled from different continents are closely related and form monophyletic groups, except for *C. colombica*, which shows a closer affinity to *Trachyxiphium guadalupense*, the type of that genus. Generic circumscriptions for *Callicostella* and *Trachyxiphium* clearly need reassessment. Interestingly, four type species, *Lepidopilidium portoricense*, *Lepidopilum scabrisetum*, *Stenodesmus tenuicuspis*, and
Stenodictyon wrightii, grouped together in a single well-supported clade (Fig. 5, node 34). If this group is supported by future studies, and its taxonomic circumscription is formalized at the generic level, the clade would be named Lepidopilum, the oldest name among the four. It is also clear that the traditional separation of Lepidopilum and Lepidopilidium by their daltoniaceous and hookeriaceous peristomes, respectively, is not corroborated by molecular phylogenetic data (compare Fig. 5).

Our phylogeny agrees with Buck (1987) in that the broad genus Hookeriopsis; i.e., including Brymela, Thamniopsis and Trachyxiphium, is heterogeneous. However, the clades resolved by molecular data do not correspond to these genera. Other genera currently ascribed to this family that need to be included in future studies are Amblytropis, Helicoblepharum, and Hookeriopsis. Re-evaluations of generic boundaries within Pilotrichoideae, in conjunction with molecular phylogenetic studies, are also needed.

2.4.3 Evolution of Habitat Preference and Gametophytic Versus Sporophytic Characters in Hookeriales

Habitat reconstructions suggest that most clades in this order contain generalist species, with few clades restricted to a single substrate; for example, Hookeria and Diploneuron (strictly terricolous, character 7, Fig. 7). In contrast to analyses of the hypnalean family Neckeraeae (Olsson et al., 2009b), where phylogenetic habitat specialization was observed, no pattern of habitat preferences at the familial level are resolved within the Hookeriales.
Results from reconstructing morphological traits show that the common ancestor of the Hookeriales likely had elimbate leaves with elongate cells (length/width > 3:1), a single costa, and conspicuously striated lower outer exostome surfaces lacking a median furrow (Figs. 5 and 6). Limbate leaves were derived from elimbate leaves at least four times in the Hookeriales (nodes 2, 14, 21, and 39). Limbate leaves represent a synapomorphy for the Hypopterygiaceae (node 2), a clade in the Daltoniaceae comprising \textit{Daltonia}, \textit{Distichophyllum}, and \textit{Leskeodon} (node 14), the genus \textit{Calyptrochaeta} (node 21), and the genus \textit{Cyclodictyon} (node 39) in the Pilotrichaceae.

The MRCA of the Hookeriales is reconstructed as having a single costa that was lost in \textit{Hookeria} (node 25) and probably in \textit{Crossomitrium} (node 24), and regained in the Pilotrichaceae as double (node 26). In fact, all clades basal to the core pleurocarps (Bell et al., 2007); i.e., Rhizogoniales, Bryales, etc., have single leaf nerves. Presence of single-nerved leaves is therefore likely a sympleisomorphy within the pleurocarps. Miller (1971) suggested that a strong leaf nerve (whether single or double) is primitive within pleurocarpous mosses whereas the ecostate condition is derived. Robinson (1975) proposed evolution in the opposite direction; that is, from ecostate to costate. Our results suggest that the evolution of costae within pleurocarpous mosses was more complex than either of these simpler scenarios suggest. Current sampling within the Hypnales was limited, but it seems that leaf nerves in pleurocarps evolved from the sympleisomorphic unicostate state to ecostate or short bicostae. The long bicostate leaves
in *Callicostella* (Pilotrichaceae, Hookeriales) and some species of *Chaetomitrium* (Symphyodontaceae, Hypnales) are secondarily developed from ecostate leaves. In other words, the double costae are new innovations and not homologous to the leaf midrib in unicostate taxa. In fact, the ecostate and doubly costate leaves in the Hypnales and Hookeriales represent homoplasy. Leaf costae appear to have been lost and gained as double in separate evolutionary events.

We reconstruct the ancestral state of leaf cell shape in Hookeriales as elongate (> 3:1). However, our analyses do not permit inferences about evolutionary transitions (all PPs values were < 0.90) of calyptra type (mitrate, cucullate) because of uncertainty associated with node 1. Nevertheless, mitrate calyptra is reconstructed as a synapomorphy for the Saulomataceae (node 7), the Daltoniaceae sensu lato (nodes 9 to 19), *Hookeria* (node 25), and the Pilotrichaceae (nodes 28 to 39). Moreover, our results suggest that, since node 20 (which includes node 27) is reconstructed as mitrate, cucullate calyptrae in the Leucomiaceae (node 27) most probably arose from mitrate types (Fig. 6).

Shifts in the selected sporophytic traits are rather frequent. Horizontal striations and median vertical furrows on the outer exostome surfaces have been lost several times in the Hookeriales. The common ancestor of Hookeriales is reconstructed to have had striated teeth and a medial furrow, but these features were lost and then regained in a number of clades; i.e., Saulomataceae (node 6), early nodes in the Daltoniaceae sensu
lato (nodes 9 and 10), the genus *Calyptrochaeta* (node 21), and the Pilotrichaceae subfamily Hypnelloideae (node 30). The Hypopterygiaceae (nodes 2 to 5) have striate teeth but no furrow, as does the genus *Hookeria* (node 25). The only genera where papillose exostome teeth without medial furrow were reconstructed as a synapomorphy were: *Daltonia* (node 19) sensu stricto, and *Pilotrichum* (node 29). Because of within-clade polymorphism, these characters provide no useful criteria for the recognition of families.

Character 1 (presence or absence of a leaf limbidium) and character 3 (leaf cell shape) are correlated. Most probably developmental linkage is behind this correlation. This developmental linkage could be understood as morphological integration, that is, covariation of multiple traits, or as developmental modularity, where parts within modules (i.e., leaves) are tightly integrated and show strong interactions (Klingenberg, 2008). The same applies to the exostome teeth. We found a correlation between characters 4 (exostome striation) and 5 (median furrow), which we cannot further explain with our current data. Exostome morphology and habitat were also correlated. The epiphytic environment presents a number of challenges to those organisms that live in it; e.g., water shortage, nutrient availability, irradiation (Zotz and Bader, 2009; Zotz and Hietz, 2001). The consensus view is that peristomes participate in the regulation of spore dispersal (Ingold, 1959). Patterson (1953), and later Lazarenko (1957), noted an "aberrant behavior in the peristome teeth" of some corticolous bryophytes. Other authors have also noted correlations between sporophytic characters and epiphytism.
(Hedenäs, 2001; Huttunen et al., 2004; Olsson et al., 2009b; Vitt, 1981). However, it is not clear how the presence or absence of a furrow in the exostome teeth could affect spore dispersal in epiphytic bryophytes.

**2.5 Concluding Remarks**

Morphological characters from the gametophytic generation have been used in the taxonomy of other plant groups such as lycopods (Wagner and Beitel, 1992), ferns (Atkinson, 1973; Smith et al., 2006; Windham and Haufler, 1986), gymnosperms (Dehgan and Dehgan, 1988), and angiosperms (pollen, i.e., Judd and Olmstead, 1994; Stuessy, 2009). Holub (1985) noted that in the lycopod family Huperziaceae the morphology of the gametophyte did not preclude that of the sporophyte and vice versa. We have shown that in Hookeriales sporophytic characters are at least as labile as gametophytic traits and a priori reliance on features restricted to one generation or the other can be misleading. Homoplasy in the sporophyte generation has also been observed in the acrocarpous moss family Funariaceae (Liu et al., 2012). We have demonstrated parallel changes and reversals in traits from both generations. Rather than relying on assumptions about the relative conservatism of morphological evolution in gametophytes versus sporophytes, evolutionary interpretations should be based on phylogenetic evidence from independent data sets.
3. Historical Diversification Patterns, Ancestral State Evolution, and Character Correlations in the Pleurocarpous Moss Genus Calyptrochaeta Desv.

This Chapter has yet to be submitted for publication in a peer-reviewed journal.

3.1 Introduction

Discerning historical diversification patterns is paramount to understand evolutionary processes such as, genetic divergence among populations, speciation, and evolutionary change in morphology, physiology and/or behavior (Pagel 1999, Rabosky 2009). Although these historical diversification patterns are often inferred from the fossil record, it is also possible to use molecular phylogenies to estimate diversification rates using model-based approaches (Rabosky 2006a, Rabosky and Lovette 2008, Rabosky and Sorhannus 2009). However, it is still challenging to draw information on extinction rates from molecular phylogenies (Rabosky 2010), though some attempts exist in the literature (Etienne et al. 2012, Morlon et al. 2011).

A number of studies have addressed diversification rates in bryophytes. Wall (2005) presented evidence of rapid radiation in the genus Mitthyridium (Calymperaceae, Dicranales), which he hypothesized could be driven by a key innovation (a shift from sexual to asexual reproduction). Huttunen et al. (2008), calculated diversification rates in Homalothecium (Brachytheciaceae, Hypnales) and also inferred a recent rapid radiation, obtaining diversification rates similar to those found by Wall (2005) for Mitthyridium.
Shaw et al. (2003b) showed phylogenetic evidence for rapid radiation in the speciose pleurocarpous moss order Hypnales, but could not reject a model of constant diversification through time for the Hookeriales. Newton et al. (2007) also calculated diversification rates in pleurocarpous mosses but did not detect the rapid radiation Shaw et al. (2003b) had observed for the Hypnales. Besides, they showed that, under a relaxed clock model, the pleurocarpous moss radiation shadowed that of angiosperms. Like Newton et al. (2007), Fiz-Palacios et al. (2011) also found evidence of moss radiation shadowing angiosperm radiation.

In liverworts, Wilson et al. (2007) have shown that, within the family Lejeuneaceae, the tropics have accumulated diversity throughout the Cenozoic and that recent temperate species in this family are nested within clades composed of mainly tropical species. These results are in accordance with the tropical conservatism (TC) hypothesis.

This TC hypothesis (Wiens and Donoghue 2004) posits that: 1) tropical climates have been environmentally stable for a longer time than temperate ones (Barron 1995) and that 2) if niches are conserved, adaptations/ transitions to temperate climates are rare (Peterson et al. 1999). Hence, under this TC scenario we expect tropical regions to concentrate more species and temperate clades to be younger than and nested within tropical ones (Wiens and Donoghue 2004). Most terrestrial biodiversity is concentrated
in the tropics (Chown and Gaston 2000) and the TC hypothesis provides a compelling explanation for this observed biodiversity pattern.

The genus *Calyptrochaeta* Desv. (Daltoniaceae, Hookeriaceae) is found almost exclusively in the Southern Hemisphere, with most of its diversity concentrated in the tropics but with some species in temperate regions (Akiyama and Suleiman 2001, Buck 1987, Churchill 1998, Fife 1991, Florschütz-de Waard and Florschütz 1979, Gradstein et al. 2005, He 1998, Hill et al. 2006, Iwatsuki 2004, Matteri and Schiavone 2002, Mohamed and Tan 1988, O’Shea 2002 and 2006, Redfearn Jr. et al. 1996, Tan and Iwatsuki 1991 and 1993, Touw 1978 and 1992). This group is an excellent candidate to test the TC hypothesis and to address historical patterns of biological evolution. For this, we need to reconstruct relationships among species in the genus and test whether *Calyptrochaeta* had a tropical origin, if successive rounds of range expansions into temperate regions have taken place, and to test if these range expansions are correlated with morphological changes.

Specifically, in this study we wish to I) estimate the timing and pattern of diversification in the moss genus *Calyptrochaeta*, II) address the directionally of geographic diversification in this genus (i.e., from tropical to temperate regions), and III) test if changes in climatic zone preferences are correlated with morphological transitions in *Calyptrochaeta*. 
3.2 Materials and Methods

3.2.1 Sampling and Molecular Protocols

The Tropicos database (www.tropicos.org) lists 33 validly published species names for the genus *Calyptrochaeta*. Of these 33 *Calyptrochaeta* species, 18 were successfully sampled for DNA. In order to address the within species morphological and geographical diversity, 58 specimens from 22 countries were sampled.

The outgroup was composed of six species of the genus *Achrophyllum* Vitt & Crosby (seven samples from three countries) and eight other genera in the family Daltoniaceae (the monotypic *Adelothecium* Mitt., *Beeveria* Fife, *Benitotania* H. Akiy., T. Yamag. & Suleiman, *Bryobrothera* Thér., and *Metadistichophyllum* Nog. & Z. Iwats., and two *Daltonia* Hook. & Taylor, two *Distichophyllum* Dozy & Molk. sensu lato, and one *Distichophyllidium* M. Fleisch. species). In total, 77 specimens were included in the analyses. Voucher information and GenBank accession numbers can be found in Appendix B. Dr. Piers Majestyk, who recently monographed the genus *Daltonia* for America (Mayestyk 2011), identified the two specimens of that genus included in our analysis. Dr. Boon Chuan Ho, who recently reviewed the genus *Distichophyllum* (Ho 2010), helped identify both specimens sampled in here from that genus. The authors confirmed identifications of *Calyptrochaeta* specimens and all other remaining taxa.

DNA was successfully amplified from samples up to 95 years old. Three DNA regions were sampled for DNA, the *trnL* (UAA) 5’ exon-*trnF* (GAA) (hereafter trnL) and
the *trnG* (UCC) intron (hereafter *trnG*) from the plastid genome, and nuclear ribosomal
ITS1-5.8-ITS2-26S (hereafter ITS). Total genomic DNA was extracted using a modified
CTAB protocol (Doyle and Doyle 1990) following Shaw (2000). Double-stranded DNA
templates were amplified by polymerase chain reaction as in Pokorny et al. (2011).
Amplification and sequencing primers can be found in Shaw et al. (2003a). Cleaned PCR
products were sequenced in the DNA Sequencing Facility
(http://www.genome.duke.edu/cores/sequencing/) at the Institute for Genome Sciences
& Policy, Duke University.

### 3.2.2 DNA Sequence Editing and Alignment

For each sample and sequenced DNA region, forward (5′–3′) and reverse (3′–5′)
sequences were assembled and checked for inaccurate base calling using Sequencher
v4.1 (GeneCodes Corporation 2000). Consensus sequences were aligned manually using
Se-Al v2.00al Carbon (Rambaut 2002) and following described alignment rules by
Kelchner (2000), while trying to minimize substitutions and indels. This approach
combines event based and similarity criteria to produce a hypothesis about the
homology of the sequenced nucleotides (Simmons 2004; Morrison 2006). Regions of
incomplete data (i.e., at the beginning and end of sequences) or ambiguous alignment
were identified and excluded from subsequent analyses. We successfully obtained 76
(98.7%) *trnL*, 73 (94.8%) *trnG*, and 66 (85.7%) ITS sequences (Appendix B). Missing data
represent 7% of the combined data matrix.
3.2.3 Phylogenetic Analyses

Bayesian Inference (BI) and Maximum Likelihood (ML) analyses were performed on the individual loci as well as the concatenated matrix. For each partition, the best-fit substitution model (under AIC) was calculated with MrModeltest v2.2 (Nylander, 2004) and PAUP* v4.0a123 (Swofford, 2002). GTR+Γ was the best-fit model selected for both ITS and trnG. For trnL and the combined data set, GTR+Γ+I was the chosen best-fit model. Analyses of these individual loci revealed no significant conflicts (i.e., ≥ 70% bootstrap support, hereafter BS, or ≥ 0.95 posterior probability, hereafter PP) among the three sampled regions (data not shown), though the topology reconstructed for trnL diverged from that reconstructed for the other two sequenced regions. All analyses were run on the Duke Shared Cluster Resource (DSCR) at Duke University (https://wiki.duke.edu/display/SCSC/DSCR).

ML analyses, including 1000 ML bootstrap replicates, were performed in GARLI v2.0 (Zwickl 2006) on the concatenated dataset under a GTR+Γ+I model. Bayesian analyses were performed in MrBayes v3.2.1 (Ronquist and Huelsenbeck 2003) for a partitioned dataset (ITS+trnG+trnL). Model parameters for each partition (i.e., each of the three sequenced regions) were sampled independently. PP distributions of trees were generated using the Metropolis-coupled Markov chain Monte Carlo method. Four runs, with four chains (10x107 generations each), were run simultaneously. Chains were sampled every 5000 generations.
Results from ML and BI runs diverged several orders of magnitude in estimated branch lengths (BL). As Brown et al. (2010) note, leaving default settings in BL and rate priors (Ratepr = Fixed, Brlenspr = Unconstrained:Exp(10.0)) can lead to this error in BL estimation when genes sampled have strikingly different mutation rates. In our case trnL is much faster than both trnG and ITS so our prior Ratepr was set to Variable=Dirichlet, but the problem persisted and BI runs in MrBayes had to be repeated with more restrictive BL and rate priors. Trial runs included highly restrictive settings (Ratepr = Dirichlet (150,150,150) and Brlenspr = Unconstrained: Exp (100.0)), which effectively constrain the parameter space explored from which to draw BL values, but result in spurious runs, given that the data cannot overcome these restrictive settings (data not shown). An effective way to fix this problem is to disentangle time from substitution rates. Thus, divergence time estimation in a BI framework is preferred in this situation.

Divergence times were estimated using BEAST v1.6.2 (Drummond and Rambaut 2007). Time constrains were imposed on nodes corresponding to the most recent common ancestor (MRCA) of all C. asplenioides samples endemic to the Mascarene Islands (i.e., La Réunion and Mauritius), the MRCA of all C. grandiretis samples endemic to Juan Fernández Islands (i.e., Robinson Crusoe and Alejandro Selkirk) and the MRCA of elimbate Daltoniaceae Adelothecium bogotense (Hampe) Mitt., and Benitotania elimbata H. Akiy., T. Yamag. & Suleiman (Fig. 9).
Both constrains on endemic “Calyptrochaeta” clades are maximum age constraints based on geological time estimates. The Mascarene archipelago is not older than 8 Myr (Schlüter and Trauth 2008). As in Pokorný et al. (2011), the node corresponding to the MRCA of Mascarene samples was constrained with a prior lognormal distribution with mean at 8 (Myr) and a SD of 1, since now submerged islands could have presented suitable habitats for the origination of C. aspleniooides endemic haplotypes up to 35 Myr (Schlüter and Trauth 2008). The Juan Fernández archipelago is thought to be younger than 6 Myr (Stuessy et al. 1984). A lognormal prior distribution with mean 2.5 (Myr) and a SD of 0.55 was chosen to constrain the node corresponding to the MCRA of C. grandiretis, since that distribution encompasses all estimated ages for emerged land in the Juan Fernández archipelago (Stuessy et al. 1984).

Fossil Adelothecium cf. bogotense is reportedly found from Dominican amber deposits (Frahm 1993). Like other present day elimbate Daltoniaceae, i.e., Adelothecium bogotense, Benitotania elimbata, and Bryobrothera crenulata (Broth. & Paris) Thér., this amber fossil presents complanate leaves with lax cells, short apiculus, long single nerve, and no limbidium. However, as in the extant A. bogotense, but unlike in Benitotania and Bryobrothera, leaf cells in the fossil are unipapillate, which is an autapomorphy for the genus Adelothecium. The fossil is smaller (leaves, on average, 1mm long) than extant Adelothecium species (leaves usually 2mm long), and cannot be ascribed to A. bogotense.
with confidence. For this reason we constrain the node corresponding to the MRCA of both *A. bogotense* and *Benitotania elimbata* with this amber fossil.

Dominican amber deposits were formed 15 to 20 Myr ago (Iturralde-Vinent and MacPhee 1996). According to Iturralde-Vinent and MacPhee (1996), “the brevity of the depositional interval (less than 5 million years) provides a temporal benchmark that can be used to calibrate rates of molecular evolution in amber taxa”. Thus, a minimum age constrain was imposed on the aforementioned node corresponding to the MRCA of *Adelothecium* and *Benitotania*. A gamma prior distribution offset to 15 Myr with shape and scale of 2 was chosen to constrain this node and accommodate any uncertainty associated to the estimated ages for Dominican amber deposits.

BI analyses in BEAST were run for 80,000,000 generations. Parameter values were sampled every 2,000 generations. A Yule process was used as the speciation prior. The program Tracer v1.5 (Rambaut and Drummond 2009) was used to estimate burnin and to examine log likelihoods of all Bayesian runs, ensuring that these runs were in the stationary phase and that adequate effective sample sizes (ESS) were attained. Calculations of the consensus tree, of the posterior probability of clades, and of the confidence intervals (CIs) around our time estimates were performed based on the trees sampled after the chains converged in TreeAnnotator v1.6.2 (Drummond and Rambaut 2007). Consensus topologies and support values from the different methodological approaches were compiled and drawn using Tree v1.3.1 (Rambaut 2009).
3.2.4 Patterns of Diversity Dynamics

Diversification rates are calculated in here using the approach proposed by Simpson et al. (2011). Their method was developed explicitly to make estimates of rates from molecular datasets comparable to those made from fossil datasets.

In this method, diversification rates are calculated by selecting a window of time (13 Myr in our case) for a set of time-calibrated phylogenies and by dividing the number of nodes within that window by the sum of all BLs within that interval, including those that produce no nodes. The result is a diversification rate that can be generally understood as the rate of adding new species per lineage–million years (species / lineage x Myr). Unlike traditional LTT methods, this method is robust to poor sampling because it is based on the ratio of nodes to branches per interval, and not on total numbers of nodes per tree, as LTT methods are (Simpson et al. 2011).

Because both our ML and BI phylogenetic reconstructions result in a highly supported topology, rather than calculating diversification rates in the post-burnin cloud of trees obtained in BEAST, as Simpson et al. (2011) propose, we have chosen a more simple approach implemented in R (R Development Core Team 2011) using a script provided by C. Simpson (pers. comm.)

In our approach, the yuleWindow function in LASER (Rabosky 2006b) is iterated only on the most optimal summary tree (rather than in a cloud of chronograms) over a timescale (55 Myr in our case). The resulting diversification rates give us an idea of the
patterns of diversification in *Calyptrochaeta* and *Achrophyllum*. However, since we lack a fossil record for these genera, it is not possible address weather sudden drops in diversification rates are due to a decrease in speciation or an increase on extinction rates.

### 3.2.5 Ancestral State Reconstructions and Correlations

Eight morphological characters (four gametophytic and four sporophytic) and occurrence in two climate zones (tropical, temperate) were binary coded (Table 4). Gametophytic characters coded include: limbidium presence (character 1: absent=0, present=1); limbidium thickness (character 2: ≤3 cells wide=0, >3 cells wide=1); cell diameter in mid leaf lamina (character 3: <40 µm=0, ≥40 µm=1); sexuality (character 4: dioicous=0, autoicous=1). Sporophytic characters coded are: distribution of seta ornamentation (character 5: absent or upper half only=0, present throughout=1); presence of seta ornamentation (character 6: absent=0, present=1); presence of conspicuous seta spines below capsule neck (character 7: absent=0, present=1); capsule orientation (character 8: erect to oblique=0, horizontal to pending=1). Habitat tolerances (climate zones) coded are: temperate (character 9: absent=0, present=1) versus tropical (character 10: absent=0, present=1).
Table 4: Character matrix. C1) limbidium presence (0: absent, 1: present); C2) limbidium thickness (0: ≤3 cells wide, 1: >3 cells wide); C3) cell diameter in mid leaf lamina (0: <40 µm, 1: ≥40 µm); C4) sexuality (0: dioicous, 1: autoicous); C5) distribution of seta ornamentation (0: absent or upper half only, 1: present throughout); C6) presence of seta ornamentation (0: absent, 1: present); C7) presence of seta spines below capsule neck (0: absent, 1: present); C8) capsule orientation (0: erect to oblique, 1: horizontal to pending); C9) temperate habitat tolerant (0: absent, 1: present); and C10) tropical habitat tolerant (0: absent, 1: present). (–) Unknown.

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61
Bayesian inference was used to reconstruct ancestral character states in the program BayesTraits (http://www.evolution.reading.ac.uk/BayesTraits.html) on 500 trees randomly sampled from the cloud of most optimal trees generated in BEAST after burnin. All reconstructions were performed on 33 nodes corresponding to well-supported (i.e., PP≥ 0.9) clades in our chronogram and defining taxonomically relevant groups. The model implemented to estimate probabilities of character state change per branch was a Multistate Markov model. For all characters we computed instantaneous forward \( q_{01} \) and backward \( q_{10} \) rates. An MCMC chain was run for 30 to 130 million iterations (burnin between 10 to 50 million) and sampled every 5 to 10 thousand generations, depending on the character being reconstructed. In order to get adequate mixing and ESS values in BayesTraits, the deviation of the rate of the normal distribution from which the rates are drawn from (ratedev parameter) has to be set so that the acceptance rate for that proposed change ideally stays between 20 and 40 percent. This was not always possible, thus outputs of runs with acceptance rates between 10 to 20 percent were also checked in Tracer v1.5 (Rambaut and Drummond 2009) for mixing and ESS values and accepted when these values were adequate. Not all

<table>
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<tr>
<th>Taxon</th>
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trees from the random set of chronograms contain the 33 target nodes, so a MRCA approach was chosen. With this approach, when a node does not exist, the minimal node containing all terminal taxa present in our target clade, plus some extra taxa, is the one reconstructed.

Six trait evolution models were compared for each character (Barker et al. 2007): both transitions and reversals possible at different rates \( q_{01} \neq q_{10} \); transitions and reversals possible at the same rate \( q_{01} = q_{10} \); no transitions possible \( q_{01} = 0 \); no reversals possible \( q_{10} = 0 \); low rate transitions possible \( 0 < q_{01} < 1 \) and \( 0 < q_{10} < 100 \); and low rate reversals possible \( 0 < q_{10} < 100 \) and \( 0 < q_{01} < 1 \). Bayes factors (BF) were calculated via harmonic mean estimators (HME) to assess optimal models (Kass and Raftery 1995). Values for BF between 0 and 2 were considered not significant, 2 to 6 were significant, 6 to 10 were strongly significant, and values greater than 10 were very strongly significant (Fig. 8). In each case, the best-fit model was then mapped for each character in the final tree using TreeGraph 2.0.42-187 beta (http://treegraph.bioinfweb.info/).

Also in BayesTraits, pair-wise correlations between morphological characters and habitat types where tested. Two models were compared (using BF), in one the two traits evolve independently, in the other model, given traits A and B at state 0, possible changes include either trait B transitioning to 1 \( (q_{12}) \) or trait A transitioning to 1 \( (q_{13}) \), and their opposites since the initial state can be 1 \( (q_{42} \) and \( q_{34} \).
Figure 8: Model selection using Bayes Factors for the reconstructed characters, them being: C1) limbidium presence; C2) limbidium thickness; C3) cell diameter in
mid leaf lamina; C4) sexuality; C5) distribution of seta ornamentation; C6) presence of seta ornamentation; C7) presence of seta spines below capsule neck; C8) capsule orientation; C9) temperate habitat tolerant; and C10) tropical habitat tolerant. BF values between 0 and 2 were considered not significant (horizontal axis cuts vertical at BF 2), between 2 and 6 as significant, 6 to 10 as strongly significant, and greater than 10 as very strongly significant. Evolutionary models describing trait evolution for each trait compared: transitions and reversals both possible at different rates ($q_{01}$=$q_{10}$); transitions and transversals at the same rate ($q_{01}$=$q_{10}$); no transitions possible ($q_{01}$=0); no reversals possible ($q_{10}$=0); transitions possible but rare (0<$q_{01}$<1); and reversals possible but rare (0<$q_{10}$<1).

In either of these two models (independent vs. correlated evolution) double transitions are not possible. If correlated evolution is detected, it is possible to test whether changes in one trait are contingent on the state of the other trait ($q_{12}$=$q_{34}$). Also, if that contingency exists ($q_{12}$=$q_{34}$), directionality can be addressed ($q_{12}$=$q_{13}$).

### 3.3 Results

#### 3.3.1 Sequence Amplification

DNA sequences were obtained for all trnL accessions except for sample LPM174. Plastid trnG only lacked four sequences (i.e., LPM113, LPM032, LPM249, LPM093) out of 77. Nuclear ITS was successfully sequenced for 66 samples. In total, the combined data matrix included 215 sequences (ca. 7% missing data) 2081 nucleotides long. Nuclear and plastid genomes provided comparable numbers of informative characters, 254 and 257 respectively.

#### 3.3.2 Phylogenetic Analyses

Both ITS and trnG showed no conflict and converged to similar topologies. On the other hand, the topology inferred from trnL is quite different (not shown).
Nevertheless, the trnL reconstruction lacks support under ML and BI, so all three regions were combined in the final runs (see section 3.2.3).

The genus *Calyptrochaeta*, as currently circumscribed, is not monophyletic. *C. grandiretis*, endemic to the Chilean Juan Fernández archipelago, is reconstructed as sister to all other *Achrophyllum* species, with maximum support in all analyses and is thus transferred to that genus (see section 3.4.5). We hereafter refer to this taxon as *A. grandirete*. All individual species sampled in the genus *Calyptrochaeta* are resolved as monophyletic, except for *C. otwayensis*, which is nested within *C. brownii* and is to be synonymized with the latter (see Discussion).

The outgroup, which is the clade labeled Daltoniaceae I in Fig. 9, converges some 55 Myr ago (Figs. 11–13). Within this clade, the genus *Achrophyllum* is monophyletic with maximum support in all reconstructions and converges 32 Myr ago. Within *Achrophyllum*, and in successive branching order, we find monophyletic *A. gradirete* sister to Chilean *A. haesselianum*, which is sister to New Zealand *A. quadrifarium*, which is sister to the *Achrophyllum* crown group, which converges 3 Myr ago and is composed of *A. anomalum*, *A. crassirete*, *A. dentatum*, and *A. magellanicum*. ML and BI support is the highest for the aforementioned nodes. Within the crown group, *A. dentatum* is sister to *A. crassirete* (85% ML BS and 1.0 PP) and *A. anomalum* is nested within *A. magellanicum* in a well-supported clade (88% ML BS and 1.0 PP).
Also within Daltoniaceae I and sister to Achrophyllum (Figs. 9, 11–13), a monophyletic clade with no ML BS and 1.0 PP converges 44 Myr ago. This clade comprises four elimbate (i.e., Adelothecium, Benitotania, Beeveria, and Bryobrothera) and six limbate taxa (with maximum support). *Metadistichophyllum rhizophorum*, known from Borneo (Malaysia) and Seram (Indonesia), is nested within the limbate Daltoniaceae.

Figure 9: Single optimal ML tree for the combined data matrix including ITS, trnG and trnL sequences (see methods for details). Numbers in grey indicate ML bootstrap support after 1000 replicates and numbers in black indicate BI PP after
burning for our BEAST runs. Sample numbers (in black) and taxon names (in grey) as in the Appendix.

All *Calyptrochaeta* samples converge over 52 Myr ago with maximum ML BS and 0.94 PP. Two main clades are resolved in this genus (Figs. 9, 11–13). *Calyptrochaeta cristata*, from New Zealand, and *C. asplenioides*, from Africa and Indian Ocean islands, forms one clade (84% ML BS and maximum PP). In the other clade we have all American, Asian, and other Australasian taxa sampled for this genus (maximum support). Both clades converge 33 Myr ago.

Samples of New Zealand *C. cristata* converge, with maximum support, 1 Myr ago. Those corresponding to African *C. asplenioides* converge, also with maximum support in all reconstructions, ca. 14 Myr. Within *C. asplenioides* two main clades (with maximum support) can be identified. On one hand, temperate South African samples converge 0.5 Myr ago. On the other, tropical Eastern African and Indian Ocean samples converge ca. 7 Myr ago.

The other main clade within *Calyptrochaeta*, comprising all American, Asian, and the remaining Australasian species, is subsequently divided into two clearly geographically delimited clades (Figs. 9, 11–13). All the exclusively New World species sampled —i.e., *C. haitensis*, *C. leptoloma*, and *C. nutans*— group in one clade (76% ML BS and maximum PP), and all the exclusively Old World taxa (except for *C. asplenioides* and *C. cristata*), plus Holantarctic *C. apiculata*, group in another clade (maximum support).
Although the exclusively New World species are reconstructed as monophyletic with high Bayesian PP (maximum support for \textit{C. haitensis} and 0.97 PP for \textit{C. nutans}), ML BS is low (no support for \textit{C. haitensis} and 70% BS for \textit{C. nutans}). These three taxa diverged $\approx$ 11 Myr ago (maximum PP and 76% ML BS) and, although \textit{C. haitensis} and \textit{C. nutans} are reconstructed as sister taxa, support from ML and BI runs is lacking (0.71 PP, no ML BS). Sister to this poorly supported clade is \textit{C. leptoloma}, a species endemic to the Juan Fernandez archipelago. Since we only have one sample of this latter species, we cannot estimate its age nor test its monophyly. Caribbean \textit{C. haitensis} converges 4 Myr ago and northern South American \textit{C. nutans} converges 3 Myr ago.

Old World taxa (with the exception of \textit{C. asplenioides} and \textit{C. cristata}), plus Holantarctic \textit{C. apiculata}, belong in a well-supported clade (maximum support in all reconstructions) that converges 20 Myr ago. Although early branching within this clade is not well resolved, it seems clear that all temperate Australasian taxa are sister to a clade including all Asian and Melanesian taxa (Figs. 9, 11–13).

Two alternative topologies are retrieved within this Old World group of species for these early-branching clades, but neither is supported. On one hand, our ML tree has Holantarctic \textit{C. apiculata} sister to all other Old World taxa. On the other, our BEAST chronogram depicts \textit{C. apiculata} as sister to temperate Australasian \textit{C. flexicollis} in a clade sister to the remaining Old World taxa.
All samples of Holantarctic *C. apiculata* converge in a maximally supported clade 1.2 Myr ago. Similarly, all samples of Australasian *C. flexicollis* belong in a maximally supported clade in all reconstructions and converge 1.9 Myr ago. All samples of Australasian *C. brownii* and *C. otwayensis* also group in a maximally supported clade (composed of nearly identical sequences), which converge 2.3 Myr ago. As previously mentioned, *C. otwayensis* is to be synonymized to *C. brownii* (see Discussion).

All Asian and Melanesian species converge 15.3 Myr ago in a well-supported clade (92 ML BS and maximum PP). The first split in this clade has all *C. spinosa* samples (native to mainland SE Asia and southern China) in a monophyletic clade (maximum ML BS and PP) sister to the remaining taxa in another well-supported clade (84% ML BS and maximum PP). The age estimated for *C. spinosa* is 3.7 Myr.

The clade sister to *C. spinosa* is divided into two highly supported sister clades (Figs. 9, 11–13). One of these two clades (96% ML BS and maximum PP) includes New Caledonian *C. marginata* in a monophyletic clade (maximum support) sister to another clade (0.86 PP and no ML BS) that includes Philippine *C. microblasta* sister to a monophyletic *C. japonica* clade (72% ML BS and maximum PP). East Asian *C. japonica* is estimated to converge 4.6 Myr ago.

The other clade (80% ML BS and maximum PP) is composed of SE Asian *C. flaccida, C. perlimbata, C. ramosa,* and *C. remotifolia.* Both *C. ramosa* and *C. remotifolia* are clearly monophyletic. Support is high for *C. ramosa* (99% ML BS and maximum PP) and,
although *C. remotifolia* has high PP (0.97), it lacks ML BS. Although *C. flaccida* and *C. perlimbata* are in the same clade, their relationship is not supported. With our current sampling, *C. ramosa* is estimated to be 0.9 Myr old and *C. remotifolia* is 7.2 Myr old.

### 3.3.3 Diversification Analysis

Over the Paleogene Period, our study group displays a general declining trend in net diversification rate. On the contrary, during the Neogene Period, the overall diversification rates increase. Also, most shifts in diversification rate coincide with well-established notable climate events in Earth history (lines A to I in Fig. 10).

**Figure 10:** Species-level diversity curve (Simpson et al. 2011) derived from the after-burnin BEAST chronogram summary tree obtained in TreeAnnotator. Diversity is roughly measured as the number of boundary crossing species each 13 Myr interval (minimum number of species set to 1). Climatic events indicated are: A) EECO, B) MECP, C) MECO, D) Oi-1 glaciation, E) Mi-1 glaciation, F) MMCO, G) MMCT, H) WAIS, and I) LPCE. (Pg) Paleogene Period. (Ng) Neogene Period.
Figure 11: BEAST chronogram with morphological character state reconstruction results for gametophyte characters (C1–C4). C1) Limbidium presence, 0: absent, 1: present. C2) Limbidium thickness, 0: ≤3 cells wide, 1: >3 cells wide. C3) Cell diameter in mid leaf lamina, 0: <40 µm, 1: ≥40 µm. C4) Sexuality, 0: dioicous, 1: autoicous. (0) in white and (1) in black. Reconstructed nodes are numbered in grey. Thickness of subtending branches is proportional to PP values.

3.3.4 Ancestral State Reconstructions and Correlations

A model where no reversals are possible (\(q_{10}=0\)) performed best (BF ≥ 2 when compared to the next best model) for characters 1, 2, 4 and 6 (Fig. 8). No loses in limbidium (C1) are detected once this character state is gained (nodes 8, 10, and 15; Fig. 11). The same applies to the thickness of the limbidium (C2), when present. Once the limbidium broadens (≥ 3 rows of cells), it does not appear to narrow (nodes 11, 21, 22, and 31; Fig. 11). Sexuality (C4) also fits a model where becoming autoicous is an irreversible state (node 16; Fig. 11). Finally, the presence of any kind of ornamentation on the sporophyte seta (C6, Fig. 12) best fits a model where reversals are not possible. The ancestral state in the study group for this character is the presence of ornamentation. Once that ornamentation disappears, it cannot be regained (nodes 3 and 7).

A model where transitions and reversals are both possible at the same rate (\(q_{01}=q_{10}\)) best fits characters 3, 8, and 9 (Fig. 8). This model was also chosen for character 10, despite the fact that it is not significantly better (BF values were not ≥ 2) than most other models, since this is the less parameter-rich model (Sullivan and Joyce, 2005).
Changes from narrower (<40 µm) to broader (≥40µm) cells in the mid-leaf lamina (C3, Fig. 11) and vice versa are equally probable, at such a low rate (0.0093) that no shifts between either character state could be reconstructed with confidence.

Shifts in sporophyte capsule orientation between erect to oblique and horizontal to pending (C8, Fig. 12), are also equally probable (rate = 0.0166); though lack of resolution in the outgroup and towards the root limits our understanding of the evolution of this character. Both climate zone preferences were also reconstructed under a \( q_{12} = q_{34} \) model (Fig. 8). The rate of change for character 9 is 0.0298 and that for character 10 is 0.0247. Thus, shifts between climatic zones can happen in either direction (Fig. 13).

Pair-wise comparisons between each morphological character and each climatic zone resulted in significant correlations for both habitat preferences coded and character 7 (presence of conspicuous seta spines below capsule neck). The constrained correlated model used to test whether changes in one trait are contingent on the state of the other trait (\( q_{12} = q_{34} \)) was not better than the unconstrained correlated model. Directionality of this change could not therefore be addressed. No other morphological characters were correlated with climate zone shifts.
Figure 13: BEAST chronogram showing habitat tolerance reconstructions (C9–C10). C9) Temperate habitat tolerant, 0: absent, 1: present. C10) Tropical habitat tolerant, 0: absent, 1: present. (0) in white and (1) in black. Reconstructed nodes are numbered in grey. Thickness of subtending branches is proportional to PP values.

3.4 Discussion

3.4.1 Timing and Patterns of Diversification in *Calyptrochaeta*

Bryophytes are known to be sensitive to their environment. They are excellent bioindicators (Chakrabortty & Paratkar, 2006) frequently used to monitor changes in airborne pollutants (Zechmeister & Hohenwallner, 2006), to assess forest integrity (Frego, 2007), and the hierarchical effects of habitat fragmentation on ecological and evolutionary processes (Pharo & Zartman, 2007). As Gignac (1992) noted for the genus *Sphagnum*, “habitat partitioning by peatmoss species indicates that climate is the most important factor affecting species niches and overshadows on a regional basis locally important ecological gradients”. Not surprisingly, bryophyte distributions can be modeled at a coarse scale from ecological information from occurrences, as Sérgio et al. (2007) and Kruijer et al. (2019) have already shown.

Results from our BEAST chronogram and our diversity rates analysis suggest patterns of diversification in our study group do not just coincidentally match notable climate events in Earth history. Though merely speculative, if bryophytes truly are sensitive to their environment and climate is indeed “the most important factor affecting species niches” (Gignac 1992), *Calyptrochaeta* may actually have a “historical memory” of these major climate change events recorded in its genome.
Diversification rate estimates for time intervals involving the root, or nodes near it, should be taken with care because of sampling limitations, error associated with divergence time estimation, and/or high extinction rates (Simpson et al. 2011). However, it is still apparent that there is a general declining trend in net diversification rate for our study group over the Paleogene Period. This decreasing diversification rate parallels the mean ocean surface temperature-cooling trend (≈ 4º C drop).

In the Paleogene, taxa within the Daltoniaceae I clade converge ≈ 55 Myr ago, at the Late Paleocene Thermal Maximum (hereafter LPTM; Zachos et al. 2001). Supported relationships among these taxa do not differ from those inferred by Pokorný et al. (2012). This climatic optimum (meaning LPTM) is part of the wider Paleocene–Eocene Thermal Maximum (PETM), which also includes the early Eocene Climatic Optimum (hereafter EECO; Zachos et al. 2001, Zachos et al. 2008). All Calyptrochaeta samples (A. gradirete excluded) converge ≈ 52 Myr ago, at the EECO (line A, Fig. 10).

Between the EECO and the end of the early mid-Eocene cooling period, MECP (line B, Fig. 10) — which took place ≈ 45 Myr ago (Pearson and Palmer 2000) — diversification rates have a negative slope for the study group. Taxa in Daltoniaceae I, excluding the genus Achrophyllum, converge ≈ 44 Myr ago, between the end of the MECP and the beginning of the Middle Eocene Climatic Optimum (hereafter MECO). The negative trend in diversification rates is reversed in this time period between the MECP and the MECO (line C, Fig. 10). The later is recognized to have taken place some 40 Myr

After the MECO and until the end of the Eocene Epoch (line D, Fig. 10), which is defined by the Eocene-Oligocene Extinction Event (hereafter EOEE) and concurrent with the Oi-1 glaciation (Liu et al. 2009, Miller et al. 1991, Zachos et al. 2001), the diversification rate declines.

Diversification rates in the study group increase soon after the Oi-1 glaciation ≈ 34 Myr ago. Both main clades in *Calyptrochaeta* (i.e., *C. asplenioides* and *C. cristata* on one hand and all other taxa sampled on the other) converge around this time. This is also when taxa sampled for the genus *Achrophyllum* (*A. gradirete* inclusive) converge.

Then, in the Oligocene-Miocene Boundary, ≈ 23 Myr ago (line E, Fig. 10) — which coincides with the Mi-1 glaciation event (Zachos et al. 2001) — we observe a new decline. Right before this glaciation event, clades including both all limbate and most elimbate (*Beeveria* excluded) Daltoniaceae sampled converge. This decline in diversification rates is then reversed and peaks 17 Myr ago (line F, Fig. 10), coinciding with the mid-Miocene climatic optimum (hereafter MMCO), which took place 18–16 Myr ago (Böhme 2003, Zachos et al. 2001, Zachos et al. 2008). At this time, the branch leading to *A. haesselianum* diverged and *Adelothecium* and *Benitotania* converged. But before that, between the Mi-1 glaciation and the MMCO, Old World *Calyptrochaeta* species (with the exception of *C. asplenioides* and *C. cristata*) plus Holantarctic *C. apiculata* converge ≈ 20 Myr ago.
Our next landmark is the mid-Miocene climatic transition (hereafter MMCT), a cooling event that took place 14 Myr ago (Lewis et al. 2008), and is also reflected as a decline in diversification rates for our study group (line G, Fig. 10). Fifteen Myr ago, between the end of the MMCO and the MMCT, all Asian and Melanesian *Calyptrochaeta* species converge. Then, at the MMCT, all *C. asplenioides* sampled converge.

Eleven Myr ago, in the Late Miocene, exclusively New World *Calyptrochaeta* species converged. Also at that time *A. quadrifarium* diverged from other *Achrophyllum* species. Later in the Late Miocene (≈ 7 Myr ago), coinciding with the intensification of the Asian monsoons and the closing of the Tethys Sea (Micheels et al. 2007, Zachos et al. 2001), SE Asian *C. remotifolia* and the tropical clade of *C. asplenioides* converge. Relationships within *C. asplenioides* and branching timing are in agreement with those retrieved by Pokorny et al. (2011).

Although we see sharp declines in diversification rate for the Neogene Period (coinciding with the Mi-1 glaciation, the peak of the MMCO, and the MMCT), the slope of the overall trend for diversification rates in the study group is positive for this time period.

The formation of the W Antarctic ice-sheet (hereafter WAIS) ≈ 6 Myr ago (Line H, Fig. 10) coincides with the onset of glaciations and a new decline in diversification rates (Zachos et al. 2001). Late in the Pliocene Epoch we see a sharp recovery, which slows down ≈ 3 Myr ago (line I, Fig. 10) coinciding with the Late Pliocene cooling event.
(hereafter LPCE), characterized by the concurrent collapse of permanent “El Niño” (Philander and Fedorov 2003), the complete closure of the Panama Seaway (Bartoli et al. 2005), and the intensification of the glaciations (Lunt et al. 2008). Caribbean *C. haitensis*, Asian *C. japonica* and *C. spinosa* (native to Mainland SE Asia and Southern China) converge in the Early Pliocene (≈ 4 Myr ago), right before the LPCE. Northern South American *C. nutans* converges at the LPCE. So does the crown group in *Achrophyllum* (i.e., *A. anomalum*, *A. crassirete*, *A. dentatum*, *A. magellanicum*). Two other species in the genus *Achrophyllum*, Chilean *A. tenuinerve* (endemic to Juan Fernández islands), and the SE Asian *A. javense*, could not be sampled for DNA. Thus, their relative position in this genus remains uncertain.

Later, in the Lower Pleistocene, *C. brownii* converges 2.3 Myr ago. SE Asian *C. ramosa*, New Zealand *C. cristata* and *C. flexicollis*, and Holantarctic *C. apiculata* follow suit and converge ≈ 1 Myr ago. Relationships and timing of divergence for *C. apiculata* are similar to those retrieved by Pokorny et al. (2011). Finally, temperate South African *C. asplenioides* converges in the Middle Pleistocene.

### 3.4.2 Evolution of Habitat Preference and Geographic Diversification in *Calyptrochaeta*

For a TC scenario to be supported, habitat tolerances for older clades should be reconstructed as preferentially tropical, and temperate clades should be relatively recent and nested within ancestrally tropical clades. It is worth noting that, what we are reconstructing in our phylogeny is not ancestral areas, but tolerance to different climatic
zones (e.g., tropical climates lack the seasonal regimes that characterize temperate regions of the world and that impose constrains in the physiology of organisms that inhabit these temperate regions).

Since reconstructions of habitat preference (either temperate, C9, or tropical, C10) in our study group best fit a model were transitions and reversals are possible at the same rate and ancestral states of nodes towards the root could not be ascribed to either tropical or temperate tolerant with confidence (PP < 0.9), we lack clear evidence in favor of exclusively unidirectional movements from tropics to temperate climate regions, as the TS hypothesis predicts.

However, we do reconstruct at least one temperate clade nested within an older tropical clade with confidence. Asian C. japonica (node 30, Fig. 13) is nested within the clade subtended by node 26 (ancestrally tropical and ≈ 13 Myr old). This node is basal to all Asian and Melanesian Calyptoachaeta species (with the exception of C. spinosa).

Node 20 (Fig. 13) defines a clade comprising tropical and temperate Calyptoachaeta species found mainly in Asia and Australasia. Although not significant, support for this node being ancestrally temperate is high; which, together with the selected model of character evolution (q01=q10), hints towards a more complex scenario than the one the TS hypothesis proposes, where temperate species can indeed be quite old and give rise to tropical ones. This could mean that, through history, tropical regions may have not been as stable as it appears (Norris et al. 2002), niche conservatism is not
the rule in *Calyptrochaeta* (Evans et al. 2009), or that climatic-niche evolution is fast in this genus (Kozak and Wiens 2010).

### 3.4.3 Evolution of Gametophyte and Sporophyte Characters in *Calyptrochaeta*

Gametophytes in the genus *Calyptrochaeta* have asymmetrical, shortly acuminate to apiculate, leaves arranged in a more or less complanate fashion (often in six rows) along either orthotropic or plagiotropic branching stems (Ho and Kruijer 2007). Leaves in *Calyptrochaeta* have an entire, often distinct, border (limbidium) and a short forked nerve (costa). Leaf lamina cells are smooth, generally thick-walled, and relatively hexagonal to isodiametric. Clusters of filiform gemmae can be found in leaf axils in many instances. Species in the genus can be autoicous or dioicous. Calyptrae are fringed (Buck 1998, Streimann 2000).

Sporophytes in *Calyptrochaeta* have thick, papillose to hairy, setae. Capsules are oval in shape and can be erect to pendant. The annulus detaches when the conic operculum dehisces. Teeth of the exostome are lanceolate, furrowed, with high ventral lamellae laterally projected, and high basal membranes. Endostomial cilia are either absent or rudimentary (Buck 1998, Streimann 2000).

Among the morphological characters reconstructed for *Calyptrochaeta*, six fit best a model where either transitions (q_{01}=0) or reversals (q_{10}=0) are not possible. These two models are basically the same. Directionality of change depends on a) our a priori
assignation of values 0 and 1 to binary states for any given character, and b) the inferred ancestry in our study group.

In the case of characters 5 and 7 (distribution of seta ornamentation and presence of spines below the capsule neck, respectively), a priori assigned state 1 coincides with the ancestral condition inferred for our study group and the best-fit model forbids transitions from state 0 to state 1. The opposite applies for characters 1, 2, 4, and 6 (limbidium presence, limbidium thickness, sexuality, and presence of seta ornamentation, respectively), where the best-fit model suggests that reversals do not occur ($q_{10} = 0$). For this set of characters (1–2 and 4–7) only one direction of change is possible, which does not directly translate to irreversibility. In order to address irreversibility (i.e., Dollo’s law; Bull and Charnov 1985), aside from detecting this pattern of character loss, we would need to infer genetic and/or developmental constrains underlying the observed pattern (Collin and Miglietta 2008, Goldberg and Igić 2008), which is beyond the scope of this paper.

We confirm, in agreement with Pokorny et al. (2012), that the presence of a leaf border is the ancestral state in *Calyptrochaeta* and that its absence is the ancestral state for Daltoniaceae I. We also show that a limbidium less than 3 cells wide is the ancestral condition in *Calyptrochaeta*, and that broader limbidia have appeared a number of times, e.g., in temperate New Zealand *C. cristata* (node 11, Fig. 11), *C. flexicollis* (node 22), and
Holantarctic *C. apiculata* (node 21), and in the clade subtended by node 31, which includes tropical SE Asian *C. flaccida*, *C. perlimbata*, *C. ramosa*, and *C. remotifolia*.

Fuegian *C. odontoloma* (not sampled) also presents rather broad limbidia and is probably closely related to New Zealand *C. flexicollis*, if not a synonym of the latter. Also unsampled, *C. mollis* resembles *C. apiculata*, although the leaf border is thin (≤ 3 cell rows) in *C. mollis*. Its placement with respect to *C. apiculata* and other Southern temperate species remains to be tested.

Taxonomic placement and monophyly of (unsampled) Sri Lankan *C. lucida*, Australian *C. brassii* (very similar to *C. isophylla*), SE Asian *C. enervis* (could be synonym to *C. perlimbata*), *C. isophylla*, *C. parviretis*, and *C. rodundifolia* (possibly another synonym of *C. perlimbata*), and Melanesian *C. subremotifolia* remains uncertain, though probably they all belong somewhere in the clade subtended by node 24.

On the basis of our reconstruction of “limbidium presence” as the ancestral condition in the genus *Calyptrochaeta*, we exclude *C. boniana* from the genus (see section 3.4.5). The type description of this taxon states this species has no leaf border and has a 4-times plicate leaf base without any trace of a nerve (Bescherelle 1894). A. Touw has identified (http://plants.jstor.org/specimen/pc0026171), and B.-C Ho has confirmed (pers. comm.), that the isotype held at the Museum of Natural History in Paris (P) is *Garovaglia powellii* Mitt. subsp. *densifolia* (Thwaites & Mitt.) During, which is in a different order of pleurocarpous mosses (Pterobryaceae, Hypnales).
The ancestral state for character 4 in both Daltoniaceae I and *Calyptrochaeta* is dioicous. We only observe a transition to an autoicous condition in the exclusively New World clade of *Calyptrochaeta* (node 16), which includes Chilean *C. leptoloma* (Juan Fernández I.), Andean *C. nutans*, and Caribbean *C. haitensis*. We predict other autoicous New World taxa, such as Brazilian *C. albescens*, Colombian *C. deflexa*, Andean *C. mniadelphus*, and *C. setigera* (native to the West Indies, Venezuela and Brazil), will also be nested in this New World clade. It also remains to be tested whether these unsampled species are monophyletic (e.g., *C. mniadelphus* probably is a synonym of *C. nutans*).

As for the sporophyte, and in particular characters referring to the seta (5–7), the ancestor to *Calyptrochaeta* (node 9) is reconstructed to have had a seta ornamented throughout its length with spines below the neck of the capsule. Then, *C. asplenioides* (node 12, Fig. 12), *C. apiculata* (node 21), and *C. brownii* (node 23) would have lost both the spines below the neck and the ornamentation at the base of the seta, only retaining papillae in the upper half. Spines are also lost in the clade that node 27 subtends, which includes *C. japonica*, *C. marginata*, and *C. microblasta*; though setae are still ornamented throughout its length in this clade. The sporophyte of *C. marginata* is unknown (thus the uncertain reconstructions for node 28). We hypothesize the seta in this species has similar ornamentation to that of *C. japonica* and *C. microblasta* (i.e., with papillae throughout its length and no spines below the neck of the capsule).
Characters 3 and 8 (cell diameter in mid-leaf lamina and capsule orientation, respectively) best fit a model were both transitions and reversals are possible at the same rate. Ancestors of clades comprising all Achrophyllum (node 2) and both C. asplenioides and C. crista (node 10) are reconstructed to have had broad mid-leaf lamina cells (diameter ≥ 40 µm). The ancestors of all other Calyptrochaeta species (node 15) and the remaining Daltoniaceae I (node 6) had smaller leaf cells (diameter < 40 µm). No changes are detected in nodes nested within clades subtended by nodes 6 and 15. The capsule in the ancestor of Calyptrochaeta (node 9) is reconstructed as horizontal to pendulous. Within Calyptrochaeta, a single change to an erect capsule is inferred for the ancestor of C. apiculata (node 21, Fig. 12).

### 3.4.4 Correlations between Morphological Characters and Climate Zones in Calyptrochaeta

Only character 7, presence of spines below the neck of the capsule, is strongly correlated with either habitat preference reconstructed. This correlation is probably the result of developmental linkage (Klingenberg 2008) or pleiotropy (Wagner & Zhang 2012), though direct selection is also a possibility. It is important to note that the main function of moss sporophytes is to produce and disperse meiotic spores. Most temperate clades in Calyptrochaeta lose the spines below the capsule. These spines could reduce predation of developing capsules, especially in tropical areas. Another possibility is that these spines increase water retention, which could facilitate water-mediated dispersal of spores (hydrochory) in tropical clades. Additionally, partitioning habitat preferences
into, e.g., temperature and precipitation ranges, via niche modeling, could help understand physiological constrains restricting character evolution in *Calyptrochaeta*.

### 3.4.5 Proposed New Nomenclatural Changes


3.4 Concluding Remarks

In this study we have I) estimated the timing and patterns of diversification for the moss genus *Calyptrochaeta*. Diversification rate appears to have declined through the Paleogene and increased throughout the Neogene Period. We also have II) inferred that shifts between tropical and temperate regions happen at similar rates in both directions. Also, although we have found no evidence supporting the tropical conservatism hypothesis in *Calyptrochaeta*, we could not reject it with confidence. Finally, we have III) found correlations between morphological transitions and climatic preference shifts in this genus. Causation could not be established for these correlations.
4. Phylogeographic Patterns in Two Southern Hemisphere Species of *Calyptrochaeta*

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4.1 Introduction

Mosses, liverworts, and hornworts (bryophytes in the broad and informal sense) are haploid-dominant land plants that disperse sexually by small unicellular spores and asexually either by specialized vegetative propagules or by fragments of whole plants. Dispersal is likely primarily by wind but can also be mediated by animals (Heinken et al. 2001; Cronberg et al. 2006; Parsons et al. 2007; Rudolphi 2009). Sexual and vegetative dispersal both appear to be effective but, perhaps surprisingly, these processes rarely yield ubiquitous ranges (Schuster 1983; Tan and Pócs 2000; Frahm 2008). As Crum (1972) speculated and Hutsemekers et al. (2008) have shown, bryophyte species may be able to disperse across the landscape, but they are subsequently limited by their ability to establish viable populations. Establishment, rather than dispersal per se, may limit the distributions of spore producing plants such as bryophytes.

Transoceanic distributions, like those documented in angiosperms and other organisms (Raven and Axelrod 1974), can also be found in bryophytes (Tan and Pócs 2000). However, unlike in angiosperms, many such disjunctions occur at the infraspecific level in bryophytes — for example, more than 50 moss species have wide intercontinental distributions in circumpolar sub-Antarctic regions (Schuster 1983). The
fact that many of these disjunct distributions occur at the infraspecific level, together with the overall age estimated for bryophytes (Wellman et al. 2003), has led to an oft-cited view that species in this group are extremely old and evolve morphological disparity at an extremely slow rate (Frey et al. 1999; Frahm 2008); also, a recent analyses of nucleotide substitution rates suggest that molecular evolution may be lower in mosses than in vascular plants (Stenøien 2008). Nevertheless, recent long-distance dispersal (LDD) could also account for these broad distributions (Muñoz et al. 2004; Heinrichs et al. 2009a). Indeed, recent phylogenetic analyses of liverworts (Hartmann et al. 2006; Heinrichs et al. 2006; Feldberg et al. 2007; Heinrichs et al. 2009b; Vanderpoorten et al. 2009) and mosses (McDaniel and Shaw 2003; Newton et al. 2007; Huttunen et al. 2008; Vanderpoorten et al. 2008) indicate that intercontinental disjunctions may be best explained by dispersal, rather than by ancient vicariance. Some studies have documented morphologically cryptic phylogenetic structure within widespread taxonomic species (Shaw 2001; Heinrichs et al. 2009a).

Species richness appears to be highest in South America and South East Asia.

*Calyptrochaeta asplenioides* (Brid.) Crosby is a widespread African endemic found in tropical and subtropical moist forests in South Africa, Democratic Republic of the Congo, Rwanda, Tanzania, Comoros, Madagascar, La Réunion, and Mauritius. This species grows on a variety of substrates including rocks and soil in shaded and moist habitats. It is also found as an epiphyte on tree trunks and twigs, among roots, and on decaying wood. It occasionally has filamentous vegetative gemmae in the gametophytic leaf axils and, although it often produces gametangia, it rarely forms sporophytes. The gametophytes are dioicous (male and female buds on different plants, Fig. 14). The distribution (Fig. 15) and ecology of this species is based on floristic treatments (Magill and van Rooy 1998; O'Shea 2006) and examination of specimens from the following herbaria: BOL, DUKE, EGR, H, NY, and PRE.

Crosby (1976) noted that samples of *C. asplenioides* display significant morphological variation in leaf size, shape and areolation that appear related to geographical distribution. The observation that plants from islands in the Indian Ocean are morphologically different from those of South Africa (Crosby 1976) suggests that there may be geographically correlated phylogenetic structure within this relatively widespread African species.
Figure 14: Geographical morphological variation observed in *C. asplenioides*. Apices of vegetative leaves from Rwanda (Pócs 9112/H), Madagascar (Pócs, Magill & Lafarge 90115/Q), D. R. Congo (Pócs 9129/S), Tanzania (Thomas 3626D-2), Comoros (Pócs, Magill & Rupf 9268/BL), South Africa (Arts RSA27/11), and La Réunion (Pócs 9680/N). A gynoecium with perichaetial leaves protecting the archegonia (Pócs 9112/H), and an androecium with perigonial leaves protecting the antheridia (Arts RSA27/11).

*Calyptrochaeta apiculata* (Hook. f. & Wilson) Vitt is disjunct between South America and Australasia and has been found in Chile, Argentina, Falkland Islands (Great Britain), Marion Island (South Africa), Australia, and New Zealand (Fife 1991, 1995; He 1998; Matteri and Schiavone 2002). It also appears to have been recently introduced in Great Britain, where it escaped from the Tresco Abbey Botanical Garden.
in the Isles of Scilly, Cornwall (Smith 2004; Hill et al. 2006). We confirmed the
distribution based on specimens from the following herbaria: BM, CHR, H, NY, and S
(Fig. 15). This species is reported from a number of substrata, including creek and
waterfall banks, bases and stems of shrubs and tree ferns, decayed logs, and on low
rocks on shaded to semi-shaded sandy seepage areas or moist ground, usually from sea
level to 400m in coastal areas (Streimann 2000).

Three varieties have been described within Calyptraea apiculata, the typical
variety, C. apiculata var. apiculata, and two others, C. apiculata var. tasmanica (Fife 1995)
apiculata differs from typical plants by its strongly papillose seta and by the more
delicate iridescent leaves. On the other hand, Matteri (1975) states that C. apiculata var.
spathulata, which is restricted to the Colchagua Province, O’Higgins Region, Chile,
presents spathulate leaves larger than those of the typical variety. Whether these
varieties reflect an underlying phylogenetic pattern remains to be tested.

Testing alternative biogeographic hypotheses requires phylogenetic data with
estimates of divergence times (Edwards and Beerli 2000; Donoghue and Moore 2003;
Sanderson et al. 2004; Renner 2005). Australasia separated from East Antarctica some 80
Myr ago and South America split from West Antarctica ca. 30 Myr ago (Dalziel 1992).
Madagascar started its drift from continental Africa some 160 Myr ago (Rabinowitz et al.
1983). The islands of Mauritius and La Réunion, which are not older than 8 Myr
(Schlüter and Trauth 2008), are located along plate fracture zones. The apparent systematic age progression along the ridge may be the outcome of southward crack propagation through the oceanic lithosphere, rather than resulting from mantle plume activity (Sheth 2005). The disjunct distributions of *C. apiculata* and *C. asplenioiides* can be explained in terms of continental drift or long-distance dispersal. The current island distribution of *C. asplenioiides* must, in part at least, reflect recent long distance dispersal (LDD), but it remains to be determined whether its origin predates the split of Madagascar from continental Africa. In order to assess whether the disjunct distributions of this species is best explained by dispersal vs. vicariance events involving continental drift, we estimate divergence times.

Figure 15: Distribution (not to scale) of *C. apiculata* (triangles) and *C. asplenioiides* (stars).
The fossil record of bryophytes has provided some insights into the timing of their evolution and diversification (Feldberg et al. 2007; Heinrichs et al. 2007; Newton et al. 2007; Wilson et al. 2007), but there are no known fossils of *Calyptrochaeta* species. The ages of oceanic volcanic islands can be used as time constrains if endemic haplotypes are identified (Warren et al. 2003; Renner 2005; Warren et al. 2006; Leaché and Mulcahy 2007; Bloor et al. 2008; Sequeira et al. 2008). This approach rests on the assumption that the haplotypes diversified in situ. Two previous analyses in moss (Wall 2005) and liverwort (Devos and Vanderpoorten 2009) genera rely on this approach. Huttunen et al. (2008) used a different approach for the moss genus *Homalothecium* Schimp., using estimated substitution rates (as opposed to dating of nodes using fossils or island ages) to infer a chronogram. Ideally, DNA regions ticking at a constant rate would be the perfect choice but, in their absence, an uncorrelated lognormal relaxed clock (Drummond et al. 2006) can be implemented in BEAST (Drummond and Rambaut 2007). However, as already noted, nucleotide substitution rates may be lower in mosses than in vascular plants (Stenøien 2008), which makes the choice of any particular rate for mosses, inferred from work on tracheophytes difficult to justify. We here present a novel alternative to these approaches.

The basic idea is to join the estimation of a chronogram for our focal group to that of a larger group that includes it, and which can be constrained using fossil data. One issue to contend with is that the more inclusive analysis should utilize a lineage
birth-death model appropriate for interspecific reconstructions whereas our

*Calyptrochaeta* data, with population sampling, should be modeled after the coalescent.

For that reason, our strategy links the two independent reconstructions rather than using a single concatenated data set. Rate estimates can then be drawn from the fossil-constrained tree to generate a chronogram for our focal group. The genus *Calyptrochaeta* belongs to the pleurocarpous moss order Hookeriales (Buck et al. 2005), which together with the orders Hypnodendrales, Ptychomniiales and Hypnales make up the "core pleurocarp" clade (Bell et al. 2007), classified as the superorder Hypnanae (Goffinet et al. 2008). Newton et al. (2007) dated the diversification of pleurocarpous mosses from fossil data and estimated ages for supported nodes in their tree. We utilize a data set with extensive sampling within the Hookeriales, and also more limited sampling from the other three orders of core pleurocarps. This data set was recently used by B.-C. Ho (2010) for phylogenetic analyses of the order Hookeriales.

We use age estimates (and errors associated to them) from Newton et al. (2007) as priors on our Hookeriales tree, and to that we link our population level sampling of *Calyptrochaeta*. We address the following questions. I) Is there phylogeographic structure among African populations of *C. asplenioides*? II) Can the disjunct distribution of *C. asplenioides* between mainland Africa and the Indian Ocean Islands be explained by vicariance associated with continental drift? III) If the disjunction is more recent and therefore better explained by dispersal, can we infer directionality to that dispersal? IV)
Can we detect more than one dispersal event? V) Does the morphological variation observed in *C. asplenioides* correspond to phylogenetic structure? With regard to *C. apiculata*, VI) Do American and Australasian populations of *C. apiculata* differ at the molecular level? VII) If so, do these differences match the varieties described for this species? VIII) Is the intercontinental distribution of this species likely a reflection of vicariance resulting from the break-up of Gondwana?

4.2 Materials and Methods

4.2.1 Sampling and Molecular Protocols

DNA was sampled from 60 *Calyptrochaeta* specimens from 16 countries. Appendix C provides voucher information and GenBank accession numbers. Thirty-seven samples correspond to *C. asplenioides* from eight countries (its entire distribution as known to-date) and 13 to *C. apiculata* from four countries. It was not possible to obtain recent collections of *C. apiculata* suitable for molecular analyses from Marion Island (South Africa), nor from Argentina. The remaining *Calyptrochaeta* specimens included in this study comprise three *C. brownii* (Dixon) J. K. Bartlett from Australia, one *C. cristata* (Hedw.) Desv. from New Zealand, one *C. flaccida* (Broth.) Z. Iwats., B.C. Tan & Touw from Philippines, three *C. japonica* (Cardot & Thér.) Z. Iwats. & Nog. from Japan, one *C. microblasta* (Broth.) B.C. Tan & H. Rob. from Philippines, and one *C. ramosa* (M. Fleisch.) B.C. Tan & H. Rob. from Indonesia. Finally, *Daltonia splachnoides* (Sm.) Hook. & Taylor from Ireland and *D. marginata* Griff. from Australia were included in the analysis as
outgroups. The authors confirmed the identifications of all *Calyptrochaeta* samples, while Dr. Piers Majestyk, who has been monographing the genus *Daltonia* throughout its distribution, identified the two specimens of that genus included in our analysis.

Specimens from which DNA was successfully amplified were between four and 50 years old. Nucleotide sequences were obtained for three DNA regions: the trnL (UAA) 5’ exon-trnF (GAA) locus (hereafter trnL) and the trnG (UCC) intron (hereafter trnG) from the plastid genome, and the ITS1-5.8-ITS2-26S region (hereafter, ITS) of nuclear ribosomal DNA. All samples included in the molecular analyses were examined microscopically in order to assess any geographically correlated morphological variation.

Total genomic DNA was extracted using a modified CTAB protocol (Doyle and Doyle 1990) as described in Shaw (2000). Double-stranded DNA templates were amplified by polymerase chain reaction (PCR), employing 35 cycles of 30 sec at 95ºC, 45 sec at 50ºC and 1 min at 72ºC, preceded by an initial 1 min melting step at 95ºC and followed by a final extension period of 7 min at 72ºC. Amplification and sequencing primers are described in Shaw et al. (2003).

### 4.2.2 Morphological Observations

All specimens of *C. apiculata* and *C. asplenioides* included in the molecular analyses were examined to assess morphological variation. From each specimen we examined two to five shoots; sterile and, when possible, bearing gametangia. Permanent
slides where prepared. Formal sampling with statistical analyses was not performed; rather, attention was directed at characters that putatively differ between plants from different geographic regions. Photographs provided (Fig. 14) reflect a synthesis of these multiple but informal observations.

4.2.3 DNA Sequence Editing and Alignment

For each sample and sequenced DNA region, forward (5’-3’) and reverse (3’-5’) sequences were assembled and checked for inaccurate base calling using Sequencher v4.1 (GeneCodes Corporation 2000). Consensus sequences were aligned manually using Se-Al v2.00al Carbon (Rambaut 2002) trying to minimize substitutions and indels by combining event-based and similarity criteria to produce a hypothesis about the homology of the nucleotide characters (Simmons and Freudenstein 2003; Simmons 2004; Morrison 2006).

Regions of ambiguous alignment and incomplete data (i.e., at the beginning and end of sequences) were identified and excluded from subsequent analyses using the program MacClade v4.06 OS Xk (Maddison and Maddison 2003). We were successful obtaining 60 (93%) \textit{trnL} sequences, 51 (82%) \textit{trnG} sequences, and 48 (77%) ITS sequences (Appendix C). Missing data represent 14% of the combined data matrix. Nodes on the phylogenetic reconstruction that are directly relevant to the present study are highly supported, so this level of missing data was not problematic.
4.2.3 Data Analyses

Comparative DNA sequence data analyses (Table 5) to detect shared polymorphisms and fixed differences were performed with SITES (Hey and Wakeley 1997).

**Table 5: Comparative DNA sequence data analyses results. Fixed differences above the diagonal. Shared polymorphisms below.**

<table>
<thead>
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<th>Taxon</th>
<th>1</th>
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<th>7</th>
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<th>10</th>
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Phylogenetic analyses employing maximum likelihood (ML) as an optimality criterion (Felsenstein 1981) were conducted using GARLI v0.96b (Zwickl 2006). All genes were combined in a single concatenated matrix as no conflicts were identified with ≥ 70% bootstrap support when each gene was analyzed separately (data not shown).

Clade support was estimated by performing 600 bootstrap replicates under the same model also in GARLI v0.96b. Divergence times were estimated with Bayesian Inference (BI) as implemented in the cross-platform program BEAST v1.5.4 (Drummond and Rambaut 2007).
Table 6: Model selection results for each dataset and partition (nDNA vs. pDNA) using the Akaike Information Criterion (Akaike 1973, 1974) as implemented in the program MrModeltest (Nylander 2002). GTR, General time-reversible (Rodriguez et al 1990); HKY, Hasegawa-Kishino-Yano (Hasegawa et al. 1985). Base—base frequencies (A, C, G); Nst—number of substitution types (1, 2, 6); Rmat—rate matrix (AC, AG, AT, CG, CT); Tratio—transversion ratio; Shape—value of $\alpha$ in gamma distribution; Pinvar—proportion of invariant characters.

<table>
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<tr>
<th>Taxon Set</th>
<th>Partition</th>
<th>Model</th>
<th>-ln likelihood</th>
<th>Optimized Parameters</th>
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<td>Base</td>
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<td>Rmat</td>
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<tr>
<td>Hypnanae</td>
<td>nDNA</td>
<td>GTR+I+Γ</td>
<td>18853.8613</td>
<td>(0.1396 0.3495 0.3246)</td>
<td>6</td>
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<tr>
<td>Hypnanae</td>
<td>pDNA</td>
<td>GTR+I+Γ</td>
<td>14950.0635</td>
<td>(0.4046 0.0846 0.0914)</td>
<td>6</td>
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<td>Calyptrochaeta</td>
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<td>GTR+Γ</td>
<td>5857.5684</td>
<td>(0.3107 0.2168 0.1968)</td>
<td>6</td>
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<tr>
<td><em>C. asplenioides</em></td>
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<td>GTR+I</td>
<td>1490.6787</td>
<td>(0.1721 0.3422 0.2987)</td>
<td>6</td>
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<tr>
<td><em>C. asplenioides</em></td>
<td>pDNA</td>
<td>GTR+I</td>
<td>1760.1832</td>
<td>(0.4068 0.1198 0.1327)</td>
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<td>HKI</td>
<td>1062.6068</td>
<td>(0.1753 0.3608 0.2878)</td>
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<tr>
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<td>HKI+I</td>
<td>1485.8422</td>
<td>(0.4165 0.1076 0.1310)</td>
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</tr>
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</table>
The best-fit substitution model for both ML and BI analyses was determined with the aid of MrModeltest v2.2 (Nylander 2004) and PAUP* v4.0b (Swofford 2002). The Akaike Information Criterion (Akaike 1973, 1974) was used to evaluate the optimal model (Table 6).

We first addressed whether substitution rates obtained from the literature, or ages for relevant islands in the Indian Ocean obtained from geological data, provided better constrains for our analyses. We also tested whether a strict clock model applies to our data. Model comparisons were performed on a subset of the Calyptrochaeta sampling (comprising all C. asplenioides specimens together with C. cristata, which was resolved in preliminary ML analyses as closest to C. asplenioides, as the outgroup) and we used Bayes factors to evaluate alternative models (Kass and Raftery 1995), calculated with TRACER v1.5 (Rambaut and Drummond 2009). We partitioned our data into nuclear and plastid DNA. As in Huttunen et al. (2008), the ITS substitution rate was fixed at $1.4 \times 10^{-2}$ substitutions per site per Myr, estimated from green algae and land plants (Bakker et al. 1995), and the plastid DNA rate set at $5.0 \times 10^{-4}$ substitutions per site per Myr (Palmer 1991; Schnabel and Wendel 1998). These authors (Huttunen et al. 2008) chose a normal distribution with a narrow standard deviation ($SD_{nDNA} = 5.0 \times 10^{-4}$, $SD_{pDNA} = 1.0 \times 10^{-4}$) for their rate priors. Preliminary runs with our dataset indicated that this distribution was too stringent; we therefore used a lognormal distribution with a standard deviation of one.
For analyses using island age to calibrate the chronogram, we constrained the node corresponding to the MRCA (most recent common ancestor) for all *C. asplenioides* samples from La Réunion and Mauritius (that is, the Mascarene islands), plus sample G067 from Madagascar (because this last sample was included in that clade; see Fig 16). This constraint on maximum age is based on the geological estimates for the volcanic islands (Schlüter and Trauth 2008), since at least two *C. asplenioides* haplotypes unique to the Mascarene islands are divergent enough so that they could have originated and diversified in situ (Fig. 16). The prior assigned to this node had a lognormal distribution with an 8 Myr mean and a SD of 1 (Fig. 14). An alternative hypothesis; i.e., that these haplotypes originated on the so-called Mascarene Plateau, a vast submerged land area, part continental and part volcanic and composed of the Cargados Carajos, Nazareth, and Saya de Malha Banks, is unlikely. Although the existence of these now-submerged banks indicates that there could have been islands older than 8 Myr, it does not appear that they were still emergent at the time the present Mascarenes appeared. Moreover, *C. asplenioides* currently occupies montane habitats at least 500 to 2300 meters above sea level. Geological evidence (Rabinowitz et al. 1983; Burke 1996; Parson and Evans 2005; Sheth 2005) indicates that no earlier islands in the archipelago likely had montane habitats with appropriate paleoclimates during the last 8 Myr, so these previous islands were unlikely to have been a source of plants from which current populations in La Réunion and Mauritius could have been derived. In any case, the prior distribution used
in the analyses does not exclude this possibility (ages up to 35 Myr could be inferred for the constrained node).

Components that differ in the models of evolution we compared are combinations of two time-constraints (island age vs. assumed substitution rates), two clock models (strict vs. relaxed uncorrelated lognormal), and four tree models (coalescent with constant size population, coalescent under a Bayesian skyline with five groups [one for *C. cristata* and four for the four main haplotype groups detected in *C. asplenioides*], coalescent under an extended Bayesian skyline, and speciation under a birth-death process). These models are listed and compared in Table 7.

Four independent MCMC analyses were each run for 100,000,000 generations for every model. Parameter values were sampled every 50,000 generations and convergence and acceptable mixing of the sampled were checked using the program TRACER v1.5 (Rambaut and Drummond 2009). Overall, the model using the mean substitution rates from the literature (Huttunen et al. 2008), an uncorrelated lognormal relaxed clock (Drummond et al. 2006), and a coalescent Bayesian skyline (Drummond et al. 2005) with five groups, performed best (Table 7). The reconstructions based on population sampling within *C. asplenioides* and *C. apiculata* were joined to that for the order Hookeriales (Ho 2010). The earliest diverging nodes in the latter dataset were constrained using the dates and confidence intervals inferred by Newton et al. (2007) in their fossil-constrained chronogram for the pleurocarpous mosses.
Table 7: Model selection using Bayes factors calculated with TRACER v1.5. The time constrain in our trees was either island age (8 Myr, hereafter IA) or mean substitution rate (hereafter MR) obtained in the literature (see Materials and Methods; the following rates were used: nDNA 0.014, pDNA 0.0005). Two clock models were compared. The first was an uncorrelated lognormal relaxed clock (hereafter ULRC), the second a strict clock (hereafter SC). Four tree models were compared. The first is a coalescent tree model with constant size population (hereafter CCS), the second is a coalescent tree model that uses a Bayesian skyline with five groups (one for \textit{C. cristata} and four for the four main haplotypes found in \textit{C. asplenioide}s, hereafter CBS), the third is a coalescent tree model that uses an extended Bayesian skyline (hereafter CEBS). Lastly, a speciation tree model using a birth-death process (hereafter SBDP) was also implemented. Bayes factors in bold case indicate a model significantly better than the one to which it is being compared. Comparisons between model combinations (time constraint/clock model/tree model) should be read across rows; comparisons above and below the diagonal are analogous but values differ in sign depending on which is tested as the better model (i.e., Bayes factors test whether one model is significantly better than another). Overall, the model using the mean substitution rates from the literature, an uncorrelated lognormal relaxed clock and a coalescent Bayesian skyline, performs better than other models.

| Time constrain | Clock Model | Tree Model | lnP(M|D) | S.E. | Bayes Factors |
|---------------|-------------|------------|--------|-----|---------------|
|               |             |            | IA ULRC | IA ULRC | IA MR ULRC | IA MR ULRC | IA MR SC ULRC |
| IA            | ULRC        | CCS        | -3,301,997 | +/- 0.829 | -3,206 | -0.39 | -4,879 | -3,088 | 1,815 | 2,13 | 1,614 | 1,456 |
| IA            | ULRC        | CBS        | -3,298,791 | +/- 0.563 | 3,206  | 2,815 | -1,674 | 0,117 | 5,02  | 1,075 | 1,591 | 1,749 |
| IA            | ULRC        | CEBS       | -3,298,791 | +/- 0.563 | 3,206  | 2,815 | -1,674 | 0,117 | 5,02  | 1,075 | 1,591 | 1,749 |
| IA            | ULRC        | SBDP       | -3,301,607 | +/- 0.576 | 0.39   | -2,815 | -4,489 | -2,698 | 2,205 | -1,74 | -         |
| MR            | ULRC        | CBS        | -3,297,118 | +/- 0.529 | 4,879  | 1,674 | 4,489  | 1,791 | 6,694 | 2,749 | 3,265 | 3,423 |
| MR            | ULRC        | CEBS       | -3,298,791 | +/- 0.563 | 3,206  | 2,815 | -1,674 | 0,117 | 5,02  | 1,075 | 1,591 | 1,749 |
| MR            | ULRC        | SBDP       | -3,301,607 | +/- 0.576 | 0.39   | -2,815 | -4,489 | -2,698 | 2,205 | -1,74 | -         |
| MR            | SC          | CCS        | -3,303,812 | +/- 0.443 | -1,815 | -5,02 | -2,205 | -6,694 | -4,903 | -      | 3,945 | 3,429 | 3,271 |
| MR            | SC          | CBS        | -3,299,866 | +/- 0.573 | 2,13   | -1,075 | 1,74   | -2,749 | -0.958 | 3,945 | -      | 0,516 | 0,674 |
| MR            | SC          | CEBS       | -3,300,838 | +/- 0.472 | 1,614  | -1,591 | 1,224  | -3,265 | -1,474 | 3,429 | -      | 0,516 | 0,158 |
| MR            | SC          | SBDP       | -3,300,540 | +/- 0.381 | 1,456  | -1,749 | 1,066  | -3,423 | -1,632 | 3,271 | 0,674 | 0,158 | -      |
The constrained nodes were the origin of Hypnodendrales (point estimate [PE] = 69.79 Myr, confidence interval [CI] = 58.98-100.59 Myr), origin of the Ptychomniales (PE = 82.03 Myr, CI = 72.21-100.44 Myr), and origin of the Hypnales+Hookeriales clade (PE = 183.61 Myr, CI = 130.75-165.1 Myr). The selected distribution for these time constraints was a gamma distribution with a standard deviation that included the confidence intervals. Substitution rate estimates for the population level datasets were then drawn from this fossil-constrained tree to simultaneously estimate divergence times and infer phylogenetic relationships for *C. aspleniooides* and *C. apiculata*. As noted previously, *C. cristata* was used as the outgroup for *C. aspleniooides*; *C. brownii* was used as the outgroup for *C. apiculata* based on preliminary ML analyses (see Fig. 16). It should be noted that the tree model selected for both *C. aspleniooides* and *C. apiculata* was the coalescent model under a Bayesian skyline. In the case of the species level dataset (Hookeriales) we compared the Yule and birth-death process with default settings and used Bayes Factors to select the best fit; this being the Yule process.

Four independent MCMC analyses were each run for 100,000,000 generations for every dataset, and parameter values were sampled every 50,000 generations. After discarding the initial 10% of steps (burnin), the four independent runs were combined to obtain an estimate of the posterior probability distribution of divergence dates for each node in the phylogenetic reconstructions using TreeAnnotator v1.5.4 (Drummond and Rambaut 2007). These estimates are shown in a chronogram built with FigTree v1.2.1.
(available at http://tree.bio.ed.ac.uk/software/figtree/ and also as part as of the BEAST suite of programs, Drummond et al. 2007). The complete data matrix is available in TreeBASE (study number S11229).

4.3 Results

4.3.1 Molecular Patterns

The concatenated matrix includes 78% constant characters, 5.6% singletons, and 16.5% parsimony informative characters. All the *Calyptrochaeta* sequences form a clade relative to the two *Daltonia* sequences included as outgroups (Fig. 16). The two focal species, *C. apiculata* and *C. asplenioides*, are also resolved individually as monophyletic with maximal support from both ML and Bayesian analyses (Figs. 16–18). *Calyptrochaeta cristata* is resolved as sister to *C. asplenioides* (98 % ML bootstrap support [BS]). Other species of *Calyptrochaeta* included here are sister to *C. apiculata* in a clade with 100 % ML BS.

*Calyptrochaeta apiculata* samples lack geographic structure and sequences yield very short (or zero) branch lengths in the ML topology. There were only five parsimony informative characters across the three loci and haplotypes are shared among Chile, New Zealand, and Australia. The plant sampled from the Isles of Scilly, Great Britain has a multilocus haplotype identical to that of samples from both Australia and Chile (Figs. 16, 17).
Figure 16: Single optimal ML tree for the combined data matrix including ITS, trnG and trnL sequences, reconstructed under the GTR + Γ substitution model (see methods for details). Numbers indicate ML bootstrap support after 600 replicates. Clades A and B are *C. apiculata* and *C. asplenioides*, respectively. Sample numbers as in Appendix C.
Calyptrochaeta asplenioides, in contrast, displays clear geographic structure and highly supported intraspecific clades (Figs. 16, 18). Haplotype groups can be identified from South Africa (100% ML BS and 0.9995 PP), eastern Africa (Democratic Republic of the Congo, Rwanda and Tanzania; 84% ML BS and 0.9803 PP), Comoros (97% ML BS and 0.9995 PP) and the Mascarene Islands (90% ML BS and 0.9995 PP). In the ML topology length of branches among clades is clearly larger than that of branches within clades (Fig. 16), which are practically non-existent. Branch length for the South Africa clade is 0.0072 substitutions/site. The remaining C. asplenioides samples group in a clade with a stem branch length of 0.072 substitutions/site. The stem branch length for the clade with samples from eastern Africa is 0.0037 substitutions/site. This clade is sister to all samples from Indian Ocean islands. The Mascarene Islands clade, on a stem branch of length 0.0086 substitutions/site, appears nested within samples from Comoros and Madagascar.

For the comparative DNA sequence data analyses (Table 5) C. apiculata was divided into two groups that comprise sequences from Australasia versus South America. Sequences from C. asplenioides were divided into South Africa versus its remaining distribution. None of our compared groupings shared any polymorphic nucleotides. There is one fixed difference between South African C. asplenioides and all other sequences obtained for this taxon. There were no fixed differences between New and Old World C. apiculata.
Figure 17: *Calyptrochaeta apiculata* BEAST chronogram. Branch width scaled to show PP (thin branches are not supported, thick ones have significant support). Numbers are estimated ages, in million years, for supported nodes; 95% confidence interval in square brackets. Sample numbers as in Appendix C.

### 4.3.2 Dating Estimates

Sequences of *C. apiculata* (Fig. 17) are estimated to have diverged from those of *C. brownii* 21.73 Myr ago (95% CI = 6.68-32.56 Myr). All *C. apiculata* sequences converge 1.83 Myr ago (95% CI = 0.32-2.26 Myr) and sequences of *C. brownii* converge 0.71 Myr ago (95% CI = 0.04-1.5 Myr). *Calyptrochaeta asplenioides* haplotypes (Fig. 18) coalesce 18.18 Myr ago (95% CI = 6.78-21.2 Myr). South African samples could have converged 4.52 Myr ago (95% CI = 0.02-1.71 Myr). The divergence of the eastern Africa clade from those containing samples from the Indian Ocean Islands is estimated at 5.99 Myr ago (95% CI = 3.58-10.41 Myr), and all samples from eastern Africa converge 0.89 Myr ago (95% CI = 0.01-0.89 Myr). All Indian Ocean sequences coalesce 4.88 Myr ago (95% CI = 2.99-8.94 Myr). Samples from Comoros and one of the haplotypes from Madagascar coalesce 0.21
Myr ago (95% CI = 0.28-19.59 Myr) ago. Finally, the samples from La Réunion and Mauritius Islands coalesce 0.51 Myr ago (95% CI = 0.18-1.61 Myr).

**Figure 18:** *Calyptrochaeta asplenioides* BEAST chronogram. Branch width scaled to show PP (thin branches are not supported, thick ones have significant support). Numbers are estimated ages, in million years, for supported nodes; 95% confidence interval in square brackets. Sample numbers as in Appendix C.

### 4.3.3 Morphological Patterns

Within *C. asplenioides* there is variation in gametophyte morphology that corresponds to phylogenetic structure. Plants from continental Africa have a short muro composed only of marginal cells, whereas samples from the Mascarenes have a larger apiculus with cells from both the margin and lamina (Fig. 14). Leaves from
Madagascar are intermediate between these extremes. Plants can have one to four rows of linear cells forming a margin. This margin is wider in samples from La Réunion (Fig. 14). Similar wide margins were also observed in plants from Mauritius (not shown). Because the laminar cells adjacent to elongated marginal cells are notably shorter in plants from continental Africa, the margin is more clearly defined in these plants than in those from most Indian Ocean islands (Fig. 14). On the other hand, *C. apiculata* shows no phylogenetic structure underlying morphological differences detected in Tasmanian plants (G060 & G101).

### 4.4 Discussion

*Calyptrochaeta asplenioides* is mainly structured into four clades with haplotypes from South Africa, eastern Africa, Madagascar plus Comoros, and the Mascarene Islands (Figs. 15–16, 18). The earliest divergence within *C. asplenioides* divides samples collected above and below the Tropic of Cancer (i.e., South Africa versus farther to the north). Our ML tree (Fig. 16) shows that South African plants are as distinct from eastern African and Indian Ocean plants as *C. brownii* is from *C. microblasta* plus *C. flaccida*, *C. japonica*, and *C. ramosa*. On morphological grounds, the latter are distinct species. In addition, there is one fixed difference (Table 5) between tropical and temperate collections of *C. asplenioides*, suggesting that plants in this clade do not exchange genes. Although we were able to document geographically correlated morphological variation within *C. asplenioides*, this variation appears to be continuous and does not warrant
taxonomic subdivision by itself; i.e., leaves in South African plants present a short mucro (as opposed to the long mucro found in Mascarene leaves), but so do eastern African, Comorian and Madagascan leaves (Fig. 14). Also, Mascarene plants present a wide limbidium in their leaves (Fig. 14), but plants from Madagascar and Comoros do not, nor do those from eastern Africa. Leaf morphology of plants from eastern Africa, Comoros and Madagascar is intermediate between the morphological extremes represented by South African and Mascarene plants. Whether the morphological variation observed in *C. asplenioides* correlates with local adaptation to different niches remains to be tested.

Haplotypes of *C. asplenioides* appear to coalesce 18.18 Myr ago (early Miocene). Madagascar started its separation from Africa between 160 (Upper Jurassic) and 130 (Lower Cretaceous) Myr ago (Rabinowitz et al. 1983), raising the possibility that the distribution of this species reflects vicariance associated with continental drift. Also, the observation that plants from islands in the Indian Ocean are reported to differ morphologically (Fig. 14) from those of South Africa (Crosby 1976) suggests that a hypothesis of vicariance should be addressed. However, our divergence time estimate indicates that the diversification of *C. asplenioides* is far too recent to be explained by continental drift, and the hypothesis of vicariance caused by continental drift requires such ancient divergence that we consider our data sufficient to reject it in favor of more recent dispersal.
Migration appears to be directional from mainland (eastern) Africa to the Indian Ocean islands. From a parsimony perspective, dispersal may have taken place just once, but we cannot reject the alternative hypothesis that there may have been more than one dispersal event since bootstrap support is lacking for the critical nodes (Fig. 16). Moreover, there could have been multiple dispersals of the same haplotype. It is interesting to note that this pattern of migration is unlikely with present-day wind currents (Woodberry et al. 1989). However, Ali & Huber (2010) propose that, in the Paleogene, ocean and wind currents were reversed. Only when Madagascar reached its present location in the early Miocene did the currents shift to the patterns now observed. Our ML reconstruction and our time intervals agree with a scenario in which C. asplenioides migrated from mainland Africa into Indian Ocean islands in the Paleogene. Subsequently, when wind currents shifted in the Miocene its populations became isolated and started to diverge, giving rise to the phylogeographic pattern we now observe.

Calyptrochaeta apiculata has a broad and highly disjunctive distribution that encompasses southern South America and Australasia (Fig. 15). An important goal of this study was to address whether populations from South America were different from those in Australasia. Also, we assessed whether this species is old enough to have undergone vicariant divergence associated with the break-up of Gondwana. Even without establishing a date for the divergence of New and Old World populations, the
finding that highly disjunct populations are little, if at all, differentiated (Fig. 16) suggests that the species has dispersed relatively recently between Chile and Australia. No fixed differences were identified between populations from these two distant regions of the world. Tasmanian plants of C. apiculata (G060 & G101 in our sampling) may have a more papillose seta and somewhat more delicate leaves than average but they are not genetically differentiated at the loci we sequenced. Since no materials of C. apiculata var. spathulata could be sampled for DNA, we cannot assess its validity.

It appears that all haplotypes of C. apiculata could coalesce in the Pliocene-Pleistocene limit (1.83 Myr ago); that is, even given the uncertainty of our dating, confidence intervals on the root node for this species do not encompass a time frame that is consistent with divergence associated with continental drift. The latter scenario would require a divergence of at least 35-80 Myr ago (Sanmartín and Ronquist 2004). The near absence of molecular differentiation between New and Old World populations, combined with our estimate of divergence time, strongly implies that the disjunct range of C. apiculata must be more recent than any continental drift hypothesis could allow. We therefore conclude that the disjunct distribution of C. apiculata reflects recent intercontinental dispersal rather than vicariance related to the break-up of Gondwana.

Frey et al. (1999) found a similar low level of genetic diversity and absence of differentiation between South American and Australasian populations in the moss Lopidium concinnum (Hook. f.) Wilson. They interpreted these observations as an
example of “stenoevolution”; that is, “speciation in geological times” (Frey et al. 1999). However, this interpretation was apparently based on the a priori assumption that the disjunct distribution of *L. concinnum* resulted from an ancient, continuous range broken up by continental drift. Thus, the lack of genetic divergence within *L. concinnum* illustrated that “no significant differing pathways of evolution had been followed within the 80-60 million years” (Frey et al. 1999) since the breakup of Gondwana. The very low levels of molecular variation observed in *Lopidium*, and also in *C. apiculata*, prohibit a complete understanding of their biogeographical history, but given the lack of genetic divergence between New and Old World populations, it seems more parsimonious to interpret these disjunctive ranges as dispersal-based. The time range we estimate for the origin of *C. apiculata* supports the interpretation of recent dispersal between South America and Australasia in *C. apiculata*.

The moss *Pyrrhobryum mnioides* (Hook.) Manuel and the liverwort genus *Monoclea* Hook. have geographic distributions that include wet temperate forests of southern South America and Australasia. Unlike in *C. apiculata*, no haplotypes were shared between New and Old World populations of *P. mnioides* (McDaniel and Shaw 2003). McDaniel & Shaw (2003) estimated divergence between South American and Australian/New Zealand populations at approximately 80 Myr ago, consistent with vicariant divergence associated with the break-up of Gondwana. Meißner et al. (1998) did not estimate divergence times for *Monoclea* but reasoned from presumed
substitution rates that the New and Old World divergence was likely ancient and could be explained by continental drift. Other cases of intercontinental disjunction that could be interpreted as relictual and resulting from continental drift have been shown to more likely reflect LDD. These include the mosses, Hypopterigium (Pfeiffer 2000) and Weymouthia (Quandt et al. 2001), and liverworts, Bryopteris (Nees) Lindenb. (Hartmann et al. 2006), Marchesinia Gray (Heinrichs et al. 2009b), Herbertus Gray (Feldberg et al. 2007) and Plagiochila (Dumort.) Dumort. (Heinrichs et al. 2006).

Newton et al. (2007) dated the diversification of the pleurocarpous mosses and estimated the age of the node defining the order Hookeriales, which includes the genus Calyptrochaeta, as approximately 75 Myr ago. Our estimates point towards a relatively recent origin and diversification of this order (in the context of mosses being an ancient group of land plants that predate vascular plants). Based on topological and chronological estimates, the Hookeriales do not appear to be an early diverging lineage of mosses. If hypotheses of vicariance in the context of the breakup of the Gondwanan continent are to be addressed, early diverging groups might be better subjects for future research. These might include for example acrocarpous taxa in the Dicranales, Funariales, and Bryales.

The present results provide strong evidence that some disjunct distributions that could be interpreted as consequences of continental drift are more likely due to recent dispersal. Understanding current distributions and biogeographical history are
obviously linked to dispersal and establishment abilities. Both *C. apiculata* and *C. asplenioides* are dioicous, with archegonia and antheridia on separate gametophytic plants and sporophytes (the result of sexual reproduction) are not common in either species. Both, however, like most mosses, can reproduce vegetatively. Spore sizes overlap (Magill and van Rooy 1998; Streimann 2000), but *C. apiculata* has slightly larger spores (16-24 µm) than *C. asplenioides* (12-17 µm). Both taxa can grow on a variety of substrates, although they appear to be restricted to a set of particular environments or microclimates. Larger spores may be less likely to travel long distances but more likely to become established because of greater energy reserves (Söderström and During 2005). *Calyptrochaeta apiculata* frequently occurs in disturbed and other open habitats, possibly promoting establishment after dispersal. *Calyptrochaeta asplenioides*, in contrast, seems to be restricted to relatively pristine tropical montane humid forests. These habitats have been drastically reduced over the last few decades in mainland Africa (Albertine Rift and Eastern Arc Mountains) and Indian Ocean Islands (Comoros, Madagascar) because of agricultural expansion (Burgess et al. 2004). Sampling in this study is insufficient to test the hypothesis that population size in *C. apiculata* has decreased in historical time, but future research could examine molecular population patterns for a signature of demographic decline.

It seems clear that the disjunct distributions of *C. apiculata* and *C. asplenioides* are better explained by recent dispersal rather than ancient vicariance. Assertions about
relict distributions must always be put in a temporal context, which is now possible through molecular and statistical approaches. In particular, if vicariance of bryophytes is to be addressed in the Southern Hemisphere, early diverging lineages are better candidates than relatively recent crown groups. Pleurocarpous mosses in the relatively recent orders, Hypnales and Hookeriales, may be too recent to exemplify vicariance patterns associated with continental drift (Heinrichs et al. 2007; Newton et al. 2007).

4.5 Concluding Remarks

To summarize (see questions in the introductory section), I) *C. asplenioides* presents a clear phylogeographic structure in which a clade of haplotypes from the Mascarene Islands is sister to a clade of haplotypes from Madagascar and Comoros; both these clades are then sister to a clade comprising haplotypes from eastern Africa; finally, these three tropical clades are sister to a temperate clade of South African haplotypes. II) Our analyses show that the current distribution of *C. asplenioides* can be explained, in its entirety, in terms of long distance dispersal. III) This dispersal is directional and fits a model where populations migrated from mainland Africa to islands in the Indian Ocean. IV) Our analyses do not allow us to know whether dispersal happened just once or multiple times between mainland Africa and Indian Ocean islands. V) Although our data support that South African haplotypes of *C. asplenioides* have been isolated for quite some time, it is not desirable to describe them as different species because morphological differences appear to be continuous. VI) *C. apiculata* hardly shows any
haplotype diversity throughout its distribution. VII) *C. apiculata* var. *tasmanica* is not genetically different from the type variety of this species. We could not obtain specimens of *C. apiculata* var. *spathulata* so we cannot assess the validity of this variety. VIII) We can refute vicariance as the mechanism leading to the current distribution of *C. apiculata*. Calyptrochaeta apiculata and *C. aspleniooides* display very much contrasting phylogeographic patterns.
5. General Conclusions

Many evolutionary processes are influenced by environmental variation over space and time, including genetic divergence among populations, speciation, and evolutionary change in morphology, physiology, or behavior.

In here we have shown that, in Hookeriales, characters both from the sporophyte and the gametophyte are labile, and that a priori reliance on features restricted to one generation or the other to infer relationships can be misleading. Homoplasy in the sporophyte generation is not exclusive to pleurocarpous mosses and has also been observed in the acrocarpous family Funariaceae (Liu et al., 2012).

Since we have demonstrated parallel changes and reversals in traits from both sporophytes and gametophytes, it is obvious that evolutionary interpretations about the relative conservatism of morphological evolution in gametophytes versus sporophytes should be based on independent phylogenetic evidence in a case-by-case basis.

We have also estimated patterns and timing of diversification in the genus *Calyptrochaeta*. As a result we have shown that this genus experienced a decline in net diversification rates from its origin until the end of the Paleogene Period, followed by an increase in diversification rate during the Neogene. Divergence time estimation is not exempt of error and our results should be taken more as an exploratory exercise than a fact. It is, however, remarkable how cleanly shifts in estimated diversification rates coincide over, and over again, with notable climate events in Earth’s history.
We have also revealed that, in *Calyptrochaeta*, shifts between tropical and temperate regions are possible at equivalent rates in either direction. Though we could not refute the TC hypothesis, our data favor a different scenario where temperate species can indeed be quite old and give rise to tropical ones. Besides, we have established correlations between climatic preference shifts and morphological transitions.

Finally, we have exposed the clear phylogenetic structure underlying the distribution of *C. asplenioides*, we have evidenced how this distribution can be explained in terms of long distance dispersal, and we have showed directionality of this dispersal from mainland Africa to Indian Ocean islands. For *C. apiculata* we have revealed an overall lack of phylogenetic structure, probably as a result of ongoing gene flow, and we have proven long distance dispersal is the mechanism underlying its Holantarctic distribution.

In all, bryophytes may have slower mutation rates than most vascular plants and they may seem morphologically static, but they are far from being “unmoving, unchanging sphinxes of the past” (Crum, 1972).
Appendix A

Voucher information and GenBank accession numbers for 122 taxa (123 samples, double Hookeria acutifolia); rps4: all available, 37 (30%) new; trnLF: 122 available, 39 (32%) new; nad5: 115 available, 31 (25%) new; ITS: 105 available, 78 (63%) new; 26S: 109 available, 47 (38%) new. Total: 574 (93%) out of 615 (5 markers x 123 exemplars) available, 232 (38%) new. (a) Hypnalean taxa once associated with the Hookeriales; (h) other Hypnalean taxa; (*) type species of respective genera; and (—) missing sequences. Newly generated sequences are in bold.

Outgroup taxa—Euptychium cuspidatum (*), New Caledonia, A.E. Newton 5373 (BM), AY631144, DQ194209, DQ200890, HQ443747, —. Garovaglia powellii, Unknown, A.E. Newton 6496 (BM), DQ296008, DQ194217, DQ200894, HQ443748, —. Hampeella pallens (*), Australia, Queensland, H. Streimann 64195 (H), AY306921, AM990371, FM161266, FM161109, AY452439. Hypnodendron vitiense, Australia, N.E. Bell 480 (BM), AY524471, AY524499, AY524526, FM161142, —. Spiridens camusii, New Caledonia, N.E. Bell 416 (BM), AY524475, AY524503, AY524530, HQ443771, —.

Ingroup taxa—Achrophyllum crassirete, Chile, J.-P. Frahm 21-10 (BONN), HQ443812, HQ443849, HQ443781, HQ443706, HQ443887. Achrophyllum quadrifarium (*), New Zealand, W. Frey & T. Pfeiffer 98-T2 (CHR), AY449660, HQ443850, AY452316, HQ443707, HQ443888. Actinodontium adscendens (*), Thailand, J.-P. Frahm 2006401 (BONN), HQ443813, —, HQ443782, HQ443708, —. Actinodontium spruce, French Guiana,
W.R. Buck 37977 (NY), AY306855, AY306689, AY452317, **HQ443709**, AY452397.

*Adelothecium bogotense* (*), Brazil, Vital & W.R. Buck 19649 (NY), AY306856, AY306690, AY452318, EF680784, AY452398. *Ancistrodes genuflexa* (*), Chile, I. Holz & J. Franzaring CH 00-154 (NY), AY306863, AY306697, AY452319, **HQ443710**, AY452399.

*Arbusculohypopterygium arbuscula* (*), Chile, I. Holz & J. Franzaring CH 00-80 (NY), AY449665, AY449671, AY452366, EF680789, AY452445. *Beeveria distichophylloides* (*), New Zealand, A.J. Fife 11150 (NY), AY306867, AY306701, AY452320, **HQ443711**, AY452400.


(NY), AF143062, AF161155, AY908629, HQ443726, HQ443898. Cyathophorum bulbosum (*), Australia, H. Streimann 55638 (NY), AY306889, AY306723, AY452339, —, AY452422.

borneense (a), Brunei, B.C. Tan 95-1060 (NY), AY306898, AY306732, AY452348, HQ443732, AY452430. Diploneuron connivens (*), Jamaica, M.R. Crosby 13732 (NY), AY306899, AY306733, AY908457, —, AY452431. Diploneuron diatomophilum, Cuba, W.R. Buck 23312 (NY), AY306870, AY306704, AY452326, HQ443733, AY452408. Distichophyllidium nymanianum (*), West Malaysia, Mohamed & Damanhuri 1118, Musci Malaysiani Exsiccati, fasc. 2: #29 (NY); Indonesia (Sulawesi), F. Müller S81 (DR), AY306901, AY306735, AY452350, HQ443734, HQ443908.

Distichophyllum carinatum, Germany, M. Nebel et al. MTB 8527/3 (STU), HQ443831, HQ443864, HQ443798, HQ443735, HQ443909.

Distichophyllum cuspidatum, West Malaysia, B.C. Tan 89-1356 (NY), HQ443832, HQ443865, —, HQ443736, HQ443910. Distichophyllum flaccidum, Chile, W.R. Buck 46275 (NY), HQ443833, HQ443866, HQ443799, HQ443737, HQ443911. Distichophyllum maibarae, China, D.G. Long 33943 (E), HQ443834, HQ443867, HQ443800, HQ443738, HQ443912. Distichophyllum malayense, West Malaysia, L. Hedenås MY92-533 (S), HQ443835, HQ443868, HQ443801, HQ443739, HQ443913. Distichophyllum microcarpum, New Zealand, H. Streimann 51286 (S), HQ443836, HQ443869, HQ443802, HQ443740, HQ443914. Distichophyllum mniifolium, South Africa, K. Hylander 10602 (S), HQ443837, HQ443870, HQ443803, HQ443741, HQ443915. Distichophyllum paradoxum, USA, Hawaii, T. Flynn 5151 (NY), AY306900, AY306734, AY452349, HQ443742, AY452432. Distichophyllum pulchellum, New Zealand, H. Streimann 51380 (NY), AY306902, AY306736, AY452351, EF680791, AY452433. Distichophyllum rigidicaule var. gabonense,


Pilotrichum bipinnatum (rej. lectotype), French Guiana, I. Holz FG 00-33 (NY), AY306976, AY306810, AY452378, HQ443765, AY452459. Pilotrichum procerum, Dominica, A. Schäfer-Verwimp 17941 (NY), AY306978, AY306812, AY452379, HQ443766, HQ443929. Pleurozium schreberi (h*), USA, B.W. Thornton 35 (DUKE), AY908281, HQ443881, AY908642, AJ288349 + AJ288563, —. Pterobryon densum (h*), Colombia, E.L. Linares & S.P. Churchill 3649 (MO); Honduras, B.H. Allen 12002 (BONN), AF143013, AF161106, AY908693, HQ443767, HQ443930. Rhynchostegiopsis tunguraguana (*), Colombia, P. Ramirez P7690 (NY), AY306986, AY306820, HQ443809, —, AY452463. Rhytidiadelphus triquetrus (h), USA, Thornton 20a (DUKE), AY908279, HQ443882, AY908636, HQ443768, HQ443931. Rutenbergia madagassa (h*), Madagascar, Fisher 33 (BM), AY524486, AY524514, AY524542,
HQ443769, —. *Sauloma tenella* (*), Australia, H. Streimann 59726 (NY), AY306987, AY306821, AY452384, HQ443770, AY452464. *Schimperobryum splendidissimum* (*), Chile, I. Holz & J. Franzaring Ch 00-156 (NY), AY306988, AY306822, AY452385, EF680807, AY452465. *Stenodesmus tenuicuspis* (*), Colombia, B.R. Ramírez et al. 8328 (MO), AY908610, HQ443883, AY908453, —, —. *Stenodictyon pallidum*, Dominican Republic, W.R. Buck 7940 (NY), AY306997, AY306831, HQ443810, HQ443772, AY452466.

*Stenodictyon wrightii* (*), Ecuador, W.R. Buck 10014 (NY), AY306998, AY306832, AY452386, HQ443773, AY452467. *Symphyodon imbricatifolius* (a), Brazil, A. Schäfer-Verwimp 14747 (NY), AY306999, AY306833, AY452387, HQ443774, AY452468.


(BONN), AM990449, AM990449, FM161338, FM161234, —. *Trachyphonium guadalupense* (*) , Trinidad, N. Djan-Chékar 94-670a (NY), HQ443848, HQ443885, —, —, AY452476.

Appendix B

Voucher information and GenBank accession numbers for the 35 taxa (77 samples) included in this study in the following order: taxon name (in italics), authorship, lab number, locality, collector and collector number (in italics), herbarium (inside brackets), and ITS, trnG, and trnL GenBank numbers. Newly generated sequences in bold. (*) Type specimen. (—) Missing sequences. ITS: 66 sequences available, 37 new (56%); trnG: 73 sequences available, 51 new (≈ 70%); trnL: 76 sequences available, 45 new (≈ 60%). Total: 215 sequences available (93%), 133 new (58%).

Outgroup taxa — Achrophyllum anomalum (Schwägr.) H. Rob., Aa598, Chile (VIII), Larrain 26248A (CONC), HQ613475, JQ943447, HQ613672. A. crassirete (Matteri) Matteri, Ac470, Chile (X), Frahm 21-10 (Herb. Frahm), HQ443706, JQ943448, HQ443849. A. dentatum (Hook. f. & Wilson) Vitt & Crosby, BBH02, Australia (NSW), Streimann 61075 (NY), AY306687, EF657200, EF680783. A. grandirete (Broth.) L. Pokorny & A.J. Shaw, LPM113, Chile (Juan Fernández I.), Hatcher & Engel 240 (NY), JQ943418, —, JQ943537; LPM114, Chile (Juan Fernández I.), Hatcher & Engel 181 (NY), JQ943419, JQ943468, JQ943513. A. haesselianum (Matteri) Matteri, LPM240, Chile (XII), Shaw 13641 (DUKE), JQ943445, JQ943499, JQ943543. A. magellanicum (Besch.) Matteri var. magellanicum, B599, Chile (XI), Crosby 16206 (L), HQ613477, JQ943450, HQ613674. A. magellanicum var. oligodontum (Matteri) Matteri, LPM249, Chile (XII), Buck 43467 (DUKE), JQ943446, —, JQ943544. A. quadrifarium (Sm.) Vitt & Crosby, BBH01, New Zealand, Streimann 51258

Beeveria *distichophylloides* (Broth. & Dixon) Fife, **BBH92**, New Zealand, *Fife 11150* (NY), 

HQ443711, **JQ943454**, AY306701. Benitotania elimbata H. Akiy., T. Yamag. & Suleiman, 

LPM138, Malaysia (Sabah), Bell 11 (H), **JQ943426**, **JQ943475**, AY449669. Bryobrothera *crenulata* (Broth. & Paris) Thér., **BBH25**, Australia (QLD), Streimann 57716 (NY), 

HQ443713, **JQ943453**, HQ443852. Daltonia marginata Griff., **JY23**, Australia (QLD), 

Streimann & Pócs 64700 (EGR), GQ905869, GQ906056, GQ906126. Da. splachnoides (Sm.) Hook. & Taylor, **GOM038**, Ireland, Hakelier s.n. (S), GQ905879, GQ906018, GQ906137.


Malaysia (Sabah), Bell 110 (H), **JQ943424**, **JQ943473**, **JQ943518**. 

*C. apiculata* (Hook. f. & Wilson) Vitt—**GOM002**, Chile (XII), *Buck 46252* (NY), 

HQ398623, HQ398670, HQ398719; **GOM113**, UK, *Long & Patton 12025* (BM), HQ398631, 

HQ398680, HQ398729; **LPM012**, New Zealand, *Macmillan 98/20* (CHR), HQ398633,
C. asplenioideae (Brid.) Crosby—GOM003, France (Reunion I.), Pócs 9680/N (NY),
HQ398634, HQ398683, HQ398732; GOM067, Madagascar, Pócs, Magill & LaFarge-
England 90115/Q (EGR), HQ398637, HQ398686, HQ398736; GOM068, Mauritius, Rui 107
(EGR), HQ398638, HQ398687, HQ398737; GOM069, Comoros, Magill & Pócs 11064a
(EGR), HQ398639, HQ398688, HQ398738; GOM070, South Africa, Arts s.n. (EGR),
HQ398640, HQ398689, HQ398739; GOM099, Dem. Rep. Congo, Pócs 9129/S (EGR),
HQ398659, HQ398705, HQ398759; LPM003, Tanzania, Pócs, Faden, Harris, Csontos &
Csontos 6257/E (EGR), HQ398660, HQ398706, HQ398763; LPM007, South Africa,
Heddderson 15035 (BOL), HQ398661, HQ398707, HQ398765.

C. brownii (Dixon) J.K. Bartlett—BBH14, Australia (NSW), Streimann 60613 (MO),
HQ398664, HQ398709, AY306707; GOM004, Australia (VIC), Streimann 58403 (NY),
HQ613478, JQ943456, HQ613675; GOM102 (C. otwayensis Streimann), Australia (VIC),
Curnow & Lepp 1374 (NY), HQ398662, HQ398710, HQ398768; LPM064 (*C. otwayensis),
Australia (VIC), Streimann 58384 (MO), JQ943414, JQ943461, JQ943505; LPM115, New
Zealand, Kantak 230 (NY), JQ943420, JQ943469, JQ943514; LPM151, Australia (VIC), Dell
91 (MEL), JQ943435, JQ943485, JQ943529; LPM152, Australia (TAS), Dell 94 (MEL),
JQ94346, JQ943486, JQ943530; LPM154 (C. otwayensis), Australia (VIC), Dell 90 (MEL),
JQ943437, JQ943487, JQ943531; LPM222 (*C. otwayensis Streimann), Australia (VIC),
Streimann 58384 (L), JQ943444, JQ943498, JQ943542.

C. cristata (Hedw.) Desv.—GOM111, New Zealand, Fife 7143 (NY), HQ398665,
HQ398712, HQ398770; LPM038, New Zealand, Frahm I-11 (MO), JQ943413, JQ943460,
JQ943504.

C. flaccida (Broth.) Z. Iwats., B.C. Tan & A. Touw—Cf525, Philippines, Linis s.n.
(SING), HQ398666, HQ398713, HQ398771.

C. flexicollis (Mitt.) Vitt—LPM032, New Zealand, Visch s.n. (MO), JQ943412, —,
JQ943501; LPM033, New Zealand, Vitt 8772A (MO), —, JQ943458, JQ943502; LPM093,
New Zealand, Paul s.n. (CHR), JQ943416, —, JQ943511; LPM208, New Zealand, Fife 6092
(MICH), —, JQ943497, JQ943541.

C. haitensis (H.A. Crum & Steere) Crosby—LPM065, Honduras, Allen 12026
(MO), —, JQ943462, JQ943506; LPM066, Honduras, Allen 12181 (MO), —, JQ943463,
JQ943507; LPM068, Costa Rica, Crosby & Crosby 6583 (MO), —, JQ943464, JQ943508.

C. japonica (Cardot & Thér.) Z. Iwats. & Nog.—GOM062, Japan, Osada s.n. (S),
HQ398667, HQ398715, HQ398773; LPM028, China (Guizhou), Crosby 15726 (MO),
JQ943411, JQ943457, JQ943500; LPM104, Japan, Takaki 7216 (MICH), JQ943417,
JQ943467, JQ943512; LPM132, Japan, Schofield 52518 (DUKE), JQ943423, JQ943472,
JQ943517; LPM146, Japan, Yamaguchi 31308 (HIRO), JQ943433, JQ943483, JQ943527.
C. leptoloma (Broth.) H. Rob. — **LPM185**, Chile (Juan Fernández I.), Skottsberg & Skottsberg 301 (S), —, **JQ943494, JQ943538**.

C. marginata (Thér.) Pursell & W.D. Reese — **LPM139**, New Caledonia, Bell 10 (H), JQ943427, JQ943476, JQ943520; **LPM140**, New Caledonia, Bell 18 (H), JQ943428, JQ943477, JQ943521.

C. microblasta (Broth.) B.C. Tan & H. Rob. — **Cm533**, Philippines, *Linis* 1459-05 (SING), HQ398668, **JQ943455**, HQ398775.

C. nutans (Hampe) S.P. Churchill — **LPM035**, Peru, Opisso & Gazis 1530 (MO), —, JQ943459, JQ943503; **LPM119**, Ecuador, Gradstein & Sipman 10123 (NY), **JQ943421**, JQ943470, JQ943515.

C. perlimbata (Dixon) B.C. Tan & B.C. Ho — **LPM166**, Papua New Guinea, Touw 17063 (L), —, **JQ943490, JQ943534**.

C. ramosa (M. Fleisch.) B.C. Tan & H. Rob. — **B597**, Indonesia (Java), Ho 07-007 (SING), **JQ943410, JQ943449**, HQ398776; **LPM163**, Indonesia (Flores), Touw & Snoek 23126 (L), —, **JQ943488, JQ943532, LPM164**, Indonesia (Flores), Touw & Snoek 23093 (L), JQ943438, JQ943489, JQ943533.

C. remotifolia (Müll. Hal.) Z. Iwats., B.C. Tan & A. Touw — **LPM137**, Malaysia (Sabah), Bell 75 (H), **JQ943425, JQ943474, JQ943519, LPM142**, Thailand, Akiyama, Kanzaki, Irie & Ando 239 (HYO), —, **JQ943479, JQ943523, LPM143**, Thailand, Akiyama, Kanzaki, Irie & Ando 249 (HYO), **JQ943430, JQ943480, JQ943524, LPM145**, Thailand,
Akiyama, Kanzaki, Irie & Ando 407 (HYO), JQ943432, JQ943482, JQ943526; LPM170, 
Indonesia (Sumatra), Touw & Snoek S25311 (L), JQ943439, JQ943491, JQ943535; LPM172, 
Indonesia (Flores), Touw & Snoek 23240 (L), JQ943440, JQ943492, JQ943536; LPM174, 
Indonesia (Bali), Touw & Snoek 22405 (L), JQ943441, JQ943493, —. 

C. spinosa (Nog.) Ninh—LPM081, China (Guizhou), Crosby 15977 (MO), —, 
JQ943465, JQ943509; LPM130, China (Hainan), Redfearn Jr, Reese, Lin, Wu, Li & Wang 
35826 (DUKE), JQ943422, JQ943471, JQ943516; LPM141, Thailand, Akiyama, Kanzaki, Irie 
& Ando 132 (HYO), JQ943429, JQ943478, JQ943522; LPM144, Thailand, Akiyama, Kanzaki, 
Irie & Ando 306 (HYO), JQ943431, JQ943481, JQ943525.
Appendix C

Voucher information for Calyptrochaeta and Daltonia samples included in the molecular analyses (herbaria in brackets), and GenBank accession data in the following sequence: ITS, trnG, trnL (— indicates the sequence was not obtained, italics for preexisting GenBank numbers). Capitals before sample numbers (in bold) indicate who performed the DNA extraction (B= B. C. Ho; G= G. Oliván; J= J. Yu; L= L. Pokorny; WB= W. Buck).


Calyptrochaeta apiculata — **G002**, Chile, Buck 46252 (NY), HQ398623, HQ398670, HQ398719; **G060**, Australia, TAS, Streimann 40080 (S), HQ398624, HQ398671, HQ398720; **G100**, Australia, NSW, Streimann 39651 (NY), HQ398625, HQ398672, HQ398721; **G101**, Australia, TAS, Streimann 40068 (NY), HQ398626, HQ398673, HQ398722; **G104**, Chile, Buck 45641 (NY), —, HQ398674, HQ398723; **G105**, Chile, Buck 45965 (NY), HQ398627, HQ398675, HQ398724; **G106**, Chile, Buck 46077 (NY), —, HQ398676, HQ398725; **G107**, Chile, Buck 46104 (NY), HQ398628, HQ398677, HQ398726; **G109**, Chile, Buck 46218 (NY), HQ398629, HQ398678, HQ398727; **G110**, Chile, Goffinet 6972 (NY), HQ398630, HQ398679, HQ398728; **G113**, GB, Channel Islands, Long & Patton 12025 (BM), HQ398631, HQ398680, HQ398729; **L009**, GB, Falkland Islands, Engel 2910 (H), HQ398632,
Calyptrochaeta asplenioides — G003, La Réunion, Póc 9680/N (NY), HQ398634, HQ398683, HQ398732; G064, La Réunion, Póc 9612/M (EGR), HQ398635, HQ398684, HQ398733; G065, La Réunion, Kis 9434/El (EGR), HQ398636, —, HQ398734; G066, Tanzania, Póc & Knox 89052/AM (EGR), —, HQ398685, HQ398735; G067, Madagascar, Póc, Magill & Lafarge 90115/Q (EGR), HQ398637, HQ398686, HQ398736; G068, Mauritius, Rui 107 (EGR), HQ398638, HQ398687, HQ398737; G069, Comoros, Magill & Póc 11064 (EGR), HQ398639, HQ398688, HQ398738; G070, South Africa, Arts RSA27/11 (EGR), HQ398640, HQ398689, HQ398739; G071, Comoros, Póc, Magill & Rupf 9268/BL (EGR), HQ398641, HQ398690, HQ398740; G072, Comoros, Magill & Póc 11051 (EGR), HQ398642, HQ398691, HQ398741; G073, Comoros. Magill & Póc 11094 (EGR), HQ398643, HQ398692, HQ398742; G074, Comoros, Magill & Póc 11101 (EGR), HQ398644, HQ398693, HQ398743; G075, La Réunion, Kis & Póc 9613/D (EGR), HQ398645, HQ398694, HQ398744; G077, La Réunion, Kis 9427/El (EGR), HQ398646, HQ398695, HQ398745; G079, La Réunion, Kis 9605/C (EGR), HQ398647, HQ398696, HQ398746; G080, La Réunion, Kis 9612/N (EGR), HQ398648, —, HQ398747; G081, La Réunion, Kis 9654/T (EGR), HQ398649, HQ398697, HQ398748; G082, La Réunion, Konya 9601/D (EGR), HQ398650, HQ398698, HQ398749; G083, La Réunion, Orban 9436/ED (EGR), —, HQ398699, HQ398750; G084, La Réunion, Orban 9436/EM (EGR), HQ398651,

Calyptrochaeta cristata—G111, New Zealand, Fife 7143 (NY), HQ398665, HQ398712, HQ398770.

Calyptrochaeta flaccida—B525, Philippines, Linis s. n. (SING), HQ398666, HQ398713, HQ398771.

Calyptrochaeta japonica—G061, Japan, Mizutani 15156 (S), —, HQ398714, HQ398772; G062, Japan, Osada (S), HQ398667, HQ398715, HQ398773; G063, Japan, Inoue (EGR), —, HQ398716, HQ398774.

Calyptrochaeta microblasta—B533, Philippines, Linis 1459-05 (SING), HQ398668, HQ398717, HQ398775.

Calyptrochaeta ramosa—B597, Indonesia, Java, Ho 07-007 (SING), HQ398669, HQ398718, HQ398776.
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Biography

Cristina Isabel Pokorny Montero was born in London (Ontario, Canada), on December 11th, 1978. She obtained her B.Sc. (Licenciatura), in Biological Sciences, on July of 2001 and her M.Sc. (DEA), in Evolutionary Biology and Biodiversity, on March of 2004, both at Universidad Autónoma the Madrid (Spain). Her Master Thesis title is “Bryological study of the city of Trento (Italy)”. Among her publications it is worth mentioning “Disentangling knots of rapid evolution: origin and diversification of the moss order Hypnales”, “Molecular evolution and diversification of the moss Family Daltoniaceae (Hookeriales, Bryophyta) with emphasis on unraveling the phylogeny of Distichophyllum and its allies”, “Phylogenetic analyses of morphological evolution in the gametophyte and sporophyte generations of the moss order Hookeriales”, “Phylogeographic Patterns in Two Southern Hemisphere Species of Calypetrochaeta (Daltoniaceae, Bryophyta)”, “Genetic structure and genealogy in the Sphagnum subsecundum complex (Sphagnaceae: Bryophyta)”, and “Schistidium lancifolium (Kindb.) Blom & Schistidium pulchrum Blom new to Italy”.

Since 2001 she has earned awards from Duke University (Graduate School and Department of Biology), Sigma Xi, International Association of Bryologists (as a member), American Bryological and Lichenological Society (as a member), Fulbright Commission, Fundación Ramón Areces, Spanish government (FPU-MEC), Universidad Autónoma de Madrid, and Museo Tridentino di Scienze Naturali, among others.