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Evolutionary dynamism in bryophytes: Phylogenomic inferences confirm rapid radiation in the moss family Funariaceae

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ABSTRACT

Rapid diversifications of plants are primarily documented and studied in angiosperms, which are perceived as evolutionarily dynamic. Recent studies have, however, revealed that bryophytes have also undergone periods of rapid radiation. The speciose family Funariaceae, including the model taxon *Physcomitrella patens*, is one such lineage. Here, we infer relationships among major lineages within the *Entosthodon-Physcomitrium* complex from virtually complete organellar exomes (i.e., 123 genes) obtained through high throughput sequencing of genomic libraries enriched in these loci via targeted locus capture. Based on these extensive exonic data we (1) reconstructed a robust backbone topology of the Funariaceae, (2) confirmed the monophyly of *Funaria* and the polyphyly of *Entosthodon*, *Physcomitrella*, and *Physcomitrium*, and (3) argue for the occurrence of a rapid radiation within the *Entosthodon-Physcomitrium* complex that began 28 mya and gave rise more than half of the species diversity of the family. This diversification may have been triggered by a whole genome duplication and coincides with global Eocene cooling that continued through the Oligocene and Miocene. The Funariaceae join a growing list of bryophyte lineages whose history is marked by at least one burst of diversification, and our study thereby strengthens the view that bryophytes are evolutionarily dynamic lineages and that patterns and processes characterizing the evolution of angiosperms may be universal among land plants.

1. Introduction

Bryophytes (mosses, liverworts, and hornworts) differ significantly from other extant land plants by two plesiomorphic traits: a dominant gametophyte and an unbranched sporophyte that remains physically dependent on the maternal plant. Despite the rather simple architecture of their vegetative body (Goffinet and Buck, 2012), bryophytes are diverse, with as many as 20,000 extant species (Crosby et al., 1999; Söderström et al., 2016). Their position in the land plant tree of life (Cox et al., 2014; Wickett et al., 2014) and simple architecture (Schofield and Crum, 1972) may have led to their perception as primitive, "unmoved sphinxes from the past" (Crum, 1972). Furthermore, their rate of molecular evolution has been suggested as slower than in other plants (Stenøien, 2008), and broad disjunctions of conspecific populations assumed to result from vicariance were further interpreted as reflecting poor evolutionary potential (Frey et al., 1999). Although the view of bryophytes being sphinxes has generally been abandoned (e.g., Laenen et al. 2014), bryophytes continue to be labelled as primitive (e.g., Pedersen and Palmgren, 2017) and early (e.g., Liu et al. 2014) land plants, and to be implicitly perceived as slowly evolving.

This perception of bryophytes contrasts strongly with that of angiosperms, which are typically viewed as an evolutionarily dynamic lineage whose phylogenetic history is marked by episodically rapid diversifications (Givnish, 2010). Such rapid successions of divergences may be triggered by extrinsic or intrinsic events (Linder, 2008). They may follow dramatic shifts in selection resulting from climatic change or from novel ecological opportunities such as those provided by the rise of the Mediterranean climate meganiche (Guzmán et al., 2009;

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Table 1
List of species and specimens included in this study, with the DNA number identifying the accession, its country or origin, collector and collection number and herbarium, and the SRA accession (SAMN07945352 to SAMN07945432 published here for the first time). Duplicated libraries performed to assess accuracy of sequencing are marked with a "b" after the ID number.

Taxon	ID	Collector and number	Accession SRA
FUNARIACEAE			
Aphanorrhegma *serratum* (Wilson & Hook.) Sull.	3305	USA, Arkansas, *Buck* 49500, NY	SAMN07945352
Entosthodon *americanus* (Lindb.) Fife	3894	Canada, Alberta, *Goffinet* 4347, CONN	SAMN07945356
E. attenuatus (Dicks.) Bryhn	3479	Ireland, *Long* 38559, CONN	SAMN07945357
	3835	Portugal, *Schäfer-Verwimp* 33687, CONN	SAMN07945358
E. bergianus (Hornsch.) Müll. Hal.	3509	South Africa, Western Cape Province, *Hedderson* 15250, BOL	SAMN07945359
E. clavatus Mitt.	3545	South Africa, Northern Cape Province, *Goffinet* 10178, CONN	SAMN07945360
	3895	South Africa, Western Cape Province, *Hedderson* 16864, BOL	SAMN07945361
	3896	South Africa, Northern Cape Province, *Goffinet* 11523, CONN	SAMN07945362
E. duriaei Mont.	3843	Spain, *Long* 38230 & Runasinghe, CONN	SAMN07945363
E. hungaricus (Boros) Loeske	3177	Austria, *Frahm* 70325, B	SAMN07945364
	3838	Hungary, *Sabovljevic* & Papp s.n., herb. *Sabovljevic*	SAMN07945365
E. lindigii (Hampe) Mitt.	3546	Peru, *Cano* et al. 5049, NY	SAMN07945366
E. muhlenbergii (Turner) Fife	3893	Hungary, *Sabovljevic* & Papp s.n., herb. *Sabovljevic*	SAMN07945367
E. obtusus (Hedw) Lindb.	3347	Ireland, *Holyoak* 04-87, NY	SAMN07945368
	3347b	Ireland, *Holyoak* 04-87, NY	SAMN07945369
E. planoconvexus (E.B. Bartram) Grout	3114	USA, California, *Shevock* 26542, CAL	SAMN07945370
E. radians (Hedw.) Müll. Hal.	3837	New Caledonia, *B. Shaw* 17548	SAMN07945371
E. smithhurstii (Broth. & Geh.) Paris	3465	Australia, *Downing* et al. s.n., CONN	SAMN07945372
Entosthodon sp.	3726	India, *Villarreal* et al. 1322, CONN	SAMN07945373
E. subintegrus (Broth.) H.A. Miller, H. Whittier & B. Whittier	3840	USA, Hawaii, *Wilding* 224, BOL	SAMN07945374
Funaria *arctica* (Berggr.) Kindb.	3544	Canada, Nunavut, *LaFarge* 13722, ALTA	SAMN07945375
	3833	Canada, Nunavut, *Björk* 28223, UBC	SAMN07945376
F. flavicans Michx.	4092	USA, North Carolina, *Shaw* s.n., CONN	SAMN07945377
F. hygrometrica Hedw.	3111	USA, North Carolina, *Goffinet* 9344, CONN	SAMN07537336
	3179	Germany, *Schuette* s.n., CONN	SAMN07945378
	3388	Japan, *Itouga* s.n., CONN	SAMN07945379
	3476	USA, Connecticut, *Goffinet* 9027, CONN	SAMN07945380
	3515	USA, Connecticut, *Budke* 142, CONN	SAMN07945381
	3632	USA, Connecticut, *Budke* 145, CONN	SAMN07945382
	3891	USA, Connecticut, *Goffinet* 9278, CONN	SAMN07945383
F. hygrometrica var. *calvescens* (Schwägr.) Mont.	3633	France, *Wilding* 34b, CONN	SAMN07945384
F. microstoma Bruch ex Schimp.	3834	China, *Miehe* et al. s.n., CONN	SAMN07945385
F. polaris Bryhn	3542	Russia, *Fedosov* s.n., CONN	SAMN07945386
Funaria sp.	3393	China, Yunnan, *Goffinet* et al. 9934, CONN	SAMN07945387
Funaria sp.	3514	Ethiopia, *Hylander* 5948, CONN	SAMN07945388
Funaria sp.	3541	Ethiopia, *Hylander* 5947, CONN	SAMN07945389
Funaria sp.	3882	Chiina, Yunnan, *Goffinet* 9947, CONN	SAMN07945390
Goniomitrium *africanum* (Müll. Hal.) Broth.	4081	South Africa, Northern Cape Province, *Goffinet* 11515, CONN	SAMN07945391
Physcomitrella *magdalenae* De Sloover	3844	Rwanda, *Buchbender* RWA-VB-0107	SAMN07945394
P. patens (Hedw.) Bruch & Schimp.	3139	Canada, British Columbia, *Spribille* s.n., CONN	SAMN07537376
	3403	USA, California, *Mishler* s.n., CONN	SAMN07945395
Physcomitrellopsis africana Broth. & Wager ex Dixon	3142	South Africa, Eastern Cape Province, *Goffinet* 10326, CONN	SAMN07945396
Physcomitridium *readeri* (Müll. Hal.) G.Roth	3892	France, *Infante* & *Heras*, VIT	SAMN07945397
Physcomitrium *collenchymatum* Gier	3178	USA, Arkansas, *Buck* 49499, NY	SAMN07945398
	3480	USA, Missouri, *Budke* 185, CONN	SAMN07945399
Physcomitrium eurystomum Sendtn.	3392	Germany, *Frahm* 10979, BONN	SAMN07945431
	3392b	Germany, *Frahm* 10979, BONN	SAMN07945432
P. hookeri Hampe	3409	USA, Missouri, *Budke* 177B, CONN	SAMN07945400
	3412	USA, Kansas, *Budke* 195, CONN	SAMN07945401
P. immersum Sull.	3176	USA, North Carolina, *Shaw* 4827. DUKE	SAMN07945402
P. japonicum (Hedw.) Mitt.	3411	Japan, *Akiyama* 22212, CONN	SAMN07945403
	3413	Japan, *Tamura* s.n., CONN	SAMN07945404
	3508	China, Shanghai, *Goffinet* 9782, CONN	SAMN07945405
	3539	China, Shanghai, *Goffinet* 9785, CONN	SAMN07945406
	3551	China, Shanghai, *Goffinet* 9783, CONN	SAMN07945407
	3816	China, Shanghai, *Goffinet* 9782, CONN	SAMN07945408
P. pyriforme (Hedw.) Hampe	3118	USA, Connecticut, *Goffinet* 9704, CONN	SAMN07945409
	3387	Russia, Moskow, *Fedosov* s.n. , CONN	SAMN07945410
	3404	USA, Missouri, *Budke* 187, CONN	SAMN07945411
	3410	USA, Missouri, *Budke* 177A, CONN	SAMN07945412
	3496	USA, Connecticut, *Goffinet* s.n., CONN	SAMN07945413
	3555	Spain, *Segarra-Moragues* s.n., CONN	SAMN07945414
	3727	USA, North Carolina, *Goffinet* 11123, CONN	SAMN07945415
	3728	USA, North Carolina, *Goffinet* 11127, CONN	SAMN07945416
	3787	Ireland, *Bosanquet* s.n., CONN	SAMN07945417
	3798	USA, North Carolina, *Buck* 63007, CONN	SAMN07945418
	3883	USA, Maine, *Buck* 54976, NY	SAMN07945419
	3886	USA, North Carolina, *Tripp* 4197, NY	SAMN07945420
Physcomitrium sp.	3814	South Africa, Western Cape Province, *Wilding* 217, BOL	SAMN07945421
Physcomitrium sp.	3817	South Africa, Eastern Cape Province, *Goffinet* 11574, CONN	SAMN07945422
Physcomitrium sp.	3842	*Shaw* 17,401, DUKE	SAMN07945423

(*continued on next page*)

Table 1 (*continued*)

Taxon	ID	Collector and number	Accession SRA
P. spathulatum Müll. Hal.	3549	South Africa, Eastern Cape Province, *Goffinet* 10312, CONN	SAMN07945424
P. sphaericum (C. Ludw.) Fürnr.	3115	China, Yunnan, *Goffinet* 9815, CONN	SAMN07945425
	3141	China, Yunnan, *Goffinet* 9827, CONN	SAMN07945426
	3550	China, Yunnan, *Goffinet* 9828, CONN	SAMN07945427
	3672	China, Guangdong, *Liu* 13003, CONN	SAMN07945428
	3815	China, Yunnan, *Goffinet* 9823, CONN	SAMN07945429
P. subsphaericum Schimp.	3556	Mexico, *Villarreal* 1218, CONN	SAMN07945430
OUTGROUP			
Timmia austriaca Hedw.	3619	Sweden, *Hedenäs* et al. s.n., CONN	SAMN07537408
Timmia norvegica J.E. Zetterst.	3578	Norway, *Hedenäs* s.n., CONN	SAMN07537410
Bryobartramia schelpei Hedd.	4083	South Africa, *Goffinet* 11520 & 11521, CONN	SAMN07945353
Encalypta intermedia Jur.	3219	USA, Nevada, *Shevock* et al. 27671, NY	SAMN07537327
Chamaebryum pottioides Thér. & Dixon	3573	South Africa, Northern Cape Province, *Goffinet* 11517, CONN	SAMN07537309
	3630	South Africa, Northern Cape Province, *Goffinet* et al. 11503, CONN	SAMN07537308
	3630b	South Africa, Northern Cape Province, *Goffinet* et al. 11503, CONN	SAMN07945354
Costesia spongiosa Thér.	4086	Chile, *Larrain* 36906, CONN	SAMN07537311
Gigaspermum repens (Hook.) Lindb.	3583	South Africa, Northern Cape Province, *Goffinet* 11535, CONN	SAMN07537338
Lorentziella imbricata (Mitt.) Broth.	3419	USA, Texas, *Rushing* s.n., CONN	SAMN07945392
Oedipodiella australis (Wager & Dixon) Dixon	4082	South Africa, Eastern Cape Province, *Goffinet* 11561, CONN	SAMN07945393
Discelium nudum (Dicks.) Brid.	3220	Sweden, *Dynesius* s.n., CONN	SAMN07537322
	3220b	Sweden, *Dynesius* s.n., CONN	SAMN07945355

Linder, 2003) or following dispersal to young islands (Vitales et al., 2014). Increases in speciation rates may also follow large scale or whole genome duplication (WGD) resulting from autopolyploidy or allopolyploidy (Abbott et al., 2013; Pease et al., 2016; Soltis et al., 2016), which would broaden the templates for genetic, hence morphological, physiological or ontogenetic innovation (Rensing, 2014; Wang et al., 2012).

Despite the long held view of their evolutionary stasis, bryophytes are increasingly emerging as dynamic lineages with much of their diversity arising following successive bursts of diversification since the mid Mesozoic (Laenen et al., 2014). Some of these rapid radiations may be linked, for example, to the rise of angiosperm forests, which provided new habitats for epiphytes (Scheben et al., 2016; Silva et al., 2017), or may have been triggered by WGD, such as in *Sphagnum* (Devos et al., 2016; Shaw et al., 2016) and in the Hypnales (Shaw et al., 2003; Newton et al., 2007; Johnson et al., 2016b). Furthermore, broad geographic distributions resulting from rather recent (i.e., post Pangaea or Gondwana) events (e.g., Lewis et al., 2014) may be followed by allopatric speciation (e.g., Medina et al., 2012, 2013). These patterns and processes may account for much of the extant diversity of lineages of mosses, such as the Funariaceae. This family likely incurred one or more past WGD in its history (Rensing et al., 2007), is globally distributed, and holds many narrow endemics (Fife, 1982).

The Funariaceae have long provided model taxa for developmental (e.g. Wettstein, 1925; French and Paolillo, 1975) and, more recently, genomic and evo-devo studies (Lang et al., 2016). The family arose early in the diversification of mosses with arthrodontous peristomes (Cox et al., 2014, 2004) and comprises now approximately 255 species (Crosby et al., 1999). Its history is marked by a first split segregating the Funarioideae and Pyramiduloideae (Werner et al., 2007), followed by the split between *Funaria* and the rapidly diversifying *Entosthodon-Physcomitrium* (E-P) clade (Liu et al., 2012). The latter comprises a large assemblage of species exhibiting a broad range of sporophyte morphologies likely resulting from recurrent reduction, as best exemplified by the polyphyly of the genus *Physcomitrella* (Liu et al., 2012). The potentially extensive homoplasy in these traits called for inferences from alternative characters. Variation in ten loci sampled from all three genome compartments proved, however, insufficient to robustly resolve the relationships among lineages within the E-P clade (Liu et al. 2012).

Here we seek to further strengthen the reconstruction of the evolutionary history of the Funariaceae, resolve the radiation within the Funarioideae and provide a time frame for their diversification. We sampled all organellar exons via liquid phase bait-mediated hybridization and sequenced 91 multiplexed enriched libraries, allowing for a cost-effective sampling of extensive loci across taxa, and resulting in near maximum coverage and significant depth, compared to retrieving organellar loci from randomly sequenced genomic libraries (e.g. Liu et al. 2014; Shaw et al. 2016). We compiled genome-scale data for 78 Funariaceae and 13 outgroup samples via high-throughput sequencing of genomic libraries enriched in all coding genes of the plastid and mitochondrial genomes. The exonic dataset should overcome the limitations of the ten organellar and nuclear loci targeted by Liu et al. (2012), considering that the organellar exomes contain a rich phylogenetic signal in the Funariaceae (Liu et al., 2013). The alignment of plastid and mitochondrial protein coding genes should be relatively unambiguous due to codon homology and absence of paralogy, given that the mitochondrial and plastid genomes seem to be maternally inherited in bryophytes (Jankowiak et al., 2005; Natcheva and Cronberg, 2007). Since meiosis is monoplastidic (Brown and Lemmon, 2016), no regular recombination occurs during the life cycle, hence phylogenetic inference from (at least) the plastid data should not be affected by other, more complex, phenomena such as hybridization and incomplete lineage sorting. This approach, however, is a tradeoff, since it offers only partial insight (i.e., maternal) into, for example, the affinities of allopolyploids. Nevertheless, these extensive exonic data provide a robust dataset for a) reconstructing the backbone topology of the Funariaceae, b) testing the monophyly of major genera within the Funariaceae, and c) assessing timing of diversification of the E-P clade.

2. Material and methods

We sampled 78 specimens from the Funariaceae, employing their closest relatives as outgroups up to a total of 91 samples (Table 1). To assess the reliability of the methodology for library preparation and sequencing error, some specimens were represented by two samples (*Chamaebryum pottioides*; *Discelium nudum*; *Entosthodon obtusus* and *Physcomitrium eurystomum*). Since wild populations of Funariaceae develop gametophytes in small clumps, typically insufficient for genomic extraction, moss samples were cultured from spores to acquire sufficient tissue for DNA extraction. Total genomic DNA was extracted using the NucleoSpin Plant II Midi kit (Macherey-Nagel. Düren, Germany) following the manufacturer's instructions. Before library preparation we verified that the extractions were not contaminated by amplifying a plastid locus (typically *psbA-trnH*) via PCR and comparing its sequence to the one obtained from DNA extracted from the original voucher. These PCRs were performed in a final volume of 25 μL with 0.15 mL of GoTaq DNA polymerase (Promega, Madison WI, USA), 1 μL of 10 mM

dNTP mix, 1 μL of each primer (10 mM) and 1 μL of DNA extract. PCR consisted of a 3 min hot start at 94 °C, followed by 40 cycles of denaturation (1 min, 94 °C), annealing (1 min, 50 °C) and extension (1 min, 70 °C), ended by a final extension step of 10 min. PCR products were cleaned used the ExoSAP-IT protocol (USB-Affymetrix, Cleveland OH, USA) and sequenced on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City CA, USA).

Library preparation was performed following the TruSeq Nano DNA Library Prep HT Protocol of 550 bp insert (Illumina. San Diego, CA, USA) using an adequate combination of adaptors for multiplexing. Aliquots of all the libraries were brought to equimolar concentrations, pooled together and used for the Targeted High-Throughput Sequencing protocol (Arbor Biosciences, Ann Arbor, MI, USA). A capture solution was assembled using the library master mix, the hybridization master mix and a customized bait solution (MYbaits. Arbor Biosciences. Ann Arbor, MI, USA) designed from all mitochondrial and plastid exons of several moss species, including *Physcomitrella patens*. The pooled and enriched libraries were sequenced using the Illumina MiSeq platform (Illumina. San Diego, CA, USA).

The paired-end, demultiplexed reads were filtered and cleaned by running Trimmomatic (Bolger et al., 2014) on the raw data, and then assembled into contigs for each of the mitochondrial and plastid coding genes using HybPiper (Johnson et al., 2016a). The datasets of the demultiplexed, unassembled reads are deposited in the NCBI Sequence Read Archive (Table 1). The sequences for each organellar locus were aligned separately using the plugin MUSCLE (Edgar, 2004) implemented in Geneious v. 7.1.3. (Biomatters. Auckland, New Zealand). Single-locus trees were obtained and inspected visually to exclude highly divergent sequences resulting from contamination or remaining low quality reads. Given their low impact on the final matrices (0.0016% of the plastid and 0.0008% of the mitochondrial matrix) indels were not coded. Since each organellar genome is considered uniparentally inherited in mosses, and hence behaves as a single locus, data from all loci within a genome were concatenated. We performed phylogenetic reconstructions of the three datasets using Bayesian Inference and Maximum Likelihood optimality criteria. Bayesian analyses were run using MrBayes v. 3.2.4 (Ronquist and Huelsenbeck, 2003) implementing the partitioning and models suggested by PartitionFinder (Lanfear et al., 2012). The MCMC algorithm was run for 10^7 generations using four runs and two chains. The convergence of the different chains was checked using Tracer (Drummond and Rambaut, 2007). Posterior probabilities (PP) were estimated from the consensus of all but the first 20% (burn-in) of trees after visual inspection of Tracer results. Maximum Likelihood analyses were run for the three datasets using RAxML v. 8.1.17 (Stamatakis, 2006), implementing the partitions suggested by PartitionFinder under the GTR model. Support for splits was estimated from split frequencies in the consensus of all optimal ML trees obtained from 100 bootstrap pseudoreplicates (GTRCAT model). Phylogenetic trees were visualized using FigTree version 1.4.2 (Rambaut and Drummond, 2010). Given the large amount of data and the potential effect of different biases operating at the genomic level (Liu et al., 2014) we only considered a node to have high support values if both the Bayesian Posterior Probability and the ML bootstrap were high (over 0.95 and 90 respectively). Single-locus and concatenated alignments are available from the Dryad Digital Repository (https://http://dx.doi.org/10.5061/dryad.78h27).

To estimate a time frame for the radiation of the Funariaceae, we reduced the plastid dataset to 10 genes with complete data (*atpA*, *atpB*, *chlN*, *psaA*, *psbB*, *rbcL*, *rpL2*, *rpoB*, *rps3* and *rps18*) and single-locus topologies approximating the one resulting from ML and BI analyses. The genes were concatenated and partitioned following the same criteria used on the complete dataset. We used a mutation rate of 5×10^{-4} substitutions/site/million years based on Palmer (1991). This rate has been extensively used in bryophytes (e.g. Huttunen et al., 2008; Shaw et al., 2010; Pokorny et al., 2011; Lewis et al., 2014) and confirmed independently for the *rbc*L gene in hornworts by Villarreal et al. (2012). As an additional calibration, we used the 95% confidence interval of age estimates (calibration II) of the internal nodes of the subtree containing *Aphanorrhegma*, *Physcomitrella*, *Entosthodon*, *Funaria*, *Gigaspermum* and *Chamaebryum* proposed by Laenen et al. (2014). In order to prevent BEAST from changing the topology, we imposed a modified, ultrametric version of the tree obtained under RAxML and locked the topology (Hsiang et al., 2015; Prum et al., 2015). Five independent runs of 10,00,00,000 generations were run sampling the MCMC chain every 10,000 generations and, after the inspection of Tracer results, merged discarding the initial 10% as burn-in.

3. Results and discussion

Sequencing multiplexed enriched genomic libraries yielded 96% of all the plastid protein coding sequences for the 91 samples (average coverage 95.4%, standard deviation 20%). All the sequences of the mitochondrial exons were recovered except a fragment of the *atp9* gene (total chondrome average coverage is 99%, with a standard deviation of 7%). The average pairwise distance among Funarioideae for the whole plastid exome is 4.3% (3.2% StDev), and for the mitochondrial exome 0.9% (1.3% StDev). The low level of variation among Funarioideae sequences is compensated by the size of the matrix (i.e., 79 plastid and 38 mitochondrial exonic sequences), yielding a total of 7887 variable sites (13.3%) in the plastome and 1028 variable sites in the chondrome (3.5%) with a relatively low percentage of missing data (1.3% in the plastid dataset and 0.3% in the mitochondrial), and no exclusion of sites of ambiguous homology across the sequences aligned within the coding frame. Finally, the organellar genomes, being likely uniparentally inherited and free of recombination, should exhibit concordant phylogenetic signal among loci, and since the loci are all linked, the signal should be cumulative across the exomes. Hybridization is known to occur within the Funariaceae (e.g. McDaniel et al., 2010; Beike et al., 2014), hence the phylogenetic hypothesis presented here may only reflect the maternal history. Nevertheless, our inferences provide the first robust resolution of evolutionary history for the Funariaceae. We provide further evidence for the polyphyly of *Entosthodon* and *Physcomitrium* and unambiguous evidence of a rapid radiation, characterizing the evolution of these genera, which is estimated to have begun approximately 28 million years ago (mya).

Phylogenetic inferences from plastid exons (Fig. 1) are congruent with those from the mt exons (Supplementary materials, trees 5 and 6) but are more robustly supported, with all but one internal backbone node maximally supported (i.e., PP = 1, and BT = 100%; Fig. 1). The phylogenomic backbone is consistent with that proposed by Werner et al. (2007) and Liu et al. (2012) in resolving an early split between the Pyramiduloideae and the Funarioideae, as well as placing *Funaria* sister to the remaining members of the family, i.e., the *Entosthodon-Physcomitrium* clade (E-P clade). The robust segregation of *Entosthodon* from *Funaria* settles the controversy regarding the status of the former, often regarded as weakly differentiated morphologically from, and hence potentially congeneric with, the latter (Lindberg, 1879). The two genera have been distinguished by the symmetry of the capsule and double peristome (e.g., McIntosh, 2007) but such a dichotomy is artificial. Instead, *Funaria* differs from *Entosthodon* (and *Physcomitrium*) by the compound and revoluble versus simple annulus (Fife, 1982; 1985; Liu et al., 2012). Such an annulus is also present in *Afoninia dahurica*, a rare species endemic to East Siberia (Ignatov et al., 2015). Nuclear ITS and plastid *trn*L-F and *psb*A-trnH loci resolve *Afoninia* as sister to the E-P clade (Ignatov et al., 2015), and hence this annulus type may be plesiomorphic in the Funarioideae.

Our phylogenomic inferences also provide the first robust resolution of relationships within the E-P clade, which consists of two sister lineages, here referred to as the *Physcomitrellopsis* clade and the *Entosthodon-Physcomitrium* complex (E-P complex), whose rapid radiation is the main focus of this study. *Entosthodon* is mostly a paraphyletic group subtending the clade comprising *Physcomitrium*, within which the

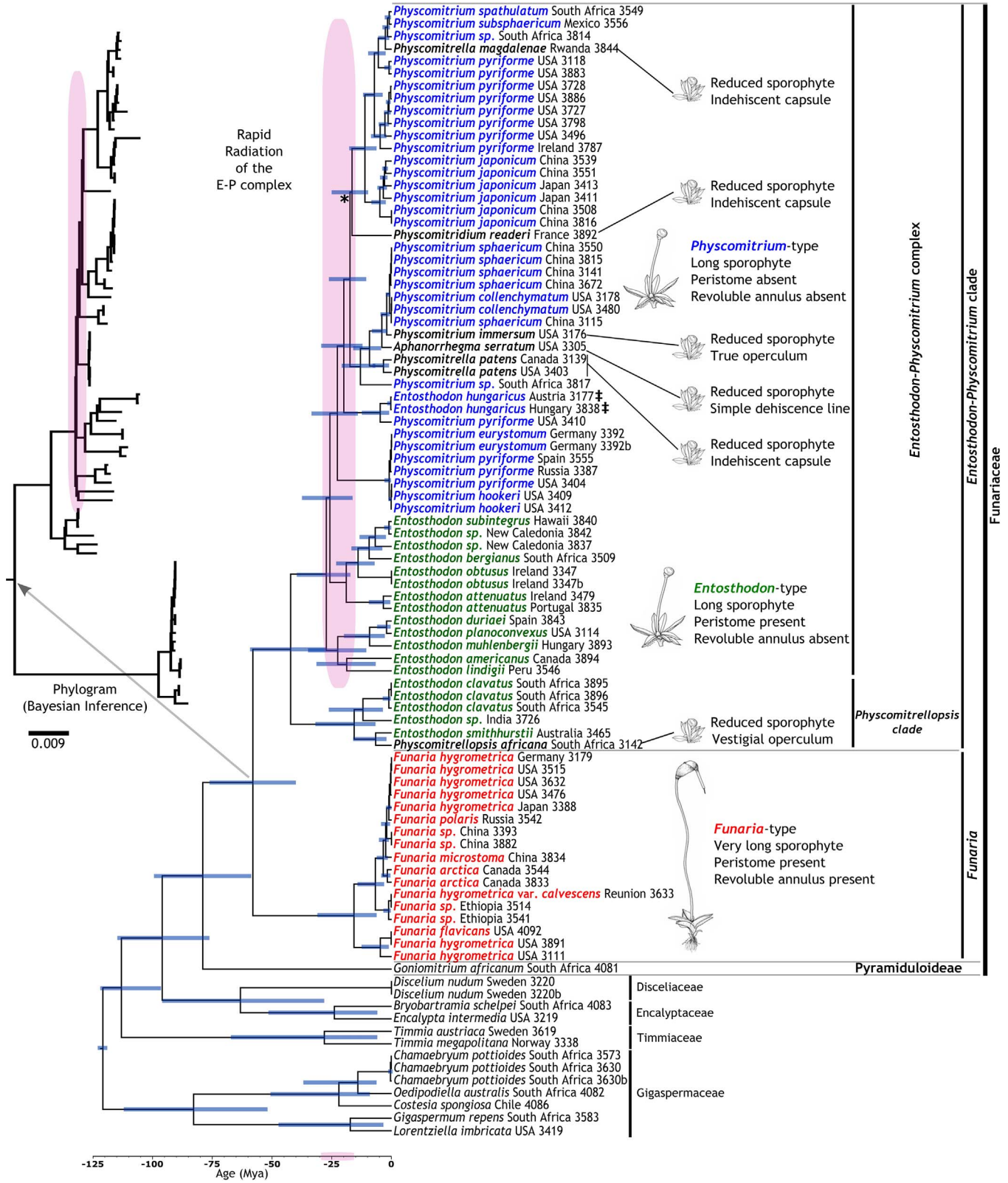

Fig. 1. Bayesian chronogram of 78 specimens of Funariaceae and their outgroups. The topology is based on the analysis of 83 coding genes of the plastid genome using both BI and ML criteria. Branch lengths of the Bayesian phylogram of the Funariaceae are shown on the left. The Funarioideae are split into two clades: *Funaria* and the E-P clade, while the latter is further divided into the *Physcomitrellopsis* clade and the E-P complex, the group that resulted from a rapid radiation (pink highlight). Internal node support values of all the higher hierarchy lineages are maximally supported with 1.0 Posterior Probability and 100 ML bootstrap with the only exception of the node marked with * (bootstrap of 76). The color code reflects the architectures of the sporophyte of the genera *Funaria* (red), *Entosthodon* (blue), and *Physcomitrium* (red), although these should be understood as generalizations. *Entosthodon hungaricus* (‡) shows sporophytes with intermediate characters and is likely an intergeneric hybrid between *Entosthodon* and *Physcomitrium*. Species with highly reduced sporophytes within the E-P complex appear in black. The Divergence Time Estimate was obtained after the analysis of a subset of ten genes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

genera *Aphanorrhegma*, *Physcomitrella* and *Physcomitridium* are nested (Fig. 1). Although the peristome may be present or absent within species of the *Entosthodon* lineages, it is consistently absent from *Physcomitrium*. The lineages in the E-P complex are heterogeneous in terms of sporophyte size, sporangium symmetry, and differentiated dehiscence, with independent trait reduction or loss being highly probable (Liu et al., 2012). The polarity of transformations and the frequency of parallel changes can only be inferred in the context of more complete taxon sampling and inclusion of nuclear loci.

Hybridization is known to occur in the Funariaceae, it has been observed *in vivo* (see Natcheva and Cronberg, 2004), triggered *in vitro* (Wettstein, 1925, 1924), and demonstrated as the process giving rise to several allegedly allopolyploid species (Beike et al., 2014; McDaniel et al., 2010). Hybridization may also account for the origin of *E. hungaricus*, a species whose morphology combines traits typical of the genus *Entosthodon* and *Physcomitrium* (Brugués and Ruiz, 2010; Cano et al., 1999) yet consistently resolved by plastid and mitochondrial data as a member of the *Physcomitrium* group (Fig. 1). At least one other case of intergeneric hybridization is known from the Funariaceae, namely *Funariophyscomitrella* F. Wettst. (Wettstein, 1924). Hybridization may have a significant impact on the inference of relationships, particularly when organellar and nuclear histories are compared. It is important to recognize that the position of hybrid taxa in an organellar phylogeny reflects only this maternal history, given that organellar genomes are free of recombination. Hence our evolutionary hypothesis must also be tested against, and the circumscription of the lineages refined based on, inferences from an extensive set of unlinked nuclear loci to identify hybrid taxa and their parental species.

The Funariaceae diverged from their common ancestor with the Disceliaceae and Encalyptaceae about 95 mya, but much of the extant diversity, which largely belongs to the E-P complex, arose within the last 28 my (Fig. 1). The phylogenetic shape of the E-P clade, and in particular of the E-P complex, bears the signatures of rapid diversification, with a dense succession of internal nodes joined by extremely short branches (Fig. 1, phylogram insert). No explicit criteria (in terms of age, time span or diversity output) have been formally accepted as characterizing a “rapid radiation” (Linder, 2008), but the diversification of the *Entosthodon-Physcomitrium* complex, which started around 28 mya and gave rise to about 150 extant species, resembles well-accepted radiations in flowering plants, whether very recent and moderately to very speciose (e.g. Klak et al., 2004 Knope et al., 2012; Pease et al., 2016; Vitales et al., 2014) to older and very prolific (e.g. Fior et al., 2013; Nürk et al., 2013; Zhang et al., 2014).

The timing of the diversification of the E-P complex coincides with global Eocene cooling that continued through the Oligocene and Miocene (Liu et al., 2009) and may have been facilitated by a prior WGD that, based on signatures in the *Physcomitrella* genome, is estimated to have occurred prior to the Oligocene (Rensing et al., 2007). The Miocene was a period of active diversification of bryophytes (Shaw et al., 2010), other land plants (e.g. Estep et al., 2014), and lichens (e.g. Leavitt et al., 2012). All genomic or transcriptomic investigations in mosses have revealed signatures of ancient WGD, and these may be linked, for example, to the subsequent radiation of the peat mosses (Devos et al., 2016; Shaw et al., 2016) and Hypnales (Johnson et al., 2016a, 2016b). WGD events in the Funariaceae may have resulted from autopolyploidy, which is common within extant species (Fritsch, 1991), or through hybridization, which is also common among natural populations (Natcheva and Cronberg, 2004). It also may have provided the genetic basis for morphological evolution but no apparent key morphological innovation characterizes the E-P complex. Indeed, the body plan of Funariaceae is very simple, and that of the E-P complex is further simplified by the reduction of the sporophyte and the partial or complete loss of the peristome and other mechanisms of capsule dehiscence and spore dispersal (Fig. 1).

Species boundaries within the E-P complex and many of their geographic distributions are ambiguous, and in need of critical study. A revision of the African species of *Entosthodon* based on morphological and phylogenetic inferences revealed that species may have rather narrow geographic distributions and perhaps ecological niches (Wilding, 2015). It is possible that the radiation within the complex is an adaptive radiation to a diversity of microhabitats, similar to the potentially adaptive variation found within *Funaria hygrometrica* in Spain (Magdy et al., 2016). Hence the diversification of the complex may be driven by ecophysiological adaptations (not reflected in the morphology), which could result in the rapid ecological isolation of populations, accentuated by the ephemeral or short-lived annual life cycle.

Rapid diversifications may be triggered by events intrinsic (e.g., novel trait) or extrinsic (e.g., climate change) to organisms (Linder, 2008). Within flowering plants, these phenomena may be the consequence of reproductive isolation resulting from shifts in pollination syndrome or from dispersal limitation, and be enhanced in plants that are annuals, monoecious and characterized by small population sizes (Givnish, 2010). The diversification of the Funariaceae may be facilitated by similar life history traits, such as hermaphroditism (Eppley et al., 2007), high inbreeding (e.g., Taylor et al., 2007; Perroud et al., 2011; Klips, 2015), and a short-lived vegetative phase (Fife, 1982). The geographic distribution of several Funariaceae species spans more than one continent (e.g., Fife, 1982, 1985; Wilding and Hedderson, 2011), suggesting high dispersal ability even when the ecological niche seems rather narrow (e.g., *Physcomitrella sensu lato*; Medina et al., 2015), although the ability of their spores to resist wind-mediated long distance dispersal is not known. For example, spores of *Physcomitrium turbinatum*, a putative North American endemic currently considered conspecific with the widespread *P. pyriforme* (McIntosh, 2007), do not resist desiccation for more than two years (Meyer, 1941) whereas those of *Funaria hygrometrica* remain viable for at least 11 years (Hoffman, 1970). Thus, despite the lower than expected spore settling velocity for *P. pyriforme* (Zanatta et al., 2016), the species may not be able to establish following long distance dispersal. This observation would be consistent with, for example, the polyphyly of allopatric *P. pyriforme* populations sampled here. Thereby, isolation due to limited dispersal could be a factor facilitating the diversification of the E-P complex.

The Funariaceae thus join a growing list of bryophyte lineages whose history is marked by at least one burst of diversification (Feldberg et al., 2014; Laenen et al., 2014; Shaw et al., 2010; Wall, 2005). Considering that WGD events and extensive gene family expansions have been demonstrated, bryophytes are not qualitatively different from vascular plants in terms of the processes that can shape their evolution and their responses to ecological opportunities. The morphological simplicity of bryophytes challenges the detection of diagnostic characters and obscures homoplasy so that complexes of bryophyte species may be opaque to taxonomic resolution based on morphological traits (i.e., cryptic; Bickford et al., 2007; Adams et al., 2014). Phylogenetic evidence for allopatric speciation and endemism is, however, growing (e.g., Medina et al., 2013; Renner et al., 2017), and rates of speciation in bryophytes may have been underestimated based on inferences from variation in morphological traits. Whilst much of our understanding of plant speciation and diversification is based solely on studies of angiosperms (Rensing, 2017), it is becoming evident that the intrinsic and extrinsic factors driving their evolution may also apply to bryophytes.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2017.12.002.

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