

Phylogeny, taxonomy and diversification events in the Caliciaceae

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Abstract Although the high degree of non-monophyly and parallel evolution has long been acknowledged within the mazaediate Caliciaceae (Lecanoromycetes, Ascomycota), a natural re-classification of the group has not yet been accomplished. Here we constructed a multigene phylogeny of the Caliciaceae-Physciaceae clade in order to resolve the detailed relationships within the group, to propose a revised classification, and to perform a dating study. The few characters present in the available fossil and the complex character evolution of the group affects the interpretation of morphological traits and thus influences the assignment of the fossil to specific nodes in the phylogeny, when divergence time analyses are carried out. Alternative fossil assignments resulted in very different time estimates and the comparison with the analysis based on a secondary calibration demonstrates that the most likely placement of the fossil is close to a terminal node rather than a basal placement in the *Calicium* clade. Our dating analysis show two successive events giving rise to main clades of mazaediate taxa within the Caliciaceae, in the Upper-Lower Cretaceous boundary and in the Paleocene. As a result of this study, *Cyphelium* is synonymized with *Calicium*, *Acolium* is resurrected, and the new genera *Allocalicium* and *Pseudothelomma* are described. Twelve new combinations are proposed: *Acolium karelicum*, *Acolium marcianum*, *Allocalicium adaequatum*, *Calicium carolinianum*, *Calicium lecideinum*, *Calicium lucidum*, *Calicium notarissii*,

Calicium pinicola, *Calicium trachyliodes*, *Pseudothelomma occidentale*, *Pseudothelomma ocellatum* and *Thelomma brunneum*. A key for the mazaedium-producing Caliciaceae is included.

Keywords *Allocalicium* gen. nov. · *Calicium* fossil · Divergence time estimates · Lichens · Multigene · *Pseudothelomma* gen. nov

Introduction

Caliciaceae is one of several ascomycete groups characterized by producing prototunicate (thin-walled and evanescent) asci and a mazaedium (an accumulation of loose, maturing spores covering the ascoma surface). These are traits connected with passive dispersal of the ascospores. For a long time, Caliciaceae was classified together with all other mazaediate and otherwise similar fungi in the order Caliciales, a presumably monophyletic group among ascomycete fungi, until Tibell (1984) suggested that most of the group was a highly polyphyletic assemblage of taxa, which had developed a mazaedium and passive spore dispersal independently. Subsequent phylogenetic studies supported this view (Gargas and Taylor 1995; Gargas et al. 1995; Wedin and Tibell 1997; Lumbsch et al. 2004, 2009; Hibbett et al. 2007; Tehler et al. 2009; Prieto et al. 2013) showing that mazaediate fungi are clearly dispersed over the phylogenetic tree of Ascomycota.

The family Caliciaceae belongs to the Lecanoromycetes (Wedin and Tibell 1997), the largest class of lichenized Fungi (Kirk et al. 2008). Despite the substantial recent advances in the understanding of Lecanoromycetes evolution at both supraordinal level (e.g. Lumbsch et al. 2004; Wedin et al. 2005; Miadlikowska et al. 2006, 2014) and lower levels (i.e. family and genera, Baloch et al. 2010; Gaya et al. 2012;

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Divakar et al. 2013; Singh et al. 2013; Otálora et al. 2014; Westberg et al. 2015; Resl et al. 2015) numerous groups are phylogenetically poorly understood and in great need of further study, and the otherwise well-known Caliciaceae is one of these. Caliciaceae forms a group with the non-mazaediate Physciaceae (Wedin et al. 2000, 2002). Although the Caliciaceae and Physciaceae have been suggested to form the suborder Physciineae in the Teloschistales (Miadlikowska et al. 2006; Hibbett et al., 2007; Kirk et al. 2008; Lumbsch and Huhndorf 2010), recent classifications tend to raise the Caliciaceae-Physciaceae group to ordinal level (Gaya et al. 2012) using the name Caliciales. The opinion on the family delimitation varies somewhat. If one prefers treating all Caliciaceae and Physciaceae as one family, the name Physciaceae was proposed for conservation (Wedin and Grube 2002) and should then be used. Recently, however, a two-family concept tends to be preferred (Gaya et al. 2012); Caliciaceae, which includes the non-mazaediate genera with *Bacidia*-type asci (the *Buellia*-group of Rambold et al. 1994; e.g. the buellioid genera, *Dirinaria* and *Pyxine*; Wedin et al. 2002; Miadlikowska et al. 2006, 2014; Gaya et al. 2012), together with all mazaediate genera in this group, and Physciaceae, which includes taxa belonging to the “*Physcia*-group” of Rambold et al. (1994), characterized by *Lecanora*-type asci. Within the Caliciaceae the recognition of two subfamilies has recently been proposed (Gaya et al. 2012): Calicioideae and Buellioideae, but this was very preliminary, based on a small taxon sampling, and it was rather unclear what other taxa should be included in the two groups in addition to the sampled ones.

Tibell (2003) studied the generic delimitations in the mazaediate Caliciaceae based on combined ITS and LSU rDNA data, where many details on the relationships within this group were revealed. Five well-supported clades were identified and informally called the *Tholurna*-clade, *Calicium glaucellum*-clade, *Calicium hyperelloides*-clade, *Calicium viride*-clade and the *Cyphelium tigillare*-clade (in the ITS tree, the *Acolium*-clade was added, making a total of six clades). Perhaps the most interesting results were that both *Calicium* and *Cyphelium* in the sense of Tibell (1984) were found to be non-monophyletic. In the ITS tree, *Cyphelium inquinans* and *C. karelicum* (the *Acolium*-clade) formed a group with the monotypic *Acroscyphus* and *Texosporium*, and a clade of *Calicium adaequatum* and *Tholurna dissimilis* (the *Tholurna*-clade), but *Cyphelium tigillare* (type of *Cyphelium*) and *C. notarisii* did not belong within that group. *Calicium* species were further distributed in four of the six clades from the ITS tree. Tibell expanded these investigations in a larger paper on Himalayan *Calicium* species (Tibell 2006) using ITS rDNA sequences, where he identified four well-supported clades: clade I, including the *Calicium glaucellum* and *C. hyperelloides* clades of Tibell (2003), clade II, including *Cyphelium* s. str. and three species of *Calicium*, clade III,

including the *Calicium viride* group (*Calicium* s. str.), and finally clade IV which includes the *Acolium*-clade, *Tholurna*-clade, and an odd *Calicium* species, *C. nobile*. These four clades have unclear relationships to each other, and most lack obvious phenotypic characteristics to delimit them.

It is clear that the natural relationships within the mazaediate Caliciaceae remain unresolved, and that several of the mazaediate genera as currently understood are non-monophyletic and thus unnatural. It is still unclear whether some of these mazaediate groups are more closely related to non-mazaediate groups within the Caliciaceae-Physciaceae clade (Caliciales sensu Gaya et al. 2012).

Adding a temporal dimension to the phylogeny may improve the phylogenetic reconstruction of phenotypic evolution, by detecting causal events or processes in the underlying phylogenetic diversity, and further by establishing a universal time-framework for biological classification that will facilitate studies in comparative evolution (Avisé 2009). Divergence time estimation has become increasingly prominent in evolutionary biology, including in the study of several groups of Ascomycota (Amo de Paz et al., 2011; Gueidan et al. 2011; Prieto and Wedin 2013; Beimforde et al. 2014; Divakar et al. 2015; Gaya et al. 2015). Information used to calibrate a phylogenetic tree is obtained from three principal sources: (1) geological events; (2) estimates from independent molecular dating studies; and (3) the fossil record, which normally is the major source of calibration points (Forest 2009). There are, however, several major complications in fungal dating analyses, among which the scarcity of fossils (Berbee and Taylor 2010), the correct interpretation of the fossils available (Kaasalainen et al. 2015), and in particular the unclear assignment of fossils to specific nodes in the phylogeny (Forest 2009), are potential sources of errors.

One fossil belonging to *Calicium* was described from Baltic amber dating back 55–35 million years ago (Rikkinen 2003). The specimen is embedded in amber and is composed of a single detached ascoma with numerous spores. The ascoma consists of a smooth stalk with a broadly obconical capitulum and a well-developed mazaedium without any visible pruina (something that could have been lost in the preservation process), and with apparently roughly ornamented spores. Although this fossil, in combination with other fossils, was used in several dated phylogenies as a calibration point (Prieto and Wedin 2013; Beimforde et al. 2014) and the assignment to *Calicium* is not disputed, the exact position within *Calicium* is uncertain as there is no distinct morphological trait suggesting a clear affinity to any group of extant species.

In the present study, we produce a multilocus phylogeny of the Caliciaceae-Physciaceae clade to generate a hypothesis of the natural relationships in this group, and to test current generic delimitations. We achieve this through phylogenetic analyses using five molecular markers including a broad taxon

sampling of the Caliciaceae-Physciaceae covering a wide range of groups of mazaedia-producing representatives and non-mazaedioid taxa from the Caliciaceae-clade. We conducted molecular dating analyses in order to understand the temporal framework of the evolution of the group. In this process, we performed different analyses with alternative positions of the *Calicium* fossil and compared with an analysis carried out with a secondary calibration as a way to evaluate the phylogenetic affinity of the *Calicium* fossil.

Material and methods

Taxon sampling

In this study we focused on the mazaediate members of the Caliciaceae; we also included members of the Physciaceae and the non-mazaediate members of the Caliciaceae (the *Buellia*-group of Rambold et al. 1994). One member of the Teloschistaceae was used as outgroup (Table 1).

Molecular techniques

DNA was extracted using DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. The following five regions were amplified: nuITS, nuLSU, mtSSU, and the protein coding genes β -tubulin and mcm7.

We amplified the nuITS with the primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990), and the nuLSU with LR0R (Rehner and Samuels 1994), LR3, LR3R, LR6, LR7 (Vilgalys and Hester 1990), PRI1 and PRI2 (Prieto et al. 2010) and nuLSU-155-5' (Döring et al. 2000) in different combinations. We also designed some specific primers for the nuLSU region in order to avoid photobiont amplifications (LSU-JLT-1F: 5'-CTCAGTAACGGCGAGTGAAG-3', LSU-JLT-1R: 5'-TCCGGCACCTTAACCTCAC-3' and LSU-JLT-2R: 5'-CCATCCGAAAACATCTGGA-3'). The mtSSU region was amplified with mtSSU1 and mtSSU3R (Zoller et al. 1999). We used the primers Mcm7-709for and Mcm7-1348rev (Schmitt et al. 2009) for amplification of the Mcm7 region and in some cases we carried out a nested PCR using 1 μ l of the PCR product and the internal primers Mcm7-CalicF and Mcm7-CalicR (Prieto et al. 2013). The protein coding β -tubulin was amplified using the primers Bt3-LM and Bt10-LM (Myllys et al. 2001).

PCR amplifications were performed using Illustra™ Hot Start Mix RTG PCR beads (GE Healthcare, UK) in a 25 μ l volume, containing 3 to 6 μ l of diluted genomic DNA, 10 μ M of each primer and distilled water. Amplifications were performed using the following program: initial denaturation at 95 °C for 15 min, followed by 35 cycles of 95 °C for 45 s, 54–56 °C for 50 s, 72 °C for 1 min, followed by a final

extension at 72 °C for 5 min. PCR products were subsequently purified using the enzymatic method Exo-sap-IT (USB Corporation, Santa Clara, California, USA) or when multiple bands were amplified, products were size-fractionated on a 1 % agarose gel run in TBE buffer, stained with GelRed™ (Biotium Inc.), visualized over a UV trans-illuminator, excised and purified using QIAquick spin columns (Qiagen). The purified PCR products were sequenced using the same amplification primers.

Sequences were assembled and edited using Sequencher v. 4.10.1. (Genes Codes Corporation, Ann Arbor) and deposited in GenBank (Table 1). Subsequently, sequences were aligned manually using MacClade 4.01 (Maddison and Maddison 2001) and translated to amino acids in the protein coding loci. Ambiguous regions (sensu Lutzoni et al. 2000) and introns were delimited manually and excluded from phylogenetic analyses. Additionally, we used Gblocks 0.91b (Castresana 2000) to identify the ambiguous regions. Since the Maximum Likelihood results were very similar between Gblocks and manually constructed matrices we used the latter for the rest of analyses.

Phylogenetic analyses

Each individual gene region was analysed using maximum likelihood-based inference (ML) as implemented in RAXML ver. 8.1.11 (Stamatakis 2014) with a GTRGAMMA model for tree inference. Bootstrapping was performed with a GTRCAT model and 1000 replicates. In order to check for gene-tree incongruence we compared maximum likelihood bootstrap values (ML-BS) between the individual gene trees considering a conflict among clades when a supported clade (bootstrap support >70 %) for one marker was contradicted with significant support by another. Because no supported nodes were in conflict, the data were combined into a single concatenated data matrix.

The combined maximum likelihood (ML) analyses were run with 7 distinct partitions (nuITS, nuLSU, mtSSU, first and second codon positions of the mcm7 and β -tubulin and the third codon position of the mcm7 and β -tubulin), using a GTRGAMMA model of molecular evolution and rate heterogeneity with unlinked parameters and 1000 bootstrap replicates.

To select partitions and its optimal models of nucleotide substitution, we used PartitionFinder version 1.0.1 (Lanfear, 2012) with unlinked branch lengths option and the Akaike Information Criterion (AIC) for model selection. The GTR model (Rodríguez et al. 1990) with an estimated proportion of invariable sites and with a gamma distribution was selected for nuITS, nuLSU and mtSSU, third codon position of the β -tubulin and first and second codon positions of the mcm7, while HKY (Hasegawa et al. 1985) and SYM (Zharkikh 1994) with an estimated proportion of invariable sites and

Table 1 Specimens used for the study, with GenBank accession numbers. Entries for newly obtained sequences are in boldface. Specimen data are given with collection number and location of voucher

Species name	Voucher information	GenBank accession number				
		mtSSU	mcm7	nuITS	nuLSU	β-tubulin
<i>Acolium inquinans</i>	Wedin 6352 (UPS)	AY143404	JX000161	AY450583	AY453639	KX529023
<i>Acolium karelicum</i>	Hermansson 16472 (UPS)	-	KX529045	KX512897	KX512879	-
<i>Acroscyphus sphaerophoroides</i>	Obermayer 6077 (UPS)	KX512984	KX529029	KX512898	-	-
<i>Alloalcalicum adaequatum</i>	Spribille 14143 (UPS)	KX512986	-	KX512906	KX512859	KX528996
<i>Amandinea coniops</i>	Nordin 6113 (UPS)	KX512978	-	-	KX512865	KX528998
<i>Amandinea punctata</i>	Wedin 2/3/96 (UPS)	AY143399	KX529025	KX512899	AY340536	-
<i>Anaptychia ciliaris</i>	Wedin 6429 (UPS)	AY143400	KX529054	AY143391	KX512894	-
<i>Anaptychia runcinata</i>	Santesson 32628 (UPS)	KX512977	KX529034	-	-	-
<i>Baculifera remensa</i>	Prieto (S)	KX512962	-	-	KX512881	-
<i>Buellia badia</i>	Westberg 09–079 (S)	KX512963	-	KX512900	KX512880	KX529008
<i>Buellia disciformis</i>	Wedin 6155 (BM)	JX000116	JX000152	AY143392	JX000082	-
<i>Buellia dispersa</i>	Ryan 21685 (S)	-	KX529035	-	-	-
<i>Buellia elegans</i>	Hansen (S)	KX512993	-	KX512901	-	KX528988
<i>Buellia erubescens</i>	Wetmore 95879 (S)	KX512969	-	KX512902	KX512874	KX529004
<i>Buellia frigida</i>	Westberg (S)	KX512992	-	KX512903	KX512852	-
<i>Buellia tesserata</i>	Tehler 7323 (S)	-	KX529050	KX512904	KX512885	-
<i>Calicium abietinum</i>	Tibell 25061 (UPS)	KX512971	KX529041	KX512905	KX512872	KX529003
<i>Calicium adpersum</i>	Prieto 3037 (S)	KX512949	KX529055	KX512907	KX512895	KX529022
<i>Calicium chlorosporum</i>	Tibell 25012 (UPS)	KX512956	KX529059	-	KX512892	-
<i>Calicium chlorosporum</i>	Thor 20859 (UPS)	KX512955	-	-	-	-
<i>Calicium corynellum</i>	Prieto (S)	KX512985	KX529048	KX512908	KX512855	KX528989
<i>Calicium denigratum</i>	Prieto (S)	KX512965	KX529044	KX512909	KX512878	-
<i>Calicium glaucellum</i>	Wedin 8563 (S)	KX512980	KX529032	KX512910	KX512864	-
<i>Calicium lecideinum</i>	Prieto (S)	KX512961	KX529046	KX512911	KX512882	KX529009
<i>Calicium lenticulare</i>	Tibell 23284 (UPS)	KX512979	KX529033	KX512912	-	KX528997
<i>Calicium montanum</i>	van den Boom 23445 (UPS)	-	-	KX529069	KX512853	-
<i>Calicium nobile 1</i>	Tibell 21968 (UPS)	KX512988	KX529060	KX512913	KX529070	-
<i>Calicium nobile 2</i>	Tibell 23396 (UPS)	KX512987	KX529061	KX512914	KX529071	-
<i>Calicium notarisii</i>	Prieto 3007 (S)	KX512960	KX529047	KX512915	KX512883	KX529011
<i>Calicium pinicola 1</i>	Lendemer & Knudsen 14982 (UPS)	KX512972	KX529040	KX512916	KX512871	KX529015
<i>Calicium pinicola 2</i>	Thor 19856 (UPS)	KX512991	KX529066	KX512917	KX512887	KX529014
<i>Calicium quercinum</i>	Tibell 22287 (UPS)	-	-	KX512918	KX512854	-
<i>Calicium salicinum</i>	Prieto (S)	KX512982	KX529027	KX512919	KX512861	KX528991
<i>Calicium tigillare</i>	Prieto 3038 (S)	JX000123	JX000162	JX000104	JX000088	KX529002
<i>Calicium trabinellum</i>	Wedin 8517 (S)	-	KX529026	KX512920	KX512858	KX528995
<i>Calicium trachylioides</i>	Nordin 4002 (UPS)	KX512959	KX529058	KX512933	KX529072	KX529018
<i>Calicium verrucosum</i>	Tibell 23198 (UPS)	-	KX529030	-	-	-
<i>Calicium viride</i>	Wedin 24/4 2000	AY584696	JX000153	HQ650703	AY340538	KX529013
<i>Dermaticum fallax</i>	Brusse 4944 (S)	-	-	KX512921	KX512866	-
<i>Dimelaena oreina</i>	Lendemer 4193 (S)	KX512976	KX529036	KX512922	KX512867	KX528999
<i>Dimelaena radiata</i>	Nash 41396 (S)	-	KX529049	KX512923	KX512884	-
<i>Diplotomma alboatrum</i>	Prieto 3034 (S)	KX512966	KX529043	KX512924	KX512877	KX529007
<i>Diplotomma venustum</i>	Westberg 10–176 (S)	KX512968	-	KX512925	-	KX529005
<i>Dirinaria applanata</i>	Seaward 109735 (S)	KX512990	-	KX512926	KX512856	-
<i>Heterodermia speciosa</i>	Wetmore (S)	KX512975	KX529037	KX512927	KX512868	KX529000
<i>Heterodermia vulgaris</i>	Frisch 11/Ug1226 (UPS)	KX512989	-	KX512928	KX512857	-

Table 1 (continued)

Species name	Voucher information	GenBank accession number				
		mtSSU	mcm7	nuITS	nuLSU	β -tubulin
<i>Phaeophyscia ciliata</i>	Prieto (S)	KX512958	KX529051	KX512929	KX512886	KX529012
<i>Phaeophyscia orbicularis</i>	Prieto 3012 (S)	KX512967	-	KX512930	KX512876	-
<i>Physcia aipolia</i>	Wedin 6145 (UPS)	AY143406	KX529052	KX512931	AY300857	KX529021
<i>Physcia tenella</i>	Odelvik & Hellström 0827 (S)	KX512974	KX529038	KX512932	KX512869	-
<i>Pseudothelomma ocellatum</i> 1	Tehler 8063 (S)	KX512957	KX529062	KX512934	KX512862	KX529019
<i>Pseudothelomma ocellatum</i> 2	Hermansson 18662 (UPS)	KX512952	KX529063	KX512935	KX512891	KX529020
<i>Pseudothelomma occidentale</i>	Nash 29968 (UPS)	KX529073	KX529057	-	-	KX529074
<i>Pyxine coccoes</i>	Prieto (S)	KX512964	-	KX512936	-	KX529010
<i>Pyxine soredata</i>	Wetmore 91254 (S)	KX512973	KX529039	KX512937	KX512870	KX529001
<i>Tetramelas chloroleuca</i>	Westberg 10–001 (S)	-	-	KX512938	KX512875	KX529006
<i>Tetramelas phaeophysciae</i>	Nordin 6896 (UPS)	-	-	KX512939	-	-
<i>Tetramelas pulverulentus</i>	Nordin 6368 (UPS)	KX512983	-	KX512940	KX512860	KX528990
<i>Texosporium sancti-jacobi</i>	Rosentreter & De Bolt 6514 (UPS)	KX512981	KX529031	KX512941	KX512863	KX528994
<i>Thelomma mammosum</i> 1	Tibell 23775 (UPS)	KX512954	KX529067	KX512942	KX512888	KX529016
<i>Thelomma mammosum</i> 2	Hernández et al. 2002 (UPS)	KX512953	KX529065	KX512943	KX512851	KX529017
<i>Thelomma santessonii</i> 1	Nordin 4011 (UPS)	KX512951	KX529064	KX512944	KX512889	-
<i>Thelomma santessonii</i> 2	Nash 38262 (UPS)	KX512950	-	KX512945	KX512890	-
<i>Tholurna dissimilis</i>	Wedin 6330 (UPS)	AY143407	KX529053	AY143397	KX512893	KX528992
<i>Tornabea scutellifera</i>	Tibell 23833 (UPS)	KX512970	KX529042	KX512946	KX512873	-
<i>Xanthoria elegans</i>	Odelvik 04532 (S)	KX512948	KX529056	KX512947	KX512896	KX529024

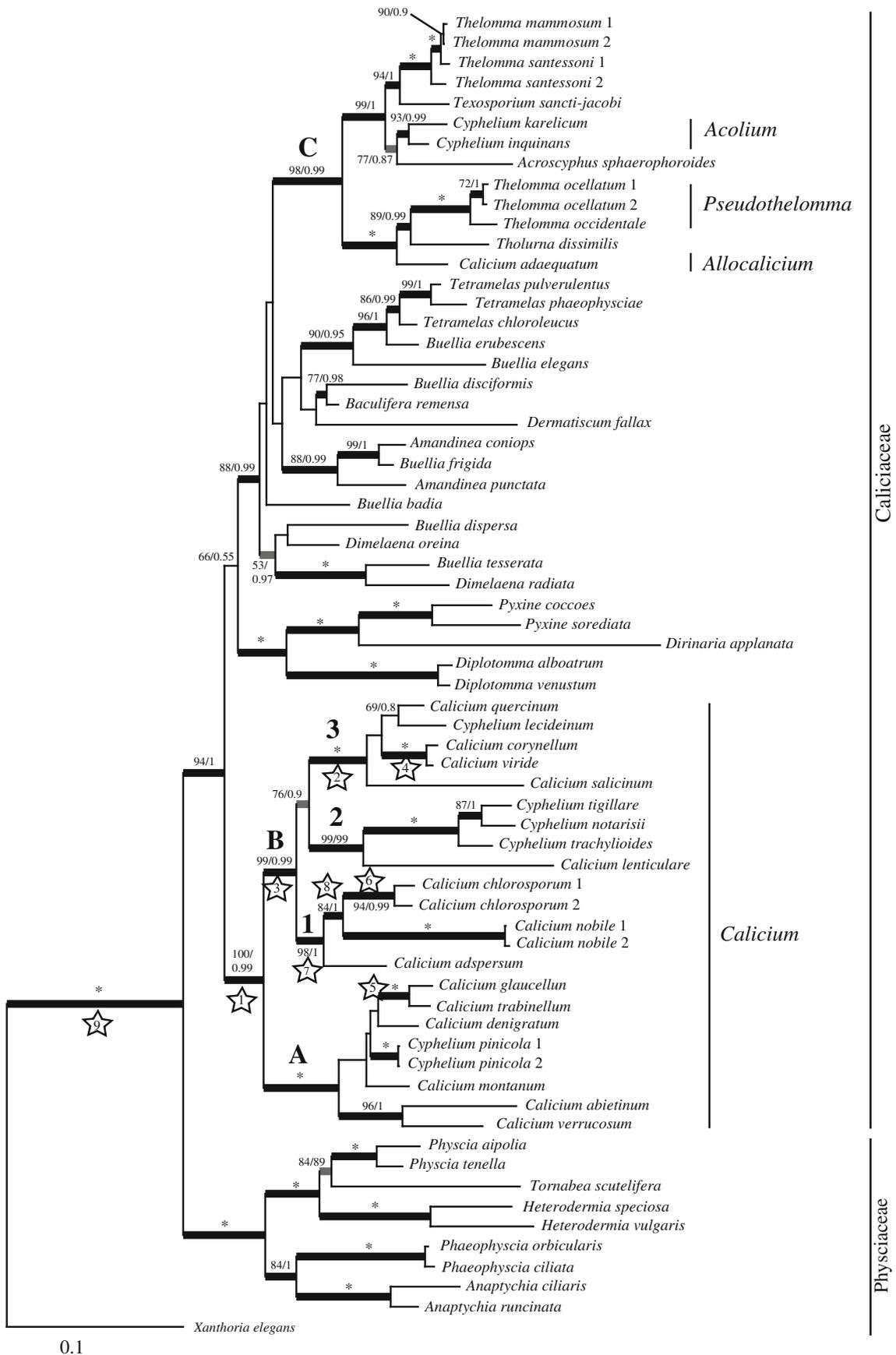
with a gamma distribution were selected for the third codon position of the mcm7 and for the first and second position of the β -tubulin, respectively. Bayesian inference was carried out through Markov Chain Monte Carlo (MCMC) sampling, as implemented in MrBayes 3.2.3 (Ronquist et al. 2011). The analyses consisted of two parallel searches, each with four chains, run for 10 M generations, and initiated with random starting trees. The chains were sampled every 1 K generations from the posterior distribution. A burn-in sample of 25,000 trees was discarded for each run. The remaining 150,000 trees (pooled from both independent runs) were used to assemble a majority rule consensus tree and to estimate branch lengths and Posterior Probabilities (PPs). To determine if the chains had converged, verify if mixing was appropriate, and choose a suitable burn-in, we plotted the log-likelihood values against the time generation with Tracer v.1.5.0 (Rambaut and Drummond 2007). We assumed stationarity of the chains when log-likelihood values reached the same stable equilibrium value for each independent run (Huelsenbeck and Ronquist 2001) and when average standard deviation of split frequencies across runs dropped below 0.01. We also tested the convergence with AWTY program (Nylander et al. 2008; Wilgenbusch et al. 2004). Maximum likelihood, Bayesian analysis and the selection of models were run on the CIPRES Science Gateway v. 3.3 (Miller et al. 2010).

Divergence time estimates and node calibration

We implemented a Bayesian Markov chain Monte Carlo algorithm for estimating divergence times using data from five gene loci using the BEAST v1.8.2 software package (Drummond et al. 2012). The tree topology and divergence times were estimated simultaneously.

We carried out nine different analyses with different positions of the fossil: A1 (clade A + B), A2 (clade B3), A3 (clade B), A4 (*C. corynellum*-*C. viride*), A5 (*Calicium glaucellum*-*C. trabinellum*), A6 (*C. chlorosporum*), A7 (clade B1), A8 (*C. nobile*-*C. chlorosporum*) (Fig. 1). For the analysis A9 we used a secondary calibration based on Prieto and Wedin (2013) using Scenario 2 (i.e. without the *Alectoria succini* fossil, which has recently been re-investigated by Kaasalainen et al. 2015 and identified as a plant remain), dating the ingroup node (Fig. 1) at 132–199 Mya.

In all cases, we had 7 different unlinked partitions (as in the ML analysis), with the GTR + I + G substitution model for each partition except for the third codon position of the mcm7 for which we selected a KKY + I + G model. We used the uncorrelated lognormal relaxed clock model, which allows rates of molecular evolution to be uncorrelated across the tree. We implemented a Yule tree prior.



◀ **Fig. 1** Best tree from RAxML with bootstrap support (ML-BS) and posterior probabilities (PP) obtained in the Bayesian analysis. The support values are ordered as ML-BS/PP. Supported clades by both analyses (ML-BS ≥ 70 , pp. ≥ 0.95) are marked with black thicker branches and with grey thicker branches when the node is only supported by one of the two analyses. An asterisk over a branch indicates that this internode has a ML-BS value and PP of 100 %. Numbers within stars indicate the nodes used for fossil calibration and correspond to Scenarios shown in Tab. 2. Newly circumscribed genera are marked in the right part of the figure

We constrained the calibration points (fossil calibrated node) with an exponential distribution dated at 35 Mya with mean = 20 and offset = 35. For the secondary calibration (analysis A9) we constrained the ingroup node with a uniform distribution with lower value of 132 Mya and upper value of 199 Mya (based on Scenario 2 from Prieto and Wedin 2013). For each analysis, we ran a first relaxed log-normal clock with default priors to estimate prior distributions to be used in a second analysis that was used to estimate priors for the final analysis. Distributions and parameters used for each analysis are specified in Table 2; priors not specified here were set to the default values.

BEAST analyses were run for 40 million generations, logging parameters and trees every 1000 generations. Convergence, mixing, and effective sample sizes (ESS) of parameters were checked using Tracer v1.5.0 (Rambaut and Drummond 2007). A burn-in of 1000 trees was removed from each analysis. The remaining trees were used to generate a maximum clade credibility tree with TreeAnnotator v1.8.2 (Drummond et al. 2012).

Results and discussion

A total of 227 sequences were generated for this study (Tab. 1). The combined data set consisted of 66 taxa and 3303 unambiguously aligned sites, 382 for the nuITS, 1021 for the nuLSU, 634 for the mtSSU, 693 for the β -tubulin and 573 for the mcm7. The maximum likelihood tree with bootstrap support and posterior probabilities from Bayesian analysis is depicted in Fig. 1. The nine divergence time analyses are compared in Table 2, and discussed below. Divergence time estimates of the analysis A9 (using secondary calibration) are shown in Fig. 2 with 95 % highest posterior density (HPD) for each node.

Phylogenetic results

This is the most complete taxon sampling conducted in a study of Caliciaceae s. str. to date, and it enables us to draw a number of conclusions and undertake several taxonomic changes that are now supported by a five-marker phylogeny (Fig. 1). The two families Physciaceae and Caliciaceae in the

sense of Gaya et al. (2012) are recovered as monophyletic. Although this study does not focus on Physciaceae, the sampled genera in this family are monophyletic and the relationships between them are fully resolved. Among the non-mazaediate Caliciaceae, relationships are not fully resolved, probably due to the fact that *Buellia* s. lat. is a heterogeneous and species-rich group from which we included comparatively few representatives. Some well-supported results are worth noting. A clade comprising *Diplotomma*, *Dirinaria* and *Pyxine* (also present in Miadlikowska et al. 2014) is resolved. *Buellia tesserata* and *B. dispersa* form a group with a non-monophyletic *Dimelaena*, which deserves further study. The inclusion of *Buellia frigida* within *Amandinea* should also be investigated further. The genus *Tetramelas*, characterized by the pigmented parts of the spore wall consisting of a thick proper wall and a thin (less than half as thick as the proper wall) irregularly cracked perispore (Nordin 2004), is also monophyletic and closely related to *B. elegans* (as also suggested by Nordin and Tibell 2005) and *B. erubescens*.

None of the mazaediate genera within Caliciaceae are monophyletic as currently circumscribed (except monotypic genera). Traditionally, *Calicium* and *Cyphelium* have been distinguished mainly by the presence of stalked apothecia in *Calicium* and sessile or immersed apothecia in *Cyphelium*. Here we can see that representatives of these two genera are present in the two main clades within Caliciaceae, and none form a monophyletic group, thus demonstrating that presence/absence of a stalk does not characterize natural groups within this family, a fact already suggested by Tibell (2003). We find it useful to denominate the three clades with mazaediate representatives as clade A, B and C, respectively (Fig. 1). Clade A comprises the *Calicium glaucellum* and *Calicium hyperelloides* clades of Tibell (2003), but nested within this group is also *Cyphelium pinicola*. All members of this clade currently classified in *Calicium* are distinguished by having a thick, hyaline outermost part of the stalk (see e.g. Tibell 1975; p. 79, Fig. 16). *Cyphelium pinicola* has sessile ascumata, but already Tibell (1969) pointed out its isolated position among the European *Cyphelium* species, with quite different excipular and spore structure. *Cyphelium pinicola* is similar, however, in spore ornamentation (irregular cracks or areolae) to the other species of clade A.

Clade A form a well-supported monophyletic group together with clade B. Clade B comprises three distinct subclades. Subclade B1 includes *Calicium adspersum*, *C. chlorosporum* and *C. nobile*, which share the presence of a distinctive yellow pruina (rarely brown) on the lower surface of the capitulum and on the mazaedium, and distinctly ornamented spores with spirally arranged ridges. In the previous ITS phylogeny by Tibell (2006), *C. nobile* grouped at the base of Tibell's Clade IV (the *Tholurna dissimilis*, *Calicium adaequatum*, *Texosporium*, *Acrosyphus* and the *Acolium*-clade group). Our results place this species in a more coherent

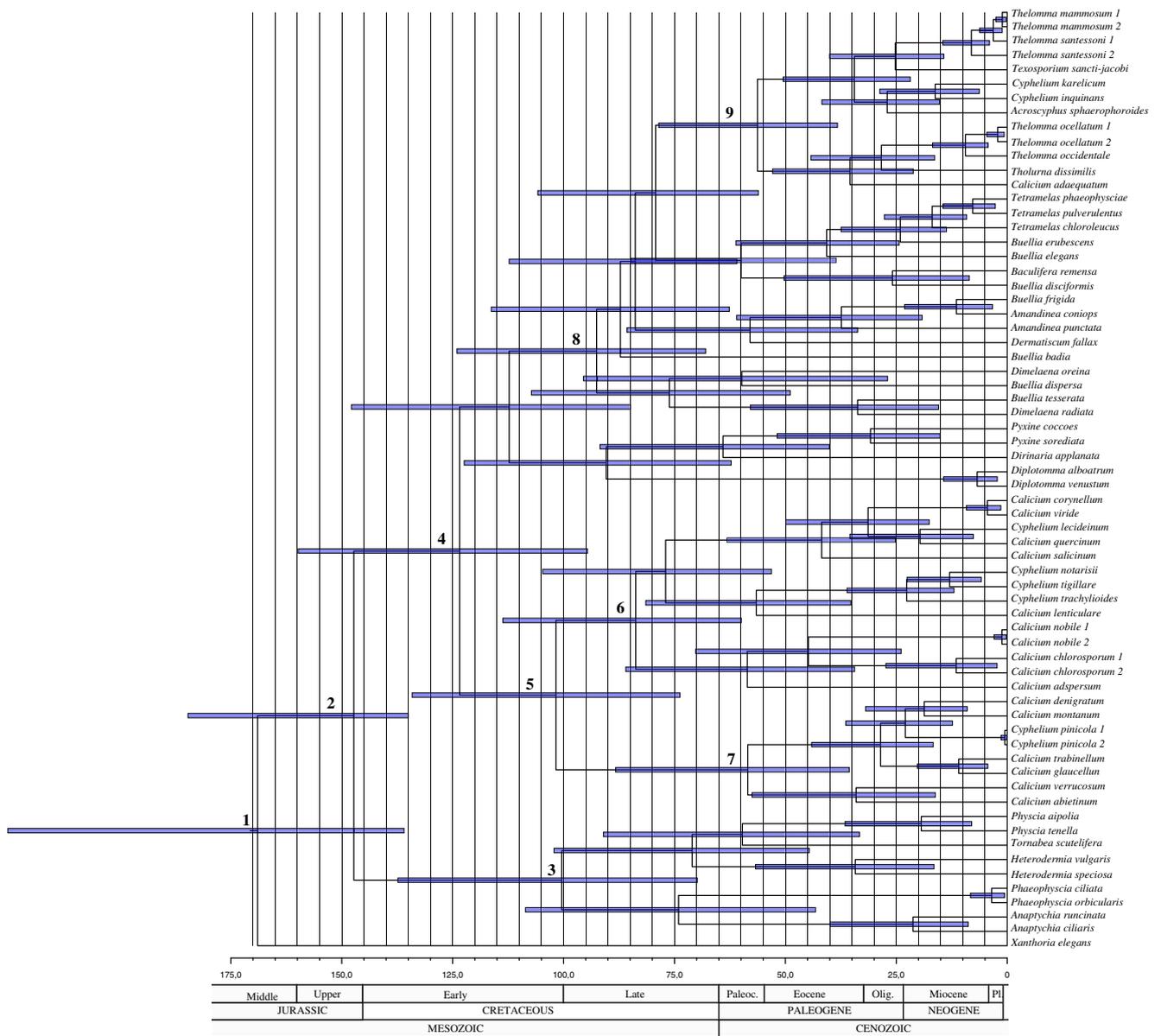


Fig. 2 Maximum clade credibility chronogram for Caliciaceae–Physciaceae. The chronogram is the result from the BEAST analysis using a secondary calibration (analysis A9). Each node represents the

median divergence time estimate and bars show their associated 95 % credibility interval. Numbers corresponding to dated groups shown in Table 2 are written above the nodes

position, which is supported by four single-marker phylogenies (β -tubulin missing), and by the concatenated analysis. It should be noted that *Calicium nobile* has one of the longest branches in the tree, but also that the position and identity was verified by two sequenced samples.

Subclade B2 includes *Cyphelium* s. str. (type *C. tigillare*) which is morphologically well-characterized, having immersed apothecia with a very thin excipulum, comparatively small, obovate to pyriform asci, smooth spores, and by growing on dry wood in comparatively exposed situations. Sister to *Cyphelium* s. str. is *Calicium lenticulare*. Subclades B1 and B2 correspond to Clade II from Tibell (2006), which do not form a monophyletic group in our analysis.

Subclade B3 corresponds to Clade III from Tibell (2006) and includes the *Calicium viride* group (*Calicium* s. str.). This group is comparatively well characterized morphologically. *Calicium viride* (the type of *Calicium*) and the closely related *C. corynellum*, *C. salicinum*, and *C. quercinum*, all have comparatively large and sturdy, stalked ascomata and spores with distinct spiral ornamentation. This well-supported group also includes the non-stalked *Cyphelium lecideinum*, which shares the very distinct spiral spore ornamentation characteristic for subclade B3.

Clade C includes the *Acolium*-clade and the *Tholurna*-clade of Tibell (2003), and the rather odd *Calicium adaequatum*. This group corresponds to Tibell’s (2006) Clade IV, but contains also

all *Thelomma* species included here, although these do not form a monophyletic group. *Thelomma mammosum* (the type of *Thelomma*) and *T. santessonii* are closely related, and together with *T. californicum* and *T. siliceum* (not included here) these form a morphologically and chemically well-characterized group of saxicolous species growing in coastal areas, *Thelomma* s. str. They are closely related to *Texosporium sancti-jacobi*, and share the presence of immersed sessile ascomata with a laterally reduced exciple. *Texosporium*, however, is unique in having a distinct spore ornamentation formed by paraphyses that adhere to the surface of the spores (Tibell and von Hofsten 1968). *Texosporium* also has a unique ecology among mazaediate lichens, growing on dung, soil and detritus in grassland soil crust communities (McCune and Rosentreter 1992).

Thelomma ocellatum and *T. occidentale* are closely related species, which are morphologically and chemically very similar (Tibell 1976) and grow on dry wood in exposed locations. Thus, they are quite different from *Thelomma* s. str., and group together with *Calicium adaequatum* and *Tholurna dissimilis*. *Tholurna dissimilis* produces radially protruding podetia, which carries apically situated ascomata. Tibell (1984) noted that *Tholurna* was not similar to the other species with podetia (*Acrosyphus*) in several characteristics, which is supported by our phylogeny. *Calicium adaequatum* is the only species with stalked apothecia within this clade, and Tibell (2003) commented on that *C. adaequatum* shared the presence of a campanulate capitulum with *Tholurna*, and a very similar strong surface ornamentation of the spores. Both species grow on twigs, and have actually a rather similar general appearance, forming small clumps of podetia or stalked ascomata. Several independent investigations have now suggested that *Calicium adaequatum* and *Tholurna* form a group, and we can here conclude that *Thelomma ocellatum* and *T. occidentale* also belong to this group. It is difficult to argue for treating these four rather distinct taxa (*Calicium adaequatum*, *Tholurna*, and *Thelomma ocellatum* + *T. occidentale*) in one genus as the two first share few obvious unique morphological traits with the two latter. Including *C. adaequatum* in *Tholurna* is not an option as this genus would become paraphyletic. We thus suggest describing new genera for both *C. adaequatum*, and for the *Thelomma ocellatum* + *T. occidentale* group (see below).

Cyphelium sensu Tibell (1984) is very heterogeneous, with species ending up in four places in the tree. Already Tibell (1971) remarked that *Cyphelium* probably was heterogeneous, which was further supported by the early SSU rDNA based phylogeny by Wedin et al. (2000). Tibell suggested (Tibell 2003: 1415) that *Cyphelium inquinans* and *C. karelicum* should be recognized as a separate genus, for which the name *Acolium* (Ach.) S. Gray is available, but this has not been taken up. The recognition of *Acolium* is however clearly supported in our analysis. *Acolium* is a small group of species growing on wood or bark, and are further characterized by a

dark excipulum that is strongly thickened at the base and ornamented spores. They have a distinct greyish-brownish thallus, sessile to somewhat immersed ascomata, and a grey pruina on the rim of the excipulum. *Acolium* forms a group with *Thelomma* s. str. and *Texosporium*. The core group of *Cyphelium* (*C. tigillare*, *C. trachyloides*, and *C. notarisii*) is a group of morphologically similar and closely related species growing on dry and exposed wood, which are characterized by immersed apothecia with a very thin excipulum and smooth spores. *Cyphelium* s. str. is nested within the majority of *Calicium*, in clade A + B, as are *Cyphelium pinicola* and *C. lecideinum*. *Cyphelium lucidum* is not included in our multigene phylogeny, but we have added the ITS sequences present in GenBank (EF551163, EF551164 and EF551165) to our ITS alignment and conducted a maximum likelihood analysis in order to check the position of the species. The resulting tree shows that *C. lucidum* is likely to be closely related to *Calicium adpersum* in Clade B1 although without support (results not shown).

Although there are some morphologically quite distinct subgroups within *Calicium* that could be given generic rank, we would still be left with a number of poorly characterized, potentially monotypic genera needing names. Accepting *Cyphelium* in any sense would make *Calicium* paraphyletic. We instead suggest including *Cyphelium* s. str. (the *Cyphelium tigillare*-group), *Cyphelium lecideinum*, *Cyphelium pinicola*, and *Cyphelium lucidum*, in *Calicium*. This results in a phylogenetically distinct *Calicium* that, however, is rather difficult to characterize morphologically. We finally note that the subfamily classification proposed by Gaya et al. (2012) becomes superfluous, as the Calicioideae now corresponds to the genus *Calicium* only.

Divergence time estimates

The phylogenetic assignment of fossils is frequently difficult as these may lack diagnostic characteristics for living clades, because the fossils are genuinely primitive or because derived characteristics are not preserved in the fossil (already noted by Hennig 1981). The additional problems of non-monophyletic extant taxa and parallel evolution of traits clearly also frequently prevents an unambiguous fossil assignment, as in the case present here. Although the fossil clearly belongs to *Calicium* (see Table 3 for a comparison with similar mazaediate genera), the relationship with a specific group within *Calicium* is not possible to infer based on the few visible characters that the fossil possesses. Thus, we instead wanted to explore the phylogenetic placements of the fossil, by comparing different alternative and arbitrary positions with a scenario based on a secondary calibration.

We obtained very different time estimates when using different alternative fossil positions and when using a secondary calibration. The credibility intervals of the alternative

Table 3 Discernable characters of the *Calicium* fossil described by Rikkinen (2003) and comparison with similar calicioid groups. Bold text denotes differences

Family	Genera	Lichenized	Thallus	Apothecia	Mazaedium	Capitulum shape	Ascomata colour	Spore septation	Spore ornamentation	Spore shape
Fossil Caliciaceae	<i>Calicium</i>	?	?	stalked	well developed	broadly obconical	black	1-septate	distinct	ellipsoidal
	<i>Acolium</i>	lichenized/ lichenicolous	episubstratic	sessile	well developed	short cylindrical to lenticular	black	1-septate	distinct	ellipsoidal
	<i>Allocalcium</i>	lichenized	immersed	stalked	well developed	cylindrical to bell-shaped	dark	1-septate	distinct	ellipsoidal
Coniocybaceae	<i>Calicium</i>	lichenized	immersed/ episubstratic	stalked/sessile/ immersed	well developed	lenticular to obconical	black	1-septate	distinct	ellipsoidal
	<i>Chaenotheca</i>	lichenized	immersed/ episubstratic	stalked	well developed	spherical to obconical	medium to pale brown	Non-septate (rarely septate)	faint/smooth	spherical to ellipsoidal-cylindrical
Microcaliciaceae	<i>Scerophora</i>	lichenized	immersed	stalked	well developed	spherical to obconical	pale	Non-septate	faint/smooth	spherical
	<i>Microcalicium</i>	saprotrophic/ lichenicolous/ algal-parasite	-	stalked/sessile	well developed	narrowly conical, spherical	greenish/brownish	1–7 septate	distinct	cylindrical
Mycocaliciaceae	<i>Chaenothecopsis</i>	saprotrophic/ lichenicolous/ algal-parasite	-	stalked/sessile	not mazaediate	obovoid to lenticular	black to brownish black	Non-septate (rarely septate)	smooth	ellipsoidal
	<i>Mycocalicium</i>	saprotrophic	-	stalked	not mazaediate	obovoid to lenticular	black to dark brown	Non septate	smooth	ellipsoidal
Phaeocaliciaceae	<i>Phaeocalicium</i>	saprotrophic	-	stalked	not mazaediate	obovoid to lenticular or strongly compressed	black to blackish brown	Non-septate or 1–3 septate	smooth	ellipsoidal/fusiform
	<i>Stenocybe</i>	saprotrophic	-	stalked	not mazaediate	obovoid to subspherical	black or plumbeous	3–7 septate	smooth	fusiform
Sphinctrimaceae	<i>Sphinctrina</i>	lichenicolous	-	sessile/shortly stalked	well developed	spherical	black	Non-septate (rarely septate)	distinct	spherical

estimates were in many instances non-overlapping (see Tab. 2 for selected nodes). The results obtained placing the fossil at the base of clade A + B (A1), clade B3 (A2), clade B (A3) and clade B1 (A7) resulted in younger ages (Tab. 2) than the analysis with the fossil placed in more terminal nodes as A4, A5 and A6. All these analyses differ considerably from the results of other dating studies (Prieto and Wedin 2013; Beimforde et al. 2014) which obtained much older or younger ages for the crown of Caliciales (166 Mya in Scenario 2 from Prieto and Wedin 2013 and ca. 160 Mya in Beimforde et al. 2014) and from those obtained based on a secondary calibration (our analysis A9). Analysis A8 is the most similar to the secondary calibration analysis suggesting that the most likely placement of the fossil is close to a terminal node and thus, a basal placement in the *Calicium* clade can likely be rejected.

As the secondary calibration analysis is based on a previous study with a robust fossil record using five fossil calibration points (Prieto and Wedin 2013), we believe this analysis is more reliable than any estimate based on one single fossil only, thus justifying basing our discussion on the chronogram resulting from this analysis (Fig. 2). In our dating analysis based on a secondary calibration the split between the Caliciales sensu Gaya et al. 2012 (the Caliciaceae-Physciaceae clade) and the Teloschistales took place in the Middle Jurassic, around 171 Mya. The age for the Physciaceae crown group in A9 is estimated at 102 Mya, and the Caliciaceae crown group at 126 Mya, thus comparable to the estimate in Prieto and Wedin (2013) and estimates produced by independent studies in other lichen families (Amo de Paz et al., 2011; Beimforde et al. 2014; Divakar et al. 2015; Gaya et al. 2015). The age of the A + B clade (main mazaediate clade) is estimated at 103 Mya (95 % HPD = 80–131 Mya) with two diversification events within the group; a first event at 85 Mya giving rise to clade B, and a second event at 60 Mya giving rise to clade A. This last event coincides with the time of appearance of the mazaediate taxa included in the Buellioi d clade (clade C, 59 Mya).

The diversification of the Physciaceae during the Late Cretaceous (102 Mya) in our secondary calibration analysis (A9) is congruent with diversification dates for other lichen families as Teloschistaceae (98 Mya, Gaya et al. 2015) and Parmeliaceae (109 Mya, Amo de Paz et al., 2011) while the diversification of the Caliciaceae took place somewhat earlier, during the Early Cretaceous (126 Mya). The mazaediate clades arose during two different periods, at the Early-Late Cretaceous boundary and in the Paleocene. The first event is close to the Cretaceous thermal maximum (89–112 Mya) where the diversification of the family Teloschistaceae was recently suggested to have taken place (Gaya et al. 2015). The second event took place close to the Paleocene–Eocene Thermal Maximum, 55.8 million years ago (Röhl et al. 2000).

In this latter period two independent events took place, a diversification episode within the *Calicium* clade coincident with an independent origin of the mazaediate clade within the Buellia-clade (clade C).

The main diversification events of mazaediate taxa took place during the Paleocene, Eocene and Oligocene as it has been reported for taxa within the lichen family Parmeliaceae (Divakar et al. 2015). The parallel, independent gains of the mazaedium in the history of the Ascomycota have already been demonstrated (Prieto et al. 2013). The appearance of other mazaediate groups is congruent with the diversification events found here. For example the Coniocybomyces crown is dated at 106 Mya and the Microcaliciales at 40 Mya (Prieto and Wedin 2013). Thus, it is probable that around this time climatic events on earth could have favored the independent appearance of these groups of taxa characterized by the presence of a mazaedium. What external factors could have promoted the appearance and diversification of mazaediate groups? As most of the extant species are epiphytes, growing on trunks or decorticated stumps of both conifers and deciduous trees, one possible explanation could be the appearance and diversification of conifers and angiosperms in the Early Mesozoic and Early Cenozoic respectively (Leslie et al. 2012; Magallón et al. 2015; Silvestro et al. 2015) and the change of the landscape this must have produced.

We conclude that utilizing a fossil with a poorly understood taxonomic affinity within the group under study is clearly problematic. One way to improve the accuracy of molecular time estimates is to use multiple fossil constraints (Graur and Martin 2004; Hedges and Kumar 2004; Rutschmann et al., 2007; Sauquet et al. 2012). However, the very small number of well-preserved and identifiable lichen fossils is a limiting factor in dating studies, and in our particular case there are currently no more available fossils. Another approximation is to use secondary calibrations, obtained from previous studies (Graur and Martin 2004; Sauquet et al. 2012). In this study we conclude that using such previously obtained dates gives us results that are comparable with other dating studies, whereas assigning the single relevant fossil to arbitrary placements results in strongly deviant dates. By comparing the inferred divergence dates with widely accepted dates (usually based on a robust fossil record) we here provide insight into the role of unreliable fossils in date estimates. Although this could be seen as a circular argument, the use of secondary calibrations can in reality serve as evaluation of the phylogenetic affinity of a fossil. In our case we can discard several alternative placements investigated, suggesting that the fossil is likely to be member of a younger clade as the *C. chlorosporum*-*C. nobile* and thus discarding its basal placement. This result will contribute to future dating studies, since it provides a good guide about which taxa to include in such studies and where to place the fossil best.

Taxonomy

Acolium (Ach.) Gray, Nat. Arr. Brit. Pl. I: 482 (1821).

Type species: *Calicium tympanellum* Ach. (lectotype, designated by Tibell 1984) = *Acolium inquinans* (Sm.) A. Massal.

Ca 5 species: *Acolium chloroconium* Tuck., *A. inquinans* (Sm.) A. Massal., *A. karelicum* (Vain.) M. Prieto and Wedin, *A. marcianum* (B. de Lesd.) M. Prieto and Wedin, *A. sessile* (Pers.) Arnold.

New combinations in *Acolium*:

Acolium karelicum (Vain.) M. Prieto and Wedin, comb. nov. (MB 817532) Basionym: *Cyphelium lucidum* var. *karelicum* Vain., Acta Soc. Fauna Fl. Fenn. 57(I): 20 (1927).

Acolium marcianum (B. de Lesd.) M. Prieto and Wedin, comb. nov. (MB 817533) Basionym: *Cyphelium marcianum* B. de Lesd., Bull. Soc. Bot. France 55: 420 (1908).

Comments: This is a small group of former *Cyphelium* species predominantly growing on bark or wood. *Acolium* is characterized by a dark excipulum that is strongly thickened at the base. Species placed here have a distinct grey-brown thallus (or are lichenicolous and do not form an independent thallus), they have sessile to somewhat immersed ascomata, and several have also a grey pruina on the rim of the excipulum. Although not investigated here, *Acolium chloroconium* is very similar to and presumably closely related to *A. inquinans* (Tibell and Ryan 2004) and is best placed here as *Cyphelium* is no longer available. *Acolium sessile* and *A. marcianum*, both lichenicolous on species of *Pertusaria* s. lat. and not investigated molecularly by us, also seem best placed here as they share the basally thickened excipulum with the other species. The placement of these three species needs confirmation from sequenced material.

Tibell (1984) designated *Calicium tympanellum* (a synonym of *Cyphelium inquinans*) as type species of *Acolium*. Both the other species originally included by Acharius (1808) in *Calicium* [unranked] *Acolium* (*C. turbinatum* and *C. stigonellum*) are synonyms of *Sphinctrina turbinata*. Several authors (Fink 1911; Clements and Shear 1931) assumed that the rankless subdivision *Acolium* was published in Acharius (1810), which make the type species selected by Fink and Clements and Shear not correctly designated.

Acroscyphus Lév., Anns. Sci. Nat., sér. 3 5: 262 (1846).

Type species: *Acroscyphus sphaerophoroides* Lév.

Comments: *Acroscyphus* is a distinct monotypic genus which is easily distinguished from the sister group *Acolium* by the dactyliform, fruticose thallus and the laterally reduced excipulum.

Calicium Pers., in Romer and Usteri, Ann. Bot. 7: 20 (1794).

Type species: *Calicium viride* Pers. (lectotype, designated by Fink, Contr. U.S. Nat. Herb. 14(1): 46, 1910).

At least 34 species: *C. abietinum* Pers., *C. adpersum* Pers., *C. carolinianum* (Tuck.) M. Prieto and Wedin, *C. chlorosporum* F. Wilson, *C. constrictum* Tibell, *C. corynellum* (Ach.) Ach., *C. denigratum* (Vain.) Tibell, *C. glaucellum* Ach., *C. hyperelloides* Nyl., *C. indicum* Tibell, *C. laevigatum* Tibell, *C. lecideinum* (Nyl.) M. Prieto and Wedin, *C. lenticulare* Ach., *C. leucochlorum* Tuck., *C. lucidum* (Th.Fr.) M. Prieto and Wedin, *C. montanum* Tibell, *C. nobile* Tibell, *C. notarissii* (Tul.) M. Prieto and Wedin, *C. parvum* Tibell, *C. pinastri* Tibell, *C. pinicola* (Tibell) M. Prieto and Wedin, *C. pyriforme* Tibell, *C. quercinum* Pers., *C. robustellum* Nyl., *C. salicinum* Pers., *C. sequoiae* Williams and Tibell, *C. tenuisporum* Tibell, *Calicium tigillare* (Ach.) Pers., *C. trabinellum* Ach., *C. trachylioides* (Nyl. ex Branth and Rostr.) M. Prieto and Wedin, *C. tricolor* F. Wilson, *C. verrucosum* Tibell, *C. victorianum* (F. Wilson) Tibell, *C. viride* Pers.

New combinations in *Calicium*:

Calicium carolinianum (Tuck.) M. Prieto and Wedin, comb. nov. (MB 817549) Basionym: *Acolium carolinianum* Tuck., Gen. lich. (Amherst): 237 (1872).

Calicium lecideinum (Nyl.) M. Prieto and Wedin, comb. nov. (MB 817534) Basionym: *Trachylia lecideina* Nyl., Mem. Soc. Sci. Nat. Cherbourg 3: 199 (1855).

Calicium lucidum (Th.Fr.) M. Prieto and Wedin, comb. nov. (MB 817535) Basionym: *Trachylia lucida* Th.Fr., Öfvers. K. VetenskAkad. Förh. 12: 18 (1855).

Calicium notarissii (Tul.) M. Prieto and Wedin, comb. nov. (MB 817538) Basionym: *Acolium notarissii* Tul., Ann. Sci. Nat., Bot., sér. 3 17: 81 (1852).

Calicium pinicola (Tibell) M. Prieto and Wedin, comb. nov. (MB 817536) Basionym: *Cyphelium pinicola* Tibell, Svensk Bot. Tidskr. 63: 477 (1969).

Calicium trachylioides (Nyl. ex Branth and Rostr.) M. Prieto and Wedin, comb. nov. (MB 817537) Basionym: *Spilomium trachylioides* Nyl. ex Branth and Rostr., Lichenes Daniae: 141 (1869).

Comments: *Calicium* in this emended version includes both species with stalked (*Calicium* in the traditional sense) and sessile or immersed ascomata. The stalked species differ from *Allocalicium* in the shape of the capitulum and the colour of the stalk. Although phylogenetically very distinct, we do not currently know of any morphological or chemical character that is unique for *Calicium* in our sense, and here, more work is needed. *Cyphelium* Ach. (type *Lichen tigillaris* Ach.) is a new synonym to *Calicium*. For more synonyms, see Tibell (1984). *Calicium carolinianum*, usually placed in *Thelomma*, was not considered closely related to *Thelomma* s. str. nor to the *T. ocellatum* group (*Pseudothelomma*, see below) by Tibell (1976), and we agree with him. Tibell (1976, 1984), however, points out similarities between *T. carolinianum* and species placed by him in *Calicium* and *Cyphelium*, and

we suggest placing it in *Calicium* until molecular investigations can clarify its position.

Texosporium Nádvl., in Tibell and Hofsten, *Mycologia* 60: 557 (1968).

Type species: *Acolium sancti-jacobi* Tuck.

Comments: *Texosporium* grows on dung, soil and detritus in grassland soil crust communities, and differs from the related *Thelomma* by the ecology and the very distinct spore ornamentation formed by paraphyses that adhere to the surface of the spores (Tibell and von Hofsten 1968).

Thelomma A. Massal., *Atti Reale Ist. Veneto Sci. Lett. Arti*, ser 3, 5: 268 (1860).

Type species: *Cyphelium mammosum* Hepp, in Hartung 1857: 147 (1857).

Ca 5 species: *T. brunneum* (W.A. Weber) M. Prieto and Wedin, *T. californicum* (Tuck.) Tibell, *T. mammosum* (Hepp) A. Massal., *T. santessonii* Tibell, *T. siliceum* (Fée) Tibell.

New combination in *Thelomma*:

Thelomma brunneum (W.A. Weber) M. Prieto and Wedin, comb. nov. (MB 817539) Basionym: *Cyphelium brunneum* W.A. Weber, *Bryologist* 70: 199 (1967).

Comments: *Thelomma* differs from the related *Texosporium* by growing on rocks in Mediterranean areas, and by lacking the distinctive spore ornamentation that characterizes *Texosporium*. The newly described *Pseudothelomma* differs in having a thin cortex, lacking the granular crystals that intersperse the usually thick cortex in *Thelomma*, and in the ecology as *Pseudothelomma* grows on decorticated wood. The generic placement of *Thelomma brunneum* needs verification from sequenced material. *Thelomma brunneum* was hitherto placed in *Cyphelium*, but as this genus is no longer available, *Thelomma* seems the best place as it share the ecology (growing on rocks in Mediterranean areas of North America).

Tholurna Norman, *Flora* (Regensburg) 44: 409 (1861).

Type species: *Tholurna dissimilis* (Norman) Norman.

Comments: *Tholurna* is a rare monospecific genus characterized by its cushion-like colonies, formed of dense clustered podetia carrying one ascoma each apically. The capitulum is campanulate, and the spores have a very distinctive ornamentation of spirally arranged ridges, two characters similar to *Allocalicium*.

New genera

Allocalicium M. Prieto and Wedin gen. nov. (MB 817540).

Type species: *Allocalicium adaequatum* (Nyl.) M. Prieto and Wedin.

Diagnosis: thallus immersed. Ascomata with olive brown stalks forming small clumps; capitulum campanulate and dark; spores 1-septate with a distinct ornamentation of spirally arranged ridges.

Etymology: Allo-, greek prefix meaning different or strange, which denotes a difference with *Calicium* while indicating an overall morphological similarity.

Description:

Thallus immersed. Ascomata distinctly stalked, 8–10 times as high as the diameter of the stalk, without pruina and with dark capitulum and pale grey to olive brown stalk. Capitulum cylindrical to distinctly bell-shaped. Excipulum 20–35 μm thick, with a distinct thickening in the upper part. Outermost part of the excipulum brown, consisting of isodiametric medium brown cells, 3–5 μm diam. Inner part of excipulum 10–17 μm thick, consisting of intertwined hyphae with swollen walls. Hypothecium medium brown, up to 40 μm high, consisting of intertwined, brown, thin walled hyphae. Stalk rather short to long, inner part of periclinally arranged but intertwined hyphae of thick walls; outermost layer medium brown with periclinally arranged brown hyphae. Stalk and excipulum 1+ dark blue. Asci cylindrical with uniseriately or sometimes biseriately arranged spores. Spores 1-septate, 9–11 \times 4.5–5.5 μm , with a distinct ornamentation of spirally arranged ridges. Habitat: on thin branches of *Alnus incana*, *Populus* and *Salix* along streams and in well-lit situations in swampy areas with high humidity.

New combinations in *Allocalicium*:

Allocalicium adaequatum (Nyl.) M. Prieto and Wedin, comb. nov. (MB 817541) Basionym: *Calicium adaequatum* Nyl., *Flora*, Regensburg 52: 409 (1869).

Comments: *Allocalicium* is distinguished from the stalked species in the unrelated *Calicium* by the pale stalks, the dark, cylindrical to distinctly bell-shaped capitulum, and the ecology. As already commented on, *Allocalicium* shares the campanulate form of the capitulum with the closely related *Tholurna*, and they both have very similar spore ornamentation.

Pseudothelomma M. Prieto and Wedin gen. nov. (MB 817542).

Type species: *Pseudothelomma ocellatum* (Körb.) M. Prieto and Wedin.

Diagnosis: thallus crustose, grey, with a thin cortex that lacks crystals. Ascomata immersed in verrucae, flat, sometimes with a green-(yellow) pruina on the mazaedia; exciple laterally thin; spores 1-septate. Chemical compounds: occasionally usnic acid (in thallus) and epanorin and rhizocarpic acid in the hymenium and mazaedium.

Etymology: the name of this new genus is based on the resemblance to *Thelomma* where the species have been included until now.

Description:

Thallus crustose, well developed, verrucose or subareolate, grey; one species with globular, short-stalked isidia in black patches on the thallus surface. Cortex thin, without granular crystals. Ascomata immersed in verrucae. Mazaedium black, flat, sometimes with a greenish or greenish-yellow pruina.

Excipulum not sclerotized, poorly developed laterally. Hypothecium blackish brown. Asci cylindrical, with uniseriately arranged spores, 1-septate, blackish-brown, constricted at the septum, with a slightly uneven surface. Chemistry: thallus K-, C-, KC-, PD-, medulla I- or I+ dark blue; occasionally usnic acid in the thallus, and epanorin and rhizocarpic acid in the hymenium and mazaedium. Habitat: on exposed dry lignum, particularly on wooden posts.

2 species: *Pseudothelomma ocellatum* (Körb.) M. Prieto and Wedin, *P. occidentale* (Herre) M. Prieto and Wedin.

New combinations in *Pseudothelomma*:

Pseudothelomma occidentale (Herre) M. Prieto and Wedin, comb. nov. (MB 817544) Basionym: *Cyphelium occidentale* Herre, Proc. Wash. Acad. Sci. 12: 62 (1910).

Pseudothelomma ocellatum (Körb.) M. Prieto and Wedin, comb. nov. (MB 817543) Basionym: *Acolium ocellatum* Körb., Parerga lichenol. 3: 285 (1861).

Comments: *Pseudothelomma* is a small, distinct group of species with immersed ascomata, growing on dry and exposed lignum. It differs from the otherwise similar *Acolium* by having a thin and non-sclerotized excipulum. It further differs from the likewise similar *Thelomma* by the ecology, and the thin, crystal-free cortex.

Key to mazaedium-producing Caliciaceae sensu Prieto & Wedin.

- 1 Fruticose lichens, forming proper podetia of thalline origin.....2
- 1 Crustose lichens, with stalked, sessile or immersed apothecia.....3
- 2 (1) Thallus stout, sparingly branched, pale greyish, ascomata immersed in branch-tips. Spores 2-celled with constricted septum, smooth. On rocks or epiphytic.....*Acrosyphus sphaerophoroides*
- 2 Thallus of radially protruding dark grey to brownish podetia, ascomata campanulate on apices of finger-like protrusions. Epiphytic on twigs.....*Tholurna dissimilis*
- 3 (1) Apothecia stalked.....4
- 3 Apothecia sessile or immersed.....5
- 4 (3) Apothecia with a pale grey to pale olive stalk and a dark, cylindrical to campanulate capitulum. Epiphytic on twigs or thin branches.....*Allocalcium adaequatum*
- 4 Apothecia not with a pale stalk together with a dark, campanulate capitulum.....*Calicium pro parte*
- 5 (3) Spores smooth, apothecia immersed with a very thin excipulum (section). On dry wood or (rarely) bark.....*Calicium* (“*Cyphelium*” *tigillare*-group)
- 5 Spores with a ± distinct ornamentation, apothecia immersed or sessile with a distinct excipulum which is thick, at least at the base. On bark, wood or rocks.....6

- 6 (5) Spore ornamentation very distinct, formed by paraphyses that adhere to the surface of the spores. On dung, detritus or soil in grassland and soil crust communities.....*Texosporium sancti-jacobi*
- 6 Spore ornamentation made up by ridges or surface cracks. On rock, bark, wood, or lichenicolous.....7
- 7 (6) On rock (*Thelomma californicum* occasionally on wood)8
- 7 On bark or wood, or lichenicolous on *Pertusaria*.....9
- 8 (7) Apothecia immersed in thallus verrucae.....*Thelomma*
- 8 Apothecia sessile.....*Calicium lecideinum*
- 9 (7) Apothecia sessile to somewhat immersed, with a grey pruina on the rim of the excipulum, and a dark excipulum that is strongly thickened at the base (section). Thallus distinct, greyish-brownish. On wood or bark, or lichenicolous on *Pertusaria* spp.....*Acolium*
- 9 Apothecia and thallus varying.....10
- 10 (9) On dry and exposed wood, with immersed apothecia.....11
- 10 On wood or bark, apothecia sessile.....*Calicium* spp. (former *Cyphelium* spp)
- 11 (10) Thallus verrucose, greyish, excipulum thin and hyaline throughout, asci cylindrical.....*Pseudothelomma*
- 11 Thallus smooth, areolate, dull reddish-brown, excipulum thick and ± sclerotized at the base, asci pyriform.....*Calicium carolinianum*

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