The insights into the evolutionary history of Translucidithyrium: based on a newly-discovered species

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Abstract

During the field studies, a Translucidithyrium-like taxon was collected in Xishuangbanna of Yunnan Province, during an investigation into the diversity of microfungi in the southwest of China. Morphological observations and phylogenetic analysis of combined LSU and ITS sequences revealed that the new taxon is a member of the genus Translucidithyrium and it is distinct from other species. Therefore, Translucidithyrium chinense sp. nov. is introduced here. The Maximum Clade Credibility (MCC) tree from LSU rDNA of Translucidithyrium and related species indicated the divergence time of existing and new species of Translucidithyrium was crown age at 16 (4–33) Mya. Combining the estimated divergence time, paleoecology and plate tectonic movements with the corresponding geological time scale, we proposed a hypothesis that the speciation (estimated divergence time) of T. chinense was earlier than T. thailandicum. Our findings provided new insights into the species of Translucidithyrium about ecological adaptation and speciation in two separate areas.

Keywords

Divergence time, morphological characteristics, new species, Phaeothecoidiellaceae, phylogeny, speciation, taxonomy

Introduction

The sooty blotch and flyspeck fungi are widespread species and commonly occur on the surface of leaves, stems and fruits in tropical and subtropical zones (Yang et al. 2010; Gleason et al. 2011; Hongsanan et al. 2017; Zeng et al. 2018). Although these
fungi do not directly harm host plants, they may affect the economic value of fruit sales ability and reduce photosynthesis in plants (Gleason et al. 2011). Sooty blotch fungi can form dark mycelial mats, whereas flyspeck fungi lack mycelial mats, form shiny and small, black spots (Batzer et al. 2005; Yang et al. 2010; Gleason et al. 2011; Zhang et al. 2015; Singtripop et al. 2016; Hongsanan et al. 2017). However, these fungi are poorly known, because of the difficulty in obtaining the strain which grows slowly (Yang et al. 2010; Hongsanan et al. 2017; Zeng et al. 2018).

Phaeothecoidiellaceae K.D. Hyde & Hongsanan was introduced by Hongsanan et al. (2017) and accommodated three genera Chaetothyrina, Houjia and Phaeothecoidiella in the order Capnodiales. Currently, it includes eight genera: Chaetothyrina, Exopassalora, Houjia, Nowamyes, Phaeothecoidiella, Rivilata, Sporidesmajora and Translucidithyrium (Hongsanan et al. 2020). Members of Phaeothecoidiellaceae are related to sooty blotch and flyspeck fungi and characterised by thyriothecia with setae, bitunicate asci and 1-septate ascospores (Singtripop et al. 2016; Hongsanan et al. 2017; Zeng et al. 2019; Hongsanan et al. 2020). Chaetothyrina is morphologically similar to the family Micropeltidaceae (Reynolds and Gilbert 2005), but is distinguishable by its brown upper wall of ascomata (Wu et al. 2019; Zeng et al. 2019). The genus Rivilata is placed in this family on the basis of morphological characters by Doilom et al. (2018). The Nowamyes was introduced as a new genus in the new family Nowamycetaceae by Crous et al. (2019) and Hongsanan et al. (2020) placed this genus into Phaeothecoidiellaceae by phylogenetic analysis. Hongsanan et al. (2020) listed Houjia, Exopassalora, Sporidesmajora and Phaeothecoidiella as asexual genera in Phaeothecoidiellaceae.

Translucidithyrium X.Y. Zeng & K.D. Hyde (2018) was introduced as a monotypic genus in Phaeothecoidiellaceae, which is represented by T. thailandicum X.Y. Zeng & K.D. Hyde (2018). It was characterised by epiphytes on the reverse of living leaves, semi-transparent ascomata, globose to subglobose asci and fusiform ascospores with verrucose and appendages. Ascospores germinated on MEA (Malt Extract Agar Medium) within 24 h. The colonies slowly grow on media, white to grey, circular and villiform (Zeng et al. 2018).

Liu et al. (2017) used the molecular clock approach to estimate the divergence time of the order Capnodiales crown age at 151–283 Mya (million years ago). Zeng et al. (2019) estimated the divergence time of the family Phaeothecoidiellaceae crown age at 40–60 Mya. The molecular clock approach for estimating divergence time might be used to predict speciation, historical climate change or other environmental events (Hélène and Arne 2014; Louca and Pennell 2020).

In this study, we collected an extraordinary new species of Translucidithyrium in Xishuangbanna, Yunnan Province, China. We described the morphological characteristics and built a phylogenetic tree to determine the classification of the new taxon. We compared and analysed the estimated divergence time of Translucidithyrium with the environmental changes around the corresponding time range to propose the evolutionary history hypothesis of Translucidithyrium distributed in two different regions (China and Thailand).
Methods

Morphological

Fresh living leaves with olivaceous dots were collected at Xishuangbanna, China (21°55′51″N, 101°15′08″E, 540 m alt.) and delivered to the laboratory for observation. According to Wu et al. (2014), the collected samples were processed and examined by microscopes: the photos of ascomata were taken by using a compound stereomicroscope (KEYENCE CORPORATION V.1.10 with camera VH-Z20R). Hand sections were made under a stereomicroscope (OLYMPUS SZ61) and mounted in water and blue cotton and photomicrographs of fungal structures were taken with a compound microscope (Nikon ECLIPSE 80i). The single spore isolation was implemented by the methods of Choi et al. (1999) and Chomnunti et al. (2014). Germinated spores were individually transferred to PDA (Potato Dextrose Agar Medium) and incubated at 26 °C for 48 h. Colony characteristics were observed and measured after 4 weeks at 26 °C. Images used for figures were processed with Adobe Photoshop CC v. 2015.5.0 software (Adobe Systems, USA). The holotype was deposited at the herbarium of IFRD (International Fungal Research & Development Centre; Research Institute of Resource Insects, Kunming), reference number IFRD 9208. The ex-type strain was deposited at IFRDCC, reference number IFRDCC 3000.

DNA isolation, amplification and sequencing

According to the manufacturer’s instructions, genomic DNA was extracted from mycelium growing on PDA at room temperature by using the Forensic DNA Kit (OMEGA, USA). The primer pair LR0R and LR5 was used to amplify the large subunit (LSU) rDNA (Vilgalys and Hester 1990). The primer pair ITS5 and ITS4 was used to amplify the internal transcribed spacer (ITS) rDNA (White et al. 1990). The primer pair NS1 and NS4 was used to amplify the partial small subunit (SSU) rDNA (White et al. 1990). The PCR reactions were in accordance with instructions from Golden Mix, Beijing TsingKe Biotech Co. Ltd, Beijing, China: initial denaturation at 98 °C for 2 min, then 30 cycles of 98 °C denaturation for 10 s, 56 °C annealing for 10 s and 72 °C extension for 10 s (ITS and SSU) or 20 s (LSU) and a final extension at 72 °C for 1 min. All PCR products were sequenced by Biomed (Beijing, China).

Sequences alignments and phylogenetic analysis

BioEdit version 7.0.5.3 (Hall 1999) was used to re-assemble sequences generated from forward and reverse primers for obtaining the integrated sequences. Sequences were downloaded from GenBank using data from the publications of Zeng et al.
(2018), Crous et al. (2019), Hongsanan et al. (2020) and Renard et al. (2020) and aligned using BioEdit version 7.0.5.3 (Hall 1999); in addition, sequences were adjusted manually.

Maximum Likelihood (ML) analysis was conducted by using RAxMLGUI v.1.0 (Silvestro and Michalak 2012). Aligned sequences were input into the software and Dothidea sambuci was selected as the outgroup taxon. One thousand non-parametric bootstrap iterations were employed with the “ML + rapid bootstrap” tools and “GTR-GAMMA” arithmetic.

For Bayesian analysis, MrModeltest 2.3 (Nylander 2004) was used to estimate the best-fitting model for the combined LSU and ITS genes. Posterior probabilities (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) were determined by Markov Chain Monte Carlo (MCMC) sampling in MrBayes v.3.2 (Ronquist and Huelsenbeck 2003). Six simultaneous Markov chains were run for 2,000,000 generations; trees were printed every 1,000 generations; trees were sampled every 100 generations. The first 5,000 trees submitted to the burn-in phase and were discarded; the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree (Cai et al. 2006, 2008; Liu et al. 2012).

Fossil calibrations and divergence time estimations

The fossil Protographum luttrelli (Renard et al. 2020) was used to calibrate the divergence time of Asterotexiales and Aulographaceae (normal distribution, mean = 119.0, SD = 3.7). The secondary calibration from the family Phaeothecoidiellaceae with a crown age of 58 Mya (normal distribution, mean = 50.0, SD = 6.1) was used (Zeng et al. 2019). The additional secondary calibration of Capnodiales was used, based on the result from Liu et al. (2017) (normal distribution, mean = 217.0, SD = 40.0).

Divergence time analysis was carried out using BEAST v1.8.4 (Drummond et al. 2012). Aligned LSU sequence data were loaded into the BEAUti v1.10.4 for generating an XML file. An uncorrelated relaxed clock model (Drummond et al. 2006) with a lognormal distribution of rates was used for the analysis. We used a Yule Process tree prior (Yule 1925; Gernhard 2008), which assumes a constant speciation rate per lineage and a randomly-generated starting tree. The length of chain was set as 50 million generations and sampling parameters were set at every 5,000 generations in MCMC. Subsequent divergence time analysis was carried out using BEAST v.1.10.4 (Drummond et al. 2012). Tracer v.1.7.1 was used to check the effective sample sizes (ESS) and acceptable values were higher than 200. The .log files and .tree files generated by BEAST were combined in LogCombiner v1.10.4 after removing a proportion of states as burn-in. The Maximum Clade Credibility (MCC) tree was given by obtained data and was estimated in TreeAnnotator v.1.10.4 (Liu et al. 2017; Zeng et al. 2019, 2020; Renard et al. 2020).

The phylogenetic tree and MCC tree were visualized in FigTree v.1.4.3 (Rambaut 2012) and Adobe Illustrator CS6 v. 16.0.0 (Adobe Systems, USA).
Table 1. Selected taxa in this study with their corresponding GenBank accession numbers. The newly-generated sequences are shown in bold.

| No. | Species                          | Voucher /strain no. | LSU         | ITS         |
|-----|----------------------------------|---------------------|-------------|-------------|
| 1   | Acidomyces acidophilus           | MH1085              | JQ172741    | JQ172741    |
| 2   | Asterina phenacis                | TH 589              | GU586217    | –           |
| 3   | Asterotexiaceae sp.              | VUL.535             | MG844162    | –           |
| 4   | Aulographum sp.                  | VUL.457             | MG844158    | –           |
| 5   | Batcheloromyces proteae          | CBS 110696          | JF746163    | JF746163    |
| 6   | Bauolnius comptiacensis          | CBS 123031          | GQ852580    | –           |
| 7   | Brunonosphearrla protearum       | CPC 16338           | GU214397    | GU214626    |
| 8   | Buellia minimula                 | Lendemer 42237(NY)  | XX449061    | –           |
| 9   | Camarosporula persooniae         | CBS 116258          | JF770461    | JF770449    |
| 10  | Capnobotryella renispora         | CBS 214.90          | GU214400    | DQ491515    |
| 11  | Capnodium coffeae                | CPC 15368           | GU214402    | GU214628    |
| 12  | Chaetothyrina guttulata          | CBS 121621          | KJ564331    | EF679363    |
| 13  | Chaetothyrina guttulata          | CBS 125988          | KJ564334    | HM148097    |
| 14  | Chaetothyrina musarum            | MFLUCC 15–0383      | KU710171    | –           |
| 15  | Cladosporium herbarum            | CBS 121011          | KJ564331    | EF679363    |
| 16  | Cladosporium hillianum           | CBS 170.54          | DQ678057    | AY213640    |
| 17  | Cladosporium ramotenellum        | CBS 100496          | GU301817    | AY128703    |
| 18  | Conidiocarpus (Phragmocapnias) betle | MFLUCC 10–0050      | –           | –           |
| 19  | Deveria stauraphora              | ATCC 200934         | KF901963    | AF393723    |
| 20  | Discomonium aciculare            | CBS 204.89          | GU214419    | AY725520    |
| 21  | Duthidea sambuci                 | AFTOL-ID 274        | AY544681    | DQ491505    |
| 22  | Duthisterina pini                | CBS 121011          | JX901821    | JX901734    |
| 23  | Elasticomyces elasticus          | CCFEE 5547          | KF309991    | –           |
| 24  | Exopassalora zambiae             | YHJN13              | GQ433631    | GQ433628    |
| 25  | Extremus adstrictus              | TRN96               | KF100022    | –           |
| 26  | Friedmanniomycyes edoliticipus   | MFLUCC 5199         | KF310007    | JN885547    |
| 27  | Hispidoconidioma alpinum         | L2–1/2              | FJ997286    | FJ997285    |
| 28  | Hortaea borneriic               | CBS 100496          | GU301817    | AY128703    |
| 29  | Houjia yanglingensis             | YHJN13              | GQ433631    | GQ433628    |
| 30  | Lecanosticta pini                | CBS 871.95          | GQ852598    | –           |
| 31  | Lembosia albersii               | MFLUCC 13–0377      | KM386982    | –           |
| 32  | Lembosia sp.                     | VUL.644             | MG844165    | –           |
| 33  | Leptoxyphium cacuminum           | MFLUCC 10–0049      | JN832602    | –           |
| 34  | Melanodothia carici             | CBS 860.72          | GU214431    | GU214638    |
| 35  | Microcyclosporella mai           | CPC 16171           | GU570545    | GU570528    |
| 36  | Microxyphium citri               | CBS 451.66          | KF902094    | –           |
| 37  | Morenoisa calamicola             | MFLUCC 14–1162      | NG059779    | NR154210    |
| 38  | Neopseudocercosporella capsellae | CPC 127.29          | KF251830    | KF251326    |
| 39  | Novamyces globulus               | CBS 144598          | MN162196    | MN161935    |
| 40  | Parapenidiella taramiensis       | CPC 23534           | KJ869211    | KJ869154    |
| 41  | Neodrevia corneliae              | CPC 15382           | GU214414    | GU214633    |
| 42  | Neodrevia hilliana               | CBS 128219          | HQ599606    | HQ599605    |
| 43  | Neodrevia saxithorae             | CBS 127.29          | KF251630    | KF251326    |
| 44  | Neodrevia saxithorae             | CBS 127.29          | KF251830    | KF251326    |
| 45  | Nowamyces globulus               | CBS 144598          | MN162196    | MN161935    |
| 46  | Nowamyces piperitae              | CBS 143490          | MN162200    | MN161944    |
| 47  | Peniophellia tasmaniensis        | CBS 124991          | KF901844    | KF901522    |
| 48  | Pseudolata cucullifera           | CBS 111318          | KF901938    | KF901613    |
| 49  | Peniophellia columnum            | CBS 486.80          | EU019274    | KF901630    |
| 50  | Pirociliella selutina            | CBS 101950          | EU019274    | EU017483    |
| 51  | Petrophila incerta               | TRN 77              | –           | –           |
| 52  | Phaeophleospora eugeniase        | CPC 15159           | KF902095    | KF901742    |
| No. | Species                          | Voucher /strain no. | LSU         | ITS         |
|-----|----------------------------------|---------------------|-------------|-------------|
| 53  | Phaeothecoidea eucalypti         | CBS 120831          | KF901848    | KF901526    |
| 54  | Phaeotheciodella illinoensis     | CBS 125223          | GU117901    | GU117897    |
| 55  | Phaeotheciodella missouriensis   | CBS 125222          | AY598917    | AY598878    |
| 56  | Phleoaspera maculans             | CBS 115123          | GU214670    | GU214670    |
| 57  | Piednia hortae                   | CBS 480.64          | GU214466    | GU214467    |
| 58  | Piednia quintansilvae            | CBS 327.63          | GU214468    | –           |
| 59  | Pseudocercospora vitis           | CPC 11595           | GU214483    | GU269829    |
| 60  | Pseudoramichloridium henryi      | CBS 124775          | KF442561    | KF442521    |
| 61  | Pseudotaeniolina globosa         | CCFEE 5734          | KF310010    | KF309976    |
| 62  | Pseudoveronaea obelavata         | CBS 132086          | JQ622102    | –           |
| 63  | Racodium rugestre                | L346                | EU048583    | GU067666    |
| 64  | Racodium rugestre                | L424                | EU048582    | GU067669    |
| 65  | Ramichloridium apiculatum        | CBS 113265          | AY90776     | AY90763     |
| 66  | Ramularia endophylla             | CBS 124973          | KP894141    | KP894248    |
| 67  | Ramularia pusilla                | CBS 125078          | GQ852653    | –           |
| 68  | Ramulipora sorghi                | CBS 110578          | GU214696    | GU214696    |
| 69  | Readerellia mirabilis            | CBS 125000          | KF251836    | KF251332    |
| 70  | Recurvorhizys mirabilis          | CBS 119434          | GU250372    | FJ415477    |
| 71  | Repetophragma zygopetalii        | VIC42946            | KT732418    |              |
| 72  | Schizothyrium poni               | CBS 486.50          | EF134948    | EF134948    |
| 73  | Scoclemistigina mangiferae       | CBS 125467          | GU253877    | GU269870    |
| 74  | Scoletia spongiosa              | CBS 325.33          | GU214696    | GU214696    |
| 75  | Septoria citida                  | USO 378994          | JF700954    | JF700932    |
| 76  | Septoria lycmaciae               | CBS 123794          | KF251972    | KF251468    |
| 77  | Sonderenia eucalyptorum          | CBS 120220          | KP901822    | KP901505    |
| 78  | Sphaerulina myriadea             | CBS 124646          | JF770468    | JF770455    |
| 79  | Sporidemogina peniulianensis     | CBS 125229          | MH874965    | MF951287    |
| 80  | Sterella araguata                | CBS 105.75          | EU019250    | EU019250    |
| 81  | Teratocladus variculatus         | CBS 113093          | GU214669    | GU214669    |
| 82  | Teratosphaeria glob Illinois     | CBS 1217.07         | GU323213    | KP091728    |
| 83  | Toxocladusporium iriaceae        | CBS 185.58          | EU040243    | EU040243    |
| 84  | Toxocladusporium rubrigenum      | CBS 124158          | FJ790305    | FJ790287    |
| 85  | Translucidithyrium chinense      | IFRDCC 3000         | MT659404    | MT659671    |
| 86  | Translucidithyrium thailandicum  | MFLUCC 16–0362      | MG930048    | MG930045    |
| 87  | Trichospermum myri                 | CBS 437.68          | GU323216    | –           |
| 88  | Trochophora simplex              | CBS 124744          | GU253880    | GU269872    |
| 89  | Uveobrunia communis              | CBS 114238          | EU019267    | AY725541    |
| 90  | Verrucariospora fori              | CCFEE 5459          | GU250390    | KF309981    |
| 91  | Xenocladusporium catenatum        | GMW 22113           | JN712570    | JN712502    |
| 92  | Zasmidium ciliate                 | CBS 146.36          | EU041878    | EU041821    |
| 93  | Zygophiala cryptogena            | OH4_1Aa             | FJ417517    | FJ425208    |
| 94  | Zygophiala tardicrescens         | MWA1a               | EF164901    | AV598856    |
| 95  | Zygophiala wisconsinensis        | OH4_9A1c            | FJ417518    | FJ425209    |

**Results**

**Phylogenetic study**

The dataset of combined LSU and ITS sequences comprised 1350 characters after alignment. Bayesian Inference, in total, generated 20,001 trees and the average standard deviation of split frequencies reached 0.0096. A total of 15,001 trees were finally used to calculate posterior probabilities. Phylogenetic analysis showed that the new collection clusters with *T. thailandicum* with 100% Maximum Likelihood bootstrap support and 1.00 posterior probabilities (Fig. 1).
Taxonomy and evolution history of *Translucidithyrium*

**Figure 1.** The topology shows family relationships of Capnodiales, based on combined LSU and ITS dataset analysis. Bootstrap values of Maximum Likelihood higher than 60% are shown on the left, while values of Bayesian posterior probabilities above 80% are shown on the right. New species is given in bold. Clades of the key species or family are given in bold. The tree is rooted with *Dothidea sambuci* (Dothideaceae, Dothideales).

**Taxonomy**

*Translucidithyrium chinense* H. X. Wu & X. H. Li, sp. nov.

Index Fungorum number: IF 557843

Facesoffungi number: FoF 09429

Figures 2, 3

**Etymology.** Refer to the location of species, China.

**Holotype.** IFRD9208

**Description.** Epiphytic on living leaves, ascomata with papillate. Superficial hyphae absent. Sexual morph: Ascomata solitary or scattered, 480–870 μm diam. (\(\bar{x} = 741\) μm, \(n = 6\)), 65–82 μm high (\(\bar{x} = 72\) μm, \(n = 8\)), olivaceous to brown, slightly semi-transparent under highlighted background, circular to suborbicular, with slightly prominent papilla, membranous, without ostiole (Fig. 2A–C). Peridium 8.3–10 μm thick, (\(\bar{x} = 9\) μm, \(n = 11\)), composed of irregular, meandering, interwoven arranged cells, two layers: from brown to hyaline, outer layer composed of closely-arranged cells, brown; inner layer composed of hyaline, oblong, subdense arranged cells, poorly developed at the base (Fig. 2D–F). Asci evenly distributed and parallel arranged in hamathecium (Fig. 2D–F), 65–90 × 51–81 μm (\(\bar{x} = 77 \times 60\) μm, \(n = 10\)), 8-spored, bitunicate, hyaline, with an ocular chamber, ovoid at immature state, globose to subglobose at mature
Figure 2. *Translucidithryium chinense* (IFRD 9208, holotype) A plant leaves B ascocoma on leaves surface C squash of ascocoma at 20 times amplification D cross section of ascocoma in blue cotton at 20 times amplification E, F cross section of ascocoma in blue cotton at 40 times amplification G asci at 100 times amplification H–K asci in blue cotton at 100 times amplification L ascospore at 100 times amplification M–P ascospore in blue cotton at 100 times amplification. Scale bars: 200 μm (B); 100 μm (C, D); 50 μm (E, F); 20 μm (G–K); 10 μm (L–P). We slightly adjusted the contrast, saturation and hue of images and removed the contaminants around main object in images in PS software without obscuration, erasure or distortion of any information existing in the original document.
state, lacking pedicel, paraphyses absent (Fig. 2G–K). *Ascospores* 41–65 × 10–13 μm (\(\bar{x} = 50 \times 11 \mu m, n = 20\)), irregularly overlapping, hyaline, ovoid at young state, fusiform with both ends tapered at mature state, 1-septate, constricted at the septum, upper cell a little larger than lower, with guttules at both ends, verrucose (Fig. 2L–P).

**Asexual morph:** Undetermined.
**Cultural characteristics.** Ascospores germinating on MEA at 36 h after spore-isolation, germinating on PDA at 48 h after spore-isolation. Colonies slow growing on MEA and PDA, irregular, villiform, convex, white on surface, yellow to brown at base. After a long period of growth, the pigments produced by culture discolor the medium, roots generate at the bottom (Fig. 3A–D). Culture hyphae hyaline, branched, constricted at the septum, 3 μm wide (Fig. 3E, F).

**Material examined.** CHINA, Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Xishuangbanna Botanical Garden; 21°55’51″N, 101°15’08″E, 540 m alt.; 21 Apr 2019; Haixia Wu and Xinhao Li leg; collected on living leaves of *Alpinia blepharocalyx* (IFRD 9208, holotype), ex-type living culture (IFRDCC 3000).

**Notes.** This new species is morphologically similar to *Translucidithyrium thailandicum* in having semi-transparent and largish ascomata, globose asci and hyaline ascospores with 1-septate. However, *Translucidithyrium chinense* has a slightly papilla thyriothecium with weaker transmittance and ascospores with guttules at both ends, while *T. thailandicum* has a flattened thyriothecium with higher transmittance and ascospores with appendages at both ends; besides, the size of ascomata and asci of *T. chinense* are slightly larger than those of *T. thailandicum* (795 μm vs. 621 μm; 77 μm vs. 64 μm). The cultural characteristics of both species are different: the culture of *T. chinense* grows more slowly, has roots inserting into medium and turn the bottom brown. Phylogenetically, *T. chinense* clusters with *T. thailandicum* as a distinct clade with high support (100% ML/1.00 PP, Fig. 1).

**Divergence times estimates.** The Maximum Clade Credibility (MCC) tree was similar to the major lineages in the Bayesian and ML trees. The crown age of

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**Figure 4.** The MCC tree with divergence times estimates of Phaeothecoidiellaceae obtained from a Bayesian approach (BEAST). Numbers at nodes indicate posterior probabilities (pp) for node support; bars correspond to the 95% highest posterior density (HPD) intervals. The key species are given in blue.
Translucidithyrium showed 16 Mya (4–33), which was earlier than the divergence time of most genera in Phaeotheciodiellaceae. The estimated divergence time of Phaeotheciodiellaceae from Zeng et al. (2019) is 58 Mya, which corresponds to our results.

**Discussion**

*Translucidithyrium thailandicum* was found in the north of Thailand (Zeng et al. 2018). *Translucidithyrium chinense* was found in the Xishuangbanna Region, southwest of China, which lies on the northern border of a rainforest with rich microfungal resources. The new species is characterised by brown to olivaceous ascomata and slightly semi-transparent, subglobose asci without pedicel and fusiform ascospores with verrucose and guttules (Fig. 2). *T. chinense* is introduced as a new species in *Translucidithyrium* by morphological and phylogenetic studies (Figs 1–3).

The ascomata of *Translucidithyrium* are different from related genera of Phaeotheciodiellaceae: *Nowamyces* has immersed ascomata, *Chaetothyrina* has ascomata with setae and *Rivilata* has subcuticular ascomata (Singtripop et al. 2016; Doilom et al. 2018; Zeng et al. 2018; Crous et al. 2019; Hongsanan et al. 2020). *Translucidithyrium* is similar to the family Schizothyriaceae in having semi-transparent ascomata, globose to subglobose asci and hyaline ascospores with guttules. Schizothyriaceae includes *Schizothyrium*, *Plochmopeltis*, *Hexagonella*, *Lecideopsis*, *Mycerema*, *Kerniomyces*, *Metathyriella*, *Myriangiella*, *Amazonotheca* and *Vonarxella* (Phookamsak et al. 2016; Wijayawardene et al. 2020). The morphology of *T. chinense* is most similar to *Lecideopsis* by having globose asci and 1-septate ascospores, but *Lecideopsis* has a short pedicel at the bottom of the asci (Phookamsak et al. 2016; Zeng et al. 2018). Phylogenetically, *Translucidithyrium* formed a long clade and clustered within the family Phaeotheciodiellaceae. It indicated the existing certain genetic distance amongst *Translucidithyrium*, Phaeotheciodiellaceae and Schizothyriaceae. Phaeotheciodiellaceae and Schizothyriaceae are poorly studied families (Batzer et al. 2008; Phookamsak et al. 2016; Singtripop et al. 2016; Hongsanan et al. 2017; Zeng et al. 2018). Therefore, more fresh specimens with molecular data are needed to confirm the classification of *Translucidithyrium*, Phaeotheciodiellaceae and Schizothyriaceae.

Zuckerkandl and Pauling (1962) suggested that the number of differences amongst amino acids was proportional to species divergence time. We estimated the divergence time using BEAST analysis. The divergence time of *Translucidithyrium* crown age was estimated at 16 Mya (4–33), which was earlier than the crown ages of *Chaetothyrina* at 2 Mya (0–5), the crown ages of *Repetophragma* at 9 Mya (2–20), the crown ages of *Nowamyces* at 7 Mya (1–20) and the crown ages of *Phaeotheciodiella* at 4 Mya (0–14) within Phaeotheciodiellaceae (Fig. 4). The divergence time of *Translucidithyrium* is earlier than other genera in Phaeotheciodiellaceae. We estimate that the long divergence time should affect the genetic variation (Pauling 1964; Hall and Hallgrímsson 2008). Additionally, the evolutionary molecular clock approach confirmed the long clades of *Translucidithyrium* in the phylogenetic tree (Fig. 1).
Historical events amongst different biological groups could then be compared with the dates of plate tectonic movements and paleoecology, according to the corresponding geological time scale (Lomolino et al. 2006; Berbee and Taylor 2010). Through relevant studies on the Qinghai-Tibet Plateau, it was found that the time of intense tectonic uplift and denudation is concentrated in 60–35 Mya, 25–17 Mya, 12–8 Mya and 5 Mya. Global cooling might have an impact on climate change in East Asia, especially at 15 Mya and 8 Mya (Lu et al. 2010). Rising plateaus and global cooling were drying up Asia (Liu 2000; Garzione et al. 2015). The time of the Qinghai-Tibet Plateau uplift and global cooling corresponded to the interval of the species in *Translucidithyrium* divergence time. We predict that the speciation of *T. chinense* was earlier than the speciation of *T. thailandicum*, as the divergence of *Translucidithyrium* was related to the Qinghai-Tibet Plateau uplift and global cooling. According to the evolution history of *Translucidithyrium*, it could be speculated that the speciation of *T. chinense* was earlier than *T. thailandicum*. With the climate becoming colder and with increased drought, *T. chinense* migrated from China to Thailand gradually to find a suitable area, then *T. thailandicum* formed. Due to the end of global cooling, the distribution pattern of *Translucidithyrium* in two different countries formed. Increasing fresh collections and application of new methodologies may result in modified conclusions.

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