A taxonomic study of *Nemania* from China, with six new species

Yin Hui Pi¹,², Si Han Long¹, You Peng Wu¹, Li Li Liu¹, Yan Lin¹, Qing De Long¹, Ji Chuan Kang³, Ying Qian Kang⁴, Chu Rui Chang¹, Xiang Chun Shen¹,², Nalin N. Wijayawardene¹,⁵,⁶, Xu Zhang¹, Qi Rui Li¹,²

¹ State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang 550004, China ² The High Efficacy Application of Natural Medicinal Resources Engineering Center of Guizhou Province (The Key Laboratory of Optimal Utilization of Natural Medicine Resources), School of Pharmaceutical Sciences, Guizhou Medical University, University Town, Guian New District, Guizhou, China ³ Engineering and Research Center for Southwest Bio-Pharmaceutical Resources of National Education Ministry of China, Guizhou University, Guiyang, Guizhou 550025, China ⁴ Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education of Guizhou and Guizhou Talent Base for Microbiology and Human Health, School of Basic Medical Sciences, Guizhou Medical University, Guiyang, China ⁵ Center for Yunnan Plateau Biological Resources Protection and Utilization, College of Biological Resource and Food Engineering, Qujing Normal University, Qujing, Yunnan 655011, China ⁶ Section of Genetics, Institute for Research and Development in Health and Social Care, No: 393/3, Lily Avenue, Off Robert Gunawardane Mawatha, Battaramulla 10120, Sri Lanka

**Corresponding author:** Qi Rui Li (lqrnd2008@163.com)

**Academic editor:** Ekaphan Kraichak  |  Received 9 June 2021  |  Accepted 29 July 2021  |  Published 24 August 2021

**Citation:** Pi YH, Long SH, Wu YP, Liu LL, Lin Y, Long QD, Kang JC, Kang YQ, Chang CR, Shen XC, Wijayawardene NN, Zhang X, Li QR (2021) A taxonomic study of *Nemania* from China, with six new species. MycoKeys 83: 39–67. [https://doi.org/10.3897/mycokeys.83.69906](https://doi.org/10.3897/mycokeys.83.69906)

**Abstract**

During an investigation of Xylariaceae from 2019 to 2020, isolates representing eight *Nemania* (Xylariaceae) species were collected from Yunnan, Guizhou and Hainan Provinces in China. Morphological and multi-gene phylogenetic analyses, based on combined ITS, α-actin, rpb2 and β-tubulin sequences, confirmed that six of them are new to science, viz. *Nemania camelliae*, *N. changningensis*, *N. cyclobalanopsina*, *N. feicuiensis*, *N. lishuicola* and *N. rubi*; one is a new record (*N. caries*) for China and one is a known species (*N. diffusa*). Morphological descriptions and illustrations of all species are detailed. In addition, the characteristics of *Nemania* are summarised and prevailing contradictions in generic concepts are discussed.

**Keywords**

phylogeny, six new species, taxonomy, Xylariaceae
Introduction

*Nemania* Gray was established by Gray (1821) for a heterogeneous assemblage of taxa and was affiliated with *Xylariaceae* Tul. & C. Tul. Since the early taxonomic description of this genus was ambiguous, taxonomists have often regarded some species of *Nemania* as synonyms of *Hypoxylon* Bull. For example, *Nemania angusta* (Petch) Y.M. Ju & J. D. Rogers was regarded as a synonym of *Hypoxylon angustum* Petch. (Miller 1961; Whalley et al. 1983; Ju and Rogers 2002). Subsequently, the generic concept of *Nemania* was modified by Pouzar (1985a, b) and Petrini and Rogers (1986). Granmo et al. (1999) and Ju and Rogers (2002) provided a comprehensive background to *Nemania* and accepted 37 species. Sánchez-Ballesteros et al. (2000) used the internal transcribed spacers (ITS) sequence to perform a phylogenetic study of *Nemania*, which supported the segregation of *Nemania* from *Hypoxylon*. However, their conclusion was based only on ITS sequences and *Xylaria* Hill & Schrank was not included in this study. Hence, the generic placement of *Nemania* in the *Xylariaceae* was unclear. Hsieh et al. (2005) used β-tubulin and α-actin to evaluate the phylogenetic relationship of several xylariaceous genera. It was found to be particularly useful in xylariaceous fungi as limited success in using ribosomal DNA genes to delineating genera and resolving generic relationships (Tang et al. 2007). Tang et al. (2007) re-established the phylogenetic relationships of *Nemania* with related genera, based on the combined dataset of ITS and rpb2 which supported the separation of *Nemania* from *Hypoxylon*. However, Tang et al. (2007) stated that *Nemania* is closely related to *Xylaria* and phylogenetically distinct from *Annulohypoxylon* Y.M. Ju et al., *Daldinia* Ces. & De Not. and *Hypoxylon*. Ultimately, the boundaries of the genus became relatively clear and *Nemania* has been accepted as a distinct genus in *Xylariaceae* (Ju and Rogers 2002). The major morphological characteristics of *Nemania* include dark brown to black stromata, carbonaceous or at least brittle and not yielding pigments in 10% potassium hydroxide (KOH) (Ju and Rogers 2002), white soft tissue existing between or below the perithecia, ascospores usually pale brown and most of them have no obvious germ-slit and spore dehiscence in 10% KOH (Tang et al. 2007).

*Nemania* accepted 37 species by 2002, which occurs mainly distributed on the rotting wood of angiosperms (Ju and Rogers 2002; Tang et al. 2007). There are a few species introduced from China in recent years. Two new species (*N. flavitextura* Y.M. Ju, H.M. Hsieh & J.D. Rogers and *N. primolutea* Y.M. Ju, H.M. Hsieh & J.D. Rogers), collected from Taiwan, were reported by Ju et al. (2005). One new species and two new record species were discovered and described by Du et al. (2016) and Ariyawansa et al. (2015) in China. Recently, two new species (*N. yunnanensis* Tibpromma & Lu and *N. aquilariae* Tibpromma & Lu), collected from Yunnan Province, China, were discovered by Tibpromma et al. (2021). Ninety-three epithets of *Nemania* are listed on Index Fungorum (2021) (accession date: 06. 2021). Only 17 species of *Nemania* with gene sequences were retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov) and morphological methods are the main distinguishing method for *Nemania*. Morphologically, it is mainly distinguished according to the germ slit, the size of the ascospores and the characteristics of the stromata.
In this study, eight species of *Nemania*, collected from Guizhou, Hainan and Yunnan Provinces in China, are introduced. Six new species are identified, based on morpho-molecular analyses, while *N. caries* is reported as a new record for China; *N. diffusa* has been previously reported from China (Du 2015). Detailed morphological descriptions, illustrations and phylogenetic information of all species are provided in this paper.

**Materials and methods**

**Collection, isolation and morphology**

Samples of rotting wood with fungi were collected from October 2019 to December 2020 in various nature reserves of Guizhou, Hainan and Yunnan Provinces, China. These samples were placed in sealed bags and the coordinates of sampling sites (such as latitude, longitude and altitude) were recorded. Specimens were taken to the laboratory for examination. Microscopic observations were made with fungi mounted in distilled water. A drop of Melzer’s Reagent was added to determine whether or not the ascus apical ring blued (the amyloid iodine reaction) and the reaction and morphology of the ring could be observed. Fragments of stroma and perithecial wall were placed in 10% KOH on a microscope slide and the extractable pigment observed. Pure cultures were obtained with the single spore isolation method (Long et al. 2019) and the cultures were grown on oatmeal agar (OA) and potato dextrose agar (PDA).

Morphological examination of fungi on the rotting wood followed the methods of Xie et al. (2020). The characteristics of the stromata were observed with an Olympus SZ61 stereomicroscope and photographed using a fitted Canon 700D digital camera. The photomicrographs of asci and ascospores were taken with a Nikon digital camera (700D) fitted to a light microscope (Nikon Ni). Adobe Photoshop CS6 was used to arrange all the microphotographs. Measurements were performed using the Tarosoft image framework (v. 0.9.0.7). At least 30 ascospores, asci and ascus apical apparatus were measured for each specimen.

To prepare herbarium materials, the colonies grown on PDA were transferred to three 1.5 ml microcentrifuge tubes filled with sterile water and stored at 4 °C or with 10% glycerol at –20 °C. Herbarium materials were deposited in the Herbarium of Guizhou Medical University (GMB) and Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN). Living cultures were deposited at Guizhou Medical University Culture Collection (GMBC).

**DNA extraction, PCR amplification and sequencing**

The BIOMIGA Fungal Genomic DNA Extraction Kit (GD2416, Biomiga, USA) was used to extract genomic DNA from fresh fungal mycelium, according to the manufacturer’s instructions. The extracted DNA was stored at –20 °C.

Target regions of internal transcribed spacers (ITS) and RNA polymerase II second largest subunit (*rpb2*) regions were amplified symmetrically using primers of ITS4/
ITS5 (White et al. 1990; Gardes and Bruns 1993) and fRPB2-5F/fRPB2-7cR (Liu et al. 1999), respectively. ACT512F and ACT783R (Hsieh et al. 2005) and T11 and T22 (Tanaka et al. 2009; Hsieh et al. 2010) primers were used for the amplification of the α-actin gene (ACT) and β-tubulin (TUB2), respectively. The components of the polymerase chain reaction (PCR) mixture and thermal cycling programme were performed as described by Pi et al. (2020). The amplified PCR fragments were sent to Sangon Biotech (Shanghai) Co., China, for sequencing. All newly-generated sequences of ITS, α-actin, rpb2 and β-tubulin regions were uploaded to the GenBank database and the accession numbers are shown in Table 1.

Sequence alignment and phylogenetic analyses

Except for newly-generated sequences, all sequences used for phylogenetic analysis were downloaded from GenBank, based on published literature and the highest hit rate of ITS in the GenBank database. Sequence data for the construction of the phylogenetic tree are listed in Table 1. Sequence alignments were generated using the MAFFT v.7.110 online programme (http://mafft.cbrc.jp/alignment/server/, Katoh and Standley 2013) under default settings. Multiple sequence alignments of ITS, α-actin, rpb2 and β-tubulin were analysed individually and in combination, manually adjusted to achieve the maximum alignment and to minimise gaps using the BioEdit v.5 (Hall 1999). The file formats were converted in ALTER (Alignment Transformation Environment) (http://www.sing-group.org/ALTER/). The Maximum Likelihood analysis was carried out with GTR+G+I model of site substitution by using RAxML 7.4.2 black box (https://www.phylo.org/, Stamatakis et al. 2008) and Bayesian Inference

Table 1. Taxa of Nemania and related genera used for phylogenetic analyses and their GenBank accession numbers.

| Species                        | Strain number | GenBank Accession number | GenBank Accession number | GenBank Accession number | GenBank Accession number | References                      |
|--------------------------------|---------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------------|
| Amphirhizina fushanensis       | HAST 91111209 (HT) | GU339496 | GQ848339 | GQ495950 | GQ452360 | Hsieh et al. (2010) |
| Am. nigrospora                 | HAST 91092308 (HT) | GU322457 | GQ848340 | GQ495951 | GQ452361 | Hsieh et al. (2010) |
| Astrocystis bambusae           | HAST 89021904 | GU322449 | GQ848386 | GQ495942 | GQ449239 | Hsieh et al. (2010) |
| As. bambusicola                | MFLUCC 17-0127 (HT) | MF67942 | MF67946 | N/A | N/A | Hyde et al. (2017) |
| As. macrospora                 | MFLUCC 14-0174 | KP297404 | KP340532 | KP406615 | N/A | Daranagama et al. (2015) |
| As. mirabilis                  | HAST 94070803 | GU322448 | GQ848385 | GQ495941 | GQ449238 | Hsieh et al. (2010) |
| Brauneperidium gracileceum     | MFLUCC 14-0011 (HT) | KP297400 | KP340528 | KP406611 | N/A | Daranagama et al. (2015) |
| B. involucratum                | MFLUCC 14-0009 | KP297399 | KP340527 | KP406610 | N/A | Daranagama et al. (2015) |
| Collodiscula bambusae          | GZUH0102 | KP054279 | KP276675 | KP276674 | N/A | Li et al. (2015b) |
| C. fangjingshanensis           | GZUH0109 (HT) | KR002590 | KR002592 | KR002589 | N/A | Li et al. (2015a) |
| C. leigongshanensis            | GZUH0107 (HT) | KP054281 | KR002588 | KR002587 | N/A | Li et al. (2015a) |
| C. tubulosa                    | GACP QR0111 (HT) | MN017302 | MN018403 | MN018405 | MN018402 | Xie et al. (2020) |
| Daldinia bambusicola           | CBS 122872 (HT) | KY610385 | KY624241 | KY615988 | KU684037 | Hsieh et al. (2005), Wendt et al. (2018) |
| Dematophora buxi               | JDR 99 | GU300070 | GQ844780 | GQ470228 | GQ398228 | Hsieh et al. (2010) |
| De. necatrix                   | CBS 349,36 | AY909001 | KY624275 | KY624310 | N/A | Pelaez et al. (2008), Wendt et al. (2018) |
| Species                  | Strain number | GenBank Accession number | References                        |
|-------------------------|---------------|--------------------------|-----------------------------------|
| DISCOYLLARIA MYRMOXOPHila | JDR 169       | GU322433 GQ844819 GQ847710 GQ438747 | Hsieh et al. (2010)               |
| ENTOLUECA MAMMAMatura  | JDR 100       | GU300007 GU844782 GQ470230 GQ398230 | Hsieh et al. (2010)               |
| Hypholoma plicatum       | CBS 122262 (HT) | JX183075 KY624280 JX183072 JX183071 | Bills et al. (2012), Wendt et al. (2018) |
| Kretzschmariella culinarum | JDR 88       | KX430043 KX430045 KX430046 KX430044 | Johnston et al. (2016)            |
| Nemania abortiva         | BSH 467 (HT)  | GU292816 GQ844768 GQ470219 GQ357423 | Hsieh et al. (2010)               |
| N. aenea                 | CBS 680.86    | AJ390427 N/A N/A N/A    | Tang et al. (2007)                |
| N. aenea var. aurouleta  | ATCC 60819    | AJ390428 N/A N/A N/A    | Tang et al. (2007)                |
| N. aquariae              | KUMCC 20-0268 (HT) | MW729422 MW717891 MW818142 MW717889 | Tilbromma et al. (2021)           |
| N. beaumontii            | HAST 405      | GU292819 GQ844772 GQ470222 GQ358994 | Wendt et al. (2018)               |
| N. bipapillata           | HAST 90086010 | GU292818 GQ844771 GQ470221 GQ358993 | Hsieh et al. (2010)               |
| N. camelliae             | GMB0067       | MW851888 MW836056 MW836030 MW836047 | This study                        |
| N. cortius               | GMB0068 (HT)  | MW851889 MW836055 MW836029 MW836046 | This study                        |
| N. chestersii            | JF 04024      | AJ390430 DQ631949 DQ480089 N/A | Tang et al. (2007, 2009)          |
| N. cellulolamponitina    | GMB0061       | MW836182 MW836058 MW836026 MW836039 | This study                        |
| N. diffusa               | HAST 91020401 | GU292817 GQ844769 GQ470220 GQ358992 | Hsieh et al. (2010)               |
| N. ficiicrius             | GMB0062 (HT)  | MW836183 MW836051 MW836025 MW836038 | This study                        |
| N. fusidisona            | GMB0063       | MW851884 MW836060 MW836022 MW836041 | This study                        |
| N. fusidisona            | GMB0066 (HT)  | MW836185 MW836059 MW836021 MW836040 | This study                        |
| N. fusidisona            | GMB0065 (HT)  | MW836186 MW836065 MW836033 MW836048 | This study                        |
| N. fusidisona            | GMB0060       | MW836187 MW836066 MW836034 MW836049 | This study                        |
| N. maritima              | HAST 89120401 (ET) | GU292822 GQ844775 GQ470225 GQ358997 | Hsieh et al. (2010), Li et al. (2015a, b) |
| N. plumbea               | JF TH-04-01   | DQ641634 DQ631952 DQ840084 N/A | Tang et al. (2007, 2009)          |
| N. primulatae            | YMJ 19102001 (ET) | EF026121 GQ844767 EF025608 EF025592 | Hsieh et al. (2010), Li et al. (2015a, b) |
| N. serpens               | HAST 255      | GU292820 GQ844773 GQ470223 GQ358995 | Hsieh et al. (2010), Li et al. (2015a, b) |
| N. sphaerostoma          | JDR 261       | GU292821 GQ844774 GQ470224 GQ35896 | Hsieh et al. (2010)               |
| N. sphaerostoma          | KUMCC 20-0267 (HT) | MW729423 MW717892 MW818141 MW717890 | Tilbromma et al. (2021)           |
| Podocoryninus mexicana   | WSP 176       | GU324762 GQ853039 GQ848480 GQ455451 | Hsieh et al. (2010)               |
| Pud. multi               | WSP 167 (HT)  | GU324761 GQ853038 GQ848489 GQ455450 | Hsieh et al. (2010)               |
| Poronia pileiformis      | WSP 8813001 (ET) | GU324760 GQ853035 GQ502720 GQ455449 | Hsieh et al. (2010)               |
| Pleurotus punctatus      | CBS 566.78 (HT) | KT281904 KY624287 KX271281 N/A | Senayake et al. (2015)            |
| Rolfellia aquila          | MCU1 51703    | KY610392 KY624285 KX271253 N/A | Wendt et al. (2018)               |
| R. merrillii             | HAST 89112601 | GU300007 GQ844781 GQ470229 GQ3589229 | Hsieh et al. (2010)               |
| R. sancta-cruciana       | HAST 90079203 | GU292824 GQ844777 GQ470227 GQ358999 | Hsieh et al. (2010)               |
| S. hirsutipes            | HAST 90482615 | GU324440 GQ848427 GQ459593 GQ438754 | Hsieh et al. (2010)               |
| S. quinquiarum           | HAST 89091608 | EF026120 GQ853021 EF025606 EF025591 | Ju et al. (2007), Hsieh et al. (2010) |
| Xylaria allantoida       | HAST 90429203 | GU324743 GQ848356 GQ502692 GQ452577 | Hsieh et al. (2010)               |
| X. apoda                 | HAST 90080084 | GU322437 GQ844825 GQ459593 GQ438751 | Hsieh et al. (2010)               |
| X. compunctum            | CBS 359.61    | KT281903 KY624230 KX271255 N/A | Senayake et al. (2015)            |
| X. cubensis              | JDR 860       | GU391523 GQ848365 GQ502700 GQ455444 | Hsieh et al. (2010)               |
| X. digitata              | HAST 919      | GU324556 GQ848338 GQ459594 GQ449245 | Hsieh et al. (2010)               |
| X. junius               | HAST 92042501 | GU322439 GQ844825 GQ459592 GQ438753 | Hsieh et al. (2010)               |

Notes: Type specimens are marked with HT (holotype), ET (epitype). N/A: sequences not available.
(BI) analysis was performed with MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001). The branch support was evaluated with a bootstrapping method of 1000 replicates (Hillis and Bull 1993). Posterior probabilities (PP) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.2 (Ronquist et al. 2012). The nucleotide substitution model was estimated by MrModeltest v.2.3 (Posada and Crandall 1998). Six simultaneous Markov chains were run for 2000000 generations and the trees were sampled each 100th generation. The first 25% of trees were discarded during the burn-in phase of each analysis. The phylogenetic trees were viewed in Figtree v.1.4.0 and arranged by Photoshop CS6. The alignments and respective phylogenetic trees were uploaded in TreeBASE (submission number: 28371).

**Results**

**Phylogenetic analyses**

The multiple-genes sequence alignments of ITS, α-actin, rpb2 and β-tubulin included 67 taxa, 2,041 positions including gaps (ITS: 1–486, α-actin: 487–677, rpb2: 678–1,715, β-tubulin: 1,716–2,041). *Daldinia bambusicola* Y.M. Ju et al. (CBS 122872) and *Hypoxylon pulicicidum* J. Fourn. et al. (CBS 122622) were selected as the outgroup taxa. A best-scoring ML tree is represented in Fig. 1. RAxML bootstrap support value ≥ 75% and Bayesian posterior probabilities (BYPP) value ≥ 0.90 are shown above the branches and indicated as thickened lines.

In the phylogenetic tree (Fig. 1), *Nemania* Gray is a sister taxon to the genera *Rosellinia* De Not., *Dematophora* R. Hartig and *Entoleuca* Syd. *Nemania* was divided into six sub-clades. In clade N1, *N. bipapillata* (Berk. & M.A. Curtis) Pouzar, *N. camelliae* sp. nov. and *N. lishuicola* sp. nov. grouped with high statistical values (100/1). In clade N2, *N. fusoidispora* Q.R. Li et al. and *N. illita* (Schwein.) Pouzar. grouped with high statistical values (100/1). Clade N3 contained the frequent species *N. diffusa* (Sowerby) S.F. Gray along with *N. cyclobalanopsina* sp. nov. grouping with high statistical values (100/1). In clade N4, *N. feicuiensis* sp. nov. with *N. abortiva* J.D. Rogers et al., *N. aquilariae* Tibpromma & Lu and *N. primolutea* Y.M. Ju et al. grouped with high statistical values (100/1). Within clade N5, *N. macrocarpa* Y.M. Ju & J.D. Rogers clustered in a well-supported sub-clade with *N. maritima* Y.M. Ju & J.D. Rogers with high statistical values (100/1). Clade N6 comprised *N. changningensis* sp. nov., *N. yunnanensis* Tibpromma & Lu, *N. caries* (Schwein.) Y.M. Ju & J.D. Rogers, *N. rubi* sp. nov., *N. plumbea* A.M.C. Tang et al., *N. chestersii* (J.D. Rogers & Whalley) Pouzar, *N. serpens* (Pers.) Gray with *N. aenea* (Nitschke) Pouzar, *N. aenea* var. *aureolutea* (L.E. Petrini & J.D. Rogers) Y.M. Ju & J.D. Rogers, *N. sphaeriostomum* (Schwein.) Lar.N. Vassiljeva & S.L. Stephenson and *N. beaumontii* (Berk. & M.A. Curtis) Y.M. Ju & J.D. Rogers grouping with high support values (100% ML, 1 BYPP).
A taxonomic study of *Nemania* from China

Figure 1. RAxML tree based on analysis of a combined dataset of ITS, α-actin, *rpb2* and β-tubulin sequences from taxa of *Nemania* and related genera. Bayesian posterior probability (PP) ≥ 0.90 is marked at the node and the maximum likelihood bootstrap support (BS) values greater than ≥ 75%; a dash (“-”) indicates a value < 0.90 (PP) or < 75% (BS). The strain number is indicated after the species name. The here-studied strains are in **bold** and new species are indicated in red.
Taxonomy

*Nemania camelliae* Y.H. Pi & Q.R. Li, sp. nov.
MycoBank No: 840086

Fig. 2

**Etymology.** Refers to the host genus name, *camellia*.

**Material examined.** CHINA, Guizhou Province, Tongren City, Fanjingshan Nature Reserve (27°47'11.41"N, 108°43'43.90"E, altitude: 515 m), on dead wood of *Camellia* sp., 15 October 2020, Y.H. Pi, 2020FJS26 (GMB0068, holotype; GMBC0068, ex-type living culture; KUN-HKAS 112689, isotype).

**Description.** Saprobic on the surface of decaying wood of *Camellia* sp. **Sexual morph:** Stromata pulvinate to effused-pulvinate, rarely perithecioid, orbicular to irregularly elongated, often coalescent; single distribution or confluent into irregularly elongated compound stromata, 1.5–4 mm long x 1–2 mm wide x 0.5–1 mm high, surface dull black, hard-textured, with inconspicuous to moderately exposed perithecial contours and usually sloping margins, internally black between ascomata, carbonaceous; subperithecial tissue black, conspicuous; does not release a coloured pigment in 10% KOH. Perithecia 0.65–0.95 mm diam. x 0.65–0.7 mm high, subglobose to depressed-spherical. Ostioles finely papillate, black, conspicuously sunken in a shallow discoid depression; ostiolar area blackish, shiny, frequently flattened. Asci 180–290 x 6–11 μm (av. = 230 x 7.5 μm, n = 30), 8-spored, unitunicate, long-cylindrical, long-stipitate, the spore-bearing parts 80–95 μm long, apically rounded with a J+, apical apparatus, 2–3 x 2.5–4 μm (av. = 2.5 x 3 μm, n = 30), jar shape. Ascospores 10–14 x 4.5–7 μm (av. = 12 x 5.5 μm, n = 30), uniseriate, unicellular, ellipsoid to slightly fusoid, inequilateral, with slightly narrow rounded ends, smooth, brown to dark brown, with a fairly conspicuous, straight, almost spore-length germ slit on the least convex side; lacking a sheath and appendage; perispore indehiscent in 10% KOH. **Asexual morph:** Undetermined.

**Culture characteristics.** The colony grows on PDA medium with a diameter of 6 cm after one week at 25 °C; white, cottony, circular, flocculent or velvety, with light yellow to slightly yellow at the centre. Not sporulating on OA nor on PDA.

**Other examined material.** CHINA, Guizhou Province, Tongren City, Fanjingshan Nature Reserve (27°42'10.26"N, 108°31'35.34"E, altitude: 426 m), on dead wood of *Camellia* sp., 16 October 2020, Y.H. Pi, 2020FJS54-1 (GMB0067), living culture, GMBC0067.

**Notes.** Phylogenetic analyses showed that *Nemania camelliae* form a distinct clade with *N. bipapillata* (82% ML, 0.97 BYPP, Fig. 1). Morphologically, *N. camelliae* is similar to *N. immersidiscus* Van der Gucht et al. in having a small discoid depression around the ostiolar papilla. However, the stromata of *N. camelliae* are entirely carbonaceous, whereas those of *N. immersidiscus* contain white soft tissue between and beneath the perithecia (Ju and Rogers 2002). Moreover, *N. immersidiscus* has slightly thinner ascospores [(10–)11–14(–16) x (4–)4.5–5.5 μm)].
A taxonomic study of *Nemania* from China

**Figure 2. *Nemania camelliae* (GMB0068, holotype) A type material B, C stromata on the surface of host D transverse section of stroma E longitudinal section of stroma F–H asci with ascospores I pigments in 10% KOH J ascospore with indehiscent perispore in 10% KOH K ascus apical apparatus (stained in Melzer’s Reagent) L, M ascospores N, O colonies on PDA (N-upper, O-lower). Scale bars: 0.5 mm (C–E); 10 μm (F–H, J–M).
**Nemania caries** (Schwein.) Y.M. Ju & J.D. Rogers, Nova Hedwigia 74(1–2): 90 (2002)

Mycobank No: 477305

Fig. 3

**Synonyms.** *Sphaeria caries* Schwein., Trans. Am. phil. Soc., New Series 4(2): 194 (1832).

*Hypoxylon caries* (Schwein.) Sacc., Syll. fung. (Abellini) 1: 393 (1882).  
*Hypoxylon balansae* Speg., Anal. Soc. cient. argent. 26(1): 30 (1888).

**Description.** Saprobic on the surface of decaying wood. **Sexual morph:** Stromata irregularly effused-pulvinate, 5.5–18 mm long × 3–9 mm wide × 0.4–0.6 mm thick, with conspicuous perithecial mounds, surface blackish-grey, carbonaceous, interior white, loosely fibrous to cottony; mature stromata lacking KOH extractable pigments. Perithecia 0.25–0.5 mm wide × 0.4–0.6 mm high, obovoid. Ostioles slightly higher than stromatal surface and with openings conic-papillate, black, inconspicuous, without encircling disc. ASCI 130–200 × 7–13 μm (av. = 150 × 9.5 μm, n = 30), 8-spored, cylindrical, unitunicate, long-stipitate, the spore-bearing parts 65–95 μm long, apically rounded with a J+, short-cylindrical apical apparatus, 1.5–2.5 × 1–2.5 μm (av. = 2 × 1.5 μm, n = 30). Ascospores 9–13.5 × 3–7 μm (av. = 11.5 × 5 μm, n = 30), brown to light brown, smooth, with an inconspicuous, straight, germ slit 1/3 spore-length, nearly equilateral, with broadly rounded ends; perispore indehiscent in 10% KOH.  

**Asexual morph:** Undetermined.

**Culture characteristics.** Colonies grow on PDA at 25 °C for two weeks, with a diameter of 4 cm. Colony on the surface is white or light orange, shallow, flat, zonnate, with irregular edges and orange on the reverse side. The colony reverse is orange. Not sporulating on OA nor on PDA.

**Material examined.** CHINA, Yunnan Province, Changning County, Lancang River Nature Reserve (25°01’13.56”N, 99°35’25.12”E, altitude: 2626 m), on dead wood, 6 October 2019, Y.H. Pi, 2019LC369 (GMB0070, KUN-HKAS 112680), living culture, GMBC0070; CHINA, Yunnan Province, Changning County, Lancang River Nature Reserve (25°01’13.33”N, 99°35’26.55”E, altitude: 2641 m), on dead wood, 6 October 2019, Y.H. Pi, 2019LC401 (GMB0069, KUN-HKAS 112682), living culture, GMBC0069.

**Known distribution.** Hawaii (Rogers and Ju 2012), Martinique (Fournier et al. 2018), Paraguay, USA (Ju and Rogers 2002), Yunnan Province, China (this paper).

**Notes.** The phylogenetic analyses show *Nemania caries* groups with *N. changningensis* with high statistical support (100% ML, 1 BYPP, Fig. 1) and the comparison calculation within the alignment found that there is a 4% difference in ITS sequences between *N. changningensis* and *N. caries*. Morphologically, *N. caries* resembles *N. colubrina* J. Fourn. & Lechat which has medium brown ascospores and a similar size of ascospores. However, *N. colubrina* differs from *N. caries* by ellipsoid-inequilateral ascospores with narrowly-rounded ends (Ju and Rogers 2002; Fournier et al. 2018). *Nemania caries* is distinguished from *N. plumbea* by its dimension of ascospores, the
Figure 3. *Nemania caries* (GMB0070) **A** type material, **B, C** stromata on the surface of host **D** transverse sections of stromata **E** longitudinal section of stroma **F–H** asci with ascospores **I** pigments in 10% KOH **J** ascospore with indehiscent perispore in 10% KOH **K** ascus apical apparatus (stained in Melzer’s Reagent) **L, M** ascospores **N, O** Colonies on PDA (**N**-upper, **O**-lower). Scale bars: 0.5 mm (**C–E**); 10 μm (**F–H, J–M**).
latter has larger ascospores (13–16 × 5.4–6.6 μm) with narrowly-rounded ends (Tang et al. 2007). The specimens we collected from the Lancang River Nature Reserve in Yunnan fit the definition of *N. caries* well and represent the first record from China.

*Nemania changningensis* Y.H. Pi & Q.R. Li, sp. nov.
MycoBank No: 840087
Fig. 4

**Etymology.** Refers to the collection location, Changning County.

**Material examined.** CHINA, Yunnan Province, Changning County, Lancang River Nature Reserve (25°01′35.02″N, 99°33′15.42″E, altitude: 2670 m), on dead wood, 3 October 2019, Y.H. Pi, 2019LC203 (GMB0056, *holotype*; GMBC0056, *ex-type living culture*; KUN-HKAS 112668, *isotype*).

**Description.** Saprobic on the surface of decaying wood. **Sexual morph:** Stromata effused-pulvinate, confluent into irregularly elongated compound stromata, up to 18–35 mm long × 2–4 mm wide × 0.3–0.5 mm high, irregularly lobed, plane or with inconspicuous perithecial mounds and sloping margins; surface covered with white tissue, persistent layer, with blackish-grey carbonaceous sub-surface showing through in places; the tissue beneath the perithecial layer inconspicuous, greyish-white in places, the underlying wood blackened; mature stromata lacking KOH extractable pigments. Perithecia 0.45–0.6 mm diam. × 0.4–0.55 mm high, subglobose to depressed-spherical. Ostioles slightly higher than stromatal surface and with openings papillate, often surrounded by white tissue, inconspicuous, black, without encircling disc. Asci 100–140 × 7–10 μm (av. = 111 × 8.5 μm, n = 30), 8-spored, unitunicate, cylindrical, short-stipitate, the spore-bearing parts 70–90 μm long, the apical apparatus of immature ascus blue in Melzer’s Reagent, but not blue in mature asci. Ascospores 10–13 × 4–6.5 μm (av. = 11.5 × 5.5 μm, n = 30), uniseriate unicellular, smooth, light brown, slightly inequilateral, with broadly rounded ends, inconspicuous or lack a germ slit; perispore indehiscent in 10% KOH. **Asexual morph:** Undetermined.

**Culture characteristics.** The colony grows slowly on the PDA with a diameter of 4.5 cm after 2 weeks at 25 °C. The colony on the surface is white, thick and flat in the middle, edges are shallow, irregular bands and rosettes. Colony reverse is orange and intermediate colour darker. Not sporulating on OA nor on PDA.

**Other examined material.** CHINA, Yunnan Province, Changning County, Lancang River Nature Reserve (25°01′30.36″N, 99°35′30.53″E, altitude: 2586 m), on dead wood, 4 October 2019, Y.H. Pi, 2019LC342 (GMB0057), living culture, GMBC0057.

**Notes.** In the phylogenetic analyses, *N. changningensis* is on a separate branch and grouped with *N. caries* with high support values (100% ML, 1 BYPP, Fig. 1). In term of ascospores dimension, *N. changningensis* resembles *N. caries*, but differs in the perithecia of *N. caries* (ovoid, 0.3–0.6 mm diam. × 0.5–0.7 mm high), in the surface not covered with white tissue and in its apical apparatus of mature asci bluing in Melzer’s Reagent (Miller 1961; Ju and Rogers 2002).
Figure 4. *Nemania changningensis* (GMB0056, **holotype**) **A** type material **B, C** stromata on the surface of host **D** transverse sections of stromata **E** longitudinal section of stroma **F–H** asci with ascospores **I** pigments in 10% KOH **J, K** asci apical apparatus (stained in Melzer’s Reagent) **L, M** ascospores **N, O** colonies on PDA (**N**-upper, **O**-lower). Scale bars: 0.5 mm (**C–E**); 10 μm (**F–H, J–M**).
Nemania cyclobalanopsina Y.H. Pi & Q.R. Li, sp. nov.
MycoBank No: 840088
Fig. 5

**Etymology.** Refers to its host, *Cyclobalanopsis glauca.*

**Material examined.** China, Yunnan Province, Changning County, Lancang River Nature Reserve (25°01’9.46”N, 99°35’29.47”E, altitude: 2623 m), on dead wood of *C. glauca*, 6 October 2019, Y.H. Pi, 2019LC357 (GMB0062, holotype; GMBC0062, ex-type living culture; KUN-HKAS 112679, isotype).

**Description.** Saprobic on the surface of decaying branches of *C. glauca* (Thunb.) Oerst. **Sexual morph:** Stromata effused-pulvinate, orbicular to ellipsoid or irregularly lobed, 6–26 mm long × 3.5–10 mm wide × 0.5–1 mm thick, occasionally confluent into larger compound stromata, with steep to sloping margins; surface light blackish, slightly blood colour; outer crust carbonaceous; interior black, entire tissue carbonaceous around the perithecia; mature stromata lacking KOH-extractable pigments. Perithecia 0.2–0.3 mm diam. × 0.38–0.46 mm high, subglobose obovoid or tubular. Ostioles higher than stromatal surface and with coarsely rounded-papillate, black, without encircling disc. Asci 90–160 × 7–11 μm (av. = 125 × 9 μm, n = 30), 8-spored, unitunicate, cylindrical, long-stipitate, the spore-bearing parts 65–85 μm long, apically rounded with a J+, short-cylindrical to slightly tubular apical apparatus stained in Melzer’s Reagent, 1.5–2.5 × 2–3 μm (av. = 2 × 2.3 μm, n = 30). Ascospores 9–14 × 4.5–7.5 μm (av. = 11 × 6 μm, n = 30), uniseriate, unicellular, ellipsoid-inequilateral with broadly rounded ends, smooth, brown to dark brown, with a conspicuous, straight germ slit slightly less than spore-length to almost spore-length on the convex side; lacking a sheath and appendage; perispore indehiscent in 10% KOH. **Asexual morph:** Undetermined.

**Culture characteristics.** Colonies on PDA medium in size with a diameter of 6 cm after two weeks at 25 °C; the surface is white, intermediate thick, cottony, dense, with undulate or ring edge, flat, low, whitish-yellow, reverse of the colony yellow at the centre. Not sporulating on OA nor on PDA.

**Other examined material.** China, Yunnan Province, Changning County, Lancang River Nature Reserve (25°52’17.40”N, 99°35’20.53”E, altitude: 1489 m), on dead wood of *C. glauca*, 4 October 2019, Y.H. Pi, 2019LC357-1 (GMB0061), living culture, GMBC0061.

**Notes.** In our phylogenetic analyses, *N. cyclobalanopsina* grouped with *N. diffusa* (100% ML, 1 BYPP, Fig. 1). Morphologically, *N. cyclobalanopsina* differs from *N. diffusa* by its blackish stromatal surfaces and coarsely rounded-papillate ostioles. Moreover, *N. diffusa* has larger perithecia (0.3–0.6 × 0.4–0.8 mm) (Granmo et al. 1999; Ju and Rogers 2002). In the multi-gene phylogenetic analysis, *N. cyclobalanopsina* appeared in a separate branch which is distinct from *N. diffusa* (Fig. 1). Moreover, there is a 3% difference in ITS sequences between *N. diffusa* and *N. cyclobalanopsina*. (Vu et al. 2019; Jeewon and Hyde 2016).
Figure 5. *Nemania cyclobalanopsina* (GMB0062, holotype) A type material B, C stromata on the surface of host D transverse sections of stromata E longitudinal sections of stromata F–H asci with ascospores I pigments in 10% KOH J ascospore with indehiscent perispore in 10% KOH K ascus apical apparatus (stained in Melzer’s Reagent) L, M ascospores N, O colonies on PDA (N-upper, O-lower). Scale bars: 0.5 mm (C–E); 10 μm (F–H, J–M).
**Nemania diffusa** (Sowerby) S.F. Gray, Nat. Arr. Brit. Pl.: 517 (1821)
MycoBank No: 477312
Fig. 6

**Synonyms.** *Sphaeria diffusa* Sowerby, Col. fig. Engl. Fung. Mushr. (London) 3(no. 25): tab. 373, fig. 10 (1802)
*Sphaeria unita* Fr., Elench. fung. (Greifswald) 2: 67 (1828)
*Sphaeria exarata* Schwein., Trans. Am. phil. Soc., New Series 4(2): 192 (1832)
*Hypoxylon exaratum* (Schwein.) Sacc., Syll. fung. (Abellini) 1: 392 (1882)
*Ustulina linearis* Rehm, Hedwigia 31(6): 310 (1892)
*Hypoxylon lilacinofuscum* Bres., Fl. Trident. Nov. 2: 43 (1892)
*Hypoxylon cohaerens* var. *brasiliense* Starbäck, Bih. K. svenska VetenskAkad. Handl., Afd. 3 27(no. 9): 8 (1901)
*Hypoxylon vestitum* Petch, Ann. R. bot. Gdns Peradeniya 8: 156 (1924)
*Nemania unita* (Fr.) Krieglst. & Enderle, Mitteilungsblatt der Arbeitsgemeinschaft Pilzkunde Niederrhein 1: 64 (1989)

**Description.** Saprobic on the surface of rotten wood. **Sexual morph:** Stromata effused-pulvinate, clear outline, ellipsoid or irregularly lobed, occasionally confluent into a larger compound stromata, 2–20 mm long × 2–9 mm wide × 0.5–1 mm thick, with conspicuous perithecial mounds, carbonaceous between the perithecia, surface dark brown or brown; the inter-perithecial tissue blackish, carbonaceous; does not release a coloured pigment in 10% KOH. Perithecia 0.3–0.55 diam. × 0.4–0.7 mm high, subglobose to obovoid. Ostioles finely conic-papillate, black, shiny. Asci 130–250 × 6–10 μm (av. = 170 × 8 μm, n = 30), 8-spored, unitunicate, cylindrical, long-stipitate, the spore-bearing parts 70–90 μm, apically rounded with a J+ apical apparatus, 1.5–2.5 × 2–3.5 μm (av. = 2 × 2.6 μm, n = 30), tubular with a faint upper rim, bluing in Melzer’s Reagent. Ascospores 9.5–13 × 4.5–7 μm (av. = 11 × 5.5 μm, n = 30), unicellular, ellipsoid-inequilateral, with narrowly-rounded ends, smooth, brown to dark brown, with a conspicuous, straight germ slit spore-length to slightly less than spore-length on the ventral side; lacking a sheath and appendage; perispore indehiscent in 10% KOH. **Asexual morph:** Undetermined.

**Culture characteristics.** Colonies grow on PDA at 25 °C for a week reaching a diameter of 5 cm. Colonies are cotton white in colour, flocculent or velvety, dense, circular, radial. On the reverse, white edge, light yellow in the middle. Not sporulating on OA nor on PDA.

**Material examined.** **CHINA,** Guizhou Province, Tongren City, Fanjingshan Nature Reserve (27°53′46.59″N, 108°431′16.29″E, altitude: 1058 m), on dead wood, 14 October 2020, Y.H. Pi, 2020FJS1 (GMB0072, KUN-HKAS 112686), living culture, GMBC0072; **CHINA,** Yunnan Province, Changning County: Lancang River Nature Reserve (21°54′17.44″N, 107°54′10.05″E, altitude: 1382 m), on dead wood, 1 October 2019, Y.H. Pi, 2019LC008 (GMB0071, KUN-HKAS 112658), living culture, GMBC0071.
Figure 6. *Nemania diffusa* (GMB0072) A specimen B, C stromata on the surface of host D transverse sections of stromata E longitudinal sections of stromata F–H asci with ascospores I pigments in 10% KOH J ascospore with indehiscent perispore in 10% KOH K ascus apical apparatus (stained in Melzer's Reagent) L, M ascospores N, O colonies on PDA (N-upper, O-lower). Scale bars: 0.5 mm (C–E); 10 μm (F–H, J–M).
Notes. The new collection morphologically resembles *N. diffusa* (Gray 1821), having effused-pulvinate carbonaceous stromata with inconspicuous perithecial mounds, brown to dark brown ellipsoid-inequilateral ascospores (9.5–13.5 × 5–6 μm), with narrowly-rounded ends and a long germ slit on the ventral side (Granmo et al. 1999; Ju and Rogers 2002). Fournier et al. (2018) predicted that *N. diffusa* might be a species complex as it is difficult to identify, based solely on morphology, thus, it should be evaluated after extensive sampling and using DNA-based taxonomy. In phylogenetic analyses of combined ITS, *rpb2*, β-tubulin and α-actin genes (Fig. 1), new collections clearly showed its close kinship with *N. diffusa*. Only a 2% difference of ITS sequences existed between our strains and *N. diffusa* (HAST 91020401, authoritative strain). Therefore, we regard the new collection as *N. diffusa*. *Nemania carbonacea* Pouzar. can be confused with *N. diffusa* by having the same dark ascospores and nearly spore-length germ slits. However, *N. carbonacea* has white, soft stromatal tissue between the perithecia (Ju and Rogers 2002).

*Nemania feicuiensis* Y.H. Pi & Q.R. Li, sp. nov.  
MycoBank No: 840089  
Fig. 7

Etymology. Refers to the collection location, Emerald Park, Chinese name of jade, feicui.

Material examined. CHINA, Hainan Province, Wuzhishan City, Emerald Park (18°48’9.64”N, 109°31’6.59”E, altitude: 352 m), on dead wood, 14 November 2020, Y.H. Pi, 2020FCGY12-2 (GMB0059, holotype; GMBC0059, ex-type living culture; KUN-HKAS 112698, isotype).

Description. Saprobic on the surface of decaying wood. Sexual morph: Stromata effused-pulvinate, superficial, orbicular to ellipsoidal or irregularly lobed, 5–27 mm long × 2.5–10 mm wide × 0.3–0.5 mm thick, surface blackish-grey, with inconspicuous perithecial outer mounds, crust weakly carbonaceous; interior black, stromatal tissue between the perithecia carbonaceous; mature stromata lacking KOH extractable pigments. Perithecia 0.3–0.55 mm diam. × 0.25–0.37 mm high, subglobose to depressed-spherical. Ostioles higher than stromatal surface and with openings slightly papillate, black, conspicuous, without encircling disc. Asci 130–180 × 7–11.5 μm (av. = 145 × 9 μm, n = 30), 8-spored, unitunicate, cylindrical, long-stipitate, the spore-bearing parts 65–85 μm long, apically rounded with a J+ apical apparatus, 1–2.5 × 2–3 μm (av. = 1.8 × 2.4 μm, n = 30), long-cylindrical. Ascospores 9.5–13 × 4–7.5 μm (av. = 11 × 6 μm, n = 30), uniseriate, unicellular, ellipsoid or slightly inequilateral, with broadly rounded ends, smooth, brown to dark brown, with a conspicuous, straight, almost spore-length germ slit on the flattened side; lacking a sheath and appendage; perispore indehiscent in 10% KOH. Asexual morph: Undetermined.

Culture characteristics. Colonies grow slowly on PDA at 25 °C for 2 weeks, with a diameter of 5 cm. Colonies are cotton white in colour, flocculent or velvety, slightly convex, circular, shallow edges, radial, white to light yellow on the reverse, light brown in the middle. Not sporulating on OA nor on PDA.
Figure 7. *Nemania feicuiensis* (GMB0059, holotype) A type material B, C stromata on the surface of host D transverse sections of stromata E longitudinal sections of stromata F–H asci with ascospores I pigments in 10% KOH J ascospore with indehiscent perispore in 10% KOH K ascus apical apparatus (stained in Melzer’s Reagent) L, M ascospores N, O colonies on PDA (N-upper, O-lower). Scale bars: 0.5 mm (C–E); 10 μm (F–H, J–M).
Other examined material. China, Hainan Province, Wuzhishan City, Emerald Park (18°47’8.26"N, 109°31’5.34"E, altitude: 426 m), on dead wood, 16 November 2020, Y.H. Pi, 2020FCGY20 (GMB0058), living culture, GMBC0058.

Notes. The phylogenetic tree (Fig. 1) shows that *N. feicuiensis* and *N. primolutea* are closely related (100% ML, 1 BYPP). In morphology, *N. feicuiensis* differs from *N. primolutea* in that the latter has luteous stromatal surface and slightly smaller ascospores (10–13 × 4.5–5.5 μm) with narrowly-rounded ends (Ju et al. 2005). Furthermore, in the multi-gene phylogenetic analysis, *N. feicuiensis* appeared in a separate branch which is distinct from *N. primolutea* (Fig. 1). *Nemania feicuiensis* is similar to *N. diffusa* in stromatal anatomy and ascospores size, but differs by ascospores shape (broadly rounded ends vs. narrowly rounded ends) and the larger perithecia of *N. diffusa* (0.3–0.6 × 0.4–0.8 mm) (Ju and Rogers 2002).

*Nemania lishuicola* Y.H. Pi & Q.R. Li, sp. nov.
MycoBank No: 840090
Fig. 8

Etymology. Refer to the host, *quercus*.

Material examined. China, Yunnan Province, Changning County: Lancang River Nature Reserve (25°01’7.93"N, 99°35’30.74"E, altitude: 2629 m), on dead bark of *Quercus* sp., 4 October 2019, Y.H. Pi, 2019LC263 (GMB0065, holotype; GMBC0065, ex-type living culture; KUN-HKAS 112673, isotype).

Description. Saprobic on the surface of decaying wood of *Quercus* sp. Sexual morph: Stromata pulvinate, attached to substrate along entire area of the base, containing one to several perithecia, frequently confluent, 1.5–4 mm long × 1–2 mm wide × 0.5–1 mm thick, with conspicuous perithecial mounds, carbonaceous between the perithecium, surface dull black and slightly shiny at maturity, the interperithecial tissue blackish, carbonaceous; not releasing a coloured pigment in 10% KOH. Perithecia 0.7–0.95 mm diam. × 0.65–0.85 mm high, subglobose to depressed-spherical. Ostioles coarsely papillate in discoid areas, ostiolar area blackish, shiny, frequently flattened, usually around a circle of white tissue. Asci 150–300 × 7–12 μm (av. = 200 × 9 μm, n = 30), 8-spored, unitunicate, cylindrical, long-stipitate, spore-bearing parts 95–130 μm long, apically rounded with a J+ apical apparatus, 2–3 × 2–3.5 μm (av. = 2.5 × 3 μm, n = 30), tubular with a faint upper rim. Ascospores 12.5–17 × 5–8.5 μm (av. = 15 × 6.5 μm, n = 30), uniseriate, unicellular, ellipsoid-inequilateral, with broadly rounded ends, smooth, brown to dark brown, with a conspicuous, straight germ slit spore-length to slightly less than spore-length on the flattened side; lacking a sheath and appendage; perispore indehiscent in 10% KOH. Asexual morph: Undetermined.

Culture characteristics. Colonies grow on PDA, a diameter of 6 cm after one week at 25 °C, white, velvety to hairy, zonate, rosette, high convex in centre, dense, white to cream from above, white irregular edge with light yellow to slightly yellow at centre from the below. Not sporulating on OA nor on PDA.
Figure 8. *Nemania lishuicola* (GMB0065, holotype) A type material B stromata on the surface of host C pigments in 10% KOH D transverse sections of stromata E longitudinal sections of stromata F ascospore with indehiscent perispore in 10% KOH G–I asci with ascospores J ascus apical apparatus (stained in Melzer’s Reagent) K, L colonies on PDA (K-upper, L-lower) M–P ascospores. Scale bars: 0.5 mm (B, D, E); 10 μm (F–J, M–P).
Other examined material. China, Yunnan Province, Changning County: Lancang River Nature Reserve (25°01’30.75”N, 99°35’21.53”E, altitude: 2608 m), on dead bark of Quercus sp., 4 October 2019, Y.H. Pi, 2019LC253 (GMB0066), living culture, GMBC0066.

Notes. Phylogenetic analyses of combined ITS, rpb2, β-tubulin and α-actin genes (Fig. 1) show that N. lishuicola has a close relationship with N. bipapillata with high support values (100 MLBP, 1% BYPP). Morphologically, N. lishuicola differs from N. bipapillata by its larger ascospores (12.5–17 × 5–8.5 μm vs. 10.5–13.5 × 4.5–6 μm) (Miller 1961; Ju and Rogers 2002).

Nemania rubi Y.H. Pi & Q.R. Li, sp. nov.
MycoBank No: 840091
Fig. 9

Etymology. Refers to the name of host genus, rubus.

Material examined. China, Guizhou Province, Pingba County (26°25’13.38”N, 106°24’25.23”E, altitude: 1255 m), on dead branches of Rubus lambertianus Ser., 5 September 2020, Y.H. Pi, 2020PB70 (GMB0064, holotype; GMBC0064, ex-type living culture; KUN-HKAS 112695, isotype).

Description. Saprobic on dead branches of R. lambertianus. Sexual morph: Stromata effused-pulvinate, irregular shape, multi-peritheciate, scattered, separate to confluent into larger compound stromata, 2.5–15 mm long × 2–9 mm wide × 0.4–0.6 mm thick; sur-
face blackish, weakly carbonaceous, with unexposed perithecial contours, uneven and ir-
regular, internally whitish between ascomata, tissue, soft-textured; not releasing a coloured pigment in 10% KOH. Perithecia 0.25–0.35 mm diam. × 0.2–0.3 mm high, subglobose. Ostioles papillate, black, obtusely conical to hemispherical, without encircling disc. Asci 85–160 × 7–11 μm (av. = 130 × 9 μm, n = 30), 8-spored, unitunicate, cylindrical, long-stipitate, spore-bearing parts 60–85 μm long, apically rounded with a J+, long-cylindrical apical apparatus, 1.5–2.5 × 2–3 μm (av. = 1.5 × 2.5 μm, n = 30). Ascospores 9–12 × 4–6 μm (av. = 10 × 4.8 μm, n = 30), uniseriate to irregularly-biseriate unicellular, smooth, olivaceous when fresh, turning brown to medium brown after a period of time, ellipsoid-
inequilateral with often broadly-rounded ends, lacking a germ slit sheath and appendage; perispore indehiscent in 10% KOH. Asexual morph: Undetermined.

Culture characteristics. Colonies grow slowly on PDA medium with a diameter of 5 cm after 10 days at 25 °C. Colonies surface were white to pale orange, circular, cot-
tony, low, dense, cottony mycelium, reverse with light orange mycelium. Not sporulating on OA nor on PDA.

Other examined material. China, Guizhou Province, Pingba County (26°25’10.24”N, 106°24’25.21”E, altitude: 1052 m), on dead wood, 5 September 2020, Y.H. Pi, 2020PB22 (GMB0063), living culture, GMBC0063.

Notes. In our phylogenetic analysis, Nemania rubi formed a distinct branch, which is sister to N. changningensis and N. caries (Fig. 1). In morphology, N. rubi is similar to...
A taxonomic study of *Nemania* from China

**Figure 9.** *Nemania rubi* (GMB0064, holotype) **A** type material **B, C** stromata on surface of host **D** transverse sections of stromata **E** longitudinal section of stromata **F–H** asci with ascospores **I** pigments in 10% KOH **J** ascospore with indeliscent perispore in 10% KOH **K** ascus apical apparatus (stained in Melzer’s Reagent) **L, M** ascospores **N, O** colonies on PDA (N-upper, O-lower). Scale bars: 0.5 mm (C–E); 10 μm (F–H, J–M).
N. caries, but is distinct in having a long-cylindrical apical apparatus and the inequilateral ascospores lacking a germ slit (Miller 1961; Ju and Rogers 2002). In addition, the perithecia of N. caries are obovoid (0.3–0.6 × 0.5–0.7 mm) and its height is greater than the width (Tang et al. 2007). The ascomata surface of N. rubi ascomata is uneven with inconspicuous perithecial mounds, which is similar to those of N. plumbea, but the latter has larger ascospores (13–16 × 5.4–6.6 μm) with germ slits on the concave side (Tang et al. 2007).

Discussion

In this study, newly-collected Nemania species from Hainan, Yunnan and Guizhou Provinces were subjected to morpho-molecular analyses. Six new species were introduced while reporting one new record from China. Nemania showed a closer affinity to Roselinia than to Kretzschmaria Fr. and Xylaria (U’Ren et al. 2016), which is also supported in the phylogenetic analysis, based on ITS, rpb2, β-tubulin and α-actin sequences. Although no asexual morphs were observed in this study, Nemania has geniculisporium-like asexual morphs which are a common character in members of Xylariaceae (Fournier et al. 2018).

Nemania forms a single branch in the phylogenetic analysis, which supports that it is a monophyletic genus. However, Nemania genus is separated into six clades (N1–N6, Fig. 1), each of which have relatively-uniform morphological characteristics. N1 clade is represented by N. bipapillata and taxa in this clade have carbonaceous interior to the stromata, ostioles encircled with a disc and dark brown ascospores with a long germ slit. The species within clade N2 are distinguished from other Nemania species with fusoid-inequilateral and pale brown ascospores and by having white soft tissues between the perithecia. The species in clades N3, N4 and N5 have little difference in morphology and may be confused. Most taxa in clades N4 and N5 have usually brown, dark brown or blackish-brown ascospores with a germ slit longer than 2/3 spore length (Granmo et al. 1999; Ju and Rogers 2002; Fournier et al. 2018). The taxa in N6 clade have light brown or medium brown ascospores with a germ slit shorter than 2/3 spore length or seemingly lacking (Ju and Rogers 2002). Interestingly, the ascospores of most taxa in N6 clade are olivaceous brown when fresh, turning medium brown after desiccation.

Separation of members of Nemania, based on morphology, is relatively difficult and confusing (Fournier et al. 2018). In some early literature, the new species lacked the description of some key morphological characteristics (Du et al. 2016). Moreover, sequences are available for only a few species in GenBank, thus species identification, based on DNA sequences, is also problematic. Hence, it is essential to re-collect old species that lack ex-type cultures and DNA sequences and to epitypify them.

The similarity of morphological features between species is high, which makes it difficult for existing morphological taxonomic features to identify species. For example, species in clade N3, which includes N. diffusa and N. cyclobalanopsina, are difficult to identify, based solely on morphological characteristics, although their ITS sequence
differences can reach more than 3% (Jeewon and Hyde 2016; Vu et al. 2019). In this clade, we tentatively use multiple-genes sequence as the main classification basis for species. Molecular data should be the main identification basis for Nemania species, especially for clade N3. It is worth noting that we should compare sequences with that from type or authoritative strains.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (32000009 and 31960005); the Fund of the Science and Technology Foundation of Guizhou Province ([2020]1Y059); the Fund of Special Project of Academic New Seedling Cultivation and Innovation Exploration in Guizhou Medical University [2018]5779-64; Guizhou Province Ordinary Colleges and Universities Youth Science and Technology Talent Growth Project [2021]154; National Natural Science Foundation of China [No. U1812403-4-4], the Fund of High-Level Innovation Talents [No. 2015-4029], the Base of International Scientific and Technological Cooperation of Guizhou Province [No. [2017]5802]; Yingqian Kang is grateful to the 111 Project (D200009) and Talent Base Project of Guizhou Province, China (FCJD2018-22).

References

Ariyawansa HA, Hyde KD, Jayasiri SC, Buyck B, Chethana KWT, Dai DQ, Dai YC, Daranagama DA, Jayawardena RS, Lücking R, Ghobad-Nejhad M, Niskanen T, Thambugala KM, Voigt K, Zhao RL, Li GJ, Doilom M, Boonmee S, Yang ZL, Cai Q, Cui YY, Bahkali AH, Chen J, Cui BK, Chen JJ, Dayarathne MC, Disanayake AJ, Ekanayaka AH, Hashimoto A, Hongsanan S, Jones EBG, Larsson E, Li WJ, Li QR, Liu JK, Luo ZL, Maharachchikumbura SSN, Mapook A, McKenzie EHC, Norphanphoun C, Konta S, Pang KL, Perera RH, Phookamsak R, Phukhamsakda C, Pinruan U, Randrianjohany E, Singtripop C, Tanaka K, Tian CM, Tibpromma S, Abdel-Wahab MA, Wanasinghe DN, Wijayawardene NN, Zhang JF, Zhang H, Abdel-Aziz FA, Wedin M, Westberg M, Ammirati JF, Bulgakov TS, Lima DX, Callaghan TM, Callac P, Chang CH, Coca LF, Dal-Forno M, Dollohofer V, Fliegerová K, Greiner K, Griffith GW, Ho HM, Hofstetter V, Jeewon R, Kang JC, Wen TC, Kirk PM, Kytövuori I, Lawrey JD, Xing J, Li H, Liu ZY, Liu XZ, Liimatainen K, Lumbsch HT, Matsumura M, Moncada B, Nunakaew S, Parnmen S, Santiago ALCMA, Sommai S, Song Y, Souza CAF, Souza-Motta CM, Su HY, Suetrong S, Wang Y, Wei SF, Wen TC, Yuan HS, Zhou LW, Réblová M, Fournier J, Camporesi E, Luangs-aard JJ, Tasanathai K, Khonsanit A, Thanakitipattana D, Somrithipol S, Diederich P, Millanes AM, Common RS, Studler M, Yan JY, Li XH, Lee HW, Nguyen TTT, Lee HB, Battistin E, Marsico O, Vizzini A, Vila J, Ercole E, Eberhardt U, Simonini G, Wen HA, Chen XH, Miettienen O, Spirin V, Hernawati (2015) Fungal diversity notes 111–252–taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 75: 27–274. https://doi.org/10.1007/s13225-015-0346-5
Bills GF, González-Menéndez V, Martín J, Platas G, Fournier J, Perşoh D, Stadler M (2012) Hypoxylon pulicicidum sp. nov. (Ascomycota, Xylariales), a pantropical insecticide-producing endophyte. PLoS ONE 7: e46687. https://doi.org/10.1371/journal.pone.0046687

Daranagama DA, Camporesi E, Tian Q, Liu X, Liu X, Chamyuang S, Stadler M, Hyde KD (2015) Anthostomella is polyphyletic comprising several genera in Xylariaceae. Fungal Diversity 73: 203–238. https://doi.org/10.1007/s13225-015-0329-6

Du ZW, Ma HX, Li Y (2016) One new record and one new variety of Nemania from China. Journal of Fungal Research 14: 22–24. http://doi.org/10.13341/j.jfr.2014.1085

Du ZW (2015) Taxonomy and Molecular Phylogeny of Kretzschmaria and Nemania from China. Master Thesis, Jilin Agricultural University, Jilin Province, China.

Fournier J, Lechat C, Courtecuisse R (2018) The genera Kretzschmariella and Nemania (Xylariaceae) in Guadeloupe and Martinique (French West Indies). Ascomycete.org 10: 1–47. http://doi.org/10.25664/art-0226

Gardes M, Brun TS (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Granmo A, Laessøe T, Schumacher T (1999) The genus Nemania s.l. (Xylariaceae) in Norden. Sommerfelta 27: 1–96.

Gray SF (1821) A natural arrangement of British plants. Baldwin, Cradock, and Joy.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98. https://doi.org/10.1021/bk-1999-0734.ch008

Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42: 182–192. https://doi.org/10.1093/sysbio/42.2.182

Hsieh HM, Ju YM, Rogers JD (2005) Molecular phylogeny of Hypoxylon and closely related genera. Mycologia 97: 844–865. https://doi.org/10.1080/15572536.2006.11832776

Hsieh HM, Lin CR, Fang MJ, Rogers JD, Fournier J, Lechat C, Ju YM (2010) Phylogenetic status of Xylaria subgenus Pseudoxylaria among taxa of the subfamily Xylarioideae (Xylariaceae) and phylogeny of the taxa involved in the subfamily. Molecular Phylogenetics and Evolution 54: 957–969. https://doi.org/10.1016/j.ympev.2009.12.015

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Hyde KD, Norphanphoun C, Abreu VP, Bazzicalupo A, Thilini Chethana KW, Clercuzio M, Dayaratne MC, Dissanayake AJ, Ekanayaka AH, He MQ, Hongsanan S, Huang SK, Jayasiri SC, Jayawardena RS, Karunarathna A, Konta S, Kušan I, Lee H, Li JF, Lin CG, Liu NG, Lu YZ, Luo ZL, Manawasinghe IS, Mapook A, Perera RH, Phookamsak R, Phukhamsakda C, Siedlecki I, Soares AM, Tennakoon DS, Tian Q, Tibpromma S, Wanasinghe DN, Xiao YP, Yang J, Zeng XY, Abdel-Aziz FA, Li WJ, Senanayake IC, Shang QJ, Daranagama DA, de Silva NI, Thambugala KM, Abdel-Wahab MA, Bahkali AH, Berbee ML, Boonmee S, Bhat DJ, Bulgakov TS, Buyck B, Camporesi E, Castañeda-Ruiz RF, Chomnunti P, Doilom M, Dovana F, Gibertoni TB, Jadan M, Jeewon R, Jones EBG, Kang JC, Karunarathna SC, Lim YW, Liu JK, Liu ZY, Plautz Jr HL, Lumyong S, Maharachchi-
A taxonomic study of *Nemania* from China

kumbura SSN, Matočec N, McKenzie EHC, Mešić A, Miller D, Pawłowska J, Pereira OL, Promputtha I, Romero AL, Ryvarden L, Su HY, Suetrong S, Tkalčec Z, Vizzini A, Wen TC, Wisitrassameewong K, Wrzonek M, Xu JC, Zhao Q, Zhao RL, Mortimer PE (2017) Fungal diversity notes 603–708: taxonomic and phylogenetic notes on genera and species. Fungal Diversity 87: 1–235. https://doi.org/10.1007/s13225-017-0391-3

Index Fungorum (2021) Index Fungorum. http://www.indexfungorum.org/names/Names.asp [Accessed on 6 June 2021]

Jeewon R, Hyde KD (2016) Establishing species boundaries and new taxa among fungi: recommendations to resolve taxonomic ambiguities. Mycosphere 7: 1669–1677. https://doi.org/10.5943/mycosphere/7/11/4

Johnston PR, Rogers JD, Park D, Martin NA (2016) *Entalbostroma erumpens* gen. et sp. nov. (Xylariaceae) from *Phormium* in New Zealand. Mycotaxon 131: 765–771. https://doi.org/10.5248/131.765

Ju YM, Rogers JD (2002) The genus *Nemania* (Xylariaceae). Nova Hedwigia 74: 75–120. https://doi.org/10.1127/0029-5035/2002/0074-0075

Ju YM, Rogers JD, Hsieh HM (2005) New *Hypoxylon* and *Nemania* species from Costa Rica and Taiwan. Mycologia 97: 562–567. https://doi.org/10.1080/15572536.2006.11832831

Ju YM, Hsieh HM, Ho MC, Szu DH, Fang MJ (2007) *Theissenia rogersii* sp. nov. and phylogenetic position of *Theissenia*. Mycologia 99: 612–621. https://doi.org/10.1080/15572536.2007.11832555

Katoh K, Standley DM (2013) MAFFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. https://doi.org/10.1093/molbev/mst010

Li QR, Kang JC, Hyde KD (2015a) Two new species of the genus *Collodiscula* (Xylariaceae) from China. Mycological Progress 14: e52. https://doi.org/10.1007/s11557-015-1075-6

Li QR, Wen TC, Kang JC, Hyde KD (2015b) A new species of *Collodiscula* (Xylariaceae) from China. Phytotaxa 205: 187–196. https://doi.org/10.11646/phytotaxa.205.3.6

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Molecular Biology and Evolution 16: 1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

Long QD, Liu LL, Zhang X, Wen TC, Kang JC, Hyde KD, Shen XC, Li QR (2019) Contributions to species of *Xylariales* in China–1. *Durotheca* species. Mycological Progress 18: e495. https://doi.org/10.1007/s11557-018-1458-6

Miller JH (1961) A Monograph of the World Species of *Hypoxylon*. University of Georgia Press, Athens, 158 pp.

Petrini LE, Rogers JD (1986) A summary of the *Hypoxylon serpens* complex. Mycotaxon 26: 401–436.

Pelaez F, González V, Platas G, Sánchez-Ballesteros J, Rubio V (2008) Molecular phylogenetic studies within the Xylariaceae based on ribosomal DNA sequences. Fungal Diversity 31: 111–134.

Pi YH, Zhang X, Liu LL, Long QD, Shen XC, Kang YQ, Hyde KD, Boonmee S, Kang JC, Li QR (2020) Contributions to species of *Xylariales* in China–4. *Hypoxylon wujiangensis* sp. nov. Phytotaxa 455: 21–30. https://doi.org/10.11646/phytotaxa.455.1.3
Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818. https://doi.org/10.1093/bioinformatics/14.9.817

Pourzar Z (1985a) Reassessment of Hypoxylon serpens-complex I. Ceska Mykologie 39: 15–25.

Pourzar Z (1985b) Reassessment of the Hypoxylon serpens-complex II. Ceska Mykologie 39: 129–134.

Rogers JD, Ju YM (2012) The Xylariaceae of the Hawaiian islands. North American Fungi 7: 1–35. https://doi.org/10.2509/naf2012.007.009

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Lartet B, Liu L, Suchard MA, Huelsenbeck JP (2012) Mrbayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Sánchez-Ballesteros J, González V, Salazar O, Acero J, Portal MA, Julian M, Rubio V, Bills GF, Polishook JD, Platás G, Mochales S, Pelaez F (2000) Phylogenetic study of Hypoxylon and related genera based on ribosomal ITS sequences. Mycologia 92: 964–977. https://doi.org/10.1080/00275514.2000.12061240

Senanayake IC, Maharachchikumbura SSN, Hyde KD, Bhat JD, Jones EBG, McKenzie EHC, Dai DQ, Daranagama DA, Dayarathne MC, Goonasekara ID, Konta S, Li WJ, Shang QJ, Stadler M, Wijayawardene NN, Xiao YP, Norphanphoun C, Li QR, Liu XZ, Bahkali AH, Kang JC, Wang Y, Wen TC, Wendt L, Xu JC, Camporesi E (2015) Towards unraveling relationships in Xylariomycetidae (Sordariomycetes). Fungal Diversity 73: 73–144. https://doi.org/10.1007/s13225-015-0340-y

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 75: 758–771. https://doi.org/10.1080/10635150802429642

Tanaka K, Hirayama K, Yonezawa H, Hatakeyama S, Harada Y, Sano T, Shirouzu T, Hosoya T (2009) Molecular taxonomy of bambusicolous fungi: Tetraplosphaeriaceae, a new pleosporalean family with Tetraploa-like anamorphs. Studies in Mycology 64: 175–209. https://doi.org/10.3114/sim.2009.64.10

Tang A, Jeewon R, Hyde KD (2009) A re-evaluation of the evolutionary relationships within the Xylariaceae based on ribosomal and protein-coding gene sequences. Fungal Diversity 34: 127–155.

Tang A, Jeewon R, Hyde KD (2007) Phylogenetic relationships of Nemania plumbea sp. nov. and related taxa based on ribosomal ITS and RPB2 sequences. Mycological Research 111: 392–402. https://doi.org/10.1016/j.mycres.2007.01.009

Tibpromma S, Zhang L, Karunarathna SC, Du TY, Phukhamsakda C, Rachakunta M, Suwanarach N, Xu J, Mortimer PE, Wang YH (2021) Volatile constituents of endophytic Fungi isolated from Aquilaria sinensis with descriptions of two new species of Nemania. Life 11: e363. https://doi.org/10.3390/life11040363

U’Ren JM, Miadlikowska J, Zimmerman NB, Lutzoni F, Stajich JE, Arnold AE (2016) Contributions of North American endophytes to the phylogeny, ecology, and taxonomy of Xylariaceae (Sordariomycetes, Ascomycota). Molecular Phylogenetics and Evolution 98: 210–232. https://doi.org/10.1016/j.ympev.2016.02.010

Vu D, Groenewald M, de Vries M, Gehrmann T, Stielow B, Eberhardt U, Al-Hatmi A, Groenewald JZ, Cardinale G, Houbraken J, Boekhout T, Crous PW, Robert V, Verkley GJM
A taxonomic study of Nemania from China

(2019) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Studies in Mycology 92: 135–154. https://doi.org/10.1016/j.simyco.2018.05.001

Wendt L, Sir EB, Kuhnert E, Heitkämper S, Lambert C, Hladki AI, Romero AI, Luangsa-ard JJ, Sritikitkulchai P, Peršoh D, Stadler M (2018) Resurrection and emendation of the Hypoxylaceae, recognised from a multigene phylogeny of the Xylariales. Mycological Progress 17: 115–154. https://doi.org/10.1007/s11557-017-1311-3

Whalley AJS, Edwards RL, Francis SM (1983) Hypoxylon gwyneddii sp. nov. from Wales. Transactions of the British Mycological Society 81: 389–446. https://doi.org/10.1016/S0007-1536(83)80091-6

White TJ, Bruns T, Lee SJWT, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Applications 18: 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Xie X, Liu LL, Shen XC, Kang JC, Hyde KD, Kang JC, Li QR (2020) Contributions to species of Xylariales in China–3. Colloidoscula tubulosa. Phytotaxa 428: 122–130. https://doi.org/10.11646/phytotaxa.428.2.6