Micromelanconis kaihuiae gen. et sp. nov., a new diaporthalean fungus from Chinese chestnut branches in southern China

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Abstract
Melanconis-like fungi are distributed in several families of Diaporthales, mainly Juglanconidaceae, Melanconidaceae, Melanconiellaceae and Pseudomelanconidaceae. A new Melanconis-like genus of Pseudomelanconidaceae was discovered on branches of Chinese chestnut (Castanea mollissima) in southern China, which was confirmed by both morphology and phylogenetic analysis of combined ITS, LSU, tef1a and rpb2 sequences. The new genus Micromelanconis is characterized by two types of conidia from natural substrate and manual media of PDA, respectively. Conidia from Chinese chestnut branches are pale brown, ellipsoid, multiguttulate, aseptate with hyaline sheath. While conidia from PDA plates are pale brown, long dumbbell-shaped, narrow at the middle and wide at both ends, multiguttulate, aseptate, and also with hyaline sheath. All Pseudomelanconidaceae species were only reported on tree branches in China until now. More interesting taxa may be discovered if detailed surveys on tree-inhabiting fungi are carried out in East Asia in the future.

Keywords
Castanea mollissima, Diaporthales, DNA phylogeny, Melanconis, systematics
Introduction

Diaporthales, a species-rich order within Sordariomycetes of Ascomycota, is characterized by perithecia with elongate beaks, often forming within stromatic tissues, deliquescent paraphyses, and asci that have a refractive apical annulus (Barr 1978; Rossman et al. 2007; Senanayake et al. 2017, 2018; Fan et al. 2018a; Jiang et al. 2020a). Species of this order inhabit a variety of substrates, including plants, soil, even living animal tissues (Barr 1978; Castlebury et al. 2002; Sogonov et al. 2008; Yang et al. 2020). Most of them are pathogens associated with plant diseases, and the rest are endophytes in healthy plants or saprobes on dead tissues (Crous et al. 2012a; Chen et al. 2016; Norphanphoun et al. 2018; Jiang et al. 2019d; Xavier et al. 2019; Zhu et al. 2020; Yang et al. 2021). Some diaporthalean fungi cause severe forest diseases, so gained attention in forest pathological studies in recent years. For example, *Cryphonectria parasitica* (Cryphonectriaceae) causes chestnut blight worldwide (Rigling and Prospero 2018; Jiang et al. 2019b); *Cytospora chrysosperma* (Cytosporaceae) causes common polar and willow cankers in China (Fan et al. 2020); *Gnomoniopsis smithogilvyi* (Gnomoniaceae) results in European chestnut fruit rot and branch canker (Shuttleworth et al. 2016; Shuttleworth and Guest 2017; Jiang and Tian 2019; Jiang et al. 2020b).

Diaporthales is well classified into families based on morphological and phylogenetic studies (Voglmayr and Jaklitsch 2014; Norphanphoun et al. 2016; Voglmayr et al. 2017; Fan et al. 2018a; Senanayake et al. 2018; Yang et al. 2018a), and up to 32 families were accepted in the order Diaporthales (Jiang et al. 2021). Specimens can be identified to specific level by morphological characters, such as transversely distoseptate brown conidia of *Coryneum* (Jiang et al. 2018b, 2019c; Senwanna et al. 2018); allantoid ascospores and conidia of *Cytospora* (Fan et al. 2020); two-guttulate fusiform conidia of *Diaporthe*-like taxa (Fan et al. 2018a; Yang et al. 2018a, b); stromatic tissues turning to purple in 3% KOH of Cryphonectriaceae species (Chen et al. 2013, 2018); dark acervular conidiomata with conspicuous central column of *Melanconis*-like taxa (Fan et al. 2016; Jaklitsch and Voglmayr 2020).

*Melanconis*-like taxa are distributed in several families of Diaporthales, mainly Juglanconidaceae, Melanconidaceae, Melanconiellaceae and Pseudomelanconidaceae, which are four morphologically similar clades in distinct phylogenetic clades within this order (Fan et al. 2018b). Species of these four families are usually discovered on branches of Betulaceae, Juglandaceae and Fagaceae, but they are not strong pathogens (Wehmeyer 1937; Du et al. 2017; Voglmayr et al. 2019).

*Castanea*, commonly known as chestnut trees, is a worldwide genus containing several economic species. Chinese chestnut (*C. mollissima*), is widely cultivated in most of the provinces in China. Previous studies have revealed that seven families (Coryneaceae, Cryphonectriaceae, Cytosporaceae, Diaporthaceae, Erythrogloeaceae, Gnomoniaceae and Pseudomelanconidaceae) of Diaporthales have been reported on branches of *Castanea*. *Coryneum castaneicola*, *C. gigasporum* and *C. suttonii* of Coryneaceae were reported on dead and diseased *Castanea mollissima* branches (Jiang et al. 2018b). *Aurantiosacculus castaneae*, *Cryphonectria neoparasitica*, *C. parasitica* and
Endothia chinensis of Cryphonectriaceae were confirmed to be Castanea mollissima canker pathogens (Jiang et al. 2019b). Cytospora ceratospermopsis, C. kuanchengensis, C. leucostoma, C. myrtagena, C. schulzeri and C. xinglongensis of Cytosporaceae were reported to be associated with Castanea mollissima branch cankers (Jiang et al. 2020c). Diaporthe eres of Diaporthaceae was discovered on dead branches of Castanea mollissima in Beijing (Yang 2018). Dendrostoma aurorae, D. castaneae, D. castaneicola, D. chinense, D. parasiticum, D. shaanxiense and D. shandongense of Erythrogloeaceae were associated with Castanea mollissima stem, branch and twig cankers (Jiang et al. 2019a). Gnomoniopsis chinensis of Gnomoniaceae caused severe stem and branch cankers only in Hebei Province (Jiang and Tian 2019; Jiang et al. 2020b). Neopseudomelanconis castaneae of Pseudomelanconidaceae was discovered on Castanea mollissima branches in Shaanxi Province (Jiang et al. 2018a).

In the present study, investigations were conducted in Castanea mollissima plantations in Hunan Province of south China. Two Melanconis-like specimens were collected on a cultivated chestnut tree. The aim of the present study was to identify the fresh collections and confirm their phylogenetic positions.

Materials and methods

Collection, examination and isolation

The fresh specimens of diseased and dead chestnut branches were collected in a Castanea mollissima plantation in Hunan Province of south China. Morphological characteristics of the conidiomata were determined under a Nikon AZ100 dissecting stereomicroscope. More than 20 fruiting bodies were sectioned, and 50 conidia were selected randomly for measurement using a Leica compound microscope (LM, DM 2500). Isolates were obtained by removing a mucoid conidial mass from conidiomata, spreading the suspension onto the surface of 1.8% potato dextrose agar (PDA), and incubated at 25 °C for up to 24 h. Single germinating conidium was removed and plated onto fresh PDA plates. Cultural characteristics of isolates incubated on PDA in the dark at 25 °C were recorded, including the colony color and conidiomata structures. Specimens were deposited in the Museum of the Beijing Forestry University (BJFC). Axenic cultures were maintained in the China Forestry Culture Collection Centre (CFCC).

DNA extraction, PCR amplification and phylogenetic analyses

Genomic DNA was extracted from colonies grown on cellophane-covered PDA, using a cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1990). DNA was estimated by electrophoresis in 1% agarose gel and the quality was measured using the NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA). Four partial loci, including the 5.8S nuclear ribosomal DNA gene with the two flanking internally tran-
scribed spacer (ITS) regions, the large subunit of the nrDNA (LSU), and the translation elongation factor 1-alpha (*tef1a*) and DNA-directed RNA polymerase II second largest subunit (*rpb2*) genes, were amplified by the following primer pairs: ITS1 and ITS4 for ITS (White et al. 1990), LR0R and LR5 for LSU (Vilgalys and Hester 1990), EF1-728F and EF2 for *tef1a* (O’Donnell et al. 1998; Carbone and Kohn 1999), and RPB2-5F and fRPB2-7cR for *rpb2* (Liu et al. 1999). The polymerase chain reaction (PCR) conditions were as follows: an initial denaturation step of 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 50 s at 48 °C (ITS, LSU) or 54 °C (*tef1a*) or 55 °C (*rpb2*), and 1 min at 72 °C, and a final elongation step of 7 min at 72 °C. PCR products were assayed via electrophoresis in 2% agarose gels. DNA sequencing was performed using an ABI PRISM 3730XL DNA Analyser with a BigDye Terminator Kit v.3.1 (Invitrogen, USA) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

For phylogenetic reconstruction, newly-generated sequences of ITS, LSU, *tef1a* and *rpb2* were initially subjected to BLAST search (BLASTn) in NCBI website (https://www.ncbi.nlm.nih.gov). Then species and their sequences from recently published articles were selected and listed in Table 1 (Crous et al. 2012b; Alvarez et al. 2016; Senanayake et al. 2017; Braun et al. 2018; Fan et al. 2018a; Jiang et al. 2020a; Wang et al. 2020). The sequence alignments of the four individual loci (ITS, LSU, *tef1a* and *rpb2*) were conducted using MAFFT 7 (http://mafft.cbrc.jp/alignment/server/index.html), manually edited in MEGA 7.0.21, and then assembled as a dataset of ITS-LSU-*tef1a-*rpb2* to infer the phylogenetic placement of our new isolates.

ML and Bayesian analysis were implemented on the CIPRES Science Gateway portal (https://www.phylo.org) using RAxML-HPC BlackBox 8.2.10 (Stamatakis 2014) and MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), respectively. For ML analyses, a GTR+GAMMA substitution model with 1000 bootstrap iterations was set. MrModeltest 2.3 was used to estimate the best nucleotide substitution model settings for each gene. Bayesian inference (BI) was performed based on the DNA data set from the results of the MrModeltest, using a Markov chain Monte Carlo (MCMC) algorithm in MrBayes 3.1.2. Two MCMC chains were run from random trees for 1000 million generations and stopped when the average standard deviation of split frequencies fell below 0.01. Trees were saved each 1000 generations. The first 25% of trees were discarded as the burn-in phase of each analysis, and the Bayesian posterior probabilities (BPPs) were calculated from the remaining trees. Phylogenetic trees were viewed with FigTree v.1.3.1 and processed by Adobe Illustrator CS5. The nucleotide sequence data of the new taxon have been deposited in GenBank (Table 1).

**Results**

The ITS, LSU, *tef1a* and *rpb2*, and combined data matrices contained 624, 867, 513, 865, and 2869 characters with gaps, respectively. The alignment comprised 92 strains, with *Nakataea oryzae* (CBS 243.76) and *Pyricularia grisea* (Ina168) from Magna-
Table 1. Details of the isolates included for molecular study used in this study.

| Species                        | Isolates       | GenBank accession numbers |
|--------------------------------|----------------|---------------------------|
|                                |                | ITS | LSU | tef1a | rpb2  |
| Apiognomonia errabunda         | AR 2813        | DQ313525 | NG027592 | DQ313565 | DQ862014 |
| Apiosporopsis carpincola       | CBS 771.79     | NA  | AF277130 | NA      | NA     |
| Apoharknessia insueta          | CBS 111377*    | JQ700683 | AY20814  | MN271820 | NA     |
|                                | CBS 114575     | MN172402 | MN172370 | MN271821 | NA     |
| Asteroxporum asterothoerum     | MFLU 15-3555   | NA  | MF190062 | NA      | NA     |
| Asteriopycnidiella tristaniopsis| CBS 132180*   | JQ65516  | JQ65522  | MN271825 | NA     |
|                                | CPC 16371      | MN172405 | MN172374 | MN271826 | NA     |
| Aurifilum marmelostoma         | CBS 129428*    | FJ80945  | MH874934 | MN271827 | MN271788 |
| Celoporthe eucalypti           | CBS 127190*    | HQ30837  | HQ30863  | HQ30850  | MN271790 |
| Celoporthe woodiana            | CBS 118785*    | DQ267131 | MN172375 | JQ824071 | MN271791 |
| Chiangraomyces baunii           | MFLUCC 17-1669 | MF190119 | MF190064 | MF377598 | MF377603 |
| Coniella africana              | CBS 114133*    | AY339344 | AY339293 | KX833600 | KX833421 |
| Coniella eucalyptorum          | CBS 112640*    | AY339338 | AY339290 | KX833637 | KX833452 |
| Coniella fusiformis            | CBS 141596*    | KX833576 | KX833397 | KX833674 | KX833481 |
| Coniella javanica              | CBS 455.68*    | KX833583 | KX833403 | KX833483 | KX833489 |
| Coryneum gigasporum            | MFLU 15-3555   | NA  | MF377591 | MF377605 | NA     |
| Coryneum umbonatum             | D201           | MH674329 | MH674337 | MH674337 | MH674333 |
| Cryptocentria decipiens        | CBS 129353     | EU42655  | MN172386 | MN271845 | MN271797 |
| Cryptomicrotia australiensis   | CBS 124007*    | GQ369457 | MN172387 | MN271851 | MN271798 |
| Cytospora chrysosperma         | CFCC 89982     | KF281261 | KP30880  | KU710952 | KU710956 |
| Cytospora elaeagni             | CFCC 89633     | KF765677 | KF765693 | KU710919 | KU710956 |
| Dendrostoma aurorae            | CFCC 52753*    | MH542498 | MH542646 | MH545447 | MH545405 |
| Dendrostoma castaneae           | CFCC 52745*    | MH542488 | MH542644 | MH545437 | MH545395 |
| Dendrostoma chinense            | CFCC 52755*    | MH542500 | MH542648 | MH545449 | MH545407 |
| Dendrostoma dispersum           | CFCC 52730*    | MH542467 | MH542629 | MH545416 | MH545374 |
| Dendrostoma melii               | CFCC 52102*    | MG682072 | MG682012 | MG682052 | MG682032 |
| Dendrostoma noui                 | CFCC 52106*    | MG682073 | MG682013 | MG682053 | MG682032 |
| Dendrostoma parasiticum         | CFCC 52762*    | MH542482 | MH542638 | MH545431 | MH545389 |
| Dendrostoma quingense           | CFCC 52732*    | MH542471 | MH542633 | MH545420 | MH545378 |
| Dendrostoma quercinum           | CFCC 52103*    | MG682077 | MG682017 | MG682057 | MG682037 |
| Dendrostoma quercus             | CFCC 52739*    | MH542476 | MH542635 | MH545425 | MH545383 |
| Dendrostoma thamnoseptum        | CFCC 52741*    | MH542486 | MH542642 | MH545435 | MH545393 |
| Dendrostoma thamnoseptum        | CFCC 52759*    | MH542504 | MH542652 | MH545453 | MH545411 |
| Diasporoporella cereciola       | CFCC 51994*    | KY82492  | KY82515  | MN271855 | NA     |
| Diasporoporella machili         | CFCC 52100*    | MG682080 | MG682020 | MG682060 | MG682040 |
| Foscariella violacea            | CFCC 52101*    | MG682081 | MG682021 | MG682061 | MG682041 |
| Fuscovascularia nigromosae      | CBS 143163*    | MK510676 | MK510686 | NA      | MK510692 |
| Holocryphia eucalypti           | CBS 124779*    | GQ303276 | GQ303307 | MN271861 | MN271802 |
| Holocryphia eucalyptorum        | CBS 142536*    | KY979772 | KY979827 | MN271862 | MN271803 |
| Holocryphia eucalyptorum        | CBS 121083     | DQ491518 | AF408361 | EU221885 | EU219295 |
| Harknessia aurea                | CBS 132119*    | JQ700685 | JQ706211 | MN271863 | NA     |
| Harknessia capensis             | CBS 111829*    | AY720719 | AY720816 | MN271864 | NA     |
| Harknessia ellipsoidea          | CBS 132121*    | JQ700687 | JQ706213 | MN271865 | NA     |
| Harknessia eucalyptorum         | CBS 342.97     | AY720745 | AF408363 | MN271866 | NA     |
| Harknessia eucalyptorum         | CBS 115842*    | MN172411 | MN172391 | MN271882 | MN271804 |
| Harknessia eucalyptorum         | CBS 132862*    | JQ862765 | JQ862755 | MN271886 | MN271805 |
| Hypoplectorus kozakoviae        | CBS 121083     | KY427148 | KY427148 | KY427217 | KY427198 |
| Hypoplectorus oblonga           | MAFF 410216    | KY427153 | KY427153 | KY427222 | KY427203 |
| Hypoplectorus pterocaryae       | MAFF 410079    | KY427155 | KY427155 | KY427224 | KY427205 |
| Lamproconium desmazieri         | MFLUCC 15-0870 | KX430134 | KX430135 | MF377591 | MF377605 |
| Macrobiotum eucalypti           | CPC 10945      | KR873244 | KR873275 | NA      | MN271810 |
| Mastigospora anisophylleae      | CBS 136421*    | KF779492 | KF777221 | MN271892 | NA     |
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The ML analysis yielded a tree with a ln likelihood value of -45806.26677 and the following model parameters: alpha = 0.298226, Π(A) = 0.241173, Π(C) = 0.258552, Π(G) = 0.275145, and Π(T) = 0.225130. For BI analyses, the general time reversible model, additionally assuming a proportion of invariant sites with gamma-distributed substitution rates of the remaining sites (GTR+I+G), was determined to be the best for the ITS, LSU, and tef1a loci by MrModeltest, whereas the most appropriate model for the rpb2 locus was the Tamura-Nei model, additionally assuming a proportion of invariant sites with gamma-distributed substitution rates of the remaining sites (TrN+I+G). The phylogeny resulting from the RAxML maximum likelihood analysis of the combined gene sequence data is shown in Fig. 1. Overall, the topologies obtained from the different phylogenetic analyses were similar, and the best scoring RAxML tree is illustrated here. The bootstrap support values above 50% of maximum likelihood analysis (ML) and Bayesian posterior probability scores (≥0.90) are noted at the nodes.

The Diaporthales separates into 32 clades, representing 32 families, and the new isolates were clustered with a well-supported clade (ML/BI = 100/1) in Pseudomelanconidaceae. The two new isolates were different from any known genera in Pseudomelanconidaceae, and represented a new genus (Fig. 1).

| Species                     | Isolates | GenBank accession numbers |
|-----------------------------|----------|---------------------------|
|                            |          | ITS  | LSU  | tef1a | rpb2 |
| Mastigosporella pigmentata  | COAD 2370* | MG587929 | MG587928 | NA    | NA   |
| Melanconiella elissi        | BPI 878343 | JQ926271 | JQ926271 | JQ926406 | JQ926339 |
| Melanconiella stipulata     | MSH      | JQ926298 | JQ926298 | JQ926431 | JQ926364 |
| Melanconis betulae          | CFCC 50471 | KT732952 | KT732971 | KT733001 | KT732984 |
| Melanconis itoana           | CFCC 50474 | KT732955 | KT732974 | KT733004 | KT732987 |
| Melanconis stibostoma       | CFCC 50475 | KT732956 | KT732975 | KT733005 | KT732988 |
| Micromelanconis kahuluiae   | CFCC 54572* | MW414473 | MW414373 | MW419880 | MW419878 |
|                            | KHS-4    | MW414474 | MW414374 | MW419881 | MW419879 |
| Nakataea oryae              | CBS 243.76 | KM484861 | DQ341498 | NA    | NA   |
| Neopseudomelanconis castaneae | CFCC 52787* | MH469162 | MH469164 | NA    | NA   |
| Phaeoappendicospora thailandensis | MFLU 12-2131 | MF190157 | MF190102 | NA    | NA   |
| Prosopidicola altaiziae     | CPC 27478 | KX228274 | KX228325 | NA    | NA   |
| Prosopidicola mexicana      | CBS 113529 | AY720709 | NA    | NA    | NA   |
| Pseudomelanconis coryae     | CFCC 52110* | MG682082 | MG682022 | MG682062 | MG682042 |
| Pseudoplagiostoma corybiæ  | CPC 14161 | GU973510 | GU973604 | GU973540 | NA   |
| Pseudoplagiostoma oldii     | CBS 115722 | GU973535 | GU973610 | GU973565 | NA   |
| Pseudoplagiostoma variable  | CBS 113067 | GU973536 | GU973611 | GU973566 | NA   |
| Pyricularia grisea           | ima168   | NA    | ABO26819 | NA    | NA   |
| Pyripora castaneae          | CFCC 53434 | MW208108 | MW208105 | MW227340 | MW218535 |
|                            | CFCC 53451 | MW208110 | MW208107 | MW227342 | MW218537 |
| Sillia karstii              | MFLU 16-2864 | KY523482 | KY523500 | NA | KYS01636 |
| Sirococcus trugae           | CBS 119626 | EU199203 | EU199136 | EF512534 | EU199159 |
| Stephagonosporium acrophilum | CBS 117025 | EU039982 | EU039993 | EU040027 | KFS70173 |
| Stilbopora longicornuata    | CBS 122529* | KFS70164 | KFS70164 | KFS70232 | KFS70194 |
| Synnemaporella aculeana     | CFCC 52094 | MG682086 | MG682026 | MG682066 | MG682046 |
| Synnemaporella toxicodendri | CFCC 52097* | MG682089 | MG682029 | MG682069 | MG682049 |
| Thaiuldomycetes bistulosus  | BCC 00018 | NA    | EF622230 | NA    | NA   |
| Tiritorella beccariana      | BCC 38312 | NA    | JQ655449 | NA    | NA   |
| Tubakia seoraksanensis      | CBS 127490* | MG591907 | KP260499 | MG592094 | NA   |
| Tubakia invernisi           | CBS 129012* | MG591879 | MG591971 | MG592064 | NA   |
| Ursicollum fallax           | CBS 118663* | DQ368755 | EF392860 | MN271897 | MN271816 |

Ex-type strains are marked by an asterisk (*) and the strains from this study are in bold.
Figure 1. Phylogram of Diaporthales from a maximum likelihood analysis based on combined ITS, LSU, tef1a and rpb2. Values above the branches indicate maximum likelihood bootstrap (left, ML BP ≥ 50%) and Bayesian probabilities (right, BI PP ≥ 0.90). The tree is rooted with *Nakataea oryzae* (CBS 243.76) and *Pyricularia grisea* (Ina168). New species proposed in the current study is in blue and the ex-type strains are marked with *. 
Figure 111. Continued.

Taxonomy

*Micromelanconis* C.M. Tian & N. Jiang, gen. nov.

MycoBank No: 838927

Etymology. Name derived from micro- and the genus name *Melanconis*.

Type species. *Micromelanconis kaibuiae* C.M. Tian & N. Jiang.

Description. Sexual morph: not observed. Asexual morph: Conidiomata acervular, conspicuous, immersed in host bark to erumpent, covered by brown to blackish exuding conidial masses at maturity. Central column beneath the disc more or less conical. Conidiophores unbranched, aseptate, cylindrical, pale brown, smooth-walled. Conidiogenous cells annellidic, occasionally with distinct annellations and collarettes.
Conidia hyaline when immature, becoming pale brown, ellipsoid, multiguttulate, aseptate, with hyaline sheath. Conidiomata formed on PDA after three weeks, randomly distributed, and black. Conidiophores unbranched, septate, cylindrical, pale brown, smooth-walled. Conidiogenous cells annellidic. Conidia pale brown, long dumbbell-shaped, narrow at the middle and wide at both ends, multiguttulate, aseptate, with hyaline sheath.

**Notes.** *Micromelanconis* is the third genus after *Neopseudomelanconis* and *Pseudomelanconis* in the family *Pseudomelanconidaceae* (Fig. 1). *Micromelanconis* is united in this family based on the *Melanconis*-like conidiomata, and pale brown conidia with conspicuous hyaline sheath. *Micromelanconis* produces two types of conidia from natural branches and manual media respectively, which differs from *Neopseudomelanconis* and *Pseudomelanconis* (Fan et al. 2018a; Jiang et al. 2018a). Additionally, *Neopseudomelanconis* is characterized by its septate conidia (Jiang et al. 2018a).

*Micromelanconis kaihuiae* C.M. Tian & N. Jiang, sp. nov.

MycoBank No: 838928

Figures 2, 3

**Etymology.** Named after Kaihui Yang, a Chinese heroine; Kaihui is also the name of the town where holotype was collected.

**Description.** **Sexual morph:** not observed. **Asexual morph:** Conidiomata acervular, 350–800 μm diam., conspicuous, immersed in host bark to erumpent, covered by brown to blackish exuding conidial masses at maturity. Central column beneath the disc more or less conical. Conidiophores unbranched, aseptate, cylindrical, pale brown, smooth-walled. Conidiogenous cells annellidic, occasionally with distinct annellations and collarettes, 12.4–47.1 × 1.2–3.8 μm. Conidia hyaline when immature, becoming pale brown, ellipsoid, multiguttulate, aseptate, 7.6–10.3 × 3.1–4.1 μm, L/W = 2–3.2, with hyaline sheath, 1 μm.

**Culture characters.** Colony on PDA at 25 °C irregular, grey olivaceous, margin becoming diffuse, aerial hyphae short, dense, surface becoming imbricate, growth limited and ceasing after two weeks. Conidiomata formed after three weeks, randomly distributed, black. Conidiophores unbranched, septate, cylindrical, pale brown, smooth-walled. Conidiogenous cells annellidic, 9.1–18.5 × 2.5–5.3 μm. Conidia pale brown, long dumbbell-shaped, narrow at the middle and wide at both ends, multiguttulate, aseptate, 10.4–13.5 × 4–5 μm, L/W = 2.3–3.3, with hyaline sheath, 1.5 μm.

**Specimens examined.** China, Hunan Province, Changsha City, Changsha County, Kaihui Town, chestnut plantation, 40°24’32.16”N, 117°28’56.24”E, 262 m asl, on stems and branches of *Castanea mollissima*, Tian Chengming and Ning Jiang, 10 November 2020 (BJFC-S1831, holotype; ex-type culture, CFCC 54572 = KH5-3). *Ibid.* (BJFC-S1832, KH5-4).

**Notes.** *Micromelanconis kaihuiae* on *Castanea mollissima* (Fagaceae, Fagales) is phylogenetically close to *Neopseudomelanconis castaneae* on *Castanea mollissima* and
Figure 2. Morphology of *Micromelanconis kaihuiae* on branches of *Castanea mollissima* (BJFC-S1831) 
A, B habit of conidiomata on a branch C transverse section of conidiomata D longitudinal section through conidiomata E conidiogenous cells attached with conidia F, G conidia. Scale bars: 100 μm (C, D); 10 μm (E–G).

*Pseudomelanconis caryae* on *Carya cathayensis* (Juglandaceae, Juglandales) (Fig. 1). All these three species are discovered on tree branches in China, and share similar morphological characters in having pale brown conidia with conspicuous hyaline sheath. *Micromelanconis kaihuiae* and *Neopseudomelanconis castaneae* even share the same host. However, they can be easily distinguished based on conidia shape, color and overall size of conidia (*M. kaihuiae*, pale brown, ellipsoid and aseptate conidia, 7.6–10.3 × 3.1–4.1 μm; pale brown, long dumbbell-shaped and aseptate conidia, 10.4–13.5 × 4–5 μm *vs.* *N. castaneae*, brown, ellipsoid to oblong and septate conidia, 18–21.5 × 4.8–7 μm *vs.* *P. caryae*, pale brown, ellipsoid to oblong and aseptate conidia, 12.5–16 × 4–5 μm) (Fan et al. 2018a; Jiang et al. 2018a). Furthermore, *M. kaihuiae* is separated
Micromelanconis kaihuiae gen. et sp. nov.

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from *N. castaneae* by 51/490 bp (10.4%) differences in ITS and 12/563 bp (2.1%) differences in LSU, and from *P. caryae* by 56/490 bp (11.4%) differences in ITS and 6/563 bp (1.1%) differences in LSU.

**Key to Pseudomelanconidaceae genera and species**

1. On *Carya* of Juglandaceae, conidia ellipsoid to oblong and aseptate. ............
   
   .................................................................................................................. *Micromelanconis kaihuiae*

2. On *Castanea* of Fagaceae. ..........................................................................

   – Conidia aseptate .................................................. *Pseudomelanconis castaneae*

   – Conidia septate ............................................. *Neopseudomelanconis castaneae*

**Discussion**

Diaporthales is a well-studied order based on integrated approaches of morphology and phylogeny in recent years (Castlebury et al. 2002; Rossman et al. 2007; Voglmayr and Jaklitsch 2014; Alvarez et al. 2016; Senanayake et al. 2017, 2018; Voglmayr et al. 2017; Braun et al. 2018; Fan et al. 2018a; Jiang et al. 2020a). Thirty-two accepted families are monophyletic and supported by morphological characters; four of them
contain *Melanconis*-like fungi, namely Juglanconidaceae, Melanconidaeae, Melanconiellaceae and Pseudomelancnoidaceae (Fan et al. 2018a). The *Melanconis*-like fungi were similar in their asexual morph, but well-separated in the phylogeny and their hosts (Voglmayr et al. 2012, 2017, 2019; Fan et al. 2018a, b; Jaklitsch and Voglmayr 2020). In the present study, a new genus and species were clustered in the family Pseudomelancnoidaceae (Fig. 1), and differed from the other *Melanconis*-like genera by its long dumbbell-shaped conidia formed on PDA plates.

Hosts are useful taxonomic information in some families of Diaporthales, such as Coryneaceae, Cryphonectriaceae, Erythrogloeaceae and Gnomoniaceae (Voglmayr et al. 2012; Jaklitsch and Voglmayr 2019; Roux et al. 2020; Wang et al. 2020; Yang et al. 2020). Hosts are important to separate *Melanconis*-like genera. *Juglanconis* inhabit *Juglans* and *Pterocarya* of Juglandaceae, *Melanconiella* and *Melanconis* occur only on the plant family Betulaceae (Voglmayr et al. 2012, 2017, 2019; Fan et al. 2018b; Jaklitsch and Voglmayr 2020). *Melanconis* species are discovered only on *Alnus* and *Betula*, while *Melanconiella* occurs in the subfamily Coryloideae with the exception of *M. betulae* and *M. decorahensis* on *Betula* (Voglmayr et al. 2012; Du et al. 2017; Fan et al. 2018a). Species of Pseudomelancnoidaceae inhabit *Carya* of Juglandaceae, and *Castanea* of Fagaceae (Fan et al. 2018a; Jiang et al. 2021). More interesting *Melanconis*-like may be revealed by more detailed surveys on tree-inhabiting fungi in the future.

**Acknowledgements**

This study is financed by the National Natural Science Foundation of China (Project No.: 31670647). We are grateful to Chungen Piao and Minwei Guo (China Forestry Culture Collection Center, Chinese Academy of Forestry, Beijing) for support of strain preservation during this study.

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