Pseudodidymellaceae fam. nov.: Phylogenetic affiliations of mycopappus-like genera in Dothideomycetes

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Abstract: The familial placement of four genera, Mycodidymella, Petrakia, Pseudodidymella, and Xenostigmina, was taxonomically revised based on morphological observations and phylogenetic analyses of nuclear rDNA SSU, LSU, tef1, and rpb2 sequences. ITS sequences were also provided as barcode markers. A total of 130 sequences were newly obtained from 28 isolates which are phylogenetically related to Melanommataceae (Pleosporales, Dothideomycetes) and its relatives. Phylogenetic analyses and morphological observation of sexual and asexual morphs led to the conclusion that Melanommataceae should be restricted to its type genus Melanomma, which is characterised by ascomata composed of a well-developed, carbonaceous peridium, and an ascothelea-like coelomycetous asexual morph. Although Mycodidymella, Petrakia, Pseudodidymella, and Xenostigmina are phylogenetically related to Melanommataceae, these genera are characterised by epi-phyllous, lenticular ascomata with well-developed basal stroma in their sexual morphs, and mycopappus-like propagules in their asexual morphs, which are clearly different from those of Melanommataceae. Pseudodidymellaceae is proposed to accommodate these four genera. Although Mycodidymella and Xenostigmina have been considered synonyms of Petrakia based on sexual morphology, we show that they are distinct genera. Based on morphological observations, these genera in Pseudodidymellaceae are easily distinguished by their synasexual morphs: sigmoid, multi-septate, thin-walled, hyaline conidia (Mycodidymella); globose to ovoid, dicytosporous, thick-walled, brown conidia with cellular appendages (Petrakia); and clavate with a short rostrum, dictyosporous, thick-walled, brown conidia (Xenostigmina). A synasexual morph of Pseudodidymella was not observed. Although Alpinaria was treated as member of Melanommataceae in a previous study, it has hyaline cells at the base of ascomata and pseudoceystidial, confluent conidomata which is atypical features in Melanommataceae, and is treated as incertae sedis.

Key words: Foliar pathogen, Synasexual morph, Systematics.

Taxonomic novelties: New family: Pseudodidymellaceae A. Hashim. & Kaz. Tanaka; New species: Melanomma japonicum A. Hashim. & Kaz. Tanaka, Pseudodidymella minima A. Hashim. & Kaz. Tanaka; New combination: Xenostigmina aceris (Dearn. & Barthol.) A. Hashim. & Kaz. Tanaka.

Available online 13 July 2017; http://dx.doi.org/10.1016/j.simyco.2017.07.002.

INTRODUCTION

The family Melanommataceae (Pleosporales) was proposed for its type genus, Melanomma (Winter 1887). Currently, more than 20 genera with diverse ecological and morphological features are recognised in this family (Tian et al. 2015). Petrakia and Xenostigmina have epi-phyllous, lenticular ascomata with well-developed basal stroma, mycopappus-like propagules, and pet- rakia- or stigmina-like synasexual morphs, and were also accepted in Melanommataceae (Funk 1986, Funk & Dorworth 1988, Crous 1998, Crous et al. 2009, Butin et al. 2013, Tian et al. 2015). Subsequently, two additional genera, Mycodidymella and Pseudodidymella, were reported to be phylogenetically related to this family (Gross et al. 2017), although their morphological features were clearly different from those of Melanomma, which is characterised by carbonaceous ascomata, trabecular pseudoparaphyses, and ascothelea-like coelomycetous asexual morphs (Barr 1987, 1990, Lumbsch & Huhndorf 2007, Kirk et al. 2008, Tian et al. 2015, Jaklitsch & Voglmayr 2017).

The genus Petrakia was originally characterised by sporodochial conidiomata and muriform, brown conidia with cellular, hyaline appendages (Sydow & Sydow 1913, Butin et al. 2013). Recently, the complete life cycle of Pe. echinata, which is the type species and a known causal agent of leaf blotch disease of Acer spp., was revealed (Butin et al. 2013). Subsequently, phylogenetic analysis using large subunit nrDNA sequences indicated that this genus is related to Melanommataceae or Pleomassariaceae (Dothideomycetes; Butin et al. 2013).

Xenostigmina zilleri, the type species of the genus, is a known pathogen that causes brown spot disease in Acer macrophyllum in Canada (Funk 1986). This species was originally described as Cercosporella aceris (Dearnless 1917). Redhead & White (1985) introduced Mycopappus, and transferred two species to this genus, i.e. C. aceris and C. alni. The type species of Myco- pappus, Mycop. alni, was suggested to be a member of Scle- robiinaeae (Helotiales, Leotiomycetes) based on its sclerotial morph and phylogenetic analyses using ITS sequences (Takahashi et al. 2006). Mycopappus aceris was excluded from this genus, because the synasexual morph of this species is the dothideomycetous taxon X. zilleri (Funk & Dorworth 1988, Crous 1998, Wei et al. 1998, Crous et al. 2009). According to phylo- genetic analysis, this genus was accepted as Melanommataceae (Phookamsak et al. 2014, Tian et al. 2015).

The genera Mycodidymella and Pseudodidymella are also members of Melanommataceae that produce mycopappus-like propagules in their asexual morphs (Wei et al. 1997, 1998, Gross et al. 2017). The genus Mycodidymella, which is based on the type species Mycod. aesculi, is known as a pathogen of
concentric ring spot disease in Aesculus turbinata (Wei et al. 1999). The life cycle of Mycod. aesculi is similar to those of Petakria and Xenostigma, except it has sigmoid and hyaline conidia in its synasexual morph. Although the synasexual morph of Petakria seems to be clearly different from that of Mycodidymella and Xenostigma, the latter two genera were synonymised with an older name, Petakria (Jaklitsch & Voglmayr 2017).

The monotypic genus Pseudodidymella was established for Pseudod. fagi (Wei et al. 1997). The species was found to be associated with brown leaf spots of Fagus crenata in Japan, and was originally characterised by lenticular ascomata with a well-developed basal stroma and a pycnopoiospora-like asexual morph, which is characterised by sporodochial conidiomata and conidia with appendages (Wei et al. 1997). Mycodidymella is morphologically similar to this genus, but can be distinguished by its pycnopoiospora-like asexual morph (Wei et al. 1998). Gross et al. (2017) discovered Pseudod. fagi on F. sylvatica in Switzerland and suggested that the pycnopoiospora-like asexual morph has mycopappus-like propagules rather than individual conidia. Thus, morphological delimitation of these two genera is problematic and requires further research. According to a phylogenetic study using ITS sequences (Gross et al. 2017), four genera with mycopappus-like propagules (Mycodidymella, Petakria, Pseudodidymella, and Xenostigma) formed a strongly supported clade within Melanomataceae sensu lato; however, familial placement and generic validity of each genus remain unresolved.

During our ongoing studies of ascomycetous fungi in Japan (Tanaka et al. 2010, 2011, 2015; Hashimoto et al. 2015a, b, 2016, 2017), we collected strains which are morphologically similar or phylogenetically related to Melanomataceae sensu lato. The main objectives of the present study were to clarify familial placement of genera in this family, and establish a taxonomic framework of Melanomataceae sensu lato based on morphological observations and molecular phylogenetic analyses of small subunit nrDNA (18S; SSU), large subunit nrDNA (28S; LSU), translation elongation factor 1-α (tef1), and DNA-directed RNA polymerase II second largest subunit (rpb2) sequences. ITS sequences were also obtained as DNA barcode markers.

MATERIALS AND METHODS

Isolates

All fungal structures were studied in preparations mounted in distilled water. Morphological characters were observed by differential interference and phase contrast microscopy (Olympus BX53, Japan), and images captured with an Olympus digital camera (DP21, Japan). A total of 28 single-spore isolates were used for morphological observation and phylogenetic analyses (Table 1).

DNA isolation, amplification and phylogenetic analysis

DNA extraction was carried out with an ISOLPLANT II kit (Nippon Gene, Japan) based on the manufacturer’s protocol. Sequences of SSU, ITS, LSU, and tef1 and rpb2 were amplified by PCR with the primer pairs NS1/NS4, ITS1/ITS4 (White et al. 1990), LR0R/LR7 (Rehner & Samuels 1994, Vilgalys & Hester 1990), EF1-983F/EF1-2218R (Rehner & Buckley 2005), and IRPB2-5F/IRPB2-7cR (Liu et al. 1999), respectively. Amplifications were performed in 25 μL volumes that consisted of 2 μL DNA extract, 2.5 μL of 10 × TEMPase Buffer I, 10 mM dNTP mix, 1 μL of each 20-μm primer, 25 mL MgCl2, 14.5 μL MilliQ water, and 0.5 μL TEMPase Hot Start DNA polymerase (Ampliqon, Denmark). PCRs were carried out on a PC 320 thermo-cycler (ASTEC, Japan) as follows: 95 °C for 15 min; followed by 35 cycles of 1 min at 94 °C, 1 min at the designated annealing temperature (42.2 °C for SSU, 61.5 °C for ITS, 46 °C for LSU, 60 °C for tef1, and 58 °C for rpb2), and 1 min at 72 °C; and a final denaturation of 7 min at 72 °C. The PCR products were directly sequenced at SolGent (South Korea).

Newly generated sequences were deposited in GenBank (Table 1). Sequences of 73 taxa of Pleosporales and Hysteriiales were also phylogenetically analysed (Table 1). Hysteriolum pulicicare and Hysteroberetrum mori (Hysteriaceae, Hysteriiales) were used as outgroups. All sequences were aligned using the MUSCLE algorithm as implemented in the program MEGA v. 5 (Tamura et al. 2011). Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian methods. The optimal substitution models for each dataset were estimated by Kakusan4 (Tanabe 2011) based on the Akaike information criterion (AIC; Akaike 1974) for ML analysis and Bayesian information Criterion (BIC; Schwarz 1978) for the Bayesian analysis. The ML analysis was performed using TreeFinder Mar 2011 (Jobb 2011) based on the models selected with the AICc4 parameter (a proportional model among genes and codons): J2+G for SSU; GTR+G for LSU; F81+G for the tef1 first codon position, J1ef+G for the tef1 second codon position, and J2+G for the tef1 third codon position; and J2+G for the rpb2 first codon position, J1+G for the rpb2 second codon position, and J2+G for the rpb2 third codon position. Bootstrap percentages (BPs) were obtained by 1000 bootstrap replications.

Bayesian analysis was performed with MrBayes v. 3.2.2 (Ronquist et al. 2012) with substitution models for different regions selected with the BIC4 parameter (proportional model among loci and codons): K80+G for SSU; GTR+G for LSU; F81+G for the tef1 first codon position, J1ef+G for the tef1 second codon position, and J2+G for the tef1 third codon position; and J2+G for the rpb2 first codon position, J1+G for the rpb2 second codon position, and J2+G for the rpb2 third codon position. Two simultaneous, independent runs of Metropolis-coupled Markov chain Monte Carlo (MCMC) were performed for 2 M generations with trees sampled every 1 000 generations. Convergence of the MCMC runs assessed from the average standard deviation of split frequencies (<0.01) and effective sample size scores (all >100) using MrBayes v. 3.2.2 and Tracer v. 1.6 (Rambaut et al. 2014), respectively. The first 25 % of trees were discarded as burn-in, and the remaining trees were used to calculate 50 % majority rule trees and determine posterior probabilities (PPs) for individual branches. The alignment was submitted to TreeBase under study number S20165.

Morphology

Colony characteristics of cultures grown on 2 % potato dextrose agar (PDA; Difco, France) were observed after 3 wk incubation at 20 °C in the dark. Colours were noted based on those described by Rayner (1970).

To induce sexual or asexual fructification in culture, 5 mm square mycelial agar discs were placed on water agar that included sterilised natural substrate, such as Aesculus turbinata.
RESULTS

Phylogeny

The ML and Bayesian phylogenetic analyses were conducted using an aligned sequence dataset composed of 941 nucleotides from SSU, 1,276 from LSU, 886 from \textit{tefI}, and 1,021 from \textit{rpb2}. The alignment contained a total of 73 taxa, which consisted of 59 from SSU, 1,276 from LSU, 886 from \textit{tefI}, and 1,021 from \textit{rpb2} (Table 1 and 2). No significant conflict was observed among individual gene phylogenies, but the familial and generic nodes mostly lacked significant support in SSU and LSU phylogenetic trees generated (data not shown). However, this combined dataset provided higher confidence values for the familial level than did those of the individual gene trees (data not shown). Of the 3,824 characters included in the alignment, 1,205 were variable and 2,844 were conserved. The ML tree with the highest log likelihood (26638.727) is shown in Fig. 1. The topology recovered by the Bayesian analysis was almost identical to that of the ML tree, except for the position of \textit{Aposphaeria corallinolutea}, \textit{Bertiella macrosperma}, \textit{Herpotrichia macrotricha}, and \textit{Fagus crenata} leaves and rice straw, and the plates were incubated at 20 °C for 2 wk in the dark. When the substrate was colonised, the plates were incubated at 20 °C under blacklight blue illumination for 2 mo to observe sporulation. Cultures were deposited in the Westerdijk Fungal Biodiversity Institute (CBS), the Japan Collection of Microorganisms (JCM), and the Genebank Project of NARO, Japan (MAFF). Specimens were deposited in the Herbarium of Hirosaki University, Fungi (HHUF).

Table 1. Specimens, isolates and new sequences used in this study.

| Species | Original no. | Specimen no. | Strain no. | Host/substrate | GenBank accession no. |
|---------|--------------|--------------|------------|----------------|-----------------------|
| Alpinaria rhododendri | KT 2520 | HHUF 30554 | CBS 142901 | Rhododendron brachycarpum | LC203314 LC203360 LC203388 LC203416 LC203335 |
| Melanomma japonicum | KT 2076 | HHUF 30539 | CBS 142902 | dead wood | LC203290 LC203366 LC203392 LC203318 |
| | KT 3028 | HHUF 30540 | CBS 142903 | Fagus crenata | LC203291 LC203337 LC203365 LC203393 LC203319 |
| | KT 3425 | HHUF 30541 | CBS 142904 | F. crenata | LC203292 LC203338 LC203366 LC203394 LC203320 |
| | – | HHUF 26520 | CBS 142905 | = JCM 13124 = MAFF 239634 |
| Me. pulvis-pyrius | KT 2110 | HHUF 30542 | CBS 142906 | Acer sp. | LC203294 LC203340 LC203368 LC203396 LC203322 |
| | KT 2113 | HHUF 30543 | CBS 142907 | Dead wood | LC203295 LC203341 LC203369 LC203397 LC203323 |
| | AH 375 | HHUF 30544 | CBS 142908 | F. crenata | LC203296 LC203342 LC203370 LC203398 LC203324 |
| | KH 27 | HHUF 30545 | CBS 142909 | Dead wood | LC203297 LC203343 LC203371 LC203399 LC203325 |
| | KH 77 | HHUF 30546 | CBS 142910 | Dead wood | LC203298 LC203344 LC203372 LC203400 LC203326 |
| | KH 86 | HHUF 30547 | CBS 142911 | Dead wood | LC203299 LC203345 LC203373 LC203401 LC203327 |
| Mycodidyrella aesculi | KT 3060 | HHUF 30549 | CBS 142913 | Aesculus turbinata | LC203301 LC203347 LC203375 LC203403 LC203329 |
| | H 2610 | HHUF 22892 | CBS 142914 | A. turbinata | LC203302 LC203348 LC203376 LC203404 LC194192 |
| | H 2620 | – | CBS 142915 | A. turbinata | LC203303 LC203349 LC203377 LC203405 LC203330 |
| | AH 560 | HHUF 30550 | CBS 142916 | A. turbinata | LC203304 LC203350 LC203378 LC203406 LC203331 |
| Petraokia echinata | – | – | CBS 133072 | Acer pseudoplatanus | LC203305 LC203351 LC203379 LC203407 – |
| | – | – | CBS 133070 | A. pseudoplatanus | LC203306 LC203352 LC203380 LC203408 – |
| Pseudodidyrella fagi | KT 3058 | HHUF 30515 | CBS 142917 | F. crenata | LC203307 LC203353 LC203381 LC203409 LC150785 |
| | KT 3074-3 | HHUF 30516 | CBS 142918 | F. crenata | LC203308 LC203354 LC203382 LC203410 LC150786 |
| | RF 5 | HHUF 30517 | CBS 142919 | F. crenata | LC203309 LC203355 LC203383 LC203411 LC150788 |
| | H 2579 | HHUF 22903 | MAFF 245740 | F. crenata | LC203310 LC203356 LC203384 LC203412 LC150787 |
| | AH 561 | HHUF 30553 | CBS 142920 | F. crenata | LC203311 LC203357 LC203385 LC203413 LC203332 |
| Pseudod. minima | KT 2918 | HHUF 30551 | CBS 142921 | Fagus japonica = MAFF 246249 | LC203312 LC203358 LC203386 LC203414 LC203333 |
| | AH 556 | HHUF 30552 | CBS 142922 | F. japonica | LC203313 LC203359 LC203387 LC203415 LC203334 |
| Xenostigmina aceris | – | – | CBS 124109 | Acer macrophyllum | LC203315 LC203361 LC203389 LC203417 – |
| | – | CBS 115685 | Acer sp. | LC203316 LC203362 LC203390 LC203418 – |
| | – | CBS 115686 | Acer sp. | LC203317 LC203363 LC203391 LC203419 – |

1 “H”: holotype, “P”: paratype.
2 Sequences generated in this study are shown in bold.

Alpinaria rhododendri is deposited in the Herbarium of Hirosaki University, Fungi (HHUF).
| Species name                      | Family              | Strain no. | SSU             | LSU             | tef1            | rpb2            |
|----------------------------------|---------------------|------------|-----------------|-----------------|-----------------|-----------------|
| Alpinaria rhododendri incertae sedis | ANM 73              | –          | –               | GU385198        | –               | –               |
| A. rhododendri incertae sedis    | CBS 141994E        | KY190004   | KY189973        | KY190009        | KY189989        |
| Alternaria alternata             | Pleosporaceae       | DQ678031   | DQ678022        | DQ677927        | DQ677980        |
| Aposthena corallinolutea incertae sedis | CBS 131287F      | –          | JF740330        | –               | –               |
| Bartellia macrospora             | incertae sedis      | IL 5005    | –               | GU385150        | –               | –               |
| Bevenykkella pulmonaria           | incertae sedis      | CBS 283.531 | KY190005       | GU301894        | –               |GU371768        |
| Byssochroenia jamaicana          | incertae sedis      | SMH 1403   | –               | GU385152        | GU327746        |
| B. rhodomphala incertae sedis    | GKM L153N           | –          | GU385157        | GU327747        |
| B. salebrosa incertae sedis      | SMH 2387            | –          | GU385162        | GU327748        |
| B. schiedemayeriana              | incertae sedis      | SMH 3157   | –               | GU385163        | GU327745        |
| B. siamensis inermis             | incertae sedis      | MFLUCC 10-0099F | KT289897  | KT289895        | KT962059        | KT962061        |
| B. villosa incertae sedis        | GKM 204N            | –          | GU385151        | GU327751        |
| Corynespora cassicola            | Corynesporascaceae  | CBS 100822 | GU296144        | GU301808        | GU349052        |GU371742        |
| Cyclothyriella rubronotata       | Cyclothyriellaceae  | CBS 141486E| KK650507        | KK650544        | KK650519        |KK650574        |
| Gammamyces piceae                | incertae sedis      | CBS 141555 | KY190006        | KY189976        | KY190011        |KY189992        |
| Herpotrichia diffusa             | incertae sedis      | CBS 250.62 | DQ678019        | DQ678071        | DQ677915        |DQ677968        |
| H. juniperi incertae sedis       | CBS 200.31          | DQ678029   | DQ678080        | DQ677925        | DQ677978        |
| H. macrotricha incertae sedis    | GKM 196N            | –          | GU385176        | GU327755        |
| H. vaginatispora incertae sedis  | MFLUCC 13-0865F     | KT934256   | KT934252        | KT934260        |
| Hysterium pulicale               | Hysteriaceae        | CBS 123377 | FJ161161        | FJ161201        | FJ161109        |FJ161127        |
| Hysterotheicum moni              | Hysteriaceae        | CBS 123563 | FJ161155        | FJ161196        | FJ161104        |
| Leptosphaeria doliolum           | Leptosphaeraceae    | CBS 505.75 | GU296159        | GU301827        | GU349069        |KT938960        |
| Lophiotales arundinis             | Lophiotalesarundinis| CBS 621.86 | DQ782383        | DQ782384        | DQ782387        |DQ782388        |
| Massaria inquinans               | Massariaceae        | CBS 125591E| HO599442        | HO599400        | HO599340        |
| Massarina eburnea                | Massariaceae        | CBS 473.64 | GU296170        | GU301840        | GU349040        |GU371732        |
| Melanomma populinia              | Melanommataceae     | CBS 543.70F| EU754031        | EU754130        | –               |–               |
| M. populina incertae sedis       | Melanommataceae     | CBS 350.82 | –               | JF740265        | –               |
| M. pulvis-pyrius incertae sedis  | Melanommataceae     | CBS 124080F| GU456302        | GU456323        | GU456265        |GU456350        |
| M. pulvis-pyrius                 | Melanommataceae     | CBS 109.77 | FJ201987        | FJ201986        | GU456274        |GU456359        |
| M. pulvis-pyrius                 | Melanommataceae     | CBS 371.75 | FJ201989        | FJ201988        | GU349019        |GU371798        |
| Muniformistrickeria rubi incertae sedis | MFLUCC 15-0681F | KT934257   | KT934253        | KT934261        |
| Neopodophiaceae saccacites       | Lentitheciaceae     | MAAF 239646E| AB524548        | AB524599        | AB539111        |AB539098        |
| Nigrograna olbiqua               | Nigrogranaceae      | CBS 1414757 | KK650512        | KK650558        | KK650530        |KK650579        |
| Phragmophila atra                | incertae sedis      | MFLUCC 15-0021 | KP698729  | KP698725        | –               |
| Praetumpfunga obtocens           | incertae sedis      | CBS 141474F| KY190008        | KY189984        | KY190009        |KY190000        |
| Prothoerasia betulinum           | Pleomassariaceae    | CBS 279.74 | DQ678027        | DQ678078        | DQ677923        |KT216532        |
| Prothoerasia canba               | Pleomassariaceae    | KT 2083-1  | AB553646        | AB553760        | –               |
| Pseudostictricella               | incertae sedis      | MFLUCC 13-0764F | KT934258 | KT934254        | KT934262        |
| Tumularia rubrifaciata           | incertae sedis      | CBS 256.84 | –               | GU301851        | GU349006        |

*H*: ex-holotype, *P*: ex-paratype, *E*: ex-epitype.
Fig. 1. Maximum-likelihood (ML) tree of Melanomataceae sensu stricto and Pseudodidymellaceae with its relatives. ML bootstrap percentages (BP) greater than 60% and Bayesian posterior probabilities (PP) above 0.95 are presented at the nodes as ML BP/Bayesian PP. A hyphen ("-"), indicates values lower than 60% BP or 0.95 PP, and a node not present in the Bayesian analysis is shown with "x". Ex-holotype, paratype, epitype, strains are indicated with a superscript H, P and E, respectively. The newly obtained sequences are shown in bold. The scale bar represents nucleotide substitution per site.

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Phragmopephala atra, Pseudostickeria murigormis and Sar-Imanas shirakamiense.

Monophyly of the genera with mycopappus-like propagules (Mycodidymella, Petrakia, Pseudodidymella, and Xenostigmata) was well-supported (91 % ML BP/ 1.00 Bayesian PP). Although these four genera are phylogenetically related to Melanommataceae sensu lato, their morphological and ecological features are clearly distinct from those of the type genus Melanomma. Therefore, we establish a new family, Pseudodidymellaceae, to accommodate these genera with mycopappus-like propagules.

Results from phylogenetic analyses of this study indicate that Alpinaria, formerly classified in Melanommataceae sensu lato (Jaklitsch & Voglmayr 2017), is phylogenetically distant from Melanommataceae sensu stricto (Fig. 1), but its familial placement is unresolved.

**Taxonomy**

Two families, including a new family (Pseudodidymellaceae), four genera, and seven species, including two new species and one new combination (Melanomma janicicum, Pseudodidymella minima, and Xenostirgmina aceris) are described below.

**Melanommataceae** G. Winter [as ‘Melanommeeae’], Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 1.2: 220. 1887.

Saprobic on dead twigs of woody plants. Sexual morph: Ascomata globose to ovoid, immersed to superficial, gregarious, ostiolate. Peridium composed of thick-walled, pseudoparenchymatous, hyaline to brown cells. Pseudoparaphyses trabeculate, septate, branched and anastomosed. Asci bitunicate, fissitunicate, cylindrical, 8-spored. Ascospores olive brown, sometimes with paler ends, strongly or slightly curved, multi-septate, smooth. Asexual morph: Conidiomata pycnidial, globose to subglobose, superficial, black, ostiolate. Peridium composed of elongate, brown cells. Conidiophores absent. Conidiogenous cells holoblastic, ampitopous to cylindrical, hyaline. Conidia ellipsoidal, hyaline, smooth, aseptate.

Type genus: Melanomma Nitschke ex Fuckel.

**Notes**: Melanommataceae was established by Winter (1887). Byssosophaira, Keissleriella, Melanomma, Ostropella, and Strickeria have been referred to as members of Melanommataceae, and this family was characterised by gregarious ascomata composed of well-developed, carbonaceous or coriaceous peridium, trabecular pseudoparaphyses, and asphaeria-like coelomycetes asexual morphs (Barr 1987). This familial concept was supported in “Outline of Ascomycota – 2007” for 18 genera (Lumbsch & Huhndorf 2007).

A study by Mugambi & Huhndorf (2009) on LSU and tef1 sequences showed that Melanommataceae is composed of Byssosophaira, Herpotrichia, Melanomma, and Pseudotrichia, and previous familial concepts did not reflect natural relationships. Several genera, such as Keissleriella and Ostropella, were phylogenetically scattered in other Pleosporales (Mugambi & Huhndorf 2009, Zhang et al. 2012, Tanaka et al. 2015), and Strickeria was placed in Sporocadaceae (Xylariales, Sordariomycetes) (Jaklitsch et al. 2016a). It was clear that the traditional concept of Melanommataceae is polyphyletic and needed revision (Kirk et al. 2008, Mugambi & Huhndorf 2009, Hyde et al. 2013). Later, two genera, Tumularia (as Monotosporella) and Phragmocephala, which have mononematous or synnematous conidiophores in their asexual morphs, were reported in Melanommataceae (Schoch et al. 2009, Su et al. 2015). Wijayawardene et al. (2012, 2014) also listed additional dematiaceous genera, Exosporiella and Nigrolentilocus, as members of this family without molecular evidence. A broad concept of Melanommataceae was proposed by Tian et al. (2015) and Jaklitsch & Voglmayr (2017), and Mycodidymella, Petrakia and Xenostigmata were treated as members of this family. However, the results of our phylogenetic analyses and morphological observations indicate that Melanommataceae should be restricted to its type genus, Melanomma.

**Melanomma** Nitschke ex Fuckel, Jb. nassau. Ver. Naturk. 23–24: 159. 1870 (1869–1870).

Synonym: Moriolopis Norman ex Keissl., Nytt Mag. Natur. 66: 88. 1927.

Saprobic on dead twigs of woody plants. Sexual morph: Ascomata globose to ovoid, immersed or erumpent to superficial, gregarious, with a short ostiolar neck. Peridium composed of thick-walled, pseudoparenchymatous, hyaline to brown cells. Pseudoparaphyses trabecular, septate, branched and anastomosed. Asci bitunicate, fissitunicate, cylindrical, 8-spored. Ascospores olive brown, sometimes with paler ends, strongly or slightly curved, multi-septate, smooth. Asexual morph: Conidiomata pycnidial, globose to subglobose, superficial, black, with a papillate ostiole. Peridium composed of elongate, brown cells. Conidiophores absent. Conidiogenous cells holoblastic, ampitopous to cylindrical, hyaline. Conidia ellipsoidal, hyaline, smooth, aseptate.

Type species: Melanomma pulvis-pyrius (Pers.) Fuckel.

**Notes**: The genus Melanomma was established by Fuckel (1870). Species in this genus are known to be saprobic on decaying plant material or weak plant pathogens (Chesters 1938, Holm 1957, Zhang et al. 2008). Melanomma pulvis-pyrius is a well-studied, widespread species in this genus. However, other species have rarely been reported or have not been recorded since their initial description. Only a few species have received modern taxonomic treatment (Holm 1957, Mathiassen 1989, 1993, Barr 1990), although approximately 300 epithets are listed in Index Fungorum (http://indexfungorum.org). Asexual morphs of this genus were reported to be asphaeria-like coelomycetes or Nigrolentilocus (Ichinoe 1970, Shivanesan 1984, Castañeda-Ruiz et al. 2001, Sánchez & Bianchinotti 2015, Tian et al. 2015).

**Melanomma japonicum** A. Hashim. & Kaz. Tanaka, sp. nov. MycoBank MB819613; Fig. 2.

**Etymology**: Referring to its country of origin, Japan.

Saprobic on dead twigs of woody plants. Sexual morph: Ascomata globose to ovoid, superficial, gregarious, 190–320 μm diam, 200–340 μm high. Ostiolar neck short papillate, composed of carbonaceous, thick-walled, black cells. Peridium 40–60 μm thick of two layers at side; outer layer 25–40 μm thick of elongate, thin-walled, 12–20 × 3–4 μm, brown cells; inner layer...
12.5–30 μm thick of globose to rectangular, 10–17.5 × 5–7 μm, hyaline cells; base of ascomata 40–53 μm thick, of two layers; outer layer 15–30 μm thick of elongate, thin-walled, 3.5–7.5 × 3.5–5 μm, brown cells; inner layer 10–30 μm thick of globose to rectangular, 7–10.5 × 6–9 μm, brown cells. Pseudoparaphyses trabeculate, 0.5 μm wide, septate, branched and anastomosed. Asci bitunicate, fissitunicate, cylindrical, 73–105 × 5.5–9 μm (x = 89.9 × 7 μm, n = 26), with a short stipe (7–16 μm long, x = 10.3 μm, n = 20), apically rounded with an ocular chamber, 8-spored. Ascospores fusiform, with broad rounded ends, 12–19 × 3–7 μm (x = 15.1 × 4.6 μm, n = 151), l/w 2.5–4.9 (x = 3.4, n = 151), 3-septate, with a primary septum nearly median (0.44–0.57, x = 0.51, n = 75), olive brown, sometimes with paler ends, constricted at the septa, smooth. Asexual morph: Conidiomata pycnidial, globose to subglobose, up to 230 μm high in section, 150–250 μm diam, semi-immersed, solitary. Ostiolar neck short papillate, composed of thick-walled, black cells. Peridium 12–33.5 μm wide, composed of 8.5–16.5 × 3.5–7.5 μm, rectangular, brown cells. Conidiophores reduced to conidiogenous cells. Conidiogenous cells holoblastic, 8–13.5 × 2–3 μm, cylindrical, hyaline, smooth. Conidia cylindrical with rounded ends, 3–4 × 2.5–2.5 μm (x = 3.3 × 2.2 μm, n = 50), l/w 1.1–2.1 (x = 1.5, n = 50), hyaline, smooth, aseptate, guttulate when young.

Culture characteristics: Colonies on PDA attaining 25–27 mm diam within 21 d in the dark, floccose, centrally raised, smoke grey (Rayner 1970), grey olivaceous at centre; reverse smoke grey, grey olivaceous at margin (Fig. 8A); asexual morph formed. Specimens examined: Japan, Aomori, Hakodate, Okiagetai, on dead twigs of woody plant, 15 Apr. 2006, K. Tanaka, KT 2076 (HHUF 30539 paratype, ex-
paratype living culture CBS 142902; Akita, Kazuno, Hachimantai, Yakeyama, Mousen pass, on dead twigs of Fagus crenata, 24 Jun. 2012, K. Tanaka, KT 3028 (HHUF 30540, ex-paratype, paratype living culture CBS 142903); Kagoshima, Tanumizu, Mt. Oonogara, on dead twigs of Fagus crenata, 25 Oct. 2013, K. Tanaka, KT 3425 (HHUF 30541, ex-paratype living culture CBS 142904); Aomori, Hakkoda, near Yunotai, on dead twigs of woody plant, 21 Jul. 2001, Y. Harada (HHUF 265520 holotype designated here, ex-holotype living culture CBS 142905 = JCM 13124 = MAFF 239634).

Notes: This species is morphologically closest to *Me. pulvis-pyrius* in ascospore size, but the size of conidia of this species is slightly longer and slenderer (3–4 μm vs. (2–)12.5–3.5 μm long; 1.1–2.1 vs. 1.0–1.7 length/width). ITS sequences of these two species differed by 13 positions with one gap.

Melanomma pulvis-pyrius (Pers.) Fuckel, Jb. nassau. Ver. Naturk. 23–24: 160. 1870 (1869 – 1870). Fig. 3.

Saprobic on dead twigs of woody plants. Sexual morph: Ascomata globose to ovoid, 210–310(–410) μm diam. Ostiolar neck short papillate, composed of carbonaceous cells. Peridium 75–88 μm thick of two layers at side; outer layer 35–45 μm thick; inner layer 30–40 μm thick, 65–75 μm thick at base. Pseudoparaphyses trubeculate, 1–1.5 μm wide. Asci 71–92 × 5–8.5 μm (X = 82.1 × 6.3 μm, n = 14), with a short stipe (5–8 μm long; X = 5.7 μm, n = 12). Ascospores 11.5–15 × 4–5 μm (X = 13 × 4.2 μm, n = 75), Iw 2.5–3.6 (X = 3.1, n = 75), 3-septate, with a primary septum nearly median (0.45–0.58, X = 0.50, n = 75). Asexual morph: Conidiomata pycnidial, globose to subglobose, 160–300 μm diam, with a papillate ostiolar neck. Peridium 18.5–22 μm wide, composed of 4–16.5 × 2.5–5 μm, rectangular, brown cells. Conidiophores reduced to conidigenous cells. Conidigenous cells holoblastic, 8–17.5 μm × 1.5–4 μm, cylindrical, hyaline, smooth. Conidia cylindrical with rounded ends, (2–)2.5–3.5 × 2–2.5(–3) μm (X = 2.9 × 2.3 μm, n = 50), Iw 1.0–1.7 (X = 1.3, n = 50), hyaline, smooth, asepitate, guttulate when young.

Culture characteristics: Colonies on PDA attaining 22–24 mm diam within 21 d, floccose, fasciculate, centrally raised, pale olivaceous grey; reverse greyish sepia, olivaceous buff at margin (Fig. 8B); asexual morph formed.

Specimens examined: Japan, Aomori, Minamitsugaru, Owani, on dead twigs of Acer mono var. maynii, 1 Jul. 2006, K. Tanaka, KT 2110 (HHUF 30542, culture CBS 142906); Hitoshi, Zatoishi, on dead twigs of woody plant, 8 Jul. 2006, H. Yonezawa, KT 2113 (HHUF 30543, culture CBS 142907); Noheji, near Mt. Ebusi, on dead twigs of Fagus crenata, 2 Sep. 2015, A. Hashimoto et al., AH 375 (HHUF 30544, culture CBS 142908); Nishimeya, Oschirousawa stream, on dead twigs of woody plant, 25 Jun. 2007, K. Hirayama et al., KH 27 (HHUF 30545, culture CBS 142909); on dead twigs of woody plant, 21 Jul. 2007, K. Hirayama et al., KH 77 (HHUF 30546, culture CBS 142910); Kawaratai, Ooka-wazoe, on dead twigs of woody plant, 28 Aug. 2007, K. Hirayama et al., KH 86 (HHUF 30547, culture CBS 142911); on dead twigs of woody plant, 30 Aug. 2008, K. Hirayama et al., KH 197 (HHUF 30548, culture CBS 142912).

Notes: The above specimens were identified as *Me. pulvis-pyrius*, the type species of *Melanomma*. The size of ascospores in our materials was almost identical to that of *Me. pulvis-pyrius* reported by Holm (1957), who observed the neotype of this species. The rpb2 sequences of our isolates were identical or had one or two differences compared with those of *Me. pulvis-pyrius* (GU456350) obtained from the ex-epitotype culture (CBS 124080).

M melanomma pulvis-pyrius is a well-studied species in *Melanomma*; its taxonomy and ontogeny of sexual morphs have been described (Chesters 1938), and it has been reported worldwide (Holm 1957, Sivanesan 1984, Vassilieva 1987, Vasyagina et al. 1987, Romero 1998, Mathiassen 1989, 1993, Zhang et al. 2008, Mugambi & Huhndorf 2009, Jaklitsch & Voglmayr 2017). However, this is the first report of *Me. pulvis-pyrius* from Japan. This species was epitypified by Zhang et al. (2008) based on a specimen collected from *Salix caprea* in France.

In the phylogenetic tree, *Me. pulvis-pyrius* clustered with *Me. populinia* (CBS 543.70 and CBS 350.82) with moderate to strong support (93 % ML BP/1.00 Bayesian PP). Because we could not compare the characters of these two species, further study is needed in the future to confirm whether these two species are conspecific.

Pseudodidymellaceae A. Hashim. & Kaz. Tanaka, fam. nov. MycoBank MB819614.

Parasitic on living leaves of woody plants. Sexual morph: Ascomata subglobose to lenticular, immersed, ostiolate. Peridium pale brown to brown, distinctly thickened at base. Pseudoparaphyses septate, branched and anastomosed. Asci bitunicate, fissitunicate, cylindrical, 8-spored. Ascospores fusiform with rounded ends, straight, 1-septate, hyaline, smooth. Spermatia cylindrical, hyaline. Asexual morph: Propagules ephiphyllous, white to yellowish, globose to subglobose, multicellular, with numerous, flexuous, cylindrical, multi-septate hyphal appendages, detached at stroma-like base composed of sub-globose to oblong, hyaline to yellow cells. Synasexual morph: Conidiomata sporodochial, superficial. Stromata composed of globose to subglobose cells. Conidiophores reduced. Conidigenous cells annelidic or holoblastic. Conidia clavate, scomploid or rounded to oval or broadly ellipsoidal, phragmosporous to muriform, hyaline to brown, falcate to sigmoid.

Type genus: Pseudodidymella C.Z. Wei et al.

Notes: Mycodidymella, Petrikaia, Pseudodidymella, and Xenostigma have mycopappus-like propagules in their life cycles. Although sexual morphs of these genera were reported, and several molecular studies were performed, the phylogenetic placement of these genera remains unresolved (Crous et al. 2009, Butin et al. 2013, Li et al. 2016, Gross et al. 2017). According to the multi-locus phylogenies, these genera are closely related to each other (Li et al. 2016, Gross et al. 2017, Jaklitsch & Voglmayr 2017). Based on phylogenetic study, Phookamsak et al. (2014) proposed to include Petrikaia and Xenostigma in Melanommataceae. Tian et al. (2015) accepted these two genera in Melanommataceae in a subsequent study. In our study, the monophyly of these four genera with mycopappus-like propagules was strongly supported (91 % ML BP/1.00 Bayesian PP; Fig. 1). Therefore, we introduce a new family, Pseudodidymellaceae, to accommodate the above four genera. Species in this family bear several common features, including sexual morphs with lenticular and subcuticular ascomata erumpent from host tissue, asexual morphs with mycopappus-like propagules, and with or without a synasexual morph that has sporodochial conidiomata. Pseudodidymellaceae can be distinguished from Melanommataceae sensu stricto based on the presence of mycopappus-like propagules.
**Mycodidymella** C.Z. Wei et al., Mycologia 90: 336. 1998.  
**Synonym:** Blastostroma C.Z. Wei et al., Mycologia 90: 337. 1998.

Parasitic on living leaves of woody plant. Sexual morph: Ascomata subglobose to lenticular, immersed, ostiolate. Peridium with rim-like side wall, composed of rectangular, thin-walled, pale brown cells, well-developed at base. Pseudoparaphyses septate, branched and anastomosed. Asci bitunicate, fissitunicate, cylindrical, 8-spored. Ascospores fusiform, 1-septate, hyaline, smooth. *Spermatia* cylindrical, hyaline. Asexual morph: Propagules epiphyllous, white to yellowish, globose to subglobose, multicellular; main bodies subglobose to oblong, bearing numerous, unbranched, flexuous, cylindrical, multi-septate hyphal appendages. Synasexual morph: Conidiomata sporodochial, white to yellowish. Stromata composed of globose to subglobose cells. Conidiophores absent. Conidiogenous cells holoblastic, hyaline. Conidia falcate to sigmoid, hyaline, multi-septate, obtuse at the apex, truncate at the base.

**Type species:** *Mycodidymella aesculi* C.Z. Wei et al.

**Mycodidymella aesculi** C.Z. Wei et al., Mycologia 90: 336. 1998.  
**Fig. 4.**  
**Synonyms:** Blastostroma aesculi C.Z. Wei et al., Mycologia 90: 338. 1998.  
*Mycopappus aesculi* C.Z. Wei et al., Mycologia 90: 336. 1998.

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**Melanomma pulvis-pyrius.** A, B. Ascomata on substrate.  
C. Ascoma in longitudinal section.  
D. Lateral peridium of ascoma.  
E. Basal peridium of ascoma.  
F. Pseudoparaphyses.  
G. Ascus.  
H. Apex of ascus.  
I. Stipe of ascus.  
J, K. Ascospores.  
L. Germinating ascospore.  
M, N. Conidiomata in culture.  
O. Conidioma in longitudinal section.  
P. Peridium of conidioma.  
Q, R. Conidiogenous cells.  
S. Conidia.  
T. Germinating conidia.

Fig. 3. Scale bars: A, M = 500 μm; B, N = 200 μm; C, O = 100 μm; D, E, P = 10 μm; F–L, Q–T = 5 μm.
Parasitic on living leaves of Aesculus turbinata. Sexual morph: Ascomata subglobose to lenticular, solitary to 3–5 grouped, immersed, up to 210 μm high, 260–380 μm diam. Ostiolar neck short papillate, composed of thick-walled, black cells. Peridium 17.5–27.5 μm thick at side, with rim-like side wall, composed of rectangular, thin-walled, 10–13.5 x 6–9 μm, pale brown cells, at base 105–140 μm thick, composed of 8.5–11.5 x 6.5–8.5 μm, hyaline to pale brown cells. Pseudoparaphyses numerous, trabeculate, 0.8–1.3 μm wide, septate, branched and anastomosed. Ascii bitunicate, fissitunicate, cylindrical, 45.5–60 x 7–12.5 μm (x = 53.3 x 10 μm, n = 20), with or without a short stipe, apically rounded with an ocular chamber, 8-spored. Ascospores fusiform with rounded ends, straight, 16–21.5 x 3–4.5 μm (x = 18.6 x 3.9 μm, n = 21), with 4.3–5.3 μm (x = 4.7, n = 21), with a septum nearly median (0.44–0.55, x = 0.51, n = 21), constricted at the septum, hyaline, smooth, guttulate when young. Spermatia 3–5 x 1–2 μm (x = 3.6 x 1.5 μm, n = 50), with 7.5–10 μm diam cells; hyphal appendages 15 to 37, unbranched, cylindrical, 3–7-septate, 72–150 x 3.5–5.5 μm (x = 111.5 x 4.6, n = 30). Synasexual morph: Conidiomata sporodochial, white to yellowish. Stromata 15–20 μm thick, composed of hyaline, glose to subglose cells. Conidiophores reduced to conidigenous cells. Conidigenous cells holoblastic, hyaline, smooth, 9–12 x 4.5–5.5 μm. Conidia falcate to sigmoid, 57–94 x 5.5–8.5 μm (x = 75.8 x 6.8, n = 50), hyaline, 8–13-septate, obtuse at the apex, truncate at the base.

Culture characteristics: Colonies on PDA attaining 31–40 mm diam within 21 d, velvety, floccose, centrally raised, buff, grey oliveaceous at centre; reverse buff; grey oliveaceous at centre (Fig. 8C); spermatial, asexual and synasexual morphs formed.

Specimens examined: Japan, Aomori, Minamitsugaru, Owani, on living leaves of Aesculus turbinata, 12 Aug. 2012. K. Tanaka et al., KT 306 (HHUF 30549, culture CBS 142913); Nishimeya, Kawaratai, Ookawazoe, near Annmon waterfall, 22 Aug. 1995, C. Z. Wei & Y. Harada (HHUF 22892 holotype of Blastostroma aesculi); 10 Sep. 2016, A. Hashimoto, AH 560 (HHUF 30550, culture CBS 142914); Hirakawa, Ikarigaseki, on living leaves of Aesculus turbinata, 18 Apr. 1995, C. Z. Wei & Y. Harada, H 2610 (HHUF 22892 holotype of Mycodidymella aesculi, ex-holotype living culture CBS 142914); on living leaves of Aesculus turbinata, 19 Apr. 1995, C. Z. Wei & Y. Harada, H 2620 (culture CBS 142915).

Notes: The genus Mycodydymella was established to accommodate a single species, Mycod. aesculi, and this species causes large concentric leaf spots on Aesculus turbinata in Japan (Wei et al. 1998). This species is morphologically characterised by lenticular ascomata and 1-septate, hyaline ascospores in the sexual morph, mycopappus-like propagules in the asexual morph, and blastostroma-like sigmoid conidia in the synasexual morph. The sexual morph of this species morphologically resembles those of Didymella or Pseudodidymella. Wei et al. (1998) assigned this genus to Phaeosphaeriaceae based on morphology. Later, familial placement of this genus was treated as incertae sedis in Dothideomycetes (Lumbsch & Huhndorf 2007). Recently, Butin et al. (2013) described the sexual morph of Pe. echinata, which is the type species of Petroka; they found that the sexual morphology of Petroka matches that of Mycodydymella and thus synonymised Mycodydymella with Petroka (Butin et al. 2013). This proposal was accepted by subsequent studies (Tian et al. 2015, Li et al. 2016, Jaklitsch & Voglmayr 2017). However, Mycod. aesculi was not included in their analyses. Our phylogenetic study revealed that their monophyletic status was not supported in any analyses (below 60 % ML BP/0.95 Bayesian PP, Fig. 1). We retained Mycodydymella as a natural genus in Pseudodidymellaceae (discussed below).

Pseudodidymella C.Z. Wei et al., Mycologia 89: 496. 1997. Synonym: Pycnopleiospora C.Z. Wei et al., Mycologia 89: 496. 1997.

Parasitic on living leaves of Fagus spp. Sexual morph: Ascomata subglobose to lenticular, solitary to grouped, immersed, ostiolate. Peridium composed of rectangular, thin-walled, pale brown cells, well-developed at base. Pseudoparaphyses septate, branched and anastomosed. Asci bitunicate, fissitunicate, cylindrical, 8-spored. Ascospores fusiform with rounded ends, 1-septate, hyaline, smooth. Spermatia cylindrical, hyaline. Asexual morph: Propagules epiphyllous, white to yellowish, globose to subglobe, 200–565 μm diam (x = 331.9 μm, n = 30); main bodies subglobe to oblong, 85–193 x 116–228 μm (x = 127.6 x 152.4, n = 30), composed of 7.5–10 μm diam cells; hyphal appendages 19 to 37, unbranched, flexuous, cylindrical, 3–7-septate, 72–150 x 3.5–5.5 μm (x = 111.5 x 4.6, n = 30). Synasexual morph: Conidiomata sporodochial, white to yellowish. Stromata 15–20 μm thick, composed of hyaline, glose to subglose cells. Conidiophores reduced to conidigenous cells. Conidigenous cells holoblastic, hyaline, smooth, 9–12 x 4.5–5.5 μm. Conidia falcate to sigmoid, 57–94 x 5.5–8.5 μm (x = 75.8 x 6.8, n = 50), hyaline, 8–13-septate, obtuse at the apex, truncate at the base.

Culture characteristics: Colonies on PDA attaining 31–40 mm diam within 21 d, velvety, floccose, centrally raised, buff, grey oliveaceous at centre; reverse buff; grey oliveaceous at centre (Fig. 8C); spermatial, asexual and synasexual morphs formed.

Specimens examined: Japan, Aomori, Minamitsugaru, Owani, on living leaves of Aesculus turbinata, 12 Aug. 2012. K. Tanaka et al., KT 306 (HHUF 30549, culture CBS 142913); Nishimeya, Kawaratai, Oikawazoe, near Annmon waterfall trail, on living leaves of Aesculus turbinata, 4 Oct. 1995, C. Z. Wei & Y. Harada (HHUF 23078 holotype of Blastostroma aesculi); 10 Sep. 2016, A. Hashimoto, AH 560 (HHUF 30550, culture CBS 142914); Hirakawa, Ikarigaseki, on living leaves of Aesculus turbinata, 18 Apr. 1995, C. Z. Wei & Y. Harada, H 2610 (HHUF 22892 holotype of Mycodidymella aesculi, ex-holotype living culture CBS 142914); on living leaves of Aesculus turbinata, 19 Apr. 1995, C. Z. Wei & Y. Harada, H 2620 (culture CBS 142915).

Notes: The genus Mycodydymella was established to accommodate a single species, Mycod. aesculi, and this species causes large concentric leaf spots on Aesculus turbinata in Japan (Wei et al. 1998). This species is morphologically...
the original description (Wei et al. 1997) seemed to misinterpret over-mature propagules. They also confirmed that *Pseudodidyrmella* is phylogenetically related to other mycopappus-forming genera, such as *Mycocididymella*, *Petrakia*, and *Xenostigmina*, based on the ITS phylogeny. Thus, morphological delimitation of *Pseudodidyrmella* and *Mycocididymella* is problematic and requires further research. In the present study, we recollected *Pseudodidymella* from its type locality, and compared the fresh materials to the holotype of *Py. fagi*. Based on morphological and phylogenetic comparisons of these specimens, we also conclude that *Wei et al. (1997)* misinterpreted the pieces of broken overmatured mycopappus-like propagules (Fig. 5A and AB) as conidia of *Pseudodidymella*, but *Pseudodidymella* actually has mycopappus-like propagules in its asexual morph.

Species in this genus bear common features, with more than 60 hyphal appendages in mycopappus-like propagules. Although other related genera have sporodochial synasexual morphs, no synasexual morph is known from *Pseudodidymella* (Wei et al. 1997, Gross et al. 2017, present study). Morphologically, *Pseudodidymella* resembles *Mycocididymella*, but can be distinguished based on the rim-like walls of the ascomata, and numerous hyphal appendages in the asexual morph.

**Pseudodidymella fagi** C.Z. Wei et al., Mycologia 89: 496. 1997.

*Fig. 5.*

**Synonym:** Pyncnopleiospora fagi C.Z. Wei et al., Mycologia 89: 496. 1997.

Parasitic on living leaves of *Fagus crenata*. Sexual morph: Ascomata subglobose to lenticular, solitary to 3–5 grouped, immersed, up to 175 μm high, 200–300 μm diam. Ostiolar neck short papillate, composed of thick-walled, black cells. **Peridium** 20–22 μm thick at side, composed of rectangular, thin-walled, 7.5–10.5 × 6.5–8.5 μm, pale brown cells, at base 58–67 μm thick, composed of 10–13.5 × 5–11.5 μm, hyaline to pale brown cells. **Pseudoparaphyses** numerous, 1–2 μm wide, septate, branched and anastomosed. Asci bitunicate, fissitunicate, cylindrical, 49–76.5 × 10–14 μm (× 60.3 × 11.5 μm, n = 20), with a short stipe (3.5–8 μm long, × 6.1 μm, n = 20), apically rounded with an ocular chamber, 8-spored. Ascosporas fusiform with rounded ends, straight, 18.5–24 × 4–5 μm (× 20.5 × 4.3 μm, n = 20), I/w 4.3–6.6 (× 4.8, n = 20), with a septum nearly median (0.47–0.58, × 0.52, n = 20), constricted at the septum, hyaline, smooth, glutulate when young. Spermatia 3–5 × 1–1.5 μm (× 3.9 × 1.2 μm, n = 50), I/w 2.1–4.8 (× 3.3, n = 50), cylindrical, hyaline. Asexual morph: **Propagules** epiphylous, white to yellowish, globose, 290–500 μm diam (× 387.2 μm, n = 30); main bodies globose, 160–315 μm diam (× 227.4 μm, n = 30), composed of subglobose, hyaline to yellow, 11.5–15 × 7.5–11.5 μm cells; hyphal appendages 63 to 138, unbranched, flexuous, cylindrical, 1–4-septate, 67–133 × 3–5 μm (× 97.1 × 3.7 μm, n = 52).

**Culture characteristics:** Colonies on PDA attaining 27–38 mm diam within 21 d, floccose, plane, smoke grey; reverse honey to isabelline (Fig. 5E); asexual morph formed.

*Specimens examined:* **Japan**, Aomori, Nakatsugaru, Onikawabe, on living leaves of *Fagus crenata*, 12 Aug. 2012, K. Tanaka et al., KT 3058 (HHUF 30515, culture CBS 142917 = MAFF 245738); Nishimeya, Okawazoe, near Annmon waterfall, on living leaves of *Fagus crenata*, 2 Sep. 2012, K. Tanaka et al., KT 3074-3 (HHUF 30516, culture CBS 142918 = MAFF 245739); 2 Sep. 2012, R. Fujimoto et al., RF 5 (HHUF 30517, culture CBS 142919 = MAFF 245741); 10 Sep. 2016, A. Hashimoto, AH 561 (HHUF 30553, culture CBS 142620); Hirakawa, Kikugaseki, on living leaves of *Fagus crenata*, 28 Apr. 1995, C. Z. Wei & Y. Harada, H 2579 (HHUF 22903, **holotype** living culture MAFF 245740); artificial inoculation on leaves of *Fagus crenata*, 30 Sep. 1996, C. Z. Wei (HHUF 23672, **holotype** of *Pyncnopleiospora*).

Notes: This species was originally reported to cause brown leaf spots on *Fagus crenata* in Japan. More recently, it was reported from a new host, *F. sylvatica* (Gross et al. 2017). To elucidate its host spectrum, further surveys for this fungus and other species on *Fagus* is needed.

**Pseudodidymella minima** A. Hashim. & Kaz. Tanaka, sp. nov. MycoBank MB819615. *Fig. 6.*

**Etymology:** Referring to the smaller-sized propagules observed in this species.

Parasitic on living leaves of *Fagus japonica*. Sexual morph: Unknown. Asexual morph: **Propagules** epiphylous, white to yellowish, globose, 110–220 (–240) μm diam (× 164.4 μm, n = 60); main bodies globose, multicellular, 78–168 μm diam (× 115 μm, n = 60), composed of subglobose, 7.5–10 μm diam, hyaline to yellow cells; hyphal appendages 65 to 135, unbranched, flexuous, cylindrical, 1–2-septate or rarely aseptate, 27–44 × 3–6 μm (× 35.5 × 4.4 μm, n = 59).

**Culture characteristics:** Colonies on PDA attaining 32–38 mm diam within 21 d, floccose, plane, smoke grey; reverse honey to isabelline (Fig. 6E); asexual morph formed.

*Specimens examined:* **Japan**, Iwate, Hanamaki, near Dai spa, on living leaves of *Fagus japonica*, 9 Oct. 2011, K. Tanaka, KT 2918 (HHUF 30551, **holotype** designated here; ex-holotype living culture CBS 142921 = MAFF 246249); 3 Sept. 2016, A. Hashimoto, AH 556 (HHUF 30552, ex-paratype living culture CBS 142922).

Notes: This species on *Fagus japonica* is easily distinguished from *Pseudod. fagi* on *F. crenata* by its much smaller propagules. Sequence differences between these two species were found at six nucleotide positions with one gap in the ITS sequences.

We did not observe the sexual or synasexual morph of *Pseudod. minima*. Further surveys are therefore needed to reveal the ecological features of this species.

**Xenostigmina aceris** (Deam. & Barthol.) A. Hashim. & Kaz. Tanaka, comb. nov. MycoBank MB821403.

**Basionym:** *Cercosporella aceris* Deam. & Barthol., Mycologia 9: 362. 1917.

**Synonyms:** *Mycopappus aceris* (Deam. & Barthol.) Redhead & G.P. White, Canad. J. Bot. 63: 1430. 1985.

*Petrakia aceris* (Deam. & Barthol.) Jaklitsch & Voglmayr, Sydowia 69: 90. 2017.

**Stigmatina zilleri** A. Funk, Canad. J. Bot. 65: 482. 1987.

**Xenostigmina zilleri** (A. Funk) Crous, Mycol. Mem. 21: 155. 1998.

**Mycosphaerella mycopappi** A. Funk & Dorworth, Canad. J. Bot. 36: 362. 1917.

**Notes:** This new combination is described as *Xenostigmina aceris* (Dearn. & Barthol.) Jaklitsch & Voglmayr, Sydowia 69: 90. 2017. Further studies are therefore needed to reveal the ecological features of this species.
Fig. 5. Pseudodidymella fagi. A, B. Ascomata on substrate. C. Ascoma in longitudinal section. D. Peridium of ascoma. E. Ascus. F. Apex of ascus. G. Stipe of ascus. H, I. Ascospores. J–L. Spermatogonia in culture. M. Spermatogonium in longitudinal section. N. Peridium of spermatogonium. O, P. Spermatogenous cells. Q, R. Spermatia. S, T. Leaves of Fagus crenata with necrotic brown spots. U, V. Propagules on the leaf surface. W, X. Propagules. Y–AB. Appendages of propagule. A–I from HHUF 22903. J–R from culture CBS 142917 = MAFF 245738. S, T from HHUF 30553. U, X, AA, AB from HHUF 30516. V, W, Z from HHUF 23672. Y from HHUF 30517. Scale bars: A, J, T = 500 μm; B, K, L, U, V = 250 μm; C, M, W, X = 50 μm; D, E, N, Y–AB = 10 μm; F–I, O–R = 5 μm.
Didymella mycopappi (A. Funk & Dorworth) Crous, Mycol. Mem. 21: 152. 1998.

Notes: Xenostigmina zilleri is the name that has been commonly used for this pathogen, although the epithet of Cercosporella aceris is older than that of Stigmina zilleri (Crous 1998, Crous et al. 2009, Phookamsak et al. 2014, Tian et al. 2015, Gross et al. 2017). Therefore, we proposed a new combination, Xenostigmina aceris.

Incertae sedis

Alpinaria Jaklitsch & Voglmayr, Sydowia 69: 84. 2017.

Saprobic on dead twigs of woody plants. Sexual morph: Ascomata globose to ovoid, immersed to superficial, gregarious, sometimes confluent, ostiolate. Peridium composed of elongate, thin-walled, brown cells, at base composed of elongate, hyaline cells. Pseudoparaphyses septate, branched and anastomosed. Asci bitunicate, cylindrical, 8-spored. Ascospores fusiform, multi-septate, smooth. Asexual morph: Conidiomata pseudopycnidial, globose to cylindrical, sometimes deformed, septe, confluent, multi-loculate, scattered, semi-immersed, black, with one to two non-papillate ostiole. Peridium rectangular, brown cells. Conidiophores absent. Conidiogenous cells holoblastic, cylindrical, hyaline, smooth. Conidia cylindrical with rounded ends, hyaline, smooth, aseptate.

Type species: Alpinaria rhododendri (Niessl) Jaklitsch & Voglmayr.

Alpinaria rhododendri (Niessl) Jaklitsch & Voglmayr, Sydowia 69: 84. 2017. Fig. 7.

Basionym: Cucurbitaria rhododendri Niessl, Verh. Nat. Ver. Brünn 10: 200. 1872.

Synonyms: Gibberidea rhododendri (Niessl) Petr., Ann. Mycol. 32: 330. 1934; nom. illegit.

Melanomma rhododendri Rehm, Ber. Naturhist. Ver. Augsburg 26: 48. 1881.
**Gibberidea rhododendri** (Rehm) Petr., Krypt. Forsch. (München) 2: 160. 1931.

**Gibberidea rhododendri** (Rehm) Kirschst., Hedwigia 81: 206, 1944; nom. illegit.

Saprobic on dead twigs of ericaceous plants. Sexual morph: Ascomata globose to ovoid, immersed, becoming largely erumpent to superficial, gregarious, sometimes confluent, 140–190 μm high, 110–250 μm diam. Ostiolar neck short papillate, composed of carbonaceous, thick-walled, black cells. Peridium 55–75 μm thick at side composed of elongate, thin-walled, 12–13 × 5–6.5 μm, brown cells, 87–102 μm thick at base composed of elongate, thin-walled, 4–6 μm diam, hyaline cells. Pseudoparaphyses trabeculate, 1–1.5 μm wide, septate, branched and anastomosed. Asci bitunicate, cylindrical, 100–116 × 7–9 μm (x = 109.5 × 7.8 μm, n = 11), with a short stipe (3.5–10 μm long, x = 7 μm, n = 11). Ascospores fusiform, 13–21 × 5–6 μm (x = 16.5 × 5.6 μm, n = 50), l/w 2.2–4.2 (x = 3.0, n = 50), 3-septate, with a primary septum nearly median (0.42–0.57, x = 0.50, n = 50) and constricted, smooth, without sheath. Asexual morph: Conidiomata pseudopycnidial, globose to cylindrical, sometimes deformed, septate, confluent, multiloculate, scattered, semi-immersed, black, up to 190 μm high, 110–250 μm diam. Ostiolar neck mainly single, occasionally two, non-papillate. Peridium 20–25 μm wide, composed of 7.5–11.5 × 5–7 μm, rectangular, brown cells. Conidiophores reduced to conidiogenous cells. Conidiogenous cells holoblastic, 6–10.5 × 3–4.5 μm, cylindrical, hyaline, smooth. Conidia cylindrical with rounded ends, 2–4 × 1–2 μm (x = 3 × 1.6 μm, n = 50), l/w 1.1–2.6 (x = 1.9, n = 50), hyaline, smooth, aseptate, guttulate when young.

**Culture characteristics**: Colonies on PDA attaining 26–31 mm diam within 21 d, velvety, wet, olivaceous black, smoke grey at margin; reverse olivaceous black at centre (Fig. 6F); asexual morph formed.

Specimen examined: **Japan**, Iwate, Hachimantai, Yakeyama, near Goshogake spa, on leaf bud of *Rhododendron brachycarpum*, 9 Jul. 2008, Y. Harada, KT 2520 (HHUF 30554; culture CBS 142901).

Notes: The ascospore size in the material mentioned above is identical to that of *A. rhododendri* reported by Jaklitsch &
Voglmayr (2017), who designated the epitype of this species. The ITS, tef1 and rpb2 sequences from our material are completely identical to those from the ex-epitype strain of this species (CBS 141994). This species has been reported from twigs or buds of Rhododendron spp. in the Asia (R. chrysanthum; Müller 1959), Europe (R. ferrugineum and R. hirsutum; Jaklitsch & Voglmayr 2017), and North America (Rhododendron sp.; Mugambi & Huhndorf 2009). In addition, we collected this species on R. brachycarpum from the subalpine zone in Japan. Alpinaria rhododendri appears to be a relatively common species in the subalpine to alpine zone worldwide.

Alpinaria was recently established to accommodate a single species A. rhododendri, which was transferred from Melanomma because this species is phylogenetically distinct from the type species of Melanomma, and possesses ascomata with a roughened surface view of textura prismatica and textura angularis (Jaklitsch & Voglmayr 2017). Furthermore, they treated the genus as a member of Melanommataceae (Jaklitsch & Voglmayr 2017). Although no asexual morph was reported for this species (Müller 1959, Mugambi & Huhndorf 2009, Jaklitsch & Voglmayr 2017), we newly observed its asexual morph in culture (Fig. 7L–S). As a result of our observation of the asexual morph, as well as the sexual morph, we clarified that this species has atypical features for Melanommataceae; its ascomata are composed of hyaline cells at the base, and are pseudopycnidial. Confluent conidiomata are not found in sexual/sexual morphs of Melanommataceae. In our phylogenetic tree, the genus placement is confirmed outside Melanommataceae sensu stricto (Fig. 1). Therefore, we treat Alpina as incertae sedis in Pleosporales in this study; additional taxa related to this genus will be needed to resolve its familial placement.

**DISCUSSION**

Re-circumscription of Melanommataceae sensu stricto

Melanommataceae has been extensively studied in recent years based on phylogenetic evidence (Mugambi & Huhndorf 2009, Schoch et al. 2009, Wijayawardene et al. 2012, 2014, Butin et al. 2013, de Gruyter et al. 2013, Su et al. 2015, Tian et al. 2015, Li et al. 2016, Gross et al. 2017, Jaklitsch & Voglmayr 2017). The characters emphasised for members of this family include a carbonaceous peridium of ascomata and trabecular pseudoparaphyses. These species are known saprobes on decaying plant material, or, rarely, as plant pathogens. The familial concept of Melanommataceae was revised and expanded after in a study by Mugambi & Huhndorf (2009), who applied a molecular approach. A recent monograph of Melanommataceae was based on morphological and multi-gene phylogenetic data (Tian et al. 2015). Although monophyly of Melanommataceae was confirmed in previous studies, statistical support for Melanommataceae sensu lato was lacking (Mugambi & Huhndorf 2009).
2009, Schoch et al. 2009, Tian et al. 2015). Additionally, previous authors did not examine the asexual morphs, although various asexual morphs, such as those with mononematous, synnematous, and pycnidial conidiomata, are known to occur in this family. Two of the most striking genera are Petraokia and Xenostigma, which have mycopappus-like propagules as asexual morphs, and were reported to be folliculicolous necrotrophs (Funk 1986, Funk & Dorworth 1988, Crous 1998, Crous et al. 2009, Butin et al. 2013), whereas species of Melanomma, the type genus of this family, have aspathophoria-like pycnidial asexual morphs and are known to be saprobies on twigs of various plant hosts (Chesters 1938, Romero 1998, Zhang et al. 2008). Our multi-gene phylogenetic analyses of this family clearly showed the poly- and paraphyletic nature of Melanommataceae sensu lato (Fig. 1), and morphological observations of sexual and asexual morphs led to the conclusion that Melanommataceae should be restricted to the type genus Melanomma. In addition, four genera with mycopappus-like propagules in their asexual morphs (Mycodeidymella, Petraokia, Pseudodidymella, and Xenostigma) are separated from Melanommataceae sensu stricto, and we thus establish a new family, Pseudodidymellaceae, to accommodate these genera.

Relationships among genera in Pseudodidymellaceae

Mycodeidymella and Xenostigma are retained as natural genera in the present study. Butin et al. (2013) found that the sexual morph of Mycodeidymella is similar to that of Petraokia, and thus recognised Petraokia in a broad sense and included Mycodeidymella as a synonym. This treatment was supported by a later study (Li et al. 2016). Gross et al. (2017) showed these three genera are closely related based on an ITS phylogeny, but no taxonomic conclusions about their generic validities were made. Recently, Jaklitsch & Voglmayr (2017) proposed that Mycodeidymella and Xenostigma are synonyms of Petraokia. They considered that phylogenetic relatedness of Xenostigma and Petraokia, and morphological similarity of the sexual morph and mycopappus-like propagules among these genera are strong arguments for synonymising them (Jaklitsch & Voglmayr 2017). Our phylogenetic analysis including Mycodeidymella as well as Xenostigma and Petraokia clarified that their monophyletic status was not well supported in any analyses (below 60 % ML BP/ 0.95 Bayesian PP, Fig. 1). Their sexual morphs are superficially similar as indicated by Jaklitsch & Voglmayr (2017), but Mycodeidymella has deeper and more well-developed ascomata (up to 210 μm high) than those of Petraokia (up to 150 μm high) and Xenostigma (up to 100 μm high). Additionally, their morphological characters of their synasexual morphs are also different: hyaline, up to 20 μm thick sporodochia, holoblastic conidiogenous cells, and sigmoid, multi-septate, thin-walled, hyaline conidia (Myco didymella; this study); brown, up to 30 μm thick sporodochia, annellidic conidiogenous cells, and globose to ovoid, dictyosporous, thick-walled, brown conidia with cellular appendages (Petraokia; Butin et al. 2013, Li et al. 2016); and brown to black, up to 45 μm high sporodochia, holoblastic conidiogenous cells, and clavate with a short rostrum, dictyosporus, thick-walled, brown conidia (Xenostigma; Funk 1986, Crous 1998). Therefore, we treat these genera as distinct based on morphological differences of sexual and synasexual morphs.

Synasexual morphs of these three genera are produced after leaves fall in late autumn (Funk & Dorworth 1988, Wei et al. 1997, Butin et al. 2013, Gross et al. 2017). Conidia of synasexual morphs were not observed on overwintered leaves for Petraokia and Mycodeidymella, and their function in the disease cycle during the winter season has not been clarified (Wei et al. 1997, Butin et al. 2013). No synasexual morph is known from Pseudodidymella, despite their close relationship to the other three genera. Further studies on the Pseudodidymella synasexual morph are needed to elucidate the whole life cycle of this genus and produce robust taxonomic classifications for Pseudodidymellaceae.

Form and function of mycopappus-like propagules

The genus Mycappus was established based on its type species Mycappus alni (on Alnus, Betula, Crataegus, and Pyrus; Redhead & White 1985, Braun et al. 2000, Takahashi et al. 2006), which produces epiphyllous, multicellular propagules in its asexual morph (Redhead & White 1985). Later, three species were assigned to in this genus: Mycappus aceris (on Acer macrophyllum; Redhead & White 1985), Mycappus aesculi (on Aesculus turbinata; Wei et al. 1998), and Mycappus quercus (on Quercus acutissima; Suto & Kawai 2000). Two species, Mycappus alni and Mycappus quercus, produce microconidia and sclerotia in culture (Redhead & White 1985, Suto & Kawai 2000), and the sexual morph of the latter species is characterised by stipitate apothecia and inoperculate asci (Suto & Suyama 2005). Mycopappus alni was suggested to be a member of Sclerotiniaceae (Helotiales, Leotiomyces) based on its sclerotial morph and phylogenetic analyses using ITS sequences (Takahashi et al. 2006). The two other species, Mycappus aceris and Mycappus aesculi, were excluded from Mycopappus sensu stricto, because their sexual morphs belong to the dothideomycetous taxa, namely Xenostigma aceris (Funk & Dorworth 1988, Crous 1998, Crous et al. 2009) and Mycopappus aesculi (Wei et al. 1998), respectively. Morphological differences in mycopappus-like propagules among these lineages were indicated in a previous study (Suto & Kawai 2000). The main bodies of sclerotinaceous species (Mycop. aesculi and Mycapp. quercus) are composed of multi-septate claviform cells (Suto & Suyama 2005, Takahashi et al. 2006), whereas those of dothideomycetous species (Mycop. aesculi and X. aceris) are composed of aseptate globose cells (Redhead & White 1985, Wei et al. 1998). The morphological resemblance of mycopappus-like propagules between leotiomycetous and dothideomycetous lineages appears to be the result of convergent evolution due to similar ecological function, such as rain-splash dispersal across the leaf surface. A similar situation was reported in two phylogenetically distinct genera, Spiroplana (Dothideomycetes) and Siprosphaera (Leotiomyces), which have spirally coiled, buoyant conidia that resulted in adaptation to water dispersal in terrestrial or aeroaquatic environments (Voglmayr et al. 2011).

The mycopappus-like propagules of Pseudodidymellaceae may contribute to secondary infection of host leaves with high inoculum potential. Wei et al. (1998) suggested that this morph plays an important role in disease development. Morphological variation of the propagules at the generic level was observed, but the taxonomic significance was not been examined in several studies (Redhead & White 1985, Wei et al. 1998, Butin et al.
Future studies

The asexual genus Seifertia on Rhododendron spp. is characterised by synnematous conidiomata with cladosporium-like conidia (Li et al. 2016). Phylogenetic relatedness of this genus to members of Pseudodidymellaceae was suggested (Li et al. 2016, Gross et al. 2017). However, we prefer to not include this species in Pseudodidymellaceae and place it incertae sedis, because of the lack of mycopappus-like propagules in the life cycle. This genus might represent a new family; however, analysis of its sexual morph and further taxa related to this genus are needed to determine its familial placement. Another genus, Alpinaria, was originally established to accommodate the type species, A. rhododendri, which was segregated from Melanomma (Jaktlitsch & Voglmayr 2017). They regarded the genus as a member of Melanommataceae, based on phylogenetic analyses (Jaktlitsch & Voglmayr 2017). In the present study, we newly observed the asexual morph of Alpinaria, which had not been reported in previous studies (Müller 1959, Holm 1968, Mugambi & Huhndorf 2009, Jaktlitsch & Voglmayr 2017). According to our phylogenetic analyses and morphological observations, this species is distantly related to Melanommataceae sensu stricto (Fig. 1) and has atypical features for Melanommataceae, such as hyaline cells at the base of ascomata and pseudopycnidal conidiomata. Several melanomma-like fungi that possess well-developed carbonaceous ascomata may have evolved several times within Pleosporales, such as in Cyclothyriellaceae, Ohieniaceae, Nigrogranaeae, Teichosporaceae, Thyridariaceae (Jaktlitsch & Voglmayr 2016, Jaktlitsch et al. 2016b). It seems that familial circumscriptions based merely on sexual morph characters is insufficient to distinguish the members of Melanommataceae sensu lato.

The present study revealed unexpected diversity of Melanommataceae sensu Tian et al. (2015). Our approaches, which combined morphological features of both sexual and asexual morphs with molecular phylogenetic analyses, enabled a recircumscription of Melanommataceae sensu stricto and the establishment of Pseudodidymellaceae. To build a comprehensive taxonomic framework, further discovery of more specimens along with additional morphological and molecular data would help elucidate other unresolved lineages of Melanommataceae sensu lato.

ACKNOWLEDGEMENTS

This work was supported by funding from the Japan Society for the Promotion of Science (JSPS 26291034, 15H04491, 16J02743, and 16K07474). We thank Y. Harada for his help with collection of fungal specimens, and anonymous reviewers for their valuable comments and suggestions.

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