

# 全生活環に基づく子の菌類の 新たな分類体系構築

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## 1. 緒言

菌類は動物や植物に並ぶ主要な分類群の一つである。菌類は抗生物質、発酵食品や酒類の嗜好品など人類に有用な側面をもたらすが、水虫や肺炎などの真菌症、文化財や建築物の腐朽の原因菌や、農作物の減収に関わる植物病原菌などの経済的・社会的に脅威となる側面ももつ。これらの点から菌類は我々の生活に密接な関わり合いをもたらす存在であると考えられるが、把握できている菌類の種数は推定 150 万種のうちの 10 % にも満たず、生物多様性の把握は不十分な状態である (Hawksworth 1991, 2001)。この問題の原因の一つに菌類の分類学上の特殊性が存在する。

菌類最大のグループである子のう菌類は、その生活環の有性世代と無性世代で、それぞれ異なる形態をもつ。菌類の多型的生活環は、地衣類のスペルマチアとのつながりを指摘した Tulasne (1851) によって初めて報告された。その後の研究で de Bary (1854) は *Eurotium herbariorum* と *Aspergillus glaucus* が同一種の有性世代および無性世代の関係にあることを明らかにすることで同様の結論を示した。Selecta fungorum carpologia において、Tulasne & Tulasne (1861, 1863, 1865) の詳細な観察に基づく一連の研究は、高等菌類が有性世代と無性世代で明らかに異なった形態をもつことを示した。これらの研究により、不完全菌類が独立した分類群ではなく、高等菌類の別の姿であるという認識が一般に受け入れられた。しかしながら、有性世代が不明な分類群は、子のう菌類や担子菌類と同一のカテゴリーに並べることができないため、不完全菌類 (Fungi imperfecti) として扱われた (Fuckel 1873)。この分類学的提案は Saccardo (1882) の Sylloge Fungorum に採用された。

これまでに不完全菌類について分類体系を構築するためのいくつかの試みがなされてきた。Saccardo (1892) は不完全菌類の子実体の形態に着目して糸状不完全菌と分生子果不完全菌の 2 つに大別し、これをさらに分生子の形、細胞数や色に基づいて細分化した。分生子形成様式を重視した初期の研究は、Vuillemin (1910, 1911) によってなされた。その後、分生子形成様式は不完全菌類の分類学的再検討の中で、属レベルの分類形質として採用された (Mason 1928, 1933, 1937, 1941, Hughes 1953a, 1976, Subramanian 1971, Tubaki 1958, Cole & Samson 1979)。しかしながら、いずれの体系も子実体の連続的な変化を有する中間形質の発見、分生子形成様式が必ずしも系統を反映しない事例、分生子の隔壁数や付属物の数に基づく属の分類が系統を反映しない事例が報告されていることから人為的なものであることが明らかとなった (Okada & Tubaki 1958, Kendrick 1979a, b, Nag Raj 1993, Samuels & Seifert 1995, Okada 1998, Crous *et al.* 2012, Hashimoto *et al.* 2015a)。

子のう菌類は不完全菌類とは別に伝統的に有性世代の子実体の形態学的特徴に基づき分類されてきた (Luttrell 1951, Ainsworth *et al.* 1973, Wehmeyer 1975, von Arx & Muller 1975, Eriksson 1981, Reynolds 1981, von Arx 1981, Barr 1987, 1990, Hawksworth 1994). 近年では, 分子系統解析による裏付けのもとで門, 綱レベルの整備がされつつある (Lutzoni *et al.* 2004, Blackwell *et al.* 2006, James *et al.* 2006, Spatafora *et al.* 2006, Schoch *et al.* 2009). 一方で, 現行の分類体系は有性世代によって科を特徴付けているため, 無性世代しか知られていない不完全菌類の多くは分子系統解析に頼らなければ系統を推定できない状況にある (Shenoy *et al.* 2007; Crous *et al.* 2009). 全生活環に基づく分類体系構築の試みはすでに行われてきた (Malloch 1970, Malloch & Cain 1972, Kendrick 1979a, b, Sugiyama 1987). たとえば類似した有性世代の特徴をもつ閉鎖子のう菌類の *Microascaceae* と *Trichocomaceae* は, それらの無性世代の形態学的特徴から明瞭に区別された (scopulariopsis 型 vs. penicillium/aspergillus 型) (Malloch 1970, Malloch & Cain 1972). しかしながら, それらの試みの中で全生活環が判明している分類群が少ないこと, 形態学的特徴のみで不完全菌類の系統は推定できないことから, 全生活環に基づく分類体系は限定的にしか構築されなかった. 分子系統解析が登場した現在においても, 不完全菌類の属や種は系統を反映しない人為的な特徴付けに基づくため, 不完全菌類を従前の分類体系のシステムに単純に組み込むことはできない.

近年の分子系統解析に基づく子のう菌類の研究は多くの隠された系統群を明らかにし, いくつかの科を再検討している (Crous *et al.* 2015, Guatimosim *et al.* 2015, Knapp *et al.* 2015, Tanaka *et al.* 2015). しかしながら子のう菌類には構成する属が少ないため情報の乏しい科や, 系統的に明らかに異なるものの有性世代のわずかな違いでのみ区別されている科が少なからずある. また有性世代の形態学的特徴を過大評価しているために, 系統的に区別すべき分類群を見過ごしている可能性もある. すなわちこのようなケースにおいて, 分類群を正しく特徴付けるためには, 有性世代と従来無視されてきた無性世代の両世代によって, 科の枠組みを構築していく必要がある. そこで本研究では, (i) 未整理のままにある不完全菌類の属および種を形態観察と分子系統解析に基づき整理すること, (ii) 菌類多様性調査から未発見の系統群を新たに見出すことで情報の乏しい科や属の形態および生態情報を充実させること, (iii) 有性世代によって特徴付けられてきた従前の科に無性世代の情報を加えることで, 従前の科の概念の妥当性を再検討し, 科を両世代によって特徴付けることの重要性を示すことを目的とした.

## 2. *Pseudolachnea* 様菌類の分類学的再検討

### ABSTRACT

*Pseudolachnea* 属と *Pseudolachnella* 属の分類には問題が挙げられている。先行研究では両者を同属と見なす意見がある一方で、分生子の隔壁数のみに基づき区別できる属として見なす意見もある。本研究では *pseudolachnea* 様菌類 26 菌株について形態観察と ITS-LSU-*tef1* 領域の配列に基づく分子系統解析を行った。結果として、それらのタクサは 4 属に区別された。すなわち、*Pseudolachnea* 属、*Pseudolachnella* 属、そして新属である *Neopseudolachnella* 属と *Pseudodinemasporium* 属である。*Pseudolachnea* 属と *Pseudolachnella* 属の両属の単系統性は確かめられたが、それらの属を区別する特徴は分生子果の壁構造にあることを見いだした。分生子果の基部および外皮層の厚さのような違いは、分生子の隔壁数よりもそれらの属を区別する上で有用であると考えられた。*Neopseudolachnella* 属は *Pseudolachnea* 属や *Pseudolachnella* 属に分生子の形態が類似しているが、後者の 2 属のメンバーと異なり、分生子果に外皮層を欠く点で区別された。*Pseudodinemasporium* 属は *Dinemasporium* 属に類似した形態の分生子をもつが、後者に比べて著しく発達した分生子果の外皮層をもつ点で区別された。*Neopseudolachnella* 属に 3 新種 (*N. acutispora*, *N. magnispora*, *N. uniseptata*), *Pseudodinemasporium* 属に 1 新種 (*P. fabiforme*), *Pseudolachnella* 属に 8 新種 (*P. asymmetrica*, *P. botulispora*, *P. brevicoronata*, *P. campylospora*, *P. complanata*, *P. falcatispora*, *P. fusiformis*, *P. pachyderma*) の計 12 新種が記載された。

### INTRODUCTION

*Pseudolachnea* is a coelomycetous genus typified by *P. hispidula* (Ranojević 1910). This genus is characterised by acervular conidiomata surrounded by numerous black setae and one-septate conidia with an appendage at both ends, and the constituent species occur on twigs or wood of various angiosperms (Ranojević 1910, Sutton 1980, Nag Raj 1993). The sexual state of this genus has not been reported but it has been suggested to belong in the *Chaetosphaeriaceae*, *Sordariomycetes* from DNA sequence data (Crous *et al.* 2012).

Several genera with a morphological resemblance to *Pseudolachnea* are known. *Pseudolachnella* (Teng 1936) is a genus segregated from *Pseudolachnea* and was established to accommodate a species with multiseptate conidia, *Pseudolachnea scolecospora*. For the same reason Hino & Katumoto (1958) proposed a new genus *Chaetopatella* for *pseudolachnea*-like species with phragmoconidia, probably without knowledge of the publication of *Pseudolachnella*. Sutton (1977, 1980),

however, thought that conidial septation is not an important character for generic circumscription among *Pseudolachnea*, *Pseudolachnella* and *Chaetopatella* and merged the latter two genera with *Pseudolachnea*. On the other hand, Nag Raj (1993) divided *Pseudolachnea* sensu Sutton (1977, 1980) into *Pseudolachnea* with one-septate conidia and *Pseudolachnella* with multiseptate conidia and synonymised *Chaetopatella* with *Pseudolachnella*. The broad generic concept of *Pseudolachnea* (Sutton 1977, 1980) has been used by several authors (Castañeda 1987, Muthumary 1987, Kiffer & Morelet 1999, Kirk et al. 2008), whereas narrower interpretation of *Pseudolachnea* and *Pseudolachnella* placing emphasis on the differences in conidial septation (Teng 1936) also has been accepted (Nag Raj 1993, Mel'nik 1997, Zhao et al. 2004, Sato et al. 2008). In a phylogenetic study based on the nuc rDNA internal transcribed spacers 1 and 2 (ITS) and large subunit (LSU), Crous et al. (2012) clarified that *Pseudolachnea* is closely related but distinct from *Dinemasporium*, which also has a similar morphology but is characterised by aseptate conidia. However, *Pseudolachnella* was not included in their study (Crous et al. 2012) and validity of the circumscription of *Pseudolachnea* and *Pseudolachnella* based merely on the morphological differences in conidial septation remains uncertain.

During our ongoing studies of coelomycetous fungi in Japan (Endo et al. 2008, Hatakeyama et al. 2008, Sato et al. 2008, Yonezawa & Tanaka 2008, Kamiyama et al. 2009, Tanaka et al. 2010, 2011, Hashimoto et al. 2015) we have collected 26 strains of pseudolachnea-like species. In addition to typical species that show a good match with the generic concepts of *Pseudolachnea* and *Pseudolachnella*, several atypical species similar to these genera in conidial morphology but with undeveloped conidiomata also were found among our materials. The main goals of the present study were to clarify phylogenetic relationships between *Pseudolachnea* and its relatives and to evaluate the morphological circumscription of these genera as emphasised by conidial septation. For these purposes we conducted phylogenetic analyses of nuc rDNA ITS and partial LSU and the translation elongation factor 1 $\alpha$  gene (*tef1*) and examined the morphology of our newly collected materials from Japan.

## **MATERIAL AND METHODS**

### **Isolation and morphological observation**

All fungal structures except conidiomata were observed in preparations mounted in distilled water. Conidiomata were fixed in formalin-acetoalcohol, sectioned with a freezing microtome (HM 400R, MICROM, Germany) and mounted in lactophenol



cotton blue. Morphological characters were observed with differential interference and phase contrast microscopy (Olympus BX53), and images were captured with an Olympus digital camera (DP20). A total of 26 single-spore isolates were used for morphological observations and phylogenetic analysis (Table 1). Colony characters were observed from cultures grown on potato dextrose agar (PDA; Difco) after 3 wk at 20 °C in the dark. Colours were designated as described by Rayner (1970). To induce conidiomatal formation in culture, 5 mm squares of mycelial agar were placed on water agar including sterilised natural substrate, such as rice straw, banana leaves, pine needles and hydrangea leaves, and the plates were incubated at 20 °C for 2 wk in the dark. When the substrate were colonised, the plates were transferred to an incubator at 20 °C under blacklight blue illumination for 2 mo and observed for fructifications. Cultures were deposited the NITE Biological Resource Centre (NBRC), and the Genbank Project, NARO, Japan (MAFF). Specimens were deposited in the fungus herbarium of Hirosaki University (HHUF).

### Phylogenetic analyses

DNA extraction was carried out with an ISOPLANT II kit (Nippon Gene, Japan) in accordance with the manufacturer's protocol. Partial LSU including D1-D2 region, the complete ITS region (ITS1-5.8S-ITS2) and partial *tef1* were amplified by PCR with the primer pairs LR0R/LR7 (Rehner & Samuels 1994), ITS1/ITS4 (White *et al.* 1990) and EF983F/EF2218R (Rehner & Buckley 2005). Amplifications were performed in 25- $\mu$ L volumes consisting of 2  $\mu$ L DNA extract, 2.5  $\mu$ L of 10  $\times$  TEMPase buffer I, 2.5  $\mu$ L dNTPs mix, 1  $\mu$ L each 20 pM primer, 1  $\mu$ L MgCl<sub>2</sub>, 14.5  $\mu$ L MilliQ water and 0.5  $\mu$ L TEMPase Hot Start DNA polymerase (Ampliqon, Denmark). PCRs were carried out on a PC 320 thermo-cycler (ASTECC, Japan) with this protocol: 95 °C for 15 min, followed by 35 cycles of 1 min at 94 °C, 1 min at 46.2 °C (for 28S), 61.5 °C (for ITS), or 60 °C (for *tef1*), 1 min at 72 °C and a final denaturation step of 7 min at 72 °C. Amplified DNA was sequenced directly at SolGent (South Korea).

The novel sequences generated from 26 isolates were deposited in GenBank (Table 1). Two *Dinemasporium* species, *D. cruciferum* and *D. strigosum*, were selected as outgroups following Crous *et al.* (2012). These sequences were aligned with those of three phylogenetically related taxa, *Brunneodinemasporium brasiliense*, *Dendrophoma cytisporoides* and *Pseudolachnea fraxini*, downloaded from GenBank with the MUSCLE algorithm implemented in MEGA 5 (Tamura *et al.* 2011). Phylogenetic analyses were conducted based on maximum likelihood (ML) and Bayesian methods. The optimum substitution models for each dataset were estimated by Kakusan4 (Tanabe 2011), based on the Akaike information criterion (AIC; Akaike 1974) for ML analyses and the Bayesian information criterion (BIC;

Schwarz 1978) for the Bayesian analyses. ML analyses were performed with TreeFinder Mar 2011 (Jobb 2011) based on the models selected by AICc4 (separate model among genes and proportional model among codons, J2+G+I for 28S, J2+G for ITS, F81+G for the first codon of *tef1*, J1+G for the second codon of *tef1*, and J2+G for the third codon of *tef1*). Bootstrap proportions (BP) were obtained by 1 000 bootstrap replications. Bayesian analyses were performed with MrBayes 3.2.2 (Ronquist *et al.* 2012) with substitution models for different genes selected by BIC4 (i.e. proportional model among genes and among codons), GTR+G+I for 28S, GTR+G for ITS, F81+G for the first codon of *tef1*, JC69+G for the second codon of *tef1*, and HKY85+G for the third codon of *tef1*. Two simultaneous, independent runs of Metropolis-coupled Markov chain Monte Carlo (MCMC) were performed for 2 000 000 generations with trees sampled every 1 000th generation. Convergence of the MCMC procedure was assessed from the average standard deviation of split frequencies (< 0.01) and effective sample size scores (all > 100) using MrBayes and Tracer 1.6 (Rambaut *et al.* 2014), respectively. The first 25% trees were discarded as burn-in, and the remaining trees were used to calculate 50% majority rule trees and to determine posterior probabilities (PP) for individual branches.

## RESULTS

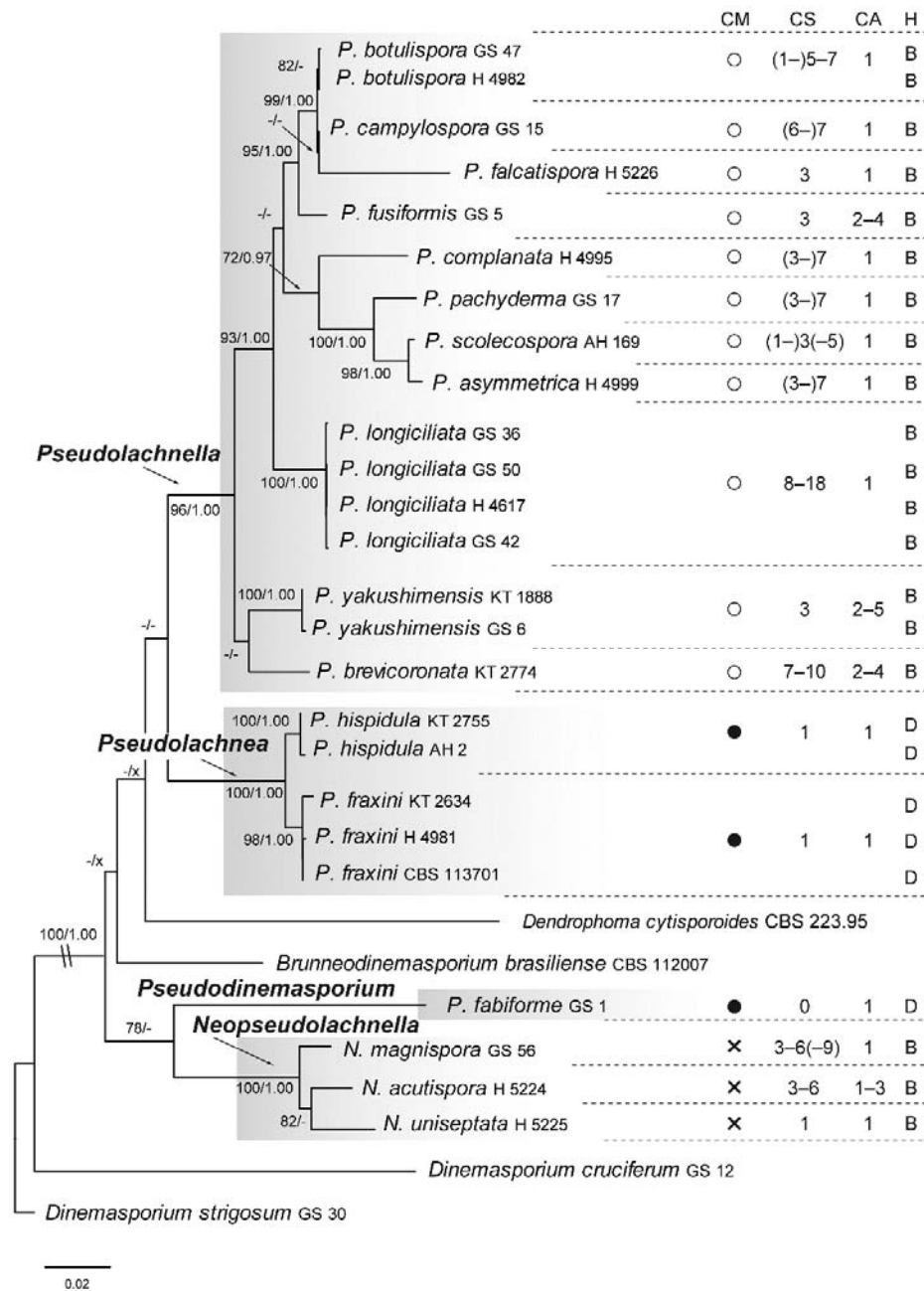
BLAST query with LSU sequences of pseudolachnea-like strains indicated that they are closely related and belong to the *Chaetosphaeriaceae*, *Sordariomycetes*. The sequence alignment comprised 808 nucleotide positions from 28S, 511 from ITS and 905 from *tef1* and consisted of sequence data for 26 strains obtained in the present study and for three species obtained from GenBank. Of the 2 224 characters included in the alignment, 463 were variable and 1 716 were conserved sites. The ML tree with the highest log likelihood (27550.0151) is illustrated (Fig. 1). The topology of the Bayesian analysis was almost identical to that of the ML tree, except the placement of *Dendrophoma*, which was basal to the *Pseudodinemasporium-Neopseudolachnella* clade. *Pseudolachnea* and *Pseudolachnella* were both monophyletic with 100%/1.00 and 96%/1.00 ML BP/Bayesian PP support, respectively, and they were grouped in a single clade without strong support. Two novel lineages, *Neopseudolachnella* and *Pseudodinemasporium*, were resolved in the tree, and they are established here as new genera. *Neopseudolachnella* was sister to a clade of *Pseudodinemasporium* with 78% ML BP, although this relationship was not supported (< 0.95) in the Bayesian analysis (Fig. 1). A total of 12 new species (i.e. three in *Neopseudolachnella*, one in *Pseudodinemasporium*, eight in *Pseudolachnella*) are described below.

**Table 1.** Specimens, isolates, and Genbank accession numbers of species used in this study.

Species	Original no.	Specimen no.	Strain no.	Host/substrate	Locality	GenBank no.		
						28S	ITS	EF1 $\alpha$
<i>Brunneodinemasporium brasiliense</i>	-	CBS H-20948 <sup>H</sup>	CBS 112007 = INIFAT CO2/51	Decaying leaf	Brazil	JQ889288	JQ889272	-
<i>Dendrophoma cytisporoides</i>	-	CBS H-12004 <sup>E</sup>	CBS 223.95	<i>Rhododendron</i> sp.	Netherlands	JQ889289	JQ889273	-
<i>Dinemasporium cruciferum</i>	GS 12	HHUF 30001	MAFF 244327	<i>Arundo donax</i>	Kagoshima, JPN	<b>AB934039</b>	AB900895	<b>AB934089</b>
<i>Dinemasporium strigosum</i>	GS 30	HHUF 29961	MAFF 244355	<i>Sasa kurilensis</i>	Hokkaido, JPN	<b>AB934040</b>	AB900884	<b>AB934090</b>
<i>Neopseudolachnella acutispora</i>	SH 50 (= H 5224)	HHUF 29727 <sup>H</sup>	MAFF 244358 = NBRC 110122	<i>Pleioblastus chino</i>	Aomori, JPN	<b>AB934041</b>	<b>AB934065</b>	<b>AB934091</b>
<i>Neopseudolachnella magnispora</i>	GS 56	HHUF 29977 <sup>H</sup>	MAFF 244359 = NBRC 110123	<i>Sasa kurilensis</i>	Aomori, JPN	<b>AB934042</b>	<b>AB934066</b>	<b>AB934092</b>
<i>Neopseudolachnella uniseptata</i>	SH 73 (= H 5225)	HHUF 29728 <sup>H</sup>	MAFF 244360 = NBRC 110124	-	Aomori, JPN	<b>AB934043</b>	<b>AB934067</b>	<b>AB934093</b>
<i>Pseudodinemasporium fabiforme</i>	GS 1 (= H 5219)	HHUF 29716 <sup>H</sup>	MAFF 244361 = NBRC 110125	<i>Betula platyphylla</i>	Ibaraki, JPN	<b>AB934044</b>	<b>AB934068</b>	<b>AB934094</b>
<i>Pseudolachnea fraxini</i>	-	CBS H-20953 <sup>H</sup>	CBS 113701	<i>Fraxinus excelsior</i>	Sweden	JQ889301	JQ889287	-
	SH 112 (= H 4981)	HHUF 28762	MAFF 244363	<i>Clematis florida</i>	Aomori, JPN	<b>AB934045</b>	<b>AB934069</b>	<b>AB934095</b>
	KT 2634	HHUF 30116	MAFF 244362	<i>Artemisia</i> sp.	Aomori, JPN	<b>AB934046</b>	<b>AB934070</b>	<b>AB934096</b>
<i>Pseudolachnea hispidula</i>	AH 2	HHUF 30117	MAFF 244364	<i>Morus bombycis</i>	Aomori, JPN	<b>AB934047</b>	<b>AB934071</b>	<b>AB934097</b>
	KT 2755	HHUF 30118	MAFF 244365	<i>Morus bombycis</i>	Aomori, JPN	<b>AB934048</b>	<b>AB934072</b>	<b>AB934098</b>
<i>Pseudolachnella asymmetrica</i>	SH 180 (= H 4999)	HHUF 28777 <sup>H</sup>	MAFF 244366 = NBRC 110126	<i>Phyllostachys nigra</i> var. <i>henonis</i>	Fukuoka, JPN	<b>AB934049</b>	<b>AB934073</b>	<b>AB934099</b>
<i>Pseudolachnella botulispora</i>	GS 47	HHUF 29969 <sup>H</sup>	MAFF 244367 = NBRC 110127	<i>Phyllostachys aurea</i>	Shizuoka, JPN	<b>AB934050</b>	<b>AB934074</b>	<b>AB934100</b>
	SH 28 (= H 4982)	HHUF 28776 <sup>P</sup>	MAFF 244368	<i>Phyllostachys pubescens</i>	Iwate, JPN	<b>AB934051</b>	<b>AB934075</b>	<b>AB934101</b>
<i>Pseudolachnella brevicoronata</i>	KT 2774	HHUF 30119 <sup>H</sup>	MAFF 244369 = NBRC 110128	<i>Sasa</i> sp.	Iwate, JPN	<b>AB934052</b>	<b>AB934076</b>	<b>AB934102</b>
<i>Pseudolachnella campylospora</i>	GS 15	HHUF 30002 <sup>H</sup>	MAFF 244370 = NBRC 110129	bamboo	Hiroshima, JPN	<b>AB934053</b>	<b>AB934077</b>	<b>AB934103</b>
<i>Pseudolachnella complanata</i>	SH 77 (= H 4995)	HHUF 28282 <sup>H</sup>	MAFF 244371 = NBRC 110130	bamboo	Kanagawa, JPN	<b>AB934054</b>	<b>AB934078</b>	<b>AB934104</b>
<i>Pseudolachnella falcatispora</i>	SH 182 (= H 5226)	HHUF 29732 <sup>H</sup>	MAFF 244372 = NBRC 110131	<i>Sinobambusa tootsik</i>	Hiroshima, JPN	<b>AB934055</b>	<b>AB934079</b>	<b>AB934105</b>
<i>Pseudolachnella fusiformis</i>	GS 5 (= H 5222)	HHUF 29725 <sup>H</sup>	MAFF 244373 = NBRC 110132	bamboo	Nara, JPN	<b>AB934056</b>	<b>AB934080</b>	<b>AB934106</b>
<i>Pseudolachnella longiciliata</i>	GS 36	HHUF 29962	MAFF 244374	<i>Sasa kurilensis</i>	Hokkaido, JPN	<b>AB934057</b>	<b>AB934081</b>	<b>AB934107</b>
	GS 42	HHUF 29966	MAFF 244375	<i>Sasa kurilensis</i>	Hokkaido, JPN	<b>AB934058</b>	<b>AB934082</b>	<b>AB934108</b>
	GS 50	HHUF 29972	MAFF 244376	<i>Sasa kurilensis</i>	Aomori, JPN	<b>AB934059</b>	<b>AB934083</b>	<b>AB934109</b>
	SH 97 (= H 4617)	HHUF 27528 <sup>E</sup>	MAFF 244377	<i>Sasa kurilensis</i>	Aomori, JPN	<b>AB934060</b>	<b>AB934084</b>	<b>AB934110</b>
<i>Pseudolachnella pachyderma</i>	GS 17	HHUF 29955 <sup>H</sup>	MAFF 244378 = NBRC 110133	bamboo	Nagasaki, JPN	<b>AB934061</b>	<b>AB934085</b>	<b>AB934111</b>
<i>Pseudolachnella scolecospora</i>	AH 169	HHUF 30268	MAFF 244379	<i>Sasa</i> sp.	Gifu, JPN	<b>AB934062</b>	<b>AB934086</b>	<b>AB934112</b>
<i>Pseudolachnella yakushimensis</i>	KT 1888 (= H 5223)	HHUF 29683 <sup>H</sup>	JCM 15101	<i>Pleioblastus</i> sp.	Kagoshima, JPN	<b>AB934063</b>	<b>AB934087</b>	<b>AB934113</b>
	GS 6	HHUF 29996	MAFF 244380	bamboo	Kagoshima, JPN	<b>AB934064</b>	<b>AB934088</b>	<b>AB934114</b>

<sup>1</sup>E, H and P indicate epitype, holotype and paratype, respectively.

<sup>2</sup>Sequences generated in this study are shown in boldface.



**Fig. 1.** Maximum-likelihood (ML) tree of *Pseudolachnea* and its relatives based on a combined dataset of nuc rDNA 28S and ITS and *tef1* sequences. ML bootstrap proportion (BP) and Bayesian posterior probabilities (PP) greater than 70% and 0.95 are presented at the nodes as ML BP/Bayesian PP, respectively. A hyphen indicates values lower than 70%/0.95 ML BP/Bayesian PP, and a node not present in the Bayesian analysis is indicated with "x". Abbreviations for species characterization are indicated by: CM. conidiomatal structure (○. less developed conidiomata; ●. well developed conidiomata; ×. conidiomata lacking excipulum); CS. number of conidial septum; CA. number of conidial appendage; H. Host plant (B. bamboo; D. dicotyledon). Bar = nucleotide substitution per site.

***Neopseudolachnella*** A. Hashim. & Kaz. Tanaka, **gen. nov.** — MycoBank MB 808687.

*Etymology*: Named for the morphological similarity of its conidia to those of the genus *Pseudolachnella*.

*Conidiomata* stromatic, acervuloid, mostly scattered, superficial, ellipsoid, dark brown to black, setose; basal stroma composed of globose to subglobose cells; excipulum absent. *Setae* marginal or interspersed, arising from basal stroma, straight to slightly curved, septate, brown but pale at the apex, smooth, unbranched, thick-walled. *Conidiophores* absent or lining the basal stroma, cylindrical, smooth, hyaline or pale brown, simple. *Conidiogenous cells* cylindrical to lageniform, smooth, hyaline or pale brown. *Conidia* cylindrical to subcylindrical, acute or obtuse at the apex, truncate at the base, septate, smooth, hyaline, bearing a single to multiple appendages at each end; apical appendage central; basal appendage excentric.

*Type species*: *Neopseudolachnella acutispora* A. Hashim., Sat. Hatak. & Kaz. Tanaka

***Neopseudolachnella acutispora*** A. Hashim., Sat. Hatak. & Kaz. Tanaka, **sp. nov.** — MycoBank MB 808688; Fig. 2.

*Etymology*: Named for its conidia, which have an acute apex.

*Conidiomata* stromatic, acervuloid, mostly scattered or sometimes 2–3 grouped, superficial to subcuticular, ellipsoid or irregular, 450–1300 µm diam, up to 280 µm high, dark brown to black, with white conidial mass in center, setose; basal stroma 7.5–12 µm thick, composed of globose to subglobose, 2–4 µm diam cells; excipulum absent. *Setae* arising from basal stroma, straight or curved, septate with 9–25 µm intervals, brown but pale at the apex, smooth, unbranched, thick-walled, 100–280 µm long, acute and 2.5–5 µm wide at the apex, 5–7.5 µm wide at the base. *Conidiophores* lining the basal stroma, cylindrical, smooth, hyaline, simple, 7–6 × 2.5 µm. *Conidiogenous cells* phialidic, cylindrical, hyaline, smooth, 7.5–18 × 1.5–2 µm. *Conidia* subcylindrical to fusiform, acute at the apex, truncate at the base, 3–6-septate, smooth, hyaline, 19–40 × 2–3 µm ( $\bar{x}$  = 30.7 × 2.1 µm, n = 50), l/w 7.6–20.0 ( $\bar{x}$  = 14.6, n = 50), bearing a single appendage or occasionally 1–3 appendages at each end; apical appendage 1–6 µm long ( $\bar{x}$  = 3.5 µm, n = 50), central; basal appendage 1–5.5 µm long ( $\bar{x}$  = 3.7 µm, n = 50), excentric.

Culture characteristics — Colonies on PDA attaining 24–26.5 mm diam after 21 d at 20 °C in the dark, smooth, sulcate, straw (46: Rayner 1970) (Fig. 18a).

*Specimen examined.* **JAPAN**, Aomori, Gonohe, Asamizu, on dead twigs of *Pleiblastus chino*, 2 Dec 2003, S. Hatakeyama, SH 50 = H5224 (HHUF 29727 **holotype**, ex-holotype living culture MAFF 244358 = NBRC 110122).

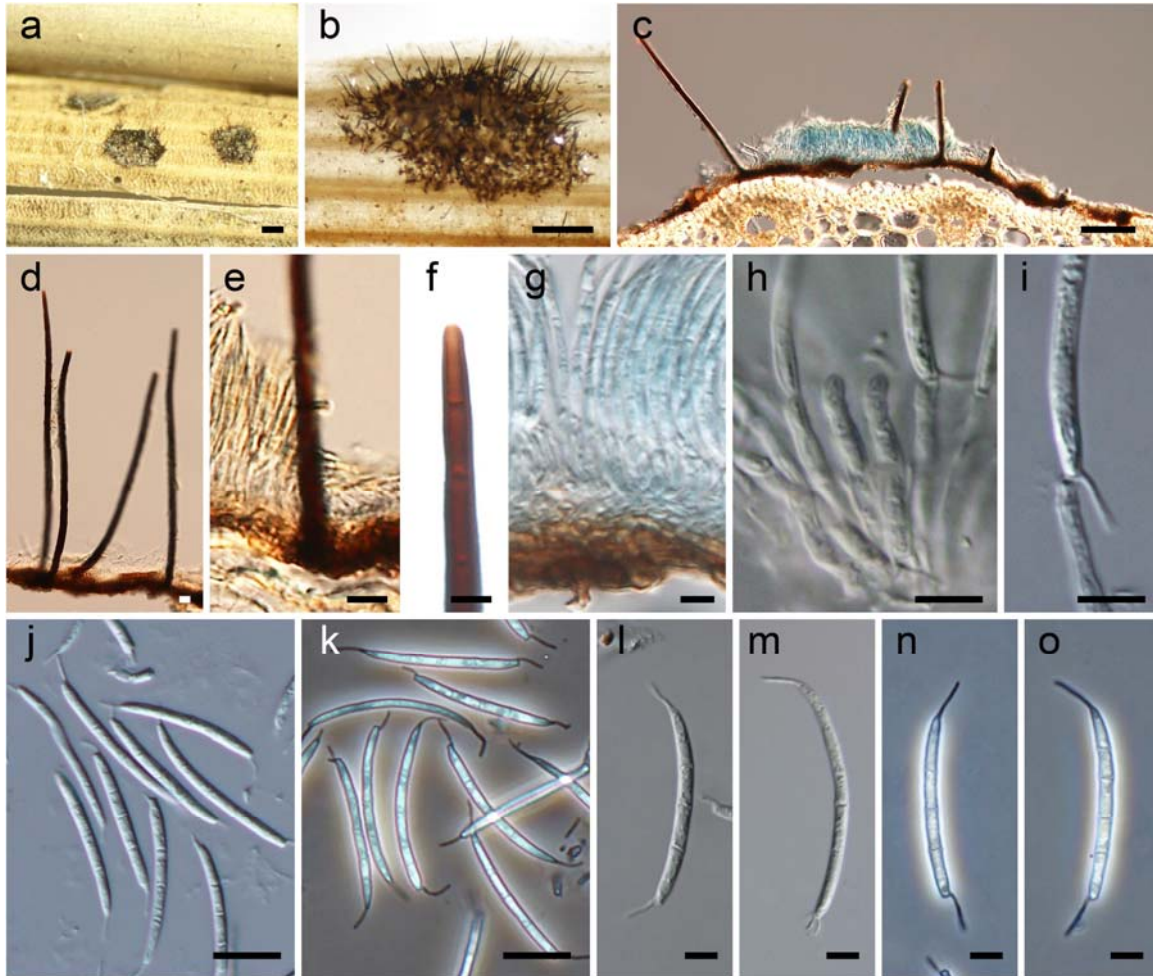
*Notes* — A new genus *Neopseudolachnella* is established to accommodate species in a fully supported clade, which is distantly related to other lineages of known genera (Fig. 1). *Neopseudolachnella* is most similar to *Pseudolachnea* and *Pseudolachnella* in having cylindrical, single- to multiseptate, hyaline conidia with bipolar appendages. However, it is clearly separated from the latter two genera by the acervuloid conidiomata lacking a peridial wall compared with the acervular conidiomata with a prominent lateral excipulum present in *Pseudolachnea* and *Pseudolachnella*. The flattened setose conidiomata of *Neopseudolachnella* superficially resemble those present in *Rattania*, but *Rattania* has sporodochial conidiomata and holoblastic conidiogenous cells sometimes extending sympodially (Prabhugaonkar & Bhat 2009). Sequence similarity between the type species of these genera (*N. acutispora* and *R. setulifera*: GU191794) was 82.2% (406/494 nt) with 4.7% (23/494 nt) gaps in their ITS regions, suggesting that they are not congeneric.

*Neopseudolachnella acutispora* has multiseptate, cylindrical conidia similar to those of *N. magnispora* but is distinguished from the latter by its smaller conidia (vs. 35–62 × 2–3 µm).

***Neopseudolachnella magnispora*** A. Hashim., G. Sato & Kaz. Tanaka, **sp. nov.**  
— MycoBank MB 808689; Fig. 3.

*Etymology*: Named for its conidia, which are the largest within this genus.

*Conidiomata* stromatic, acervuloid, mostly scattered, superficial, ellipsoid, 290–600 µm long, 150–180 µm wide, up to 100 µm high, dark brown to black, with white conidial mass in center, setose; basal stroma composed of globose to subglobose cells; excipulum absent. *Setae* marginal, arising from basal stroma, straight to slightly curved, septate, brown but pale at the apex, smooth, unbranched, thick-walled, 180–250 µm long, acute and 2.5 µm wide at the apex, 7.5–10.5 µm wide at the base. *Conidiophores* absent. *Conidiogenous cells* ovoid to lageniform, hyaline, smooth, 9–11.5 × 1.5–2 µm. *Conidia* cylindrical to subcylindrical, acute or obtuse at the apex, truncate at the base, 3–7(–9)-septate, smooth, hyaline, 35–62 × 2–3 µm ( $\bar{x}$  = 50.8 × 2.2 µm, n = 50), l/w 16.7–31.0 ( $\bar{x}$  = 23.5, n = 50), bearing an appendage at each end; apical appendage (2–)4–9 µm long ( $\bar{x}$  = 6.1 µm long, n = 50), central; basal appendage (1.5–)3–8 µm long ( $\bar{x}$  = 5.1 µm long, n = 50), excentric.

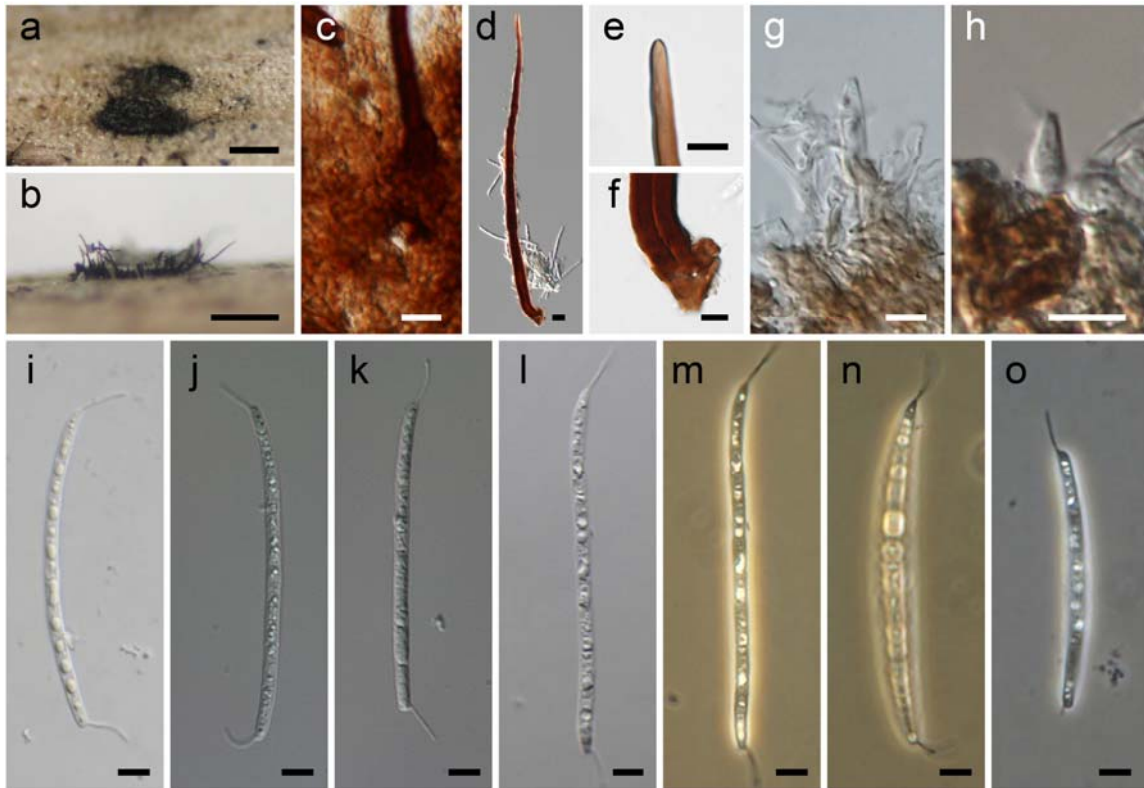


**Fig. 2.** *Neopseudolachnella acutispora* a, b. Conidiomata on host surface. c. Conidioma in longitudinal section. d. Conidiomatal setae. e. Base of conidiomatal seta. f. Apex of conidiomatal seta. g. Basal stroma of conidioma and conidiophores. h. Conidiogenous cells. i. Conidiogenous cell and immature conidium. j–o. Conidia. a–o. HHUF 29727. — Scale bars: a, b = 250  $\mu$ m; c, d = 20  $\mu$ m; e–o = 5  $\mu$ m.

**Culture characteristics** — Colonies on PDA attaining 19–24 mm diam after 21 d at 20 °C in the dark, floccose, straw (46) (Fig. 18b).

**Specimen examined.** **JAPAN**, Aomori, Nishimeya, Tsugaru pass, on dead twigs of *Sasa kurilensis*, 28 Aug 2007, H. Yonezawa et al., GS 56 (HHUF 29977 **holotype**, ex-holotype living culture MAFF 244359 = NBRC 110123).

**Notes** — The most distinctive feature of this species is that it produces the largest conidia among the three *Neopseudolachnella* species recognised in this study.



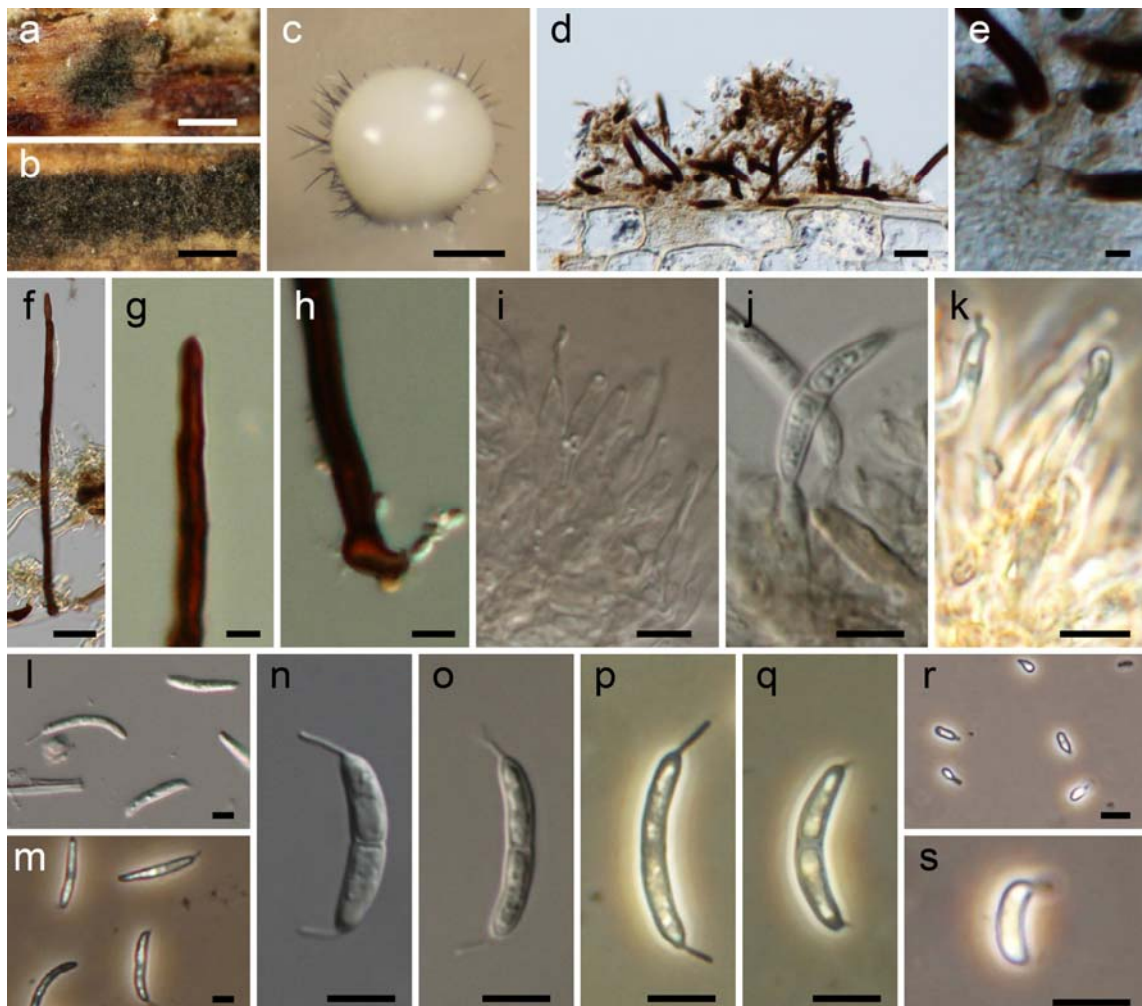
**Fig. 3.** *Neopseudolachnella magnispora*. a, b. Conidiomata on host surface. c. Basal stroma of conidioma. d. Conidiomatal seta. e. Apex of conidiomatal seta. f. Base of conidiomatal seta. g. Basal stroma of conidioma and conidiogenous cell. h. Conidiogenous cell. i–o. Conidia. a–o. HHUF 29977. — Scale bars: a, b = 500  $\mu\text{m}$ ; c, d = 10  $\mu\text{m}$ ; e–o = 5  $\mu\text{m}$ .

***Neopseudolachnella uniseptata*** A. Hashim., Sat. Hatak. & Kaz. Tanaka, **sp. nov.** — MycoBank MB 808690; Fig. 4.

*Etymology:* Named for its 1-septate conidia.

*Conidiomata* stromatic, acervuloid, mostly scattered or sometimes 2–3 grouped, superficial to subcuticular, ellipsoid or irregular, 400–780  $\mu\text{m}$  long, 290–380  $\mu\text{m}$  wide, up to 105–130  $\mu\text{m}$  high, dark brown to black, with white conidial mass in center, setose; basal stroma 5–7.5  $\mu\text{m}$  thick, composed of globose to subglobose, 2.5–6  $\mu\text{m}$  diam cells; excipulum absent. *Setae* arising from basal stroma, straight or slightly curved, 0–1-septate, brown but pale at the apex, smooth, unbranched, thick-walled, 110–195  $\mu\text{m}$  long, acute and 2.5–3  $\mu\text{m}$  wide at the apex, 4–5  $\mu\text{m}$  wide at the base. *Conidiophores* lining the basal stroma, cylindrical, smooth, pale brown, simple, 4–8  $\times$  3–3.5  $\mu\text{m}$ . *Conidiogenous cells* phialidic, cylindrical, pale brown to hyaline, smooth, 7–12  $\times$  2.5–3  $\mu\text{m}$ . *Conidia* fusiform to allantoid, obtuse at the apex, slightly truncate





**Fig. 4.** *Neopseudolachnella uniseptata*. a–c. Conidiomata in culture. d. Conidioma in longitudinal section. e. Basal stroma of conidioma. f. Conidiomatal seta. g. Apex of conidiomatal seta. h. Base of conidiomatal seta. i. Conidiophores and conidiogenous cells. j, k. Conidiogenous cells and immature conidia. l–q. Conidia. r, s. Microconidia. a–s. culture MAFF 244360. — Scale bars: a, b = 500  $\mu$ m; c = 250  $\mu$ m; d, f = 20  $\mu$ m; e, g–s = 5  $\mu$ m.

at the base, 1-septate, slightly constricted at the septum, smooth, hyaline, 10.5–19.5  $\times$  1.5–2.5  $\mu$ m ( $\bar{x}$  = 15.0  $\times$  2.0  $\mu$ m, n = 50), l/w 5.3–13.0 ( $\bar{x}$  = 7.5, n = 50), bearing an appendage at each end; apical appendage 1–4  $\mu$ m long ( $\bar{x}$  = 2.4  $\mu$ m, n = 50), central; basal appendage 0.5–4  $\mu$ m long ( $\bar{x}$  = 2.0  $\mu$ m, n = 50), excentric. Microconidia ovoid to reniform, obtuse at the apex, slightly truncate at the base, aseptate, smooth, hyaline, 2–6.5  $\times$  1–2  $\mu$ m ( $\bar{x}$  = 3.4  $\times$  1.6  $\mu$ m, n = 20), l/w 1.1–3.3 ( $\bar{x}$  = 2.1, n = 20), bearing an appendage at the apex; 1–2  $\mu$ m long ( $\bar{x}$  = 1.9, n = 20).

Culture characteristics — Colonies on PDA attaining 18–21 mm diam after 21 d at 20 °C in the dark, smooth, zonate, sulphur yellow (15) (Fig. 18c).

*Specimen examined.* JAPAN, Aomori, on *Sasa* sp., 2003, S. Hatakeyama, SH 73 = H 5225 (HHUF 29728 **holotype**, ex-holotype culture MAFF 244360 = NBRC 110124).

*Notes* — The conidia of *N. uniseptata* are morphologically distinct from those of *N. acutispora* and *N. magnispora* in terms of the number of conidial septa.

***Pseudodinemasporium*** A. Hashim. & Kaz. Tanaka, **gen. nov.** — MycoBank MB 808707.

*Etymology:* Named for its morphological similarity to the genus *Dinemasporium*.

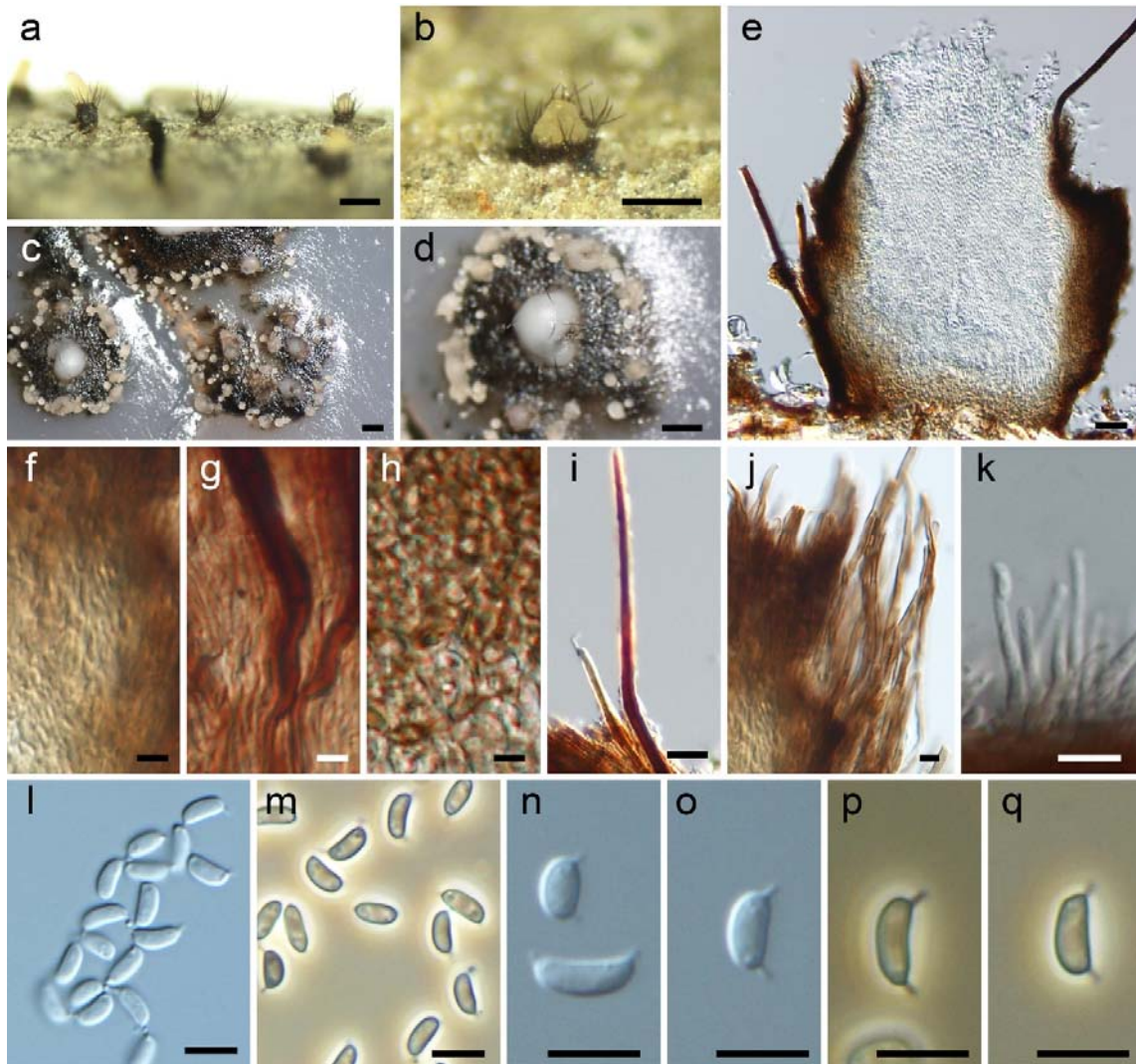
*Conidiomata* stromatic, acervular, cupulate, setose, mostly scattered to grouped, superficial, globose or ellipsoid, dark brown to black; basal stroma well-developed, composed of subglobose cells; excipulum composed of rectangular or subglobose, brown cells. *Setae* arising from excipulum, curved, septate, brown but pale at the apex, smooth, unbranched, thick-walled; inconspicuous setae pale brown, multi-septate. *Conidiophores* lining the basal stroma, cylindrical, septate, smooth, hyaline, simple or branched. *Conidiogenous cells* phialidic, cylindrical, hyaline, smooth. *Conidia* ovoid to ellipsoidal, obtuse at the apex, slightly truncate at the base, unicellular, hyaline, smooth, guttulate, bearing an appendage at each end.

*Type species:* *Pseudodinemasporium fabiforme* A. Hashim., G. Sato & Kaz. Tanaka

***Pseudodinemasporium fabiforme*** A. Hashim., G. Sato & Kaz. Tanaka, **sp. nov.** — MycoBank MB 808708; Fig. 5.

*Etymology:* Named for its bean-shaped conidia.

*Conidiomata* stromatic, acervular, cupulate, mostly scattered or sometimes 3–4 grouped, superficial, globose or ellipsoid, 130–230  $\mu\text{m}$  diam, up to 240  $\mu\text{m}$  high, dark brown to black, with white to orange conidial mass in center, setose; basal stroma well-developed, 25–35  $\mu\text{m}$  thick, composed of subglobose, 3–5  $\times$  2.5–5  $\mu\text{m}$  cells; excipulum well-developed, 12.5–20(–40)  $\mu\text{m}$  thick, composed of rectangular to subglobose cells. *Setae* arising from excipulum, curved, septate, brown but pale at the apex, smooth, unbranched, thick-walled, 170–310  $\mu\text{m}$  long, acute, 1  $\mu\text{m}$  wide at the apex, 5–7  $\mu\text{m}$  wide at the base; inconspicuous setae pale brown, multi-septate, (10–)20–50(–100)  $\mu\text{m}$  long, ca. 2  $\mu\text{m}$  wide. *Conidiophores* lining the basal stroma, cylindrical, septate, smooth, hyaline, simple or branched, 6.5–16.5  $\times$  1.5  $\mu\text{m}$ . *Conidiogenous cells* phialidic, cylindrical, hyaline, smooth, 7–12.5  $\times$  1–1.5  $\mu\text{m}$ . *Conidia* reniform, ovoid to ellipsoidal, obtuse at the apex, slightly truncate at the base, unicellular, hyaline, smooth, guttulate, 3–5  $\times$  1–2.5  $\mu\text{m}$  ( $\bar{x}$  = 4.1  $\times$  1.9  $\mu\text{m}$ , n = 50),



**Fig. 5.** *Pseudodinemasporium fabiforme*. a, b. Conidiomata on host surface. c, d. Conidiomata in culture. e. Conidioma in longitudinal section. f, g. Excipulum of conidioma. h. Basal stroma of conidioma. i. Conidiomatal setae. j. Edge of conidiomata. k. Conidiophores and conidiogenous cells. l–q. Conidia. a, b, e–k. HHUF 29716. c, d, l–q. culture MAFF 244361. — Scale bars: a–d = 250  $\mu\text{m}$ ; e, i = 20  $\mu\text{m}$ ; f–h, j–q = 5  $\mu\text{m}$ .

l/w 1.5–3.5 ( $\bar{x}$  = 2.2, n = 50), bearing an appendage at each end; appendage 0.5–1.5  $\mu\text{m}$  long ( $\bar{x}$  = 1.0  $\mu\text{m}$ , n = 44); basal appendage excentric.

Culture characteristics — Colonies on PDA attaining 18–21 mm diam after 21 d at 20 °C in the dark, floccose, violet (32) (Fig. 18d). Conidia produced in culture are similar to those on natural substrate.

*Specimen examined.* **JAPAN**, Ibaraki, Tsukuba, Tsukuba Botanical Garden, on dead twigs of *Betula platyphylla*, 4 Feb 2006, K. Tanaka, GS 1 = H 5219 (HHUF 29716 **holotype**, ex-holotype living culture MAFF 244361 = NBRC 110125).

*Notes* — A new genus *Pseudodinemasporium* is proposed here to include *P. fabiforme*, a dinemasporium-like species. Three genera resemble *Pseudodinemasporium* (viz. *Brunneodinemasporium*, *Dendrophoma* and *Dinemasporium*) all of which have setose conidiomata and one-celled conidia with a single appendage at each end. Among these genera, *Dendrophoma* is phylogenetically close to *Pseudodinemasporium* but differs from the latter in that it has conidiomata with a stipe-like basal excipulum composed of dense textura intricata (Crous *et al.* 2012). *Brunneodinemasporium* is characterised by a densely aggregated layer of brown conidiogenous cells, hyaline to pale brown conidia, and the conidial appendage is apparently separated from the conidial body by a septum (Crous *et al.* 2012); these features are absent in *Pseudodinemasporium*. *Dinemasporium* has conidiomata with a poorly developed lateral excipulum (Hashimoto *et al.* 2015), unlike *Pseudodinemasporium*, which has relatively large conidiomata with a well developed peridial wall.

***Pseudolachnea*** Ranoj., Ann. Mycol. 8:593, 1910.

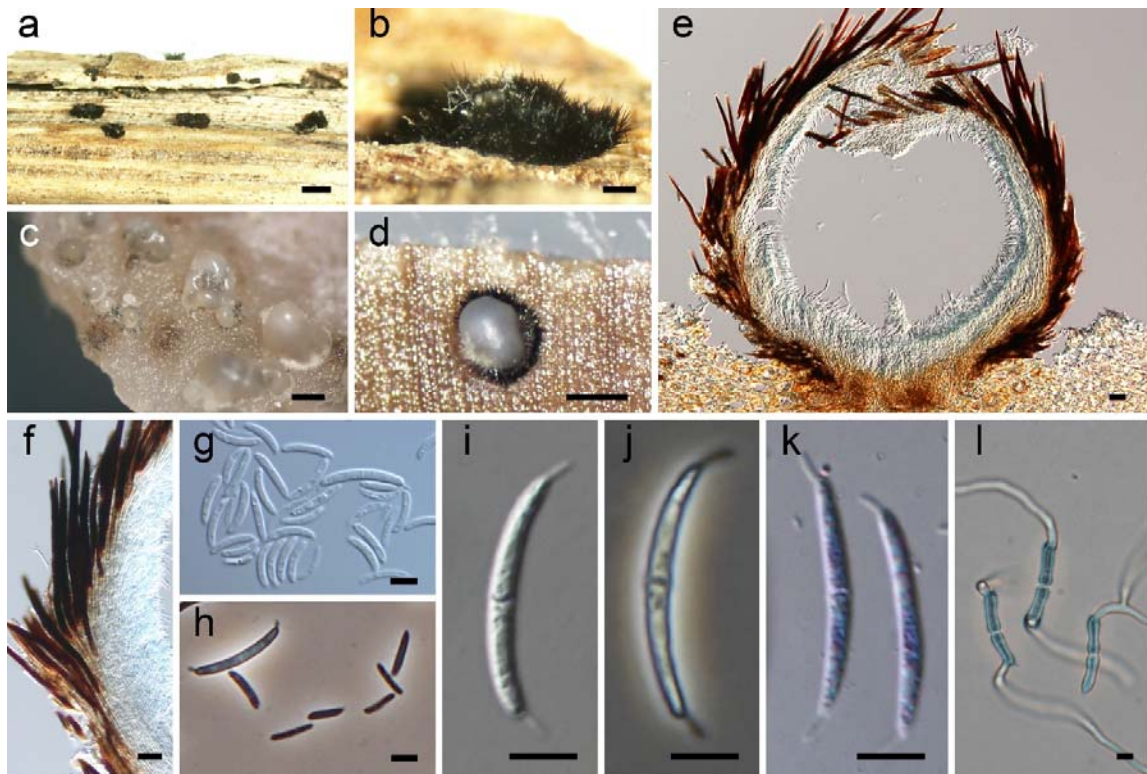
*Conidiomata* stromatic, acervular, cupulate, superficial to subepidermal origin, ellipsoidal or irregular in outline, setose, black; basal stroma composed of pale brown, globose to subglobose, thin-walled cells; excipulum well-developed, composed of pale brown, hyphal to rectangular, slightly thick-walled cells. *Setae* marginal, cylindrical, straight to curved, septate or aseptate, dark brown to brown, thick-walled, acute at the apex. *Conidiophores* arising from the upper cells of the basal stroma, branched, hyaline, smooth. *Conidiogenous cells* phialidic, cylindrical, hyaline, smooth. *Conidia* 1-septate, fusiform to allantoid, obtuse at the apex, slightly truncate at the base, hyaline, bearing a filiform appendage at each end.

*Type species: Pseudolachnea hispidula* (Schrad.) B. Sutton.

***Pseudolachnea fraxini*** Crous, Persoonia 28:135, 2012; Fig. 6.

*Conidiomata* stromatic, acervular, cupulate, setose, ellipsoidal or irregular in outline, 180–1500 µm diam, 100–450 µm high; basal stroma 12.5–75(–100) µm thick; excipulum 25–90 µm thick, well-developed. *Setae* 75–360 µm long, acute and 2.5–3 µm wide at the apex, 3.5–7.5 µm wide at the base. *Conidiophores* 10.5–12 µm long, 1.5–2 µm wide. *Conidiogenous cells* 19–16 × 2 µm. *Conidia* 14–21 × 1.5–2.5 µm ( $\bar{x}$  = 18.3 × 2.0 µm, n = 103), l/w 6.6–10.9 ( $\bar{x}$  = 8.8, n = 103), 1-septate, fusiform to allantoid, hyaline, bearing an appendage of 1–3 µm long ( $\bar{x}$  = 2.0 µm, n = 50) at each end.



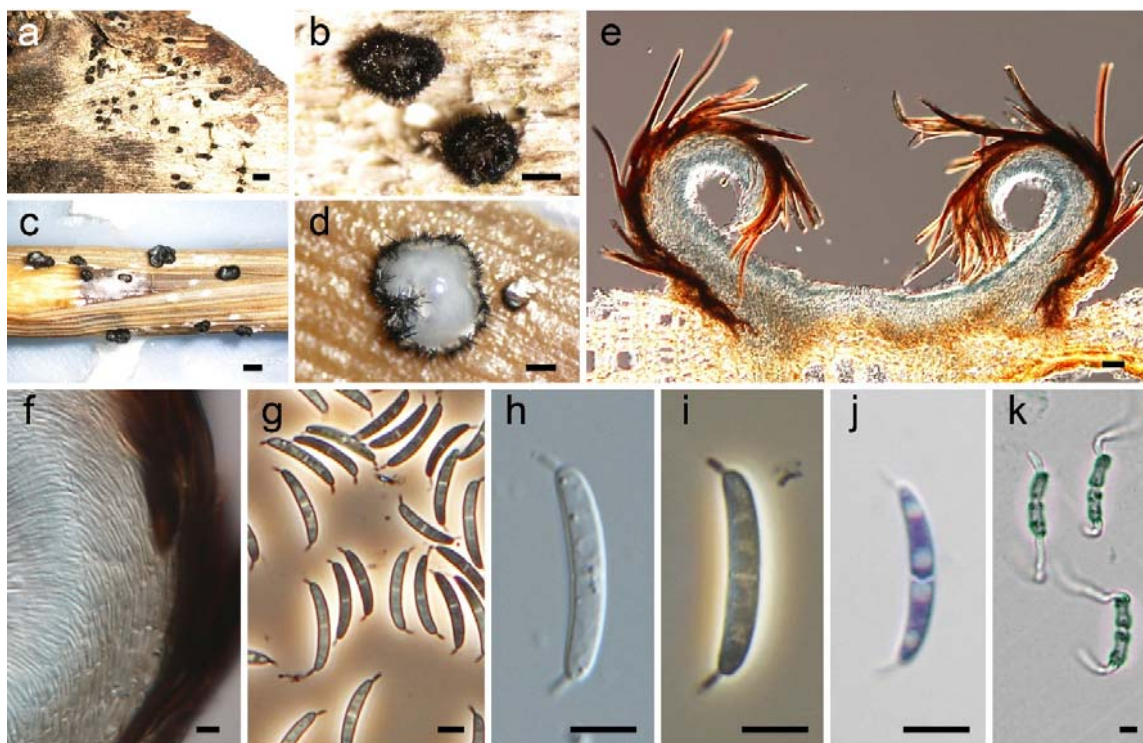


**Fig. 6.** *Pseudolachnea fraxini*. a, b. Conidiomata on host surface. c, d. Conidiomata in culture. e. Conidioma in longitudinal section. f. Excipulum of conidioma. g, h. Microconidia and conidia. i–k. Conidia. l. Germinating conidia. a, b, e, f, k, l. HHUF 30116. c, d, g, h. culture MAFF 244363. i, j. HHUF 28762. — Scale bars: a = 1000  $\mu\text{m}$ ; b–d = 250  $\mu\text{m}$ ; e, f = 20  $\mu\text{m}$ . g–l = 5  $\mu\text{m}$ .

**Culture characteristics** — Colonies on PDA attaining 27–30 mm diam after 21 d at 20 °C in the dark, smooth to floccose, white (Fig. 18e). Conidia 14–20  $\times$  2  $\mu\text{m}$  ( $\bar{x}$  = 16.7  $\times$  2.0  $\mu\text{m}$ , n = 25), l/w 7.1–10.4 ( $\bar{x}$  = 8.4, n = 25). Microconidia 7–11  $\times$  1–2  $\mu\text{m}$  ( $\bar{x}$  = 8.1  $\times$  1.3  $\mu\text{m}$ , n = 25), l/w 4.4–8.2 ( $\bar{x}$  = 5.7, n = 25), allantoid, obtuse or rarely acute at both ends, hyaline.

**Specimens examined.** **JAPAN**, Aomori, Towada, Yagami, on dead stems of *Clematis florida*, 27 May 2003, S. Hatakeyama, SH 112 = H4981 (HHUF 28762, living culture MAFF 244363); Kitatsugaru, Nakadomari, near Gongenzaki, on overwintered stems of *Artemisia* sp., 4 May 2009, K. Tanaka, KT 2634 (HHUF 30116, living culture MAFF 244362).

**Notes** — The above materials were identified as *P. species* provided by Crous *et al.* (2012), as well as sequence similarity in the ITS region.



**Fig. 7.** *Pseudolachnea hispidula*. a, b. Conidiomata on host surface. c, d. Conidiomata in culture. e. Conidioma in longitudinal section. f. Excipulum of conidioma. g–j. Conidia. k. Germinating conidia. a, b, e, f, j, k. HHUF 30118. c, d, g–i. culture MAFF 244365. — Scale bars: a, b = 500  $\mu\text{m}$ ; c, d = 250  $\mu\text{m}$ ; e = 20  $\mu\text{m}$ ; f–k = 5  $\mu\text{m}$ .

***Pseudolachnea hispidula*** (Schrad.) B. Sutton, Mycol. Pap. 141:167, 1977; Fig. 7.

*Conidiomata* stromatic, acervular, cupulate, setose, ellipsoid, 200–800  $\mu\text{m}$  diam, 600–750  $\mu\text{m}$  high; basal stroma 50–75  $\mu\text{m}$  thick; excipulum 30–62  $\mu\text{m}$  thick, well-developed. *Setae* 100–200  $\mu\text{m}$  long, acute and 2.5–3  $\mu\text{m}$  wide at the apex, 4–7.5  $\mu\text{m}$  wide at the base. *Conidiophores* 8–16  $\mu\text{m}$  long, 2  $\mu\text{m}$  wide. Conidiogenous cells 8.5–10  $\times$  2  $\mu\text{m}$ . Conidia 10.5–16(–17)  $\times$  2–2.5(–3)  $\mu\text{m}$  ( $\bar{x}$  = 14.9  $\times$  2.2  $\mu\text{m}$ , n = 70), l/w 4.8–8.2 ( $\bar{x}$  = 6.9, n = 70), 1-septate, fusiform, slightly curved, hyaline, bearing an unbranched appendage of 0.5–2.5  $\mu\text{m}$  long ( $\bar{x}$  = 1.3  $\mu\text{m}$ , n = 50) at each end.

Culture characteristics — Colonies on PDA attaining 18–22 mm diam after 21 d at 20 °C in the dark, floccose, straw (46) (Fig. 18f). Conidia 13–16(–17.5)  $\times$  2–2.5  $\mu\text{m}$  ( $\bar{x}$  = 15.0  $\times$  2.3  $\mu\text{m}$ , n = 20), l/w 5.6–7.2 ( $\bar{x}$  = 6.5, n = 20).

*Specimens examined.* **JAPAN**, Aomori, Hirosaki, Campus of Hirosaki Univ., on dead twigs of *Morus bombycis*, 27 Oct 2010, A. Hashimoto, AH 2 (HHUF 30117, living culture MAFF 244364); K. Tanaka, KT 2755 (HHUF 30118, living culture MAFF 244365).

*Notes* — Morphological features of the above materials agree with the description of *P. hispidula* provided by Nag Raj (1993). This species is distinguished from

*Pseudolachnea fraxini* by the smaller conidia compared with those of the latter (14–21 × 1.5–2.5 µm). In addition, there were 6–8 base differences in their ITS regions.

***Pseudolachnella*** Teng, Sinensia 7:775, 1936.

*Conidiomata* stromatic, acervular, cupulate, scattered to grouped, superficial, conical in sectional view, setose, black; basal stroma composed of brown, globose to subglobose, thick-walled cells; excipulum poorly developed, composed of globose to subglobose, pale brown cells. *Setae* marginal, cylindrical, straight to slightly curved, aseptate or septate, brown to dark brown, thick-walled, acute at the apex. *Conidiophores* arising from the upper cells of the basal stroma, cylindrical, branched, septate, hyaline, smooth. *Conidiogenous cells* phialidic, subcylindrical, hyaline, smooth. *Conidia* multi-septate, subcylindrical, acute or obtuse at the apex, truncate at the base, hyaline, slightly curved, smooth, bearing unbranched or branched, single to multiple appendages at each end; apical appendage central; basal appendage excentric.

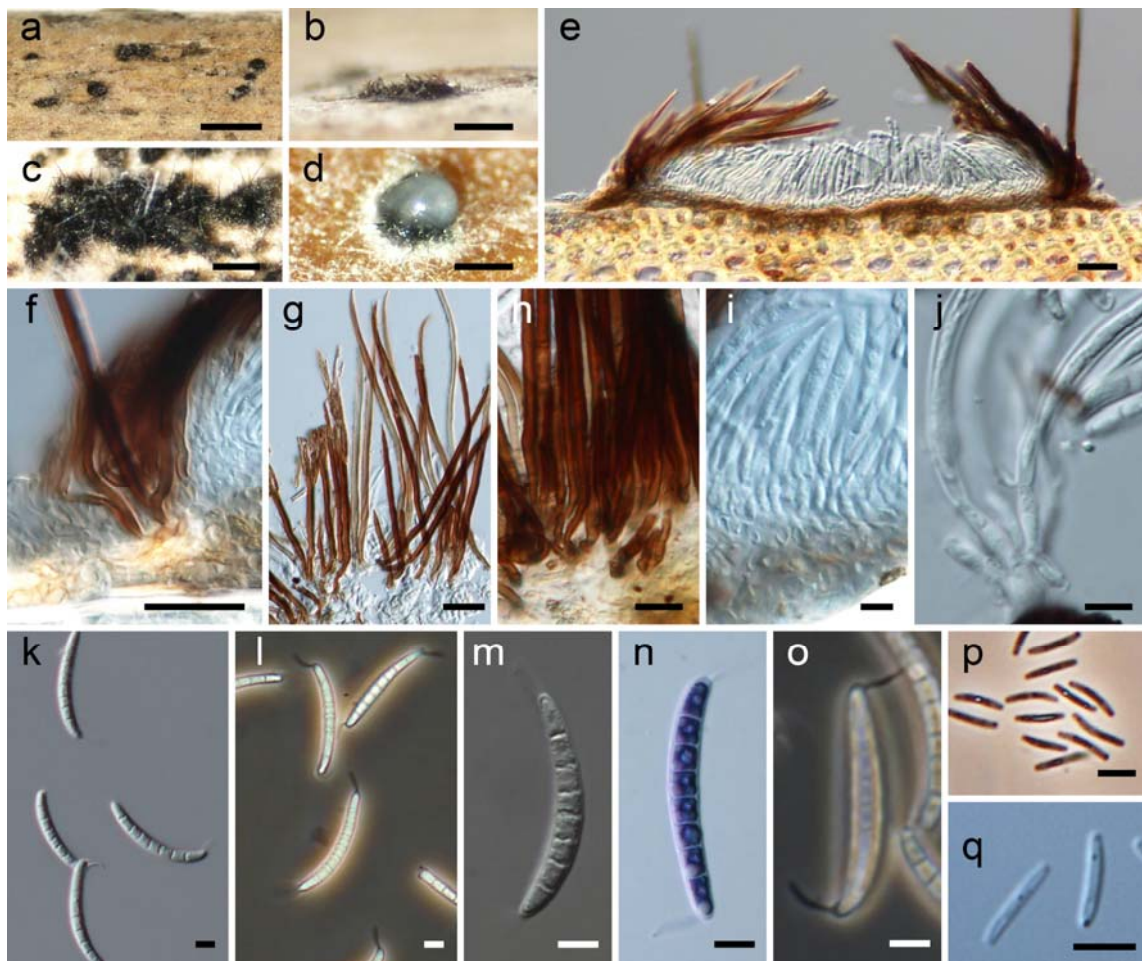
*Type species: Pseudolachnella scolecospora* (Teng & C.I. Chen) Teng, Sinensia 7:775, 1936.

***Pseudolachnella asymmetrica*** A. Hashim., Y. Harada & Kaz. Tanaka, **sp. nov.**  
— MycoBank MB 808709; Fig. 8.

*Etymology:* Named for the asymmetric orientation of the conidial appendages.

*Conidiomata* stromatic, acervular, setose, shallow-cupulate, scattered to occasionally 2–3 grouped, superficial or subepidermal origin, applanate, oval in outline, 200–670 µm long, 160–330 µm wide, 110–115 µm high, oval in sectional view, setose, black; basal stroma 4–8.5 µm thick, composed of brown, globose to subglobose, thick-walled, 2.5–5 µm diam cells; excipulum 20–30 µm thick, poorly developed, composed of globose to subglobose, pale brown cells. *Setae* marginal, cylindrical, straight or curved, aseptate, pale brown, thick-walled, 75–140 µm long, acute and 1.5–2.5 µm wide at the apex, 2–3 µm wide at the base. *Conidiophores* arising from the upper cells of the basal stroma, cylindrical, hyaline, smooth, 7 × 2 µm. *Conidiogenous cells* phialidic, cylindrical to lageniform, hyaline, smooth, 7–9 × 2–2.5 µm. *Conidia* 20.5–34 × 3–5 µm ( $\bar{x}$  = 28.7 × 4.0 µm, n = 50), l/w 4.6–9.4 ( $\bar{x}$  = 7.3, n = 50), (6–)7-septate, fusiform to allantoid, obtuse at the apex, slightly truncate at the base, hyaline, slightly curved, smooth, bearing an unbranched appendage at each end; apical appendage 3–8 µm long ( $\bar{x}$  = 5.7 µm, n = 50), central; basal appendage 3–7.5 µm long ( $\bar{x}$  = 5.0 µm, n = 50), excentric.





**Fig. 8.** *Pseudolachnella asymmetrica*. a, b. Conidiomata on host surface. c, d. Conidiomata in culture. e. Conidioma in longitudinal section. f. Basal stroma and excipulum of conidioma. g. Conidiomatal setae. h. Base of conidiomatal setae. i. Basal stroma of conidioma and conidiophores. j. Conidiogenous cells and immature conidia. k–o. Conidia. p, q. Microconidia. a, b, e–o. HHUF 28777. c, d, p, q. culture MAFF 244366. — Scale bars: a = 1000  $\mu$ m; b–d = 250  $\mu$ m; e–g = 20  $\mu$ m; h–q = 5  $\mu$ m.

**Culture characteristics** — Colonies on PDA attaining 32–35 mm diam after 21 d at 20 °C in the dark, floccose, sulphur yellow (15) (Fig. 18g).

**Specimen examined.** **JAPAN**, Fukuoka, Campus of Kyusyu Univ., on dead twigs of *Phyllostachys nigra* var. *henonis*, 27 Mar 2004, Y. Harada, SH 180 = H 4999 (HHUF 28777 **holotype**, ex-holotype living culture MAFF 244366 = NBRC 110126).

**Notes** — *Pseudolachnella asymmetrica* resembles *Pseudolachnella campylospora* in conidial morphology but has slightly broader conidia compared with those of the latter (l/w 7.3 vs. 8.3 in average). Moreover they differ at 28 positions of the ITS region and do not share a most recent common ancestor within the *Pseudolachnella* clade (Fig. 1).



***Pseudolachnella botulispora*** A. Hashim., G. Sato & Kaz. Tanaka, **sp. nov.** — MycoBank MB 808710; Fig. 9.

*Etymology*: Named for its botuliform conidia.

*Conidiomata* stromatic, acervular, setose, shallow-cupulate, scattered to occasionally 2–3 grouped, superficial, globose to oval in outline, 70–200  $\mu\text{m}$  diam, 60–110  $\mu\text{m}$  high, conical in sectional view, setose, black; basal stroma 5–6  $\mu\text{m}$  thick, composed of brown, globose to subglobose, thick-walled, 2.5–3  $\mu\text{m}$  diam cells; excipulum 27.5  $\mu\text{m}$  thick, poorly developed, composed of globose to subglobose, pale brown cells. *Setae* marginal, cylindrical, straight or curved, aseptate or rarely septate, brown to dark brown, paler toward the apex, thick-walled, 25–140  $\mu\text{m}$  long, acute and 1.5–3  $\mu\text{m}$  wide at the apex, 3–5.5  $\mu\text{m}$  wide at the base. *Conidiophores* arising from the upper cells of the basal stroma, branched, hyaline, smooth, 4.5–6.5  $\times$  2–3.5  $\mu\text{m}$ . *Conidiogenous cells* phialidic, cylindrical, hyaline, smooth, 4.5–12.5  $\times$  2–4  $\mu\text{m}$ . *Conidia* 22–35  $\times$  4–6  $\mu\text{m}$  ( $\bar{x}$  = 28.2  $\times$  4.9  $\mu\text{m}$ , n = 50), l/w 4.4–7.6 ( $\bar{x}$  = 5.8, n = 50), (1–)5–7-septate, cylindrical to allantoid, obtuse at the apex, truncate at the base, hyaline, slightly curved, smooth, bearing an unbranched appendage at each end; apical appendage 2–8  $\mu\text{m}$  long ( $\bar{x}$  = 4.8  $\mu\text{m}$ , n = 50), central; basal appendage 1.5–8  $\mu\text{m}$  long ( $\bar{x}$  = 5.2  $\mu\text{m}$ , n = 50), excentric.

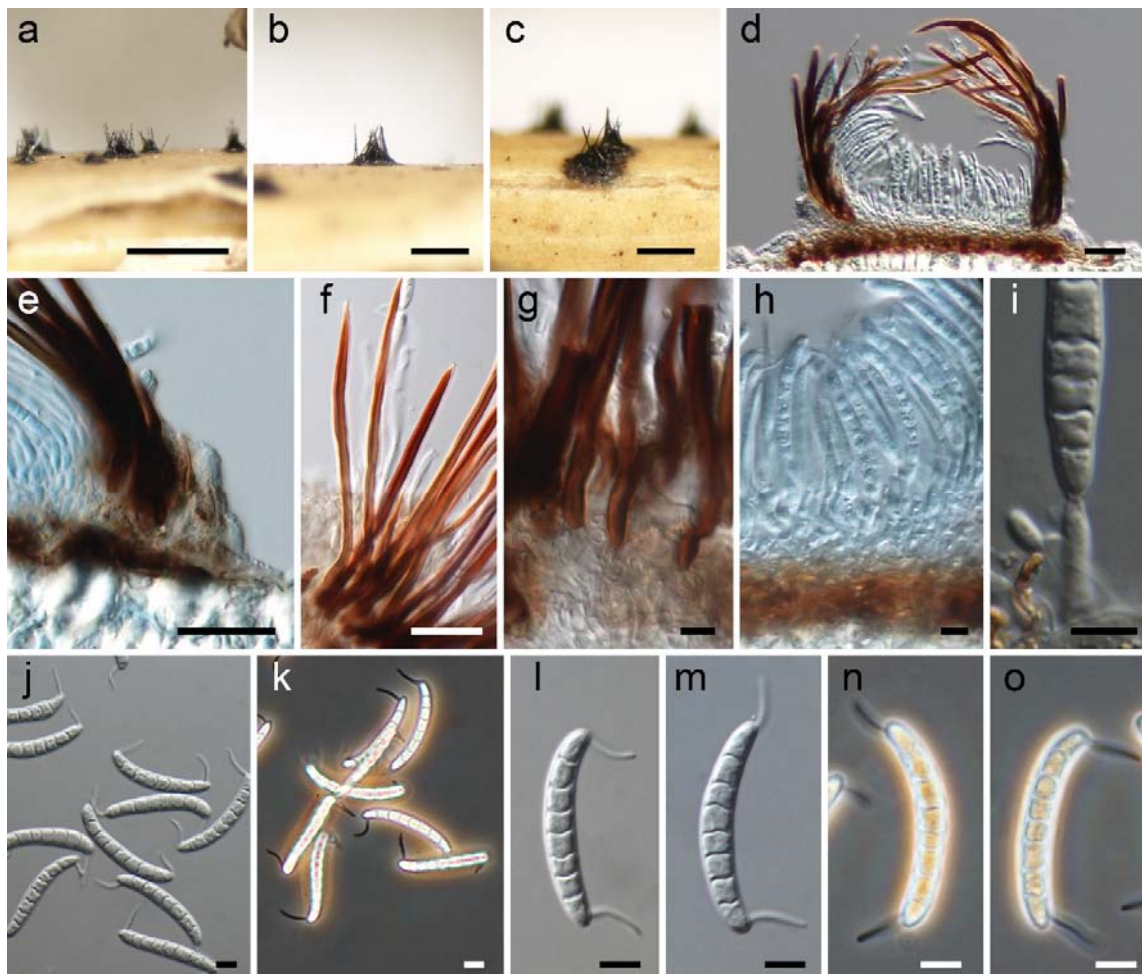
*Culture characteristics* — Colonies on PDA attaining 45–50 mm diam after 21 d at 20 °C in the dark, smooth, white (Fig. 18h).

*Specimens examined*. **JAPAN**, Shizuoka, Suntou, Nagaizumi, Fuji Bamboo Garden, on dead twigs of *Phyllostachys aurea*, 19 Aug 2007, G. Sato & K. Tanaka, GS 47 (HHUF 29969 **holotype**, ex-holotype living culture MAFF 244368 = NBRC 110127); Iwate, Ueda, Campus of Iwate Univ., on dead twigs of *Phyllostachys pubescens*, 17 Feb 2003, K. Tanaka & Y. Harada, SH 28 = H 4982 (HHUF 28776 **paratype**, ex-paratype living culture MAFF 244369).

*Notes* — In terms of the similar conidia size and number of conidial septa, this species is related to *Pseudolachnella asymmetrica* and *Pseudolachnella campylospora* but is distinguished from these two species by the relatively broader conidia. The length/width mean value of conidia is 5.8 in *P. botulispora*, 7.3 in *P. asymmetrica*, and 8.3 in *P. campylospora*.

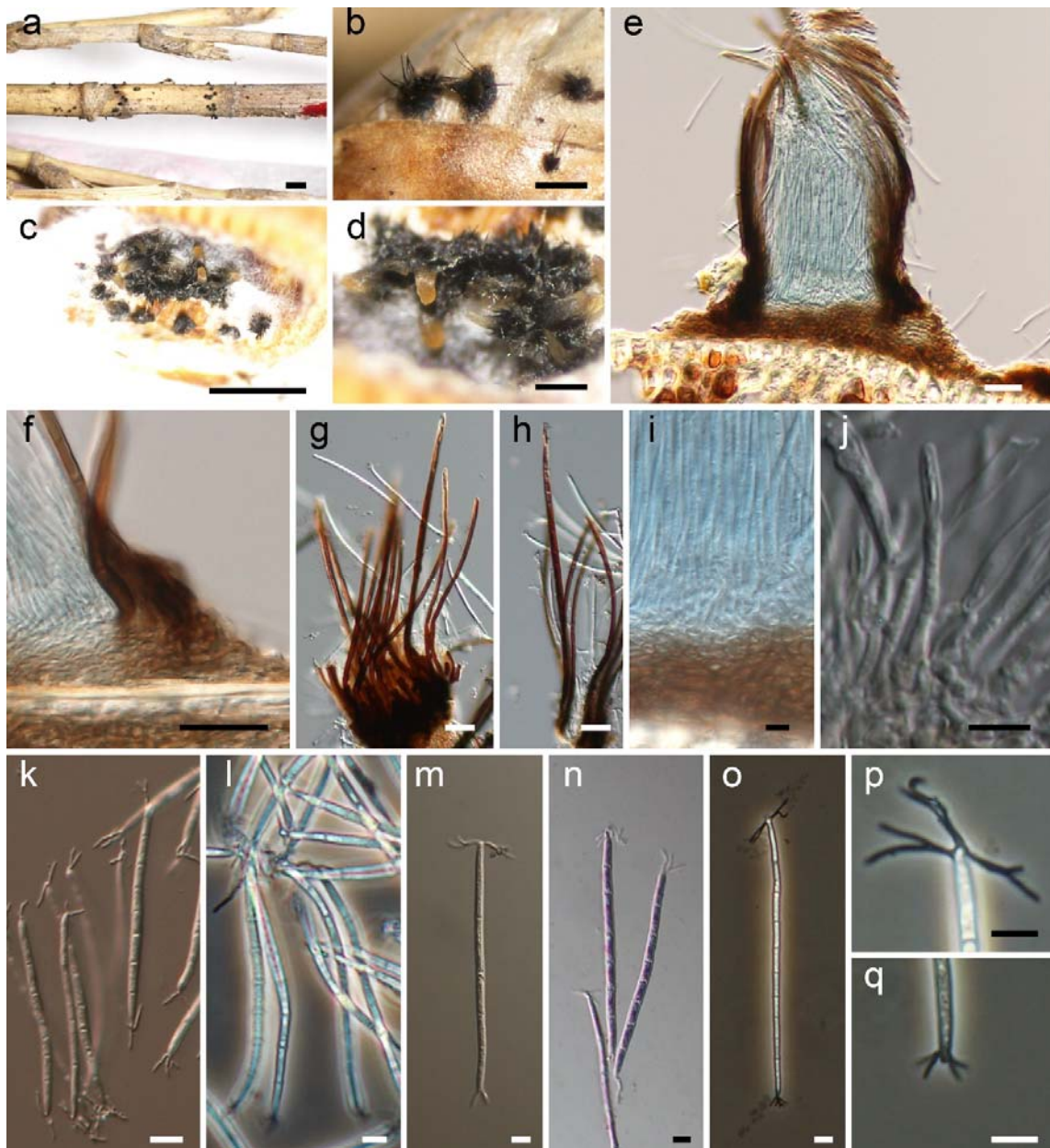
***Pseudolachnella brevicoronata*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB 808711; Fig. 10.

*Etymology*: Named for its resemblance to *Pseudolachnella coronata* (I. Hino & Katum.) Nag Raj, but with shorter conidia.



**Fig. 9.** *Pseudolachnella botulispora*. a–c. Conidiomata on host surface. d. Conidioma in longitudinal section. e. Basal stroma and excipulum of conidioma. f. Conidiomatal setae. g. Base of conidiomatal setae. h. Basal stroma of conidioma and conidiophores. i. Conidiogenous cell and immature conidium. j–o. Conidia. a–o. HHUF 29969. — Scale bars: a = 1000  $\mu\text{m}$ ; b, c = 250  $\mu\text{m}$ ; d–f = 20  $\mu\text{m}$ ; g–o = 5  $\mu\text{m}$ .

*Conidiomata* stromatic, acervular, setose, shallow-cupulate, scattered to 7–11 grouped, superficial or subepidermal origin, oval to ellipsoid in outline, 90–450  $\mu\text{m}$  diam, 130–300  $\mu\text{m}$  high, broadly cylindrical to conical in sectional view, setose, black; basal stroma 20–30(–37.5)  $\mu\text{m}$  thick, composed of pale brown, globose to subglobose, thin-walled, 2–5  $\mu\text{m}$  diam cells; excipulum 12.5–20  $\mu\text{m}$  thick, poorly developed, composed of globose to subglobose, pale brown cells. *Setae* marginal, cylindrical, straight or curved, multi-septate, dark brown, paler toward the apex, thick-walled, 100–160  $\mu\text{m}$  long, acute and 2.5–3  $\mu\text{m}$  wide at the apex, 3–7  $\mu\text{m}$  wide at the base. *Conidiophores* arising from the upper cells of the basal stroma, unbranched, hyaline, smooth, 7.5–9  $\mu\text{m}$  long, 2–2.5  $\mu\text{m}$  wide. *Conidiogenous cells* phialidic, cylindrical, hyaline, smooth, 8.5–15  $\times$  1.5–2  $\mu\text{m}$ . *Conidia* 62–90.5  $\times$  2–3  $\mu\text{m}$  ( $\bar{x}$  =



**Fig. 10.** *Pseudolachnella brevicoronata*. a, b. Conidiomata on host surface. c, d. Conidiomata in culture. e. Conidioma in longitudinal section. f. Basal stroma and excipulum of conidioma. g, h. Conidiomatal setae. i. Basal stroma of conidioma and conidiophores. j. Conidiogenous cells and immature conidia. k–o. Conidia. p. Apical appendages. q. Basal appendages. a, b, e–j, m–q. HHUF 30119. c, d, k, l. culture MAFF 244369. — Scale bars: a, c = 1000  $\mu\text{m}$ ; b, d = 250  $\mu\text{m}$ ; e–h = 20  $\mu\text{m}$ ; i–q = 5  $\mu\text{m}$ .

72.5  $\times$  2.3  $\mu\text{m}$ , n = 50), l/w 29.0–39.0 ( $\bar{x}$  = 32.1, n = 50), 7–10-septate, cylindrical to fusiform, obtuse at the apex, truncate at the base, hyaline, straight or slightly curved, smooth, bearing 2–4 unbranched or branched appendages at each end; apical

appendages 5–15.5  $\mu\text{m}$  long ( $\bar{x}$  = 8.2  $\mu\text{m}$ ,  $n$  = 50), branched, central; basal appendages 2–5.5  $\mu\text{m}$  long ( $\bar{x}$  = 3.8  $\mu\text{m}$ ,  $n$  = 50), unbranched or branched, excentric.

Culture characteristics — Colonies on PDA attaining 24–29 mm diam after 21 d at 20 °C in the dark, floccose, pale luteous (11) (Fig. 18i).

*Specimens examined.* **JAPAN**, Iwate, Hanamaki, near Dai-onsen, on dead twigs of *Sasa* sp., 25 Jun 2011, K. Tanaka, KT 2774 (HHUF 30119 **holotype**, ex-holotype living culture MAFF 244369 = NBRC 110128).

*Notes* — *Pseudolachnella brevicoronata* and *Pseudolachnella coronata* both share several morphological features in their conidia, for example, the relatively larger size (more than 60  $\mu\text{m}$  long), with more than seven septa, and with multiple appendages. However, *P. brevicoronata* has smaller conidia than those of the latter (vs. 70–110  $\mu\text{m}$  long; Hino & Katumoto 1958, Nag Raj 1993).

***Pseudolachnella campylospora*** A. Hashim., G. Sato & Kaz. Tanaka, **sp. nov.** — MycoBank MB 808712; Fig. 11.

*Etymology:* Named for its curved conidia.

*Conidiomata* stromatic, acervular, setose, shallow-cupulate, scattered to occasionally 2–3 grouped, superficial, globose to oval in outline, 180–580  $\mu\text{m}$  diam, 70–180  $\mu\text{m}$  high, conical in sectional view, setose, black; basal stroma 5–11.5  $\mu\text{m}$  thick, composed of brown, globose to subglobose, thick-walled, 2.5–5  $\mu\text{m}$  diam cells; excipulum 25–40  $\mu\text{m}$  thick, poorly developed, composed of globose to subglobose, pale brown cells. *Setae* marginal, cylindrical, straight or slightly curved, aseptate, brown to dark brown, thick-walled, 95–215  $\mu\text{m}$  long, acute and 1.5–3  $\mu\text{m}$  wide at the apex, 4  $\mu\text{m}$  wide at the base. *Conidiophores* arising from the upper cells of the basal stroma, cylindrical, hyaline, smooth, 6.5–20  $\times$  2.5–3  $\mu\text{m}$ . *Conidiogenous cells* phialidic, cylindrical to lageniform, hyaline, smooth, 7–11.5  $\times$  2  $\mu\text{m}$ . *Conidia* 25.5–35.5  $\times$  3–5  $\mu\text{m}$  ( $\bar{x}$  = 29.7  $\times$  3.7  $\mu\text{m}$ ,  $n$  = 50), l/w 5.7–10.8 ( $\bar{x}$  = 8.3,  $n$  = 50), (6–)7-septate, fusiform to allantoid, obtuse at the apex, slightly truncate at the base, hyaline, slightly curved, smooth, bearing an unbranched appendage at each end; apical appendage (4–)5–9  $\mu\text{m}$  long ( $\bar{x}$  = 7.0  $\mu\text{m}$ ,  $n$  = 50), central; basal appendage (3–)4–9  $\mu\text{m}$  long ( $\bar{x}$  = 5.8  $\mu\text{m}$ ,  $n$  = 50), excentric.

*Culture characters* — Colonies on PDA attaining 53–56 mm diam after 21 d at 20 °C in the dark, floccose, white (Fig. 18j).

*Specimen examined.* **JAPAN**, Hiroshima, Otake, on dead twigs of bamboo, 28 Mar 2007, G. Sato, GS 15 (HHUF 30002 **holotype**, ex-holotype living culture MAFF 244370 = NBRC 110129).

*Notes* — *Pseudolachnella campylospora* formed a strongly supported (99%/1.00 ML BP/Bayesian PP) monophyletic clade with *Pseudolachnella falcatispora* and *P. botulispora* (Fig. 1). However, *P. campylospora* (with seven-septate conidia) can be easily distinguished from *P. falcatispora* (with three-septate conidia). *Pseudolachnella campylospora* is differentiated from *P. botulispora* by its slender conidia as noted above (see Notes on *P. botulispora*).

***Pseudolachnella complanata*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB 808713; Fig. 12.

*Etymology*: Named for its flattened conidiomata.

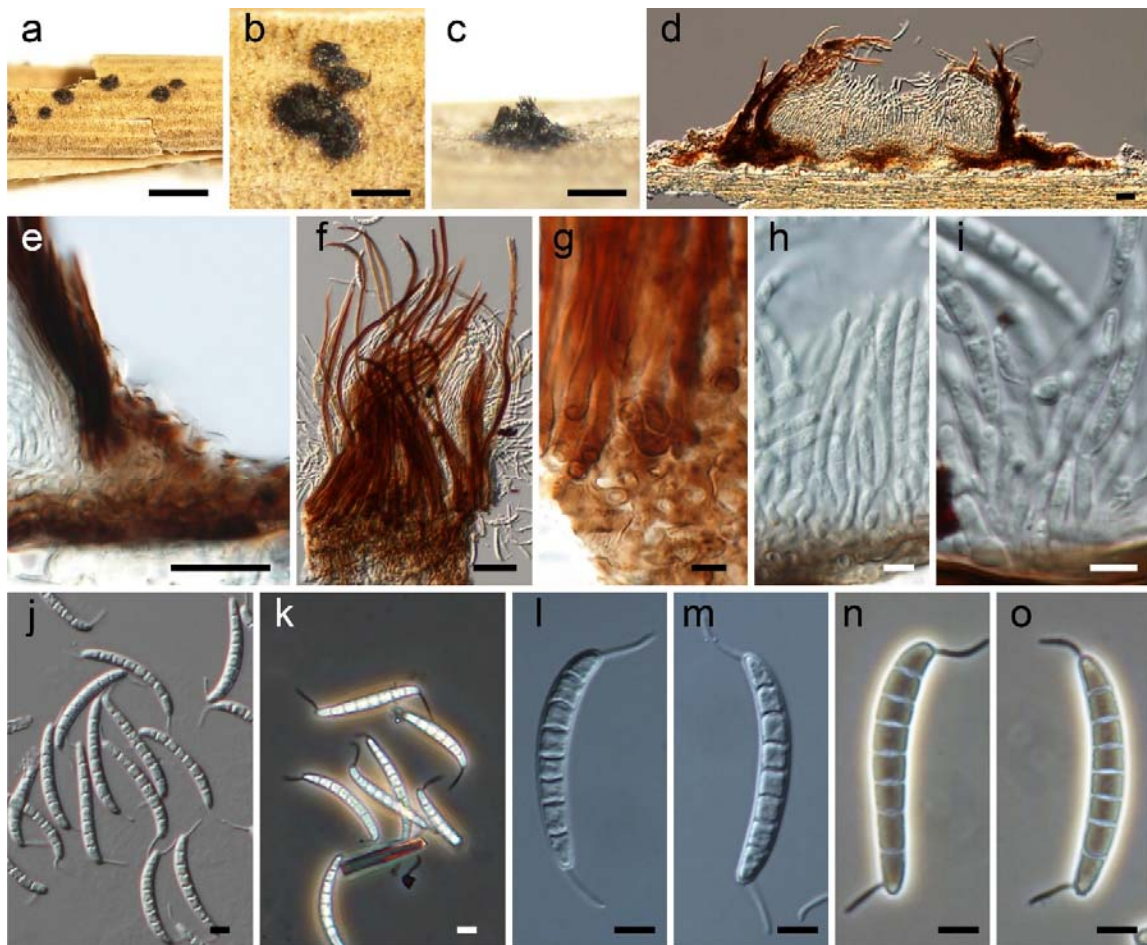
*Conidiomata* stromatic, acervular, setose, shallow-cupulate, scattered, superficial, applanate, oval in outline, 500–1500 µm long, 300–700 µm wide, 70–180 µm high, conical in sectional view, setose, black; margin entire 160–260 µm wide, without setae; basal stroma 7.5–17.5 µm thick, composed of brown, globose to subglobose, thick-walled, 2.5–7.5 µm diam cells; excipulum 20–40 µm thick, poorly developed, composed of globose to subglobose, pale brown cells. *Setae* arising from slightly inside of the margin, cylindrical, curved, 3–5-septate, pale brown, thick-walled, 90–260 µm long, acute and 1.5–2.5 µm wide at the apex, 4–6 µm wide at the base. *Conidiophores* lining the upper cells of the basal stroma, cylindrical, hyaline, smooth, 7.5–12 × 2 µm. *Conidiogenous cells* phialidic, cylindrical to lageniform, hyaline, smooth, 7–12.5 × 2–2.5 µm. *Conidia* (22–)26–40 × 2–3 µm ( $\bar{x}$  = 33.3 × 2.5 µm, n = 50), l/w 8.7–19.1 ( $\bar{x}$  = 14.3, n = 50), (3–)7-septate, fusiform to allantoid, obtuse at the apex, slightly truncate at the base, hyaline, slightly curved, smooth, bearing an unbranched appendage at each end; apical appendage 1–2 µm long ( $\bar{x}$  = 1.5 µm, n = 50), central; basal appendage 1–2 µm long ( $\bar{x}$  = 1.6 µm, n = 50), excentric.

*Culture characters* — Colonies on PDA attaining 23–31 mm diam after 21 d at 20 °C in the dark, floccose, zonate, primrose (66) (Fig. 18k).

*Specimen examined*. **JAPAN**, Kanagawa, Hakone, Yumoto, near Fukujyuin, on dead twigs of bamboo, 23 Dec 2003, T. Iijima, SH 77 = H 4995 (HHUF 28282 **holotype**, ex-holotype living culture MAFF 244371 = NBRC 110130).

*Notes* — The most characteristic feature of this species is the compressed conidiomata. Although similar flattened conidiomata are present in *Pseudolachnella pachyderma*, the latter has somewhat longer conidia (30–44 × 2.5–3 µm) and longer conidial appendages (2.5–7 µm). ITS sequence differences between these two taxa were found at 18 positions.



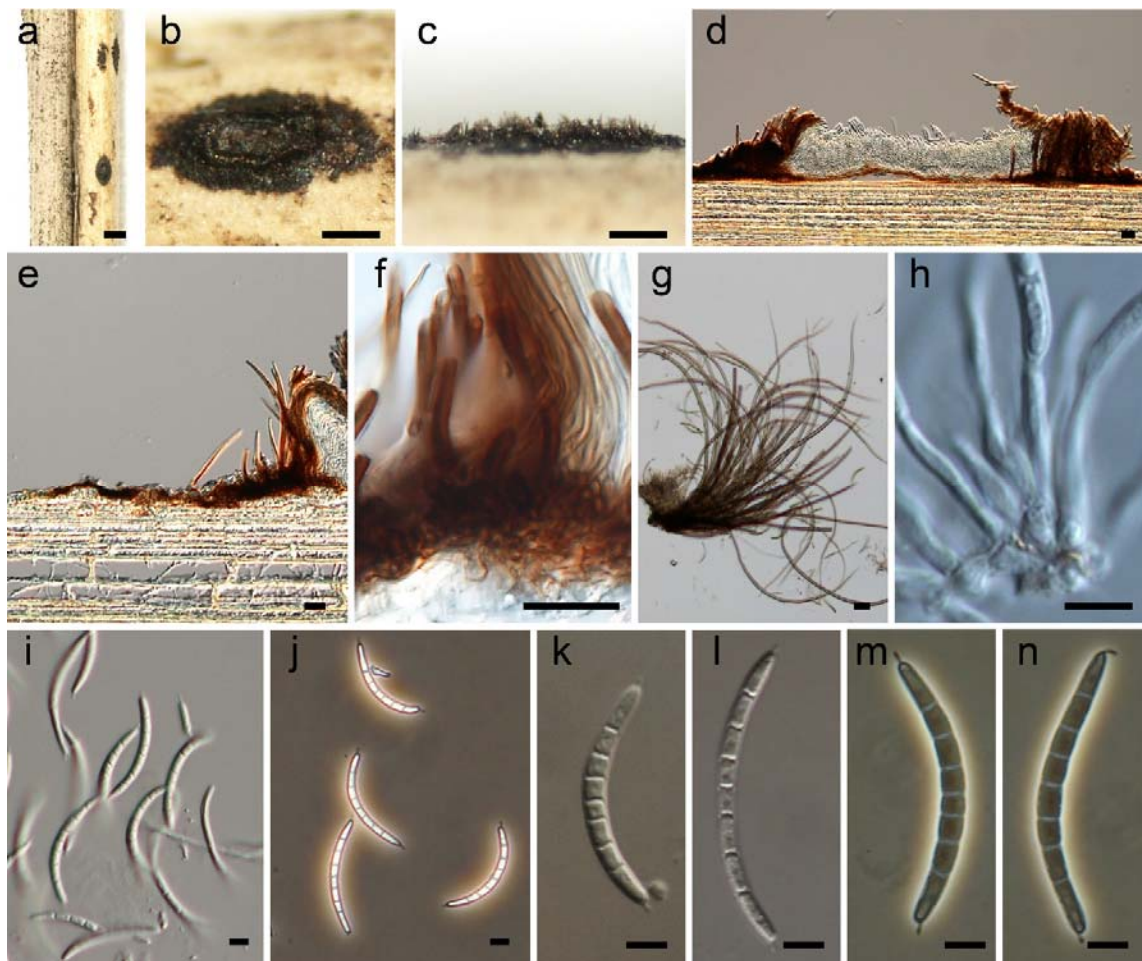


**Fig. 11.** *Pseudolachnella campylospora*. a–c. Conidiomata on host surface. d. Conidioma in longitudinal section. e. Basal stroma of conidioma and excipulum of conidioma. f. Conidiomatal setae. g. Base of conidiomatal setae. h. Basal stroma of conidioma and conidiophores. i. Conidiogenous cells and immature conidia. j–o. Conidia. a–o. HHUF 28282. — Scale bars: a = 1000  $\mu$ m; b, c = 250  $\mu$ m; d–f = 20  $\mu$ m; g–o = 5  $\mu$ m.

***Pseudolachnella falcatispora*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB 808714; Fig. 13.

**Etymology:** Named for its sickle-shaped conidia.

*Conidiomata* stromatic, acervular, setose, shallow-cupulate, scattered, superficial, applanate, oval in outline, 200–400  $\mu$ m diam, up to 1450  $\mu$ m diam in grouped conidiomata, 110–155  $\mu$ m high, papillate in sectional view, setose, black; basal stroma 5–6.5  $\mu$ m thick, composed of brown, globose to subglobose, thick-walled, 2.5–4  $\mu$ m diam cells; excipulum 15–30  $\mu$ m thick, poorly developed, composed of globose to subglobose, pale brown cells. *Setae* marginal, cylindrical, curved, aseptate, brown to dark brown, thick-walled, 90–185  $\mu$ m long, acute and 2.5–3  $\mu$ m wide at the apex, 3–5  $\mu$ m wide at the base. *Conidiophores* absent. *Conidiogenous*



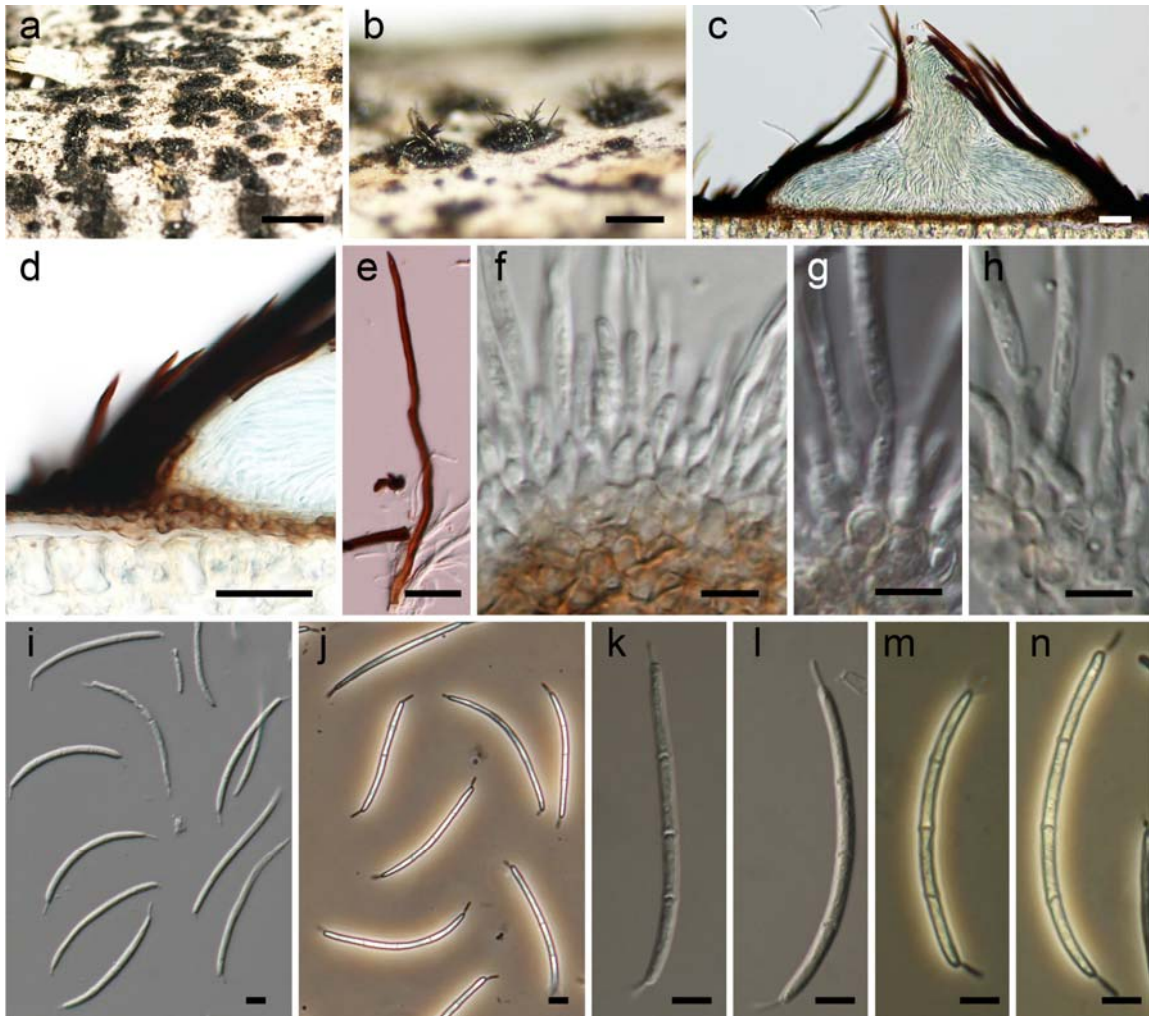
**Fig. 12.** *Pseudolachnella complanata*. a–c. Conidiomata on host surface. d. Conidioma in longitudinal section. e. Basal stroma of conidioma. f. Basal stroma and excipulum of conidioma. g. Conidiomatal setae. h. Conidiogenous cell and immature conidia. i–n. Conidia. a–n. HHUF 28282. — Scale bars: a = 1000  $\mu\text{m}$ ; b, c = 250  $\mu\text{m}$ ; d–g = 20  $\mu\text{m}$ ; h–n = 5  $\mu\text{m}$ .

*cells* phialidic, cylindrical to lageniform, hyaline, smooth, 16–19  $\times$  4.5–5  $\mu\text{m}$ . *Conidia* 28–44  $\times$  2–3  $\mu\text{m}$  ( $\bar{x}$  = 35.4  $\times$  2.3  $\mu\text{m}$ , n = 50), l/w 10.3–21.1 ( $\bar{x}$  = 15.8, n = 50), 3-septate, falcate, obtuse at the apex, truncate at the base, hyaline, slightly curved, bearing an unbranched appendage at each end; apical appendage 2–4  $\mu\text{m}$  long ( $\bar{x}$  = 2.9  $\mu\text{m}$ , n = 50), central; basal appendage 2–4  $\mu\text{m}$  long ( $\bar{x}$  = 2.7  $\mu\text{m}$ , n = 50), excentric.

**Culture characters** — Colonies on PDA attaining 39–40 mm diam after 21 d at 20  $^{\circ}\text{C}$  in the dark, floccose, white (Fig. 18l).

**Specimen examined.** **JAPAN**, Hiroshima, on dead culms of *Sinobambusa tootsik*, 29 Mar 2004, H. Araki, SH 182 = H 5226 (HHUF 29732 **holotype**, ex-holotype culture MAFF 244372 = NBRC 110131).





**Fig. 13.** *Pseudolachnella falcatispora*. a, b. Conidiomata on host surface. c. Conidioma in longitudinal section. d. Basal stroma and excipulum of conidioma. e. Conidiomatal setae. f. Basal stroma of conidioma and conidiogenous cells. g, h. Conidiogenous cells and immature conidia. i–n. Conidia. a–n. HHUF 29732. — Scale bars: a = 1000  $\mu\text{m}$ , b = 250  $\mu\text{m}$ , c–e = 20  $\mu\text{m}$ , f–n = 5  $\mu\text{m}$ .

**Notes** — *Pseudolachnella falcatispora* bears a strong resemblance to *Pseudolachnella scolecospora* in conidial morphology, but the basal stroma of conidiomata is less developed (5–6.5  $\mu\text{m}$  thick) than that of the latter species (6.5–12.5  $\mu\text{m}$  thick). Sequence differences between these two taxa were found at 28 and 29 sites in ITS and *tef1*, respectively. *Pseudolachnella indica*, described from culms of a *Panicum* sp. (Nag Raj 1993), also is similar to *P. falcatispora*, but *P. indica* has conidiophores up to 20  $\mu\text{m}$  long and slightly smaller conidia (24–38  $\times$  2.5–5  $\mu\text{m}$ ,  $\bar{x}$  = 31.8  $\times$  2.2  $\mu\text{m}$ ; Nag Raj 1993). No sequence information for *P. indica* was available for the molecular analyses.



***Pseudolachnella fusiformis*** A. Hashim., G. Sato & Kaz. Tanaka, **sp. nov.** — MycoBank MB 808715; Fig. 14.

*Etymology*: Named for its fusiform conidia.

*Conidiomata* stromatic, acervular, setose, shallow-cupulate, scattered to occasionally 2–3 grouped, superficial, globose to oval in outline, 260–490 µm diam, 80–190 µm high, conical in sectional view, setose, black; basal stroma 5–6 µm thick, composed of brown, globose to subglobose, thick-walled, 2.5–3 µm diam cells; excipulum 27.5 µm thick, poorly developed, composed of globose to subglobose, pale brown cells. *Setae* marginal, cylindrical, straight or curved, aseptate or septate, pale brown to dark brown, paler toward the apex, thick-walled, 35–75 µm long, acute and 2.5–4 µm wide at the apex, 4–7.5 µm wide at the base. *Conidiophores* absent. *Conidiogenous cells* phialidic, cylindrical to doliiform, hyaline, smooth, 5–7 × 3–4 µm. *Conidia* 15–20 × 4–6.5 µm ( $\bar{x}$  = 26.9 × 5.4 µm, n = 50), l/w 2.5–4.0 ( $\bar{x}$  = 3.2, n = 50), 3-septate, fusiform to oval, obtuse at the apex, truncate at the base, hyaline, straight, smooth, bearing 2–4 branched or unbranched appendages at each end; apical appendages 3.5–11.5 µm long ( $\bar{x}$  = 6.9 µm, n = 50), central; basal appendages 3–9 µm long ( $\bar{x}$  = 5.8 µm, n = 50), excentric.

Culture characters — Colonies on PDA attaining 17.5–30 mm diam after 21 d at 20 °C in the dark, floccose, dark brick (82) (Fig. 18m).

*Specimen examined*. **JAPAN**, Nara, Kamikitayama, Gyoujyagaeri tunnel, on dead sheath of bamboo, 14 Aug 2006, G. Sato, GS 5 = H 5222 (HHUF 29725 **holotype**, ex-holotype living culture MAFF 224373 = NBRC 110132).

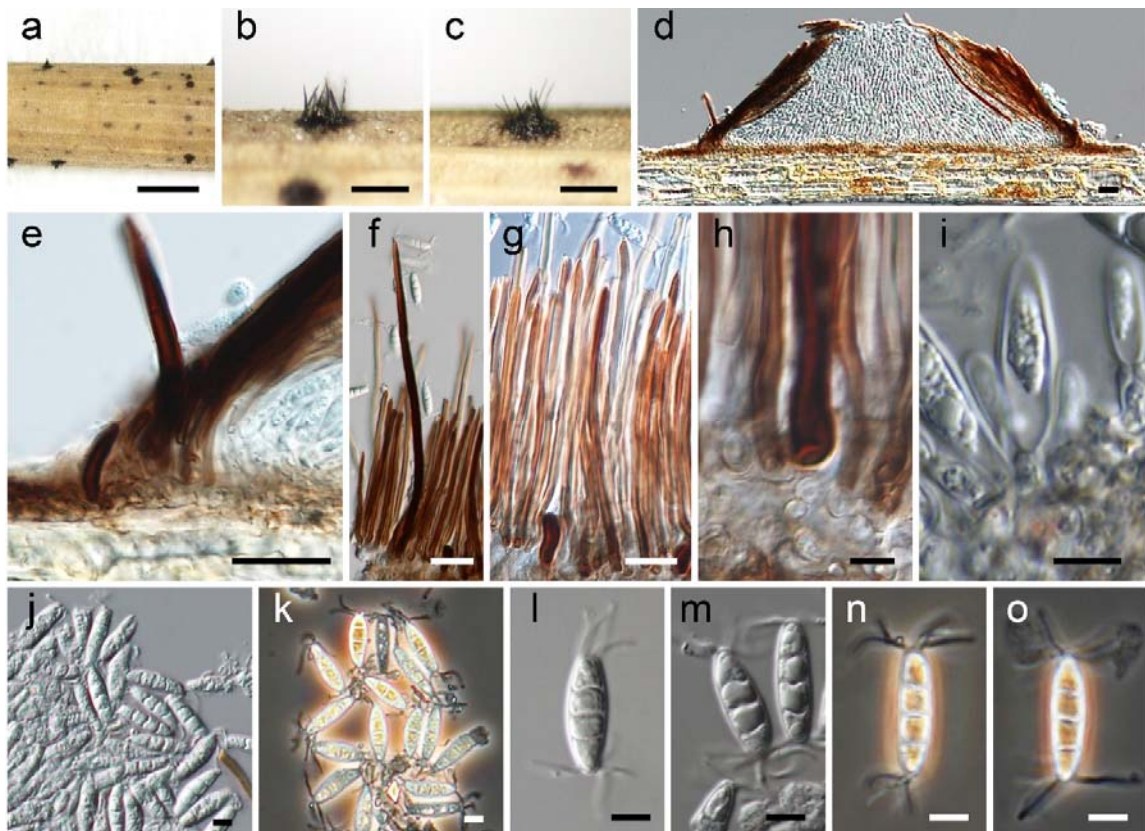
*Notes* — In addition to eight species previously recognised in *Pseudolachnella* (Nag Raj 1993, Zhao *et al.* 2004, Sato *et al.* 2008), eight new species are described in this study. Among these 16 species, *P. fusiformis* has the smallest and relatively plump conidia.

***Pseudolachnella longiciliata*** (I. Hino & Katum.) Nag Raj, Coelomycetous anamorphs with appendage bearing conidia:725, 1993; Fig. 15.

*Basionym*: *Chaetopatella longiciliata* I. Hino & Katum., J. Jpn. Bot. 33:239, 1958.

*Synonym*: *Pseudolachnea longiciliata* (I. Hino & Katum.) B. Sutton, The Coelomycetes:461, 1980.

*Conidiomata* stromatic, acervular, setose, cupulate, globose to oval in outline, 220–420 µm diam, 270–490 µm high, mucronate to ampliform in sectional view; basal

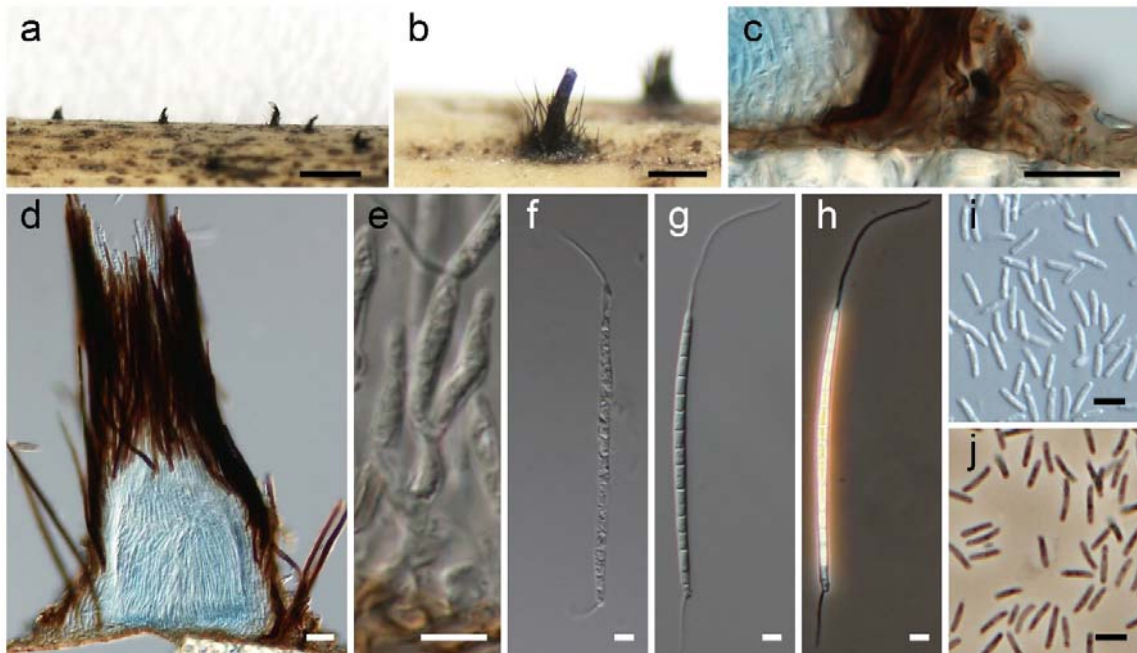


**Fig. 14.** *Pseudolachnella fusiformis*. a–c. Conidiomata on host surface. d. Conidioma in longitudinal section. e. Basal stroma and excipulum of conidioma. f, g. Conidiomatal setae. h. Base of conidiomatal setae. i. Conidiogenous cells and immature conidia. j–o. Conidia. a–o. HHUF 29725. — Scale bars: a = 1000  $\mu\text{m}$ ; b, c = 250  $\mu\text{m}$ ; d–g = 20  $\mu\text{m}$ ; h–o = 5  $\mu\text{m}$ .

stroma 6.5–14  $\mu\text{m}$  thick; excipulum 22.5–47.5  $\mu\text{m}$  thick, poorly developed. *Setae* 185–320  $\mu\text{m}$  long, acute and 2–3  $\mu\text{m}$  wide at the apex, 5–10  $\mu\text{m}$  wide at the base. *Conidiophores* 7.5–10  $\mu\text{m}$  long, 2–2.5  $\mu\text{m}$  wide. *Conidiogenous cells* 6–19  $\times$  2  $\mu\text{m}$ . *Conidia* 61.5–87  $\times$  (2–)2.5–5  $\mu\text{m}$  ( $\bar{x}$  = 74.4  $\times$  3.0  $\mu\text{m}$ , n = 60), l/w 15.7–35.5 ( $\bar{x}$  = 25.4, n = 60), 8–18-septate, cylindrical, hyaline, smooth, bearing an unbranched appendage at each end; apical appendage 8.5–40  $\mu\text{m}$  long ( $\bar{x}$  = 21.0  $\mu\text{m}$ , n = 62); basal appendage 4–23  $\mu\text{m}$  long ( $\bar{x}$  = 9.5  $\mu\text{m}$ , n = 64).

**Culture characters** — Colonies on PDA attaining 24–27 mm diam after 21 d at 20  $^{\circ}\text{C}$  in the dark, floccose, isabelline (65) (Fig. 18n).

**Specimens examined.** **JAPAN**, Hokkaido, Isl. Rishiri, Mikaeridai, on dead twigs of *Sasa kurilensis*, 30 Jul 2007, G. Sato & K. Tanaka, GS 36 (HHUF 29962, living culture MAFF 244374); Kutsugata, Mt. Rishiri (8 stage), on dead twigs of *Sasa kurilensis*, 28 Jul 2007, G. Sato & K. Tanaka, GS 42 (HHUF 29966, living culture MAFF 244375); Aomori, Nishimeya, Tsugaru pass, on dead twigs of *Sasa kurilensis*, 28 Aug 2007, H. Yonezawa *et al.*, GS 50 (HHUF 29972, living culture MAFF 244376); Towada, near Tsuta spa, on dead sheath of *Sasa kurilensis*, 3 Aug 2002, S. Hatakeyama, SH 97 =



**Fig. 15.** *Pseudolachnella longiciliata*. a, b. Conidiomata on host surface. c. Basal stroma and excipulum of conidioma. d. Conidioma in longitudinal section. e. Conidiophores, conidiogenous cells and immature conidia. f–h. Conidia. i, j. Microconidia. a–e, g, h. HHUF 27528. i, j. culture MAFF 244376. f. YAM 21812. — Scale bars: a = 1000  $\mu$ m; b = 250  $\mu$ m; c, d = 20  $\mu$ m; e–j = 5  $\mu$ m.

H 4617 (HHUF 27528 **epitype**, ex-epitype living culture MAFF 244377); Akita, Kitaakita-gun, Mt. Nyutozan, on dead twigs of *Sasa kurilensis*, 4 Aug 1957, H. Muroi (YAM 21812 **holotype**).

**Notes** — This species is readily distinguished from others in *Pseudolachnella* by its mucronate to ampliform conidiomata up to 490  $\mu$ m high and the 8–18-septate conidia with longer apical and shorter basal appendages. The species has been reported from culms of bamboo (e.g. *Bambusa* sp., *Sasa kurilensis*, and *Yushania vigens*) in the subarctic regions of Asia, such as northern Japan (Hino & Katumoto 1958), Kunashir Island in the Northern Territory (Mel'nik 1997) and Sakhalin in Russia (Mel'nik pers comm) and in mountainous areas at high elevations in China (Zhao *et al.* 2004) and India (Muthumary 1987). It appears to be a relatively common species in northern Japan but could not be found in the subtropical area in our study. The holotype (YAM 21812) of *Chaetopatella longiciliata* was compared with our materials, and an epitype was designated in the present study. This species, the type of the genus *Chaetopatella* (Hino & Katumoto 1958), was nested within the clade of *Pseudolachnella* typified by *P. scolecospora* (Teng 1936; Fig. 1). The synonymy of *Chaetopatella* with *Pseudolachnella* was confirmed with molecular evidence for the first time, although it was already suggested by Nag Raj (1993) on the basis of morphological evidence.

***Pseudolachnella pachyderma*** A. Hashim., G. Sato & Kaz. Tanaka, **sp. nov.** — MycoBank MB 808716; Fig. 16.

*Etymology*: Named for its thick-walled conidiomata.

*Conidiomata* stromatic, acervular, setose, shallow-cupulate, scattered to 2–3 grouped, superficial, applanate, oval in outline, 200–450 µm diam, 75–100 µm high, conical in sectional view, setose, black; margin entire, 60–100 µm wide, without setae; basal stroma 10–20 µm thick, composed of brown, globose to subglobose, thick-walled, 2.5–5 µm diam cells; excipulum 25–40 µm thick, poorly developed, composed of globose to subglobose, pale brown cells. *Setae* arising from slightly inside of the margin, cylindrical, straight to slightly curved, septate, dark brown, thick-walled, 95–190 µm long, acute and 2.5 µm wide at the apex, 3–4 µm wide at the base. *Conidiophores* lining the upper cells of the basal stroma, cylindrical, hyaline, smooth, 7–20 × 2–3.5 µm. *Conidiogenous cells* phialidic, cylindrical to lageniform, hyaline, smooth, 6–12 × 2 µm. *Conidia* 30–44 × 2.5–3 µm ( $\bar{x}$  = 37.4 × 2.6 µm, n = 50), l/w 10.0–18.0 ( $\bar{x}$  = 14.5, n = 50), (3–)7-septate, subcylindrical to fusiform, obtuse at the apex, truncate at the base, hyaline, slightly curved, smooth, bearing an unbranched appendage at each end; apical appendage 2.5–7 µm long ( $\bar{x}$  = 4.5 µm, n = 50), central; basal appendage 2.5–7 µm long ( $\bar{x}$  = 4.8 µm, n = 50), excentric.

Culture characters — Colonies on PDA attaining 34–40 mm diam after 21 d at 20 °C in the dark, smooth, buff (45) (Fig. 18o)

*Specimen examined*. JAPAN, Nagasaki, Isahaya, on dead twigs of bamboo, 30 Mar 2007, G. Sato, GS17 (HHUF 29955 **holotype**, ex-holotype living culture MAFF 2444378 = NBRC 110133).

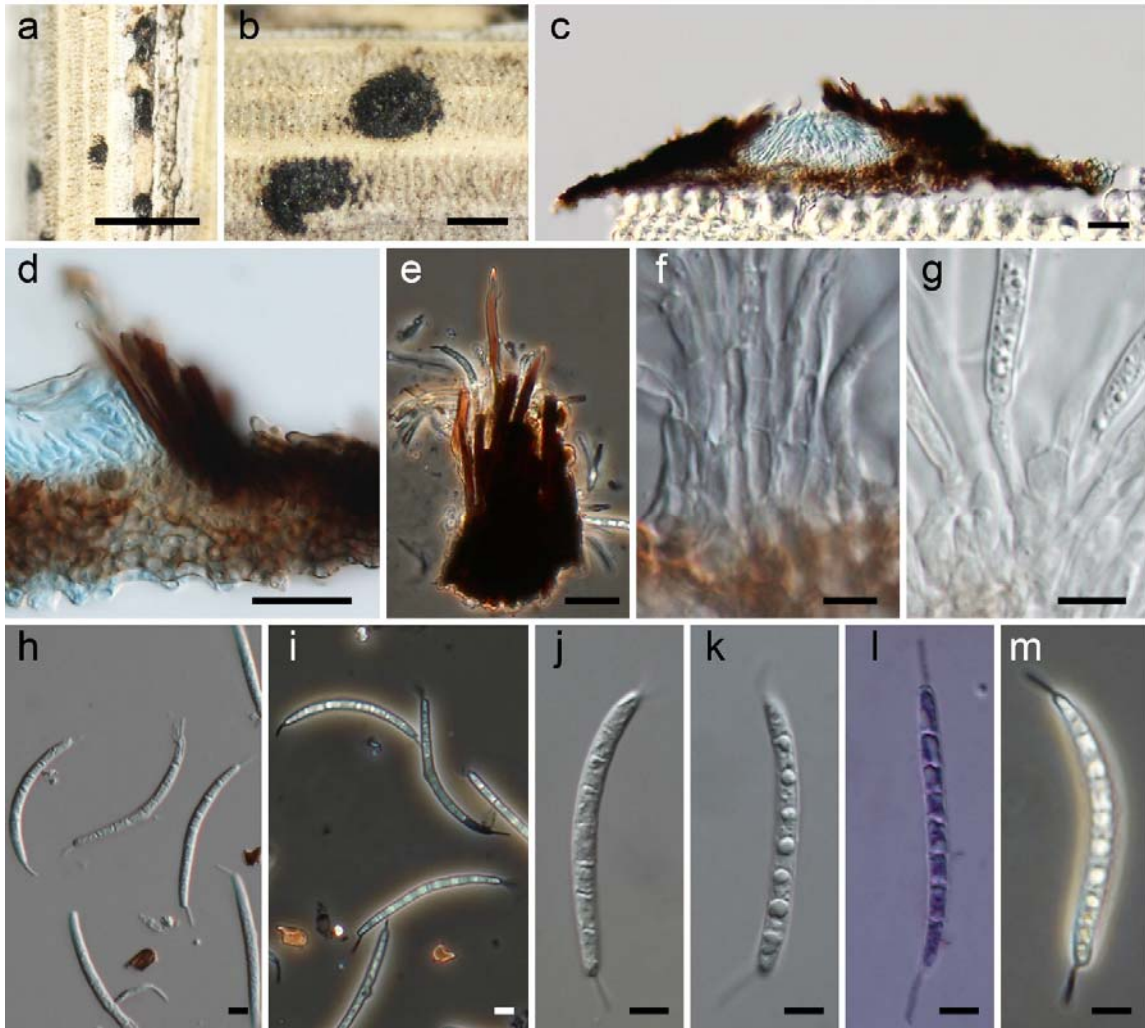
*Notes* — This species is characterised by the flattened conidiomata with a relatively thick peridial wall. The conidia of *P. pachyderma* overlap in size with those of *Pseudolachnella falcatispora* and *Pseudolachnella scolecospora*, but the latter two species have consistently three-septate conidia. It is phylogenetically close to *Pseudolachnella asymmetrica* (Fig. 1), but the latter has smaller and broader conidia (20.5–34 × 3–5 µm).

***Pseudolachnella scolecospora*** (Teng & C.I. Chen) Teng, Sinensia 7:775, 1936; Fig. 17.

*Basionym*: *Pseudolachnea scolecospora* Teng & C.I. Chen, Contrib. Biol. Lab. Sci. Soc. China, Bot. Ser. 8:277, 1933.

*Conidiomata* stromatic, acervular, setose, shallow-cupulate, oval in outline, 490–1000 µm long, 280–400 µm wide, 100–140 µm high; basal stroma 6.5–12.5 µm thick; excipulum 15–37.5 µm thick, poorly developed. *Setae* 130–180 µm long, acute and

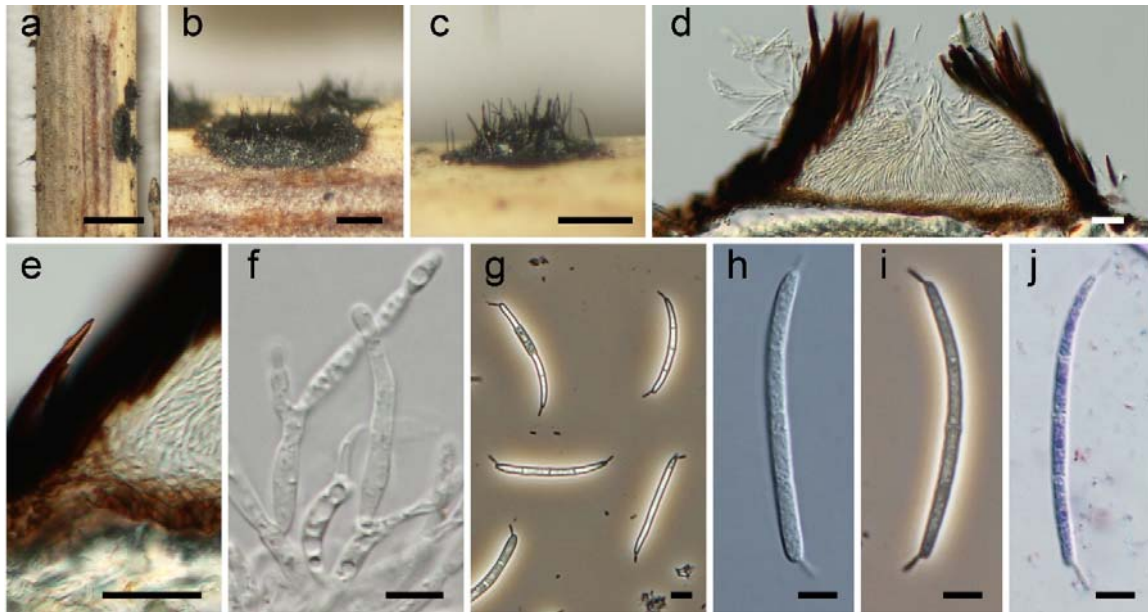




**Fig. 16.** *Pseudolachnella pachyderma*. a, b. Conidiomata on host surface. c. Conidioma in longitudinal section. d. Basal stroma and excipulum of conidioma. e. Conidiomatal setae. f. Basal stroma of conidioma and conidiophores. g. Conidiogenous cells and immature conidia. h–m. Conidia. a–m. HHUF 29955. — Scale bars: a = 1000  $\mu\text{m}$ . b = 250  $\mu\text{m}$ . c–e = 20  $\mu\text{m}$ . f–m = 5  $\mu\text{m}$ .

2–3  $\mu\text{m}$  wide at the apex, 3–4  $\mu\text{m}$  wide at the base. *Conidiophores* 10–23  $\times$  2–2.5  $\mu\text{m}$ . *Conidiogenous cells* 7–16.5  $\times$  1.5–3  $\mu\text{m}$ . *Conidia* 31–44.5  $\times$  2–3  $\mu\text{m}$  ( $\bar{x}$  = 37.6  $\times$  2.4  $\mu\text{m}$ , n = 50), l/w 12.3–19.5 ( $\bar{x}$  = 15.8, n = 50), (1–)3(–5)-septate, subcylindrical, hyaline, slightly curved, smooth, bearing an unbranched appendage at each end; apical appendage 1.5–3  $\mu\text{m}$  long ( $\bar{x}$  = 2.3  $\mu\text{m}$ , n = 50), central; basal appendage 1.5–3.5  $\mu\text{m}$  long ( $\bar{x}$  = 2.4  $\mu\text{m}$ , n = 50), excentric.

Culture characters — Colonies on PDA attaining 20–41 mm diam after 21 d at 20  $^{\circ}\text{C}$  in the dark, smooth, white (Fig. 18p).



**Fig. 17.** *Pseudolachnella scolecospora*. a–c. Conidiomata on host surface. d. Conidioma in longitudinal section. e. Basal stroma and excipulum of conidioma. f. Conidiophores, conidiogenous cells and immature conidia. g–j. Conidia. a–j. HHUF 30268. — Scale bars: a = 1000  $\mu\text{m}$ ; b, c = 250  $\mu\text{m}$ ; d, e = 20  $\mu\text{m}$ ; f–j = 5  $\mu\text{m}$ .

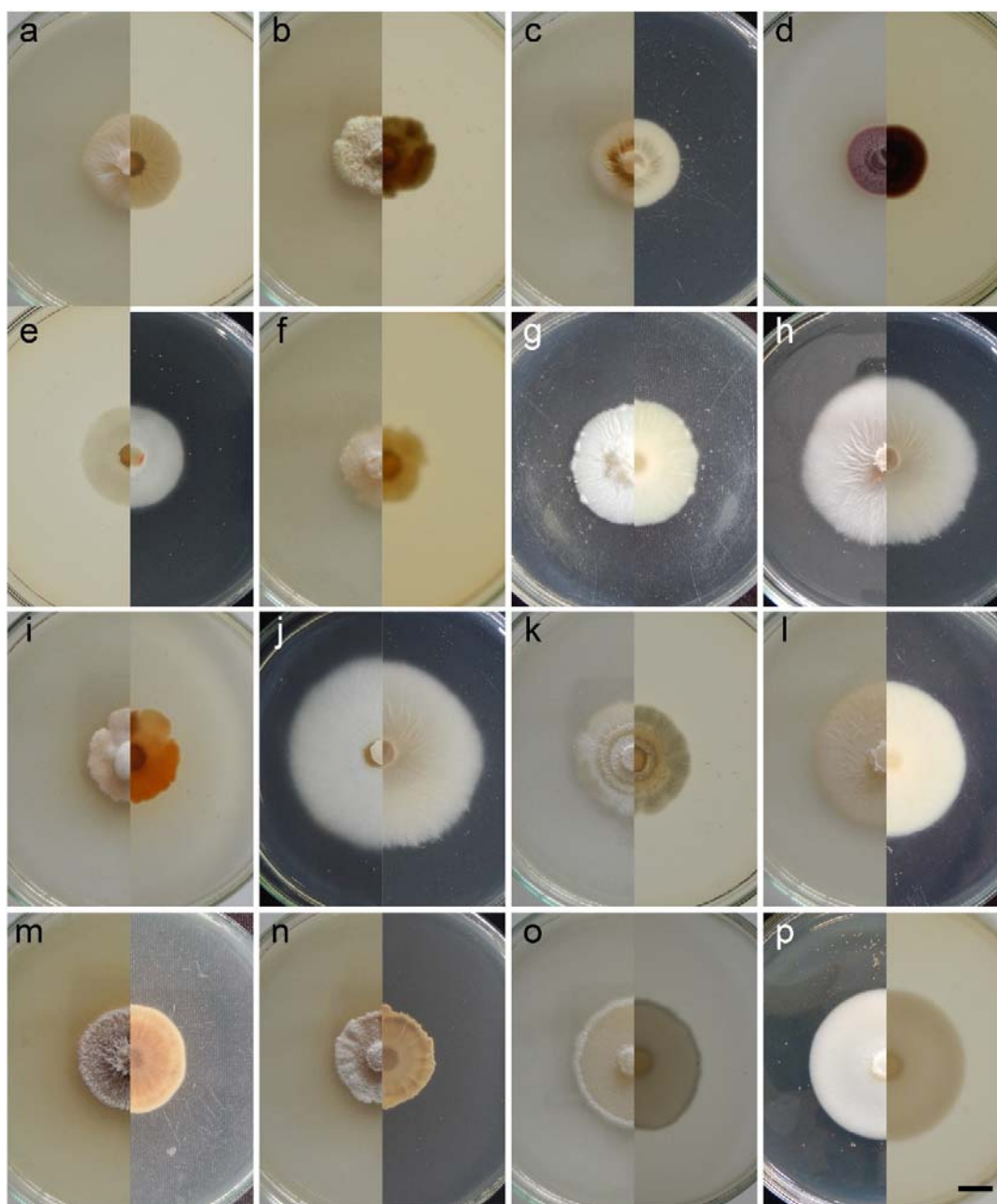
**Specimen examined.** **JAPAN**, Gifu, Tonomachi, near Kashimori Park, on dead twigs of *Sasa* sp., 27 Mar 2013, A. Hashimoto, AH 169 (HHUF 30268, living culture MAFF 244379).

**Notes** — The size of conidia in our material is slightly smaller than those of *P. scolecospora* reported by Nag Raj (1993) as  $\bar{x} = 39.5 \times 2.5 \mu\text{m}$ , but the length:width ratio of the conidia is identical. This species originally was described as a species of *Pseudolachnea* (Teng & Ling 1933) but later was transferred to a new genus *Pseudolachnella* as the type species (Teng 1936). The generic validity of *Pseudolachnella* was confirmed in the current study on the basis of molecular data for the first time (Fig. 1).

***Pseudolachnella yakushimensis*** G. Sato, Kaz. Tanaka & Hosoya, *Mycoscience* 49:392, 2008.

**Specimens examined.** **JAPAN**, Kagoshima, Isl. Yakushima, Shiratani-unsuikyo, on dead twigs of *Pleioblastus* sp., 19 Oct 2005, K. Tanaka & T. Hosoya, KT 1888 = H 5223 (HHUF 29683 **holotype**, ex-holotype living culture NBRC 15101); Nagata, on dead twigs of bamboo, 15 Mar 2007, H. Yonezawa & K. Tanaka, GS 6 (HHUF 29996, living culture MAFF 244380).

**Notes** — For description and illustration of this species see Sato *et al.* (2008). Two strains of *P. yakushimensis* grouped with *P. brevicoronata* without significant support.



**Fig. 18.** Colony characters of species sued in this study on PDA within 3 wk at 20 °C in the dark (left: surface, right: reverse). a. *Neopseudolachnella acutispora* (MAFF 244358). b. *N. magnispora* (MAFF 244359). c. *N. uniseptata* (MAFF 244360). d. *Pseudodinemasporium fabiforme* (MAFF 244361). e. *Pseudolachnea fraxini* (MAFF 244363). f. *Pseudolachnea hispidula* (MAFF 244365). g. *Pseudolachnella asymmetrica* (MAFF 244366). h. *Pseudolachnella botulispora* (MAFF 244367). i. *Pseudolachnella brevicoronata* (MAFF 244369). j. *Pseudolachnella campylospora* (MAFF 244370). k. *Pseudolachnella complanata* (MAFF 244371). l. *Pseudolachnella falcatispora* (MAFF 244372). m. *Pseudolachnella fusiformis* (MAFF 244373). n. *Pseudolachnella longiciliata* (MAFF 244377). o. *Pseudolachnella pachyderma* (MAFF 244378). p. *Pseudolachnella scolecospora* (MAFF 244379). — Scale bar: 1 cm.

In both taxa the conidia have multiple appendages, but those of *P. yakushimensis* are considerably smaller ( $15\text{--}33 \times 2\text{--}3 \mu\text{m}$  vs.  $62\text{--}90.5 \times 2\text{--}3 \mu\text{m}$ ).

## DISCUSSION

Phylogenetic relationships of pseudolachnea-like species with acervulous conidiomata surrounded by black setae and appendaged conidia were assessed on the basis of morphology and DNA sequences for LSU, ITS and *tef1*. A total of 24 strains, excluding two strains used as outgroup taxa, and three species for which sequence data were obtained from GenBank were classified in four genera: *Pseudolachnea*, *Pseudolachnella*, including two new genera *Neopseudolachnella* and *Pseudodinemasporium*.

The main purpose of this study was to assess the monophyly of *Pseudolachnea* (Ranojović 1910) and *Pseudolachnella* (Teng 1936). These genera previously have been distinguished mainly by their conidial septation (i.e. one-septate conidia in *Pseudolachnea* and multiseptate conidia in *Pseudolachnella* [Teng 1936]). However, whether the question these genera should be recognised as natural taxa is controversial. Several authors have accepted them as distinct genera (Nag Raj 1993, Mel'nik 1997, Zhao *et al.* 2004, Sato *et al.* 2008), whereas Sutton (1977, 1980) regarded them as congeneric and this opinion often has been followed (Castañeda 1987, Muthumary 1987, Kiffer & Morelet 1999, Kirk *et al.* 2008). Our study clearly indicated that these two genera are natural groups because they formed two distinct clades in the ML tree (Fig. 1). *Pseudolachnea*, comprising *P. hispidula* (the type species of this genus) and *P. fraxini* both with one-septate conidia, was highly supported and the clade of *Pseudolachnella* containing 11 species with 3- to 18-septate conidia also received high ML BP and Bayesian PP support (Fig. 1). This result might indicate that conidial septation is of phylogenetic importance for these genera. However, we considered that this criterion alone is insufficient to delineate them, because more radical differences were found from morphological comparison of the taxa. The acervular conidiomata with numerous setae are superficially identical in both genera, but *Pseudolachnea* has larger conidiomata than those of *Pseudolachnella*. These differences in external appearance seem to be due to the morphology of the conidiomatal structure. In *Pseudolachnea*, the conidiomata are composed of a thick basal stroma up to 100  $\mu\text{m}$  and a well developed excipulum up to 90  $\mu\text{m}$  wide, whereas conidiomata in *Pseudolachnella* have a thin basal stroma (mostly up to 20  $\mu\text{m}$ , rarely to 37.5  $\mu\text{m}$ ) and less-developed excipulum (up to 47.5  $\mu\text{m}$ ). In general, a generic concept based on Saccardoan spore morphology such as colour and septation is recognised as uninformative in many fungal genera (e.g. Hirayama & Tanaka 2011). Therefore it would not be unexpected if a *Pseudolachnea*



species with multiseptate conidia or a *Pseudolachnella* species with one-septate conidia is found in the future, as in the case of *Neopseudolachnella*. In addition to their morphological differences, host preference in these genera might correlate with their phylogenetic distinction. Sato *et al.* (2008) noted that *Pseudolachnea* mainly occurs on twigs or wood of various angiosperms, but *Pseudolachnella* exclusively occurs on bamboo. This tendency was confirmed in the present study; four strains of *Pseudolachnea* were collected from several dicots such as *Artemisia*, *Clematis* and *Morus*, whereas all 16 strains of *Pseudolachnella* containing the eight new species were collected from bamboo genera, such as *Phyllostachys*, *Pleioblastus*, *Sasa* and *Sinobambusa* (Fig. 1). Further collection of these fungal genera will be required to clarify the relationship between their evolution and host preference.

A new species of *Pseudolachnella*, *P. guaviyunis*, was described from *Myrcianthes pungens* trees (*Myrtaceae*; Crous *et al.* 2014). However, phylogenetic analysis with ITS (KJ834524) and LSU (KJ834525) sequences of this species indicated that it is distantly related to *Pseudolachnella* and resides in a basal position of *Dinemasporium* clade (data not shown). Morphologically several characters of *P. guaviyunis*, (i.e. the conidiomata with well developed basal stroma, up to 85 µm thick, and lacking conidiomatal setae, pale brown conidiophores, and hyaline to pale brown conidia with smooth to verruculose ornamentation) do not fit with the generic concept of *Pseudolachnella* proposed in our study. Therefore *P. guaviyunis* should be excluded from *Pseudolachnella* and a new genus might be required for this species.

Our study revealed the existence of two novel genera, *Neopseudolachnella* and *Pseudodinemasporium*. *Neopseudolachnella* includes three new species having one- to multiseptate conidia similar to those of *Pseudolachnea* or *Pseudolachnella* but has acervuloid conidiomata with an undeveloped basal stroma lacking an excipulum unlike those species of the latter two genera. Similarly *Pseudodinemasporium* has conidia resembling those of *Dinemasporium*, but can be distinguished from the latter by the conidiomata composed of a well developed peridial structure. These morphological observations of *Neopseudolachnella* and *Pseudodinemasporium*, as well as their molecular phylogenetic relationships, further reinforce our conclusion that differences in conidiomatal structure between *Pseudolachnea* and *Pseudolachnella* are more reliable indicators of evolutionary relationship than conidial septation is (Fig. 1). Some polyphyletic genera could be subdivided on the basis of anatomical differences of conidiomata, such as *Immersidiscosia*, a segregate from *Discosia sensu lato* (Tanaka *et al.* 2011), but there are also instances in which various conidiomatal morphologies such as sporodochial, synnemata and pycnidia are found in one natural genus (e.g. *Nectria*; Hirooka *et al.* 2012). Detailed morphological observation of asexual fructifications in

combination with molecular evidence will be needed to identify characters that support generic segregation.

A great variability of morphological features was observed in the conidial appendages of *Pseudolachnella* species. Many species in this genus have a single, unbranched appendage at both ends of the conidia. In contrast, some *Pseudolachnella* species, for example, *P. brevicoronata* (Fig. 10), *P. fusiformis* (Fig. 14) and *P. yakushimensis* (Sato *et al.* 2008), have multiple, branched or unbranched appendages on the conidia and they are distributed randomly among other species of the genus that have bipolar-appendaged conidia (Fig. 1). This result shows that conidial appendage morphology is of limited importance for definition of genera and assessment of their phylogenetic relationships. This conclusion is in agreement with that of Hashimoto *et al.* (2015), who noted that *Dinemasporium*, *Diarimella* and *Stauronema*, formerly characterised, respectively, by bipolar unbranched, branched multiple and bipolar and laterally appendaged conidia, should be treated as the same genus, *Dinemasporium*.

Phylogenetic analyses with DNA sequences in the present study clearly showed that *Pseudolachnea*, *Pseudolachnella*, *Neopseudolachnella* and *Pseudodinemasporium* belong to the *Chaetosphaeriaceae* (*Sordariomycetes*), although it was not our purpose to resolve the phylogenetic position of these four genera within the family. *Chaetosphaeriaceae* comprises morphologically less differentiated teleomorphic and considerably diverged anamorphic genera (Réblová 2000), and the representative genus *Chaetosphaeria* is apparently polyphyletic (Fernández *et al.* 2006, Jeewon *et al.* 2009). Several morphological and phylogenetic studies have been conducted based on sexual (e.g. Atkinson *et al.* 2007), or both sexual and asexual states (e.g. Réblová & Winka 2000, Fernández *et al.* 2006), to establish a natural classification of genera or species in this family, but all anamorphic genera used in these studies are hyphomycetes. There are 32 anamorphic hyphomycetous genera listed as members of the *Chaetosphaeriaceae* (Wijayawardene *et al.* 2012), and coelomycetous genera are only rarely known with a few exceptions (i.e., *Infundibulomyces* [Somrithipol *et al.* 2008] and *Dinemasporium* and its related genera [Crous *et al.* 2012]). It is thought that several coelomycetous genera for which phylogenetic information is presently lacking, such as *Conicomycetes* and *Zinzipegasa* (Nag Raj 1993), may have affinities with *Chaetosphaeriaceae* as judged from their morphological similarity with *Pseudolachnea* and its relatives. Further discovery of new coelomycetous lineages belonging to this family combined with phenotypic and genotypic analyses may contribute to an improved understanding of natural relationships among taxonomically complex members of the *Chaetosphaeriaceae*.

### 3. 亜熱帯地方で得られた 4 種の未記載種の報告

#### ABSTRACT

日本における菌類多様性調査の中で *Aquasubmersa* 属, *Conicomycetes* 属, *Lophiotrema* 属, *Pseudolachnella* 属の未記載種 4 種を見いだした。 *Aquasubmersa* 属は淡水性で分生子殻状の分生子果と無色単細胞の分生子によって特徴付けられる不完全菌である。本研究では本属菌の新種である *A. japonicum* を記載し, 本属菌の有性世代を初めて明らかにした。 *Conicomycetes* 属はシンネマ状の分生子果と先端に 1 本の付属糸をもつ分生子によって特徴付けられる不完全菌類である。新種 *C. pseudotransvaalensis* は本属の基準種 *C. transvaalensis* に形態的に類似するが, 後者よりもやや大きい分生子をもつ点で区別できた。LSU 領域に基づく分子系統解析により, 本属菌が *Chaetosphaeria* 科 (ケトスファエリア目, フンタマカビ綱) のメンバーであることを初めて明らかにした。 *Lophiotrema* 属の新種である *L. fallopiae* はイタドリから分離された。本種は slit 状の孔口をもつ子のう果, 柄の短い子のうおよび pleurophoma 型の無性世代をもつ点で *Lophiotrema* 属の属概念に当てはまる。 *Lophiotrema fallopiae* は *L. vagabundum* に子のう胞子の点で形態的に類似するが, 後者よりも太い子のう胞子をもつことで区別できた。 *Pseudolachnella brevifusiformis* はリュウキュウチクから得られた。本種の分生子の形態は *P. brevifusiformis* に類似するが, 本種は *Pseudolachnella* 属の中でも最小の分生子をもつことから新種として記載した。

***Aquasubmersa japonicum*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — *Index Fungorum* number IF551422; Fig. 19

*Etymology*: named after its country of origin, Japan.

*Saprobic* on dead wood. **Sexual state**: *Ascomata* 180–210(–300)  $\mu\text{m}$  high, 115–175(–230)  $\mu\text{m}$  diam., scattered to grouped, subglobose, immersed to semi-immersed. *Ostiole* 10–16  $\mu\text{m}$  high, short papillate, without clypeus. *Peridium* 20–30  $\mu\text{m}$  thick, composed of 4–7 layers of polygonal, 8–12.5  $\times$  2.5–5.5  $\mu\text{m}$ , thin-walled cells. *Hamathecium* numerous, 2–4.5  $\mu\text{m}$  wide, hyaline, septate, branched. *Asci* (60–)66–93  $\times$  (4.5–)5.5–8  $\mu\text{m}$  ( $\bar{x}$  = 76  $\times$  6.2  $\mu\text{m}$ ,  $n$  = 17), bitunicate, 8-spored, biseriate, cylindrical, with a short stipe (9–14  $\mu\text{m}$  long), apically rounded with an ocular chamber. *Ascospores* 20–26  $\times$  4–5.5(–6)  $\mu\text{m}$  ( $\bar{x}$  = 23.2  $\times$  4.6  $\mu\text{m}$ ,  $n$  = 65), l/w (3.7–)4–6(–6.5) ( $\bar{x}$  = 5.1,  $n$  = 65), broadly fusiform with rounded ends, straight, with a septum mostly median (0.45–0.54;  $\bar{x}$  = 0.50,  $n$  = 65), hyaline, smooth, guttulate when young, with an entire sheath; sheath when fresh condition diffuse, gelatinous, up to 10  $\mu\text{m}$  wide, later becoming sharply delimited firm sheath of 1.5–4  $\mu\text{m}$  thick.

**Asexual state:** *Conidiomata* pycnidial, globose to subglobose, up to 215  $\mu\text{m}$  high in section, 115–195  $\mu\text{m}$  diam., scattered, semi-immersed, solitary, black to brown. *Ostiole* 53–80  $\mu\text{m}$  high, short papillate, circular, dark-brown, centre. *Peridium* 20–25  $\mu\text{m}$  thick, composed of 9.5–12.5  $\times$  3–7.5  $\mu\text{m}$ , polygonal, hyaline to brown cells; *Conidiophores* reduced. *Conidiogenous cells* holoblastic, 9–12  $\times$  5.5–6.5  $\mu\text{m}$ , lageniform, hyaline, smooth. *Conidia* ellipsoidal, 17–22(–23)  $\times$  (7–)8–9  $\mu\text{m}$  ( $\bar{x}$  = 19.4  $\times$  8.4  $\mu\text{m}$ , n = 50), l/w 1.9–2.7 ( $\bar{x}$  = 2.3, n = 50), hyaline, smooth, guttulate when young.

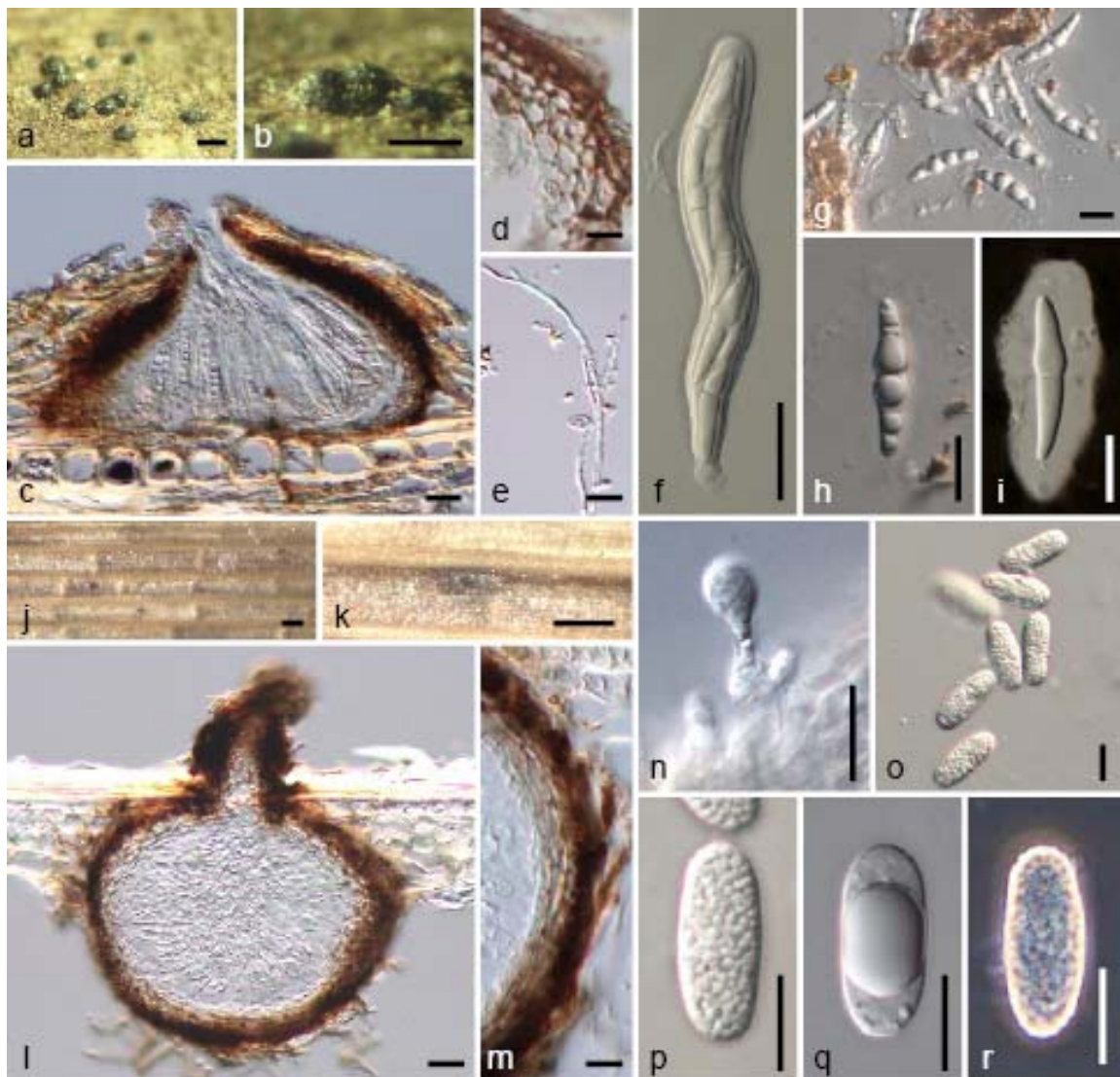
Cultural characteristics — Ascospores formed in culture are similar to those on natural substrate.

*Specimens examined.* **JAPAN**, Okinawa, Isl. Iriomote, Oomija-river, on submerged wood, 12 July 2011, K. Tanaka & K. Hirayama, KT 2813 (HHUF 30468, **paratype**; ex-paratype living culture = MAFF 245218). Okinawa, Isl. Ishigaki, Banna park, on dead wood, 16 July 2011, K. Tanaka & K. Hirayama, KT 2862 (HHUF 30469, **holotype**; ex-holotype living culture = MAFF 245219). *ibid.*, KT 2863 (HHUF 30470, **paratype**; ex-paratype living culture = MAFF 245220)

*Notes* — The genus *Aquasubmersa* was established to accommodate a freshwater coelomycete, *A. mircensis*, by Zhang *et al.* (2012). The genus was characterised by the pycnidia, which have papilla, a peridium composed of thin-walled cells, and unicellular conidia; no sexual morph was reported.

Phylogenetic analyses including 18S and 28S rDNA sequence data, placed *A. mircensis* in *Pleosporales*, but the familial position was not clarified (Zhang *et al.* 2012). Result of Blast search of GenBank suggested that this genus has a close phylogenetic affinity with *Lophiotrema* (*Lophiotremataceae*). *Lophiotrema* can be distinguished from *Aquasubmersa* by having a compressed, slit-like ostiole, and a peridium composed of parallel rows of elongate cells. This is the first report of sexual morph of *Aquasubmersa*.

The asexual characters of *A. japonicum* show a good match with the generic concepts of *Aquasubmersa*. The conidia of *A. japonicum* differ in being slightly larger than those of *A. mircensis* (18.3  $\times$  9.4  $\mu\text{m}$ ; Zhang *et al.* 2012). ITS sequence differences between our materials of *A. japonicum* and the ex-type strain of *A. mircensis* (NR 121545) were found at 27–28 positions with three gaps.



**Fig. 19.** *Aquasubmersa japonica* a, b Appearance of ascomata on substrate c Ascoma in longitudinal section d Peridium. e Pseudoparaphyses f Ascus g, h Ascospores with mucilaginous sheath I Ascospore in India ink j, k Conidiomata in culture l Conidioma in longitudinal section m Peridium of conidioma n Conidiogenous cell and immature conidium o–r Conidia. a, b, e, h, i from HHUF 30469; c, d, g from HHUF 30470; f from HHUF 30468; j–r from culture MAFF 245220. — Scale bars: a, b = 200  $\mu\text{m}$ , c, f, l = 20  $\mu\text{m}$ , d, e, g–i, m–r = 10  $\mu\text{m}$ , j, k = 300  $\mu\text{m}$

***Conicomycetes pseudotransvaalensis*** A. Hashim., G. Sato et Kaz. Tanaka, **sp. nov.**  
— Index Fungorum number: IF551057; Fig. 21.

*Etymology:* named after its morphological similarity to *Conicomycetes transvaalensis*.

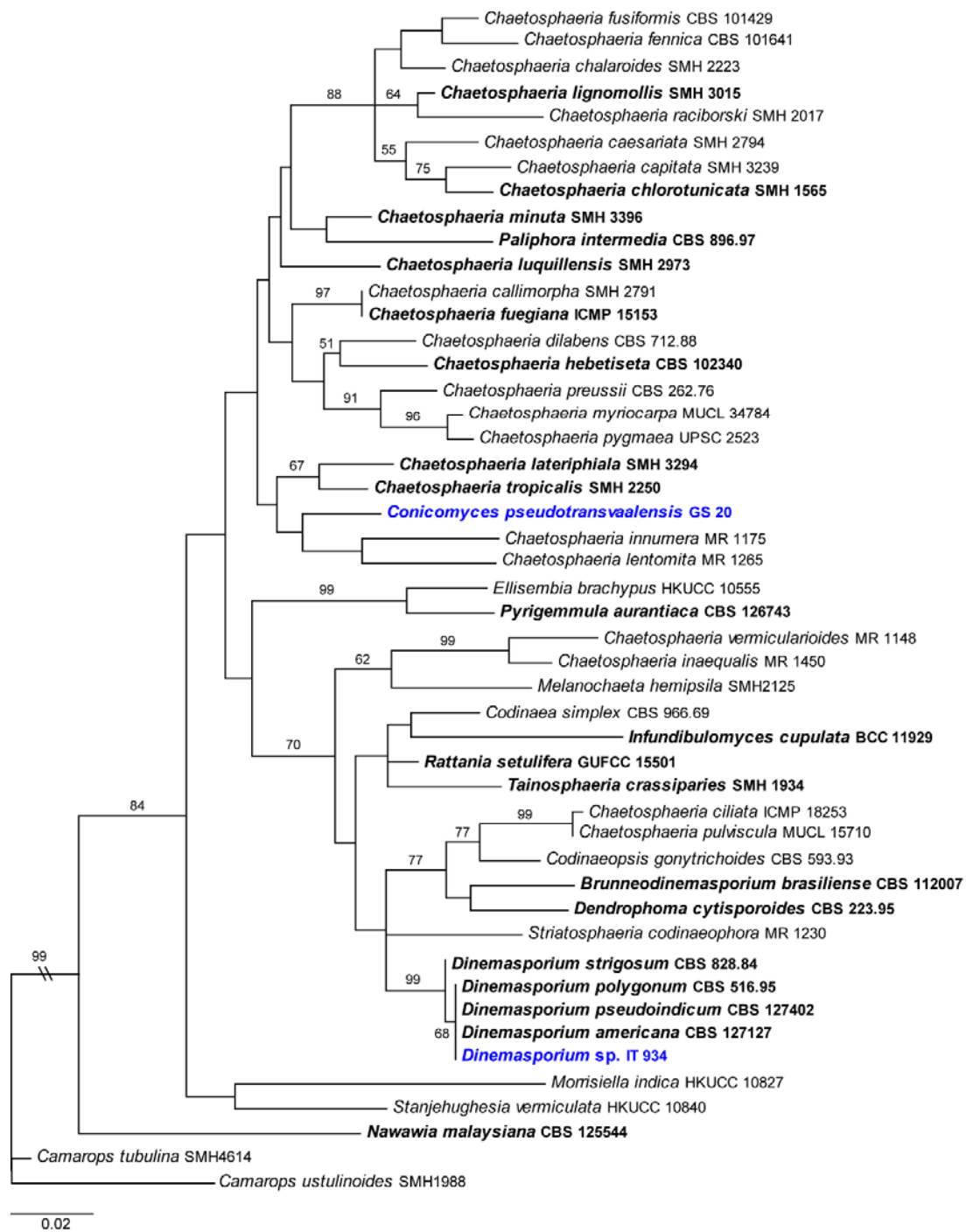
*Saprobic* on *Machilus japonica*. **Sexual state:** Unknown. **Asexual state:** *Conidiomata* stromatic, synnematos, scattered, superficial, cone-shaped, black to

brown, setose, up to 780  $\mu\text{m}$  high, 260–360  $\mu\text{m}$  wide at the base, cornuted with a head and a stipe; head slightly swollen, 65–100  $\mu\text{m}$  wide, bearing a concave conidial hymenium; stipe cylindrical or contorted, black to dark brown, 30–100  $\mu\text{m}$  wide, composed of rectangular, thin-walled, brown, 1.5–2.5  $\mu\text{m}$  wide cells of *texura porrecta*. *Setae* arising from stroma or stipe, straight or curved, erect, septate, brown but pale at the apex, thick-walled, smooth, unbranched, up to 450  $\mu\text{m}$  long, acute and 2.5–4  $\mu\text{m}$  wide at the apex, 5–7  $\mu\text{m}$  wide at the base. *Conidiophores* arising from inner elements of the stipe and lining of the conidiomata, hyaline to pale brown, unbranched or branched, up to 40  $\mu\text{m}$ . *Conidiogenous cells* phialidic, cylindrical, hyaline to pale brown, smooth, 16–88  $\times$  2.5–3  $\mu\text{m}$ . *Conidia* claviform, slightly obtuse at the apex, slightly truncate at the base, 15–22-septate, hyaline, smooth, guttulate, 105–170  $\times$  7.5–10  $\mu\text{m}$  ( $\bar{x}$  = 150.8  $\times$  8.8  $\mu\text{m}$ , n = 50), l/w 12–22.7 ( $\bar{x}$  = 17.4, n = 50), bearing an unbranched appendage at the apex; appendage 40–80  $\mu\text{m}$  long ( $\bar{x}$  = 59.8  $\mu\text{m}$ , n = 50), excentric.

Cultural characteristics — Conidia in culture are similar to those on natural substrate.

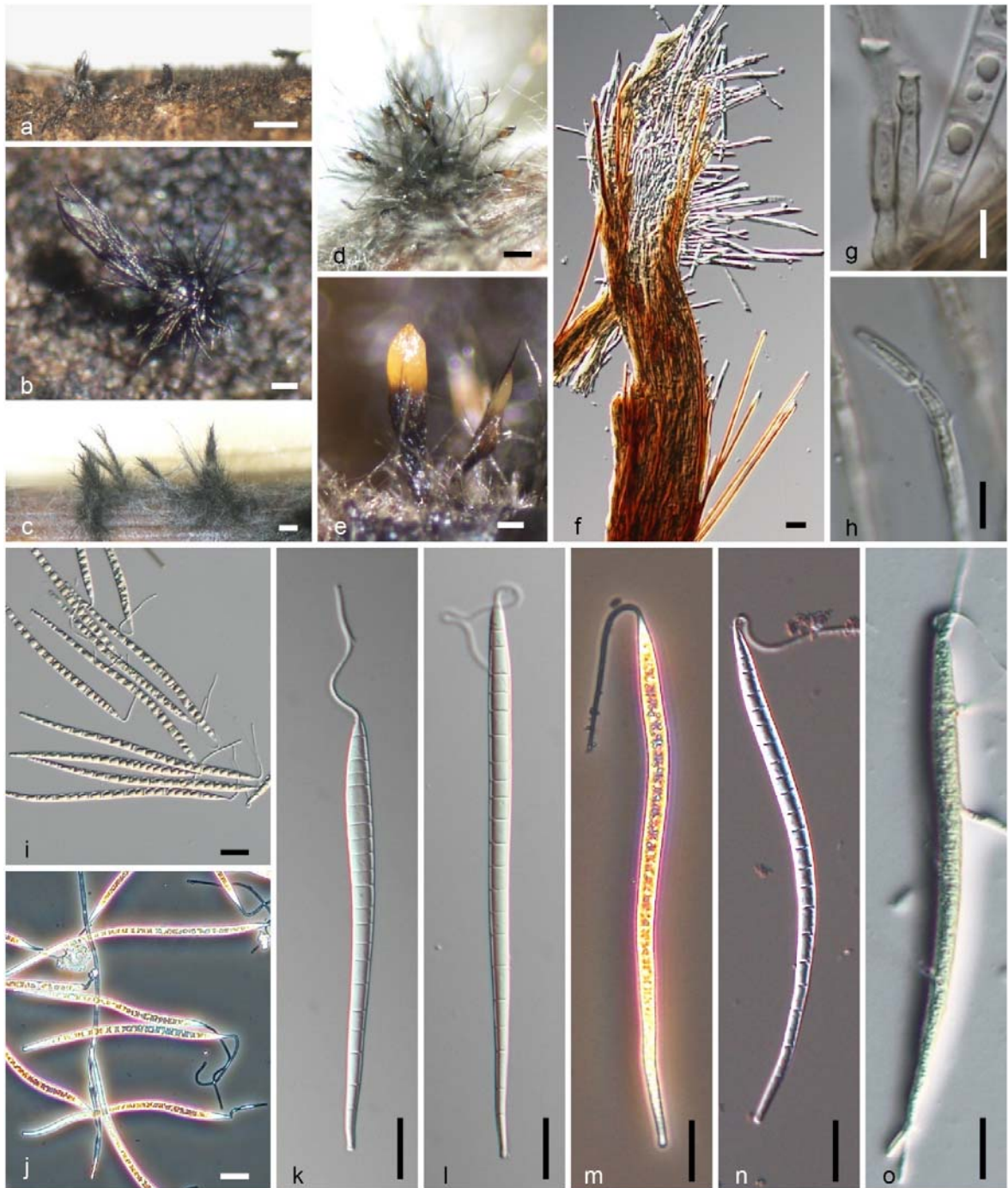
*Specimen examined*: **JAPAN**, Kagoshima, Yakushima, Yakusugi Land, dead twigs of *Machilus japonica*, 15 March 2007, K. Tanaka & H. Yonezawa, GS 20 (HHUF 29956, **holotype**); ex-holotype living culture MAFF 244767). GenBank ITS: LC001710; LSU: LC001708.

*Notes* — The genus *Conicomycetes* was established to accommodate *C. transvaalensis* having synnematosus conidiomata and apically appendaged conidia (Sinclair *et al.* 1983). *Conicomycetes* currently contains three described species (Sinclair *et al.* 1983, Illman & White 1984, Seifert 1999), but no molecular studies have been undertaken for the genus. Morphologically *C. pseudotransvaalensis* is similar to *C. transvaalensis* in having large conidia more than 100 $\mu\text{m}$  long, but the latter has slightly long and slender conidia with more septation (122–200  $\times$  5.5–7.5  $\mu\text{m}$ , l/w 25, 19–29-septate; Nag Raj 1993). Based on a megablast search, the closest hits to the LSU sequence of *C. pseudotransvaalensis* are *Chaetosphaeria fuegiana* (GenBank EF063574; Identities= 729/754 (96.7 %), Gaps 5/754 (0.7 %)), *Chaetosphaeria hebetiseta* [GenBank AF178549; Identities=723/754 (95.9 %), Gaps 5/754 (0.7 %)] and *Chaetosphaeria dilabens* [GenBank AF178557; Identities=720/751 (95.9 %), Gaps=5/751 (0.7 %)]. These results clearly indicate that the genus is a member of *Chaetosphaeriaceae* (*Sordariomycetes*), as previously suggested by Hashimoto *et al.* (2015b) based on morphological grounds. In Fig. 20. *pseudotransvaalensis* clusters in *Chaetosphaeriaceae* and is related to species of *Chaetosphaeria sensu lato*.



**Fig. 20.** Phylogram generated from Maximum likelihood analysis based on LSU sequence data. Maximum Likelihood bootstrap support values greater than 50 % are indicated above or below the nodes. The ex-typic species are in bold. New isolates are in blue. The tree is rooted with *Camarops tubulina* SMH 4614 and *Camarops ustulinoides* SMH 1988.





**Fig. 21.** *Conicomyces pseudotransvaalensis*. a, b Conidiomata on host surface c-e Conidiomata in culture f Conidioma in longitudinal section g, h Conidiogenous cells i-n Conidia o Germinating conidium a, b, f, h, k, l from HHUF 29956 (holotype); c-e, g, i, j, m-o from MAFF 244767 (ex-holotype culture). — Scale bars: a, c = 500  $\mu$ m, b, e = 100 $\mu$ m, d = 250  $\mu$ m, f, i-o = 20  $\mu$ m, g, h = 5  $\mu$ m.



***Lophiotrema fallopieae*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB817597; Fig. 22.

*Etymology:* Referring to the genetic name of host plant.

Saprobic on dead stem of *Fallopia japonica*. **Sexual morph:** *Ascomata* 200–300 µm high, 180–250 µm diam., scattered, immersed, erumpent at the neck, subglobose in section. *Ostiolar neck* up to 40 µm high, compressed, composed of carbonaceous, black, thick-walled cells, without clypeus. *Peridium* 17.5–25.5 µm thick, 5–8 layers, composed of polygonal to elongate, thin-walled, 8–13 × 2.5–3 µm cells. *Pseudoparaphyses* numerous, trabeculate, 1–1.5 µm wide, septate, branched. *Asci* (77–)93–125 × (5.5–)7–9 µm ( $\bar{x}$  = 105.8 × 7.6 µm, n = 10), 8-spored, numerous, bitunicate, fissitunicate, cylindrical, with a short stipe (5–11 µm long,  $\bar{x}$  = 7.4 µm, n = 10), apically rounded with an ocular chamber. *Ascospores* 19–24 × 4.5–6 µm ( $\bar{x}$  = 21.7 × 5.2 µm, n = 50), l/w 3.7–5.3 ( $\bar{x}$  = 4.2, n = 50), fusiform with rounded ends, straight, 1-septate, constricted, with a primary septum nearly median [0.45–0.55,  $\bar{x}$  = 0.51, n = 50], hyaline, smooth, guttulate when young, with an entire gelatinous sheath (2.5–6 µm wide at sides). **Asexual morph:** *Conidiomata* pycnidial, globose to subglobose, up to 200 µm high in section, 120–180 µm diam., scattered, semi-immersed, solitary, black. *Peridium* 10–14 µm wide; outer layers composed of 7.5–10.5 × 4–7.5 µm, subglobose, brown cells; inner layers composed of 5–7.5 × 0.5–1.5 µm, “porrecta”, hyaline cells. *Conidiophores* reduced. *Conidiogenous* cells holoblastic, 7–9 × 2–4 µm, lageniform, hyaline, smooth. *Conidia* ellipsoidal with rounded ends, 2–3.5 × 1–2 µm ( $\bar{x}$  = 2.7 × 1.5 µm, n = 60), l/w 1.2–2.5 ( $\bar{x}$  = 1.8, n = 60), hyaline, aseptate, smooth.

Culture characteristics — Colonies on PDA attaining 12.5–15.5 mm diam within 21 d at 20 °C in the dark.

*Specimens* examined: **JAPAN**, Aomori, Hirakawa, Kuzukawa, near Aseishi-river, on dead stem of *Fallopia japonica*, 5 Sep 2010, K. Tanaka, KT 2748 (HHUF 30506 **holotype**); ex-type living culture, MAFF 245612. GenBank SSU: LC149911; ITS: LC149913; LSU: LC149915.

*Notes* — The characters of *L. fallopieae* fit into the generic concept of *Lophiotrema* in having compressed ostiolar necks, a peridium composed of *textura angularis*, and cylindrical asci with short stipes. This species is similar to *L. nucula*, the type species of *Lophiotrema*, but *L. nucula* has shorter ascospores [17–21(–25) × (4–)5–6.5 µm; Zhang et al. 2009]. *Lophiotrema fallopieae* is phylogenetically close to *L. vagabundum*, but *L. vagabundum* has slightly narrower ascospores (20–26 × 4–5.5 µm, l/w 4.3–5.9; Tanaka & Harada 2003).



**Fig. 22.** *Lophiotrema fallopii*. a, b Appearance of ascomata on substrate. c Ascoma in longitudinal section. d Peridium of ascoma. e, f Ascus. g Ascus apex. h Pseudoparaphyses. i–k Ascospores. l Ascospore with gelatinous sheath (in Indian ink). m Germinating ascospore. n, o Conidiomata in culture. p Conidioma in longitudinal section. q Peridium of conidioma. r, s Conidiogenous cells. t, u Conidia. v Germinating conidium. a–m From HHUF 30506 (holotype); n–v from MAFF 245612 (ex-type culture). Scale bars a = 1 mm, b, n, o = 250  $\mu$ , c, p = 20  $\mu$ m, d–f, q = 10  $\mu$ m, g–m, r–v = 5  $\mu$ m.

***Pseudolachnella brevifusiformis*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB 815299; Fig. 24.

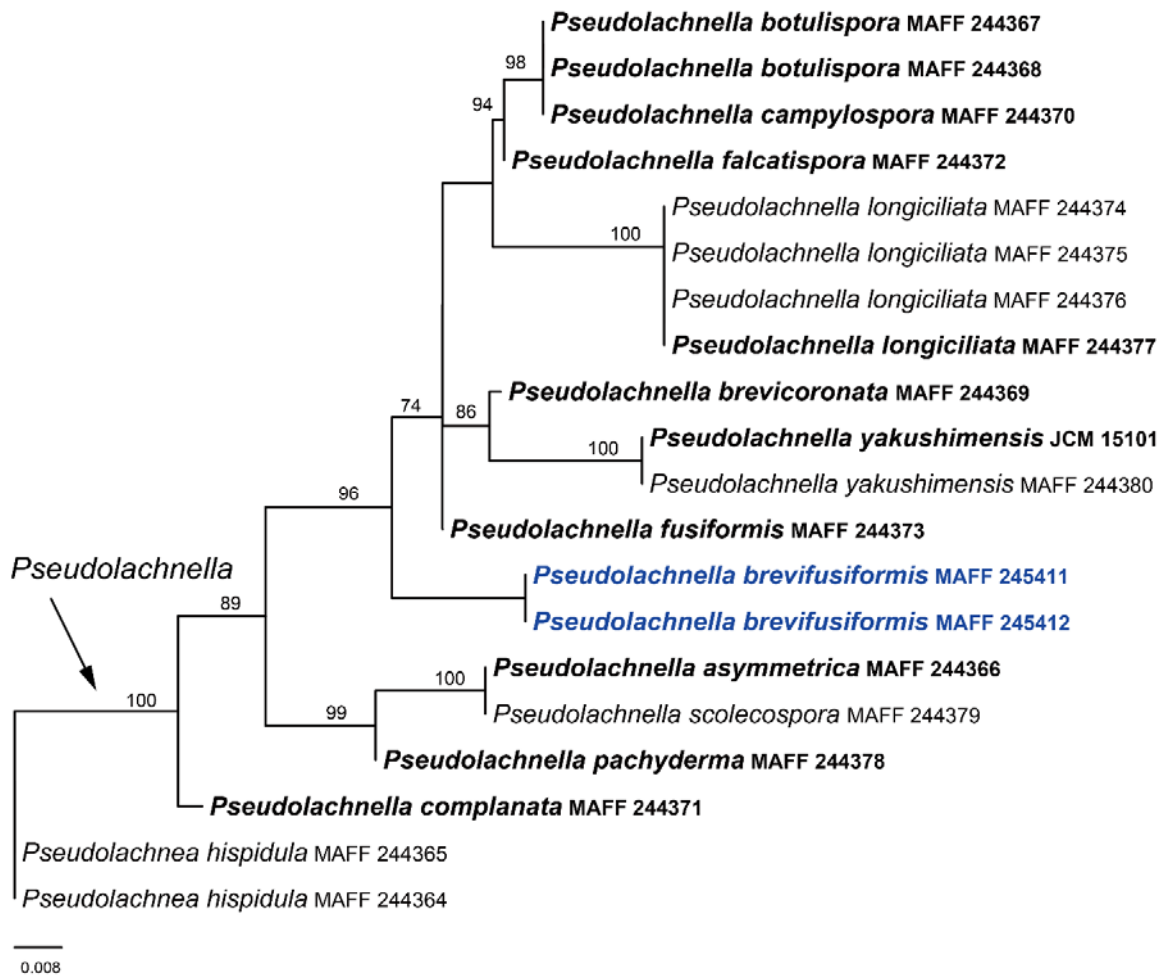
*Etymology*: named after its resemblance to *Pseudolachnella fusiformis*, but with smaller conidia.

**Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* stromatic, acervular, setose, shallow-cupulate, superficial, globose to oval in outline, up to 295  $\mu\text{m}$  high, (325–)450–700(–895)  $\mu\text{m}$  diam., scattered to occasionally 2–5 grouped, conical in sectional view; basal stroma 6.5–15  $\mu\text{m}$  thick, composed of brown, globose, thick-walled, 2–2.5  $\mu\text{m}$  diam. cells; excipulum 30–44.5(–50)  $\mu\text{m}$  thick, poorly developed, composed of globose, pale brown cells. *Setae* marginal, cylindrical, straight to slightly curved, aseptate, brown to dark brown, thick-walled, (315–)380–520  $\mu\text{m}$  long, acute and 2–3.5  $\mu\text{m}$  wide at the apex, 3–4  $\mu\text{m}$  wide at the base. *Conidiophores* absent. *Conidiogenous cells* phialidic, cylindrical to lageniform, hyaline, smooth, 6.5–14  $\times$  1.5–2.5  $\mu\text{m}$ . *Conidia* (9.5–)10.5–18(–19)  $\times$  2–3.5  $\mu\text{m}$  ( $\bar{x}$  = 14.0  $\times$  2.9  $\mu\text{m}$ ,  $n$  = 78), l/w 3.4–7.6(–8.7) ( $\bar{x}$  = 5.0,  $n$  = 78), (1–)3-septate, clavate to cylindrical, obtuse at the apex, truncate at the base, hyaline, smooth, bearing (2–)3–6 unbranched appendages at each end; apical appendage (2.5–)3–6  $\mu\text{m}$  long ( $\bar{x}$  = 4.3  $\mu\text{m}$ ,  $n$  = 61), central; basal appendage (2.5–)3–5.5(–6.5)  $\mu\text{m}$  long ( $\bar{x}$  = 4.0  $\mu\text{m}$ ,  $n$  = 61), eccentric.

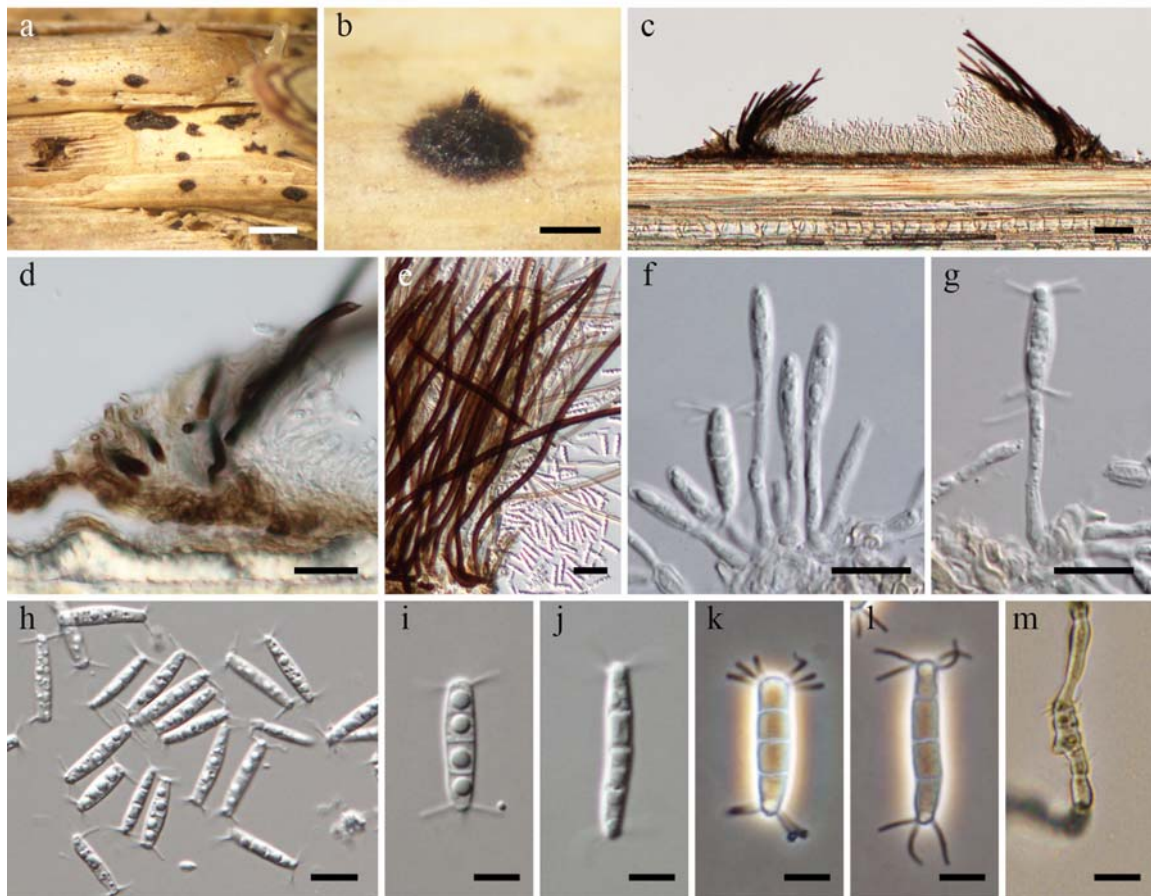
*Specimens examined*: **JAPAN**, Okinawa, Kunigami, Yona, Mt. Fuenchiji, on dead sheath of *Pleioblastus linearis*, 19 May 2015, K. Tanaka *et al.*, KT 3536 (HHUF 30495, **holotype**; single conidium isolate = MAFF 245411); *ibid.*, KT 3537 (HHUF 30496, **paratype**; single conidium isolate = MAFF 245412).

*Notes*: In terms of the similar conidial size and multiple conidial appendages, *Pseudolachnella brevifusiformis* resembles *P. fusiformis*, but can be distinguished from the latter by its smaller conidia (vs. 15–20  $\times$  4–6.5  $\mu\text{m}$ ; Hashimoto *et al.* 2015b). In addition, there were 25 base differences with 12 gaps in their ITS sequence data. *Pseudolachnella brevifusiformis* was collected from *Pleioblastus linearis*. The phylogenetic tree for *Pseudolachnella* is presented in Fig. 23.

*Pseudolachnella ryukyuensis* was also recorded from same host plant (Hino & Katumoto 1958, Nag Raj 1993). Morphologically, *P. brevifusiformis* has smaller conidia, as compared with those of the latter (vs. 30–40  $\times$  2.5–3  $\mu\text{m}$ ; Nag Raj 1993).



**Fig. 23.** Maximum-likelihood tree of *Pseudolachnella* spp. based on analysis of ITS sequence data. Bootstrap values greater than 50 % are presented at the nodes. New taxa are in blue exetypes in bold.



**Fig. 24.** *Pseudolachnella brevifusiformis* a, b Appearance of conidiomata on substrate c Conidioma in longitudinal section d Excipulum of conidioma e Conidiomatal setae f, g Conidiogenous cells and immature conidia h–l Conidia m Germinating conidium a–d, i, k, m from HHUF 30495 (holotype); e–h, j, l from HHUF 30496 (paratype). — Scale bars: a = 1 mm, b = 250 $\mu$ m, c = 50 $\mu$ m, d, e = 20 $\mu$ m, f–h, m = 10 $\mu$ m, i–l = 5  $\mu$ m.





## 4. *Cryptocoryneum* 属菌の分類学的再検討

### ABSTRACT

*Cryptocoryneum* 属菌について、形態観察と分子系統解析に基づき分類学的再検討を行った。*Cryptocoryneum* 属菌 18 菌株はアフリカ産の 2 菌株、ヨーロッパ産の 3 菌株、日本産の 13 菌株を含み、それらは ITS-*tef1* 領域の配列を用いて系統解析された。*Cryptocoryneum* 属菌は *Lophiotrema* 属 (*Lophiotremataceae*, *Pleosporales*) と *Aquasubmersa* 属 (*incertae sedis*, *Pleosporales*) に系統的に近縁であることが分子系統解析から示唆された。*Cryptocoryneum fasciculatum*, *C. hysteroioides*, *Torula uniformis* の正基準標本を観察した結果、これらの種は優先権のある *C. hysteroioides* と同種であることが確かめられた。*Cryptocoryneum hysteroioides* は長らく *C. condensatum* のシノニムとして認識されてきたが、これらの 2 種は同属内の別種であると考えられた。本研究では広義 *C. condensatum* に形態的に類似したいくつかの隠蔽種を見いだしたが、それらは分生子の大きさ、分生子のアームの数、隔壁数によって区別された。以上の形態学的特徴は *Cryptocoryneum* 属内の種の特徴付けに有用であると見なされた。*Cryptocoryneum akitaense*, *C. brevicondensatum*, *C. congregatum*, *C. japonicum*, *C. longicondensatum*, *C. paracondensatum* および *C. pseudorilstonei* の計 7 新種が記載された。

### INTRODUCTION

The asexual genus *Cryptocoryneum* is characterised by having stromatic sporodochia, cheiroid conidia, and conidial arms that are developed downward from the cap cells (Schoknecht & Crane 1977). Since the establishment of this genus, 17 taxa have been described worldwide. Most species of this genus occur on dead wood, with some species rarely reported from leaf litter (Kirk 1983) or arthropod dung (Talbot 1952). As the sexual stage of this genus has not been reported and no molecular study has been performed, the phylogenetic placement of this genus remains unresolved (Wijayawardene *et al.* 2012).

The most well-studied and ubiquitous species in this genus, *C. condensatum* has been presently known from Asia, Australia, Europe, North America and South America (Hughes 1958, Ellis 1971, Schoknecht & Crane 1977, Katumoto 1988, von Heftberger *et al.* 1997, Mel'nik 2000, Silva *et al.* 2015). *Cryptocoryneum rilstonei* has also been reported in Africa, Australia, Europe and South America (Ellis 1972, Hughes 1978, Kirk 1982, Silva *et al.* 2015). However, other species of *Cryptocoryneum* have not been recorded since their first descriptions.

During our ongoing studies of anamorphic fungi in Japan (Endo *et al.* 2008, Hatakeyama *et al.* 2008, Sato *et al.* 2008, Yonezawa & Tanaka 2008, Kamiyama *et al.* 2009, Tanaka *et al.* 2010, 2011, 2015, Hashimoto *et al.* 2015a, b) we have collected 18 strains of *Cryptocoryneum* including several undescribed species. The main objective of the present study was to describe these new species. In this study, we conducted phylogenetic analysis of *Cryptocoryneum* species based on nuclear rDNA ITS and *tef1* and performed a morphological examination to provide a robust taxonomic framework.

## **MATERIALS AND METHODS**

### ***Isolation and morphological observation***

All fungal structures were observed in preparations mounted in distilled water. Morphological characters were observed with differential interference and phase contrast microscopy (Olympus BX53), and images were captured with an Olympus digital camera (DP20 and DP21). A total of 18 single-spore isolates including five additional strains preserved in the Centraalbureau voor Schimmelcultures (CBS) were used for morphological observations and phylogenetic analysis (Table 2). Colony characters were observed from cultures grown on potato dextrose agar (PDA; Difco) after 3 weeks at 20 °C in the dark. Colours were assessed as described by Rayner (1970). To induce conidial formation in culture, 5 mm squares of mycelial agar were placed on water agar including sterilised natural substrate, such as rice straws, banana leaves, pine needles, and hydrangea leaves, and the plates were incubated at 20 °C for 2 weeks in the dark. When the substrate was colonised, the plates were incubated at 20 °C under blacklight blue illumination for 2 months to observe the sporulation. Cultures were deposited in NBRC and MAFF. Specimens were deposited in HHUF.

The holotypes of *C. fasciculatum*, *C. hysteroioides*, *C. rilstonei*, and *Torula uniformis* were borrowed from G, PRM, IMI, NYS, respectively. The type specimens of *C. erumpens*, *C. fasciculatum* var. *olivaceum*, *C. scopiforme*, and *C. simmonsii* were located at the herbarium PAD. However, our requests for loans of these materials were not permitted, and it was encouraged to visit the herbarium to examine the type materials instead. Although we could not examine these type specimens, we have provided morphological information of these species based on the literature.

**Table 2. Specimens, isolates and new sequences obtained in this study**

Species	Original no.	Specimen no.	Strain no.	Host/substrate	Locality	GenBank no.	
						ITS	<i>TEF1</i>
<i>Cryptocoryneum akitaense</i>	KT 3019	HHUF 30477 <sup>H</sup>	MAFF 245365 = NBRC 111758	<i>Rhododendron brachycarpum</i>	Akita, JPN	LC096154	LC096136
<i>Cryptocoryneum brevicondensatum</i>	yone 152	HHUF 30478 <sup>H</sup>	MAFF 245366 = NBRC 111759	dead wood	Aomori, JPN	LC096155	LC096137
<i>Cryptocoryneum condensatum</i>	4418b	UPS F-632989	CBS 113959	<i>Salix fragilis</i>	Uppland, SWE	LC096156	LC096138
	-	-	CBS 122629	plant debris	Bragança, PRT	LC096157	LC096139
	-	-	CBS 122633	plant debris	Cataluynya, ESP	LC096158	LC096140
<i>Cryptocoryneum congregatum</i>	KT 2892	HHUF 30479 <sup>H</sup>	MAFF 245367 = NBRC 111760	<i>Fagus crenata</i>	Aomori, JPN	LC096159	LC096141
<i>Cryptocoryneum hysteroioides</i>	no 1527	G 00266173 <sup>a</sup>	-	<i>Quercus or Mali</i>	Locality unknown	-	-
	-	NYSf 3296 <sup>b</sup>	-	dead wood	New York, USA	-	-
	-	PRM 155687	-	dead wood	Locality unknown	-	-
	-	PRM 155688 <sup>H</sup>	-	dead wood	Reichenberg, GER	-	-
<i>Cryptocoryneum japonicum</i>	KT 2961	HHUF 30480	MAFF 245368	<i>Fagus crenata</i>	Aomori, JPN	LC096160	LC096142
	KT 3291	HHUF 30481	MAFF 245369	<i>Acer japonicum</i>	Aomori, JPN	LC096161	LC096143
	KT 3300	HHUF 30482 <sup>H</sup>	MAFF 245370 = NBRC 111761	<i>Fagus crenata</i>	Aomori, JPN	LC096162	LC096144
<i>Cryptocoryneum longicondensatum</i>	KT 3413	HHUF 30483	MAFF 245371	<i>Fagus crenata</i>	Aomori, JPN	LC096163	LC096145
	yone 36	HHUF 30484	MAFF 245372	<i>Acer</i> sp.	Aomori, JPN	LC096164	LC096146
	yone 157	HHUF 30485	MAFF 245373	dead wood	Aomori, JPN	LC096165	LC096147
	KT 2913	HHUF 30486 <sup>H</sup>	MAFF 245374 = NBRC 111762	<i>Fagus crenata</i>	Aomori, JPN	LC096166	LC096148
<i>Cryptocoryneum paracondensatum</i>	KT 3487	HHUF 30487	MAFF 245375	<i>Fagus crenata</i>	Aomori, JPN	LC096167	LC096149
	KT 3071	HHUF 30488	MAFF 245376	<i>Fagus crenata</i>	Aomori, JPN	LC096168	LC096150
	KT 3241	HHUF 30489 <sup>H</sup>	MAFF 245377 = NBRC 111763	<i>Fagus crenata</i>	Aomori, JPN	LC096169	LC096151
<i>Cryptocoryneum pseudorilstonei</i>	-	HHUF 30490 <sup>H</sup>	CBS 114518	<i>Ischyrolepis subverticellata</i>	Western Cape, ZAF	LC096171	LC096153
<i>Cryptocoryneum rilstonei</i>	-	IMI 39939 <sup>H</sup>	-	<i>Fraxinus excelsior</i>	England, GB	-	-
<i>Cryptocoryneum</i> sp.	-	-	CBS 113641	<i>Elegia equisetacea</i>	Western Cape, ZAF	LC096170	LC096152

H, holotype.

a, holotype of *Cryptocoryneum fasciculatum*.

b, holotype of *Torula uniformis*.

### ***Phylogenetic analyses***

DNA extraction was carried out with an ISOPLANT II kit (Nippon Gene, Japan) in accordance with the manufacturer's protocol. The complete ITS region (ITS1-5.8S-ITS2) and partial *tef1* were amplified by PCR with the primer pairs ITS1/ITS4 (White *et al.* 1990) and EF983F/EF2218R (Rehner & Buckley 2005), respectively. Amplifications were performed in 25 mL volumes consisting of 2 mL DNA extract, 2.5 mL 10×TEMPase buffer I, 2.5 mL dNTPs mix, 1 mL each 20 pM primer, 1 mL MgCl<sub>2</sub>, 14.5 mL MilliQ water, and 0.5 mL TEMPase Hot Start DNA polymerase (Ampliqon, Denmark). PCRs were carried out on a PC 320 thermo-cycler (ASTECH, Japan) with this protocol: 95 °C for 15 min, followed by 35 cycles of 1 min at 94 °C, 1 min at 61.5 °C (for ITS), or 60 °C (for *tef1*), 1 min at 72 °C, and a final denaturation step of 7 min at 72 °C. The PCR products were sequenced directly at SolGent (South Korea).

The novel sequences generated from 18 isolates of *Cryptocoryneum* were deposited in GenBank (Table 2). Two *Lophiotrema* strains, *L. neoarundinaria* KT 856 and KT 2200, were selected as outgroups. These sequences were aligned with the MUSCLE algorithm implemented in MEGA 5 (Tamura *et al.* 2011). Phylogenetic analyses were conducted using ML and Bayesian methods. The optimum substitution models for each dataset were estimated by Kakusan4 (Tanabe 2011), based on AIC (Akaike 1974) for ML analysis and BIC (Schwarz 1978) for the Bayesian analysis. ML analysis was performed with TreeFinder Mar 2011 (Jobb 2011) based on the models selected by AICc4 (separate model among genes and proportional model among codons, J2ef+G for ITS, F81+G for the first codon of *tef1*, TVM+H for the second codon of *tef1*, and J2+G for the third codon of *tef1*). BPs were obtained by 1 000 bootstrap replications. Bayesian analysis was performed with MrBayes 3.2.2 (Ronquist *et al.* 2012) with substitution models for different regions selected by BIC4 (i.e. proportional model among loci and among codons), K80+G for ITS, F81+G for the first codon of *tef1*, JC69+H for the second codon of *tef1*, and GTR+G for the third codon of *tef1*. Two simultaneous, independent runs of MCMC were performed for 2 000 000 generations with trees sampled every 1 000th generation.

Convergence of the MCMC procedure was assessed from the average standard deviation of split frequencies (<0.01) and effective sample size scores (all>100) using MrBayes and Tracer 1.6 (Rambaut *et al.* 2014), respectively. The first 25% trees were discarded as burn-in, and the remaining trees were used to calculate 50% majority rule trees and to determine PPs for individual branches.



## RESULTS

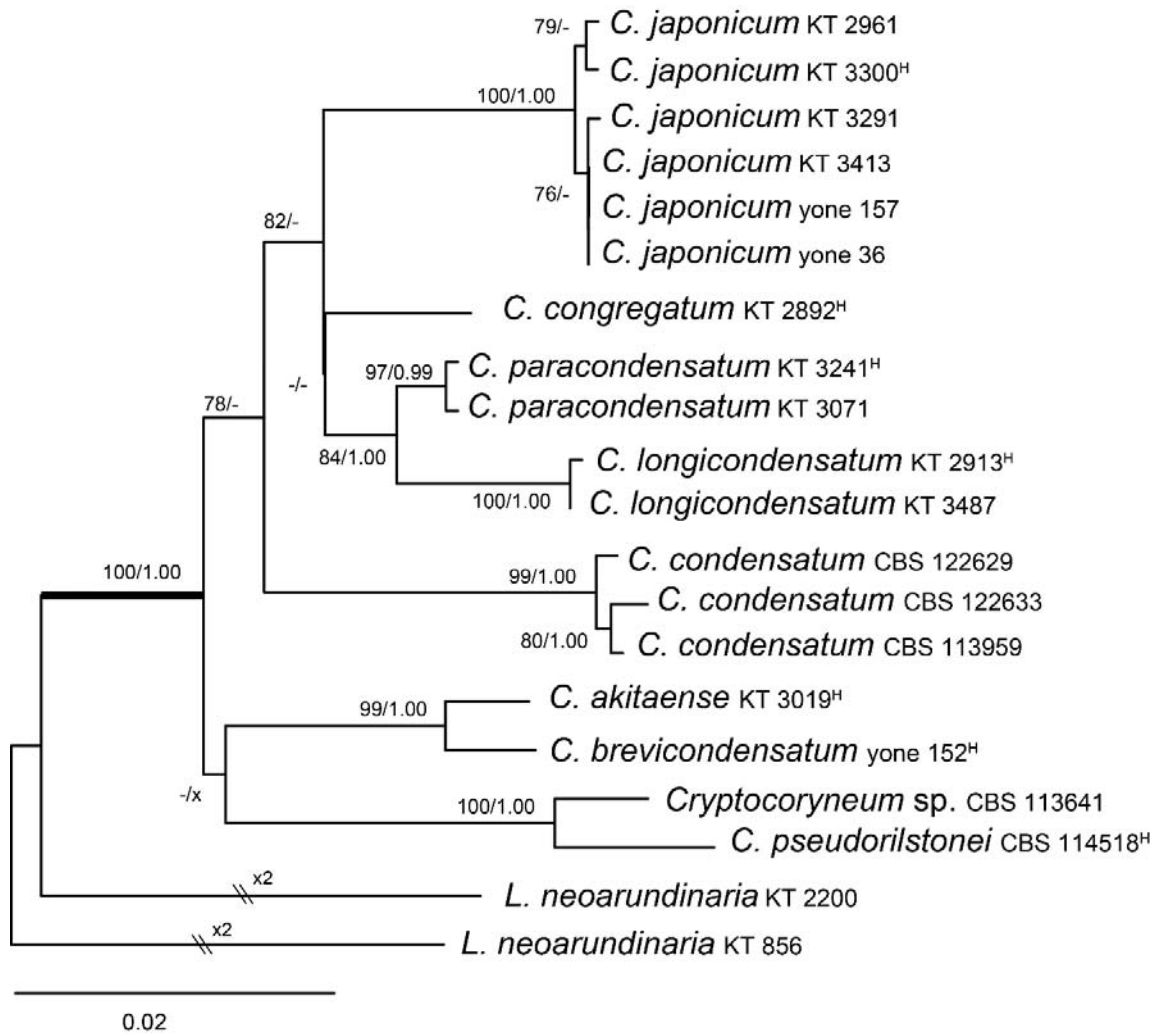
A Blast search of GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) with ITS sequences of *Cryptocoryneum* strains revealed their close relationship to *Lophiotremataceae* (*Dothideomycetes*). ML and Bayesian phylogenetic analyses of 20 strains including two outgroup sequences were conducted using an aligned sequence dataset comprising 543 nucleotide positions from ITS and 896 from *tef1*. Of the 1 439 characters included in the alignment, 218 were variable and 1 220 were conserved. The ML tree with the highest log likelihood (−3356.8744) is shown in Fig. 25. The topology of the Bayesian analysis was almost identical to that of the ML tree. In both trees, the monophyly of the genus *Cryptocoryneum* was highly supported (100% ML BP and 1.00 Bayesian PP) (Fig. 25). The alignment was submitted to TreeBASE with the study number S18427. Seven new species, as well as three known species are described below.

***Cryptocoryneum*** Fuckel, Fungi Rhenani Exsiccati Cent. XV–XVI: 25. 1865.

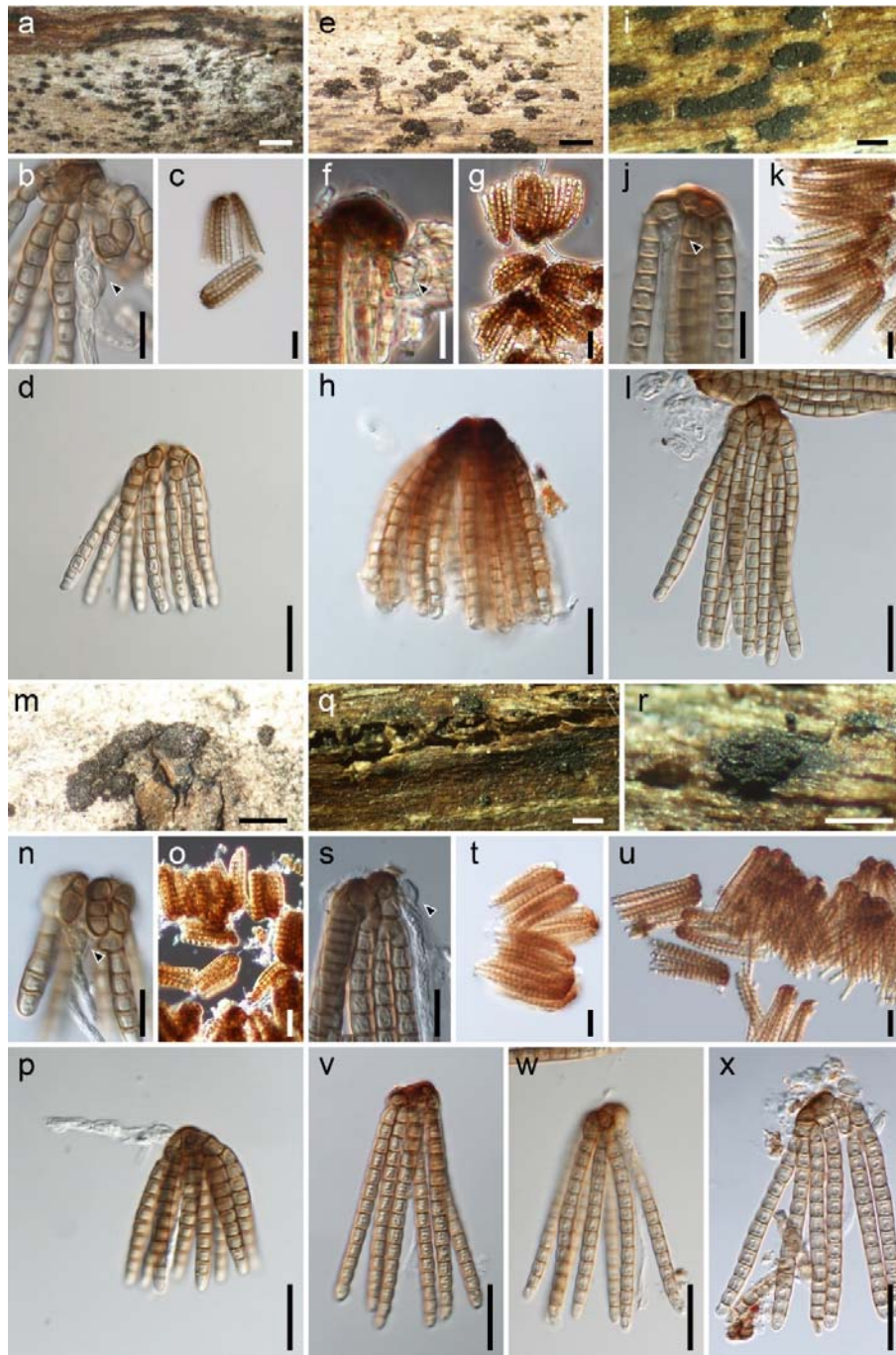
*Saprobic* on dead woody plants. **Sexual morph:** unknown. **Asexual morph:** *Sporodochia* pulvinate, often confluent, dark brown to black. *Conidiophores* arising from the stromatic cells, straight, simple, septate, hyaline to pale brown, smooth. *Conidiogenous cells* monoblastic, cylindrical to oblong, terminal, determinate, hyaline to pale brown. *Conidia* solitary, acrogenous, branched, cheiroid, with dark brown to black cap cells firmly united together, multi-armed; *basal cells* brown, cuneiform, smooth, thin-walled; *arms* cylindrical, pale brown, branched at base, smooth, multi-septate.

*Type species:* *Cryptocoryneum fasciculatum* Fuckel [= *C. hysterooides* (Corda) Peyronel]

*Note* — *Cryptocoryneum*, which was established by Fuckel (1865) to accommodate *C. fasciculatum* (= *C. hysterooides*), is characterised by the production of conidia developing downward from the cap cells. Seventeen taxa were listed in Index Fungorum (<http://www.indexfungorum.org/>). However, two species, *C. bigeminum* and *C. obovatum*, have been transferred to *Eversia* and *Bactrodesmium*, respectively (Ellis 1963, Schoknecht & Crane 1977), and one subspecies, *C. fasciculatum* subsp. *olivaceum* has been excluded from this genus by Peyronel (1918). *Cryptocoryneum lignicola* described by Prostakova (1966) should also be excluded from the genus, because it does not have cheiroid conidia. Thus, the genus presently comprises 13 taxa. A key to species accepted in *Cryptocoryneum* is given.



**Fig. 25.** Maximum-likelihood (ML) tree of *Cryptocoryneum* spp. based on a combined dataset of nrDNA ITS and TEF1 sequences. ML bootstrap proportion (BP) and Bayesian posterior probabilities (PP) greater than 70% and 0.95 are presented at the nodes as ML BP/ Bayesian PP, respectively. A hyphen ("-") indicates values lower than 70 %/0.95 ML BP/Bayesian PP, and a node not present in the Bayesian analysis is shown with x. The tree is with *Lophiotrema* spp. as outgroup. H = ex-holotype strain.



**Fig. 26.** *Cryptocoryneum* spp. a–d *C. akitaense*. e–h *C. brevicondensatum*. i–l *C. condensatum*. m–p *C. congregatum*. q–x *C. hysterioides*. a, e, i, m, q, r Conidiomata on natural substrate. b, f, j, n, s Conidiogenous cells (arrow head). c, d, g, h, k, l, o, p, t–x Conidia. a–d from HHUF 30477; e–h from HHUF 30478; i–l from UPS F-632989; m–p from HHUF 30479; q–t, v from no 1527 (holotype of *C. fasciculatum*); u, w from NYSf 3296 (holotype of *Torula uniformis*); x from PRM 155688 (holotype of *C. hysterioides*). — Scale bars: a, e, i, m, q = 500  $\mu$ m, b, f, j, n, s = 10  $\mu$ m, c, d, g, h, k, l, o, p, t–x = 20  $\mu$ m, r = 250  $\mu$ m.

***Cryptocoryneum akitaense*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB 815317; Fig. 26a–d.

*Etymology*: named after its type locality.

*Saprobic* on dead twigs of *Rhododendron brachycarpum*. **Sexual morph**: unknown. **Asexual morph**: *Sporodochia* pulvinate, (90)130–300(440)  $\mu\text{m}$  diam., (30)40–50  $\mu\text{m}$  high, often confluent, dark brown to black. *Conidiophores* arising from the stromatic cells, straight, simple, septate, hyaline to pale brown, smooth. *Conidiogenous cells* monoblastic, cylindrical to oblong, terminal, determinate, hyaline to pale brown 7.5–8.5  $\times$  5–7  $\mu\text{m}$ . *Conidia* solitary, acrogenous, branched, cheiroid, (34)38–61(67)  $\times$  19–72  $\mu\text{m}$  ( $\bar{x}$  = 49.1  $\times$  38.6  $\mu\text{m}$ , n = 54), l/w 0.7–2.6 ( $\bar{x}$  = 1.4, n = 54), with brown cap cells firmly united together, with 6–15 arms ( $\bar{x}$  = 10, n = 54); *basal cells* brown, cuneiform, smooth, thin-walled, 5–8(9.5)  $\mu\text{m}$  wide ( $\bar{x}$  = 6.5  $\mu\text{m}$ , n = 31); *arms* cylindrical, hyaline to pale brown, branched at base, smooth, 7–13-septate ( $\bar{x}$  = 10, n = 55), (34)38–60.5  $\mu\text{m}$  long ( $\bar{x}$  = 46.2  $\mu\text{m}$  long, n = 55), 4–7  $\mu\text{m}$  wide at base, 3–4  $\mu\text{m}$  wide at apex.

Culture characters — Colonies on PDA 29–31 mm diam. after 21 d at 20 °C in the dark, velvety, smoke grey (105); reverse similar (Fig. 27u); without sporulation.

*Specimen examined*. **JAPAN**, Akita, Kazuno, Hachimantai, Yakeyama, Mousen pass, on dead twigs of *Rhododendron brachycarpum*, 24 June 2012, K. Tanaka, KT 3019 (HHUF 30477 **holotype**, ex-holotype living culture MAFF 245365 = NBRC 111758).

*Note* — *Cryptocoryneum akitaense* is phylogenetically close to *C. brevicondensatum* (Fig. 25), but can be distinguished from the latter by its shorter and wider conidia [vs. (44.5)47–68.5(72)  $\times$  17–48(57)  $\mu\text{m}$ , l/w 0.9–3.5 in the latter].

***Cryptocoryneum brevicondensatum*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB 815318; Fig. 26e–h.

*Etymology*: named after its resemblance to *Cryptocoryneum condensatum*, but with shorter conidia.

*Saprobic* on dead twigs of woody plant. **Sexual morph**: unknown. **Asexual morph**: *Sporodochia* pulvinate, (130)200–510  $\mu\text{m}$  diam., (30)40–50  $\mu\text{m}$  high, often confluent, dark brown to black. *Conidiophores* arising from the stromatic cells, straight, simple, septate, hyaline to pale brown, smooth. *Conidiogenous cells* monoblastic, cylindrical to oblong, terminal, determinate, hyaline to pale brown, 9–10  $\times$  4–6.5  $\mu\text{m}$ . *Conidia* solitary, acrogenous, branched, cheiroid, (44.5)47–68.5(72)  $\times$  17–48(57)  $\mu\text{m}$  ( $\bar{x}$  = 57.5  $\times$  34.2  $\mu\text{m}$ , n = 50), l/w 0.9–3.5 ( $\bar{x}$  = 1.8, n = 50), with dark brown to black cap cells firmly united together, with 6–14 arms ( $\bar{x}$  = 9, n = 50); *basal cells* brown,

cuneiform, smooth, thin-walled, 6.5–12(14)  $\mu\text{m}$  wide ( $\bar{x}$  = 9.2  $\mu\text{m}$ , n = 50); *arms* cylindrical, pale brown, branched at base, smooth, 8–12-septate ( $\bar{x}$  = 10, n = 50), (37.5)41–60(64)  $\mu\text{m}$  long ( $\bar{x}$  = 50.3  $\mu\text{m}$  long, n = 50), 4–7  $\mu\text{m}$  wide at base, 3–5  $\mu\text{m}$  wide at apex.

Culture characters — Colonies on PDA 25–27 mm diam. after 21 d at 20 °C in the dark, velvety, grey olivaceous (107) to olivaceous black (108); reverse almost black (Fig. 27v); without sporulation.

*Specimen examined.* **JAPAN**, Aomori, Nishimeya, Ooshirosawa stream, on dead wood, 21 July 2007, H. Yonezawa, yone 152 (HHUF 30478 **holotype**; ex-holotype living culture MAFF 245366 = NBRC 111759).

*Note* — *Cryptocoryneum brevicondensatum* is similar to *C. condensatum* in conidial morphology, but can be distinguished from the latter by its smaller conidia with more septa [vs. (64)69–91(97)  $\times$  19.5–58(70)  $\mu\text{m}$ , with 13–19(21)-septa]. ITS and *tef1* sequences between these two species differed in 15 positions with nine gaps, and 35–39 positions with three gaps, respectively.

***Cryptocoryneum condensatum*** (Wallr.) E.W. Mason & S. Hughes ex S. Hughes, Canadian Journal of Botany 36: 758. 1958; Fig. 26i–l.

*Basionym:* *Hormiscium condensatum* Wallr., Flora Cryptogamica Germaniae 2: 186 (1833)

*Synonym:* *Cryptocoryneum condensatum* (Wallr.) E.W. Mason & S. Hughes, The natural history of the Scarborough district 1: 161 (1953) nom. nud. (Art. 33, Melbourne Code)

*Saprobic* on woody plants. **Sexual morph:** unknown. **Asexual morph:** *Sporodochia* pulvinate, (110)300–640(830)  $\mu\text{m}$  diam., often confluent, dark brown to black. *Conidiophores* arising from the stromatic cells, straight, simple, septate, hyaline to pale brown, smooth. *Conidiogenous cells* monoblastic, cylindrical to oblong, terminal, determinate, hyaline to pale brown, 9.5–10  $\times$  3–3.5  $\mu\text{m}$ . *Conidia* solitary, acrogenous, branched, cheiroid, (64)69–91(97)  $\times$  19.5–58(70)  $\mu\text{m}$  ( $\bar{x}$  = 76.8  $\times$  35.8  $\mu\text{m}$ , n = 50), l/w 1.1–4.4 ( $\bar{x}$  = 2.4, n = 50), with dark brown to cap cells firmly united together, with 5–10 arms ( $\bar{x}$  = 7, n = 50); *basal cells* brown, cuneiform, smooth, thin-walled, 5–9.5  $\mu\text{m}$  wide ( $\bar{x}$  = 7.1  $\mu\text{m}$ , n = 50); *arms* cylindrical, brown, branched at base, smooth, 13–19(21)-septate ( $\bar{x}$  = 16, n = 50), 64–89.5  $\mu\text{m}$  long ( $\bar{x}$  = 73.8  $\mu\text{m}$  long, n = 50), 3–6.5(8)  $\mu\text{m}$  wide at base, 2–6.5  $\mu\text{m}$  wide at apex.

Culture characters — Conidia formed in culture are similar (72–89  $\times$  27–42  $\mu\text{m}$ ,  $\bar{x}$  = 79.2  $\times$  35.0  $\mu\text{m}$ , n = 5) to those on natural substrate. Colonies on PDA 32–34 mm



diam. after 21 d at 20 °C in the dark, floccose, grey olivaceous (107); reverse almost black (Fig. 27w).

*Specimens examined.* **SWEDEN**, Uppland, Dalby par., the isle Kofsan., on *Salix fragilis*, K. Holm & L. Holm 4418b (UPS F-632989; culture CBS 113959); **PORTUGAL**, Bragança, Cova, da Lûa, on plant debris, November 2007, J. Capilla, R. Castañeda, C. Silvera (culture CBS 122629); **SPAIN**, Catalunya, La Garrotxa, Salt Sellent, on plant debris, October 2007, D. Garcia, G. Siso (culture CBS 122633).

*Notes:* The length of conidia, the number of conidial septa and width of conidial arms in the materials mentioned above almost matched those of *C. condensatum* reported by Schoknecht & Crane (1977), who observed the holotype of *C. condensatum*.

*Cryptocoryneum condensatum* is very similar to *C. paracondensatum* in having conidia overlapping in size, but can be separated from the latter by relatively longer conidial arms (64–89.5 µm vs. 52–85.5 µm). Colonies of *C. condensatum* grow rapidly (32–34 mm; Fig. 27w) as compared to those of *C. paracondensatum* (21–26 mm; Fig. 27aa). In addition, sequences of these two species differed at 12–13 positions with 8–9 gaps in the ITS, and 25–29 positions with three gaps and eight amino acid substitutions in the *tef1*.

***Cryptocoryneum congregatum*** A. Hashim. & Kaz. Tanaka, **sp. nov.** —  
MycoBank MB 815319; Fig. 26m–p.

*Etymology:* named for its congested conidial arms.

*Saprobic* on dead twigs of *Fagus crenata*. **Sexual morph:** unknown. **Asexual morph:** *Sporodochia* pulvinate, 190–630(850) µm diam., 65–130 µm high, often confluent, dark brown to black. *Conidiophores* arising from the stromatic cells, straight, simple, septate, hyaline to pale brown, smooth. *Conidiogenous cells* monoblastic, cylindrical to oblong, terminal, determinate, hyaline to pale brown, 9–16 × 5 µm. *Conidia* solitary, acrogenous, branched, cheiroid, (31)40–54 × 16–40(48.5) µm ( $\bar{x}$  = 46.2 × 28.1 µm, n = 50), l/w 0.9–3.1 ( $\bar{x}$  = 1.8, n = 50), with dark brown cap cells firmly united together, with 3–9 arms ( $\bar{x}$  = 6, n = 50); *basal cells* brown, cuneiform, smooth, thin-walled, 5–9(11.5) µm wide ( $\bar{x}$  = 7 µm, n = 50); *arms* cylindrical, brown, branched at base, smooth, (5)7–11-septate ( $\bar{x}$  = 9, n = 48), (30)34–53 µm long ( $\bar{x}$  = 42.4 µm long, n = 50), 4–6(8) µm wide at base, 3–4.5 µm wide at apex.

Culture characters — Conidia formed in culture are slightly larger [(40.5)42–55(61) × (15)22–44 µm,  $\bar{x}$  = 49.5 × 32.1 µm, n = 20] than those on natural substrate. Colonies on PDA 21–22 mm diam. after 21 d at 20 °C in the dark, floccose, smoke grey (105) to grey olivaceous (107); reverse similar (Fig. 27x).

*Specimens examined.* **JAPAN**, Aomori, Nishimeya, Shirakami Natural Science Park (Hirosaki Univ.), on dead twigs of *Fagus crenata*, 17 September 2011, K. Tanaka *et al.* KT 2892 (HHUF 30479, **holotype**; ex-holotype living culture MAFF 245367 = NBRC 111760).

*Notes* — *Cryptocoryneum congregatum* resembles *C. akitaense*, but has slightly smaller conidia with fewer arms [vs. (34)38–61(67) × 19–72 µm, with up to 15 arms]. Sequence differences between these two species were found at eight positions with one gap in the ITS, and 34 positions with seven amino acid substitutions in the *tef1*.

*Cryptocoryneum congregatum* has resemblance to *C. neolitseae* in conidial size and number of conidial septation, but the two species can be distinguished by the colour of conidiophores (vs. black in the latter; Hansford 1956).

***Cryptocoryneum hysteroioides*** (Corda) Peyronel, Nuovo Giornale Botanico Italiano 25: 449. 1918; Fig. 26q–x.

*Basionym:* *Torula hysteroioides* Corda, Icones fungorum hucusque cognitorum 1: 9, t. 2:139. 1837.

*Synonyms:* *Hormiscium hysteroioides* (Corda) Sacc., Sylloge Fungorum 4: 264. 1886.

*Exosporium hysteroioides* (Corda) Höhn., Sitzungsberichte der Akademie der Wissenschaften, mathematisch-naturwissenschaftliche Klasse Abt. I 111: 1035. 1902.

*Cryptocoryneum fasciculatum* Fuckel, Fungi Rhenani Exsiccati Cent. XV–XVI: 25, Fung. Rhen. no 1527. 1865.

*Torula uniformis* Peck, Annual Report on the New York State Museum of Natural History 33 (4): 27. 1880.

*Hormiscium uniforme* (Peck) Sacc., Sylloge Fungorum 4: 263. 1886.

*Saprobic* on woody plants. **Sexual morph:** unknown. **Asexual morph:** *Sporodochia* pulvinate, (115)280–500(670) µm diam., (50)65–82 µm high, often confluent, dark brown to black. *Conidiophores* arising from the stromatic cells, straight, simple, septate, hyaline to pale brown, smooth. *Conidiogenous cells* monoblastic, cylindrical to oblong, terminal, determinate, hyaline to pale brown, 7–9 × 4–5 µm. *Conidia* solitary, acrogenous, branched, cheiroid, (60)65–80 × 13.5–48(59) µm ( $\bar{x}$  = 70.6 × 32 µm, n = 57), l/w 1.1–5.2 ( $\bar{x}$  = 2.5, n = 57), with dark brown cap cells firmly united together, with 4–10 arms ( $\bar{x}$  = 6, n = 57); *basal cells* brown, cuneiform, smooth, thin-walled, 5–11 µm wide ( $\bar{x}$  = 7 µm, n = 57); *arms* cylindrical, brown, branched at base, smooth, 11–16(18)-septate ( $\bar{x}$  = 14, n = 57), (49)55–76(79) µm long ( $\bar{x}$  = 66.1 µm long, n = 102), 3–7 µm wide at base, 2.5–7 µm wide at apex.

*Specimens examined.* Locality unknown, on *Quercus* or *Malus*, date unknown, L. Fuckel no 1527 (G 00266173; holotype of *C. fasciculatum*); **USA**, Schenectady county, New York, Village of Quaker Street, June date unknown, C. H. Peck (NYSf 3296; holotype of *Torula uniformis*); **Germany**, Reichenberg, on dead wood, July date unknown, A. C. J. Corda (PRM 155688, **holotype** of *C. hysteroioides*); Locality unknown, on dead wood, date unknown, A. C. J. Corda (PRM 155687).

*Notes* — *Cryptocoryneum hysteroioides* has been considered a synonym of *C. condensatum*, with the latter regarded as the type species of the genus (Hughes 1958, Ellis 1971, Schoknecht & Crane 1977, Kirk 1983, von Heftberger *et al.* 1997, Mel'nik 2000, da Silva *et al.* 2015). However, we do not consider these two species to be conspecific. *Cryptocoryneum hysteroioides* differs from *C. condensatum* in having shorter conidia with fewer septa [vs. up to 91(97)  $\mu\text{m}$  long, with up to 19(21) septa]. The synonymy of *C. fasciculatum* and *T. uniformis* under *C. hysteroioides* was confirmed by our morphological examination of their type specimens, and therefore, we have redesignated *C. hysteroioides* (= *C. fasciculatum*) as the type species of *Cryptocoryneum*. Fresh material and cultures of *C. hysteroioides* are still needed to clarify its exact phylogenetic position within *Cryptocoryneum*.

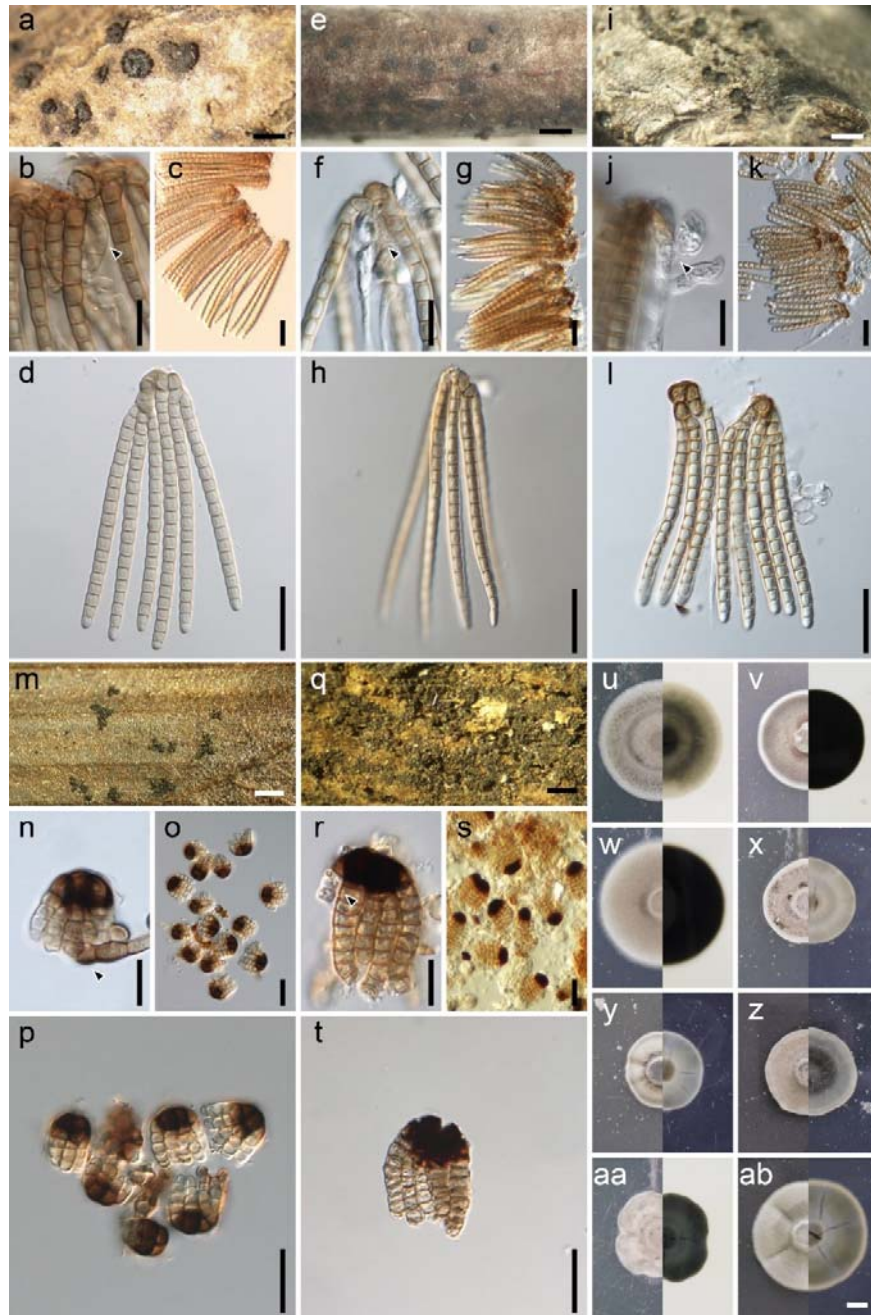
***Cryptocoryneum japonicum*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB 815320; Fig. 27a–d.

*Etymology:* named after its country of origin, Japan.

*Saprobic* on dead twigs of woody plants. **Sexual morph:** unknown. **Asexual morph:** *Sporodochia* pulvinate, (80)190–360(440)  $\mu\text{m}$  diam., (50)60–65  $\mu\text{m}$  high, often confluent, dark brown to black. *Conidiophores* arising from the stromatic cells, straight, simple, septate, pale brown to hyaline, smooth. *Conidiogenous cells* monoblastic, cylindrical to oblong, terminal, determinate, hyaline to pale brown, 6–9.5(17)  $\times$  3–6  $\mu\text{m}$ . *Conidia* solitary, acrogenous, branched, cheiroid, (67)70–100(107)  $\times$  (11)17–62(98)  $\mu\text{m}$  ( $\bar{x}$  = 87.1  $\times$  36.5  $\mu\text{m}$ , n = 85), l/w 1.0–5.4(9.0) ( $\bar{x}$  = 3.0, n = 85), with dark brown cap cells firmly united together, with 3–10 arms ( $\bar{x}$  = 6, n = 85); *basal cells* brown, cuneiform, smooth, thin-walled, 4–11  $\mu\text{m}$  wide ( $\bar{x}$  = 6.3  $\mu\text{m}$ , n = 85); *arms* cylindrical, pale brown, branched at base, smooth, 13–24(27)-septate ( $\bar{x}$  = 20, n = 82), 66–99  $\mu\text{m}$  long ( $\bar{x}$  = 82.7  $\mu\text{m}$  long, n = 82), 3–6.5  $\mu\text{m}$  wide at base, 2–4  $\mu\text{m}$  wide at apex.

*Culture characters* — Conidia formed in culture are similar [(68)76–98(125)  $\times$  (17)24–64(87)  $\mu\text{m}$ ,  $\bar{x}$  = 85.9  $\times$  46.4  $\mu\text{m}$ , n = 34] to those on natural substrate.

Colonies on PDA 19–22 mm diam. after 21 d at 20 °C in the dark, floccose, radiately sulcate, grey olivaceous (107); reverse similar (Fig. 27y).



**Fig. 27.** *Cryptocoryneum* spp. a–d *C. japonicum*. e–h *C. longicondensatum*. i–l *C. paracondensatum*. m–p *C. pseudorilstonei*. q–t *C. rilstonei*. a, e, i, m, q Conidiomata on natural substrate. b, f, j, n, r Conidiogenous cells (arrow head). c, d, g, h, k, l, o, p, s, t Conidia. u–ab Colony characters of *Cryptocoryneum* spp. used in this study on PDA within 3 wk at 20 °C in the dark (right: reverse, left: upper), u *C. akitaense*. v *C. brevicondensatum*. w *C. condensatum*. x *C. congregatum*. y *C. japonicum*. z *C. longicondensatum*. aa *C. paracondensatum*. ab *C. pseudorilstonei*. a from HHUF 30484; b–d from HHUF 30482; e–h from HHUF 30486; i–l from HHUF 30489; m–p from HHUF 30490; q–t from IMI 39939; u from MAFF 245365; v from MAFF 245366; w from CBS 113959; x from MAFF 245367; y from MAFF 245370; z from MAFF 245374; aa from MAFF 245377; ab from CBS 113641. — Scale bars: a, e, i, m, q = 500 µm, b, f, j, n, r = 10 µm, c, d, g, h, k, l, o, p, s, t = 20 µm, u–ab = 1 cm

*Specimens examined.* **JAPAN**, Aomori, Towada, Okuse, Tsuta-spa, on dead twigs of *Fagus crenata*, 6 November 2011, K. Tanaka *et al.*, KT 2961 (HHUF 30480, **paratype**; ex-paratype living culture MAFF 245368); Nishimeya, Shirakami, Toranosawa trail, on dead twigs of *Acer japonicum*, 11 August 2014, K. Tanaka, KT 3291 (HHUF 30481, **paratype**; ex-paratype living culture MAFF 245369); *ibid.* on dead twigs of *F. crenata*, 11 August 2014, K. Tanaka, KT 3300 (HHUF 30482, **holotype**; ex-holotype living culture MAFF 245370 = NBRC 111761); *ibid.* on dead twigs of *F. crenata*, 19 October 2013, K. Tanaka *et al.*, KT 3413 (HHUF 30483, **paratype**; ex-paratype living culture MAFF 245371); Takakuramori, on dead twigs of *Acer* sp., 24 June 2006, H. Yonezawa, yone 36 (HHUF 30484, **paratype**; ex-paratype living culture MAFF 245372); Chisan dam, on dead wood, 21 July 2007, H. Yonezawa, yone 157 (HHUF 30485, **paratype**; ex-paratype living culture MAFF 245373).

*Notes* — The conidia of *C. japonicum* have a slight resemblance to those of *C. longicondensatum*, but differ in having longer arms with more septa (up to 88  $\mu\text{m}$  long with up to 21 septa in *C. longicondensatum*). Sequence similarities between these two species were 98.5 % (519/527) in the ITS and 96.3 % (948/984) in the *tef1*.

*Cryptocoryneum japonicum* and *C. simmonsii* have overlapping conidial lengths (90–100  $\mu\text{m}$  in the latter; Saccardo 1920), but can be distinguished by the number of conidial septa (8–10 in *C. simmonsii*; Saccardo 1920).

Our six isolates of *C. japonicum* differed by only two positions in ITS sequences, and three positions without no amino acid substitution in *tef1* sequences. Morphological features of these isolates were completely identical.

***Cryptocoryneum longicondensatum*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB 815321; Fig. 27e–h.

*Etymology*: named after its resemblance to *Cryptocoryneum condensatum*, but with longer conidia.

*Saprobic* on dead twigs of *Fagus crenata*. **Sexual morph**: unknown. **Asexual morph**: *Sporodochia* pulvinate, 180–360(460)  $\mu\text{m}$  diam., (45)75–95  $\mu\text{m}$  high, often confluent, dark brown to black. *Conidiophores* arising from the stromatic cells, straight, simple, septate, pale brown to hyaline, smooth. *Conidiogenous cells* monoblastic, cylindrical to oblong, terminal, determinate, hyaline to pale brown, (3)9–12  $\times$  3–6(11)  $\mu\text{m}$ . *Conidia* solitary, acrogenous, branched, cheiroid, (69)74–92  $\times$  (12)17–66(96)  $\mu\text{m}$  ( $\bar{x}$  = 81.6  $\times$  39.7  $\mu\text{m}$ , n = 70), l/w 1.0–4.8(6.9) ( $\bar{x}$  = 2.5, n = 70), with brown cap cells firmly united together, with 3–6(8) arms ( $\bar{x}$  = 5, n = 70); *basal cells* brown, cuneiform, smooth, thin-walled, 4–11  $\mu\text{m}$  ( $\bar{x}$  = 7.1  $\mu\text{m}$ , n = 70); *arms* cylindrical, pale brown, branched at base, smooth, (14)16–21-septate ( $\bar{x}$  = 18, n = 50), (67)70–88  $\mu\text{m}$  long ( $\bar{x}$  = 78.9  $\mu\text{m}$  long, n = 65), 3–6  $\mu\text{m}$  wide at base, 2–3.5  $\mu\text{m}$  wide at apex.

Culture characters — Colonies on PDA 22–26 mm diam. after 21 d at 20 °C in the dark, velvety, smoke grey (105) to grey olivaceous (107); reverse similar (Fig. 27z); without sporulation.

*Specimens examined.* **JAPAN**, Aomori, Ajigasawa, Shirakami line, near Akaishigawa trail, on dead twigs of *Fagus crenata*, 21 September 2011, K. Tanaka *et al.*, KT 2913 (HHUF 30486, **holotype**; ex-holotype living culture MAFF 245374 = NBRC 111762); Towada, Okuse, Tsuta-spa, on dead twigs of *F. crenata*, 14 September 2014, K. Tanaka, KT 3487 (HHUF 30487, **paratype**; ex-paratype living culture MAFF 245375).

*Notes* — Conidial dimension of *C. longicondensatum* overlaps with that of *C. condensatum*, but the former is distinguishable by having a tendency to fewer conidial arms (5–10 arms). Sequence differences between these two species were found at 11–12 of 518 nucleotide positions with 9–10 gaps in the ITS, and 31–35 of 896 nucleotide positions with seven amino acid substitutions in the *tef1*. Although *C. longicondensatum* formed a moderately supported clade with *C. paracondensatum* in the phylogenetic tree (84%/1.00 ML BP/Bayesian PP) (Fig. 25), it differs from the latter in having relatively longer conidia (vs. 58–88.5 µm long in the latter).

***Cryptocoryneum paracondensatum*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB 815322; Fig. 27i–l.

*Etymology:* named after its resemblance to *Cryptocoryneum condensatum*.

Saprobic on dead twigs of *Fagus crenata*. **Sexual morph:** unknown. **Asexual morph:** *Sporodochia* pulvinate, (160)190–340 × 140–250 µm diam., (40)50–97 µm high, often confluent, dark brown to black. *Conidiophores* arising from the stromatic cells, straight, simple, septate, hyaline to pale brown, smooth. *Conidiogenous cells* monoblastic, cylindrical to oblong, terminal, determinate, hyaline to pale brown, 6–11(14.5) × 3–6(8) µm. *Conidia* solitary, acrogenous, branched, cheiroid, 58–88.5 × (13)15–57(72) µm ( $\bar{x}$  = 72.3 × 31 µm, n = 100), l/w 1.0–4.8 ( $\bar{x}$  = 2.7, n = 100), with dark brown to black cap cells firmly united together, with 3–8(11) arms ( $\bar{x}$  = 5, n = 100); basal cells brown, cuneiform, smooth, thin-walled, 5.5–11.5(14) µm wide ( $\bar{x}$  = 8 µm, n = 100); arms cylindrical, pale brown, branched at base, smooth, (12)14–19(21)-septate ( $\bar{x}$  = 16, n = 98), 52–85.5 µm long ( $\bar{x}$  = 67.9 µm long, n = 98), 3.5–7 µm wide at base, 2–4 µm wide at apex.

Culture characters — Colonies on PDA 21–26 mm diam. after 21 d at 20 °C in the dark, floccose, radiately sulcate, smoke grey (105) to grey olivaceous (107); reverse greenish grey (110) (Fig. 27aa); without sporulation.

*Specimens examined.* **JAPAN**, Aomori, Minamitsugaru, Owani, on twigs of *Fagus crenata*, 12 August 2012, K. Tanaka *et al.*, KT 3071 (HHUF 30488, **paratype**; ex-paratype living culture MAFF 245376);



ibid. on dead twigs of *Fagus crenata*, 11 May 2013, K. Tanaka, KT 3241 (HHUF 30489, **holotype**; ex-holotype living culture MAFF 245377 = NBRC 111763).

*Note* — In terms of conidial size, *C. paracondensatum* is morphologically similar to *C. hysteroioides*, but differs in conidial septation, with the latter having 11–16(18)-septate conidia.

***Cryptocoryneum pseudorilstonei*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB 815323; Fig. 27m–p.

*Etymology*: named after its resemblance to *Cryptocoryneum rilstonei*.

*Saprobic* on *Ischyrolepis subverticellata*. **Sexual morph**: unknown. **Asexual morph**: *Sporodochia* pulvinate, 160–260(390)  $\mu\text{m}$  diam., often confluent, dark brown to black. *Conidiophores* arising from the stromatic cells, straight, simple, septate, hyaline to pale brown, smooth. *Conidiogenous cells* monoblastic, cylindrical to oblong, terminal, determinate, hyaline to pale brown, 6–10  $\times$  6.5–10  $\mu\text{m}$ . *Conidia* solitary, acrogenous, branched, cheiroid, (14.5)17–25  $\times$  14–27  $\mu\text{m}$  ( $\bar{x}$  = 19.5  $\times$  18.8  $\mu\text{m}$ , n = 50), l/w 0.7–1.6 ( $\bar{x}$  = 1.1, n = 50), with dark brown to black cap cells firmly united together, with 4–10 arms ( $\bar{x}$  = 7, n = 50); *basal cells* brown, cuneiform, smooth, thin-walled, 4–8.5  $\mu\text{m}$  wide ( $\bar{x}$  = 6.4  $\mu\text{m}$ , n = 50); *arms* cylindrical, hyaline to pale brown, branched at base, smooth, 2–6-septate ( $\bar{x}$  = 4, n = 47), 14–22(24.5)  $\mu\text{m}$  long ( $\bar{x}$  = 17.8  $\mu\text{m}$  long, n = 47), 3.5–7  $\mu\text{m}$  wide at base, 2.5–5  $\mu\text{m}$  wide at apex.

*Culture characters* — Colonies on PDA 28–29 mm diam. after 21 d at 20 °C in the dark, floccose, radiately sulcate, smoke grey (105); reverse similar (Fig. 27ab); with abundant sporulation.

*Specimen examined*. **SOUTH AFRICA**, Western Cape, Kirstenbosch, National Botanical Garden, on *Elegia equisetacea*, 3 December 2001, S. Lee (HHUF 30490, dried culture specimen made from of CBS 113641, **holotype**, ex-holotype living culture CBS 113641).

*Notes* — The strain CBS 113641 was originally deposited as *C. rilstonei*, but is clearly different from *C. rilstonei* in conidial size. *Cryptocoryneum pseudorilstonei* has resemblance to *C. rilstonei* in dark brown to black cap cells in the conidia, but can be distinguished from the latter by smaller conidia [vs. (18)21–40  $\times$  17–32.5  $\mu\text{m}$ ].

In the phylogenetic tree, *C. pseudorilstonei* clustered with *Cryptocoryneum* sp. (CBS 114518). The strain CBS 114518 was also originally deposited as *C. rilstonei*. Sequence differences between these two strains were found at two positions with three gaps in the ITS and 18 positions without amino acid substitution in the *tef1*. We could not observe morphological features of CBS 114518, because it did not sporulate in culture. Therefore, we tentatively judged that the isolates CBS 114518

and CBS 113641 are different species, and treated the strain CBS 114518 as *Cryptocoryneum* sp.

*Cryptocoryneum pseudorilstonei* is similar to *C. parvulum* in conidial features, but the latter has wellinflated conidiophores (da Silva *et al.* 2015) unlike those of *C. pseudorilstonei*.

***Cryptocoryneum rilstonei*** [as 'rilstonii'] M.B. Ellis, Mycological Papers 131: 2 (1972); Fig. 27q–t.

*Saprobic* on twigs of *Fraxinus excelsior*. **Sexual morph:** unknown. **Asexual morph:** *Sporodochia* pulvinate, 140–260(330)  $\mu\text{m}$  diam., often confluent, dark brown to black. *Conidiophores* arising from the stromatic cells, straight, simple, septate, hyaline to pale brown, smooth. *Conidiogenous cells* monoblastic, terminal, determinate, hyaline to pale brown. *Conidia* solitary, acrogenous, branched, cheiroid, (18)21–40  $\times$  17–32.5  $\mu\text{m}$  ( $\bar{x}$  = 30.4  $\times$  24.1  $\mu\text{m}$ , n = 50), l/w 0.7–1.9 ( $\bar{x}$  = 1.3, n = 50), with dark brown to black cap cells firmly united together, with 5–9 arms ( $\bar{x}$  = 7, n = 50); *basal cells* brown, cuneiform, smooth, thin-walled, 5.5–10  $\mu\text{m}$  ( $\bar{x}$  = 7  $\mu\text{m}$ , n = 50); *arms* cylindrical, pale brown, branched at base, smooth, (3)5–10-septate, ( $\bar{x}$  = 7, n = 50), (12)17–35  $\mu\text{m}$  long ( $\bar{x}$  = 24.1  $\mu\text{m}$ , n = 23), 3–6  $\mu\text{m}$  wide at base, 3–5  $\mu\text{m}$  wide at apex.

*Specimen examined.* **UK**, England, Cornwall, Perranzabuloe, Lambourne Hill, on *Fraxinus excelsior*, 3 March 1950, M.B. Ellis (IMI 39939, **holotype**).

*Notes* — One of the most diagnostic features of this species within *Cryptocoryneum* is the presence of well-developed dark brown to black cap cells in the conidia (Fig. 27r–t). No culture of *C. rilstonei* is presently available and this species was, therefore, not included in the phylogenetic analysis.

### Key to *Cryptocoryneum* species

1. Conidia with dark cap cells ..... 2
- 1\*. Conidia without dark cap cells ..... 4
  
2. Conidiophores cylindrical ..... 3
- 2\*. Conidiophores inflated; conidia 12–23 × 10.5–15 µm, with dark cap cells, 4 arms, 3–4-septate ..... ***C. parvulum*** (Silva *et al.* 2015)
  
3. Conidia up to 40 µm long; (18)21–40 × 17–32.5 µm, with 5–9 arms, (3)5–10-septate ..... ***C. rilstonei*** (this study)
- 3\*. Conidia up to 25 µm long; (14.5)17–25 × 14–27 µm, with 4–10 arms, 2–6-septate ..... ***C. pseudorilstonei*** (this study)
  
4. Conidia mostly more than 60 µm long ..... 5
- 4\*. Conidia mostly less than 60 µm long ..... 13
  
5. Conidia less than 25-septate ..... 6
- 5\*. Conidia more than 25-septate; 200–350 µm × 8 µm, 35–40-septate ..... ***C. aureum*** (Viala 1891)
  
6. Conidia mostly more than 90 µm long ..... 7
- 6\*. Conidia mostly less than 90 µm long ..... 8
  
7. Conidia up to 145 µm long; 100–145 × 19–31 µm, 7–11-septate ..... ***C. millettiae*** (Sawada 1943)
- 7\*. Conidia up to 100 µm long; 90–100 × 5 µm, 8–10-septate ..... ***C. simmonsii*** (Saccardo 1920)
  
8. Conidia more than 70 µm long, and mostly more than 12-septate ..... 9
- 8\*. Conidia less than 70 µm long, and mostly less than 12-septate; (44.5)47–68.5(72) × 17–48(57) µm, with 6–14 arms, 8–12-septate ..... ***C. brevicondensatum*** (this study)
  
9. Conidia 70–100 µm long, and conidial arms more than 75 µm long ..... 10
- 9\*. Conidia 60–90 µm long, and conidial arms less than 75 µm long ..... 11
  
10. Average of conidial arm length more than 80 µm long, and up to 24-septate; conidia (67)70–100(107) × (11)17–62(98) µm ( $\bar{x}$  = 87.1 × 36.5 µm), with 3–10 arms, 13–24(27)-septate ..... ***C. japonicum*** (this study)
- 10\*. Average of conidial arm length less than 80 µm long, and up to 20-septate; conidia (69)74–92 × (12)17–66(96) µm ( $\bar{x}$  = 81.6 × 39.7 µm), with 3–6(8) arms, (14)16–21-septate ..... ***C. longicondensatum*** (this study)
  
11. Conidia up to 89 µm long, and up to 19-septate ..... 12
- 11\*. Conidia up to 80 µm long, and up to 16-septate; (60)65–80 × 13.5–48(59) µm, with 4–10 arms, (49)55–76(79) µm long, 11–16(18)-septate ..... ***C. hysterooides*** (this study)
  
12. Average of conidial arms more than 75 µm long, and mostly 7 arms; conidia (64)69–91(97) × 19.5–58(70) µm ( $\bar{x}$  = 76.8 × 35.8 µm), with 5–10 arms ( $\bar{x}$  = 7), 64–89.5 µm long ( $\bar{x}$  = 73.8 µm long) ..... ***C. condensatum*** (this study)
- 12\*. Average of conidial arms less than 75 µm long, and mostly 5 arms; conidia 58–88.5 × (13)15–57(72) µm ( $\bar{x}$  = 72.3 × 31 µm), with 3–8(11) arms ( $\bar{x}$  = 5), 52–85.5 µm long ( $\bar{x}$  = 67.9 µm long) ..... ***C. paracondensatum*** (this study)
  
13. Conidia up to 50–61 µm long, up to 5–10-septate ..... 14
- 13\*. Conidia up to 22–40 µm long, up to 4–8-septate ..... 16

14. Conidiophores hyaline to pale brown . . . . .	15
14*. Conidiophores black; conidia 40–60 × 7–8 μm, with 5–10-septate . . . . .	<b>C. neolitseae</b> (Hansford 1956)
15. Conidia up to 54 μm long, and up to 9 arms; conidia (31)40–54 × 16–40(48.5) μm, with 3–9 arms, (5)7–11-septate . . . . .	<b>C. congregatum</b> (this study)
15*. Conidia up to 61 μm long, and up to 15 arms; conidia (34)38–61(67) × 19–72 μm, with 6–15 arms, 7–13-septate . . . . .	<b>C. akitaense</b> (this study)
16. Conidia more than 23 μm long. . . . .	17
16*. Conidia less than 23 μm long; 21–22 × 3.5 μm, 5-septate . . . . .	<b>C. scopiforme</b> (Saccardo 1892)
17. Conidia up to 44 μm long; 25–44 × 6–7 μm, 4–8-septate . . . . .	<b>C. bombacis</b> (Hennings 1908)
17*. Conidia up to 28 μm long; 23–28 × 2.3–3.6 μm, 5–7-septate . . .	<b>C. psammae</b> (Oudemans 1892)

## DISCUSSION

Species of *Cryptocoryneum* have been chiefly identified by their conidial size (von Heftberger *et al.* 1997, Mel'nik 2000). Our study indicates that the separation of species in *Cryptocoryneum* solely on the basis of previous criteria is difficult. For example, conidia of *C. condensatum* bear a strong resemblance to those of *C. brevicondensatum*, *C. paracondensatum* and *C. longicondensatum* in conidial size. These four species were successfully distinguished by comparing detailed structures of their conidia, such as the number of conidial arms and conidial septa. We conclude that these minute morphological differences are useful for species delimitation within *Cryptocoryneum*. Similar examples have been reported for species in coelomycetous genera such as *Dinemasporium* and *Pseudolachnella* Teng (*Chaetosphaeriales*, *Sordariomycetes*) (Hashimoto *et al.* 2015a, b).

*Cryptocoryneum* was originally introduced based on *C. fasciculatum* (Fuckel 1865). Hughes (1958) regarded *C. fasciculatum*, *C. hysterooides* (= *Torula hysterooides* Corda 1837) and *T. uniformis* (Peck 1880) as synonymous with *C. condensatum* (= *Hormiscium condensatum*; Wallroth 1833) because conidial features of these species overlapped in their original descriptions. His treatment was accepted by subsequent researchers and *C. condensatum* has since been regarded as the type species of the genus (Ellis 1971, Schoknecht & Crane 1977, Kirk 1983, Katamoto 1988, von Heftberger *et al.* 1997, Mel'nik 2000, da Silva *et al.* 2015). Our morphological reassessment of *C. fasciculatum* and *T. uniformis* has confirmed that these two species should be reduced to synonyms of *C. hysterooides*. *Cryptocoryneum hysterooides*, however, should be separated from *C. condensatum* as a distinct species on the basis of their minute morphological differences; we therefore redesignate *C. hysterooides* as the type species.

In the present study, we described seven new species of *Cryptocoryneum*. Six of these species, except *C. pseudorilstonei*, were originally misidentified as *C. condensatum* on the basis of their morphological resemblance. Although *C. condensatum* was considered to have a worldwide distribution on various plant substrata (Schoknecht & Crane 1977, Kirk 1983, Katumoto 1988, von Heftberger *et al.* 1997, da Silva *et al.* 2015), a robust re-identification of *C. condensatum sensu lato* based on detailed morphological characteristics and molecular analysis will be needed to reveal several cryptic species within this species complex. It is expected that *C. condensatum sensu stricto* is distributed in a limited area and has a particular host preference.

To date, the phylogenetic placement of *Cryptocoryneum* has remained unresolved because a sexual stage of this genus has not been reported and no molecular study has been conducted (Wijayawardene *et al.* 2012). Our phylogenetic analysis based on ITS and *tef1* sequences indicates that *Cryptocoryneum* is phylogenetically related to *Lophiotrema* species (*Lophiotremataceae*; Hirayama & Tanaka 2011). However, we could not further resolve phylogenetic relationships between *Cryptocoryneum* and *Lophiotremataceae*. Additional taxa related to *Lophiotremataceae* and additional gene regions, such as nuclear rDNA SSU and LSU and the second largest subunit of RNA polymerase II (*rpb2*), will be required to determine the familial placement of *Cryptocoryneum*.

## 5. 広義 *Lophiotrema* 科の分類学的再検討

### ABSTRACT

*Lophiotrema* 科 (*Pleospora* 目, クロイボタケ綱) について、形態学的特徴と SSU-ITS-LSU-*tef1-rpb2* 領域の配列に基づき分類学的再検討を行った。 *Lophiotrema* 科とその近縁なグループの種について新たに 208 配列を決定した。 系統解析の結果に基づき、 *Lophiotrema* 科は *Lophiotrema* 属と 5 つの新属である *Atrocalyx*, *Crassimassarina*, *Cryptoclypeus*, *Galeaticarpa*, *Pseudocryptoclypeus* を含むことが判明した。 *Lophiotrema* 科内の各属はトサカ状の孔口をもつ、もしくはもたない子のう果と分生子殻状の分生子果によって特徴付けられた。 *Aquasubmersa* 科, *Cryptocoryneum* 科, *Hermatomyces* 科の 3 新科が提案された。 *Lophiotrema* 科のメンバーとして認識されてきた乳頭突起状の子のう果孔口と分生子殻状の分生子果をもつ *Aquasubmersa* 属と、スポロドキア状の分生子果と二型性 (レンズ状と円筒形) の分生子をもつ *Hermatomyces* 属は、それぞれ *Aquasubmersa* 科と *Hermatomyces* 科に收容された。 子座性のスポロドキアと cap 細胞から下向きに伸長するアームをもつ手のひら状の分生子で特徴付けられる *Cryptocoryneum* 属は *Cryptocoryneaceae* に收容された。 *Antealophiotrema* 属と *Pseudolophiotrema* 属の 2 新属が設立されたが、その系統的な位置は不明であった。 *Antealophiotrema* 属は *Lophiotrema* 属に形態的に類似した子のう果をもつが、著しく発達した子のう果殻壁と monodictys 型の無性世代をもつ点で後者の属と区別された。 *Pseudolophiotrema* 属は *Lophiotrema* 属に類似するが、薄い殻壁をもつ点で区別された。 本研究では 3 新科, 2 新属, 8 新種, 2 新組み合わせを提唱し、それらの種について記載した。

### INTRODUCTION

*Lophiotremataceae* was originally established as a monotypic family comprising the type genus *Lophiotrema* (Hirayama & Tanaka 2011). This genus is relatively common, known from throughout the world, and new species are continually being discovered (Mathiassen 1993, Tanaka & Harada 2003, Zhang et al. 2009, Liu et al. 2015, Hyde et al. 2016). Species in *Lophiotrema* are characterised by immersed ascomata with a crest-like ostiolar neck and cylindrical asci. Although the taxonomic validity of *Lophiotremataceae* has been confirmed by molecular studies, only a few of its members were known until recently (Hyde et al. 2013).

Several major taxonomic and phylogenetic investigations have been carried out to discover missing lineages in *Ascomycota* (Zhang et al. 2012, Ariyawansa et al. 2015, Liu et al. 2015, Doilom et al. 2016, Hashimoto et al. 2016, Hyde et al. 2016, Li et al. 2016). In these studies, three interesting genera, *Aquasubmersa*, *Cryptocoryneum*, and *Hermatomyces*, were analysed phylogenetically suggesting a close relationship to *Lophiotremataceae*. *Aquasubmersa* was initially described as a coelomycetous genus (Zhang et al. 2012). Later a second species of this genus, *A.*



*japonicum* with sexual and asexual morphs, was reported (Ariyawansa et al. 2015). The asexual genus *Hermatomyces* is characterised by having sporodochial conidiomata and dimorphic, i.e. lenticular and cylindrical, conidia (Chang 1995). Phylogenetic analyses using sequences of nuclear rDNA small subunit (18S; SSU), internal transcribed spacer (ITS) and large subunit (28S; LSU) regions and translation elongation factor 1- $\alpha$  (*tef1*) and DNA-directed RNA polymerase II second largest subunit (*rpb2*) genes have suggested that *Aquasubmersa* and *Hermatomyces* are closely related to *Lophiotrema* (Doilom et al. 2016, Tibpromma et al. 2016). On the basis of their phylogenetic studies, Doilom et al. (2016) and Tibpromma et al. (2016) proposed that *Aquasubmersa* and *Hermatomyces* are additional members of *Lophiotremataceae*. Another genus, *Cryptocoryneum*, is characterised by having stromatic sporodochia and cheiroid conidia (Schoknecht & Crane 1977).

During our ongoing studies of ascomycetous fungi in Japan (Tanaka et al. 2010, 2011, 2015, Hashimoto et al. 2015a, b, 2016), we have collected 57 strains morphologically or phylogenetically related to *Lophiotremataceae*. The main objectives of the present study were to establish a taxonomic framework within *Lophiotremataceae sensu lato* and to evaluate the circumscription of this family based on morphological observations and phylogenetic analyses of SSU, ITS, and LSU nuclear rDNA and *tef1*, and *rpb2* gene sequences.

## **MATERIALS AND METHODS**

### **Isolation and morphological observation**

All fungal structures were observed in preparations mounted in distilled water. Morphological characters were observed by differential interference and phase contrast microscopy (Olympus BX53), with images captured with an Olympus digital camera (DP21). A total of 57 single-spore isolates were used for morphological observations and phylogenetic analyses (Table 1). Colony characteristics of cultures grown on potato dextrose agar (PDA; Difco) were observed after 3 wk cultivation at 20 °C in the dark. Colours were noted as described by Rayner (1970). To induce sexual or asexual fructification in culture, 5-mm squares of mycelial agar were placed on water agar including sterilised natural substrate, such as rice straws and pine needles, 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 months to observe sporulation. Cultures were deposited in the Japan Collection of Microorganisms (JCM), the NITE Biological Resource Centre (NBRC), and the Genbank Project, NARO, Japan (MAFF). Specimens were deposited in the fungus herbarium of Hirosaki University (HHUF).

## Phylogenetic analyses

DNA extraction was carried out with an ISOPLANT II kit (Nippon Gene, Japan) in accordance with the manufacturer's protocol. Partial SSU, complete ITS, and partial LSU nuclear rDNA regions and partial *tef1* and partial *rpb2* genes 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 fRPB2-5F/fRPB2-7cR (Liu et al. 1999), respectively. Amplifications were performed in 25  $\mu$ L volumes consisting of 2  $\mu$ L DNA extract, 2.5  $\mu$ L of 10  $\times$  TEMPase Buffer I, 10 mM dNTP mix, 1  $\mu$ L of each 20-pM primer, 25 mM MgCl<sub>2</sub>, 14.5  $\mu$ L MilliQ water, and 0.5  $\mu$ L TEMPase Hot Start DNA polymerase (Ampliqon, Denmark). PCRs were carried out on a PC 320 thermocycler (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, with a final denaturation step of 7 min at 72 °C. The PCR products were sequenced directly at SolGent (South Korea).

Newly generated sequences were deposited in GenBank (Table 3). Sequences of 32 taxa from *Amniculicolaceae*, *Anteagloniaceae*, *Aquasubmersaceae*, *Hermatomycetaceae*, *Lophiotremataceae*, *Pseudoastrophaeriellaceae*, *Testudinaceae*, and *Tetraplosphaeriaceae* were also phylogenetically analysed (Table 4). *Lophiostoma crenatum* and *L. arundinis* (*Lophiostomataceae*) were used as outgroups. All sequences were aligned using the MUSCLE algorithm as implemented in MEGA 5 (Tamura et al. 2011). Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian methods. The optimum substitution models for each dataset were estimated by Kakusan4 (Tanabe 2011) based on the Akaike information Criterion (AIC; Akaike 1974) for the ML analysis and the 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 (proportional model among genes and proportional model among codons), namely, TN93ef+G for SSU, TN93+G for LSU, J2ef+G for ITS, F81+G for the *tef1* first codon position, TIMef+G for the *tef1* second codon position, J2+G for the *tef1* third codon position, J2+G for the *rpb2* first codon position, JC69+G for the *rpb2* second codon position, and TN93ef+G for the *rpb2* third codon position. Bootstrap proportions (BPs) were obtained by 1 000 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 (i.e. proportional model among loci and among codons): K80+G for SSU, SYM+G for LSU, SYM+G for ITS, F81+G for the *tef1* first

codon position, GTR+G for the *tef1* second codon position, GTR+G for the *tef1* third codon position, GTR+G for the *rpb2* first codon position, HKY85+G for the *rpb2* second codon position, and SYM+G for the *rpb2* third codon position. Two simultaneous, independent Metropolis-coupled Markov chain Monte Carlo (MCMC) runs were performed for 2 M generations with trees sampled every 1 000 generations. Convergence of the MCMC procedure was assessed from the average standard deviation of split frequencies (< 0.01) and effective sample size scores (all > 100) using MrBayes 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 to determine posterior probabilities (PPs) for individual branches. The alignment was submitted to TreeBase under study number S19310.

## RESULTS

The ML and Bayesian phylogenetic analyses were conducted using an aligned sequence dataset comprising 970 nucleotide positions from SSU, 1 275 from LSU, 340 from ITS, 909 from *tef1*, and 1 023 from *rpb2*. The alignment contained a total of 89 taxa, which consisted of 84 taxa (94.4 %) in SSU, 89 (100 %) in LSU, 71 (79.8 %) in ITS, 81 (91 %) in *tef1*, 73 (82 %) in *rpb2* (Table 1, 2). No significant conflict was observed among individual gene phylogenies, allowing the five genes to be combined into a single dataset. This combined dataset provided higher confidence values for the familial level than did those of the individual gene trees (data not shown). ITS1 was excluded from the analyses because it contained too many ambiguously aligned regions. Of the 4 517 characters included in the alignment, 1 307 were variable and 3 189 were conserved. The ML tree with the highest log likelihood (−31261.0019) is shown in Fig. 28. The Bayesian likelihood score was −31334.0529. The topology recovered by the Bayesian analysis was almost identical to that of the ML tree except for the position of *Anteagloniaceae*.

**Table 3 Specimens, isolates and new sequence accessions used in this study**

Species	Family <sup>a</sup>	Original no.	Specimen no. <sup>b</sup>	Strain no.	GenBank Accession no. <sup>c</sup>				
					SSU	LSU	<i>tef1</i>	<i>rpb2</i>	ITS
<i>Antealophiotrema brunneosporum</i>	IS	-	CBS H-20222 <sup>H</sup>	CBS 123095	<b>LC194298</b>	<b>LC194340</b>	<b>LC194382</b>	<b>LC194419</b>	<b>LC194474</b>
<i>Aquasubmersa japonica</i>	Aqu	KT 2813	HHUF 30468 <sup>P</sup>	MAFF 245218	LC061581 <sup>1</sup>	LC061586 <sup>1</sup>	<b>LC194383</b>	<b>LC194420</b>	LC061591 <sup>1</sup>
<i>A. japonica</i>	Aqu	KT 2862	HHUF 30469 <sup>H</sup>	MAFF 245219	LC061582 <sup>1</sup>	LC061587 <sup>1</sup>	<b>LC194384</b>	<b>LC194421</b>	LC061592 <sup>1</sup>
<i>A. japonica</i>	Aqu	KT 2863	HHUF 30470 <sup>P</sup>	MAFF 245220	LC061583 <sup>1</sup>	LC061588 <sup>1</sup>	<b>LC194385</b>	<b>LC194422</b>	LC061593 <sup>1</sup>
<i>Atrocalyx acutisporus</i>	Lop	KT 2436	HHUF 30504 <sup>H</sup>	MAFF 245613 = NBRC 112316	<b>LC194299</b>	<b>LC194341</b>	<b>LC194386</b>	<b>LC194423</b>	<b>LC194475</b>
<i>A. lignicola</i>	Lop	-	CBS H-20221 <sup>H</sup>	CBS 122364	<b>LC194300</b>	<b>LC194342</b>	<b>LC194387</b>	<b>LC194424</b>	<b>LC194476</b>
<i>Crassimassarina macrospora</i>	Lop	KH 152	HHUF 30512 <sup>P</sup>	MAFF 245617	<b>LC194301</b>	<b>LC194343</b>	<b>LC194388</b>	<b>LC194425</b>	<b>LC194477</b>
<i>C. macrospora</i>	Lop	KT 1764	HHUF 29084 <sup>H</sup>	JCM 13096 = MAFF 239606	<b>LC194302</b>	<b>LC194344</b>	<b>LC194389</b>	<b>LC194426</b>	<b>LC194478</b>
<i>Cryptoclypeus oxysporus</i>	Lop	KT 2772	HHUF 30507 <sup>H</sup>	MAFF 245614 = NBRC 112317	<b>LC194303</b>	<b>LC194345</b>	<b>LC194390</b>	<b>LC194427</b>	<b>LC194479</b>
<i>C. ryukyuensis</i>	Lop	AH 342	HHUF 30510 <sup>P</sup>	MAFF 245616	<b>LC194304</b>	<b>LC194346</b>	<b>LC194391</b>	<b>LC194428</b>	<b>LC194480</b>
<i>C. ryukyuensis</i>	Lop	KT 3534	HHUF 30509 <sup>H</sup>	MAFF 245615 = NBRC 112318	<b>LC194305</b>	<b>LC194347</b>	<b>LC194392</b>	<b>LC194429</b>	<b>LC194481</b>
<i>Cryptocoryneum akitaense</i>	Cry	KT 3019	HHUF 30477 <sup>H</sup>	MAFF 245365 = NBRC 111758	<b>LC194306</b>	<b>LC194348</b>	LC096136 <sup>2</sup>	<b>LC194430</b>	LC096154 <sup>2</sup>
<i>C. brevicondensatum</i>	Cry	yone 152	HHUF 30478 <sup>H</sup>	MAFF 245366 = NBRC 111759	<b>LC194307</b>	<b>LC194349</b>	LC096137 <sup>2</sup>	<b>LC194431</b>	LC096155 <sup>2</sup>
<i>C. condensatum</i>	Cry	-	UPS F-632989	CBS 113959	<b>LC194308</b>	<b>LC194350</b>	LC096138 <sup>2</sup>	<b>LC194432</b>	LC096156 <sup>2</sup>
<i>C. condensatum</i>	Cry	-	-	CBS 122629	<b>LC194309</b>	<b>LC194351</b>	LC096139 <sup>2</sup>	<b>LC194433</b>	LC096157 <sup>2</sup>
<i>C. condensatum</i>	Cry	-	-	CBS 122633	<b>LC194310</b>	<b>LC194352</b>	LC096140 <sup>2</sup>	<b>LC194434</b>	LC096158 <sup>2</sup>
<i>C. congregatum</i>	Cry	KT 2892	HHUF 30479 <sup>H</sup>	MAFF 245367 = NBRC 111760	<b>LC194311</b>	<b>LC194353</b>	LC096141 <sup>2</sup>	<b>LC194435</b>	LC096159 <sup>2</sup>
<i>C. japonicum</i>	Cry	KT 2961	HHUF 30480 <sup>P</sup>	MAFF 245368	<b>LC194312</b>	<b>LC194354</b>	LC096142 <sup>2</sup>	<b>LC194436</b>	LC096160 <sup>2</sup>
<i>C. japonicum</i>	Cry	KT 3291	HHUF 30481 <sup>P</sup>	MAFF 245369	<b>LC194313</b>	<b>LC194355</b>	LC096143 <sup>2</sup>	<b>LC194437</b>	LC096161 <sup>2</sup>
<i>C. japonicum</i>	Cry	KT 3300	HHUF 30482 <sup>H</sup>	MAFF 245370 = NBRC 111761	<b>LC194314</b>	<b>LC194356</b>	LC096144 <sup>2</sup>	<b>LC194438</b>	LC096162 <sup>2</sup>
<i>C. japonicum</i>	Cry	KT 3413	HHUF 30483 <sup>P</sup>	MAFF 245371	<b>LC194315</b>	<b>LC194357</b>	LC096145 <sup>2</sup>	<b>LC194439</b>	LC096163 <sup>2</sup>
<i>C. japonicum</i>	Cry	yone 36	HHUF 30484 <sup>P</sup>	MAFF 245372	<b>LC194316</b>	<b>LC194358</b>	LC096146 <sup>2</sup>	<b>LC194440</b>	LC096164 <sup>2</sup>
<i>C. japonicum</i>	Cry	yone 157	HHUF 30485 <sup>P</sup>	MAFF 245373	<b>LC194317</b>	<b>LC194359</b>	LC096147 <sup>2</sup>	<b>LC194441</b>	LC096165 <sup>2</sup>
<i>C. longicondensatum</i>	Cry	KT 2913	HHUF 30486 <sup>H</sup>	MAFF 245374 = NBRC 111762	<b>LC194318</b>	<b>LC194360</b>	LC096148 <sup>2</sup>	<b>LC194442</b>	LC096166 <sup>2</sup>
<i>C. longicondensatum</i>	Cry	KT 3487	HHUF 30487 <sup>P</sup>	MAFF 245375	<b>LC194319</b>	<b>LC194361</b>	LC096149 <sup>2</sup>	<b>LC194443</b>	LC096167 <sup>2</sup>

Table 3 (Continued)

Species	Family	Original no.	Specimen no.	Strain no.	GenBank Accession no.				
					SSU	LSU	<i>tef1</i>	<i>rpb2</i>	ITS
<i>C. paracondensatum</i>	Cry	KT 3071	HHUF 30488 <sup>P</sup>	MAFF 245376	<b>LC194320</b>	<b>LC194362</b>	LC096150 <sup>2</sup>	<b>LC194444</b>	LC096168 <sup>2</sup>
<i>C. paracondensatum</i>	Cry	KT 3241	HHUF 30489 <sup>H</sup>	MAFF 245377 = NBRC 111763	<b>LC194321</b>	<b>LC194363</b>	LC096151 <sup>2</sup>	<b>LC194445</b>	LC096169 <sup>2</sup>
<i>C. pseudorilstonei</i>	Cry	-	HHUF 30490 <sup>H</sup>	CBS 113641	<b>LC194322</b>	<b>LC194364</b>	LC096152 <sup>2</sup>	<b>LC194446</b>	LC096170 <sup>2</sup>
<i>Cryptocoryneum</i> sp.	Cry	-	-	CBS 114518	<b>LC194323</b>	<b>LC194365</b>	LC096153 <sup>2</sup>	<b>LC194447</b>	LC096171 <sup>2</sup>
<i>Galeaticarpa aomoriensis</i>	Lop	KT 2563	HHUF 30505 <sup>H</sup>	MAFF 245618 = NBRC 112319	<b>LC194324</b>	<b>LC194366</b>	<b>LC194393</b>	<b>LC194448</b>	<b>LC194482</b>
<i>Hermatomyces iriomotensis</i>	Her	KH 361	HHUF 30518 <sup>H</sup>	MAFF 245730 = NBRC 112471	<b>LC194325</b>	<b>LC194367</b>	<b>LC194394</b>	<b>LC194449</b>	<b>LC194483</b>
<i>H. tectonae</i>	Her	KT 2450	HHUF 30520	MAFF 245731	<b>LC194326</b>	<b>LC194368</b>	<b>LC194395</b>	<b>LC194450</b>	<b>LC194484</b>
<i>H. tectonae</i>	Her	AH 276	HHUF 30521	MAFF 245732	<b>LC194327</b>	<b>LC194369</b>	<b>LC194396</b>	<b>LC194451</b>	<b>LC194485</b>
<i>H. tectonae</i>	Her	AH 314	HHUF 30522	MAFF 245733	<b>LC194328</b>	<b>LC194370</b>	<b>LC194397</b>	<b>LC194452</b>	<b>LC194486</b>
<i>H. tectonae</i>	Her	KH 329	HHUF 30523	MAFF 245734	<b>LC194329</b>	<b>LC194371</b>	<b>LC194398</b>	<b>LC194453</b>	<b>LC194487</b>
<i>H. tectonae</i>	Her	KH 356	HHUF 30524	MAFF 245735	<b>LC194330</b>	<b>LC194372</b>	<b>LC194399</b>	<b>LC194454</b>	<b>LC194488</b>
<i>H. tectonae</i>	Her	KH 390	HHUF 30525	MAFF 245736	<b>LC194331</b>	<b>LC194373</b>	<b>LC194400</b>	<b>LC194455</b>	<b>LC194489</b>
<i>H. tectonae</i>	Her	KH 409	HHUF 30526	MAFF 245737	<b>LC194332</b>	<b>LC194374</b>	<b>LC194401</b>	<b>LC194456</b>	<b>LC194490</b>
" <i>Lophiotrema boreale</i> "	IS	-	-	CBS 114422 = JCM 14136	<b>LC194333</b>	<b>LC194375</b>	<b>LC194402</b>	<b>LC194457</b>	<b>LC194491</b>
<i>L. eburnoides</i>	Lop	KT 1424-1	HHUF 30079 <sup>H</sup>	JCM 17826 = MAFF 242970	LC001706 <sup>3</sup>	LC001707 <sup>3</sup>	<b>LC194403</b>	<b>LC194458</b>	LC001709 <sup>3</sup>
<i>Lophiotrema fallopiiae</i>	Lop	KT 2748	HHUF 30506 <sup>H</sup>	MAFF 245612	LC149911 <sup>4</sup>	LC149915 <sup>4</sup>	<b>LC194404</b>	<b>LC194459</b>	LC149913 <sup>4</sup>
<i>L. neoarundinaria</i>	Lop	KT 856	HHUF 27547	MAFF 239461	AB524455 <sup>5</sup>	AB524596 <sup>5</sup>	AB539109 <sup>6</sup>	AB539096 <sup>6</sup>	AB524786 <sup>5</sup>
<i>L. neoarundinaria</i>	Lop	KT 1034	HHUF 30015	NBRC 106239	AB524457 <sup>5</sup>	AB524598 <sup>5</sup>	<b>LC194405</b>	<b>LC194460</b>	<b>LC194492</b>
<i>L. neoarundinaria</i>	Lop	KT 2200	HHUF 30014	NBRC 106238	AB524456 <sup>5</sup>	AB524597 <sup>5</sup>	AB539110 <sup>6</sup>	AB539097 <sup>6</sup>	AB524787 <sup>5</sup>
<i>L. neohysterioides</i>	Lop	KH 17	HHUF 30511	MAFF 245619	<b>LC194334</b>	<b>LC194376</b>	<b>LC194406</b>	<b>LC194461</b>	<b>LC194493</b>
<i>L. neohysterioides</i>	Lop	KT 588	HHUF 27368	MAFF 245620	<b>LC194335</b>	<b>LC194377</b>	<b>LC194407</b>	<b>LC194462</b>	<b>LC194494</b>
<i>L. neohysterioides</i>	Lop	KT 713	HHUF 27328	JCM 17673	AB618701 <sup>7</sup>	AB619019 <sup>7</sup>	<b>LC194408</b>	<b>LC194463</b>	<b>LC194495</b>
<i>L. neohysterioides</i>	Lop	KT 756	HHUF 27330	MAFF 239457	AB618702 <sup>7</sup>	AB619020 <sup>7</sup>	<b>LC194409</b>	<b>LC194464</b>	<b>LC194496</b>
<i>L. nucula</i>	Lop	-	-	CBS 627.86 = JCM 14132	AB618703 <sup>7</sup>	AB619021 <sup>7</sup>	<b>LC194410</b>	<b>LC194465</b>	<b>LC194497</b>
<i>L. vagabundum</i>	Lop	KH 164	HHUF 30077	JCM 17674	AB618704 <sup>7</sup>	AB619022 <sup>7</sup>	<b>LC194411</b>	<b>LC194466</b>	<b>LC194498</b>
<i>L. vagabundum</i>	Lop	KH 172	HHUF 30078	JCM 17675	AB618705 <sup>7</sup>	AB619023 <sup>7</sup>	<b>LC194412</b>	<b>LC194467</b>	<b>LC194499</b>
<i>L. vagabundum</i>	Lop	KT 664	HHUF 27323	MAFF 239456	AB618706 <sup>7</sup>	AB619024 <sup>7</sup>	<b>LC194413</b>	<b>LC194468</b>	<b>LC194500</b>
<i>L. vagabundum</i>	Lop	KT 3310	HHUF 30508	MAFF 245621	<b>LC194336</b>	<b>LC194378</b>	<b>LC194414</b>	<b>LC194469</b>	<b>LC194501</b>

Table 3 (Continued)

Species	Family	Original no.	Specimen no.	Strain no.	GenBank Accession no.				
					SSU	LSU	<i>tef1</i>	<i>rpb2</i>	ITS
<i>L. vagabundum</i>	Lop	-	F-634236	CBS 113975 = JCM 14138	AB618707 <sup>7</sup>	AB619025 <sup>7</sup>	<b>LC194415</b>	<b>LC194470</b>	<b>LC194502</b>
" <i>Massarina albocarnis</i> "	Lop	-	-	CBS 119345	<b>LC194337</b>	<b>LC194379</b>	<b>LC194416</b>	<b>LC194471</b>	<b>LC194503</b>
<i>Pseudocryptoclypeus yakushimensis</i>	Lop	KT 2186	HHUF 30503 <sup>H</sup>	MAFF 245622 = NBRC 112320	<b>LC194338</b>	<b>LC194380</b>	<b>LC194417</b>	<b>LC194472</b>	<b>LC194504</b>
<i>Pseudolophiotrema elymicola</i>	IS	KT 1450	HHUF 28984 <sup>H</sup>	JCM 13090 = MAFF 239600	<b>LC194339</b>	<b>LC194381</b>	<b>LC194418</b>	<b>LC194473</b>	<b>LC194505</b>

<sup>a</sup>Abbreviation of family names: Aqu (*Aquasubmersaceae*), Cry (*Cryptocoryneaceae*), Her (*Hermatomycetaceae*), IS (*incertae sedis*), Lop (*Lophiotremataceae*).

<sup>b</sup>"H" : holotype, "P": paratype.

<sup>c</sup>Sequences with <sup>1</sup> from Ariyawansa et al. (2015), <sup>2</sup> from Hashimoto et al. (2016), <sup>3</sup> from Liu et al. (2015), <sup>4</sup>from Hyde et al. (2016), <sup>5</sup> from Tanaka et al. (2009), <sup>6</sup>from Schoch et al. (2009), <sup>7</sup> from Hirayama & Tanaka (2011)



Table 4 Specimens, isolates and GenBank accession numbers of species used in the phylogenetic study

Species	Family <sup>a</sup>	Specimen no. <sup>b</sup>	Strain no.	GenBank Accession no. <sup>c</sup>				
				SSU	LSU	<i>tef1</i>	<i>rpb2</i>	ITS
<i>Amniculicola immersa</i>	Amn	CBS H-20226 <sup>H</sup>	CBS 123083	GU456295	FJ795498	GU456273	GU456358	-
<i>A. parva</i>	Amn	CBS H-20227 <sup>H</sup>	CBS 123092	GU296134	GU301797	GU349065	-	-
<i>Anteaglonium abbreviatum</i>	Ant	ANM 925.1	-	-	GQ221877	GQ221924	-	-
<i>A. globosum</i>	Ant	ANM 925.2 <sup>H</sup>	-	-	GQ221879	GQ221925	-	-
<i>A. globosum</i>	Ant	SMH 5283 <sup>P</sup>	-	-	GQ221911	GQ221919	-	-
<i>A. parvulum</i>	Ant	MFLU 16-0473	MFLUCC 14-0815	KU922912	KU922911	KU922919	-	-
<i>A. parvulum</i>	Ant	MFLU 16-0472	MFLUCC 14-0817	KU922914	KU922913	-	-	-
<i>A. parvulum</i>	Ant	MFLU 16-0474	MFLUCC 14-0821	KU922916	KU922915	KU922921	-	-
<i>A. parvulum</i>	Ant	MFLU 16-0470	MFLUCC 14-0823	KU922918	KU922917	KU922922	-	-
<i>A. thailandicum</i>	Ant	MFLU 16-0471 <sup>H</sup>	MFLUCC 14-0816	KU922910	KU922909	KU922920	-	-
<i>Aquasubmersa mircensis</i>	Aqu	MFLU 111001 <sup>H</sup>	MFLUCC 11-0401 = IFRDCC 2572	JX276956	JX276955	-	-	JX276954
<i>Byssolophis sphaerioides</i>	IS	-	IFRDCC 2053	GU456296	GU456318	GU456263	GU456348	-
<i>Hermatomyces tectonae</i>	Her	MFLU 15-3437 <sup>H</sup>	MFLUCC 14-1140	KU712465	KU764695	KU872757	KU712486	KU144917
<i>H. tectonae</i>	Her	MFLU 15-3438 <sup>P</sup>	MFLUCC 14-1141	KU712466	KU764696	KU872758	-	KU144918
<i>H. tectonae</i>	Her	MFLU 15-3439 <sup>P</sup>	MFLUCC 14-1142	KU712467	KU764697	-	KU712487	KU144919
<i>H. thailandica</i>	Her	MFLU 15-3440 <sup>H</sup>	MFLUCC 14-1143	KU712468	KU764692	KU872754	KU712488	KU144920
<i>H. thailandica</i>	Her	MFLU 15-3441 <sup>P</sup>	MFLUCC 14-1144	KU712469	KU764693	KU872755	KU712489	KU144921
<i>H. thailandica</i>	Her	MFLU 15-3442 <sup>P</sup>	MFLUCC 14-1145	KU712470	KU764694	KU872756	KU712490	KU144922
<i>Lepidosphaeria nicotiae</i>	Tes	-	CBS 101341	-	DQ678067	DQ677910	DQ677963	-
<i>Lophiostoma arundinis</i>	Lps	-	CBS 621.86	DQ782383	DQ782384	DQ782387	DQ782386	AJ496633
" <i>Lophiotrema</i> " <i>bambusae</i>	Lpt	MFLU 11-0150	MFLUCC 10-0558	KX672159	KX672154	KX672162	KX672161	KX672149
<i>L. crenatum</i>	Lps	-	CBS 629.86	DQ678017	DQ678069	DQ677912	DQ677965	-
<i>Polyposphaeria fusca</i>	Tet	HHUF 29399 <sup>H</sup>	JCM 13175 = MAFF 239685	AB524463	AB524604	-	-	AB524789
<i>Pseudoastrosphaeriella bambusae</i>	Pse	MFLU 11-0155 <sup>H</sup>	MFLUCC 11-0205	KT955455	KT955475	KT955437	KT955414	-
<i>P. longicolla</i>	Pse	MFLU 11-0207 <sup>H</sup>	MFLUCC 11-0171	-	KT955476	KT955438	KT955420	-
<i>P. thailandensis</i>	Pse	MFLU 11-0145 <sup>H</sup>	MFLUCC 10-0553	KT955456	KT955477	KT955439	KT955411	-
<i>Pseudotetraploa curviappendiculata</i>	Tet	HHUF 28582 <sup>H</sup>	JCM 12852 = MAFF 239495	AB524467	AB524608	-	-	AB524792
<i>Quadricrura septentrionalis</i>	Tet	HHUF 28781 <sup>P</sup>	CBS 125429	AB524474	AB524615	-	-	AB524799

Table 4 (Continued)

Species	Family <sup>a</sup>	Specimen no. <sup>b</sup>	Strain no.	GenBank Accession no. <sup>c</sup>				
				SSU	LSU	<i>tef1</i>	<i>rpb2</i>	ITS
<i>Tetrapla sasicola</i>	Tet	HHUF 27566 <sup>H</sup>	JCM 13167 = MAFF 239677	AB524490	AB524631	-	-	AB524807
<i>Triplosphaeria maxima</i>	Tet	HHUF 29390 <sup>H</sup>	JCM 13172 = MAFF 239682	AB524496	AB524637	-	-	AB524812
<i>Ulospora bilgramii</i>	Tes	-	CBS 101364	DQ678025	DQ678076	DQ677921	DQ677974	-
<i>Verruculina enalia</i>	Tes	-	BCC 18402	GU479771	GU479803	GU479864	GU479836	-

<sup>a</sup>Abbreviation of family names: Amn (*Amniculicolaceae*), Ant (*Anteaglioniaceae*), Aqu (*Aquasubmersaceae*), Her (*Heratomyetaceae*), Tes (*Testudinaceae*), Tet (*Tetraplosphaeriaceae*), Pse (*Pseudoastrospheariellaceae*), Lps (*Lophiostomataceae*), Lpt (*Lophiotremataceae*), IS (*incertae sedis*).

<sup>b</sup>“H” : holotype, “P”: paratype.

In the phylogenetic tree shown in Fig. 1, *Lophiotremataceae* forms a moderately supported clade (77 % ML BP/1.00 Bayesian PP) (Fig. 28). The clade corresponding to *Lophiotrema sensu stricto* includes *L. eburnoides*, *L. fallopieae*, *L. neoarundinaria*, *L. neohysterioides*, *L. nucula*, and *L. vagabundum*. The results of the phylogenetic analyses suggest that four species previously placed in *Lophiotrema*, i.e., *L. boreale*, *L. brunneosporum*, *L. lignicola*, and *L. bambusae* (Mathiassen 1993, Zhang et al. 2009, Hyde et al. 2016), should be excluded from the genus. The first two species, *L. boreale* and *L. brunneosporum*, lie outside of *Lophiotremataceae*, but their familial placements are unresolved (Fig. 28). On the basis of these results, a new genus, *Antealophiotrema*, is established for *L. brunneosporum*. *Lophiotrema lignicola* is transferred to the new genus *Atrocalyx*, and a new combination, *Atrocalyx lignicola*, is proposed. The generic placement of *L. bambusae* and *L. boreale* remains unresolved because we were unable to examine any material of these species.

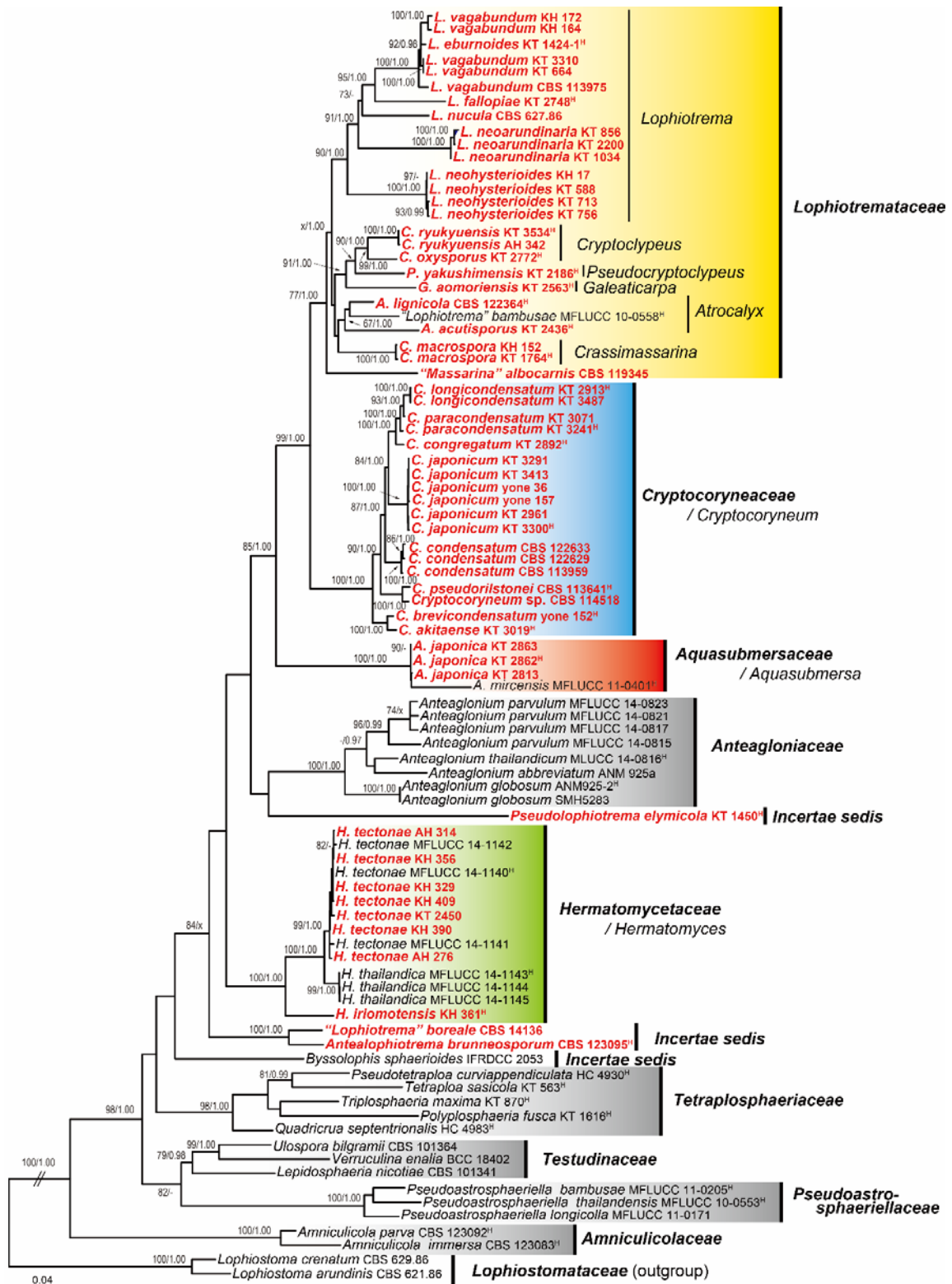
Four families, including the three new families, *Aquasubmersaceae*, *Cryptocoryneaceae*, and *Hermatomycetaceae*, 10 genera including seven new genera, nine species including seven new species, and two new combinations are described below.

***Aquasubmersaceae*** A. Hashim. & Kaz. Tanaka, **fam. nov.** — MycoBank MB819235.

Saprobic on woody plants. **Sexual morph:** *Ascomata* scattered to grouped, subglobose, semi-immersed, with a papillate ostiolar neck. *Peridium* composed of flattened, thin-walled, polygonal cells. *Pseudoparaphyses* septate, branched. *Asci* bitunicate, cylindrical, with a short pedicel, 8-spored. *Ascospores* hyaline, broadly fusiform with rounded ends, septate. **Asexual morph:** *Conidiomata* pycnidial, globose to subglobose, scattered, semi-immersed, ostiolate. *Conidiophores* absent. *Conidiogenous cells* holoblastic, lageniform, hyaline, smooth. *Conidia* hyaline, ellipsoidal, smooth.

*Type genus:* *Aquasubmersa* K.D. Hyde & Huang Zhang, *Cryptog. Mycol.* 33: 340. 2012.

**Fig. 28.** Maximum-likelihood tree of *Aquasubmersaceae*, *Cryptocoryneaceae*, *Hermatomycetaceae* and *Lophiotremataceae* based on SSU-ITS-LSU-*tef1-rpb2* sequences. ML bootstrap proportion (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 strains are indicated in H. The newly obtained sequences annotated in bold and red. The scale bar represents nucleotide substitution per site. ▶



Notes — Previous phylogenetic studies using SSU and LSU rDNA sequences placed *Aquasubmersa* in *Pleosporales* (Zhang et al. 2012, Ariyawansa et al. 2015). This genus was treated as a member of *Lophiotremataceae* in a recent study (Doilom et al. 2016). The results of our phylogenetic analyses based on SSU-ITS-LSU-*tef1-rpb2* sequences strongly support (99% ML BP/1.00 Bayesian PP) this genus as sister to a clade comprising *Lophiotremataceae* and *Cryptocoryneaceae* (Fig. 28). To accommodate the genus *Aquasubmersa*, we introduce a new family, *Aquasubmersaceae*. Species in *Aquasubmersaceae* share several common features, such as ascomata having a papillate ostiolar neck and pycnidial conidiomata. *Lophiotremataceae* differs from *Aquasubmersaceae* in having ascomata with a compressed, slit-like ostiole and aseptate. *Cryptocoryneaceae* and *Hermatomycetaceae*, whose sexual morphs are still undetermined, can be easily distinguished from *Aquasubmersaceae*, which has ellipsoidal conidia, because two families have sporodochial conidiomata and cheiroid or lenticular conidia, respectively.

***Aquasubmersa*** K.D. Hyde & Huang Zhang, *Cryptog. Mycol.* 33: 340. 2012.

*Type species: Aquasubmersa mircensis* Huang Zhang & K.D. Hyde, *Cryptog. Mycol.* 33: 340. 2012.

Notes — The type species of this genus, *A. mircensis*, was reported only as an asexual morph (Zhang et al. 2012). Ariyawansa et al. (2015) subsequently introduced the second species, *A. japonica*, with sexual and asexual morphs. Both species occurred on submerged woody plants.

***Cryptocoryneaceae*** A. Hashim. & Kaz. Tanaka, **fam. nov.** — MycoBank MB819237.

Saprobic on various plants. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* sporodochial, pulvinate, often confluent, dark brown to black. *Conidiophores* arising from the stromatic cells, simple, septate, hyaline to pale brown. *Conidiogenous cells* monoblastic, cylindrical to oblong, terminal, determinate, hyaline to pale brown. *Conidia* solitary, acrogenous, branched, cheiroid, with dark brown to black cap cells firmly united together, multi-armed; basal cells brown, cuneiform, smooth, thin-walled; arms cylindrical, pale brown, branched at base, smooth, multi-septate.

*Type genus: Cryptocoryneum* Fuckel, *Fungi Rhenani Exsiccati Cent.* XV–XVI: 25. 1865.

Notes — *Cryptocoryneum* is characterised by having stromatic sporodochia, cheiroid conidia, and conidial arms that are developed downward from the cap cells (Schoknecht & Crane 1977). This genus was recently taxonomically and phylogenetically reassessed by Hashimoto *et al.* (2016). A Blast search with ITS sequences of species in this genus suggested a close relationship with *Lophiotremataceae*, but their familial position remains unresolved (Hashimoto *et al.* 2016). According to our results, *Cryptocoryneum* forms a lineage that is phylogenetically distinct from *Lophiotremataceae* (Fig. 28). Although the sexual morph of *Cryptocoryneum* has not been determined, asexual morphs of this genus are phenotypically different from the coelomycetous asexual morph of *Lophiotremataceae* (Leuchtmann 1985, this study). We therefore introduce the new family *Cryptocoryneaceae* to accommodate the genus *Cryptocoryneum*.

***Cryptocoryneum*** Fuckel, Fungi Rhenani Exsiccati Cent. XV–XVI: 25. 1865.

*Type species: Cryptocoryneum fasciculatum* Fuckel, Fungi Rhenani Exsiccati Cent. XV–XVI: 25. Fung. Rhen. 1527. 1865. [= *Cryptocoryneum hysterooides* (Corda) Peyronel, Nuovo Giornale Botanico Italiano 25: 449. 1918.]

Notes — For further information on this genus, see Hashimoto *et al.* (2016).

***Hermatomycetaceae*** A. Hashim. & Kaz. Tanaka, Locq. ex A. Hashim. & Kaz. Tanaka, fam. nov. — MycoBank MB819238.

*Hermatomycetaceae* Locq., Mycologie générale et structural: 202. 1984. nom. inval. (Art. 36.1, Melbourne Code).

Saprobic on various plants. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* sporodochial, pulvinate, dark brown to black. *Conidiophores* mononematous, pale brown. *Conidiogenous cells* monoblastic, integrated, terminal, cylindrical. *Conidia* dimorphic; lenticular conidia ellipsoidal, muriform; cylindrical trans-septate, hyaline to brown.

*Type genus: Hermatomyces* Speg., Anal. Mus. nac. B. Aires, Ser. 3 13: 445. 1911.

Notes — The family *Hermatomycetaceae* sensu Locq. was informally proposed as a provisional name (Locquin 1984). *Hermatomycetaceae*, which should be regarded as a natural taxon, is established here to accommodate the single genus *Hermatomyces*. Two of the most striking features of *Hermatomyces* are the sporodochial conidiomata and the dimorphic conidia, the latter existing in lenticular and cylindrical forms (Chang 1995). As a sexual state of this genus has not been



reported and a phylogenetic study has not been performed, this genus has long been treated as “*incertae sedis*” within *Ascomycota* (Wijayawardene et al. 2012). Doilom et al. (2016) and Tibpromma et al. (2016) have suggested that this genus belongs to *Lophiotremataceae* on the basis of their phylogenetic analyses using SSU, LSU, *tef1*, and *rpb2*. Our analyses using additional species of *Lophiotremataceae* and its related taxa revealed that *Hermatomyces* is distantly related to *Lophiotremataceae* (Fig. 28).

***Hermatomyces*** Speg., Anal. Mus. nac. B. Aires, Ser. 3 13: 445. 1911.

Saprobic on various plants. **Sexual morph:** Undermined. **Asexual morph:** *Conidiomata* sporodochial, pulvinate, often confluent, dark brown to black. *Conidiophores* mononematous, septate, pale brown. *Conidiogenous cells* monoblastic, integrated, terminal, cylindrical. *Conidia* dimorphic; lenticular conidia ellipsoidal, muriform, dark brown to black at central cells, pale brown at peripheral cells; cylindrical conidia composed of black peripheral cells and dark brown upper cells, hyaline at lower cells.

*Type species: Hermatomyces tucumanensis* Speg., Anal. Mus. nac. B. Aires, Ser. 3 13: 446. 1911.

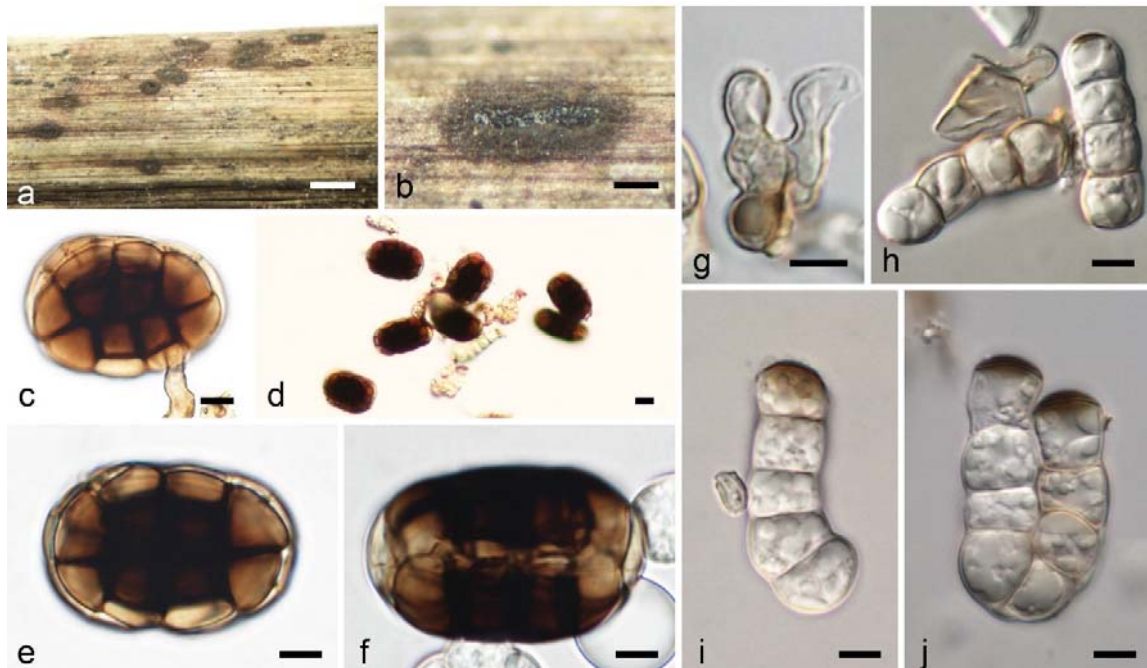
Notes — This genus was established by Spegazzini (1911) to accommodate *H. tucumanensis*. Since its establishment, ten additional taxa have been described worldwide (Spegazzini 1911, Hughes 1953, Rao & de Hoog 1986, Castañeda-Ruiz & Heredia 2000, Leão-Ferreira et al. 2013, Prasher & Prasher 2014, Doilom et al. 2016, Tibpromma et al. 2016). Most species of this genus occur on angiosperms and monocots, with a few rarely reported from ferns (Castañeda-Ruiz & Heredia 2000) or gymnosperms (Mel'nik 2000). The most widespread species in the genus, *H. sphaericus*, has been reported from Africa, Eurasia, and Central and South America (Spegazzini 1911, Hughes 1953, Chang 1995, Matsushima 1993, Mel'nik 2000, Barbosa & Gusmão 2011).

***Hermatomyces iriomotensis*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB819239; Fig. 29.

*Etymology:* Referring to the collection site.

Saprobic on woody plant. **Sexual morph:** Undermined. **Asexual morph:** *Conidiomata* sporodochial, pulvinate, often confluent, dark brown to black. *Conidiophores* mononematous, short, pale brown, smooth, 2.5–3 µm wide, 17.5–22 µm high, sometimes reduced to conidiogenous cells. *Conidiogenous cells*

monoblastic, terminal, integrated. *Conidia* dimorphic, lenticular and cylindrical. *Lenticular conidia* solitary, elliptical to almost round in one plane, smooth, muriform, with pale peripheral cells surrounding central dark brown to black cells,  $30\text{--}36 \times 20\text{--}27 \mu\text{m}$  ( $\bar{x} = 33.6 \times 23.5 \mu\text{m}$ ,  $n = 50$ ),  $18\text{--}24 \mu\text{m}$  thick ( $\bar{x} = 21.9 \mu\text{m}$ ,  $n = 30$ ) in lateral view. *Cylindrical conidia* straight to curved, with one to two columns, 3–7-septate,



**Fig. 29.** *Hermatomyces iriomotensis*. a, b. Conidiomata on substrate; c. Conidiogenous cell and immature lenticular conidium; d–f. Lenticular conidia (f lateral views); g. Conidiogenous cells and immature cylindrical conidium; h–j. Cylindrical conidia; a–j from HHUF 30518 (holotype). — Scale bars: a = 1 mm; b = 200  $\mu\text{m}$ ; c, e–j = 5  $\mu\text{m}$ ; d = 10  $\mu\text{m}$ .

hyaline, sometimes pale brown at apical cell, constricted at the septa,  $20.5\text{--}33 \times 7\text{--}12.5 \mu\text{m}$  ( $\bar{x} = 28.7 \times 9.6 \mu\text{m}$ ,  $n = 20$ ).

**Culture characteristics** — Colonies on PDA attaining 24–28 mm diam within 21 d at 20 °C in the dark, floccose, centrally raised, straw (46: Rayner 1970) to grey olivaceous (107); reverse smoke grey (105) to grey olivaceous (107) (Fig. 39a); no sporulation observed.

**Specimen examined.** **JAPAN**, Okinawa, Isl. Iriomote, near Tropical botanic garden, on dead twigs of woody plant, 13 Jul. 2011, K. Hirayama & K. Tanaka, KH 361 (HHUF 30518, ex-holotype living culture MAFF 245730 = NBRC 112471).

**Notes** — Lenticular conidia of this species resemble those of *H. uniseriatum*, but *H. iriomotensis* can be distinguished from the latter species by the presence of 3–7-

septate cylindrical conidia arranged in 1–2 columns (vs. 2–3-septate and one row in the latter; Leão-Ferreira et al. 2013). *Hermatomyces iriomotensis* differs from *H. krabiensis*, which has cylindrical conidia with swollen lower cells (Tibpromma et al. 2016). Additionally, ITS sequences between these two taxa differed at seven positions.

**Lophiotremataceae** K. Hiray. & Kaz. Tanaka, Mycoscience 52: 405. 2011.

Saprobic on various plants. **Sexual morph:** *Ascomata* immersed, erumpent at the apex, subglobose. *Ostiolar neck* crest-like or rarely papillate, mostly elongate and laterally compressed. *Peridium* composed of rectangular to globose cells. *Pseudoparaphyses* septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, with a short stipe, 8-spored. *Ascospores* fusiform to broadly fusiform, hyaline, smooth. **Asexual morph:** *Conidiomata* pycnidial, globose to subglobose, scattered, semi-immersed, ostiolate. *Peridium* composed of subglobose to angular, brown cells. *Conidiophores* absent. *Conidiogenous cells* holoblastic or phialidic, cylindrical to ampliform, hyaline. *Conidia* ellipsoidal to cylindrical with rounded ends, hyaline, smooth, aseptate or multi-septate.

*Type genus:* *Lophiotrema* Sacc., *Michelia* 1(no. 3): 338. 1878.

Notes — As originally circumscribed, *Lophiotremataceae* was a monotypic family comprising the genus *Lophiotrema* (Zhang et al. 2009, Hirayama & Tanaka 2011, Hyde et al. 2013). A somewhat broader familial concept for *Lophiotremataceae* was adopted by Doilom et al. (2016) and Tibpromma et al. (2016), who considered the family to comprise *Aquasubmersa*, *Hermatomyces*, and *Lophiotrema* on the basis of phylogenetic studies. However, the results of our morphological examination and phylogenetic analyses using SSU, ITS, LSU, *tef1*, and *rpb2* sequences suggest that this family encompasses *Lophiotrema* and five new genera.

Because *Aquasubmersa* and *Hermatomyces* were placed outside of *Lophiotremataceae* in our phylogenetic tree (Fig. 28), we treat these genera as *Aquasubmersaceae* and *Hermatomycetaceae*, respectively. One species of *Lophiotrema* (*L. lignicola*) was grouped with *Atrocalyx*, a new genus in *Lophiotremataceae*, while two species (*Lophiotrema boreale* and *L. brunneosporum*) were placed outside of *Lophiotremataceae* entirely.

Marincowitz et al. (2008) have suggested that "*Massarina albocarnis*" (CBS 119345) has a phylogenetic affinity with *Lophiotrema* based on Blast results involving ITS and LSU sequences. However, Beier et al. (2015), who observed the holotype specimen of *M. albocarnis*, has indicated that this species belongs to *Diaporthe* (*Sordariomycetes*). The isolate CBS 119345 may thus be misidentified.

Unfortunately, we were unable to examine any morphological features of CBS 119345 because it did not sporulate in culture.

**Lophiotrema** Sacc., *Michelia* 1(no. 3): 338. 1878.

*Type species: Lophiotrema nucula* (Fr.) Sacc., *Michelia* 1(no. 3): 338. 1878.

Notes — For further information on this genus, see Holm & Holm (1988), Tanaka & Harada (2003), Zhang *et al.* (2009) and Hirayama & Tanaka (2011). In the present study, we found that *Lophiotrema sensu stricto* should be limited to species having ascomata with a slit-like ostiole and an ascomatal wall of uniform thickness, asci with a short stipe, and pycnidial asexual morphs. Although *L. brunneosporum* with a monodictys-like asexual morph was sister to “*Lophiotrema*” *boreale* in our phylogenetic tree (Fig. 28), these two taxa were distinct from *Lophiotremataceae sensu stricto* (Fig. 28). Consequently, they should be treated as distant lineages in *Dothideomycetes*. We were unable to morphologically examine *L. boreale* from an isolate of this species (CBS 114422) or the original specimen used for isolation. Further examination is required to clarify the taxonomic placement of this species.

**Atrocalyx** A. Hashim. & Kaz. Tanaka, **gen. nov.** — MycoBank MB819240.

*Etymology:* From the Latin *atro-*, meaning black, and *calyx*, meaning cap.

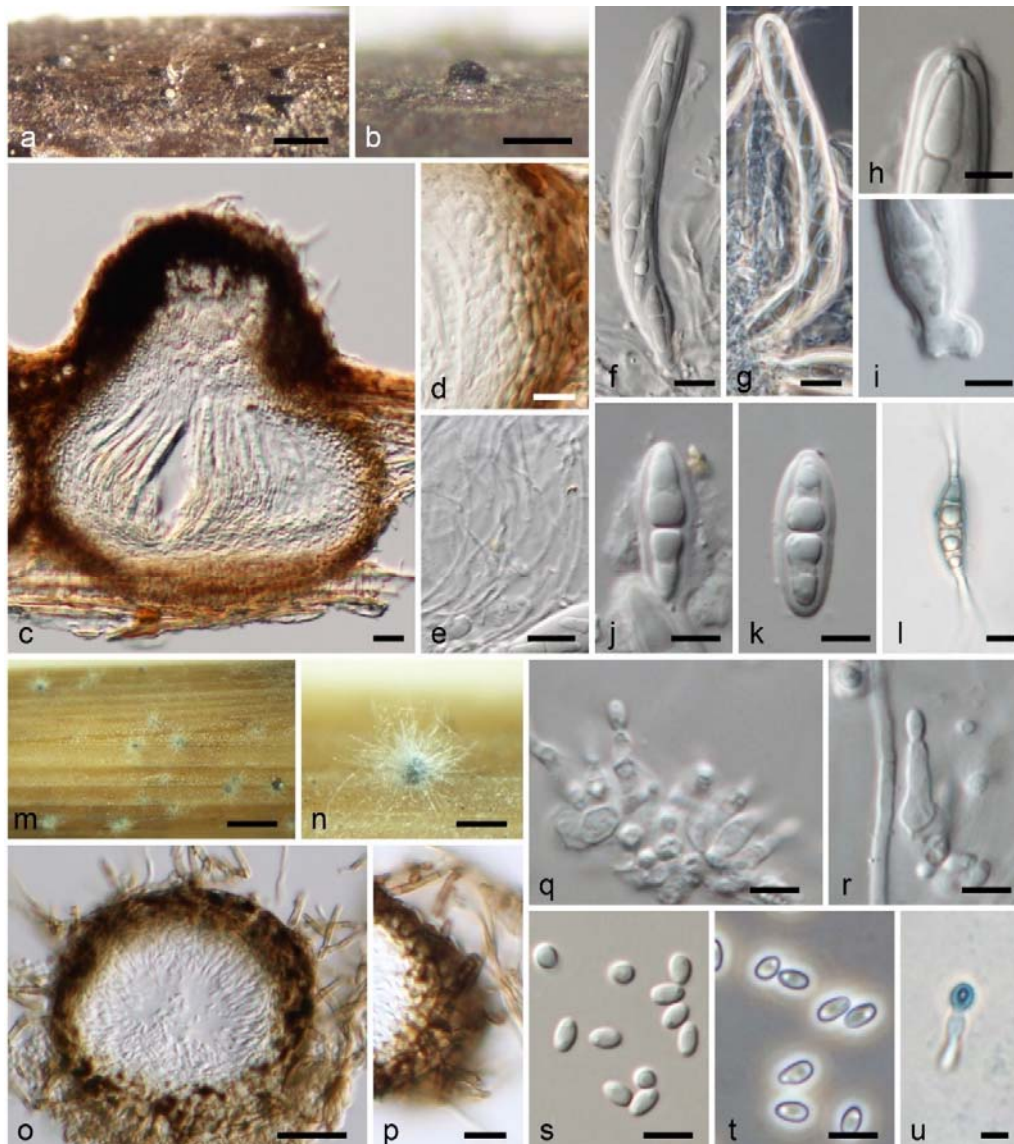
Saprobic on woody plants. **Sexual morph:** *Ascomata* solitary to grouped, semi-immersed to immersed. *Ostiolar neck* crest-like, elongate and laterally compressed, surrounded by dark brown hyphae. *Peridium* composed of 2 layers at side. *Pseudoparaphyses* septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 8-spored. *Ascospores* broadly fusiform, hyaline, 1-septate, smooth. **Asexual morph:** *Conidiomata* pycnidial, globose to subglobose, superficial, black, ostiolate. *Peridium* composed of elongate, brown cells. *Conidiophores* absent. *Conidiogenous* cells holoblastic, ampliform to cylindrical, hyaline. *Conidia* ellipsoidal, hyaline, smooth, aseptate.

*Type species: Atrocalyx acutisporus* A. Hashim. & Kaz. Tanaka.

Notes — The new genus *Atrocalyx* is established to accommodate *A. lignicola* (formerly *L. lignicola*) and a new species, *A. acutisporus*. These two species are characterised by a crest-like, elongated and laterally compressed ostiolar neck (Fig. 30b, 31b) surrounded by a well-developed peridium (up to 62.5 µm) (Fig. 30c, 31c). “*Lophiotrema*” *bambusae*, which was recently introduced as a species in *Lophiotrema* (Hyde *et al.* 2016), was nested within a moderately supported clade (67 % ML BP/1.00 Bayesian PP) along with these two species in our phylogenetic

tree (Fig. 28). Because we could not observe any material of “*Lophiotrema*” *bambusae*, the generic placement of this species is pending.

The genus is morphologically similar to *Lophiotrema*, but can be distinguished from the latter by its well-developed peridium around the ostiolar neck and base (vs. a poorly developed peridium up to 25 µm thick; Holm & Holm 1988).



**Fig. 30.** *Atrocalyx acutisporus*. a, b. Appearance of ascomata on substrate; c. Ascoma in longitudinal section; d. Peridium of ascoma; e. Pseudoparaphyses; f, g. Asci; h. Ascus apex; i. Ascus stipe; 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, t. Conidia; u. Germinating conidium; a–l from HHUF 30504 (holotype); m–u from MAFF 245613 (ex-holotype culture). — Scale bars: a = 500 µm; b = 200 µm; c, o = 20 µm; d–g, p = 10 µm; h–l, q–u = 5 µm; m = 1 mm; n = 250 µm.

***Atrocalyx acutisporus*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB819241; Fig. 30.

*Etymology:* Referring to the ascospores with acute ends.

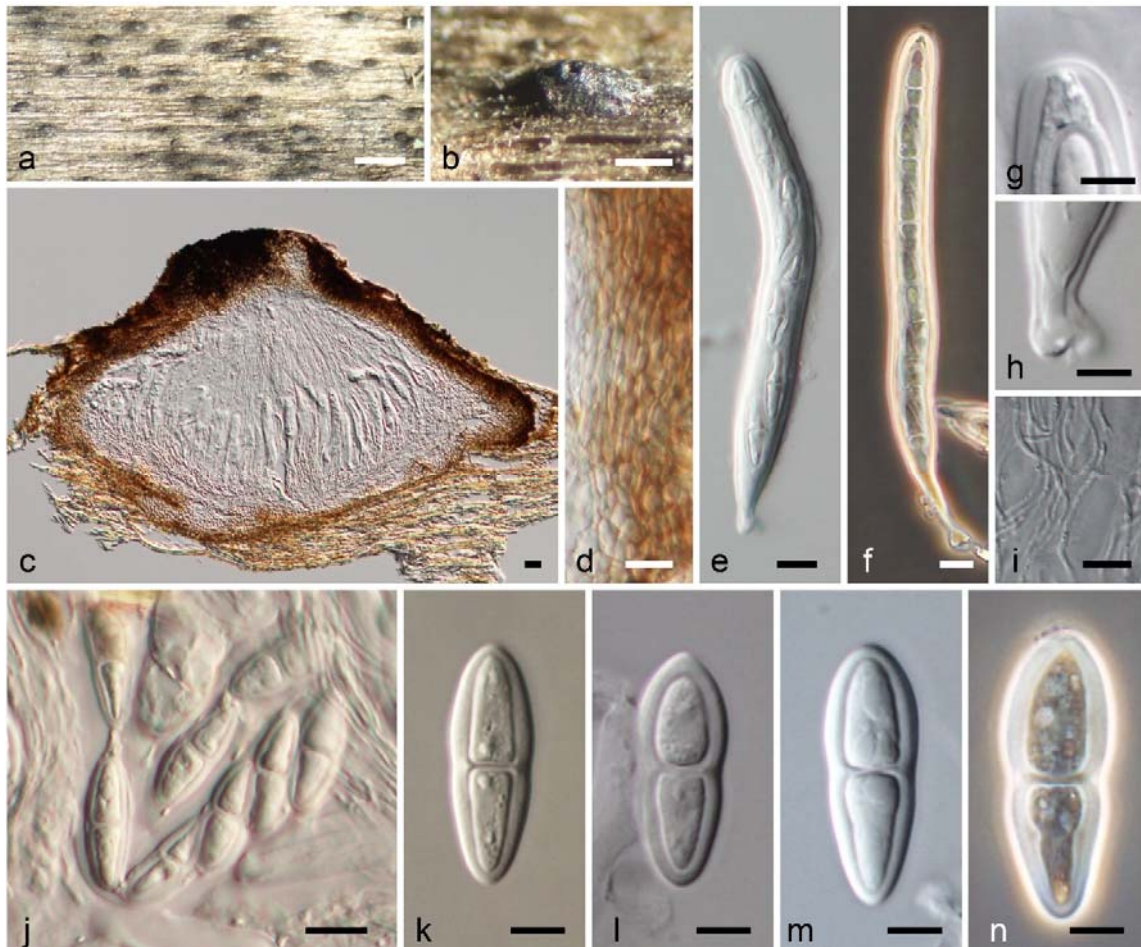
Saprobic on dead twigs of woody plant. **Sexual morph:** *Ascomata* ellipsoidal, solitary to 2–4 grouped, immersed, 110–140  $\mu\text{m}$  high, 190–210  $\mu\text{m}$  diam. *Ostiolar neck* crest-like, elongated and laterally compressed, surrounded by dark brown hyphae. *Peridium* 20–30  $\mu\text{m}$  thick at side, composed of 2 layers; outer layer 11–17  $\mu\text{m}$  thick, composed of elongated, thin-walled, 8.5–12.5  $\times$  2.5–3  $\mu\text{m}$ , brown cells; inner layer 15–17  $\mu\text{m}$  thick, composed of globose to rectangular, 7.5–9  $\times$  4–5  $\mu\text{m}$ , hyaline cells; near the ostiole 37.5–45  $\mu\text{m}$  thick, composed of globose, brown to black cells; 19–37.5  $\mu\text{m}$  thick at the base, composed of globose to rectangular, 1.8–2.5  $\mu\text{m}$  diam cells. *Pseudoparaphyses* numerous, 1–1.5  $\mu\text{m}$  wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, (66.5–)75–89.5  $\times$  8–11  $\mu\text{m}$  ( $\bar{x}$  = 80.8  $\times$  9.2  $\mu\text{m}$ , n = 10), with a short stipe (4–7.5  $\mu\text{m}$  long,  $\bar{x}$  = 5.5  $\mu\text{m}$ , n = 10), apically rounded with an ocular chamber, 8-spored. *Ascospores* broadly fusiform with acute ends, 13.5–18(–20)  $\times$  3–4(–5.5)  $\mu\text{m}$  ( $\bar{x}$  = 15.9  $\times$  3.7  $\mu\text{m}$ , n = 50), l/w 3.7–5.1 ( $\bar{x}$  = 4.4, n = 50), hyaline, with a septum nearly median (0.44–0.59,  $\bar{x}$  = 0.51, n = 50), slightly constricted at the septum, smooth, with an entire gelatinous sheath up to 2  $\mu\text{m}$  thick. **Asexual morph:** *Conidiomata* pycnidial, globose to subglobose, up to 145  $\mu\text{m}$  high, 60–130  $\mu\text{m}$  diam, 2–3 grouped, superficial, black, with a papillate ostiolar neck. *Peridium* 7.5–10  $\mu\text{m}$  thick, composed of 3–4 layers of 7.5–13  $\times$  1.5–3  $\mu\text{m}$ , elongated, brown cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, 7.5–12  $\times$  1.5–3  $\mu\text{m}$ , ampliform to cylindrical, hyaline, smooth. *Conidia* ellipsoidal, 3–4  $\times$  1.9–2  $\mu\text{m}$  ( $\bar{x}$  = 3.5  $\times$  2.0  $\mu\text{m}$ , n = 50), l/w 1.5–2.1 ( $\bar{x}$  = 1.8, n = 50), hyaline, smooth, aseptate, guttulate when young.

Culture characteristics — Colonies on PDA attaining 24–25 mm diam within 21 d at 20 °C in the dark, floccose, radiately, smoke grey (105); reverse olivaceous grey to olivaceous black (108) (Fig. 39b); asexual morph formed.

*Specimen examined.* **JAPAN**, Okinawa, Isl. Iriomote, Sono trail, on dead twigs of woody plant, 29 Sept. 2007, K. Tanaka & H. Yonezawa, KT 2436 (HHUF 30504 **holotype**, ex-holotype living culture MAFF 245613 = NBRC 112316)

Notes — *Atrocalyx acutisporus* superficially resembles *A. lignicola*, but can be distinguished from the latter by its smaller ascospores (13.5–18(–20)  $\times$  3–4(–5.5)  $\mu\text{m}$  vs. 20–26  $\times$  4–5.5(–6)  $\mu\text{m}$ , respectively). ITS sequence differences between these two species were found at 16 of 529 nucleotide positions, with two gaps.





**Fig. 31.** *Atrocalyx lignicola*. a, b. Appearance of ascomata on substrate; c. Ascoma in longitudinal section; d. Peridium of ascoma; e, f. Asci; g. Ascus apex; h. Ascus stipe; i. Pseudoparaphyses; j–n. Ascospores; a–n from CBS H-20221 (holotype). — Scale bars: a = 1 mm; b = 250  $\mu$ m; c = 20  $\mu$ m; d–f, i = 10  $\mu$ m; g, h, j–n = 5  $\mu$ m.

***Atrocalyx lignicola*** (Ying Zhang, J. Fourn. & K.D. Hyde) A. Hashim. & Kaz. Tanaka, **comb. nov.** — MycoBank MB819242; Fig. 31.

**Basionym:** *Lophiotrema lignicola* Ying Zhang, J. Fourn. & K.D. Hyde, *Fungal Divers.* 38: 238. 2009.

Saprobic on dead twigs of *Populus* sp. **Sexual morph:** *Ascomata* ellipsoidal, 330–380  $\mu$ m high, 350–600  $\mu$ m long, 230–400  $\mu$ m wide, solitary to 4–5 grouped, semi-immersed. *Ostiolar neck* crest-like, elongated and laterally compressed, surrounded by dark brown hyphae. *Peridium* 27.5–45  $\mu$ m thick at side, composed of 2 layers; outer layer 15–20  $\mu$ m thick, composed of elongated, thin-walled, 10–12.5  $\times$  3.5–5  $\mu$ m, brown cells; inner layer 15–22  $\mu$ m thick, composed of globose to rectangular, 5–6.5  $\mu$ m diam, hyaline cells; near the ostiole 57–62.5  $\mu$ m thick, composed of dark



brown cells; at base 35–46  $\mu\text{m}$  thick, composed of globose to rectangular, 2.5–4(–6)  $\mu\text{m}$  diam, brown to black cells. *Pseudoparaphyses* numerous, 1–1.5  $\mu\text{m}$  wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 100–146  $\times$  12.5–17  $\mu\text{m}$  ( $\bar{x}$  = 125.2  $\times$  13.4  $\mu\text{m}$ , n = 11), with a short stipe (4–18  $\mu\text{m}$  long,  $\bar{x}$  = 8.4  $\mu\text{m}$ , n = 11), apically rounded with an ocular chamber, 8-spored. *Ascospores* broadly fusiform with rounded ends, 20–26  $\times$  6.5–9.5  $\mu\text{m}$  ( $\bar{x}$  = 22.3  $\times$  7.4  $\mu\text{m}$ , n = 50), hyaline, with a septum nearly median (0.45–0.54,  $\bar{x}$  = 0.50, n = 50), slightly constricted at the septum, smooth, with an entire gelatinous sheath up to 2  $\mu\text{m}$  thick. Senescent ascospores 3-septate, yellowish. **Asexual morph:** Undetermined.

Culture characteristics — Colonies on PDA attaining 23–26 mm diam within 21 d at 20 °C in the dark, velvety, plane, smoke grey (105); reverse olivaceous black (108) (Fig. 39c); asexual morph formed.

*Specimen examined.* BELGIUM, Hainaut, Orval, ruisseau de Williers, on decorticated trunk of *Populus* sp., 29 Sept. 2006, J. Fournier (CBS H-20221 **holotype**, ex-holotype living culture CBS 122364)

Notes — *Atrocalyx lignicola* was first described as a species of *Lophiotrema* on the basis of its 1-septate, hyaline, strongly constricted ascospores (Zhang et al. 2009). According to our morphological observations of the holotype specimen, however, this species is not typical of the genus *Lophiotrema*; in particular, *A. lignicola* possesses ascomata with well-developed peridium (Fig. 31c).

***Crassimassarina*** A. Hashim. & Kaz. Tanaka, **gen. nov.** — MycoBank MB819243

*Etymology:* After its morphological similarity to *Massarina*, but with well-developed ascomatal wall.

Saprobic on dead twigs of woody plants. **Sexual morph:** *Ascomata* solitary to grouped, immersed to erumpent, subglobose. *Ostiolar neck* papillate, without slit-like ostiole, composed of carbonaceous, thick-walled, black cells. *Peridium* composed of rectangular, thin-walled cells. *Pseudoparaphyses* trabeculate, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 8-spored. *Ascospores* broadly fusiform with rounded ends, straight, 1-septate, hyaline, smooth. **Asexual morph:** *Conidiomata* pycnidial, globose to subglobose, scattered, semi-immersed, solitary, black, ostiolate. *Peridium* composed of subglobose to rectangular, brown cells. *Conidiophores* absent. *Conidiogenous cells* holoblastic, cylindrical, hyaline. *Conidia* cylindrical with rounded ends, hyaline, smooth, multi-septate.

*Type species:* *Crassimassarina macrospora* A. Hashim. & Kaz. Tanaka.

***Crassimassarina macrospora*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB819244; Fig. 32.

**Etymology:** Referring to the large ascospores.

Saprobic on dead twigs of *Cornus controversa*. **Sexual morph:** *Ascomata* solitary to 4–5 grouped, immersed, erumpent at the apex, subglobose in section, 450–620  $\mu\text{m}$  high, 380–700  $\mu\text{m}$  diam. *Ostiolar neck* papillate, without slit-like ostiole, composed of carbonaceous, thick-walled, black cells. *Peridium* 25–60  $\mu\text{m}$  thick, composed of 6–8 layers of rectangular, thin-walled, 5–12.5  $\mu\text{m}$  diam cells, surrounded by brown hyphae (2–3  $\mu\text{m}$  thick). *Pseudoparaphyses* numerous, trabeculate, 1–1.5  $\mu\text{m}$  wide, septate, branched and anastomosed. *Asci* numerous, bitunicate, fissitunicate, cylindrical, 165–200  $\times$  25–33  $\mu\text{m}$  ( $\bar{x}$  = 183.5  $\times$  27.9  $\mu\text{m}$ , n = 7), with a short stipe (7.5–26  $\mu\text{m}$  long,  $\bar{x}$  = 16.8  $\mu\text{m}$ , n = 7), apically rounded with an ocular chamber, 8-spored. *Ascospores* broadly fusiform with rounded ends, straight, (29.5–)33–42  $\times$  9–15  $\mu\text{m}$  ( $\bar{x}$  = 37.8  $\times$  13.0  $\mu\text{m}$ , n = 55), l/w 2.1–4.0 ( $\bar{x}$  = 2.9, n = 55), 1(–3)-septate, with a submedian primary septum (0.51–0.58(–0.71),  $\bar{x}$  = 0.53, n = 55), strongly constricted at the septum and midpoints of each cell, hyaline, smooth, guttulate when young, with an entire gelatinous sheath (1–3  $\mu\text{m}$  wide at sides). **Asexual morph:** *Conidiomata* pycnidial, globose to subglobose, up to 240  $\mu\text{m}$  high, 190–250  $\mu\text{m}$  diam, scattered, semi-immersed, solitary, black, with a papillate ostiolar neck. *Peridium* 15–20  $\mu\text{m}$  thick, composed of 4–6 layers of 4–7  $\mu\text{m}$  diam, subglobose to rectangular, brown cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, 6–14  $\times$  3–5  $\mu\text{m}$ , cylindrical, hyaline, smooth. *Conidia* cylindrical to with rounded ends, (32–)36–55(–58)  $\times$  6–8  $\mu\text{m}$  ( $\bar{x}$  = 44.1  $\times$  7.2  $\mu\text{m}$ , n = 50), l/w 4.7–8.1 ( $\bar{x}$  = 6.2, n = 50), hyaline, smooth, 3(–7)-septate, guttulate when young.

**Culture characteristics** — Colonies on PDA attaining 36–40 mm diam within 21 d, velvety, plane, smoke grey (105), grey olivaceous (107) at margin; reverse olivaceous black (108) (Fig. 39d, e); asexual morph formed.

**Specimen examined.** **JAPAN**, Ibaraki, Tsukuba, Amakubo, Tsukuba botanical garden, on dead twigs of *Cornus controversa*, 20 Nov. 2004, Y. Oki, KT 1764 (HHUF 29084 **holotype**, ex-holotype living culture JCM 13096 = MAFF 239606); *ibid.*, KT 1765 (HHUF 29085 **paratype**); Aomori, Minamitsugaru, Owani, on dead twigs of woody plant, 28 Jun. 2008, K. Hirayama & K. Tanaka, KH 152 (HHUF 30512 **paratype**, ex-paratype living culture MAFF 245617).

**Fig. 32.** *Crassimassarina macrospora*. a, b. Appearance of ascomata on substrate; c. Ascoma in longitudinal section; d. Peridium of ascoma; e. Pseudoparaphyses; f, g. Asci; h. Ascus apex; i. Ascus stipe; j–m. Ascospores; n. Ascospore with a gelatinous sheath (in india ink); o, p. Conidiomata in culture; q. Conidioma in longitudinal section; r. Peridium of conidioma; s. Conidiogenous cells; t–v. Conidia (u in trypan blue); a, c–f, j, k, n from HHUF 29084 (holotype); b, g–i, l, m from HHUF 30512 (paratype); o–s, u, v from JCM 13096 = MAFF 239606 (ex-holotype culture); t from MAFF 245617 (ex-paratype culture). — Scale bars: a, o = 1 mm; b, p = 250  $\mu\text{m}$ ; c, q = 20  $\mu\text{m}$ ; d–g, m, n, r = 10  $\mu\text{m}$ ; h–l, s–v = 5  $\mu\text{m}$ .



Notes — This genus is morphologically similar to *Massarina* and genera in *Pleomassariaceae sensu lato* (Barr 1982, Tanaka et al. 2005, 2015) in having large, immersed ascomata with a short papillate ostiolar neck and relatively large ascospores. However, *Crassimassarina* is different from *Massarina* in having a well-developed ascomatal wall; it differs from pleomassariaceous genera in having trabeculate pseudoparaphyses and hyaline ascospores. The asexual morph of *Crassimassarina* resembles that of *Stagonospora* in regards to its pycnidial conidiomata, conidiophores with reduced conidiogenous cells, and multi-septate cylindrical conidia; however, the latter genus differs from *Crassimassarina* in having phialidic conidiogenous cells (Quaedvlieg et al. 2013, Tanaka et al. 2015). *Massarina* and *Stagonospora* (*Pleosporales*, *Massarinaceae*) are phylogenetically distinct lineages from *Lophiotremataceae* containing *Crassimassarina*.

*Crassimassarina* can be distinguished from other genera in *Lophiotremataceae* by its ascomata lacking a slit-like ostiole, an ascomatal peridium composed of carbonaceous cells (Fig. 32c), and multi-septate, large conidia (Fig. 32t–v).

ITS sequences of the two examined isolates of *C. macrospora* differed at only two positions, with two gaps. Morphological features and culture characteristics of these isolates were completely identical (Fig. 32k, l, 39d, e).

***Cryptoclypeus*** A. Hashim. & Kaz. Tanaka, **gen. nov.** — MycoBank MB819245.

*Etymology*: Referring to the ascomata covered by a less-developed clypeus.

Saprobic on dead twigs of bamboo. **Sexual morph**: *Ascomata* solitary to grouped, immersed. *Ostiolar neck* crest-like, elongated, laterally compressed, with a slit-like ostiole and less-developed clypeus. *Peridium* composed of rectangular, thin-walled, pale brown cells. *Pseudoparaphyses* septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 8-spored. *Ascospores* fusiform, multi-septate, hyaline, smooth. **Asexual morph**: *Conidiomata* pycnidial, globose to subglobose, grouped, immersed, ostiolate. *Peridium* composed of subglobose to rectangular, brown cells. *Conidiophores* absent. *Conidiogenous cells* phialidic, ampliform to cylindrical. *Conidia* cylindrical with angular ends, hyaline, smooth, 1-septate.

*Type species*: *Cryptoclypeus ryukyuensis* A. Hashim. & Kaz. Tanaka.

Notes — Two species of *Cryptoclypeus* share common characteristics, e.g., ascomata with a less-developed clypeus (up to 450 µm wide), and a peridium composed of rectangular cells (Fig. 33c, d and Fig. 34c, d). In the phylogenetic tree, they formed a well-supported clade in *Lophiotremataceae* (99 % ML BP/1.00 Bayesian PP) (Fig. 28).

The sexual morph of *Cryptoclypeus* is similar to that of *Tetraploa* (*Tetraplosphaeriaceae*), which also has ascomata with a clypeus and narrowly fusiform ascospores (Tanaka et al. 2009). *Cryptoclypeus*, however, can be distinguished from the latter genus by having a well-developed neck with a slit-like ostiole. The asexual morph of *Cryptoclypeus* superficially resembles that of *Bambusicola* (*Bambusicolaceae*), but is easily distinguishable from the latter by having phialidic conidiogenous cells rather than annellidic ones (Dai et al. 2012).

***Cryptoclypeus oxysporus*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB819246; Fig. 33.

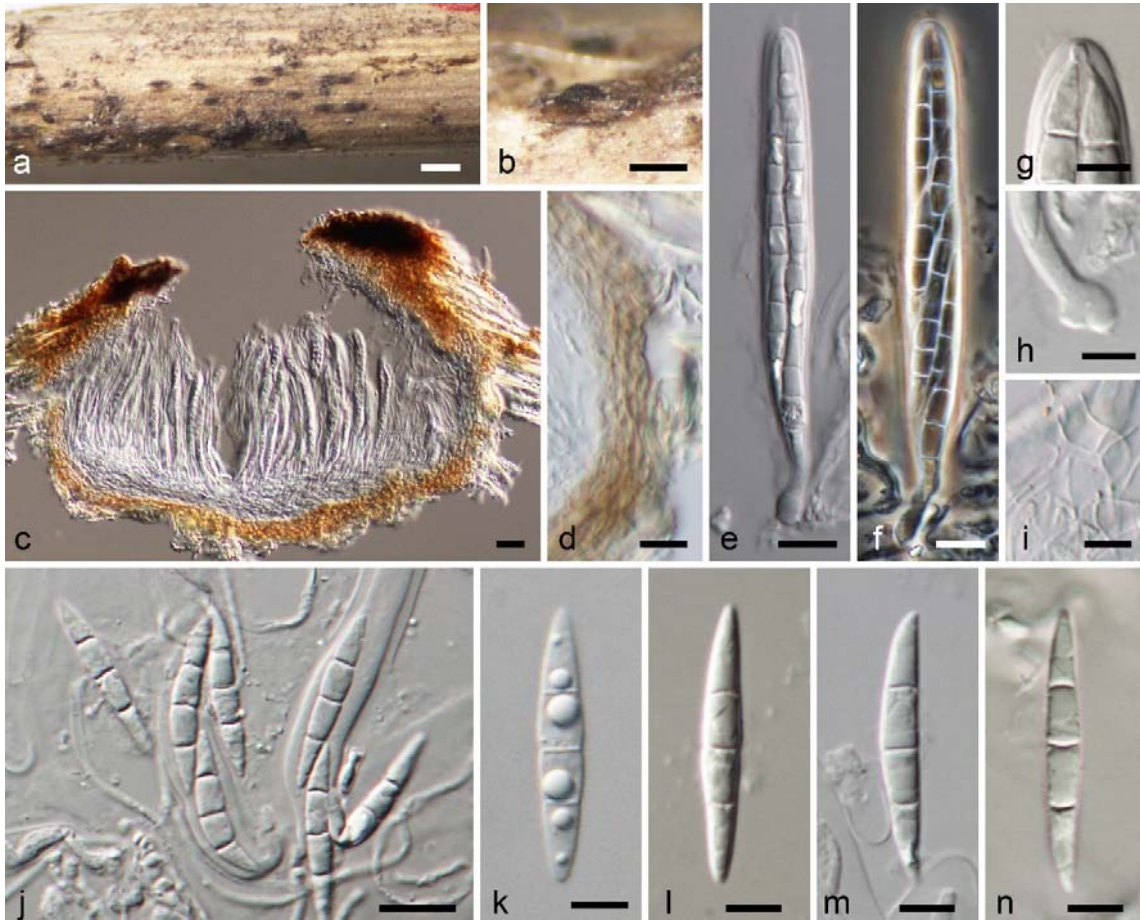
*Etymology*: Referring to the sharp ascospores.

Saprobic on dead culm of *Sasa* sp. **Sexual morph**: *Ascomata* ellipsoidal, solitary, immersed, 245–235  $\mu\text{m}$  high, 180–240  $\mu\text{m}$  diam. *Ostiolar neck* crest-like, elongated, laterally compressed, with less-developed clypeus (230–320  $\mu\text{m}$  wide). *Peridium* uniform, 15–17.5  $\mu\text{m}$  thick, composed of rectangular, thin-walled, 6.5–14  $\times$  2.5–4.5  $\mu\text{m}$ , pale brown cells. *Pseudoparaphyses* numerous, 0.5–1  $\mu\text{m}$  wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 71–100  $\times$  6–9  $\mu\text{m}$  ( $\bar{x}$  = 85.8  $\times$  7.7  $\mu\text{m}$ , n = 10), with a short stipe (5.5–11  $\mu\text{m}$  long,  $\bar{x}$  = 7.8  $\mu\text{m}$ , n = 10), apically rounded with an ocular chamber, 8-spored. *Ascospores* fusiform with acute ends, straight, 19–27  $\times$  3–4.5  $\mu\text{m}$  ( $\bar{x}$  = 23.3  $\times$  3.2  $\mu\text{m}$ , n = 50), l/w 5.5–8.9 ( $\bar{x}$  = 7.2, n = 50), 3-septate, slightly constricted at the primary septum nearly median (0.46–0.55,  $\bar{x}$  = 0.50, n = 50), hyaline, smooth. **Asexual morph**: Undetermined.

**Culture characteristics** — Colonies on PDA attaining 24–28 mm diam within 21 d at 20 °C in the dark, velvety, radiately, centrally raised, smoke grey (105) to grey olivaceous (107); reverse olivaceous black (108) (Fig. 39f); no sporulation observed.

*Specimen examined*. **JAPAN**, Iwate, Hanamaki, near Dai spa, on dead culm of *Sasa* sp., 25 Jun. 2011, K. Tanaka, KT 2772 (HHUF 30507 **holotype**, ex-holotype living culture MAFF 245614 = NBRC 112317).





**Fig. 33.** *Cryptoclypeus oxysporus*. a, b. Appearance of ascomata on substrate; c. Ascoma in longitudinal section; d. Peridium of ascoma; e, f. Asci; g. Ascus apex; h. Ascus stipe; i. Pseudoparaphyses; j–n. Ascospores; a–n from HHUF 30507 (holotype). — Scale bars: a = 1 mm; b = 250  $\mu$ m; c = 20  $\mu$ m; d–f, i = 10  $\mu$ m; g, h, j–n = 5  $\mu$ m.

**Notes** — This species can be distinguished from *C. ryukyuensis* by its slightly larger ascospores with acute ends (Fig. 33k–n) (19–27  $\times$  3–4.5  $\mu$ m vs. 15–24  $\times$  3–4.5  $\mu$ m, with rounded ends in the latter species; Fig. 34k–m). ITS sequences between these two species differed at 17–18 positions, with eight gaps.

***Cryptoclypeus ryukyuensis*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB819247; Fig. 34.

**Etymology:** Referring to the collection site.

Saprobic on dead twigs of *Pleioblastus linearis*. **Sexual morph:** Ascomata ellipsoidal, solitary to 3–5 grouped, immersed, 220–240  $\mu$ m high, 330–380  $\mu$ m diam. *Ostiolar neck* crest-like, elongated, laterally compressed, with less-developed black clypeus

(380–450  $\mu\text{m}$  wide). *Peridium* uniform, 22.5–25  $\mu\text{m}$  thick at side, composed of rectangular, thin-walled, 10–22.5  $\times$  5–6.5  $\mu\text{m}$ , pale brown cells. *Pseudoparaphyses* numerous, 1–1.5  $\mu\text{m}$  wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 72.5–100.5  $\times$  6–10  $\mu\text{m}$  ( $\bar{x}$  = 87.0  $\times$  7.8  $\mu\text{m}$ , n = 20), with a short stipe (4–9  $\mu\text{m}$  long,  $\bar{x}$  = 6.5  $\mu\text{m}$ , n = 20), apically rounded with an ocular chamber, 8-spored. *Ascospores* fusiform with rounded ends, straight, 15–24  $\times$  3–4.5  $\mu\text{m}$  ( $\bar{x}$  = 19.7  $\times$  3.8  $\mu\text{m}$ , n = 50), l/w (3.8–)4.3–6.6 ( $\bar{x}$  = 5.2, n = 50), 3-septate, slightly constricted at the primary septum nearly median (0.45–0.53,  $\bar{x}$  = 0.50, n = 50), hyaline, smooth, guttulate when young. **Asexual morph:** *Conidiomata* pycnidial, globose to subglobose, up to 230  $\mu\text{m}$  high, 230–340  $\mu\text{m}$  diam, 3–5 grouped, immersed. *Ostiolar neck* up to 50  $\mu\text{m}$  high, carbonaceous, papillate. *Peridium* 17.5–27.5  $\mu\text{m}$  thick, composed of 7–10 layers of 4–8  $\times$  2.5–6.5  $\mu\text{m}$ , subglobose to rectangular, brown cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, 7–12  $\times$  2–3  $\mu\text{m}$ , ampliform to cylindrical, hyaline, smooth. *Conidia* cylindrical with rounded ends, 11.5–15  $\times$  2–2.5  $\mu\text{m}$  ( $\bar{x}$  = 12.9  $\times$  2.1  $\mu\text{m}$ , n = 50), l/w 5.1–7.0(–8.3) ( $\bar{x}$  = 6.3, n = 50), hyaline, smooth, 1-septate, guttulate when young.

Culture characteristics — Colonies on PDA attaining 28–30 mm diam within 21 d at 20 °C in the dark, velvety, radiately, centrally raised, smoke grey (105) to grey olivaceous (107); reverse greenish black (124) (Fig. 39g, h); no sporulation observed.

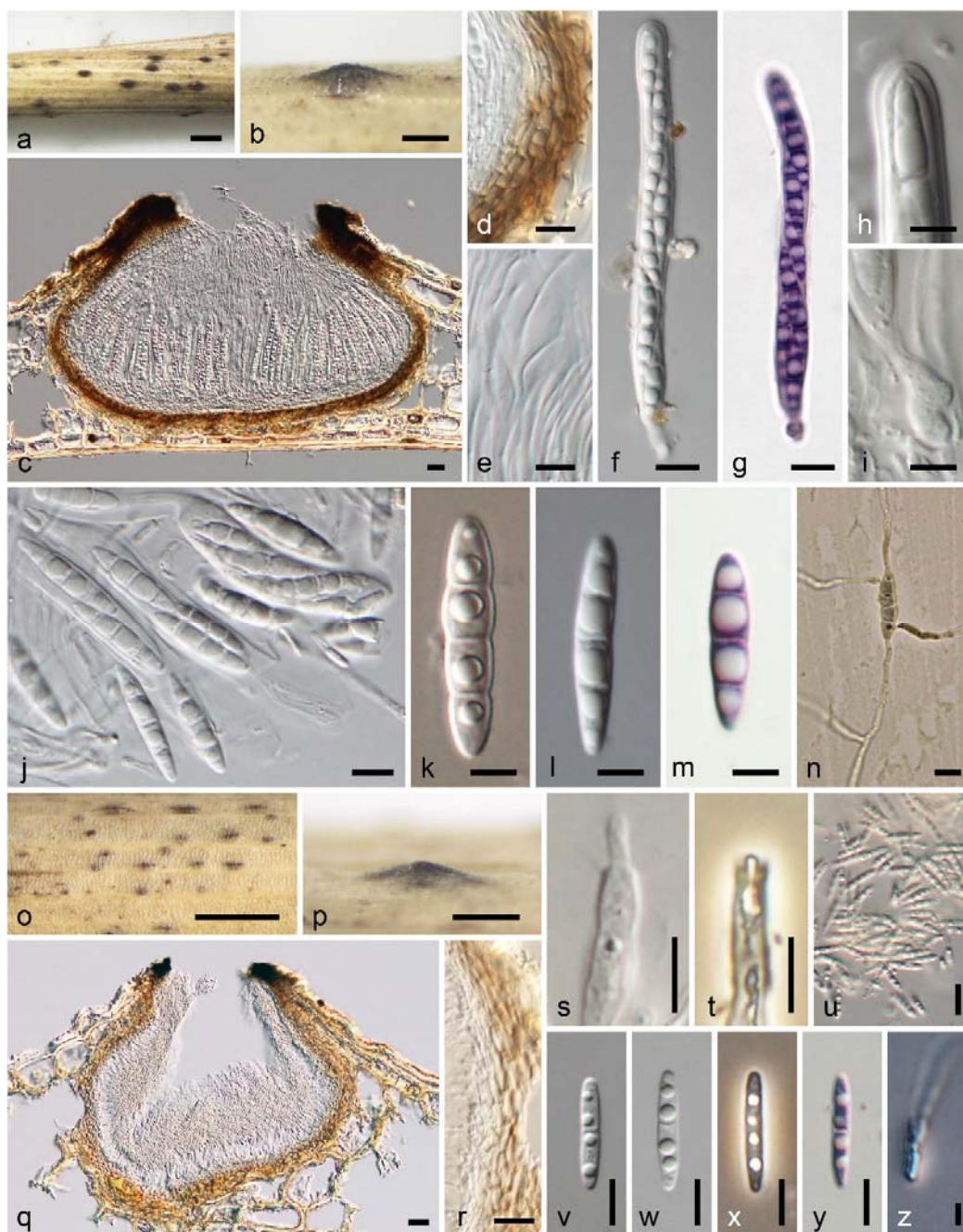
*Specimen examined.* JAPAN, Okinawa, Kunigami-son, Okuma, Mt. Yonaha, on dead culm of *Pleioblastus linearis*, 18 May 2015, A. Hashimoto et al., AH 342 (HHUF 30510 **paratype**, ex-paratype living culture MAFF 245616); Yona, Mt. Fuenchiji, on dead culm of *Pleioblastus linearis*, 19 May 2015, K. Tanaka et al., KT 3534 (HHUF 30509 **holotype**, ex-holotype living culture MAFF 245615 = NBRC 112318).

Notes — Ex-holotype and ex-paratype isolates of *C. ryukyuensis* are derived from sexual and asexual morphs, respectively. Unfortunately, a connection between sexual and asexual forms of this species could not be confirmed in culture. Nonetheless, *rpb2* sequences were completely identical between the two strains, while ITS sequences differed at only one position and *tef1* sequences differed at two, neither of which caused an amino acid substitution. Culture characteristics were also identical (Fig. 39f, g). We therefore regard these isolates as conspecific.

***Galeaticarpa*** A. Hashim. & Kaz. Tanaka, **gen. nov.** — MycoBank MB819248.

*Etymology:* From the Latin *galea*, meaning helmet, and *carpa*, meaning ascomata.





**Fig. 34.** *Cryptoclypeus ryukyuensis*. a, b. Appearance of ascomata on substrate; c. Ascoma in longitudinal section; d. Peridium of ascoma; e. Pseudoparaphyses; f, g. Asci (g in trypan blue); h. Ascus apex; i. Ascus stipe; j–m. Ascospores (m in trypan blue); n. Germinating ascospore; o, p. Conidiomata on substrate; q. Conidioma in longitudinal section; r. Peridium of conidioma; s, t. Conidiogenous cells; u–y. Conidia (y in trypan blue); z. Germinating conidium; a–n from HHUF 30509 (holotype); o–z from HHUF 30510 (paratype). — Scale bars: a, o = 1 mm; b, p = 250  $\mu$ m; c, q = 20  $\mu$ m; d–g, j, n, r, u = 10  $\mu$ m; h, i, k–m, s, t, v–z = 5  $\mu$ m.

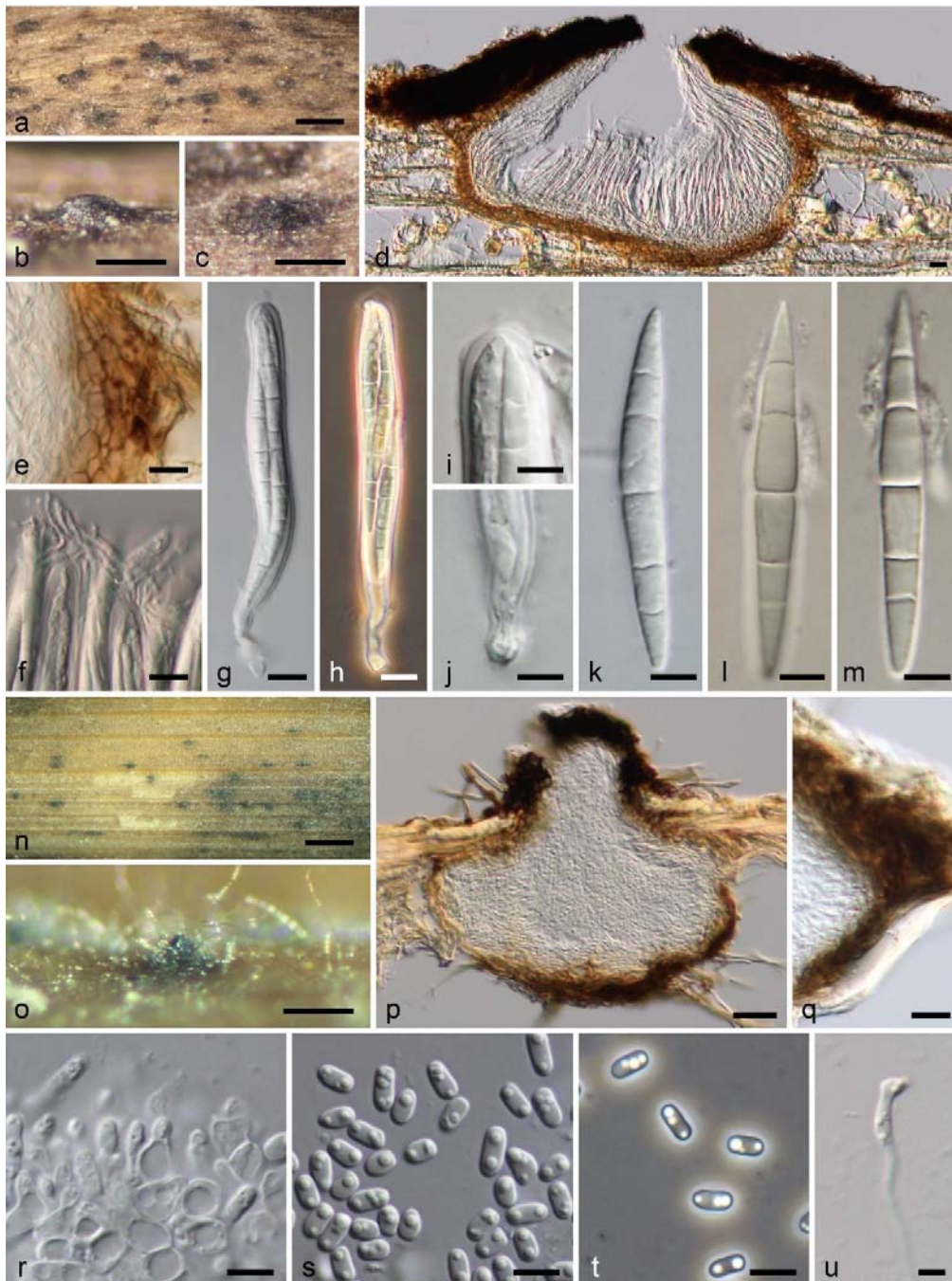
Saprobic on woody plants. **Sexual morph:** *Ascomata* solitary to grouped, immersed to erumpent, subglobose. *Ostiolar neck* elongate, laterally compressed, surrounded by well-developed clypeus. *Peridium* composed of rectangular, brown cells. *Pseudoparaphyses* septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 8-spored. *Ascospores* fusiform, multi-septate, hyaline, smooth. **Asexual morph:** *Conidiomata* pycnidial, globose to subglobose, grouped, immersed. *Ostiolar neck* carbonaceous, papillate. *Peridium* composed of rectangular, pale brown to brown cells. *Conidiophores* absent. *Conidiogenous cells* holoblastic, cylindrical, hyaline, smooth. *Conidia* ellipsoidal with rounded ends, hyaline, smooth, aseptate.

*Type species:* *Galeaticarpa aomoriensis* A. Hashim. & Kaz. Tanaka.

***Galeaticarpa aomoriensis*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB819249; Fig. 35.

*Etymology:* Referring to the collection site.

Saprobic on dead twigs of woody plant. **Sexual morph:** *Ascomata* ellipsoidal, solitary to 4–5 grouped, immersed to erumpent, subglobose, 265–285  $\mu\text{m}$  high, 370–400  $\mu\text{m}$  diam. *Ostiolar neck* crest-like, elongated, laterally compressed, surrounded by well-developed clypeus (620–750  $\mu\text{m}$  wide). *Peridium* uniform, 15–26  $\mu\text{m}$  thick, composed of 3–5 layers of rectangular, thin-walled,  $7.5 \times 3\text{--}6.5$   $\mu\text{m}$ , brown cells. *Pseudoparaphyses* numerous, 1–1.5  $\mu\text{m}$  wide, septate, branched and anastomosed. *Asci* numerous, bitunicate, fissitunicate, cylindrical,  $78\text{--}102 \times 8.5\text{--}11$   $\mu\text{m}$  ( $\bar{x} = 93.5 \times 9.7$   $\mu\text{m}$ ,  $n = 11$ ), with a short stipe (4–11  $\mu\text{m}$  long,  $\bar{x} = 7.2$   $\mu\text{m}$ ,  $n = 11$ ), apically rounded with an ocular chamber, 8-spored. *Ascospores* fusiform with acute ends, straight,  $31\text{--}47 \times 4\text{--}5.5$   $\mu\text{m}$  ( $\bar{x} = 40.6 \times 4.7$   $\mu\text{m}$ ,  $n = 50$ ), l/w (5.8–)7.2–10.5 ( $\bar{x} = 8.7$ ,  $n = 50$ ), 5-septate, with a primary septum nearly median (0.46–0.57,  $\bar{x} = 0.51$ ,  $n = 50$ ), slightly constricted at the primary septum, hyaline, smooth. **Asexual morph:** *Conidiomata* pycnidial, flask-shaped, up to 135  $\mu\text{m}$  high in section, 210–250(–420)  $\mu\text{m}$  diam, 3–5 grouped, semi-immersed. *Ostiolar neck* 32–50  $\mu\text{m}$  high, carbonaceous, papillate. *Peridium* 10–12.5  $\mu\text{m}$  wide, composed of 2–3 layers of  $10\text{--}12 \times 3\text{--}4$   $\mu\text{m}$ , rectangular, pale brown to brown cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic,  $5.5\text{--}11 \times 2.5\text{--}3$   $\mu\text{m}$ , cylindrical, hyaline, smooth. *Conidia* ellipsoidal with rounded ends,  $3.5\text{--}6 \times 1.8\text{--}2.2$   $\mu\text{m}$  ( $\bar{x} = 4.5 \times 2.0$   $\mu\text{m}$ ,  $n = 50$ ), l/w 1.8–3.0 ( $\bar{x} = 2.2$ ,  $n = 50$ ), hyaline, smooth, aseptate, guttulate when young.



**Fig. 35.** *Galeaticarpa aomoriensis*. a–c. Appearance of ascomata on substrate; d. Ascoma in longitudinal section; e. Peridium of ascoma; f. Pseudoparaphyses; g, h. Asci; i. Ascus apex; j. Ascus stipe; k–m. Ascospores; n, o. Conidiomata in culture; p. Conidioma in longitudinal section; q. Peridium of conidioma; r. Conidiogenous cells; s, t. Conidia; u. Germinating conidium; a–m from HHUF 30505 (holotype); n–u from MAFF 245618 = NBRC 112319 (ex-holotype culture). — Scale bars: a, n = 1 mm; b, c, o = 250  $\mu$ m; d, p = 20  $\mu$ m; e–h, q = 10  $\mu$ m; i–m, r–u = 5  $\mu$ m.

Culture characteristics — Colonies on PDA attaining 17–19 mm diam within 21 d at 20 °C in the dark, velvety, plane, smoke grey (105); reverse chestnut (40), brown vinaceous (84) pigment produced (Fig. 39i); asexual morph formed.

*Specimen examined.* **JAPAN**, Aomori, Nishimeya, Shirakami, Oshirosawa stream, on dead twigs of dead woody plant, 30 Aug. 2008, K. Tanaka et al., KT 2563 (HHUF 30505 **holotype**, ex-holotype living culture MAFF 245618 = NBRC 112319).

*Notes* — A new monotypic genus, *Galeaticarpa*, is proposed here for species having ascomata with a clypeus, cylindrical asci with a short stipe, and fusiform, multi-septate, hyaline ascospores. These morphological characters are similar to those of *Astrosphaeriella*, but *Galeaticarpa* can be distinguished from *Astrosphaeriella* by its crest-like ostiolar neck and uniformly developed peridium (vs. a poorly developed peridium at the base in *Astrosphaeriella*; Chen & Hsieh 2004, Phookamsak et al. 2015). These two genera are distantly related and belong to the families *Lophotremataceae* and *Astrosphaeriellaceae*, respectively.

*Cryptoclypeus*, *Galeaticarpa*, and *Pseudocryptoclypeus* are morphologically similar to one other in having ascomata with a clypeus, but *Galeaticarpa* can be distinguished from these other genera by its most striking features—a well-developed clypeus (up to 750 µm wide) (Fig. 35a–d) and flask-shaped conidiomata (Fig. 35p, q). In addition to phenotypic differences, *Cryptoclypeus* and *Pseudocryptoclypeus* tend to occur exclusively on bamboos, while *Galeaticarpa* occurs on woody plants. Several bambusicolous fungi have been reported to be phylogenetically unrelated to genera and species on other host plants, even though they have morphological similarities with those groups (Tanaka et al. 2009, Hashimoto et al. 2015b). Further discovery of new lineages related to these genera is needed to clarify the relationship between their evolution and host preference.

***Pseudocryptoclypeus*** A. Hashim. & Kaz. Tanaka, **gen. nov.** — MycoBank MB819250.

*Etymology:* After its morphological similarity to *Cryptoclypeus*.

Saprobic on bamboo. **Sexual morph:** *Ascomata* scattered to grouped, immersed. *Ostiolar neck* crest-like, elongate, laterally compressed, with less-developed clypeus. *Peridium* composed of compressed to rectangular, thin-walled, brown cells. *Pseudoparaphyses* septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 8-spored. *Ascospores* fusiform with acute ends, multi-septate, hyaline, smooth. **Asexual morph:** *Conidiomata* pycnidial, globose to subglobose, grouped, immersed, ostiolate. *Peridium* composed of rectangular, pale brown cells. *Conidiophores* absent. *Conidiogenous cells* holoblastic, ampliform to



cylindrical, hyaline. *Conidia* cylindrical with rounded ends, hyaline, smooth, 1-septate.

*Type species: Pseudocryptoclypeus yakushimensis* A. Hashim. & Kaz. Tanaka.

***Pseudocryptoclypeus yakushimensis*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB819251; Fig. 36.

*Etyymology:* Referring to the collection site.

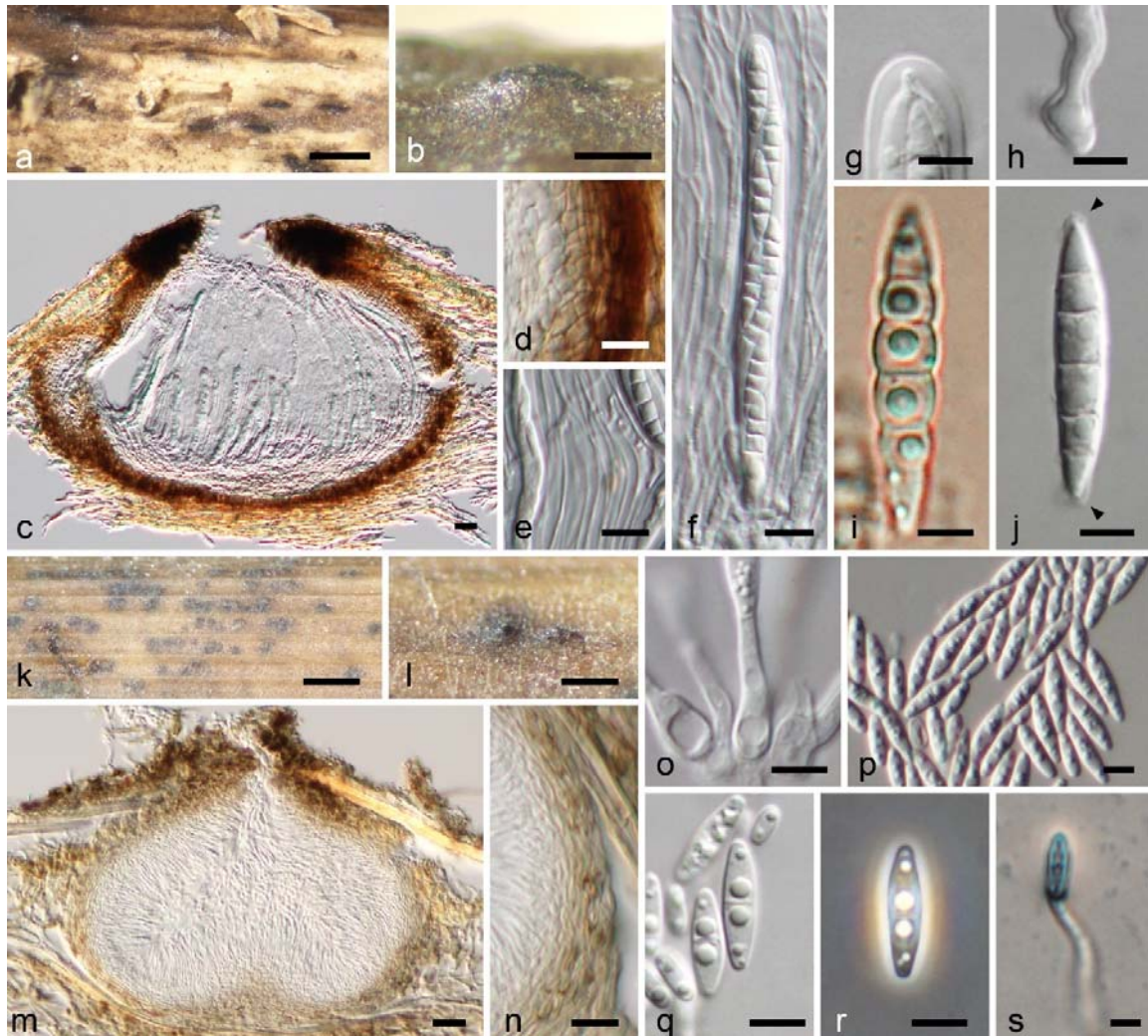
Saprobic on dead culms of bamboo. **Sexual morph:** *Ascomata* ellipsoidal, 4–5 grouped, immersed, 325–380  $\mu\text{m}$  high, 250–260  $\mu\text{m}$  diam. *Ostiolar neck* crest-like, elongated, laterally compressed, with less-developed clypeus (200–315  $\mu\text{m}$  wide). *Peridium* 17.5–25  $\mu\text{m}$  thick of 2 layers at side; outer layers 10–17.5  $\mu\text{m}$  thick of compressed, thin-walled, 12–17  $\times$  1.5–4.2  $\mu\text{m}$ , brown cells; inner layers of 7.5–10  $\mu\text{m}$  thick, rectangular, 5–9  $\times$  4–6  $\mu\text{m}$ , pale brown cells. *Pseudoparaphyses* 0.5–1  $\mu\text{m}$  wide, septate, branched, anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 92.5–127  $\times$  7.5–10  $\mu\text{m}$  ( $\bar{x}$  = 104.8  $\times$  8.2  $\mu\text{m}$ , n = 15), with a short stipe (5–15  $\mu\text{m}$  long,  $\bar{x}$  = 8.2  $\mu\text{m}$ , n = 15), apically rounded with an ocular chamber, 8-spored. *Ascospores* fusiform with acute ends, straight, 20–32.5  $\times$  3–5  $\mu\text{m}$  ( $\bar{x}$  = 25.4  $\times$  4.0  $\mu\text{m}$ , n = 50), l/w 4.7–8.1 ( $\bar{x}$  = 6.4, n = 50), 5-septate, with a primary septum nearly median (0.44–0.57,  $\bar{x}$  = 0.50, n = 50), slightly constricted at the primary septum, hyaline, smooth, with gelatinous pad at each end. **Asexual morph:** *Conidiomata* pycnidial, globose to subglobose, up to 375  $\mu\text{m}$  high, 400–480  $\mu\text{m}$  diam, 5–6 grouped, immersed. *Ostiolar neck* carbonaceous, papillate, 47.5–77.5  $\mu\text{m}$  high. *Peridium* 10–15  $\mu\text{m}$  thick, composed of 3 layers of 7.5–10  $\times$  0.8–1.0  $\mu\text{m}$ , rectangular, pale brown cells. *Conidiophores* absent. *Conidiogenous cells* holoblastic, 12–16  $\times$  2.5–3  $\mu\text{m}$ , ampliform to cylindrical, hyaline, smooth. *Conidia* cylindrical with rounded ends, 9–14  $\times$  2.5–3.5  $\mu\text{m}$  ( $\bar{x}$  = 12.0  $\times$  3.0  $\mu\text{m}$ , n = 50), l/w 3.3–4.8 ( $\bar{x}$  = 4.0, n = 50), hyaline, smooth, 1-septate, guttulate when young.

Culture characteristics — Colonies on PDA attaining 32–52 mm diam within 21 d at 20 °C in the dark, floccose, plane, smoke grey (105); reverse grey olivaceous (107) to olivaceous black (108) (Fig. 39j); asexual morph formed.

*Specimen examined.* **JAPAN**, Kagoshima, Isl. Yakushima, Nagata, on dead culms of bamboo, 16 Mar. 2007, K. Tanaka & H. Yonezawa, KT 2186 (HHUF 30503 **holotype**, ex-holotype living culture MAFF 245622 = NBRC 112320).

Notes — *Pseudocryptoclypeus* is similar to *Cryptoclypeus* in having a less-developed clypeus in ascomata, multi-septate ascospores, pycnidial conidiomata, and 1-septate, hyaline conidia. *Pseudocryptoclypeus* has an ascomatal peridium

composed of two-layers and holoblastic conidiogenous cells (Fig. 36m, o) rather than the 1-layered ascomatal wall and phialidic conidiogenous cells of *Cryptoclypeus* (Fig. 34q, s, t). Although these two genera constituted a highly supported clade (90 % ML BP/1.00 Bayesian PP) in our phylogenetic tree (Fig. 28), they had 43–45 base differences with 61–63 gaps in their ITS regions.



**Fig. 36.** *Pseudocryptoclypeus yakushimensis*. a, b. Appearance of ascomata on substrate; c. Ascoma in longitudinal section; d. Peridium of ascoma; e. Pseudoparaphyses; f. Ascus; g. Ascus apex; h. Ascus stipe; i, j. Ascospores (j arrowheads indicate gelatinous pad); k, l. Conidiomata in culture; m. Conidioma in longitudinal section; n. Peridium of conidioma; o. Conidiogenous cells; p–r. Conidia; s. Germinating conidium; a–j from HHUF 30503 (holotype); k–s from MAFF 245622 = NBRC 112320 (ex-holotype culture). — Scale bars: a, k = 1 mm; b = 200  $\mu$ m; c, m = 20  $\mu$ m; d–f, n = 10  $\mu$ m; g–j, o–s = 5  $\mu$ m; l = 250  $\mu$ m.

### *Incertae sedis*

***Antealophiotrema*** A. Hashim. & Kaz. Tanaka, **gen. nov.** — MycoBank MB819252.

*Etymology*: Formerly belonging to *Lophiotrema*.

Saprobic on woody plants. **Sexual morph**: *Ascomata* subglobose to depressed ellipsoidal. *Ostiolar neck* crest-like, elongate, laterally compressed. *Peridium* composed of globose to rectangular, hyaline to brown cells, with brown hyphae at side. *Pseudoparaphyses* septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical to clavate, 4–8-spored. *Ascospores* narrowly fusiform, 1-septate, brown, smooth. **Asexual morph**: *Mycelium* superficial, superficial hyphae brown. *Conidiophores* absent. *Conidiogenous cells* holoblastic, integrated, terminal, brown, truncate, solitary, acrogenous. *Conidia* obovoid, black, muriform.

*Type species*: *Antealophiotrema brunneosporum* (Ying Zhang, J. Fourn. & K.D. Hyde) A. Hashim. & Kaz. Tanaka.

***Antealophiotrema brunneosporum*** (Yin. Zhang, J. Fourn. & K.D. Hyde) A. Hashim. & Kaz. Tanaka, **comb. nov.** — MycoBank MB819253; Fig. 37.

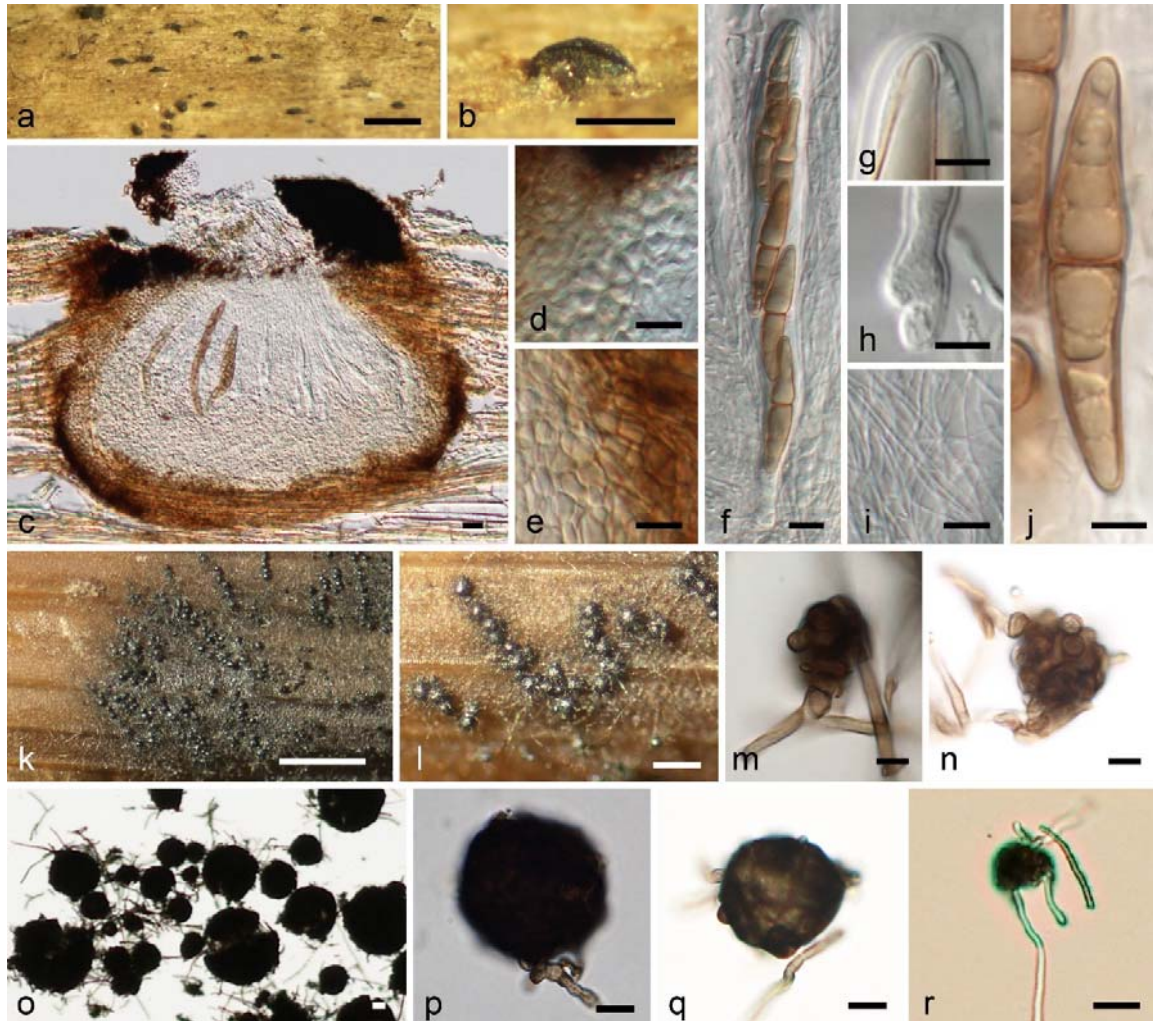
*Basionym*: *Lophiotrema brunneosporum* Ying Zhang, J. Fourn. & K.D. Hyde, Fungal Divers. 38: 240. 2009.

Saprobic on decorticated wood of *Salix* sp. **Sexual morph**: *Ascomata* subglobose to depressed ellipsoidal, up to 380 µm high, 460–530 µm diam. *Ostiolar neck* crest-like, elongated, laterally compressed. *Peridium* ununiform, 42.5–62.5 µm thick, composed of 2 layers; outer layers 22.5–25 µm thick, composed of moderately thick-walled, rectangular, (5–)7–9 × 5–7.5 µm, brown cells, with brown hyphae; inner layers 20–32.5 µm thick of thin-walled, rectangular, 7–9 × 4–7.5 µm, hyaline to pale brown cells; at base 27.5–35 µm thick, of globose to rectangular, 3.5–7 × 3.5–4 µm, pale brown cells. *Pseudoparaphyses* numerous, 0.8–1.5 µm wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical to clavate, 119–148 × 13–15 µm ( $\bar{x}$  = 138.2 × 14.3 µm, n = 8), with a short stipe (8.5–16 µm long,  $\bar{x}$  = 10.9 µm, n = 8), apically rounded with an ocular chamber, with biseriate 4–8 ascospores. *Ascospores* narrowly fusiform with slightly rounded ends, 34.5–48 × 6.5–10 µm ( $\bar{x}$  = 39.8 × 8.0 µm, n = 32), l/w 4.1–6.1 ( $\bar{x}$  = 5.0, n = 32), with a septum nearly median (0.44–0.54,  $\bar{x}$  = 0.49, n = 50), strongly constricted at the septum, brown, smooth. **Asexual morph**: *Mycelium* superficial, superficial hyphae brown. *Conidiophores* absent. *Conidiogenous cells* holoblastic, integrated, terminal, brown, truncate, solitary, acrogenous. *Conidia* obovoid, black, 27.5–85 µm diam ( $\bar{x}$  = 52.0 µm, n = 57), muriform.

Culture characteristics — Colonies on PDA attaining 12–17 mm diam within 21 d at 20 °C in the dark, floccose, centrally raised, grey olivaceous (107); reverse olivaceous black (108) (Fig. 39k); asexual morph formed.



*Specimen examined.* **FRANCE**, Ariège, Rimont, Las Muros, on decorticated wood of *Salix* sp., 24 Sept. 2006, J. Fournier & K.D. Hyde (CBS H-20222 **holotype**, ex-holotype culture CBS 123095)



**Fig. 37.** *Antealophiotrema brunneosporum*. a, b. Appearance of ascomata on substrate; c. Ascoma in longitudinal section; d. Peridium of ascoma near ostiole; e. Peridium of ascoma at side; f. Ascus; g. Ascus apex; h. Ascus stipe; i. Pseudoparaphyses; j. Ascospore; k, l. Conidia in culture; m, n. Conidiogenous cells and immature conidia; o–q. Conidia; r. Germinating conidium; a–j from CBS H-20222 (holotype); k–r from CBS 123095 (ex-holotype culture). — Scale bars: a, k = 1 mm; b, l = 250  $\mu$ m; c, o, r = 20  $\mu$ m; d–f, i, p, q = 10  $\mu$ m; g, h, j, m, n = 5  $\mu$ m.

**Notes** — The transfer of *L. brunneosporum* to *Antealophiotrema* is based on its morphological features, as it differs from *Lophiotrema sensu stricto* in having a well-developed peridium (up to 62.5  $\mu$ m thick, Fig. 37c) as well as a monodictys-like asexual morph (Fig. 37k–r). In our phylogenetic tree, this species and "*Lophiotrema*" *boreale* (CBS 114422) formed a fully supported clade (100 % ML BP/1.00 Bayesian

PP) outside of *Lophiotremataceae* and are recognised as a lineage distinct from *Lophiotrema sensu stricto* (Fig. 28). We treat *Antealophiotrema* as “*incertae sedis*” in *Pleosporales* at present; additional taxa related to this monotypic genus will be required to resolve its familial placement.

***Pseudolophiotrema*** A. Hashim. & Kaz. Tanaka, **gen. nov.** — MycoBank MB819254.

*Etymology:* After its morphological similarity to *Lophiotrema*.

Saprobic on herbaceous plants. **Sexual morph:** *Ascomata* grouped, immersed, globose. *Ostiolar neck* crest-like, elongate, laterally compressed. *Peridium* composed of compressed, thin-walled, pale brown cells. *Pseudoparaphyses* numerous, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 8-spored. *Ascospores* fusiform, 1-septate, hyaline, smooth. **Asexual morph:** Undetermined.

*Type species:* *Pseudolophiotrema elymicola* A. Hashim. & Kaz. Tanaka.

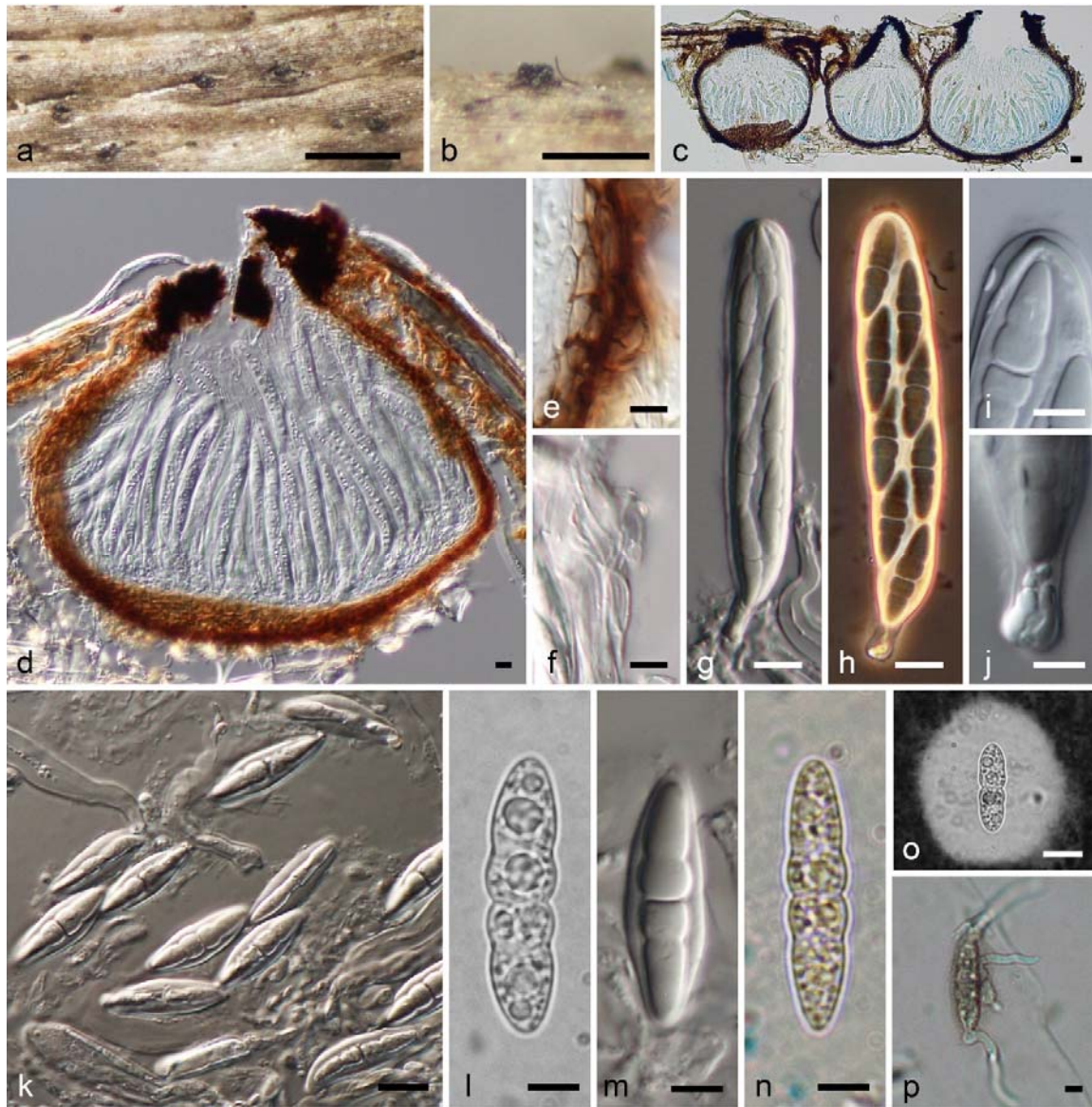
***Pseudolophiotrema elymicola*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB819255; Fig. 38.

*Etymology:* Referring to the generic name of the host plant.

Saprobic on dead leaves of *Leymus mollis*. **Sexual morph:** *Ascomata* grouped, immersed, globose, 200–300  $\mu\text{m}$  high, 190–340  $\mu\text{m}$  diam. *Ostiolar neck* crest-like, elongated, laterally compressed. *Peridium* uniform, 10–12.5  $\mu\text{m}$  thick composed of compressed, thin-walled, 5–12  $\times$  2–3.5  $\mu\text{m}$ , pale brown cells. *Pseudoparaphyses* numerous, 1.5–2  $\mu\text{m}$  wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 82–108  $\times$  10–17  $\mu\text{m}$  ( $\bar{x}$  = 92.4  $\times$  13.9  $\mu\text{m}$ , n = 11), with a short stipe (5–8  $\mu\text{m}$  long,  $\bar{x}$  = 6.3  $\mu\text{m}$ , n = 10), apically rounded with an ocular chamber, 8-spored. *Ascospores* fusiform with acute ends, straight, 20–28  $\times$  4.5–7  $\mu\text{m}$  ( $\bar{x}$  = 22.7  $\times$  5.5  $\mu\text{m}$ , n = 50), l/w 3.2–5.1 ( $\bar{x}$  = 4.2, n = 50), with a septum nearly median (0.47–0.56,  $\bar{x}$  = 0.51, n = 50), strongly constricted at the septum and midpoint of each cell, hyaline, smooth, with a gelatinous sheath. **Asexual morph:** Undetermined.

Culture characteristics — Colonies on PDA attaining 18–19 mm diam within 21 d at 20 °C in the dark, velvety, plane, radiately, smoke grey (105); reverse grey olivaceous (107) (Fig. 39I); sexual morph formed.

*Specimen examined.* **JAPAN**, Hokkaido, Yufutsu, on dead leaves of *Leymus mollis*, 1 Sep. 2003, Y. Harada, KT 1450 (HHUF 28984 **holotype**, ex-holotype culture JCM 13090 = MAFF 239600).



**Fig. 38.** *Pseudolophiotrema elymicola*. a, b. Appearance of ascomata on substrate; c, d. Ascoma in longitudinal section; e. Peridium of ascoma; f. Pseudoparaphyses; g, h. Asci; i. Ascus apex; j. Ascus stipe; k–n. Ascospores; o. Ascospore with a gelatinous sheath (in india ink); p. Germinating ascospore; a–m, o, p from HHUF 28984 (holotype); n from JCM 13090 = MAFF 239600 (ex-holotype culture). — Scale bars: a = 500  $\mu$ m; b = 250  $\mu$ m; c, d = 20  $\mu$ m; e–h, k, o, p = 10  $\mu$ m; i, j, l–n = 5  $\mu$ m.

**Notes** — *Pseudolophiotrema* is quite similar to *Lophiotrema* in having a crest-like ostiolar neck, a peridium of uniform thickness, cylindrical asci with a short stipe, and 1-septate hyaline ascospores with a gelatinous sheath. However, it can be separated



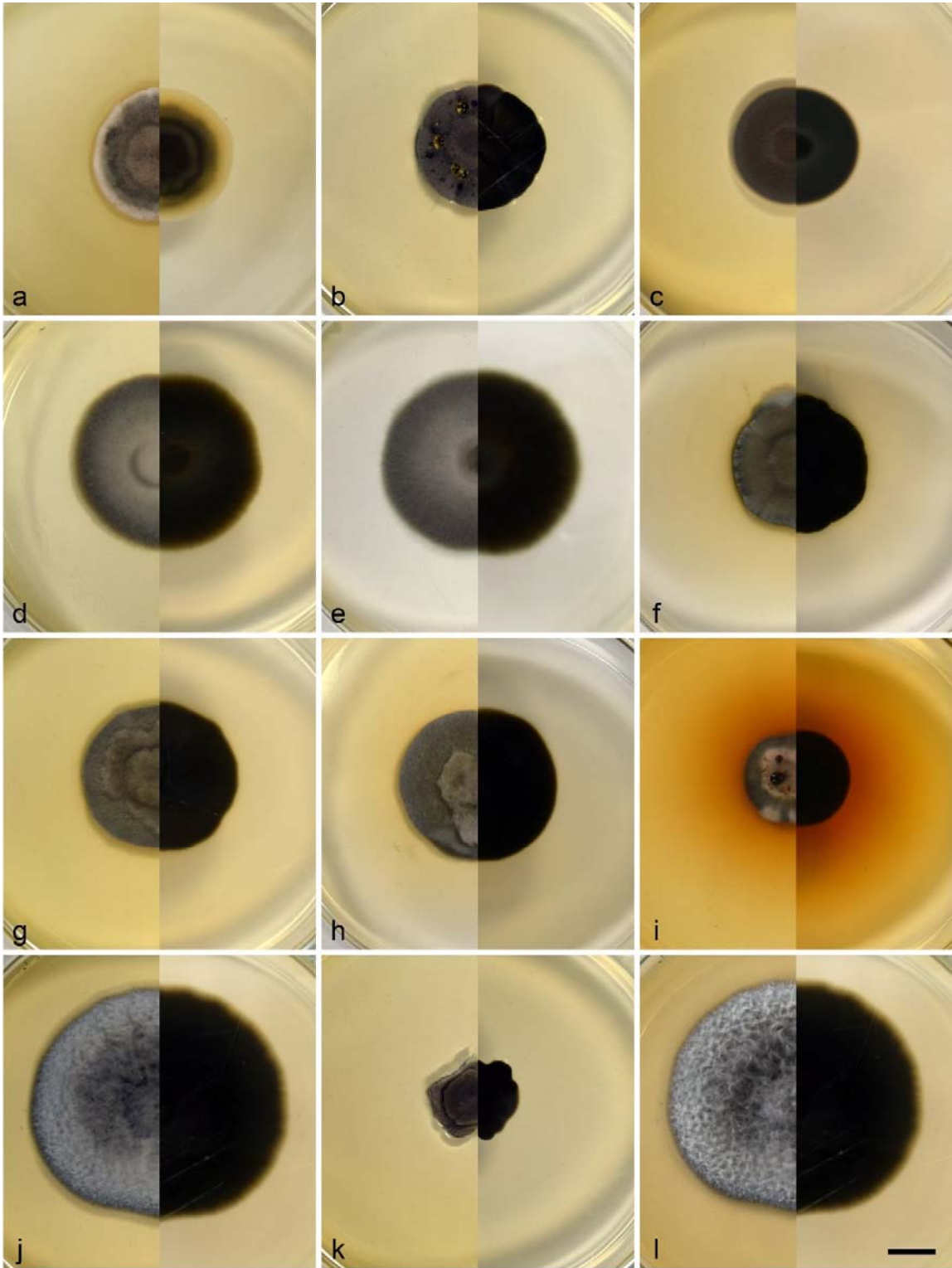
from the latter genus by its thinner ascomatal wall (10–12.5  $\mu\text{m}$  thick; Fig. 38d, e) composed of compressed cells vs. the 20–30- $\mu\text{m}$  thick wall made up of rectangular cells in *Lophiotrema* (Holm & Holm 1988).

In our phylogenetic tree, the genus *Pseudolophiotrema* was placed completely outside of *Lophiotremataceae*, and was also separate from the families *Cryptocoryneaceae* and *Aquasubmersaceae* (Fig. 28). The familial placement of *Pseudolophiotrema* remains unclear. Additional taxa belonging to this genus are needed to fully understand its taxonomic affiliations within *Pleosporales*.

## DISCUSSION

*Lophiotremataceae* was established by Hirayama & Tanaka (2011) to accommodate *Lophiotrema*. Subsequently, *Aquasubmersa* and *Hermatomyces* were recognised as additional members of this family on the basis of molecular phylogenetic analyses (Ariyawansa et al. 2015, Doilom et al. 2016, Hyde et al. 2016). A phylogenetic relationship between *Cryptocoryneum* and *Lophiotremataceae* was also suggested by a Blast search of ITS sequences of species in that genus (Hashimoto et al. 2016). Although tree topologies generated in previous molecular studies have suggested that the above-mentioned genera might be members of *Lophiotremataceae*, statistical support for a *Lophiotremataceae sensu lato* clade has been relatively weak (Ariyawansa et al. 2015, Doilom et al. 2016, Hashimoto et al. 2016, Hyde et al. 2016). Our results do not support the monophyly of *Lophiotremataceae sensu lato* as recognised in several previous studies (Fig. 28). Instead, the various asexual morphs found in *Aquasubmersa*, *Cryptocoryneum*, and *Hermatomyces* belong to separate families whose monophyly is strongly supported (Fig. 28). We thus consider *Lophiotremataceae* to be restricted to *Lophiotrema* and five new genera (*Atrocalyx*, *Crassimassarina*, *Cryptoclypeus*, *Galeaticarpa*, and *Pseudocryptoclypeus*), all of which have ascomata with or without slit-like ostioles and pycnidial conidiomata. We also conclude that *Aquasubmersa* (a freshwater lineage having papillate ascomatal ostioles and pycnidial conidiomata with 1-celled conidia), *Cryptocoryneum* (having

**Fig. 39.** Colony characters of lophiotremataceous species used in this study on PDA within 3 wk at 20 °C in the dark (left: upper, right: reverse). a. *Hermatomyces iriomotensis* (MAFF 245730 = NBRC 112471, ex-holotype culture); b. *Atrocalyx acutisporus* (MAFF 245613 = NBRC 112316, ex-holotype culture); c. *A. lignicola* (CBS 122364, ex-holotype culture); d. *Crassimassarina macrospora* (JCM 13096 = MAFF 239606, ex-holotype culture); e. *C. macrospora* (MAFF 245617, ex-paratype culture) f. *Cryptoclypeus oxysporus* (MAFF 245614 = NBRC 112317, ex-holotype culture); g. *C. ryukyuensis* (MAFF 245615 = NBRC 112318, ex-holotype culture); h. *C. ryukyuensis* (MAFF 245616, ex-paratype culture); i. *Galeaticarpa aomoriensis* (MAFF 245618 = NBRC 112319, ex-holotype culture); j. *Pseudocryptoclypeus yakushimensis* (MAFF 245622 = NBRC 112320, ex-holotype culture); k. *Antealophiotrema brunneosporum* (CBS 123095, ex-holotype culture); l. *Pseudolophiotrema elymicola* (JCM 13090 = MAFF 239600, ex-holotype culture). — Scale bar: a–l = 1 cm.



sporodochial conidiomata with cheiroid conidia), and *Hermatomyces* (having sporodochial conidiomata with lenticular and/or cylindrical conidia), which have provisionally been regarded as genera of *Lophiotremataceae*, should be placed in their own families given their different asexual forms.

Except for *Crassimassarina*, genera accepted in the present study as members of *Lophiotremataceae* (i.e. *Lophiotrema sensu stricto* and the newly introduced genera *Atrocalyx*, *Cryptoclypeus*, *Galeaticarpa*, and *Pseudocryptoclypeus*) have features that are generally consistent with traditional generic concepts of *Lophiotrema sensu lato* (Holm & Holm 1988, Tanaka & Harada 2003, Zhang et al. 2009, Hirayama & Tanaka 2011). In contrast, *Crassimassarina* has some characteristics atypical of *Lophiotremataceae*: large, immersed ascomata with a papillate ostiolar neck, resembling those of genera in *Pleomassariaceae sensu lato* (Barr 1982, Tanaka et al. 2005), as well as pycnidial conidiomata with multi-septate cylindrical conidia resembling those of *Stagonospora* (Quaedvlieg et al. 2013, Tanaka et al. 2015). The phylogenetic placement of this morphologically distinct lineage in *Lophiotremataceae* led us to conduct detailed observations of the above lophiotremataceous genera. These observations revealed morphological variations among ascomata with slit-like ostioles in *Lophiotremataceae*, such as ascomata with a distinct clypeus around the ostiolar neck (in *Cryptoclypeus*, *Galeaticarpa* and *Pseudocryptoclypeus*, Fig. 33c, Fig. 34c, Fig. 35d, Fig. 36c), ascomata with a well-developed peridium (*Atrocalyx*, Fig. 30c, Fig. 31c), and ascomata with a less-developed peridium of a uniform thickness up to 25  $\mu\text{m}$  (*Lophiotrema*; Holm & Holm 1988). These morphologically distinguishable groups constitute phylogenetically distinct lineages that also correspond to their differing asexual forms (Fig. 30o, Fig. 32q, Fig. 34q, Fig. 35p, Fig. 36m). For example, the sexual morphs of *Cryptoclypeus*, *Galeaticarpa*, and *Pseudocryptoclypeus* have similar morphological features, but these genera can be separated from one another according to the morphology of their conidiomata or conidiogenous cells (see Notes on each genus). Consequently, we treat these five genera as separate from *Lophiotrema sensu stricto* on the basis of holomorph morphology and phylogenetic relationships. *Antealophiotrema brunneosporum*, originally misidentified as a species in *Lophiotrema* because of its morphological resemblance to that genus (Zhang et al. 2009), is only distantly related to *Lophiotremataceae*. This species is clearly different from *Lophiotrema* in regard to the anatomical characteristics of its peridium and its monodictys-like asexual morph. The result of our present study strongly confirms that the presence of a slit-like ostiole is an unreliable character for delimitation of generic relationships.

Traditionally, the slit-like ostiole of the ascomata has been especially emphasised as a useful character for familial circumscription in ascomycetes (Chesters & Bell 1970). In earlier studies, *Lophiotrema* was placed in *Lophiostomataceae* according to this criterion (Chesters & Bell 1970, Leuchtman 1985, Holm & Holm 1988, Barr 1992). Zhang et al. (2009) suggested that *Lophiotrema* is phylogenetically distinct from *Lophiostomataceae* but could not find any morphological differences between

their sexual morphs. Hirayama & Tanaka (2011) reevaluated the phylogenetic significance of several morphological features used for characterisation of each genus in previous studies. After determining that ascus shape and length of ascus stipe are reliable taxonomic indicators to delineate these two genera, they established *Lophiotremataceae* to accommodate *Lophiotrema* (Hirayama & Tanaka 2011). Our results, however, strongly confirm that several lophiotrema-like species, i.e. *Antealophiotrema brunneosporum*, "*Lophiotrema*" *boreale*, and *Pseudolophiotrema elymicola*, deviate from *Lophiotremataceae*. These species were originally misidentified or provisionally identified as species in *Lophiotrema* on the basis of their morphological resemblance to the genus (Mathiassen 1989, 1993, Zhang et al. 2009), but were found to be phylogenetically separate from *Lophiotrema sensu stricto* in this study. Previous morphological circumscriptions of *Lophiotremataceae* and *Lophiotrema* (Holm & Holm 1988, Mathiassen 1989, 1993, Tanaka & Harada 2003, Zhang et al. 2009, Hirayama & Tanaka 2011, Ariyawansa et al. 2015, Doilom et al. 2016, Hyde et al. 2016) were obviously fairly broad concepts that did not reflect their phylogenetic relationships. We additionally discovered several morphological variants of ascomata having slit-like ostioles (e.g. *Atrocalyx*, *Cryptoclypeus*, *Galeaticarpa*, and *Pseudocryptoclypeus*), revealed the phylogenetic position of a genus without slit-like ostioles in *Lophiotremataceae* (i.e., *Crassimassarina*), and observed asexual morphs of genera belonging to *Lophiotremataceae*. More precise morphological examination of both sexual and asexual morphs in this family will be needed to define familial concepts of *Lophiotremataceae* and its relatives.

The phylogenetic significance of slit-like ostioles of ascomata should also be reevaluated because this phenotypic character is now known in several families, such as *Aigialaceae* (Suetrong et al. 2009), *Ligninsphaeriaceae* (Zhang et al. 2016), and *Lophiostomataceae* (Thambugala et al. 2015). The slit-like ostiole should be regarded as a character that has evolved multiple times independently within *Dothideomycetes*, similar to the parallel evolution of hysterothecial ascomata in *Anteagloniaceae* (Mugambi & Huhndorf 2009), *Gloniaceae* (Boehm et al. 2009), *Hysteriaceae* (Boehm et al. 2009), *Lophiostomataceae* (Thambugala et al. 2015), and *Mytilinidiaceae* (Boehm et al. 2009).

Recent molecular studies on *Dothideomycetes* have revealed hidden lineages and prompted revision of several families in this class (Crous et al. 2015, Guatimosim et al. 2015, Knapp et al. 2015, Tanaka et al. 2015). Although multiple molecular systematic studies, mainly of pathogenic fungi of woody plants (Phillips et al. 2013, Slippers et al. 2013, Alves et al. 2014, Fan et al. 2015, Trakunyingcharoen et al. 2015), have generated a robust phylogeny for *Botryosphaeriales*, many additional new lineages in this group, including endophytes and saprophytes, have been discovered from various niches (Thambugala et al. 2014, 2016, Crous et al. 2015, Linaldeddu et al. 2016, Osorio et al. 2016, Wyka & Broders 2016). Drawing on many recent collections, our study has revealed previously unrecognised diversity within



*Lophiotremataceae* and its relatives and the potential taxonomic importance of the asexual morphs in defining families. To build a comprehensive taxonomic framework, further morphological surveys based on additional collections together with more molecular data are needed.

## 6. 広義 *Melanomma* 科の分類学的再検討

### ABSTRACT

*Mycodidymella* 属, *Petrakia* 属, *Pseudodidymella* 属, *Xenostigmina* 属の4属の科の所属について, 形態観察と SSU-LSU-*tef1-rpb2* 領域の分子系統解析に基づき分類学的に検討した. *Melanomma* 科 (プレオスポラ目, クロイボタケ綱) に系統的に近縁な 28 菌株から新たに 128 配列が得られた. 分子系統解析と有性世代および無性世代の形態観察の結果, *Melanomma* 科は著しく発達した殻壁で構成される子のう果と aposphaeria 型の分生子果の無性世代で特徴付けられる *Melanomma* 属の単一のグループに限定されるべきであると結論づけた. *Mycodidymella* 属, *Petrakia* 属, *Pseudodidymella* 属, *Xenostigmina* 属は系統的に *Melanomma* 科に近縁であるが, それらは葉上生, レンズ状で, 子座基部が著しく発達した子のう果と mycopappus 型のプロパギュールを無性世代にもつ点で *Melanomma* 属と明らかに異なった. *Pseudodidymella* 科がこれら4属を收容するために設立された. *Mycodidymella* 属は有性世代の形態学的特徴に基づき *Petrakia* 属のシノニムとしてみなされてきたが, 本研究では *Mycodidymella* 属は区別すべき分類群であるとみなした. *Pseudodidymella* 科属菌はそれらのシアナモルフによって容易に区別することができた. すなわち, 糸状, 多隔壁, 無色の分生子 (*Mycodidymella*), 球形から卵形, 石垣状, 褐色, 細胞性の付属物をもつ分生子 (*Petrakia*), 棍棒形, 先端にくちばし状突起, 石垣状, 褐色の分生子 (*Xenostigmina*). *Pseudodidymella* 属のシアナモルフは観察されなかった. *Alpinaria rhododendri* は *Melanomma* 科のメンバーとして見なされてきたが, 本科には典型的ではない無色の細胞で構成された子のう果の基部と融合性の偽分生子殻をもつ点で, 科の所属は不明なままであった. 計1新科, 2新種を提唱し, それらの種について記載した. DNA バーコードマーカーとして ITS 領域の配列を決定した.

### INTRODUCTION

*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 epiphyllous, lenticular ascomata with well-developed basal stroma, mycopappus-like propagules, and petrakia- 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 aposphaeria-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 1913, Butin *et al.* 2013). Recently, the complete life cycle of *Pe. echinata*, which is the type species and a known causal agent of leaves 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).

*Xenostigmia 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 *Mycopappus aceris*. The type species of *Mycopappus*, *Mycop. alni*, was suggested to be a member of *Sclerotiniaceae* (*Helotiales*, *Leotiomyces*) based on sclerotial stage and phylogenetic analyses using ITS sequences (Takahashi *et al.* 2006). *Mycopappus aceris* was excluded from the genus, because the sexual 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 phylogenetic 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.* 1998). The life cycle of *Mycod. aesculi* is similar to those of *Petrakia* and *Xenostigmia*, except it has sigmoid and hyaline conidia in its synasexual morph. Although the synasexual morph of *Petrakia* seems to be clearly different from that of *Mycodidymella* and *Xenostigmia*, the latter two genera were synonymised with older name *Petrakia* (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 well-developed basal stroma and a pycnopleiospora-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 pycnopleiospora-like asexual morph (Wei *et al.* 1998). Gross *et al.* (2017) discovered *Pseudod. fagi* on *F. sylvatica* in Switzerland and suggested that the pycnopleiospora-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 phylogenetic study using ITS sequences (Gross *et al.* 2017), four genera with mycopappus-like propagules (*Mycodidymella*, *Petrakia*, *Pseudodidymella*, and *Xenostigmia*) formed a strongly supported clade within *Melanommataceae sensu lato*; however, familial placement and generic validity of each genus were not resolved.

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 *Melanommataceae 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 *Melanommataceae 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- $\alpha$  (*tef1*), and DNA-directed RNA polymerase II second largest subunit (*rpb2*) sequences. ITS sequences were also obtained as barcode markers.

## **MATERIALS AND METHODS**

### **Isolation and morphological observation**

All fungal structures were observed 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 5). Colony characteristics of cultures grown on potato dextrose agar (PDA; Difco, France) were observed after a 3-wk cultivation 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 squares of mycelial agar were placed on water agar that included sterilised natural substrate, such as *Aesculus turbinata* and *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 months to observe the sporulation. Specimens were deposited in the Herbarium of Hirosaki University, Fungi (HHUF).

## Phylogenetic analyses

DNA extraction was carried out with an ISOPLANT 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 fRPB2-5F/fRPB2-7cR (Liu *et al.* 1999), respectively. Amplifications were performed in 25  $\mu$ L volumes that consisted of 2  $\mu$ L DNA extract, 2.5  $\mu$ L of 10 $\times$  TEMPase Buffer I, 10 mM dNTP mix, 1  $\mu$ L of each 20-pM primer, 25 mM MgCl<sub>2</sub>, 14.5  $\mu$ L MilliQ water, and 0.5  $\mu$ L TEMPase Hot Start DNA polymerase (Ampliqon, Denmark). PCRs were carried out on a PC 320 thermocycler (ASTECH, 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 5). Sequences of 72 taxa of *Pleosporales* and *Hysteriales* were also phylogenetically analysed (Table 6). *Hysterium pulicare* and *Hysterobrevium mori* (*Hysteriaceae*, *Hysteriales*) were used as outgroups. All sequences were aligned using the MUSCLE algorithm as implemented in the program MEGA 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): J1+G for SSU; GTR+G for LSU; J1+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 proportions (BPs) were obtained by 1 000 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; SYM+G for LSU; GTR+G for the *tef1* first codon position, SYM+G for the *tef1* second codon position, and GTR+G for the *tef1* third codon position; and GTR+G for the *rpb2* first codon

**Table 5.** Specimens, isolates and new sequences used in this study.

Species	Original no.	Specimen no. <sup>a</sup>	Host/substrate	GenBank Accession no. <sup>b</sup>				
				SSU	LSU	<i>tef1</i>	<i>rpb2</i>	ITS
<i>Alpinaria rhododendri</i>	KT 2520	HHUF 30554	<i>Rhododendron brachycarpum</i>	LC203314	LC203360	LC203388	LC203416	LC203335
<i>Melanomma japonicum</i>	KT 2076	HHUF 30539 <sup>P</sup>	dead wood	LC203290	LC203336	LC203364	LC203392	LC203318
	KT 3028	HHUF 30540 <sup>P</sup>	<i>Fagus crenata</i>	LC203291	LC203337	LC203365	LC203393	LC203319
	KT 3425	HHUF 30541 <sup>P</sup>	<i>F. crenata</i>	LC203292	LC203338	LC203366	LC203394	LC203320
	-	HHUF 26520 <sup>H</sup>	dead wood	LC203293	LC203339	LC203367	LC203395	LC203321
<i>Me. pulvis-pyrius</i>	KT 2110	HHUF 30542	<i>Acer</i> sp.	LC203294	LC203340	LC203368	LC203396	LC203322
	KT 2113	HHUF 30543	dead wood	LC203295	LC203341	LC203369	LC203397	LC203323
	AH 375	HHUF 30544	<i>F. crenata</i>	LC203296	LC203342	LC203370	LC203398	LC203324
	KH 27	HHUF 30545	dead wood	LC203297	LC203343	LC203371	LC203399	LC203325
	KH 77	HHUF 30546	dead wood	LC203298	LC203344	LC203372	LC203400	LC203326
	KH 86	HHUF 30547	dead wood	LC203299	LC203345	LC203373	LC203401	LC203327
	KH 197	HHUF 30548	dead wood	LC203300	LC203346	LC203374	LC203402	LC203328
<i>Mycodidymella aesculi</i>	KT 3060	HHUF 30549	<i>Aesculus turbinata</i>	LC203301	LC203347	LC203375	LC203403	LC203329
	H 2610	HHUF 22892 <sup>H</sup>	<i>A. turbinata</i>	LC203302	LC203348	LC203376	LC203404	LC194192
	H 2620	-	<i>A. turbinata</i>	LC203303	LC203349	LC203377	LC203405	LC203330
	AH 560	HHUF 30550	<i>A. turbinata</i>	LC203304	LC203350	LC203378	LC203406	LC203331
<i>Petrakia echinata</i>	-	-	<i>Acer pseudoplatanus</i>	LC203305	LC203351	LC203379	LC203407	-
	-	-	<i>A. pseudoplatanus</i>	LC203306	LC203352	LC203380	LC203408	-
<i>Pseudodidymella fagi</i>	KT 3058	HHUF 30515	<i>F. crenata</i>	LC203307	LC203353	LC203381	LC203409	LC150785
	KT 3074-3	HHUF 30516	<i>F. crenata</i>	LC203308	LC203354	LC203382	LC203410	LC150786
	RF 5	HHUF 30517	<i>F. crenata</i>	LC203309	LC203355	LC203383	LC203411	LC150788
	H 2579	HHUF 22903 <sup>H</sup>	<i>F. crenata</i>	LC203310	LC203356	LC203384	LC203412	LC150787
	AH 561	HHUF 30553	<i>F. crenata</i>	LC203311	LC203357	LC203385	LC203413	LC203332
<i>Pseudod. minima</i>	KT 2918	HHUF 30551 <sup>H</sup>	<i>Fagus japonica</i>	LC203312	LC203358	LC203386	LC203414	LC203333
	AH 556	HHUF 30552 <sup>P</sup>	<i>F. japonica</i>	LC203313	LC203359	LC203387	LC203415	LC203334
<i>Xenostigmina zilleri</i>	-	-	<i>Acer macrophyllum</i>	LC203315	LC203361	LC203389	LC203417	-
	-	-	<i>Acer</i> sp.	LC203316	LC203362	LC203390	LC203418	-
	-	-	<i>Acer</i> sp.	LC203317	LC203363	LC203391	LC203419	-

<sup>a</sup>“H” : holotype, “P”: paratype.<sup>b</sup> holotype of *Blastostroma aesculi*.<sup>c</sup> holotype of *Pycnopleiospora fagi*.



**Table 6.** GenBank accession numbers of species used in the phylogenetic study.

Species name	Family	Strain No. <sup>a</sup>	GenBank Accession no.			
			SSU	LSU	<i>tef1</i>	<i>rpb2</i>
<i>Alpinaria rhododendri</i>	<i>incertae sedis</i>	ANM 73	-	GU385198	-	-
<i>Alternaria alternata</i>	<i>Pleosporaceae</i>	CBS 916.96 <sup>E</sup>	DQ678031	DQ678082	DQ677927	DQ677980
<i>Aposphaeria corallinolutea</i>	<i>incertae sedis</i>	CBS 131287 <sup>H</sup>	-	JF740330	-	-
<i>A. populina</i>	<i>Melanommataceae</i>	CBS 543.70 <sup>E</sup>	EU754031	EU754130	-	-
<i>A. populina</i>	<i>Melanommataceae</i>	CBS 350.82	-	JF740265	-	-
<i>Bambusicola massarinia</i>	<i>Bambusicolaceae</i>	MFLUCC 11-0389 <sup>H</sup>	JX442041	JX442037	KP761725	KP761716
<i>Bertiella macrospora</i>	<i>incertae sedis</i>	IL 5005	-	GU385150	-	-
<i>Beverwykella pulmonaria</i>	<i>incertae sedis</i>	CBS 283.53 <sup>H</sup>	-	GU301804	-	GU371768
<i>Biatriospora marina</i>	<i>Nigrogranaceae</i>	CY 1228	GQ925835	GQ925848	GU479848	GU479823
<i>Byssosphaeria jamaicana</i>	<i>incertae sedis</i>	SMH 1403	-	GU385152	GU327746	-
<i>B. rhodomphala</i>	<i>incertae sedis</i>	GKM L153N	-	GU385157	GU327747	-
<i>B. salebrosa</i>	<i>incertae sedis</i>	SMH 2387	-	GU385162	GU327748	-
<i>B. schiedermayeriana</i>	<i>incertae sedis</i>	SMH 3157	-	GU385163	GU327745	-
<i>B. siamensis</i>	<i>incertae sedis</i>	MFLUCC 10-0099 <sup>H</sup>	KT289897	KT289895	KT962059	KT962061
<i>B. villosa</i>	<i>incertae sedis</i>	GKM 204N	-	GU385151	GU327751	-
<i>Corynespora cassiicola</i>	<i>Corynesporascaceae</i>	CBS 100822	GU296144	GU301808	GU349052	GU371742
<i>Cyclothyriella rubronotata</i>	<i>Cyclothyriellaceae</i>	CBS 419.85	-	GU301875	GU349002	GU371728
<i>Herpotrichia diffusa</i>	<i>incertae sedis</i>	CBS 250.62	DQ678019	DQ678071	DQ677915	DQ677968
<i>H. juniperi</i>	<i>incertae sedis</i>	CBS 200.31	DQ678029	DQ678080	DQ677925	DQ677978
<i>H. macrotricha</i>	<i>incertae sedis</i>	GKM 196N	-	GU385176	GU327755	-
<i>H. vaginatispora</i>	<i>incertae sedis</i>	MFLUCC 13-0865 <sup>H</sup>	KT934256	KT934252	KT934260	-
<i>Hysterium pulicare</i>	<i>Hysteriaceae</i>	CBS 123377	FJ161161	FJ161201	FJ161109	FJ161127
<i>Hysterobrevium mori</i>	<i>Hysteriaceae</i>	CBS 123563	FJ161155	FJ161196	FJ161104	-
<i>Leptosphaeria doliolum</i>	<i>Leptosphaeriaceae</i>	CBS 505.75	GU296159	GU301827	GU349069	KT389640
<i>Lophiostoma arundinis</i>	<i>Lophiostomataceae</i>	CBS 621.86	DQ782383	DQ782384	DQ782387	DQ782386
<i>Massaria inquinans</i>	<i>Massariaceae</i>	CBS 125591 <sup>E</sup>	HQ599442	HQ599400	HQ599340	-
<i>Massarina eburnea</i>	<i>Massarinaceae</i>	CBS 473.64	GU296170	GU301840	GU349040	GU371732
<i>Melanomma pulvis-pyrius</i>	<i>Melanommataceae</i>	CBS 124080 <sup>E</sup>	GU456302	GU456323	GU456265	GU456350
<i>M. pulvis-pyrius</i>	<i>Melanommataceae</i>	CBS 109.77	FJ201987	FJ201986	GU456274	GU456359
<i>M. pulvis-pyrius</i>	<i>Melanommataceae</i>	CBS 371.75	FJ201989	FJ201988	GU349019	GU371798
<i>Muriformistrickeria rubi</i>	<i>incertae sedis</i>	MFLUCC 15-0681 <sup>H</sup>	KT934257	KT934253	KT934261	-
<i>Neophiosphaerella sasicola</i>	<i>Lentitheciaceae</i>	MAFF 239644 <sup>E</sup>	AB524458	AB524599	AB539111	AB539098
<i>Phaeosphaeria oryzae</i>	<i>Phaeosphaeriaceae</i>	CBS 110110 <sup>E</sup>	GQ387530	GQ387591	-	KF252193
<i>Phragmocephala atra</i>	<i>incertae sedis</i>	MFLUCC 15-0021	KP698729	KP698725	-	-
<i>Prosthemia betulinum</i>	<i>Pleomassariaceae</i>	CBS 279.74	DQ678027	DQ678078	DQ677923	KT216532
<i>Pseudostrickeria muriformis</i>	<i>incertae sedis</i>	MFLUCC 13-0764 <sup>H</sup>	KT934258	KT934254	KT934262	-
<i>Pseudotrachia mutabilis</i>	<i>incertae sedis</i>	SMH 5288	-	GU385210	-	-
<i>Roussoella verrucispora</i>	<i>Thyridariaceae</i>	CBS 125434 <sup>H</sup>	AB524481	AB524622	AB539115	AB539102
<i>Sarimanas shirakamiense</i>	<i>incertae sedis</i>	KT 3000 <sup>H</sup>	LC001712	LC001715	-	-
<i>Seifertia azaleae</i>	<i>incertae sedis</i>	DAOM 239136	-	EU030276	-	-
<i>S. shangrilaensis</i>	<i>incertae sedis</i>	MFLUCC 16-0238 <sup>H</sup>	KU954102	KU954100	KU954101	-
<i>Sporormiella minima</i>	<i>Sporormiaceae</i>	CBS 524.50	DQ678003	DQ678056	DQ677897	DQ677950
<i>Teichospora trabicola</i>	<i>Teichosporaceae</i>	CBS 140730 <sup>E</sup>	-	KU601591	-	KU601600
<i>Tumularia tuberculata</i>	<i>incertae sedis</i>	CBS 256.84	-	GU301851	GU349006	-

<sup>a</sup>“H” : holotype, “P”: paratype, “E” epitype.

position, GTR+G for the *rpb2* second codon position, and GTR+G for the *rpb2* third codon position. Two simultaneous, independent runs of Metropolis-coupled Markovchain Monte Carlo (MCMC) were performed for 2 M generations with trees sampled every 1000 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.

## RESULTS

The ML and Bayesian phylogenetic analyses were conducted using an aligned sequence dataset composed of 969 nucleotides from SSU, 1 330 from LSU, 926 from *tef1*, and 1 026 from *rpb2*. The alignment contained a total of 72 taxa, which consisted of 56 taxa (77.8 %) in SSU, 72 (100 %) in LSU, 60 (83.3 %) in *tef1*, 50 (69.4 %) in *rpb2* (Table 5, 6). No significant conflict was observed among individual gene phylogenies, allowing the four genes to be combined into a single dataset. This combined dataset provided higher confidence values for the familial level than did those of the individual gene trees (data not shown). Of the 4 251 characters included in the alignment, 1 280 were variable and 2 900 were conserved. The ML tree with the highest log likelihood (−27528.4609) is shown in Fig. 40. The Bayesian likelihood score was −27459.4697. The topology recovered by the Bayesian analysis was almost identical to that of the ML tree, except for the position of *Aposphaeria corallinolutea*, *Herpotrichia macrotricha* and *Tumularia tuberculata*.

Monophyly of the genera with mycopappus-like propagules (*Mycodidymella*, *Petrakia*, *Pseudodidymella*, and *Xenostigmia*) was well-supported (99 % 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. 40), but its familial placement is unresolved.

Two families, including a new family (*Pseudodidymellaceae*); four genera; and six species, including two new species (*Melanomma japonicum* and *Pseudodidymella minima*) are described below.

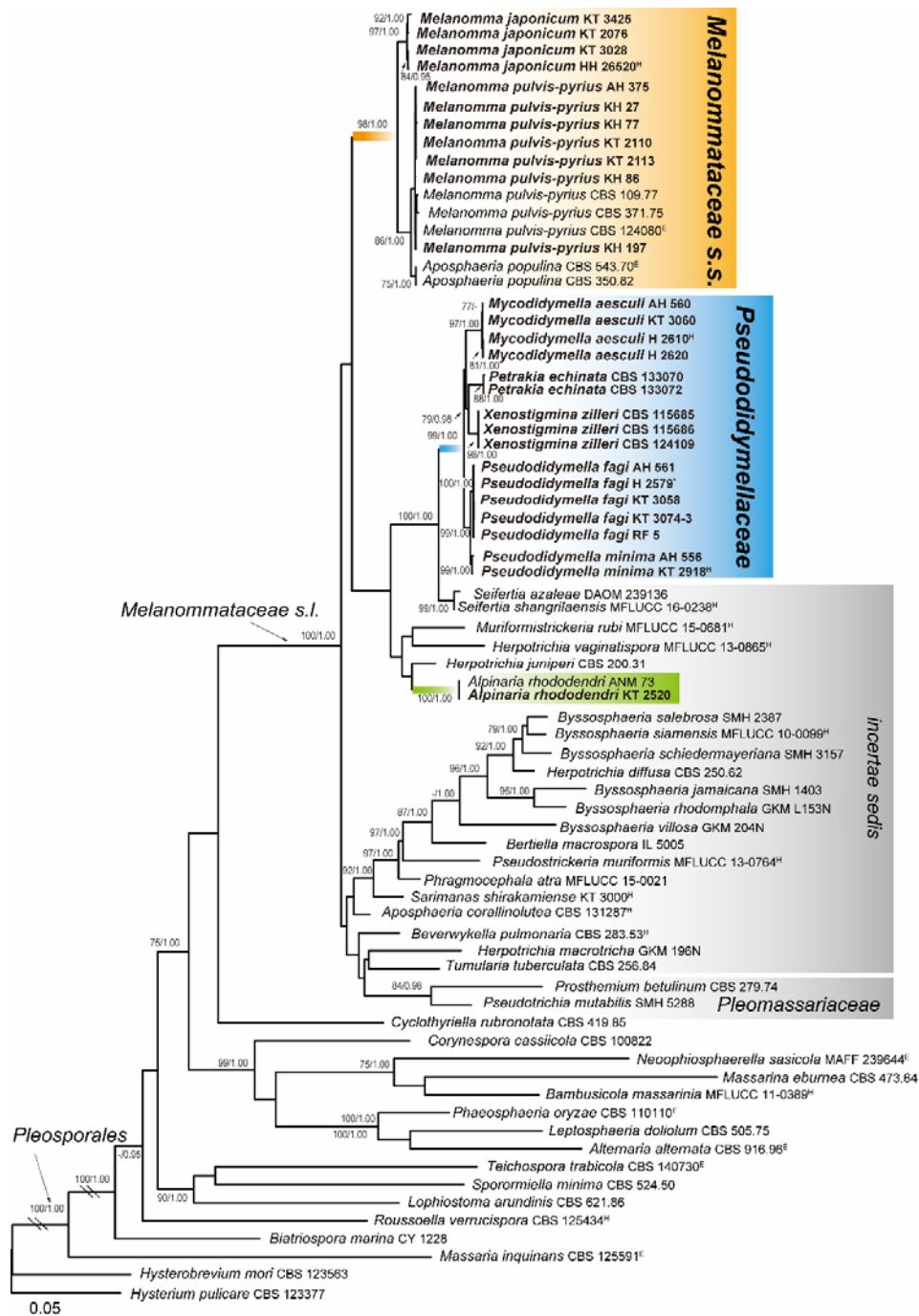
**Melanommataceae** G. Winter [as 'Melanommeae'], Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 1.2: 220. 1887.

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, multi-septate, smooth. **Asexual morph:** *Conidiomata* pycnidial, globose to subglobose, superficial, black, ostiolate. *Peridium* composed of elongate, brown cells. *Conidiophores* absent. *Conidiogenous cells* holoblastic, ampliform to cylindrical, hyaline. *Conidia* ellipsoidal, hyaline, smooth, aseptate.

*Type genus: Melanomma pulvis-pyrius* (Pers.) Fuckel, Jb. Nassau. Ver. Naturk. 23-24: 160. 1877.

*Notes* — *Melanommataceae* was established by Winter (1887). *Byssosphaeria*, *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 aposphaeria-like coelomycetous 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 *Byssosphaeria*, *Herpotrichia*, *Melanomma*, and *Pseudotrachia*, and previous familial concepts did not reflect natural relationships. Several genera, such as *Keissleriella* and *Ostropella*, were phylogenetically scattered in other *Pleosporales* families (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 *Xenostigmina* were treated as members of this family. However, the results of our phylogenetic analyses and morphological observation indicate that *Melanommataceae* should be restricted to its type genus, *Melanomma*.



**Fig. 40.** Maximum-likelihood tree of *Melanommataceae sensu strict* and *Pseudodidymellaceae* with its relatives. ML bootstrap proportion (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, epitype strains are indicated in <sup>H</sup> and <sup>E</sup>, respectively. Ex-holotype strains of *Pycnoplectospora fagi* is indicated in <sup>\*</sup>. The newly obtained sequences annotated in bold. The scale bar represents nucleotide substitution per site.

**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, ampliform to cylindrical, hyaline, smooth. *Conidia* ellipsoidal, hyaline, smooth, aseptate.

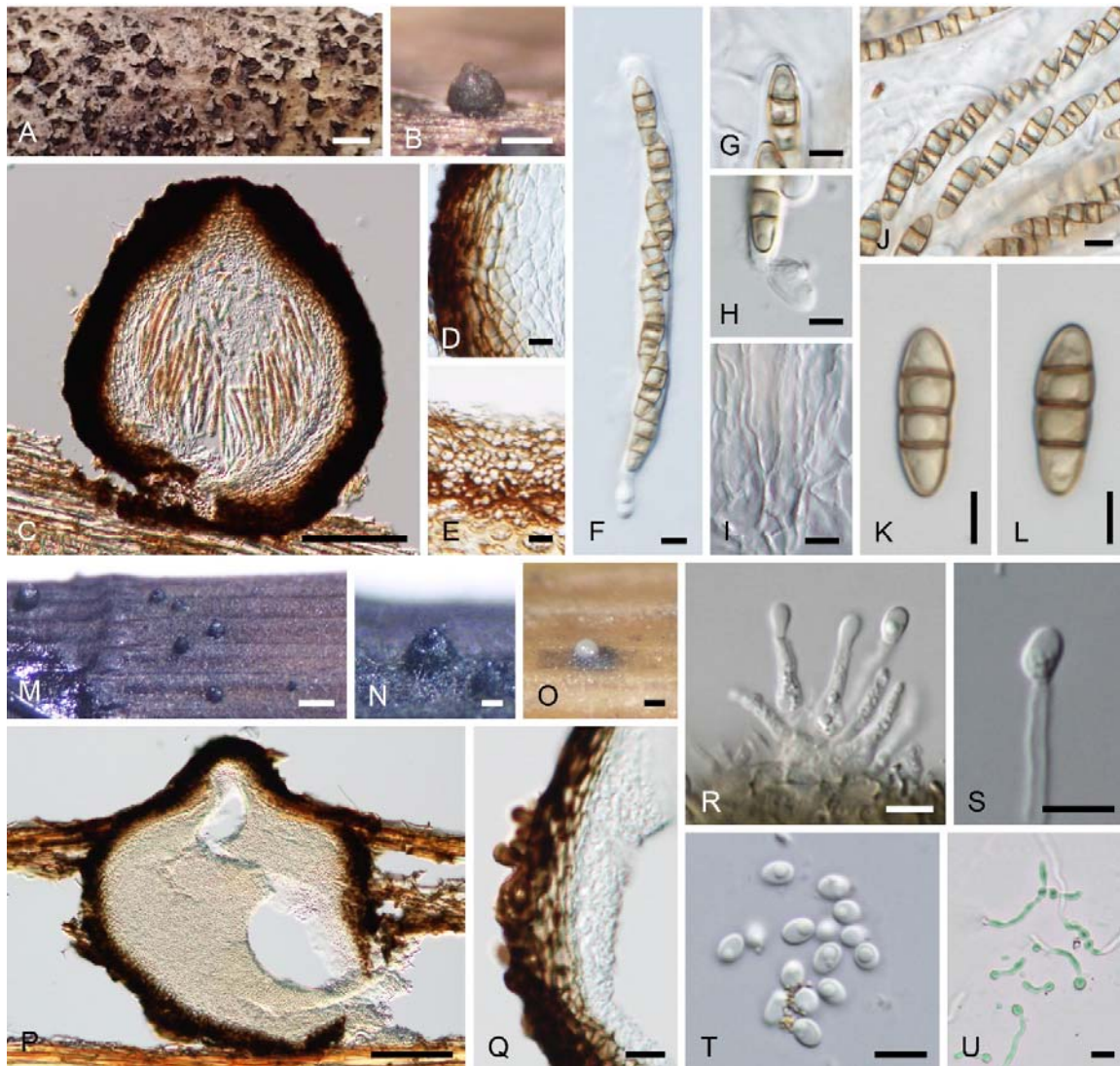
*Type species:* *Melanomma pulvis-pyrius* (Pers.) Fuckel, Jb. nassau. Ver. Naturk. 23–24: 160. 1870 (1869–1870)

*Notes* — The genus *Melanomma* was established by Fuckel (1870). Species in this genus are known to be saprobes 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 have been listed in Index Fungorum (<http://indexfungorum.org>). Asexual morphs of this genus were reported to be aposphaeria-like coelomycetes or *Nigrolentilocus* (Ichinoe 1970, Sivanesan 1984, Castañeda-Ruiz *et al.* 2001, Sánchez & Bianchinotti 2015, Tian *et al.* 2015).

***Melanomma japonicum*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB 819613; Fig. 41.

*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.

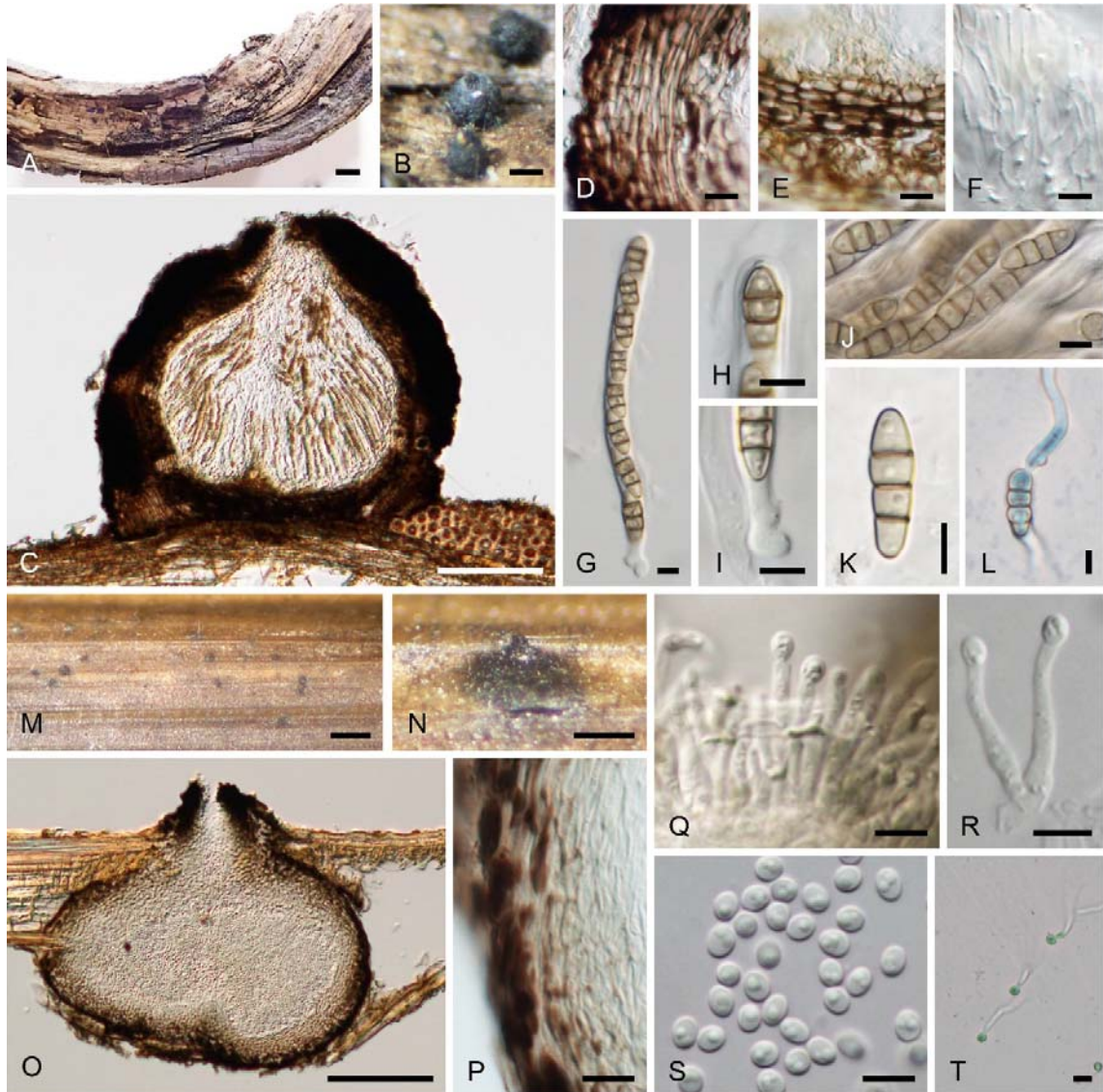


**Fig. 41.** *Melanomma japonicum*. A, B Ascomata on substrate; C Ascoma in longitudinal section; D Lateral peridium of ascoma; E Basal peridium of ascoma; F Ascus; G Apex of ascus; H Stipe of ascus; I Pseudoparaphyses; J–L Ascospores; M–O Conidiomata in culture; P Conidioma in longitudinal section; Q Peridium of conidioma; R, S Conidiogenous cells; T Conidia; U Germinating conidia. A, C–J from HHUF 26520; B, K, L HHUF 30540; M–O from culture KT 3028; P–U from culture JCM 13124. Scale bars: A = 500  $\mu\text{m}$ ; B = 200  $\mu\text{m}$ ; C, N–P = 100  $\mu\text{m}$ ; D, E, G–M, R–U = 5  $\mu\text{m}$ ; F, Q = 10  $\mu\text{m}$ .

*Pseudoparaphyses* trabeculate, 0.5  $\mu\text{m}$  wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 73–105  $\times$  5.5–9  $\mu\text{m}$  ( $\bar{x}$  = 89.9  $\times$  7  $\mu\text{m}$ , n = 26), with a short stipe (7–16  $\mu\text{m}$  long,  $\bar{x}$  = 10.3  $\mu\text{m}$ , n = 20), apically rounded with an ocular chamber, 8-spored. *Ascospores* fusiform, with broad rounded ends, straight to slightly curved, 12–19  $\times$  3–7  $\mu\text{m}$  ( $\bar{x}$  = 15.1  $\times$  4.6  $\mu\text{m}$ , n = 151), l/w 2.5–4.9 ( $\bar{x}$  = 3.4, n = 151), 3-septate, with a primary septum nearly median (0.44–0.57,  $\bar{x}$  = 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  $\mu\text{m}$  high in section, 150–250  $\mu\text{m}$  diam, semi-immersed, solitary. *Ostiolar neck* short papillate, composed of thick-walled, black cells. *Peridium* 12–33.5  $\mu\text{m}$  wide, composed of 8.5–16.5  $\times$  3.5–7.5  $\mu\text{m}$ , rectangular, brown cells. *Conidiophores* reduced to



**Fig. 42.** *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. A–F, J–L from HHUF 30544; G–I from HHUF 30543; M–Q, T from culture KH 97; R, S from culture AH 375. Scale bars: A, M = 500  $\mu\text{m}$ ; B, N = 200  $\mu\text{m}$ ; C, O = 100  $\mu\text{m}$ ; D, E, P = 10  $\mu\text{m}$ ; F–L, Q–T = 5  $\mu\text{m}$ .

conidiogenous cells. *Conidiogenous cells* holoblastic, 8–13.5 × 2–3 µm, cylindrical, hyaline, smooth. *Conidia* cylindrical with rounded ends, 3–4 × 2–2.5 µm ( $\bar{x}$  = 3.3 × 2.2 µm, n = 50), l/w 1.1–2.1 ( $\bar{x}$  = 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 (105: Rayner 1970), grey olivaceous (107) at centre; reverse smoke grey (105), grey olivaceous (108) at margin (Fig. 47a); asexual morph formed.

*Specimens examined.* **JAPAN**, Aomori, Hakkoda, Okiagetai, on dead twigs of woody plant, 15 Apr. 2006, K. Tanaka, KT 2076 (HHUF 30539 paratype); Akita, Kazuno, Hachimantai, Yakeyama, Mousen pass, on dead twigs of *Fagus crenata*, 24 Jun. 2012, K. Tanaka, KT 3028 (HHUF 30540 paratype); Kagoshima, Tarumizu, Mt. Oonogara, on dead twigs of *Fagus crenata*, 25 Oct. 2013, K. Tanaka, KT 3425 (HHUF 30541 paratype); Aomori, Hakkoda, near Yunotai, on dead twigs of woody plant, 21 Jul. 2001, Y. Harada, (HHUF 26520 **holotype**, ex-holotype living culture 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 more slender (3–4 µm vs. (2–)2.5–3.5 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. 42.

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* trabeculate, 1–1.5 µm wide. *Asci* 71–92 × 5–8.5 µm ( $\bar{x}$  = 82.1 × 6.3 µm, n = 14), with a short stipe (5–8 µm long,  $\bar{x}$  = 5.7 µm, n = 12). *Ascospores* 11.5–15.5 × 4–5 µm ( $\bar{x}$  = 13 × 4.2 µm, n = 75), l/w 2.5–3.6 ( $\bar{x}$  = 3.1, n = 75), 3-septate, with a primary septum nearly median (0.45–0.58,  $\bar{x}$  = 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 conidiogenous cells. *Conidiogenous cells* holoblastic, 8–17.5 × 1.5–4 µm, cylindrical, hyaline, smooth. *Conidia* cylindrical with rounded ends, (2–)2.5–3.5 × 2–2.5(–3) µm ( $\bar{x}$  = 2.9 × 2.3 µm, n = 50), l/w 1.0–1.7 ( $\bar{x}$  = 1.3, n = 50), hyaline, smooth, aseptate, guttulate when young.

Culture characteristics — Colonies on PDA attaining 22–24 mm diam within 21 d, floccose, fasciculate, centrally raised, pale olivaceous grey (120); reverse greyish sepia (106), olivaceous buff (89) at margin (Fig. 47b); asexual morph formed.

*Specimens examined.* **JAPAN**, Aomori, Minamitsugaru, Owani, on dead twigs of *Acer mono* var. *mayrii*, 1 Jul. 2006, K. Tanaka, KT 2110 (HHUF 30542); Hirosaki, Zatoishi, on dead twigs of woody plant, 8 Jul. 2006, H. Yonezawa, KT 2113 (HHUF 30543); Noheji, near Mt. Eboshi, on dead twigs of *Fagus crenata*, 2 Sep. 2015, A. Hashimoto *et al.*, AH 375 (HHUF 30544); Nishimeya, Ooshirosawa stream, on dead twigs of woody plant, 25 Jun. 2007, K. Hirayama *et al.*, KH 27 (HHUF 30545); on dead twigs of woody plant, 21 Jul. 2007, K. Hirayama *et al.*, KH 77 (HHUF 30546); Kawaratai, Ookawazoe, on dead twigs of woody plant, 28 Aug. 2007, K. Hirayama *et al.* KH 86 (HHUF 30547); on dead twigs of woody plant, 30 Aug. 2008, K. Hirayama *et al.*, KH 197 (HHUF 30548).

*Notes* — The above specimens were identified as *Me. pulvis-pyrius*, the type species of *Melanomma*. The size of ascospores in our material 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-epitype culture (CBS 124080).

*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 *Aposphaeria populina* (CBS 543.70 and CBS 350.82) with moderate to strong support (86 % 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 MB 819614.

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* epiphyllous, white to yellowish, globose to subglobose, multicellular, with numerous, flexuous, cylindrical, multi-septate hyphal appendages, detached at stroma-like base composed of subglobose to oblong, hyaline to yellow cells. **Synasexual morph:** *Conidiomata* sporodochial, superficial. *Stromata* composed of globose to subglobose cells. *Conidiophores* reduced. *Conidiogenous cells* annellidic

or holoblastic. *Conidia* clavate, sigmoid or rounded to oval or broadly ellipsoidal, phragmosporous to muriform, hyaline to brown, falcate to sigmoid.

*Type genus: Pseudodidymella* C.Z. Wei, Y. Harada & Katum.

*Notes* — *Mycodidymella*, *Petrakia*, *Pseudodidymella*, and *Xenostigmina* 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, Buntin *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 *Petrakia* and *Xenostigmina* 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 (97 % ML BP/ 1.00 Bayesian PP; Fig. 40). 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.

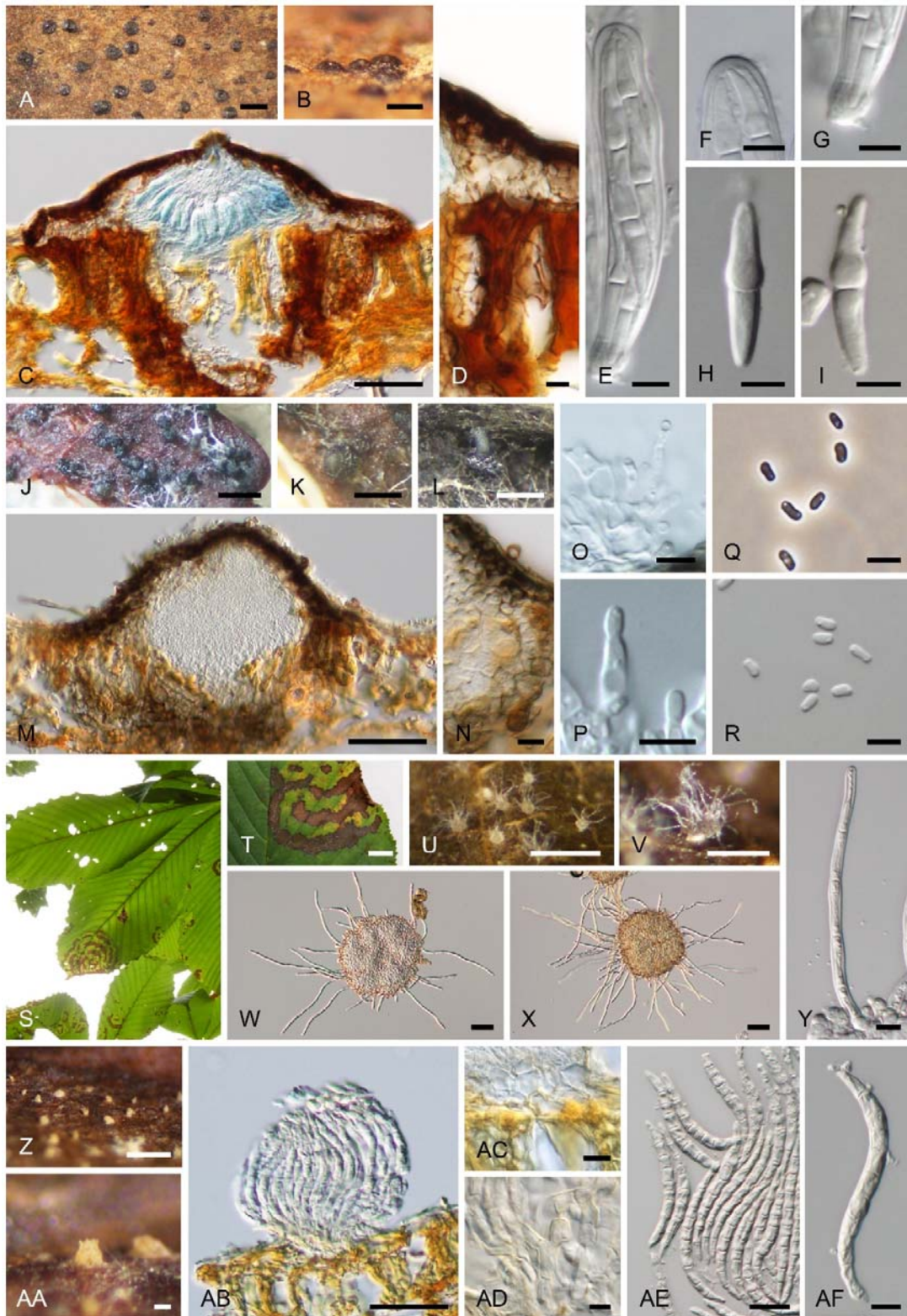
***Mycodidymella*** C.Z. Wei, Y. Harada & Katum., Mycologia 90(2): 336. 1998.

*Synonym: Blastostroma* C.Z. Wei, Y. Harada & Katum., Mycologia 90(2): 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, Y. Harada & Katum.





◀ **Fig. 43.** *Mycodidymella aesculi*. 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 *Aesculus turbinata* with necrotic brown spots; U, V Propagules on the leaf surface; W, X Propagules; Y Appendage of propagule; Z, AA Sporodochia on the leaf surface; AB Sporodochium in longitudinal section; AC Stroma of sporodochium; AD Conidiogenous cells; AE, AF Conidia. A–I from HHUF 22892; J–R from culture culture KT 3060; S, T from HHUF 30550; U–Y from HHUF 30549; Z–AF from HHUF 23078. Scale bars: A, J, T, Z = 500 µm; B, K, L, U, V = 250 µm; C, M, AA, AB, W, X = 50 µm; D, E, N, Y, AC, AE, AF = 10 µm; F–I, O–R, AD = 5 µm.

***Mycodidymella aesculi*** C.Z. Wei, Y. Harada & Katum., *Mycologia* 90: 336. 1998; Fig. 43.

*Synonyms:* *Blastostroma aesculi* C.Z. Wei, Y. Harada & Katum., *Mycologia* 90: 338. 1998.

*Mycopappus aesculi* C.Z. Wei, Y. Harada & Katum., *Mycologia* 90: 336. 1998.

*Petrakia aesculi* (C.Z. Wei, Y. Harada & Katum.) Jaklitsch & Voglmayr, *Sydowia* 69: 91. 2017

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 × 6–9 µm, pale brown cells, at base 105–140 µm thick, composed of 8.5–11.5 × 6.5–8.5 µm, hyaline to pale brown cells. *Pseudoparaphyses* numerous, trabeculate, 0.8–1.3 µm wide, septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 45.5–60 × 7–12.5 µm ( $\bar{x}$  = 53.3 × 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 × 3–4.5 µm ( $\bar{x}$  = 18.6 × 3.9 µm, n = 21), l/w 4.3–5.3 ( $\bar{x}$  = 4.7, n = 21), with a septum nearly median (0.44–0.55,  $\bar{x}$  = 0.51, n = 21), constricted at the septum, hyaline, smooth, guttulate when young. *Spermatia* 3–5 × 1–2 µm ( $\bar{x}$  = 3.6 × 1.5 µm, n = 50), l/w 1.7–3.7 ( $\bar{x}$  = 2.5, n = 50), cylindrical, hyaline. **Asexual morph:** *Propagules* epiphyllous, white to yellowish, globose to subglobose, 200–565 µm diam ( $\bar{x}$  = 331.9 µm, n = 30); main bodies subglobose to oblong, 85–193 × 116–228 µm ( $\bar{x}$  = 127.6 × 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 × 3.5–5.5 µm ( $\bar{x}$  = 111.5 × 4.6, n = 30). **Synasexual morph:** *Conidiomata* sporodochial, white to yellowish. *Stromata* 15–20 µm thick, composed of hyaline, globose to subglobose cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, hyaline, smooth, 9–12 × 4–5.5 µm. *Conidia* falcate to sigmoid, 57–94 × 5.5–8.5 µm ( $\bar{x}$  = 75.8 × 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 (45), grey olivaceous (107) at centre; reverse buff (45); grey olivaceous (107) 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 3060, (HHUF 30549); Nishimeya, Kawaratai, Ookawazoe, 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 Sept. 2016, A. Hashimoto, AH 560 (HHUF 30550); Ikarigaseki, on living leaves of *Aesculus turbinata*, 18 Apr. 1995, C. Z. Wei & Y. Harada, H 2610 (HHUF 22892 **holotype** of *Mycodidymella aesculi*); on living leaves of *Aesculus turbinata*, 18 Apr. 1995, C. Z. Wei & Y. Harada, H 2620.

*Notes* — The genus *Mycodidymella* 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 *Petrakia*; they found that the sexual morphology of *Petrakia* matches that of *Mycodidymella* and thus synonymised *Mycodidymella* with *Petrakia* (Butin *et al.* 2013). This proposal was accepted by subsequent studies (Tian *et al.* 2015, Li *et al.* 2016). Jaklitsch & Voglmayr (2017) proposed that *Mycodidymella* is a synonym of *Petrakia*, according to their morphological resemblances of sexual morph and mycopappus-like propagules. However, our phylogenetic study revealed that their monophyletic status was not supported in any analyses (below 60 % ML BP/ 0.95 Bayesian PP, Fig. 40). We retained *Mycodidymella* as a natural genus in *Pseudodidymellaceae* (discussed below).

***Pseudodidymella*** C.Z. Wei, Y. Harada & Katum., Mycologia 89: 496. 1997.

*Synonym:* *Pycnopleiospora* C.Z. Wei, Y. Harada & Katum., Mycologia 89: 496. 1997.

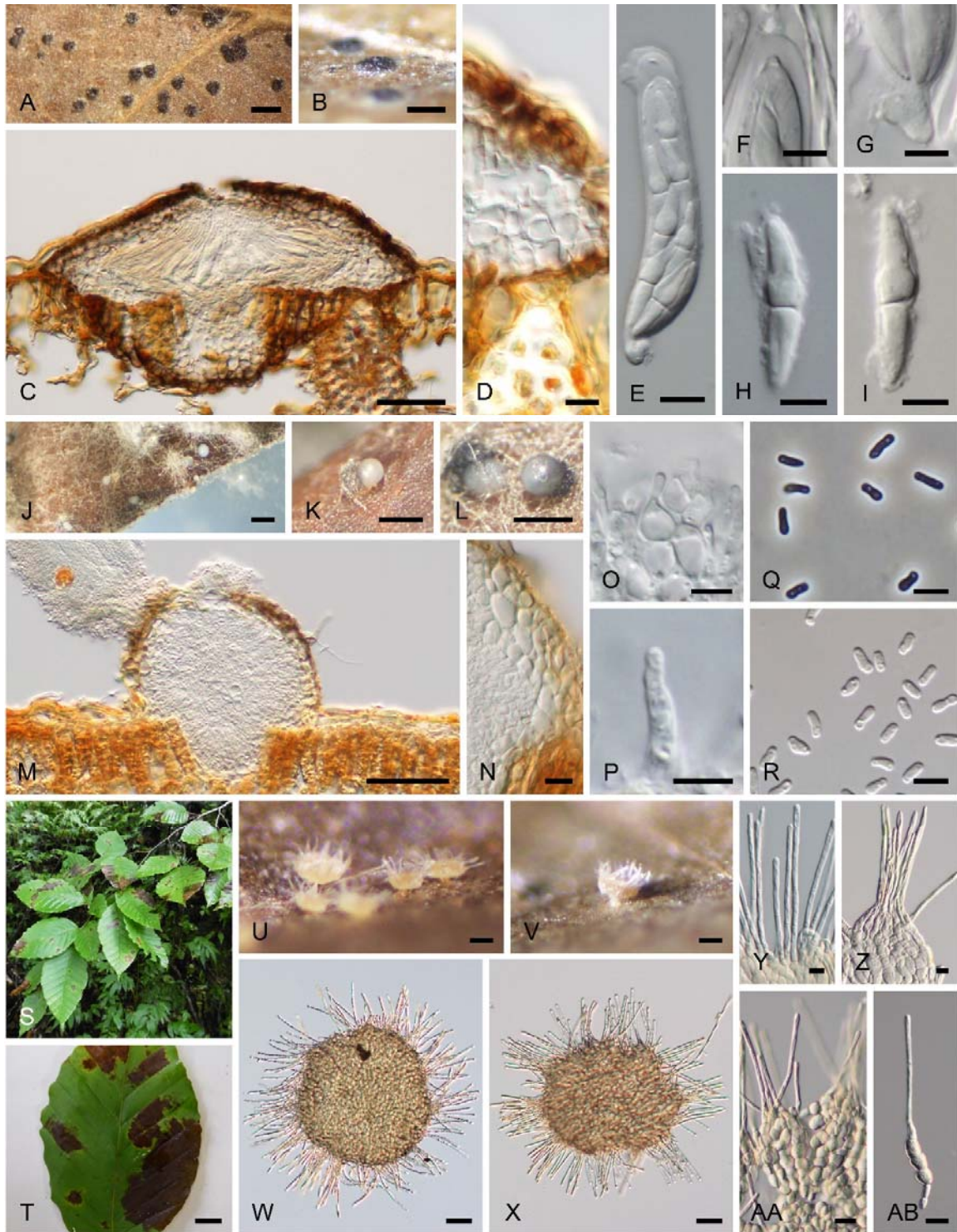
Parasitic on living leaves of *Fagus* spp. **Sexual morph:** *Ascomata* subglobose to lenticular, solitary to grouped, immersed, ostiolate. *Peridium* composed of rectangular, thin-walled, pale brown cells, well-developed at base. *Pseudoparaphyses* septate, branched and anastomosed. *Asci* bitunicate, fissitunicate, cylindrical, 8-spored. *Ascospores* fusiform with rounded ends, 1-

septate, hyaline, smooth. *Spermatia* cylindrical, hyaline. **Asexual morph:** Propagules epiphyllous, white to yellowish, globose, multicellular; sclerotia-like multicellular structures globose, subglobose, hyaline to yellow, bearing numerous, unbranched, flexuous, multi-septate hyphal appendages.

*Type species: Pseudodidymella fagi* C.Z. Wei, Y. Harada & Katum., Mycologia 89: 496. 1997.

*Notes* — The genus *Pseudodidymella*, based on the type species *Pseudod. fagi*, has lenticular ascomata and a pycnopleiospora-like asexual morph which is characterised by sporodochial conidiomata and appendage-bearing conidia (Wei *et al.* 1997). Because its sexual morph morphologically resembles that of *Didymella*, this genus was considered a member of *Phaeosphaeriaceae* (Wei *et al.* 1997). The sexual morph of this genus superficially resembles that of *Mycodidymella*, but it can be distinguished based on its pycnopleiospora-like asexual morph (Wei *et al.* 1998). Since then, this genus has been treated as *incertae sedis* in *Dothideomycetes* (Lumbsch & Huhndorf 2007). Gross *et al.* (2017) discovered *Pseudod. fagi* on *Fagus sylvatica* in Switzerland; they noted that the asexual morph of this species was previously recorded as *Pycnopleiospora*, but actually has mycopappus-like propagules rather than individual conidia, and the original description (Wei *et al.* 1997) seemed to misinterpret over-mature propagules. They also confirmed that *Pseudodidymella* is phylogenetically related to other mycopappus-forming genera, such as *Mycodidymella*, *Petrakia*, and *Xenostigmina*, based on the ITS phylogeny. Thus, morphological delimitation of *Pseudodidymella* and *Mycodidymella* is problematic and requires further research. In the present study, we recollected *Pseudod. fagi* 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. 44AA, 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, this study). Morphologically, *Pseudodidymella* resembles *Mycodidymella*, but can be distinguished based on the rim-like walls of the ascomata, and numerous hyphal appendages in the asexual morph.



◀ **Fig. 44.** *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 culture KT 3058; 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.

***Pseudodidymella fagi*** C.Z. Wei, Y. Harada & Katum., Mycologia 89: 496. 1997; Fig 44.

**Synonym:** *Pycnopleiospora fagi* C.Z. Wei, Y. Harada & Katum., 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 ( $\bar{x}$  = 60.3 × 11.5 µm, n = 20), with a short stipe (3.5–8 µm long,  $\bar{x}$  = 6.1 µm, n = 20), apically rounded with an ocular chamber, 8-spored. *Ascospores* fusiform with rounded ends, straight, 18.5–24 × 4–5 µm ( $\bar{x}$  = 20.5 × 4.3 µm, n = 20), l/w 4.3–5.6 ( $\bar{x}$  = 4.8, n = 20), with a septum nearly median (0.47–0.58,  $\bar{x}$  = 0.52, n = 20), constricted at the septum, hyaline, smooth, guttulate when young. *Spermatia* 3–5 × 1–1.5 µm ( $\bar{x}$  = 3.9 × 1.2 µm, n = 50), l/w 2.1–4.8 ( $\bar{x}$  = 3.3, n = 50), cylindrical, hyaline. **Asexual morph:** *Propagules* epiphyllous, white to yellowish, globose, 290–500 µm diam ( $\bar{x}$  = 387.2 µm, n = 30); main bodies globose, 160–315 µm diam ( $\bar{x}$  = 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 ( $\bar{x}$  = 97.1 × 3.7 µm, n = 52).

Culture characteristics — Colonies on PDA attaining 27–37 mm diam within 21 d, velvety, plane, buff (45) to olivaceous black (108) at centre; reverse buff (45) to olivaceous black (108) at centre (Fig. 47e); spermatial and asexual morphs formed.

**Specimens examined.** **JAPAN**, Aomori, Nakatsugaru, Onikawabe, on living leaves of *Fagus crenata*, 12 Aug. 2012, K. Tanaka *et al.*, KT 3058 (HHUF 30515); Nishimeya, Ookawazoe, near Annmon waterfall trail, on living leaves of *Fagus crenata*, 2 Sep. 2012, K. Tanaka *et al.*, KT 3074-3 (HHUF 30516); 2 Sep. 2012, R. Fujimoto *et al.*, RF 5 (HHUF 30517); 10 Sep. 2016, A. Hashimoto, AH 558 (HHUF 30553); Ikarigaseki, on living leaves of *Fagus crenata*, 28 Apr. 1995, C. Z. Wei & Y. Harada, H 2579 (HHUF 22903 **holotype** of *Pseudodidymella fagi*); artificial inoculation on leaves of *Fagus crenata*, 30 Sep. 1996, C. Z. Wei (HHUF 23672; **holotype** of *Pycnopleiospora fagi*).

*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, additional survey of this fungus on other *Fagus* species is needed in the future.

***Pseudodidymella minima*** A. Hashim. & Kaz. Tanaka, **sp. nov.** — MycoBank MB 819615; Fig. 45.

*Etymology*: Referring to its smaller size propagules observed in this species.

Parasitic on living leaves of *Fagus japonica*. **Sexual morph**: Unknown. **Asexual morph**: *Propagules* epiphyllous, white to yellowish, globose, 110–220(–240)  $\mu\text{m}$  diam ( $\bar{x}$  = 164.4  $\mu\text{m}$ , n = 60); sclerotia-like structures globose, multicellular, 78–168  $\mu\text{m}$  diam ( $\bar{x}$  = 115  $\mu\text{m}$ , n = 60), composed of subglobose, 7.5–10  $\mu\text{m}$  diam hyaline to yellow cells; hyphal appendages 65 to 135, unbranched, flexuous, cylindrical, 1–2-septate or rarely aseptate, 27–44  $\times$  3–6  $\mu\text{m}$  ( $\bar{x}$  = 35.5  $\times$  4.4  $\mu\text{m}$ , n = 59).

Culture characteristics — Colonies on PDA attaining 32–38 mm diam within 21 d, floccose, plane, smoke grey (105); reverse honey (64) to Isabelline (65) (Fig. 47d); 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**); 3 Sept. 2016, A. Hashimoto, AH 556 (HHUF 30552 paratype).

*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.

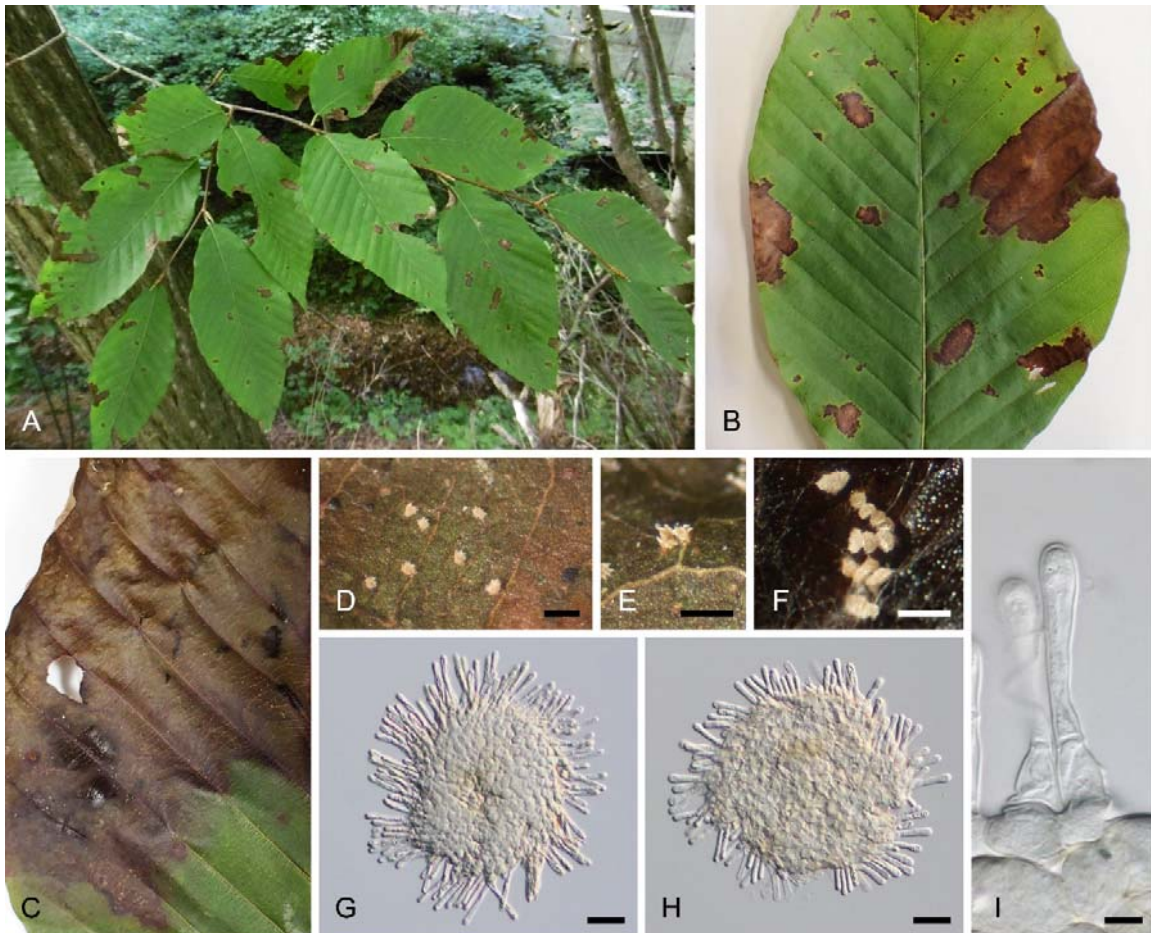
We did not observe sexual and synasexual morph of *Pseudod. minima*. Further survey is needed in the future to reveal ecological features of this species.

### ***Incertae sedis***

***Alpinaria*** Jaklitsch & Voglmayer, 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, septate, confluent, multiloculate, scattered, semi-immersed, black, with one to two non-papillate ostiole. *Peridium* rectangular, brown cells. *Conidiophores*





**Fig. 45.** *Pseudodidymella minima*. A–C Leaves of *Fagus japonicum* with necrotic brown spots; D–F Propagules on the leaf surface; G, H Propagules; I Appendages of propagule. A–C, H from HHUF 30552; D, E, G, I from HHUF 30551; F from culture KT 2918. Scale bars: D–F = 250 µm; G, H = 50 µm; I = 5 µm.

absent. *Conidiogenous cells* holoblastic, cylindrical, hyaline, smooth. *Conidia* cylindrical with rounded ends, hyaline, smooth, aseptate.

*Type species: Alpinaria rhododendri* (Niessl) Jalitsch & Voglmayer

***Alpinaria rhododendri*** (Niessl) Jalitsch & Voglmayer, *Sydowia* 69: 84. 2017; Fig. 46.

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

*Synonyms: Melanomma rhododendri* Rehm, *Ber. Naturhist. Ver. Augsburg* 26: 48. 1881.

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





**Fig. 46.** *Alpinaria rhododendri*. A, B Ascomata on substrate; C Ascoma in longitudinal section; D Lateral peridium of ascoma; E Ascus; F Apex of ascus; G Stipe of ascus; H Pseudoparaphyses; I–K Ascospores; L, M Conidiomata in culture; N Conidiomata in longitudinal section; O Peridium of conidiomata; P, Q Conidiogenous cells; R Conidia; S Germinating conidia. A–K from HHUF 30554; L–S from culture KT 2520. Scale bars: A, L = 500  $\mu$ m; B, C, M, N = 100  $\mu$ m; D, E, O = 10  $\mu$ m; F–K, P–S = 5  $\mu$ m.

*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  $\mu$ m high, 110–250  $\mu$ m diam. Ostiolar neck short papillate, composed of carbonaceous, thick-walled, black cells. *Peridium* 55–75  $\mu$ m thick at side composed of elongate, thin-walled, 12–13  $\times$  5–6.5  $\mu$ m, brown cells, 87–102  $\mu$ m thick at base composed of elongate, thin-walled, 4–6  $\mu$ m diam, hyaline cells. *Pseudoparaphyses* trabeculate, 1–1.5  $\mu$ m wide, septate, branched and anastomosed. *Asci* bitunicate, cylindrical, 100–118  $\times$  7–9  $\mu$ m ( $\bar{x}$  = 109.5  $\times$  7.8  $\mu$ m,

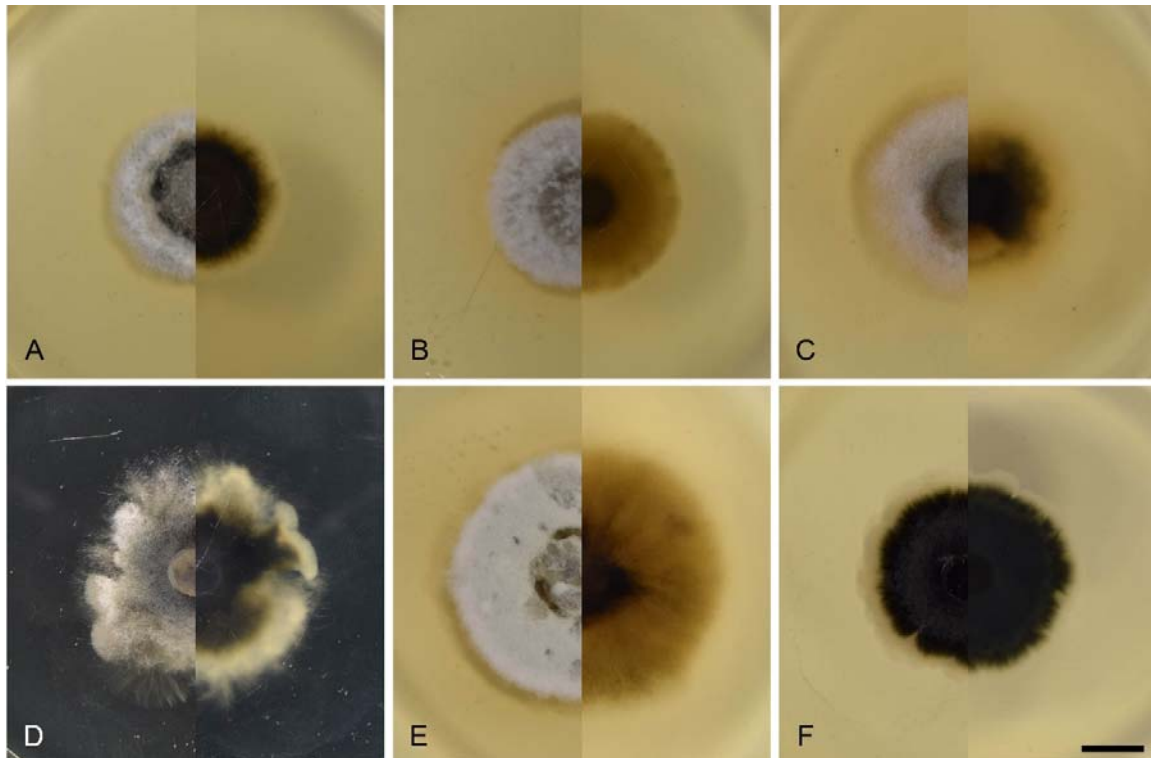
n = 11), with a short stipe (3.5–10  $\mu\text{m}$  long,  $\bar{x}$  = 7  $\mu\text{m}$ , n = 11). *Ascospores* fusiform, 13–21.2  $\times$  5–6  $\mu\text{m}$  ( $\bar{x}$  = 16.5  $\times$  5.6  $\mu\text{m}$ , n = 50), l/w 2.2–4.2 ( $\bar{x}$  = 3.0, n = 50), 3-septate, with a primary septum nearly median (0.42–0.57,  $\bar{x}$  = 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  $\mu\text{m}$  high, 110–250  $\mu\text{m}$  diam. *Ostiolar neck* mainly single, occasionally two, non-papillate. *Peridium* 20–25  $\mu\text{m}$  wide, composed of 7.5–11.5  $\times$  5–7  $\mu\text{m}$ , rectangular, brown cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, 6–10.5  $\times$  3–4.5  $\mu\text{m}$ , cylindrical, hyaline, smooth. *Conidia* cylindrical with rounded ends, 2–4  $\times$  1–2  $\mu\text{m}$  ( $\bar{x}$  = 3  $\times$  1.6  $\mu\text{m}$ , n = 50), l/w 1.1–2.6 ( $\bar{x}$  = 1.9, n = 50), hyaline, smooth, aseptate, guttulate when young.

Culture characteristics — Colonies on PDA attaining 26–31 mm diam within 21 d, velvety, wetty, olivaceous black (108), smoky grey (105) at margin; reverse olivaceous black (108) at centre (Fig. 47f); 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).

*Notes* — The size of ascospores in the material mentioned above was 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 were completely identical to those from the epitype strain of this species (CBS 141994). The 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 subalpine zone in Japan. *Alpinaria rhododendri* appears to be relatively common species in subalpine to alpine zone worldwide.

*Alpinaria* was recently established to accommodate single species *A. rhododendri*, which is transferred from *Melanomma* because the species is phylogenetically distinct from the type species of *Melanomma*, and possesses roughened surface view ascomata composed of prismatic and angular cells (Jaklitsch & Voglmayr 2017). 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. 46L–S). Result of our detailed observation of asexual morph as well as sexual morph clarified this species has atypical features for *Melanommataceae*; its ascomata composed of hyaline cells at the base, and pseudopycnidial, confluent conidiomata are not found in sexual/asexual morphs of



**Fig. 47.** Colony characters of *Melanomma* spp. and *Pseudodidymellaceae* spp. used in this study on PDA within 3 wk at 20 °C in the dark (left: upper, right: reverse). A *Melanomma japonicum* (JCM 13124 = MAFF 239634, ex-holotype culture); B *Me. pulvis-pyrius* (culture AH 375); C *Mycodidymella aesculi* (culture H 2510); D *Pseudodidymella fagi* (MAFF 245740, ex-holotype culture of *Pycnopleiospora fagi*); E *Pseudod. minima* (culture KT 2918); F *Alpinaria rhododendri* (culture KT 2520). Scale bar: a–f = 1 cm.

*Melanommataceae*. In our phylogenetic tree, the genus confirmed its placement in outside of *Melanommataceae sensu stricto* (Fig. 40). We treat *Alpinaria* 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 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 a study by Mugambi & Huhndorf (2009), who applied a molecular approach. A recent monograph of *Melanommataceae* was based on morphological and multi-gene phylogenetic studies (Tian *et al.* 2015). Although monophyly of *Melanommataceae* was confirmed in previous studies, statistical support for *Melanommataceae sensu lato* was not strong (Mugambi & Huhndorf 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, synnematos, and pycnidial conidiomata, are known in this family. Two of the most striking genera are *Petrakia* and *Xenostigmina*, which have mycopappus-like propagules in asexual morphs and were reported to be necrotrophs of leaves (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 aposphaeria-like pycnidial asexual morphs and are known to be saprobes on twigs of various plants (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. 40), 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 (*Mycodidymella*, *Petrakia*, *Pseudodidymella*, and *Xenostigmina*) are separated from *Melanommataceae sensu stricto*, and we thus establish a new family, *Pseudodidymellaceae*, to accommodate these genera.

### **Relationships among genera in *Pseudodidymellaceae***

*Mycodidymella* and *Xenostigmina* are retained as natural genera in the present study. Butin *et al.* (2013) found that the sexual morph of *Mycodidymella* is similar to that of *Petrakia* and thus recognised *Petrakia* in a broad sense and included *Mycodidymella* 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 *Mycodidymella* and *Xenostigmina* are synonyms of *Petrakia*. They considered that phylogenetic relatedness of *Xenostigmina* and *Petrakia*, and morphological resemblances of sexual morph and mycopappus-like propagules among these genera are strong arguments for synonymising them (Jaklitsch & Voglmayr 2017). Our phylogenetic analysis including *Mycodidymella* as well as *Xenostigmina* and *Petrakia* clarified that their monophyletic status was not well supported in any analyses (below 60 % ML BP/ 0.95 Bayesian PP, Fig. 40). Their sexual morphs are superficially similar as indicated by Jaklitsch & Voglmayr (2017), but *Mycodidymella* has deeper and well-

developed ascomata (up to 210  $\mu\text{m}$  high) than those of *Petrakia* (up to 150  $\mu\text{m}$  high) and *Xenostigmina* (up to 100  $\mu\text{m}$  high). Additionally, their morphological character of synasexual morphs are also different; hyaline, up to 20  $\mu\text{m}$  thick sporodochia, holoblastic conidiogenous cells, and sigmoid, multi-septate, thin-walled, hyaline conidia (*Mycodidymella*; this study); brown, up to 30  $\mu\text{m}$  thick sporodochia, annellidic conidiogenous cells, and globose to ovoid, dictyosporus, thick-walled, brown conidia with cellular appendages (*Petrakia*; Butin *et al.* 2013, Li *et al.* 2016); and brown to black, up to 45  $\mu\text{m}$  high sporodochia, holoblastic conidiogenous cells, and clavate with a short rostrum, dictyosporus, thick-walled, brown conidia (*Xenostigmina*; Funk 1986, Crous 1998). Therefore, we treated these genera as distinct based on morphological differences of sexual and synasexual morphs.

Synasexual stages 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 stages were not observed on overwintered leaves for *Petrakia* and *Mycodidymella*, 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 *Mycopappus* was established based on its type species *Mycop. 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: *Mycop. aceris* (on *Acer macrophyllum*; Redhead & White 1985), *Mycop. aesculi* (on *Aesculus turbinata*; Wei *et al.* 1998), and *Mycop. quercus* (on *Quercus acutissima*; Suto & Kawai 2000). Two species, *Mycop. alni* and *Mycop. 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 stage and phylogenetic analyses using ITS sequences (Takahashi *et al.* 2006). The two other species, *Mycop. aceris* and *Mycop. aesculi*, were excluded from *Mycopappus sensu stricto*, because their sexual morphs belong to the dothideomycetous taxa *Xenostigmina zilleri* (Funk & Dorworth 1988, Crous 1998, Crous *et al.* 2009) and *Mycodidymella 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 sclerotiniaceous species (*Mycop. alni* and *Mycop. quercus*) are composed of multi-septate claviform cells (Suto & Kawai 2000, Suto & Suyama 2005, Takahashi *et al.* 2006), whereas those of dothideomycetous species (*Mycod. aesculi* and *X. zilleri*) 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 *Spirosphaera* (*Leotiomycetes*), 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 stage 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.* 2013, Gross *et al.* 2017, Jaklitsch & Voglmayr 2017). Our observations revealed that morphological features of propagules differed between *Mycodidymella*, *Petrakia*, and *Xenostigmina* (with few appendages), and *Pseudodidymella* (with numerous appendages). The hyphal appendages of *Pseudodidymella* could enhance fungal encounters with *Fagus* leaves that have conspicuous wax ornamentation (Denk 2003), as is the case of asexual fungi with conidial appendages (Nag Raj 1993, Hashimoto *et al.* 2015a). The morphological variation of propagules is also observed at the species level: *Pseudod. fagi* on *F. crenata* has a larger main bodies with longer appendages (Fig. 44W, X), and *Pseudod. minima* on *F. japonica* has a smaller main bodies with shorter appendages (Fig. 45G, H). These morphological variations of their propagules may be correlated with presence (in *F. japonica*) or absence (in *F. crenata*) of leaf papillae (Denk 2003) as a result of adaptation to host surface.

A phoma-like stage is known in the life cycle in *Petrakia* (Butin *et al.* 2013). This stage is also observed in *Mycodidymella* and *Pseudodidymella* after fructification of mycopappus-like propagules (Fig. 43J–R, 44J–R). The conidia-like structures of this stage seem to be spermatia, because they do not germinate in water agar or glucose agar.

## **Speciation through host switching and host jumping**



Plant pathogens frequently infect phylogenetically related hosts (Jackson 2004, Giraud *et al.* 2008, Walker *et al.* 2010, 2012, Mejía *et al.* 2011). The genus *Pseudodidymella* was originally established as a monotypic genus composed of the type species *Pseudod. fagi*, which was reported to be a pathogen of *F. crenata* (*Fagaceae*, *Fagales*) in Japan (Wei *et al.* 1997). Most recently, this species was re-discovered and reported to be a disease agent of *F. sylvatica* in Germany and Switzerland (Gross *et al.* 2017). A new species of this genus, *Pseudod. minima*, occurs on *F. japonica*. Members of *Pseudodidymella* appear to be host-specific on *Fagus*. Close host/fungus associations and coevolution were reported in members of *Gnomoniaceae*, *Phaeosphaeriaceae*, and *Sclerotiniaceae* (Jackson 2004, Walker *et al.* 2012, Ertz *et al.* 2015). Although ITS sequences of *Pseudod. fagi* were 100 % identical among isolates from *F. crenata* and *F. sylvatica* (Gross *et al.* 2017), those of *Pseudod. minima* differed from *Pseudod. fagi* based on six nucleotide positions and one gap in ITS sequences (this study). This result was compatible with host phylogeny: *F. crenata* and *F. sylvatica* are closely related to each other, but *F. japonica* is phylogenetically distantly related to the other species (Denk *et al.* 2005).

Alternatively, three genera, *Mycodidymella*, *Petrakia*, and *Xenostigmia*, are host-specific for *Acer* spp. or *Aesculus* (*Sapindaceae*, *Sapindales*), which are distantly related to *Fagales* (APG IV 2016). It has been recognised that several plant pathogens switch to unrelated host plants (Reddy *et al.* 1998, Takamatsu *et al.* 2000, Jackson 2004). Gross *et al.* (2017) also found that host switching occurred in members of *Pseudodidymellaceae*, and members of this family evolutionarily diversified by host switching. Similar evolutionary processes that led to speciation through host jumping are known from *Clavicipitaceae*, which includes plant pathogens, insect pathogens, and mycoparasites (Kepler *et al.* 2012).

### Future studies

The asexual genus *Seifertia* on *Rhododendron* spp. is characterised by synnematos 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* (Jaklitsch & Voglmayr 2017). They regarded the genus as a member of *Melanommataceae*, based on phylogenetic analyses (Jaklitsch & Voglmayr 2017). In the present study, we newly observed asexual morph of *Alpinaria*,

which had not been reported in previous studies (Müller 1959, Mugambi & Huhndorf 2009, Jaklitsch & Voglmayr 2017). According to our phylogenetic analyses and morphological observation, this species is distantly related to *Melanommataceae sensu stricto* (Fig. 40) and has atypical features for *Melanommataceae*, such as hyaline cells at the base of ascomata and pseudopycnidial conidiomata. Several melanomma-like fungi that possess well-developed carbonaceous ascomata may have evolved several times within *Pleosporales*, such as in *Cyclothyriellaceae*, *Ohleriaceae*, *Nigrogranaceae*, *Teichosporaceae*, *Thyridariaceae* (Jaklitsch & Voglmayr 2016, Jaklitsch *et al.* 2016b). It seems that the familial circumscription based on 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 re-circumscription of *Melanommataceae sensu stricto* and 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*.

## 6. 総合考察

本研究では従前の有性世代を重視した分類体系に基づく *Chaetosphaeria* 科, 広義 *Lophiotrema* 科および広義 *Melanomma* 科を有性世代に加え無性世代の形態観察および分子系統解析に基づき再検討した。

### *Chaetosphaeria* 科の事例

本科に所属する菌類は多様な無性世代の形態を有するものの, 有性世代の形態には一様な共通性がみられることから単一属の *Chaetosphaeria* 属としてまとめられてきた (Fernández *et al.* 2006). カップ状の子実体で特徴付けられる系統不明の不完全菌類 72 菌株が, 本科に所属することを分子系統解析により見いだした (2 章および 3 章). 従前の *Chaetosphaeria* 科の科概念は主にモノネマ状の単純な無性世代をもつものとして認識されてきたが (Réblová & Winka 2000, Fernández *et al.* 2006), *Pseudolachnea* 様菌類のように分生子果を形成する複雑な形態的特徴をもつ無性世代の系統群も含まれることを明らかにし, 無性世代の形態学的特徴を科の系統情報に加えることの重要性を示すことができた. このことは従前の分類体系に無性世代を加えることで, 科概念が補強される事例にあたる. これにより, 将来的に不完全菌類であっても形態学的情報から系統推定ができるようになると考えられる.

### 広義 *Lophiotrema* 科の事例

*Lophiotrema* 科は *Lophiotrema* 属の単一属で構成される点で情報の乏しい科である. 本科は当初トサカ状の孔口をもつ子のう果と柄が短い二重壁子のうによって特徴付けられる系統群として認識されてきた (Hirayama & Tanaka 2011). 分子系統解析に基づく後続の研究により *Aquasubmersa* 属, *Cryptocoryneum* 属および *Hermatomyces* 属が本科に近縁であることが示唆されてきた (Ariyawansa *et al.* 2015, Doilom *et al.* 2016, Hashimoto *et al.* 2016, Tibpromma *et al.* 2016). しかしながら, それらの属の系統的な所属は明確にされてきておらず, *Lophiotrema* 科は再検討する必要があると考えられた. 本研究では *Lophiotrema* 科に系統的に近いと考えられる 57 菌株を分子系統解析に基づき見いだした (3 章, 4 章および 5 章). 系統解析の結果, *Lophiotrema* 科は *Lophiotrema* 属と 5 つの新属を含むグループとして再定義され, 本科のメンバーはトサカ状の孔口をもつ, もしくはもたない子のう果と分生子殻状の分生子果によって特徴付けられることを明らかにした. *Aquasubmersa* 科, *Cryptocoryneum* 科, *Hermatomyces* 科は無性世代の特徴に基づき狭義 *Lophiotrema* 科と区別された. 従来 *Lophiotrema* 属の分

類形質として見なされてきたトサカ状の孔口をもつ子のう果はプレオスポ目内で独立して複数回獲得されたことが示唆された。これは *Lophiotrema* 科に所属する属や種のメンバー不足によって、形態学的特徴を十分に理解できなかったことが原因であると考えられた。以上のことは全生活環の形態学的特徴に基づき科が分割された事例であり、無性世代によって科概念が補強された事例でもあると考えられる。不完全菌類のみで構成される科である *Cryptocoryneum* 科と *Hermatomyces* 科は無性世代によってのみ構成される科の事例と考えることができる。

### 広義 *Melanomma* 科の事例

*Melanomma* 科は有性世代の形態学的特徴と分子系統解析に基づき、*Melanomma* 属を含む 20 属から構成される生態的にも形態的にも多様なグループとして特徴づけられている (Tian *et al.* 2015)。本科にはモノネマやシンネマの分生子果をもつ菌や分生子果不完全菌類など多様な形態の無性世代のグループが含まれる (de Gruyter *et al.* 2013, Tian *et al.* 2015, Li *et al.* 2016)。特に *Petrakia* 属および *Xenostigmina* 属は無性世代に mycopappus 型プロパギュールを生活環にもつ点で、本科の基準属である *Melanomma* 属と明らかに異なる (Crous *et al.* 2009, Butin *et al.* 2013, de Gruyter *et al.* 2013, Tian *et al.* 2015)。本研究では *Melanomma* 科 (プレオスポ目, クロイボタケ綱) に系統的に近縁な 28 菌株を見いだした (6 章)。分子系統解析と有性世代および無性世代の形態観察の結果、*Melanomma* 科は著しく発達した殻壁で構成される子のう果と aposphaeria 型の分生子果の無性世代で特徴付けられる *Melanomma* 属の単一のグループに限定されるべきであると結論づけた。葉上生、レンズ状で、子座基部が著しく発達した子のう果と mycopappus 型のプロパギュールを無性世代にもつ点で、*Pseudodidymella* 属、*Mycodidymella* 属、*Petrakia* 属、*Xenostigmina* 属は狭義 *Melanomma* 科とは区別され、新科 *Pseudodidymella* 科に収容された。これら 5 属の不明瞭であった属の境界線はシンアナモルフの形態学的特徴に基づき再定義された。*Alpinaria rhododendri* は *Melanomma* 科のメンバーとして見なされてきたが、本科には典型的ではない無色の細胞で構成された子のう果の基部と融合性の偽分生子殻をもつ点で、科の所属は不明なままであった。このことは、従前の科概念が有性世代を過大評価したため、本来区別されるべき科が見過ごされていた事例にあたると思われる。

従来の子のう菌類の分類体系は、伝統的に有性世代を重視して構築されてきた。本研究では両世代に基づき科概念を再構築することで、有性世代を過大評価したため見過ごされてきた科や、無性世代のみで構築される科、全生活環に基づき科概念が補強された科の事例を見いだした。これらの結果は、有性世代に偏った従前の科の枠組みが不完全であることを示唆する。科の枠組みの情報に無性世代の形質情報を加えることで、伝統

的に系統的な情報をもたなかった無性世代の形態情報からでも系統を推定できるようになり, 子とう菌類の進化の歴史を無性世代からも考察できる分類体系の基盤が構築されることが考えられる. それゆえ, 子とう菌類の自然分類構築のために, 全生活環に基づく新しい分類体系の基盤構築が今後必要である.





## 7. 摘要

*Chaetosphaeria* 科 (ケトスファエリア目, フンタマカビ綱), *Lophiotrema* 科 (プレオスポラ目, クロイボタケ綱), *Melanomma* 科 (プレオスポラ目, クロイボタケ綱) が分類学的に再検討された. 分生子果不完全菌類である *Conicomycetes* 属, *Pseudolachnea* 属および *Pseudolachnella* 属は *Chaetosphaeria* 科に所属することが明らかにされた. 分生子の隔壁数を重視した *Pseudolachnea* 属 (1 隔壁) と *Pseudolachnella* 属 (3 隔壁以上) の属概念は, 不十分であり, 分生子殻の微細構造が属を区別する形態形質として有用であることが示唆された. 分生子殻を欠き, 1 ~ 多隔壁の分生子をもつ *Neopseudolachnella* 属および著しく発達した殻壁と単細胞の *dinemasporium* 型の分生子をもつ *Pseudodinemasporium* 属が設立された. *Chaetosphaeria* 科内の属分類において無性世代は重要視されてこなかったが, 無性世代の形態学的多様性から本科における無性世代の分類学的重要性を示した. 加えて本科には分生子果に剛毛をもち, 分生子に付属糸をもつ無性世代のグループが多い傾向を見いだした. 形態観察と SSU, ITS, LSU, *tef1*, *rpb2* 領域の配列に基づき *Lophiotrema* 科とその近縁なグループ (*Aquasubmersa* 属, *Cryptocoryneum* 属, *Hermatomyces*, 属) が分類学的に再検討された. 狭義 *Lophiotrema* 科の属はトサカ状の孔口をもつ, もしくはもたない子のう果と分生子殻状の分生子果によって特徴付けられる. 新科 *Aquasubmersa* 科, *Cryptocoryneum* 科, *Hermatomyces* 科が無性世代の形態学的特徴の違いから提案された. *lophiotrema* 型の子のう果をもつ菌はプレオスポラ目内で複数回獲得されたことが示唆された. それゆえ, 従前のトサカ状の孔口のある子のう果を重視した *Lophiotrema* 科/属の概念は, 有性世代の系統的有用性を過大評価していると考えられた.

広義 *Melanomma* 科 (プレオスポラ目, クロイボタケ綱) に含まれる *Mycodidymella* 属, *Petrakia* 属, *Pseudodidymella* 属, *Xenostigmata* 属の 4 属の科の所属について形態観察と SSU, LSU, *tef1*, *rpb2* 領域の配列に基づく分子系統解析に基づき分類学的に再検討した. 結果として, *Melanomma* 科は著しく発達した殻壁で構成される子のう果と *apospheeria* 型の分生子果の無性世代で特徴付けられる *Melanomma* 属の単一のグループに限定されるべきであると考えられた. *Mycodidymella* 属, *Petrakia* 属, *Pseudodidymella* 属, *Xenostigmata* 属は葉上生, レンズ状で, 子座基部が著しく発達した子のう果と *mycopappus* 型のプロパギュールを無性世代にもつ点で *Melanomma* 属と明らかに異なるため, 新科 *Pseudodidymella* 科に收容された. *Pseudodidymella* 科の属の境界線がシミアナモルフに基づき再定義された.

本研究では総計 4 新科, 9 新属, 32 新種, 2 新組み合わせを提唱し, それらの種について記載し, DNA バーコードマーカーとして ITS 領域の配列を決定した.



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