***Crassiperidium* (Pleosporales, Dothideomycetes), a new ascomycetous genus parasitic on *Fagus crenata* in Japan**

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Abstract

A new genus, *Crassiperidium*, is established to accommodate two new species, *C. octosporum* and *C. quadrisporum*, found on twigs of the *Fagus crenata* in Japan. *Crassiperidium* is characterized by globose to depressed globose ascomata with a well-developed ascomatal wall at the sides, clavate asci, broadly fusiform, hyaline ascospores, pycnidial conidiomata, and cylindrical, multi-septate, hyaline conidia produced by annellidic conidiogenous cells. Phylogenetic analyses of a combined dataset of SSU and LSU nrDNA, and RPB2 revealed that *Crassiperidium* is close to Cyclothyriellaceae (Pleosporales, Dothideomycetes), but its familial placement was unresolved. The two new species of *Crassiperidium* have similar-sized ascospores and conidia, but the asci are 8-spored in *C. octosporum* and 4-spored in *C. quadrisporum*. Phylogenetic analyses of *Crassiperidium* species based on the sequences of ITS, TEF1, RPB2, and a combined dataset of these three loci support the validity of these two species.

Key words – 3 new taxa – Ascomycota – Cyclothyriellaceae – Phylogeny – *Pseudoasteromassaria* – Taxonomy

Introduction

During the ongoing studies on ascomycetous fungi growing on *Fagus* spp. in Japan (e.g., Hashimoto et al. 2016, 2017a), two undescribed species belonging to the Pleosporales were collected. The morphological characteristics of these species, such as globose to depressed globose ascomata with well-developed ascomatal wall at the sides, clavate asci, broadly fusiform ascospores, pycnidial conidiomata, and cylindrical, multi-septate, hyaline conidia are similar to those of *Pseudoasteromassaria*. The monotypic genus *Pseudoasteromassaria* (type species: *P. fagi*) found from the twigs of *Fagus crenata* in Japan, was recently described and was placed in Latoruaceae following a phylogenetic analysis using sequences of the small and large subunit

nrDNA (SSU and LSU) (Ariyawansa et al. 2015). On the other hand, preliminary BLAST analysis using the LSU sequences of these fungi suggests that they have phylogenetic affinities with *Cyclothyriella* and *Massariosphaeria* belonging to Cyclothyriellaceae (Jaklitsch & Voglmayr 2016).

Pleosporales is the largest order in the Dothideomycetes, and the taxonomic and phylogenetic relationships of each family/genus within this order are well established (e.g., Zhang et al. 2012, Hyde et al. 2013, Tanaka et al. 2015, Liu et al. 2017, Jaklitsch et al. 2018). Owing to their importance in forest ecosystems, ecologically diverse fungi occurring on *Fagus* spp., such as *F. crenata*, *F. grandifolia*, and *F. sylvatica* are listed and well-documented (Baird et al. 2007, Unterseher & Schnittler 2010, Hosoya et al. 2018), but our newly collected fungi on *F. crenata* do not match any of the previously known genera in Pleosporales. Therefore, we establish a new genus, *Crassiperidium*, to accommodate two new species, *C. octosporum* and *C. quadrisporum*.

Materials & Methods

Isolates and morphology

Microscopic observations and single spore isolations were performed as described by Tanaka et al. (2015). The position of the primary septum of spores was noted using the decimal system (Shoemaker 1984). Specimens are deposited in the Herbarium of Hirosaki University Fungi (HHUF). Single ascospore/conidium cultures were deposited at the Japan Collection of Microorganisms (JCM) and the GenBank Project, NARO (MAFF). Colony characteristics were recorded from growth on potato dextrose agar (PDA) after 2 weeks at 20°C in the dark and colony colors were noted according to Rayner (1970). To observe the asexual morph in culture, sporulation was promoted by placing a small piece of mycelial culture on rice straw agar (Tanaka & Harada 2003).

DNA extraction and phylogenetic analyses

DNA was extracted from 12 isolates in total using the ISOPLANT II kit (Nippon Gene, Tokyo, Japan) following the manufacturer's instruction. The sequence of SSU, the internal transcribed spacer region of ribosomal DNA (ITS), LSU, translation elongation factor 1- α (TEF1), and RNA polymerase second largest subunit (RPB2) was amplified by PCR using primer sets NS1/NS4, ITS1/ITS4 (White et al. 1990), LR0R/LR7 (Rehner & Samuels 1994), EF-983F/EF-2218R (Rehner & Buckley 2005), and fRPB2-5F/fRPB2-7cR (Liu et al. 1999), respectively. PCR was carried out in 25 μ L including 2.5 μ L of 10 $\times$ TEMPase Buffer I, 1 μ L of each 20 pM primer, 25 mM MgCl₂, 2.5 μ L of dNTP mix, 14.5 μ L of ultra pure water and 0.5 μ L of TEMPase Hot Start DNA Polymerase (Ampliqon, Denmark). Amplifications of each region were performed on a PC 320 thermo-cycler (ASTEC, Japan) as follows: 95°C for 15 min; 35 cycles of 1 min at 94°C, 1 min at annealing temperature (42.2°C for SSU, 61.5°C for ITS, 46°C for LSU, 60°C for TEF1, 55°C for RPB2), 1 min at 72°C; 72°C for 7 min. PCR products were sequenced at SolGent (South Korea). Newly generated sequences were deposited in GenBank (Table 1). Primary analyses of SSU-LSU-RPB2 sequences from 35 taxa of Pleosporales were analyzed to clarify the familial/generic placement of the fungus (Table 1, Appendix 1). *Lophiostoma macrostomum* and *L. arundinis* (Lophiostomataceae) were used as outgroups for the primary analyses. Secondary analyses were conducted on single gene phylogenetic analyses for ITS, TEF1, and RPB2, and the combination of these three from 12 taxa of our newly obtained strains to assess the species boundary. Sequences were aligned using an algorithm implemented in MEGA v. 5.2.2 (Tamura et al. 2011). Phylogenetic analyses were conducted using maximum-likelihood (ML) and Bayesian methods. The optimum substitution models for each data set were estimated using Kakusan4 software (Tanabe 2011) based on the Akaike information criterion (AIC; Akaike 1974) for ML analysis and on Bayesian information criterion (BIC; Schwarz 1978) for the Bayesian analysis. ML analysis was performed using the RAxML-HPC2 v. 8.2.10 on the CIPRES Science Gateway (Miller et al. 2015, Stamatakis 2014) based on models selected with the AICc4 parameter (a separate codon separate

model for primary analyses and the equalrate codon equalrate model for secondary analyses). The GTR+G model was optimal for all partitions in the primary and secondary dataset. Bootstrap proportions (BPs) were obtained using 1,000 bootstrap replications. Bayesian analysis was performed with MrBayes v. 3.2.6 on the CIPRES Science Gateway (Miller et al. 2015, Ronquist et al. 2012), using substitution models containing the BIC4 parameter (i.e., proportional codon proportional model for each analyses). K80+G was used for SSU, SYM+G for LSU, and GTR+G for RPB2 in the first dataset. The second dataset used K80+G for ITS, F81+H for the first and second position of RPB2, HKY85+H for the third codon position, F81+H for the TEF1 first codon position, JC69 for the second position, and HKY85+H for the third position. Two simultaneous and independent Metropolis-coupled Markov chain Monte Carlo (MCMC) runs were performed for 2 M generations with the tree sampled for every 1,000 generations of the analyses. Convergence of the MCMC procedure was assessed from the average standard deviation of split frequencies (< 0.01) and the effective sample size scores (all > 100) using MrBayes and Tracer v. 1.6 (Rambaut et al. 2014), respectively. The first 25% of the trees were discarded as burn-in, and the remainder were used to calculate the 50% majority-rule trees and to determine the posterior probabilities (PPs) for individual branches. These alignments were submitted to TreeBASE under study number S23305.

Table 1 Culture and GenBank accession number of *Crassiperidium* spp. used in this study.

Taxon	Stain no.	GenBank accession no.				
		SSU	ITS	LSU	TEF1	RPB2
<i>C. octosporum</i>	KT 2144 = JCM 17827 = MAFF 242971	LC373084	LC373096	LC373108	LC373120	LC373132
<i>C. octosporum</i>	KT 2894 = MAFF 246399	LC373085	LC373097	LC373109	LC373121	LC373133
<i>C. octosporum</i>	KT 3008 = MAFF 246400	LC373086	LC373098	LC373110	LC373122	LC373134
<i>C. octosporum</i>	KT 3029 = MAFF 246401	LC373087	LC373099	LC373111	LC373123	LC373135
<i>C. octosporum</i>	KT 3046 = MAFF 246402	LC373088	LC373100	LC373112	LC373124	LC373136
<i>C. octosporum</i>	KT 3188 = MAFF 246403	LC373089	LC373101	LC373113	LC373125	LC373137
<i>C. octosporum</i>	KT 3468 = MAFF 246404	LC373090	LC373102	LC373114	LC373126	LC373138
<i>C. octosporum</i>	KT 3604 = MAFF 246405	LC373091	LC373103	LC373115	LC373127	LC373139
<i>C. octosporum</i>	KT 3605 = MAFF 246406	LC373092	LC373104	LC373116	LC373128	LC373140
<i>C. octosporum</i>	MM 9 = MAFF 246407	LC373093	LC373105	LC373117	LC373129	LC373141
<i>C. quadrisporum</i>	KT 2798-1 = JCM 32442 = MAFF 246397	LC373094	LC373106	LC373118	LC373130	LC373142
<i>C. quadrisporum</i>	KT 2798-2 = MAFF 246398	LC373095	LC373107	LC373119	LC373131	LC373143

Results

Phylogenetic analyses

For the first analysis, ML and Bayesian phylogenetic analyses were conducted using an aligned sequence dataset comprising 983 nucleotide positions from SSU, 1,284 from LSU, and 993 from RPB2. Of the 3,260 characters included in the alignment, 998 were variable and 2,262 were conserved. This combined dataset provided higher confidence values for the familial and generic levels than those of individual gene trees (data not shown). The ML tree with the highest log likelihood (−21060.538) is shown in Fig. 1. The Bayesian likelihood score was −20408.695. The topology recovered by the Bayesian analysis was identical to that of the ML tree. *Crassiperidium* spp. formed a sister clade to *Cyclothyriella* and *Massariosphaeria* in Cyclothyriellaceae (Fig. 1), but this relationship was not well-supported (Fig. 1).

Alignment of the second analysis was based on 515 nucleotide positions from ITS, 1,032 from RPB2, and 942 from TEF1. Of the 2,489 characters included in the alignment, 92 were variable and 2,397 were conserved. Three individual and combined phylogenies showed almost

identical tree topologies with branches supported by > 90% ML BP and > 0.95 Bayesian PP and indicated two distinct operational taxonomic units (Fig. 2). The ML tree with the highest log likelihood (–858.532 in ITS, –1554.488 in RPB2, –1407.289 in TEF1, and –3886.896 in ITS-RPB2-TEF1) is shown in Fig. 2. The Bayesian likelihood score was –877.519 in ITS, –1559.689 in RPB2, –1411.493 in TEF1, and –3871.473 in ITS-RPB2-TEF1. All the topology recovered by Bayesian analysis was identical to that of the ML tree. Two monophyletic clades of *Crassiperidium* (*C. octosporum* and *C. quadrisporum*) were fully supported by both ML and Bayesian analyses in all datasets (Fig. 2).

Taxonomy

Crassiperidium M. Matsum. & Kaz. Tanaka, gen. nov.

Index Fungorum number: IF555726; Facesoffungi number: FoF05595

Etymology – From the Latin *Crassi-*, meaning thick, in reference to the thick ascomatal wall.

Sexual morph: *Ascomata* scattered, immersed, depressed globose to globose, ostiolate. *Ascomatal wall* composed of rectangular to polygonal, pale brown to brown cells. *Hamathecium* numerous, cellular, septate. *Asci* fissitunicate, cylindrical to clavate, pedicellate, 4–8-spored. *Ascospores* broadly fusiform, straight, thick-walled, with a submedian septate, 1–3-septate, hyaline, smooth-walled. Asexual morph: *Conidiomata* pycnidial, scattered, immersed, globose to subglobose, ostiolate. *Conidiomatal wall* composed of angular, pale brown to brown cells. *Conidiophores* absent. *Conidiogenous cells* annellidic, doliiform to ampulliform. *Conidia* cylindrical, truncate at the base, multi-septate, hyaline, smooth, without sheath.

Type species – *Crassiperidium octosporum* M. Matsum. & Kaz. Tanaka

Notes – The new genus *Crassiperidium* is similar to *Asteromassaria* in Pleomassariaceae. However, the asexual morph of *Asteromassaria* differs from that of *Crassiperidium* in having acervular conidiomata and crescentic to falcate, multi-septate, brown conidia (Sivanesan 1984, Wijayawardene et al. 2016). *Crassiperidium* resembles *Pseudoasteromassaria*, which has similar sexual and asexual morphs and the same host plant (Ariyawansa et al. 2015). However, *Pseudoasteromassaria* is a member of Latoruaceae and is distinguished from *Crassiperidium* in having pigmented ascospores and phialidic conidiogenesis (Ariyawansa et al. 2015).

Crassiperidium octosporum M. Matsum. & Kaz. Tanaka, sp. nov.

Fig. 3

Index Fungorum number: IF555727; Facesoffungi number: FoF05593

Etymology – Referring to the asci with 8 spores.

Sexual morph: *Ascomata* scattered, immersed, depressed globose, 300–500 µm high, 750–1200 µm diam., with a central ostiole. *Ascomatal wall* 7.5–12.5 µm thick at the base, 130–160 µm thick at sides, composed of rectangular to polygonal, 6–14 × 3–9 µm cells. *Hamathecium* comprising numerous, 1–2 µm wide, hyaline, septate, branched, cellular, pseudoparaphyses. *Asci* 157.5–202.5 × 26–35 µm ($\bar{x}$ = 178.7 × 29.9 µm, n = 13), 8-spored, fissitunicate, cylindric to clavate, pedicellate (29–49.5 µm long), apically rounded. *Ascospores* 31–44 × 12–17 µm ($\bar{x}$ = 37.7 × 14.1 µm, n = 85), l/w 2.1–3.6 ($\bar{x}$ = 2.7, n = 85), uni- to bi-seriate, hyaline, broadly fusiform, mostly straight, thick-walled, 1–3-septate, with a submedian primary septum (0.50–0.56, $\bar{x}$ = 0.53, n = 76), smooth-walled, with an entire sheath. Asexual morph (nature): *Conidiomata* pycnidial, scattered, immersed to superficial, depressed globose, 430–550 µm high, 680–790 µm diam., ostiolate. *Conidiomatal wall* 15–28 µm thick at the base, 30–53 µm thick at sides, composed of rectangular to polygonal, 6–15 × 3–7.5 µm, pale brown cells. *Conidia* 53.5–65 × 10–13 µm ($\bar{x}$ = 59.2 × 11.7 µm, n = 30), l/w 4.4–6.1 ($\bar{x}$ = 5.1, n = 30), cylindrical, truncate at the base, 7-septate, hyaline, smooth-walled, without a sheath. Asexual morph (culture): *Conidiomata* pycnidial, scattered, immersed to superficial, globose, 490–620 µm high, 420–450 µm diam., ostiolate. *Conidiomatal wall* 60–77.5 µm thick at the base, 22.5–70 µm thick at sides. *Conidiophores* absent. *Conidiogenous cells* 7.5–11.5 × 4–5 µm, annellidic, doliiform to ampulliform. *Conidia* (41–)46–70(–74) × 10–14.5 µm ($\bar{x}$ =

57.5 × 11.6 μm, n = 109), l/w 3.2–6.6 ($\bar{x}$ = 4.8, n = 109), cylindrical, truncate at the base, 7-septate, hyaline, smooth-walled, without a sheath.

Figure 1 – Maximum-likelihood tree based on combined SSU, LSU and RPB2 sequence. Bootstrap support values and Bayesian posterior probabilities greater than 75 % and 0.95 are given above each node. The tree is rooted to *Lophiostoma* spp. New species are shown in bold. The scale bar represents nucleotide substitutions per site.

Figure 2 – Maximum-likelihood tree of *Crassipерidium* species based on each ITS (upper left), TEF1 (upper right) and RPB2 (bottom left), and combined sequences (ITS-TEF1-RPB2; bottom right). Bootstrap support values and Bayesian posterior probabilities greater than 75 % and 0.95 are given above each node. The scale bar represents nucleotide substitutions per site.

Figure 3 – *Crassiperidium octosporum* (a–d, f, g, j, k HHUF 30080. e, h, i, l HHUF 30603. m, n MAFF 246407. o, p MAFF 246402. q MAFF 246406. r–t MAFF 246404). a, b Ascomata on host surface. c Ascoma in longitudinal section. d Ascomatal wall. e Pseudoparaphyses. f, g Asci. h–k Ascospores. l Germinating ascospore. m, n Conidiomata formed in culture. o Conidioma in longitudinal section. p Conidiomatal wall. q Conidiogenous cells. r–t Conidia. Scar bars: a, m = 1 mm, b, n = 500 μ m, c, o = 100 μ m, d–l, p–t = 10 μ m.

Culture characteristics – Colonies on PDA 20–30 mm diam. after 2 weeks at 20°C in the dark, floccose, White to Greyish Sepia (106, Rayner 1970); reverse White and Greyish Sepia (106) to Grey Olivaceous (107).

Material examined (all on twigs of *Fagus crenata*) – JAPAN, Iwate, Hanamaki, near Mt. Hayachine, 12 August 2006, K. Tanaka, S. Hatakeyama, Y. Suzuki and H. Yonezawa (HHUF 30080, holotype designated here), ex-holotype culture (ex-ascospore) KT 2144 = JCM 17827 = MAFF 242971; Aomori, Nishimeya, The Shirakami Natural Science Park (Hirosaki University), 17 September 2011, K. Tanaka, A. Hashimoto and Y. Sasaki (HHUF 30597), culture (ex-conidium) KT 2894 = MAFF 246399; Aomori, Nishitsugaru, Ajigasawa, mini Shirakami, 4 June 2012, K. Tanaka and T. Shirouzu (HHUF 30598), culture (ex-conidium) KT 3008 = MAFF 246400; Akita, Kazuno, Hachimantai, Mt. Yakeyama, Mousen pass, 24 June 2012, K. Tanaka (HHUF 30599), culture (ex-conidium) KT 3029 = MAFF 246401; Hokkaido, Furano, Yamabe, The University of Tokyo Hokkaido Forest, 18 June 2012, Y.S. Takahashi (HHUF 30600), culture (ex-conidium) KT 3046 = MAFF 246402; Aomori, Hakkoda, Jougakura, 4 November 2012, K. Tanaka (HHUF 30601), culture (ex-conidium) KT 3188 = MAFF 246403; Akita, Hachimantai, Kamazawa, Gosyogake hot spring trail, 22 June 2014, K. Tanaka (HHUF 30602), culture (ex-conidium) KT 3468 = MAFF 246404; Nagano, Shiga Heights, near Hasuike, 22 September 2015, K. Tanaka (HHUF 30603), culture (ex-ascospore) KT 3604 = MAFF 246405; *ibid.* (HHUF 30604), culture (ex-conidium) KT 3605 = MAFF 246406; Aomori, Hirosaki, Bunkyo-cho, Hirosaki University, 27 August 2013, M. Matsumura (HHUF 30605), culture (ex-conidium) MM 9 = MAFF 246407.

Notes – *Crassiperidium octosporum* is similar to *C. quadrisporum*, but differs from the latter in having asci with 8 spores and larger conidia ($57.5 \times 11.6 \mu\text{m}$ vs. $48.6 \times 10.1 \mu\text{m}$ in culture). Furthermore, the sequences of these two species are different at 19, 25, and 33 positions in ITS, TEF1, and RPB2, respectively.

Crassiperidium quadrisporum M. Matsum. & Kaz. Tanaka, sp. nov.

Fig. 4

Index Fungorum number: IF555728; Facesoffungi number: FoF05594

Etymology – Referring to the asci with 4 spores.

Sexual morph: *Ascomata* scattered, immersed, depressed globose to globose, 410–500 μm high, 720–810 μm diam., with a central ostiole. *Ascomatal wall* 15–35 μm thick at the base, 100–162 μm thick at sides, composed of rectangular to polygonal, $8\text{--}20 \times 5\text{--}13 \mu\text{m}$ cells. *Hamathecium* comprising numerous, 1–2 μm wide, hyaline, septate, branched, cellular, pseudoparaphyses. *Asci* $145\text{--}216 \times 20\text{--}37.5 \mu\text{m}$ ($\bar{x} = 180.8 \times 26.3 \mu\text{m}$, $n = 20$), 4-spored, fissitunicate, cylindric to clavate, pedicellate (25–55 μm long), apically rounded. *Ascospores* $30\text{--}41 \times 11\text{--}15.5 \mu\text{m}$ ($\bar{x} = 33.8 \times 13.2 \mu\text{m}$, $n = 50$), l/w 2.1–3.0 ($\bar{x} = 2.6$, $n = 50$), uniseriate, hyaline, broadly fusiform, mostly straight, thick-walled, 1–3-septate, with a submedian primary septum (0.51–0.56, $\bar{x} = 0.53$, $n = 42$), smooth-walled, with an entire sheath. Asexual morph (culture): *Conidiomata* pycnidial, scattered, immersed, globose, 320–400 μm high, 280–360 μm diam., ostiolate. *Conidiomatal wall* 20–62.5 μm thick, composed of rectangular to polygonal, $5\text{--}23 \times 2\text{--}7 \mu\text{m}$, pale brown to brown cells. *Conidiophores* absent. *Conidiogenous cells* $8\text{--}15 \times 4\text{--}8 \mu\text{m}$, annellidic, doliiform to ampulliform. *Conidia* $38\text{--}57 \times 8\text{--}12 \mu\text{m}$ ($\bar{x} = 48.6 \times 10.1 \mu\text{m}$, $n = 60$), l/w 4.0–5.9 ($\bar{x} = 4.8$, $n = 60$), cylindrical, truncate at the base, 7-septate, hyaline, smooth-walled, without a sheath.

Culture characteristics – Colonies on PDA 25–35 mm diam. after 2 weeks at 20°C in the dark, floccose, White; reverse White and Fuscous Black (104) or Black.

Material examined – JAPAN, Aomori, Nishimeya, The Shirakami Natural Science Park (Hirosaki University), on twigs of *Fagus crenata*, 2 July 2011, K. Tanaka, A. Hashimoto and Y. Sasaki (HHUF 30596, holotype designated here), ex-holotype cultures (ex-ascospore) KT 2798-1 = JCM 32442 = MAFF 246397, KT 2798-2 = MAFF 246398.

Notes – This species is easily distinguished from *C. octosporum* by its 4-spored asci. Generally, the number of ascospores per ascus is considered as a useful character for species characterization in Pleosporales (Voglmayr & Jaklitsch 2011, Hirayama et al. 2014).

Figure 4 – *Crassiperidium quadrisporum* (a–l HHUF 30596. m–t MAFF 246397). a, b Ascomata on host surface. c Ascoma in longitudinal section. d Ascomatal wall. e Pseudoparaphyses. f, g Asci. h–k Ascospores. l Germinating ascospore. m, n Conidiomata formed in culture. o Conidioma in longitudinal section. p Conidiomatal wall. q Conidiogenous cell. r–t Conidia. Scar bars: a, m = 1 mm, b, n = 500 μ m, c, o = 100 μ m, d–l, p–t = 10 μ m.

Discussion

Pleosporales is the largest order in Dothideomycetes. Monographic revision of this order based on the type specimens along with phylogenetic analyses has been published by Zhang et al.

(2012). Following this publication, many comprehensive works on Pleosporales have been published (e.g., Jaklitsch et al. 2016, Hashimoto et al. 2017b, 2018, Hyde et al. 2017, Voglmayr & Jaklitsch 2017, Koukol et al. 2018). Currently, 400 genera in 75 families are known in Pleosporales (Wijayawardene et al. 2018), but *Crassiperidium* does not match any of the previously known genera in this order.

Morphologically, *Crassiperidium* is most similar to *Pseudoasteromassaria* (Ariyawansa et al. 2015) in that both have globose to depressed globose ascomata with a well-developed ascomatal wall at the sides, pycnidial conidiomata, cylindrical, multi-septate, hyaline conidia, and the same host plant (*Fagus crenata*). However, *Pseudoasteromassaria* has pigmented ascospores and phialidic conidiogenous cells, and phylogenetically belongs to Latoruaceae, which is a member of Massarineae (Tanaka et al. 2015). *Crassiperidium* resembles *Asteromassaria* in having broadly fusiform ascospores and *Fagus* as the host plant, but the latter genus typified by *A. macrospora* has acervular conidiomata and crescentic to falcate, multi-septate, brown conidia (Sivanesan 1984), and is regarded as belonging to Pleomassariaceae (Hyde et al. 2013). Phylogenetically, *Crassiperidium* forms a sister clade to *Cyclothyriella* and *Massariosphaeria* in Cyclothyriellaceae (Fig. 1), but this genus differs from the latter genera that have pigmented ascospores in their sexual morph and phialidic conidiogenesis in their asexual morph (Jaklitsch & Voglmayr 2016).

Our study shows that the new genus *Crassiperidium*, is phylogenetically close to Cyclothyriellaceae from analyses of molecular data based on SSU, LSU, and RPB2 sequences, but their relationship was not supported (Fig. 1). Therefore, placement of *Crassiperidium* at the family level remains unresolved at this time. This genus should thus be treated as a member of an undescribed new family, but further collections of its related lineages are needed to clarify the familial affiliation of this genus.

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Appendix 1 Sequence accessions in GenBank used for phylogenetic analysis

Taxon	Stain no.	GenBank accession no.		
		SSU	LSU	RPB2
<i>Acrocalymma walkeri</i>	CBS 257.93	FJ795495	FJ795454	FJ795471
<i>Bambusicola massarinia</i>	MFLUCC 11-0389	JX442041	JX442037	KP761716
<i>Bimuria novae-zelandiae</i>	CBS 107.79	AY016338	AY016356	DQ470917
<i>Brevicollum hyalosporum</i>	MAFF 243400	LC271236	LC271239	LC271249
<i>Coniothyrium palmarum</i>	CBS 400.71	DQ678008	EU754153	DQ677956
<i>Corynespora cassiicola</i>	CBS 100822	GU296144	GU301808	GU371742
<i>Crassiparies quadrisporus</i>	KH 111	LC100017	LC100025	LC271251
<i>Cyclothyriella rubronotata</i>	CBS 141486	KX650507	KX650544	KX650574
<i>Didymella exigua</i>	CBS 183.55	EU754056	EU754155	GU371764
<i>Halojulella avicenniae</i>	BCC 18422	GU371831	GU371823	GU371787
<i>Lentithecium fluviatile</i>	CBS 123090	FJ795492	FJ795450	FJ795467
<i>Leptosphaeria doliolum</i>	CBS 505.75	GU296159	GU301827	KT389640
<i>Lophiostoma arundinis</i>	CBS 621.86	DQ782383	DQ782384	DQ782386
<i>Lophiostoma macrostomum</i>	KT 635	AB521731	AB433273	JN993484
<i>Macrodiplodiopsis desmazieri</i>	CBS 221.37	DQ678013	DQ678065	DQ677961
<i>Massarina eburnea</i>	CBS 473.64	GU296170	GU301840	GU371732
<i>Massariosphaeria phaeospora</i>	CBS 611.86	GU296173	GU301843	GU371794
<i>Melanomma japonicum</i>	CBS 142905	LC203293	LC203339	LC203395
<i>Melanomma pulvis-pyrius</i>	CBS 124080	GU456302	GU456323	GU456350
<i>Morosphaeria ramunculicola</i>	JK 5304B	GU479760	GU479794	GU479831
<i>Prosthemium betulinum</i>	CBS 279.74	DQ678027	DQ678078	DQ677976
<i>Stemphylium botryosum</i>	CBS 714.68	KC584603	KC584345	AF107804
<i>Trematosphaeria pertusa</i>	CBS 122368	FJ201991	FJ201990	GU371801