

# Taxonomy and phylogeny of *Cryptocoryneum* (Pleosporales, Dothideomycetes)

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**Abstract** Species of *Cryptocoryneum* were taxonomically reassessed on the basis of morphological observations and the results of molecular phylogenetic analysis. Eighteen isolates of *Cryptocoryneum* species, namely two strains from Africa, three from Europe, and 13 from Japan, were phylogenetically analysed using sequences of nuclear rDNA internal transcribed spacers (ITS) and the partial sequence of the translation elongation factor 1 $\alpha$  gene (*TEF1*). The phylogenetic analysis indicated that *Cryptocoryneum* species formed a monophyletic clade and were closely related to *Lophiotrema* (Lophiotremataceae) and *Aquasubmersa* (*incertae sedis*) in the Pleosporales (Dothideomycetes). We examined holotype specimens of *C. fasciculatum*, *C. hysteroioides*, and *Torula uniformis* and concluded that these species are conspecific, with *C. hysteroioides* having priority. Although *C. hysteroioides* has long been regarded as a synonym of *C. condensatum*, we consider *C. hysteroioides* to be a distinct species within the genus. We found several cryptic species that were morphologically similar to *C. condensatum sensu lato*, but that could be separated on the basis of conidial size and the number of conidial arms and conidial septa, characters that seem to be informative for species delimitation within

*Cryptocoryneum*. A total of seven new species, namely *C. akitaense*, *C. brevicondensatum*, *C. congregatum*, *C. japonicum*, *C. longicondensatum*, *C. paracondensatum*, and *C. pseudorilstonei*, are described and illustrated. A key to species accepted in *Cryptocoryneum* is provided.

**Keywords** Ascomycota · Hyphomycetes · Lophiotremataceae

## Introduction

The asexual genus *Cryptocoryneum* Fuckel is characterised by having stromatic sporodochia, cheiroid conidia, and conidial arms that are developed downward from the cap cells (Schoknecht and 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* (Wallr.) E.W. Mason, has been presently known from Asia, Australia, Europe, North America, and South America (Hughes 1958; Ellis 1971; Schoknecht and Crane 1977; Katumoto 1988; von Hefberger et al. 1997; Mel'nik 2000; da Silva et al. 2015). *Cryptocoryneum rilstonei* M.B. Ellis has also been reported in Africa, Australia, Europe, and South America (Ellis 1972; Hughes 1978; Kirk 1982; da 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 and Tanaka 2008; Kamiyama et al. 2009; Tanaka

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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 the translation elongation factor 1 $\alpha$  gene (*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 1). Colony characters were observed from cultures grown on potato dextrose agar (PDA; Difco, USA) 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 the NITE Biological Resource Centre (NBRC) and the Ministry of Agriculture, Forestry, and Fisheries (MAFF). Specimens were deposited in the herbarium of Hirosaki University, Fungi (HHUF).

The holotypes of *C. fasciculatum* Fuckel, *C. hysterioides* (Corda) Peyronel, *C. rilstonei*, and *Torula uniformis* Peck were borrowed from G, PRM, IML, NYS, respectively. The type specimens of *C. erumpens* Sacc., *C. fasciculatum* var. *olivaceum* Sacc., *C. scopiforme* E. Bommer, M. Rousseau & Sacc., and *C. simmonsii* Sacc. 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.

### 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 and Buckley 2005), respectively. Amplifications were performed in 25 mL volumes consisting of 2 mL DNA extract, 2.5 mL 10 $\times$  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 (ASTEC, 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 (Korea).

The novel sequences generated from 18 isolates of *Cryptocoryneum* were deposited in GenBank (Table 1). Two *Lophiotrema* strains, *L. neoarundinaria* KT 856 and KT 2200, were selected as outgroups. These sequences were aligned with the MUSCLE algorithm implemented in the program molecular evolutionary genetic analysis (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 ML analysis and the Bayesian information criterion (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*). Bootstrap proportions (BP) 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 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

### Molecular phylogenetic analyses

A Blast search of GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) with ITS sequences of *Cryptocoryneum* strains

**Table 1** Specimens, isolates and new sequences obtained in this study

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

H, holotype

a, holotype of *Cryptocoryneum fasciculatum*

b, holotype of *Tortula uniformis*

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. 1. 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. 1). The alignment was submitted to TreeBASE with the study number S18427. Seven new species, as well as three known species are described below.

## Taxonomy

***Cryptocoryneum*** Fuckel, Fungi Rhenani Exsiccata 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* (Cooke & Ellis) S. Hughes and *C. obovatum* Oudem, have been transferred to *Eversia* J.L. Crane & Schokn. and *Bactrodesmium* Cooke, respectively (Ellis 1963; Schoknecht and 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.

**1. *Cryptocoryneum akitaense*** A. Hashim. & Kaz. Tanaka, **sp. nov.** Fig. 2a–d

*Holotype:* HHUF 30477

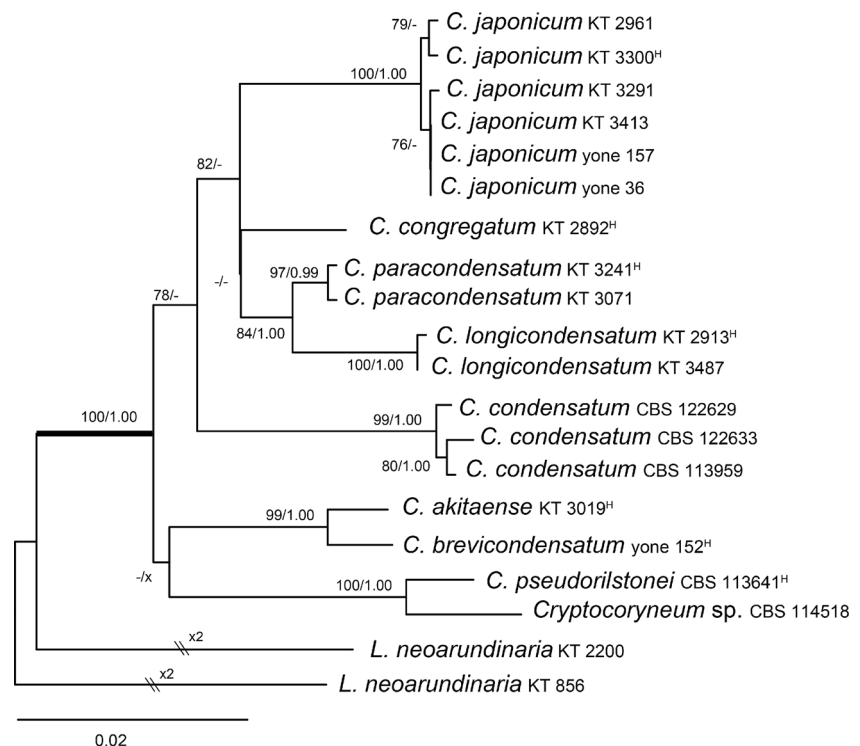
*Mycobank:* MB 815317

*Etymology:* named after its type locality.

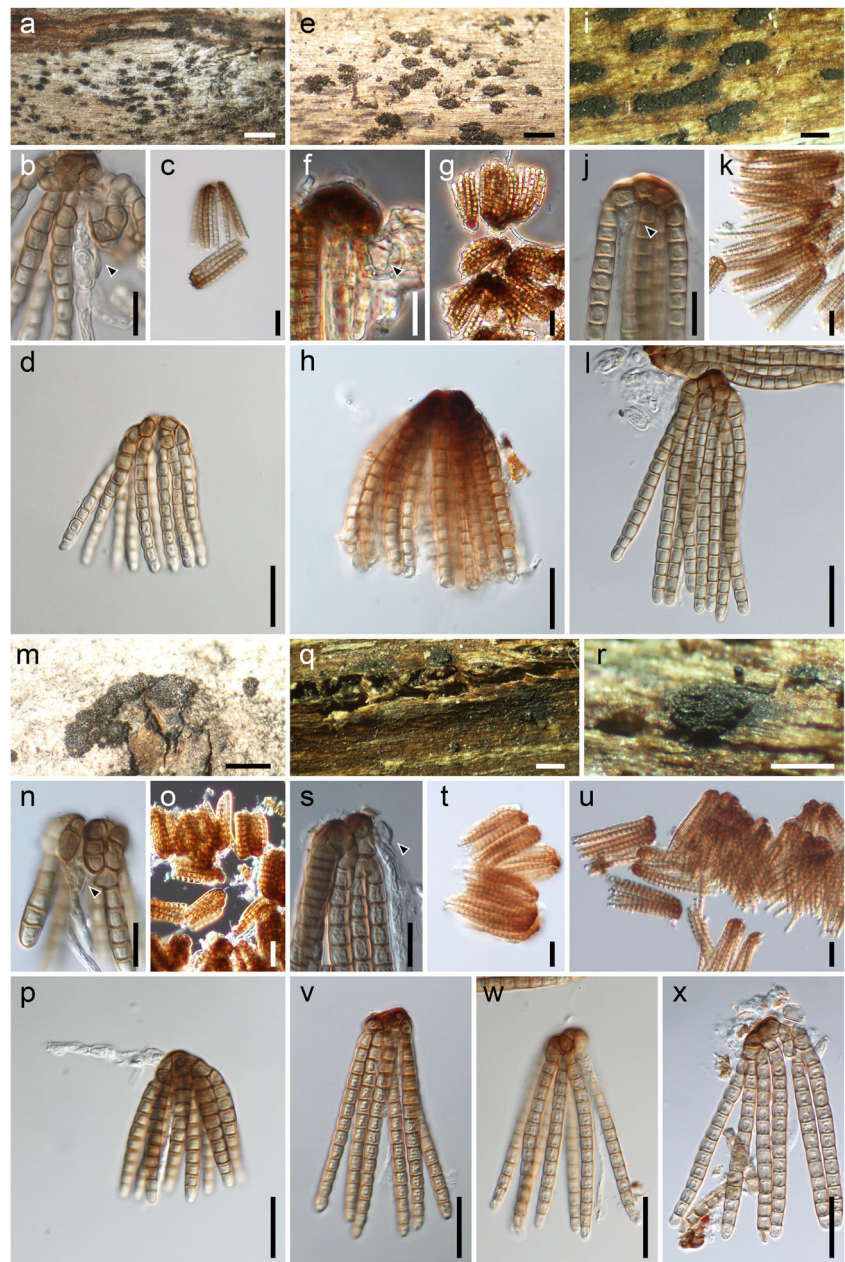
*Saprobic* on dead twigs of *Rhododendron brachycarpum*.

**Sexual morph:** unknown. **Asexual morph:** *Sporodochia* pulvinate, (90)130–300(440) μm diam., (30)40–50 μm high, often confluent, dark brown to black. *Conidiophores* arising

**Fig. 1** 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 ×. The tree is with *Lophiotrema* spp. as outgroup. H = ex-holotype strain



**Fig. 2** *Cryptocoryneum* spp. a–d *C. akitaense*. e–h *C. brevicondensatum*. i–l *C. condensatum*. m–p *C. congregatum*. q–x *C. hysteroioides*. **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. hysteroioides*). 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



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  $\mu$ m. *Conidia* solitary, acrogenous, branched, cheiroid, (34)38–61(67)  $\times$  19–72  $\mu$ m ( $\bar{x}$  = 49.1  $\times$  38.6  $\mu$ 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)  $\mu$ m wide ( $\bar{x}$  = 6.5  $\mu$ 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  $\mu$ m long ( $\bar{x}$  = 46.2  $\mu$ m long, n = 55), 4–7  $\mu$ m wide at base, 3–4  $\mu$ m wide at apex.

*Cultural characteristics*: Colonies on PDA 29–31 mm diam. after 21 d at 20  $^{\circ}$ C in the dark, velvety, smoke grey

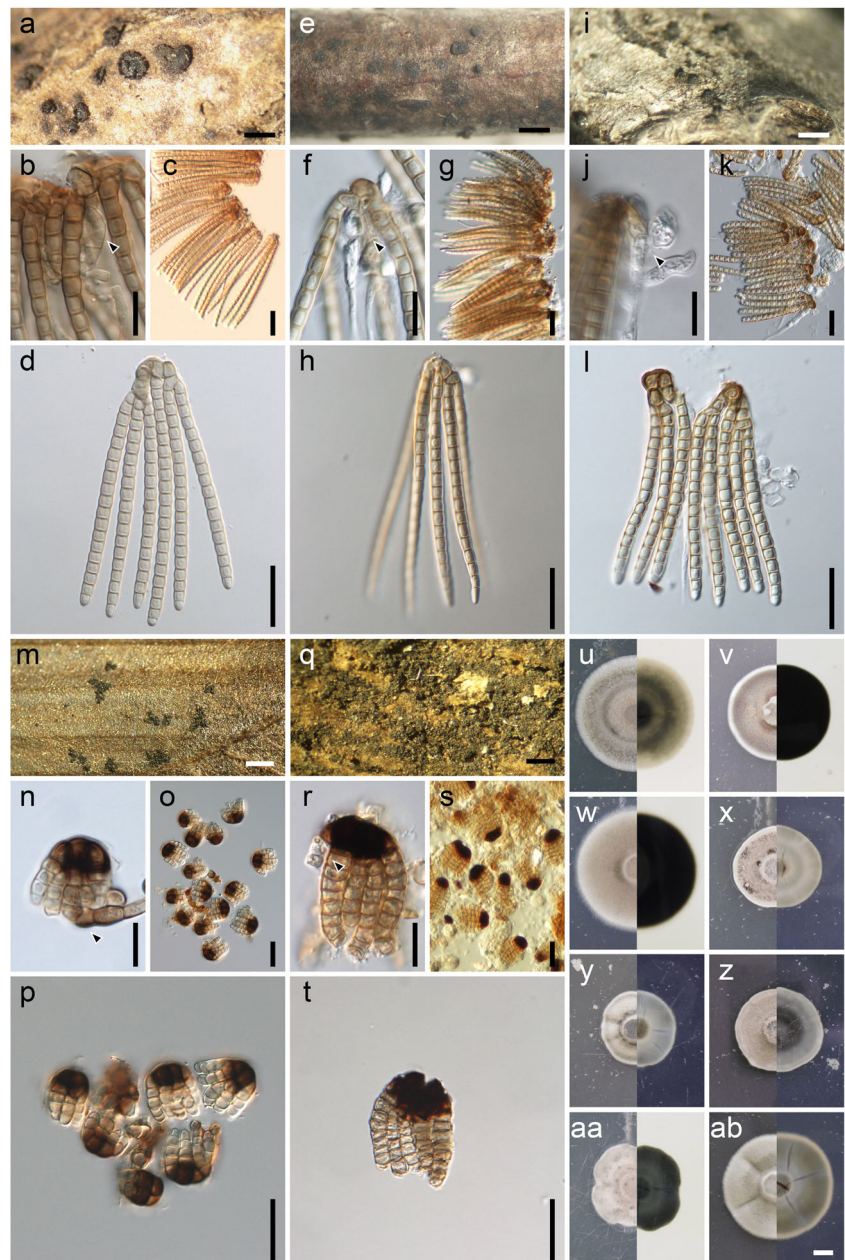
(105: Rayner 1970); reverse similar (Fig. 3u); without sporulation.

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

Note: *Cryptocoryneum akitaense* is phylogenetically close to *C. brevicondensatum* A. Hashim. & Kaz. Tanaka (Fig. 1), but can be distinguished from the latter by its shorter and wider conidia [vs. (44.5)47–68.5(72)  $\times$  17–48(57)  $\mu$ m, l/w 0.9–3.5 in the latter].

**2. *Cryptocoryneum brevicondensatum* A. Hashim. & Kaz. Tanaka, sp. nov.** Fig. 2e–h

**Fig. 3** *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



*Holotype*: HHUF 30478

*Mycobank*: MB 815318

*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 µm diam., (30)40–50 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, 9–10 × 4–6.5 µm. *Conidia* solitary, acrogenous, branched, cheiroid, (44.5)47–68.5(72) × 17–48(57) µm ( $\bar{x}$  =

57.5 × 34.2 µm, 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) µm wide ( $\bar{x}$  = 9.2 µm, n = 50); *arms* cylindrical, pale brown, branched at base, smooth, 8–12-septate ( $\bar{x}$  = 10, n = 50), (37.5)41–60(64) µm long ( $\bar{x}$  = 50.3 µm long, n = 50), 4–7 µm wide at base, 3–5 µm wide at apex.

*Cultural characteristics*: 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. 3v); without sporulation.

*Material examined*: JAPAN, Aomori, Nishimeya, Ooshirosawa stream, on dead wood, 21 July 2007, H.

Yonezawa (yone 152=HHUF 30478, **holotype** designated here; ex-holotype living culture MAFF 245366=NBRC 111759).

Notes: *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) × 19.5–58(70) μm, 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.

**3. *Cryptocoryneum condensatum*** (Wallr.) E.W. Mason & S. Hughes ex S. Hughes, Canadian Journal of Botany 36: 758 (1958) Fig. 2i–l

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

≡ *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) μm 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 × 3–3.5 μm. *Conidia* solitary, acrogenous, branched, cheiroid, (64)69–91(97) × 19.5–58(70) μm ( $\bar{x}$  = 76.8 × 35.8 μm, 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 μm wide ( $\bar{x}$  = 7.1 μm, n = 50); *arms* cylindrical, brown, branched at base, smooth, 13–19(21)-septate ( $\bar{x}$  = 16, n = 50), 64–89.5 μm long ( $\bar{x}$  = 73.8 μm long, n = 50), 3–6.5(8) μm wide at base, 2–6.5 μm wide at apex.

**Cultural characteristics:** Conidia formed in culture are similar (72–89 × 27–42 μm,  $\bar{x}$  = 79.2 × 35.0 μm, 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. 3w).

**Material 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 Lúa, 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 and Crane (1977), who observed the holotype of *C. condensatum*.

*Cryptocoryneum condensatum* is very similar to *C. paracondensatum* A. Hashim. & Kaz. Tanaka 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. 3w) as compared to those of *C. paracondensatum* (21–26 mm; Fig. 3aa). 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*.

**4. *Cryptocoryneum congregatum*** A. Hashim. & Kaz. Tanaka, **sp. nov.**

Fig. 2m–p

**Holotype:** HHUF 30479

**Mycobank:** MB 815319

**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.

**Cultural characteristics:** 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. 3x).

**Material 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** designated here; 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. neolitiseae* Hansf. in conidial size and number of conidial septation, but the two species can be distinguished by the color of conidiophores (vs. black in the latter; Hansford 1956).

**5. *Cryptocoryneum hysterooides*** (Corda) Peyronel, Nuovo Giornale Botanico Italiano 25: 449 (1918) Fig. 2q–x

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

≡ *Hormiscium hysterooides* (Corda) Sacc., Sylloge Fungorum 4: 264 (1886)

≡ *Exosporium hysterooides* (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)  $\mu\text{m}$  diam., (50)65–82  $\mu\text{m}$  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, 7–9  $\times$  4–5  $\mu\text{m}$ . *Conidia* solitary, acrogenous, branched, cheiroid, (60)65–80  $\times$  13.5–48(59)  $\mu\text{m}$  ( $\bar{x}$  = 70.6  $\times$  32  $\mu\text{m}$ , 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  $\mu\text{m}$  wide ( $\bar{x}$  = 7  $\mu\text{m}$ , n=57); *arms* cylindrical, brown, branched at base, smooth, 11–16(18)-septate ( $\bar{x}$  = 14, n=57), (49)55–76(79)  $\mu\text{m}$  long ( $\bar{x}$  = 66.1  $\mu\text{m}$  long, n=102), 3–7  $\mu\text{m}$  wide at base, 2.5–7  $\mu\text{m}$  wide at apex.

**Material 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. hysterooides*); Locality unknown, on dead wood, date unknown, A. C. J. Corda (PRM 155687).

**Notes:** *Cryptocoryneum hysterooides* has been considered a synonym of *C. condensatum*, with the latter regarded as the type species of the genus (Hughes 1958; Ellis 1971; Schoknecht and 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 hysterooides* 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. hysterooides* was confirmed by our morphological examination of their type specimens, and therefore, we have redesignated

*C. hysterooides* (= *C. fasciculatum*) as the type species of *Cryptocoryneum*. Fresh material and cultures of *C. hysterooides* are still needed to clarify its exact phylogenetic position within *Cryptocoryneum*.

**6. *Cryptocoryneum japonicum*** A. Hashim. & Kaz. Tanaka, **sp. nov.** Fig. 3a–d

**Holotype:** HHUF 30482

**Mycobank:** MB 815320

**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.

**Cultural characteristics:** 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. 3y).

**Material 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** designated here; 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* A. Hashim. & Kaz. Tanaka, 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 µm 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.

**7. *Cryptocoryneum longicondensatum*** A. Hashim. & Kaz. Tanaka, **sp. nov.** Fig. 3e–h

*Holotype*: HHUF 30486

*Mycobank*: MB 815321

*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) µm diam., (45)75–95 µm 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 × 3–6(11) µm. *Conidia* solitary, acrogenous, branched, cheiroid, (69)74–92 × (12)17–66(96) µm ( $\bar{x}$  = 81.6 × 39.7 µm, 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 µm ( $\bar{x}$  = 7.1 µm, n = 70); *arms* cylindrical, pale brown, branched at base, smooth, (14)16–21-septate ( $\bar{x}$  = 18, n = 50), (67)70–88 µm long ( $\bar{x}$  = 78.9 µm long, n = 65), 3–6 µm wide at base, 2–3.5 µm wide at apex.

*Cultural characteristics*: 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. 3z); without sporulation.

*Material 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** designated here; 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* A.

Hashim. & Kaz. Tanaka in the phylogenetic tree (84%/1.00 ML BP/Bayesian PP) (Fig. 1), it differs from the latter in having relatively longer conidia (vs. 58–88.5 µm long in the latter).

**8. *Cryptocoryneum paracondensatum*** A. Hashim. & Kaz. Tanaka, **sp. nov.** Fig. 3i–l

*Holotype*: HHUF 30489

*Mycobank*: MB 815322

*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.

*Cultural characteristics*: 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. 3aa); without sporulation.

*Material 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** designated here; ex-holotype living culture MAFF 245377 = NBRC 111763).

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

**9. *Cryptocoryneum pseudorilstonei*** A. Hashim. & Kaz. Tanaka, **sp. nov.** Fig. 3m–p

*Holotype*: HHUF 30490

*Mycobank*: MB 815323

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

*Saprobic* on *Elegia equisetacea*. **Sexual morph**: unknown. **Asexual morph**: *Sporodochia* pulvinate, 160–260(390) µm 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 × 6.5–10 µm. *Conidia* solitary, acrogenous, branched, cheiroid, (14.5)17–25 × 14–27 µm ( $\bar{x}$  = 19.5 × 18.8 µm, 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 µm wide ( $\bar{x}$  = 6.4 µm, n = 50); *arms* cylindrical, hyaline to pale brown, branched at base, smooth, 2–6-septate ( $\bar{x}$  = 4, n = 47), 14–22(24.5) µm long ( $\bar{x}$  = 17.8 µm long, n = 47), 3.5–7 µm wide at base, 2.5–5 µm wide at apex.

**Cultural characteristics:** 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. 3ab); with abundant sporulation.

**Material 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** designated here; 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 × 17–32.5 µm].

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* S.S. Silva et al. in conidial features, but the latter has well-inflated conidiophores (da Silva et al. 2015) unlike those of *C. pseudorilstonei*.

**10. *Cryptocoryneum rilstonei*** [as *rilstonii*] M.B. Ellis, Mycological Papers 131: 2 (1972) Fig. 3q–t

**Saprobic** on twigs of *Fraxinus excelsior*. **Sexual morph:** unknown. **Asexual morph:** *Sporodochia* pulvinate, 140–260(330) µm 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 × 17–32.5 µm ( $\bar{x}$  = 30.4 × 24.1 µm, 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 µm ( $\bar{x}$  = 7 µm, n = 50); *arms* cylindrical, pale brown, branched at base, smooth, (3)5–10-

septate, ( $\bar{x}$  = 7, n = 50), (12)17–35 µm long ( $\bar{x}$  = 24.1 µm, n = 23), 3–6 µm wide at base, 3–5 µm wide at apex.

**Material 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. 3r–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* (da 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  $\mu\text{m}$  long, and up to 16-septate; (60)65–80  $\times$  13.5–48(59)  $\mu\text{m}$ , with 4–10 arms, (49)55–76(79)  $\mu\text{m}$  long, 11–16(18)-septate . . . . . *C. hysteroioides* (this study)
12. Average of conidial arms more than 75  $\mu\text{m}$  long, and mostly 7 arms; conidia (64)69–91(97)  $\times$  19.5–58(70)  $\mu\text{m}$  ( $\bar{x}$  = 76.8  $\times$  35.8  $\mu\text{m}$ ), with 5–10 arms ( $\bar{x}$  = 7), 64–89.5  $\mu\text{m}$  long ( $\bar{x}$  = 73.8  $\mu\text{m}$  long) . . . . . *C. condensatum* (this study)
- 12\*. Average of conidial arms less than 75  $\mu\text{m}$  long, and mostly 5 arms; conidia 58–88.5  $\times$  (13)15–57(72)  $\mu\text{m}$  ( $\bar{x}$  = 72.3  $\times$  31  $\mu\text{m}$ ), with 3–8(11) arms ( $\bar{x}$  = 5), 52–85.5  $\mu\text{m}$  long ( $\bar{x}$  = 67.9  $\mu\text{m}$  long) . . . . . *C. paracondensatum* (this study)
13. Conidia up to 50–61  $\mu\text{m}$  long, up to 5–10-septate . . . 14
- 13\*. Conidia up to 22–40  $\mu\text{m}$  long, up to 4–8-septate . . . 16
14. Conidiophores hyaline to pale brown . . . . . 15
- 14\*. Conidiophores black; conidia 40–60  $\times$  7–8  $\mu\text{m}$ , with 5–10-septate . . . . . *C. neolitseae* (Hansford 1956)
15. Conidia up to 54  $\mu\text{m}$  long, and up to 9 arms; conidia (31)40–54  $\times$  16–40(48.5)  $\mu\text{m}$ , with 3–9 arms, (5)7–11-septate . . . . . *C. congregatum* (this study)
- 15\*. Conidia up to 61  $\mu\text{m}$  long, and up to 15 arms; conidia (34)38–61(67)  $\times$  19–72  $\mu\text{m}$ , with 6–15 arms, 7–13-septate . . . . . *C. akitaense* (this study)
16. Conidia more than 23  $\mu\text{m}$  long. . . . . 17
- 16\*. Conidia less than 23  $\mu\text{m}$  long; 21–22  $\times$  3.5  $\mu\text{m}$ , 5-septate . . . . . *C. scopiforme* (Saccardo 1892)
17. Conidia up to 44  $\mu\text{m}$  long; 25–44  $\times$  6–7  $\mu\text{m}$ , 4–8-septate . . . . . *C. bombacis* (Hennings 1908)
- 17\*. Conidia up to 28  $\mu\text{m}$  long; 23–28  $\times$  2.3–3.6  $\mu\text{m}$ , 5–7-septate . . . . . *C. psammae* (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* Lév. 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. hysteroioides* (= *Torula hysteroioides* Corda; Corda 1837) and *T. uniformis* Peck (Peck 1880) as synonymous with *C. condensatum* (= *Hormiscium*

*condensatum* Wallr.; 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 and Crane 1977; Kirk 1983; Katumoto 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. hysteroioides*. *Cryptocoryneum hysteroioides*, however, should be separated from *C. condensatum* as a distinct species on the basis of their minute morphological differences; we therefore redesignate *C. hysteroioides* 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 and 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 and 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 18S and 28S and the second largest subunit of RNA polymerase II, will be required to determine the familial placement of *Cryptocoryneum*.

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## References

- Akaike H (1974) A new look at the statistical model identification. IEEE Trans Autom Contr 19:716–723

- Corda ACJ (1837) *Icones fungorum hucusque cognitorum*, vol 1. J.G. Calve, Prague
- Endo M, Hatakeyama S, Harada Y, Tanaka K (2008) Description of a coelomycete *Ciliochorella castaneae* newly found in Japan and notes on its distribution and phylogeny. *Nippon Kingakukai Kaiho* 49:115–120 (in Japanese)
- Ellis MB (1963) Dematiaceous hyphomycetes. IV. *Mycol Pap* 87:1–42
- Ellis MB (1971) Dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew
- Ellis MB (1972) Dematiaceous hyphomycetes. XI. *Mycol Pap* 131:1–25
- Fuckel L (1865) *Fungi Rhenani Exsiccati Cent. XV–XVI:1401–1600*
- Hansford CG (1956) Australian Fungi. III. New species and revisions. *Proc Linn Soc N S W* 81:23–51
- Hashimoto A, Sato G, Matsuda T, Hirayama K, Hatakeyama S, Harada Y, Shirouzu T, Tanaka K (2015a) Molecular taxonomy of *Dinemasporium* and its allied genera. *Mycoscience* 56:86–101
- Hashimoto A, Sato G, Matsuda T, Matsumura M, Hatakeyama S, Harada Y, Ikeda H, Tanaka K (2015b) Taxonomic revision of *Pseudolachnea* and *Pseudolachnella* and establishment of *Neopseudolachnella* and *Pseudodinemasporium* gen. nov. *Mycologia* 107:383–408
- Hatakeyama S, Tanaka K, Harada Y (2008) Bambusicolous fungi in Japan (7): a new coelomycetous genus, *Versicolorisporium*. *Mycoscience* 49:211–214
- Hennings PA (1908) *Fungi S. Paulensis IV a cl. Puttemans collecti*. *Hedwigia* 48:1–20
- Hirayama K, Tanaka K (2011) Taxonomic revision of *Lophiostoma* and *Lophiotrema* based on reevaluation of morphological characters and molecular analyses. *Mycoscience* 52:401–412
- Hughes SJ (1958) Revisionses hyphomycetum aliquot cum appendice de nominibus rejciendis. *Can J Bot* 36:727–836
- Hughes SJ (1978) New Zealand Fungi 25. Miscellaneous species. *N Z J Bot* 16:311–370
- Jobb G (2011) *Treefinder* Mar 2011. Available at <http://www.treefinder.de>
- Kamiyama M, Hirayama K, Tanaka K, Mel'nik VA (2009) Transfer of *Asterosporium orientale* to the genus *Prosthemium* (Pleosporales, Ascomycota): a common coelomycetous fungus with stellate conidia occurring on twigs of *Betula* spp. *Mycoscience* 50:438–441
- Katamoto K (1988) Materials for the fungus flora of Japan (43). *Trans Mycol Soc Japan* 29:359–362
- Kirk PM (1982) New or interesting microfungi. V. Microfungi colonizing *Laurus nobilis* leaf litter. *Trans Br Mycol Soc* 78:293–303
- Kirk PM (1983) New or interesting microfungi. X. Hyphomycetes on *Laurus nobilis* leaf litter. *Mycotaxon* 18:259–298
- Mel'nik VA (2000) *Definitorium fungorum Rossiae. Classis hyphomycetes. Vol. 1. Fam. Dematiaceae*, Nauka, Sankt-Peterburg (in Russian)
- Oudemans CAJA (1892) Contributions à la flore mycologique des Pays-Bas. XIV. *Nederl kruidk Archf, Ser. 2*, 6:1–65
- Peck CH (1880) Report of the Botanist. *Ann Rep N Y State Mus Nat Hist* 33:11–49
- Peyronel B (1918) Micromiceti di Val Germanasca. *Nuovo G Bot Ital* 25: 405–464
- Prostakova M (1966) Species fungorum novae in plantis fructiferis et bacciferis moldaviae. *Infektsioznye Zabolevaniya Kulturnykh Rastenii Moldavii Infekts Zabol kul'tur Rast Mold* 6:10–12 (in Russian)
- Rambaut A, Suchard MA, Xie W, Drummond AJ (2014) *Tracer 1.6*. Available at <http://beast.bio.ed.ac.uk/Tracer>
- Rayner RW (1970) A mycological color chart. Commonwealth Mycological Institute and British Mycological Society, Kew
- Rehner SA, Buckley E (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97:84–98
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 61:539–542
- Saccardo PA (1892) *Sylloge Fungorum omnium hucusque cognitorum* 10. Published by the author, Berlin
- Saccardo PA (1920) *Mycetes Boreali-Americani a cl. Doct. J.R. Weir (Spokane, Washington) an. MCMXIX communicati*. *Nuovo G Bot Ital* 27:75–88
- Sato G, Tanaka K, Hosoya T (2008) Bambusicolous fungi in Japan (8): a new species of *Pseudolachnella* from Yakushima Island, southern Japan. *Mycoscience* 49:392–394
- Sawada K (1943) Descriptive catalogue of Formosan fungi. Part IX. *Rept Dep Agr Gov Res Inst Formos* 86:1–178
- Schoknecht JD, Crane JL (1977) Revision of *Torula* and *Hormiscium* Species. *Torula occulta*, *T. diversa*, *T. elasticae*, *T. bigemina* and *Hormiscium condensatum* reexamined. *Mycologia* 69:533–546
- Schwarz G (1978) Estimating the dimension of a model. *Ann Stat* 6:461–464
- da Silva SS, Gusmão LFP, Castañeda-Ruiz RF (2015) *Cryptocoryneum parvulum*, a new species on *Araucaria angustigolia* (Brazilian pine). *Mycotaxon* 130:465–469
- Talbot PHB (1952) Dispersal of fungus spores by small animals inhabiting wood and bark. *Trans Br Mycol Soc* 35:123–128
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Mol Biol Evol* 28:2731–2739
- Tanabe AS (2011) Kakusan4 and Aminosan: two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. *Mol Ecol Resour* 11:914–921
- Tanaka K, Endo M, Hirayama K, Okane I, Hosoya T, Sato T (2011) Phylogeny of *Discosia* and *Seimatosporium* and introduction of *Adisco* and *Immersidisco* genera nova. *Persoonia* 26:85–98
- Tanaka K, Hirayama K, Yonezawa H, Sato G, Toriyabe A, Kudo H, Hashimoto A, Matsumura M, Harada Y, Kurihara Y, Shirouzu T, Hosoya T (2015) Revision of the *Massarineae* (Pleosporales, Dothideomycetes). *Stud Mycol* 82:75–136
- Tanaka K, Mel'nik VA, Kamiyama M, Hirayama K, Shirouzu T (2010) Molecular phylogeny of two coelomycetous fungal genera with stellate conidia, *Prosthemium* and *Asterosporium*, on Fagales trees. *Botany* 88:1057–1071
- von Heftberger M, Mayrhofer H, Scheuer C (1997) Neuere Funde von *Cryptocoryneum condensatum* (Hyphomycetes, mitosporic Pilze) aus Österreich und Slowenien. *Mitt Naturwiss Ver Steiermark* 127: 61–64
- Viala P (1891) *Monographie du pourridié (Dematophora)*. Librairie de L'Académie de Médecine, Montpellier
- Wallroth CFW (1833) *Flora Cryptogamica Germaniae II*. J.L. Schrag, Norimbergae
- White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: A guide to methods and applications*. Academic, New York, pp 315–322
- Wijayawardene DNN, McKenzie EHC, Hyde KD (2012) Towards incorporating anamorphic fungi in a natural classification-checklist and notes for 2011. *Mycosphere* 3:157–228
- Yonezawa H, Tanaka K (2008) A second species of *Neoheteroceras* and additional characters of the genus. *Mycoscience* 49:152–154