



## Camarosporium sensu stricto in Pleosporinae, Pleosporales with two new species

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### Abstract

Three coelomycete species with muriform conidia were collected in Italy and subjected to morpho-molecular analyses. In morphology, all species are similar to *Camarosporium sensu stricto*. Based on morphology, one was identified as *C. spartii* and the other two taxa are introduced as new species, viz. *C. clematidis* and *C. robbiniicola*. Maximum likelihood, maximum parsimony and Bayesian analyses of the combined LSU, SSU and ITS dataset show that the three taxa cluster with *C. quaternatum*, the type species of *Camarosporium*, with high bootstrap and PP values. All four species can be considered as *Camarosporium sensu stricto*. *Cucurbitaria elongata* groups in the *Camarosporium* clade near to *Camarosporium robbiniicola* and is considered that it is not congeneric with *Cucurbitaria sensu stricto* (*Cucurbitariaceae*).

**Keywords:** asexual states, coelomycetes, molecular phylogeny, morphology

### Introduction

Coelomycetes are asexual fungi that produce conidia in cavities, *i.e.* pycnidial, acervular or stromatic conidiomata (Sutton 1980, Nag Raj 1993, Wijayawardene *et al.* 2012a), and are an important group in agriculture and phytopathology (Wijayawardene *et al.* 2012c). Since Hughes’ (1953) significant publication on conidial development and classification, considerable research has been carried out on the taxonomy of conidial fungi, *i.e.* coelomycetes and hyphomycetes. Sutton (1980) and Nag Raj (1993) are the main classic monographs on coelomycetous fungi, and are largely based on morphology as the criteria for identification. Sutton (1980) introduced six sub-orders to accommodate coelomycetous taxa based on the shape of conidiomata (*i.e.* pycnidial or stromatic) and conidial development (*i.e.* blastic or thallic). Even though this classification system was realized to be highly artificial from a phylogenetic perception (de Gruyter *et al.* 2009, Aveskamp *et al.* 2010, Wijayawardene *et al.* 2013a, b), it is still an outstanding work for identification of coelomycetes. However, recent molecular studies (Dai *et al.* 2012, de Gruyter *et al.* 2012, Crous *et al.* 2013, Wijayawardene *et al.* 2013a, b) have shown a better resolution of taxonomic placement of coelomycetous taxa with links to their sexual morphs (Kendrick 1979). The asexual coelomycetes with established links to sexual morphs must now also be given a single name (Chomnunti *et al.* 2011, Hawksworth 2012, Wijayawardene *et al.* 2014).

The genus *Camarosporium* was introduced by Schulzer (1870: 649) with *C. quaternatum* Schulzer as the type species. Currently, there are more than 500 names listed in the genus (Index Fungorum 2014). During our collection of coelomycetes in Italy (Wijayawardene *et al.* 2013b, 2014), we collected several camarosporium-like species and carried out morpho-molecular studies. In this study we report on molecular data analyses of a combined dataset of large subunit rDNA (LSU), small subunit rDNA (SSU) and internal transcribed spacer (ITS) for three taxa that shows them to belong to *Pleosporinae*, *Pleosporales*.

De Not., the type species of *Pyrenochaeta* (ex-type strain CBS 407.76, *fide* de Gruyter *et al.* 2009). *Pyrenochaeta acicola* (CBS 122789, *fide* de Gruyter *et al.* 2009), *Pyrenochaeta quercina* (CBS 115095, *fide* de Gruyter *et al.* 2010) and *Pyrenochaetopsis decipiens* (CBS 343.85, *fide* de Gruyter *et al.* 2010) also clustered in the same clade.

We have re-examined the specimen of *Cucurbitaria elongata* (MFLU 13-0085 *fide* Hyde *et al.* 2013), from which MFLUCC 14-0260 was isolated (Ariyawansa unpub.). We also compared the morphology with illustrations in Saccardo (1883) and Mirza (1968) (Table 2). *Cucurbitaria elongata* has ascospores with a wide range of dimensions. Other major characters such as number of septa (and muriform conidia), colour of the spore (dark brown), spore arrangement (uniseriate), number of spores in ascus (mainly 8) are similar.

**TABLE 2.**—Comparison of morphology of *Cucurbitaria elongata*

Host	Ascomata	Ascospores ( $\mu\text{m}$ )	Ascospores ( $\mu\text{m}$ )	References
<i>Robinia pseudoacacia</i>		120–150 $\times$ 12–14	25–28 $\times$ 10–12	Saccardo 1883
<i>R. pseudoacacia</i>	650–950 $\times$ 500–775	140–255 $\times$ 14–18	21–38 $\times$ 8–12	Mirza 1968
<i>Cytisus scoparius</i>		135–155 $\times$ 10–15	19–23 $\times$ 8–11	Hyde <i>et al.</i> 2013 (MFLU 13-0085)

*Cucurbitaria berberidis*, the type species of *Cucurbitaria* is morphologically similar to *C. elongata* (Mirza 1968, Doilom *et al.* 2013, Hyde *et al.* 2013). Doilom *et al.* (2013) reported *C. berberidis* with a pyrenochaeta-like asexual state from pure culture. *Cucurbitaria elongata* (MFLU 13-0085) has not produced an asexual state in culture (Ariyawansa unpub.).

Saccardo (1884) mentioned that *Cucurbitaria elongata* is the sexual state of *Camarosporium robiniae*. Mirza (1968) and Ellis & Ellis (1985) did not agree with this link but accepted that *Cucurbitaria elongata* has a *Camarosporium* asexual state. Mirza (1968) established the link between *Camarosporium* and *Cucurbitaria elongata* based on culture methods and observed pycnidia in the culture of *Cucurbitaria elongata*. The spores produced in the pycnidia are very similar (13–21  $\times$  7–10  $\mu\text{m}$ ) with those in our collection of *Camarosporium robiniae* (18–28  $\times$  7–11  $\mu\text{m}$ ).

In this study we accept *Camarosporium* as a distinct genus, which is accommodated in *Pleosporinae*, *Pleosporales*, as it groups away from *Cucurbitariaceae* and *Leptosphaeriaceae*. However, there are only a few *Camarosporium* species that have been subjected to molecular based identification and analyses. Hence, it is essential to re-collect and carry out both morpho-molecular analyses to clarify the generic concept for *Camarosporium sensu stricto* to identify other camarosporium-like coelomycetes.

## Acknowledgments

The Mushroom Research Foundation (MRF), Chiang Rai Province, Thailand is acknowledged for providing Postgraduate Scholarship support for Nalin N. Wijayawardene and K.W.T. Chethana. Erio Camporesi thanks Giancarlo Lombardi for his invaluable help in the collecting programme and identifying host plants. Nalin N. Wijayawardene thanks Dhanushka Udayanga for his help to carry out molecular analyses. Appreciation is extended to the Guizhou Province Research Fund No. 20113045 for funding all molecular studies and Mae Fah Luang University grant for studying *Dothideomycetes* (no. 56101020032).

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