A preliminary account of the *Cucurbitariaceae*

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Abstract: Fresh collections, type studies and molecular phylogenetic analyses of a multigene matrix of partial nuSSU-ITS-LSU rDNA, *rpb2*, *tef1* and *tub2* sequences were used to evaluate the boundaries of *Cucurbitaria* in a strict sense and of several related genera of the *Cucurbitariaceae*. Two species are recognised in *Cucurbitaria* and 19 in *Neocucurbitaria*. The monotypic genera *Astragalicola*, *Cucitella*, *Parafenestella*, *Protofenestella*, and *Seltsamia* are described as new. *Fenestella* is here included as its generic type *F. fenestrata* (= *F. princeps*), which is lecto- and epitypified. *Fenestella mackenzei* and *F. ostryae* are combined in *Parafenestella*. Asexual morphs of *Cucurbitariaceae*, where known, are all pyrenochaeta- or phoma-like. Comparison of the phylogenetic analyses of the ITS-LSU and combined matrices demonstrate that at least *rpb2* sequences should be added whenever possible to improve phylogenetic resolution of the tree backbone; in addition, the *tef1* introns should be added as well to improve delimitation of closely related species.

Key words: *Ascomycota*, *Dothideomycetes*, new taxa, *Phoma*, phylogenetic analysis, *Pleosporales*, *Pyrenochaeta*, pyrenomycetes.
Taxonomic novelties: New genera: *Astragalicola* Jaklitsch & Voglmayr, *Cucitella* Jaklitsch & Voglmayr, *Parafenestella* Jaklitsch & Voglmayr, *Protofenestella* Jaklitsch & Voglmayr, *Seltsamia* Jaklitsch & Voglmayr; **New species:** *Astragalicola amorpha* Jaklitsch & Voglmayr, *Cucitella opali* Jaklitsch & Voglmayr, *Cucurbitaria oromediterranea* Jaklitsch & Voglmayr, *Neocucurbitaria acanthocladae* Jaklitsch & Voglmayr, *N. aetnensis* Jaklitsch & Voglmayr, *N. cinereae* Jaklitsch & Voglmayr, *N. cisticola* Jaklitsch & Voglmayr, *N. juglandicola* Jaklitsch & Voglmayr, *N. populi* Jaklitsch & Voglmayr, *N. rhamnicola* Jaklitsch & Voglmayr, *N. rhamnioides* Jaklitsch & Voglmayr, *N. ribicola* Jaklitsch & Voglmayr, *N. vachelliae* Jaklitsch & Voglmayr, *Parafenestella pseudoplatani* Jaklitsch & Voglmayr, *Protofenestella ulmi* Jaklitsch & Voglmayr, *Seltsamia ulmi* Jaklitsch & Voglmayr; **New combinations:** *Neocucurbitaria rhamni* (Nees : Fr.) Jaklitsch & Voglmayr, *Parafenestella mackenziei* (Wanas. *et al.*) Jaklitsch & Voglmayr, *Parafenestella ostryae* (Wanas. *et al.*) Jaklitsch & Voglmayr; **Epitypifications (basionyms):** *Sphaeria rhamni* Nees, *Fenestella princeps* Tul. & C. Tul., *Valsa fenestrata* Berk. & Broome.

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INTRODUCTION

The family *Cucurbitariaceae* was described by Winter (1885; as *Cucurbitarieae*), who listed *Cucurbitaria*, *Gibbera*, *Gibberidea*, *Nitschkia* and *Otthia* as members of this family. He used the family for non-stromatic pyrenomycetes forming ascomata "in lawns", i.e., more or less grouped and superficial on the substrate or on a hypostroma when present. Arx & Müller (1975) incorporated the family in the *Pleosporaceae*. Over the years the family was reduced to *Cucurbitaria*, while Barr (1987) included also *Cucurbidothis*, *Otthia*, *Rhytidiella* and *Syncarpella*. This concept (excluding *Otthia*) was presented by Doilom *et al.* (2013), who also included *Pyrenochaeta* and *Pyrenochaetopsis* following earlier phylogenetic analyses (Aveskamp *et al.* 2010, de Gruyter *et al.* 2010, 2012). They also epitypified the generic type of *Cucurbitaria*, *C. berberidis*, using material collected in Austria. However, *Cucurbidothis pityophila* does not belong to the *Cucurbitariaceae*. It has a putative coniothyrium-like asexual morph intimately associated with ascomata. According to Valenzuela-Lopez *et al.* (2018) this species (represented by strain CBS 149.32) is a member of the *Didymosphaeriaceae*, albeit with a very long branch in their phylogenetic tree. *Cucurbidothis* was often treated as a synonym of *Curreya* (Arx & Müller 1975, Arx & van der Aa 1983). The generic type of the latter, *C. conorum*, has not been collected recently. Also this fungus may not be a member of the *Cucurbitariaceae*, judging from, e.g., the biseriate arrangement of ascospores in clavate asci and some stromatic tissues surrounding the ascomata. Barr (1981) had even combined *C. conorum* in *Pleospora*. Other species assigned to *Curreya*, *C. acaciae*, *C. austroafricana*, *C. grandicipis* and *C. proteae* belong to *Teichospora* in the *Teichosporaceae* (Jaklitsch *et al.* 2016). *Rhytidiella* and *Syncarpella* differ from all fungi identified in the *Cucurbitariaceae* by cylindrical to vermiform phragmospores (see Doilom *et al.* 2013) and ecologically by inducing cankers (Barr & Boise 1989, Zalasky 1975). No DNA data are available for these genera.

Cucurbitaria is one of the oldest genera of ascomycetes separated from *Sphaeria*. The genus, as defined by its type species, *C. berberidis*, is characterised by tuberculate perithecioid ascomata with basally thickened and elongated peridium sitting on a common subiculum often termed hypostroma and erumpent from bark in groups, by cylindrical fissitunicate asci with uniseriate arrangement of the brown muriform ascospores, and a pyrenochaeta- or, more generalised, phoma-like asexual morph. This and other species of *Cucurbitaria* are usually regarded as saprotrophs or necrotrophs (Doilom *et al.* 2013, Mirza 1968).

A vague original definition of the genus *Cucurbitaria* led to misuse of the generic name for many unrelated genera of

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pyrenomycetes. Therefore, Index Fungorum (June 2017) lists 465 epithets including 34 infraspecific taxa, of which at least 340 do not belong to the *Cucurbitariaceae*. Owing to Kuntze (1898) more than 220 combinations in *Cucurbitaria* represent nectria-like fungi (*Hypocreales*); others are homonyms, illegitimate names or erratic entries, many others belong to different genera. Welch (1926) studied morphologically type materials present in American herbaria, commented on many taxa and accepted only five species in *Cucurbitaria* (*C. arizonica*, *C. berberidis*, *C. caraganae*, *C. elongata* and *C. laburni*). He synonymised many names, excluded others from the genus and determined that type material of most species was inadequate for unequivocal interpretation. Barr (1990a) accepted 11 species for North America. The latest comprehensive monographic study of the genus was performed by Mirza (1968) in the pre-molecular period. He studied 28 species, of which he described six new ones, creating two homonyms, cultured eight species and reported that six asexual genera, *Camarosporium*, *Coniothyrium*, *Hendersonia*, *Leptophoma*, *Phoma* and *Pyrenochaeta*, were associated with sexual morphs of this genus. In pure culture he found several developmental conidial stages including diplodia-like morphs. In recent years the connection of asexual morphs to their sexual morphs has proven to be phylogenetically informative at the generic to even ordinal level in the *Dothideomycetes* (Crous 2009, Crous *et al.* 2009, Slippers *et al.* 2013, Hyde *et al.* 2013, Jaklitsch & Voglmayr 2016). However, the respective genera are often polyphyletic, mainly because morphological delimitation of similar genera offering few easily recognisable and little varying features or which are incompletely studied regarding their life cycles, is difficult or sometimes impossible, and therefore unrelated fungi are subsumed under a common generic name (Crous *et al.* 2009). For example, phoma-like genera such as *Pleurostromella* (Petrak 1922), *Pleurophoma*, *Pleurophomella*, *Pyrenochaeta* and others are morphological variants for the same asexual morphs that have been associated with the *Cucurbitariaceae*, but they can be also found in many other families of the *Pleosporales* (de Gruyter *et al.* 2012, Jaklitsch & Voglmayr 2016).

For many *Cucurbitaria* species a camarosporium-like asexual morph was determined by morphology and culture studies (Mirza 1968, Sivanesan 1984). However, *Camarosporium* appears to be unavailable for these fungi after the epitypification of its type species, *C. quaternatum* by Crous & Groenewald (2017). Recently, *Camarosporium* s. lat. was treated by Wanasinghe *et al.* (2017a), who combined some of the most common species, particularly those on fabaceous hosts such as *Cucurbitaria caraganae*, *C. elongata* and *C. laburni*, in their new genus *Camarosporidiella* (*Camarosporidiellaceae*).

A few species once in *Cucurbitaria* have recently been identified as belonging to different genera, e.g., *Cucurbitaria bicolor*, which is a synonym of *Thyronectria rhodochlora* in the *Nectriaceae*, *Hypocreales* (Checa *et al.* 2015), while *Cucurbitaria obducens*, *C. piceae* and *C. rhododendri* belong to the *Melanommataceae* (Jaklitsch & Voglmayr 2017).

The phylogenetic studies cited above suggest that only few taxa remain in *Cucurbitariaceae* s. str. *Pyrenochaeta* has been attributed to *Cucurbitariaceae*, as *Cucurbitaria berberidis* produces a pyrenochaeta-like asexual morph, but also other *Pyrenochaeta* spp., e.g. *P. cava* and *P. nobilis*, and *Pyrenochaetopsis* spp. were identified as members of the *Cucurbitariaceae* (Chen *et al.* 2015, de Gruyter *et al.* 2010, 2012). Recently, Wanasinghe *et al.* (2017b) placed two of them (*P. quercina*, *P. unguis-hominis*) in their new genus *Neocucurbitaria*. Most recently, Valenzuela-Lopez *et al.* (2018) performed an extensive study of phoma- and pyrenochaeta-like coelomycetes, studying more than 350 strains mostly from the CBS and the UTHSC, including many new isolates from medical environments. They established several new families and genera, recognised many *Phoma* spp. in various genera of the *Didymellaceae*, as had been partly also shown in earlier works (see, e.g., Chen *et al.* 2015). In the *Cucurbitariaceae* Valenzuela-Lopez *et al.* (2018) combined *Pyrenochaeta cava*, *P. hakeae* and *P. keratinophila* in *Neocucurbitaria*, clarified the concept of and epitypified *Pyrenochaeta quercina*, the basionym of *N. quercina*, and described the new species *Neocucurbitaria aquatica* and *N. irregularis*. They also described the new monotypic genus *Allocucurbitaria*, and for *Plenodomus corni*, earlier also known as *Pyrenochaeta corni* (Boerema *et al.* 1996) and for the new species *P. italica*, based on a strain previously identified as *Pyrenochaeta corni*, they described the new genus *Paracucurbitaria*. Valenzuela-Lopez *et al.* (2018) excluded all other species of *Pyrenochaeta* that had been recognised by Wanasinghe *et al.* (2017b) as belonging to the *Cucurbitariaceae* from the family erecting several new genera and families. They also excluded *Pyrenochaeta* s. str. from the *Cucurbitariaceae* and erected a new family for *Pyrenochaetopsis*.

In our present work we include the genera *Allocucurbitaria*, *Cucurbitaria*, *Neocucurbitaria*, *Paracucurbitaria*, and the five new monotypic genera *Astragalicola*, *Cucitella*, *Parafenestella*, *Protofenestella*, and *Seltsamia*. The genus *Fenestella* is included as its generic type *F. fenestrata* (= *F. princeps*), which is lecto- and epitypified in order to stabilize its name and phylogenetic position.

MATERIALS AND METHODS

Isolates and specimens

All isolates used in this study originated from ascospores or conidia (where noted) of fresh specimens. Strain identifiers including NCBI GenBank accession numbers of gene sequences used to compute the phylogenetic trees are listed in Table 1. Strain acronyms other than those of official culture collections are used here primarily as strain identifiers throughout the work. Representative isolates have been deposited at the Westerdijk Fungal Biodiversity Centre, Utrecht, The Netherlands (CBS culture collection). Details of the specimens used for morphological investigations are listed in the Taxonomy section under the respective descriptions. The following cultures were sequenced but not further treated here: *Phaeosphaeria* (*Amarenomyces*) *ammophilae*: Sweden, Halland: Varberg, Apelviken, sandy beach, from old leaves of *Ammophila arenaria*, 31 Oct. 2015, S. Lund, det. and comm. O. Eriksson (WU 36958; culture AA); *Plenodomus hendersoniae*: Austria, Steiermark, Deutschlandsberg, Koralmgebiet, forest road to Grünangerhütte from the north, before the wooden bridge over the Schwarze Sulm, on *Salix appendiculata*, 16 May 2015, G. Friebes (WU 36959; culture LTO). Herbarium acronyms are according to Thiers (2017). Freshly collected specimens have been deposited in the Fungarium of the Department of Botany and Biodiversity Research, University of Vienna (WU).