



## A family level rDNA based phylogeny of Cucurbitariaceae and Fenestellaceae with descriptions of new *Fenestella* species and *Neocucurbitaria* gen. nov.

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### Abstract

The taxonomy of the family Cucurbitariaceae and its allies, especially Fenestellaceae has received little attention despite its broad relevance. To further clarify phylogenetic relationships between the two families, new collections were made and samples examined morphologically. Morphometric observations reveal two new *Fenestella* species that are different from other known species and are introduced as *Fenestella ostryae* sp. nov. and *F. mackenziei* sp. nov. The asexual morph of *F. ostryae* is also established from single spore cultures and described. Phylogeny inferred from a combined dataset of ITS, LSU and SSU rDNA sequence also reveals a close association of the new taxa to *F. fenestrata*, but they are distinct. Sequence analyses also support our previous assumption that Fenestellaceae should be synonymized with Cucurbitariaceae. *Pyrenochaeta quercina*, *Pyrenochaeta unguis-hominis* and an additional new cucurbitaria-like isolate constitute a strongly supported monophyletic lineage within Cucurbitariaceae. To better resolve intergeneric level relationships and improve taxonomic issues within Cucurbitariaceae, a new genus, *Neocucurbitaria* gen. nov. is introduced with *Neocucurbitaria acerina* as a new species.

**Key words** – Asexual morph – Dictyospores – Dothideomycetes – Italy – phylogeny – sexual morph

### Introduction

The family Cucurbitariaceae is a well-supported monophyletic family in the order *Pleosporales* (Doilom et al. 2013, Hyde et al. 2013, Wijayawardene et al. 2014, Li et al. 2016). The family was introduced by Winter (1885) and typified by *Cucurbitaria berberidis*. Cucurbitariaceae

is characterized by aggregated, ostiolate ascomata, which are seated on a basal stromatic structure, fissitunicate and cylindrical asci, pigmented, phragmosporous or muriform ascospores and a necrotrophic or saprobic lifestyle on woody plants (Hyde et al. 2013). Intergeneric classification based on phenotypes within Cucurbitariaceae has often been controversial. For example, Barr (1987) considered 'turbinate or globose or ovoid ascomata, with warted or nearly smooth surfaces, cylindrical or slightly clavate or oblong asci, symmetric and ellipsoid or fusoid or asymmetric and oblong or elongate ascospores' as general features of Cucurbitariaceae and considered the family to belong in Pleosporales. Considering the above phenotypic features, genera such as *Cucurbitaria*, *Cucurbitidothis*, *Othia*, *Rhytidiella* and *Syncarpella* were also included in the family. Later phylogenetic studies have shown that Cucurbitariaceae is a heterogeneous group and recent studies have excluded some genera from this family and referred other genera to the family (see the notes under Cucurbitariaceae in Hyde et al. 2013 and Doilom et al 2013).

Doilom et al. (2013) revisited the family Cucurbitariaceae based on DNA sequence data in databases to verify its phylogenetic affinities, examination of type species and links to asexual morphs. They epitypified *Cucurbitaria berberidis* with molecular data and a pyrenochaeta-like asexual morph, illustrated *Curreya*, *Rhytidiella* and *Syncarpella* from their holotypes and discussed their familial affinities. Hyde et al. (2013) also provided a comprehensive transcript to this family with illustrations. Wijayawardene et al. (2014) included *Cucurbitidothis*, *Cucurbitaria*, *Curreya*, *Pyrenochaeta*, *Pyrenochaetopsis*, *Rhytidiella* and *Syncarpella* as conventional genera in Cucurbitariaceae. However, Cucurbitariaceae members still comprise a large number of epithets in Index Fungorum (Doilom et al. 2013) and only a small number of species have DNA sequence data in GenBank. Thambugala et al. (2015) have introduced a new genus, *Neocurreya* for *Curreya austroafricana*, *C. grandicipis* and *C. proteae* in Floricolaceae (Pleosporales) based on evidence from morphology and phylogeny. The placement of the type species of *Curreya*, *C. conorum* is still unclear as the latter has not yet been cultured and there is no DNA sequence data in databases to verify their phylogenetic affinities. In addition, DNA sequence is also unavailable for species of *Cucurbitidothis*, *Rhytidiella* and *Syncarpella*. The taxonomy of these similar and closely related genera warrants further investigations based on fresh collections and phylogenetic inferences.

We have recently revisited the taxonomy of various members of pleosporaceous Dothideomycetes, with the intention of providing a better understanding of their phylogeny based on both morphology and multi-gene phylogenetic analyses. During collections and examination, we have isolated taxa from Italy which belong to the Cucurbitariaceae based on our preliminary phylogenetic analyses. Subsequently, with further morphological and molecular characterization, we present an account of a new genus, *Neocucurbitaria* and rectify the familial placement of *Fenestella* with two new species. The new taxa are compared with other species in Cucurbitariaceae and comprehensive descriptions and micrographs are provided and supported with DNA based phylogenies.

## Materials & Methods

### Sample collection, morphological studies and isolation

Fresh fungal material was collected from Forlì-Cesena Province in Italy and brought to the laboratory in Zip lock plastic bag. Samples were examined with a Motic SMZ 168 Series microscope. Hand sections of the fruiting structures were mounted in water for microscopic studies and photomicrography. The taxa were examined using a Nikon ECLIPSE 80i compound microscope and photographed with a Canon 550D digital camera fitted to the microscope. Indian ink was added to water mounts to show the presence of a gelatinous sheath around the ascospores. Measurements were made with the Tarosoft (R) Image Frame Work program and images used for figures processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems, USA).

Single ascospore isolation was carried out following the method described in Chomnunti et al. (2014). Germinated spores were individually transferred to potato dextrose agar (PDA) plates

and grown at 16°C in the daylight. Colony colour and other characters were observed and measured after three weeks. The specimens are deposited at the Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand. Living cultures are deposited at the Culture Collection of Mae Fah Luang University (MFLUCC). Faces of Fungi and Index Fungorum numbers are provided as outlined in Jayasiri et al. (2015) and Index Fungorum (2017) and new species are justified based on recommendations outlined by Jeewon & Hyde (2016).

### **DNA extraction and PCR amplification**

Fungal isolates were grown on PDA for 3–4 weeks at 25 °C and total genomic DNA was extracted from 50 to 100 mg of axenic mycelium scraped from the edges of the growing culture (Wu et al. 2001; Jeewon et al. 2002). Mycelium was ground to a fine powder with liquid nitrogen and DNA was extracted using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux, P.R. China) following the instructions of the manufacturer. DNA to be used as template for PCR were stored at 4 °C for use in regular work and duplicated at -20 °C for long term storage.

DNA sequence data was obtained from the partial sequences of three genes, the internal transcribed spacers (ITS1, 5.8S, ITS2), large subunit (28S, LSU) and small subunit rDNA (18S, SSU). ITS regions were amplified using primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). LSU was amplified using the primers LROR (5'-TCCTGAGGGAACTTCG-3') and LR5 (5'-ACCCGCTGAACTTAAGC-3') (Vilgalys & Hester 1990, Rehner & Samuels 1994). SSU was amplified using primers NS1 (5'-GTAGTCATATGCTTGTCTC-3') and NS4 (5'-CTTCCGTC AATTCCTTAAAG-3') (White et al. 1990).

Polymerase chain reaction (PCR) was carried out in a volume of 25 µl which contained 12.5 µl of 2× Power Taq PCR MasterMix (Biotek Co., China), 1 µl of each primer (10 µM), 1 µl genomic DNA and 9.5 µl deionized water. PCR thermal cycle program for gene amplifications were as follows: initialization at 95 °C for 5 mins, followed by 35 cycles of denaturation at 95 °C for 90 s, annealing at 55 °C (ITS and LSU) and 48 °C (SSU) for 1 min., elongation at 72 °C for 1 min. and final extension at 72 °C for 10 mins. PCR amplicons was verified on 1 % agarose electrophoresis gels stained with ethidium bromide. The amplified PCR fragments were sent to a commercial sequencing provider (BGI, Ltd Shenzhen, P.R. China). The nucleotide sequence data acquired were deposited in GenBank (Table 1).

### **Sequencing and sequence alignment**

Other sequences used in the analyses (Table 1) were obtained from GenBank based on blast searches in GenBank and published data (de Gruyter et al. 2010, Doilom et al. 2013, Ariyawansa et al. 2015, Crous et al. 2015). The multiple alignments of all sequences, as well as the reference sequences were automatically generated with MAFFT v. 7 (<http://mafft.cbrc.jp/alignment/server/index.html>; Katoh & Standley 2013), and were improved manually when necessary using BioEdit v. 7.0.5.2 (Hall 1999). Ambiguous regions were excluded from the analyses and gaps were treated as missing data.

### **Phylogenetic analysis**

Phylogenetic analyses of both individual and combined aligned data were performed under maximum-likelihood, maximum parsimony and Bayesian criteria. The sequence alignments were converted to NEXUS file (. nex) for maximum parsimony and Bayesian analyses using ClustalX2 v. 1.83 (Thompson et al. 1997). The NEXUS file was prepared for MrModeltest v. 2.2 after deleting the symbols ="ABCDEFGHIJKLMNQRSTUWXYZ" (Nylander 2004) in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002). For the Randomized Accelerated Maximum Likelihood (RAxML) analysis, sequence alignments were converted to PHYLIP file (. phy) using ALTER (alignment transformation environment: <http://sing.ei.uvigo.es/ALTER/>; 2016).

Parsimony analysis was carried with the heuristic search option in PAUP v. 4.0b10 with the following parameter settings: characters unordered with equal weight, random taxon addition, branch swapping with tree bisection-reconnection (TBR) algorithm, branches collapsing if the maximum branch length was zero. Alignment gaps were treated as missing characters in the analysis of the combine data set, where they occurred in relatively conserved regions. Trees were inferred using the heuristic search option with 1000 random sequence additions, with maxtrees set at 5000. Descriptive tree statistics for parsimony; Tree Length (TL), Consistency Index (CI), Retention Index (RI), Relative Consistency Index (RC) and Homoplasy Index (HI) were calculated for trees generated under different optimality criteria. The Kishino-Hasegawa tests (Kishino & Hasegawa 1989) were performed in order to determine whether trees were significantly different. Maximum parsimony bootstrap values (MP) equal or greater than 75 % are given above each node (Fig. 1). Other details pertaining to analyses (e.g. consideration of TT ratios, comparison of tree topologies, selection of outgroups etc.) are outlined in Jeewon et al. (2003, 2004, 2013).

The evolutionary models for Bayesian analysis and maximum-likelihood were selected independently for each locus using MrModeltest v. 2.3 (Nylander 2004) under the Akaike Information Criterion (AIC) implemented in PAUP v. 4.0b10. GTR+I+G model is resulted in each locus for Bayesian analysis and maximum-likelihood by AIC in MrModeltest as the best-fit model.

Bayesian analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronqvist 2001) to evaluate Bayesian posterior probabilities (BYPP) (Rannala & Yang 1996; Zhaxybayeva & Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC). Six simultaneous Markov chains were run for 5,000,000 generations and trees were sampled every 1000th generation. The distribution of log-likelihood scores was examined to determine stationary phase for each search and to decide if extra runs were required to achieve convergence, using the program Tracer 1.5 (Rambaut & Drummond 2007). First 20 % of generated trees were discarded and remaining 80 % of trees were used to calculate posterior probabilities of the majority rule consensus tree.

Maximum likelihood trees were generated using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis 2008, 2014) in the CIPRES Science Gateway platform (Miller et al. 2010). Maximum likelihood bootstrap values (ML) equal or greater than 75 % are given above each node (Fig. 1).

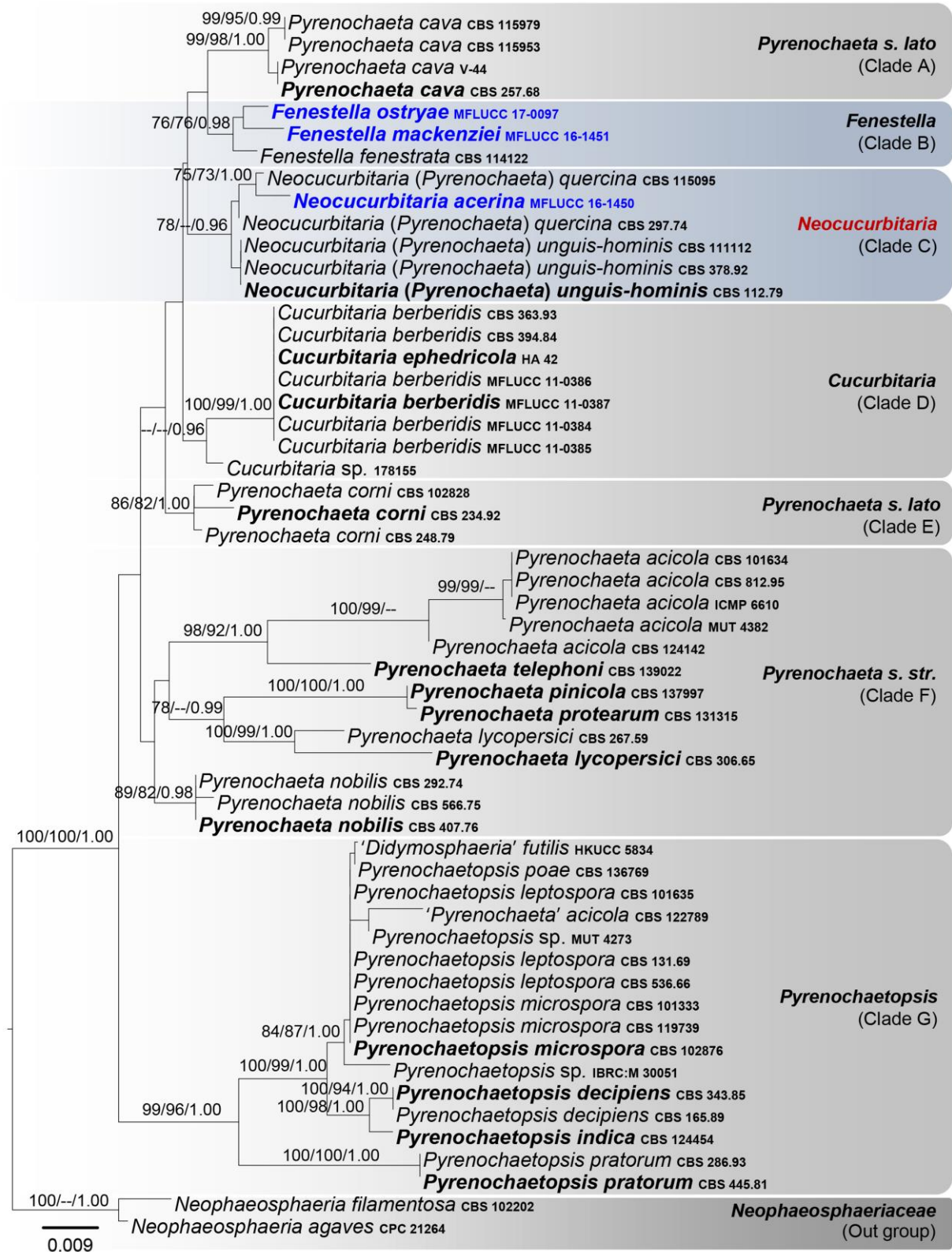
Phylograms were visualized with FigTree v1.4.0 program (Rambaut 2012) and reorganized in Microsoft power point (2007) and Adobe Illustrator® CS5 (Version 15.0.0, Adobe®, San Jose, CA). The finalized alignment and tree were deposited in TreeBASE, submission ID: 20603 (<http://www.treebase.org/>).

## Results and Discussion

### Phylogenetic analysis

The combined LSU, SSU and ITS gene dataset comprised 55 sequences with strains from Cucurbitariaceae and our new strains. After exclusion of ambiguous regions, the combined dataset included 2293 positions (LSU: 1–850; SSU: 851–1808; ITS: 1809–2293) including gaps. RAxML analysis yielded a best scoring tree (Fig. 1) with a final ML optimization likelihood value of -6683.634221. The matrix had 335 distinct alignment patterns, with 23.06% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.253143, C = 0.212693, G = 0.281258, T = 0.252906; substitution rates AC = 0.424225, AG = 1.978950, AT = 5.795696, CG = 5.267144, CT = 13.194889, GT = 1.000; proportion of invariable sites I = 0.794639; gamma distribution shape parameter  $\alpha$  = 0.647201. The maximum parsimonious dataset consists of 2018 constant characters, 233 parsimony-informative and 42 parsimony-uninformative characters. The parsimony analysis of the data matrix resulted in five thousand equally parsimonious trees with a length of 650 steps (CI = 0.575, RI = 0.788, RC = 0.454, HI = 0.425) in the first tree. The Bayesian and MP trees recovered did not contradict the ML tree topology for the strongly supported branches of the combined gene data set and hence only the ML tree is shown (Fig. 1).

In this study, we have included taxa representing all Cucurbitariaceae genera which are available in GenBank (Fig. 1). *Cucurbitaria elongata* (AFTOL-ID 1568, MFLUCC 14–0260) and



**Fig. 1** - RAxML tree based on analysis of a combined dataset of LSU, SSU and ITS partial sequence data. Bootstrap support values for ML, MP higher than 75 % and BYPP greater than 0.95 are given above each branch respectively. The ex-type and reference strains are in bold. The new isolates are in blue. The tree is rooted to *Neophaeosphaeria agaves* and *N. filamentosa* (Neophaeosphaeriaceae).

*Pyrenochaeta romeroi* (CBS 252.60, CBS 122784) did not group with members of Cucurbitariaceae in Pleosporinae (data not shown). Thus, we excluded them from the final data set.

**Table 1** Taxa used in the phylogenetic analysis and their corresponding GenBank numbers. The newly generated sequences are indicated in bold.

Taxon	Culture accession no.	GenBank accession no.*		
		ITS	LSU	SSU
<i>Cucurbitaria berberidis</i>	CBS 363.93	JF740191	GQ387606	GQ387545
<i>C. berberidis</i>	CBS 394.84	NA	GQ387605	GQ387544
<i>C. berberidis</i>	MFLUCC 11–0384	NA	KC506793	KC506797
<i>C. berberidis</i>	MFLUCC 11–0385	NA	KC506794	KC506798
<i>C. berberidis</i>	MFLUCC 11–0386	NA	KC506795	KC506799
<i>C. berberidis</i>	MFLUCC 11–0387	NA	KC506796	KC506800
<i>C. ephedricola</i>	HA 42	NA	KT313007	KT313005
<i>Cucurbitaria</i> sp.	178155	NA	FJ215698	FJ215704
<i>Didymosphaeria futilis</i>	HKUCC 5834	NA	GU205219	GU205236
<i>Fenestella fenestrata</i>	CBS 114122	NA	GU205220	GU205238
<b><i>Fenestella mackenziei</i></b>	<b>MFLUCC 16–1451</b>	<b>KY563071</b>	<b>KY563074</b>	<b>KY563077</b>
<b><i>F. ostryae</i></b>	<b>MFLUCC 17–0097</b>	<b>KY563072</b>	<b>KY563075</b>	<b>KY563078</b>
<b><i>Neocucurbitaria acerina</i></b>	<b>MFLUCC 16–1450</b>	<b>KY563073</b>	<b>KY563076</b>	<b>KY563079</b>
<i>N. (Pyrenochaeta) quercina</i>	CBS 115095	NA	GQ387619	GQ387558
<i>N. (Pyrenochaeta) quercina</i>	CBS 297.74	NA	GQ387620	GQ387559
<i>N. (Pyrenochaeta) unguis-hominis</i>	CBS 111112	NA	GQ387623	GQ387562
<i>N. (Pyrenochaeta) unguis-hominis</i>	CBS 112.79	NA	GQ387622	GQ387561
<i>N. (Pyrenochaeta) unguis-hominis</i>	CBS 378.92	EU930010	GQ387621	GQ387560
<i>Neophaeosphaeria agaves</i>	CPC 21264	NR_137833	KF777227	NA
<i>N. filamentosa</i>	CBS 102202	JF740259	GQ387577	GQ387516
<i>Pyrenochaeta acicola</i>	CBS 101634	NA	GQ387603	GQ387542
<i>P. acicola</i>	CBS 122789	NA	EU754204	EU754105
<i>P. acicola</i>	CBS 124142	NA	GQ387604	GQ387543
<i>P. acicola</i>	CBS 812.95	NA	GQ387602	GQ387541
<i>P. acicola</i>	ICMP 6610	KT309953	KT309741	NA
<i>P. acicola</i>	MUT 4382	KJ395501	KJ395497	NA
<i>P. cava</i>	CBS 115953	NA	GQ387607	GQ387546
<i>P. cava</i>	CBS 115979	AY853248	EU754198	EU754099
<i>P. cava</i>	CBS 257.68	JF740260	EU754199	EU754100
<i>P. cava</i>	V-44	NA	KF590167	NA
<i>P. corni</i>	CBS 102828	NA	GQ387609	GQ387548
<i>P. corni</i>	CBS 234.92	NA	EU754176	EU754077
<i>P. corni</i>	CBS 248.79	NA	GQ387608	GQ387547
<i>P. lycopersici</i>	CBS 267.59	JF740261	GQ387612	GQ387551
<i>P. lycopersici</i>	CBS 306.65	NR_103581	EU754205	EU754106
<i>P. nobilis</i>	CBS 292.74	NA	GQ387615	GQ387554
<i>P. nobilis</i>	CBS 407.76	EU930011	EU754206	EU754107
<i>P. nobilis</i>	CBS 566.75	NA	GQ387616	GQ387555
<i>P. pinicola</i>	CPC 23455	KJ869152	KJ869209	NA
<i>P. protearum</i>	CBS 131315	JQ044434	JQ044453	NA
<i>P. telephoni</i>	CBS 139022	KM516291	KM516290	KR260987
<i>Pyrenochaetopsis decipiens</i>	CBS 165.89	NA	GQ387625	GQ387564
<i>P. decipiens</i>	CBS 343.85	NA	GQ387624	GQ387563
<i>P. indica</i>	CBS 124454	NA	GQ387626	GQ387565
<i>P. leptospora</i>	CBS 101635	JF740262	GQ387627	GQ387566
<i>P. leptospora</i>	CBS 131.69	NA	GQ387629	GQ387568
<i>P. leptospora</i>	CBS 536.66	NA	GQ387628	GQ387567



<i>P. microspora</i>	CBS 101333	NA	GQ387630	GQ387569
<i>P. microspora</i>	CBS 102876	NA	GQ387631	GQ387570
<i>P. microspora</i>	CBS 119739	NA	GQ387632	GQ387571
<i>P. poae</i>	CBS 136769	KJ869117	KJ869175	NA
<i>P. pratorum</i>	CBS 286.93	JF740264	JF740331	NA
<i>P. pratorum</i>	CBS 445.81	NR_11162 3	GU238136	GU238228
<i>Pyrenochaetopsis</i> sp.	IBRC:M 30051	KF730241	KF803343	KF803344
<i>Pyrenochaetopsis</i> sp.	MUT 4273	KJ395500	KJ395496	NA

\*NA: No sequence available in GenBank.

All *Pyrenochaeta cava* strains analyzed herein (CBS 115979, CBS 115953, CBS 257.68 and V-44) cluster in a highly supported monophyletic clade (99% ML / 98% MP / 1.00 BYPP, clade A, Fig 1) in Cucurbitariaceae. *Fenestella* species analyzed here also constitute a clade (clade B) with 76% ML / 76% MP / 0.98 BYPP (Fig. 1). *Fenestella fenestrata* (CBS 114122) is basal to *F. ostryae* MFLUCC 16–1451 and *F. mackenziei* MFLUCC 16–0184 which are new taxa in this study.

*Pyrenochaeta quercina* (CBS 297.74, CBS 115095), *P. unguis-hominis* (CBS 112.79, CBS 378.92, CBS 111112) and our new strain (MFLUCC 16–1450) grouped in a separate clade (clade C) with 78% ML and 0.96 BYPP, but with >75% in MP analysis. The sexual morph of *Pyrenochaeta* is understudied and the phylogenetic affiliation of *Neocucurbitaria* with one of the *Pyrenochaeta quercina* (asexual morph) strains (CBS 115095) in this subclade is particularly interesting. The subclade is significantly supported in all statistical analyses in this study (75% ML / 73% MP / 1.00 BYPP, Fig 1). However, DNA sequence data of *P. quercina* (CBS 115095) analysed herein is an unverified sequence as it is not from the type material. Given that the relationship between the two taxa is undetermined, we introduce our isolate as a new species (*Neocucurbitaria acerina*) in clade C (Fig. 1). Given the phylogenetic results obtained herein with *Pyrenochaeta* in clade C, we believe that it would taxonomically more appropriate to establish a new genus, *Neocucurbitaria* to accommodate these species.

The strains of the type genus (*Cucurbitaria*) of the family clustered in a clade comprises six strains of *Cucurbitaria berberidis* i.e. ex-epitype strain (MFLUCC 11-0384), iso-epitype strains (MFLUCC 11-0384, MFLUCC 11-0385, MFLUCC 11-0386, MFLUCC 11-0387), representative strains (CBS 363.93, CBS 394.84) and *C. ephedricola* (HA 42). It is interesting to note that all *Cucurbitaria berberidis* isolates and *C. ephedricola* cluster together and could not be segregated based on our phylogenetic analyses. In the *Cucurbitaria* group only 28S and 18S rDNA sequence data are used (which are rather too conserved for species delineation) and hence the strains are phylogenetically indistinguishable. However, they are morphologically different (Ariyawansa et al. 2015). Only *Cucurbitaria berberidis* (CBS 394.84) has ITS sequence data available and it would be wise to consider or evaluate the utility of several other protein genes such as DNA-directed ribosomal polymerase II (RPB2) or translation elongation factor alpha 1 (*tef1- $\alpha$* ) to further elucidate phylogenetic relationships among this group of fungi with fresh collections.

Three strains of *Pyrenochaeta corni* (CBS 234.92, CBS 248.79, CBS 102828) grouped in a clade with 86% ML / 82% MP / 1.00 BYPP statistical support (clade E, Fig. 1). *Pyrenochaeta nobilis* isolates (CBS 292.74, CBS 407.76, CBS 566.75) grouped together with strong support (89% ML / 82% MP / 0.98 BYPP, Fig. 1), but surprisingly its affinity with other *Pyrenochaeta* species within clade F (characterised as the *Pyrenochaeta sensu stricto* clade) is unresolved (no support). A similar phylogenetic scenario is observed with *P. corni* characterized as the *Pyrenochaeta sensu lato* clade. Apart from the *Pyrenochaeta nobilis* group, clade F comprised a few subclades with significant statistical support i.e. *Pyrenochaeta acicola* (CBS 812.95, CBS 101634, CBS 124142, ICMP 6610, MUT 4382) with 99% ML & MP support and *Pyrenochaeta telephoni* (CBS 139022) with 98% ML / 92% MP / 1.00 BYPP support and two strains of *Pyrenochaeta lycopersici* (CBS 267.59, CBS 306.65) with 100% ML and 99% MP / 1.00 BYPP statistical support.

The *Pyrenochaetopsis* species are well-supported in all analyses with good statistical support i.e. 99% ML / 96% MP / 1.00 BYPP (Fig. 1, clade G). Clade G comprises *Pyrenochaetopsis decipiens* (CBS 165.89, CBS 343.85), *P. indica* (CBS 124454), *P. leptospora* (CBS 131.69, CBS 536.66, CBS 101635), *P. microspora* (CBS 101333, CBS 102876, CBS 119739), *P. pratorum* (CBS 286.93, CBS 445.81) and *P. poae* (CBS 136769).

### Taxonomy

**Cucurbitariaceae** G. Winter, Rabenhorst's Kryptogamen-Flora, Pilze - Ascomyceten 1(2): 308 (1885)

= Fenestellaceae M.E. Barr, Mycologia 71(5): 952 (1979)

Type – *Cucurbitaria* Gray, A natural arrangement of British plants 1: 519 (1821)

Type species – *Cucurbitaria berberidis* (Pers.) Gray, A natural arrangement of British plants 1: 519 (1821)

**Fenestella** Tul. & C. Tul., Selecta Fungorum Carpologia: Xylariei- Valsei- Spaeriei 2: 207 (1863)

Type species – *Fenestella princeps* Tul. & C. Tul., Selecta Fungorum Carpologia: Xylariei- Valsei- Spaeriei 2: 207 (1863)

Notes – *Fenestella* was introduced by Tulasne & Tulasne (1863) and is typified by *F. princeps*. The genus was introduced to accommodate Dothideomycetes species forming valsoid ascostromata and brown to reddish-brown, muriform ascospores (Barr 1979, Hyde et al. 2013, Phookamsak & Hyde 2015). *Fenestella* is relatively poorly studied and the type species of the genus could not be located. Therefore, Phookamsak & Hyde (2015) revisited the family Fenestellaceae and transferred the genus *Lojkania* Rehm to Testudinaceae, maintaining *Fenestella* in Fenestellaceae.

*Fenestella* can be distinguished from other genera in Cucurbitariaceae by its arrangement of locules in the ascostromata and the peridium structure (Doilom et al. 2013, Phookamsak & Hyde 2015). Although, these characters can distinguish *Fenestella* from other genera in Cucurbitariaceae, they are inappropriate for generic delineation (Phookamsak & Hyde 2015). It is not significant to separate them at the family level (Phookamsak & Hyde 2015). However, Phookamsak & Hyde (2015) maintained the monotypic genus *Fenestella* in Fenestellaceae due to the lack of a modern taxonomic description of the genus and limited molecular data. In this study, two new taxa are introduced which are morphologically typical of *Fenestella* viz. *F. ostryae* sp. nov. and *F. mackenziei* sp. nov. Phylogenetically, *F. ostryae* and *F. mackenziei* cluster together with good support and share a close phylogenetic affinity to *F. fenestrata* (CBS 114122) within the family Cucurbitariaceae. With this updated phylogeny where Fenestellaceae is nested in between Cucurbitariaceae and insufficient morphological grounds to support Fenestellaceae as an independent family, we propose *Fenestella* to be transferred to Cucurbitariaceae and Fenestellaceae be synonymized with Cucurbitariaceae.

The asexual morph of *Fenestella* has been reported as the coelomycetous genus *Pleurostromella* having oblong, hyaline, aseptate conidia (Brefeld 1891, Huhndorf & Glawe 1990, Wijayawardene et al. 2012, Hyde et al. 2013, Phookamsak & Hyde 2015). However, the generic type of *Pleurostromella*, *P. ulmicola* lacks molecular data to confirm its phylogenetic affinity and the link between sexual and asexual morphs (*Fenestella* and *Pleurostromella*) has not yet been clarified. In this study, the asexual morph of *F. ostryae* was recovered from culture (PDA), confirming the coelomycetous asexual status of *Fenestella*. The asexual morph of *Fenestella* resembles the genus *Pyrenochaeta*, but they are phylogenetically distinct.

***Fenestella ostryae*** Wanas., Camporesi, E.B.G. Jones & K.D. Hyde, **sp. nov.**

Figs 2-3

Index Fungorum Number – IF552830

Facesoffungi Number – FoF 02900

Etymology – Name reflects the host genus *Ostrya*.

Holotype – MFLU 16–0184

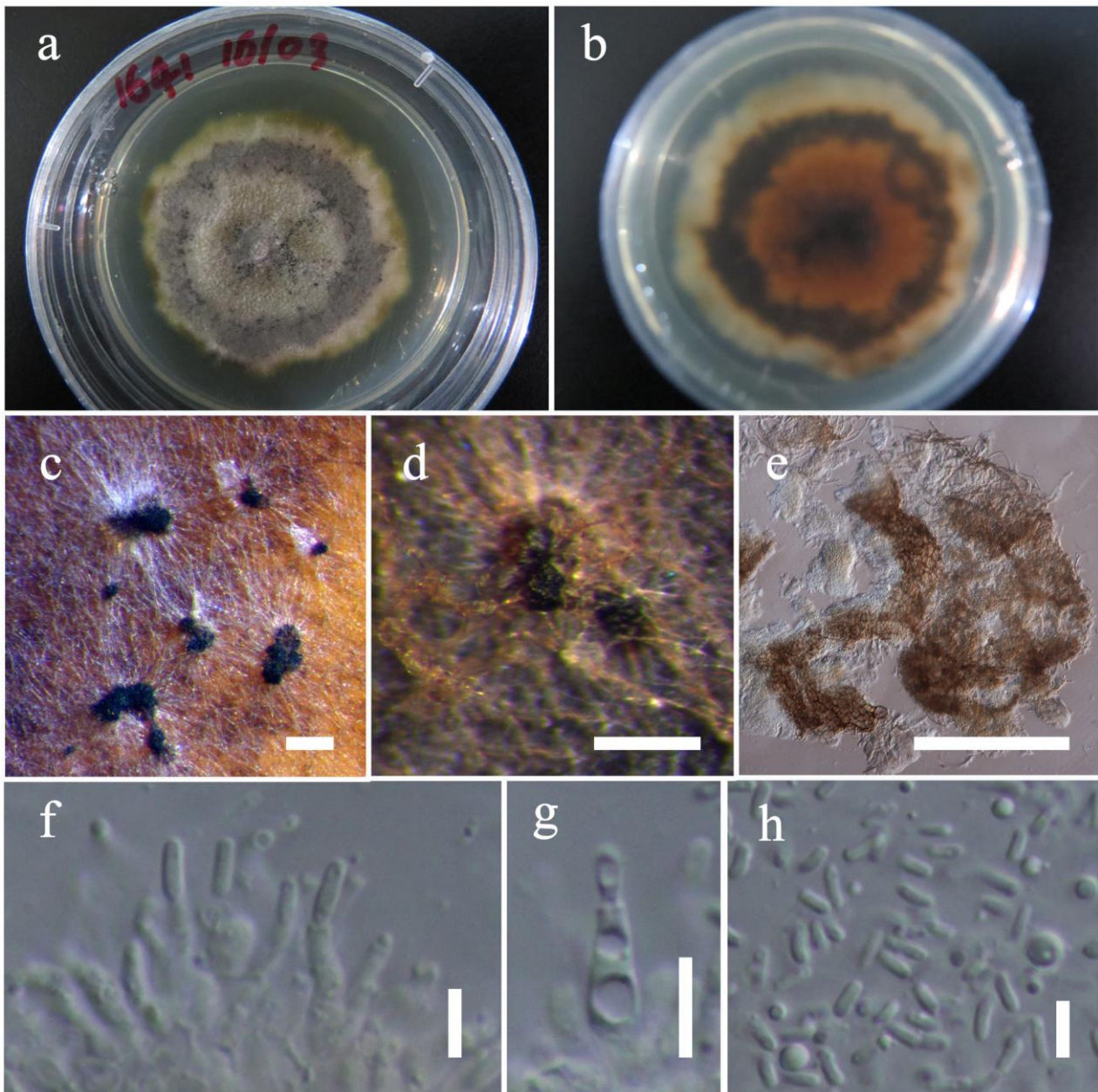




**Fig. 2** – *Fenestella ostryae* (MFLU 16–0184, holotype). **a** Appearance of ascostromata on host substrate. **b** Horizontal section through ascostromata illustrating the multilocules at the upper layers. **c** Vertical section through ascostroma. **d** Peridium. **e** Pseudoparaphyses. **f–i** Asci. **j–o** Ascospores. Scale bars: b, c = 200  $\mu$ m, d, f–o = 20  $\mu$ m, e = 5  $\mu$ m.

*Saprobic* on dead twigs of *Ostrya carpinifolia* Scop. **Sexual morph:** *Ascostromata* 300–550  $\mu$ m high, 450–800  $\mu$ m diam. ( $\bar{x}$  = 431.6  $\times$  620.4  $\mu$ m, n = 5), solitary, immersed to erumpent through host periderm, visible as raised, circular areas with a central sunken area, with blackened ostiolar dots, with 1–5 locules in a valsoid configuration. *Locules* 250–450  $\mu$ m high, 350–500  $\mu$ m diam. ( $\bar{x}$  = 351.6  $\times$  400.1  $\mu$ m, n = 5), clustered or gregarious, immersed in valsoid groups beneath periderm of host, multi-loculate, ovoid to obpyriform, glabrous, covered by vegetative hyphae, dark brown to black. *Peridium* 35–60  $\mu$ m wide at the base, 20–40  $\mu$ m wide at the sides, comprising 4–6





**Fig. 3** – Asexual morph of *Fenestella ostryae* forming on PDA after 8 weeks (MFLU 16–0184, holotype) **a, b** Culture on PDA (a = from above, b = from below). **c, d** Conidiomata on colony. **e** Squashed conidiomata. **f, g** Mature and immature conidia attached to conidiogenous cells. **h** Mature and immature conidia. Scale bars: c–e =200 µm, f–h =5 µm.

layers, outer layer pigmented, comprising reddish-brown to dark brown, thin-walled cells of *textura angularis*, inner layer composed of hyaline, flattened, thin-walled cells of *textura angularis*. *Hamathecium* comprising numerous, 2–3.5 µm wide, filamentous, branched, septate, pseudoparaphyses. *Asci* 190–210 × 20–30 µm ( $\bar{x}$  = 202.2 × 24.9 µm, n = 30), 8-spored, bitunicate, fissitunicate, cylindrical to cylindrical-clavate, with a 10–40 µm long pedicel, apically rounded, with a minute ocular chamber. *Ascospores* 40–50 × 15–20 µm ( $\bar{x}$  = 45.6 × 18.1 µm, n = 40), overlapping uni-seriate, muriform, ellipsoidal to broadly fusiform, hyaline to pale brown when young, becoming dark brown at maturity, paler at the extreme ends, with 6–12 transverse septa and 3–4 longitudinal septa, slightly constricted at the central septum, upper part larger than the lower part, guttulate in each cell, ends remaining cone-shaped, with pointed ends. **Asexual morph:** Coelomycetous, phoma-like. *Pycnidia* solitary to confluent, on upper surface or submerged in agar, globose to subglobose, ostiole apapillate or papillate, olivaceous to olivaceous-black, the wall with pseudoparenchymatous cells. *Conidiophores* hyaline, cylindrical, arising from the inner wall of the

conidioma. *Conidiogenous cells*  $5\text{--}7 \times 2\text{--}4 \mu\text{m}$  ( $\bar{x} = 6.6 \times 2.9 \mu\text{m}$ ,  $n = 20$ ), hyaline, enteroblastic, phialidic, doliiform to cylindrical, straight or slightly curved, indeterminate, discrete, or integrated, septate. *Conidia*  $2.8\text{--}4 \times 1\text{--}1.5 \mu\text{m}$  ( $\bar{x} = 3.4 \times 1.2 \mu\text{m}$ ,  $n = 30$ ), hyaline, aseptate, cylindrical/ellipsoidal to allantoid, not often guttulate, smooth-walled.

Culture characteristics – Colonies on PDA reaching 2 cm diam. after 30 days at 16 C, circular, margin white at first, dark grey outer and creamy center after 4 weeks, flat on the surface, without aerial mycelium, reverse iron to greenish grey. Hyphae septate branched, hyaline, thin, smooth-walled.

Known distribution – Italy, on dead twigs of *Ostrya carpinifolia* (Betulaceae).

Material examined – Italy, Forlì-Cesena, Galeata, Strada San Zeno, on dead aerial twigs of *Ostrya carpinifolia*, 11 January 2014, Erio Camporesi, IT 1641 (MFLU 16–0184, **holotype**) **isotype** in BBH, ex-type living culture, MFLUCC 17–0097.

Notes – *Fenestella ostryae* closely resembles *F. fenestrata* in having immersed, multi-loculate ascostromata, cylindrical to cylindrical-clavate asci and ellipsoidal to broadly fusiform ascospores, which are paler at the extreme ends. Their phylogenetic relationship remains uncertain due to the unavailability of molecular data from the type material of *F. fenestrata*. However, *F. fenestrata* has comparatively larger ascostromata and longer asci than *F. ostryae* (see Table 2).

In this study, *Fenestella mackenziei* groups as a sister taxon to *F. ostryae* which was also collected from Forlì-Cesena Province in Italy. They are morphologically similar in ascal and ascospores characteristics, i.e. shape and septation, but morphometric dimensions are considerably different (see Table 2). In addition, *Fenestella ostryae* has multi-loculate ascostromata and *F. mackenziei* has uni-loculate ascostromata. ITS sequence comparison also reveals 19 base pair differences between the two taxa and hence we introduce them as two new species which is in concordance to Jeewon & Hyde (2016).

***Fenestella mackenziei*** Wanas., Camporesi, E.B.G. Jones & K.D. Hyde, **sp. nov.**

Fig. 4

Index Fungorum Number – IF552831

Facesoffungi Number – FoF 02901

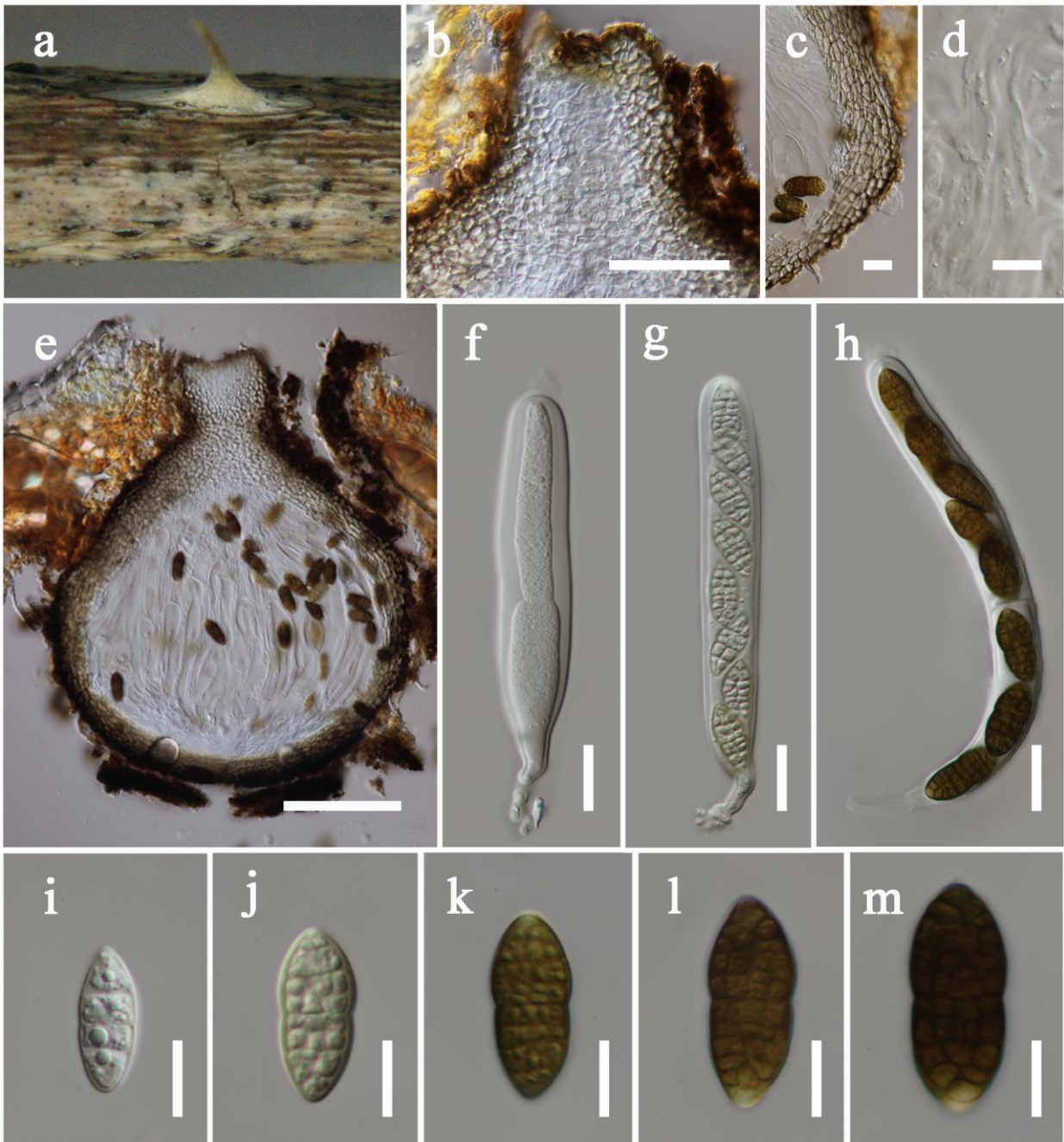
Etymology – In honour of Dr. Eric Hugh Charles McKenzie for his immense contribution to mycology

Holotype – MFLU 16–0181

*Saprobic* on dead twigs of *Rosa canina* L. **Sexual morph:** *Ascomata* 250–350  $\mu\text{m}$  high, 300–450  $\mu\text{m}$  diam. ( $\bar{x} = 337.8 \times 364.8 \mu\text{m}$ ,  $n = 5$ ), solitary or scattered, immersed to semi-erumpent through host tissues, visible as raised, circular areas with a central sunken area, with blackened ostiolar dots, globose to subglobose in shape, glabrous, dark brown to black, ostiolate. *Ostiole* 60–90  $\mu\text{m}$  high, 50–70  $\mu\text{m}$  diam. ( $\bar{x} = 78.2 \times 58.9 \mu\text{m}$ ,  $n = 5$ ), black, smooth, with ostiolar canal filled with hyaline cells. *Peridium* 20–30  $\mu\text{m}$  wide at the base, 30–60  $\mu\text{m}$  wide at the sides, comprising 4–6 layers, outer layer pigmented, comprising reddish-brown to dark brown, thick-walled cells of *textura angularis*, inner layer composed of hyaline, flattened, thin-walled cells of *textura angularis*. *Hamathecium* comprising numerous, 2–2.5  $\mu\text{m}$  wide, filamentous, branched, septate, pseudoparaphyses. *Asci* 140–200  $\times$  20–25  $\mu\text{m}$  ( $\bar{x} = 168.2 \times 22.6 \mu\text{m}$ ,  $n = 30$ ), 8-spored, bitunicate, fissitunicate, cylindrical to cylindrical-clavate, with a 10–20  $\mu\text{m}$  long pedicel, apically rounded, with a minute ocular chamber. *Ascospores* 25–32  $\times$  11–15  $\mu\text{m}$  ( $\bar{x} = 30.1 \times 13.1 \mu\text{m}$ ,  $n = 40$ ), overlapping uni-seriate, muriform, ellipsoidal to broadly fusiform, hyaline to pale brown when young, becoming dark brown at maturity, paler at the extreme ends, with 6–10 transverse septa and 2–4 longitudinal septa, slightly constricted at the central septum, upper part larger than the lower part, guttulate in each cell, ends remaining cone-shaped, with pointed ends. **Asexual morph:** Undetermined.

Culture characteristics – Colonies on PDA reaching 2 cm diam. after 30 days at 16°C, circular, margin white at first, dark grey outer and creamy center after 4 weeks, flat on the surface, without aerial mycelium, reverse iron to greenish-grey. Hyphae septate branched, hyaline, thin, smooth-walled.





**Fig. 4** – *Fenestella mackenziei* (MFLU 16-0181, **holotype**). **a** Appearance of ascomata on host substrate. **b** Section of ascoma. **c** Peridium. **d** Pseudoparaphyses. **e-h** Asci. **i-n** Ascospores. Scale bars: b = 100  $\mu$ m, c, e-n = 20  $\mu$ m, d = 5  $\mu$ m.

Known distribution – Italy, on dead twigs of *Rosa canina* (Rosaceae).

Material examined – Italy, Forlì-Cesena, San Benedetto in Alps, dead aerial twigs of *Rosa canina*, 26 December 2013, Erio Camporesi, IT 1601 (MFLU 16–0181, **holotype**) **isotype** in BBH, ex-type living culture, MFLUCC 16–1451.

Notes – See above notes under *Fenestella ostryae*.

***Neocucurbitaria*** Wanas., E.B.G. Jones & K.D. Hyde, **gen. nov.**

Index Fungorum Number – IF552832

Facesoffungi Number – 02902

Etymology – Name reflects the fact that although morphologically similar, it is distinct from the genus *Cucurbitaria*

**Table 2.** Synopsis of *Fenestella* species with morphological features discussed in this study.

Species	Ascomata/ Ascostromata ( $\mu\text{m}$ )	Number of locules	Asci ( $\mu\text{m}$ )	Ascospores		
				Size ( $\mu\text{m}$ )	Transvers e septa	Longitudin al septa
<i>Fenestella fenestrata</i> <sup>1</sup>	550–680 high 850–1530 diam.	2–5	176–330 $\times$ 24.5– 32	39–53 $\times$ 15– 20	7–8	3–4
<i>Fenestella mackenziei</i> <sup>2</sup>	250–350 high 300–450 diam.	1	140–200 $\times$ 20–25 stipe 10-20 long	25–32 $\times$ 11– 15	6–10	2–4
<i>Fenestella ostryae</i> <sup>2</sup>	300–550 high 450–800 diam.	1–5	190–210 $\times$ 20–30 stipe 10-40 long	40–50 $\times$ 15– 20	6–12	3–4
<i>Fenestella princeps</i> <sup>1</sup>		Multi- loculate	300–440 $\times$ 23–28 stipe 30-90 long	50–62 $\times$ 16– 23	3–6	3–4

<sup>1</sup> Phookamsak & Hyde (2015), <sup>2</sup> This study

*Saprobic* on dicotyledons, trees and shrubs in terrestrial habitat or *parasitic* on human skin.

**Sexual morph:** Ascomata black, superficial to semi-immersed, confluent, gregarious, sometimes scattered beneath the host periderm or on decorticated wood, fully or partly erumpent, globose, uniloculate, with an ostiole. *Ostiole* central, slightly sunken, short and broad papillate, inconspicuous at the surface, black, smooth, filled with hyaline periphyses. *Peridium* comprising 6–10 layers, outer most layer heavily pigmented, thin-walled, comprising blackish to dark brown amorphous layer, middle layer heavily pigmented, thick-walled, comprising blackish to dark brown loosely packed cells of *textura angularis*, inner layer composed 3–4 layers, reddish-brown to hyaline, cells towards the inside comprising lighter, flattened, thick-walled cells of *textura angularis*. *Hamathecium* comprising numerous, filamentous, branched, septate pseudoparaphyses. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical, short-pedicellate, apex rounded with a minute ocular chamber. *Ascospores* overlapping uniseriate, muriform, mostly ellipsoidal, 6–8 transverse septa, with a longitudinal septum, deeply constricted at the middle septum, slightly constricted at remaining septa, initially hyaline, becoming pale brown at maturity, symmetrical, slightly paler ends, conical and narrowly rounded at the ends. **Asexual morph:** Coelomycetous. *Conidiomata* pycnidial, solitary to confluent, globose to subglobose, papillate ostiolate. *Ostiole* single, central, circular. *Setae* abundant around the ostiole, erect, dark brown to black, thick-walled. *Conidiogenous cell* doliiform to cylindrical, septate, hyaline. *Conidia* hyaline, aseptate, cylindrical/ellipsoidal, smooth-walled (Sutton 1980).

Type species – *Neocucurbitaria unguis-hominis* (Punith. & M.P. English) Wanas., E.B.G. Jones & K.D. Hyde

Notes – De Notaris (1849) introduced *Pyrenochaeta* to accommodate *P. nobilis* as the type species and twelve other closely related taxa, which are characterized by branched, filiform, septate, acropleurogenous conidiophores (Schneider 1979, de Gruyter et al. 2010). A number of *Pyrenochaeta* species e.g. *P. cava*, *P. corni* and *P. quercina* are important either as endophytes, saprobes or pathogens such as *P. lycopersici* in agriculture and forestry (de Gruyter et al. 2010, Clergeot et al. 2012). Furthermore, they can be human pathogens that cause infection of human skin and nails e.g. *Pyrenochaeta unguis-hominis* (Toh et al. 2016).

The taxonomy of *Pyrenochaeta* species has been studied by de Gruyter et al. (2010), but their pleomorphism has received little attention, and thus, the basic biology of this group is not fully understood. De Gruyter et al. (2010) accepted *Cucurbitaria* as the sexual morph of *Pyrenochaeta*. However, in this study *Pyrenochaeta nobilis*, the type species of *Pyrenochaeta*, is phylogenetically distant from *Cucurbitaria berberidis*, the type species of *Cucurbitaria*, and this was accepted in Doilom et al. (2013). Our phylogeny also indicates that the affinities of *P. nobilis* are uncertain as its isolates constitute an independent lineage distinct from other species. The taxonomy of this species needs revision with more fresh collections as there are only slight morphologically differences with other species.

In this study, *Pyrenochaeta quercina* (CBS 297.74, CBS 115095) and *P. unguis-hominis* (CBS 112.79, CBS 378.92, CBS 111112) cluster as sister groups in a well-supported clade (78% ML and 0.96 BYPP, clade C, Fig. 1), with one of our newly isolated sexual morphs. This new isolate (*Neocucurbitaria acerina*) is shown to be the sexual morph of pyrenochaeta-like species and differs from other sexual morphs recorded in Cucurbitariaceae (see the notes under *Neocucurbitaria acerina*). *Pyrenochaeta quercina* strains are not from the type material and it is difficult to infer an accurate relationship between *P. quercina* and our new sexual isolate. *Pyrenochaeta unguis-hominis* also differs from the type species, *P. nobilis*, in conidiogenesis. *Pyrenochaeta unguis-hominis* has phoma-like conidiogenous cells, whereas *P. nobilis* has acropleurogenous conidiogenesis. In addition, *Pyrenochaeta unguis-hominis* has comparatively smaller conidia ( $3\text{--}3.5\ \mu\text{m} \times 1\text{--}1.5\ \mu\text{m}$ ) than *P. nobilis* ( $4.8\text{--}7.5\ \mu\text{m} \times 1\text{--}1.5\ \mu\text{m}$ ). Based on DNA sequence data coupled with morphological evidence we introduce *Neocucurbitaria* to accommodate *Neocucurbitaria acerina*, *Pyrenochaeta quercina* and *P. unguis-hominis*.

*Neocucurbitaria acerina* Wanas., Camporesi, E.B.G. Jones & K.D. Hyde **sp. nov.**

Fig. 5

Index Fungorum Number – IF552833

Facesoffungi Number – FoF 02903

Etymology – Name reflects the host genus *Acer*.

Holotype – MFLU 16–2710

*Endophytic* or *saprobic* on *Acer campestre*, trees and shrubs in terrestrial habitat. Sexual morph: *Ascomata* 250–350  $\mu\text{m}$  high 300–450  $\mu\text{m}$  diam. ( $\bar{x} = 329.6 \times 399.7\ \mu\text{m}$ ,  $n = 10$ ), black, superficial to semi-immersed, confluent, gregarious, sometimes scattered beneath the host periderm or on decorticated wood, fully or partly erumpent, globose, uniloculate, with an ostiole. *Ostiole* 50–70  $\mu\text{m}$  long 70–100  $\mu\text{m}$  diam., centrally located, slightly sunken, short and broad papillate, inconspicuous at the surface, black, smooth, filled with hyaline periphyses. *Peridium* 25–30  $\mu\text{m}$  wide at the base, 50–70  $\mu\text{m}$  wide in sides, thick, comprising 6–10 layers, outer most layer heavily pigmented, thin-walled, comprising blackish to dark brown amorphous layer, middle layer heavily pigmented, thick-walled, comprising blackish to dark brown loosely packed cells of *textura angularis*, inner layer composed 3–4 layers, reddish brown to hyaline, cells towards the inside lighter, flattened, thick-walled cells of *textura angularis*. *Hamathecium* comprising numerous, 2–2.5  $\mu\text{m}$  ( $n = 40$ ) wide, filamentous, branched septate, pseudoparaphyses. *Asci* 100–110  $\times$  10–12  $\mu\text{m}$  ( $\bar{x} = 105.8 \times 10.6\ \mu\text{m}$ ,  $n = 40$ ), 8-spored, bitunicate, fissitunicate, cylindrical, short-pedicellate, apex rounded with a minute ocular chamber. *Ascospores* 15–20  $\times$  6–9  $\mu\text{m}$  ( $\bar{x} = 19.4 \times 7.1\ \mu\text{m}$ ,  $n = 50$ ), overlapping uniseriate, muriform, mostly ellipsoidal, 6–8 transverse septa, with a longitudinal septum, deeply constricted at the middle septum, slightly constricted at remaining septa, initially hyaline, becoming pale brown at maturity, symmetrical, slightly paler ends, conical and narrowly rounded at the ends. Asexual morph: Undetermined.

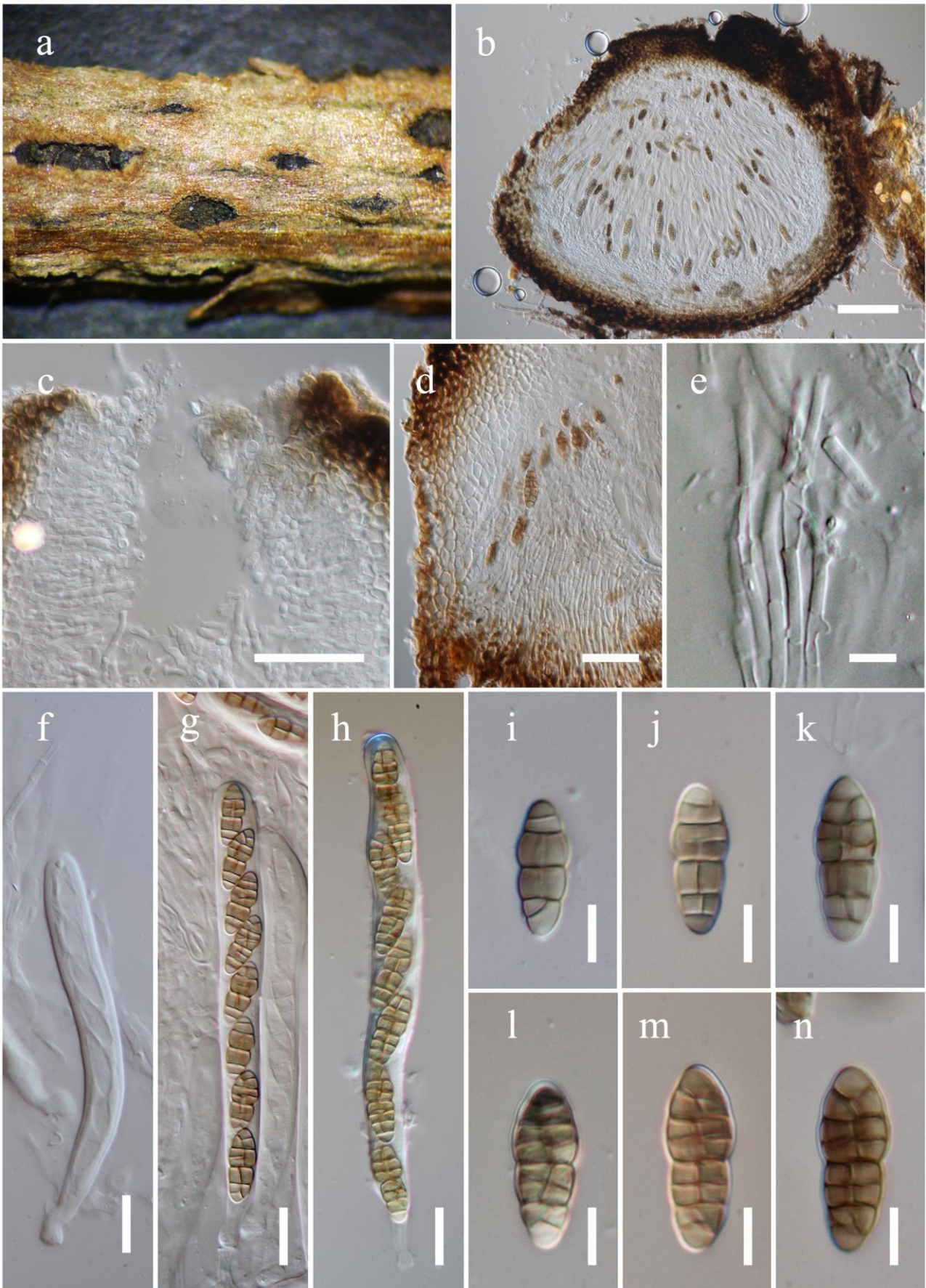
Culture characteristics – Colonies on PDA reaching 3 cm diam. after 30 days at 16 °C, circular, smooth margin white at first, dark lava after 4 weeks flat on the surface, without aerial mycelium, reverse grayish black. Hyphae septate branched, hyaline, thin, smooth-walled.

Known distribution – Italy, on dead twigs of *Acer campestre* L. (Sapindaceae)

Material examined – Italy, Forlì-Cesena, Fiumicello di Premilcuore, on dead aerial twigs of *Acer campestre*, 20 November 2013, Erio Camporesi, IT1527 (MFLU 16–2710, **holotype**), **isotype** in BBH, living culture, MFLUCC 16–1450.

Notes – In this study, we introduce a novel taxon as a possible sexual morph of *Pyrenochaeta quercina* from Italy on *Acer campestre* based on a combined gene analyses of ITS, LSU and SSU sequence data. Our new species resembles *Cucurbitaria* in having semi-immersed, globose ascomata with a central ostiole, short-pedicellate asci with a minute ocular chamber and overlapping uniseriate, pale brown, muriform ascospores. However, there are differences as *Neocucurbitaria acerina* has mostly solitary ascomata, ostiole with hyaline periphyses, smaller asci ( $<110 \times 12\ \mu\text{m}$ ) and smaller ascospores ( $<20 \times 9\ \mu\text{m}$ ) with 1-longitudinal septum, while





**Fig. 5** – *Neocucurbitaria acerina* (MFLU 16-2710, holotype). a. Appearance of ascomata on host substrate. b. Vertical section of ascoma. c. Close up of ostiole. d. Peridium. e. Pseudoparaphyses. f–h. Asci. i–n. Ascospores. Scale bars: b = 100  $\mu$ m, c, d = 50  $\mu$ m, e, i–n = 10  $\mu$ m, f–h = 20  $\mu$ m.

*Cucurbitaria* species have gregarious ascomata, ostiole without periphyses, larger asci (>150 × 12 µm) and larger ascospores (>20 × 9 µm) with more than a 1-longitudinal septum (Table 3).

***Neocucurbitaria quercina*** (Kabát & Bubák) Wanas., E.B.G. Jones & K.D. Hyde, **comb. nov.**

Index Fungorum Number – IF552834

Facesoffungi Number – FoF 02904

Basionym – *Pyrenochaeta quercina* Kabát & Bubák, Hedwigia 52: 342 (1912)

Notes – See above notes under *Neocucurbitaria*.

***Neocucurbitaria unguis-hominis*** (Punith. & M.P. English) Wanas., E.B.G. Jones & K.D. Hyde **comb. nov.**

Index Fungorum Number – IF552835

Facesoffungi Number – FoF 02905

Basionym – *Pyrenochaeta unguis-hominis* Punith. & M.P. English, Trans. Br. mycol. Soc. 64(3): 539 (1975)

Notes – See above notes under *Neocucurbitaria*.

**Table 3.** Synopsis of *Cucurbitaria berberidis*, *C. ephedricola* and *Neocucurbitaria acerina* species with morphological features discussed in this study.

Species	Ascomata (µm)	Periphyses	Asci (µm)	Ascospore (µm)
<i>Cucurbitaria berberidis</i> <sup>1</sup>	Less commonly solitary 400–900 high 400–600 diam.	Absent	180–200 × 20–307–9-transverse septa	23–32 × 9–14 3–4-longitudinal septa 20–30 × 10–15
<i>Cucurbitaria ephedricola</i> <sup>2</sup>	Less commonly solitary 300–800 diam.	Absent	150–200 × 13–205–9-transverse septa	1–2-longitudinal septa 15–20 × 6–9
<i>Neocucurbitaria acerina</i> <sup>3</sup>	Commonly solitary 250–350 high 300–450 diam.	Hyaline	100–110 × 10–126–8-transverse septa	1-longitudinal septum

<sup>1</sup> Doilom et al. (2013), <sup>2</sup> Ariyawansa et al. (2015), <sup>3</sup> This study

### Acknowledgments

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