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, Protocenestella and Seltsamia are described as new. Fenestella is here included as its generic type F. fenisetra (= F. prinscis), which is lepto- and epitypified. Fenestella mackenziei and F. ostryae are combined in Parafenestella. Asexual morphs of Cucurbitariaceae, where known, are all pyrenochoeta- or phoma-like. Comparison of the phylogenetic analyses of the ITS-LSU and combined matrices demonstrates 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, Pyrenochoeta, pyrenomycetes. Taxonomic novelties: New genera: Astragalicola Jaklitsch & Voglmayr, Cucitella Jaklitsch & Voglmayr, Parafenestella Jaklitsch & Voglmayr, Protocenestella Jaklitsch & Voglmayr, Seltsamia Jaklitsch & Voglmayr; New species: Astragalicola amorpha Jaklitsch & Voglmayr, Cucitella opal Jaklitsch & Voglmayr, Cucurbitaria oromediterranea Jaklitsch & Voglmayr, Neocucurbitaria acanthocladae Jaklitsch & Voglmayr, N. aetnensis Jaklitsch & Voglmayr, N. cinereae Jaklitsch & Voglmayr, N. cisticola Jaklitsch & Voglmayr, N. judiandica Jaklitsch & Voglmayr, N. populi Jaklitsch & Voglmayr, N. rhamnicola Jaklitsch & Voglmayr, N. rhamnioides Jaklitsch & Voglmayr, N. ribicola Jaklitsch & Voglmayr, N. vachelliae Jaklitsch & Voglmayr, Parafenestella pseudoplantata Jaklitsch & Voglmayr, Protocenestella ulmi Jaklitsch & Voglmayr, Seltsamia ulmi Jaklitsch & Voglmayr; New combinations: Neocucurbitaria rhamni (Nees: Fr.) Jaklitsch & Voglmayr, Parafenestella mackenziei (Wanas. et al.) Jaklitsch & Voglmayr, Parafenestella ostreyae (Wanas. et al.) Jaklitsch & Voglmayr; Epitypifications (basionyms): Sphaeria rhamni Nees, Fenestella princeps Tul. & C. Tul., Valsa fenisetra Berk. & Broome.

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INTRODUCTION

The family Cucurbitariaceae was described by Winter (1885; as Cucurbitariaceae), who listed Cucurbitaria, Gibbera, Gibberidella, Nitischka 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 Cucurbitothid, Otthia, Rhytidella and Syncarpella. This concept (excluding Otthia) was proposed by Doilom et al. (2013), who also included Pyrenochoeta and Pyr enochaeotopsis following earlier phylogenetic analyses (Aveskamp et al. 2010, de Gruyter et al. 2010, 2012). They also epitypified the generic type of Cucurbitaria, C. berediris, using material collected in Austria. However, Cucurbitothid 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. Cucurbitothis 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. austrofaciana, C. grandicpis and C. proteae belong to Telichospora in the Telichosporaceae (Jaklitsch et al. 2016). Rhyti diella and Syncarpella differ from all fungi identified in the Cucurbitariaceae by cylindrical to veriform 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. berediris, is characterised by tuberculate perithecoid 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 pyrenochoeta- 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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regarding their life cycles, is difficult and little varying features or which are incompletely studied. Genera are often polyphyletic, mainly because morphological and therefore unrelated fungi are subsumed under a common genus. Jaklitsch & Voglmayr (2016).

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 diplodiella-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 Hypocreales, 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 Cucurbitariae Valenzuela-Lopez et al. (2018) combined Pyrenochaeta cava, P. hakeae and P. keratinophila in Neocucurbitaria, clarified the concept of and eptypified 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 Cucurbitariae from the family erecting several new genera and families. They also excluded Pyrenochaeta s. str. from the Cucurbitariae 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 Astragalicala, Cucelitia, Parafenosetella, Protolenasperella, and Seftelamia. The genus Fenestella is included as its generic type F. fenestrata (= F. princeps), which is lecto- and eptypified 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 (Amar- enomycetes) ammophila: Sweden, Halland: Varberg, Apelvik, sandy beach, from old leaves of Ammophila arenaria, 31 Oct. 2015, S. Lund, det. and comm. O. Eriksson (WU 36958; culture AA); Plenodomus hentsoniae: Austria, Steiermark, Deutschlandsberg, Korallegemeit, forest road to Grünangerhütte from the north, before the wooden bridge over the Schwarze Sulm, on Salix appendiculata, 16 May 2015, G. Friesel (WU 36959; culture LTO). Herbarium acronyms are according to Thiers (2017). Freshly collected specimens have been deposited in the Fungalum of the Department of Botany and Biodiversity Research, University of Vienna (WU).
| Taxon | Host/substrate | Strain | Specimen voucher no. | ITS | LSU | SSU | rpb2 | tef1 | tub2 |
|------|---------------|--------|----------------------|-----|-----|-----|------|------|------|
| Allocucurbitaria botulispora | Superficial tissue | CBS 142452 | CBS H-23028 | holo | LT592932 | LN907416 | – | LT593070 | – | LT593001 |
| Alternaria alternata | Arachis hypogaea | CBS 916.96 = AFTOL-ID 1610 | IMI 254138 | epi | KF465761 | DQ678082 | KC584507 | KC584375 | DQ677927 | – |
| Astragalica ampha | Astragalus angustifolius | CBS 142999 = C227a | WU 35994 | holo | MF795753 | MF795753 | – | MF797595 | MF798542 | MF795883 |
| Coniothyrium palmarum | Chamaerops humilis petioles | CBS 400.71 = AFTOL-ID 1379 | CBS H-10891 | holo | AY270207 | JX681084 | AY642513 | DQ677966 | DQ677903 | KT387992 |
| Cucitella opali | Acer opalus | CBS 142405 = FV | WU 35995 | holo | MF795754 | MF795754 | MF795837 | MF795796 | MF795843 | MF795884 |
| Cucurbitaria berberidis | Berberis vulgaris | CBS 130007 = CB1 = MFLUCC 11-0384 | WU 35997 | holo | MF795761 | MF795761 | MF795800 | MF795799 | MF795846 | MF795887 |
| C. oromediterranea | Berberis cretica | CB2 | WU 35989 | – | MF795763 | MF795763 | MF795805 | MF795851 | MF795892 |
| Didymella exigua | Rumex arifolius | CBS 183.55 | CBS H-20123 | neo | GU237794 | EU754155 | EU754056 | EU874850 | – | GU237525 |
| Dothidotthia symphoricarpi | Symphoricarpus rotundifolius | CBS 119687 | BPI 871823 | epi | JF740260 | EU754199 | EU754056 | EU874850 | – | GU237525 |
| Fenestella fenestrata | Alnus glutinosa | CBS 143001 = FP9 | WU 35996 | epi | MF795765 | MF795765 | MF795807 | MF795853 | MF795893 |
| Leptosphaeria doliolum | Urtica dioica | CBS 505.75 | CBS H-13882 | JF740205 | GU301827 | GU296159 | KT389640 | GU349060 | JF740144 |
| Leptosphaerulina australis | Eugenia aromatica | CBS 317.83 | CBS H-13890 | – | GU237829 | GU296160 | GU371790 | GU349070 | GU237540 |
| Massarina eburnea | Fagus sylvatica | CBS 473.64 | – | – | AF383959 | GU301840 | GU296170 | GU371732 | GU349040 |
| Neocucurbitaria acanthocladae | Genista acanthoclada | CBS 142398 = C225 | WU 35997 | holo | MF795766 | MF795766 | MF795808 | MF795854 | MF795894 |
| N. acerina | Acer pseudoplatanus | C26a | WU 35999 | – | MF795767 | MF795767 | MF795809 | MF795855 | MF795895 |
| N. aetnensis | Genista aetnensis | CBS 142402 = C261 | WU 3629 | holo | MF795769 | MF795769 | MF795811 | MF795857 | MF795897 |
| N. aquatica | Sea water | CBS 297.74 | CBS H-16102 | holo | LT623221 | EU754177 | – | LT623278 | – | LT623238 |
| N. cava | Unknown | CBS 115979 | CBS H-16102 | holo | LT623221 | EU754177 | – | LT623278 | – | LT623234 |
| N. cinerea | Genista cinerea | CBS 142406 = KU9 | WU 36931 | holo | MF795771 | MF795771 | MF795813 | MF795859 | MF795899 |
| N. cisticola | Cistus monspeliensis | CBS 142402 = C264 | WU 36932 | holo | MF795772 | MF795772 | – | MF795814 | MF795860 | MF795900 |
| N. hakeae | Hakea sp. | CBS 142109 | CBS H-22894 | holo | KY173436 | KY173526 | – | KY173595 | – | KY173613 |
| N. irregularis | Subcutaneous tissue | CBS 142791 | CBS H-23029 | holo | LT592916 | LN907372 | – | LT593054 | – | LT592985 |

(continued on next page)
| Taxon                  | Host/substrate                       | Strain               | Specimen voucher no. | Ex-type status | GenBank accession numbers |
|-----------------------|--------------------------------------|----------------------|----------------------|-----------------|--------------------------|
|                       |                                      |                      |                      |                 | ITS | LSU | SSU | rpB2 | tef1 | tub2 |
| *N. juglandicola*     | Juglans regia CBS 142390 = BW6      | WU 36933             | holo                 | MF79577        | MF795773 | –   | MF795815 | MF795861 | MF795901 |
| *N. keratinophila*    | Man corneal scrapings CBS 121759    | CBS H-20122          | holo                 | EUB85415       | LT623215 | –   | LT623275 | LT623236 |
| *N. populi*           | Populus sp. CBS 142393 = C28        | WU 36934             | holo                 | MF795774       | MF795774 | –   | MF795816 | MF795862 | MF795902 |
| *N. quercina*         | Quercus robur CBS 115095            | CBS H-23205          | neo                  | LT623220       | GQ387619 | GQ387558 | LT623277 | LT623237 |
| *N. rhamni*           | Rhamnus frangula CBS 142391 = C1    | WU 36935             | epi                  | MF795775       | MF795775 | MF795838 | MF795863 | –       |
|                       | Rhamnus frangula C12                 | WU 36936             |                      | MF795776       | MF795776 | MF795864 | MF795903 | –       |
|                       | Rhamnus frangula C13                 | WU 36944             |                      | MF795777       | MF795777 | MF795819 | MF795865 | MF795904 |
|                       | Rhamnus frangula C190                | WU 36945             |                      | MF795778       | MF795778 | MF795820 | MF795866 | –       |
|                       | Rhamnus saxatilis C277               | WU 36943             |                      | MF795779       | MF795779 | MF795821 | MF795867 | MF795905 |
| *N. rhamnicola*       | Rhamnus lycioides CBS 142396 = C185 | WU 36946             | holo                 | MF795780       | MF795780 | –   | MF795822 | MF795868 | MF795906 |
|                       | Rhamnus alaternus KRx                | WU 36947             |                      | MF795781       | MF795781 | MF795823 | MF795869 | MF795907 |
| *N. rhamnioides*      | Rhamnus myrtifolius CBS 142395 = C118| WU 36948             | holo                 | MF795782       | MF795782 | MF795824 | MF795870 | MF795908 |
|                       | Rhamnus saxatilis ssp. prunifolius C222| WU 36949             |                      | MF795783       | MF795783 | MF795839 | MF795871 | MF795909 |
|                       | Rhamnus saxatilis ssp. prunifolius C233| WU 36950             |                      | MF795784       | MF795784 | MF795825 | MF795872 | MF795910 |
| *N. rubicola*         | Ribes rubrum CBS 142394 = C55       | WU 36951             | holo                 | MF795785       | MF795785 | MF795840 | MF795873 | MF795911 |
|                       | Ribes rubrum C155                    | WU 36952             |                      | MF795786       | MF795786 | –   | MF795828 | MF795874 | MF795912 |
| *N. unguis-hominis*   | Agapornis sp. Lung CBS 111112        |                      | –                    | LT623222       | GQ387623 | –   | LT623279 | LT623239 |
| *N. vachelliae*       | Vachellia gummosa CBS 142397 = C192 | WU 36953             | holo                 | MF795787       | MF795787 | –   | MF795829 | MF795875 | MF795913 |
| *Neopyrenochaeta*     | Waterpipe CBS 812.95                 |                      |                      | MF795788       | MF795788 | –   | MF795830 | MF795876 | MF795914 |
| *N. fragariae*        | Fragaria ananassa CBS 101634        |                      |                      | MF795789       | MF795789 | –   | MF795831 | MF795877 | –       |
| *N. in florescentiae* | Protea neriifolia CBS 119222        |                      | 5867                  | LT903672       | GQ387608 | –   | LT903673 | LT900365 |
| *N. telephoni*        | Screen of a mobile phone CBS 139022 |                      | MCC H1001            | KM516291       | KM516290 | –   | LT717685 | LT717678 |
| *Neopyrenochaetopsis* | Superficial tissue CBS 143033       |                      |                      | LT592923       | LN907861 | –   | LT593061 | LT592992 |
| Paracuratibaria*      | Fraixinus excelsior with bacterial canker CBS 248.79 |                      |                      | LT623219       | EU754176 | EU754077 | LT623274 | LT623235 |
| *P. italica*          | Olea europaea CBS 234.92             |                      |                      | LT623219       | EU754176 | –   | LT623274 | LT623235 |
| Parafenestella*       | Rosa canina MFLUCC 16-1451          |                      | KY563071            | –              | –   | –   | –   | –       |
| *P. ostryae*          | Ostrya carpinifolia MFLUCC 17-0097   |                      | KY563072            | –              | –   | –   | –   | –       |
| *P. pseudoplatani*    | Acer pseudoplatanus CBS 142392 = C26| WU 36954             | holo                 | MF795788       | MF795788 | –   | MF795830 | MF795876 | MF795914 |
| *Phaeosphaeria*       | Ammophila arenaria AA                | WU 36958             |                      | MF795789       | MF795789 | –   | MF795831 | MF795877 | –       |
| *P. oryzae*           | Oryza sativa CBS 110110             |                      | KF251186            | KF251689       | GQ387530 | –   | KF252193 | KF252680 |
| *Phaeosphaeriopsis*   | Ruscus aculeatus CBS 1563.86        |                      | –                    | KF251919       | KF251702 | GQ387531 | KF252206 | KF253155 |
| Phoma herbarum        | Rosa multiflora CBS 615.75          |                      | –                    | FJ427022       | EU754186 | EU754087 | KP304020 | –       |
| Plerodomus hendersoniae| Salix appendiculata LTO             | WU 36959             |                      | MF795790       | MF795790 | –   | MF795832 | MF795878 | –       |
| Pleospora herbarum    | Medicago sativa leaf CBS 191.86     |                      |                      | DQ491516       | DQ247804 | DQ247812 | DQ471090 | AY749032 |
| Taxon                              | Host/substrate               | Strain          | Specimen voucher no. | Ex-type status | GenBank accession numbers |
|-----------------------------------|------------------------------|-----------------|----------------------|----------------|--------------------------|
|                                   |                              |                 |                      |                |                           |
| P. terrestris                     | Soil                         | CBS 282.72      |                      | holo           | LT623228 LT623216 –      | LT623287 – LT623246 |
|                                   |                              |                 |                      |                | LT623228               | LT623287 – LT623246 |
| Pyrenochaeta nobilis              | Laurus nobilis leaves        | CBS 407.76 = AFTOL-ID 1856 |                  | neo            | MF795792 MF795792       | MF795834 MF795880 MF795916 |
| Pyrenochaetopsis americana        | Unknown                      | UTHSC D16-225  |                      | holo           | LT592912 LN907368 –     | LT593050 – LT592981 |
|                                   |                              |                 |                      |                | LT593050 –             | LT593015              |
| P. botulispora                    | Respiratory tract            | CBS 142458      |                      | holo           | LT592946 LN907441 –     | LT593085 – LT593015 |
|                                   |                              |                 |                      |                | LT593085 –             | LT593015              |
| P. confuens                       | Deep tissue/fluids           | CBS 142459      |                      | holo           | LT592950 LN907446 –     | LT593089 – LT593019 |
| P. globosa                        | Superficial tissue           | CBS 143034      |                      | holo           | LT592934 LN907418 –     | LT593072 – LT593003 |
| P. leiptospora                    | Secale cereale               | CBS 101635      |                      | epi            | MF795793 MF795793 MF795841 MF79581 MF795917 MF795917 |
| P. ubeniformis                    | Superficial tissue           | CBS 142461      |                      | holo           | LT592935 LN907420 –     | LT593074 – LT593004 |
| Seltsamia ulmi                    | Ulmus glabra                 | CBS 143002 = L.150 | WU 36957 | holo | MF795794 MF795794 MF795794 MF795836 MF795882 MF795918 |
| Staurosporahelia aptrotii         | Lycium sp.                   | CBS 483.95      |                      | holo           | KY929149 GU301806 GU296141 – | GU349044 – |
| Trematosphaeria pertusa           | Fraxinus excelsior           | CBS 122368      |                      | epi            | AB809646 FJ201990 FJ201991 genome2 genome2 genome2 |
| Xenopyrenochaetopsis pratorum     | Lolium perenne               | CBS 445.81      |                      | iso            | JF740263 GU238136 GU238228 KT389671 – | KT389846 |

1 Ex-epitype of Alternaria tenuis Nees.
2 Sequence retrieved from genome deposited at JGI-DOE (http://genome.jgi.doe.gov/).
Cultures preparation and phenotype analysis

Cultures were prepared and maintained as described previously (Jaklitsch 2009) except that CMD (CMA: Sigma, St Louis, Missouri; supplemented with 2 % (w/v) D-(+)-glucose-monohydrate) or 2 % malt extract agar (MEA; 2 % w/v malt extract, 2 % w/v agar-agar; Merck, Darmstadt, Germany) was used as the isolation medium. Cultures used for the study of asexual morph-morphology were grown on CMD or MEA at 22 ± 3 °C, rarely SNA (Nirenberg 1976) for conidiation assessment, in darkness. Microscopic observations were made in tap water except where noted. Morphological analyses of microscopic characters were carried out as described by Jaklitsch (2009). Methods of microscopy included stereomicroscopy using a Nikon SMZ 1500 and Nomarski differential interference contrast (DIC) using the compound microscopes Nikon Eclipse E600 or Zeiss Axio Imager.A1 equipped with a Zeiss Axiocam 506 colour digital camera. Images and data were gathered using a Nikon Coolpix 4500 or a Nikon DS-U2 digital camera and measured by the NIS-Elements D v. 3.0 or 3.22.15 or Zeiss ZEN Blue Edition software. For certain images of ascospores the stacking software Zerene Stacker v. 1.04 (Zerene Systems LLC, Richland, WA, USA) was used. Measurements are reported as maxima and minima in parentheses and the mean plus and minus the standard deviation of a number of measurements given in parentheses.

DNA extraction and sequencing methods

The extraction of genomic DNA was performed as reported previously (Voglmyar & Jaklitsch 2011, Jaklitsch et al. 2012) using the DNeasy Plant Mini Kit (QiAgen GmbH, Hilden, Germany) or the modified CTAB method of Riehlmüller et al. (2002).

The following loci were amplified and sequenced: the complete internally transcribed spacer region (ITS1-5.8S-ITS2) and a ca. 900 bp fragment of the large subunit nuclear ribosomal DNA (nLSU rDNA) as a single fragment with primers V9G (de Hoog & Gerrits van den Ende 1998) and LRS (Vilgalys & Hester 1990); a ca. 1.0–1.4 kb fragment of the small subunit nuclear ribosomal DNA (nSSU rDNA) with primers SL1 (Landvik et al. 1997) and NSSU1088 (Kauff & Lutzoni 2002); a ca. 1.2 kb fragment of the RNA polymerase II subunit 2 (rbp2) gene with primers RRPB2-5f and RRPB2-7cr (Liu et al. 1999) or dRPB2-5f and dRPB2-7r (Voglmyar et al. 2016a); a ca. 1.2–1.3 kb fragment of the translation elongation factor 1-alpha (tef1) gene with primers EF1-728F (Carbone & Kohn 1999) and TEF1LLErev (Jaklitsch et al. 2005) or EF1-2218R (Rehner & Buckley 2005); and a ca. 0.7 kb fragment of the beta tubulin (tub2) gene with primers T1 O’Donnell & Cigelnik 1997 or T1HV (Voglmyar et al. 2016b) and BtHV2r (Voglmyar et al. 2016b, 2017). PCR products were purified using an enzymatic PCR cleanup (Welte et al. 1994) as described in Voglmyar & Jaklitsch (2008). DNA was cycle-sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington, U.K.) with the same primers as in PCR; in addition, primers ITS4 (White et al. 1990), and LR3 (Vilgalys & Hester 1990) were used for the ITS-LSU region. In some cases the tef1 was cycle-sequenced with internal primers TEF1_INTF (forward; Jaklitsch 2009) and TEF1_INT2 (reverse; Voglmyar & Jaklitsch 2017). Sequencing was performed on an automated DNA sequencer (3730xl Genetic Analyzer, Applied Biosystems).

Analysis of sequence data

For the phylogenetic analyses, combined matrices of ITS-LSU, SSU, rpb2, tef1 and tub2 sequences were produced. GenBank sequences of selected families of Pleosporales from the suborder Pleosporineae were selected according to Hyde et al. (2013) and recent additions (Crous & Groenewald 2017, Valenzuela-Lopez et al. 2018, Wanasinghe et al. 2017b) and supplemented with GenBank nucleotide sequences of some additional taxa. For some strains for which the whole genome data are available, sequences were retrieved from JGI-DOE (http://genome.jgi.doe.gov). Two representative taxa (Massarina eburnea, Trematosphaeria pertusa) from the suborder Massarineae were selected as outgroup (Tanaka et al. 2015). All alignments were produced with the server version of MAFFT (www.ebi.ac.uk/Tools/mafft), checked and refined using BioEdit v. 7.0.9.0 (Hall 1999). Due to alignment problems, the nucleotide characters at the very 5’ end of the ITS1 were excluded for all taxa outside Cucurbitaliaceae, Pyrenochoaetopsidaceae and Pyremocheta nobilis. For phylogenetic analyses, two matrices were produced, one comprising only ITS-LSU sequences and a second combined matrix of ITS-LSU, SSU, rpb2, tef1 and tub2, containing only accession for which, in addition to the LSU, at least rpb2 or tef1 were available. The ITS-LSU matrix contained 1 649 nucleotide characters and the combined matrix 6 058 nucleotide characters: 1 697 from the ITS-LSU, 1 002 from the SSU, 1 070 from rpb2, 1 453 from tef1, and 836 from tub2. Prior to phylogenetic analyses, the approach of Wiens (1998) was applied to test for significant levels of localised incongruence among the markers used for the combined analysis, using the level of bootstrap support (Sung et al. 2007) as described in Jaklitsch & Voglmyar (2014). For this, the 70 % maximum parsimony (MP) bootstrap consensus trees from 100 bootstrap replicates calculated for each individual partition, with the same parameters given below and with each replicate limited to 1 million rearrangements, were compared. These bootstrap trees were also used for an evaluation of the phylogenetic resolution of the individual markers; but for this the 50 % bootstrap support was implemented.

Maximum parsimony (MP) analysis of the combined matrices was performed using a parsimony ratchet approach. For this, a nexus file was prepared using PRAP v. 2.0b3 (Müller 2004), implementing 1 000 ratchet replicates with 25 % of randomly chosen positions upweighted to 2, which was then run with PAUP v. 4.0a156 (Swofford 2002). The resulting best trees were then loaded in PAUP and subjected to heuristic search with TBR branch swapping (MULTREES option in effect, steepest descent option not in effect). Bootstrap analysis with 1 000 replicates was performed using 5 (ITS-LSU matrix) or 10 (combined multigene matrix) rounds of replicates and subsequent TBR branch swapping (MULTREES option in effect, steepest descent option not in effect) during each bootstrap replicate, with each replicate limited to 1 million rearrangements in the ITS-LSU matrix. In all MP analyses molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data; the COLLAPSE command was set to minbrlen.

Maximum likelihood (ML) analyses were performed with RAxML (Stamatakis 2006) as implemented in raxmlGUI v. 1.3 (Silvestro & Michalak 2012), using the ML + r bootstrap setting and the GTR+GAMMA substitution model with 1 000
bootstrap replicates. The matrix was partitioned for the individual gene regions, and substitution model parameters were calculated separately for them.

For evaluation and discussion of bootstrap support, values below 70 % were considered low, between 70 and 90 % medium/ moderate, and above 90 % high.

RESULTS

Molecular phylogeny

Test for localised incongruence among the markers
In the MP bootstrap tree of tub2, Neocucurbitaria rhamnioides C118 was placed basal to the N. rhamnioides - N. rhammcola clade with high support, which was in conflict with the bootstrap trees of all other markers; in the combined analyses the clade with high support, which was in contrast to ITS-LSU and tub2; therefore excluded for N. rhamnioides C118. No additional significant topological conflicts were observed between the bootstrap trees of the various genes, indicating the absence of significant incongruence and combinability of the loci (Wiens 1998).

Phylogenetic analyses
Of the 1 649 nucleotide characters of the ITS-LSU matrix, 211 were parsimony informative. Maximum parsimony analyses revealed 2720 MP trees 843 steps long, one of which is shown in Fig. 1. The MP trees were identical in the deeper nodes, but within Cucurbitariaceae several nodes especially within Cucurbitaria and Neocucurbitaria collapsed to a polytomy in the strict consensus tree (marked by asterisks in Fig. 1).

Of the 6 056 nucleotide characters of the combined matrix, 1471 were parsimony informative (319 of ITS-LSU, 44 of SSU, 506 of rpb2, 345 of tef1, and 257 of tub2). Maximum parsimony analyses revealed 64 MP trees 9 817 steps long, one of which is shown as Fig. 2. Topologies of the MP trees were identical except for a few deeper nodes in Cucurbitariaceae and a polytomy of Neocucurbitaria cava, N. populi and N. juglandicola (marked by asterisks in Fig. 2).

Comparison of the phylogenetic analyses of the ITS-LSU matrix with the combined matrix shows a significant increase of resolution within Cucurbitariaceae in the latter. While the closely related Cucurbitaria berberidis and C. oremediterranea were not resolved in the ITS-LSU tree (Fig. 1), they received high to maximum support in the combined analyses (Fig. 2); likewise, the weakly (59 % MP) to unsupported (ML) Neocucurbitaria rhammiocola (Fig. 1) received maximum support (Fig. 2). In addition, internal support of many other nodes increased substantially; most notably to mention the high (MP) to maximum (ML) support for Cucurbitariaceae and the genus Neocucurbitaria which had low and no significant support, respectively, in the ITS-LSU tree. Also many nodes of the backbone within Neocucurbitaria received medium to high support in the combined analyses, which were unsupported in the ITS-LSU analyses; for instance, the monophyly of the three species on Rhamnus (Fig. 2).

Comparison of the phylogenetic resolution of the individual markers
Comparison of the bootstrap trees of the individual markers used for evaluation of localised incongruence revealed also highly interesting insights into their phylogenetic resolution and support. Only the most relevant outcomes with respect to Cucurbitariaceae are discussed here (data not shown). As expected, the SSU has too little phylogenetic information and almost all nodes within Pleosporineae lack significant support; it is therefore not further discussed here. With the other markers, the Cucurbitariaceae are only resolved by rpb2 and ITS-LSU, receiving low (64 %) and medium (83 %) support, respectively. Backbone support within Cucurbitariaceae is generally highest with rpb2, followed by ITS-LSU and tub2; however, the deeper nodes within Cucurbitariaceae are unsupported with all markers. The genus Neocucurbitaria receives medium support by the tub2 (72 %) and the ITS-LSU (82 %) analyses, and high support (99 %) by the rpb2 analyses. Within Neocucurbitaria, rpb2 consistently revealed a high support for all main clades and is superior to ITS-LSU and tub2, where many of these clades were not resolved in ITS-LSU and tub2, where many of these clades were received only low to medium support. The genus Cucurbitaria received medium support (88 %) in tub2, high support (98 %) in ITS-LSU and maximum support in rpb2. However, with these markers the two closely related Cucurbitaria species remained unresolved in ITS-LSU and were only partially resolved in rpb2 (C. berberidis, 95 %), but fully resolved in tub2 (C. berberidis, 54 %; C. oremediterranea, 99 %). Neocucurbitaria rhamnica received low (64 %), medium (83 %) and maximum support by ITS-LSU, tub2 and rpb2, respectively, and N. rhamnioides received high support by ITS-LSU (90 %) and rpb2 (92 %). In the tef1 analyses all deeper nodes of the tree were not supported, but it is the best marker for resolution of closely related species; it is the only marker where the species pairs Cucurbitaria berberidis/ C. oremediterranea and Neocucurbitaria rhamniocola/ N. rhamnioides were resolved with maximum support.

Morphology

It is noted that most representatives of the Cucurbitariaceae studied here have true paraphyses. Hamathecial threads with free apices among immature ascii are necessary to assess this feature. However, in the materials of several species no immate ascii were present, therefore we term the hamathelial threads ?paraphyses due to uncertainty.

Taxonomy

Cucurbitariaceae G. Winter [as Cucurbitarieae]. Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 1: 308. 1885.

Ascomata immersed in bark, erumpent, often becoming superficial, scattered or gregarious in or on a subiculum or in a volsoid pseudostroma, perithecioid, globose, subglobose, turbinate, lenticular or pyriform, brown to black; surface verruculose to coarsely tubercular. Ostioles inconspicuous or papillate to cylindrical, ostiolar canal periphysate. Peridium pseudoparenchymatous, usually brown. Hamathecium comprising numerous hyaline, filiform, septate and often anastomosing paraphyses, sometimes possibly pseudoparaphyses. Asci cylindrical to oblong, bitunicate, fissitunicate, with an ocular chamber and typically with a short stipe, containing 4–8 ascospores in uni- to partly biseriate arrangement. Ascospores ellipsoid, fusoid or oblong, brown, muriform, rarely with a gelatinous sheath, sometimes with appendage cells. Asexual morphs coelomycetous, phoma- or pyrenochaeta-like.
Fig. 1. Phylogram of one of 2720 MP trees 843 steps long (CI = 0.518, RI = 0.750), obtained by PAUP from an analysis of the ITS-LSU matrix of Cucurbitariaceae, Neopyrenochaetaceae and Pseudopyrenochaetaceae, with the latter selected as outgroup according to Fig. 2. MP and ML bootstrap support above 50 % are given at the first and second position, respectively, above or below the branches. Strains formatted in bold were isolated and sequenced in the current study; ex-type strains are indicated by a superscript T. Nodes that collapsed in the strict consensus of all 2 720 MP trees are marked by an asterisk (*). Note the lack of internal support for most backbone nodes, and the lack of resolution for closely related taxa.
Fig. 2. Phylogram of one of 64 MP trees 9817 steps long (CI = 0.345, RI = 0.625), obtained by PAUP from an analysis of the combined matrix (SSU-ITS-LSU, Cucurbitariaceae). 64 MP trees are marked by an asterisk (*).

Strains formatted in bold were isolated and sequenced in the current study; ex-type strains are indicated by a superscript T. Nodes that collapsed in the strict consensus of the

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**Cucurbitariaceae**

- Neocucurbitaria rhamnoides C222
- Neocucurbitaria rhamnoides CBS 142395
- Neocucurbitaria rhamnica CBS 142396
- Neocucurbitaria rhamnica KRx
- Neocucurbitaria rhamni C190
- Neocucurbitaria rhamni C133
- Neocucurbitaria rhamni C112
- Neocucurbitaria rhamni CBS 142391
- Neocucurbitaria rhamni C277
- Neocucurbitaria ribicola CBS 142394
- Neocucurbitaria ribicola C155
- Neocucurbitaria acerina C26a
- Neocucurbitaria acerina CBS 142403
- Neocucurbitaria irregularis CBS 142791
- Neocucurbitaria keratinophila CBS 121759
- Neocucurbitaria aquatica CBS 297.74
- Neocucurbitaria unguiis-hominis CBS 111112
- Neocucurbitaria vachelliae CBS 142397
- Neocucurbitaria populi CBS 142393
- Neocucurbitaria cisticola CBS 142402
- Neocucurbitaria hakeae CPC 2892
- Neocucurbitaria aetensis C270
- Neocucurbitaria aetensis CBS 142404
- Neocucurbitaria cinereae CBS 142408
- Neocucurbitaria acanthocladae CBS 142398
- Neocucurbitaria quercina CBS 115095

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**Pleosporales**

- Cucurbitariaceae
- Pleosporaceae
- Coniothyriaceae
- Phaeosphaeriaceae
- Didymellaceae
- Leptosphaeriaceae
- Pleosporaceae
- Dacrymycetaceae
- Pseudopyrenochaetaceae
- Xylohyphaceae

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**Pleosporinae**

- Neopyrenochaetae
- Neocucurbitariae
- Coniothyriaceae
- Phaeosphaeriaceae
- Didymellaceae
- Leptosphaeriaceae
Saprobic on wood, bark and leaves or fungicolous, sometimes pathogenic on humans, also isolated from soil, possibly endophytic in plants.

Type genus. – Cucurbitaria.

Notes: In most taxa, particularly of Cucitella, Fenestrella, Neo-cucurbitaria and Parafenestella, where the study of the hama-theicum was possible, we detected paraphyses with free apices among immature asci. Cucurbitaria may have pseudopara- phy ses, but this has not been re-assessed.

Cucurbitaria Gray, Nat. Arr. Brit. Pl. (London) 1: 519. 1821.

Synonym: Crotonocarpia Fückel, Jb. nassau. Ver. Naturk. 23–24: 163. 1870 (1869–1870).

Ascomata erumpent from bark, scattered or aggregated in clusters on a subiculum, globose to turbinate, brown to black; apex obtuse, surface usually coarsely warted. Ostiolar openings inconspicuous, central, sunken, sometimes visible as a minute, light-coloured areas. Peridium firm and thick, pseudoparenchymatous, brown to black outside, lighter-coloured to the inside, typically thickened and often distinctly elongated basally. Hamathecium of branched ? paraphyses. Asci cylindrical, bitunicate, fissitunicate, with a short stipe, a simple or knob-like base, and a distinct ocular chamber; containing 8 ascospores in uniseriate arrangement. Ascospores ellipsoid, straight, muriform, slightly constricted at the median septum, golden-, reddish- to dark brown, smooth.

Asexual morph pyrenochaeta-like. Pycnidia on natural hosts and in artificial culture with apical setae, superficial (or immersed in agar), more or less globose, brown to black. Peridium thin, pseudoparenchymatous, brown. Conidiogenous cells phialidic, cylindrical to lageniform, formed on simple or basally branched conidiophores and on basal hyaline cells in nature and in artificial culture. Conidia produced acropleurogenously, i.e. at one side of the conidiophore on phialides or pegs, and terminally. Conidia 1-celled, oblong, cylindrical or ellipsoid, straight or curved, hyaline to pale brownish, guttulate.

Type species: Cucurbitaria berberidis (Pers.) Gray.

Notes: Most of the generic synonyms of Cucurbitaria listed in Species Fungorum and by Doilm et al. (2013) are different fungi or require reassessment, therefore we list only Crotonocarpia. For Gymnocepes and Megaloceps see Jaklitsch & Voglmayr (2017); also Gibberidea does not belong here (unpubl. results).

Cucurbitaria berberidis (Pers.) Gray, Nat. Arr. Brit. Pl. (London) 1: 519. 1821.

Basionym: Sphaeria berberidis Pers., Neues Mag. Bot. 1: 83. 1794. : Fr.: Syst. Mycol. 2 (2): 415. 1823.

Synonymy: Hypoxylon berberidis (Pers. : Fr.) J. Kickx f., Rech. Serv. Fl. Crypt. Fland. 1: 18. 1841.

Crotonocarpia moriformis Fückel, Jb. nassau. Ver. Naturk. 23–24: 163. 1870 (1869–1870).

Phoma berberidis Sacc., Michelia 1(no. 2): 259. 1878.

Pyrenochaeta berberidis (Sacc.) Brunaud, Act. Soc. linn. Bordeaux, Trois. sér. 40: 83. 1886.

Gibberidea berberidis (Pers. : Fr.) Rabenh. ex Kuntze, Revis. gen. pl. (Leipzig) 3(2): 481. 1898.

Cucurbitaria moriformis (Fückel) M. E. Barr, Mycotaxon 29: 503. 1987.

See Doilm et al. (2013) for description and typification.

Material examined: Austria, Kärnten, St. Margareten im Rosental, Drau-Auen, grid square 9452/1, on branches of Berberis vulgaris, 31 Dec. 2002, W. Jaklitsch W.J. 2043 (WU 39966); Wogradra, grid square 9452/3, on Berberis vulgaris, 14 Apr. 2006, W. Jaklitsch W.J. 2901 (WU 35985); ibid., 30 Apr. 2011, W. Jaklitsch (WU 31405 epitype; ex-epitype-culture CBS 130007 = CB1, second isolate CB); Vienna, 3rd district, Botanical Garden, on branches of Berberis sp., 14 Mar. 2016, W. Jaklitsch & H. Voglmayr (WU 35987; culture CBS 142401). Belgium, Sint-Hubrechtis-Lille, Neerpet, on branch of Berberis vulgaris sp. atropupurea, 26 Jan. 2014, P. Bormans (WU 35986; culture C39).

Notes: Cucurbitaria eruphodica sensu Aiyawansa et al. (2015), discussed in Wanasinghe et al. (2017b), is clearly C. berberidis. The authors only produced SSU and LSU (accessions KT313005, KT313007) of their single isolate HA 42, and they are identical in composition and length with those of C. berberidis strain CBS 394. 84. Their specimen was collected in the Italian region Emilia Romagna and neither the collector nor the authors were obviously able to identify the host. According to Flora Italiana (http://fluriq.altervista.org/flora/taxa/floraindice.php) no Ephedra occurs in Emilia Romagna. There is no basis to select arbitrarily a name out of numerous Cucurbitaria names (see e.g. Mirza 1968) without knowing the host. Wanasinghe et al. (2017b) argued that their specimen differed in morphology from C. berberidis. However, it may be poorly developed, but otherwise we do not see much difference. We suggest that the authors re-check the morphology of their specimen.

Cucurbitaria oromediterranea Jaklitsch & Voglmayr, sp. nov., MycoBank MB822999. Fig. 3.

Etymology: Referring to its occurrence in ommediteanan regions.

Ascomata (300–)430–620(–750) μm (n = 67) diam, (300–)500–630(–650) μm (n = 15) high, scattered or aggregated in small groups, erumpent-superficial, globose, subglobose to turbinate or pulvinate, often collapsing from above, with sunken centre, sometimes laterally fused, black, with surface coarsely cracked into plates; seated on a subiculum of thick-walled, brown 2.5–6 μm wide hyphae continuing in the wood. Ostioles (59–)80–120(–135) μm (n = 15) long, (55–)71–108(–115) μm (n = 15) wide at the apex, usually indistinct at the surface, sometimes indistinctly papillate, sometimes with reddish-brown centre. Peridium (77–)114–233(–268) μm (n = 15) wide at base, (53–)63–127(–150) μm (n = 15) at the sides; outer layer narrow, dark brown to black, opaque, coarsely warted, of thick-walled cells immersed in a dark amorphous resinous mass; inner layer thick, particularly at the base, consisting of brown, thin-walled, pseudoparenchymatous cells (4.5–)6.5–18(–26.5) × (3.5–)5.5–11(–15) μm (n = 30). Hamathecium of moderately branched, 1–3.5(–4) μm wide ?paraphyses. Asci (144–)165–225(–260) μm (n = 35), cylindrical, bitunicate, fissitunicate, with a short stipe and a simple or knob-like base, narrow walls with endotunica thickened at the apex and a distinct ocular chamber; containing 8 ascospores in uniseriate arrangement. Ascospores (22–)25–33(–40) × (9.3–)11.3–14.5(–17.2) μm, l/w (1.9–)2.1–2.5(–2.8) (n = 76), ellipsoid, straight, with (6–)7(–8)–12 transverse and 2–4 longitudinal septa, slightly constricted at the median primary septum, ends rounded to subacute, first hyaline to yellowish, turning golden-, reddish- to dark brown, in 3 % KOH dark brown to nearly black, smooth. Pycnidia on natural hosts (73–)110–235(–330) μm (n = 43) diam, scattered, superficial, globose, dark brown to black, with apical setae.

Cultures and asexual morph: A 90 mm Petri dish containing CMD, inoculated at the side and incubated in the dark at 22 °C entirely covered by mycelium after 4–6 wk. Colony dark olive brown to nearly black, margin hyaline, not or indistinctly zonate,
Fig. 3. A–J, K–W, Y–F1. Cucurbitaria oromediterranea. J, X, C. berberidis (WU 35987). A, B. Ascomata in face view. C, D. Ascoma in vertical section showing thickened basal peridium. E, F. Peridium in vertical section, showing opaque warts in E. G. Subicular hyphae. H–J. Ascii. K, L. Ascus tips showing ocular chamber. M. Hamathecium. N–X. Ascospores. Y. Pycnidium on natural substrate. Z–F1. Asexual morph on CMD at 22 °C after 2–3 wk. Z. Pycnidium. A1. Setae. B1–D1. Conidiophores and phialides. E1, F1. Conidia. A, C–G, L, M, Q–T, W, U 35988; B, N–P, U, V, Y–F1, WU 35991/C86; H, I, WU 35989; K, WU 35990. Scale bars: A, B = 0.5 mm; C, D = 150 μm; E, Y, Z = 50 μm; F, M–X, C1 = 10 μm; G, K, L, B1 = 7 μm; H–J, A1 = 20 μm; D1–F1 = 5 μm.
odour indistinct. Pycnidia (70–)90–125 μm (n = 17) diam, usually numerous, formed within 2 wk in the centre, in a concentric zone or scattered over the whole colony, superficial or immersed in agar, first hyaline to greyish, turning black, globose with a small papilla or pycniform, with protruding cells and setae at the surface; extruded conidial drops hyaline to greyish brown. Setae concentrated at the apex, up to 60 μm long and 7 μm thick at their bases, greyish to dark brown, with rounded ends. Peridium thin, of thin-walled pale brown cells (4.7–)6.5–10(–15) μm diam (n = 25) forming a t. angularis, outside darker, thick-walled and more rounded, inside lined by a layer of angular hyaline cells giving rise to conidiophores or phialides. Conidiophores hyaline, simple or branched near the base into two or several branches, each with lateral or unicellular branches forming solitary terminal phialides. Phialides (5.3–)7.0–9.8(–10.7) × (1.6–)1.7–2.2(–2.5) (n = 23), cylindrical, sinuous or lageniform. Conidia (2.5–)3.3–4.0(–4.5) × (0.9–)1.1–1.7(–2.1) μm, l/w (1.6–)2.2–3.3(–4.8) (n = 64), 1-celled, oblong, cylindrical or ellipsoid, straight or slightly curved, often attenuated toward one end, hyaline to pale brownish, containing two subterminal guttules.

Habitat: On wood and bark of Berberis spp., known from B. aetnensis, B. cretica and B. hispanica.

Distribution: At elevations from above ca. 900 m in the Mediterranean.

Holotype: Greece. Crete, Omalos plain, heading to Selinikitos Giro, N 35 19 20 E 23 54 22, elev. 1 120 m, on twigs of Berberis cretica, 5 Jun. 2015, W. Jaklitsch & H. Voglmayr (WU 35988; ex-holotype culture CBS 142399 = C229).

Other material examined: Greece. Crete, Omalos, on twigs of Berberis cretica, 26 Jun. 2013, W. Jaklitsch (WU 35989; culture CB2); path to a waste dump off the road to Omalos, on Berberis cretica, soc. Thyronectria caudata, Thiuridum sp., 28 Nov. 2011, W. Jaklitsch (WU 32130). Italy. Sicily, Enna, south side, elev. ca. 1 900 m, roadside, on twigs of Berberis aetnensis, 17 Jun. 2016, W. Jaklitsch & H. Voglmayr (WU 35993; culture C265). Spain. Andalusia, Granada, La Zuzia, Cerro del Trevenque, above the Jardín Botánico de la Conquijera, above 1 600 m, on twigs of Berberis hispanica, 14 May 2014, W. Jaklitsch & J. Tello (WU 35992; culture CB3); same data, elev. ca. 1 700 m, soc. Thyronectria caudata, S. Tello & W. Jaklitsch (WU 33428); ca. 1 600 m, on twigs of Berberis hispanica, 14 May 2014, W. Jaklitsch & S. Tello (WU 35991; culture CB6); Jaén, Jaén, La Pandera, N 37°37’54” W 3°46’00”, 1 800 m, on Berberis hispanica, 12 May 2014, S. Tello, W. Jaklitsch, J. Extrada & D. Merino (WU 33429; culture C29).

Notes: Cucurbitaria oromediterranea is virtually indistinguishable from C. berberidis in its sexual and asexual morphs and cultures, only ascospores are on average more reddish brown in C. oromediterranea and ascomatal pustules are often less conspicuous due to a lower number of ascomata per group. However, multigene phylogeny clearly separates it from C. berberidis (Fig. 2), and both species can be reliably distinguished by tef1 and tub2 sequences where they differ in 26 (including 3 gaps) and 12 fixed nucleotide substitutions, respectively. Cucurbitaria oromediterranea is the specific host of Thyronectria caudata (Jaklitsch & Voglmayr 2014) and, as inferred from the distribution of the latter, it occurs at high elevations in the African, Asian and European Mediterranean region. Thus, Cucurbitaria berberidis and C. oromediterranea have clearly different ecological requirements. The hamathecial threads have free rounded ends, but their development is unclear, therefore we term them ?paraphyses. In culture on CMD we found conidiophores only in entirely mature pycnidia, not in young ones.

Astragalicola Jaklitsch & Voglmayr, gen. nov., MycoBank MB823000.

Etymology: Referring to its occurrence on Astragalus.

Only known as asexual morph. Pycnidia scattered or aggregated in groups on wood and bark, more or less superficial, globose, non-papillate, black; contents with a waxy to gelatinous consistency; ostiolar opening apical; peridium pseudoparenchymatous. Phialides and conidiophores densely aggregated at inner side. Conidiophores simple or branched once. Phialides lageniform to cylindrical or sigmoid. Conidia formed on phialides, their base cells or lateral pegs on conidiophores, oblong or narrowly ellipsoid, straight or curved, 1-celled, hyaline, guttulate.

Type species: Astragalicola amorpha Jaklitsch & Voglmayr.

Notes: The genus Astragalicola differs from Phoma by the presence of conidiophores and from Pyrenochaeta by the lack of setae. Erection of a new genus was necessary following phylogenetic analysis, as it forms a separate clade.

Astragalicola amorpha Jaklitsch & Voglmayr, sp. nov., MycoBank MB823001. Fig. 4.

Etymology: Referring to its sole occurrence as asexual morph.

Pycnidia (140–)195–281(–336) μm diam (n = 34), aggregated in groups on bark or scattered on wood, superficial with bases often immersed, sometimes laterally fused, globose, non-papillate, with a minute apical ostiolar opening, more or less smooth, black, contents olivaceous, with a waxy to gelatinous consistency; peridium ca. 20–70 μm thick, outer layer unevenly dark brown pigmented, of thick-walled cells (4–)5.5–10(–16.5) μm diam (n = 30) forming t. angularis to globulosa, inner layer pseudo-parenchymatous, hyaline, giving rise to densely aggregated phialides and conidiophores. Conidiophores up to ca. 45 μm long, simple or branched once. Phialides (5.3–)6.2–9.3(–11) × (1.4–)1.8–2.3(–2.5) μm, l/w (2.1–)2.8–4.9(–6) (n = 30), lageniform to cylindrical, often curved or sigmoid. Conidia formed on phialides and laterally on their base cells or lateral pegs on conidiophores, (2.3–)2.5–3.0(–3.5) × (1.0–)1.2–1.4(–1.6) μm, l/w (1.7–)1.9–2.4(–2.8) (n = 83), oblong or narrowly ellipsoid, straight or slightly curved, 1-celled, hyaline, with 1–2 or more minute guttules, sometimes attenuated toward one end.

Culture: Colony radius 35 mm on CMD after 15 d at 22 °C; colony circular, olivaceous with pale margin, radial structure, few pycnidia forming at the near margin; odour indistinct.

Habitat: on stems of Astragalus angustifolius.

Distribution: Greece (Crete), only known from the holotype location.

Holotype: Greece. Crete, Psiloritis, at the margin of the Nida plateau, on basal stem parts of Astragalus angustifolius, intimately associated with a reddish Fusarium sp. (holomorph; reddish-violaceous ascomata containing bicellular ascospores); soc. Camarosporium sp. (C227, C227b), a member of the Lophiostomataceae (C227c) and a Scopinella sp., 8 Jun. 2015, W. Jaklitsch & H. Voglmayr (WU 35994; ex-holotype culture CBS 142399 = C227a, from conidia).

Notes: Cucurbitaria astragali P. Karst. & Har. is no probable candidate for an earlier name of this fungus, as it was described from Astragalus monspessulanus, a herbaceous host, and its
putative asexual morph was identified as a *Hendersonia* with large, 4-septate conidia (Karsten & Hariot 1890). *Phoma astragalii* Ellis & Kellerm. is a *nomen nudum* fide Boerema et al. (2004; p. 61); *Phoma astragalii* Cooke & Harkn. is now known as *Stagonosporopsis astragalii* (Cooke & Harkn.) Aveskamp et al. (Aveskamp et al. 2010). *Phoma astragalicolica* Hollós has much larger conidia (6–8 × 2.5–3 μm) than *Astragalicolica amorpha* and occurs in leaves (Saccardo 1913). *Phoma astragalina* (Gonz. Fr.) Boerema & Kesteren differs from *Astragalicolica amorpha* by narrower conidia and occurrence on various herbaceous hosts in south-western Asia (Boerema & Kesteren 1981, Boerema et al. 1994).

*Cucitella* Jaklitsch & Voglmayr, gen. nov., MycoBank MB 823002.

*Etymology*: Based on a combination of the generic names *Cucurbitaria* (Cuc) and *Fenestella* (tella).

Ascomata depressed subglobose to pyriform, black, immersed in bark on subiculum, erumpent through cracks in the periderm, aggregated or laterally fused in clusters forming compact pustules causing small bumps on the bark surface. Ascomatal apices often papillate, black, rounded or apically flattened, with circular or angular outline. *Peridium* pseudoparenchymatous, inner side lined by densely stacked simple or basally branched conidiophores with lateral pegs and solitary terminal, narrowly lageniform to subcyllindrical phialides. *Conidia* formed on pegs and phialides, 1-celled, cylindrical to allantoid, hyaline, smooth. In pycnidia formed in culture conidia were only produced on sessile phialides.

*Type species*: *Cucitella opali* Jaklitsch & Voglmayr.

*Note*: Compact pustules and ascospores with a relatively large number of septa and lighter ends suggest a generic affiliation with *Fenestella*, but the multigene phylogeny disproves this hypothesis.

*Cucitella opali* Jaklitsch & Voglmayr, sp. nov., MycoBank MB 8823003. Fig. 5.

*Etymology*: Due to its occurrence on *Acer opalus*.

Ascomata (230–)305–537(–600) μm (n = 14) diam, ca. 160–430 μm high, depressed subglobose, globose to pyriform, black, immersed in bark on a brownish subiculum, erumpent through cracks in the periderm, tightly aggregated or laterally fused in clusters of up to ca. 10, forming compact pustules causing small bumps on the bark surface. Ascomatal apices often papillate, (44–)65–105(–115) μm (n = 16) diam, black, rounded or apically flattened, with circular or angular outline. Subiculum consisting of 2–6 μm wide, thick-walled, hyaline, greyish brown to medium brown hyphae. *Peridium* 15–55 μm thick, thin at the base, thickest around the ostioles, consisting of a thick-walled *t. angularis*, dark brown outside becoming lighter brown to yellowish or hyaline toward the inner side, formed by cells (3.5–)5.2–9.5(–13.7) μm (n = 60) diam. *Hamathecium* consisting of numerous branched, 1–3(–4) μm wide paraphyses with free ends among immature asci. Asci (183–)189–205(–216) × (21.2–)22.3–27.2(–29.2) μm (n = 12), cylindrical, bitunicate, fissitunicate, with an ocular chamber, short stipe and a simple or knob-like base, containing...
Fig. 5. Cucitella opali (WU 35995/CBS 142405). A–O. Sexual morph (WU 35995). A. Pustule of ascomata in bark with two ascomata in vertical section. B. Peridium in vertical section. C. Subicular hyphae. D. Ascus apex. E–H. Asci (exotunica broken in G). I–O. Ascospores (I young). P–S. Asexual morph on natural substrate. P, Q. Pycnidia. R. Cluster of short conidiophores with phialides. S. Conidia. T–Z. Asexual morph in culture (CBS 142405 on CMD after 5–6 d at 22 °C). T. Pycnidia. U. Pycnidial wall. V, W. Conidiogenous cells. X–Z. Conidia. D, F, I, O. in 3 % KOH. Scale bars: A, P = 150 μm; B–D, J–O, U = 10 μm; E–H = 25 μm; I, R = 7 μm; Q = 250 μm; S, V–Z = 3 μm; T = 70 μm.
Conidia cylindrical latter up to ca. 50 pseudoparenchymatous, consisting of dark brown thick-groups in bark cracks, globose, collapsing cupulate, shiny black. diam, occurring in association with ascomata, aggregated in small ends, smooth; slightly to distinctly darker in 3 % KOH when mature.

Asci narrow, branched and anastomosing paraphyses. Peridium pseudoparenchymatous, consisting of dark brown thick-walled cells, inside lined by densely stacked conidiophores, the latter up to ca. 50 μm long, simple or branched at the base, with lateral pegs and terminal solitary, narrowly lageniform to sub-cylindrical phialides 4.5–7(–7.8) × (1.5–)1.7–2.5(–2.8) μm (n = 10). Conidia formed on pegs and phialides, (3–)3.4–4.2(–5) × (0.9–)1.1–1.3(–1.5) μm, l/w (2.3–)2.7–3.8(–4.5) μm (n = 27), 1-celled, cylindrical to allantoid, hyaline, smooth.

Cultures and asexual morph: Colony radius on CMD 23–26 mm after 20 d at 22 °C; colony first hyaline, turning olivaceous to nearly black, margin hyaline, aerial hyphae inconspicuous, odour indistinct to slightly unpleasant. Pycnidia (after 5–6 d) 40–120 μm diam, forming within a few days in large numbers, immersed to superficial, evenly scattered or aggregated in small groups, globose to pyriform, papillate, first hyaline, slowly turning olivaceous. Peridium consisting of a thin, moderately thick-walled, olivaceous brown t. globulosa-angularis of (4–)5.7–10(–13.5) μm (n = 45) wide cells; inner side lined by olivaceous cells giving rise to phialides. Phialides (3.8–)4.3–6(–7) × (2.7–)3.4–4.4(–5.4) μm (n = 16), sessile, crowded, mostly subglobose, less commonly lageniform or conical. Conidia (2.8–)3.3–4.6(–6.3) × (1–)1.1–1.3(–1.5) μm, l/w (2.2–)2.7–4(–5.6) μm (n = 70), 1-celled, allantoid, cylindrical to sigmoid, hyaline, containing 0–2 small guttules, smooth.

Habitat: In bark of Acer opalus.

Distribution: Europe, only known from the type location.

Holotype: France, Rougon, Gorges du Verdon, at the tunnels, on a twig of Acer opalus, 29 Jul. 2011, H. Voglmayr (WU 35995; ex-holotype culture CBS 142405 = FV).

Notes: Ascospores of this species resemble those of Parafenestella pseudoplatani but are distinctly smaller. In contrast to the latter, C. opali forms compact pustules on the natural host.

Fenestella Tul. & C. Tul., Selecta Fungorum Carpologia: Xylariaceae Valsei-Spaeriei 2: 207. 1863, emend. Jaklitsch & Voglmayr.

Ascomata immersed in bark, aggregated on a subiculum and sometimes on a crumbly stromatic crust, forming a pustular pseudostroma appearing as bumps, causing irregular ruptures of the host periderm; upper surface filled by a brown to black crumbly disc with inconspicuous sunken or slightly projecting papillate ostioles or lacking a disc and then filled by more or less convergent papillate to cylindrical ostiolar necks; as a final stage of development sometimes entire pseudostroma becoming superficial on inner bark or wood. Ascomata depressed subglobose to pyriform or distorted by mutual pressure, often obliquely oriented toward a common centre. Peridium pseudoparenchymatous. Hamathecium consisting of narrow, branched and anastomosing paraphyses. Asci cylindrical to oblong, bi- and fissitunicate, containing 4–8 mostly uniseriate ascospores. Ascospores fusoid to ellipsoid or oblong, brown with lighter end cells, with or without hyaline appendage cells. Asexual morphs: phoma-like where known; none detected in the generic type.

Type species: Fenestella fenestrata (Berk. & Broome) J. Schröt.

Notes: Huhndorf & Glawe (1990) obtained a pycnidial asexual morph in culture, which was produced from ascospores of a putative Fenestella from an American Acer sp., but did not name it. The phylogenetic relationship of that fungus with Fenestella s. str. is however unclear.

Pending further studies, the genus Fenestella is here only included with its type species in order to fix its name by lecto- and epitypification and to clarify the positions of some other morphologically similar fungi, which phylogenetically do not belong to the genus.

Fenestella bipapillata. a species included by Phookamsak & Hyde (2015), was relegated to Dickeyporthe by Jaklitsch & Barr (1997).

Fenestella fenestrata (Berk. & Broome) J. Schröt., in Cohn, Krypt.-Fl. Schlesien (Breslau) 3.2(4): 435. 1897(1908), Fig. 6. Basionym: Valsa fenestrata Berk. & Broome, Ann. Mag. nat. Hist., Ser. 3: 3: 366. 1859.

Synonym: Fenestella princeps Tul. & C. Tul., Selecta Fung. Carp. 2: 207. 1863.

Pseudostroma (0.9–)1.3–2.2(–2.9) mm (n = 66), up to 0.7, rarely 0.9 mm high, subglobose, flat conical to pulvinate at flat base, immersed beneath the periderm of the host forming up to 0.5 mm high bumps or, after removal of the periderm more or less superficial on the inner bark or wood, with circular to more or less elliptic outline, sometimes confluent with one or two other stromata, containing 2–10(–12) ascomata, depending on development conditions, surrounded by subiculum hyphae and sometimes by a thin dark crumbly stromatic crust; central upper disc flat and sunken in fissures of the periderm, brown to black, sometimes with lighter spots, smooth or tuberculate, usually without clearly discernible ostiolar openings, but the latter sometimes appearing as flat brown or black discs ca. 180–330 μm diam, often becoming exposed by erosion of the uppermost layer, consisting of a narrow brown wall, whitish internal tissue of the ostiolar canal and a dark mass of ascospores or empty canal in their centre. Ascomata (390–)520–830(–)1 140 μm (n = 68) diam, depressed subglobose to pyriform or distorted by mutual pressure, often obliquely oriented toward a common centre, collapsing from the sides. Ostioles eccentric, vertical or oblique and convergent. Peridium ca. 15–50 μm thick, pseudoparenchymatous; cells (5–)6.5(–)11(–)15.5 μm (n = 94) diam in section and in surface view, thick-walled, dark brown outside to nearly hyaline inside, and with subicular hyphae originating on its surface. Subicular hyphae 2–6 μm wide, sometimes inflated to 8 μm, pale brown, darker toward the peridium, thick-walled. Hamathecium consisting of numerous branched and anastomosing, 1–4 μm wide paraphyses with free ends. Asci (256–)290–352(–377) × (22.3–)24–30.8(–36) μm (n = 22), cylindrical to oblong, bitunicate, fissitunicate, with an ocular chamber, a short and narrow stipe and simple base, containing (4–)8 mostly uniseriate ascospores. Ascospores (36.5–)49.3–65(–73) × (14.2–)18–25(–31) μm, l/w (2.1–)2.4(–)2.9(–3.7) μm (n = 116) including the hyaline cellular appendages, first hyaline, with a conspicuously thick wall, a median primary septum and a canal through the wall of each end,
Fig. 6. Fenestella fenestrata. A, B. Pseudostroma pustules in face view (B, vertically cut half pustule). C, Valsoid group of exposed ascomata in remnants of subiculum. D, E. Sectioned pseudostromata in (oblique) side view showing ascomata and eroded ostioles (shiny patches in E are due to a glue previously used for attachment to a sheet). F–H. Ascus apices (F, immature). I–K. Peridium (I, J, in face view; K, in vertical section). L–O. Asci. P–A1. Ascospores (in R, S, V with hamathecial threads in the background and free end in S, in X without appendage cells). K, S, W, Y, Z. in 3 % KOH. A, D, H, J, K, N, O, V–A1, lectotype PC 0084495; B, I, P–S, epitype WU 35996; C, E–G, L, T, U, PC 0084493; M, PC 0084496. Scale bars: A, E = 500 μm; B–D = 300 μm; F–H, K, P–W, Y–A1 = 15 μm; I, J, X = 10 μm; L–O = 30 μm.
developing 2–4 additional main septa, turning pale or yellowish brown, when mature fusoid to ellipsoid or oblong, symmetric or slightly inequilateral, slightly constricted at the primary septum, dark to blackish brown, with 13–20 transverse and 4–6 (–7) longitudinal septa. Terminal cells often paler, with or without a hyaline, ca. 2–7 μm long, rounded or longish, rarely acute appendage cell. Ascospores turning blackish brown to black in 3 % KOH and slightly to strongly swelling with septa often becoming indistinct, appendages remaining hyaline. Asexual morph not observed; none detected in culture.

**Typification and other comments:** Lectotype of *Valsa fenestrata* and *Fenestella princeps* here designated: UK, England, Wiltshire, Spye Park, on twigs of *Alnus glutinosa*, Mar. 1859, C.E. Broome (PC 0084495; MBT378881, MBT378882). Epitype of *Valsa fenestrata* and *Fenestella princeps*, here designated: Austria, Oberösterreich, Raab, Wetzlitzach, on twigs of *Alnus glutinosa*, soc. effete *Diaporthia sp.*, 29 Apr. 2017, H. Voglmayr (WU 35996; MBT378883, MBT378884; ex-epitype culture CBS 143001 = FP9).

**Background:** Tulasne & C. Tulasne (1863) based their new genus *Fenestella* on *Valsa fenestrata* and used the new species epithect *princeps* for it, apparently because they disliked having the same word in both the genus and species name. Berkeley & Broome (1859) had described *Valsa fenestrata* citing two specimens, one from Quercus in Otton Wood, Leicestershire, leg. A. Bloxam, and one from *Alnus glutinosa* in Spye Park, Wiltshire, leg. C. E. Broome, March 1859. The *Quercus* specimen is extant in K as K(M) 233193. Barr (1990b) argued that *Valsa fenestrata* is a *nomen dubium*, because obtuse ascospores illustrated by Berkeley & Broome (1859) for the *Quercus* specimen indicate that two species were present. However, Tulasne & C. Tulasne (1863) explicitly referred to the *Alnus* specimen from Spye Park, which they received from Broome. In conclusion, *F. fenestrata* and *F. princeps* are the same species and its host is *Alnus glutinosa*. Broome’s specimen from Spye Park was obviously separated into the two fragments PC 0084494 and PC 0084495 (both extant in PC). PC 0084494 only contains the sexual morph of a *Cystospora* with ascospores (11.8–12.5–15–16.2) μm (3–4) × (4–4.5) μm, Iw (2.8–3.2–4.1–4.7) μm (n = 30), while PC 0084495 contains *F. fenestrata*. Therefore, we here designate the latter as lectotype of *Valsa fenestrata* and *Fenestella princeps* and epitypify these names with a fresh specimen from *Alnus glutinosa*. Nomenclaturally *F. princeps* is the generic type, but the species epithect *fenestrata* is older and has therefore priority. Tulasne & C. Tulasne (1863) cited also own material, which they collected in April 1860 on *Alnus glutinosa* in France near Paris (PC 0084493); this specimen corroborates the link to Berkeley & Broome’s (1859) protologue plus specimen and thus conspecificity. They also noted that they did not see Berkeley’s *Quercus* specimen and did not find the fungus on *Quercus* in France, but argued that there is apparently no difference to the *Alnus* material. Even if the fungus on *Quercus* was a different species, lectotypification makes this irrelevant. Tulasne & C. Tulasne (1863) also subsumed a specimen from Otth, provisionally named *Valsa macrospora* by him (PC 0084496) from *Alnus glutinosa*, under *F. princeps*. All cited materials were deposited in PC by Tulasne & C. Tulasne in 1873.

Phookamsak & Hyde (2015) examined and presented Otth’s material (PC 0084496) as typical for *F. princeps* and differentiated it from *F. fenestrata*, which they based on two specimens from GZU, by “multiloculate stromata” and smaller ascospores and asci in the former. It is not clear, whether PC 0084496 is conspecific with *F. fenestrata*, because the “multiloculate stroma” seem to be those of an effete *Melanconis* sp. (with white to yellowish ectostromatic discs and ca. 3–10 discrete circinate black ostioles) colonised by a *Fenestella* with narrowly fusoid ascospores, which, on the other hand, considerably overlap in size with those of other material cited above; our measurements of this material correspond well to those of Phookamsak & Hyde (2015). In any case, in none of the other specimens including type material ascomata were found to be immersed in a *Melanconis* pseudostroma. The epitype from Austria corresponds well to the British and French materials, and the culture derived from it is used to infer the phylogenetic position of *F. fenestrata*.

Other specimens examined: France, SW Paris, Chaville, on dead twigs of *Alnus glutinosa*, Apr. 1860, L. R. Tulasne (PC 0084493). Switzerland, on dead branches of *Alnus sp.*, autumn 1861, G. Otth (PC 0084496; as *Valsa macrospora*, cited in Delectum Otitianum Fungorum Thunensium, no. 33 fide Tul. & C. Tul. 1863).

**Notes:** In all available type and authentic materials (PC 008493, PC 008495, WU 35996) asci are only partially developed and the interior of ascomata appears white and few asci with ascospores are present, or they are over-mature and empty. Hymathelial threads with free ends were seen in all specimens, but their development is unknown, therefore it is not clear whether the term paraphyses or pseudoparaphyses applies. Direct association with *Diaporthia* and the thin stromatic encasement of ascomata, which is often present, may suggest that *Fenestella fenestrata* is fungicolous on *Diaporthia*. If the material of PC 0084496 is conspecific, then *Melanconis* is another host genus of the fungus. Study of the materials cited above has made clear that the number of ascoma per pseudostroma but also ascospore size are no good criteria to distinguish among species. We found the following variation in ascospore size (measurements include appendage cells): In the lectotype (PC 0084495) ascospores vary between (36.5–41.5–58.5–63.7) μm (14.8–16.4–21.7–22.8) μm and (45.7–53.5–67–73) μm (15.3–19.2–25.7–30) μm among two ascomata. In Tulasne’s material (PC 0084493) ascospores vary between (37.8–45.7–66.7–69) μm (14.2–16.5–25.9–27.2) μm and (45.5–51.6–66.5) μm (18.3–20.3–28.2–31) μm among two ascomata. Summarized ascospore measurements of the epitype are (47.5–53.2–63–67.5) μm (16–19–23.3–25) μm. Some spores may be slightly compressed in mounts and some may not be entirely mature or aberrant, the typical ascospore size is interpolated as (42–45–65 × 17–25 μm. Appendage cells are sometimes distinctly elongated, suggesting that germination occurs preferentially at these loci. Ascospore colour is strongly dependent on development, age of the specimen, mounting medium and method of microscopy.

**Neocucurbitaria** Wanas et al., Mycosphere 8(4): 408. 2017, emend. Jaklitsch & Voglmayr.

Ascomata immersed in and erumpent from bark or superficial in bark fissures on inner bark or wood, scattered or aggregated in varying groups, sometimes confluent in masses, globose, subglobose, pyriform or collapsing-discoid or turbinate, sometimes deeply cupulate, brown to black, disposed on or surrounded by subiculum; surface verruculose, warted or irregularly or radially cracked. Ascomatal apices variable, even within species, rounded or flat, papillate or non-papillate, sometimes with radial cracks, furrowed, stellate or irregularly tuberculate, brown, black, reddish or yellow, containing a minute central ostiolar opening.
Ostiolar canal periphysate. Subiculum consisting of 2–7 μm wide, thick-walled, hyaline, greyish to dark brown hyphae usually forming loose mats, sometimes forming compact masses agglutinating ascomata. Peridium pseudoparenchymatous, usually less than 100(–120) μm thick, consisting of encrusted thick-walled, pale to blackish brown cells with encrusted pigment at the outer side, often intermingled with subicular hyphae, becoming lighter to hyaline and thinner-walled towards the inner side; sometimes ostiolar region fortified by a hyaline layer. Hamathecium formed by numerous branched, 1–3(–4) μm wide paraphyses with free ends. Asci cylindrical, oblong or subclavate, bitunicate, fissitunicate, with an ocular chamber, short stipe and a simple or knob-like base, containing 4–8 ascospores in uniseriate, sometimes partly biseriate arrangement. Ascospores ellipsoid, fusoid, oblong to subclavate, usually slightly constricted at the simple or knob-like base, containing 4–6(–10)12.5 μm (n = 34) wide cells becoming lighter and thinner-walled towards the inner side; particularly in the ostiolar region often forming a golden yellow outer and a brown inner layer. Hamathecium formed by numerous branched, 1–3 μm wide paraphyses with free ends. Asci (135–)153–183(–191) × (13.2–)13.4–17.3(–19.5) μm (n = 24), cylindrical, bitunicate, fissitunicate, with an ocular chamber, short stipe and a simple or knob-like base, containing 6–8 (obliquely) uniseriately arranged ascospores. Ascospores (21–)22–27(–30.5) × (9.0–)9.8–12.2(–14.4) μm, l/w (1.9–)2.1–2.4(–2.6) (n = 51), ellipsoid, slightly constricted at the median primary septum, upper half not or scarcely enlarged, first pale to yellowish brown, turning dark brown at maturity, darkening in 3 % KOH. Peridium 20–65 μm thick, pseudoparenchymatous, consisting of thick-walled medium brown to olivaceous, (4–)6–10(–12.5) μm (n = 34) wide cells becoming lighter and thinner-walled towards the inner side; particularly in the ostiolar region often forming a golden yellow outer and a brown inner layer. Hamathecium formed by numerous branched, 1–3 μm wide paraphyses with free ends. Asci (98–)104–138(–143) × (18.2–)18.6–23.5(–26.5) μm (n = 32) diam, mostly irregularly disposed, containing 6–8(–10)12 ascospores, ellipsoid, oblong to subclavate, sometimes attenuated toward one end, 1-celled, hyaline, with 1–2 subterminal guttules, smooth.

Asexual morph on natural hosts: Pycnidia superficial on inner bark or wood on variably developed subiculum, often scattered among ascomata or erumpent in dense fascicles through bark, globose, subglobose to collapsing discoid to cupulate, often with a minute apical papilla; peridium pseudoparenchymatous, dark brown. Surface smooth and glabrous or bearing some hyphal outgrowths. Inner side lined with hyaline cells giving rise to densely arranged phialides and simple conidiophores. Co-nidiophores simple, bearing lateral pegs and solitary terminal phialides. Phialides lageniform to cylindrical, straight or curved. Conidia formed on phialides and on lateral pegs of conidiophores, oblong, allantoid or ellipsoid, sometimes attenuated toward one end, 1-celled, hyaline, with 1–2 subterminal guttules, smooth.

Asexual morph in CMD culture: Pycnidia superficial on or immersed in agar, globose, conical to pulvinate or nearly cylindrical, with light rounded papilla, first usually olivaceous, darkening with time, with a light, often eccentric opening. Pycnidial wall formed by a thin t. angularis-globulosa of rather thin- to thick-walled subhyaline, olivaceous to brown cells; surface sometimes with brown hyphal appendages, lacking setae. Inner side of the peridium lined by globose to angular idiogenous cells and sometimes short, simple or basally branched conidiophores. Phialides sessile and crowded on base cells or terminally on conidiophores, subglobose, broadly conical or lageniform to cylindrical, straight, curved or sigmoid. Conidia formed on phialides and pegs on the sides of conidiophores, oblong, allantoid to ellipsoid or drop-like, straight or slightly curved, 1-celled, hyaline, sometimes dilute brownish in age, typically containing 0–2, sometimes more, subterminal guttules, smooth. Probably saprotrophic on wood and bark of trees and shrubs, sometimes parasitic on human skin.

Type species: Neocucurbitaria unguis-hominis (Punith. & M.P. English) Wanas. et al.

Note: The generic description is here enlarged in order to represent the whole morphological variation shown by the members studied here.

Neocucurbitaria acanthocladae Jaklitsch & Voglmayr, sp. nov., MycoBank MB823004. Fig. 7.

Eymology: For its occurrence on Genista acanthoclada.

Ascomata (157–)284–443(–471) μm (n = 26) diam, immersed in and erumpent from bark in variable groups of up to 10 individuals, very variable in size and shape, more or less globose or pyriform, brown, surrounded by cream, pale brown or yellowish to yellow-brown subiculum. Ascomatal apices (88–)115–182(–235) μm (n = 32) diam, highly variable, convex or rounded or more commonly flat and furrowed or stellate, brown, black or yellow. Subiculum sometimes forming compact masses agglutinating ascomata, consisting of ca. 2–7 μm wide, thick-walled, hyaline to pale brown hyphae encrusted by yellow particles dissolving in 3 % KOH. Peridium 20–65 μm thick, pseudoparenchymatous, consisting of thick-walled medium brown to olivaceous, (4–)6–10(–12.5) μm (n = 34) wide cells becoming lighter and thinner-walled towards the inner side; particularly in the ostiolar region often forming a golden yellow outer and a brown inner layer. Hamathecium formed by numerous branched, 1–3 μm wide paraphyses with free ends. Asci (135–)153–183(–191) × (13.2–)13.4–17.3(–19.5) μm (n = 24), cylindrical, bitunicate, with an ocular chamber, short stipe and a simple or knob-like base, containing 6–8 (obliquely) uniseriately arranged ascospores. Ascospores (21–)22–27(–30.5) × (9.0–)9.8–12.2(–14.4) μm, l/w (1.9–)2.1–2.4(–2.6) (n = 51), ellipsoid, slightly constricted at the median primary septum, upper half not or scarcely enlarged, first pale to yellowish brown, turning dark brown, with 7–10(–11) transverse and 2–3 longitudinal septa, smooth.

Cultures and asexual morph: colony radius on CMD at 22 °C ca. 6 mm after 1 wk, 26 mm after 55 d; colony olivaceous, grey-brown to dark grey, with radial rays; odour sour-yeasty. Pycnidia 45–118 μm diam, forming after ca. 1 wk in small numbers, mostly immersed in agar, solitary, irregularly disposed; pycnidial peridium thin, formed by thick-walled, greyish-olivaceous, angular cells (4–)6.5–10.8(–12.8) μm (n = 27) diam; inner side lined by hyaline cells giving rise to conidiogenous cells. Conidiogenous cells (4.5–)5.0–6.5(–7.5) × (2.2–)2.7–4.3(–4.9) μm (n = 25), sessile, crowded, phialidic, lageniform to subglobose, often with elongated neck. Conidia (2.8–)3.3–3.8(–3.9) × (1.2–)1.3–1.5(–1.7) μm, l/w (2–)2.3–2.8(–2.9) (n = 32), 1-celled, oblong to allantoid, hyaline, containing 0–2 subterminal guttules, smooth.

Habitat: on wood and bark of Genista acanthoclada.

Distribution: Southern Europe, only known from the type location in Crete.

Holotype: Greece. Crete, Agios Ioannis, heading to Zonianna, 35° 19' 24.7N 24° 46' 47.2E. elev. 465 m, on branch of Genista acanthoclada, soc. Platystomum sp., 8 Jun. 2015, W. Jaklitsch & H. Voglmayr (WU 35997; ex-holotype culture CBS 142938 = C225).

Notes: Unlike any other species of Neocucurbitaria, N. acanthocladae is characterised by a light coloured peridium and yellow stellate ostioles. The latter are to some extent shared with N. aethensis, which differs by host, black ascomata tending to be more erumpent and ascospores with different septation. The phylogenetically related N. cinereae has also different ascospore characters. These three species occur on Genista spp.
Fig. 7. Neocucurbitaria acanthocladae. A–R. Sexual morph (WU 35997). A–C. Ascomata in face view (C. ostiolar area). D. Ascoma with black furrowed ostiole. E. Obliquely disposed ascoma with yellow-brown subiculum and yellow apex. F. Ostiolar area with yellow-brown surrounding wall. G. Peridium in vertical section. H. Subicular hypha. I. Hamathecium. J–L. Asci. M. Ascus apex. N–R. Ascospores (N, R. young). S–W. Asexual morph in culture (CBS 142398 on CMD after 7–10 d at 22 °C). S. Pycnidial wall. T, U. Conidiogenous cells. V, W. Conidia. F, I–R. in 3 % KOH. Scale bars: A, B = 200 μm; C = 70 μm; D, E = 100 μm; F = 30 μm; G, J–L = 20 μm; H, I, S = 10 μm; M–R = 5 μm; T–W = 3 μm.
Fig. 8. Neocurcubitaria acerina. A–N. Sexual morph. A–C. Ascomata in face view. D. Peridium in vertical section. E. Ascus apex. F–K. Ascospores. L–N. Asci. O–T. Asexual morph in culture (CMD, after 4–7 d at 22 °C). O. Pycnidia. P. Pycnidial wall. Q. Pycnidial appendages. R. Conidiogenous cells. S, T. Conidia. Scale bars: A, B = 150 μm; C = 100 μm; D, L–N = 10 μm; E, G, P, R = 5 μm; F, H–K, Q = 7 μm; O = 100 μm; S, T = 3 μm.
Neocucurbitaria acerina Wanas. et al., Mycosphere 8(4): 410. 2017. Fig. 8.

Ascomata (177–)202–338(–410) μm (n = 20) diam and high, scattered or variably aggregated in small groups, immersed below bark epidermis, becoming free upon shredding of the bark, with bases usually immersed, sitting on an inconspicuous subciculum of ca. 2–5 μm wide, thick-walled brown hyphae also originating at ascomatal sides, becoming seta-like near the ostiolum; black, more or less globose with rounded apical papilla (43–)53–95(–110) μm diam (n = 15), collapsing from the sides or base; surface verruculose to nearly smooth. Peridium ca. 20–60 μm thick, of a thick-walled dark brown t. angularis becoming lighter and thinner-walled towards inner side, formed by (3.5–)5–9.5(–12) μm (n = 47) long cells; pigment in outer cell layers coarsely encrusted; near the ostiolum inside fortified by a hyaline t. angularis. Hathematieum formed by a dense tissue of numerous richly branched, 1–3.5 μm wide paraphyses with free ends. Asci (95–)103–136(–157) × (10.5–)11.7–13.5(–14.7) μm (n = 32), cylindric, bitunicate, fissitunicate, with an ocular chamber, short stipe and a simple or knob-like base, containing 8 uni- partially biseriately arranged ascospores. Ascospores (14–)17.5–22.4(–25.8) × (5.6–)6.5–7.7(–8.7) μm, l/w (2.2–)2.6–3.1–3.6 (n = 64), fusoid-oblong-subclavate, slightly constricted at the median primary septum, upper half slightly enlarged, first yellow-brown, soon turning dark brown, with (5–)6–8(–9) transverse and 1–2 longitudinal septa, smooth. Pycnidia scattered on bark, 60–150 μm diam, subglobose to collapsing discoid.

Cultures and asexual morph in culture (on CMD after 4–7 d at 22 °C): colony radius 8–11 mm after 1 wk, 14–18 mm after 2 wk; mycelium dense, flat hyphal, turning greyish-olivaceous or dark grey-brown, finally black; long aerial hyphae forming strands; odour indistinct to slightly unpleasant. Pycnidia (43–)153–115(–141) μm (n = 20) diam, formed after a few days, numerous, scattered to aggregated, globose to pulvinate, olivaceous, turning black within a week. Pycnidial wall formed by a coarse t. angularis of rather thinly-walled, (3.5–)5–10(–12.5) μm (n = 30) long cells; surface with brown hyphal appendages; inner side lined by hyaline cells giving rise to conidigenous cells. Conidigenous cells sessile, crowded, phalidic, very variable,lageniform to subglobose, (4.0–)5.0–7.5(–9.5) × (1.6–)1.7–3.9(–5.3) μm. Conidia (2.2–)2.5–3.3(–4.0) × (0.9–)1.1–1.5(–1.9) μm, l/w (1.6–)2.6–3.6 (n = 84), 1-celled, oblong to narrowly ellipsoid, straight or slightly curved, hyaline, typically containing 2 subterminal guttules, smooth.

Habitat: on wood and bark of Acer spp., known from A. campestris and A. pseudoplatanus.

Distribution: Europe (Austria, Italy).

Material examined: Austria. Niederösterreich, Lunz-Mittersee, on dead bark of a standing trunk of Acer pseudoplatanus, 10 May 2016, H. Voglmayr (WU 35998); culture CBS 142403 = C255, Vienna, Strebersdorf, Krottenhofgasse, on a dead, partly detecated branch of Acer pseudoplatanus, asexual morph, soc. Paraf estella pseudoplatani, 17 Nov. 2013, W. Jaklitsch (WU 35999; culture C26ia from conidia).

Notes: Apparently there is no old name of Cucurbitaria or Fen estella described from Acer that may be used for this species. The following types were examined or information was gathered:

In the holotype of Cucurbitaria acerina Fuckel (G 00266375(I)), from Herbier Fuckel 1894 in Herbier Barby Boissier), collected by Fuckel on Acer campestris in Germany, ascomata are immersed in rows and erumpent from bark, they are depressed subglobose, non-papillate, contain hamathecium of branched 1–3 μm wide threads, 6–8-spored, cylindrical, fissitunicate asci ca. 154 × 15 μm, with spores uniseriate, partly biseriate in the middle. Ascospor es are (18–)21–25(–26.5) × (8–)8.7–10.2(–11.3) μm, l/w (1.9–)2.2–2.7(–3.2) (n = 41), narrowly ellipsoid to fusoid, pale to medium brown, with 3–7 transverse and 1(–2) longitudinal septa; in 3 % KOH slightly darker, greyish brown, with more distinct, dark, conspicuous septa, distinctly constricted at the median septum, upper part slightly wider. Ascospore characteristics like shape, light colour, usually less and more distantly set septa, which are darker and thicker than the wall, suggest that C. acerina is different from N. acerina.

Cucurbitaria protracta Fuckel (holotype G 00266414), from Herbier Fuckel 1894, collected from thin twigs of Acer campestre, is very similar to C. acerina and possibly only a form of that species. It differs from the latter by slightly smaller ascospores, (15.4–)19–23(–24) × (6.5–)7.7–9.7(–10.6) μm, l/w (2–)2.2–2.7(–3.1) (n = 47), which have usually only 3 transverse septa and one longitudinal septum, but may have up to 6 transverse septa; they are light to medium brown, with thick and dark septa.

Cucurbitaria homalaes (Fr.) Sacc., basionym Melogramma homalaum Fr., described from Acer pseudoplatanus, is a thyridaria-like fungus, according to our examination of type material in UPS.

No type material of Cucurbitaria negundinis G. Winter, described from Acer negundo, has been located. According to Mirza (1968) the species is characterised by larger, esp. broader ascospores (26.7–37 × 9–12 μm or 20–26 × 9–11 μm, from 2 specimens) having 3–7 (mostly 3, 4 or 5) transverse and 1 longitudinal septa, and camarosporium-like conidia associated on the natural host may point to a position outside the Cucurbitariaceae.

Fenestella frir (Fr.) Sacc., based on Sphaeria frir Fr., also described from Acer negundo: Fries (1823) cited the specimen Scleromyces. Sacc. Exs. 227 labelled Sphaeria coronata in UPS in the protologue of Sphaeria frir. It contains empty perithecia with cylindrical necks. It is thus no cucurbitariaceous fungus, and no further interpretation is possible. Another collection labelled as Sphaeria frir by Fries is extant in UPS, but it is likely a later collection, i.e., no type material. According to the description by Currey (1859), Fenestella mougetti (Pers. ex Curr.) Sacc., described from Acer pseudoplatanus in France, may match N. acerina, but the name is illegitimate due to the sanctioned name Sphaeria mougetti Fr., the basionym of Sphaeriaeformella mougetti (Fr.) Sacc.

Neocucurbitaria aetnensis Jaklitsch & Voglmayr, sp. nov., MycoBank MB823005. Fig. 9.

Etymology: As it has been only found on the volcano Etna, on Genista aetnensis.

Ascomata (195–)240–393(–513) μm (n = 41) diam, immersed in bark, erumpent through fissures, crowded in small or large groups, or solitary, globose, subglobose or pyriform, sometimes collapsing at the sides, with rounded, furrowed or tuberculate, yellow, reddish or black apical papilla (92–)198–212 μm (n = 30) diam, or with radial cracks or non-papillate; ostiole periphysate. Surface grey to black, verruculose. Peridium 22–120 μm thick, pseudoparenchymatous, composed of 2–3 layers of (4–)6–10.5(–15.3) μm (n = 41) long cells, hyaline and thin-walled in a narrow inner layer, pale (greyish-) brown to subhyaline and thin- to moderately thick-walled in a broad middle layer and dark brown to opaque, thick-walled and intermingled.
Fig. 9. Neocucurbitaria aesthensis. A–Q. Sexual morph. A–C. Ascomata in face view. D. Ascomata in vertical section. E. Peridium and a subicular hypha in vertical section. F. Part of ostiole with periphyses. G. Ascus apex. H–J. Asci. K–Q. Ascospores (K. young). R–W. Asexual morph in culture (CMD, after 6–7 d at 22 °C). R. Pycnidia. S. Pycnidal wall. T, U. Conidiogenous cells. V, W. Conidia. H–Q. in 3 % KOH. A, B, D–P, T, WU 36929/CBS 142404; C, Q–S, U–W. WU 36930/C270. Scale bars: A–D = 200 μm; E, H–J = 15 μm; F = 10 μm; G, Q, S, T = 5 μm; K–P = 7 μm; R = 70 μm; U–W = 3 μm.
with subicular hyphae in narrow outer layer partly tending to be thicker toward the base; pigment encrusted. Basal and lateral subiculum consisting of 2–7 μm wide, thick-walled dark brown hyphae. Hamathecium consisting of numerous branched, 1–3 μm wide paraphyses with free ends. Ascii (114–)125–154(–182) × (13.7–)14.5–17.5(–19) μm (n = 19), cylindrical, bitunicate, fissitunicate, with an ocular chamber, short stipe and a simple or knob-like base, containing 6–8 ascospores in (obliquely) uniseriate arrangement. Ascospores (19.3–)20.3–23.6(–26.3) × (8.3–)9.9–10.5(–11) μm, l/w (1.9–)2.1–2.4(–2.5) (n = 50), ellipsoid with broadly rounded ends, slightly constricted at the median primary septum, upper half sometimes slightly enlarged, medium to dark brown, with 5–7 transverse and 1–2 longitudinal septa, smooth.

Pycnidia co-occurring with ascomata, 45–105 μm diam, globose to collapsing-cupulate, shiny black, finely papillate.

Cultures and asexual morph: Colony radius 20 mm on CMD after 1 mo at 22 °C; colony dense, first greyish, turning dark olivaceous brown to nearly black with hyaline or olivaceous margin, more or less zonate, with radial grooves, sometimes nearly stellate, white crystals forming on the surface; odour indistinct. Pycnidia (after 6 d) 38–147 μm diam, forming within a few days in the centre and/or at the colony margin, immersed to nearly superficial, densely but singly disposed or in small groups, more or less globose, olivaceous to nearly black, usually surrounded by brown, often submoniliform hyphae; conidia emerging in whitish drops. Peridium consisting of a thin t. angularis-globulosa of moderately thick-walled, olivaceous to subhyaline, guttulate cells (4–)5–9(–11) μm (n = 50) diam; inner side lined by hyaline cells giving rise to phialides. Phialides (3.2–)3.8–6.2(–7.7) × (1.9–)2.1–4(–5.7) μm (n = 36), sessile, crowded, lageniform to globose. Conidia (2.8–)3–3.7(–4.2) × (1.1–)1.3–1.7(–2.1) μm, l/w (1.7–)1.9–2.6(–3) (n = 58), 1-celled, oblong, straight or slightly curved, hyaline, partly becoming dillute brownish when aged, containing 0–2, sometimes more, subterminal guttules, smooth.

Habitat: On wood and bark of Genista aetnensis.

Distribution: Europe, Italy, only known from the type location.

Holotype: Italy. Sicily, Etna, east side, near Zafferana Etnea, on corticated, 1.5–2.5 cm thick branches of Genista aetnensis, 17 Jun. 2016, W. Jaklitsch & H. Voglmayr (WU 36929; ex-holotype culture CBS 142404 = C261).

Other material examined: Italy, Sicily, Etna, north side, on branch of Genista aetnensis, 18 Jun. 2016, W. Jaklitsch & H. Voglmayr (WU 36930; culture C270).

Notes: For distinction from N. acanthocladae see notes under this species. Neocucurbitaria cinerea differs, e.g., by smaller ascospores from N. aetnensis.

Neocucurbitaria cinerea Jaklitsch & Voglmayr, sp. nov., MycoBank MB823006. Fig. 10.

Etymology: For its occurrence on Genista cinerea.

Ascomata (162–)195–305(–338) μm (n = 23) diam, 160–440 μm high, erumpent from bark, aggregated in large groups on a subiculum or scattered, globose, subglobose or pyriform, sometimes vertically elongated, black, with verruculose surface; apex usually distinctly papillate, (58–)68–106(–132) μm (n = 20) diam, rounded to irregularly warted, reddish, dark brown or black, sometimes finely cracked; ostiole periphysate. Peridium 25–85 μm thick, consisting of a thick-walled, dark brown and heavily encrusted outer t. angularis and a rather thin-walled hyaline inner layer, formed by cells (2.8–)4.8–9.3(–12.5) μm (n = 75) diam. Basal subiculum consisting of 3–6 μm wide, thick-walled, light to dark brown hyphae. Hamathecium consisting of numerous, branched and anasto- mosing, 1–3(–4) μm wide paraphyses. Ascii (115–)118–130(–135) × (11.5–)12–14(–14.8) μm (n = 16), cylindrical, bitunicate, fissitunicate, with an ocular chamber, short stipe and a simple or knob-like base, containing 8, rarely 4 ascospores in (obliquely) uniseriate arrangement. Ascospores (17–)17.8–19.3(–20.5) × (7.2–)8–9(–10) μm, l/w (2.1–)2.3–2.6 (n = 47), ellipsoid, slightly constricted at the median primary septum, upper half slightly enlarged, first hyaline with 1–3 septa, turning pale brown and eventually dark brown, with (3–)5–7 transverse and 1–2 longitudinal septa, smooth.

Pycnidia co-occurring with ascomata, 44–103 μm diam, globose, shiny black, finely papillate.

Cultures and asexual morph: Colony radius 9 mm after 5 d on CMD at 22 °C; mycelium first colourless, turning olivaceous to greyish brown, later dark olivaceous to nearly black with radially disposed spots; odour slightly unpleasant. Pycnidia 54–141 μm diam, formed within a few days, aggregated in groups of up to 10, turning dark olivaceous to nearly black, more or less globose, fusing laterally, papillate. Pycnidial wall thin, formed by thick-walled, olivaceous brown, angular to globose or ellipsoid cells (3.7–)5.5–9.5(–11.5) μm (n = 37) diam; inner side lined by hyaline cells giving rise to conidigenous cells. Conidiogenous cells (3–)4–6.6(–7.8) × (2–)2.3–4.6(–4.6) μm (n = 21), sessile, crowded, phialidic, broadly conical, subglobose or lageniform. Conidia (3–)3.5–4.3(–4.8) × (1.4–)1.6–2(–2.5) μm, l/w (1.6–)1.9–2.5(–2.8) (n = 48), 1-celled, oblong, sometimes attenuated toward one end, hyaline, containing 2 or more minute guttules, smooth.

Habitat: In bark of Genista cinerea.

Distribution: Europe, Spain, only known from the type locality.

Holotype: Spain, Andalusia, Granada, near Montefrío, 37°20′54″N, 4°18′4″W, elev. 785 m, on partly decorticated twig of Genista cinerea, soc. Diaporthes, sp. 11 May 2014, W. Jaklitsch (WU 36931; ex-holotype culture CBS 142406 = KU9).

Notes: Neocucurbitaria cinerea, the third species of this genus described from a Genista, differs from N. acanthocladae and N. aetnensis by smaller ascospores and from the former also by ascospore septation. Ascomata of N. cinerea tend to occur in large groups and they only rarely have yellowish apices, which are not stellate.

Neocucurbitaria cistica Jaklitsch & Voglmayr, sp. nov., MycoBank MB823047. Fig. 11.

Etymology: For its occurrence on Cistus.

Ascomata (147–)195–310(–372) μm (n = 24) diam, scattered or aggregated in small groups below the host epidermis on a subiculum, erumpent through bark fissures, subglobose to pyriform, collapsing at the sides, with a distinct shiny apical papilla (44–)52–83(–88) μm (n = 16) diam, the latter flattened at the top and circular or angular in outline. Surface grey to black, verrucos. Subiculum consisting of 2–7 μm wide, thick-walled, dark brown hyphae. Peridium 15–55 μm thick, consisting of a thick-walled t. angularis, dark brown to opaque outside, becoming lighter (to subhyaline in the ostiolar region) and thinner-walled
Fig. 10. Neocucurbitaria cinereae. A–P. Sexual morph (WU 36931). A, B. Ascomata in face view. C. Ascoma in vertical section. D. Peridium in vertical section. E. Apex of immature ascus. F. Hamathecium. G–J. Asci (G. immature). K–P. Ascospores (K, L. young). Q–U. Asexual morph in culture (CBS 142406 on CMD after 5–6 d at 22 °C). Q. Pycnidia. R. Pycnidial wall. S. Conidiogenous cells. T, U. Conidia. E–J, L–P. in 3 % KOH. Scale bars: A, B, Q = 250 μm; C = 100 μm; D, F, R–T = 7 μm; E, U = 3 μm; G–J = 15 μm; K–P = 5 μm.
towards inner side, formed by cells (4–)5–9(–12) μm (n = 30) diam. *Hamathecium* formed by numerous, richly branched, 1–2.5(–3) μm wide paraphyses with numerous free ends. *Asci* (115–)130–159(–166) × (14.3–)15–17(–18) μm (n = 18), cylindrical, bitunicate, fissitunicate, with an ocular chamber, short stipe and a simple or knob-like base, containing 8 obliquely uniseriately arranged ascospores. *Ascospores* (20–)21.3–24.3(–26.3) × (9–)9.7–10.8(–11.4) μm, l/w (2–)2.1–2.4(–2.7) (n = 45), fusoid to ellipsoid, often with pointed and paler ends, constricted at the median primary septum, upper half slightly enlarged, pale brown when young, turning dark (reddish) brown, with 6–7 transverse and 1–3 longitudinal septa, smooth. *Cultures and asexual morph*: Colony radius on CMD 26 mm after 24 d at 22 °C; colony circular, first hyaline, turning pale dull brown with greyish dots in the centre, odour indistinct. *Pycnidia* (after 5 d) 58–147 μm diam, forming within a few days,
scattered, more or less globose, first hyaline, turning olivaceous; conidia emerging in whitish drops. Peridium consisting of a thin t. globulos-angulosa of moderately thick-walled, (4.3–) 6.8–11.8(–14.2) μm (n = 34) wide cells; inner side lined by hyaline cells giving rise to phialides and short 1–2-celled conidiophores. Phialides (4.5–)5.2–8.2(–10) × (1.8–)2.1–4.3(–6.3) μm (n = 30), sessile, crowded, or solitary terminally on conidiophores, lageniform to subglobose. Conidia formed on phialides and lateral pegs on conidiophores, (2.9–)3.2–4(–4.5) × (1.1–)1.3–1.6(–1.9) μm, l/w (2–)2.3–2.9(–3.6) (n = 40), 1-celled, oblong, straight or slightly curved, hyaline, containing 0–2 subterminal guttules, smooth.

Habitat: On wood and bark of Cistus monspelienis.

Distribution: Spain, La Gomera, only known from the type location.

Holotype: Spain, La Gomera, SE Vallehermoso, at the Mirador de Alojera, on a twig of Cistus monspelienis, 23 Mar. 2016, H. Voglmayr (WU 63932; ex-holotype culture CBS 142402 = C244).

Notes: No Cucurbitaria or Pyrenochaeta has been described from Cistus. Phoma cisti Brunaud, described from Cistus salvi- folius in France, has minute subovoid conidia to 3 μm long (Saccardo 1892, p. 153), while conidia of P. cistina, described from Cistus laurifolius in Kew, England, are larger, 6–7 × 2.5 μm. The latter was combined in Phomopsis by Grove (1917).

Neocucurbitaria juglandicola Jaklitsch & Voglmayr, sp. nov., MycoBank MB823007. Fig. 12.

Eymology: For its occurrence on Juglans.

Ascomata (177–)180–260(–320) μm diam (n = 12), (177–)192–246(–265) μm high (n = 8), immersed in bark, becoming visible in bark fissures, scattered or aggregated in small groups, depressed globose to pyriform, with or without a rounded apical papilla, black, with verruculose to nearly smooth surface, basally and laterally surrounded by subiculum of ca. 2–5 μm wide, thick-walled brown hyphae. Peridium to ca. 60 μm thick, consisting of a thick-walled dark brown t. angularis becoming lighter and thinner-walled towards inner side, formed by (4–)5–9.5–(11) μm (n = 20) long cells. Hamathecium formed by branched, 1–3 μm wide paraphyses. Ascii (79–)95–124(–133) × (11.7–)13–16.7(–17) μm (n = 14), oblong, bitunicate, fissitunicate, with a distinct ocular chamber, short stipe and a simple or knob-like base, containing 8 uni- or biseriately arranged ascospores. Ascospores (15.5–)17.7–21.5(–26.8) × (8.0–)8.8–10.2(–11.0) μm, l/w (1.8–)1.9–2.2–2.6 (n = 61), ellipsoid, straight or slightly curved, slightly constricted at the median primary septum, upper half often slightly enlarged, first pale brown, turning medium to dark brown, with 5–7(–8) transverse and 1–2 longitudinal septa, smooth.

Asexual morph on the natural host: Pycnidia (53–)64–115(–142) μm diam (n = 16), scattered below the host epidermis on subicular hyphae, also in association with ascocarps, globose, sometimes with a blunt rounded apical papilla. Peridium consisting of a thin brown t. angularis of (3–)4.5–7(–7.5) μm (n = 30) long cells, lined at the interior with a layer of hyaline angular to rounded cells. Phialides lageniform to cylindrical, (4.3–)5.0–6.8–7.2) × (1.4–)1.7–2.3(–2.5) μm (n = 14), formed on the hyaline inner cells or singly terminally on short, to ca. 30 μm long, simple, 1–3 celled conidiophores. Conidia formed on phialides and on lateral pegs on conidiophores, (2.2–)2.5–3.0(–3.2) × (1.3–)1.4–1.5(–1.6) μm, l/w (1.5–)1.6–2(–2.2) (n = 12), 1-celled, oblong to ellipsoid, hyaline, smooth.

Cultures and asexual morph in culture: Colony radius on CMD at 22 °C 27 mm after 1 mo, mycelium zonate, dense, first pale greyish, dull dark brown with numerous pycnidial dots in the centre, margin uneven; odour indistinct. Pycnidia 40–90 μm diam, forming in the centre within a few days, immersed in agar or superficial, scattered or aggregated in small numbers, green-olivaceous to black, globose to nearly cylindrical, with a light, often eccentric opening; conidial drops white to greyish-brown. Pycnidial peridium thin, consisting of a thick-walled t. angularis-prismatic of (4–)6–11(–14.5) μm (n = 32) long cells with encrusted olivaceous pigment; inner side lined by hyaline cells giving rise to conidiogenous cells. Conidiogenous cells sessile, crowded, mostly subglobose, also lageniform, (3.5–)4.5–6.2(–6.5) × (1.8–)2–4(–6) μm (n = 18). Conidia (2.5–)3.3–4.1–4.5(–) × (1.1–)1.2–1.5–(2.2) μm, l/w (1.9–)2.4–3.1(–3.6) (n = 45), 1-celled, oblong to allantoid or sigmoid, hyaline, containing 0–2 subterminal guttules, smooth.

Habitat: On bark of Juglans regia.

Distribution: Only known from the type location in Vienna, Austria.

Holotype: Austria, Vienna 22nd district, at AGES, Spargelfeldstraße 191, on twig of Juglans regia, sec. Cytospora sp., 13 Feb. 2015, R. Moosbeckhofer, comm. B. Wegen (WU 36933; ex-holotype culture CBS 142390 = BW6).

Notes: This species is apparently different from Cucurbitaria juglandis. Type material in G (G00266380) does not contain any sexual morph but plenty of two fungi, which Fuckel regarded as asexual states of C. juglandis: many conspicuous pustules of a Diplodia, and a fungus with 1-celled hyaline conidia. The latter are produced on phialides (5.7–)6.5–10(–10.8) × 2–3.5 μm (n = 6) and measure (3.5–)4–5(–6) × (2–)2.2–2.7(–3.1) μm, l/w (1.5–)1.7–2(–2.5) (n = 24) and thus differ from conidia of N. juglandicola by shape and size. On the herbarium label a nearly ellipsoid ascus with biseriate spore arrangement and ascospores are described, which are pale yellow, measure 26–28 × 8–10 μm, have 6–8 transverse septa and are muriform, which agrees with Fuckel (1871) but does not suggest that the fungus belongs to Cucurbitaria. Phylogenetically N. juglandicola is close to but distinct from N. cava (cf. Figs 1, 2 and Valenzuela-Lopez et al. 2018). It should be noted that branch length of N. cava in Fig. 2 is certainly significantly underestimated due to the lack of the phylogenetically highly informative tef1 and much shorter tub2 sequences (333 vs. 673 bp) available for N. cava.

Neocucurbitaria populii Jaklitsch & Voglmayr, sp. nov., MycoBank MB823008. Fig. 13.

Eymology: For its occurrence on Populus.

Ascomata (200–)215–347(–405) μm (n = 20) diam, ca. 250–350 μm high, densely aggregated in numbers of up to ca. 30 in pustules erumpent from bark, on ample subiculum between and below them, more or less globose to pyriform, sometimes laterally collapsing, later with a distinct apical papilla (44–)58–101(–133) μm (n = 24) diam. Surface black, verruculose to nearly smooth. Subiculum consisting of ca. 2–6 μm wide, thick-walled olive-brown hyphae penetrating bark and wood. Peridium 20–60 μm thick, consisting of a thick-walled dark brown t.
Fig. 12. Neocucurbitaria juglandicola (A–S) and Cucurbitaria juglandis (T). A–J. Sexual morph (WU 36933). A. Ascomata in face and lateral view. B–G. Ascospores. H–J. Asci. K–N. Asexual morph on natural substrate (WU 36933). K. Pycnidia in face view. L. Peridium. M. Conidiophores and phialides. N. Conidia. O–S. Asexual morph in culture (CBS 142390 on CMD after 4–6 d at 22 °C). O. Pycnidia. P. Peridium. Q. Conidiogenous cells. R, S. Conidia. T. Conidia from Cucurbitaria juglandis type material G00266380 (in 3 % KOH). Scale bars: A, K, O = 100 µm; B–G, L, P = 7 µm; H–J, M = 10 µm; N, R–T = 3 µm; Q = 5 µm.
Fig. 13. *Neocucurbitaria populi*. A–Q. Sexual morph (WU 36934). A, B. Ascomata in face view. C. Ascoma in vertical section. D. Peridium in vertical section. E. Ascus apex. F. Subiculum. G, L–Q. Ascospores. H–K. Asci. R–Z. Asexual morph in culture (CBS 142393 on CMD after 5 d at 22 °C). R. Pycnidia (left with conidial drop). S, W–Z. Conidia. T. Pycnidial wall and conidiogenous cells. U, V. Conidiogenous cells. F–Q. in 3 % KOH. Scale bars: A, B = 500 μm; C, R = 100 μm; D, F = 10 μm; E, G, U, Y, Z = 5 μm; H–K = 15 μm; L–Q, S, T, V–X = 7 μm.
angularis becoming lighter and thinner-walled towards inner side, formed by cells (3.5–)5–9.5(–12) μm (n = 35) diam. Hamathecium formed by numerous branched, 1–3 μm wide paraphyses. Asci (120–)130–159(–178) × (15.8–)16.5–19.5(–22.2) μm (n = 24), cylindrical, bitunicate, fissitunicate, with an ocular chamber, short stipe and a simple or knob-like base, containing 8 (obliquely) uniseriately arranged ascospores. Ascospores (21.4–)22.3–28.5(–32.2) × (9.5–)10.5–13.5(–15.5) μm, l/w (1.8–)1.9–2.3(–2.6) (n = 37), ellipsoid, slightly constricted at the median primary septum, upper half often slightly enlarged, first yellow- to golden brown, soon turning dark to blackish brown, with (5–)7–8(–10) transverse and 2–4 longitudinal septa, smooth.

Cultures and asexual morph: Colonies on CMD at 22 °C reaching a growth radius of ca. 15 mm after 1 mo, mycelium soon turning brown, finally black, odour indistinct. Pycnidia ca. 73–205 μm diam, forming after a few days, partly immersed in agar, dark olivaceous to black, globose, longish, sometimes fusing, with central ostiole, papillate or not, often covered by aerial hyphae. Pycnidial peridium 10.5(–)11.5 μm, l/w (1.8–)1.9–2.3(–2.6) (n = 37), ellipsoid, with central ostiole, papillate or not, covering the central hyphae.

Distribution: Northern Europe, only the type location in Sweden.

Holotype: Sweden, Skåne, Åhus, Ripa, Motocrossbanan, on branch of cultivated Populus sp., 3 Mar. 2013. S.-Å. Hanson Herb SÅH 2013-020 (WU 36934, ex-holotype culture CBS 142383 = C28).

Notes: Yellow particles on ascomata in Fig. 13A, B are parts of a undetermined fungus. Two species of Cucurbitaria have been described from Populus. One is C. populina (Bacc. & P. Avetta) Rehm from Populus nigra in Italy (Rehm in Schnabl 1892). This is a younger homonym of C. populina (Pers.) Quél. (Quellet 1863) and therefore illegitimate and unavailable. The latter belongs to Cytospora according to Species Fungorum. The second one is C. staphula Dearn. ex R.H. Arnold & R.C. Russell from Populus balsamifera, P. trichocarpa and P. tremuloides in North America. Cucurbitaria staphula differs from Neocucurbitaria populi by larger, biseriately arranged ascospores of different shape (lower end extended and often pointed), a different asexual morph (Macrophoma tunefaciens) and by occurrence on galls (Arnold & Russell 1960, Arnold 1974). The phylogenetically closely related N. juglandicola differs from N. populi in smaller ascospores having only 1–2 longitudinal septa, and by 33 and 31 nucleotide substitutions in tef1 and rp2, respectively.

Neocucurbitaria rhamni (Nees : Fr.) Jaklitsch & Voglmayr, comb. nov., MycoBank MB823009. Fig. 14.

Basionym: Sphaeria rhamni Nees, Syst. Pilze (Würzburg): 299. 1817.

Synonyms: Sphaeria rhamni Nees, Syst. Myc. 2: 417. 1823. Cucurbitaria rhamni (Nees : Fr.) Fuckel, Jb. nassau. Ver. Naturk. 23–24: 174. 1870(1869–1870).

Ascomata (200–)268–432(–676) μm diam (n = 149), (90–)120–222(–333) μm high (n = 82), aggregated in highly variable groups of 2 to more than 200, or scattered singly on an inconspicuous to well-developed subiculum of olivaceous- to dark-brown, thick-walled, 2–6.5 μm wide hyphae, erumpent to superficial on wood and bark, mostly becoming visible in bark fissures, first subglobose, soon collapsing at the top to become turbinate or discoid, shiny black, carbonaceous, surface irregularly and coarsely tubercular. Ostiolar openings usually inconspicuous, whitish, or surrounded by a reddish to blackish papilla (39–)57–83(–92) μm diam (n = 18) delimited by a narrow concentric depression. Peridium (38–)49–83(–110) μm thick (n = 46), pseudoparenchymatous, consisting of a brown to nearly black outer layer of thick-walled incrusted cells and a broad pale brown to hyaline thinned-inner layer; peridial cells (3–)5–9.5(–15.5) μm diam (n = 106). Hamathecium of 1.5–4.5 wide, branched paraphyses. Asci (115–)118–152(–162) × (12.0–)12.3–14.8(–16.1) μm (n = 15), cylindrical to subclavate, bitunicate, fissitunicate, with a short stipe and a knob-like base, narrow walls with endotunica thickened at the apex and a distinct ocular chamber, containing 4–8 uni- or biseriately arranged ascospores. Ascospores (15.7–)18–22(–24.8) × (7.2–)8.2–9.8(–12) μm, l/w (1.7–)2.0–2.5(–2.8) (n = 87), fusoid to ellipsoid, straight, with obsolete to subulate ends, upper part often broader, with 3–7 transverse and 1–2 longitudinal septa, golden to dark brown, end cells often paler, constricted at the primary septum, less at other septa, smooth.

Asexual morph on the natural host: Pycnidia (50–)69–122(–157) μm diam (n = 56), scattered on variably developed subiculum of 2.5–6 μm wide brown hyphae on wood, accompanying ascomata, or erumpent in dense fascicles through bark, globose, sometimes collapsing becoming cupulate, with a small papilla, black, smooth and glabrous. Peridium dark brown, consisting of cells (4–)6–10(–12) μm diam (n = 30) forming L. angularis to L. prismaticalis, lined inside by a layer of hyaline cells giving rise to densely arranged conidiophores or phialides. Conidiophores simple, up to ca. 35 μm long, 1–3–celled, with solitary terminal phialides and lateral pegs typically present at the upper end of conidiophore cells. Phialides (4.8–)5.5–8.0(–9.5) × (2.0–)2.1–2.7(–3.2) μm, l/w (2.2–)2.3–3.5(–4.5) (n = 28), lageniform to subcylindrical. Conidia (2.5–)2.8–3.3(–3.5) × (1.3–)1.4–1.7(–2.1) μm, l/w (1.4–)1.7–2.2(–2.5) (n = 65), ellipsoid, oblong, often attenuated toward one end (drop-like), 1-celled, hyaline, containing 1–2 guttules, smooth.

Cultures and asexual morph in culture: Colony radius 33–45 mm after 1 mo on CMD at 22 °C; colony dense, dark olivaceous brown, usually with conspicuous radial texture, aerial hyphae and odour indistinct. Pycnidia mainly formed in the centre. On SNA after 1 wk at 22 °C pycnidia ca. 100–160 μm diam, more or less superficial on agar concentrated in the colony centre; peridium thin, forming a L. angularis of (olivaceous) brown cells; setae lacking. Phialides formed on conidiophores arising from an inner layer of hyaline pseudoparenchymatous cells or formed directly on the latter. Conidiophores simple or with sparse single branches. Phialides (4.5–)5.8–8.4(–8.7) × (1.7–)2.0–2.6(–2.8) μm, l/w (2.0–)2.3–4.2(–5.1) (n = 12), lageniform to cylindrical. Conidia (2.5–)2.7–3.3(–3.7) × 1.5–1.7(–1.9) μm, l/w (1.5–)1.7–2.1(–2.3) (n = 31), oblong or ellipsoid, often attenuated toward one end. On MEA after 3 wk conidia becoming slightly larger and inflated.
Fig. 14. Neocucurbitaria rhamni. A–D. Ascomata in face view. E, F. Peridium in vertical section (inner layer in F). G, H. Asci. I. Ascus apex showing ocular chamber. J–P. Ascospores. Q–Z. Asexual morph on the natural hosts. Q, R. Pycnidia. S. Pycnidial wall in section. T–X. Conidiophores and phialides. Y, Z. Conidia. A1–F1. Asexual morph in culture at 22 °C (A1–E1, after 7 d on SNA; F1, after 20 d on MEA). A1–C1. Conidiophores and phialides. D1–F1. Conidia. M–P, S–F1. in 3 % KOH. A, D, A1–F1, WU 36936/C112; B, Q, Y, Z, WU 36944; C, M. Lectotype B 700016439; E–L, WU 36941; N–P, R–V, WU 36935; W, X, WU 36939. Scale bars: A, B = 300 μm; C, D, Q, R = 150 μm; E–H, S = 15 μm; I, U, V, X, Y, A1–C1 = 5 μm; J–P, T = 7 μm; W, Z, D1–F1 = 3 μm.
(2.5–)3.0–4.0(–5.0) × (1.7–)2.0–2.5(–2.9) μm, l/w (1.2–)1.3–1.9(–2.4) (n = 21); also phialides becoming inflated to subglobose.

Hosts: On dead attached or broken branches of *Rhamnus frangula*, *R. cathartica*, and *R. saxatilis*, typically near attachment areas of thin dead branches or twigs; also reported from *R. alpina* (Mirza 1968).

Distribution: Europe, also reported from Canada on *Rhamnus cathartica* (Barr 1990a).

Typification: In the herbarium B two specimens are extant, which have the same handwriting of T.F.L. Nees von Esenbeck, but do not bear any collection data. *Sphaeria rhamni* B 70 0016438! contains a few stromata of *Diaporthe fibrosa* on *Rhamnus cathartica*. However, B 70 0016439! bears the script “Sphaeria rhamni mihi” as given in the protologue, indicating that this is the material, which Nees von Esenbeck himself identified as his taxon *Sphaeria rhamni* and likely used as the original material for the description of his taxon. We therefore here designate B 70 0016439 as lectotype of *Sphaeria rhamni* (MBT378885). The material agrees with the current concept of *Cucurbitaria rhamni*, as it contains coarsely tuberculate, more or less discoid ascomata both scattered and in loose or dense groups; more than 100 ascomata are present on the smaller piece of the specimen. As asci are only present in fragments and there are more than one species on *Rhamnus* spp., we here epitypify *Sphaeria rhamni*. *Epitype*, here designated: *Austria*, Kärnten, St. Margareten im Rosental, Dullach, Drau-Auen, grid square 9452/1, on twigs of *Rhamnus frangula*, 11 Jul. 2013, W. Jaklitsch (WU 36935; MBT378886; ex-epitype culture CBS 142393 = C1).

Other material examined: *Austria*, Kärnten, St. Margareten im Rosental, alluvial forest at the brook Tumpit, grid square 9452/4, on twigs of *Rhamnus frangula*, 7 Jun. 2014, W. Jaklitsch (WU 36936; culture C112 from conidia); shrubs in village area, grid square 9452/4, on twigs of *R. frangula*, 28 Oct. 1995, W. Jaklitsch W.J. 773 (WU 36937); ibidem, on decorticated wood of *Rhamnus cathartica*, 2 Dec. 1995 (WU 36938); Zabrde, grid square 9452/4, 7 Sep. 2014, W. Jaklitsch (WU 36939; culture C130). Niederösterreich, Engelhartstetten, at Schloss Hof, on a partly decorticated branch of *Rhamnus cathartica*, 17 Jun. 2011, H. Voglmayr & I. Greilhuber (WU 36940); Klausen-Leopoldsdorf, near Ranzenbach, on twigs of *R. frangula*, 28 Mar. 2016, W. Jaklitsch (WU 36941; culture C242); Mauerbach, near the cemetery, grid square 77/63, on twigs of *R. frangula*, 25 Aug. 2001, W. Jaklitsch W.J. 1777 (WU 36942); Mödling, Eichkogel, on thin twigs of *Rhamnus saxatilis*, 12 Nov. 2016, H. Voglmayr & I. Greilhuber (WU 36943; culture C277). *Norway*, Aust-Agden, Froland kommune, Ytre Laurvaks, on twigs of *R. frangula*, 3 Oct. 2014, W. Jaklitsch (WU 36944; culture C133). *Spain*, Madrid, Somosiera, Dehesa de Somosiera, at the Arroyo de la Dehesa, N 41°7′29.55" W 3°34′44.88", elev. 1400 m, on twigs of *R. frangula*, 13 Apr. 2015, W. Jaklitsch, J. Checa, M. Blanco, Á. López & F. J. Rejos (WU 36945; culture C190).

Notes: Mirza (1968) listed *Diplotria frangulae* Fuckel, *Camposporium rhamni* Allescher and *Microdiplodia rhamni* Fuckel as putative asexual morphs detected by association on natural hosts, but the true asexual morph is phoma-like with transition to pyrenocheata-like, based on unicellular hyaline conidia (both morphs), absence of setae (phoma-like), short conidiophores (pyrenocheata-like). The hamathecium consists probably of true paraphyses rather than pseudoparaphyses, as free rounded ends have been seen among young asci. On *Rhamnus cathartica* ostioral apices of *Diaporthe fibrosa* sometimes mimic ascomata of *C. rhamni*. In the lectotype some aberrant ascospores up to 27.7 × 12.6 μm are present. For comparison among the three species recognized on *Rhamnus* spp. see notes under *N. rhamnioides*.

**Neocucurbitaria rhamnicola** Jaklitsch & Voglmayr, sp. nov., MycoBank MB823010. Fig. 15. **Etymology:** For its occurrence on *Rhamnus*.

Ascomata (195–)256–375(–452) μm diam (n = 46), (91–)115–175(–215) μm high (n = 20), scattered or aggregated in small groups in bark fissures on some brown subiculum on inner bark layers and wood, subglobose with flattened top, collapsing-discoid with a central depression and reddish to black central papilla (33–)58–90(–97) μm diam (n = 33), sometimes deeply cupulate lacking a papilla, surface smooth to verruculose, black. *Peridium* (38–)41–76(–97) μm thick (n = 23), pseudoparenchymatous, outer layer dark brown, pigment incrusted, inner layer yellowish to hyaline; cells thick-walled, (4–)5–8(–12) μm (n = 47). *Subiculum* consisting of 2–6.5 μm wide brown, thick-walled hyphae. *Hamathecium* consisting of 1–3 μm wide branched ?paraphyses. *Asci* (98–)109–140(–150) × (11–)12–16(–18) μm (n = 25), cylindrical to narrowly clavate, bitunicate, fisitunicate, thick-walled, with a distinct ocular chamber, a short stipe and a simple to knob-like base, 8 (obliquely) uniseriately to biseriately arranged ascospores. *Ascospores* (15.5–)18–22(–26.5) × (7–)8.5–10.7(–11.7) μm, l/w (1.7–)1.9–2.3(–2.7) μm (n = 60), ellipsoid to fusoid, constricted at the median septum, upper part often slightly enlarged, lower often attenuated toward the base when 3-septate, with 3–7 transverse and 1(–2) longitudinal septa, medium to dark brown, darkening in 3 % KOH, smooth.

**Cultures and asexual morph:** colony radius 12 mm after 8 d, 45 mm after 37 d on CMD; colony dark olive brown to nearly black, with radial texture; odour indistinct; pycnidia formed in small numbers. Colony diam 7 mm after 8 d on MEA at 22 °C, colony brown, turning nearly black, finely zonate, completely covered by pycnidia and white aerial hyphae; *pycnidia* more or less in radial rows, laterally fused, 50–210 μm diam, with hyaline to olivaceous conidial drops. *Peridium* thin, subhyaline, pale olivaceous to brownish, consisting of a *t. angularis* of thin-walled, angular to subglobose cells (6.0–)6.5–11(–15) × (5–)6–8.5(–9.5) μm (n = 30), with 2–4 celled, pale brown hyphal outgrowths to ca. 30 × 5–7 μm. *Phialides* (4.0–)5.0–7.3(–8.7) × (2.0–)2.5–4.3(–6.0) μm, l/w (1.1–)1.4–2.6(–3.4) μm (n = 39), lageniform, often basally inflated, arranged in dense clusters. *Conidia* (2.5–)2.8–3.7(–4.3) × (1.1–)1.4–1.8(–2) μm, l/w (1.3–)1.7–2.4(–2.6) μm (n = 46), 1-celled, hyaline, oblong or narrowly ellipsoid, straight or curved, also drop-like, often with 1–2 guttules, sometimes scar distinctly truncate.

**Habitat:** On wood and bark of *Rhamnus* spp., known from *R. alaternus* and *R. lycioides*.

**Distribution:** Southern Europe (Spain).

**Holotype:** Spain, Guadalajara, Chiloeches, Finca Roma, below El Viso, N 40°32′6.27″ W 3°13′4.26″, elev. 750 m, on branches of *Rhamnus lycioides*, 10 Apr. 2015, W. Jaklitsch, J. Checa, Á. López & F. J. Rejos (WU 36946; ex-holotype culture CBS 142396 = C185).

Other material examined: *Spain*, Andalusia, N Castellar, on a branch of *Rhamnus alaternus*, 5 Apr. 2014, W. Jaklitsch (WU 36947; cultures KFrx, KFr).
Fig. 15. Neocucurbitaria rhamnicola. A–C. Ascomata in face view. D–G. Asci. H. Ascus apex showing ocular chamber. I. Hamathecium. J. Ascoma in vertical section. K–P. Ascospores. Q–W. Asexual morph in culture (on MEA after 8 d at 22 °C). Q. Pycnidia. R. Hyphal appendage on pycnidal wall. S. Pycnidial wall in face view. T–V. Conidiophores and conidiogenous cells. W. Conidia. R–W. in 3 % KOH. A, D, I–M, Q–W, WU 36946/CBS 142396. B, C, E–H, N–P, WU 36947. Scale bars: A, B = 300 μm; C = 100 μm; D = 15 μm; E–G = 10 μm; H, T, U = 5 μm; I, K–P, R, S = 7 μm; J = 50 μm; Q = 200 μm; V, W = 3 μm.
Neocucurbitaria rhamnioides Jaklitsch & Voglmayr, sp. nov.

**Etymology:** For its resemblance of Neocucurbitaria rhamni.

Ascomata (163–)255–360(–426) μm diam (n = 92), (97–)125–240(–390) μm high (n = 67), scattered or aggregated in groups of up to ca. 30 individuals, in bark fissures on inner bark layers and wood, also singly erumpent-superficial on bark, subglobose, collapsing-discoid or turbinate with a central papilla (33–)40–92(–157) μm diam (n = 36), sometimes deeply cupulate lacking papilla; surface finely verruculose, developing more or less radial cracks on the upper part of the ascoma, black. **Peridium** ca. 30–80 μm thick, pseudoparenchymatous, outer layer dark brown, pigment incrusted, inner layer yellowish to hyaline; cells thick-walled, (4–)4.5–8(–12) μm diam (n = 46). Basal **subiculum** consisting of 2–6.5 μm wide brown, thick-walled hyphae, sometimes ascending to the top of ascoma, but not originating from upper ascomatal parts. **Hamathecium**

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**Fig. 16.** Neocucurbitaria rhamnioides. A–C. Ascomata in face view. D. Peridium in vertical section. E. Subicular hyphae. F. Ascus apex after fissitunicate dehiscence. G. Ascus base. H–J. Asci. K–O. Ascospores. P–U. Asexual morph on the natural substrate. P. Pycnidium. Q, R. Conidiophores and phialides. S–U. Conidia. J, O, Q, R, U, in 3 % KOH. A, D, I, K, N, O, Q–U, WU 36948; B, E, J, L, M, P, WU 36949; C, F–H, WU 36950. Scale bars: A = 300 μm; B, C = 150 μm; D–F, H–J = 15 μm; G, K, M–O, Q = 7 μm; L, R–T = 5 μm; P = 50 μm; U = 2 μm.

**Neocucurbitaria rhamnioides** Jaklitsch & Voglmayr, sp. nov., MycoBank MB823011. Fig. 16.

**Etymology:** For its resemblance of Neocucurbitaria rhamni.
consisting of 1–3.5 μm wide branched paraphyses. 

Asci (92–) 103–129(–146) × (11)15–13(–14.5) μm (n = 20), cylindrical, bitunicate, fissitunicate, thick-walled, with a distinct ocular chamber, a short stipe and a simple to knob-like base, containing 8 ascospores in (obliquely) uniseriate arrangement. Ascospores (14–)17–22(–25.5) × (7.5–)7.5–10(–11) μm, l/w (1.8–) 2.1–2.4(–2.7) (n = 70), ellipsoid to fusoid, constricted at the median septum, upper part often slightly enlarged, lower often attenuated toward the base when 3-septate, with 3–7 transverse and 1(–2) longitudinal septa, medium to dark brown, darkening in 3% KOH, smooth.

Asexual morph on natural hosts: Pycnidia (43–) 62–110(–141) μm diam (n = 30), superficial on inner bark or wood on usually inconspicuous subiculum, scattered among ascomata, globose, often with a minute apical papilla; peridium pseudoparenchymatous, dark brown, bearing some hyphal outgrowths on the outer side. Inner side lined with hyaline cells giving rise to phialides and simple, to ca. 25 μm long conidiophores. Conidiophores bearing lateral pegs and solitary phialides terminally. Phialides (4.3–)5.0–7.7(–8.7) × (1.5–) 1.7–2.3(–2.7) (n = 18), lageniform to cylindrical, straight or curved. Conidia (2.5–3.0–3.5–3.8) × (1.0–)1.1–1.3(–1.5) μm, l/w (2.3–)2.4–2.9(–3.1) (n = 44), oblong to allantoid, 1-celled, hyaline, with 1–2 subterminal guttules.

Cultures on CMD at 22 °C: colony radius up to 28 mm after 3 wk; colony circular, dark olivaceous brown, dense, with radial texture, sometimes finely zonate; odour indistinct.

Habitat: on wood and bark of Rhamnus spp., known from R. myrtifolius and R. saxatilis.

Distribution: Mediterranean Europe.

Holotype: Spain, Córdoba, Las Lagunillas, La Tiñosa, 37°22′56″N, 4°15′5.8″W, 1 360 m, on twigs of Rhamnus myrtifolius, 17 May 2014, S. Tello (WU 36948; ex-holotype culture CBS 142395 = C118).

Other material examined: Greece, Crete, Omalos, on twigs of Rhamnus saxatilis sp. prunifolius, 5 Jun. 2015, W. Jaklitsch & H. Voglmayr (WU 36949; culture C222); near Askifou, on twigs of Rhamnus saxatilis sp. prunifolius, 6 Jun. 2015, W. Jaklitsch & H. Voglmayr (WU 36950; culture C223).

Notes: Neocucurbitaria rhannoides differs from N. rhamni in the geographical distribution and lack of conspicuous tubercles on ascomata. A distinction from N. rhannica is difficult, but the former tends to develop fine cracks in the central ascomatal surface and occurs on other species of Rhamnus. Ascospor shape and septation depend on the specimen, as in one specimen ascospores with 3 transverse septa and a strongly attenuated lower part may be common, and in another little-varying, 7-septate ascospores may be common. Ascomata on bark are often associated with lichens and may be mistaken for lichen apothecia at first sight. Species recognition is primarily based on the statistical support of the monophyletic lineages, but hosts and geography help to distinguish among them. Both species differ by 17 and 20 fixed nucleotide substitutions in tef1 and rp2, respectively.

Neocucurbitaria ribicola Jaklitsch & Voglmayr, sp. nov. MycoBank MB823012. Fig. 17, 18.

Eymology: For its occurrence on Ribes.

Ascomata (250–)335–542(–628) μm diam (n = 41), (220–) 295–456(–516) μm high (n = 24), scattered or aggregated in small groups or fused into amorphous masses in bark fissures on inner bark layers or wood, sometimes surrounding the whole twig in groups of more than 50 individuals, arranged vertically, obliquely or parallel to the host surface, globose, subglobose to pyriform, collapsing from the side when old. Surface olivaceous to dull brown, covered by subicular hyphae except for the apical papilla, rarely glabrous and then verruculose. Apical papillae (44–)125–210(–265) μm diam (n = 29), often convergent, black, usually stout, rounded in section or more often sulcate, consisting of several radial lobes, or irregularly cracked. Peridium 40–90 μm thick, usually thickened around the papilla to ca. 130 μm, pseudoparenchymatous, consisting of a broad dark brown to black outer layer consisting of thick-walled cells with walls incrusted by coarse amorphous pigment particles, and a narrow inner layer consisting of thinner-walled, brown to hyaline cells; cells (4–)6–11(–13) μm diam (n = 39). Subicular hyphae 2.5–6.5 μm wide, olivaceous to dark brown, thick-walled, short and seta-like around the ostiole, long and scarcely branched on other parts of the ascoma, penetrating into host tissues. Hamathecium consisting of branched, 1–2.5(–3) μm wide ? paraphyses. Asci (150–)158–198(–202) × 14.8–18.2(–20.2) μm (n = 11), cylindrical, bitunicate, fissitunicate, thick-walled, with a distinct ocular chamber often containing refractive bodies, a short stipe and simple or knob-like base, containing 6–8 ascospores in uniseriate arrangement. Ascospores (20–) 21.5–26.3(–30) × (9–)9.8–11.7(–13) μm, l/w (1.8–) 2.0–2.5(–2.7) (n = 71), broadly ellipsoid or broadly fusiform, with the upper part usually broader, with (3–)5–8(–9) transverse and 1–3 longitudinal septa, constricted at the median septum, yellowish brown when young, turning dark to blackish brown at maturity, smooth, containing large guttules when young and vital. Pycnidia uncommon on the natural host, scattered among ascomata, globose, with a minute apical papilla, black, more or less smooth, ca. 70–100 μm diam.

Cultures and asexual morph in culture: Colony radius on CMD at 22 °C 21 mm after 18 d, 47 mm after 2 mo; 5–6 mm 7 d after reconstitution from −80 °C. Colony greyish olivaceous to brown with hyaline margin, finely zonate, odour indistinct to slightly unpleasant. Pycnidia numerous, (33–)38–54(–70) μm (n = 25) diam, globose, with large light rounded papilla 12–30 μm diam, pale olivaceous, darkening with time, scattered or densely disposed around the inoculation plug within a few days, later in additional zones and in radial rows, on the agar surface and immersed in the agar, producing whitish milky conidial drops. Peridium consisting of a thin olivaceous brown l. angularis of (3.5–)4.5–7.5(–10) μm (n = 43) long cells; surface with olivaceous hyphal outgrowths. Inner side of the peridium lined by globose to angular hyaline cells giving rise to conidia, lageniform, straight, curved or sigmoid phialides (5.7–)6–8(–9.2) × (2.1–) 2.3–3(–3.3) μm (n = 15) and short, 1–3-celled, simple conidiophores. Conidia formed on hyaline base cells, sessile phialides, single terminal phialides and pegs on the sides of the conidiophores, (2.6–)3.2–4(–5) × (1.2–)1.4–1.8(–2.4) μm, l/w (1.4–)1.9–2.6(–3.3) (n = 120), 1-celled, hyaline, oblong to ellipsoid or drop-like, with 1–2 subterminal guttules, smooth. On MEA pycnidia becoming larger, up to ca. 130 μm diam, densely aggregated-confluent in the centre, superposed by long white aerial hyphae. After 10 d no conidiogenous cells detectable, and aberrant conidia up to 6 × 2 μm present.

Habitat: on partly decorticated twigs and branches of Ribes rubrum, sometimes associated with Thyroneuctria berolinensis.

Distribution: Central Europe, uncommon.

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Holotype: Austria, Kärnten, St. Margareten im Rosental, village area, grid square 9452/4, on twigs of Ribes rubrum, 2 May 2014, W. Jaklitsch (WU 36951; ex-holotype culture CBS 142394 = C55).

Other material examined: Austria, Oberösterreich, Schärding, Enzenkirchen, Landersberg, grid square 7648/1, on twig of Ribes rubrum, partly covered by algae and ascomata of Thyronectria berolinensis, 21 Mar. 2015, H. Voglmayr (WU 36952; culture C155).

Notes: Neocucurbitaria ribicola is an uncommon species or easily overlooked due to its inconspicuous ascomata, which are covered by hyphae or other fungi. In addition, dead twigs of Ribes are usually inhabited by many other fungi. The holotype
Pycnidial wall. C = 10 μm. quite well for strongly overlapping size range. Mirza smaller than in C. ribicola (Niessl 1872), is apparently a different fungus, as it differs from longitudinal septa. The latter were described by Niessl (1872) as ascomata, a conspicuously thick peridium, absence of a fur-

(WU 36951) contains less material than WU 36952, but is better developed, containing more intact ascii.

Another species described from Ribes, Cucurbitaria ribis (Niessl 1872), is apparently a different fungus, as it differs from C. ribicola by centrally depressed, shiny black and glabrous ascomata, a conspicuously thick peridium, absence of a fur-

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18.0–22.2(–26.2) × (6.8–)7.5–9.3(–10.7) μm, l/w (1.9–)2.2–2.6(–3.2) (n = 40), ellipsoid to fusoid with rounded ends or clavate, slightly constricted at the median primary septum, upper half usually slightly enlarged, with (4–)6–7 transverse and 1–2 longitudinal septa, first pale brown, soon turning dark brown, end cells sometimes slightly lighter, smooth.

Colonies on CMD at 22 °C reaching a growth radius of ca. 9 mm after 10 d, ca. 19 mm after 22 d, mycelium dense, colourless, turning olivaceous from the centre, aerial hyphae often numerous, partly immersed in agar, scattered to aggregated, more or less globose, with central papilla, sometimes laterally fused, pale olive when young, soon turning dark olive to black; conidia emerging in whitish to olivaceous drops.

Peridium consisting of a thin t. prismatica-angularis of thick-walled cells (2.5–)5–10(–14) μm (n = 43) diam; inner side lined by hyaline cells giving rise to conidiophores and phialides. Conidiophores simple or basally divided into several nearly parallel branches with lateral pegs and solitary terminal phialides. Phialides (4.8–)6.7–10.0(–10.5) × (1.8–)2.0–2.5(–2.8) μm, l/w (2.1–)3.1–4.6(–5.3) (n = 25), lageniform. Conidia (2.6–)3.4–4.5(–5.1) × (1.2–)1.3–1.7(–2.1) μm, l/w (2.1–)2.3–2.9(–3.5) (n = 49), oblong to allantoid, sometimes pinched, 1-celled, hyaline, containing 0–2 subterminal guttules, smooth.

Habitat: On wood and bark of Vachellia gummifera.

Distribution: Morocco, only known from the type location

Holotype: Morocco, Agadir, Ait Melloul, behind the Institut Agronomique et Vétérinaire Hassan II (IAV), on branch of Vachellia (Acacia) gummifera, soc. Diaporthe sp., Eutypa sp., 6 May 2015, W. Jaklitsch, M. Mokhtari & M. Louay (WU 36953; ex-holotype culture CBS 142397 = C192).

Notes: Other Cucurbitaria species described from Acacia spp. comprise Cucurbitaria arizonica, described from Acacia greggii A. Gray (as “Acacia grayii”, now known as Senegalia greggii), also Cucurbitaria halimodendri (cf. Mirza 1968) and Cucurbitaria pakistanica (Petrak & Ahmad 1954) from Acacia modesta, now known as Senegalia modesta, in Pakistan. Ascospores of C. arizonica have only three transverse septa (Barr 1990a), C. halimodendri belongs to Camarosporidiella (Wanasinghe et al. 2017a), and C. pakistanica (Petrak & Ahmad 1954) is described with ascospores having 3–5 transverse septa and a single longitudinal septum. Type material of C. pakistanica from W (W 1980/07242!) contains mostly a Diplodia sp. and some black,
verruculose, turbinate to collapsed discoid ascomata 175–355 μm diam, erumpent from bark in dense groups on brown subiculum, with a peridium of large (to 19 μm diam) dark brown, thick-walled cells forming t. angularis-globulosa, mostly immature cylindrical asci and numerous, to 4.5 μm thick hama-thecial threads. Ascospores are uniseriately arranged, (18–22) × (8.7–10.2) μm, l/w (1.8–2.3) (n = 20), ellipsoid to oblong, with 3–5 transverse and longitudinal septa, constricted at the median primary septum, hyaline to pale or yellowish brown when young, turning greyish brown and finally dark to blackish brown at maturity, smooth.

Parafenestella Jaklitsch & Voglmayr, gen. nov., MycoBank MB823014.

Etymology: Owing to its phylogenetic neighbourhood to Fenestella.

Ascomata scattered or variably aggregated below the host epidermis becoming visible in bark fissures, subglobose, globose to pyriform, sometimes collapsing-discoid upon drying when immature or becoming vertically pinched, black, often apically white inside, usually surrounded by subicular hyphae. *Apical papilla* black, rounded or oblong in section, often flattened. *Peridium* pseudoparenchymatous. *Hamathecium* consisting of numerous branched and anastomosing paraphyses. *Asci* cylindrical, bitunicate, fissitunicate, thick-walled, with an ocellar chamber, a short stipe and simple or knob-like base, containing 6–8 ascospores in (obliquely overlapping) uniseriate arrangement. Ascospores ellipsoid with upper part slightly wider, with often subacute and lighter ends, several transverse and longitudinal septa, constricted at the median primary septum, hyaline to pale or yellowish brown when young, turning greysih brown and finally dark to blackish brown at maturity, smooth.

Fig. 20. Neocucurbitaria vachelliae. A–M. Sexual morph (WU 36953). A–C. Ascomata in face view. D. Peridium in vertical section. E. Ascus apex. F. Subiculum. G–J. Ascospores (young in G). K–M. Asci. N–S. Asexual morph in culture (CBS 142397 on CMD after 6–7 d at 22 °C). N. Pycnidia with conidial drops. O. Pycnidial wall. P, Q. Conidiophores and phialides. R, S. Conidia. E, G–J, L, M. in 3 % KOH. Scale bars: A, B = 200 μm; C, N = 100 μm; D, K = 15 μm; E, O, P = 7 μm; F, L, M = 10 μm. G–J, Q–S = 5 μm.
Asexual morph in culture: Pycnidia more or less globose, olivaceous, green to black; surface often roughened by hyphal appendages. Peridium pseudoparenchymatous. Phialides formed on hyaline base cells or apically on short simple or basally branched conidiophores, lageniform to cylindrical or subglobose. Conidia 1-celled, oblong or allantoid, sometimes attenuated towards one end or pinched, hyaline, guttulate, smooth.

Ecology: Apparently fungicolous or saprobic in bark.

Type species: Parafenestella pseudoplatani Jaklitsch & Voglmayr.

Notes: The genus Parafenestella differs from Fenestella phylogenetically, by absence of well-delimited pseudostromata and ascosporae, which show a transition from fenestella- to (neo) cucurbitaria-like. Asexual morph in culture: Phialides formed on hyaline base cells or apically on short simple or basally branched, up to 30 μm long conidiophores, (4.3–)5.5–7.5–(8.8) × (1.5–)2–2.5–3(−3) μm (n = 39), lageniform to cylindrical or subglobose. Conidia (3.2–)3.5–4(−4.5) × (1.0–)1.2–1.5–(1.7) μm, l/w (2–)2.5–3.1(−3.4) (n = 59), 1-celled, oblong or allantoid, sometimes attenuated towards one end or pinched, hyaline, with 2 subterminal drops, smooth.

Habitat: In Diaporthus pseudostromata on dead branches of Acer pseudoplatanus.

Distribution: Central Europe (Austria); only known from the type locality.

Holotype: Austria, Vienna, Strebersdorf, Krottenhofgasse, on branch of Acer pseudoplatanus, 17 Nov. 2013, W. Jaklitsch (WU 36954; ex-holotype culture CBS 142392 = C26, C26T).

Notes: In the holotype the asexual morph of Neocucurbitaria acerina is also present. Due to minor differences it is difficult to differentiate between these two asexual morphs morphologically. Data referring to the natural substrate are recorded from pycnidia densely clustered on Diaporthus pseudostromata. Subglobose conidiogenous cells from pycnidia in culture were not included in measurements. For other species described in Cucurbitaria and Fenestella on Acer see notes under Neocucurbitaria acerina.

Parafenestella mackenziei (Wanas. et al.) Jaklitsch & Voglmayr, comb. nov., MycoBank MB823016.

Basionym: Fenestella mackenziei Wanas. et al., Mycosphere 8: 407. 2017.

Notes: This species was described as a new species in Fenestella by Wanasinghe et al. (2017a) from Rosa canina in Italy. It clusters with P. pseudoplatani rather than with Fenestella fenestra (Fig. 1), therefore we combine it in Parafenestella.

Parafenestella ostryae (Wanas. et al.) Jaklitsch & Voglmayr, comb. nov., MycoBank MB823017.

Basionym: Fenestella ostryae Wanas. et al., Mycosphere 8: 404. 2017.

Note: This species was described from Ostrya carpinifolia in Italy; see Wanasinghe et al. (2017a) for descriptions, illustrations and additional data of this and the foregoing species.

Protofenestella Jaklitsch & Voglmayr, gen. nov., MycoBank MB823018.

Etymology: A primitive form of Fenestella, characterised by random and indefinite disposition of its ascomata on natural substrate.

Ascomata globose, pyriform, depressed subglobose to lenticular, immersed below the host epidermis, solitary or randomly disposed, loosely or densely aggregated in often large ill-defined groups, not forming defined pustules, inconspicuous at the bark surface, surrounded by subiculum. Ostioles inconspicuous, short and blunt conical. Peridium pseudoparenchymatous, covered with subicular hyphae outside, becoming lighter and thinner-walled toward inner side. Hamathecium formed by numerous richly branched ?paraphyses. Asci cylindrical, bitunicate, fissitunicate, with an ocular chamber, short stipe and a simple or knob-like base, containing 6–8 ascosporae in (obliquely overlapping) uniseriate arrangement. Ascosporae (21.8–)24.5–29.5–(31.5) × (11–)11.5–14–(16) μm, l/w (1.6–)1.9–2.3–(2.5) (n = 32), ellipsoid with upper part slightly wider, with often subacute and sometimes lighter ends, (7–)8–12–(13) transverse and 3–4 longitudinal septa, strongly constricted at the median primary septum, yellowish brown when young, turning greyish brown and finally blackish brown at maturity, smooth. Pycnidia co-occurring with ascomata, crowded on a subiculum in Diaporthus pseudostromata, globose, black, 60–150 μm diam, variably collapsing.

Cultures and asexual morph in culture: Growth radius ca. 14 mm after 2 wk on CMD at 22 °C. Colony dense, without distinct odour, pale, mycelium remaining colourless but colony appearing greyish brown due to numerous pycnidia starting to form within 24 h after inoculation. Pycnidia 43–90 μm diam, concentrated and aggregated around the inoculation plug, more scattered with distance from the centre, more or less globose, first pale olivaceous, slowly turning green to nearly black, surface roughened by hyphal appendages, with colourless to pale olivaceous conidial drop. Peridium consisting of a green t. angularis to t. prismatica of moderately thick-walled, (3.5–)5–8–(10) μm (n = 41) long cells. Phialides formed on hyaline base cells or apically on short simple or basally branched, up to 3.1–3.4(−3.4) (n = 59), 1-celled, oblong or allantoid, sometimes attenuated towards one end or pinched, hyaline, with 2 subterminal drops, smooth.

Habitat: In Diaporthus pseudostromata on dead branches of Acer pseudoplatanus.
Fig. 21. Parafenestrella pseudoplatani (WU 36954/CBS 142392). A–M. Sexual morph. A–C. Ascomata in face view. D. Peridium and subicular hyphae in vertical section. E. Ascus apex. F. Ascus stipe and base. G–J. Ascospores. K–M. Asci (young in K). N–U. Asexual morph. N. Pycnidia on natural substrate. O. Pycnidia. P. Pycnidial wall. Q, R. Phialides from natural substrate. S–U. Conidia. Q, S–U. From culture (CMD, after 7 d at 22 °C). D–F, I–M. O, Q, R. in 3 % KOH. Scale bars: A–C = 200 μm; D, K–M = 20 μm; E, G–J, P–R = 7 μm; F = 10 μm. N = 100 μm. O = 70 μm. S, T = 5 μm; U = 3 μm.
Ascomata wall distinctly thicker than septa except for the ends containing narrow canals; later brown to dark brown, with several transverse and longitudinal septa; not to distinctly constricted at the median to slightly eccentric primary septum; ends often lighter coloured, brownish to hyaline, often protruding and subacute, pierced by a pore and often with a hyaline roundish to cylindrical cellular appendage at one or both ends; turning blackish brown in 3 % KOH.

Asexual morph in culture: Pycnidia more or less globose, papillate, nearly black. Peridium thin pseudoparenchymatous. Phialides sessile, clustered in small groups or short chains, subglobose to lageniform. Conidia 1-celled, oblong to allantoid or ellipsoid, hyaline, guttulate, smooth.

Ecology: Probably saprobic in bark.

Type species: Protofenestella ulmi Jaklitsch & Voglmayr.

Note: Protofenestella differs from Fenestella phylogenetically and by non-clustered ascomata.

Protofenestella ulmi Jaklitsch & Voglmayr, sp. nov., MycoBank MB823019, Fig. 22.

Etymology: For its occurrence on Ulmus.

Ascomata (390–)475–675(–780) μm (n = 41) diam, ca. 200–500 μm high, globose, pyriform, depressed subglobose to lenticular, immersed below the host epidermis, solitary or randomly disposed, loosely or densely aggregated in often large ill-defined groups, not forming defined pustules, inconspicuous at the bark surface, surrounded by ample subiculum consisting of thick-walled, hyaline, silvery to pale brown, 2–5 μm wide hyphae, tending to be darker brown toward the peridium. Ostioles inconspicuous, short and blunt conical, sometimes yellow inside, concealed beyond (100–)115–285(–500) μm (n = 21) wide, pale greyish to brown, circular to longish cracks at the bark surface, often darkened by ascospore deposits or when old. Peridium 15–45 μm thick, consisting of a t. angularis of (2.5–)4–8(–10) μm (n = 53) wide cells, thick-walled and dark brown with inhomogenously disposed pigment and densely covered with subicular hyphae outside, becoming lighter and thinner-walled toward inner side. Inner ascomatal surface appearing whitish when old and effete. Hamathecium formed by numerous richly branched, 1–3 μm wide ?paraphyses. Ascii (184–)214–294(–325) × (20–)23.5–29.5(–32.5) μm (n = 27), cylindrical, bitunicate, fissitunicate, with an ocular chamber, short stipe and a simple or knob-like base, containing (4–)8 (oblquely) uniseriately arranged, sometimes partly overlapping ascospores. Ascospores (32–)38–52(–63) × (14.2–)16–21.3(–25) μm, l/w (1.8–)2.1–2.8(–3.3) (n = 117), ellipsoid to fusoid, symmetric to slightly asymmetric, first hyaline to yellowish, with 3–4(–5) transverse septa, developing a longitudinal septum; wall distinctly thicker than septa except for the ends containing narrow canals; later medium (olivaceous)-brown to dark brown, with 12–18(–20) transverse and 3–7 longitudinal septa, with a thin, rarely slightly swelling perispore, lacking a sheath; not to distinctly constricted at the median to slightly eccentric primary septum; ends often lighter coloured, brownish to hyaline, often protruding and subacute, pierced by a pore and often with a hyaline roundish to cylindrical cellular appendage up to 4 μm long at one or both ends; turning blackish brown in 3 % KOH.

Cultures and asexual morph: Colony radius on CMD 30 mm after 23 d at 22 °C; colony greyish olive, turning unevenly brown; aerial hyphae scant, odour indistinct. Pycnidia (after 6 d) 40–100 μm diam, forming within a few days in small numbers, scattered, more or less globose, papillate, nearly black. Peridium consisting of a thin, rather thin-walled, olivaceous brown t. angularis of (2.7–)5–8.5(–10) μm (n = 44) wide cells; inner side lined by hyaline cells giving rise to phialides. Phialides (4.3–)4.9–6.6(–8) × (2.2–)3–4.5(–5) μm (n = 17), sessile, clustered in small groups or short chains, subglobose to lageniform. Conidia (2.9–)3.5–4.5(–5.4) × (1.3–)1.6–2(–2.2) μm, l/w (1.7–)1.9–2.7(–3.5) (n = 46), 1-celled, oblong to allantoid or ellipsoid, hyaline, containing 2 or more small guttules, smooth.

Habitat: In bark of Ulmus spp.

Distribution: Europe (Austria, Sweden).

Holotype: Austria, Vienna 10th district, Unterlaa, on branches of Ulmus minor, soc. Diplodia sp., Thryonecrtia rhodochlora and Nigrograna sp., 14 Mar. 2015, R. Moosbeckhofer G00485, comm. B. Wergen/I. Greilhuber (WU 36955; ex-holotype culture CBS 143000 = FP5).

Other material examined: Austria, Vienna 21st district, Marchfeldkanalweg, near Felix Slavik Straße, on branches of Ulmus minor, soc. Diplodia sp., 10 May 2003, W. Jaklitsch W.J. 2115 (WU 36956). Sweden, Uppland: Balingsta par., Boräs, ca. 50 m W of the old house, chopped off branches of Ulmus glabra, soc. Kirschsteiniothelia aethiops, Nectria sp., several coelomycetes, Hypoxylon and myxomycete spores, K. & L. Holm, 29 Mar. 1988 (UPS F-178445, as Fenestella fenestra; culture UPSC 2554-55 = CBS 114422); Delby par., Tuna, S of the western farm, at the feet of the cliff, on twig of Ulmus glabra, K. & L. Holm, 17 Apr. 1988 (UPS F-178444, as Fenestella fenestrae).

Notes: Phylogenetically, Protofenestella ulmi is unrelated to Fenestella (Figs 1, 2). Although ascospore morphology agrees perfectly with Fenestella, P. ulmi differs morphologically from the latter genus in that ascomata do not form defined clusters. All available materials contain either scant or immature/overmature ascomata that are accompanied by several other fungi, some of which form erumpent clusters of grouped ascomata. Well-developed asci were only found in WU 36955, therefore this specimen serves as holotype. Ascospore measurements include appendages, the latter are in total up to 7 μm long. Pleomassaria ulmica differs from P. ulmi by ascospores with fewer longitudinal septa and the presence of a swelling sheath. See also notes under Seltsamia ulmi.

Seltsamia Jaklitsch & Voglmayr, gen. nov., MycoBank MB823020.

Etymology: Based on the German word “seiltsam” for strange, because the morphology of the fungus, esp. the ascospore sheath, is atypical for Cucurbitariaceae.

Ascomata pyriform, black, immersed singly or in valsoid groups beneath periderm above ascomata of its host, upright or oblique with convergent ostiolar necks, surrounded by subiculum, forming bumps, becoming visible through bark fissures. Ostiolar necks forming stout papillae. Peridium leathery, black, pseudoparenchymatous, 3-layered. Hamathecium consisting of branched ?paraphyses. Asci cylindrical, with a distinct ocular chamber, a slightly elongated stipe and a simple base, containing 8 uni- to partly biseriately arranged ascospores. Ascospores fusoid to subclavate, with the upper part slightly widened, first yellow, with 3 main septa, later brown, finally with numerous transverse and longitudinal septa, surrounded by a swelling sheath around each hemisphere. Asexual morph unknown.
Habitat: Fungicolous, e.g., on Hapalocystis bicaudata on Ulmus glabra.

Type species: Seltsamia ulmi Jaklitsch & Voglmayr.

Seltsamia ulmi Jaklitsch & Voglmayr, sp. nov., MycoBank MB823021. Fig. 23.

Etymology: For its occurrence on Ulmus.

Ascomata (480–)524–760–(870) μm diam (n = 14), pyriform, black, immersed singly or in vasoïd groups of up to ca. 10 individuals, upright or oblique, usually with ostiolar necks convergent to a common centre, surrounded by subiculum, slightly lifting the bark forming bumps, becoming visible through bark fissures. Ostiolar necks forming more or less stout papillae 150–300 μm diam, when young whitish to yellowish inside. Peridium leathery, black, ca. 40–160 μm thick, consisting of a narrow opaque outer layer of dark brown thick-walled cells (4.8–)8–14.5–(17) μm diam (n = 60), a median brown t. angularis of thin-walled and similarly sized cells, and an inner layer of brown compressed cells. Subicular hyphae originating on the ascospore surface, densely woven, making measurement wide, agglutinated, branched ?paraphyses. Asci (310–)346–419–(457) × (30–)35–44–(52.5) μm (n = 32), cylindrical, with a distinct ocular chamber, a slightly elongated stipe and a simple base, containing 8 uni- to partly biseriately arranged spores. Ascospores (52.5–)58.5–67–(71.5) × (16–)18.8–22.5–(26) μm, l/w (2.5–)2.8–3.3–(3.7) (n = 85), fusoid to subclavate, with the upper part slightly widened, first yellow, with 3 main septa, later brown, finally with numerous, ca. 17–25 transverse and 3–7 longitudinal septa, surrounded by a sheath around each hemisphere, quickly swelling and losing contours in water.

Cultures: Mycelium filling a 90 mm Petri dish on MEA, when centrally inoculated, after 36 d at room temperature. Colony dense, dark olive-brown, odour indistinct, no asexual morph detectable.

Habitat: On Hapalocystis bicaudata on corticated Ulmus glabra.

Distribution: Only known from the holotype in Norway.

Holotype: Norway. Aust-Agder, Froland kommune, Ytre Lauv-rak, associated with Hapalocystis bicaudata on corticated twigs of Ulmus glabra, soc. Stylonectria wegeliana, 3 Oct. 2014, H. Voglmayr & W. Jaklitsch (WU 36957; ex-holotype culture CBS 143002 = L150). Notes: This is an exceptional species. Its home in the Cucurbitariaceae as following from molecular phylogeny is unexpected. Morphologically, the swelling ascospore sheath would suggest a pleomassaria-like fungus, clustering of ascocoma in association with other pyrenomycetes on the other hand. Fenestella Pleomassaria ulmica (basionym Cucurbitaria ulmica Fuckel) has smaller ascospores and fewer septa, viz. 38.5–48.5 × 11–16.5 μm and 8–13 transverse, 2–3 longitudinal septa (cf. Barr 1982).

Fuckel (1870) described C. ulmica with 8–10 septate muriform ascospores of 36 × 11 μm. Type material of C. ulmica (from Ulmus minor in Schloßpark Reichartshausen at Oestrich-Winkel, Germany) was distributed as Fungi Rhenani 2170. All specimens from G (G 00127253, G 00127254, G 00127255) and W (W 2015-01917, W 1922-12005) examined contain over-mature, effete, depauperate material. No ascospores as given above were found. In G 00127254 we found a few collapsed-discoid ascomata and few small brown spores with 3–4/1 septa, 14.4–18.6 × 6.4–8.7 μm and some Dothidodidiothcia cf. ramulicola. Also G 00127255 contains a few ascomata of a Dothidodidiothcia sp. In W 1922-12005 some Diplopia sp. is present.

Petrak (1922) reported a specimen collected in Podhorn, Czech Republic from Ulmus sp., which he identified as Cucurbitaria ulmica. He described ascospores as blackish brown with a very variable size, 30–60 × 14–24 μm and mostly 9–14/1–2 septa. By association on the natural host he described the putative asexual morph Pleurostromella ulmica having conidia 2–3 × 0.75–1.2 μm, thus it is neither the asexual morph of Protofenestella ulmi (conidia ca. 3–5 × 1.3–2.2 μm) nor of S. ulmi, which did not produce an asexual morph in culture. All other species described from Ulmus under Cucurbitaria or Fenestella (C. naucosa (Fr.) Fuckel, C. ulmea P. Karst., Fenestella ulmica Ellis & Everh.) were described with much smaller ascospores having much fewer septa than S. ulmi.

DISCUSSION

Molecular Phylogeny

Although many earlier phylogenetic trees presented for the Pleosporales (e.g. Zhang et al. 2012, Hyde et al. 2013) pretended having been calculated using multigene matrices, they were basically constructed by using LSU (and SSU) sequences, as there is still lack of protein-coding markers for the vast majority of taxa. Even in most recent papers relevant to the topic (Crous & Groenewald 2017, Wanasinghe et al. 2017b) primarily or exclusively ribosomal markers were used to construct trees, on which taxonomic conclusions were built. Although ITS, which was also used by these authors, improves resolution, it does not offer firm and reliable criteria to decide where to draw lines between families and genera. Resolution and statistical support of clades is also highly depending on the number and selection of taxa. Wanasinghe et al. (2017b) included in Cucurbitariaceae many Pyrenochaeta and Pyrenochaetopsis spp., which are shown by multigene analyses not to be part of the family (see below).

We performed an analysis based on ITS-LSU alone to elucidate the phylogenetic position of Neocucurbitaria acerina and of the new Fenestella spp. described by Wanasinghe et al. (2017b); as shown in Fig. 1, Parafenestella, with the three species P. mackenzeri, P. ostryae and P. pseudoplatani, received low (54 % MP) to moderate (83 % ML) support. However, taxonomic conclusions based on ITS-LSU alone are in part ambiguous or problematic, as, e.g., Pyrenochaeta nobilis may be judged as...
Fig. 23. Seltsamia ulmi (WU 36957). A. Horizontal section at the ostiolar level. B. Horizontal section at the ascomatal level. C, D. Ascoma in vertical section. E. Peridium in vertical section showing remnants of subicular hyphae. F–H. Asci (young in F). I. Ascus apex showing ocular chamber. J–Q. Ascospores (showing swelling sheath in J and K). E, J. in 3% KOH. Scale bars: A–C = 200 μm; D = 100 μm; E–H, K = 25 μm; I = 10 μm; J, L–Q = 15 μm.
belonging to the Cucurbitariaceae or not. Cucurbitaria berberidis and C. oromediterranea cannot be resolved with ITS-LSU, and also the genus Neocucurbitaria receives no support (Fig. 1).

Only inclusion of protein-coding phylogenetic markers considerably improves resolution and offers more stable and reliable topologies and hence bases for taxonomic inferences. Valenzuela-Lopez et al. (2018) included rpb2 and tub2 sequences for their phylogenetic work on phoma- and pyrenochaeta-like fungi, improving the framework for placing these coelomycetes in Cucurbitariaceae, Didymellaceae and other families and genera. Within Cucurbitariaceae, they added the two new genera Allocucurbitaria and Paracucurbitaria, recognized several additional species in Neocucurbitaria, and stabilised names such as N. cava (earlier known as Pyr-nochaeta cava) by typification.

We included the taxa recognised by Valenzuela-Lopez et al. (2018) in Cucurbitariaceae in our multigene analyses represented by Fig. 2. As a result, Cucurbitariaceae are highly supported, and sister group relationship of the generic type Pyrenochaeta nobilis to all other Cucurbitariaceae receives low to moderate support. The family is subdivided into several well supported clades: In Cucurbitaria we presently recognise only two taxa from Berberis spp.: C. oromediterranea is phylogenetically clearly separated from C. berberidis, and can be molecularly distinguished by 26 and 12 diagnostic nucleotide substitutions in tef1 and tub2 sequences, respectively. Neocucurbitaria is the largest clade receiving high to maximum support in the multigene analysis. There are several highly supported clades in Neocucurbitaria. Two of them are host-specific, N. acanthocladae, N. aetnensis and N. cinereae on Genista spp. and N. rhamni, N. rhannicola and N. rhanniiodes on Rhamnus spp.; on the other hand the highly supported clade containing N. cisticola, N. juglandicola and N. populi among others consists of species occurring on unrelated hosts. There is also considerable morphological variation within Neocucurbitaria, but, e.g., the various shapes of ascomatal apices including ostiolar areas from inconspicuous and rounded to papillate or irregularly tubercular, furrowed or stellate, may occur within a single species (see, e.g., N. acanthocladae). Furthermore, furrowed or stellate ostiolar areas also occur in other subclasses, e.g., N. ribicola.

A third major clade, the Fenestella clade (Fenestella and Parafenestella) is here only treated rudimentarily. Additional phylogenetic data to be included in a future work may show more clearly why erection of the genus Parafenestella is justified. Seltsamia is phylogenetically distinct from Allocucurbitaria, Astrapalicola and the “Fenestella clade”. Clustering of Cucitella with Paracucurbitaria in the multigene tree is weakly supported (54 %) in MP and unsupported in ML analyses, i.e. the position of the former is uncertain within the Cucurbitariaceae, warranting its status as a distinct genus. Comparison of the bootstrap trees of the individual markers show that especially the rpb2 gene contributes substantially to the phylogenetic resolution of many internal as well terminal nodes, whereas the tef1 especially contributes to resolution of closely related species. The same has also been observed in other lineages of Pleosporales, e.g. the Massarineae (Voglmayr & Jaklitsch 2017). This may be partly due to the high number of parsimony informative characters of rpb2 compared to the other markers, but also to the fact that rpb2 is a protein coding gene, which enables a highly reliable alignment compared to ITS and tef1 introns, which become difficult to align over a wider range of taxa due to frequent indels and subsequent genomic rearrangements, adding a substantial amount of homoplasies. In addition, the ITS-region, although established as the primary barcode of fungi for pragmatic reasons (Schoch et al. 2012), usually shows significantly less variation between closely related taxa than, e.g., the tef1 introns (e.g. Voglmayr et al. 2017), which therefore provide a far better resolution in case of closely related species. For these reasons, it is highly recommended that rpb2 and tef1 introns are sequenced and used in combined analyses with the ITS-LSU rDNA. However, as seen from the combined analyses (Fig. 2), additional suitable protein-coding genes will be necessary to further improve the resolution of many nodes.

Morphology

Although phylogenetic analyses are the major argument for generic delimitation, there are, however, also morphological features that characterize genera. We summarise these characters here:

Astrapalicola: is only known from the asexual morph with rather large pycnidia having olivaceous contents with waxy to gelatinous consistency.

Cucurbitaria: produces conspicuously and coarsely warted ascomata with thick, basally thickened and elongated wall. Asexual morphs form setose pycnidia on the natural substrates and in artificial culture.

Cucitella: is fenestella-like, forms compact pustules and ascosporas with several transverse and longitudinal septa, dark brown with lighter ends. It is therefore unfortunately only safely distinguishable from Fenestella phylogenetically.

Fenestella: forms pseudostromata; ascospores have many septa difficult to count. Peridium as in Neocucurbitaria.

Neocucurbitaria: ascomatal peridium thin, when thickened then only slightly in the upper part; pycnidia non-setose.

Parafenestella: ascomata aggregated or not, not forming well-delimited pseudostromata, papillate, ascospores fenestella- to (neo)cucurbitaria-like.

Protofenestella: like Fenestella, but ascomata immersed and evenly effused, not forming well-defined groups or pseudostromata.

Seltsamia: pleomassaria-like; ascospores with a bipartite swelling gelatinous sheath.

Asexual morphs in the Cucurbitariaceae

Since de Gruyter et al. (2010) Pyrenochaeta belongs to the Cucurbitariaceae. Pyrenochaeta differs from Phoma by setose pycnidia, while conidiophores commonly occur in Pyrenochaeta but rarely in Phoma (Sutton 1980). Phoma s. str. belongs to the Didymellaceae (de Gruyter et al. 2012). Schneider (1979) monographed Pyrenochaeta and neotypified its generic type, P. nobilis (ex-neotype culture CBS 407.76), collected in Italy on Laurus leaves. Its pycnidia are setose in nature and in artificial culture. Although P. berberidis, the asexual morph name and now a synonym of C. berberidis, has the same features, Schneider (1979) did not accept it in Pyrenochaeta, but included P. quercina and P. unguis-hominis, both of which are now in Neocucurbitaria (Wanasinghe et al. 2017b, Valenzuela-Lopez et al. 2018). Three other species, N. cava, N. hakeae and N. keratinophila, hitherto classified in Pyrenochaeta, were combined in Neocucurbitaria by
Valenzuela-Lopez et al. (2018), who also placed Pyrenochaeta corni in their new genus *Paracucurbitaria* and excluded *Pyrenochaeta nobilis* from the *Cucurbitariaceae*.

Valenzuela-Lopez et al. (2018) reported abundant setae in the description of the family *Cucurbitariaceae*, the generic description of *Neocucurbitaria* and in *N. quercina*, citing an old description for that species. In culture of *N. quercina*, however, they found very short, thin-walled pycnidial setae rounded at the top, which may rather be interpreted as hyphal outgrowths. We found setose pycnidia only in asexual morphs of *Cucurbitaria* s. str. Asexual morphs of all other representatives of the *Cucurbitariaceae* we studied may be called phoma-like, because they produce pycnidia that may have hyphal appendages but lack setae. However, pycnidia in nature show a marked tendency to produce conidiophores with lateral pegs and lageniform phialides (acropleurogenous), but in culture conidiophores are often absent and only sessile, nearly globose conidigenous cells are formed, i.e. the “two different ways of conidiation” as addressed, e.g., by de Gruyter et al. (2010), are in fact only dependent on external/environmental conditions for one and the same species. This fact has already been surmised for *Nigrograna mackinnonii*, which did not form conidiophores in culture (de Gruyter et al. 2012), while pycnidia of other *Nigrograna* species produced conidiophores on the natural substrate (Jaklitsch & Voglmayr 2016). Thus, formation of pycnidia in artificial culture represents an unnatural condition, and conidiophores may be produced or not. In conclusion, presence or absence of conidiophores in pycnidia formed in culture is no taxonomic criterion on the generic and even species level.

Hosts and Distribution

It is difficult to specifically collect fungi belonging to *Cucurbitariaceae*. Much more common are camarosporium-like fungi, which formerly belonged to *Cucurbitaria*, particularly *Camarosporiella*, *Camarosporium*, *Neocamarosporium*, *Paracamarosporium* (incl. *Pseudocamarosporium*; see, e.g., Crous & Groenewald 2017, Wasanisinghe et al. 2017a), *Staurosphaeria*, or taxa of the *Melanommataceae* (Jaklitsch & Voglmayr 2017), are difficult to differentiate from the *Cucurbitariaceae* morphologically, and they may occur on the same hosts. As an example, we collected three phylogenetically different but morphologically similar fungi on *Pyrus* not included in this work. As a consequence, every single specimen has to be cultured and sequenced to be certain about phylogenetic relationships and generic affinities.

Sexual morphs of *Cucurbitariaceae* occur on wood and bark of various trees and shrubs and less commonly on tougher herbaceous plants with lignified tissues (*Astragalus*) and appear to be host-specific (Mirza 1968 pro parte and this work). Asexual morphs may occur on such substrates, but those for which no sexual morph is known, also occur on various other substrates, particularly on leaves but also in soil and in medical environments (Valenzuela-Lopez et al. 2018). Based on current data, *Cucurbitariaceae* occur in temperate and Mediterranean climates. More collecting is necessary to draw a more complete picture.

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