**Asterotexis cucurbitacearum**, a poorly known pathogen of Cucurbitaceae new to Costa Rica, Grenada and Panama

Yuriza Guerreroa, Tina Antje Hofmannb,c, Carl Williamsa, Marco Thinesb,d and Meike Piepenbringb

aFacultad de Ciencias Agropecuarias, Universidad de Panamá, Chiriquí, Panamá; bInstitute of Ecology, Evolution and Diversity, Goethe-University, Siesmayerstr. 70, 60323 Frankfurt, Germany; cFacultad de Ciencias Naturales y Exactas, Universidad Autónoma de Chiriquí, 0427 David, Chiriquí, Panamá; dBiodiversity and Climate Research Centre (BiK-F), Senckenberganlage 25, 60325 Frankfurt (Main), Germany

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A pathogenic bitunicate ascomycetous fungus, identified as *Asterotexis cucurbitacearum*, was found repeatedly on living leaves of *Cucurbita pepo* (gourd) and *Sechium edule* (chayote) in Panama. *A. cucurbitacearum* represents a new report for Panama and *S. edule* is a new host plant for this fungus. Based on an examination of herbarium specimens, this species is also reported for the first time from Costa Rica and Grenada. Cells of the scutellum extending to the base of the fruiting structure and producing an appressorium on the leaf surface are documented for the first time. As shown by LSU sequence data, *A. cucurbitacearum* represents a divergent, hitherto molecularly unsampled, phylogenetic clade of the Dothideomycetes. The closest relatives, for which sequence data are available, are members of the Pleosporomycetidae and Dothideomycetes incertae sedis with sequence similarity below 90%, as shown by BLAST search. A preliminary neighbour-joining analysis indicates that *A. cucurbitacearum* probably belongs to the Dothideomycetidae (incertae sedis).

**Keywords:** appressorium; Asterinaceae; Dothideomycetes; infection structures; morphology; phytopathology

**Introduction**

Numerous cultivated species of Cucurbitaceae are of economic significance, with *Citrullus lanatus* L. (watermelon), *Cucumis melo* L. (e.g. honeydew melon), *Cucumis sativus* L. (cucumber), *Cucurbita pepo* L. (gourd) and *Sechium edule* (Jacq.) Sw. (chayote) being important crops in Panama and Costa Rica. Fungal pathogens that are known to parasitize members of Cucurbitaceae in Costa Rica and Panama are listed by Esquivel (1982), Farr and Rossman (2010), Gamboa (1989), McGuire and Crandall (1967), Piepenbring (2006) and Toler et al. (1959). During an investigation of pathogenic fungi infecting cultivated species of Cucurbitaceae in Panama, a bitunicate ascomyete, identified as *Asterotexis cucurbitacearum*, was discovered on leaves of *Cucurbita pepo* and *Sechium edule*, forming ascomata on the abaxial sides of the leaves and causing premature dieback in the case of heavy infections. The process of identification was difficult as *A. cucurbitacearum* is rarely mentioned in the phytopathological literature. The most detailed but incomplete description with illustrations of this species was published by Müller and von Arx (1962). *Asterotexis cucurbitacearum* has been cited as *Rhagadolobium cucurbitacearum* (Rehm) Theiss. & Syd. in the past. It fundamentally differs from species of *Rhagadolobium* spp. (Parmulariaceae) by the absence of an internal stroma.

According to Kirk et al. (2008) and Inacio and Cannon (2008), the genus *Asterotexis* includes two species and belongs to the Asterinaceae, which have been shown to represent a distinct lineage related to species of Venturiaceae by Hofmann et al. (2010). Sequence data are lacking for species of *Asterotexis*, as well as phytopathological studies. This situation is typical of tropical, plant parasitic microfungi that cannot be cultured (Piepenbring et al. 2011). The aim of the present study is a characterization of the neglected pathogen, *A. cucurbitacearum*, based on morphological investigation and partial nrLSU sequence data.

**Materials and methods**

Specimens of *Asterotexis cucurbitacearum* on living leaves of *Cucurbita pepo* and *Sechium edule* (Cucurbitaceae) were collected in Panama, province of Chiriqui, between 2005 and 2009. The material was analyzed in fresh and in dry condition with a Zeiss Axioskop 2 plus bright-field light microscope with phase contrast. Measurements are given as mean values ± standard deviation of 20 measurements with extreme values in brackets.
Specimens of *A. cucurbitacearum* from Costa Rica and Grenada were obtained from the US National Fungus Collections (BPI) in Beltsville, Maryland, USA. The Panamanian specimens are deposited at the herbarium of the University of Panama (PMA), the Botanische Staatsammlung München (M) or the Herbarium Senckenbergianum (FR) in Frankfurt, Germany.

Molecular sequence data were obtained from the specimen ppMP 1188 (M-0141224). DNA extraction and PCR were carried out using the most suitable method, as evaluated by Telle and Thines (2008). PCR conditions were as described therein, except for setting the extension time to 60 s. A sequence of 811 bp of the D1-D2 region of the nrLSU has been deposited in GenBank (GenBank accession number HQ610510). Values of similarity were obtained with the help of the BLAST search tool. A preliminary neighbour-joining analysis (results not shown) was performed with MEGA4 (Tamura et al. 2007) including the sequences most similar to those of *A. cucurbitacearum* and to “Mycosphaerella pneumatophorae” (accession number FJ176856), which is the sequence found to be most similar to the one of *A. cucurbitacearum* by the BLAST search.

**Results**

**Morphology and taxonomy**

*Asterotexis cucurbitacearum* (Rehm) Arx [as 'cucurbitarum'], Fungus, Wageningen 28: 6 (1958): Figures 1 and 2.

Basionym: Dothidella cucurbitacearum Rehm, Hedwigia 36: 376 (1897). Synonym: Rhagadolobium cucurbitacearum (Rehm) Theiss. & Syd., Annales Mycologici 12(3): 275 (1914). Type on unknown Cucurbitaceae, Brazil, Blumenau, May 1887, E. Ule 1415 (syntype S F47805, n. v.), Rio de Janeiro, May 1887, E. Ule 676 (syntype S F7565, n. v.).

Ascomata on abaxial sides of leaves, extending along secondary and tertiary veins, irregular to star-shaped, 1–7(-8) mm diameter, sometimes coalescing and covering entire leaf parts, causing dieback of leaves. Ascomata superficial, scutellate, brown to blackish, scutellum formed by radially arranged rows of cells, one cell layer thick, opening with numerous irregular fissures, cells of the scutellum in surface view rectangular to isodiametric, 5–10 × 4–5(-6) μm, 3–5 μm high, brown, smooth. Individual cells of the scutellum forming one hyaline, aseptate hypha each, of approximately 3 μm in diameter, through ascogenous cavity towards epidermis of host; base of each cell producing appressorium firmly attached to host cuticle. Appressoria single or close to each other in small groups, globose and cone-shaped, ovoid to elongate in surface view, about 8–13 × 5–7 μm, brown, each appressorium forms a central hyaline penetration peg perforating cuticle and wall of host cell. Inter- and intracellular hyphae within epidermal cells of host indistinct, scattered, hyaline, difficult to distinguish in dry specimens. Asci cylindrical to clavate, 8-spored, bitunicate, (36-)40–48(-49) × 11–15 (-16) μm, hyaline, vertically orientated within ascoma. Ascogenous hyphae at base of asci difficult to distinguish. Ascospores ellipsoidal to slipper-shaped, uniseptate, septum central, (13-)14–17 × 6–7 μm, upper cell globose, lower cell smaller, cylindrical, both cells hyaline to slightly brownish, smooth. Germinating ascospores and anamorph not observed.

*A. cucurbitacearum* on *S. edule* is frequently overgrown by the hyphomycete *Septofusidium variabilis* (Matsush.) Samuels.

Material examined: On *Sechium edule*, COSTA RICA, Cartago Province, Tapanti, October 1941, R. Vicente s.n. (BPI 646286). PANAMA, Chiriquí Province, Boquete District, border of Caldera River, 2 September 2005, Y. Guerrero & C. Williams 6 (PMA); Renancimiento District, border of road to Piedra Candela, 8 October 2005, M. Piepenbring 3640 (PMA); the same locality, 21 July 2006, T. A. Hofmann et al. ppMP 1188 (M-0141224, PMA); the same locality, 18 January 2007, T. A. Hofmann.
Figure 2. *Asterotexis cucurbitacearum* on *Sechium edule*, as seen by light microscopy. (A) Scutellum of a crushed ascoma in surface view (Guerrero 6). Scale bar = 40 µm. (B) Part of a transverse section of a fruiting body on the surface of host tissue, with a scutellum, hyphae with appressoria, and asci with ascospores (Guerrero 6 and Kirschner 3349). Haustorial hyphae could not be distinguished in herbarium specimens. Scale bar = 20 µm.

et al. ppMP 1255 (M-0141225, PMA); Dolega District, Los Algarrobos, Casa de la Alemana, 13 September 2007, T. A. Hofmann 537 (PMA); Boqueron District, Cordillera, Finca of Alexis Guerra, with hyperparasitic *Septofusidium variabilis* (det. R. Kirschner), 24 August 2009, R. Kirschner 3349 (= SH 14) (FR, PMA).

**On Cucurbita pepo**, PANAMA, Chiriquí Province, David District, Chiriquí, Facultad de Ciencias Agropecuarias, 15 December 2005, Guerrero 27 (PMA).

**On Cucurbita sp.** ("pumpkin"), GRENA DA (Lesser Antilles), St. George’s, 1912-13, R. Thaxter s.n. (BPI 653102).

Host plants and distribution: On *Cayaponia americana* (Plum. ex Lam.) Cogn. in the Dominican Republic (Ciferri 1956); on *Cucurbita moschata* Duchesne (as *Pepo moschata* Britton) in Venezuela (Chardon and Toro 1934); on *Cucurbita pepo* in Brazil (Mendes et al. 1998), Trinidad & Tobago (Baker and Dale 1951), and Panama (new record); on *Cucurbita sp.* in Brazil (Mendes et al. 1998) and Grenada (new record); on *Gurania* sp. in the Dominican Republic (Ciferri 1956); on *Sechium edule* in Costa Rica (new record) and Panamá (new record); on *Trichosanthes* sp. in the Dominican Republic (Ciferri 1956). Farr & Rossman (2010) do not report *S. edule* as host plant, so *S. edule* probably is a previously not reported host of *A. cucurbitacearum*. The hyphomycete *Septofusidium variabilis* is reported for the first time for Panama.

**Sequence data**

A BLAST search based on 811 bp of the D1-D2 region of the nrLSU of *Asterotexis cucurbitacearum* shows a maximum similarity of 87% with 100% coverage with the sequence of a species classified as “*Mycosphaerella pneumatophorae*” (accession number FJ176856) and published by Schoch et al. (2009a). Next similar sequences with 85% and lower values of similarity correspond to species belonging to Pleosporomycetidae (Hysteriales) or Dothideomycetes incertae sedis (Patellariales). A preliminary phylogenetic hypothesis (not shown) obtained by applying the NJ method indicates that *A. cucurbitacearum* might belong to the Dothideomycetidae (incertae sedis).

**Discussion**

**Morphology**

Some cells of the scutellum of *Asterotexis cucurbitacearum* grow towards the leaf surface, through the locule filled with asci, form an appressorium at the base, and penetrate the wall of the epidermal host cell. This unique morphology of the infection process is documented here for the first time. Theissen and Sydow (1914) interpreted the drop-like structures at the ascomatal base as brown conidia rather than appressoria. Conidia-forming structures are not known for *A. cucurbitacearum* and were not observed during our investigation. According to Müller and von Arx (1962), *A. cucurbitacearum* forms hyaline surface mycelium with lateral brown appressoria. However, the Costa Rican, Grenadian and the Panamanian material of *A. cucurbitacearum* does not contain surface mycelium with lateral appressoria. Such structures were never described by authors who investigated type material of *A. cucurbitacearum*, for example Rehm (1897) or Theissen and Sydow (1914). The hyaline surface mycelium with brown appressoria observed by Müller and von Arx (1962) probably belongs to a hyperparasitic or phytopathogenic fungus growing on or close to *A. cucurbitacearum* on *C. pepo*.

**Sequence data**

The partial nrLSU of *A. cucurbitacearum* was most similar (87%) to an accession filed under “*Mycosphaerella pneumatophorae*” (accession number FJ176856) contributed by Schoch et al. (2009a). According to Aptroot (2006), the species *M. pneumatophorae* Kohlm. is a
synonym of *Collemopsidium pneumatophorae* (Kohlm.) Aptroot, belonging to the Xanthopyreniaceae within Chaetothyriomycetidae (Eurotiomycetes). In Schoch et al. (2009a), the species called “*M. pneumatophorae*” (FJ176856) is related to members of Phaeotrichaceae and Venturiaceae, two families with an uncertain placement in actual phylogenies of Dothideomycetes (Schoch et al. 2009b). Therefore, *M. pneumatophorae* certainly does not represent a species of *Mycosphaerella* (Dothideomycetidae). The molecular data also indicate that the genus *Asterotexis* is apparently not closely related to Asterinaceae as previously thought, but represents a poorly known lineage of tropical microfungi in the Dothideomycetidae (incertae sedis). A more concise systematic position cannot be defined because sequence data of closely related taxa are not available.

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