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Acremonium exuviarum sp. nov., a lizard-associated fungus with affinity to *Emericellopsis*

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Abstract: In a survey of cycloheximide-tolerant fungi growing from shed reptile skins, an *Acremonium*-like fungus was isolated that was distinctive in producing relatively large conidia in chains from phialides that tended to collapse after forming a number of conidia. Phylogenetic analysis based on ribosomal internal transcribed spacer and β-tubulin sequences revealed that the isolate represented an undescribed and relatively phylogenetically isolated member of the clade containing the teleomorph genus *Emericellopsis* as well as related anamorphs in *Acremonium* and *Stanjemonium.* The species is here described as *Acremonium exuviarum.* It has been isolated only on a single occasion and its ecology is unknown. Discovery of such new species in the pharmaceutically important *Emericellopsis* clade is potentially of practical significance.

Taxonomic novelty: *Acremonium exuviarum* Sigler, Zuccaro, Summerbell & Paré sp. nov.
Key words: *Acremonium, Emericellopsis, Corucia zebrata,* skink, veterinary

INTRODUCTION

An *Acremonium*-like fungus was isolated from shed dorsal skin of a captive female Solomon Island, or prehensile-tailed, skink (*Corucia zebrata* Gray) during a survey of the mycobiota from healthy reptiles (Paré *et al*. 2003). The objective of the survey was to examine freshly shed exuviae from 127 healthy captive reptiles for the presence of the *Chrysosporium* Corda anamorph of *Nannizziopsis vriesii* (Apinis) Currah, recently identified as an agent of cutaneous mycosis in reptiles (Paré *et al.* 1997, Nichols *et al.* 1999, Thomas *et al*. 2002). Although that survey used a medium containing cycloheximide to selectively recover *Chrysosporium* species and onygenalean fungi, there was a high frequency of recovery of saprotrophic fungi. *Acremonium* species, for example, were recovered at a frequency of 13 % from shed skin samples. The *Acremonium* isolate from the skink demonstrated unusual features, so its identification was investigated in some detail. The relatively large round conidia and branching phialide structure suggested a possible *Emericellopsis* F.H. Beyma lineage and the isolate was included in a broader study of terrestrial- and marine-associated *Emericellopsis* species and related anamorphs (Zuccaro *et al.* 2004, this volume). Morphological and phylogenetic analyses confirm that the skink isolate is distinct and that it has affinities within *Emericellopsis.*

MATERIALS AND METHODS

Details on the methods for sampling of shed skin samples, specimen submission and culture are provided in Paré *et al.* (2003). The Solomon Island skink was an adult wild-caught female (coded CZ-F) housed at the Steinhart Aquarium, San Francisco, CA. Shedding skin was removed with forceps aseptically from the dorsal area and the right flank. The isolate described here was obtained from the dorsal skin and deposited as UAMH 9995 in the University of Alberta Microfungus Collection and Herbarium (UAMH), Edmonton, Alberta, Canada. A subculture is deposited in Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands, as CBS 113360. Colony descriptions are based on potato-dextrose agar (PDA, Difco Laboratories, Detroit, MI, U.S.A.) at 25 and 30 °C, 2 % malt extract agar (containing Difco malt extract) at 22 °C, and oatmeal-salts agar (OAT; Kane *et al*. 1997). Herbarium specimens were prepared by growing the fungus on PDA and OAT overlaid with a cellophane membrane (Carmichael 1961). Colour names are according to Kornerup & Wanscher (1978). Cycloheximide tolerance was evaluated on Mycosel agar (MYC, Difco). Microscopic morphology was examined from Riddell slide culture preparations incubated at 25 °C and mounted in lacto-fuchsin or polyvinyl alcohol, following the method and recipes described in Kane *et al.* (1997). The nutrient medium was cereal

agar made with Heinz (H.J. Heinz, Pittsburgh, PA, U.S.A.) mixed cereal, also manufactured as Pablum mixed cereal.

DNA extraction, amplification and sequencing methods are as described in Zuccaro *et al.* (2004). Sequences of the nuclear ribosomal internal transcribed spacer (ITS) region (555 characters), inclusive of the intervening 5.8S rDNA, as well as a portion of the β-tubulin locus (347 characters) were aligned manually (GENEDOC; Nicholas *et al.* 1997) and combined to form a matrix of 902 characters. The final alignment comprised fifteen taxa including three *Bionectria* Speg. species used as outgroup. Relationships among strains included in the present phylogenetic analysis are shown in Fig. 1. These are a subset of those examined by Zuccaro *et al.* (2004; provenance provided in their Table 1). Each genetic region was partitioned within the matrix to allow independent analyses and partition homogeneity tests (Farris *et al.* 1994, Swofford 2000). All phylogenetic analyses were performed using PAUP 4.0b10 (Swofford 2000). Maximum parsimony settings included: heuristic searches with random sequence addition (10–50 replicates) using the tree-bisection reconnection (TBS) branch-swapping algorithm with and without successive weighting (Farris 1969). Bootstrap analyses and partition heterogeneity tests were performed using up to 1000 replicates. Maximum likelihood (ML) with quartet puzzling (Strimmer & von Haeseler 1996) was performed to account for any rate heterogeneity existing between the partitions. Substitution rate-matrix parameters and nucleotide frequencies were estimated from the data and from site-specific rates calculated according to the general time-reversible + site-specific rates (GTR + SS) substitution model implemented in PAUP. Likelihood calculations were made using the least squares method and the number of puzzling steps varied between 10 000 and 50 000.

RESULTS

Acremonium exuviarum sequences displayed a high degree of homology with those of *Emericellopsis* and *Stanjemonium* W. Gams, O'Donnell, Schroers & C.M. Chr. species (Fig. 1). ITS region similarities ranged from 95–99 % within the group, with the sequence from *A. exuviarum* matching others at the 96–97 % level. The values of similarity dropped to 76–77 % when this sequence was compared to those from species of *Bionectria*, the sister group of *Emericellopsis* (Rossman *et al.* 2001). The molecular link to *Emericellopsis* is more pronounced when the β-tubulin "intron 3" sequences are compared. *Emericellopsis* and *Stanjemonium* species have a very short intron 3 sequence, varying in length between 49 and 52 base pairs. The intron 3 sequence from *A. exuviarum* is also very short and has a high similarity to that of *E. synnematicola* P.N. Mathur & Thirum. and *E. salmosynnemata* Grosklags & Swift.

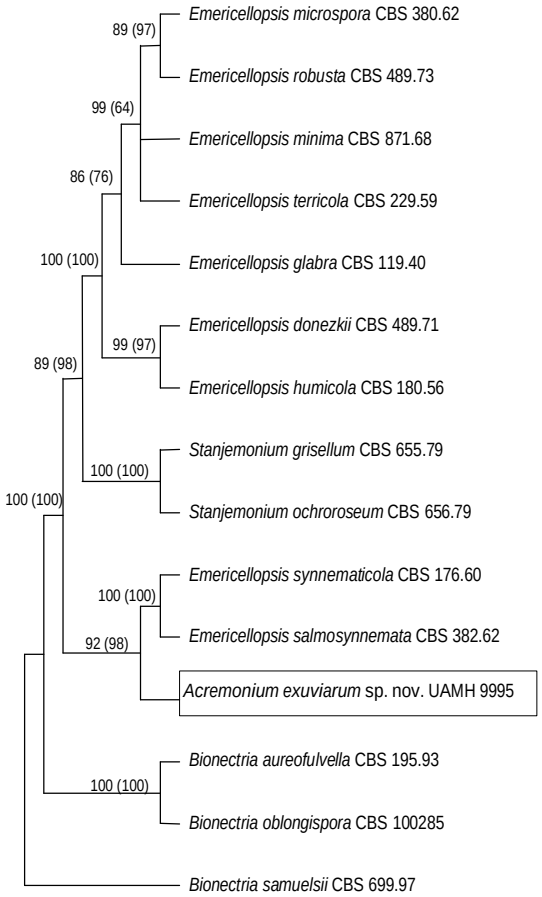


Fig 1. The relationship of *Acremonium exuviarum* sp. nov with *Emericellopsis* based upon combined nuclear ribosomal internal transcribed spacer (ITS)-5.8S and β-tubulin sequence analysis. Strict consensus cladogram of two most parsimonious trees of 351 steps. Bootstrap values are shown above the branches with quartet puzzling supports given in brackets.

Separate maximum parsimony analyses of the ITS and β-tubulin genes generated similar topological trees, but did not resolve the placement of some species (data not shown). Partition homogeneity tests between ITS1 and ITS2, and between the whole ITS region and the β-tubulin gene, revealed no significant differences (P = 0.3 and P = 0.37 respectively) between the distributions of tree lengths, allowing these datasets to be combined. Maximum parsimony analysis of the combined dataset generated two most parsimonious trees of length 351 steps (confidence index [CI] = 0.821, retention index [RI] = 0.867, rescaled consistency index [RC] = 0.711 and homoplasy index [HI] = 0.179; parsimony-informative characters [PIC] = 180) from a single tree-island. Figure 1 shows the strict consensus of these two trees and the bootstrap support values for each node. A consensus tree of similar topology was generated after ML-quartet puzzling analysis. This analysis estimated the relative rates for the two genetic regions (0.733 for the ITS and 1.4 for the β-tubulin gene) so that any rate hetero-

geneity between them could be taken into account. In both analyses, *A. exuviarum* formed a strongly supported clade with *E. synnematicola* P.N. Mathur & Thirum. and *E. salmosynnemata* Grosklags & Swift (92 % bootstrap support and 98 % quartet puzzling support).

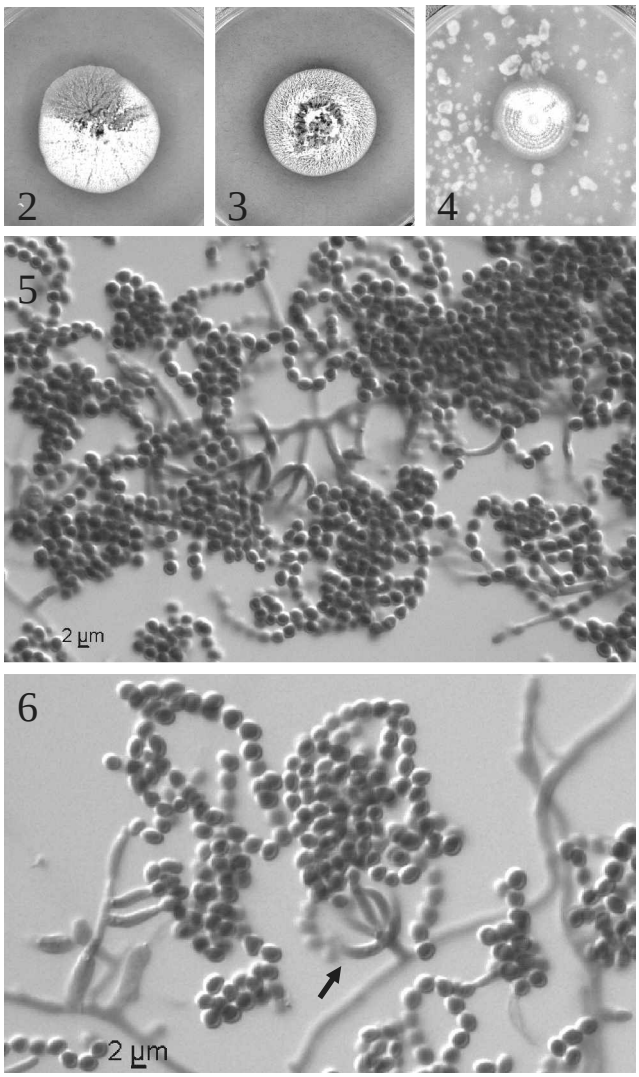
Acremonium exuviarum Sigler, Zuccaro, Summerbell & Paré, **sp. nov.** MycoBank MB500101. Figs 2–8.

Etymology: *exuvia* (L) referring to cast off skins.

Coloniae in agaro moderatim rapide crescunt 25–30 °C, cremeae vel pallide aurantiacae, sulcatae et elevatae, vel planae et zonatae, saepe partes texturae variae formantes, pars aversa pallide grisea; incrementum tardum ad 37 °C. Hyphae hyalinae, septatae, leves, angustae, 0.5–2 µm latae, interdum fila formantes. Phialides simplices vel (2–)3(–4) verticillatae in conidiophoro brevi non distincto; tenuiter curvatae, 3.5–20(–40) × 1.3–3 µm; cellulae terminales postea collabuntur. Conidia catenis vel capitulis mucidis connexa, hyalina, levia, ovoidea vel subglobosa, 3–4 × 2–3 µm. Chlamydosporae absunt; teleomorphosis ignota.

Holotypus: Colonia exsiccata (UAMH 9995); cultura viva (UAMH 9995; CBS 113360).

Colonies on PDA after 10 d at 25 and 30 °C similar, 3.2–3.5 cm diam, yellowish white (4A2) to orange-white (6B2), slightly elevated at the center, with few to many radial folds, felty, finely zonate, sometimes forming sectors of white funiculose hyphae or glabrous, reverse pale grey, lacking diffusible pigments. Growth slower at 22 °C (2.8 cm diam on PDA; 2 cm on 2 % MEA). Colonies on 2 % MEA thin, hyaline. On OAT, colonies after 10 d at 25°C 2.5 cm diam, flat, zonate, powdery, heavily sporulating, pale orange (5A3) in the centre, becoming thin, orange white (5A2) at the margin; colonies at 22 °C thinner, not zonate. Growth strongly inhibited at 35 °C (5 mm after 14 d on PDA). Good growth on medium with cycloheximide. *Hyphae* in slide culture preparations (as per Kane *et al.* 1997) are hyaline, septate, smooth, narrow, 0.5–2 µm wide, sometimes aggregating in strands. *Conidiogenous cells* are phialides attached singly on the mycelium or borne in verticils of 2–4 (commonly 3) on short undifferentiated conidiophores. *Phialides* are cylindrical, slightly tapered at the tip, without visible collarettes, often slightly curved especially when borne in verticils, 3.5–20(–40) µm long (av. 9.2 µm) and 1.3–3 µm wide (av. 1.7 µm); terminal cells sometimes collapsing.



Figs 2–4. Colonies of *Acremonium exuviarum* (UAMH 9995) after 10 d. 2. PDA 25 ºC. 3. PDA 30 ºC. 4. OAT 25 ºC. 5-6. Conidia formed in chains. Note phialides are single or borne in verticils (arrow) of (2–)3(–4) on short undifferentiated conidiophores. Preparations are stained with lactofuchsin. Scale bar = 2 µm.

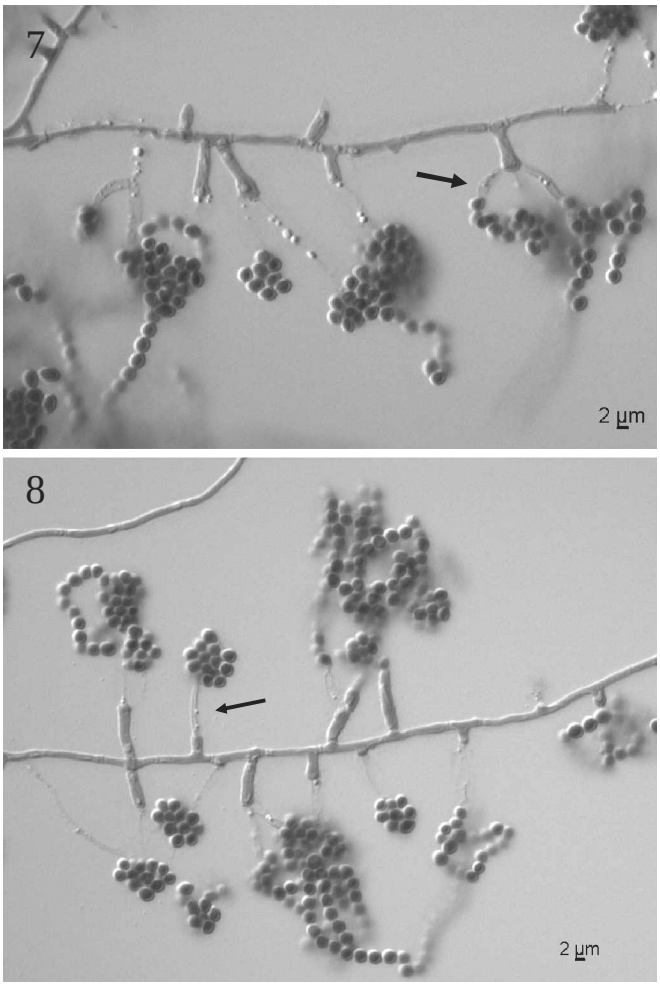
Conidia are formed in loose chains which may collapse into heads and are hyaline, smooth, ovoid to subglobose, 3–4 × 2–3 µm. *Chlamydospores* and teleomorph not observed.

DISCUSSION

The morphological features of *A. exuviarum* are unusual among members of the *Emericellopsis* clade. Conidia are formed in loose chains and phialides appear to collapse in age. Collapsing conidiogenous cells are described for *Stanjemonium* species, but these cells produce a single conidium and collapse soon after this conidium is mature. This may be an adaptation to limited availability of free moisture in the desert or semi-desert soils from which most *Stanjemonium* isolates have been collected. Consideration was given to placing our new taxon in *Stanjemonium*,

but this was rejected on both morphological (multiconidial vs. uniconidial phialides) and phylogenetic grounds. The *Stanjemonium* species grouped in a subclade well separated from the branch bearing *A. exuviarum*. Based on a broad analysis of *Emericellopsis* and related anamorphs, Zuccaro *et al.* (2004) have argued that *Stanjemonium* could either be taken up for all named anamorphs in the *Emericellopsis* clade, or it could be one of four genera accommodating anamorphs within the clade. We follow their decision to retain *Acremonium* for most anamorph species with affinities to *Emericellopsis*. The occurrence of collapsing phialides in *A. exuviarum*, however, provides support for their observation that these may not be distinctive at the generic level.

Acremonium exuviarum is the only member of the *Emericellopsis* clade known to produce conidia in chains. The production of catenulate conidia by a single member of this clade support the suggestion by Gams (1971) that this feature has little or no taxonomic significance above the species level in the hypocrealean species that comprise the bulk of the genus *Acremonium*.



Figs 7, **8.** *Acremonium exuviarum* (UAMH 9995) in slide culture preparation stained with lacto-fuchsin showing disintegration of terminal conidiogenous cells. Scale bar = 2 µm.

This parallels the situation seen in related hypocrealean genera such as *Fusarium* Link (O'Donnell *et al.* 1998), *Lecanicillium* Zare & W. Gams (2001) and *Clonostachys* Corda (Schroers 2001), where very closely related taxa may form conidia either in chains or in mucoid heads.

Acremonium exuviarum is represented only by its type, so little is known about its ecology. *Emericellopsis* species are traditionally considered to be soil inhabitants; however recent work has indicated that these organisms are associated with a range of substrates, including brown algae (Zuccaro *et al*. 2003, 2004) and wood (Lai Ka Pang, pers. comm.). As shown by Zuccaro *et al*. (2004), the terrestrial species of *Emericellopsis* tested so far express cellulases and polyphenoloxidase, suggesting a potential to be involved in plant and wood decay. Although the expression of cellulase was not tested in the same manner for *A. exuviarum*, this fungus was slightly cellulolytic when grown on OAT overlaid with a cellophane membrane (Carmichael 1961). *Acremonium exuviarum* was isolated from an adult wild-caught female skink in active shed. It is unknown how long the animal had been in captivity at the Steinhart Aquarium in San Francisco. Veterinarians and keepers were requested to sample only from animals that appeared healthy and free of skin lesions and to collect the samples aseptically. The animal may have been exposed to the fungus on material provided for climbing; this often consists of pieces of wood. *Corucia zebrata*, a member of the *Scincidae*, is native to the tropical rain forests of the Solomon Islands where it is arboreal. Large powerful claws and a long prehensile tail enable it to cling to trees and it is often found in trees of old growth forests (de Vosjoli 1993). However, the possibility that *A. exuviarum* simply derived from air spora settled on the lizard's skin cannot be excluded.

The *Emericellopsis* clade is of unusual pharmaceutical significance. It includes significant sources of cephalosporin C and related compounds (Backus & Orpurt 1961) as well as of other metabolites of interest such as bergofungin peptaibols (Berg *et al.* 1999). It is hoped that elucidation of new biodiversity within this group will lead to investigation of the taxa involved for compounds of potential interest in medicine and industry.

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