

Disseminated Mycosis in Veiled Chameleons (*Chamaeleo calypttratus*) Caused by *Chamaeleomyces granulomatis*, a New Fungus Related to *Paecilomyces viridis*[∇]

Lynne Sigler,^{1*} Connie Fe C. Gibas,² Branko Kokotovic,³ and Mads F. Bertelsen⁴

University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden and Medical Microbiology and Immunology, Edmonton, AB, Canada T6G 2E1¹; University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden, Edmonton, AB, Canada T6G 2E1²; Division of Veterinary Diagnostics and Research, National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, DK-1790 Frederiksberg, Denmark³; and Center for Zoo and Wild Animal Health, Copenhagen Zoo, Roskildevej 38, DK-2000 Frederiksberg, Denmark⁴

Received 28 May 2010/Returned for modification 10 June 2010/Accepted 14 July 2010

An outbreak of disseminated granulomatous disease occurred in a group of veiled chameleons (*Chamaeleo calypttratus*) in a zoo collection. An adult female and six offspring developed large granulomas in multiple organs and were euthanized. At necropsy, roughly spherical yellow-to-white nodules 1 to 3 mm in diameter were grossly visible in the liver and other organs. Histopathology revealed fungal elements that were spherical to ovoid in shape, fragments of slender to irregularly swollen hyphae, and occasional conidia produced on phialides. Fungal isolates were initially suspected on the basis of morphology results to represent *Paecilomyces viridis*, a species known only from one outbreak of fatal mycosis in carpet chameleons (*Furcifer lateralis*). Data obtained from morphological studies and from phylogenetic analyses of nuclear ribosomal rRNA (rDNA) sequence data revealed the Danish chameleon isolates to be a related undescribed anamorphic species within the family Clavicipitaceae that includes many insect pathogens. *Chamaeleomyces granulomatis* gen. et sp. nov. is given as the name for the newly described fungus, and *P. viridis* is transferred to the new genus as *Chamaeleomyces viridis* comb. nov. *Chamaeleomyces* species are distinguished by having basally swollen phialides tapering to a narrow neck, conidia in fragile chains, and pale green to greenish-gray colonies. Both species are dimorphic, producing a transitory yeast stage characterized by ovoid-to-subglobose or subcylindrical yeast-like cells. *Chamaeleomyces* species appear to be rare but aggressive pathogens of chameleons.

Disseminated, often fatal, mycoses caused by *Paecilomyces* species have previously been reported in many reptiles, including lizards and crocodiles; *P. lilacinus* and *P. variotii* are the main species involved (14). *Paecilomyces viridis* was first reported in 1964 as the cause of disseminated fungal infection in 4 of 50 carpet chameleons (*Furcifer lateralis*; formerly *Chamaeleo lateralis*) that were sent from Madagascar to Paris for experimental use (16, 17). The fungus was isolated from blood and multiple organs, including liver and spleen, and it produced yeast-like cells *in vivo* but not *in vitro*. There have been no subsequent reports of *P. viridis* causing mycosis in any reptile, and two reports of human infection have not been well substantiated and probably concerned infections by *P. variotii* (4). A recent outbreak of disseminated granulomatous disease in a group of veiled chameleons (*Chamaeleo calypttratus*) housed at the Copenhagen Zoo yielded fungal isolates with morphologies resembling those of *P. viridis*. However, a DNA sequence from the internal transcribed spacer (ITS) region of the nuclear ribosomal rRNA (rDNA) gene from one isolate showed only 94% similarity to that of a *P. viridis* sequence obtained from GenBank (AY624194, strain CBS 348.65, ex-type culture). This result prompted further investigation into

the relationship between *P. viridis* and the new chameleon isolates and other *Paecilomyces* species.

Recent taxonomic studies have shown the genus *Paecilomyces* to be polyphyletic. The type species, *P. variotii*, is placed within the ascomycete family Trichocomaceae (Eurotiales) (12). Many other *Paecilomyces* species, including the pathogenic species *P. lilacinus* and *P. viridis*, group within the family Clavicipitaceae *sensu lato* (Hypocreales), comprising many arthropod- and grass-associated fungi (7, 13, 14). Some of these *Paecilomyces* species have been reclassified in the genus *Isaria*, but *P. lilacinus* and *P. viridis* are unrelated to *Isaria* species and their relationships to other members of the family have not been resolved (7, 14). In a recent multigene phylogenetic study, clavicipitalean fungi were reclassified into three families, namely, Clavicipitaceae *sensu stricto*, Cordycipitaceae, and Ophiocordycipitaceae, but *P. viridis* was not examined in that study (20).

Data obtained from morphological studies and phylogenetic analyses of rDNA sequences provide evidence that the Danish chameleon isolates represent a new species related to *P. viridis*. Both species are here described in the new genus *Chamaeleomyces* within the family Clavicipitaceae *sensu stricto*.

* Corresponding author. Mailing address: University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden & Medical Microbiology and Immunology, Edmonton, AB, Canada T6G 2E1. Phone: 780-987-4811. Fax: 780-987-4141. E-mail: lynne.sigler@ualberta.ca.

[∇] Published ahead of print on 21 July 2010.

CASE REPORTS

An approximately 2-year-old female veiled chameleon was acquired from a member of the public and maintained with a male in a breeding facility at the Copenhagen Zoo. Twelve

months after its acquisition, the female produced 17 hatchlings. The hatchlings were group housed for 2 months, after which seven were culled. Two additional animals were euthanized at 6 months of age, and two were shipped to another zoo. The remaining six young chameleons were housed in separate vivaria. The temperature was maintained at 22 to 28°C with 45°C in a basking spot, and animals were fed live insects (two-spotted crickets [*Gryllus bimaculatus*], migratory locusts [*Locusta migratoria*], mealworms [*Tenebrio molitor*], greater wax moth larvae [*Galleria mellonella*], house flies [*Musca domestica*], and blue bottle flies [*Calliphora vomitoria*]) as well as finely chopped greens.

At 9 months of age, three of the animals presented with focal raised lesions along the lips. Initial biopsy specimens were nondiagnostic, and as animals showed no signs of discomfort, no treatment was attempted. Over the next couple of months, the lesions grew in size and number, and the three animals were euthanized. After approximately 10 months, the remaining three animals showed similar signs, and were euthanized. The dam died shortly after ovipositioning of a subsequent clutch.

All seven chameleons were subjected to a gross necropsy, and representative samples of major organs were collected in 10% buffered formalin, processed routinely for histopathology, sectioned at 6- μ m intervals, and stained with hematoxylin and eosin as well as methenamine silver and periodic acid-Schiff. Selected tissue samples were collected aseptically for fungal and bacterial culture. Additional skin samples were collected and frozen at -20°C.

Gross postmortem and histological findings were similar for all seven animals; observations are presented as the number of positive findings per number of animals examined (Fig. 1, Fig. 2A; Table 1). Multiple discrete raised lesions were present along the margins of the lips (6/7) (Fig. 1A and B) and within the oral cavity (6/6). Gross joint swelling with loss of bone and cartilage and an increased amount of thick, yellow, opaque fluid was seen in the hip (3/7), elbow (3/7), and carpus (2/7) as well as in intercoccygeal joints (4/7) (Fig. 1C and D). Roughly spherical yellow-white nodules of 1 to 3 mm in diameter were present in the liver (7/7), lung (3/7), and kidney (3/7), but the numbers differed greatly among animals (Fig. 1E to G). Histological findings consisted of granulomas of various chronicities, ranging from clusters of large macrophages to dense fibrous granulomas affecting a range of organs (Fig. 1H). Spherical-to-ovoid fungal elements 2 to 4 μ m in diameter and fragments of slender to irregularly swollen hyphae were identified in all cases (Fig. 1I, 2A). Rarely, conidia borne on phialides were observed. The affected organs included liver (7/7), skin (7/7), oral mucosa (6/6), joint or bone (5/5), muscle (4/4), lung (4/7), intestine (1/5), spleen (1/5), fat body (1/4), kidney (3/6), heart (1/7), and oviduct or ovary (2/4). The adult female stood out in several aspects: the granulomas present were larger and surrounded by more fibrous tissue; there was massive infection of the oviduct as well as ovaria; and the presence of conidia borne on phialides was more commonly observed (Fig. 2A [circle]). Aerobic bacterial cultures of multiple organ samples from multiple animals yielded no growth.

Fungal cultures performed on specimens of lungs, livers, and joints at the National Veterinary Institute, Frederiksberg, Denmark, grew green to greenish-gray colored molds from five

animals. Isolates were referred to the University of Alberta Microfungus Collection and Herbarium (UAMH), Edmonton, Canada, for identification.

MATERIALS AND METHODS

Five isolates having *Paecilomyces*-like morphologies were deposited in UAMH (Table 1). Two isolates from lung specimens were identified as *Penicillium* species and excluded as contaminants. Colonial features and growth rates were evaluated using single-point inoculation on plates with potato dextrose agar (PDA; BD Diagnostic Systems, Sparks, MD) incubated at 30°C and 35°C for 14 days. The presence of a yeast-like stage was assessed by streaking a single colony onto a PDA plate incubated at 35°C and by examining colonies under a dissecting microscope daily for 4 days. Moist yeast-like colonies were restreaked to new plates as required. Tolerance to cycloheximide was determined by comparing growth on mycosel agar containing cycloheximide (BD) (400 μ g⁻¹) with that seen on phytone yeast extract agar (BD) lacking cycloheximide. Color terms and codes are derived from the color standards of Kornerup and Wanscher (9). Micromorphological features were determined from slide culture preparations by the use of cereal agar (18) as the sporulation medium and incubation for 5 to 7 days at 25°C. The same methods were used to study *P. viridis* UAMH 2994, an authentic isolate received from the Pasteur Institute as isolate no. 849 from G. L. G. Segretain, which was revived from freeze-dried stock material.

Sequences of the ITS and partial large subunit (LSU) regions were obtained for three chameleon isolates (UAMH 11028, 11176, and 11178) and for UAMH 2994 by employing previously described methods for DNA extraction, amplification, and sequencing (18, 19). Briefly, DNA was extracted using an E.Z.N.A. SP fungal DNA kit (United Bioinformatica Inc., Saskatoon, SK, Canada). The ITS-to-LSU region was amplified with primer pair BMBC-R (10) and LR7, and sequences were obtained for ITS with primers BMBC-R, ITS1, ITS2, and ITS4 (21) and for LSU with primers LROR, LR3R, LR5, LR7, and LR16 [<http://www.biology.duke.edu/fungi/mycolab/primers.htm>]. The partial small subunit (SSU) region for UAMH 11028 was amplified using primer pair NS1 and NS8 and sequenced with forward primers NS11mun, NS13mun, and NS15mun and reverse primers NS2, NS4, and NS6 as previously described (19). PCR mixtures were subjected to 30 cycles on a Perkin Elmer GeneAmp thermal cycler (Applied Biosystems, Foster City, CA). Cycle sequencing was done using a BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems) and run on an ABI 377 prism automated sequencer (Applied Biosystems). Sequences were edited using Sequencher version 4.8 software (Gene Codes Corp., Ann Arbor, MI). Data matrices were assembled from members of the family Clavicipitaceae sensu lato based on the results of BLAST searches and recent literature (3, 12, 20) and manually aligned using the Sequence Alignment Editor sequence alignment program (Se-Al, version 2.0a11; Department of Zoology, University of Oxford, United Kingdom [<http://tree.bio.ed.ac.uk/software/seal/>]). Maximum parsimony analyses were performed using PAUP software (version 4.0b10; Sinauer Associates, Inc., Sunderland MA [<http://paup.csit.fsu.edu>]), with gaps treated as missing data. Robustness of trees was determined by the bootstrap (BS) method, using a full heuristic search with 100 resamplings for ITS and a fast heuristic search with 1,000 resamplings for LSU and SSU. Bayesian analysis was conducted using MrBayes (version 3.1.2; Department of Scientific Computing, Florida State University, Tallahassee, FL [<http://mrbayes.csit.fsu.edu/download.php>]), based on the Markov chain-Monte Carlo method (6) and assuming a discrete gamma distribution (GTR + I + G) model. Four Markov chains were run simultaneously, and trees were sampled every 100 out of 2 million generations. The first 3,000 trees for the ITS analysis, 6,000 trees for the LSU analysis, and 4,000 trees for the SSU analysis were discarded as representing a burn-in period, and inferences of posterior probabilities (PP) were calculated from 17,001, 14,001, and 16,001 trees, respectively. The resulting trees were imported for PAUP analysis, and a 50% majority rule consensus tree was calculated. Clades that were supported by BS values \geq 70% and PP values \geq 95% were recorded. GenBank accession numbers were obtained for isolates newly sequenced.

Nucleotide sequence accession numbers. ITS sequences for *C. granulomatis* were deposited in GenBank under accession no. HM195305 (UAMH 11028), HM195306 (UAMH 11176), and HM195307 (UAMH 11178) and for *C. viridis* under accession no. HM195308 (UAMH 2994). Partial LSU sequences were deposited under accession no. HM195304 (UAMH 11028), HM635078 (UAMH 11176), HM635077 (UAMH 11178), and HM635079 (UAMH 2994). The SSU sequence was deposited under accession no. HM635076 (UAMH 11028).

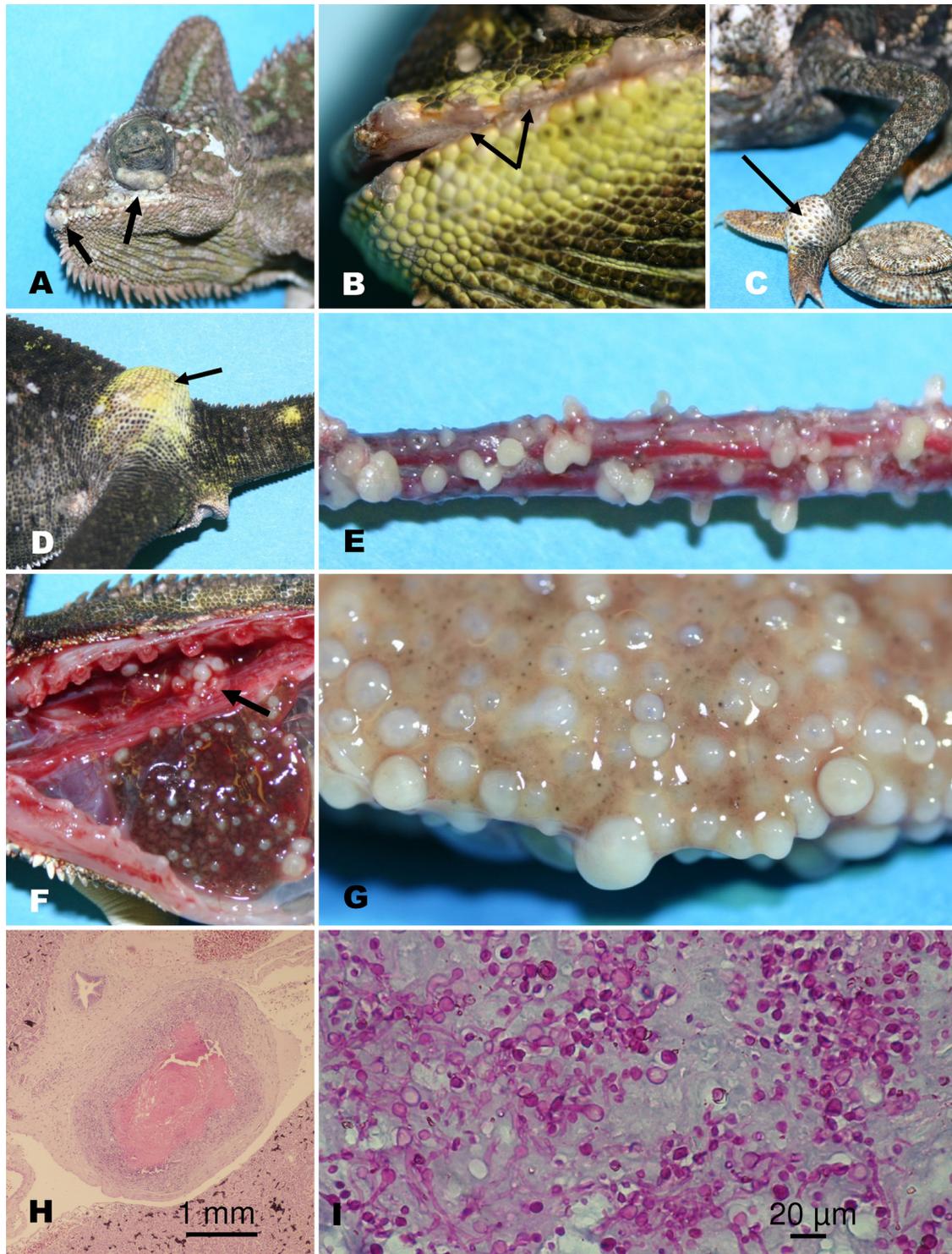


FIG. 1. Gross and histopathological appearance of lesions in veiled chameleons. (A and B) Raised lesions along margins of lips and under eye (arrows). (C) Gross swelling of carpus (arrow). (D) Grossly swollen hip joint (arrow). (E) Multiple granulomas on tongue (arrow) and liver. (F) Granulomas in lung (arrow) and liver. (G) Multiple large granulomas in liver. (H) Hematoxylin-and-eosin-stained subgross view of granuloma on surface of liver. Bar, 1 mm. (I) Periodic acid-Schiff-stained section showing numerous irregularly swollen hyphae and ovoid cells inside a granuloma in the liver. Bar, 20 μ m.

RESULTS

Colony growth rates and pigmentation and expression of a yeast-like stage differed between the Danish chameleon iso-

lates and *P. viridis* (Fig. 2B to G). The Danish isolates grew faster at 30°C and showed restricted growth at 35°C. Colonies were greenish gray (30B2 to 30B3) and velvety to slightly cottony near the center and usually produced amber exudate

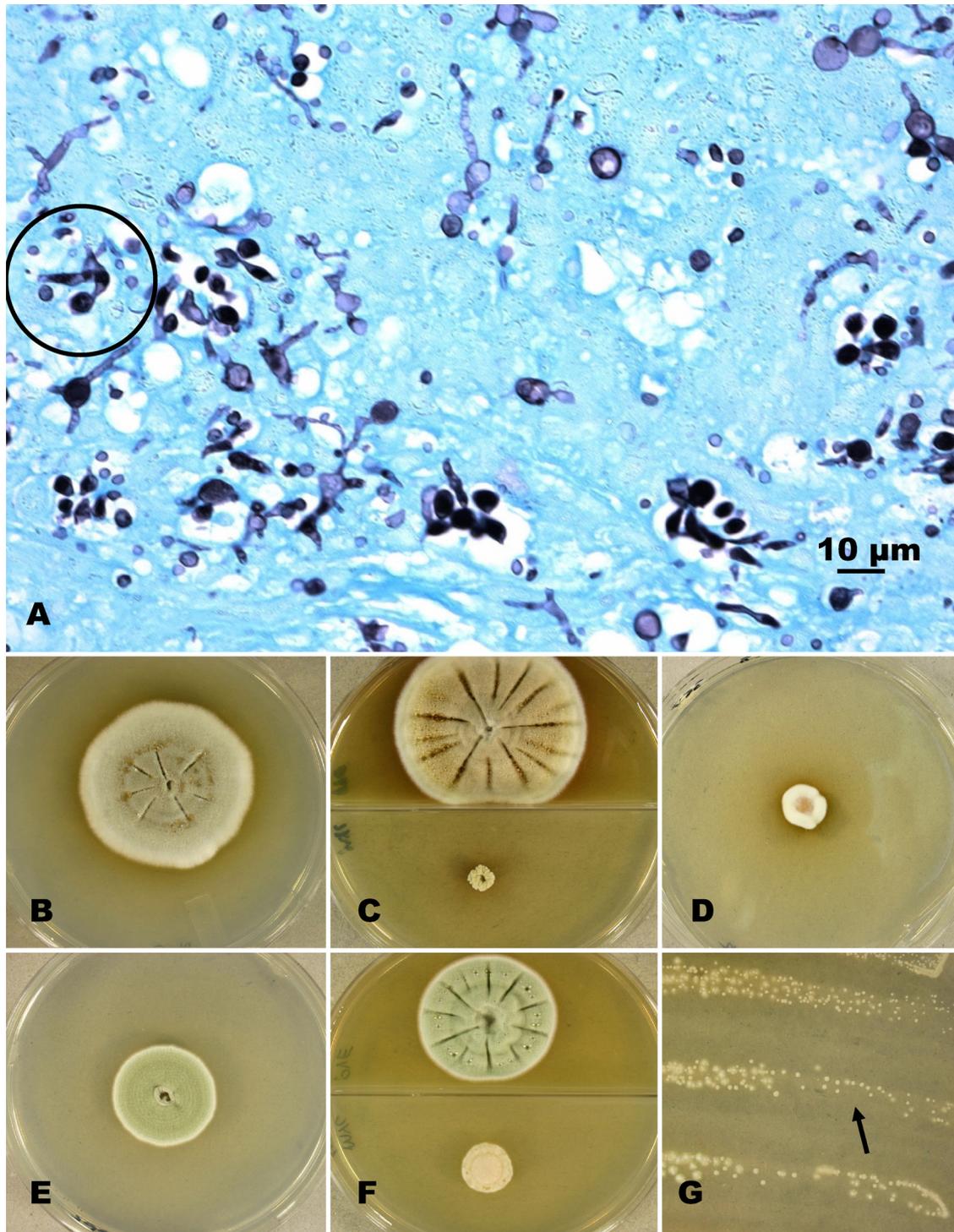


FIG. 2. (A) Methanamine silver-stained section of liver showing irregularly swollen hyphae, ovoid cells, and a putative phialide and conidium (circle). Bar, 10 μ m. (B to D) Colony of *Chamaeleomyces granulomatis* UAMH 11028 ex-type strain after 14 days of incubation. (B) Colony shown on PDA at 30°C. (C) Colonies shown on phytone-yeast extract agar (top) and mycosel agar (bottom). (D) Colony shown on PDA at 35°C. (E and F) Colonies of *Chamaeleomyces viridis* UAMH 2994 after 14 days of incubation. (E) Colony shown on PDA at 30°C. (F) Colony shown on phytone-yeast extract agar (top) and mycosel agar (bottom). (G) Moist, yeast-like colonies of *C. granulomatis* UAMH 11028 produced on PDA after 3 days of incubation at 35°C (arrow).

droplets and diffusible pigment. The colony of isolate UAMH 11178, derived from the dam animal (Table 1), was paler, yellowish white (4A2) to yellowish beige (5B2), and cottony to wooly. *P. viridis* grew more slowly at 30°C and showed no

growth at 35°C. The colony was grayish green (30C3 to 30C4), powdery and slightly zonate, and lacked diffusible pigment (Fig. 2E). All isolates grew poorly on medium containing cycloheximide (Fig. 2C and F). Three Danish chameleon isolates

TABLE 1. Summary of pathology and fungus culture results for veiled chameleons with multifocal granulomata

Animal PM no. ^a	Gender, age, wt ^b	Macroscopic granuloma result					Fungal hyphae histology result	Fungal culture	
		Lip	Liver	Lung	Kidney	Joint		Result (site)	UAMH no. ^d
z-17-08	F, 9 mo, 27 g	+	+	–	–	+	+	+ (lung)	(<i>Penicillium</i> sp.)
z-21-08	M, 9 mo, 24 g	+	+	–	–	–	+	ND ^c	
z-24-08	F, 9 mo, 44 g	+	+	+	–	+	+	+ (liver)	11176
z-94-08 ^c	F, ≥2 yr 6 mo, 60 g	–	+	+	–	–	+	+ (liver)	11178
z-102-08	M, 1 yr 7 mo, 60 g	+	+	–	+	+	+	ND	
z-15-09	M, 1 yr 10 mo, 63 g	+	+	–	+	+	+	+ (liver, joint)	11028, 11029
z-68-09	F, 2 yr 4 mo, 56 g	+	+	+	+	+	+	+ (liver, lung)	11177 (<i>Penicillium</i> sp.)

^a Postmortem identification number.

^b F, female; M, male.

^c ND, culture not done.

^d Isolates accessioned in University of Alberta Microfungus Collection and Herbarium, Edmonton, AB, Canada (UAMH); isolates in parentheses were considered contaminants and not accessed.

^e Mother of all other animals; exact age unknown.

(UAMH 11028, 11176, and 11177) demonstrated a mixture of mycelial and moist, yeast-like colonies after 3 to 4 days when streaked on PDA plates incubated at 35°C (Fig. 2G). The yeast-like growth was transitory and could be enhanced by further subculture, but colonies rapidly became mycelial upon further incubation. The more cottony isolate, UAMH 11178, remained mycelial under these conditions. The *P. viridis* isolate was restreaked several times and produced only one strongly restricted colony that demonstrated a yeast-like micromorphology. Most isolates, except UAMH 11178, also produced a mixture of yeast-like cells and conidiating mycelia in slide cultures incubated at 25°C.

Microscopically, the Danish chameleon isolates were similar to *P. viridis* but differed in conidial sizes, in the arrangements of the phialides, and in the shapes and sizes of the yeast-like cells. Conidia of the former were produced in short, sometimes curved, fragile chains and were smooth, subglobose, and sometimes wider than they were long, measuring 3 to 6.4 μm in length and 3 to 4.2 μm in width. Phialides were straight or slightly curved, were solitary or arranged in whorls of two to three, and had a single opening (monophialides) (Fig. 3A). They were inflated at the base and tapered to a narrow neck. The more cottony isolate (UAMH 11178) was atypical in producing some longer, more cylindrical phialides, conidia that were ovoid to subglobose (Fig. 3B), and helically coiled hyphae that were not present in any other isolate grown under the same conditions (Fig. 3C). The yeast-like growth consisted of ovoid to subcylindrical cells measuring 7 to 15 μm in length by 2.5 to 4.7 μm in width and producing blastic conidia at one end from a broadly tapered neck (Fig. 3D). The phialides of *P. viridis* were occasionally solitary but were more commonly arranged in verticils, and the conidia were smaller, measuring 3.2 to 4 μm in length by 2.3 to 3.2 μm in width (Fig. 4A). The yeast-like cells were ovoid to subglobose with a narrow neck and measured 4.6 to 6 μm in length by 3 to 3.5 μm in width (Fig. 4B). Some narrow undulate hyphae were occasionally present (Fig. 4C).

In the ITS analysis, the Danish chameleon isolates and *P. viridis* were placed in two subclades within a strongly supported clade (BS, 85; PP, 98) (Fig. 5, dark-gray-shaded box) that was distinct from all other groups (Fig. 5). The chameleon fungi grouped among species of the family Clavicipitaceae sensu stricto (Fig. 5, light-gray-shaded box) as described by Sung

et al. (20), but the Clavicipitaceae grouping was supported only in the Bayesian analysis (PP 100). The chameleon fungi were distinct from all species currently or formerly classified in *Paecilomyces*. The ITS data set included 45 taxa and comprised 696 characters, of which 267 were constant, 305 were parsimony informative, and 124 were non-parsimony informative. The ITS sequence for *P. viridis* was 543 nucleotides (nt), whereas those of the Danish chameleon isolates were 516 nt and identical, except for that of isolate UAMH 11178, which differed at one position.

Sequences of the LSU and SSU regions are highly conserved within this group of fungi, so few groupings received strong evidentiary support. The LSU analysis yielded results similar to the ITS data with respect to placement of the chameleon fungi among species of the Clavicipitaceae (Fig. 6, box with gray shading), but the internal nodes were poorly resolved and the grouping of the chameleon fungi received low support (61 PP). The LSU data set included 57 taxa and 1,446 characters, of which 1,099 were constant, 276 were parsimony informative, and 71 were non-parsimony informative. In the SSU analysis, the Danish chameleon fungus was in a separate lineage but its relationship to the other species was not resolved (Fig. 7). The LSU sequences were 1,269 nt for *P. viridis* and 1,350 nt and identical among the Danish chameleon fungi. The SSU sequence was 1,766 nt for isolate UAMH 11028 and identical to the sequence of *P. viridis* obtained from GenBank (AB023949, ex-type strain CBS 348.65). The SSU data set comprised 1,697 characters, of which 1,502 were constant, 121 were parsimony informative, and 74 were non-parsimony informative.

TAXONOMY

Chamaeleomyces Sigler gen. nov. Mycobank MB 518408. Etymology: associated with chameleons. Coloniae pallide virides vel griseo-virides. Hyphae hyalinae, angustae, septatae, ramosae. Conidiophora simplicia vel complexa; phialides solitariae aut verticillatae, monophialidicae, hyalinae, ad basim inflatae, raro cylindrica, ad apicem angustatae, collaria absunt. Conidia in catenis, hyalina vel pallide viridia vel olivaceo-grisea (in massa), levia, subglobosa vel ovoidea. Chlamydo-sporae absunt. Singillatim dimorphicae, producentes cellulas subglobosas ad ovoideas vel subcylindricas et instar fermenti.

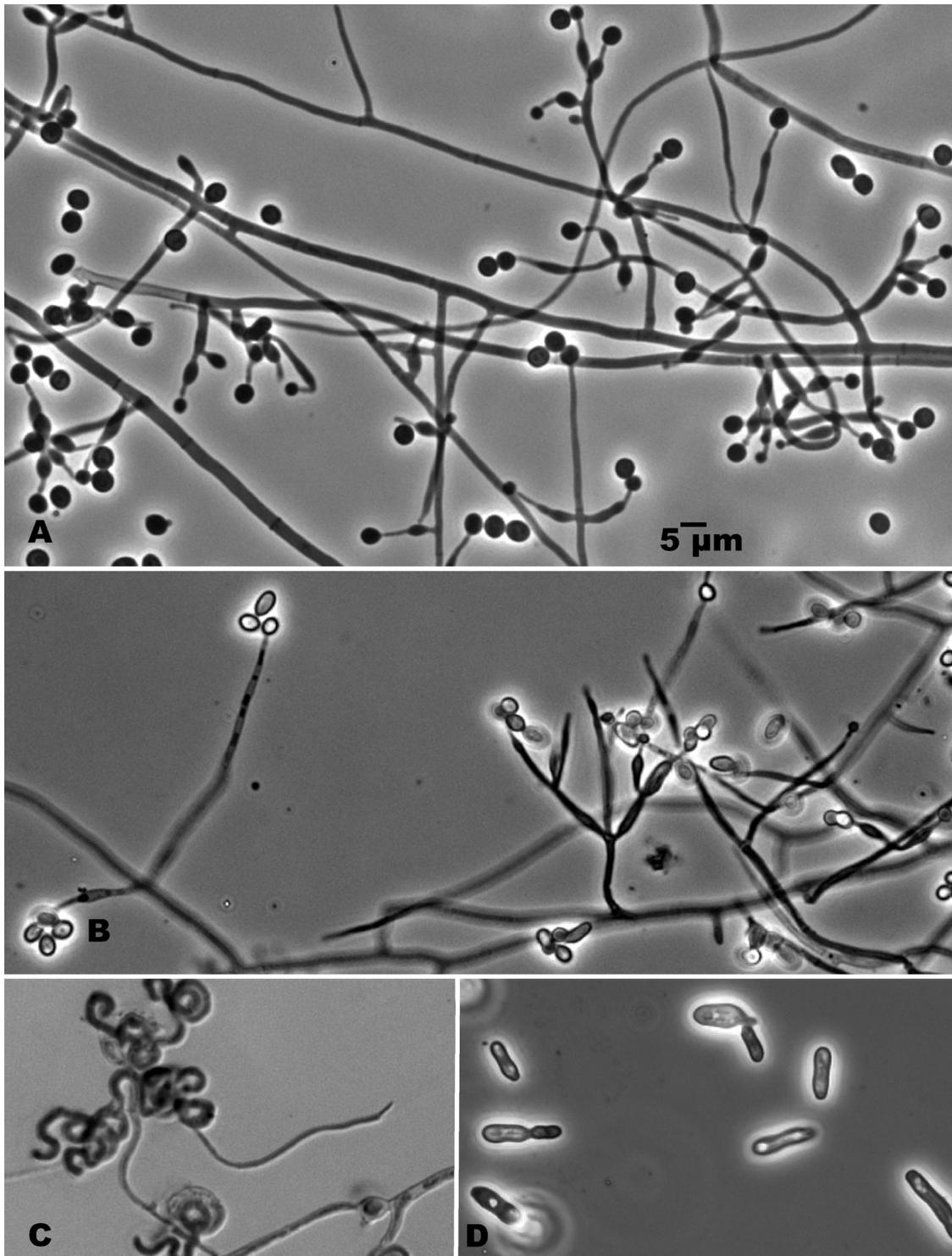


FIG. 3. (A to D) Microscopic morphology of *Chamaeleomyces granulomatis*. (A) Slide culture preparation showing basally swollen phialides and subglobose conidia in short chains (UAMH 11028). (B) Longer phialide and ovoidal conidia of the more cottony UAMH 11178 isolate. (C) Helically coiled hyphae produced by isolate UAMH 11178. (D) Yeast-like cells (also known as hyphal bodies) produced on PDA after 3 days at 35°C (UAMH 11028). Bar, 5 μ m (all panels).

Teleomorphosis ignota. Affinitas generis anamorphosis; Ascomycota; ordo Hypocreales, familia Clavicipitaceae. Species typica: *Chamaeleomyces granulomatis* Sigler.

Colonies are pale green to grayish-green. Hyphae are hya-

line, narrow, septate, branched. Conidiophores are simple or complex; phialides are solitary or verticillate, monophialidic, hyaline, inflated at the base, rarely cylindrical, tapering to a narrow tip, and lacking a collarette. Conidia are in chains and

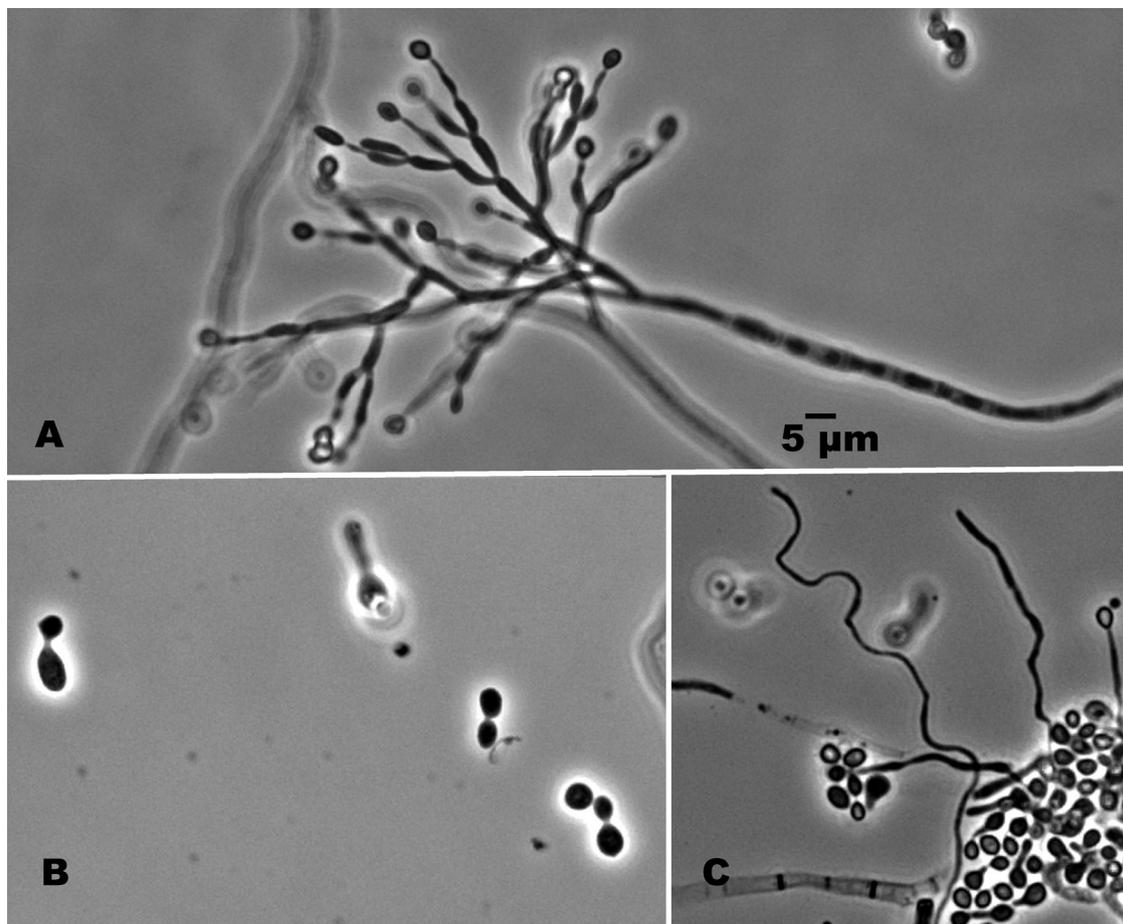


FIG. 4. (A to C) Microscopic morphology of *Chamaeleomyces viridis* (UAMH 2994). (A) Phialides arranged in verticils produced in a slide culture preparation after 7 days at 30°C. (B) Yeast-like cells produced on PDA after 3 days at 35°C. (C) Conidia, yeast-like cells, and undulate hyphae after 20 days at 30°C in slide culture preparation. Bar, 5 µm (all panels).

are hyaline to pale green or olivaceous gray (in mass), smooth, subglobose to ovoid. Chlamydo spores are absent. Isolates are dimorphic, producing subglobose to ovoid or subcylindrical yeast-like cells. Teleomorphs are unknown. *Chamaeleomyces* is an anamorphic genus placed within the order Hypocreales, family Clavicipitaceae. Type species: *Chamaeleomyces granulomatis* Sigler.

***Chamaeleomyces granulomatis* Sigler sp. nov.** Mycobank MB 518409. Etymology: causing granulomatous disease in chameleons. Coloniae moderatim celeriter crescentes ad 30°C, griseo-virides, planae vel sulcatae, velutinae vel lanosae. Incrementum tardum prope 35°C in agaro cum cycloheximido. Phialides monophialidicae, solitariae vel dupliciter vel tripliciter verticillatae, interdum aliquando curvae, ad basim inflatae, raro cylindricae, 5 ad 15 µm longae, 1.5 ad 2.7 µm apud basim, et 0.5 ad 1 µm apud apicem. Conidia in catenis brevibus et fragilibus et aliquando curvis, hyalina vel olivaceo-grisea (in massa), subglobosa vel ellipsoidea, 3 ad 6.4 µm longa et 3 ad 4.2 µm crassa. Cellulae instar fermenti dimorphicae, subcylindricae vel ovoideae, 7 ad 15 µm longae et 2.5 ad 4.7 µm crassae. Ascumata et chlamydo sporae absunt.

Colonies are moderately fast growing, 4.5 to 5 cm in diameter after 14 days at 30°C, greenish gray (30B2 to 30B3), flat to furrowed, velvety to somewhat cottony, often producing amber

exudate droplets and diffusing pigment. Growth is slow at 35°C and on medium with cycloheximide. Phialides have a single opening (monophialides) and are solitary or arranged in whorls of two to three. They are sometimes slightly curved, swollen at the base, tapering to a narrow neck, and measuring 5 to 15 µm in length, 1.5 to 2.7 µm in width at the base, and 0.5 to 1 µm in width at the tip. Conidia are in short, sometimes curved, fragile chains. They are hyaline to olivaceous gray in mass, smooth, usually subglobose, sometimes wider than they are long, and measure 3 to 6.4 µm in length and 3 to 4.2 µm in width. Rarely, longer (up to 40-µm) cylindrical phialides, ovoid to subglobose conidia measuring 2.4 to 6 µm in length by 1.9 to 3.2 µm in width, and helically coiled hyphae are produced. Yeast-like cells are subcylindrical or ovoid, 7 to 15 µm in length by 2.5 to 4.7 µm in width. Ascumata and chlamydo spores are absent.

Holotype. UAMH 11028, isolated from a specimen from the liver of a male veiled chameleon, Copenhagen, Denmark, is preserved as a dried colony and living culture.

***Chamaeleomyces viridis* (Segretain et al. ex Samson) Sigler comb. nov.** Mycobank MB 518410. Basionym: *Paecilomyces viridis* Segretain et al. ex Samson, Stud. Mycol. 6:64, 1974.

The original description and illustrations are available online under accession no. MB319126 (<http://www.mycobank.org/mycotaxo.aspx>).

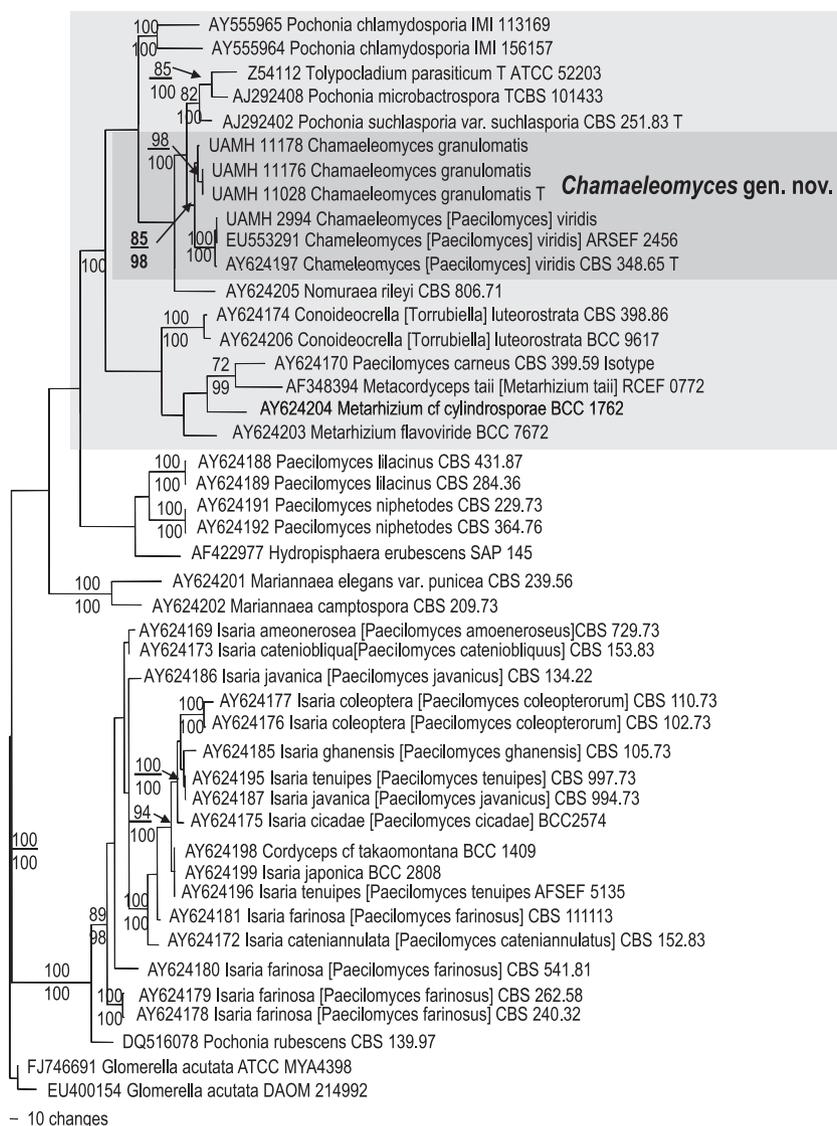


FIG. 5. One of 10 equally parsimonious trees inferred from maximum parsimony analysis of ITS rDNA sequences, showing the placement of *Chamaeleomyces* species (dark-gray-shaded area) within the family Clavicipitaceae (light-gray-shaded box). The indices for the phylogenetic tree were a consistency index (CI) of 0.499, a retention index (RI) of 0.723, and a homoplasy index (HI) of 0.501. Numbers above branches are bootstrap values (BP ≥ 70%); numbers below branches are posterior probability values (≥95%) from Bayesian analysis. GenBank accession numbers and culture collection numbers are shown for each isolate where available. T, ex-type culture.

DISCUSSION

Chamaeleomyces species appear to be very rare but aggressive pathogens of chameleons. Infections by both *C. granulomatis* and *C. viridis* are notable for their production of granulomatous lesions at multiple sites, with particular involvement of the liver. In the *C. granulomatis* outbreak, animals were raised in captivity, and the progression of infection was insidious. Lesions were first noted in three siblings after 9 months, but the remaining siblings developed symptoms over another 10-month period. The juvenile chameleons were euthanized, and only the dam died spontaneously, but the disease was at a very advanced stage in several of the juveniles. Disseminated mycosis was diagnosed at necropsy, when histopathology assays revealed the presence of ovoidal yeast-like cells and hy-

phal fragments within the abundant granulomata. Relatively often, the presence of a conidium borne on a phialide could be observed (Fig. 2A, circle). In the case of *C. viridis*, infection had a rapid onset and involved wild-caught animals (17). Four of 50 chameleons from Madagascar sent by air to the Institut Pasteur in Paris became acutely emaciated, with three dying within 3 to 5 weeks of arrival. The animals were being used experimentally for passage of the parasite *Trypanosoma thezieni*, but 2 of the 4 had not yet been inoculated. The mycosis was first revealed by the presence of abundant yeast cells in blood smears and confirmed by histopathology of nodules revealing the presence of yeast cells and filaments of various widths, by isolation of the fungus from blood and organs of all four animals, and by experimental infection in chameleons

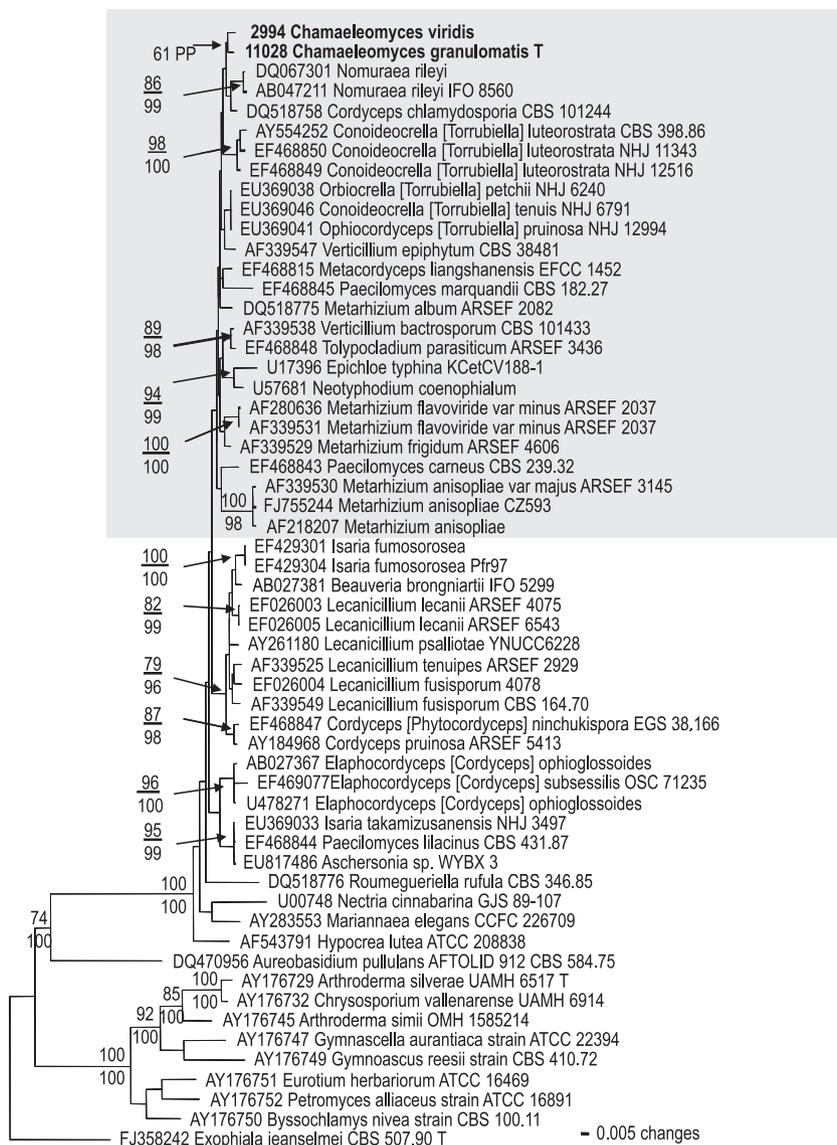


FIG. 6. One of 842 equally parsimonious trees (CI, 0.529; RI, 0.471; HI, 0.795) inferred from maximum parsimony analysis of partial large LSU rDNA sequences, showing the placement of *Chamaeleomyces* species within the Clavicipitaceae family (shaded box). Bootstrap values $\geq 70\%$ and posterior probability values $\geq 95\%$ are shown. GenBank accession numbers and culture collection numbers are shown. T, ex-type culture.

following intraperitoneal inoculation of blood or spores. Experimental infection was also obtained in a frog, but not in a mouse, by intraperitoneal inoculation.

The source of the infection in either outbreak is not clear. Segretain et al. (17) were unable to isolate *C. viridis* from food (flies or maggots) or from cages in which the animals were housed, and they suggested the possibility of either airborne exposure or intestinal invasion based on histologic evidence of yeast cells and filaments in lung and intestinal mucosa. In the case of *C. granulomatis*, only some animals developed lung lesions, suggesting that this was not the primary route of invasion. We suggest that the most likely source of infection was ingestion of infected insects (see further discussion on clavicipitalean fungi below) and intestinal invasion, followed by spread to the liver. We speculate that the dam was the first infected, that she harbored the fungus for some time, and that

the infection was transmitted to the developing eggs, which, once laid, were housed in a separate facility. The massive presence of fungal organisms in the ovary and oviduct of this animal supports this theory. The isolate (UAMH 11178) obtained from the liver of the dam expressed slightly different morphologies, including yellowish-white colonies, more elongate phialides, and the absence of a yeast stage, suggesting that the altered morphology may have resulted from host adaptation over a longer period. The ITS sequence for this isolate differed by only one nucleotide from the other *C. granulomatis* sequences, thus suggesting that intraspecific differences were not a factor, but sampling of isolates from other cohorts is needed to evaluate this finding. The exact age and provenance of the founder animal is unknown, because it was donated by a private collector to another zoo shortly prior to transfer to the collection at hand. Cutaneous lesions developed in all six



FIG. 7. One of 2 equally parsimonious trees (CI, 0.654; RI, 0.707; HI, 0.3646) inferred from maximum parsimony analysis of small subunit rDNA sequences, showing the placement of *Chamaeleomyces granulomatis* within the family Clavicipitaceae sensu lato (Hypocreales). Bootstrap values $\geq 70\%$ and posterior probability values $\geq 95\%$ are shown. GenBank accession numbers and culture collection numbers are shown. T, ex-type culture.

siblings and may represent a possible route of infection. However, this is purely speculative, as all animals were housed separately after the first 2 months, and none was introduced to naive animals.

The nodular granulomatous lesions of *C. granulomatis* are highly distinctive and appear very similar to those observed in captive Jackson's chameleons (*Chamaeleo jacksonii*) in New Zealand (see p. 537 and Fig. 11.68 in reference 14). Ten chameleons died from the infection over a period of 2 to 3 years, and histopathology revealed fungal elements (J. Potter, personal communication). The fungal culture results were mixed, with one isolate identified as a *Paecilomyces* species and others as *Penicillium* species, but *Paecilomyces* species are sometimes mistaken for *Penicillium* species, especially when colonies are green. *Candida* species were also grown. The isolates were not identified to the species level, and no conclusion was reached about which organism was the etiologic agent. However, our examination of two histopathology images kindly sent by J. Potter revealed hyphae of various widths and the presence of a putative phialide and conidium strongly similar to those shown for *C. granulomatis* in Fig. 2A (circle). This finding, plus that of the distinctive granulomata, thus provides evidence that the Jackson's chameleon infections were likely caused by *C. granulomatis*.

A major taxonomic revision based on multigene phylogenetic analyses resulted in the reclassification of *Cordyceps* and other clavicipitalean fungi into three families within the Hypocreales, namely, Clavicipitaceae sensu stricto, Cordycipitaceae,

and Ophiocordycipitaceae, and in the description of several new genera and species (20). Our ITS analysis clearly demonstrates that the two *Chamaeleomyces* species are in a distinct lineage and that they group among species now classified in the Clavicipitaceae (20) (shaded box, Fig. 5 and 6); however, the internal relationships between *Chamaeleomyces* species and other members of the Clavicipitaceae family have not been satisfactorily resolved by ITS, LSU, or SSU analyses. The Clavicipitaceae family includes *Metacordyceps* species with *Metarhizium* and *Pochonia* anamorphs, *Nomuraea rileyi*, *Paecilomyces carneus*, *P. marquandii*, and *Conoideocrella luteostrata* (formerly classified in *Torrubiella*) (8) with a *Paecilomyces cinnamomeous* anamorph. *Metarhizium* species (3) and *N. rileyi* differ from *Chamaeleomyces* species in having cylindrical phialides with short necks and yellowish to olivaceous green fast-growing and pale green slow-growing colonies, respectively. *Pochonia* species have yellowish-white colonies and produce conidia in slimy heads or in chains from slender subulate phialides, and some species develop distinctive dictyochlamydo-spores (22). Most species are entomopathogens, with *Metarhizium* and *N. rileyi* found on Lepidoptera, including moths and butterflies, *Conoideocrella* on Homoptera, including cicadas, plant hoppers, aphids, and scale insects, and *Pochonia* on Coleoptera (beetles), but some species are also common in soil. These fungi infect insects by penetration of the cuticle, followed by multiplication in the hemolymph by formation of yeast-like cells (called hyphal bodies by invertebrate pathologists), and subsequent growth into surrounding tissues by in-

vasive mycelia that kill the insect and emerge from the cadaver, where sporulation occurs. In these fungi, the expression of the yeast-like stage is nutritionally rather than thermally dependent. The elongate and subcylindrical yeast-like cells of *C. granulomatis* were produced both on PDA at 35°C and on cereal agar at 25°C and strongly resembled those described by Pendland and Boucias (15) for *N. rileyi* grown at 26°C on a complex growth medium such as Sabouraud maltose agar with yeast extract. Similarly, previous studies on *C. viridis* determined that the yeast-like growth was stimulated on both complex and defined media in the presence of antibiotic compounds, including azalomycin F, cyanein (brefeldin A), griseofulvin, and monorden (radicol) (1, 2).

Several clavicipitalean fungi have been identified as the cause of mycoses in reptiles. The most common is *Paecilomyces lilacinus*, associated with cutaneous and invasive infections in crocodylians and many other reptiles (14). *Beauveria bassiana* and *Metarhizium anisopliae* have been associated with fatal pulmonary infection in American alligators (5) (L. Sigler, unpublished data). Infections by these species are usually opportunistic, occurring in animals stressed by suboptimum housing, environmental conditions, or diet. Similarly to *C. granulomatis*, each of these fungi is known occasionally to produce conidia in tissue, allowing, in some cases, putative identification of the fungus based on histopathology (5, 11, 14) (Sigler, unpublished).

Chamaeleomyces species are distinguished from *Metarhizium*, *Paecilomyces*, and *Penicillium* species by a combination of morphological features, including the basally swollen phialides with narrow necks, conidia in fragile chains, pale green to greenish-gray colonies, and yeast-like growth. *Metarhizium*, *Paecilomyces*, and *Penicillium* species produce conidia in long chains from phialides borne on complex conidiophores that have several levels of branching. In *Metarhizium* and *Penicillium* species, conidia often aggregate in columns. The combined morphological and molecular data place these enigmatic reptile pathogens in a distinct lineage within the Clavicipitaceae family, but determination of their closest relatives and of their potential insect host(s) awaits additional sampling.

ACKNOWLEDGMENTS

We thank the reptile staff for expert handling of the animals; Jean Paré, Vivi Bille-Hansen, Steen Giese, Tim Kåre Jensen, James Wellehan, and John Potter for helpful comments; and Michael Hertwig-Jaksch for assistance with the Latin.

L. Sigler thanks the Natural Sciences and Engineering Research Council of Canada (NSERC) for financial support.

REFERENCES

1. Baráthová, H., V. Betina, and P. Nemeč. 1969. Morphological changes in fungi by antibiotics. *Folia Microbiol.* (Prague) **14**:475–483.
2. Baráthová, H., V. Betina, and L. Ulický. 1977. Regulation of differentiation of the dimorphic fungus *Paecilomyces viridis* by nitrogen sources, antibiotics and metabolic inhibitors. *Folia Microbiol.* (Prague) **22**:222–231.
3. Bischoff, J. F., S. A. Rehner, and R. A. Humber. 2009. A multilocus phylogeny of the *Metarhizium anisopliae* lineage. *Mycologia* **101**:512–530.
4. De Hoog, G. S., J. Guarro, J. Gené, and M. J. Figueras. 2000. Atlas of clinical fungi, 2nd ed. Centraalbureau voor Schimmelcultures, Utrecht, Netherlands.
5. Fromtling, R. A., S. D. Kosanke, J. M. Jensen, and G. S. Bulmer. 1979. Fatal *Beauveria bassiana* infection in a captive American alligator. *J. Am. Vet. Med. Assoc.* **175**:934–936.
6. Huelsenbeck, J. P., and F. Ronquist. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**:754–755.
7. Inglis, P. W., and M. S. Tigano. 2006. Identification and taxonomy of some entomopathogenic *Paecilomyces* spp. (Ascomycota) isolates using rDNA-ITS sequences. *Gen. Mol. Biol.* **29**:132–136.
8. Johnson, D., G. H. Sung, N. L. Hywel-Jones, J. J. Luangsa-Ard, J. F. Bischoff, R. M. Kepler, and J. W. Spatafora. 2009. Systematics and evolution of the genus *Torrubiella* (Hypocreales, Ascomycota). *Mycol. Res.* **113**:279–289.
9. Kornerup, A., and J. H. Wanscher. 1978. Methuen handbook of color, 3rd ed. Methuen, London, United Kingdom.
10. Lane, D. J., B. Pace, G. J. Olsen, D. A. Stahl, M. L. Sogin, and N. R. Pace. 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc. Nat. Acad. Sci. U. S. A.* **89**:6955–6959.
11. Liu, K., D. N. Howell, J. R. Perfect, and W. A. Schell. 1998. Morphologic criteria for the preliminary identification of *Fusarium*, *Paecilomyces*, and *Acremonium* species by histopathology. *Am. J. Clin. Pathol.* **109**:45–54.
12. Luangsa-ard, J. J., N. L. Hywel-Jones, L. Manoch, and R. A. Samson. 2005. On the relationships of *Paecilomyces* sect. *Isarioidea* species. *Mycol. Res.* **109**:581–589.
13. Luangsa-ard, J. J., N. L. Hywel-Jones, and R. A. Samson. 2004. The polyphyletic nature of *Paecilomyces sensu lato* based on 18S-generated rDNA phylogeny. *Mycologia* **96**:773–780.
14. Paré, J., and E. R. Jacobson. 2007. Mycotic diseases of reptiles, p. 527–570. In E. R. Jacobson (ed.), *Diseases and pathology of reptiles: color atlas and text*. CRC Press Inc., Boca Raton, FL.
15. Pendland, J. C., and D. C. Boucias. 1997. In vitro growth of the entomopathogenic hyphomycete *Nomuraea rileyi*. *Mycologia* **89**:66–71.
16. Samson, R. A. 1974. *Paecilomyces* and some allied hyphomycetes. *Stud. Mycol.* **6**:1–119.
17. Segretain, G., H. Fromentin, P. Destombes, E. R. Brygoo, and A. Dodin. 1964. *Paecilomyces viridis* nov. sp., dimorphic fungus, agent of a generalized mycosis in *Chamaeleo lateralis* Gray. *Compt. Rend. Acad. Sci. (Paris)* **259**:258–261. (In French.)
18. Sigler, L., and C. F. C. Gibas. 2005. Utility of a cultural method for identification of the ericoid mycobiont *Oidiodendron maius* confirmed by ITS sequence analysis. *Stud. Mycol.* **53**:63–74. <http://www.cbs.knaw.nl/publications/>.
19. Sigler, L., D. A. Sutton, C. F. C. Gibas, R. C. Summerbell, R. K. Noel, and P. C. Iwen. 2010. *Phialosimplex*, a new anamorphic genus associated with infections in dogs and having phylogenetic affinity to the Trichocomaceae. *Med. Mycol.* **48**:335–345.
20. Sung, G. H., N. L. Hywel-Jones, J. M. Sung, J. J. Luangsa-Ard, B. Shrestha, and J. W. Spatafora. 2007. Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. *Stud. Mycol.* **57**:5–63. <http://www.cbs.knaw.nl/publications/>.
21. White, T., T. Burns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p. 315–322. In D. H. Gelfand, M. A. Innis, J. J. Sninsky, and T. J. White (ed.), *PCR protocols. A guide to methods and applications*. Academic Press Inc., San Diego, CA.
22. Zare, R., W. Gams, and H. C. Evans. 2001. A revision of *Verticillium* section Prostrata. V. The genus *Pochonia*, with notes on *Rotiferophthora*. *Nova Hedwigia* **73**:51–86.