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# Molecular Characterization of Reptile Pathogens Currently Known as Members of the *Chrysosporium* Anamorph of *Nannizziopsis vriesii* Complex and Relationship with Some Human-Associated Isolates

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**In recent years, the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV), *Chrysosporium guarroi*, *Chrysosporium ophiodiicola*, and *Chrysosporium* species have been reported as the causes of dermal or deep lesions in reptiles. These infections are contagious and often fatal and affect both captive and wild animals. Forty-nine CANV isolates from reptiles and six isolates from human sources were compared with *N. vriesii* based on their cultural characteristics and DNA sequence data. Analyses of the sequences of the internal transcribed spacer and small subunit of the nuclear ribosomal gene revealed that the reptile pathogens and human isolates belong in well-supported clades corresponding to three lineages that are distinct from all other taxa within the family *Onygenaceae* of the order *Onygenales*. One lineage represents the genus *Nannizziopsis* and comprises *N. vriesii*, *N. guarroi*, and six additional species encompassing isolates from chameleons and geckos, crocodiles, agamid and iguanid lizards, and humans. Two other lineages comprise the genus *Ophidiomyces*, with the species *Ophidiomyces ophiodiicola* occurring only in snakes, and *Paranannizziopsis* gen. nov., with three new species infecting squamates and tuataras. The newly described species are *Nannizziopsis dermatitidis*, *Nannizziopsis crocodili*, *Nannizziopsis barbata*, *Nannizziopsis infrequens*, *Nannizziopsis hominis*, *Nannizziopsis obscura*, *Paranannizziopsis australasiensis*, *Paranannizziopsis californiensis*, and *Paranannizziopsis crustacea*. *Chrysosporium longisporum* has been reclassified as *Paranannizziopsis longispora*. *N. guarroi* causes yellow fungus disease, a common infection in bearded dragons and green iguanas, and *O. ophiodiicola* is an emerging pathogen of captive and wild snakes. Human-associated species were not recovered from reptiles, and reptile-associated species were recovered only from reptiles, thereby mitigating concerns related to zoonosis.**

Fungal skin disease, or dermatomycosis, is increasingly recognized in reptiles (1, 2). In the last 2 decades, fungi identified as the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) or sometimes as *Chrysosporium* species have emerged as the leading causes of fungal dermatitis in reptiles (1, 2). *N. vriesii*, an ascomycetous fungus belonging to the order *Onygenales*, family *Onygenaceae*, was isolated originally from an *Ameiva* sp. and produces a *Chrysosporium*-like anamorph in culture that is indistinguishable from that of the reptile-associated isolates (3). The absence of teleomorphs (sexual stages) in the reptile-associated CANV isolates has made their relationship with *N. vriesii* difficult to resolve.

Infection with CANV typically begins as a cutaneous disease, with lesions characterized by hyperkeratosis, necrosis, vesicles, ulcers, and crusts, and it often progresses to fatal systemic disease. While dermatomycosis in reptiles has been classically linked with stress, overcrowding, and substandard husbandry in captive animals, an experimental challenge of veiled chameleons (*Chamaeleo calyptratus*) with the CANV confirmed that it acts as a primary pathogen, at least in that reptile species (4). Breaches in cutaneous integrity facilitate infection, and the infection is contagious (4). Although these infections are primarily observed in pets and captive animals, similar types of infections have been documented recently in wild animals (5). The biology and ecological niche of the CANV remain poorly understood, but there is evidence to suggest it is not a common constituent of the reptilian cutaneous mycobiota, at least in squamates (6). With the exception of a single isolate obtained from a shed skin of a captive African rock python (*Python sebae*) (6), all isolates have been recovered from lesions of sick animals.

Infections with the CANV and *Chrysosporium* species have been documented in lizards, including chameleons (*Calumna parsonii*, *Furcifer lateralis*, *Trioceros* (formerly *Chamaeleo*) *jacksonii* (3), inland (*Pogona vitticeps*) (7–9) and coastal bearded dragons (*Pogona barbata*) (10), green iguanas (*Iguana iguana*) (11, 12), ameivas (*Ameiva* sp., *Ameiva chaitzami*) (13, 14), day geckos (*Phelsuma* sp.) (15), sungazers (*Cordylus giganteus*) (16), brown anoles (*Anolis sagrei*) (17), leopard geckos (*Eublepharis macularius*) (18), terrestrial and aquatic snakes, including brown tree snakes (*Boiga irregularis*) (19), a garter snake (*Thamnophis* sp.) (20), green anaconda (*Eunectes murinus murinus*) (21), boa constrictor (*Boa constrictor constrictor*) (22), broad-headed snake (*Hoplocephalus bungaroides*) (23), eastern massasauga rattlesnakes (*Sistrurus catenatus catenatus*) (5), tentacled snakes (*Erpeton tentaculatum*) (24), and saltwater crocodiles (*Crocodylus porosus*) (25). Additional isolates have been obtained from skin or deep dermal lesions in a corn snake (*Elaphe guttata*), a milk snake (*Lampropeltis* sp.), file snakes (*Acrochordus* sp.), water snakes (*Nerodia* spp.), and northern tuataras (*Sphenodon punctatus punc-*

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*tatus*). Infections with these pathogens have been documented in North America, Europe, Asia, and Australasia.

In 2009 and 2010, two *Chrysosporium* species were described as being CANV-like isolates. *Chrysosporium ophiodiicola* was determined as the cause of a subcutaneous granuloma in a black rat snake (*Elaphe obsoleta obsoleta*) in the United States (26), and *Chrysosporium guarroi* was identified as the cause of disseminated cutaneous disease in pet green iguanas from Spain (12). Both species were described as being related to the CANV, as judged by a comparison of their internal transcribed spacer (ITS) region rRNA gene sequences with those available in GenBank.

The objective of this study was to assess the phylogenetic relationships among CANV isolates from reptile dermal or deep lesions and with *C. guarroi*, *C. ophiodiicola*, and *N. vriesii*, as well as with other *Onygenales* using DNA sequences from the ITS and small subunit (SSU) regions of the nuclear rRNA gene. Isolates appearing to be morphologically similar to the CANV have also been cultured occasionally from human specimens, and these were included in the analyses to assess their relationships with isolates from reptiles (27, 28). Based on a synthesis of the molecular and morphological assessments, six new species in *Nannizziopsis* and three new species in the new genus *Paranannizziopsis* are described here. The new genus *Ophidiomyces* was published recently to accommodate *C. ophiodiicola*, and we provide here an expanded description to facilitate the recognition of this emerging snake pathogen (29). While the present paper was under review, Stchigel et al. (30) published a study that analyzed 19 isolates from reptile and human sources. The four *Nannizziopsis* species described in that study, including *Nannizziopsis chlamydospora*, *Nannizziopsis draconii*, *Nannizziopsis arthrosporioides*, and *Nannizziopsis pluriseptata*, differ from those described here based on a comparison of ITS sequence similarity, their analyses, and phenotypic features. They reclassified *C. guarroi* as *Nannizziopsis guarroi*, in agreement with our assessment, and described *Chrysosporium longisporum*, which we reclassify in the genus *Paranannizziopsis*.

## MATERIALS AND METHODS

Currently, 55 isolates are accessioned in the University of Alberta Microfungus Collection and Herbarium (UAMH), Edmonton, Alberta, Canada, and their provenances are listed in Table 1. Their colonial features and growth rates were recorded on potato dextrose agar plates (PDA) (BD Diagnostic Systems, Sparks, MD) and incubated at 30°C and 35°C, each for 21 days. The incubation temperature for all other procedures was 30°C. Tolerance to cycloheximide was assessed by comparing growth on Mycosel agar (BD) containing cycloheximide with that on Phytone yeast extract agar (BD) after 21 days. The isolates were grown on slide culture preparations on cereal agar for microscopic observations and on oatmeal salts agar (OAT) for 42 days or longer to attempt to induce ascomata (recipes for media can be found at www.uamh.devonian.ualberta.ca/OrderCultures.aspx). Most isolates were evaluated for their reactions in tests that have been used for the examination of dermatophytic fungi and some *Chrysosporium* species, including reactions on bromocresol purple-milk solids-glucose agar (BCP-MS-G) (prepared in-house), urease activity in urea broth (BD), and ability to perforate hairs, according to the methods and interpretations of Kane et al. (31). Reactions on BCP-MS-G were observed at 7, 10, and 14 days and were reported at 14 days. The observations reported included growth as slow, moderate, or profuse, indicator change from neutral (originally sky blue) to alkaline (purple), and the hydrolysis (clearing) of milk solids either beyond the colony border or beneath the colony.

DNA sequences were newly obtained for 44 isolates (Table 1). Five other strains that were sampled to assess their possible relationships with *N. vriesii* were *Arachnotheca glomerata* (ex-type strain UAMH 3551), *Arachniotus albicans* (ex-type strain UAMH 3102), which has been classified in the genera *Amauroascus*, *Nannizziopsis* (32), and *Arachnotheca* (in MycoBank), and *Nannizziopsis mirabilis* (ex-type strain UAMH 7712), and two unclassified *Nannizziopsis*-like isolates (strains UAMH 3124 and UAMH 4036). DNA was extracted using the UltraClean microbial DNA isolation kit (Mo Bio Laboratories, Inc., Solana Beach, CA) or the manufacturer's recommended kit for a Thermo Scientific KingFisher mL magnetic particle processor (VWR, Mississauga, Ontario, Canada). PCR was performed using the primer pairs NS1-ITS4 or ITS5-ITS4 in 10-μl reaction mixtures containing 0.1 mM each deoxynucleoside triphosphate (dNTP) (Invitrogen Canada, Inc., Burlington, Ontario, Canada), 0.8 pmol of each primer, 1 μl 10× Titanium *Taq* buffer, and 0.1 μl 50× Titanium *Taq* DNA polymerase (BD Biosciences, Mississauga, Ontario, Canada), with the following thermal cycling conditions: 95°C for 3 min, followed by 40 cycles of 95°C for 1 min, 58°C for 90 s, 72°C for 2 min, followed by a final extension of 72°C for 8 min. PCR products were directly sequenced using the BigDye Terminator v.3.1 cycle sequencing reaction kit (ABI Prism/Applied Biosystems, Streetsville, Ontario, Canada) using the PCR primers and the internal SSU primers NS2 to NS8 as needed. Sequences were edited using Sequencher version 5 software (Gene Codes Corporation, Ann Arbor, MI) and compiled using BioEdit software version 7.0.5.3 (Ibis Biosciences, Carlsbad, CA [see http://www.mbio.ncsu.edu/bioedit/bioedit.html]). SSU and ITS data matrices were assembled to include the representatives of the closest relatives, which were determined based on published phylogenies and current BLASTn searches. The SSU sequences were manually aligned. The ITS data were submitted for alignment to the Web-based utility MAFFT version 7 (see http://mafft.cbrc.jp/alignment/server/) using the L-INS-i strategy. Parsimony analyses were performed using PAUP software (version 4.0b10; Sinauer Associates, Inc., Sunderland, MA [see http://paup.csit.fsu.edu/]), with gaps treated as missing data. Bootstrap (BS) percentages were determined from 1,000 resamplings of the data set using the full heuristic search option and random sequence addition, with the number per replicate limited to 1 million rearrangements. Bayesian analysis was performed using MrBayes software version 3.2 (Department of Scientific Computing, Florida State University, Tallahassee, FL [see http://mrbayes.csit.fsu.edu/download.php]). DNA substitution rates were set as a flat Dirichlet (1.0, 1.0, 1.0, 1.0) by default, which allows the program to estimate the parameters while mixing among Markov chain Monte Carlo (MCMC) sampling. Two independent runs were performed on multiple processors. Each run was set for four chains of 100,000,000 MCMC generations with a sampling frequency of every 2,000 generations. The Bayesian consensus tree was directly generated after 25% burn-in. Clades that were supported by BS values of ≥75% and posterior probability (PP) values of ≥95% were recorded.

**Nucleotide sequence accession numbers.** The GenBank accession numbers for the newly generated ITS and SSU sequences are listed in Table 1. Additional ITS sequences were deposited in GenBank under accession no. KF477239 for *Amauroascus albicans* (strain UAMH 3102), KF477241 for *A. glomerata* (strain UAMH 3551), KF477243 for *N. mirabilis* (strain UAMH 7712), and KF477240 and KF477242 for the *Nannizziopsis*-like isolates UAMH 3124 and UAMH 4036.

## RESULTS

**Phylogenetic analyses of the SSU rRNA gene and ITS rRNA gene partitions.** The SSU data matrix comprised 60 taxa and 1,702 characters, of which 208 were parsimony informative. The ITS data matrix comprised 96 taxa and 857 characters, of which 416 were parsimony informative. Maximum parsimony analyses resulted in 3,641 most parsimonious trees (MPT) of score 692 for the SSU and 6,543 MPT of score 3,161 for the ITS (Fig. 1 and 2). The large number of trees found was due to the uncertainty of relationships within the *Onygenaceae* (SSU) and the treatment of the multiple identical sequences for the target groups by the

TABLE 1 Isolates examined in this study

| UAMH no.[a] | ITS subclade no. | Original name | Final name | Provenance of isolates[b] | Histopathology observations | Designations in other collections[c] | GenBank accession no. for: | | Reference no. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | ITS | SSU | |
| 3526 | I | *Nannizziopsis vriesii* | *N. vriesii* | USA: South California, soil | | | KF477197 | | |
| **3527 T** | I | *N. vriesii* | *N. vriesii* | Netherlands: skin and lungs of lizard (*Ameiva* sp.), G. A. de Vries | ND[d] | ATCC 22444, ATCC 24074, CBS 407.71, IMI 149994 | KF477198 | KF466858 | 13 |
| 6610 | II | CANV[e] | *Nannizziopsis dermatitidis* | Madagascar: cutis and muscles of day gecko (*Phelsuma* sp.), isolated in Germany, B. J. Schildger | ND | CBS C88-360 (5955) | KF477199 | | 15 |
| 7582 | II | CANV | *N. dermatitidis* | Canada, Ontario: skin biopsy specimen, M Parson's chameleon (*Calumna parsonii*), 1993 | Hyphae | | | | 3 |
| **7583 T** | II | CANV | *N. dermatitidis* | Canada, Ontario: liver and kidney, M jewel chameleon (*Furcifer lateralis*) (same origin as UAMH 7582), 1993 | Hyphae | | KF477200 | KF466859 | 3 |
| 7861 | II | CANV | *N. dermatitidis* | Canada, Ontario: skin biopsy specimen, adult M Jackson's chameleon (*Trioceros jacksonii*), L. Sigler, 1995 | Hyphae | | KF477201 | | 3 |
| 11231 | II | CANV | *N. dermatitidis* | USA, FL: skin lesion, leopard gecko (*Eublepharis macularius*), 2010 | Hyphae and arthroconidia | | JX457149 | | 18 |
| 11232 | II | CANV | *N. dermatitidis* | USA, FL: ulcerative ventral dermatitis, *E. macularius*, 2010 (same origin as UAMH 11231) | Hyphae and arthroconidia | | KF477202[f] | | 18 |
| 9664 | IIIa | CANV | *Nannizziopsis crocodili* | Australia, Gulf of Carpentaria: plaque-like skin lesion on saltwater crocodile (*Crocodylus porosus*), A. Thomas, 1999 | Hyphae and arthroconidia | | | | 25 |
| 9665 | IIIa | CANV | *N. crocodili* | Lesion on *C. porosus* (same origin as UAMH 9664) | Hyphae and arthroconidia | | KF477203[f] | | 25 |
| **9666 T** | IIIa | CANV | *N. crocodili* | Lesion on *C. porosus* (same origin as UAMH 9664) | Hyphae and arthroconidia | | KF477204 | KF466860 | 25 |
| 9908 | IIIa | CANV | *N. crocodili* | Australia, Gulf of Carpentaria: skin lesion on *C. porosus*, A. Thomas, 2000 | Hyphae | | KF477205 | | |
| **11185 T** | IIIb | CANV | *Nannizziopsis barbata* | Australia, NSW: skin of coastal bearded dragon (*Pogona barbata*) with deep ulcerative dermatitis, R. Johnson, 2009 | Hyphae | | JF323871 | KF466861 | 10 |
| 10171 | IV | CANV | *Nannizziopsis guarroi* | USA, WI: oral lesion in inland bearded dragon (*Pogona vitticeps*), J. Paré, 1992 | Hyphae | | KF477206 | KF466862 | 7 |
| 10211 | IV | CANV | *N. guarroi* | USA, FL: cutaneous scabs, *P. vitticeps*, J. Paré, 2002 | Hyphae | | KF477207 | | 7 |
| 10351 | IV | CANV | *N. guarroi* | USA, WI: amputated leg, *P. vitticeps* | Hyphae in granulomas | | | | 7 |
| 10352 | IV | CANV | *N. guarroi* | USA, WI: crusted facial lesion, *P. vitticeps* | Hyphae | | KF477208 | | |
| 10353 | IV | CANV | *N. guarroi* | USA, FL: skin lesion, *P. vitticeps*, J. Paré | Hyphae | | | | |
| 10409 | IV | CANV | *N. guarroi* | USA, NY: fatal deep lesion, *P. vitticeps* | Hyphae | | KF477209 | | |
| 10918 | IV | CANV | *N. guarroi* | Spain: tail abscess and leg, green iguana (*Iguana iguana*), M. Gallego, 2008 | Hyphae | | KF477210 | | |
| 10923 | IV | CANV | *N. guarroi* | Spain: left leg, *I. iguana*, M. Gallego, 2008 | ND | | KF477211 | | |
| 10938 | IV | CANV | *N. guarroi* | Spain: skin around eye, *I. iguana*, M. Gallego, 2008 | ND | | KF477212 | | |
| 10944 | IV | CANV | *N. guarroi* | Spain: leg of *I. iguana*, M. Gallego, 2008 | ND | | KF477213 | | |
| 10960 | IV | CANV | *N. guarroi* | Spain: lesion on tail, *I. iguana*, M. Gallego, 2008 | ND | | KF477214 | | |
| **10417 T** | V | CANV | *Nannizziopsis infrequens* | USA, IA: bronchial wash specimen, human, M, 40 yr old, $HIV^+$, 2005 | ND | | AY744467 | KF466863 | 27 |
| **7859 T** | VI | CANV | *Nannizziopsis hominis* | USA, CA: right thigh mass, human, M, $HIV^+$, 1994 | Hyphae | UTHSC 94-1427 | KF477215 | KF466864 | |
| 7860 | VI | CANV | *N. hominis* | USA, CA: right groin lesion (same patient as UAMH 7859) | ND | | | | |
| 7932 | VI | CANV | *N. hominis* | USA, CA: abscess right leg (same patient as 7859), isolated 1995 | ND | UTHSC 95-497 | | | |
| 9852 | VI | CANV | *N. hominis* | USA, MA: inguinal node, human, Nigerian, M, 32 yr old, with disseminated adenopathy, 2000 | ND | UTHSC 00-1109 | KF477216 | | |
| **5875 T** | VII | CANV | *Nannizziopsis obscura* | USA, NY: abscess right ankle, human, M, 24 yr old (isolated twice), 1982 | Hyphae and budding yeast | | KF477217 | KF466865 | 28 |
| 10439 | VIIIa | CANV | *Paranannizziopsis australasiensis* | Australia, Victoria: multifocal necrotizing skin lesions, aquatic file snake (no. 1) (*Acrochordus* sp.) | Hyphae | | KF477218 | KF466866 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 10440 | VIIIa | CANV | *P. australasiensis* | Australia, Victoria: multifocal necrotizing skin lesions, *Acrochordus* sp. (no. 2) | Hyphae | | | | |
| 11644 | VIIIa | CANV | *P. australasiensis* | New Zealand: skin lesions, northern tuatara (*Sphenodon punctatus punctatus*), F, 22 yr old, C. Harvey, 2012 | Hyphae | | KF477219 | | |
| **11645 T** | VIIIa | CANV | *P. australasiensis* | New Zealand: skin lesions, *S. punctatus punctatus*, F, 18 yr old, C. Harvey, 2012 | Hyphae | | KF477220 | | 36 |
| 11665 | VIIIa | CANV | *P. australasiensis* | New Zealand: skin lesion, deceased *Pogona barbata*, F, 3 yr old, C. Harvey, 2012 | Hyphae | | KF477221 | | |
| 11719 | VIIIa | CANV | *P. australasiensis* | New Zealand: skin lesion on thigh, juvenile *S. punctatus punctatus*, 2013 | Hyphae | | KF477222 | | |
| 10692 | VIIIb | CANV | *Paranannizziopsis californiensis* | USA, CA: skin lesion, tentacled snake (*Erpeton tentaculatum*), L. Sigler, 2006 | Hyphae and conidia | | KF477223[f] | | |
| **10693 T** | VIIIb | CANV | *P. californiensis* | USA, CA: skin lesion, *E. tentaculatum*, L. Sigler, 2006 (same origin as UAMH 10692) | Hyphae and conidia | | KF477224 | KF466867 | |
| **10199 T** | IX | CANV | *Paranannizziopsis crustacea* | Canada, Ontario: skin lesion, *E. tentaculatum* with fatal disseminated infection, L. Sigler, 2002 | Hyphae and arthroconidia | | KF477225 | KF466868 | 24 |
| 10200 | IX | CANV | *P. crustacea* | Canada, Ontario: skin, *E. tentaculatum* (same origin as UAMH 10199) | Hyphae and arthroconidia | | | | 24 |
| 10201 | IX | CANV | *P. crustacea* | Canada, Ontario: skin, *E. tentaculatum* (same origin as UAMH 10199) | Hyphae and arthroconidia | | KF477226 | | 24 |
| 6218 | X | CANV | *Ophidiomyces ophiodiicola* | USA, NY: subcutaneous nodule, tail, F corn snake (*Elaphe guttata guttata*), M. Kemna, 1986 | Hyphae and arthroconidia | | KF477227 | | |
| 6642 | X | CANV | *O. ophiodiicola* | USA, MD: fatal dermatitis, captive brown tree snake (*Boiga irregularis*) (BTS 4), originally from Guam, R. Weyant, 1990 | Hyphae and arthroconidia | | KC884267 | KF466869 | 19 |
| 6688 | X | CANV | *O. ophiodiicola* | England: subcutaneous lesions, back and underside of ball python (*Python regius*), C. Philpot, 1985 | Hyphae | | KF477228 | KF466870 | |
| 9832 | X | *Chrysosporium queenslandicum* | *O. ophiodiicola* | Germany: skin lesions, garter snake (*Thamnophis* sp.) with disseminated infection, T. Vissiennon, 1999 | Hyphae and arthroconidia | CBS 102663, CBS M253 | KF477229 | | 20 |
| 9985 | X | CANV | *O. ophiodiicola* | USA, NM: shed mid-dorsal skin, 1-yr-old African rock python (*Python sebae*), J. Pare, 2001 | ND[g] | | KF477230 | | 6 |
| 10079 | X | CANV | *O. ophiodiicola* | USA, WI: skin lesions, milk snake (*Lampropeltis* sp.), J. Paré, 2001 | ND | | KF477231 | | |
| 10296 | X | CANV | *O. ophiodiicola* | Australia, Queensland: skin lesions, mouth and head of file snake on display in crocodile farm, A. Thomas, 2003 | ND | | KF477232 | | |
| 10716 | X | CANV | *O. ophiodiicola* | USA, FL: skin biopsy specimen, wild-caught water snake (no. 9) (*Nerodia clarkii*) in captivity, C. Schuman, July 2006 | ND | | | | |
| 10717 | X | CANV | *O. ophiodiicola* | USA, FL: skin biopsy specimen, wild-caught *N. clarkii* (no. 5), C. Schuman, July 2006 | ND | | KF477233 | KF466871 | |
| 10768 | X | CANV | *O. ophiodiicola* | USA, FL: skin biopsy specimen, eastern diamondback rattlesnake (*Crotalus adamanteus*), D. Schuman, 2006 | ND | | KF477234 | | |
| 10769 | X | CANV | *O. ophiodiicola* | USA, FL: skin biopsy specimen, *N. clarkii* (no. 21), C. Schuman, October 2006 | ND | | KF477235 | | |
| 10770 | X | CANV | *O. ophiodiicola* | USA, FL: skin biopsy specimen, *N. clarkii* (no. 19), C. Schuman, 2006 | ND | | | | |
| 10949 | X | CANV | *O. ophiodiicola* | USA, CA: skin lesion, green anaconda snake (*Eunectes murinus murinus*), L. Sigler 2008 | Hyphae and arthroconidia | | KF477236 | | 21 |
| 11295 | X | CANV | *O. ophiodiicola* | Australia, Victoria: skin lesion adjacent to the cloaca, broad-headed snake (*Hoplocephalus bungaroides*), D. McLelland, 2010 | Hyphae and arthroconidia | | KF477237 | | 23 |

[a] Isolates are accessioned at the University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta, Canada (UAMH). Holotypes are indicated in bold type.
[b] M, male; F, female.
[c] ATCC, American Type Culture Collection, Manassas, VA; CBS, Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; IMI, CABI Microbial Genetic Resources, Egham, United Kingdom; UTHSC, Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, TX.
[d] ND, no data.
[e] CANV, *Chrysosporium* anamorph of *N. vriesii*.
[f] ITS sequences were obtained for comparison but not included in the phylogenetic analyses.
[g] This isolate was obtained from skin scales as part of a survey of fungi isolated from shed skin and was not examined by histopathology.

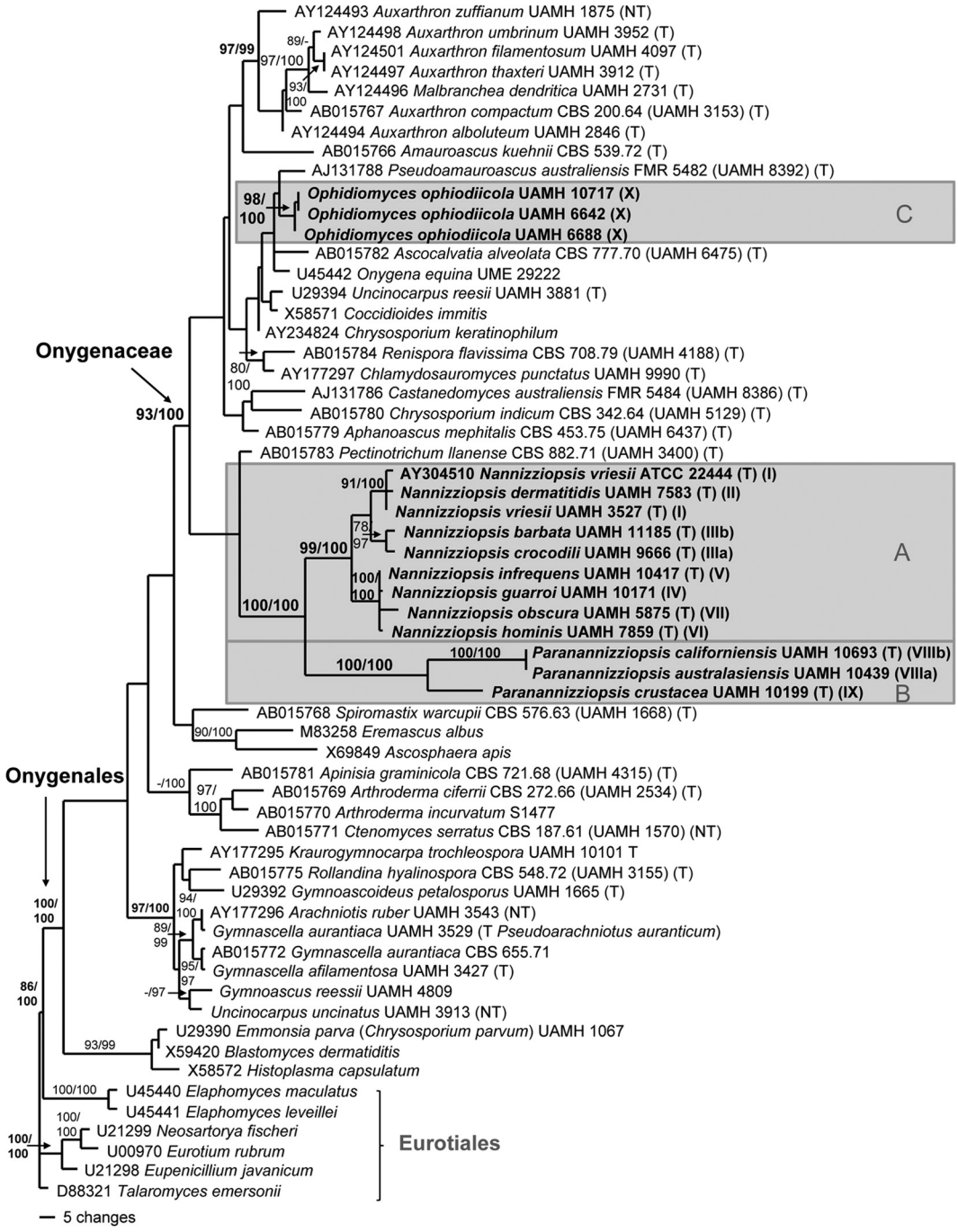


**FIG 1** One of 3,641 equally parsimonious trees inferred from maximum parsimony analysis of SSU rRNA gene sequences, showing the *Onygenaceae* and three major lineages (clades A, B, and C) corresponding to the three genera of CANV fungi, including *Nannizziopsis* (eight species), *Paranannizziopsis* (three species), and *Ophidiomyces* (monotypic). The indices for the tree were a consistency index (CI) of 0.509, retention index (RI) of 0.788, and a homoplasy index (HI) of 0.491. Bootstrap values of ≥75% and posterior probability values of ≥95% are shown. CANV fungi are labeled with species name, culture collection number, and ITS subclade numbers (I to X). GenBank accession numbers and culture collection numbers are shown where available. T, ex-type culture; NT, neotype.

branch-swapping algorithm (ITS). The topology for the MPT shown in Fig. 1 was retained in the corresponding strict consensus of all trees, except for the arrangements of some taxa within the uppermost clade of the *Onygenaceae*. Although the genetic diversity sampled for the ITS analyses was broad, the resulting tree structure was congruent with that derived from the SSU data for the target taxa. Bayesian analyses resulted in a single consensus tree for each gene region. In both the SSU and ITS analyses, the reptile isolates grouped into three individually strongly supported clades (BS of 98 to 100 and PP of 100 for each clade) designated A, B, and C that represent the genus *Nannizziopsis* and two new lineages. There was strong support for the three lineages within the family *Onygenaceae* of the *Onygenales* (Fig. 1) (BS, 93; PP, 100), and the lineages were distinct from all *Chrysosporium* species and

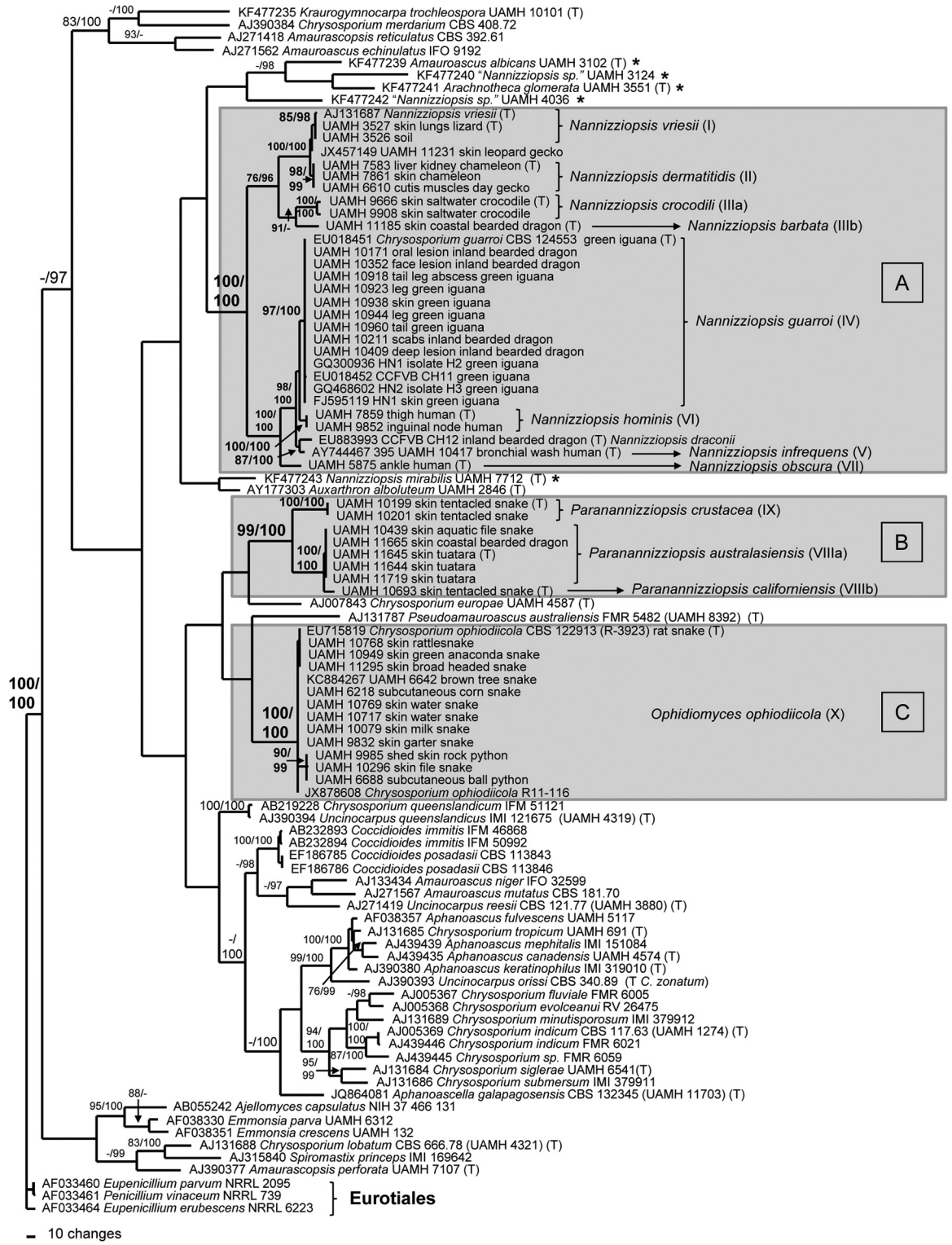


**FIG 2** One of 6,543 equally parsimonious trees (CI, 0.333; RI, 0.754; HI, 0.667) inferred from maximum parsimony analysis of ITS rRNA gene sequences, showing three major lineages (clades A, B, and C) corresponding to the three genera of CANV fungi, including *Nannizziopsis* (nine species), *Paranannizziopsis* (three species), and *Ophidiomyces* (monotypic). Bootstrap values of ≥75% and posterior probability values of ≥95% are shown above or beside the branches. CANV fungi are labeled with species name, culture collection number, and ITS subclade numbers (I to X). GenBank accession numbers and culture collection numbers are shown where available. *, Five strains sampled to assess their possible relationship with *Nannizziopsis vriesii*. T, ex-type culture.

other taxa (Fig. 1 and 2). Clade A was strongly supported as a sister to clade B (BS, 100; PP, 100) by the SSU analyses but not by ITS analysis. Clades A and B were additionally supported, individually and as sister clades, by long branches in the SSU tree, indicating substantial genetic differentiation. Both A and B clades comprised several subclades. Clade A included eight subclades encompassing the ex-type culture of *N. vriesii* (I), chameleon and gecko isolates (II), crocodile isolates (IIIa), isolates from agamid and iguanid lizards (IIIb and IV), and humans (V, VI, and VII) (Fig. 2). The ITS sequence for strain UAMH 5875 (subclade VII), confirmed using DNA extracted from two independently grown stock cultures, was unusually long, with a large insertion of approximately 150 nucleotides in the ITS1 spacer region. No strictly anamorphic reptile isolate grouped with the teleomorphic *N. vriesii*. An isolate from the leopard gecko (UAMH 11231) was an intermediate between subclades I and II. Subclade IV included the ex-type culture of *N. guarroi* and all unclassified isolates from inland bearded dragons and iguanas. An isolate from a coastal bearded dragon (UAMH 11185) was excluded and grouped as a sister to subclade IIIa. Clade B comprised three subclades. Subclade VIIIa included isolates from acrochordid snakes, tuataras, and a coastal bearded dragon. Tentacled snake isolates from a Canadian zoo and those from an American zoo grouped in separate subclades (VIIIb and IX). A sequence from the ex-type culture of *C. ophiodiicola* and all unclassified isolates from semiaquatic and terrestrial snakes were placed in clade C. A relationship of clade C with clades A and B was unresolved. Excluded from *Nannizziopsis* clade A were all species currently or formerly classified in *Arachnotheca* or *Nannizziopsis*, including *A. glomerata*, *A. albicans* (formerly *N. albicans*), and *N. mirabilis*. Moreover, there was no close relationship between these species (Fig. 2). Similarly, the two unclassified *Nannizziopsis*-like isolates did not group with any sampled species, so their sequences were deposited as *Onygenaceae* species.

Based on the new international code of nomenclature for algae, fungi, and plants, which ends the separate naming of different states of fungi (Article 59) (33), we assigned members of clade A to *Nannizziopsis* and described six new species for the lizard and human subclades. We propose the new genus *Paranannizziopsis* for members of clade B, with three new species encompassing isolates from highly aquatic squamates, tuataras, and a coastal bearded dragon. Clade C included *C. ophiodiicola* and all unclassified isolates from aquatic, semiaquatic, and terrestrial snakes that are described here as *Ophidiomyces ophiodiicola*.

**Morphology.** Although isolates from reptiles may be suspected as belonging to one of the genera described here based on the macro- and micromorphological characteristics described below, the morphological distinctions within and between clades A, B, and C were generally insufficient for a reliable identification of isolates to the species level without the use of DNA sequencing. Table 2 provides a summary of the morphological and physiological characteristics for these species. All isolates were moderately fast growing on PDA at 30°C and had yellowish-white, velvety to powdery, dense, and sometimes zonate, colonies, with uncolored to yellowish reverse (Fig. 3 and Table 2). Isolates streaked on PDA sometimes demonstrated a mixture of mycelial colonies and moist-to-glabrous transitory yeast-like colonies (Fig. 3E and K). Exudate droplets occasionally formed on the colony surfaces, but diffusible colored pigments were not produced on PDA. Thermotolerance distinguished a few species that demonstrated good growth at 35°C (e.g. Fig. 3B, J, M, P, and S). All isolates were tolerant of cycloheximide and perforated hairs. All isolates produced aleurioconidia, which are solitary conidia released by lytic dehiscence (Fig. 4 to 9). The aleurioconidia were commonly sessile, sometimes subtended by slightly swollen cells, or formed at the ends of short stalks. They were clavate or pyriform with truncate bases, occasionally subglobose or obovate, mostly single celled, and occasionally two celled. The aleurioconidia resembled those of some *Chrysosporium* and *Trichophyton* species, and this similarity has led to the misidentification of isolates in the past; however, two morphological characteristics distinguished the fungi described here. *Nannizziopsis* and *Ophidiomyces* species commonly have chains of adjacent cylindrical arthroconidia that are produced by schizolytic fragmentation of the hyphae (Fig. 4 to 7 and 9C and D). This type of arthroconidial development occurred also in *Paranannizziopsis crustacea* (Fig. 9B) but was lacking in the two other *Paranannizziopsis* species. These and other species occasionally produced rhexolytically dehiscing intercalary arthroconidia, a type of arthroconidium produced by many onygenalean fungi (Fig. 8B). The second notable characteristic, occurring in members of all three genera but not in *Chrysosporium* species or dermatophytes, was the formation of short, solitary, undulate, lateral branches that were occasionally sparsely septate (Fig. 4 to 9). Arthroconidia sometimes demonstrated budding and were found especially in the moist yeast-like colonies (Fig. 3E, 4D, and 5D). Arthroconidia were also produced *in vivo* in the epidermises of infected animals (Fig. 10A and Table 1). Some isolates produced ascomatal initials (Fig. 6J, 7D, and 7F), typically within cottony sectors, or developed infertile ascomata (Fig. 5E and 6D). With the exception of *N. vriesii*, isolates failed to produce ascospores in cultures that were grown on OAT or other media and incubated for several months. Isolates demonstrated varied responses on BCP-MS-G and in urea broth (Table 2). Variation sometimes occurred among isolates of a species or between subcultures from the powdery and cottony sectors of an individual isolate. Most *O. ophiodiicola* isolates demonstrated alkalinization, as well as strong clearing of the medium. Isolates were weakly to strongly urease positive except for a single isolate from a human source. *O. ophiodiicola* isolates often produced a mercaptan (skunk-like) odor.

## TAXONOMY

***Nannizziopsis* Currah.** Mycotaxon 24:160, 1985. MycoBank accession no. MB 25725). Type species: *Nannizziopsis vriesii* (Apinis) Currah, Mycotaxon 24:164, 1985 (subclade I) (MycoBank accession no. MB 104542). *N. vriesii* was described and illustrated by Currah (42) and Apinis (13) and is distinguished from all the other *Nannizziopsis* species described below by the production of ascomata. Ascomata (gymnothecia) produced on OAT at 30°C were composed of hyaline anastomosing asperulate hyphae containing small globose punctate-reticulate ascospores measuring 2.2 to 3 μm in diameter (Fig. 4A). Colonies on PDA were 4.5 to 5.5 cm in diameter after 21 days, velvety to slightly cottony, and furrowed. Growth was inhibited at 35°C, with colonies attaining 2.5 cm in diameter (Fig. 3A and B). Aleurioconidia were 2.5 to 6 μm long (in rare cases up to 8 μm long) and 1.5 to 2.7 μm wide (Fig. 4B). Isolates produced undulate hyphae and cylindrical fission arthroconidia measuring 2.7 to 7.3 μm long and 1.7 to 2.7 μm wide and sometimes showing yeast-like budding (Fig. 4C and D). Notes: The ex-type strain was isolated from the skin and lungs of an *Ameiva* lizard and was the only isolate in this study from a teiid lizard. No details are available on the course of infection. The

**TABLE 2** Summary of the morphological and physiological characteristics of *Nannizziopsis*, *Paranannizziopsis*, and *Ophidiomyces* species

| Species | Colony diam (cm)[f] on PDA after 21 days at: | | Cycloheximide tolerance | Conidial size (μm) (length by width)[k] | | Presence of undulate hyphal branches | Other microscopic structures detected | Reactions on BCP-MS-G medium[a] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30°C | 35°C | | Aleurioconidia[d] | Arthroconidia[e] | | | Growth | Alkalinization | Hydrolysis of milk solids | Urease activity[b] | Presence of hair perforation[c] | Other observations |
| *Nannizziopsis vriesii* | 4.5–5.5 | 2.5 | + | 2.5–6 (8) by 1.5–2.7 | 2.7–7.3 by 1.7–2.7 | + | Ascomata | ++ | − (tr Y) | H− | +/++ | + | |
| *Nannizziopsis dermatitidis* | (2.5) 3.8–4.7 | NG (<1) | + | 2.8–7.5 (9) by 1.2–3 | 2.8–9 by 1.5–3 | + | | +/++ | − (tr Y) | H+ | ++ | + | |
| *Nannizziopsis crocodili* | 4–5.5 | 1–2.7 | | 1.5–2.5 by 1.3–2.4 | 3.7–7.5 by 2–3 | + | Infertile ascomata | +/++ | − (tr Y) | H+ | ++ | + | |
| *Nannizziopsis barbata* | 5.5–6 | NG | + | 3–6.5 by 1.8–2.5 | 4.4–8.5 by 1.7–3.5 | + | Infertile ascomata | ND[g] | ND | ND | ND | + | |
| *Nannizziopsis guarroi* | 2.7–4.7 | 2.3–4 | + | 3.2–6.5 by 1.5–2.5 | 2.8–7 by 2–3.7 | + | | ++ | − (tr Y) | H− | ++ | + | |
| *Nannizziopsis infrequens* | 6 | 5 | + | 2.2–5.5 by 1.5–2.7 | Intercalary (few) 5–10.5 by 1.8–2.3 | + (few) | Ascomatal initials[h] | +++ | − | H− | ++ | + | + AccuProbe histoplasma culture identification test |
| *Nannizziopsis hominis* | 4.2–4.8 | 3–3.5 | + | 2.3–7 by 1.5–3.2 | 2.5–9 by 1.4–3.1 | + | Ascomatal initials | +++ | − (tr +)[i] | H−/H+[i] | ++ | + | + AccuProbe blastomyces culture identification[j] |
| *Nannizziopsis obscura* | 5 | 5 | + | 2.5–7 by 1.6–2.7 | 3.1–7.7 by 1.6–3.2 | + | Ascomatal initials | +++ | − (tr Y) | H− | − | + | |
| *Paranannizziopsis australasiensis* | 4.5–5 | NG | + | 3.5–8 by 1.5–2.7 | Not observed | + | Ascomatal initials | +++ | − | H− | + | + | |
| *Paranannizziopsis californiensis* | 4.8–5.2 | <1 | + | 4–8.5 by 1.8–2.6 | Not observed | + (few) | Ascomatal initials and irregularly shaped cells | +++ | − | H− | + | + | |
| *Paranannizziopsis crustacea* | 5.8–6.5 | NG | + | 4–7.5 by 2–3.5 | 3.8–9.2 by 1.9–2.7 | + | | +++ | − | H+/H−[i] | ++/+[i] | + | |
| *Ophidiomyces ophiodiicola* | 4–6 | NG (2–3.5) | + | 2.5–7.5 by 1.5–2.5 | 3–12.5 (15) by 1.5–3.5 | + | Ascomatal initials | ++/+++ | +/−[i] | H+/H−[i] | ++/−[i] | + | Odor often present |

[a] Reactions on bromocresol purple-milk solids-glucose agar are after 14 days at 30°C as described in Materials and Methods and following Kane et al. (31). Growth is profuse (+++), moderate (++), or slow (+). A change in medium color from sky blue to purple is positive alkalinization; − tr Y, sky blue medium has a trace of a yellow (acidification); H−, hydrolysis (clearing) of milk solids is beneath the colony; H+, hydrolysis of milk solids is cleared beyond the colony border.

[b] Reactions in urea broth were negative (straw yellow), weak (+, pink), or positive (++, fuchsia) after 14 days at 30°C.

[c] The presence of perforations in hairs was recorded after 14 days at 30°C (31).

[d] Aleurioconidia refers to solitary conidia released by rhexolytic dehiscence.

[e] Arthroconidia refers to conidia formed in adjacent chains by schizolytic dehiscence of the hypha, unless noted as being intercalary.

[f] Numbers in parentheses refer to colony diameters that are exceptional in some isolates. NG, no growth in most isolates tested.

[g] ND, not determined.

[h] Ascomatal initials usually occurred in cottony sectors for species where presence is noted.

[i] Varied responses occurred on BCP-MS-G and in urea among isolates of the species or between subcultures from powdery and cottony sectors of the same isolate.

[j] Positive test reported by the submitting laboratory for one isolate (UAMH 9852).

[k] Numbers in parentheses refer to exceptional dimensions at the low or high end of the size range.

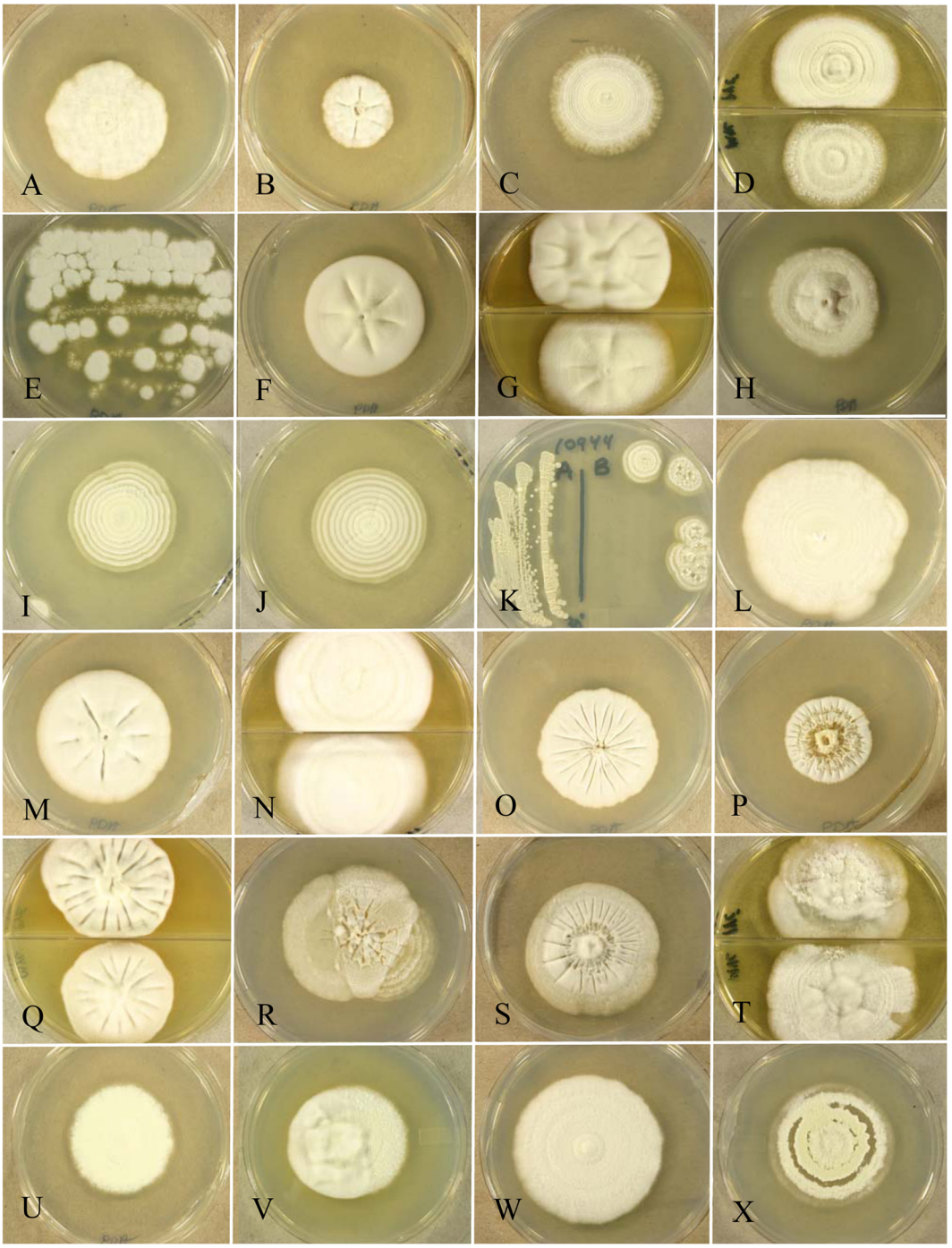


**FIG 3** Colonies of *Nannizziopsis*, *Paranannizziopsis*, and *Ophidiomyces* isolates after 21 days of incubation, except as indicated. Colonies of *N. vriesii* shown on PDA at 30°C (A) and 35°C (B). *N. dermatitidis* shown on PDA (C) and on PYE (top) and Mycosel (MYC) (bottom) (D) at 30°C. (E) *N. dermatitidis* streaked on PDA showing yeast and mold colonies after 16 days at 30°C. *N. crocodili* shown on PDA (F) and on PYE (top) and MYC (bottom) (G) at 30°C. (H). *N. barbata* shown on PDA at 30°C. *N. guarroi* shown on PDA at 30°C (I) and at 35°C (J). (K) *N. guarroi* streaked on PDA showing yeast and mold colonies after 11 days at 30°C. *N. infrequens* shown on PDA at 30°C (L) and 35°C (M) and on PYE (top) and MYC (bottom) at 30°C (N). *N. hominis* shown on PDA at 30°C (O) and 35°C (P) and on PYE (top) and MYC (bottom) at 30°C (Q). *N. obscura* shown on PDA at 30°C (R) and at 35°C (S) and on PYE (top) and MYC (bottom) (T) at 30°C. *Paranannizziopsis australasiensis* (U), *P. californiensis* (V), and *P. crustacea* (W) shown on PDA at 30°C. (X) *Ophidiomyces ophiodiicola* shown on PDA at 30°C.

isolate from soil (UAMH 3526) was the only one obtained from an inanimate substrate. Although Stchigel et al. (30) identified an isolate from human infection (RKI 04-0104) as *N. vriesii*, we suggest that it is closer to *Nannizziopsis obscura*, a human-pathogenic species (see Notes for *N. obscura*).

***Nannizziopsis dermatitidis* Sigler, Hambleton, and Paré sp. nov. (subclade II).** MycoBank accession no. MB 804604. Colonies on PDA attained 3.8 to 4.7 cm diameter after 21 days and were strongly zonate and powdery with a thin margin (Fig. 3C). Most isolates failed to grow at 35°C. Aleurioconidia were clavate to

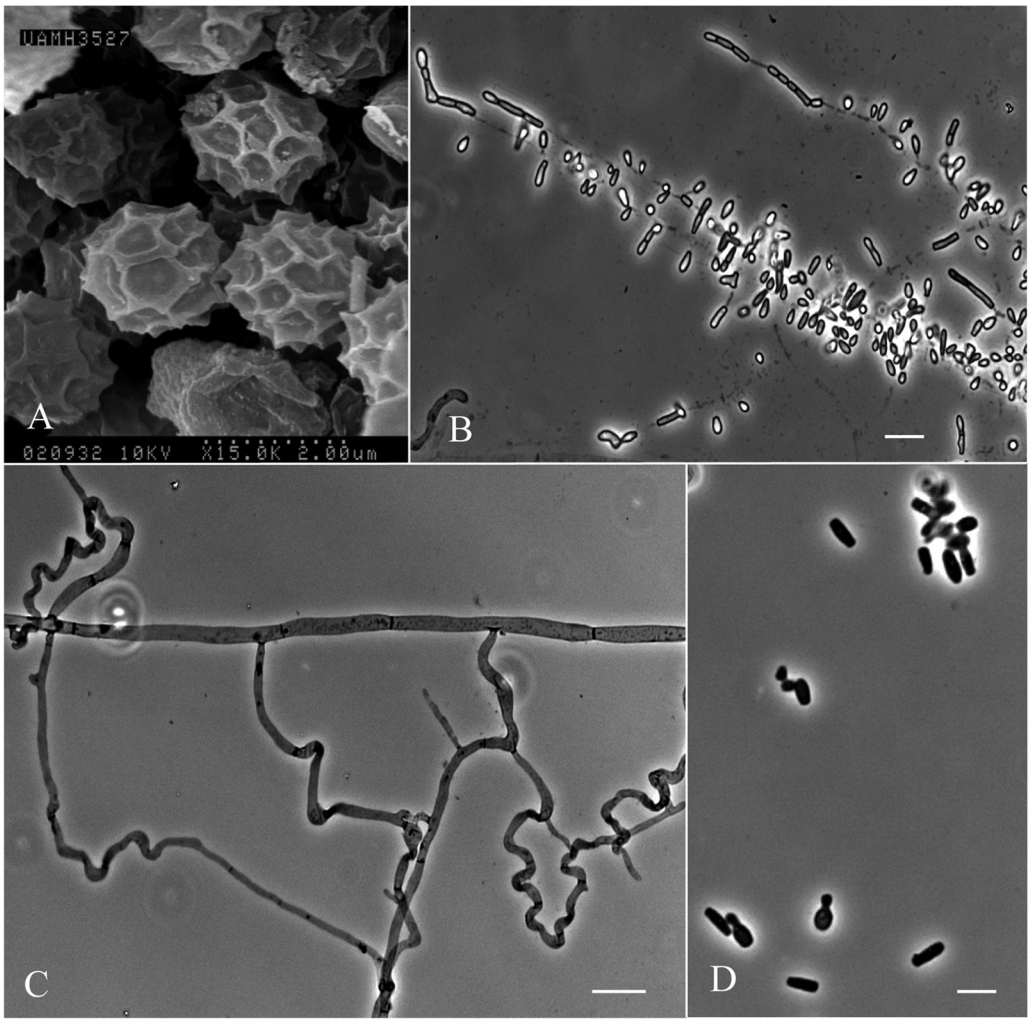


**FIG 4** Microscopic morphology of *Nannizziopsis vriesii*. (A) Scanning electron micrograph showing wall ornamentation of globose ascospores. (B and C) Slide culture preparations showing aleurioconidia, occasional arthroconidia, and undulate hyphae. (D) Arthroconidia and budding cells produced on PDA. Bars = 10 μm.

pyriform and measured 2.8 to 7.5 μm long if single-celled, up to 9 μm long if 2-celled, and 1.2 to 3 μm wide (Fig. 5A). Undulate branches and cylindrical- to slightly barrel-shaped fission arthroconidia were formed with arthroconidia measuring 2.8 to 9 μm long and 1.5 to 3 μm wide (Fig. 5B and C). Transitory yeast-like colonies grown on PDA at 30°C were composed of ovoid-to-cylindrical yeast-like cells and arthroconidia (Fig. 5D). Similarly, arthroconidia and yeast-like cells predominated in cultures of an isolate from day gecko (UAMH 6610) that were glabrous and cerebriform centrally but cottony at the periphery. Colonies of this isolate grew slowly at both 30°C (2.5 cm in diameter) and 35°C (0.8 cm in diameter). Holotype: Canada, Ontario, ex-liver and kidney of male *F. lateralis*, 24 November 1993, UAMH 7583, dried specimen and living culture. Etymology: of skin lesions. Notes: Infections in chameleons and geckos are recorded (Table 1) (3, 15, 18). In an experimental challenge of veiled chameleons, the ex-type culture of *N. dermatitidis* induced lesions confirmed by histopathology and culture (4). A day gecko isolate confirmed here as *N. dermatitidis* was reported originally as a *Trichophyton* species that caused deep dermatitis in geckos imported to Germany (15). An isolate from leopard gecko (UAMH 11231) (18) appeared to be similar to *N. dermatitidis*, but its intermediate placement between *N. vriesii* and *N. dermatitidis* in the ITS tree (Fig. 2) suggests that an analysis of additional isolates is required to resolve its taxonomic position.

***Nannizziopsis crocodili* Sigler, Hambleton, and Paré sp. nov. (subclade IIIa).** MycoBank accession no. MB 804605. Colonies on PDA were 4 to 5.5 cm in diameter, velvety to powdery, slightly to strongly zonate, and sometimes with exudate droplets after 21 days (Fig. 3F). Growth was slow at 35°C (1 to 2.7 cm diameter after 21 days). Aleurioconidia were subglobose, measuring 1.5 to 2.5 μm long and 1.3 to 2.4 μm wide, and sessile or borne on swollen cells either on the vegetative mycelium or within ascomata-like structures (pseudogymnothecia) (Fig. 5E, F, and G). The pseudogymnothecia developed within 2 to 3 weeks on both PDA and OAT and were composed of branched septate, anastomosing, and asperulate hyphae surrounding masses of conidia. No ascospores were produced in cultures incubated for several months or when available isolates were mated. Arthroconidia measuring 3.7 to 7.5 μm long and 2 to 3 μm wide were produced at low frequency and often showed germination (Fig. 5H and I). Undulate hyphae were formed

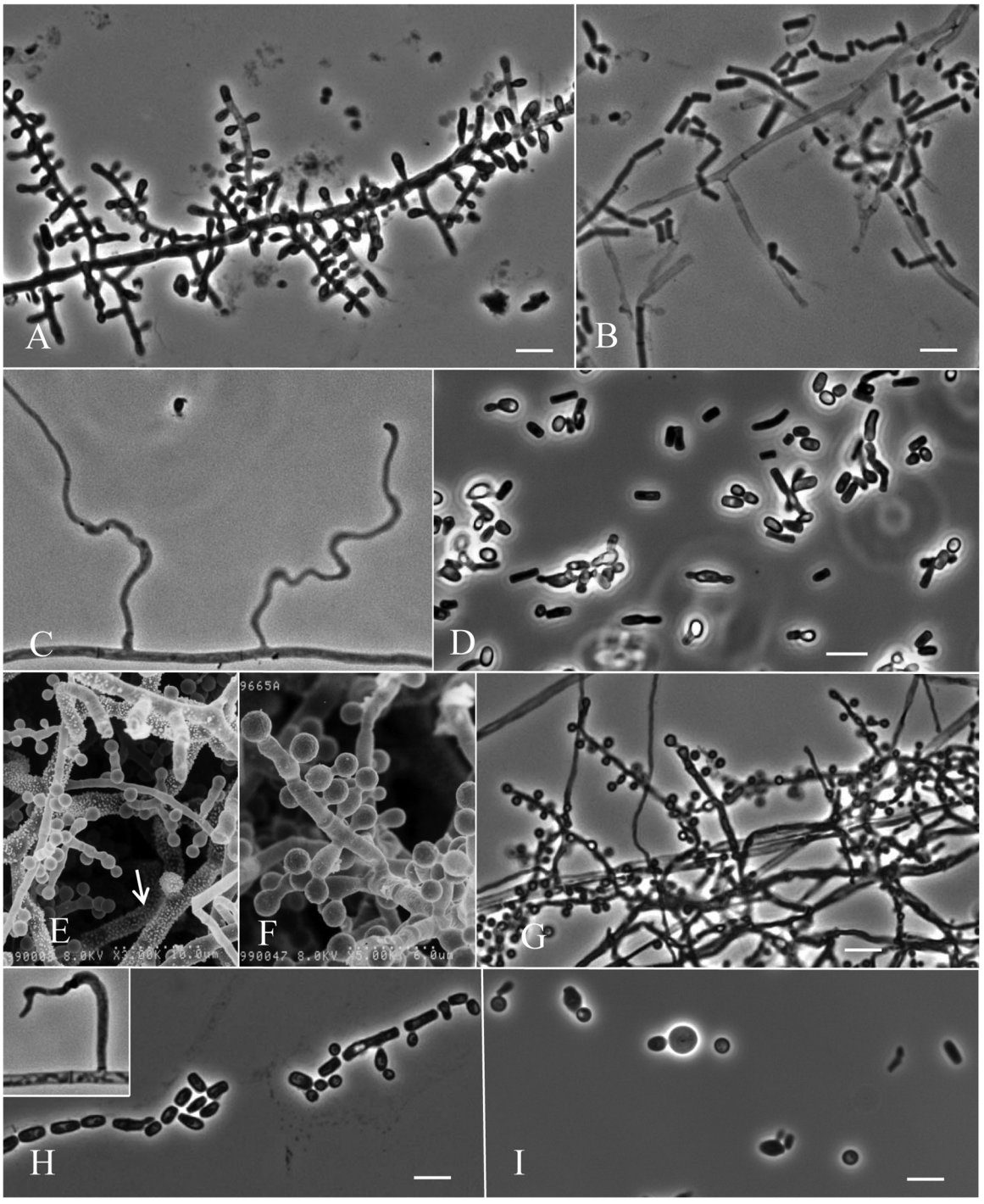


**FIG 5** Microscopic morphology of *Nannizziopsis dermatitidis* showing aleurioconidia (A), fission arthroconidia (B), and undulate hyphae (C). (D) Arthroconidia and budding cells produced on PDA. (E to I) Microscopic morphology of *Nannizziopsis crocodili*. (E and F) Scanning electron micrographs showing subglobose aleurioconidia among asperulate hyphae (indicated by arrow) of pseudogymnothecia. (G and H) Slide culture preparation showing aleurioconidia, fission arthroconidia, and an undulate hyphal branch (H inset). (I) Budding cells produced on BCP-MS-G agar. Bars = 10 μm.

(Fig. 5H, inset). Holotype: Australia, Gulf of Carpentaria, skin lesion on *C. porosus*, 1999, A. Thomas, UAMH 9666, dried specimen and living culture. Etymology: of the crocodile. Notes: This species is known only as the cause of fatal dermatitis in farmed Australian saltwater crocodiles, with hatchlings developing plaque-like lesions (25). It is distinguished from all other *Nannizziopsis* species by the small subglobose conidia often subtended by swollen cells, and by the formation of pseudogymnothecia.

***Nannizziopsis barbata* Sigler, Hambleton, and Paré sp. nov. (subclade IIIb).** MycoBank accession no. MB 804606. Colonies on PDA were 5.5 to 6 cm in diameter, powdery, flat to slightly

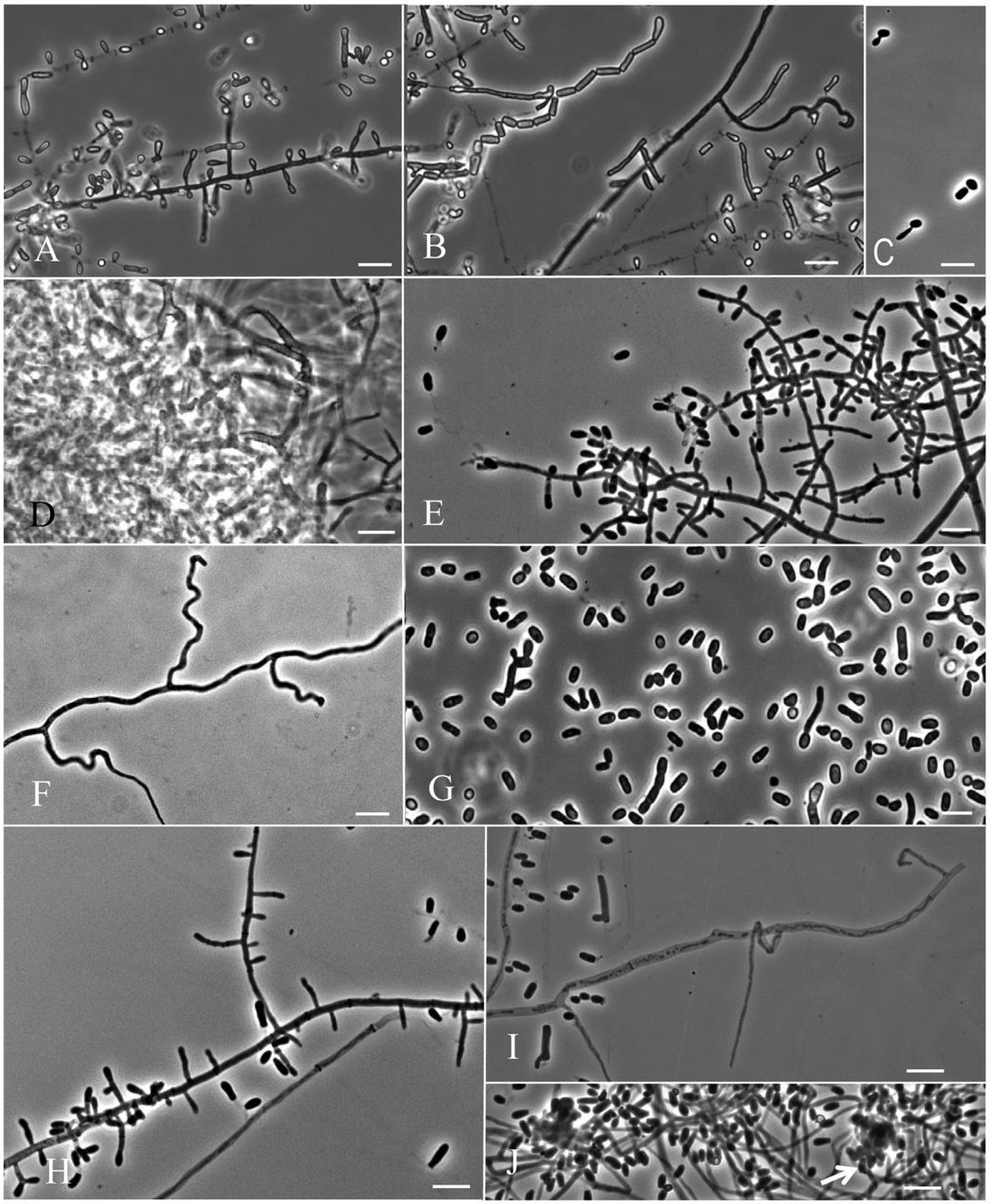


**FIG 6** Microscopic morphology of *Nannizziopsis barbata* showing aleurioconidia (A), fission arthroconidia and undulate hyphae (B), budding cells produced on PDA (C), and asperulate hyphae of a pseudogymnothecium on OAT (D). Microscopic morphology of *Nannizziopsis guarroi* showing aleurioconidia (E), undulate hyphae (F), and cylindrical arthroconidia, some of which are germinating (G). Microscopic morphology of *Nannizziopsis infrequens* showing aleurioconidia (H), undulate hyphae and rare intercalary arthroconidia (I), and ascomatal initials (arrow) (J). Bars = 10 μm.

raised and cottony at the center, but otherwise zonate after 21 days (Fig. 3H). There was no growth at 35°C. Aleurioconidia were pyriform to clavate, measured 3 to 6.5 μm long and 1.8 to 2.5 μm wide, and were sessile or borne on slightly swollen cells (Fig. 6A). Fission arthroconidia measuring 4.4 to 8.5 μm long and 1.7 to 3.5 μm wide, as well as undulate hyphae, were commonly produced (Fig. 6B). Moist colonies on PDA demonstrated budding (Fig. 6C). Infertile pseudogymnothecia composed of hyaline, anastomosing, asperulate hyphae were produced on OAT (Fig. 6D). Holotype: Australia, New South Wales, Penrith, skin lesion on *P. barbata*, 10 June 2009, UAMH 11185, dried specimen and living culture. Etymology: of the species *P. barbata*. Notes: *N. barbata* is thought to be the cause of an outbreak of infection in four *P. barbata* housed together in an outdoor enclosure at the Taronga Zoo (10). Histopathology confirmed hyphae in the skin of all four animals, the liver in two cases, and the heart of one animal. Cultures of a *Chrysosporium*-like fungus obtained from two animals were not held for follow-up investigation. However, a fifth wild-caught captive animal presented at the same clinic with ulcerated skin lesions in which histopathology sections revealed hyphae.

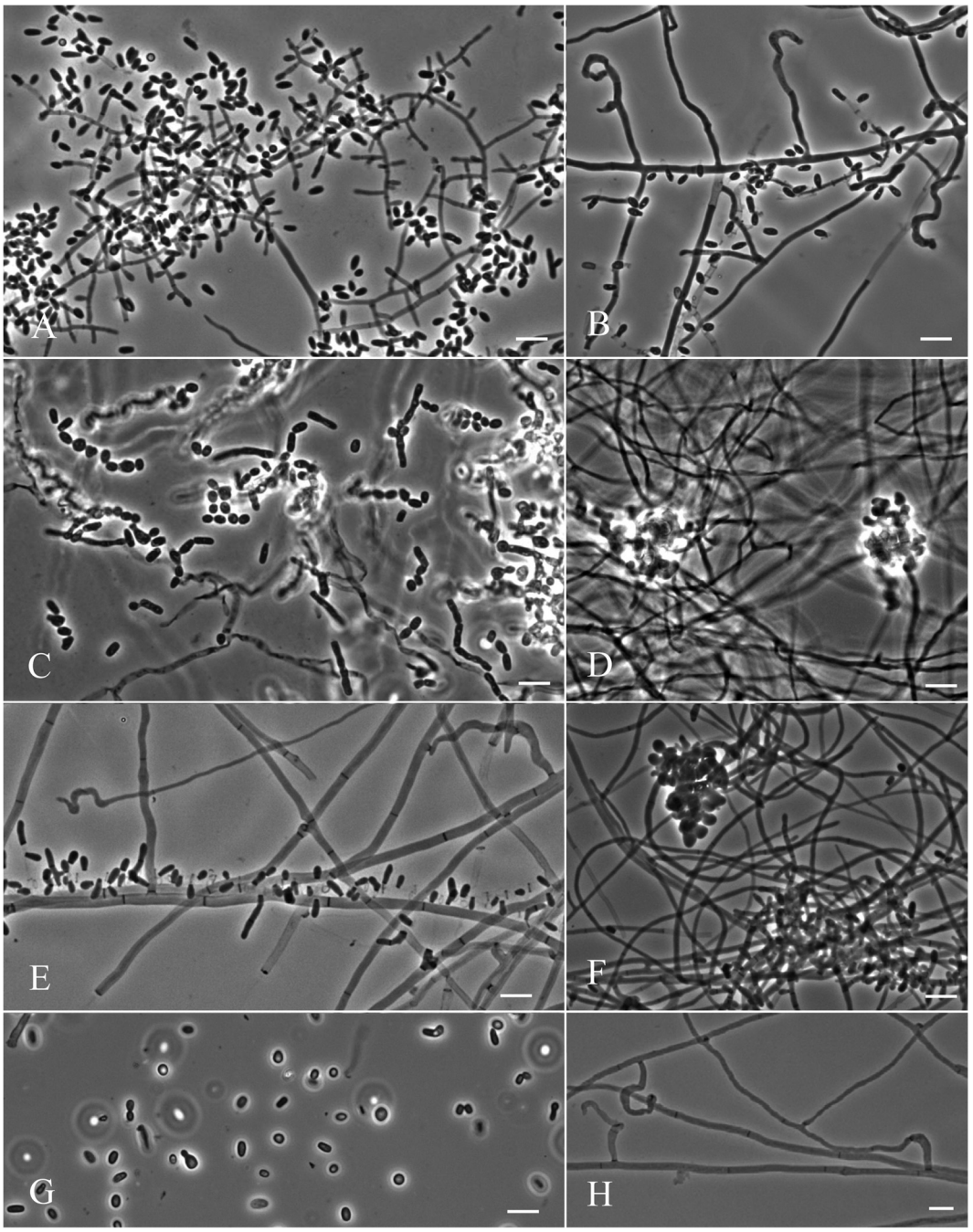


**FIG 7** Microscopic morphology of *Nannizziopsis hominis* showing aleurioconidia (A), undulate hyphae (B), fission arthroconidia (C), and ascomatal initials (D). Microscopic morphology of *Nannizziopsis obscura* showing aleurioconidia (E), ascomatal initial (F), budding cells (G), and undulate hyphae (H). Bars = 10 μm.

The ex-type isolate obtained from a skin specimen at necropsy demonstrated the highest ITS similarity (92% similarity) with *N. crocodili* isolates from Australia (Fig. 2).

***Nannizziopsis guarroi* (J. Cabañes and Abarca) J. Cabañes, Abarca, Stchigel, and Cano (30) (subclade IV).** Holotype CBS 124553. Colonies on PDA were 2.7 to 4.7 cm in diameter, powdery, sometimes sectoring to cottony, often strongly zonate, sometimes with exudate droplets (Fig. 3I). Growth at 35°C was similar, with colonies attaining 2.3 to 4 cm diameter (Fig. 3J). Aleurioconidia were clavate to pyriform and measured 3.2 to 6.5 μm long and 1.5 to 2.5 μm wide (Fig. 6E). Undulate hyphae were common (Fig. 6F). Arthroconidia in chains measured 2.8 to 7 μm long and 2 to 3.7 μm wide and sometimes showed budding in young cultures (Fig. 6G). Notes: *N. guarroi* is distinguished from other reptile-associated *Nannizziopsis* species by its slightly lower growth rate at 30°C and good growth at 35°C. Our results differ from those of Stchigel et al. (30) with respect to BCP-MS-G responses, in that repeated testing of isolates showed no pH change or slight acidification (trace yellow) rather

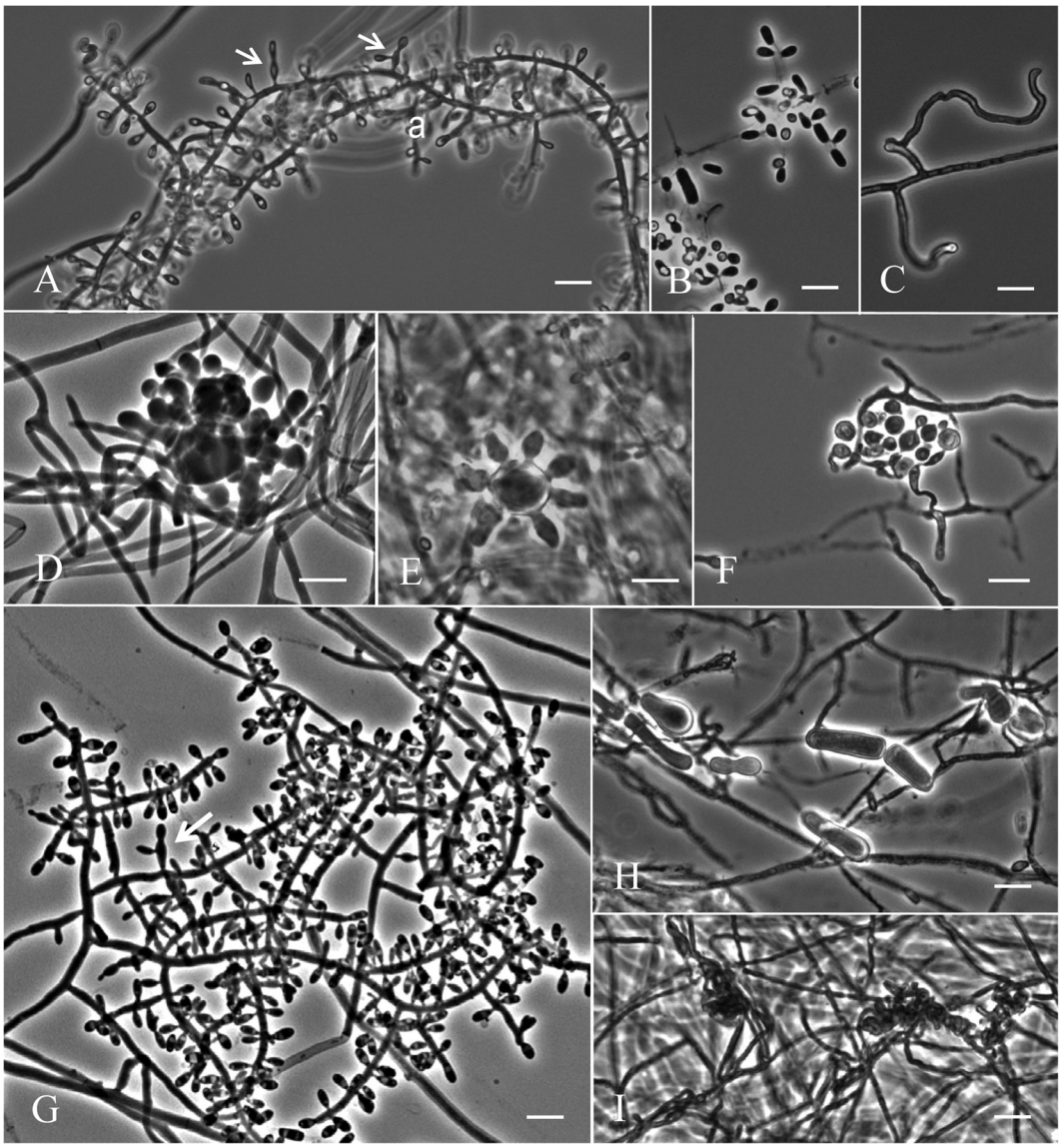


**FIG 8** Microscopic morphology of *Paranannizziopsis australasiensis* showing aleurioconidia borne sessile or subtended by a swollen cell (arrows) (A), occasional intercalary arthroconidia (B), undulate hyphae (C), ascomatal initials (D and E), and mycelium with swollen cells produced in the vicinity of the initials (F). Microscopic morphology of *Paranannizziopsis californiensis* showing aleurioconidia sometimes subtended by a swollen cell (arrow) (G) and large irregularly shaped cells (H) associated with ascomatal initials (I). Bars = 10 μm.

than alkalinization, as was reported in that study. In the ITS tree (Fig. 2), *N. guarroi* groups with *Nannizziopsis hominis* and other species from human sources, all of which grow well at 35°C. *N. guarroi* has been recorded frequently from inland bearded dragons and green iguanas that are commonly kept as pets, as well as from sungazers (*C. giganteus*) and common agama (*Agama agama*) (7–9, 11, 12, 16, 30, 34). This species is considered the etiologic agent of yellow fungus disease in inland bearded dragons, a contagious and progressive necrogranulomatous dermatomycosis first observed about 15 years ago. Two isolates from coastal bearded dragons (*P. barbata*) were excluded from *N. guarroi* and grouped in *N. barbata* (subclade IIIb) and *Paranannizziopsis australasiensis* (subclade VIIIa), respectively.

***Nannizziopsis infrequens* Sigler, Hambleton, and Paré sp. nov. (subclade V).** MycoBank accession no. MB 804608. Colonies attained 6 cm in diameter and were flat, velvety to powdery (Fig. 3L). At 35°C, colonies were 5 cm in diameter and more radially furrowed (Fig. 3M). Clavate to pyriform aleurioconidia were 2.2 to 5.5 μm long and 1.5 to 2.7 μm wide (Fig. 6H). Occasional intercalary arthroconidia, undulate hyphae, and ascomatal initials were produced but fission arthroconidia were not observed (Fig. 6I and J). Holotype: United States, Iowa, human bronchial wash specimen, 3 November 2003, UAMH 10417, dried specimen and living culture. Etymology: infrequent (in occurrence). Notes: The ex-type strain was isolated from a bronchial washing obtained from an $HIV^+$ male with pneumonia; however, the isolate was not considered to be contributing to the lung infection, and therefore no antifungal therapy was given (27). Because the patient resided in an area that is endemic for histoplasmosis, an AccuProbe test (Gen-Probe, San Diego, CA) was performed and tested positive in two different tests, albeit at lower relative light unit (RLU) values

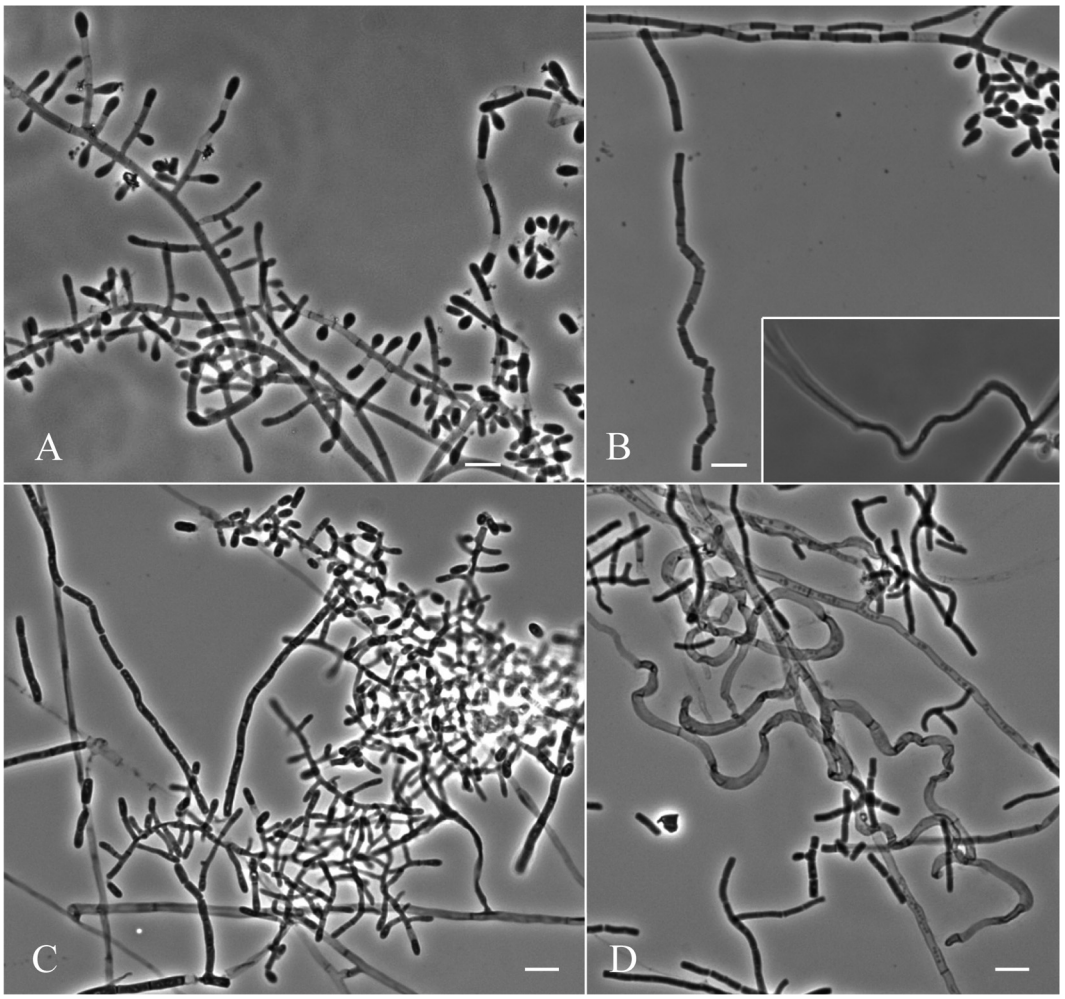


**FIG 9** Microscopic morphology of *Paranannizziopsis crustacea* showing aleurioconidia and occasional intercalary arthroconidia (A), fission arthroconidia (B), and an undulate hyphal branch (B inset). (C and D) Microscopic morphology of *Ophidiomyces ophiodiicola* showing aleurioconidia, fission arthroconidia, and numerous undulate hyphae. Bars = 10 μm.

than are typically obtained with *Histoplasma capsulatum* controls (27) (Table 2). Tests performed at the submitting laboratory indicated that the isolate grew at 40°C. The human-associated *N. infrequens* differs from the recently described reptile-associated species *N. chlamydospora* at 12 positions in the ITS region and by the absence of chlamydospores; it differs from *N. draconii* at 18 positions in the ITS region (Fig. 2), by its ability to grow at 40°C, and by the absence of alkalinity on BCP-MS-G (30).

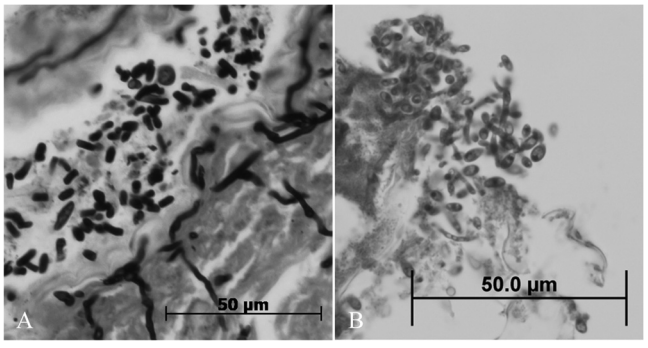


**FIG 10** Histopathological sections of skin lesions showing typical arthroconidia of *Paranannizziopsis crustacea* (A) and aleurioconidia produced at the lesion surface by *P. californiensis* (B). Image B was used with the permission of A. P. Pessier, San Diego Zoo Institute for Conservation Research, San Diego, CA.

***Nannizziopsis hominis* Sigler, Hambleton, and Paré sp. nov. (subclade VI).** MycoBank accession no. MB 804609. Colonies on PDA were 4.2 to 4.8 cm in diameter and were velvety to powdery, flat, radially furrowed or slightly zonate, and sometimes sectoring (Fig. 3O). At 35°C, colonies were 3 to 3.5 cm in diameter, velvety, and flat to strongly furrowed (Fig. 3P). Growth was similar on peptone yeast extract (PYE) after 21 days at 30°C, but a slight yellow diffusible pigment was produced (Fig. 3Q). Aleurioconidia were single-celled, rarely 2-celled, and measured 2.3 to 7 μm long and 1.5 to 3.2 μm wide (Fig. 7A). Undulate hyphae and fission arthroconidia were produced (Fig. 7B and C). Arthroconidia were cylindrical- to barrel-shaped, with rounded ends, and measured 2.4 to 9 μm long by 1.4 to 3.1 μm wide. Some ascomatal initials were present in cottony sectors (Fig. 7D). Holotype: United States, California, human thigh mass, 1994, UAMH 7859, dried specimen and living culture. Etymology: of man. Notes: *N. hominis* was isolated in 1994 from a deep muscle mass on the right thigh, right groin, buttock, and lung of an $HIV^+$ male who died 8 months after the initial isolation, presumably of complications from AIDS. The

patient received itraconazole for the fungal infection. He resided in the Sacramento, CA area, worked in business, had no history of drug use, and had no pets. The isolate from the lungs was tentatively identified initially as a *Trichophyton* species. In 2000, a white mold was isolated three times from the swollen lymph nodes of an immunocompetent Nigerian man from the Boston, MA area who presented with disseminated adenopathy following a trip to Nigeria. One of the isolates from this patient tested positive in the AccuProbe *Blastomyces* culture identification test (Table 2) and was sent to the Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, TX, for evaluation. Because of its *Chrysosporium*-like morphology, the isolate was then forwarded to the UAMH for further review. The patient was readmitted to the hospital 6 months later and found to have a disseminated fungal infection involving the heart (endocarditis), lungs, spleen, and kidneys. The fungus was not regrown, but the patient had been on itraconazole since the initial diagnosis and remained on the drug for 2 years. An isolate from disseminated disease in a Nigerian-American (GenBank accession no. HF547876, isolate UTHSC R-4317) that was identified as *N. guarroi* by Stchigel et al. (30) appears to represent another *N. hominis* isolate. The ITS sequence differs in only 3 positions, and the isolate grew at 40°C, whereas *N. guarroi* isolates did not grow at this temperature.

***Nannizziopsis obscura* Sigler, Hambleton, and Paré sp. nov. (subclade VII).** MycoBank accession no. MB 804610. Colonies growing at 30°C and 35°C were similar, attaining 5 cm in diameter, thin, often sectoring, with sectors glabrous to felty or thinly cottony, flat, or furrowed (Fig. 3R and S). Conidia were sessile or on short stalks, clavate, or pyriform, occasionally 2-celled, and measured 2.5 to 7 μm long and 1.6 to 2.7 μm wide (Fig. 7E). Ascomatal initials and undulate hyphae were produced (Fig. 7F and H). Arthroconidia measuring 3.1 to 7.7 μm long and 1.6 to 3.2 μm wide were produced in glabrous sectors and sometimes showed budding (Fig. 7G). Holotype: United States, New York, human leg abscess, 1984, UAMH 5875, dried specimen and living culture. Etymology: hidden (occurrence). Notes: *N. obscura* was isolated from two successive biopsy samples of a large tibial abscess in the right leg of a 24-year-old African-American male with a 2-month history of osteomyelitis (28). The patient's history included a trip to Africa and exposure to dust during home renovations. Histologic sections of the specimen revealed septate hyphae and budding yeasts within and around giant cells (28). The fungus was reported to grow at 37°C and on medium with cycloheximide and to produce numerous arthroconidia initially thought to resemble *Geotrichum*. It was identified as a *Chrysosporium* sp. when it was observed to also produce typical aleurioconidia (28). The patient was treated with amphotericin B over a 4-month period. An isolate causing a brain infection in an $HIV^+$ Nigerian male in Germany was identified as the CANV, but no details on the methods used to identify the fungus were provided (35). Stchigel et al. (30) identified the isolate (RKI 04-0104; GenBank accession no. HF547869) as *N. vriesii*, but its ability to grow at 40°C, low ITS similarity with other *N. vriesii* isolates (93%), and the low support for the grouping with *N. vriesii* in both phylogenetic analyses make this identification questionable. Their sequence groups with *N. obscura*, also a human pathogen, but differs at 14 positions along the ITS region.

***Paranannizziopsis* Sigler, Hambleton, and Paré gen. nov.** MycoBank accession no. MB 804611. Colonies were pale and moderately fast growing. Vegetative hyphae were narrow, branched, and septate, sometimes with racquet mycelia. Conidia (aleurioconidia) were sessile or produced on slightly swollen cells or on short stalks and were released by rhexolytic dehiscence. They were hyaline, smooth, pyriform, and clavate to obovate. Arthroconidia were absent, intercalary, or produced in adjacent chains. Undulate lateral branches were produced. No teleomorph was produced. *Paranannizziopsis* species are distinguished from *Nannizziopsis* and *Ophidiomyces* species by the uncommon occurrence or absence of fission arthroconidia (Table 2). Type species: *P. australasiensis* Sigler, Hambleton, and Paré.

***Paranannizziopsis australasiensis* Sigler, Hambleton, and Paré sp. nov. (subclade VIIIa).** MycoBank accession no. MB 804612. Colonies on PDA attained 4.5- to 5-cm diameter and were powdery or sometimes cottony, flat, or faintly zonate (Fig. 3U). There was no growth at 35°C. Aleurioconidia were sessile or were subtended by slightly swollen cells from which one or two conidia were produced (Fig. 8A). The conidia were pyriform to clavate and measured 3.5 to 8 μm long and 1.5 to 2.7 μm wide. Occasional intercalary arthroconidia and undulate hyphae were produced (Fig. 8B and C). Ascomatal initials occurred in cottony sectors and appeared as inflated cells with secondary proliferations (Fig. 8D and E). Some mycelia surrounding the ascomatal initials demonstrated swollen intercalary cells (Fig. 8F). Holotype: New Zealand, Auckland, skin lesion of *S. punctatus punctatus*, C. Harvey, 2011, UAMH 11645, dried specimen and living culture. Etymology: from Australasia. Notes: All isolates have been obtained from animals housed in zoos in Australia or New Zealand. Two aquatic file snakes (*Acrochordus* species) from the Melbourne Zoo presented with rapidly developing multifocal necrotizing skin lesions. Three isolates were associated with skin lesions in northern tuataras held in the Auckland Zoological Park, Auckland, New Zealand (36). A coastal bearded dragon (*P. barbata*) from the same zoo was diagnosed postmortem when the fungus was grown from a stored frozen biopsy specimen.

***Paranannizziopsis californiensis* Sigler, Hambleton, and Paré sp. nov. (subclade VIIIb).** MycoBank accession no. MB 804614. Colonies on PDA attained 4.5- to 5.2-cm diameter and were powdery and flat to slightly zonate (Fig. 3V). Growth at 35°C was strongly inhibited. Aleurioconidia were clavate to pyriform or obovate, measured 4 to 8.5 μm long and 1.8 to 2.6 μm wide, and were sessile or borne on a slightly swollen cell (Fig. 8G, arrow). Undulate hyphae were uncommon, and arthroconidia were not observed. Ascomatal initials occurred in cottony sectors and were associated with large irregularly shaped cells (Fig. 8H and I). The latter measured 10 to 36 μm long and 3.5 to 9.5 μm wide. Holotype: United States, California, San Diego, skin lesion of *Erpeton tentaculatum*, L. Sigler, 8 May 2006, UAMH 10693, dried specimen and living culture. Etymology: from California. Notes: *P. californiensis* was isolated from the skin or scales of three of five aquatic captive snakes (*E. tentaculatum*) housed at the San Diego Zoo. Several snakes died in the outbreak. Although both *P. californiensis* and *P. crustacea* were isolated from *E. tentaculatum*, they differ genetically (Fig. 2), morphologically (Table 2), and potentially, histopathologically. While typical cylindrical arthroconidia were observed in the tissues of lesions caused by *P. crustacea* (Fig. 10A), aleurioconidia were observed at the surface of a lesion caused by *P. californiensis* (Fig. 10B) A review of additional cases will be required to determine if the formation of aleurioconidia in tissue is a consistent finding in *P. crustacea* infections.

***Paranannizziopsis crustacea* Sigler, Hambleton, and Paré sp. nov. (subclade IX).** MycoBank accession no. MB 804615. Colonies on PDA attained 5.8 to 6.5 cm diameter and were powdery, flat, occasionally with dense downy overgrowth (Fig. 3W). There was no growth at 35°C. Aleurioconidia were clavate to pyriform or obovate, sessile or formed on short stalks, and measured 4 to 7.5 μm long and 2 to 3.5 μm wide (Fig. 9A). Undulate hyphae, fission arthroconidia, and occasional intercalary arthroconidia were produced (Fig. 9B). Arthroconidia measured 3.8 to 9.2 μm long and 1.9 to 2.7 μm wide. Holotype: Canada, Ontario, skin lesion of *E. tentaculatum*, L. Sigler, 11 June 2002, UAMH 10199, dried specimen and living culture. Etymology: crusty (lesion). Notes: *P. crustacea* caused fatal dermatitis in four captive tentacled snakes (24). On the surface of the ulcers was a crust of cellular debris containing clusters of arthroconidia. In culture, the isolates produced powdery and cottony sectors that when subcultured, differed in their preponderance of aleurioconidia and arthroconidia, respectively, and in the physiological reactions they elicited. The powdery aleurioconidial colony type was strongly urease positive and showed strong clearing of milk solids on BCP-MS-G medium, in contrast to the cottony arthroconidial type that was weakly urease positive and showed less clearing on BCP-MS-G (Table 2).

***Paranannizziopsis longispora* (Stchigel, Deanna A. Sutton, Cano, and Guarro) Sigler, Hambleton, and Paré comb. nov.** MycoBank accession no. MB 805156. Basionym: *Chrysosporium longisporum* Stchigel, Deanna A. Sutton, Cano, and Guarro, Persoonia 31:93, 2013. MycoBank accession no. MB 801990. The sequence of *C. longisporum* (GenBank accession no. HF547873, isolate UTHSC R-4380) groups closest to *P. crustacea* but differs at 9 positions in the ITS region. This level of sequence difference, combined with morphological differences, including the absence of growth at 30°C, absence of fission arthroconidia in chains, and longer conidia (3 to 13 μm long), provides support for the retention of both species (30).

***Ophidiomyces* Sigler, Hambleton, and Paré (see reference 29) (IF550166).** MycoBank accession no. MB 550166. Colonies were yellowish-white and moderately fast growing. Vegetative hyphae were narrow, branched, and septate, occasionally with racquet mycelia. Conidia were sessile or borne on short stalks and released by rhexolytic dehiscence (aleurioconidia). Aleurioconidia were hyaline, smooth, and cylindrical to clavate. Arthroconidia were formed in chains by schizolytic fragmentation of hyphae or were sometimes intercalary. Short, undulate, and sparsely septate lateral branches were common. No teleomorph is known. Type species: *O. ophiodiicola* (Guarro, Deanna A. Sutton, Wickes, and Rajeev) Sigler, Hambleton, and Paré (see reference 29) (IF550167). MycoBank accession no. MB 550167. Basionym: *C. ophiodiicola* Guarro, Deanna A. Sutton, Wickes, and Rajeev, J. Clin. Microbiol. 47:1208. MycoBank accession no. MB 506606. Holotype: United States, Georgia, Sparta, black rat snake with mycotic granuloma, 2009, CBS 122913 (isotypes FMR 9510, UTHSC 07-604 [R-3923]). Colonies on PDA were 4 to 6 cm in diameter and were velvety to powdery, dense, flat, frequently zonate, and sometimes with cottony sectors (Fig. 3X). Clear exudate droplets were often present. Most isolates failed to grow at 35°C, but two isolates (UAMH 10949 and UAMH 11295) attained a diameter of 2 to 3.5 cm after 21 days. Aleurioconidia were sessile or borne at the ends of short stalks, cylindrical to clavate, and 2.5 to 7.5 μm long and 1.5 to 2.5 μm wide (Fig. 9C). Arthroconidia were 3 to 12.5 μm long (in rare cases up to 15 μm long) by 1.5 to 3.5 μm wide (Fig. 9D). In young cultures on PDA, arthroconidia sometimes showed budding or germination. Undulate hyphae were commonly produced (Fig. 9D). Rarely, these fragmented to form chains of arthroconidia. Some isolates produced ascomatal initials in cottony sectors. Most isolates produced a strong to weak mercaptan-like odor. Notes: In addition to the mycotic granuloma in a black rat snake (26), published reports concerning *O. ophiodiicola*, and confirmed here by the sequencing of isolates, include fatal dermatitis in brown tree snakes attributed to the CANV (isolate UAMH 6642) (19), disseminated infection in a garter snake attributed to *Chrysosporium queenslandicum* (isolate UAMH 9832) (20), disseminated dermatitis in green anacondas (isolate UAMH 10949) (21), and moist dermatitis in an Australian broad-headed snake (isolate UAMH 11295) (23). Fungal dermatitis in wild-caught captive carpet snakes (*Morelia spilotes variegata*) was attributed to *Geotrichum* (37), but the development of arthroconidia at the surface of the lesions is highly characteristic of *O. ophiodiicola* infection (19). A diagnosis of CANV in a boa constrictor was based on the appearance of the skin lesions and of the hyphae in tissue (22). Most cases involve captive animals, but *O. ophiodiicola* is also associated with infections in wild snakes. The isolates from *Nerodia* species in the present study were from wild-caught snakes that were part of a zoology research study in Florida (Table 1). Six of 30 snakes became infected. A sequence (GenBank accession no. JX878608) from an isolate from one of 11 wild timber rattlesnakes (*Crotalus horridus*) from Massachusetts showed high homology with the *O. ophiodiicola* sequences described here (38) (Fig. 2). Similarly, direct PCR of five skin biopsy samples from eastern massasauga rattlesnakes yielded amplicons that showed >99% homology with *O. ophiodiicola* (GenBank accession no. EU715819) (5). Most isolates that we studied came from the United States, with others from Australia, Germany, or the United Kingdom (Table 1). Only one isolate (UAMH 9985) was not associated with infection and came from skin scales of a captive African rock python (*P. sebae*) in a southwestern zoo during a survey of shed reptile exuviae (6).

## DISCUSSION

The molecular genetic differences disclosed in this study support the reassignment of isolates of morphologically similar fungi, formerly referred to as members of the CANV complex, into three genera, two of them novel. *Nannizziopsis*, *Paranannizziopsis*, and *Ophidiomyces* are the leading causes of infectious dermatomycoses in captive reptiles. Infections caused by these fungi in reptiles are contagious, typically affecting multiple individuals, and usually present as a rapidly progressing, deep, necrotic, or granulomatous dermatomycosis that eventually disseminates and for which the outcome is usually fatal. Molecular data are pivotal in redefining and clarifying the range of susceptible hosts for each fungal species, and they allow for the detection of a trend in which each genus seems to be associated with infections within given reptile taxa. We found no evidence of *Nannizziopsis* infection in snakes, but *N. guarroi* and *N. dermatitidis* are major pathogens of lizards. We documented three *Nannizziopsis* species, *N. infrequens*, *N. hominis* (including an isolate of human origin originally determined to be *N. guarroi* [30]), and *N. obscura* from specimens or cases of human infection, but these species have not been recovered from reptiles; this somewhat mitigates zoonotic concerns associated with handling popular pet reptiles, such as green iguanas or bearded dragons, in which *N. guarroi* dermatitis is com-

mon. *N. crocodili* is recorded only from two outbreaks in farmed saltwater crocodiles in Australia in which 48 hatchlings died of infection. It may be that disease caused by *N. crocodili* is more common than is suspected, because cultures from specimens are often overgrown by rapidly growing environmental fungi, like *Fusarium* spp. or *Purpureocillium lilacinum* (25).

Reptile host specificity appears to be slightly different among *Paranannizziopsis* species. Two species were obtained from separate outbreaks of fatal dermatomycosis in tentacled snakes with very similar clinicopathological presentations. Snakes were housed in zoological institutions in California (*P. californiensis*) and Ontario, Canada (*P. crustacea*) under seemingly adequate husbandry. One of the authors of the present study (J. A. Paré) has seen tentacled snakes with a similar kind of dermatomycosis in other zoological collections, including a very recent case at the Bronx Zoo in which the fungal isolate was confirmed as *P. crustacea* by ITS sequence comparison; this suggests that the disease caused by *Paranannizziopsis* species may be emerging as a leading cause of death in these aquatic snakes. Acidifying the exhibit water has led to clinical improvement and even apparent resolution of outbreaks in some, but not all, cases (J. A. Paré, unpublished data). The host range of *P. australasiensis* is less restricted and includes reptiles as diverse as a file snake, a lizard, and the much more distantly related tuatara. The host range of species affected by *Nannizziopsis* and *Paranannizziopsis* will become better defined in the future as isolates from sick animals are sequenced and assigned to the correct fungal species.

*O. ophiodiicola* has been recovered only from snakes, and all isolates but one were from lesions. Infections of *O. ophiodiicola* progress rapidly and are frequently associated with conspicuous lesions of the head and ventral scales and with the presence of caseous brown plaques, crusts, or nodules (5, 19–23, 26, 37). Most infections were documented in captive animals that were often recently caught. The first case published under the CANV appellation was from a colony of brown tree snakes reared at the U.S. National Institutes of Health (19), but our first isolate was obtained from a subcutaneous nodule in a corn snake examined in Ithaca, NY (Table 1). Recently, snake fungal disease (SFD) has been identified as an emerging disease of wild crotalid snakes in Illinois and New England, in which affected animals often demonstrate disfiguring lesions on the face and head (39). *O. ophiodiicola* (as *C. ophiodiicola*) has been cultured repeatedly from snakes with these facial lesions, and while other fungi also have been isolated, none was known to be a snake pathogen. Table 1 lists 10 *O. ophiodiicola* isolates, all confirmed as the cause of disease in ophidians encompassing colubrids, boids, pythonids, acrochordids, and elapids; this lends strong support to the supposition that *O. ophiodiicola* is the cause of SFD in wild snakes.

The ecology and geographical distributions of *Nannizziopsis*, *Paranannizziopsis*, and *Ophidiomyces* species remain poorly understood, as all but one isolate have come from lesions in sick animals and not from the environment. A culture-based survey indicated that that these fungi are rare on the skin of healthy captive squamates (6), and these results are supported by PCR assays that failed to detect the presence of *O. ophiodiicola* (as *Chrysosporium* species) in 38 wild eastern massasauga snakes that were captured for a disease investigation survey (40). In contrast, PCR assays did detect the presence of *O. ophiodiicola* in 9 of 14 wild timber rattlesnakes from New England that presented with skin lesions (38). The reptile trade, which occurs on a worldwide scale, has obscured the provenance of *Nannizziopsis* isolates recovered from sick captive reptiles. The first *N. dermatitidis* isolates were recovered in the 1990s from sick captive day geckos imported from Madagascar to Germany (Table 1, UAMH 6610) (15) and from chameleons in Canada (3). The three cases of chameleon infection were temporally clustered and might have been from the same shipment of wild-caught lizards to Canada. The only other outbreak attributable to *N. dermatitidis* occurred 15 years later in leopard geckos from a captive breeding operation in Florida (18). While this last outbreak further supports the pathogenicity of *N. dermatitidis* in geckonid lizards, it is distinct in time and place compared to the prior documented cases. *N. guarroi* was described originally from captive green iguanas in Spain and has been isolated repeatedly from pet inland bearded dragons with yellow fungus disease in North America. The infection may have spilled over to green iguanas through the pet trade, as the first case of *N. guarroi* in green iguanas coincided temporally with the first documented European cases of yellow fungus disease in bearded dragons (8, 9). In contrast, a distinct *Nannizziopsis* species, *N. barbata*, was recovered from a wild-caught captive coastal bearded dragon (10). Coastal bearded dragons are closely related to inland bearded dragons, but they are not constituents of the commercial pet trade and they are mostly allopatric, so that the range of the two species barely overlaps.

Infections caused by *Nannizziopsis*, *Paranannizziopsis*, and *Ophidiomyces* species are contagious among reptiles. Almost all species produce cylindrical fission arthroconidia in culture, as well as in infected cutaneous tissues, and we believe these to be the primary propagules for the transmission of infection between reptiles (4, 25). In young cultures, arthroconidia are frequently present in mucoid-to-glabrous colonies in which yeast-like budding is present. In tissues, arthroconidia occur in the stratum corneum or deeper in the epidermis, or in characteristic aggregates or tufts at the surface of lesions (3, 4, 19, 24). In an experimental evaluation of pathogenicity, 12 of 20 (60%) healthy veiled chameleons developed histologically confirmed lesions following the application of *N. dermatitidis* conidia directly to intact or abraded skin (4). Infection also occurred in one of 10 animals in the environmental exposure group, and the recovery of the fungus from settle plates and cage materials indicated its potential for fast dissemination in the captive environment. While fission arthroconidia are produced by *P. crustacea*, they have not been observed in *P. australasiensis* or *P. californiensis*. The histopathology of a lesion caused by the latter species revealed the presence of aleurioconidia (Fig. 10B). Further data on *P. californiensis* infections are needed to determine whether this presentation is unusual for the species. Another distinctive morphological feature of all these species is the formation of undulate hyphal branches that may play a role in pathogenicity, at least in reptiles, by possibly aiding in attachment. The two species that have few undulate hyphae (*N. infrequens* and *P. californiensis*) also lacked fission arthroconidia, and pathogenicity is confirmed only for the latter species (Table 2).

There are limited data on the efficacy of orally administered antifungal agents in treating infections in reptiles. Lesions of *N. dermatitidis* responded to itraconazole therapy in one of two chameleons but not to ketoconazole in a third animal (3). Snakes with confirmed *O. ophiodiicola* infection treated with itraconazole or ketoconazole similarly failed to respond (20–22, 26). Most data on antifungal therapy concerns *N. guarroi*. Itraconazole failed to resolve infections in two of three bearded dragons after 6 to 8

weeks, but a third was cured with itraconazole and amputation of the affected limb (7). Only seven of 13 bearded dragons responded to itraconazole together with topical clotrimazole (9). Systemic ketoconazole therapy combined with topical terbinafine improved lesions in a single bearded dragon (12), but only one of three animals with severe infection improved with ketoconazole alone (34). The efficacy and safety of voriconazole in curing bearded dragons and girdled lizards were demonstrated in two studies (16, 41). Antifungal susceptibility testing of *N. guarroi* isolates indicated acquired resistance to itraconazole in one of 32 isolates (41).

This study demonstrated the genetic diversity of members of the CANV complex and determined that the reptile pathogens and human isolates belonged in three well-supported lineages that are distinct from all other taxa within the family *Onygenaceae*, of the order *Onygenales*. With the new genera and species classifications proposed here, it will be possible to accurately identify the major fungal species associated with reptile dermatomycosis, determine the prevalence of these fungi in the environment, evaluate whether antifungal susceptibility profiles differ among species or between genera, and develop strategies for disease prevention and therapy.

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