CZECH MYCOLOGY 77(1): 1–16, FEBRUARY 26, 2025 (ONLINE VERSION, ISSN 1805-1421)

Aspergillus nanangensis: updated description with phenotypic and ecological comparison to other species of section *Janorum*

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Sklenář F., Jurjević Ž., Hubka V. (2025): *Aspergillus nanangensis*: updated description with phenotypic and ecological comparison to other species of section *Janorum*. – Czech Mycol. 77(1): 1–16.

Aspergillus nanangensis is a recently described species, only known from a single collection from forest soil in Australia. It belongs to the less explored section *Janorum* within the subgenus *Circumdati*. It produces secondary metabolites (nanangenines), some of which possess anticancer activity. Here, we report new isolations of this species from indoor air and dust in the USA, and update the description of this species to include previously unobserved phenotypic characteristics, notably two different types of conidial heads (white and green), a typical feature of section *Janorum*. In addition to presenting new data about the ecology of this species, we review the geographic distribution of all currently recognised members of section *Janorum*. Phylogenetic analysis based on four DNA loci (ITS rDNA, *benA*, *CaM*, and *RPB2*) has confirmed that *A. nanangensis* is a well-supported species. Finally, we provide a comprehensive comparison of the distinguishing characters of *A. nanangensis* with the four remaining species accepted in section *Janorum*.

Key words: *Aspergillus janus*, fungal taxonomy, GlobalFungi, multilocus phylogeny, nanangenines.

Article history: received 13 November 2024, revised 17 January 2025, accepted 27 January 2025, published online 26 February 2025 (including Electronic supplement).

DOI: https://doi.org/10.33585/cmy.77101

Sklenář F., Jurjević Ž., Hubka V. (2025): *Aspergillus nanangensis*: aktualizovaný popis s fenotypovým a ekologickým srovnáním s ostatními druhy sekce *Janorum*. – Czech Mycol. 77(1): 1–16.

Aspergillus nanangensis je nedávno popsaný druh, známý pouze z jediného sběru z lesní půdy v Austrálii. Patří do nepříliš prozkoumané sekce *Janorum* v podrodu *Circumdati*. Produkuje sekundární metabolity (nanangeniny), z nichž některé vykazují protinádorovou aktivitu. Tato studie přináší informace o nových izolátech tohoto druhu z prachu a vnitřního ovzduší domu v USA a aktualizaci popisu tohoto druhu s uvedením dosud nepozorovaných fenotypových znaků, z nichž nejdůležitější je přítomnost dvou různých typů konidiálních hlavic (bílé a zelené), typická pro sekci *Janorum*. Spolu s novými údaji o ekologii tohoto druhu podáváme přehled geografického rozšíření všech v současnosti

uznávaných zástupců sekce *Janorum*. Fylogenetická analýza založená na čtyřech lokusech DNA (ITS rDNA, *benA*, *CaM* a *RPB2*) potvrdila, že *A. nanangensis* je dobře podpořený druh. Studie také obsahuje komplexní srovnání rozlišovacích znaků *A. nanangensis* se čtyřmi zbývajícími druhy uznanými v sekci *Janorum*.

INTRODUCTION

Aspergillus is an important genus of filamentous fungi with a rapidly increasing number of described species (Visagie et al. 2024). *Aspergillus nanangensis* belongs to the section *Janorum*, originally proposed as section *Jani* by Hubka et al. (2015) to accommodate *A. janus* and *A. brevijanus*. The name of the section was later corrected to *Janorum* by Houbraken et al. (2020) to align with Latin grammar. Historically, the two mentioned species were classified into the section *Versicolores* (Raper et Fennell 1965, Klich 1993), currently considered a series within section *Nidulantes* (Houbraken et al. 2020, Sklenář et al. 2022). The segregation of *A. janus* and *A. brevijanus* into a distinct section was supported by phylogenetic analysis and strong phenotypic evidence (Hubka et al. 2015).

The species of section *Janorum* usually produce three distinct types of conidiophores. Two types have a typical *Aspergillus* structure consisting of a stipe, a vesicle, and phialides, but differ in vesicle shape (white ones mostly spathulate to clavate; green ones mostly pyriform to subglobose) and colour of produced conidia (white vs green). The third type consists of micro- to semi-macronematous conidiophores producing so-called accessory conidia (Hubka et al. 2015). Accessory conidia are not unique to section *Janorum* but were observed in two related sections, section *Terrei* and *Flavipedes* (Balajee 2009, Hubka et al. 2015). Their function and structure were mainly studied in section *Terrei*. They lack melanin and hydrophobin layers in their cell walls, and are formed during hypoxia, submersed growth, and also during infection. It is believed that they may boost fungal dissemination during infection (Deak et al. 2009).

The isolation of *A. janus* and *A. brevijanus* has been sparsely reported, most records originating from soil (Nováková et al. 2012, Souza et al. 2019), although there are reports from hospital air in India (Singh et Singh 1999) and foodstuff in Turkey (Asan 2004). Three additional species belonging to the section were described recently, *A. yunnanensis* from soil in China by Cai et al. (2020), *A. trisporus* from soil in Brazil by Souza et al. (2019), and *A. nanangensis* from forest soil in Australia by Pitt in Crous et al. (2020). The production of accessory conidia has been reported in *A. janus*, *A. brevijanus*, and *A. trisporus*, but not in *A. yunnanensis* and *A. nanangensis*.

Aspergillus nanangensis has been shown to produce novel bioactive compounds, nanangenines (sesquiterpenoids; Lacey et al. 2019) and nanangelenin (benzazepine alkaloid; Li et al. 2020). These compounds often exhibit antibacterial, anti-inflammatory, or anticancer properties (Dhyani et al. 2022). *Aspergillus brevijanus* produces brevijanazines (Li et al. 2022), piperazine compounds which can serve as a basis for the production of drugs such as antidepressants, antipsychotics, antihistamines, antifungals, and antibiotics (Rathi et al. 2016, Kant et Maji 2021).

This article reports the isolation of two additional strains of *A. nanangensis* and presents an emended description and differential diagnosis of this species, which was recently described based on a single isolate (Crous et al. 2020). Additionally, we summarise data on the ecology and distribution of accepted species of section *Janorum*.

METHODS

Molecular studies. Total genomic DNA was isolated from 7-day old cultures with an ArchivePure DNAyeast and Gram2+ kit (5 PRIME Inc., Gaithersburg, Maryland, USA). The quality of the isolated DNA was measured on a NanoDrop 1000 Spectrophotometer. The ITS region of rDNA was amplified using forward primer ITS1 (White et al. 1990) and reverse primer NL4 (O'Donnell 1993). The partial sequence of the β-tubulin gene (*benA*) was amplified using forward primer Bt2a and reverse primer Bt2b (Glass et Donaldson 1995). The partial sequence of the calmodulin gene (*CaM*) was amplified using forward primers CF1L, CF1M, and reverse primer CF4 (Peterson 2008). The partial sequence of the RNA polymerase II second largest subunit gene (*RPB2*) was amplified using forward primer fRPB2-5F and reverse primer fRPB2-7CR (Liu et al. 1999). PCR protocols were previously described by Hubka et al. (2018). PCR products were purified with ethanol and sodium acetate as described by Sklenář et al. (2021). Purified products were sequenced with Sanger sequencing technology at Macrogen sequencing service (Amsterdam, Netherlands). The sequences were deposited in GenBank under accession numbers listed in Tab. 1.

Phylogenetic analysis. Sequences were inspected and assembled in Bioedit 7.2.5 (Hall 1999) and aligned in MAFFT 7 (Katoh et Standley 2013) using the G-INS-I strategy. To inspect the phylogenetic relationship between the strains, we performed Bayesian inference (BI) and Maximum likelihood (ML) analysis of each locus. The most suitable nucleotide substitution models were selected with jModelTest 2.1.7 (Posada 2008) using the Bayesian information criterion (BIC). The model selected for alignment of ITS sequences was TVM+I. In case of *CaM* sequences, the selected model was TrNef+G, for *benA* K80+G and for *RPB2* TrNef+G, BI was calculated in MrBayes 3.2.7 (Ronquist et al. 2012). The length of the chain was set to 40,000 generations (sufficient to reach convergence for all loci except ITS, which was run for another 30,000 generations), sampling frequency was set to 100, and burn-in to 25%. Maximum likelihood analysis was performed in IQ-TREE 2.2.0 (Minh et al. 2020) with 1000 standard bootstrap replicates. Figures representing the phylogenetic trees were created in FigTree 1.4.4 (Rambaut 2018) and edited in Adobe Photoshop CS6 (Adobe Systems Inc, San Jose, California, USA).

Biogeographic data of *Aspergillus nanangensis* and closely related species were retrieved from the GlobalFungi database (Větrovský et al. 2020) using the taxon search function with the species option.

Tab. 1. Strains of *Aspergillus* sect. *Janorum* used in phylogenetic analysis.

Species	Strain no.[a]	Provenance (locality, substrate, year of isolation, collector)	GenBank/ENA/DDBJ accession Nos.			
			ITS (LSU)[b]	*benA*	*CaM*	*RPB2*
A. brevijanus	NRRL 1935^T = ATCC 16828^T = CBS 111.46^T = IMI 16066^T = CCF 4546^T = FRR 1935^T	Mexico, Alameda, isol. ex soil, 1942, W.B. Roos	EF669582	EU014078	EF669540	EF669624
A. janus	NRRL 1787^T = ATCC 16835^T = CBS 118.45^T = IHEM 4374^T = IMI 16065^T = CCF 4549^T = FRR 1787^T	Panama, isol. ex soil, 1942 (soil was collected in 1941), J.T. Bonner	EF669578	EU014076	EF669536	EF669620
A. janus	NRRL 1936 = IMI 362226 = CCF 4550	Panama, isol. ex soil, 1942, R.D. Coghill	EF669583	EU014077	EF669541	EF669625
A. janus	FMR 15883	Unknown source		LT907912		
A. janus	CBS 128799	Ecuador, unknown source	MH865149			
A. trisporus	CCDCA FI 15^T = CML 3603^T	Brazil, Minas Gerais state, soil, 2014, V.M. Pereira	MF616388	MF616387	MN013146	MF616389
A. nanangensis	CBS 146238^T = FRR 6048^T = MST FP2251^T	Australia, Queensland, Nanango, forest soil, 2004, J.I. Pitt	MK979278	MT184783	MT184789	MT184795
A. nanangensis	CCF 5673 = CBS 145857 = EMSL 2787	USA, Washington, Bremerton, bedroom – settle plates, 2015, Ž. Jurjević	MN413243	MN412753	MN412754	MN412756
A. nanangensis	CCF 6240 = CBS 145856 = EMSL 2820	USA, Washington, Bremerton, dust in bedroom, 2015, Ž. Jurjević	MN413244	MN412752	MN412755	MN412757
A. yunnanensis	CGMCC 3.19711^T	China, Yunnan Province, farmland soil, 2017, P.P. Huang	MN066373	MN072909	MN072911	MN072913
A. yunnanensis	CGMCC 3.19712	China, Yunnan Province, farmland soil, 2017, P.P. Huang	MN066374	MN072910	MN072912	MN072914

[a] Acronyms of culture collections in alphabetic order: ATCC – American Type Culture Collection, Manassas, Virginia, USA; CBS – Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; CCDCA – Filamentous fungi culture collection of the Department of Food Science, Federal University of Lavras, Brazil; CCF – Culture Collection of Fungi at the Department of Botany of Charles University, Prague, Czech Republic; CGMCC – China General Microbiological Culture Collection Center, Beijing, China; CML – Mycological collection of Lavras, Brazil; EMSL – EMSL Analytical Inc., New Jersey, USA; FMR – Faculty of Medicine, Reus, Spain; FRR – Food Fungal Culture Collection, North Ride, Australia; IHEM – Belgian Coordinated Collections of Micro-organisms (BCCM/IHEM), Brussels, Belgium; IMI – CABI's collection of fungi and bacteria, Egham, UK; MST – Microbial Screening Technologies, Smithfield, Australia; NRRL – Agricultural Research Service Culture Collection, Peoria, Illinois, USA; UTHSCSA – Collection of Fungus Testing Laboratory, University of Texas, Health Science Center, San Antonio, USA.

[b] If available, the sequence of the entire region including both ITS and LSU is given. For some strains, only the ITS sequence is provided in GenBank.

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Phenotypic studies. To observe the macromorphological characters of the colonies, isolates were inoculated on Czapek Yeast Autolysate agar (CYA, Fluka, Buchs, Switzerland), Czapek-Dox agar (CZA), Malt Extract agar (MEA, Oxoid, Melbourne, Australia), Oatmeal agar (OA), CYA supplemented with 20% sucrose (CY20S), and Yeast Extract Sucrose agar (YES), and then incubated for 14 d at 25 °C in the dark. Colour names and codes used in the descriptions refer to Kornerup et Wanscher (1967). To determine maximum growth temperature, cultivation on MEA at higher temperatures was tested. Photographs of colony details were taken using an Olympus SZX2-ILLT dissecting microscope (Tokyo, Japan) equipped with an Olympus DP27 digital camera (Tokyo, Japan). Microscopic characters were observed and photographed using an Olympus BX51 microscope with an Olympus DP72 camera. Samples were prepared from 7-day old cultures grown on CYA and OA with lactic acid (60%) used as mounting medium. Values of micromorphological features are presented as the range of extreme values, with the mean ± SD provided in parentheses. Adobe Photoshop CS6 was used for photographic plate preparation.

RESULTS

COMPARISON OF *ASPERGILLUS* SECT. *JANORUM* SPECIES

Phylogenetic analysis

The phylogenetic analysis consisted of the BI and ML methods applied on the concatenated alignment of four DNA loci and single-gene datasets. The resulting multilocus phylogenetic tree is depicted in Fig. 1 (partitioned analysis of the concatenated alignment) and single locus trees in Electronic Supplement Figs S1–S8. The alignment of ITS sequences was 570 bp long with 71 variable and 29 parsimony-informative sites; the alignment of *benA* sequences was 521 bp long with 192 variable and 85 parsimony-informative sites; the alignment of *CaM* sequences was 735 bp long with 264 variable and 124 parsimony-informative sites; the alignment of *RPB2* sequences was 1014 bp long with 264 variable and 150 parsimony-informative sites.

Aspergillus nanangensis strains CCF 5673 and CCF 6240 clustered with the ex-type strain CBS 146238 of *A. nanangensis* and were sister to the recently described *A. yunnanensis* in all analyses. The sequences of CCF 5673 and CCF 6240 were identical to each other in all four loci and also identical to the ex-type strain (CBS 146238) in the ITS locus. They differed from the ex-type strain by two substitutions in *benA*, two substitutions in *CaM*, and six substitutions in *RPB2* genes. *Aspergillus nanangensis* and *A. yunnanensis* form a sister clade of *A. janus*. The tree topology was identical regardless of locus or method used, only the support for individual branches varied. The lineage of *A. nanangensis* was highly supported in almost all phylogenetic reconstructions except for the ML analysis of the *benA* locus, where the split between *A. nanangensis* and *A. yunnanensis* gained low support (bs = 65), and also for the ML and BI analyses of the ITS locus (bs = 55, pp = 0.74). Other splits were also generally well

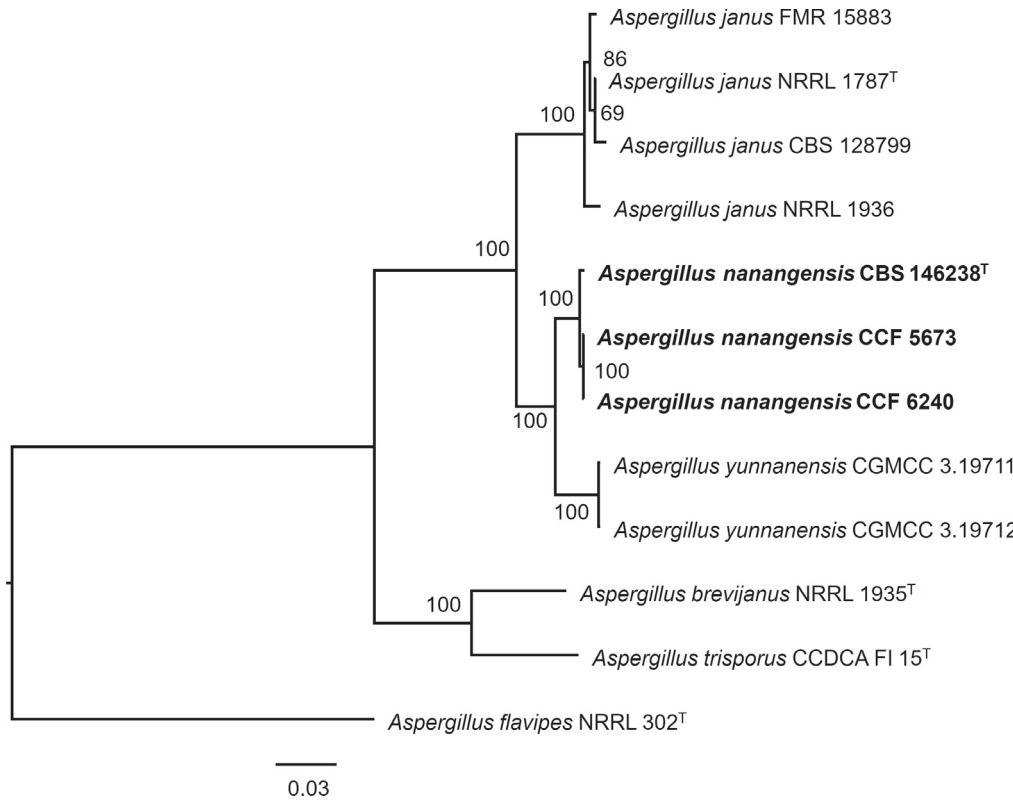

Fig. 1. Best scoring Maximum likelihood phylogenetic tree based on four DNA sequence loci (ITS, *benA*, *CaM*, *RPB2*) of section *Janorum* strains inferred in IQ-TREE 2.2.0 (Minh et al. 2020). The support values represent bootstrap values from IQ-TREE (1000 standard nonparametric replicates) and posterior probability values calculated in MrBayes 3.2.7 (Ronquist et al. 2012). Each locus was treated as a separate partition with a specific model of evolution selected in jModelTest 2.1.7 (Posada 2008) – see Methods section. Ex-type strains are indicated by a T in superscript.

supported with just several values of moderate support occurring in the ITS locus, which is known for its lower resolution power compared to the *benA*, *CaM*, and *RPB2* genes (Visagie et al. 2014).

Geographical distribution

The number of strains available from public collections belonging to section *Janorum* is very limited, making it difficult to draw meaningful conclusions about the distribution of the species. *A. janus* is the species of section *Janorum* with the highest number of public records. It has been mostly documented from soil on several continents (Czechia, Slovakia, Egypt, Turkey, Pakistan, Brazil, Panama, Ecuador, USA) and in rare cases from indoor air (India) and human-made products (Italy,

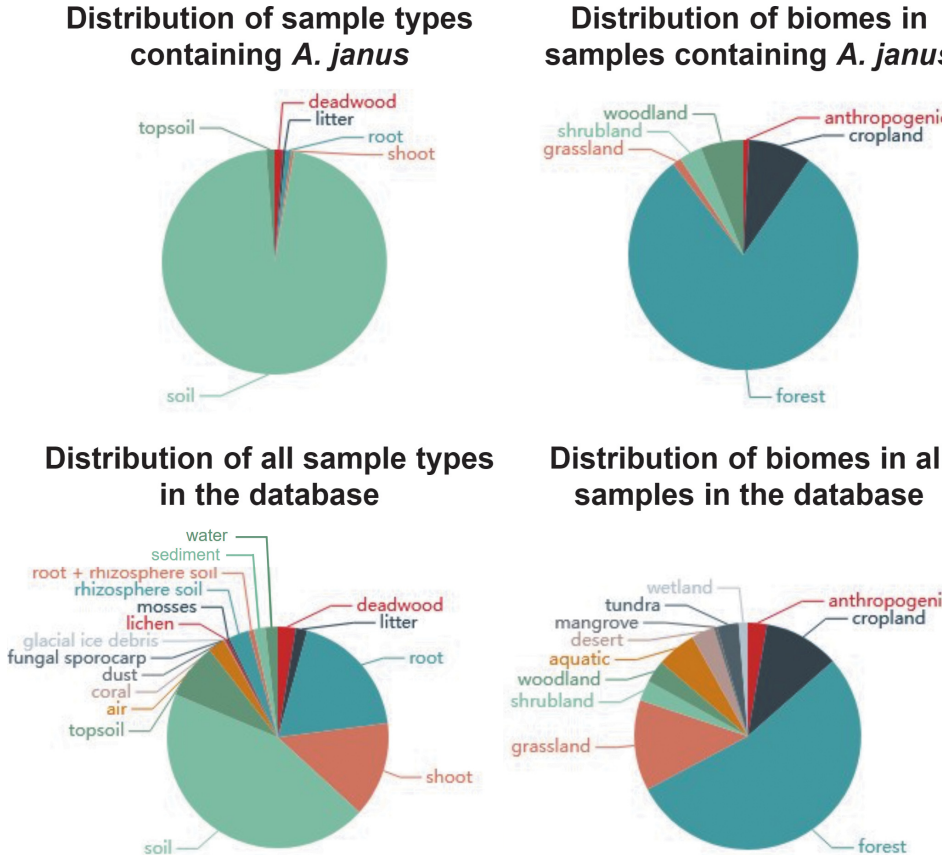

Fig. 2. Distribution of sample types and biomes in GlobalFungi database samples containing sequences of *Aspergillus janus*. This species occurs predominantly in forest soil.

USA) (Hubka et al. 2015). *Aspergillus brevijanus* (Turkey, Mexico, Colombia; Azaz et Pekel 2002, Nouripour-Sisakht et al. 2017), *A. trisporus* (Brazil; Souza et al. 2019) and *A. yunnanensis* (China; Cai et al. 2020) were all isolated solely from soil. The GenBank database (https://www.ncbi.nlm.nih.gov/genbank/, accessed 10 November 2024) contains several sequences identical to the type strain of *A. nanangensis*. The samples were isolated from the following substrates: soil (Australia; Curlevski et al. 2010), macroalga *Palmaria palmata* (France; Le Strat et al. 2023), and the two strains reported in this study from bedroom air and dust in the USA.

The challenge of limited strain availability for biogeographic analysis of most fungi has recently been mitigated by the release of the GlobalFungi database (Větrovský et al. 2020), which aggregates data from metagenomic studies worldwide and thus enables the estimation of distribution patterns of many fungal species occurring in the habitats available in the database. To explore the biogeography

of *A. nanangensis* and related species, we examined the distribution of species of section *Janorum* represented in the database. *Aspergillus janus* is the most frequently occurring species of section *Janorum* (in the database, appearing in the 249 out of 84,972 samples). *Aspergillus nanangensis* is present in 131 samples, *A. yunnanensis* in 85 samples, *A. trisporus* in 13 samples, and *A. brevijanus* is completely absent in the database. Key findings are presented in Figs 2 and 3.

Fig. 2 shows the types and biomes of samples which contained sequences of *A. janus*, compared to sample types and sample biomes of all samples in the database. This comparison shows that samples containing *A. janus* mostly come from forest soil (other sample types and biomes are much less represented in samples containing *A. janus* sequences than in all samples from the database). Other species of the section exhibit a similar distribution of sample types and biomes (data not shown), with soil and forest predominating in their respective datasets.

Fig. 3 shows the geographical distribution of *A. nanangensis*, *A. janus*, and *A. yunnanensis* according to the database (*A. brevijanus* does not occur in the database and *A. trisporus* is represented in an extremely small number of samples). *Aspergillus nanangensis* is represented by a relatively small number of samples in the database, but interestingly, the distribution is cosmopolitan, spanning across all continents except Antarctica and most climate zones. *Aspergillus janus*, the most abundant species of the section in the database, is predominantly restricted to tropical and subtropical regions, particularly in Central and South America and Central Africa according to the available data. *Aspergillus yunnanensis*, sister species of *A. nanangensis*, is, unlike *A. nanangensis*, only present in Africa, Asia, and Europe.

Phenotypic analysis

The phenotypic characteristics of two *A. nanangensis* strains from bedroom air and dust in the USA isolated in this study (CCF 5673 and CCF 6240) are summarised in the emended description (see section Taxonomy). The micro- and macromorphology of the species is displayed in Fig. 4. *Aspergillus nanangensis* produces two distinct types of conidial heads, a defining character of section *Janorum*. However, unlike most other species in the section, it does not produce accessory conidia or Hülle cells, or at least none were observed by us. It does not grow at 30 °C and produces conidiophore stipes which are longer than average in section. White conidiophores measure 800–1200 µm, while green conidiophores measure 600–900 µm. This combination of phenotypic characters is unique within the section. The differences between individual species are summarised in the differential diagnosis (Taxonomy section).

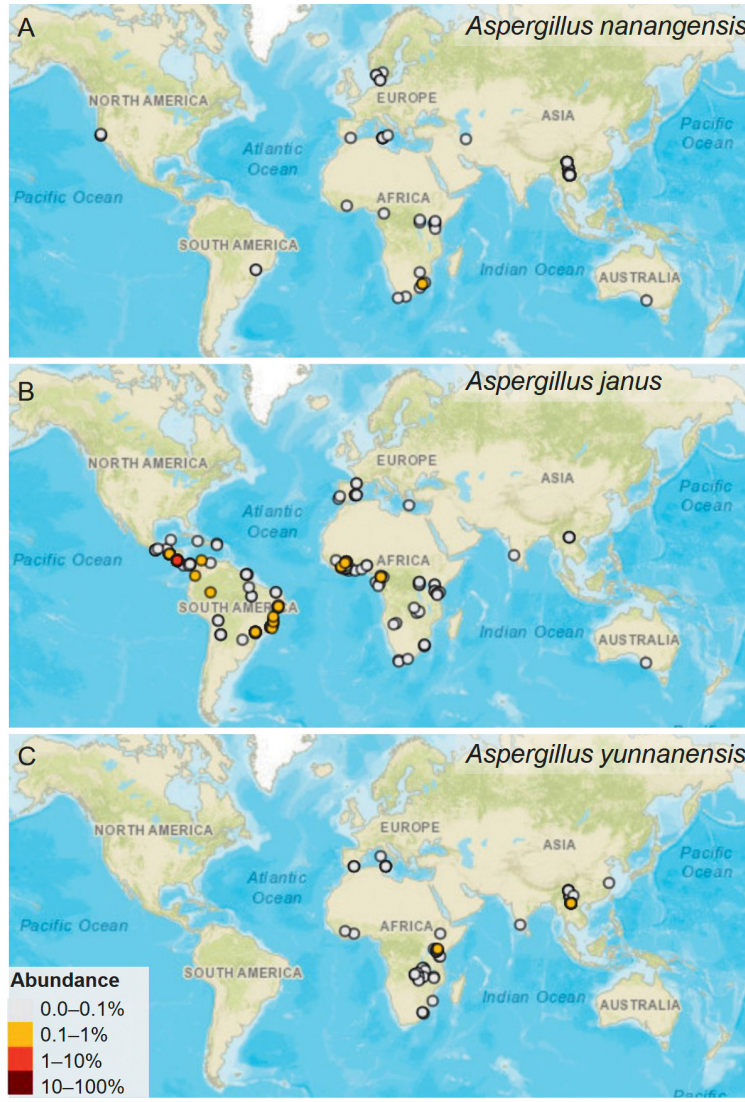

Fig. 3. Global distribution of *Aspergillus nanangensis* (A), *Aspergillus janus* (B) and *Aspergillus yunnanensis* (C) based on occurrence data. The map visualises the known geographical presence of the species as retrieved from the GlobalFungi Database (accessed 30 September 2024). This dataset contains occurrences from various studies and provides an overview of the distribution of species across different ecosystems worldwide. *Aspergillus nanangensis* is absent in many samples in the database, but its distribution is cosmopolitan and spans all continents (except Antarctica). *Aspergillus janus* occurs significantly more often in the database compared to *A. nanangensis*, but its distribution is more concentrated in the tropical and subtropical regions, although the number of samples is very limited in North America and Asia. The abundance of *A. yunnanensis* within the dataset is comparable to that of *A. nanangensis*, but it has been detected on smaller scale, with no presence in North America, South America, or Australia.

TAXONOMY

Aspergillus nanangensis Pitt, Persoonia 44: 365, 2020 Fig. 4

MycoBank: MB 836001

Type: Australia, Queensland, Nanango, from undisturbed forest soil, 2004, J.I. Pitt (holotype DAR 84903, ex-type culture CBS 146238 = FRR 6048 = MST FP2251; ITS, *benA*, *CaM*, and *RPB2* sequences GenBank MK979278, MT184783, MT184789, and MT184795).

Emended description

Colony characters (14 d at 25 °C). Colonies on CYA attaining 23–37 mm diam. (14–22 mm after 7 d), velutinous to floccose without exudate and with production of yellow to orange soluble pigment; colonies of strain CCF 5673 raised with delicately radially furrowed margins, sporulation abundant, greenish grey in centre (26D2), margins dull red (11B3), entire to lobate, reverse light brown (5D5); colonies of strain CCF 6240 umbonate, radially furrowed, pale yellow (4A3) to orange white (6A2), occasionally with dull green (26E3) sectors, sporulation abundant in some sectors only. Colonies on CZA attaining 15–20 mm diam. (8–13 mm after 7 d), velutinous, raised, greenish grey (26B2) to dull green (26D3) in colony centre, pale yellow (4A3) to almost white in marginal parts, sporulation abundant, no exudate, soluble pigment absent or light yellow, margins entire to delicately lobate, reverse light brown (5D5, 6D4) to brownish grey (6D2). Colonies on MEA attaining 18–26 mm diam. (10–18 mm after 7 d), velutinous to floccose, raised, sporulation abundant, pale yellow (4A3) to greenish grey (26B2) in colony centre, yellowish white (4A2) to almost white in marginal parts, no exudate, no soluble pigment, margins entire to delicately lobate, reverse brownish orange (5C5). Colonies on OA attaining 22–26 mm diam. (12–16 mm after 7 d), flat, floccose in centre with dark green (26F3) and white areas of sporulation, marginal zone broad, submerged, no exudate, no soluble pigment, margins entire to delicately lobate, reverse greyish yellow in centre (2C3) to yellowish white (2A2, 4A2) in margins. Colonies on CY20S attaining 30–38 mm diam. (19–23 mm after 7 d), velutinous to lanose, flat to raised, pale yellow (4A3) or orange white (6A2) to almost white, sporulation sparse to abundant, no exudate, no soluble pigment, margins entire to delicately filiform, reverse brownish orange (5C5) to greyish orange (5B4). Colonies on YES attaining 26–39 mm diam. (18–22 mm after 7 d), velutinous to lanose, raised, pale yellow (4A3) or orange white (6A2) to white, sporulation sparse to abundant, no exudate, no soluble pigment, margins entire, reverse brown (7E5) to light brown (6D5).

Colonies on MEA at 27 °C attaining 9–12 mm diam., no production of microcolonies at 30 °C.

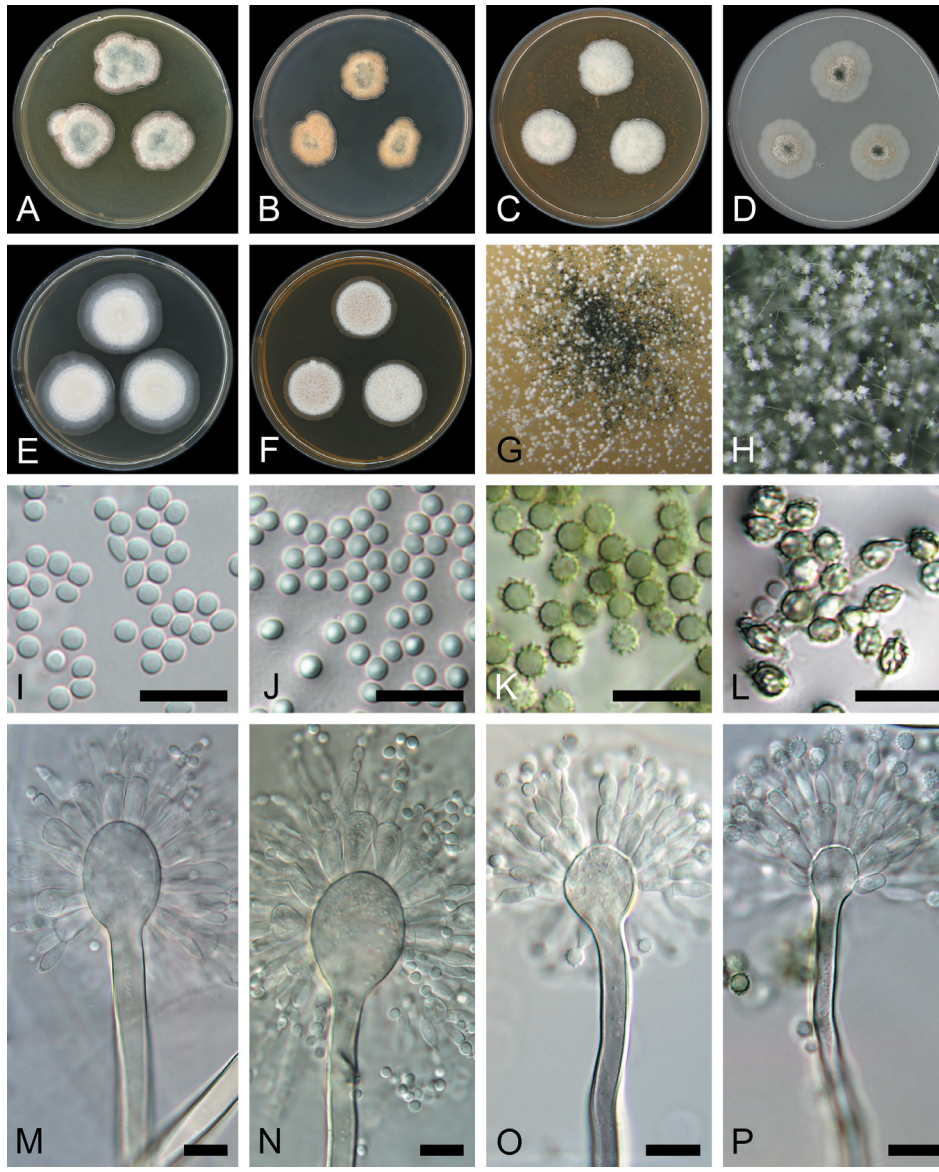

Fig. 4. Macromorphology and micromorphology of *Aspergillus nanangensis* (CCF 5673). **A** – colonies on CYA; **B** – CZA; **C** – MEA; **D** – OA; **E** – CY20S; **F** – YES. **G, H** – conidial heads. **I** – conidia from white conidiophores; **J** – conidia from white conidiophores in air bubble; **K** – conidia from green conidiophores; **L** – conidia from green conidiophores in air bubble. **M, N** – white conidiophores; **O, P** – green conidiophores. Scale bars = 10 µm. Photos F. Sklenář.

Micromorphology observed on CYA. White conidial heads loosely radiate, biseriate, stipes smooth, hyaline, 800–1200 × (4)5–7(8) µm; vesicles hyaline, elongate to subclavate, 9–15(20) µm diam.; metulae hyaline, cylindrical, 7–10 µm long, covering two-thirds up to entire vesicle surface; phialides hyaline, flask-shaped, 7–9 µm long; conidia globose to subglobose, smooth and hyaline, 2.5–3.5 (3.0 ± 0.3) × 2–2.5 (2.3 ± 0.2) µm.

Green conidial heads globose to radiate, biseriate, stipes smooth, pale brown, 600–900 × 4–5 µm; vesicles pyriform or subglobose, 9–12 µm diam.; metulae hyaline, cylindrical, 7–9 µm long, covering two-thirds of the vesicle; phialides hyaline, flask-shaped, 6–7.5 µm long; conidia globose to subglobose, echinulate and olive green en masse, 3.5–4.5 (3.9 ± 0.3) × 2.5–3 (2.8 ± 0.3) µm, without ornamentation.

On CYA, CZA and OA, green and white conidiophores are produced in comparable proportion, while the white type of conidiophores significantly predominates on MEA, CY20S, and YES. On DG18, very short (15 µm) or diminutive (not fully developed) conidiophores are formed.

Accessory conidia not observed. Ascomata and Hülle cells absent.

Distinguishing characters

Aspergillus nanangensis can be distinguished from both *A. brevijanus* and *A. janus* by slower growth on CZA (*A. nanangensis*: 15–20 mm in 14 days; *A. brevijanus*: 30–31 mm in 14 days; *A. janus*: 28–35 mm in 14 days*)* and absence of accessory conidia, which are common in both mentioned species (Hubka et al. 2015). *Aspergillus janus* produces Hülle cells which are absent in *A. nanangensis* and *A. brevijanus*. *Aspergillus brevijanus* is the only species in the section capable of growing at 37 °C. White conidial heads of *A. nanangensis* are similar to those of *A. brevijanus* in stipe length (shorter than 2 mm) and vesicle shape (elongate or subclavate, but not entirely clavate as in *A. janus*). *Aspergillus yunnanensis* differs from *A. nanangensis* by the production of Hülle cells and larger vesicles of white conidiophores (*A. yunnanensis*: 25–30 µm; *A. nanangensis*: 9–15(20) µm). In contrast to *A. nanangensis*, *A. trisporus* produces much shorter conidiophore stipes (*A. trisporus* white conidiophores: 325–740 µm, green conidiophores: 84–138 µm versus *A. nanangensis* white conidiophores: 800–1200 µm, green conidiophores: 600–900 µm). *Aspergillus trisporus* also forms clavate vesicles of white conidial heads (similar to *A. janus*, but contrasting to the elongate to subclavate heads of *A. nanangensis*) (Tab. 2).

Tab. 2. Overview of distinguishing characters of *Aspergillus nanangensis* and the other species of sect. *Janorum*.

Species	*A. janus*	*A. brevijanus*	*A. trisporus*	*A. yunnanensis*	*A. nanangensis*
Colony diam. on CZA (14 d)	28–35 mm	30–31 mm	?	?	15–20 mm
Hülle cells	present	absent	absent	present	absent
Growth at 37 °C	no	yes	no	no	no
Accessory conidia	present	present	present	absent	absent
Green stipe length	500 µm	55–120 µm	84–138 µm	300–400 µm	600–900 µm
White stipe length	1000+ µm	1000+ µm	325–740 µm	1000–2000 µm	800–1200 µm
White vesicle diameter	9–22 µm	8–18 µm	19–25 µm	25–30 µm	9–15(20) µm
White vesicle shape	spathulate to clavate, globose to pyriform	pyriform	clavate	spathulate to clavate	elongate to subclavate
Reference	Hubka et al. (2015)	Hubka et al. (2015)	Souza et al. (2019)	Cai et al. (2020)	this study

DISCUSSION

In this article, we confirm that *A. nanangensis* is a phylogenetically well-resolved species which is sister to *A. yunnanensis*. Contrary to the original description by Pitt in Crous et al. (2020), we have shown that *A. nanangensis* produces two types of conidiophores (green and white), which is a typical characteristic of section *Janorum*. In agreement with Crous et al. (2020), we did not observe accessory conidia in *A. nanangensis*, which are known to occur in *A. janus*, *A. brevijanus*, and *A. trisporus*.

Biogeographic data obtained from the GlobalFungi database suggests that the ecology of *A. nanangensis* may differ from other species in section *Janorum*. Although only a few samples in the database contain *A. nanangensis*, its distribution spans across all continents and covers a broad range of latitudes, while closely related species appear to be restricted to narrower latitudinal ranges.

Section *Janorum* remains one of the smallest sections of *Aspergillus*, yet it possesses a unique set of phenotypic characteristics and some unique secondary metabolites with potential antibacterial, anti-inflammatory and anticancer activity. Brevijanazines can potentially be used as the basis for novel drug developments (Wang et al. 2023). Recently, *A. brevijanus* was studied to elucidate the fungal biosynthesis of p-nitrobenzoic acid (PNBA), revealing that cytochrome P450 BvjF functions as a PNBA synthetase (Li et al. 2022), adding to the biotechnological potential of these species.

ACKNOWLEDGEMENTS

The study was supported by the Strategie AV21 "VP33 MycoLife – the world of fungi" project of the Czech Academy of Sciences, and the Czech Academy of Sciences Long-term Research Development Project (RVO: 61388971). František Sklenář was supported by Charles University Research Centre program UNCE/24/SCI/006.

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