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Neospadicoides australiensis (Xenospadicoidaceae), a new freshwater fungus from South Australia

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Abstract

During a survey of freshwater fungi in Scott Creek Conservation Park, South Australia, a new species of *Neospadicoides* was found and described. Phylogenetic analyses using ITS and LSU sequences from the new species assisted in species delimitation and placed it in the genus *Neospadicoides*. The morphology of the new species is compared with that of *N. thailandica* and *N. lignicola.*

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Introduction

Freshwater fungi are abundant in Australian rivers, streams, dams, and lakes. However, studies on this ecological group are scarce. During a survey of freshwater fungi growing on decaying wood in streams in Scott Creek Conservation Park, South Australia, a new species of *Neospadicoides* Z.L. Luo *et al*. was found.

Neospadicoides was introduced by Luo *et al.* (2019) for species with brown, unbranched conidiophores with acrogenous or acropleurogenous, fusiform or obovoid, septate, smooth-walled conidia within the Xenospadicoidaceae. The sexual state of *Neospadoides* species is unknown. There are currently six accepted species of *Neospadicoides* (Index Fungorum http://www.indexfungorum.org/Names/Names.asp).

Methods

Collection details and examination

Samples of submerged wood less than 5 cm in diameter were collected in Scott Creek Conservation Park, South Australia from two streams, approximately 50 cm deep with a muddy base, that flow only during winter. Samples were sealed into plastic bags for transport to the laboratory. The riparian vegetation is a mixture of native vegetation and invasive weeds.

Samples were incubated in sterile plastic containers and regularly examined for fungi over 6 months using a Leica MZ7s dissecting microscope. Any fungi observed were photographed, described, and transferred to a microscope slide with a drop of distilled water. A cover slip was added, and the slide examined using a Nikon Eclipse Ni with differential interference contrast. Photographs were taken using either a Sony RX-100 or Canon D6 camera. Measurements of characters are presented as a range and the mean (x).

Single spore isolation

Potato dextrose agar (PDA, BD micro) was autoclaved, cooled to 60 °C, 100mg/l streptomycin and 70mg/l of penicillin added as filter-sterilised stock solutions and 10 ml poured into each 60mm diameter plate.

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Table 1: List of strains analysed in this study, with GenBank accession numbers. Newly generated sequences are shown in bold.

Species	Strain	ITS	LSU
Brachysporium nigrum	MR1346	MG600388	KT991662
Brachysporium polyseptatum	DAOM 231136	-	NG_058624
Neomyrmecridium guizhouense	GZCC 20-0008	NR_170024	MT002307
Neospadicoides aquatica	MFLU 18-1605	MK828699	MK849851
Neospadicoides aquatica	S-729	MK828700	MK849852
Neospadicoides australiensis	**AD289898**	**OP796194**	**OP796193**
Neospadicoides bambusicola	MFLUCC 21-0058	MZ435866	MZ435868
Neospadicoides biseptata	GZCC 19-0448	MW133934	OP377933
Neospadicoides biseptata	GZCC 20-0419	OP377858	OP377943
Neospadicoides lignicola	MFLU 18-1606	NR_168815	MK849854
Neospadicoides thailandica	MFLU 21–0032	MZ412520	MZ412532
Neospadicoides thailandica	MFLUCC 21-0031	MZ412521	MZ412533
Neospadicoides yunnanensis	MFLU 18-2329	NR_168814	-
Neospadicoides yunnanensis	S-1499	MK828701	MK849853
Spadicoides fuscolutea	CBS 141263	KY931798	KY931826
Spadicoides fuscolutea	CBS 141262	KY931797	KY931825
Spadicoides hyalostoma	CBS 131268	KY931799	KY931827
Spadicoides hyalostoma	CBS 137793	KY931800	KY931828

Ascospores or conidia were transferred to a sterile 1.5 ml microtube with 20 µl of sterile water using a sterile microneedle. The mixture was agitated for several seconds, then transferred to a PDA plate using a pipette. Plates were incubated at room temperature and checked over 5 days for germinating spores using a Leica MZ7s dissecting microscope. Germinated spores were picked off the agar surface using a sterile needle and transferred to individual PDA plates which were incubated at room temperature.

DNA extraction, amplification, and sequencing

Approximately 50 mg of fungal mycelium was scraped from the surface of agar cultures with a sterile scalpel and the genomic DNA was isolated using a Qiagen DNeasy Plant Mini kit following the manufacturer's protocols. The final DNA extracts were eluted into 100 µl of buffer.

Primers LROR/LR5 (Rehner and Samuels 1994; Vilgalys and Hester 1990) were used to amplify the sequences from the 28S nrRNA gene (LSU) and ITS1/ITS 4 (White *et al.* 1990) were used to amplify the internal transcribed spacer region and the intervening 5.8S nr RNA gene (ITS).

Reaction mixtures contained 5 µl buffer, 1 µl (10 mM each) dNTPs, 1 µl (10mM) of each primer, 0.25 µl hotStart Taq DNA polymerase (New England Biolabs), 1 µl DNA template, and 16.75 µl sterile milliQ water.

PCR amplification was performed in an Applied Biosystems 2720 Thermo Cycler. Cycling conditions for PCR were initial denaturation at 95 °C for 3 min; 35 cycles of denaturation at 95 °C for 1 min, annealing at 52 °C (ITS) or 54 °C (LSU) for 50 s, and extension at 72 °C for 1 min; and a final extension at 72 °C for 10 min.

PCR products were observed on a 1.5% agarose electrophoresis gel stained with Gel Red (Gene Target Solutions).

The resulting amplicons were purified using a Qiagen QIAquick PCR Purifcation Kit and sequenced in both directions using the respective primers by the Australian Genome Research Facility. Raw sequence reads were assembled, examined, and edited using Sequencher v. 5.3 (Gene Codes Corporation). Newly generated sequences were submitted to NCBI GenBank under the accession numbers listed in table 1.

Phylogenetic analyses

The generated sequences for each gene were used in megablast searches (Zhang *et al.* 2000) to identify closely related sequences in NCBI's GenBank nucleotide database.

Sequences were aligned in Geneious Prime v. 2023.0.4 using MUSCLE. Alignments were imported into Mega X 10.2.6 (Stecher *et al.* 2020) to find the best substitution models for phylogenetic analyses. Genes were then concatenated and maximum-likelihood phylogenetic trees were constructed using RAXML v. 8.2.11 (Stamatakis 2014) within Geneious using the GTR + I + G substitution model and branch support values were calculated with 1000 rapid bootstrap inferences. The same alignment was analysed with MrBayes using the GTR GAMMA I substitution model (v 3.2.6, Huelsenbeck and Ronquist, 2001) within Geneious. All resulting trees were formatted in Geneious, then further edited in Adobe Illustrator v. 27.0. *Brachysporium* (Sacc.) Sacc. was used as the outgroup based on the phylogenetic analysis of Hyde et al. (2021).

Results

In the BLASTn search of ITS and LSU sequences, *Neospadicoides australiensis* is most similar to *N. lignicola* (MFLU 18-1606) and *N. thailandica* (MFLU 21-0031) with 92.9% and 98.4% similarity, respectively. A combined ITS and LSU sequence analysis (fig. 1) recovers all six species of *Neospadicoides* as a strongly supported clade sister to *Spadicoides* S. Hughes and indicates that *N. australiensis* forms a distinct lineage within the genus.

Taxonomy

Neospadicoides australiensis Fryar & D.E.A. Catches.

Index Fungorum number: IF559993; Facesoffungi number: FoF 13375; GenBank numbers: ITS: OP796194; LSU OP796193

Holotype: AD289898

Figures 2, 3

Saprobic on decaying wood in an ephemeral freshwater stream. **Sexual morph:** undetermined. **Asexual morph**: *Conidiophores* 120–276 µm long, 7.5–8.4 µm wide at the base, 3.6–4.8 µm at the apex (x = 187.2 × 7.4 x 3.9 µm, n=14), macronematous, mononematous, erect, cylindrical, unbranched, smooth, dark brown, septate, wider at the base, tapering towards the apex, solitary. *Conidiogenous cells* holoblastic, discrete, terminal and intercalary, intercalary conidiogenous cells in the upper section of the conidiophore only, brown, truncate at the apex, usually 1-celled, sometimes 2-celled, when 2-celled, constricted at the septa 7–12.5 × 3.5–5 µm (x =10.2 × 4.1 µm, n=16). *Conidia* 18–33 µm long, 10–16 µm wide (x = 25.3 × 12.4 µm, n=27), obovoid, 3-septate, thick-walled, multi-guttulate, subhyaline to light brown, smooth-walled, truncate at the base, narrow channel between the cells (Fig. 2).

Culture characteristics: On PDA after 6 months 40 mm. Margin uneven, feathered. Mycelium dark brown. Aerial hyphae lighter brown. Reverse dark brown. No staining of agar. Abundant submerged hyphae. Hyphae hyaline to brown, septate, sparsely branched, walls straight, smooth, 2–3 µm wide (fig. 3).

Material examined: AUSTRALIA, Scott Creek Conservation Park (S35° 5' 46", E138° 40' 59"), on decaying wood submerged in a stream, 26 August 2020, (AD289898, **holotype**).

Etymology: The epithet refers to the country where the species was found, Australia.

Notes: *Neospadicoides australiensis* is morphologically most similar to *N. thailandica* D.F. Bao, H.Y. Su, K.D. Hyde & Z.L. Luo (Bao *et al.* 2021) with thick-walled, clavate, dark brown conidia. However, the conidiogenous cells of *N. australiensis* are both terminal and intercalary, whereas those of *N. thailandica* are terminal only. Also, the conidia of *N. australiensis* lack a sheath and are narrower. *Neospadicoides australianesis* should also be compared to *N. lignicola* Z.L. Luo, K.D. Hyde & H.Y. Su which also has holoblastic, terminal and intercalary conidiogenous cells, but the conidia of *N. lignicola* are thin-walled and smaller (Luo *et al.* 2019).

Based on its morphology and phylogenetic placement, we therefore introduce *Neospadicoides australiensis* as a new species.

Disclosures

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Conflict of interest: The authors declare that there is no conflict of interest.

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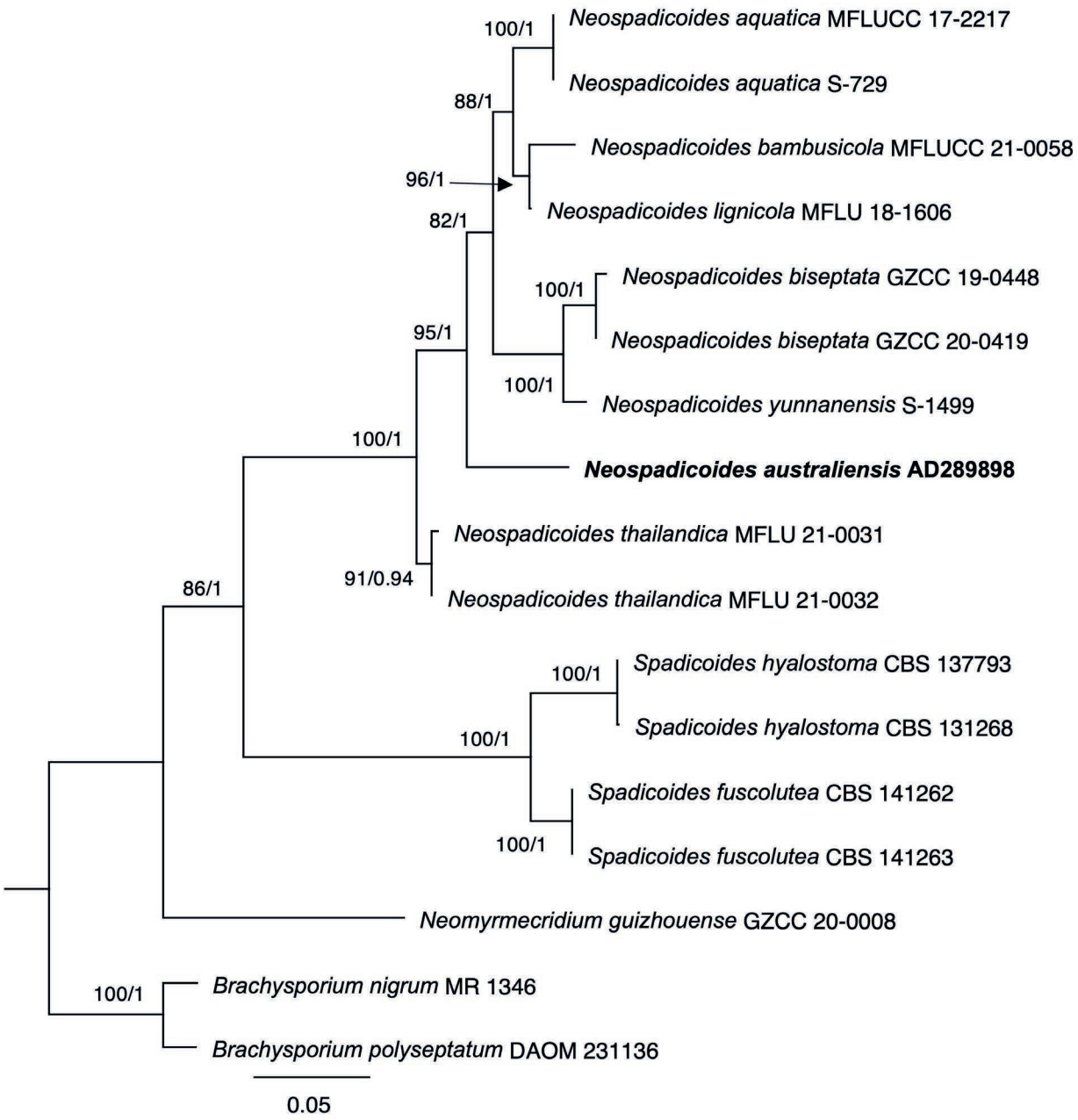

Fig. 1: Phylogram generated from maximum likelihood analysis based on combined ITS and LSU (1446 bp) sequence data of *Neospadicoides* and closely related taxa. Bootstrap support values were obtained from 1000 replicates using GTR + G + I substitution model in RAxML (8.2.11) in Geneious Prime (2022.2.2.). Bayesian analysis was performed using MrBayes (3.2.6.) in Geneious. Bootstrap values equal to or great that 70% and Bayesian posterior probabilities equal to or greater than 0.95 are given above the nodes. The scale bar indicates nucleotide sequence divergence.

Fig. 2: ***Neospadicoides australiensis*** (AD289898, **holotype**). **a** Conidiophore on wood. **b** Apex of conidiophore, conidiogenous cells and conidia. **c**, **d** Conidiophores. e-h Conidia. Scale bars: **a** = 100 µm, **b**, **e-h** = 5 µm, **c-d** = 20 µm.

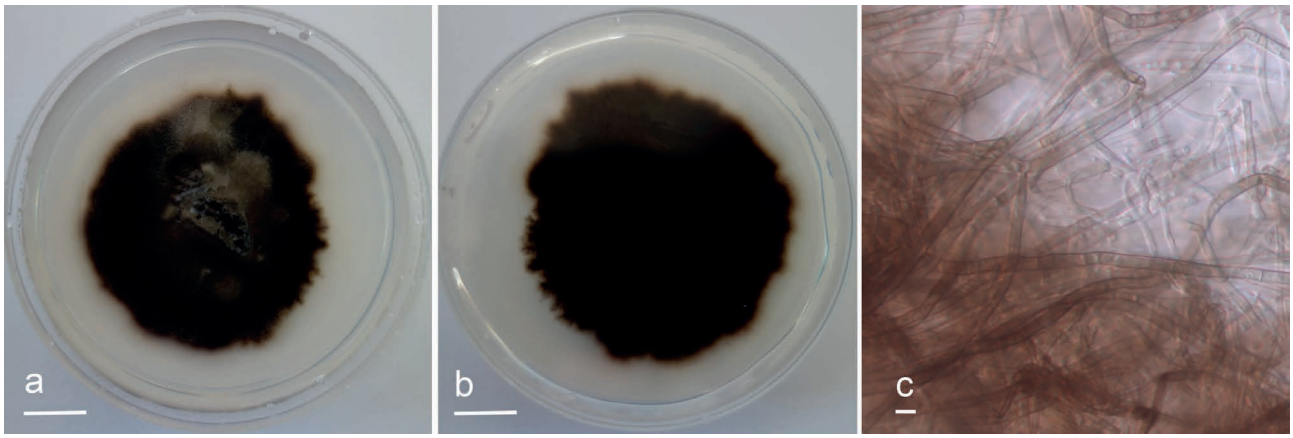

Fig. 3: Culture of *Neospadicoides australiensis* (AD289898, **holotype**) on PDA after 6 months. **a** mycelium on PDA, **b** reverse side of culture, **c** hyphae. Scale bars: a-b = 1cm, c = 5 µm.
