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Discovery of *Torula srijayewardenepurensis* sp. nov. and distribution updates of *Apiospora* and *Paradictyoarthrinium* in Sri Lanka

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Abstract

Fungal biodiversity remains an underexplored topic in Sri Lanka, particularly within microfungal communities inhabiting both anthropogenic and natural environments. During the continuous microfungal survey, three fungal isolates were obtained from the premises of the University of Sri Jayewardenepura. Based on morphology and multigene phylogeny (internal transcribed spacer: ITS, nuclear ribosomal 28S large subunit rRNA: LSU, nuclear ribosomal 18S small subunit rRNA: SSU, and translation elongation factor-1α: *tef1-α*), one of the isolates is introduced as a new species, *Torula srijayewardenepurensis*. It can be distinguished from the phylogenetically closely related *T. chromolaenae* and *T. longan* by having conidiophores reduced to conidiogenous cells and shorter conidiogenous cells. The other two isolates, identified as *Apiospora pseudorasikravindrae* and *Paradictyoarthrinium diffractum*, are supported by their morphological traits and phylogenetic relationships and represent new records for Sri Lanka. In addition, *Apiospora pseudorasikravindrae* comb. nov. (≡ *Arthrinium pseudorasikravindrae*), previously invalidly published, is validly published in this publication.

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Introduction

Fungi play crucial roles in nutrient cycling and ecosystem functioning and act as symbionts, pathogens, and decomposers[1–4]. Among them, microfungi comprise a broad range of saprobic, pathogenic, and endophytic taxa that are particularly abundant in terrestrial ecosystems, especially within decaying plant material[5]. Despite their ecological importance and potential biotechnological applications, the diversity of microfungi in tropical regions remains significantly underexplored[6,7]. Despite covering a relatively small area of 6,524,540 hectares, Sri Lanka ranks among the most biologically diverse countries in Asia, with a rich biodiversity resulting from its varied climate and topography across a wide range of ecosystems, including tropical rainforests, montane and sub-montane regions, and dry zones[8,9]. Nevertheless, fungal biodiversity studies in the country have historically been limited, and many microfungal groups remain poorly documented[10,11]. Recent studies have begun to reveal the remarkable diversity of the islandwide mycobiota; however, major gaps remain in the taxonomy, distribution, and ecological functions of many microfungal groups[12–14].

Microfungi are integral to ecosystem functioning due to their highly specialized roles in the decomposition of plant-derived substrates. As primary agents of lignocellulose decay, they secrete a diverse suite of extracellular enzymes, including cellulases, hemicellulases, laccases, and lignin peroxidases, that enable the stepwise breakdown of complex plant polymers and the subsequent mobilization of carbon and nutrients into the soil matrix[2,5,15–18]. Saprobic microfungi exhibit strong substrate specificity, often initiating colonization through hyphal penetration of senescent tissues, where they modify cell-wall architecture and accelerate litter turnover. Increasing evidence also highlights the dual ecological strategies of endophytic fungi: although asymptomatic during the host's lifespan, many taxa undergo a functional shift to saprotrophy following host senescence, rapidly exploiting the nutrient-rich tissues they previously inhabited[19–21]. This endophyte-to-saprobe transition underscores their importance as early colonizers in the decomposition continuum and as key regulators of nutrient fluxes within terrestrial ecosystems.

During a mycological survey conducted within urbanized ecosystems in Sri Lanka, several saprobic fungi were isolated from decaying leaves and plant debris in the premises of the University of Sri Jayewardenepura. Morphological characteristics combined with multi-gene phylogenetic analyses revealed a previously undescribed species, along with two other microfungal species recorded for the first time in Sri Lanka. The present findings contribute to the growing body of knowledge on tropical microfungi, and underscore the importance of local urban ecosystems as reservoirs of undocumented fungal diversity.

Materials and methods

Sample collection, morphological studies, and isolation

Woody and plant debris were collected from the University of Sri Jayewardenepura premises (6°51'06" N 79°54'31" E, 25 masl) in November 2024. The collected specimens were placed in eco-friendly high-pressure polyethylene (LDPE) zip-lock bags, and brought to the Genetics and Molecular Biology laboratory, Faculty

of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka. The specimens were initially examined using a stereomicroscope (OPTIKA SZO-T, Italy), and all the colony characteristics, including position on the host surface, shape, and color, were recorded. Following the examination, micro-morphological characteristics were photographed using a Nikon ECLIPSE N*i*-U upright compound microscope (Nikon, Japan), equipped with an Axiocam 506 color digital camera (ZEISS, Hanover, Germany). All measurements were obtained using the Tarosoft® Image Framework application, while photo plates were prepared with Adobe Photoshop CS3 Extended version 10.0 (Adobe Systems, USA).

The method described by Senanayake et al.[22] was used for the single conidial isolation. After germination, conidia were transferred to potato dextrose agar (PDA) and incubated at 25 °C overnight. Pure cultures were obtained after a subsequent subculturing process. Culture characteristic features were observed and recorded. Type specimens and other dried specimens were prepared and deposited in the Herbarium of the University of Sri Jayewardenepura (USJ-H), Sri Lanka. Isolated cultures were deposited in the fungi culture collection of the University of Sri Jayewardenepura (USJCC) and the culture collection of the Genetics and Molecular Biology Unit, Faculty of Applied Sciences, University of Sri Jayewardenepura (GMBUCC). Nomenclatural data for fungal novelty were deposited in MycoBank (www.mycobank.org).

Extraction of DNA, PCR amplification, and sequencing

Genomic DNA was extracted using fungal strains grown on PDA for 3–4 weeks at 25 °C. Axenic mycelium (80–100 mg) was harvested from actively growing cultures, and genomic DNA was extracted using the Biospin Fungal DNA Extraction Kit (Bioer Technology Co. Ltd., Hangzhou, China) according to the manufacturer's instructions. The DNA products were stored at 4 °C, with replicate samples preserved at −20 °C to ensure long-term stability. Genomic regions amplified included: the internal transcribed spacer (ITS1–5.8S–ITS2), the nuclear ribosomal 28S large subunit rRNA (LSU), the nuclear ribosomal 18S small subunit rRNA (SSU), the partial translation elongation factor 1-alpha (*tef1-α*), the partial sequence of the beta-tubulin gene (*tub2*), and the partial second largest subunit of RNA polymerase II (*rpb2*). The ITS region was amplified using ITS4 and ITS5[23], LSU with LR0R and LR5[24], and SSU with NS1 and NS4[23]. The *tef1-α* gene was amplified using EF1-983F and EF1-2218R[25], *tub2* with Bt2a and Bt2b[26], and *rpb2* with fRPB2-5F and fRPB2-7cR[27]. The PCR reaction was performed in a total volume of 25 µl, consisting of 9.5 µl sterilized distilled water, 12.5 µl of 2× Power Taq PCR MasterMix (containing 0.1 U/µl Taq DNA polymerase, and 500 µM of each dNTP: dATP, dCTP, dGTP, dTTP; Bioteke Corporation (Wuxi) Co., Ltd.), 1 µl each of forward and reverse primers (10 pM stock), and 1–2 µl of DNA template. Thermal cycle programs for LSU, SSU, ITS, *tef1-α*, *tub2*, and *rpb2* genes were followed as mentioned in Thambugala et al.[28]. PCR products were visualized on 1% agarose gels stained with ethidium bromide under UV illumination. Subsequently, PCR product purification and sequencing were performed at Genelabs Medical (Pvt) Ltd, Sri Lanka.

Phylogenetic analyses

The resulting sequences (forward and reverse) were initially checked using BioEdit v 7.0.5.3[29] and assembled with SeqMan v. 7.0.0 (DNASTAR, Madison, WI, USA). The nucleotide BLAST search (https://blast.ncbi.nlm.nih.gov) was carried out to determine the strains that have high similarities. The reference sequences used in the analyses were obtained from recent publications[30,31] (Supplementary Tables S1–S3). Single-gene datasets were aligned with Bioedit 7.1.3.0[29], and the consensus sequences were further improved with MUSCLE implemented in MEGA11[32]. Alignments were manually checked and adjusted when necessary.

Phylogenetic analyses using both maximum likelihood (ML) and Bayesian inference (BI) methods were carried out using concatenated alignments. The best-fit substitution models were chosen using MrModeltest v2.3[33] based on the Akaike Information Criterion (AIC). Maximum Likelihood analysis was conducted with RAxML-HPC2 v8.2.8[34] via the CIPRES Science Gateway[35], employing the GTR + I + G nucleotide substitution model with 1,000 bootstrap replicates. Bayesian Inference was performed in MrBayes v3.2.1[36] with six simultaneous Markov chains run for 1, 5, and 3 million generations, sampling every 100th tree for *Paradictyoarthrinium*, *Torula*, and *Apiospora*, respectively. The first 20% of sampled trees were discarded as burn-in, and the remaining 80% were used to estimate posterior probabilities and produce consensus trees. The resulting phylogenetic trees were visualized and preliminarily edited using MEGA11[32], with final graphical adjustments and layout formatting completed in Microsoft PowerPoint 2016.

Results

Phylogenetic analyses

The phylogenetic trees in this study were constructed using maximum likelihood (ML), and Bayesian inference (BI). The best maximum likelihood trees, with support values from both analyses, are shown at each corresponding node in Figs. 1–3.

Phylogenetic analyses of *Paradictyoarthrinium*

The concatenated LSU (1–867), ITS (868–1,437), and *rpb2* (1,438–2,499) sequence datasets were analyzed to identify the phylogenetic position of our newly generated sequence within *Paradictyoarthrinium*. The dataset consisted of 20 sequences, with *Nigrograna obliqua* (CBS141477) as the outgroup taxon. The ML and BI analyses yielded similar tree topologies, and therefore, only the best-scoring ML tree is presented (Fig. 1) with the likelihood value of −8,213.564 and the following model parameters: Estimated base frequencies were A = 0.250, C = 0.250, G = 0.250, and T = 0.250; substitution rates were AC = 1.00000, AG = 4.66643, AT = 1.000000, CG = 1.000000, CT = 8.25386, and GT = 1.000000; gamma distribution shape parameter: α = 0.429. The BI analysis ran for one million generations, with the average standard deviation of split frequencies reaching below 0.01 (0.008125). This analysis generated 10,000 trees, from which 8,000 were sampled after discarding 20% as burn-in. The alignment contained a total of 714 distinct patterns. All *Paradictyoarthrinium* strains formed a strongly supported clade (100% ML, 1.00 PP) sister to *Melanomma* and *Nigrograna*. The new isolate USJCC-0274 clustered within the *P. diffractum* strains (MFLUCC 12-0557, MFLUCC 24-0257, KUMCC 19-0111, UESTCC 24.0187, and MFLUCC 13-0466) with strong statistical support (99% ML/1.00 PP) (Fig. 1).

Phylogenetic analyses of *Torula*

The concatenated ITS (1–583), LSU (584–1,445), SSU (1,446–2,471), and *tef1*-α (2,471–3,367) sequence datasets were analyzed to infer the phylogenetic position of our newly generated sequences within *Torula*. The dataset consisted of 47 sequences, with *Cylindrotorula indica* (NFCCI 4836 and NFCCI 4837) as the outgroup taxa. The RAxML and Bayesian analyses produced similar tree topologies, and therefore, only the best-scoring ML tree is presented (Fig. 2) with the likelihood value of -10,479.082 and the following model parameters: Estimated base frequencies were A = 0.250, C = 0.250, G = 0.250, and T = 0.250; substitution rates were AC = 1.68626, AG = 2.49231, AT = 1.68626, CG = 1.000000, CT = 7.12644, and GT = 1.000000; gamma distribution shape parameter: α = 0.557. The BI analysis ran for 5 million generations, with the average standard deviation of split frequencies reaching below 0.01 (0.006276). This analysis generated

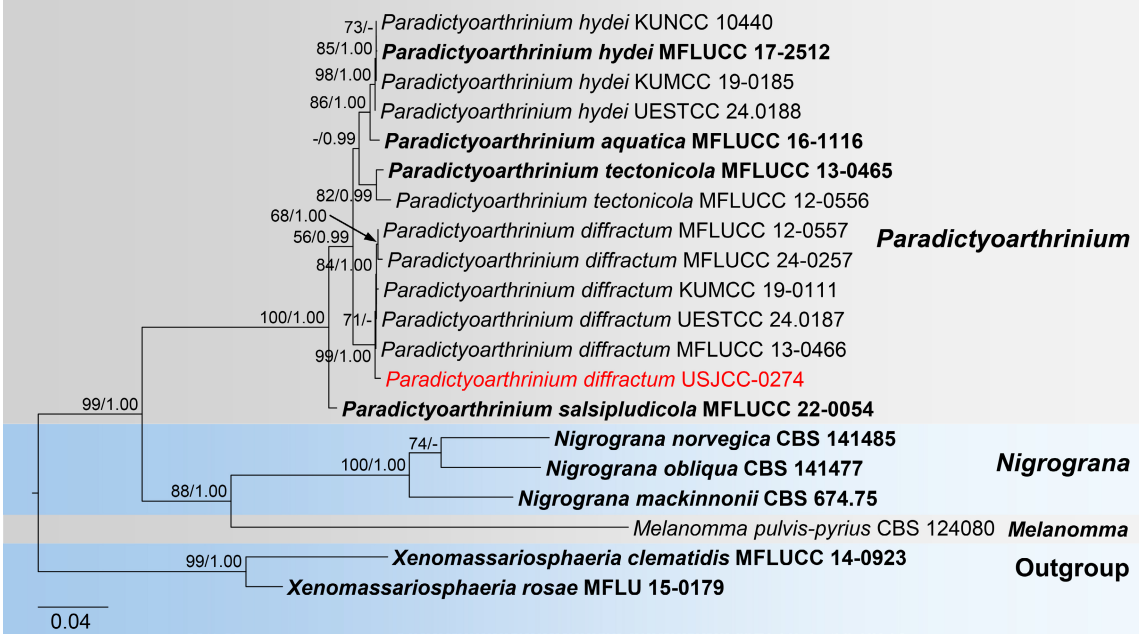

Fig. 1 Maximum likelihood (ML) tree of combined LSU, ITS, and *rpb2* sequences from *Paradictyoarthrinium* species. *Xenomassariosphaeria clematidis* MFLUCC 14-0923 and *X. rosae* MFLU 15-0179 were used as the outgroup. Maximum likelihood bootstrap support values (≥ 50%) and Bayesian posterior probability (≥ 0.90) are indicated at nodes. The newly generated strain is indicated in red, while type strains are shown in bold.

50,000 trees, from which 10,000 were sampled after discarding 20% as burn-in. The alignment contained a total of 685 distinct patterns. *Torula* strains formed a statistically well-supported clade (100% ML/1.00 PP) with five distinct clusters. The newly generated sequences (USJCC-0275 and USJCC-0276) clustered basal to the strains of *T. chromolaenae* and *T. longan* with a robust statistical support (100% ML/1.00 PP) (Fig. 2).

Phylogenetic analyses of *Apiospora*

The concatenated ITS (1–643), LSU (644–1,495), *tub2* (1,496–2,082), and *tef1-α* (2,083–3,137) sequence dataset was analyzed to determine the phylogenetic position of our newly generated sequence within *Apiospora*. The dataset included 72 sequences, including *Arthrinium caricicola* (CBS 145127) as the outgroup. The RAxML and Bayesian analyses yielded a similar tree topology, and therefore, only the best-scoring ML tree is presented (Fig. 3) with the likelihood value of −20144.9873 and the following model parameters: Estimated base frequencies were A = 0.250, C = 0.250, G = 0.250, and T = 0.250; substitution rates were AC = 1.0000, AG = 2.5276, AT = 1.0000, CG = 1.0000, CT = 4.1989, and GT = 1.000000; gamma distribution shape parameter: α = 0.732. The BI analysis ran for 3 million generations, with the average standard deviation of split frequencies reaching below 0.01 (0.009897). This analysis generated 30,000 trees, from which 6,000 were sampled after discarding 20% as burn-in. The alignment contained a total of 1,645 distinct patterns. *Apiospora* strains formed a monophyletic group with seven clusters. The newly generated sequence (USJCC-0277) clustered with the type of *A. pseudorasikravindrae* (KUMCC 20–0208), forming a sister relationship with *A. acutiapica* (KUMCC 20-0210) in a well-supported clade (89% ML; Fig. 3).

Taxonomy

Dothideomycetes O.E. Erikss. & Winka, Myconet 1(1): 5 (1997)

Pleosporales Luttr. ex M.E. Barr, Prodr. Cl. Loculoasc. (Amherst): 67 (1987)

Paradictyoarthriniaceae Doilom, J.K. Liu & K.D. Hyde, Fungal Diversity 72: 133 (2015)

Paradictyoarthrinium Matsush., Matsush. Mycol. Mem. 9: 18 (1996)

Notes: Matsushima[37] introduced *Paradictyoarthrinium* in Paradictyoarthriniaceae (Pleosporales) as a monotypic genus with *P. diffractum*. Later studies with multigene phylogeny using LSU, SSU, *tef1-α*, and *rpb2* reconfirmed the placement of *Paradictyoarthrinium*[38,39]. *Paradictyoarthrinium* species are characterized by gregarious, black, powdery colonies bearing macronematous conidiophores, which produce asymmetrically and irregularly dictyoseptate, muriform, subglobose to ellipsoidal, dark brown conidia. Currently, the genus *Paradictyoarthrinium* comprises five species: *P. aquatica*, *P. diffractum*, *P. hydei*, *P. salsipaludicola*, and *P. tectonicola*, and they are recorded in different countries, including China, India, South Africa, and Thailand[30]. The members of *Paradictyoarthrinium* are primarily saprophytes occurring on decaying wood in terrestrial, freshwater, and marine environments[40].

Paradictyoarthrinium diffractum Matsush., Matsushima Mycol. Mem. 9: 18 (1996) Fig. 4

MycoBank number: MB 415849

Saprobic on a piece of decaying wood. Sexual morph: Undetermined. Asexual morph: *Colonies* on the woody substrates are scattered, superficial, effuse, gregarious, blackish, and powdery. *Conidiophores* 15–30 × 1–3 µm ($\bar{x}$ = 19 × 2.2 µm, n = 25), macronematous, sometimes micronematous, erect to slightly curved, short, branched or unbranched, thick-walled, black, arising from hyphae, slightly constricted at the septa. *Conidiogenous cells* 3–10 × 2–7.2 µm ($\bar{x}$ = 6 × 4.6 µm, n = 25), blastic, mostly terminal, determinate, brown, smooth. *Conidia* 15–25 × 16–20 µm ($\bar{x}$ = 19 × 18 µm, n = 35), dictyoconidia, solitary or sometimes formed in chains, unevenly dictyoseptate, subglobose to broadly ellipsoidal, sometimes irregular or lobed, verrucose, dark brown to black, thick-walled.

Culture characteristics: Colonies on PDA are fast-growing, reaching 5–6 cm in diameter after two weeks at 20–25 °C, colonies sparse, circular, raised, surface slightly rough, with an irregular margin, cottony to fairly fluffy with sparse aspects, colony from above, white to cream at the centre, pale brown to dark brown at the margin; from below, dark brown to black at the centre, pale brown at the margin, mycelium white to cream with tufting.

Material examined: Sri Lanka, Western Province, Colombo District, University of Sri Jayewardenepura premises, on a piece of decaying

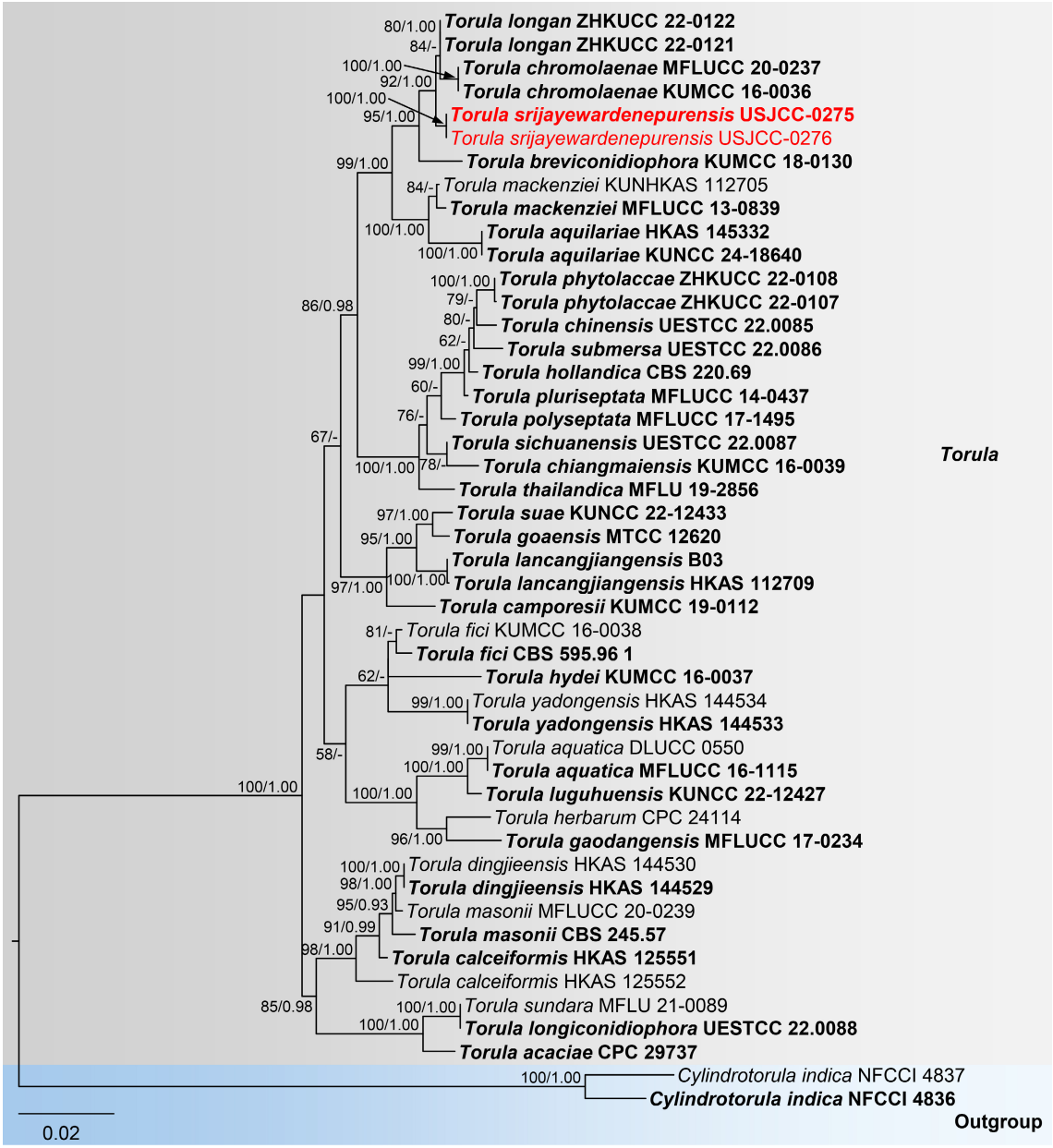

Fig. 2 Maximum likelihood (ML) tree of concatenated ITS, LSU, SSU, and *tef1-α* sequences from *Torula* species. *Cylindrotorula indica* (NFCCI 4836 and NFCCI 4837) was used as the outgroup. Maximum likelihood bootstrap support values (≥ 50%) and Bayesian posterior probability (≥ 0.90) are indicated at nodes. Newly generated strains in this study are highlighted in red, while type strains are shown in bold.

wood, 1 November 2024, Kasun M. Thambugala, KTSL009–2024 (USJ-H 255), culture USJCC-0274 (GMBUCC 24–019).

Notes: The new isolate USJCC-0274 clustered within the *Paradictyoarthrinium diffractum* clade (99% ML, 1.00 PP) in the combined LSU, ITS, and *rpb2* phylogeny. The ITS and *rpb2* sequences of isolate USJCC-0274 show 100% similarity to those of *P. diffractum* isolates GMBCC 2212, KUMCC 21-0336, and MFLUCC 24-0152, with no gaps. The dark brown to black, thick-walled conidia, which arise from branched or unbranched conidiophores and occur singly or occasionally in short chains, are unevenly dictyoseptate, subglobose to broadly ellipsoidal, sometimes irregular or lobed, and verrucose. These features agree with descriptions of *P. diffractum* in earlier studies[40,41]. *P. diffractum* has been recorded as saprobic on dead twigs in South Africa[37], dead stems of *Tectona grandis* in Thailand[42], dead spathes of *Cocos nucifera* in India[38], dead branches of *Pinus taeda* in China[40], and decaying wood of *Delonix regia* in Thailand[41]. Based on morphological and molecular evidence, the isolate is identified as *P. diffractum*. This is the first report of this species from Sri Lanka.

Torulaceae Corda, Deutschlands Flora, Abt. III. Die Pilze Deutschlands 2: 71 (1829)

Torula Pers., Ann. Bot. (Usteri) 15: 25 (1795)

Persoon[43] first introduced *Torula* in Torulaceae with *T. herbarum* as the type species. Later studies with multi-gene phylogeny, Crous et al.[44] confirmed the phylogenetic placement of *Torula* within Torulaceae (within Pleosporales). The Torula shows a wide host range and is present in both terrestrial and aquatic habitats[45–47]. *Torula* is characterized by cupulate conidiogenous cells in branched chains, subglobose, verrucose, septate conidia[44,45,48]. Currently, the phylogenetic placement of *Torula* was well established through multigene phylogeny of ITS, LSU, SSU, *tef1-α*, and *rpb2*[30,49].

Torula srijayewardenepurensis Thambugala & Tennakoon, *sp. nov*. Fig. 5

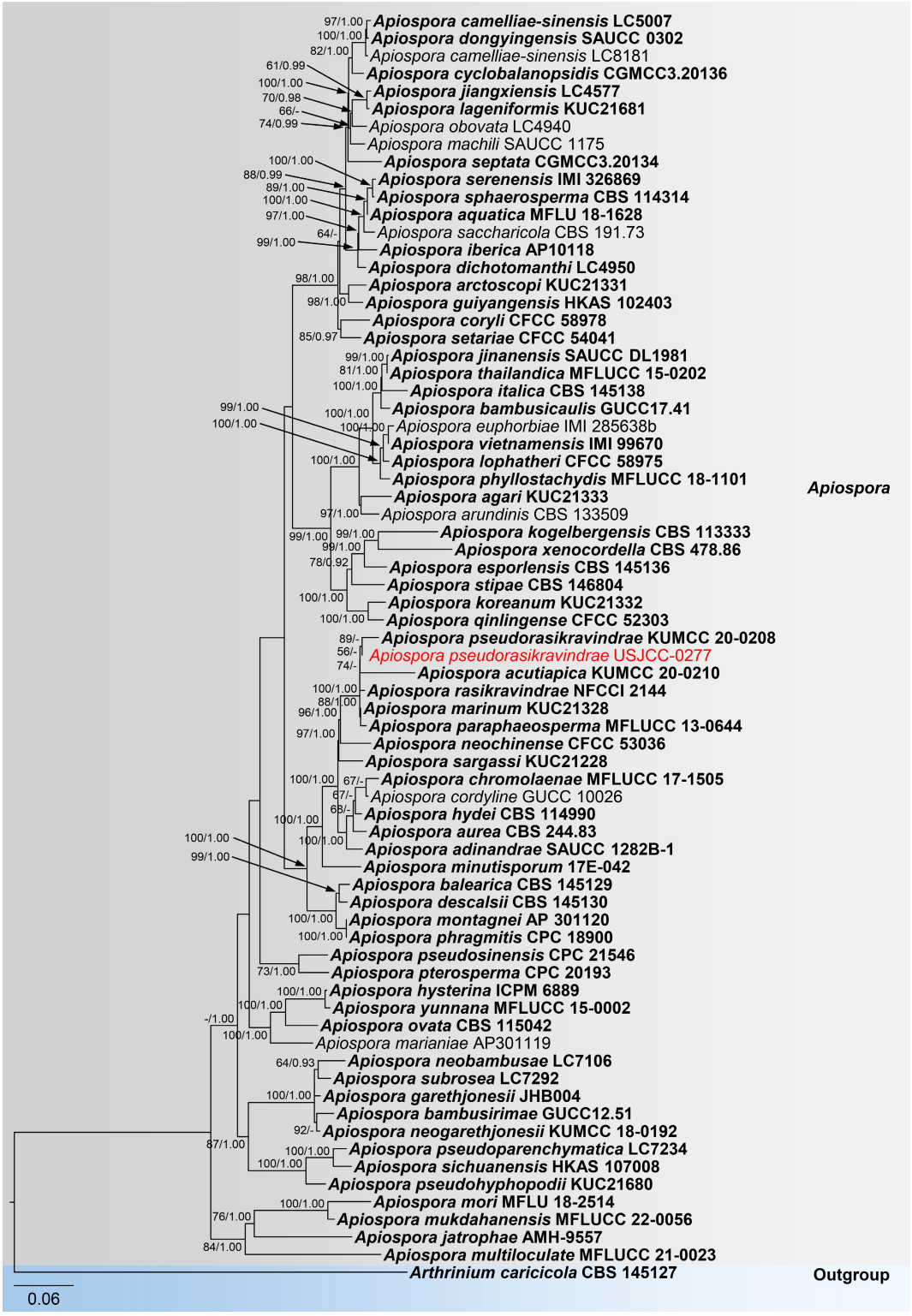

Fig. 3 Maximum likelihood (ML) tree of concatenated ITS, LSU, *tef1-α*, and *tub2* sequences from *Apiospora* species. *Arthrinium caricicola* was used as the outgroup. Maximum likelihood bootstrap support values (≥ 50%) and Bayesian posterior probability (≥ 0.90) are indicated at nodes. The newly generated strain is shown in red, while type strains are shown in bold.

MycoBank number: MB 860342

Etymology: The specific epithet *srijayewardenepurensis* refers to the University of Sri Jayewardenepura, Sri Lanka, the location where the holotype was collected.

Saprobic on a decaying unknown twig. Sexual morph: Undetermined. Asexual morph: Colonies effuse, scattered, dark brown to black, powdery, velutinous, dense to moderately dense. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 2–4 µm long × 3–5 µm wide ($\bar{x}$ = 2.5 × 3.2 µm, n = 10), solitary on mycelium, erect, mono- to poly-blastic, sympodial, subcylindrical to doliiform, terminal, brown to dark brown, smooth, thick-walled, globose to ellipsoid or sometimes coronate at apex. *Conidia* 4–18 µm long ×

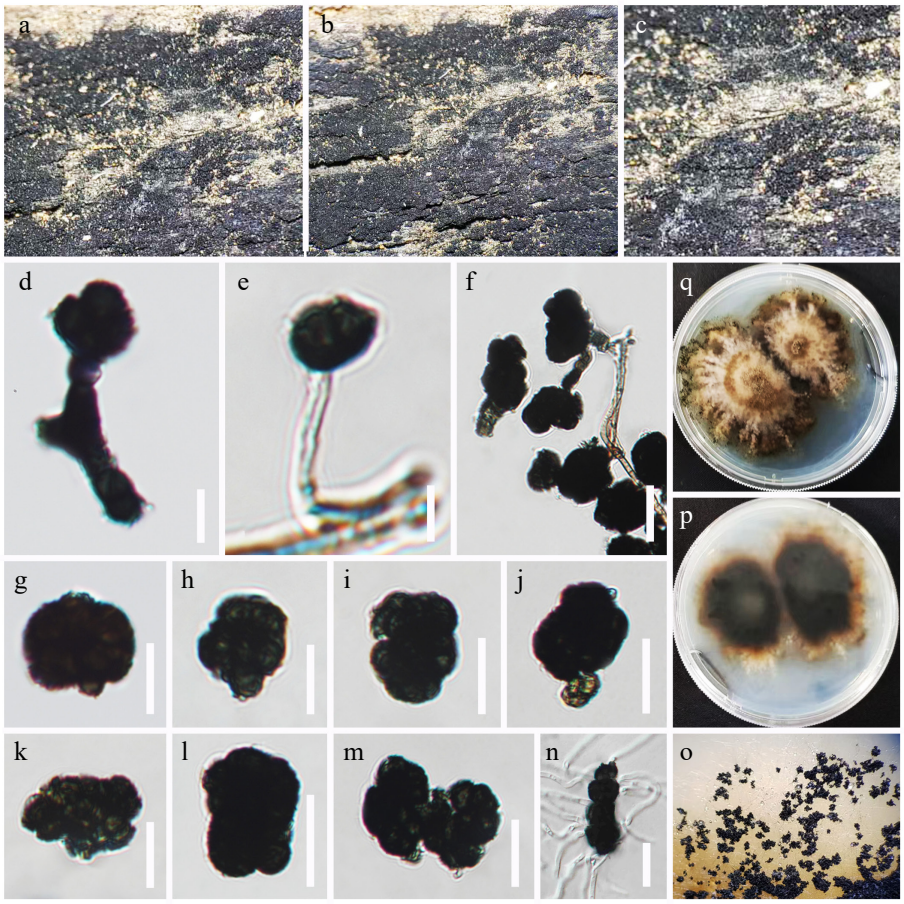

Fig. 4 *Paradictyoarthrinium diffractum* (USJ-H-255). (a)–(c) Colonies on the host substrate. (d)–(f) Conidiogenous cells and developing conidia. (g)–(m). Conidia. (n) Germinated conidium. (o) Front, and (p) the reverse views of the culture on PDA media. (q) Colonies on PDA media. Scale bars: (d)–(n) = 15 μm.

3–5 μm wide ($\bar{x}$ = 12.6 × 3.7 μm, n = 20) catenated, phragmosporous, acrogenous, brown to dark-brown, subglobose to obovoid, 1–3-septate, smooth to minutely verruculose, rounded at both ends or occasionally with a dark, terminal, coronate to cupulate apical cell, composed of moniliform cells, slightly constricted at some septa.

Culture characteristics: Colonies on PDA fast-growing, reaching 3–4 cm diameter after two weeks at 20–25 °C, colonies moderately sparse, flat, circular, with a slightly rough surface, entire edge, and well-defined margin, cottony to moderately fluffy with sparse aspects, colony from above, light brown at the centre, cream to yellowish at the margin; from below, light brown to dark brown at centre, yellowish at the margin, mycelium white to cream with tufting.

Material examined: Sri Lanka, Western Province, Colombo District, University of Sri Jayewardenepura premises, on a decaying unknown twig, 1 November 2024, Kasun M. Thambugala, KTSL008–2024 (USJ-H-252, holotype), ex-type culture USJCC-0275 (GMBUCC 24–016); *ibid.* KTSL008-2–2024 (USJ-H 253), culture USJCC-0276 (GMBUCC 24–017).

Notes: The multi-gene phylogenetic analyses (ITS, LSU, SSU, and *tef1-α* sequences) place the two isolates USJCC-0275 and USJCC-0276 as an independent lineage basal to *Torula chromolaenae* and *T. longan*, with strong support (92% ML, 1.00 BYPP). *Torula breviconidiophora* KUMCC 18-0130 forms a basal clade to *T. chromolaenae*, *T. longan*, and the lineage formed by the two new isolates. The ITS sequence of USJCC-0275 is similar to that of *T. chromolaenae* (KUMCC 16-0036; 98%, 4/523 gaps) and *T. longan* (ZHKUCC 22-0121; 99%, 1/505 gaps). The *tef1-α* sequence is similar to that of *T. chromolaenae* (KUMCC 16-0036; 98%, 2/874 gaps) and *T. mackenziei* (MFLUCC 13-0839; 98%, 2/906 gaps). The two isolates differ from *T. chromolaenae* and *T. longan* by having conidiophores reduced to conidiogenous cells and by their shorter conidiogenous cells. The conidia of *T. breviconidiophora* are medium to dark brown, 1–4-septate, with subhyaline or pale brown apical cells, which also distinguishes it from the new isolates[50]. Based on morphological and molecular evidence, the two isolates are identified as a new species, *T. srijayewardenepurensis*.

Sordariomycetes O.E. Erikss. & Winka, Myconet 1(1): 10 (1997)

Amphisphaeriales D. Hawksw. & O.E. Erikss., Syst. Ascom. 5(1): 177 (1986)

Apiosporaceae K.D. Hyde, J. Fröhl., Joanne E. Taylor & M.E. Barr, Sydowia 50(1): 23 (1998)

Apiospora Sacc., Atti Soc. Veneto-Trent. Sci. Nat., Padova, Sér. 4 4: 85 (1875)

Notes: Saccardo[51] established *Apiospora* Sacc. with *A. montagnei* as the type species. However, the taxonomic placement of *Apiospora* remained unclear with morphologically closer genera *Arthrinium* and *Neoarthrinium* due to the presence of arthrinium-like basauxic conidiogenesis[52]. Crous & Groenewald[53] later synonymized *Apiospora* with *Arthrinium* based on the one fungus-one name policy. However, later studies with additional genetic data of type species of *Apiospora* and *Arthrinium*, Pintos & Alvarado[54] were able to separate *Apiospora* and *Arthrinium* into two distinct genera. Pintos & Alvarado[54] revealed that *Arthrinium* species have variously shaped conidia and inhabit Cyperaceae and Juncaceae in temperate, cold, and alpine habitats, while most *Apiospora* species have rounded/lenticular conidia, inhabiting mainly Poaceae together

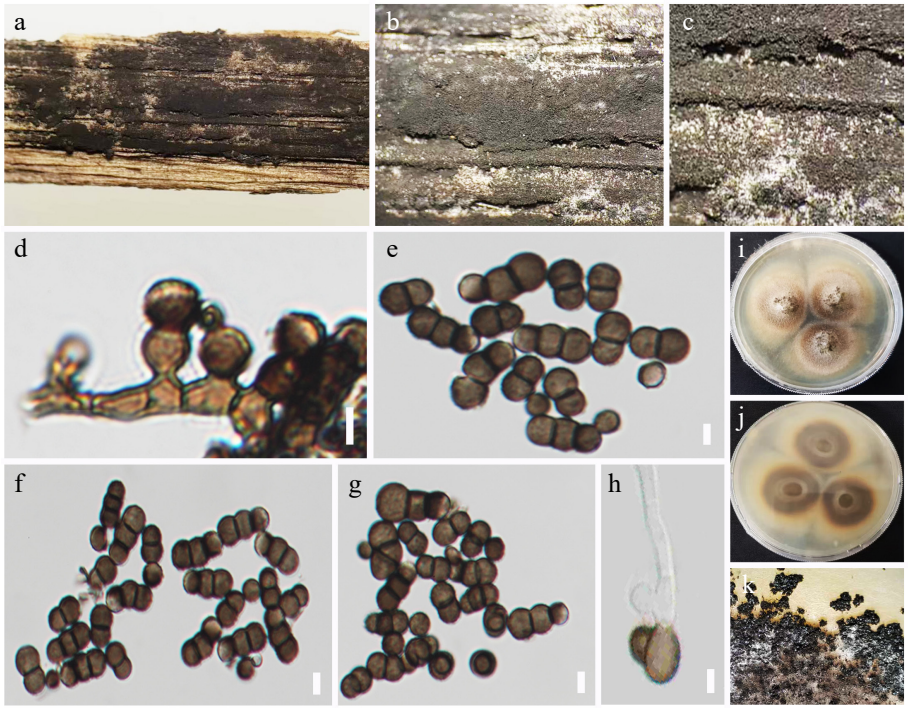

Fig. 5 *Torula srijayewardenepurensis* (USJ-H-252, holotype). (a)–(c) Colonies on the host substrate. (d) Conidiophores with conidiogenous cells. (e)–(g) Conidia. (h) Germinated conidium. (i) Front, and (j) the reverse view of the culture on PDA medium. (k) Appearance of colonies on PDA. Scale bars: (d)–(h) = 5 μm.

with other host plant families distributed in a wide range of habitats from tropical to subtropical regions. *Apiospora montagnei*, the epitype for the type species of *Apiospora*, was proposed by Pintos & Alvarado[55]. *Apiospora* is distinctly characterized by conidia that are globose, subglobose to ellipsoid, oval, or obovoid in face view, appearing lenticular in side view, and by the presence of basauxic conidiogenous cells[54].

Apiospora pseudorasikravindrae (Senan. & Cheew) Thambugala, comb. nov. Fig. 6

MycoBank number: MB 558505

≡ *Arthrinium pseudorasikravindrae* Senan. & Cheew., Frontiers Microbiol. 11 (no. 602773): 11 (2020)

Typus: China, Guangdong Province, Shenzhen City, Futian District, northwest of Futian, Bijiashan Park, on sheath of *Bambusa dolichoclada* (Poaceae), 23 September 2018, IS, SI 73 (HKAS 107669, holotype), ex-type culture, KUMCC 20-0208; *ibid* October 15, 2018, IS, SI 73-1 (HKAS 107670, paratype), ex-paratype culture KUMCC 20-0211.

Description of the new collection: *Saprobic* on clumps of *Bambusa vulgaris* (Poaceae). Sexual morph: undetermined. Asexual morph: Colonies scattered to aggregated, forming numerous black spots, superficial. Conidiophore mother cells are ampulliform, doliiform to subglobose, hyaline, smooth-walled. Conidiophores reduced to conidiogenous cells. Conidiogenous cells 5–10 × 2–5 μm ($\overline{x}$ = 7.9 × 4.5 μm, n = 30), holoblastic, develop from conidiophore mother cells. *Conidia* 4.6–9 × 7–12 μm ($\overline{x}$ = 8.6 × 10.6 μm, n = 30), lenticular in side view, globose in face view, with a pale longitudinal slit, thick-walled, brown to dark brown, finely roughened with one or two concentric pale rings.

Culture characteristics: Colonies on PDA fast-growing, reaching 5–6 cm diameter after two weeks at 20–25 °C, colonies sparse, circular, slightly raised, with a moderately rough surface, entire edge, and well-defined margin, cottony to fairly fluffy with sparse aspects, colony from above, pale brown at the centre, white at the margin; from below, pale brown at centre, white to cream at the margin, mycelium white to cream with tufting.

Material examined: Sri Lanka, Western Province, Colombo District, University of Sri Jayewardenepura premises, on clumps of *Bambusa vulgaris* Schrad. ex J.C. Wendl., 1 November 2024, Kasun M. Thambugala, KTSL007–2024 (USJ-H 254), culture USJCC-0277 (GMBUCC 24–018).

Notes: *Arthrinium pseudorasikravindrae* was described from *Bambusa dolichoclada* in Guangdong Province, China, by Senanayake et al.[56]. Following Pintos & Alvarado[55], Tian et al.[57] proposed a new combination and transferred the species to *Apiospora* as *A. pseudorasikravindrae*, based on combined LSU, ITS, *tef1-α*, and *tub2* sequence analyses. However, this name was invalid because the authors did not provide full details of the basionym reference, as required by ICN Art. 41.5. In this study, we provide a valid combination, formally establishing *Apiospora pseudorasikravindrae* based on comprehensive morpho-molecular evidence.

One of the new isolates (USJCC-0277) forms a sister lineage to *A. pseudorasikravindrae* (KUMCC 20-0208) in a well-supported clade (89% ML). Isolate USJCC-0277 shares similar morphology with KUMCC 20-0208, including ampulliform, doliiform to subglobose conidiogenous cells (5–10 × 2–5 μm vs 4–10 × 1.2–5 μm), and lenticular conidia in side view, globose in face view, with a pale longitudinal slit. The conidia are thick-walled, brown to dark brown, finely roughened, with one or two concentric pale rings (4.6–9 × 7–12 μm vs 5–10 × 5.5–11 μm). The *tub2* and *tef1-α* sequences of USJCC-0277 show 100% similarity to those of KUMCC 20-0208 with no gaps. The ITS sequence of KUMCC 20-0208, however, contains ambiguous base calls in conserved regions, which may explain the long branch in the phylogeny. Based on protein-coding gene similarity and morphological agreement, the new isolate is identified as *A. pseudorasikravindrae*. *Apiospora pseudorasikravindrae* has previously been reported only from *Bambusa dolichoclada* in China[56]. In this study, *A. pseudorasikravindrae* was collected on clumps of *Bambusa vulgaris* in Sri Lanka, representing a new host and a new geographical record.

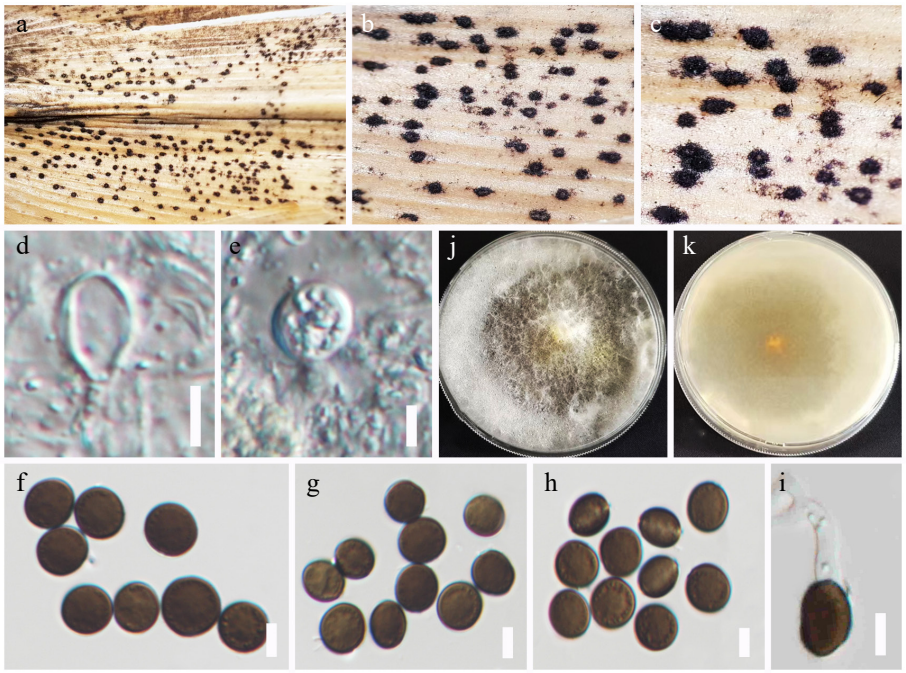

Fig. 6 *Apiospora pseudorasikravindrae* (USJ-H-254). (a)–(c) Colonies on the host substrate. (d), (e) Conidiogenous cells and developing conidia. (f)–(h) Conidia. (i) Germinated conidium. (j) Front, and (k) the reverse views of the culture on PDA media. Scale bars: (d)–(i) 5 μm.

Discussion

In this study, a novel microfungal species is introduced, *Torula srijayewardenepurensis*, and the first record of *Apiospora pseudorasikravindrae* and *Paradictyoarthrinium diffractum* from Sri Lanka are documented. The discovery of *Torula srijayewardenepurensis* sp. nov., and the new records of *Apiospora pseudorasikravindrae* and *Paradictyoarthrinium diffractum* not only add to the growing account of Sri Lankan mycobiota but also illustrate the hidden richness of microfungi in urban and anthropogenically influenced environments. The isolation of all three taxa from leaf litter collected within an urban setting underscores the immense potential of even highly modified ecosystems in harboring novel and ecologically significant fungal taxa.

This observation suggests that contrary to the widely held assumption that urban environments are uniformly low in biodiversity, certain urban niches may support a greater diversity of microfungi than expected. Rather than making broad claims, the present results indicate that these habitats may harbour previously undocumented or overlooked fungal taxa. However, more systematic and comprehensive sampling across multiple urban settings would be required to substantiate this pattern more broadly. Given that urbanization is rapidly reshaping tropical landscapes, it is essential to include urban ecosystems in fungal biodiversity assessments.

Torula species have a wide host range and occur in diverse habitats, commonly as saprobes in both aquatic and terrestrial environments across temperate to tropical regions[49,58,59]. Although more than 560 species epithets have been recorded under the genus *Torula*, many of them are considered ambiguous, having been described solely based on morphological characteristics. Although certain morphological traits differentiate each species within *Torula*, their overall morphology is quite similar, making it difficult to use these features alone for species identification[49].

Accurate delimitation of the new species, as well as verification of the new country records, required the integration of detailed morphology with multi-locus phylogenetic analyses. Neither dataset alone provided sufficient resolution, but together they yielded a consistent and well-supported taxonomic framework.

Although not all gene regions were available for every isolate, the combination of the markers that were successfully sequenced (e.g., ITS, LSU, SSU, and selected protein-coding genes such as *tub2*, *rpb2*, or *tef1-α*, depending on the taxon) provided sufficient phylogenetic resolution to clarify the taxonomic placement and relationships. The multi-locus framework, even when partially represented, strengthened the integrative approach and improved species delimitation within morphologically similar groups such as *Torula* and related genera.

Author contributions

The authors confirm their contributions to the paper as follows: conceptualization, data curation, and formal analysis: Thambugala KM, Tennakoon DS, Samarakoon MC; funding acquisition: Thambugala KM, Hongsanan S; investigation, methodology, and writing – original draft: Thambugala KM, Tennakoon DS, Samarakoon MC, Milanka I, Daranagama DA; supervision: Thambugala KM, Hongsanan S; writing – review and editing: Thambugala KM, Tennakoon DS, Samarakoon MC, Milanka I, Daranagama DA. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All the data generated and analyzed during this study are available in the article. DNA sequence data are available in the GenBank database (www.ncbi.nlm.nih.gov/genbank), and the accession numbers are provided in Supplementary Tables S1–S3.

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Conflict of interest

The authors declare that they have no conflict of interest.

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