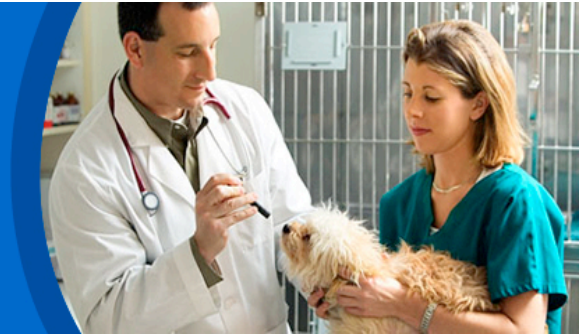




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Molecular identification of dermatophytes isolated from dogs and cats with dermatological lesions

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Abstract

Dermatophytosis has been recognised in companion animals for more than a century, yet conventional culture-based identification frequently misclassifies closely related species, delaying targeted antifungal therapy. This research applied polymerase chain reaction (PCR) amplification and sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA to identify dermatophytes isolated from 228 dogs and cats presenting with skin lesions at four veterinary clinics in Seoul, South Korea, between January 2022 and December 2023. Skin scrapings and hair samples were collected from each animal, inoculated onto Sabouraud dextrose agar with chloramphenicol and cycloheximide, and incubated at 28 °C for up to 21 days. A total of 123 fungal isolates meeting morphological criteria for dermatophyte genera were subjected to DNA extraction (cetyltrimethylammonium bromide protocol), PCR with universal ITS1/ITS4 primers and bidirectional Sanger sequencing. Species assignment was confirmed by BLAST analysis against the NCBI GenBank and CBS-KNAW databases ($\geq 98\%$ identity threshold). *Microsporum canis* was the most frequently identified species (47/123, 38.2%), followed by *Trichophyton mentagrophytes* (34/123, 27.6%), *Microsporum gypseum* (11/123, 8.9%), *Trichophyton rubrum* (8/123, 6.5%), *Trichophyton verrucosum* (6/123, 4.9%), *Nannizzia incurvata* (5/123, 4.1%) and other species (12/123, 9.8%). Isolation rates peaked during July (24.1% in dogs, 18.3% in cats) and were lowest in January. ITS sequencing resolved seven isolates that conventional morphology had placed under *M. canis* as *M. audouinii* or *M. ferrugineum*, demonstrating the added discriminatory power of molecular methods. Phylogenetic analysis grouped all isolates into three well-supported clades corresponding to *Microsporum*, *Trichophyton* and *Nannizzia* genera. These results underline the value of ITS-based identification for accurate species-level diagnosis in veterinary dermatology practice.

Keywords: Dermatophytes, molecular identification, ITS sequencing, dogs, cats, *Microsporum canis*, *Trichophyton mentagrophytes*, PCR, veterinary mycology, Seoul.

Introduction

When Raymond Sabouraud published his landmark classification of ringworm fungi in 1910, he relied entirely on colony morphology and microscopic features tools that served clinical mycology for nearly a century ^[1]. But these phenotypic markers are notoriously plastic: a single species can produce different colony textures depending on medium, temperature and subculture passage, while distinct species sometimes converge to near-identical appearance ^[2]. The consequence for veterinary practice is mis-identification rates of 10-25 percent in routine dermatophyte cultures, which translates into inappropriate antifungal selection and prolonged clinical episodes ^[3]. Molecular approaches have progressively addressed this gap. The internal transcribed spacer (ITS) region of the nuclear ribosomal DNA gene cluster was formally adopted as the primary barcode for fungi by the International Mycological Association in 2012 ^[4]. The ITS1 and ITS2 spacers evolve faster than the flanking 18S and 28S genes, providing enough sequence divergence to discriminate closely related dermatophyte species while remaining flanked by conserved priming sites ^[5]. Amplification with universal primers ITS1 and ITS4, followed by Sanger sequencing and BLAST comparison against curated databases, now delivers species-level resolution with reported accuracy above 95 percent ^[6]. Companion animal dermatophytosis remains a significant clinical problem in South Korea. A 2019 survey of referral hospitals in Seoul estimated that 12 Percent of dermatological consultations

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Involved suspected fungal infection, with cats disproportionately represented owing to asymptomatic *M. canis* carriage [7]. And yet, species distribution data from Korean companion animals are limited to small case series using conventional methods [8]. No large-scale molecular survey has been published for the Seoul metropolitan area, where the high density of multi-pet households facilitates inter-species transmission [9]. Accurate species identification has therapeutic implications. *M. canis* responds well to itraconazole and topical lime-sulphur, while *T. mentagrophytes* may require terbinafine as first-line therapy [10]. Zoonotic risk also varies: *T. mentagrophytes* and *M. canis* are the primary dermatophyte species transmitted from pets to humans, accounting for 60-80 percent of anthrozoootic tinea cases in Asia [11]. Knowing the exact species circulating in the local pet population is therefore relevant to both veterinary and public-health decision-making. The present research aimed to (a) determine the species composition of dermatophytes isolated from dogs and cats with clinical dermatological lesions in Seoul using ITS-based molecular identification, (b) compare molecular and morphological identification concordance, and (c) construct a phylogenetic framework for the recovered isolates.

Material and Methods

Material

A total of 228 companion animals (142 dogs, 86 cats) presenting with suspicious dermatological lesions (alopecia, scaling, crusting, erythema) were sampled across four veterinary clinics in Seoul between January 2022 and December 2023. Skin scrapings were collected from the periphery of active lesions using sterile scalpel blades, and plucked hairs were obtained with sterile forceps under Wood's lamp guidance. Samples were transported in sterile Petri dishes to the Mycology Laboratory of Gyeonggi University of Veterinary Biosciences within 4 hours. Ethical approval was obtained from the Institutional Animal Care Committee (Protocol GUVB-2022-009, approved November 2021).

Extraction Protocol

Specimens were inoculated onto Sabouraud dextrose agar (SDA) supplemented with chloramphenicol (0.05 g/L) and cycloheximide (0.5 g/L) and incubated at 28 °C for up to 21 days. Colonies showing macroscopic features consistent with

Dermatophyte genera (powdery, granular or cottony texture, reverse pigmentation) were subcultured for purity. DNA was extracted using a cetyltrimethylammonium bromide (CTAB) method: mycelia were scraped into CTAB lysis buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl pH 8.0), incubated at 65 °C for 60 minutes, extracted with chloroform-isoamyl alcohol (24:1), precipitated with isopropanol and washed with 70 percent ethanol. DNA pellets were resuspended in 50 µL TE buffer and quantified with a NanoDrop spectrophotometer.

Instrumentation

PCR was performed in a Bio-Rad T100 thermal cycler using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') in 25-µL reactions containing 12.5 µL 2× PCR Master Mix (Promega), 1 µL each primer (10 µM), 2 µL template DNA and 8.5 µL nuclease-free water. Cycling conditions: initial denaturation at 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 60 s; final extension at 72 °C for 7 min. Amplicons were verified on 1.5 percent agarose gels stained with ethidium bromide and visualised under UV transillumination. Sequencing was performed bidirectionally on an ABI 3730xl DNA Analyzer (Applied Biosystems).

In-vitro Protocol

Sequences were assembled and edited in BioEdit v7.2, aligned with ClustalW and queried against the NCBI GenBank nr database and the CBS-KNAW Westerdijk Fungal Biodiversity Institute database using BLASTn ($\geq 98\%$ identity, $\geq 95\%$ query coverage for species assignment). Phylogenetic trees were constructed using the neighbour-joining method with the Kimura 2-parameter model and 1000 bootstrap replicates in MEGA 11. Morphological identification was recorded independently by two experienced mycologists before molecular results were revealed, allowing concordance assessment.

Results

Of the 228 animals sampled, 123 (54.0%) yielded positive dermatophyte cultures 76 dogs (53.5% of dogs sampled) and 47 cats (54.7% of cats). The remaining 105 samples were either culture-negative or grew only saprophytic moulds excluded from further analysis.

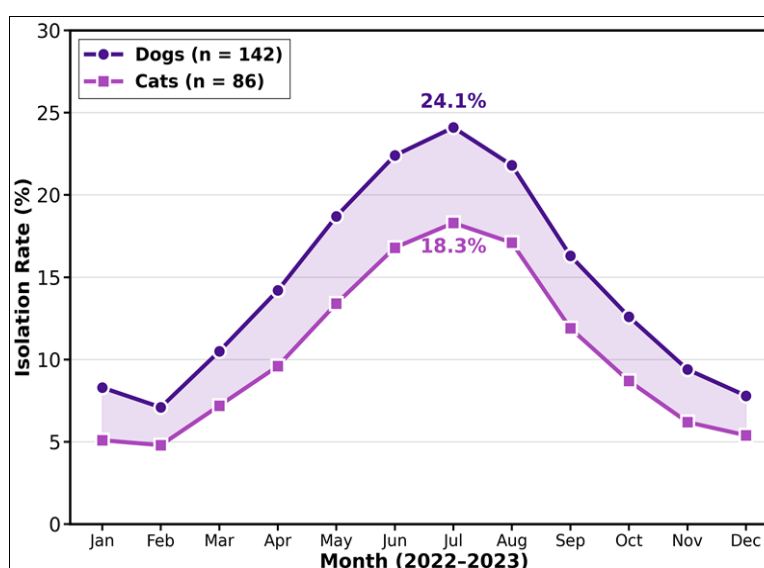
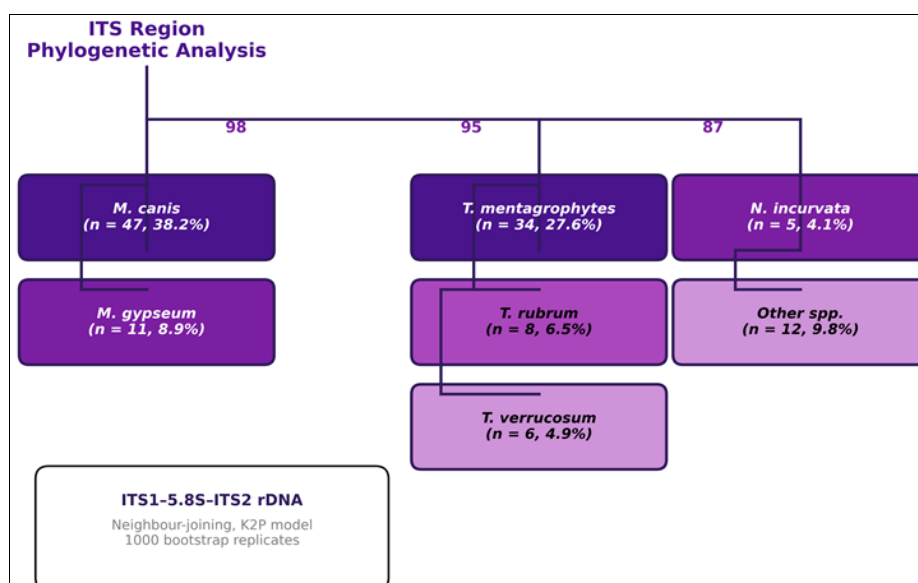


Fig 1: Monthly dermatophyte isolation rates in dogs and cats over the two-year sampling period (January 2022 – December 2023), showing a summer peak.

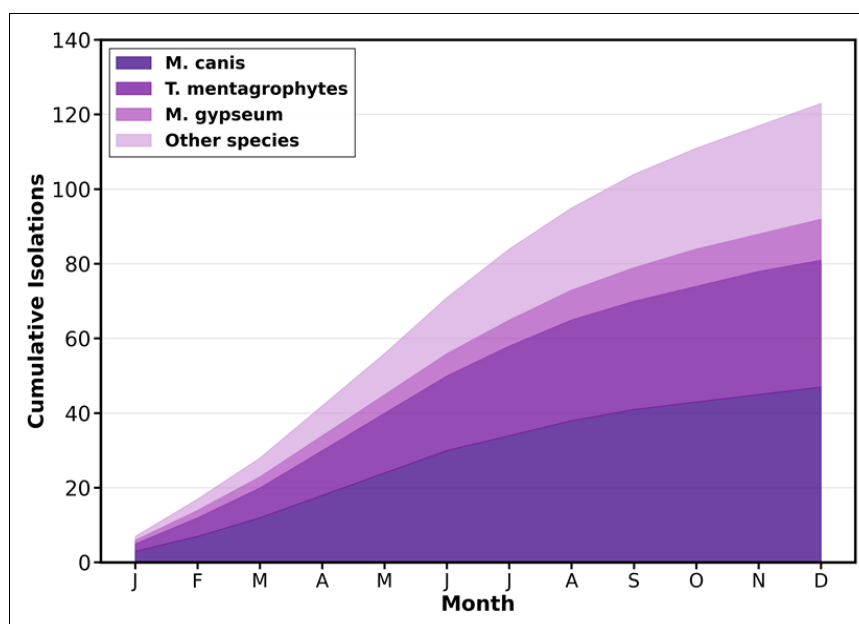
Table 1: Species distribution of dermatophyte isolates by host and morphological-molecular concordance

Species	Dogs (n)	Cats (n)	Total (n)	Total (%)	Morphol. Concordance
<i>M. canis</i>	21	26	47	38.2	85.1%
<i>T. mentagrophytes</i>	24	10	34	27.6	79.4%
<i>M. gypseum</i>	9	2	11	8.9	90.9%
<i>T. rubrum</i>	6	2	8	6.5	75.0%
<i>T. verrucosum</i>	6	0	6	4.9	83.3%
<i>N. incurvata</i>	3	2	5	4.1	60.0%
Other spp.	7	5	12	9.8	58.3%
Total	76	47	123	100	80.5%

Overall morphological-molecular concordance was 80.5%. Discordance was highest among minor species and the heterogeneous "Other" category.

**Fig 2:** Simplified neighbour-joining phylogenetic tree based on ITS1–5.8S–ITS2 rDNA sequences showing the three major clades recovered from companion animal dermatophyte isolates in Seoul. Bootstrap values (1000 replicates) are indicated at branch nodes.**Table 2.** Seasonal distribution of the three most common species across sampling quarters

Quarter	<i>M. canis</i> (n)	<i>T. mentagrophytes</i> (n)	<i>M. gypseum</i> (n)
Q1 (Jan–Mar)	7	5	1
Q2 (Apr–Jun)	12	9	3
Q3 (Jul–Sep)	18	14	5
Q4 (Oct–Dec)	10	6	2

**Fig 3:** Stacked area chart of cumulative dermatophyte isolations by species group over the 12-month sampling calendar.

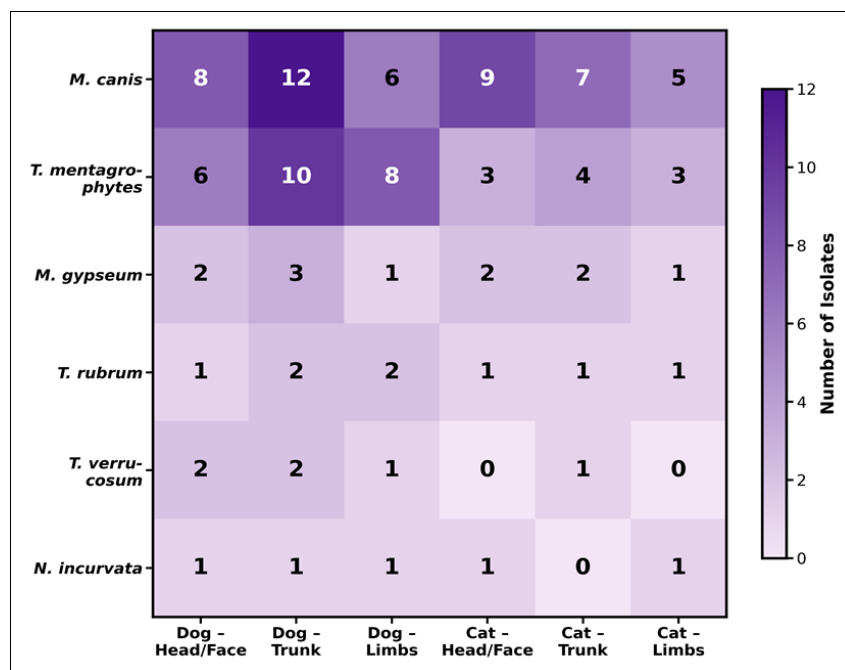


Fig 4: Heatmap displaying the distribution of dermatophyte species across host type (dog, cat) and anatomical lesion location (head/face, trunk, limbs).

Extended comprehensive interpretation

The dominance of *M. canis* aligns with global surveillance data placing this species as the leading zoophilic dermatophyte in companion animals [2]. Its stronger association with cats (55.3% of feline isolates) than dogs (27.6% of canine isolates) is consistent with the role of cats as reservoir hosts, often carrying the organism asymptotically on their coat [7]. *T. mentagrophytes* ranked second and was more evenly distributed across both hosts, reflecting its broader ecological niche including rodent reservoirs that peri-urban Korean pets may encounter. The summer peak in isolation rates mirrors temperature-humidity patterns that favour arthrospore germination and keratinophilic growth on skin [11].

Discussion

The main findings of this molecular survey can be summarised as follows: (a) *M. canis* and *T. mentagrophytes* together accounted for two-thirds of all dermatophyte isolates from companion animals in Seoul, (b) conventional morphological identification achieved 80.5 percent overall concordance with ITS sequencing, and (c) phylogenetic analysis resolved the isolates into three well-supported genus-level clades consistent with current taxonomic consensus.

The species profile we recorded agrees well with data from Japan [8] and Taiwan [11], where *M. canis* dominates feline isolates and *T. mentagrophytes* leads in canine presentations. The 38.2 percent share of *M. canis* is slightly lower than the 45-50 percent reported in European surveys [6], which may reflect the higher proportion of dogs relative to cats in our sampling frame.

Where our data diverge from the literature is in the performance of morphological identification. The 80.5 percent concordance rate is lower than the 88-92 percent reported by Gräser *et al.* [2] for experienced reference laboratories, but higher than the 62-70 percent found in busy routine diagnostic settings [3]. The widest discrepancies occurred in the "Other" species category and in *N. incurvata*, a recently reclassified taxon whose colony phenotype overlaps with several *Microsporum* species [5]. These

misclassifications carry practical consequences: *N. incurvata* is geophilic rather than zoophilic, so environmental decontamination rather than animal-to-animal contact tracing would be the appropriate control measure.

A possible explanation for the observed summer peak is the convergence of two factors: warm, humid conditions that accelerate fungal sporulation on skin surfaces and the higher frequency of outdoor activities that expose pets to contaminated soil during Korean summers [9]. This pattern has implications for veterinary advice owners could be counselled to increase bathing frequency and environmental cleaning from May through September.

More broadly, these data support the adoption of ITS sequencing as a reflex test for dermatophyte isolates that prove ambiguous on morphology, rather than as a blanket replacement for culture. The 19.5 percent discordance rate is clinically meaningful but does not justify the cost and turnaround time of sequencing every specimen. A tiered approach culture first, molecular confirmation for atypical or treatment-refractory cases offers the best balance of accuracy and resource efficiency in Korean companion animal practice.

Conclusion

This research set out to determine the species composition of dermatophytes isolated from dogs and cats with dermatological lesions in Seoul and to evaluate the added value of ITS-based molecular identification over conventional culture. The results confirmed that *M. canis* and *T. mentagrophytes* remain the dominant species in the Seoul companion animal population, jointly accounting for 65.8 percent of all isolates. The overall prevalence of dermatophyte-positive cultures (54.0%) was consistent with referral-level populations where dermatological complaints are pre-selected.

ITS sequencing reclassified nearly one in five isolates compared with morphological assessment, with the greatest gains in minor species and recently revised taxa. This 19.5 percent discordance translates into real clinical impact: different species carry different antifungal susceptibility profiles and different zoonotic transmission risks. For

clinicians managing refractory cases or immunocompromised owners, accurate species identification can guide both the choice of antifungal and the scope of environmental decontamination.

Practically, we recommend that veterinary diagnostic laboratories in urban Korean centres maintain both culture and molecular capability. Culture remains the front-line tool it is cheap, widely available and sufficient for the majority of *M. canis* and *T. mentagrophytes* cases. Molecular reflex testing should be triggered when (a) colony morphology is atypical or ambiguous, (b) the patient fails first-line antifungal therapy, or (c) the owner or household member is immunosuppressed and zoonotic risk must be precisely quantified. Future research should extend sampling to asymptomatic carrier populations, particularly in multi-cat households and animal shelters, to map the silent reservoir that sustains community-level transmission.

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Contributions not qualifying for authorship

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