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Research article

First report and new host record of *Verruciconidia persicina* from the beetle gut in India

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Abstract

Fungi associated with beetle guts represent an important but underexplored source of microbial diversity and ecological interactions. Such associations may contribute to host nutrition, defence, or adaptation to specific environments. During a survey of beetle gut-associated fungi, a fungal strain was isolated from the gut of *Platynotus excavatus* collected in Junnar, India. Based on morphological characteristics and multigene phylogenetic analysis of ITS (Internal transcribed spacer), LSU (Large Submit Unit), TEF-1α (translation elongation factor 1-alpha), and RPB2 (RNA polymerase II) sequences, the isolate was identified as *Verruciconidia persicina*. The strain (MCC 9995) clusters with the ex-type strain *V. persicina* (CBS 310.59) with 91% bootstrap support but exhibited slightly wider hyphae (up to 4.5 µm vs. 3.5 µm). This represents the first report of the genus *Verruciconidia* from an insect gut and its first record from India.

Keywords
Platynotus excavatus, pure culture, ITS, LSU, TEF-1α, RPB2, phylogeny

Introduction

In the class Insecta, order Coleoptera represents one of the most diverse taxa, including some wood-feeding beetle families, such as Scarabaeidae, Passalidae, Cerambycidae, Elateridae, and Tenebrionidae (Rojas-Jiménez and Hernández, 2015). Traditionally, most research on beetles emphasized external traits such as morphology and physiology, often overlooking their microbial symbionts. However, in recent decades, studies have increasingly highlighted the crucial role of these microbial partners in nutrition, digestion, detoxification, and ecological adaptation (Huang et al., 2012; Ziganshina et al., 2018). The family Tenebrionidae, with more than 20,000 species, is a cosmopolitan group of decomposers inhabiting forests and deserts (Cheng et al., 2022; Evans, 2023). These beetles occupy diverse microhabitats such as rotten wood, bark, stones, and decaying logs. Both larvae and adults are mainly detritivores, feeding on decaying vegetation, seeds, roots, cereals, and animal dung, while some species also exploit stored products (Thakare et al., 2012). Their versatile diet is supported by a well-developed digestive system capable of processing complex substrates, making Tenebrionids excellent models for studying gut-associated microorganisms (Muniswamy et al., 2018; Evans, 2023).

The genus *Verruciconidia* was established within the family Bionectriaceae, separated from *Acremonium* based on both morphological and multigene phylogenetic analyses (Hou et al., 2023). Primarily, seven species were accommodated in this genus, including *Verruciconidia persicina* (Nicot) L.W. Hou, L. Cai & Crous [formerly *Acremonium persicinum* (Nicot) W. Gams], *Verruciconidia verruculosa* (W. Gams & Veenb.-Rijks) L.W. Hou, L. Cai & Crous (formerly *A. verruculosum* W. Gams & Veenb.-Rijk), and five novel species characterised by verrucose conidia, namely *V. erythroxyli* L.W. Hou, L. Cai & Crous, *V. infuscata* L.W. Hou, L. Cai & Crous, *V. quercina* L.W. Hou, L. Cai & Crous, *V. siccicapita* L.W. Hou, L. Cai & Crous, and *V. unguis* L.W. Hou, L. Cai & Crous (Hou et al., 2023). A significant feature of *Verruciconidia* is the production of verrucose (warty) conidia, which distinguishes it from other genera in Bionectriaceae. However, some members, such as *V. erythroxyli*, *V. persicina*, and *V. unguis,* diverge from this pattern, producing smooth-walled conidia, thereby highlighting morphological diversity within the genus (Hou et al., 2023). The type species, *V. verruculosa*, was first isolated from agricultural soil in the Netherlands and the other species were described from a range of substrates, including soil, plant debris, air, and human nails (Hou et al., 2023). More recently, the genus was expanded further with the description of *V. oligospora* Meng M. Wang & Wei Li ter from intertidal sediment in China (Wang et al., 2025), along with two new combinations, *V. guizhouensis* (S.Qiu Tong & Y.Jun Wu) Lin Zhao bis & Crous (formerly *Acremonium guizhouense* S.Qiu Tong & Y.Jun Wu) and *V. saulensis* (Lechat & J. Fourn.) Lin Zhao bis & Crous (formerly *Lasionectria saulensis* Lechat & J. Fourn.) (Zhao et al., 2025). With these additions, *Verruciconidia* now includes ten species distributed across diverse ecological niches (Table 1).

The present study reports the first isolation and morphological characterisation of a *V. persicina* from the gut of *P. excavatus*. Additionally, we document the first record of this fungus in India. The findings highlight the significant role of beetles as novel hosts for various microbial species, underscoring the need for further research on the fungal diversity of insects.

Materials and Methods

Isolates and morphology of the mycelial colony

Beetles were collected from organic waste (19°22.338’ N, 73°88.218’ E), surface-disinfected, and dissected aseptically under a stereomicroscope. Gut tissues were suspended in 0.7% saline solution and homogenised using a sterile mortar and pestle. The homogenate was serially diluted and plated on PDA supplemented with 100 μg ml^{-1} ampicillin and 100 μg ml^{-1} streptomycin to minimise bacterial contamination (Avchar et al., 2024). Morphology of the mycelial colony was examined on potato dextrose agar (PDA), 2% malt extract agar (MEA), 2% oatmeal agar (OA), and synthetic nutrient agar (SNA) (Hou et al., 2023). The culture was deposited at the National Centre for Microbial

Resource (NCMR), India (MCC 9995). Morphological studies were conducted using an Olympus BX53 microscope (Olympus, Japan).

Table 1 – Total number of species from genus *Verruciconidia* isolated from different host/substrates.

Species	Host/substrates	Country	References
V. verruculosa	Agricultural soil	Netherland	Hou et al. 2023
V. siccicapita	Agricultural soil	Thailand	Hou et al. 2023
V. quercina	Agricultural soil	Netherlands	Hou et al. 2023
	Dead leaves of *Quercus* spp.	Netherlands	Hou et al. 2023
V. infuscata	Air	Japan	Hou et al. 2023
V. erythroxyli	Leaves of *Erythroxylum areolatum*	Cuba	Hou et al. 2023
	Leaf of *Urena lobata*	Democratic Republic of the Congo	Hou et al. 2023
V. unguis	Human nail	Netherlands	Hou et al. 2023
	Human nail	Austria	Hou et al. 2023
V. persicina	Coastal sand under *Ammophila arenaria*	France	Hou et al. 2023
	Endophyte in *Festuca ovina*	Iran	Hou et al. 2023
	Leaf of *Vitis vinifera*	Italy	Hou et al. 2023
	Tent canvas	Papua New Guinea	Hou et al. 2023
	Dead hardwood branch	United States of America	Vu et al. 2019
	***Platynotus excavatus* beetle Gut**	**India**	**Present study**
V. oligospora	Intertidal sediment of a mud beach	China	Wang et al. 2025
V. guizhouensis	Rhizosphere soil of *Capsicum annuum*	China	Zhao et al. 2025
V. saulensis	Dead leaves of *Astrocaryum* spp.	France	Zhao et al. 2026

Molecular identification and phylogenetic analysis

The extraction of genomic DNA was done according to Aamir et al. (2015). The amplification of the complete genes was performed using standard primers and PCR cycling conditions reported previously i.e. internal transcribed spacer (ITS) region (ITS1 and ITS4 primers), large subunit (LSU) region (LROR and LR5), translation elongation factor 1-alpha (EF-1α) region (983F and 2218R) and RNA polymerase II (rpb2) region (drpb2-5f and drpb2-7r) (Vilgalys and Hester, 1990; White et al., 1990; Carbone and Kohn, 1999; Liu et al., 1999). PCR products were purified using PEG-NaCl (Avchar et al., 2024) and sequenced on an ABI 3730xl DNA analyser (Applied Biosystems, Inc., Foster City, California, United States) (Avchar et al., 2024). Sequences were submitted to GenBank. All available sequences of *Verruciconidia* species, with preference for type or ex-type strains, were retrieved from GenBank and supplemented with representatives of related taxa in Bionectriaceae. Individual loci (ITS, LSU, TEF1-α, and RPB2) were aligned separately using MUSCLE (Edgar, 2004) and manually curated in MEGA 7 to remove ambiguities. Each alignment was trimmed to equal length, and the curated alignments were concatenated into a single dataset using Phylogeny.fr (Dereeper et al., 2008). Phylogenetic analyses were then performed in IQ-TREE (Nguyen et al., 2014). The best-fit model for each locus was estimated independently, while for the concatenated dataset, TNe+I+G4was selected as the optimal model under the Bayesian Information Criterion (BIC). Maximum likelihood inference was conducted with 1,000 bootstrap replicates. *Saccharata proteae* CBS 115206 was designated as an out group. The multigene dataset included sequences of ITS, LSU, TEF1-α, and RPB2 regions from the isolates analysed in this study and reference strains obtained from GenBank. Detailed information on the strains and corresponding GenBank accession numbers is provided in Table 2.

Table 2 – Sequences used in this study for phylogeny and related GenBank accession numbers.

Taxon name	Strain code	GenBank accession code			
		ITS	**LSU**	**TEF1**	**RPB2**
Verruciconidia persicina	CBS:102349	OQ429912	HQ232086	OQ471242	OQ454309
	CBS 295.70A	MH859634	HQ232075	OQ471243	OQ454310
	CBS:169.65	MH858531	HQ232072	OQ471244	OQ454311
	CBS:295.70B	MH859635	MH871389	OQ471246	OQ454313
	CBS:127298	OQ429917	OQ430168	OQ471247	OQ454314
	CBS:128826	OQ429915	OQ430166	OQ471245	OQ454312
	CBS:101712	OQ429918	OQ430169	OQ471248	OQ454315
	CBS:116385	OQ429919	OQ430170	OQ471249	OQ454316
	CBS:120889	OQ429920	OQ430171	OQ4712503	OQ454317
	CBS:310.59^{T}	OQ429921	OQ430172	OQ471251	OQ454318
	CBS:113716	OQ429922	OQ430173	OQ471252	OQ454319
	CBS:218.96	OQ429924	OQ430175	OQ471254	OQ454321
	CBS:439.66	OQ429923	HQ232083	OQ471253	OQ454320
	MCC:9995	**PV546861**	**PV546869**	**PV568052**	**PV554179**
Verruciconidia verruculosa	CBS:990.69	MH859502	MH871282	OQ471261	OQ454328
	CBS:299.81B	OQ429932	OQ430183	OQ471262	OQ454329
	CBS:989.69^{T}	OQ429933	OQ430184	OQ471263	OQ454330
Verruciconidia unguis	CBS:424.93^{T}	OQ429929	OQ430180	OQ471259	OQ454326
	CBS:378.70E	OQ429930	OQ430181	OQ471260	OQ454327
Verruciconidia erythroxyli	CBS:728.87^{T}	OQ429910	OQ430161	OQ471240.	OQ454307
	CBS:378.70D	OQ429909	OQ430160	OQ471239	OQ454306
Verruciconidia infuscata	CBS 100888^{T}	NR189499	OQ430162	OQ471241	OQ454308
Verruciconidia siccicapita	CBS378.70A^{T}	NR189501	OQ430179	OQ471258	OQ454325
Verruciconidia quercina	CBS:469.67^{T}	OQ429925	OQ430176	OQ471255	OQ454322
	CBS:183.78	OQ429926	OQ430177	OQ471256	OQ454323
	CBS:355.77	OQ429927	OQ430178	OQ471257	OQ454324
Verruciconidia. oligospora	CGMCC 3.28727^{T}	PV020696	PV020714	PV050425	PV023188
Verruciconidia. guizhouensis	SQT04	OP703289	OP740981	OP757290	OP730525
Verruciconidia saulensis	CLLG 21159	ON206617	ON206592	-	-
Saccharata proteae	CBS 115206	KF531812	GU301869	GU349030	GU357753

Results

Morphological description

Verruciconidia persicina (Nicot) L.W. Hou, L. Cai & Crous, Stud. Mycol. 105: 91 (2023) MycoBank MB 845840; *Holotype*: CBS 310.59 Basionym: *Paecilomyces persicinus* Nicot, Bull. Soc. Mycol. France74: 222. 1958. Synonym: *Acremonium persicinum* (Nicot) W. Gams, *Cephalosporium-artige Schimmelpilze* (Stuttgart): 75. 1971.

Teleomorph - Undetermined. *Chlamydospores* not observed. **Anamorph -** mycelium branched, septate, rough, hyaline, thin-walled, up to 4.5 µm wide (Fig. 1). Sporulation abundant. Conidiophores are solitary or in aggregates, straight to flexuous, often bent at lower parts, arising from superficial/submerged hyphae, unbranched or branched, often with sterile outgrowths (1–3 septa), thick-walled. Phialides terminal/lateral, cylindrical/subulate, bent, hyaline, thick-walled, smooth. Conidia aseptate, ellipsoid to ovoid, sometimes apiculate at the base, hyaline, smooth-walled, 4–6 × 2.6–3 µm (Fig. 1). **Culture characteristics:** After 14 d at 25°C: Colonies on PDA reaching 41–44 mm diam., flat, moderate aerial mycelium, felty to dusty, dirty white/rosy buff, entire margin, creamy white reverse (Fig. 1A). On MEA reaching 42–45 mm diam., flat, radially folded, floccose, salmon centre with rosy buff periphery, crenate margin, and creamy white reverse (Fig. 1B). On OA reaching 40–43 mm diam., flat, sparse aerial mycelium, dusty, white turning pale brown, entire margin, colourless reverse (Fig. 1C). On SNA reaching 30–33 mm diam., flat, sparse aerial mycelium, dusty, colourless, entire margin, and colourless reverse (Fig. 1D). No odour detected on any medium.

Fig. 1 – *Verruciconidia persicina* (ex-type culture MCC 9995). A–D. Colonies on PDA, MEA, OA, and SNA respectively, after 14 d at 25 °C. E. Conidiophores and conidial chains. F–H. Conidiophores. I. Conidia. Scale bars = 10 µm.

Material examined: India, Maharashtra, Pune District, 19°22.338' N, 73°88.218' E, from the gut of *Platynotus excavatus* beetle, August 18, 2023. Ganesh Gore (holotype CBS 310.59); ex-type living culture, MCC 9995 (National Centre for Microbial Resource India., new record for India). **GenBank numbers**: ITS = PV546861; LSU = PV546869; TEF1 = PV568052; RPB2 = PV554179. **Diagnosis**: The present isolate differs slightly from the type *V. persicina* (CBS 310.59) in having narrower mycelia (up to 3.5 µm vs. 4.5 µm).

Phylogenetic analysis

Phylogenetic analysis places MCC 9995 within the *V. persicina* clade with 91% bootstrap support (Fig. 2). Based on a megablast search of the NCBI GenBank nucleotide database, the closest hits using the ITS sequence of *V. persicina* (MCC 9995) showed the highest similarity to *V. persicina* [strain CBS:310, GenBank: OQ429921; Identities = 511/513(99%), Zero gap (0%)]. Closest hits using the LSU sequence of *V. persicina* (MCC 9995) showed the highest similarity to *V. persicina* [strain CBS:310, GenBank: MH869409; Identities = 748/751(99%), Zero gap (0%)]. Closest hits using the TEF1 sequence of *V. persicina* (MCC 9995) showed the highest similarity to *V. persicina* [strain CBS:310, GenBank: OQ471251; Identities = 782/791(99%), Zero gap (0%)]. Closest hits using the RPB2 sequence of *V. persicina* (MCC 9995) showed the highest similarity to *V. persicina* [strain CBS:310, GenBank: OQ471251; Identities = 782/791(99%), Zero gap (0%)].

Discussion

The genus *Verruciconidia* comprises filamentous fungi that exhibit broad ecological versatility, having been isolated from diverse substrates and environments. Species such as *V. verruculosa*, *V. quercina*, and *V. siccicapita* have been reported from agricultural soils, while others like *V. infuscata,* have been isolated from air, and *V. erythroxyli* from plant leaves (e.g., *Erythroxylum areolatum* and *Urena*). Notably, *V. unguis* has been reported from human nails, highlighting the genus's potential clinical relevance (Hou et al., 2023). *Verruciconidia persicina*, originally described as *P. persicinus* and later reclassified, has been isolated from a variety of less common substrates, including tent canvas, coastal sand associated with *Ammophila arenaria*, dead hardwood branches, and leaves of *Festuca ovina* and *Vitis vinifera*. The species demonstrates a wide geographic distribution, with records spanning the Netherlands, Thailand, Japan, Cuba, Democratic Republic of the Congo, Austria, France, Iran, Italy, Papua New Guinea, and the USA (Hou et al., 2023).

In the present study, *V. persicina* was isolated from the gut of the Tenebrionid beetle *Platynotus excavatus*, collected from an organic waste site in Junnar, India. This represents not only the first record of *V. persicina* from India but also the first report of any member of *Verruciconidia* associated with an insect gut. The isolate (MCC 9995) was identified based on morphology and concatenated phylogenetic analysis of ITS, LSU, TEF1-α, and RPB2 loci resolved *Verrucoconidia persicina* (MCC 9995) within the strongly supported *V. persicina* clade (91% bootstrap), confirming its conspecific placement. All analysed *Verrucoconidia* species formed well-supported, monophyletic lineages, underscoring clear species boundaries and taxonomic stability within the genus. The multilocus approach provided higher resolution than single-locus markers, emphasising its value in delineating closely related taxa in fungal systematics. Despite slight morphological differences, specifically, narrower mycelia, the Indian isolate grouped robustly with *V. persicina* (CBS 310.59) in the phylogenetic tree, confirming conspecificity.

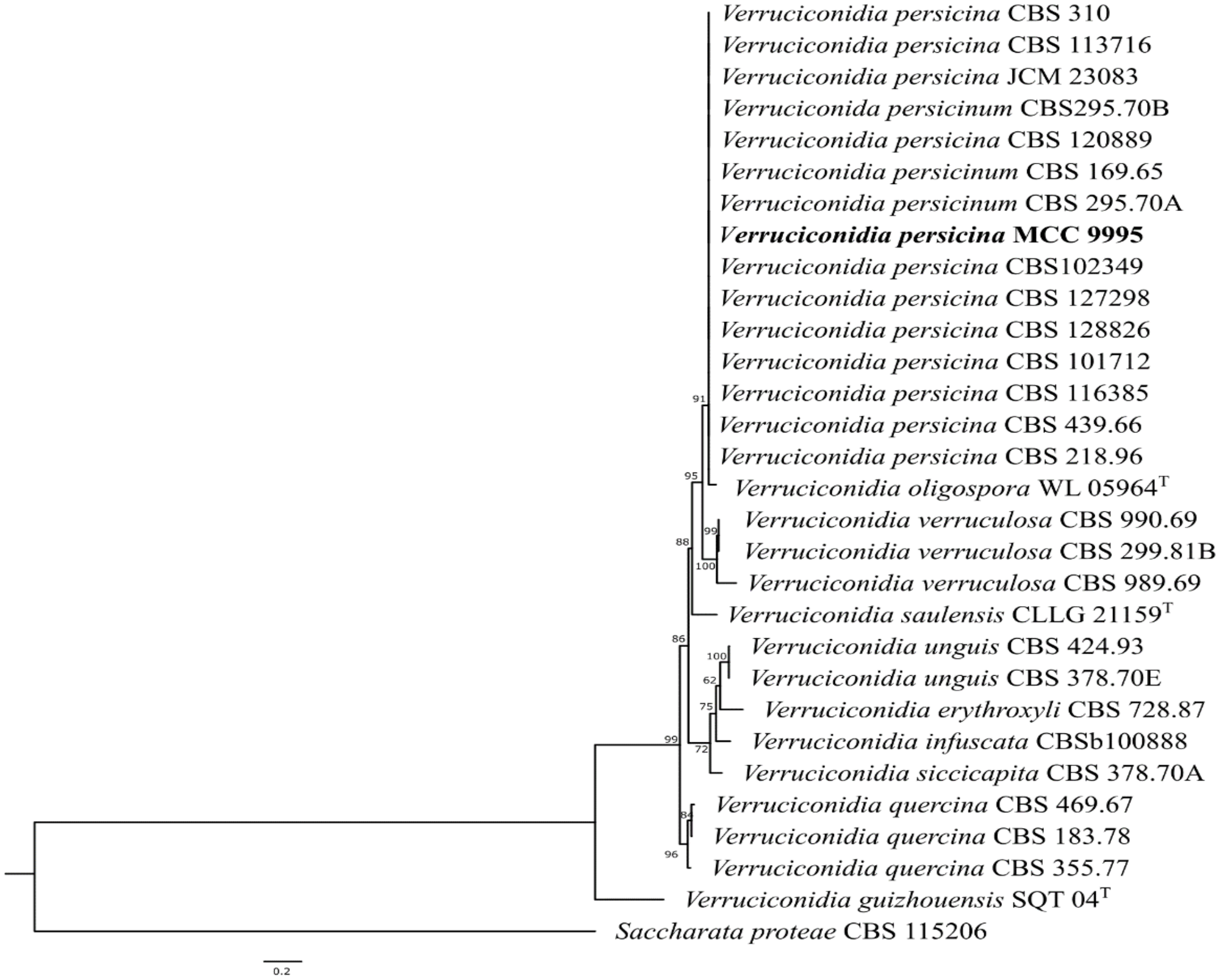

Fig. 2. – Maximum likelihood (ML) phylogenetic tree based on the concatenated dataset of ITS, LSU, TEF1-α, and RPB2 sequences, showing the placement of *Verrucoconidia persicina* (MCC 9995) among related species of the genus *Verrucoconidia*. The alignment consisted of 3,041 positions. The tree was inferred using IQ-TREE v1.6.12 under the TNe+I+G4 model selected according to the Bayesian Information Criterion (BIC). Bootstrap values (>50%) from 1,000 replicates are shown at the nodes. The tree is rooted with *Saccharata proteae* CBS 115206. The scale bar represents the number of substitutions per site.

The discovery of *V. persicina* in an insect gut environment is particularly significant. While many entomopathogenic and symbiotic fungi are well-documented among beetles, the genus *Verruciconidia* has never been previously reported in such a niche. This suggests the possibility that some *Verruciconidia* species may have broader ecological roles than previously understood, including potential involvement in the digestive processes or microbial community structure of beetles. Alternatively, the fungus may act as a transient saprobe or opportunistic coloniser of nutrient-rich gut contents. Overall, this finding expands the known ecological range of *V. persicina* and emphasises the importance of exploring under-investigated niches such as insect guts for fungal diversity. Further studies involving more extensive sampling, functional analyses, and co-occurrence data could help clarify the ecological roles and adaptations of *Verruciconidia* species in both terrestrial and host-associated environments.

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