MYCOBIOLOGY
2023, VOL. 51, NO. 3, 115–121
https://doi.org/10.1080/12298093.2023.2203973

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Neocucurbitaria chlamydospora sp. nov.: A Novel Species of the Family Cucurbitariaceae Isolated from a Stink Bug in Korea

Soo-Min Hong[a]*, Kallol Das[a]*, Seong-Keun Lim[a], Sang Jae Suh[a], Seung-Yeol Lee[a,b] and Hee-Young Jung[a,b]

[a]College of Agriculture and Life Sciences, Kyungpook National University, Daegu, Korea; [b]Institute of Plant Medicine, Kyungpook National University, Daegu, Korea

ABSTRACT

The fungal strain KNUF-22-18B, belonging to Cucurbitariaceae, was discovered from a stink bug (*Hygia lativentris*) during the investigation of insect microbiota in Chungnam Province, South Korea. The colonies of the strain KNUF-22-18B were wooly floccose, white to brown in the center on oatmeal agar (OA), and the colonies were buff, margin even, and colorless, reverse white to yellowish toward the center on malt extract agar (MEA). The strain KNUF-22-18B produced pycnidia after 60 days of culturing on potato dextrose agar, but pycnidia were not observed on OA. On the contrary, *N. keratinophila* CBS 121759^T abundantly formed superficial pycnidia on OA and MEA after a few days. The strain KNUF-22-18B produced chlamydospores subglobose to globose, mainly in the chain, with a small diameter of 4.4–8.8 µm. At the same time, *N. keratinophila* CBS 121759^T displayed a globose terminal with a diameter of 8–10 µm. A multilocus phylogeny using the internal transcribed spacer regions, 28S rDNA large subunit, β-tubulin, and RNA polymerase II large subunit genes further validated the uniqueness of the strain. The detailed description and illustration of the proposed species as *Neocucurbitaria chlamydospora* sp. nov. from Korea was strongly supported by molecular phylogeny.

ARTICLE HISTORY
Received 30 January 2023
Revised 23 March 2023
Accepted 26 March 2023

KEYWORDS
Stink bug; *Hygia lativentris*; morphology; *Neocucurbitaria chlamydospora*

1. Introduction

Cucurbitariaceae was introduced and typified by *Cucurbitaria berberidis*, and recently 17 genera such as *Allocucurbitaria*, *Astragalicola*, *Cucitella*, *Cucurbitaria*, *Curreya*, *Gemmamyces*, *Leucothyridium*, *Megaloseptoria*, *Neocucurbitaria*, *Paracucurbitaria*, *Parafenestella*, *Protofenestella*, *Pyrenochaetopsis*, *Rhytidiella*, *Seltsamia*, *Syncarpella*, and *Synfenestella* were listed as members of this family (http://www.catalogueoflife.org – accessed on 20 March 2023) [1]. The family is distinguished by aggregated, ostiolate ascomata supported by a basal stromatic structure, fissitunicate and cylindrical asci, pigmented, phragmosporous or muriform ascospores, and a necrotrophic or saprobic lifestyle on woody plants [2]. The phylogenetic analyses have demonstrated that Cucurbitariaceae is a diverse group, and more recent research has eliminated specific genera from this family, referred other genera, and found point to the fact that Cucurbitariaceae is not a monophyletic group [2,3]. However, the genus *Neocucurbitaria* was introduced to accommodate cucurbitaria-like species characterized by solitary fruiting bodies containing periphyses and muriform ascospores belongs to the family Cucurbitariaceae [4]. The new taxa *N. aquatica* and *N. irregularis* were described and several members also united *Pyrenochaeta cava*, *P. hakeae*, and *P. keratinophila* into *Neocucurbitaria*, defined and epitypified *N. quercina* under Cucurbitariaceae [5]. They also described the new monotypic genus *Allocucurbitaria*, and for *Plenodomus corni*, earlier also known as *Pyrenochaeta corni* and for the new species *P. italica*, based on a strain previously identified as *P. corni*, they described the new genus *Paracucurbitaria* [5,6].

Moreover, microbial communities connected to insects are extremely diverse, and interactions between and within microbiome members can have various implications for the fitness and behavior of insects [7,8]. It has been revealed by research into insect microbiota that multiple factors – including host genetics and the environment – influence the makeup of the microbial community [9]. In some cases, microbes are another factor that contributes to the destructive nature of invasive insects [10]. Historically, significant agricultural pests in the US and stink bugs are most prevalent

CONTACT Hee-Young Jung heeyoung@knu.ac.kr
*These authors contributed equally to this work.

in the southeastern region. These consume a variety of commercial crops, such as fruits, nuts, vegetables, and cereals, and are known as polyphagous [11–13]. The feeding habits of stink bugs result in the loss of turgidity, slowed growth, delayed maturation, dimpling of fruits, abortion of seeds and fruiting bodies, and small, shriveled seeds [11,12]. Thus, there is a possibility of transmission of microfungi through stink bugs and insects as well. Though, there is not enough information about the relationship between stink bugs and Cucurbitariaceae fungi so far. Thus, the main objective of this study was to explore the native microfungi from insects that could lead to the expansion of niches and adaptations for demanding environmental conditions. This information needs to be gathered to understand the cultural, morphological, and molecular phylogenetic relationships among these fungi in Korea.

2. Materials and methods

2.1. Sample collection and fungal strain isolation

The fungal strain used in this investigation was associated with the stink bug (*Hygia lativentris*) collected from Chungnam Province (36°52′44.8″N, 126°22′27.1″E), South Korea. The fungi were isolated following the previously described method with modification using potato dextrose agar (PDA) (Difco, Detroit, MI) plates with incubation at 25 °C for 2–3 days [14]. Single colonies were transferred to PDA plates and incubated for 4–5 days at 25 °C. The strain KNUF-22-18B was selected for additional molecular analyses based on various culture parameters. The fungal strain was kept at −80 °C in 20% glycerol for additional research.

2.2. Cultural and morphological characterization

The fungus KNUF-22-18B was grown in various media, including PDA, 2% malt extract agar (MEA; Difco, Detroit, MI), and oatmeal agar (OA; Difco, Detroit, MI), and incubated for 14 days at 25 °C [15]. The ability of the strain KNUF-22-18B to grow at cardinal temperatures was determined on PDA after seven days in darkness ranging from 5 °C to 35 °C at 5 °C intervals [5]. The fungal growth rate was evaluated, and size, color, and shape of the colonies were noted. A light microscope (BX-50; Olympus, Tokyo, Japan) was used to examine the mycological characteristics.

2.3. Genomic DNA extraction, PCR amplification, and sequencing

Total genomic DNA from strain KNUF-22-18B was extracted from fungal mycelia grown on the PDA plate using the HiGene Genomic DNA Prep Kit (BIOFACT, Daejeon, South Korea) following the manufacturer's protocol. Four loci were amplified, including the internal transcribed spacer (ITS), partial large subunit nrRNA (28S nrDNA; LSU), partial beta-tubulin (*TUB2*), and partial DNA-directed RNA polymerase II second largest subunit (*RPB2*) genes using the primer pairs ITS1F/ITS4 [16,17], LROR/LR5 [18,19], Btub2Fd/Btub4Rd [20], and RPB2-5F2/RPB2-7cR [21,22]. The quality of the PCR products was checked by running them through an ethidium bromide-stained 1.2% agarose gel electrophoresis. ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA) was used to purify the product, and it was then sent to SolGent for sequencing (Daejeon, South Korea).

2.4. Molecular phylogenetic analyses

Sequences obtained from the National Center for Biotechnology Information were used to construct phylogenetic trees (Table 1). Kimura's two-parameter model was used to eliminate ambiguous positions from alignments and to generate evolutionary distance matrices for the neighbor-joining (NJ) procedure [23]. The alignments were manually performed for each gene, and then sequences were merged by using MEGA7.0 software program. This analysis also identified nodes with filled circles in the NJ phylogenetic tree [24]. Open circles showed corresponding nodes from maximum likelihood or maximum parsimony algorithms [25,26]. Maximum likelihood and maximum parsimony techniques were used to infer the phylogenetic trees in MEGA7.0, and bootstrap values based on 1000 replicates were used to confirm the reliability of the tree [27].

3. Results

3.1. Taxonomical analysis of Neocucurbitaria chlamydospora *KNUF-22-18B*

The KNUF-22-18B strain had distinct morphological characteristics from those of allied species of *Neocucurbitaria*. Therefore, it was described as a new species.

Neocucurbitaria chlamydospora H.Y. Jung and S.Y. Lee, sp. nov. (Figure 1)

MycoBank: MB847053

Etymology: The specific epithet refers to the formation of chlamydospores.

Typus: Chungnam Province (36°52′44.8″N, 126°22′27.1″E), isolated from Stink bug (*H. lativentris*). The stock culture (NIBRFGC000509948) was deposited in the National Institute of Biological Resources as a metabolically inactive culture.

Table 1. GenBank accession numbers used for the phylogenetic analyses in this study.

Species	Strain numbers	GenBank accession numbers			
		ITS	LSU	*RPB2*	*TUB2*
Neocucurbitaria chlamydospora	KNUF-22-18B^T	**OQ060587**	**OQ060588**	**OQ148364**	**OQ148365**
N. acanthocladae	C225 = CBS 142398^T	MF795766	MF795766	MF795808	MF795894
N. acerina	C255 = CBS 142403	MF795768	MF795768	MF795810	MF795896
N. aetnensis	C261 = CBS 142404^T	MF795769	MF795769	MF795811	MF795897
N. aquatica	CBS 297.74	LT623221	EU754177	LT623278	LT623238
N. cava	CBS 257.68	JF740260	EU754199	LT717681	KT389844
N. cinereae	KU9 = CBS 142406^T	MF795771	MF795771	MF795813	MF795899
N. cisticola	C244 = CBS 142402^T	MF795772	MF795772	MF795814	MF795900
N. hakeae	CBS 142395^T	KY173436	KY173526	KY173593	KY173613
N. irregularis	CBS 142791	LT592916	LN907372	LT593054	LT592985
N. juglandicola	BW6 = CBS 142390^T	NR156358	MF795773	MF795815	MF795901
N. keratinophila	CBS 121759^T	NR137017	NG070610	LT623275	LT623236
N. populi	C28 = CBS 142393^T	MF795774	MF795774	MF795816	MF795902
N. quercina	CBS 115095^T	LT623220	GQ387619	LT623277	LT623237
N. rhamni	C133	MF795777	MF795777	MF795819	MF795904
N. rhamnicola	C185 = CBS 142396^T	MF795780	MF795780	MF795822	MF795906
N. rhamnioides	C118 = CBS 142395^T	MF795782	MF795782	MF795824	MF795908
N. ribicola	C55 = CBS 142394^T	NR156362	MF795785	MF795827	MF795911
N. salicis-albae	CBS 144611^T	NR163365	MK442535	MK442669	MK442738
N. unguis-hominis	CBS 111112	LT623222	GQ387623	LT623279	LT623239
N. vachelliae	C192 = CBS 142397^T	MF795787	MF795787	MF795829	MF795913
Pyrenochaetopsis confluens	CBS 142459^T	LT592950	LN907446	LT593089	LT593019

ITS: internal transcribed spacer regions of the rDNA; LSU: partial large subunit of 28S rDNA; *RPB2*: RNA polymerase II second largest subunit; *TUB2*: β-tubulin.
The newly generated sequences are indicated in bold.

Habitat and known distribution: Members of this genus were associated with the different hosts, substrates, and plant species in many countries such as wheat-field soil (Germany), *Hakea* sp. (Australia), human subcutaneous tissue (USA), human corneal scrapings (Spain), *Quercus robur* (Italy), *Agapornis* sp. (The Netherlands), *Frangula alnus* (Austria), *Rhamnus lycioides* (Spain), *Vachellia gummifera* (Morocco), and air sample (Wales), whereas the proposed novel species was isolated from the stink bug (*H. lativentris*) in Chungnam Province, South Korea. The ecology of the collected region was consisted of rocks mixing with soil, wild grasses, and nearby mountain with native plantation.

Cultural characteristics: The colonies of the strain KNUF-22-18B were wooly floccose, olive brown, margin even, whitish near the edge, and colorless margin, with a diameter of 41.4–43.7 mm on PDA after 14 days at 25 °C (Figure 1(A)). Colonies on OA attained 47.1–48.3 mm at 14 days at 25 °C, white, wooly floccose, mycelium white to brown in the center, and reverse brown in the center (Figure 1(B)). On MEA, the margin of the colonies was even buff (yellowish), margin colorless, reverse white to yellowish toward the center, with a diameter of 36.4–37.2 mm after 14 d at 25 °C (Figure 1(C)); Optimal temperature of growth and sporulation 25 °C; minimum temperature of growth 5 °C; maximum temperature of growth 35 °C.

Morphological characteristics: Conidiomata pycnidial, olivaceous brown to almost black, globose or flask shaped, primarily solitary or confluent, 100–400 µm in diameter on PDA, dark-brown intercellular material observed in 60-day-old cultures on PDA. The outer surface of the pycnidial wall was brown to black and roughened. Pycnidia were not observed on the OA media (Figure 1(D–F)). Chlamydospores were hyaline, mostly in chains, short-branched, smooth, thick-walled, globose to subglobose, and with a diameter of 4.4–8.8 µm ($n = 25$, $\bar{x} = 6.65$ µm) and observed mainly after 60-day-old PDA cultures (Figure 1(G,H)). Chlamydospores were rarely observed on OA and MEA media. Hyphae hyaline to brown, smooth- and thin-walled, septate, 2.1–3.3 µm wide. Conidiophores were hyaline, smooth-walled, straight to slightly curved, and branched. Conidiogenous cells integrated into the conidiophore were hyaline, smooth-walled, and had a more or less cylindrical collarette (Figure 1(I–K)). Phialoconidia produced directly from aerial mycelium, globose or subglobose, pale brown and slightly thick-walled, guttulate, 3.2–5.3 × 3.1–4.6 µm ($n = 50$, $\bar{x} = 4.3 \times 3.8$ µm) (Figure 1(L)). Conidia hyaline, aseptate, oval-to-ellipsoid, 3.1–4.2 × 1.7–3.0 µm ($n = 30$, $\bar{x} = 3.7 \times 2.3$ µm), straight or slightly curved, smooth, rounded at both ends (Figure 1(M)).

Note: The colonies of the strain KNUF-22-18B were wooly floccose, white to brown in the center, and reverse brown in the center on OA, whereas those of the closest strain *N. keratinophila* CBS 121759^T were flat, immersed mycelium pale gray olivaceous to greenish olivaceous, aerial mycelium concolorous, diffuse wooly floccose; reverse olivaceous gray to olivaceous black in the center. Another closest strain, *N. irregularis* CBS 142791^T, showed flattened, olive-brown colonies, reverse brownish gray (Table 2). The strain KNUF-22-18B produced pycnidia in 60-day-old cultures on PDA,

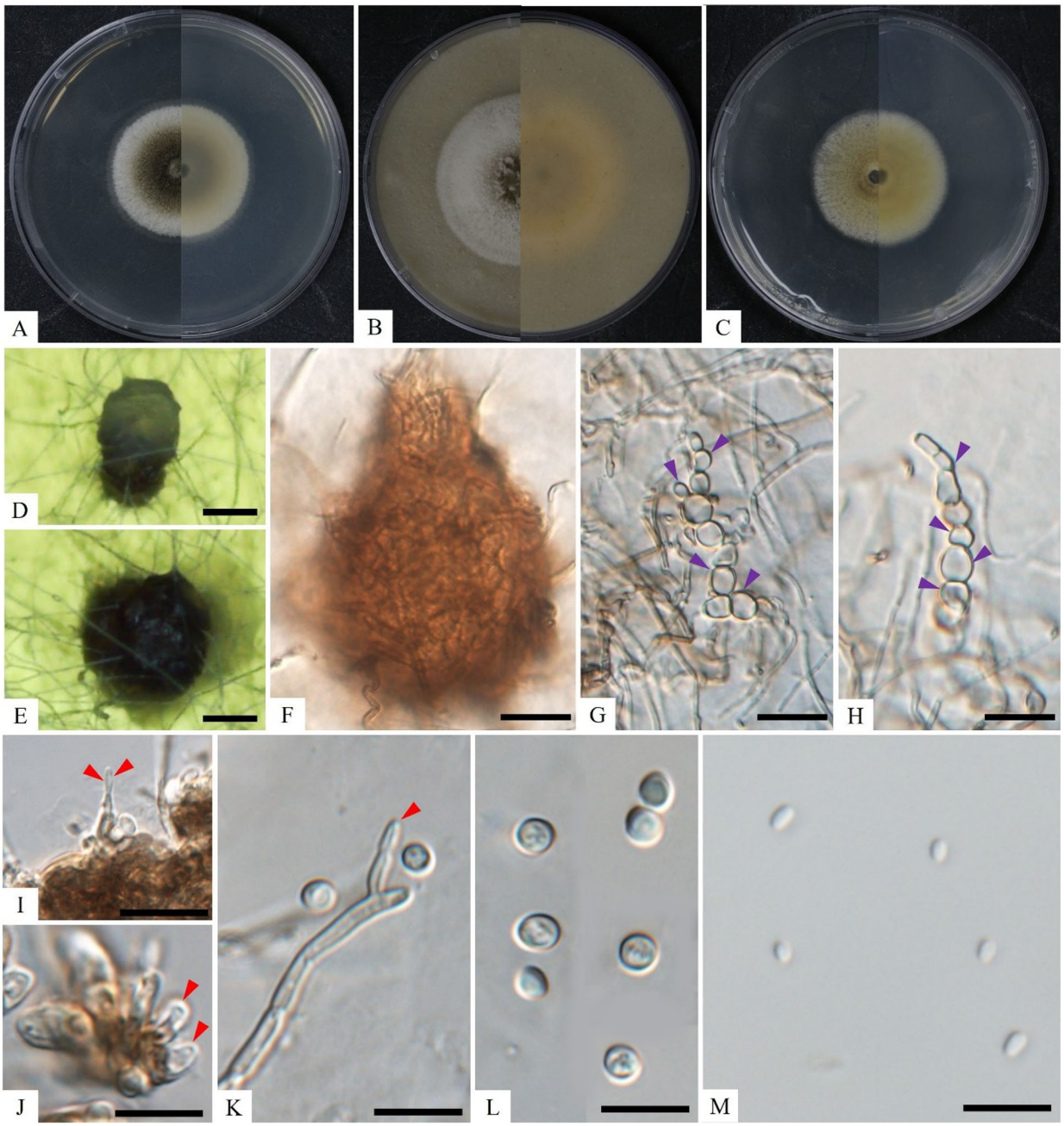

Figure 1. Cultural and morphological characteristics of KNUF-22-18B^{T}. Colony on potato dextrose (A); oatmeal (B); and malt extract agar (C) at 25 °C in 14 days, accordingly. Pycnidia on PDA (D, E); pycnidium (F); chlamydospores-like structures (G, H); conidiogenous cells and conidiophores (I–K); phialoconidia produced from aerial mycelium (L); and conidia from pycnidia (M). Arrows indicate chlamydospores (purple) and conidiogenous cells (red). Scale bars: D, E = 100 µm; F–H = 20 µm; I–M = 10 µm.

and pycnidia were not observed on OA, whereas *N. keratinophila* CBS 121759^{T} abundantly formed superficial pycnidia on OA and MEA after a few days. Chlamydospores were mainly in chains, short-branched, smooth, thick-walled, globose to subglobose, and with a small diameter of 4.4–8.8 µm and observed primarily in old PDA cultures. In the case of *N. keratinophila* CBS 121759^{T}, chlamydospores were terminal, smooth, and thick-walled, globose, 8–10 µm and mainly observed on old OA cultures but absent in *N. irregularis* and *N. acerina*. However, the conidia of the strain KNUF-22-18B (3.1–4.2 × 1.7–3.0 µm) were almost similar to those of *N. keratinophila* ((2)2.5–3(4) × 1–2 µm) and *N. irregularis* (2.5–4 × 1.5–2.0 µm), and the asexual morph was not determined for *N. acerina* (Table 2). Although the closest strain, *N. keratinophila*, produced remarkably similar conidia, the characteristics of the colonies, formation of pycnidia, and chlamydospores were different. Therefore, the morphology of strain KNUF-22-18B is distinct from the previously identified species of *Neocucurbitaria*.

3.2. Molecular phylogeny of strain KNUF-22-18B

The sequences in ITS regions (566 bp) were 97.9–99.8% similar to the reported species *N. keratinophila* CBS 123295, *N. irregularis* UTHSC DI16-229^{T},

Table 2. Morphological characteristics of *Neocucurbitaria chlamydospora* sp. nov. and comparison with the closest species of *Neocucurbitaria*.

Strain name	Cultural characteristics	Pycnidia	Chlamydospores	Conidia
N. chlamydospora KNUF-22-18B^T (in this study)	Colonies on OA, wooly floccose, white to brown in the center; reverse brown in the center. On MEA, margin even, buff, margin colorless; reverse light to yellowish.	Olivaceous brown to almost black, globose or flask shaped, 100–400 μm on PDA.	Mostly in chains, globose to subglobose, 4.4–8.8 μm, and observed mainly on old PDA.	Oval to ellipsoid, straight or slightly curved, 3.1–4.2 × 1.7–3.0 μm. Phialoconidia globose or subglobose, guttulate, 3.2–5.3 × 3.1–4.6 μm.
N. keratinophila CBS 121759^T [28]	On OA, flat, immersed, pale gray olivaceous to greenish olivaceous, diffuse wooly floccose; reverse olivaceous gray to olivaceous black in the center. On MEA, buff, immersed, olivaceous black or brown vinaceous; reverse dark hazel.	Olivaceous brown to almost black, globose or flask shaped, 100–400 μm on OA.	Terminal, globose, 8–10 μm, and observed mainly on old OA.	Ellipsoid, straight or slightly curved, few guttules, (2)2.5–3(4) × 1.0–2.0 μm. Phialoconidia globose or subglobose, 3.0–4.0 μm.
N. irregularis CBS 142791^T [5]	On OA, flattened, olive brown; reverse brownish gray. On MEA, flattened, pale yellow; reverse pale yellow to grayish yellow.	Brown, superficial, glabrous, subglobose to ovoid, 75–130 × 65–120 μm on OA.	N/A	Ellipsoidal to cylindrical, guttulate, 2.5–4.0 × 1.5–2.0 μm.
N. acerina MFLUCC 16–1450^T [4]	On PDA, circular, smooth margin white at first, dark lava after 4 weeks flat on the surface; reverse grayish black. On OA and MEA: N/A	Asexual morph: undetermined	Asexual morph: undetermined	Asexual morph: undetermined
N. aquatica CBS 297.74^T [5]	On OA, flattened, olive; reverse olive to dark gray. On MEA, flattened, yellowish gray, reverse gray.	N/A	N/A	N/A
N. unguis-hominis CBS 111112^T [29]	On OA, floccose, brown vinaceous to fawn, margin smooth; reverse variable or grayish sepia. On MEA: N/A	Brown to black, subglobose to globose, 100–200 μm on OA.	N/A	Short cylindrical, slightly curved, guttulate, 2–3 (–3.5) × 1(–1.5) μm.

N/A: not available in previous references.

N. unguis-hominis GLS20, *N. quercina* CBS 297.74, and *N. acerina* C26a. The strain KNUF-22-18B had a high similarity of 99.5–99.6% based on the LSU (793 bp) gene sequences with *N. irregularis* UTHSC DI16-229^T, *N. salicis-albae* CBS:144611^T, *N. keratinophila* CBS 121759^T, and *N. unguis-hominis* CNM-CM:8743. The *RPB2* (859 bp) gene displayed 95.0–95.9% similarity with *N. irregularis* UTHSC DI16-229^T, *N. unguis-hominis* CBS 111112, *N. acerina* C255, and *N. keratinophila* CNM-CM 8674. The *TUB2* (337 bp) gene had 93.0–95.9% similarity with *N. irregularis* UTHSC DI16-229^T, *N. acerina* C25, *N. quercina* CBS 297.74, and *N. keratinophila* CNM-CM 6401. According to the NJ phylogenetic tree constructed using the ITS regions, LSU, *RPB2*, and *TUB2* gene sequences and filled nodes in the maximum likelihood and maximum parsimony trees, the strain KNUF-22-18B was distinctly clustered with *N. keratinophila* CBS 121759^T. Nodes produced with maximum likelihood or maximum parsimony are displayed as open circles (Figure 2). Phylogenetic analyses using a combination of sequences with maximum parsimony (tree length = 1243, consistency index = 0.50, retention index = 0.62, and composite index = 0.37) were used to determine the strain's exact taxonomic position. The tree length (tree length = 1243) of multi-locus genes showed the lower bootstrap values (64) in NJ phylogenetic tree that suggested the sequences are much differed and lower bootstrap values displayed even in maximum likelihood or maximum parsimony from the closest species of *N. keratinophila* CBS121759^T. Based on a megablast search of NCBI's GenBank nucleotide database, the ITS sequence was identical to *N. keratinophila* CNM-CM 5882 (Identities = 513/527 (97%)). Closest hits using the LSU sequence are *N. keratinophila* CBS 121759 (Identities = 789/793 (99%)). The *RPB2* sequence was similar to *N. keratinophila* CNM-CM 8674 (Identities = 823/858 (96%)). The partial *TUB2* sequence had highest similarity to *N. keratinophila* CBS 121759 (Identities = 322/337 (96%)). The final alignment contained a total of 1243 characters used for the phylogenetic analyses, including alignment gaps. KNUF-22-18B is thus a far-off, unusual, and phylogenetically separate species. Phylogenetic analyses and morphological observations revealed that strain KNUF-22-18B was different from the *Neocucurbitaria* species that had previously been identified (Figure 2). As a result, it needs to be classified as a novel species in the genus and given the name *Neocucurbitaria chlamydospora* sp. nov.

4. Discussion

In previous studies, *Neocucurbitaria unguis-hominis* (syn. *Pyrenochaeta unguis-hominis*) was reported for the first time during a survey of thickened toenails [29]. The genus *Neocucurbitaria* was recently introduced to accommodate *N. unguis-hominis* (the type species of the genus), *N. quercina*, and *N. acerina* [4]. *N. keratinophila* (formerly *Pyrenochaeta keratinophila*) was reported as a case of keratitis invading the human cornea from Spain [28]. Also, *N. acerina* was found in dead aerial twigs of *Acer*

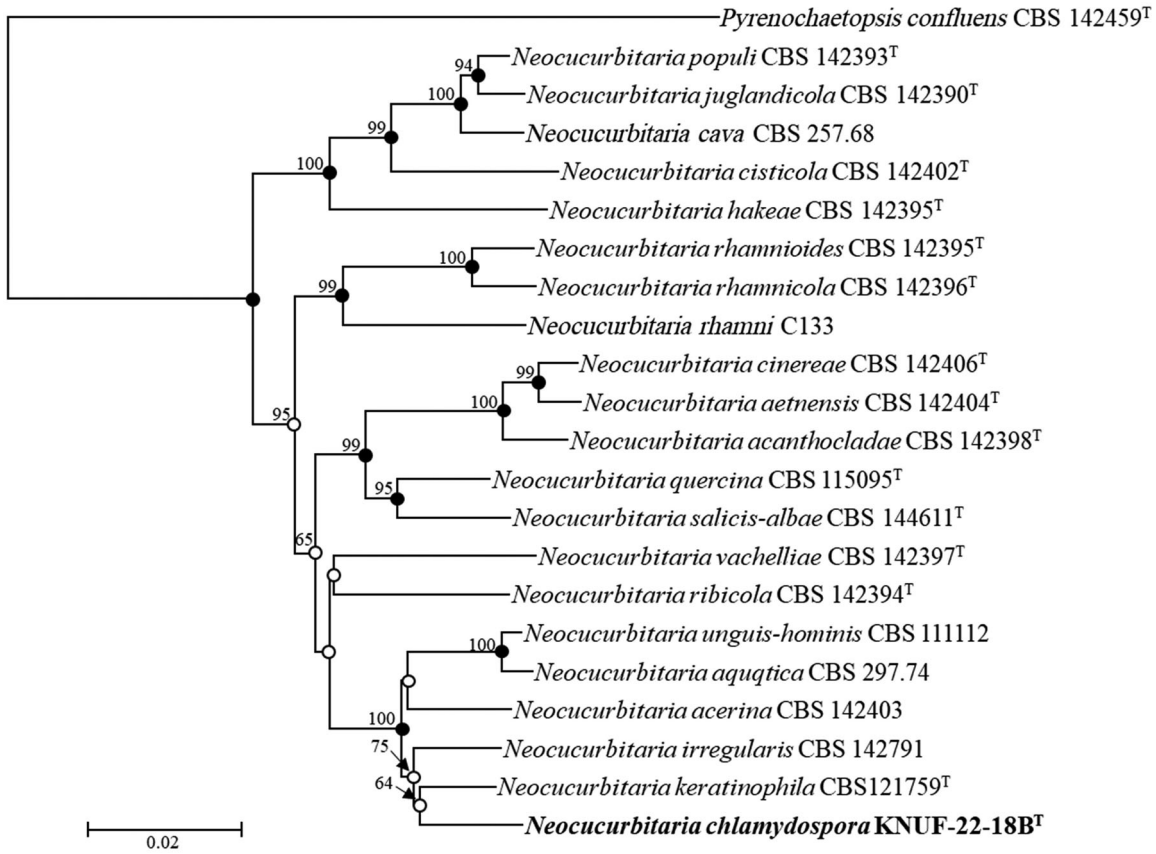

Figure 2. Neighbor-joining phylogenetic tree of KNUF-22-18B^T based on ITS, LSU, *RPB2*, and *TUB2* sequences showing the phylogenetic position among the related strains in the genus *Neocucurbitaria*. *Pyrenochaetopsis confluens* CBS 142459^T was used as an outgroup. The strain isolated in this study is indicated in bold, and the numbers above the branches represent the bootstrap values (>60%) obtained for 1000 replicates. Bar = 0.02 substitutions per nucleotide position.

campestre in Italy [4] and *N. cava* from alpine conifers in Korea [30], whereas *N. aquadulcis* and *N. variabilis* were reported from submerged plant debris in freshwaters from Spain [31]. Moreover, several *Penicillium*, *Aspergillus*, and *Cladosporium* species were isolated from insects in Korea, namely *Lixus imperessiventris*, *Muljarus japonicus*, *Meloe proscarabaeus*, and *Tribolium castaneum* [32,33]. Recently, *Oidiodendron clavatum* and *Xenoacremonium minutisporum* were found in beetles called *Cicindela transbaicalica* and *Dorcus titanus castanicolor*, respectively, in Korea [34,35]. To date, 24 species of *Neocucurbitaria* have been identified from different countries with diversified hosts (http://www.speciesfungorum.org/Names/Names.asp – accessed on 7 March 2023). So far, none of the species has been recorded from the stink bug (*H. lativentris*). Although members of the genus *Neocucurbitaria* occur in many habitats worldwide [36], the identified strain KNUF-22-18B was isolated from the stink bug (*H. lativentris*) in Korea.

In conclusion, considering all aspects of this new species, classification, etiology, pathogenicity, and ecology are essential, and the potential activities should be further investigated. According to morphological characteristics and phylogenetic analysis, the strain KNUF-22-18B is distinct from previously identified species of the genus *Neocucurbitaria*; thus, *Neocucurbitaria chlamydospora* sp. nov. is proposed as a novel species.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by a grant from the National Institute of Biological Resources, funded by the Ministry of Environment of the Republic of Korea [NIBR202231206].

ORCID

Kallol Das http://orcid.org/0000-0003-0906-3983
Seung-Yeol Lee http://orcid.org/0000-0003-1676-0330
Hee-Young Jung http://orcid.org/0000-0002-4254-3367

References

[1] Winter HG. Pilze. II. Abtheilung. Ascomyceten: Gymnoasceen und Pyrenomyceten. In: Rabenhorst GL, editor. Rabenhorst's Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz. 2nd ed. Leipzig: Kummer; 1885. p. 65–528.

[2] Hyde KD, Jones EBG, Liu JK, et al. Families of Dothideomycetes. Fungal Divers. 2013;63(1):1–313.
[3] Doilom M, Liu JK, Jaklitsch WM, et al. An outline of the family Cucurbitariaceae. Sydowia. 2013;65: 167–192.
[4] Wanasinghe DN, Phookamsak R, Jeewon R, et al. A family level rDNA based phylogeny of Cucurbitariaceae and Fenestellaceae with descriptions of new *Fenestella* species and *Neocucurbitaria* gen. nov. Mycosphere. 2017;8(4):397–414.
[5] Valenzuela-Lopez N, Cano-Lira JF, Guarro J, et al. Coelomycetous Dothideomycetes with emphasis on the families Cucurbitariaceae and Didymellaceae. Stud Mycol. 2018;90:1–69.
[6] Boerema GH, Loerakker WM, Hamers ME. Contributions towards a monograph of *Phoma* (Coelomycetes) – III. 2. Misapplications of the type species name and the generic synonyms of section *Plenodomus* (excluded species). Persoonia. 1996;16:141–189.
[7] Gurung K, Wertheim B, Falcao Salles J. The microbiome of pest insects: it is not just bacteria. Entomol Exp Appl. 2019;167(3):156–170.
[8] Jing TZ, Qi FH, Wang ZY. Most dominant roles of insect gut bacteria: digestion, detoxification, or essential nutrient provision? Microbiome. 2020; 8(1):1–20.
[9] Yao Z, Ma Q, Cai Z, et al. Similar shift patterns in gut bacterial and fungal communities across the life stages of *Bactrocera minax* larvae from two field populations. Front Microbiol. 2019;10:2262.
[10] Lu M, Hulcr J, Sun J. The role of symbiotic microbes in insect invasions. Annu Rev Ecol Evol Syst. 2016;47(1):487–505.
[11] McPherson JE, McPherson RM. Stink bugs of economic importance in America North of Mexico. Boca Raton (FL): CRC Press; 2000.
[12] Panizzi AR, Slansky F. Review of phytophagous pentatomids (Hemiptera: Pentatomidae) associated with soybean in the Americas. Fla Entomol. 1985; 68(1):184–214.
[13] Todd JW. Ecology and behavior of *Nezara viridula*. Annu Rev Entomol. 1989;34(1):273–292.
[14] Das K, Kim JH, Choi KS, et al. A new report of *Biscogniauxia petrensis* isolated from mosquitoes in Korea. Kor J Mycol. 2020;48:87–93.
[15] Crous PW, Schumacher RK, Akulov A, et al. New and interesting fungi 2. Fungal Syst Evol. 2019;3: 57–134.
[16] White TJ, Bruns T, Lee S, et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, et al., editors. PCR protocols: a guide to methods and applications. New York: Academic Press, Inc.; 1990. p. 315–322.
[17] Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol. 1993;2(2):113–118.
[18] Vilgalys R, Hester M. Rapid genetic identification, and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol. 1990;172(8):4238–4246.
[19] Rehner SA, Samuels GJ. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycol Res. 1994;98(6): 625–634.
[20] Woudenberg JHC, Aveskamp MM, de Gruyter J, et al. Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. Persoonia. 2009;22:56–62.
[21] Liu YJ, Whelen S, Hall BD. Phylogenetic relationships among ascomycetes evidence from an RNA polymerase II subunit. Mol Biol Evol. 1999;16(12): 1799–1808.
[22] Sung GH, Sung JM, Hywel-Jones NL, et al. A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): identification of localized incongruence using a combinational bootstrap approach. Mol Phylogenet Evol. 2007;44(3):1204–1223.
[23] Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980;16(2):111–120.
[24] Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4:406–425.
[25] Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol. 1981;17(6):368–376.
[26] Fitch WM. Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool. 1971;20(4):406–416.
[27] Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870–1874.
[28] Verkley GJM, Gené J, Guarro J, et al. *Pyrenochaeta keratinophila* sp. nov., aislado de una infección ocular en España. Rev Iberoam Micol. 2010;27(1): 22–24.
[29] Punithalingam E, English MP. *Pyrenochaeta unguis-hominis* sp. nov. on human toe-nails. Trans Br Mycol Soc. 1975;64(3):539–541.
[30] Eo JK, Eom AH. Community of endophytic fungi from alpine conifers on Mt. Seorak. Mycobiology. 2022;50(5):317–325.
[31] Magaña-Dueñas V, Stchigel AM, Cano-Lira JF. New coelomycetous fungi from freshwater in Spain. J Fungi. 2021;7(5):368.
[32] Lamsal K, Kim SW, Naeimi S, et al. Three new records of *Penicillium* species isolated from insect specimens in Korea. Mycobiology. 2013;41(2):116–119.
[33] Yun TS, Park SY, Yu J, et al. Isolation and identification of fungal species from the insect pest *Tribolium castaneum* in rice processing complexes in Korea. Plant Pathol J. 2018;34(5):356–366.
[34] Lee JH, Ten LN, Lim SK, et al. Molecular and morphological characteristics of a new species collected from an insect (*Cicindela transbaicalica*) in Korea. Mycobiology. 2022;50(3):181–187.
[35] Vanna R, Esther, Das K, Lee S-Y, et al. Molecular and morphological characterization of two novel species from soil and beetles (*Dorcus titanus castanicolor*) in Korea. Mycobiology. 2022;50(6):429–438.
[36] Jaklitsch WM, Checa J, Blanco MN, et al. Preliminary account of the Cucurbitariaceae. Stud Mycol. 2018;90:71–118.