

## Molecular and Morphological Characteristics of a New Species Collected from an Insect (*Cicindela transbaicalica*) in Korea

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### ABSTRACT

To exploit insect-derived fungi, insects were collected from seven different regions in Korea, including Gyeongbuk, Goryeong, and several fungi were isolated from them. A fungal strain designated 21-64-D was isolated from riparian tiger beetle (*Cicindela transbaicalica*) and morphologically identified as a species belonging to the genus *Oidiodendron*. Phylogenetic analysis using the nucleotide sequences of internal transcribed spacer (ITS) regions and the partial sequence of the large subunit of the nuclear ribosomal RNA (LSU) gene revealed the distinct phylogenetic position of the isolate among recognized *Oidiodendron* species including its closest neighbors *O. chlamydosporicum*, *O. citrinum*, *O. maius*, and *O. pilicola*. The hyphal and conidial morphology of the strain, particularly club-shaped hyphae, clearly differentiated it from its close relatives. Results indicated that 21-64-D is a novel species in the genus *Oidiodendron*, for which the name *Oidiodendron clavatum* sp. nov. is proposed.

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The genus *Oidiodendron* belonging to the family Myxotrichaceae was first described by Robak in 1932, with three species isolated from wood pulp, *Oidiodendron fuscum*, *Oidiodendron nigrum*, and *Oidiodendron rhodogenum* [1]. The taxonomic history of the reclassification of the members of the genus and the classification of its novel species have been widely reviewed and reported [2–5]. More than 30 species are currently listed in the Mycobank (<https://www.mycobank.org>) and Index Fungorum (<http://www.indexfungorum.org>) databases. The genus *Oidiodendron* has been isolated from a wide range of habitats, including soil, marine sediments, lichens, air, and various cellulosic substrates, such as litter, wood pulp, bark, moss, and paper [2,4]. Some *Oidiodendron* species were reported as ericoid mycorrhizal fungi [3,6]. The key morphological features of *Oidiodendron* are erect and well-differentiated dematiaceous conidiophores, profusely branched above, forming fertile hyphae that fragment basipetally to form arthroconidia. The primary characteristics for identification are the shape, size, and ornamentation of conidia, conidiophore length, and cultural morphology [7].

In Korea, fungi are mainly isolated from soil, plants, and mountainous areas. Many new and unreported species have been reported, and similar experiments are still being conducted. This study

attempted to isolate fungi from insects that deviate slightly from the common sources. Fungi and insects reciprocally interact establishing a wide range of symbiotic relationships, from interactions in which fungi act against insects to those in which fungi form mutualistic associations with insects [8]. In mutualistic interactions, one or both participants receive a net benefit such as dispersal, protection, or nutrition. Insect–fungus mutualisms evolved in at least 14 families of insects in six orders (Coleoptera, Blattodea, Lepidoptera, Hymenoptera, Diptera, and Hemiptera) and at least 15 orders of fungi in the Ascomycota and Basidiomycota [9]. In contrast, parasitism is the non-mutualistic form of symbiosis, taking place when only one of the organisms benefits at the expense of the other. Insect species can be infected by obligate or facultative entomopathogenic fungi which exploit them as the only or prevalent nutrient source. These fungi include over 700 species from various genera that can infect and kill several insect and mite species and they are considered as potential biocontrol agents against pest insects [10,11]. Our attempts focused on unexplored sources that were not yet tried and expected to find unreported and novel fungal species. Riparian tiger beetle (*Cicindela transbaicalica*), widely distributed in Korea and whose adults are seen from May to

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October [10], was considered a promising source of such fungi.

In this study, a specimen of *C. transbaicalica* was collected from Gyeongbuk, Goryeong-gun, in the riverside of Korea (35°39'34.4"N, 128°20'07.4"E) in April 2021, transferred to the laboratory, and stored at 4 °C until use. The insects were ground and mixed with double-distilled water, and the suspension was serially diluted. Then, 100 µL of each dilution was spread on potato dextrose agar (PDA; Difco, Detroit, MI, USA) plates and incubated at 25 °C for 2–3 days. Single colonies were transferred to fresh PDA plates and incubated at 25 °C for 4–5 days. The colony, designated 21-64-D, was selected for further molecular analysis based on its cultural characteristics. The strain was maintained in 20% glycerol at –80 °C for further study.

Genomic DNA was extracted from mycelia using the HiGene Genomic DNA prep kit (Biopact, Daejeon, South Korea). For molecular identification, internal transcribed spacer (ITS) regions were amplified using primers ITS1F and ITS4 [12], and a large subunit of the nuclear ribosomal RNA (LSU) gene was amplified using primers LROR and LR5 [13]. The amplified polymerase chain reaction products were purified using the EXOSAP-IT kit (Thermo Fisher Scientific, Waltham, MA). The purified DNA was sequenced by Macrogen Co., Ltd. (Daejeon, South Korea). The sequences of the species related to the genus *Oidiodendron* were

retrieved from the National Center for Biotechnology Information (Table 1). The sequences were initially aligned using Clustal X 2.0 [14], and phylogenetic analyses were performed using MEGA 7 [15]. The construction of phylogenetic trees was carried out using neighbor joining (NJ) [16], maximum likelihood (ML) [17], and maximum parsimony (MP) [18] algorithms.

A BLAST search of the NCBI database revealed that the LSU sequence of strain 21-64-D shared 97.9%, 97.7%, and 99.4% identity with closely related *Oidiodendron pilicola* CBS 158.76, *Oidiodendron chlamydosporicum* CBS 132.72, and *Oidiodendron truncatum* CBS 629.70, respectively. However, based on the ITS region sequence similarity, the closest neighbors of the isolate were *Oidiodendron maius* ISO.29, *Oidiodendron eucalypti* CPC32659, and *Oidiodendron tenuissimum* CBS 126945, with relatively low similarity values of 92.6%, 92.3%, and 92.1%, respectively. These results clearly indicated that the comparative analysis based on the sequence of only one gene did not allow precise identification of the closest relatives. Therefore, multilocus sequence analysis was performed using concatenated sequences of the ITS regions and the LSU gene of strain 21-64-D.

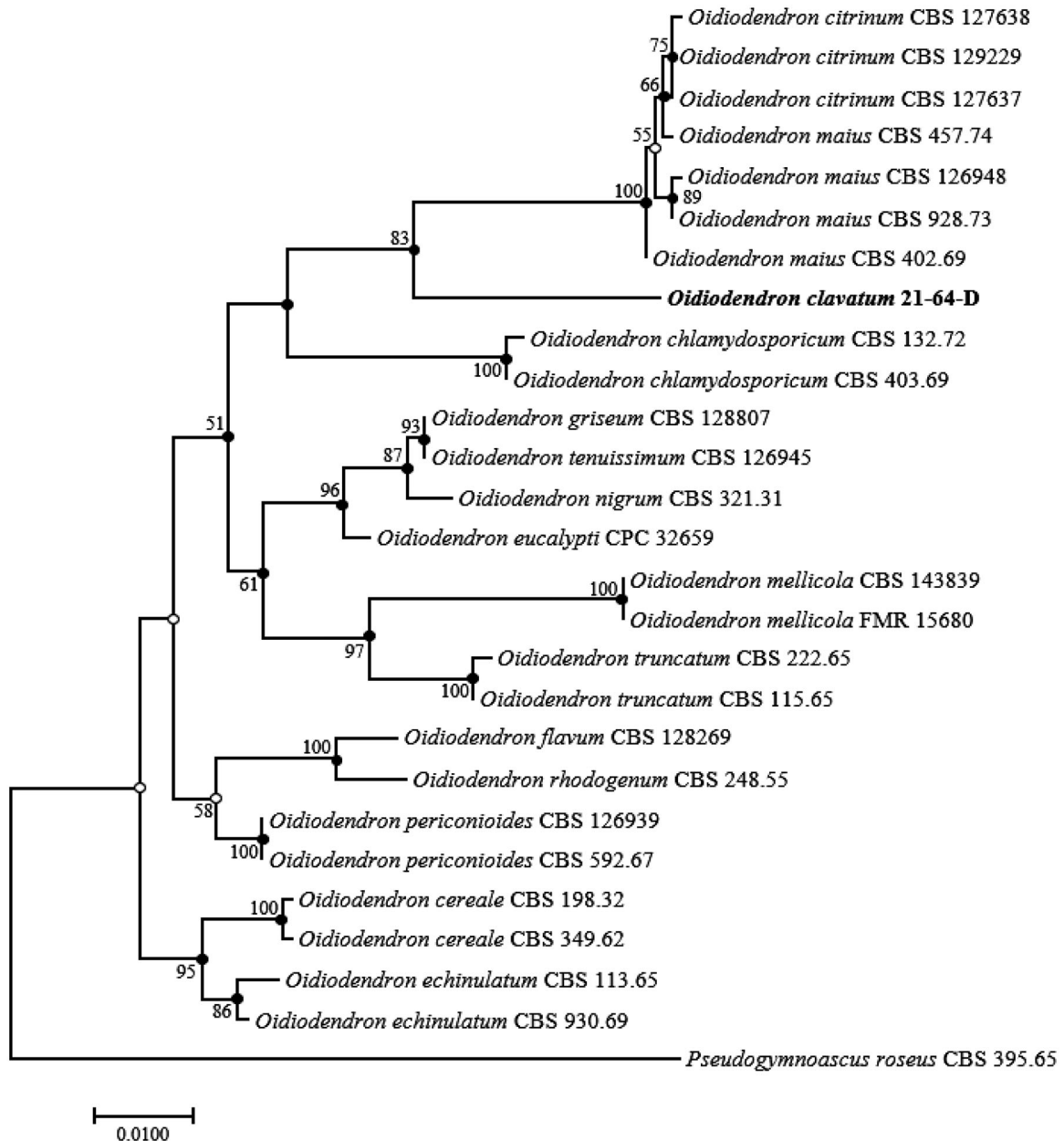
The constructed ML phylogenetic tree (Figure 1) clearly demonstrated that the isolate belongs to the genus *Oidiodendron* but is distinct from other *Oidiodendron* species. Based on the tree topology,

**Table 1.** List of species used in phylogenetic analyses along with their GenBank accession numbers.

Species	Strain	Origin	GenBank accession no.	
			ITS	LSU
<i>Oidiodendron cereale</i>	CBS 198.32 <sup>a</sup>	Netherlands; 2019	MH855282	MH866733
<i>Oidiodendron cereale</i>	CBS 349.62 <sup>a</sup>	Italy; 2019	MH858176	MH869770
<i>Oidiodendron chlamydosporicum</i>	CBS 132.72	Netherlands; 2019	MH860415	MH872153
<i>Oidiodendron chlamydosporicum</i>	CBS 403.69 <sup>a</sup>	Canada; 2019	MH859334	NG_064071
<i>Oidiodendron citrinum</i>	CBS 127637	USA; 2019	MH864648	MH876084
<i>Oidiodendron citrinum</i>	CBS 127638	USA; 2019	MH864649	MH876085
<i>Oidiodendron citrinum</i>	CBS 129229	USA; 2019	MH865252	MH876692
<b><i>Oidiodendron clavatum</i></b>	<b>21-64-D</b>	<b>Korea; 2021</b>	<b>LC683775</b>	<b>LC683776</b>
<i>Oidiodendron echinulatum</i>	CBS 113.65	Germany; 2019	MH858509	MH870143
<i>Oidiodendron echinulatum</i>	CBS 930.69	Denmark; 2019	MH859482	MH871263
<i>Oidiodendron eucalypti</i>	CPC 32659	Australia; 2018	NR_160624	NG_064544
<i>Oidiodendron flavum</i>	CBS 128269	USA; 2019	MH864869	MH876316
<i>Oidiodendron griseum</i>	CBS 128807	USA; 2019	MH865155	MH876594
<i>Oidiodendron maius</i>	CBS 126948	USA; 2019	MH864347	MH875791
<i>Oidiodendron maius</i>	CBS 402.69 <sup>a</sup>	Canada; 2019	MH859333	MH871081
<i>Oidiodendron maius</i>	CBS 457.74	Nepal; 2019	MH860869	MH872607
<i>Oidiodendron maius</i>	CBS 928.73	Sweden; 2019	MH860825	MH872559
<i>Oidiodendron mellicola</i>	CBS 143839	Spain; 2018	LT906544	LT978464
<i>Oidiodendron mellicola</i>	FMR 15680	Spain; 2018	LT906540	LT978465
<i>Oidiodendron nigrum</i>	CBS 321.31 <sup>a</sup>	Norway; 2019	MH855228	MH866680
<i>Oidiodendron periconioides</i>	CBS 126939	USA; 2019	MH864342	MH875787
<i>Oidiodendron periconioides</i>	CBS 592.67	Austria; 2019	MH859058	MH870778
<i>Oidiodendron pilicola</i>	CBS 158.76	Sweden; 2019	–	MH878525
<i>Oidiodendron rhodogenum</i>	CBS 248.55	Netherlands; 2019	MH857466	MH869009
<i>Oidiodendron tenuissimum</i>	CBS 126945	USA; 2019	MH864345	MH875789
<i>Oidiodendron truncatum</i>	CBS 222.65	Canada; 2019	MH858549	MH870187
<i>Oidiodendron truncatum</i>	CBS 115.65	Germany; 2019	MH858510	MH870144
<i>Pseudogymnoascus roseus</i>	CBS 395.65	United Kingdom; 2019	NR_165894	NG_070577

The isolated strain is shown in bold.

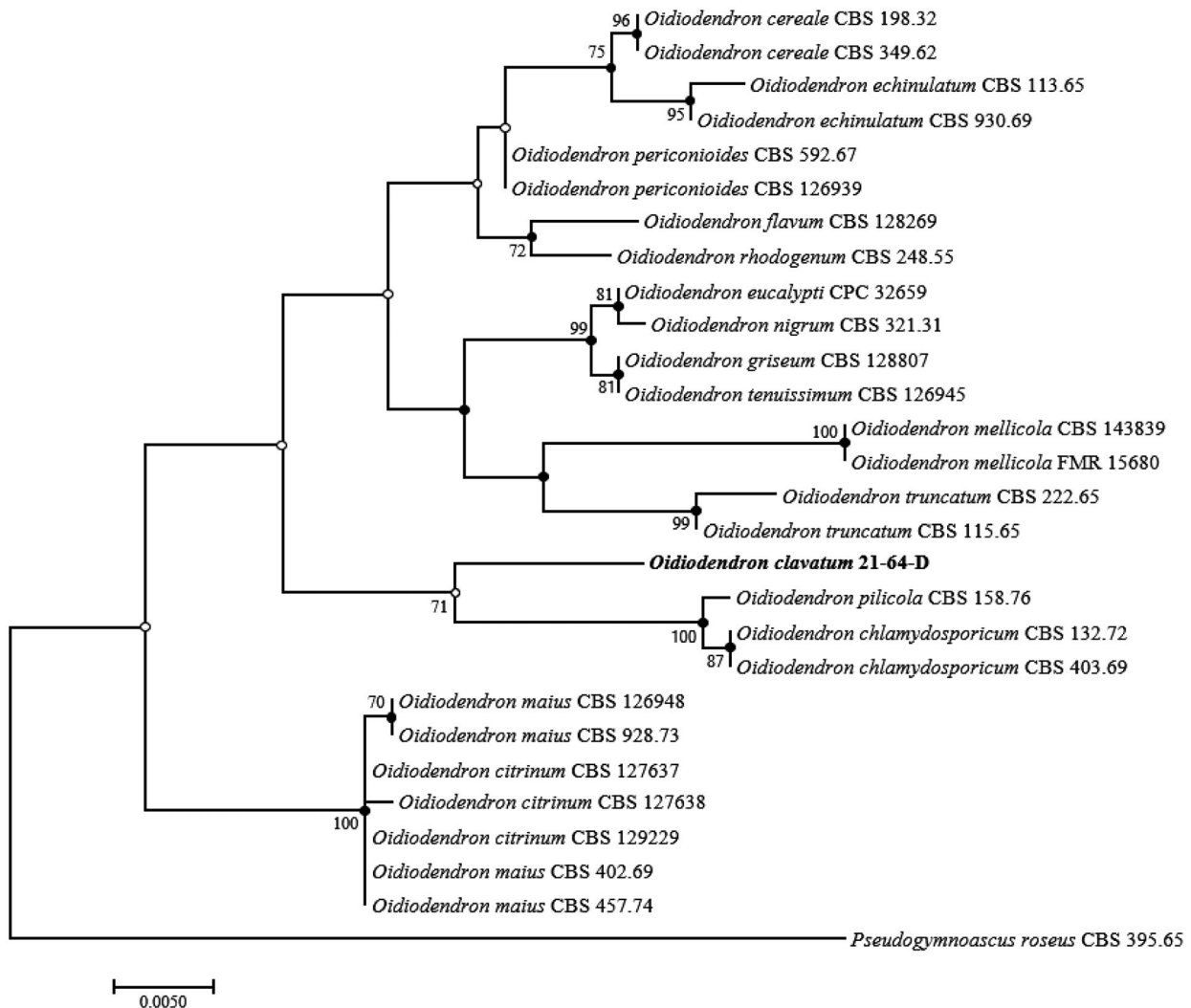
<sup>a</sup>Ex-type.



**Figure 1.** ML phylogenetic tree based on the concatenated sequences of ITS regions and the LSU gene showing the phylogenetic position of strain 21-64-D among *Oidiiodendron* species. Bootstrap values greater than 50% (based on 1000 replications) are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in trees generated using NJ and MP algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated using the NJ or MP algorithm. The isolated strain is shown in bold. *Pseudogymnoascus roseus* CBS 395.65 was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

its phylogenetically closest neighbors are *O. chlamydosporicum*, *O. maius*, and *Oidiiodendron citrinum* (an additional strain not mentioned above). Unfortunately, the ITS sequence was unavailable for *O. pilicola* in the GenBank database. Therefore, a second ML phylogenetic tree was constructed using the sequences of LSU gene to clarify the phylogenetic relationship level between strain 21-64-D and *O. pilicola*. As shown in Figure 2, 21-64-D formed a separate cluster, indicating the novelty of the isolate at the species level from *O. pilicola* and the other above-mentioned *Oidiiodendron* species. Moreover, both ML trees shared corresponding nodes with NJ

and MP trees, as indicated by filled circles in Figures 1 and 2. Accordingly, the novel strain is considered a single, novel, phylogenetically distinct *Oidiiodendron* species. Based on the topology of the phylogenetic trees, four species, *O. chlamydosporicum*, *O. citrinum*, *O. maius*, and *O. pilicola*, were selected to compare their morphological characteristics to that of 21-64-D to confirm the results of the phylogenetic analysis. The morphological features of the isolate were observed under a light microscope (BX-50, Olympus, Tokyo, Japan). Strain 21-64-D was cultured on PDA, malt extract agar (MEA; Difco), oatmeal agar (OA; Difco), and cornmeal



**Figure 2.** ML phylogenetic tree based on the sequences of the LSU gene showing the distinct position of *Oidiodendron clavatum* from *O. pilicola* and other members of the genus. Bootstrap values greater than 50% (percentage of 1000 replications) are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in trees generated using NJ and MP algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated using the NJ or MP algorithm. The isolated strain is shown in bold. *Pseudogymnoascus roseus* CBS 395.65 was used as an outgroup. Bar, 0.005 substitutions per nucleotide position.

agar (CMA; Difco), and the size, shape, and color of its colonies were recorded after 28 days of cultivation at 25 °C.

### Taxonomy

*Oidiodendron clavatum* S.Y. Lee, L.N. Ten & H.Y. Jung sp. nov. (Figure 3).

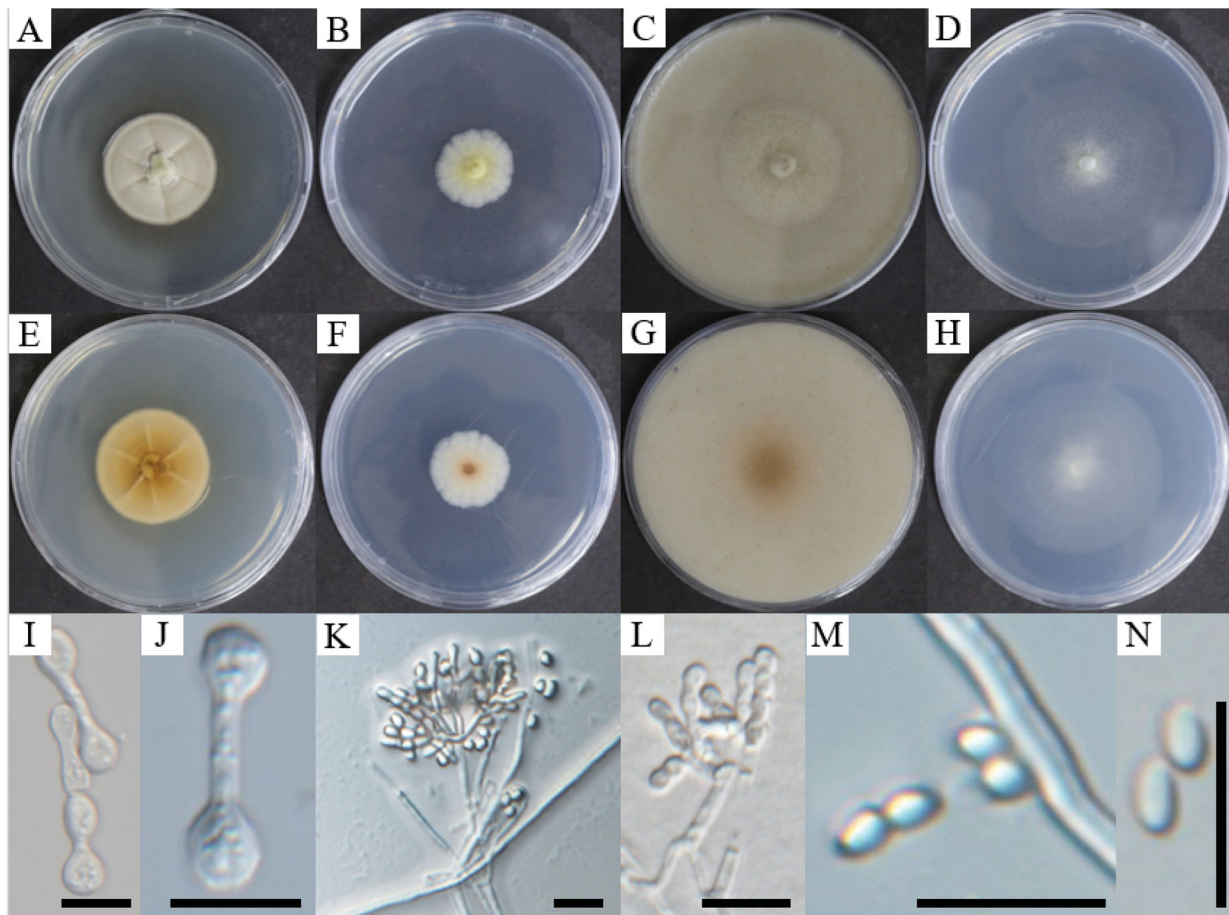
*Mycobank* no.: MB843126.

**Etymology:** The specific epithet is derived from the Latin word *clavatum* (“club”) and refers to the shape of hyphae.

**Typus:** The culture was isolated from the insects (*Cicindela transbaicalica*) in Gyeongbuk, Goryeong, Korea (35°39′34.4″N, 128°20′07.4″E). The stock culture (NIBRFGC000509256) was deposited in the National Institute of Biological Resources (NIBR) as a metabolically inactive culture.

**Habitat:** The fungus is associated with the riparian tiger beetle (*C. transbaicalica*).

**Description:** Colony growth was rapid at 25 °C on PDA, MEA, OA, and CMA. After 28 days of incubation on PDA, the colonies were light yellow, reverse pale yellow to brown in the center, and reached 34.0–34.6 mm in diameter. On MEA, the colonies were 24.7–26.4 mm in diameter and bright yellow to white and reverse white. On OA, the colonies were pale yellow aerial hypha and brown reverse, reaching 36.7–38.0 mm in diameter. On CMA, the colonies were 39.0–39.8 mm in diameter and white to dusky yellow with flat mycelium, center black graininess, and transparent white on the reverse side of the Petri dish. Additionally, lots of conidia were found with well-developed conidiophores at the centers of colonies. Conidiophores were opaque and abundant, short, smooth with about four to five conidia chains formed on top.



**Figure 3.** Cultural and morphological characteristics of *Oidiiodendron clavatum*. Colonies on PDA (A, E), MEA (B, F), OA (C, G), and CMA (D, H) after 28 days of cultivation at 25°C. Hyphae expand into club-shaped (I), club-shaped hypha (J), conidiophores (K, L), and conidia (M, N). Scale bar, 10  $\mu\text{m}$  (I–N).

Hyphae were hyaline, branched, smooth-walled, and club-shaped. Conidia was pale brownish, elliptical, and thin-walled, and the size was  $2.3 - (3.1) - 3.9 \times 1.6 - (2.0) - 2.6 \mu\text{m}$  (Figure 3).

**Notes:** *O. clavatum* (strain 21-64-D) has a close molecular phylogenetic relationship with *O. maius*, *O. citrinum*, *O. chlamydosporicum*, and *O. pilicola*. The morphological characteristics of strain 21-64-D distinguish it from closely related species based on hyphae, conidiophores, and conidia (Table 2). The average length of conidia of 21-64-D ( $3.1 \times 2.0 \mu\text{m}$ ; length-to-width ratio ( $L/W$ )=1.6) is longer than that of *O. citrinum* ( $2.8 \times 1.8 \mu\text{m}$ ;  $L/W=1.6$ ) and *O. chlamydosporicum* ( $2.5 \times 1.7 \mu\text{m}$ ;  $L/W=1.5$ ). In contrast, it is shorter than *O. maius* ( $3.3 \times 1.7 \mu\text{m}$ ;  $L/W=1.9$ ). The average width of the conidia of strain 21-64-D ( $3.1 \times 2.0 \mu\text{m}$ ;  $L/W=1.6$ ) is bigger than that of the three closely related species, *O. maius* ( $3.3 \times 1.7 \mu\text{m}$ ;  $L/W=1.9$ ), *O. chlamydosporicum* ( $2.5 \times 1.7 \mu\text{m}$ ;  $L/W=1.5$ ), and *O. citrinum* ( $2.8 \times 1.8 \mu\text{m}$ ;  $L/W=1.6$ ). Moreover, the conidial  $L/W$  values differentiate *O. clavatum* (1.6) from *O. maius* (1.9), *O. chlamydosporicum* (1.5), and *O. pilicola* (1.8). The conidia of isolate 21-64-D are pale brown, whereas the four close relatives are hyaline. The biggest difference of 21-64-D from *O. pilicola* is

the shape of conidia. *O. clavatum* has elliptical-shaped conidia, whereas *O. pilicola* produces barrel-shaped conidia. The shape of conidiophores and hyphae also clearly distinguishes the novel species from its close phylogenetic neighbors. *O. clavatum* has branched conidiophores, whereas *O. maius* and *O. citrinum* produce unbranched conidiophores. Notably, melanized chlamydo-spores are a characteristic feature of *O. chlamydosporicum*, while strain 21-64-D does not generate them. Furthermore, club-shaped hyphae are the most distinctive morphological character of *O. clavatum*, the same shape of hyphae was not observed in the closest *Oidiiodendron* species. The colony of strain 21-64-D on CMA is white to dusky yellow with black graininess in the center, additionally differentiating it from the close phylogenetic relatives. *O. maius* has off-white to gray, appressed colonies. *O. citrinum* produces yellow-green, appressed colonies, and *O. chlamydosporicum* has cream or pale gray to green-gray or brown, darker at margins, appressed colonies. In addition, the average size of the colonies on CMA clearly differentiates *O. clavatum* (39.4 mm) from *O. chlamydosporicum* (12.5 mm).

The above-mentioned morphological characteristics support the phylogenetic analysis results of

**Table 2.** Morphological comparison of *Oidiiodendron clavatum* with closely related species.

Characteristics	Form	<i>O. clavatum</i> <sup>a</sup>	<i>O. maius</i> <sup>b</sup>	<i>O. citrinum</i> <sup>b</sup>	<i>O. chlamydosporicum</i> <sup>b</sup>	<i>O. pilicola</i> <sup>b</sup>
Colony	Color	White to dusky yellow, center black graininess, reverse pale yellow to brown in the center	Off-white to gray, appressed, reverse pale gray to dark brown in the center	Yellow-green, appressed, reverse pale brown to dark in the center	Cream or pale gray to green-gray or brown, darker at margins, appressed; reverse cream near margin, becoming dark brown at the center	N/A
	Size	CMA: 39–39.8 mm at 28 days	CMA: 29–38 mm at 28 days	CMA: 30–36 mm at 28 days	CMA 9–16 mm at 28 days	N/A
Conidiophores	Color	Opacity	Dark	Melanized	Melanized	Pale olivaceous brown
	Shape	Abundant, short, smooth, branched, with hanging conidia on top	Abundant, tall, bearing masses of white conidia, unbranched, smooth	Abundant, bearing masses of yellow conidia, tall, unbranched, smooth	Intergrading between structures that are short, branched, and lightly pigmented to melanized and structures that are erect, both bear chains of hyaline conidia with melanized chlamydospores	Simple, erect, septate, thick-walled
Hyphae	Color	Hyaline	Hyaline	Hyaline	N/A	Hyaline
	Shape	Branched, smooth-walled, and club-shaped	Dichotomously branched, fragmenting to form long, undulating chains of conidia	Dichotomously branched, fragmenting to form long, undulating chains of conidia	N/A	Branched, fragmenting to form conidia
Conidia	Color	Pale brownish	Hyaline	Hyaline	Hyaline	Hyaline
	Shape	Thin-walled, elliptical	Thin-walled, subglobose to elongate	Thin-walled, subglobose to elongate	Thin-walled, globose, subglobose, or elongated	Barrel-shaped, truncate with frills at both ends, smooth, catenate
	Size (µm)	2.3 – (3.1) – 3.9 × 1.6 – (2.0) – 2.6	2.0 – (3.3) – 5.0 × 1.0 – (1.7) – 2.5	1.5 – (2.8) – 5.0 × 1.0 – (1.8) – 2.5	1.5 – (2.5) – 5.0 × 1.0 – (1.7) – 2.5	3.0 – (3.2) – 3.5 × 1.5 – (1.8) – 2.0
	L/W	1.6	1.9	1.6	1.5	1.8

N/A: not available.

<sup>a</sup>Fungal strain studied in this paper.

Sources of description [5].

strain 21-64-D, clearly confirming the novelty of the isolate at the species level among the other recognized *Oidiiodendron* species. The application of DNA sequence analysis and molecular phylogenetic analyses had a major impact on the systematics of Myxotrichaceae. In particular, a relationship between *Oidiiodendron* species and teleomorphic taxa within Myxotrichaceae was strongly confirmed by molecular analysis using ITS sequences [7]. Later, these data were successfully used by Rice and Currah [5] to perform a survey of the named *Oidiiodendron* species and related anamorphs of the genus *Myxotrichum*. Besides ITS, LSU sequences were also used for the phylogenetic analysis of *Oidiiodendron*, but two loci were applied separately from each other. In particular, phylogenetic analyses using LSU sequences were conducted to establish *O. eucalypti* [6] and *Oidiiodendron mellicola* [19]. To the authors' knowledge, there was only one attempt to use combined ITS and LSU sequences, which was carried out for the identification of isolated *Oidiiodendron* strain KNUE20T046, but a limited number of *Oidiiodendron* species were included for phylogenetic analysis [20]. The results, particularly the identification of unexpected *O. citrinum* as the close relative of strain 21-64-D, clearly indicated that molecular analysis using two concatenated genetic markers allows more accurate differentiation and classification of novel *Oidiiodendron* strains.

*Oidiiodendron* is a cosmopolitan genus whose members, as mentioned above, can be found in

various environmental niches. Strain 21-64-D was isolated from riparian tiger beetle. This is the first *Oidiiodendron* species isolated from insects to the authors' knowledge. The widespread distribution of the genus is connected with their excellent adaptive capacity, and now *Oidiiodendron* strains are considered promising sources of secondary metabolites with various biological activities [21]. Seven new compounds, named chetracins B, C, D and oidio-perazines A to D, were isolated from *O. truncatum*. Among them, chetracin B revealed cytotoxic activity in the nanomolar range against a panel of five human cancer lines [22]. This *Oidiiodendron* species is also known as a producer of oidiiodendronic acid and oidiiodendrolides A, B, and C, which are effective antifungal agents against pathogenic yeasts, *Candida albicans*, and *Cryptococcus neoformans* [23]. Two nematicides, 4-hydroxyphenylacetic and oidiolactone D, antimicrobial harzianic acid, and antibacterial dihydrosecofusicin and secofusicin were isolated from *Oidiiodendron* sp. [24], *Oidiiodendron flavum* [21], and *Oidiiodendron griseum* [25], respectively. *O. clavatum* as a novel species provides additional opportunity to expand research on bioactive compounds, and its further investigation would be worthwhile. In addition, strain 21-64-D is the first insect-associated species of the genus *Oidiiodendron*. These results extend the distribution of the genus and highlight the need for further studies on its ecological and biological roles.

In conclusion, morphological and phylogenetic analyses showed that strain 21-64-D is distinct from previously identified *Oidiiodendron* species. It should be considered a novel species within the genus with the name *O. clavatum* sp. nov.








### Disclosure statement

The authors declare no potential conflict of interest.

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