

***Clavariadelphus pakistanicus* (Gomphales): A new macrofungal record from India**

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ABSTRACT

Clavariadelphus pakistanicus is described for the first time from India based on morphological and molecular (ITS) data from Jammu and Kashmir. Complete morphological descriptions, photographs and comparisons with related species is provided and a key to the Indian species of *Clavariadelphus* is also provided

Keywords: *Abies pindrow*, *Clavariadelphaceae*, Coniferous, Jammu and Kashmir, ITS

INTRODUCTION

Genus *Clavariadelphus* Donk (*Gomphales*, *Clavariadelphaceae*) is widely distributed in temperate regions with several species restricted to mixed deciduous-conifer forests (Methven, 1990). Worldwide, this genus encompasses 28 species and is characterized by pallid brown to yellow-brown to light-yellow colouration, narrow clavate, tall, erect basidiomes, solitary growth, smooth inamyloid broadly ellipsoidal to amygdaliform basidiospores and clamped tramal hyphae with ampulliform or medallion inflations at the septa (Methven, 1990; Kirk *et al.*, 2008; Hanif *et al.*, 2014; Sher *et al.*, 2018; Huang *et al.*, 2020; Lu and Li, 2020). In India, *Clavariadelphus* is so far represented by four species *viz.*, *Clavariadelphus himalayensis* Methven, *C. mirus* (Pat.) Corner, *C. truncatus* Donk, and *C. yunnanensis* Methven (Thind and Anand, 1956; Thind and Dev, 1956; Methven, 1989).

During our studies on the macrofungi of Jammu and Kashmir, we came across an interesting species and on detailed study revealed it as *Clavariadelphus pakistanicus* Hanif & Khalid. The species is so far not reported from India. It is described in detail with morphological and molecular data.

MATERIALS AND METHODS

Morphological studies: Basidiomes of *Clavariadelphus pakistanicus* were collected from the coniferous forests of district Anantnag, Jammu and Kashmir, India. Detailed field notes are based on fresh basidiomes. Photographs of basidiomes were captured with Canon EOS1300D. Colour terms given in the description are those of Korerup and Wanscher (1978). Dried basidiomes revived in 5% aqueous KOH and stained with 1% Congo red were used for micromorphological studies. Basidiospore dimensions are given as average, Q = length/width ratio. Iodine reactions were also observed by staining the basidiospores in Melzer's reagent. Line drawings of microscopic structures were made from rehydrated material using camera lucida.

DNA extraction, PCR amplification and DNA sequencing: NucleoSpin® Plant II Kit (Macherey-Nagel) was used to isolate genomic DNA from dried basidiomes according to manufacturer's protocol. ITS 1F/ITS 4R were used to amplify ITS region (White *et al.*, 1990). PCR Thermal Cycler was used for amplification reactions (GeneAmp PCR System 9700, Applied Biosystems). PCR conditions included initial denaturation at 98°C for 30sec followed by 40 cycles at 98°C for 5sec, 60°C for 10sec, 72°C for 15sec and final extension at 72°C for 60sec. PCR products were purified and then sequenced by BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA). Sequence generated was submitted in GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) and accession number of *Clavariadelphus pakistanicus* is given in **fig. 1**.

Sequence alignment and phylogenetic analysis: Consensus sequence was generated using Bio-Edit software from ABI chromatograms of ITS region. The consensus sequence was then used for BLAST search in NCBI. The sequences from GenBank were selected based on the BLASTn results and relevant published phylogenies (Hanif *et al.*, 2014; Sher *et al.*, 2018). The alignment of the sequences was generated by MAFFT v. 7 (Katoh and Standley, 2013) and sequences were imported into MEGA-X software. Bootstrap consensus tree was inferred from 1000 bootstrap replicates using maximum likelihood (ML) analysis. *Clavaria flavostellifera* Olariaga, Salcedo, P.P. Daniëls & Kautman and *C. calabrica* Franchi & M. Marchetti were used as outgroup.

RESULTS AND DISCUSSION

Phylogenetic analysis: The ITS dataset of *Clavariadelphus pakistanicus* comprised of 38 sequences including our sequence. BLAST analysis revealed 98.31% similarity with *C. pakistanicus* (HQ379937) having query cover 99% with zero e-value. *Clavaria flavostellifera* and *C. calabrica* were used as outgroup. The sequence of studied species in phylogram is presented in bold (**Fig.1**).

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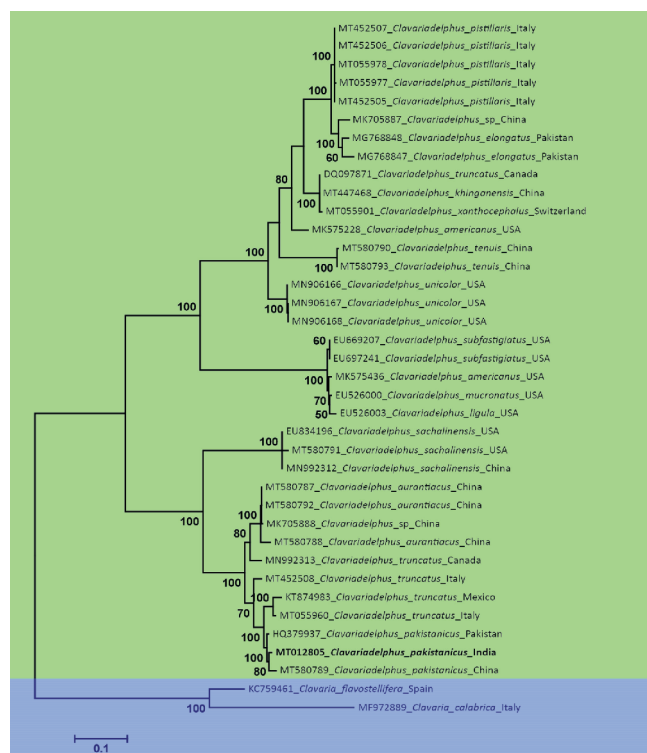


Fig. 1: Maximum Likelihood (ML) consensus tree of *Clavariadelphus pakistanicus* (MT012805) derived from the ITS dataset. *Clavaria flavostellifera* and *C. calabrica* were used as the outgroup. The new record is presented in bold.

TAXONOMY

Clavariadelphus pakistanicus Hanif & Khalid, in Hanif, Khalid & Exeter, *Botany* **92**(7): 475 (2014) (**Fig. 2 a-g**)

Basidiomes up to 60 mm long, 18–34 mm in diam., clavate to sub-cylindrical, apex obtuse when young, becoming flat when mature, with a tapering base; surface pastel red (7A4) up to two-third, elsewhere white (7A1), turning reddish brown and olive with 10% aqueous KOH and 10% FeSO₄ respectively, dry. Context white (7A1), solid and firm during younger stage becoming spongy and soft when mature, turning slight yellowish and greenish with aqueous 10% KOH and 10% FeSO₄ respectively. Odour mild. Basidia 40.0–48.0 × 8.0–9.6 μm, clavate to sub-cylindrical, 2–4 spored, multi-guttulated, smooth. Sterigmata 7.2–9.6 μm long, 2–4 in number, slightly incurved. Basidiospores 7.2–10.4 μm × 4.8–7.2 μm (av L = 8.8 μm, av W = 6.0 μm, Q = 1.4–1.5), broadly ellipsoid, apiculus present, smooth, thin walled, inamyloid, finely granular vacuolated contents present. Cystidia 24.0–36.0 × 4.8–7.2 μm, cylindrical, oil drops present, clamped at base, thin walled, smooth. Apical pellis elements 20.5–27.4 × 7.8–8.5 μm, variable in shape, apices mostly capitate to obtuse to rarely appendiculate, hyaline, thin-walled, smooth. Tramal hyphae 6.0–12.0 μm wide, septate, clamped, hyaline, branched occasionally, smooth, thin walled. Cuticle hyphae 4.0–12.0 μm wide, septate, clamped, ampulliform inflations up to 6.0 μm wide, thin walled, smooth.

Table 1: Taxa included in this study: Taxon, GenBank accession number for sequences, location, and voucher number

Taxon	ITS GenBank accession No.	Location	Voucher No.
<i>Clavariadelphus pistillaris</i>	MT452507	Italy	AMB 18611
<i>Clavariadelphus pistillaris</i>	MT452506	Italy	AMB 18610
<i>Clavariadelphus pistillaris</i>	MT055978	Italy	AMB 18597
<i>Clavariadelphus pistillaris</i>	MT055977	Italy	AMB 18596
<i>Clavariadelphus pistillaris</i>	MT452505	Italy	AMB 18595
<i>Clavariadelphus sp.</i>	MK705887	China	HKAS 57396
<i>Clavariadelphus elongatus</i>	MG768848	Pakistan	SWAT000559
<i>Clavariadelphus elongatus</i>	MG768847	Pakistan	LAH31397
<i>Clavariadelphus truncatus</i>	DQ097871	Canada	OUC99108
<i>Clavariadelphus khinganensis</i>	MT447468	China	MHKMUH.Y.
<i>Clavariadelphus xanthocephalus</i>	MT055901	Switzerland	ZTMyc54987
<i>Clavariadelphus americanus</i>	MK575228	USA	SDRussell mycomap#1288
<i>Clavariadelphus tenuis</i>	MT580790	China	MHHNU 9934
<i>Clavariadelphus tenuis</i>	MT580793	China	MHHNU 9900
<i>Clavariadelphus unicolor</i>	MN906166	USA	SDRussell mushroom observer# 112193
<i>Clavariadelphus unicolor</i>	MN906167	USA	SDRussell inaturalist# 8562538
<i>Clavariadelphus unicolor</i>	MN906168	USA	SDRussell mycomap# 3005
<i>Clavariadelphus subfastigiatus</i>	EU669207	USA	OSC 119587
<i>Clavariadelphus subfastigiatus</i>	EU697241	USA	OSC 104665
<i>Clavariadelphus americanus</i>	MK575436	USA	SDR NAMA 2017 - 129
<i>Clavariadelphus mucronatus</i>	EU526000	USA	OSC 1064138
<i>Clavariadelphus ligula</i>	EU526003	USA	OSC 1064245
<i>Clavariadelphus sachalinensis</i>	EU834196	USA	OSC 96213
<i>Clavariadelphus sachalinensis</i>	MT580791	China	MHHNU 7816
<i>Clavariadelphus sachalinensis</i>	MN992312	Canada	ANT061QFB28654
<i>Clavariadelphus aurantiacus</i>	MT580787	China	MHHNU 9256
<i>Clavariadelphus aurantiacus</i>	MT580792	China	MHHNU10085
<i>Clavariadelphus sp.</i>	MK705888	China	HKAS57396
<i>Clavariadelphus aurantiacus</i>	MT580788	China	HKAS53889
<i>Clavariadelphus truncatus</i>	MN992313	Canada	ANT130QFB28761
<i>Clavariadelphus truncatus</i>	MT452508	Italy	AMB 18612
<i>Clavariadelphus truncatus</i>	KT874983	Mexico	CB08379
<i>Clavariadelphus truncatus</i>	MT055960	Italy	AMB18580
<i>Clavariadelphus pakistanicus</i>	HQ379937	Pakistan	mh129901
<i>Clavariadelphus pakistanicus</i>	MT012805	India	SR1742
<i>Clavariadelphus pakistanicus</i>	MT580789	China	MHHNU 9282
<i>Clavaria flavostellifera</i>	KC759461	Spain	BIO-Fungi 10433
<i>Clavaria calabrica</i>	MF972889	Italy	ZTMyc58697

Habitat and phenology: Solitary to scattered or gregarious, uncommon, growing in forest of *Abies pindrow*.

Collection examined: India, Jammu and Kashmir, Kashmir, Anantnag, Daksum, Lat: 33.686692°N, Lon: 75.261327°E, Altitude 2520 m, August 2019, Uzma Altaf and Yash Pal Sharma, HBJU-792.

Edibility status: Inedible.

Distribution: *Clavariadelphus pakistanicus* has been earlier reported from Khyber Pakhtunkhwa, Pakistan growing in forests of *Abies pindrow* (Royle ex D. Don) Royle and *Pinus wallichiana* A.B. Jacks (Hanif *et al.*, 2014).

Remarks: The description of current specimen is in conformity with Hanif *et al.* (2014) except for the absence of rhizomorphic mats of the present species. Morphologically, *Clavariadelphus truncatus* shows similarities with *C. pakistanicus* but is distinguished by its clavate basidiomata that become turbinate with a truncate perforated sterile apex and has larger elliptical oblong basidiospores (9.0–13.0 × 5.0–7.0 μm). In addition, *C. pakistanicus* shows reddish brown colour whereas *C.*

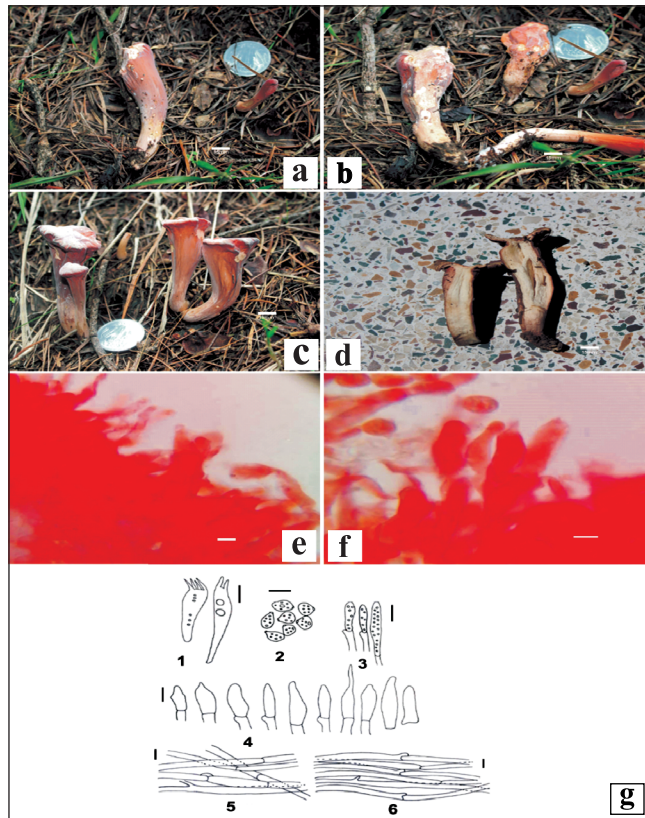


Fig. 2 (a-g): *Clavariadelphus pakistanicus*: a-c. Basidiomes in natural habitat, d. Context of specimen, e-f. Apical pellis elements, g. Line drawings: 1. Basidia, 2. Basidiospores, 3. Cystidia, 4. Apical pellis elements, 5. Tramal hyphae, 6. Cuticle hyphae. Scale bars a-d = 30 mm, e-f = 10 μ m, g (1-6) = 10 μ m.

Taxonomic key to species of *Clavariadelphus* in India

1. Basidiomes greyish-red to pastel-red, negative reaction with KOH, flesh does not stain where bruised or cut, broadly ellipsoid basidiospores (9.0-11.5 μ m \times 5.0-6.0 μ m)..... *C. himalayensis*
2. Basidiomes light brown to brown, broadly ovate basidiospores (10.0-13.0 μ m \times 6.0-8.0 μ m), flesh slowly stains brunnescent to russet on exposure.....
..... *C. mirus*
3. Basidiomes pastel-red up to 2/3, elsewhere white, reddish brown reaction with KOH, basidiospores (7.2-10.4 μ m \times 4.8-7.2 μ m)..... *C. pakistanicus*
4. Basidiomes yellow or orange ochraceous, rufescent, or cinnamon; cherry reddish reaction with KOH, basidiospores (9.5-13.5 μ m \times 5.5-7.0 μ m).... *C. truncatus*
5. Basidiomes pale yellow to pinkish yellow or cinnamon buff, yellow reactivity with KOH, broadly ellipsoid basidiospores (9.0-11.5 μ m \times 5.0-6.0 μ m).....
..... *C. yunnanensis*

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