

***Narcissea lahorensis* sp. nov. (Psathyrellaceae, Agaricales) from Pakistan**Muhammad Usman<sup>1,2\*</sup>, Aiman Izhar<sup>1,3</sup>, Abdul Nasir Khalid<sup>1,4</sup><sup>1</sup>Fungal Biology and Systematics Research Laboratory, Institute of Botany, University of the Punjab, Quaid-e-Azam Campus 54590, Lahore, Pakistan.<sup>2</sup>musmanmughal52@yahoo.com; <https://orcid.org/0000-0002-3490-058X><sup>3</sup>aimanizhar25@gmail.com; <https://orcid.org/0000-0002-8739-2881><sup>4</sup>drankhalid@gmail.com; <https://orcid.org/0000-0002-5635-8031>

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Lahore

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**Abstract:** *Narcissea lahorensis* sp. nov. is described from Pakistan based on morpho-anatomical and phylogenetic analyses using ITS and LSU regions, as a third species of the genus *Narcissea*. It is characterized by small basidiomata, with the pileus (10 – 18 mm in diam.) having a pruinose surface covered with whitish floccose veil composed of globose to sub-globose elements, deeply plicate from margin to center with dull yellow orange center, brownish black edges of lamellae, a short stipe (13 – 20 × 0.8 – 1.8 mm) without annulus and volva, limoniform to rectangular, brownish black to reddish brown basidiospores, 7.6 – 9.3 × 6.2 – 8.1 µm, and polymorphic cheilocystidia and pleurocystidia. Our analyses confirmed that it is a distinct species in the genus *Narcissea* and is described here as new to science.**INTRODUCTION**

*Psathyrellaceae* was recently split into 16 genera by Wächter and Melzer (2020) based on phylogenetic evidence and morphological characteristics. *Narcissea* Wächter & A. Melzer (2020: 1203) is one of these genera and it was represented by two species only, viz., *N. patouillardii* (Quél.) Wächter & A. Melzer (2020: 1204) and *N. cordispora* (T. Gibbs) Wächter & A. Melzer (2020: 1203) (Wächter & Melzer 2020). Previously, both belonged to the genus *Coprinus* Pers. (1797: 62) but were transferred to the genus *Coprinopsis* P. Karst. (1881: 27) as *C. patouillardii* (Quél.) Gminder (2010: 650) and *C. cordispora* (T. Gibbs) Watling & M.J. Richardson (2010: 406) (Krieglsteiner & Gminder 2010, Watling & Richardson 2010, Wächter & Melzer 2020).

The genus *Narcissea* is characterized by well-developed granular veil on pileus and tri- to polygonal basidiospores (in outline) being strongly flattened and with a central germ-pore. Other features of the genus are small basidiomata, occurrence on dung or on manured soil, polymorphic cheilocystidia and pleurocystidia, and the absence of clamps (Wächter & Melzer 2020). Here, we describe *Narcissea lahorensis*, a new species from Pakistan, based on phylogenetic and morphological analysis. This study is the continuation of our efforts to unveil the fungal diversity of the country using integrative taxonomic approaches to describe new fungal taxa.

**MATERIALS AND METHODS***Sampling Site*

Basidiomata were collected in the Quaid-e-Azam Campus of the University of the Punjab, Lahore, during the winter season. The district of Lahore is averagely 217 m above sea level and covers an area of 1772 km<sup>2</sup> while the total vegetation cover is 259 km<sup>2</sup> (Siddiqui *et al.*, 2020; Tanveer *et al.*, 2020). It has a hot semi-arid climate (Köppen climate classification BSh) with long, wet and extremely hot summers, dry and warm winters, annual monsoons and dust storms. It experiences a long rainy season (from end of June to mid-September), which increases the humidity of the area. Considerable macrofungal diversity was recorded in Lahore district with around 100 species of macrofungi (mostly of the order *Agaricales*) belonging to 11 families reported so far (Ahmad *et al.*, 1997; Tayyaba, 2020).

*Morphological characterization*

Macroscopic characteristics of the fresh basidiomata were noted in the field and some from the tagged photographs as mentioned in Hussain *et al.* (2018). The Munsell soil color chart was followed for color codes (Munsell, 1994). Microscopic features were described using a trinocular OLYMPUS CH30 microscope. The color of microscopic structures was observed in 5–10% KOH solution. Microscopic structures were stained with 1% Congo red solution. Twenty-five basidiospores, basidia, and cystidia were measured from 4 basidiomata. The abbreviation [n/m/p] indicates 'n' basidiospores measured from 'm' basidiomata of 'p' collections. Basidiospore dimensions were recorded as '(a–) b–c (–d)', where 'a' = extreme minimum value, range 'b–c' contains at least 90% of the calculated values, and 'd' = extreme maximum value of basidiospores. Fungal species names with authorities were retrieved from Index Fungorum (2021).

*Molecular characterization*

DNA extraction from fresh basidiomata followed the 2% CTAB method of Bruns (1995). Amplification of specific nc rDNA regions, including ITS and LSU was done using a Thermal cycler (Bio-RAD T100). Primers used during amplifications were ITS1F 5'-CCT GGT CAT TTA GAG GAA GTA A-3' as forward and ITS4 5'-TCC TCC GCT CTA TTG ATA TGC-3' as reverse for ITS region (>750 bp) while LROR 5'-ACC CGC TGA ACT TAA GC-3' as forward and LR5 5'-TCC TGA GGG AAA CTT CG-3' as reverse for LSU region (>1,300bp) (Gardes & Bruns, 1993; Usman & Khalid, 2020a; White *et al.*, 1990). PCR protocol consisted of 35 cycles of denaturation at 94 °C for 30 sec, annealing at 54 °C for 1 min, extension at 71 °C for 2 min while initial denaturation at 94 °C for 2 min and a final extension at 71 °C for 5 min as in Usman & Khalid (2020b).

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### Alignment and phylogenetic analyses

The obtained sequences of ITS and LSU regions for MU01-21 and MU02-21 were assembled using BioEdit v. 7.2.5. (Alzohairy, 2011). For ITS phylogenetic analyses, sequences were selected from an initial NCBI BLAST search comprising results that had identity of more than 89%; the closest one was AY461837 named as *Coprinus cordisporus* with 97% identity and 93% query cover. Then the remaining sequences of genus *Narcessea* were added in the dataset from publications not included through the initial NCBI BLAST search (Keirle *et al.*, 2004; Wächter & Melzer, 2020). In the final data set, 43 sequences were included out of which two sequences of *Coprinellus campanulatus* S. Hussain & H. Ahmad (2018: 48) were used as an outgroup (MH753666 and MH753667). For LSU phylogenetic analyses all sequences of genus *Narcessea* were added as ingroup while MW621007 named *Coprinellus andreorum* Sammut & Karich (2021: 24) was used as an outgroup taxon. For the final alignment of both region's datasets Bioedit was used for Clustal W method. Phylogenetic trees were constructed with the Maximum Likelihood (ML) method using RAXML-HPC2 v. 8.2.12 as implemented on the CIPRES portal with 1000 bootstrap replicates for both regions (Miller *et al.*, 2010; Stamatakis, 2014).

For Bayesian analyses, Markov Chain Monte Carlo (MCMC) coalescent approach implemented in BEAST v 1.10.4 (Rambaut *et al.*, 2018) was used. Four independent runs were conducted, and chain length was 1 million generations with a sampling frequency of 1000. Tracer v 1.10.4 was used for the Burn-in value determination (Rambaut *et al.*, 2018) with effective sample sizes (ESS) higher than 300. Maximum Clade Credibility (MCC) tree was generated using Log Combiner v 1.10.4 and Tree Annotator v 1.10.4 (Rambaut *et al.*, 2018). Posterior probabilities (PPs) of  $\geq 0.80$  were considered significant and mentioned at nodes. Phylogenetic trees were visualized using FigTree v. 1.4.2 (Rambaut, 2012).

## RESULTS

### Phylogenetic analyses using ITS region (Fig. 5, Table 1)

Sequences of the nc rDNA ITS region from two basidiomata collections, MU01-21 (LAH36395) and MU02-21 (LAH36970), were successfully obtained. The final dataset after multiple sequence alignment consisted of 713 and 692 base pairs, respectively. The closest sequences in the same clade were named as uncultured fungus (MW215870) and uncultured *Coprinus* (MH161295), respectively, without morphological description. These will be considered as dark taxa as those are DNA sequences obtained from environmental metabarcoding via high-throughput sequencing (HTS) (Jeszeová *et al.*, 2018; Marčiulyrienė *et al.*, 2021). The closest sister clade consists of four sequences (AY461831, AY461841, AY461816 and AY461817) assigned as *Coprinus cordispora* B clade in Wächter & Melzer (2020). Those sequences belong to dark taxa and have no morphological description for comparison. The ITS phylogenetic analyses support the distinctiveness of *N. lahorensis* from all other analyzed species with very strong bootstrap values.

### Phylogenetic analyses using LSU region (Fig. 6, Table 1)

The sequences of nc rDNA LSU region from two basidiomata collections MU01-21 (LAH36395) and MU02-21 (LAH36970) consisted of 916 and 904 base pairs, respectively. The closest sequences in the tree were identified as belonging to *Coprinus patouillardii* (FN396197 and FM876265) forming a sister clade, the species characterized by deliquescent fruiting bodies, but morphological description was not available for comparison with our material (Nagy *et al.* 2011). Other close sequences in the tree were identified as belonging to *Coprinus patouillardii* (FN396195 and FN396196) and *Coprinus cordisporus* (AF041511) with no morphological description included (Nagy *et al.*, 2011; Wächter & Melzer, 2020). Judging from phylogenetic analysis based on nc rDNA LSU region *N. lahorensis* is a distinct species with a strong bootstrap value, and clearly different from other species in the tree.

## TAXONOMY

***Narcessea lahorensis* Usman & Khalid, sp. nov. (Figs. 1–3)**

Mycobank MB 840992

**Etymology:**—The specific epithet "*lahorensis*" (Latin) refers to Lahore, the district where the type locality is placed.

**Diagnosis:**—*Narcessea lahorensis* is different from its closest species *N. patouillardii* by having short stipe, up to 20 mm in height, greyish white in color, swollen at the base, dull yellowish orange center of the pileus, dimorphic basidia, hymenial cystidia partly utriform.

**Holotype:**—PAKISTAN. Punjab, Lahore, Allama Iqbal Hall, Boys Hostel no 3, (31°29'57.60"N, 74°18'33.86"E) 213 m a.s.l, January 28, 2021, *Muhammad Usman*, MU01-21 (LAH36395), GenBank: OK161241 (ITS) and OK161263 (LSU).

### Description

*Basidiomata* small.

*Pileus* 10–18 mm in diam., fleshy, plano-convex when mature, pruinose, covered with whitish floccose veil, pale gray (2.5YR 5/1) to light gray (10YR 7/1), surface rough, deeply plicate from margin to center, center not plicate and dull yellowish orange (10YR 7/4).

*Lamellae* deliquescent, free, close, with regular lamellulae of 1 – 2 tiers, thin, fragile, light brown (5YR 6/4) with brownish black edge (5YR 3/1).

*Stipe* 13 – 20 × 0.8 – 1.8 mm, central, cylindrical, tapering upwards, swollen at base, shiny, delicate, smooth, grayish white (HueN8/0). *Annulus* and *volva* absent.

*Odor* and *taste* not recorded.



Fig. 1. A-E. Fresh basidiomata of *Narcissea lahorensis* (holotype)

M. Usman

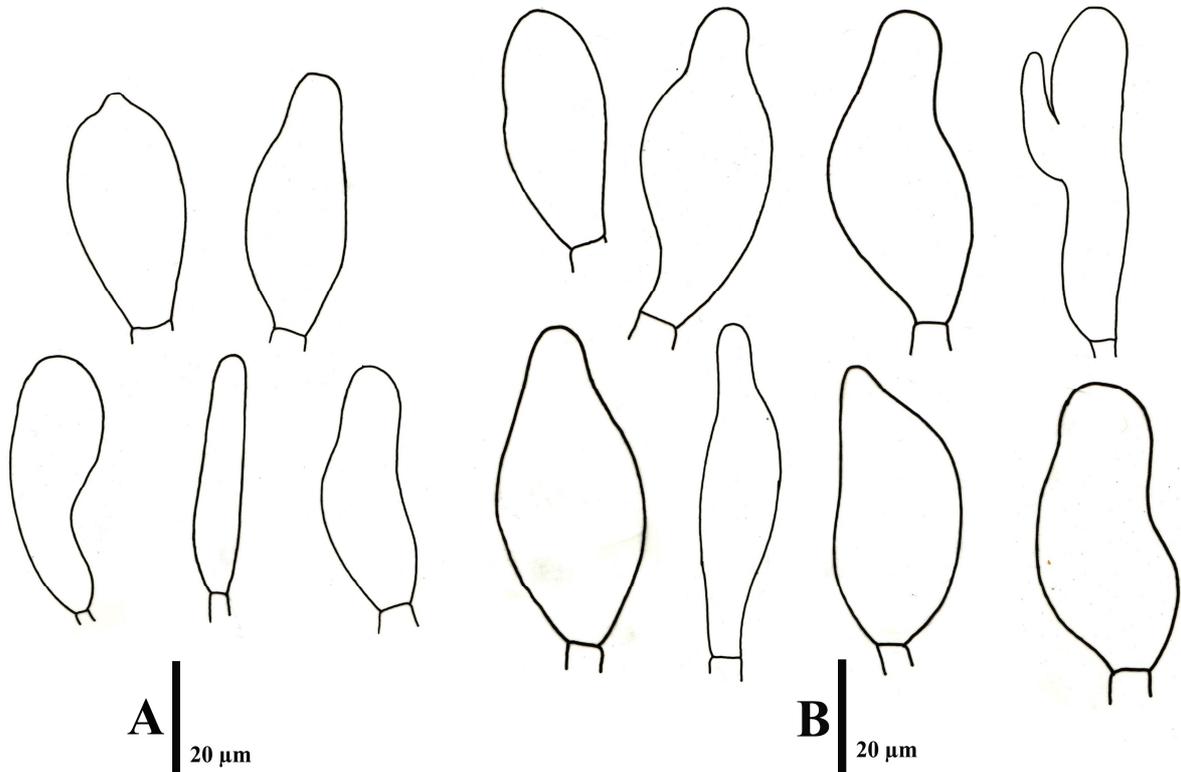


Fig. 2. Line drawings of microscopic elements of *Narcissea lahorensis* (holotype):  
A. cheilocystidia; B. pleurocystidia

M. Usman

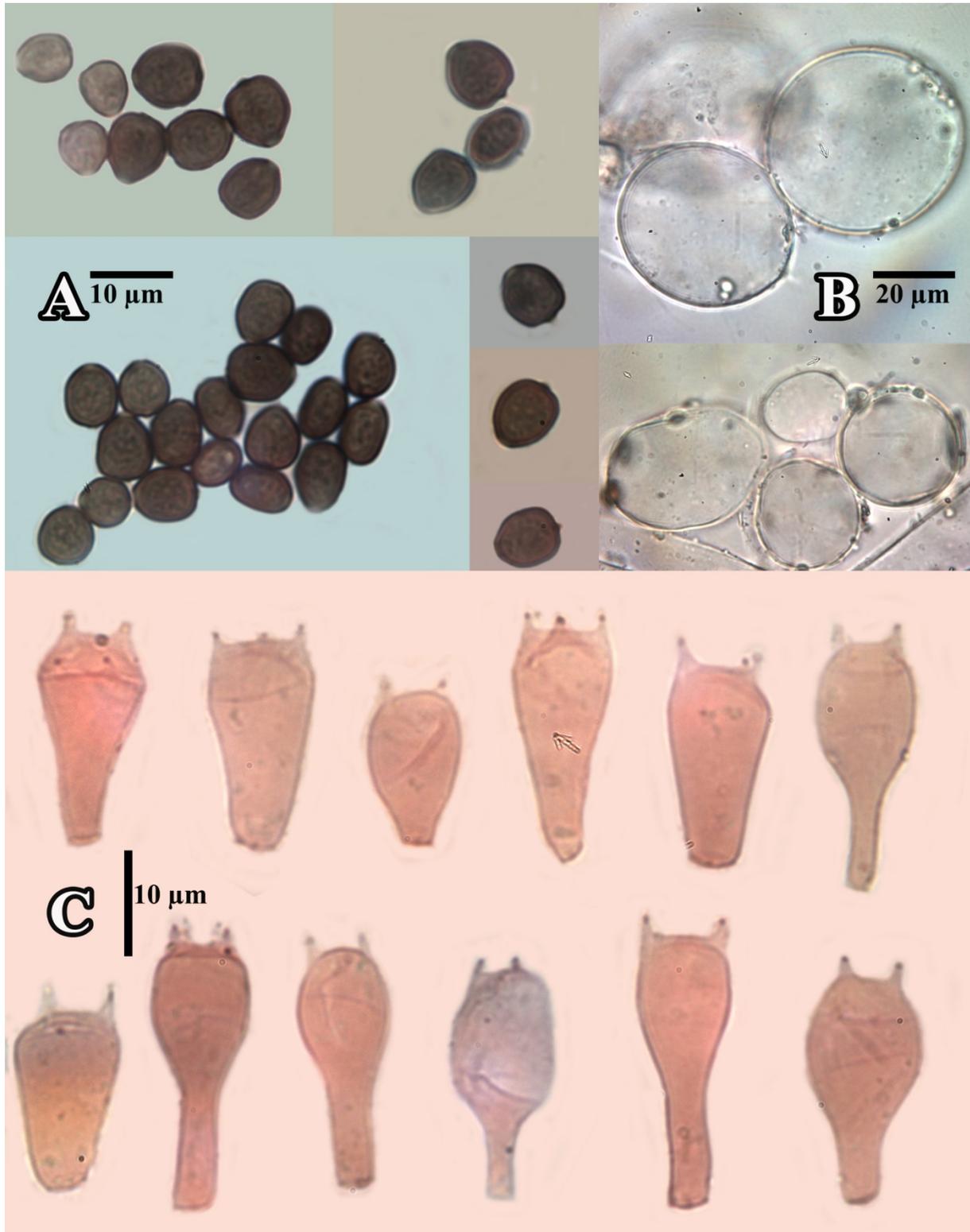


Fig. 2. A-G. *Narcissea lahorensis* (holotype): A. basidiospores; B.veil elements; C.basidia

M. Usman

*Basidiospores* [80/2/4], (6.1–) 7.6 – 9.3 (–9.8) × (5.1–) 6.2 – 8.1 (–8.7) μm, Q= 1.1 – 1.4, Q<sub>av</sub> = 1.3, limoniform to rectangular or sometimes almost heart-shaped in frontal view, oblong in profile and polar views, smooth, thick-walled, non-guttulate, with central germ pore, brownish black (7.5YR 3/1) to reddish brown (4/2).

*Basidia* 15.6 – 28.8 × 8.6 – 11.5 μm, hyaline, 4-spored (sometimes also 2-spored), dimorphic, short and long narrowly clavate to clavate.

*Cheilocystidia* 35.9 – 47.4 × 8.8 – 16.6 μm, polymorphic, some ovoid and cylindrical, others narrowly utriform to utriform, hyaline, thin-walled, oil droplets present.

*Pleurocystidia* 42.9 – 60.1 × 16.9 – 23.6 μm, partially like the cheilocystidia but larger, polymorphic, varying from utriform to pyriform, thin-walled, hyaline.

*Pileipellis* composed of hyphae 2.3 – 3.4 μm in width, branched, clamp connection absent, hyaline, thin walled.

Veil elements 25.10 – 47.05 µm in diam., globose to sub-globose, hyaline, thin-walled.

Stipitipellis composed of hyphae 3 – 7 µm in width, unbranched, clamp connection absent, hyaline, thin walled.

Habit and habitat:—Solitary to gregarious on moist soil near sanitary points, under a tree of *Mangifera indica* L. Basidiomata were collected during the winter season (January-February).

Additional specimen examined:—PAKISTAN. Punjab, Lahore, Hostel Road, (31°30'0.58"N, 74°18'32.79"E) 213 m a.s.l. February 5, 2021, *Muhammad Usman*, MU02-21 (LAH36970), GenBank: OK161242 (ITS) and OK161264 (LSU).

DISCUSSION

Recently, Wächter & Melzer (2020) have revised the classification of coprinoid fungi and erected new genera for better understanding of the family *Psathyrellaceae*. The newly proposed taxon belongs to the genus *Narcissea* which was represented by only two species so far, *N. patouillardii* and *N. cordispora*. Phylogenetically, the genus is different from its closest genera i.e. *Coprinellus* P. Karst. and *Tulosesus* Wächter & A. Melzer (Wächter & Melzer, 2020), the latter, however, considered a superfluous synonym of *Coprinellus* by Voto's online key ([www.ameronlus.it/chiavi\\_micologia.php](http://www.ameronlus.it/chiavi_micologia.php)). *Coprinellus* and *Tulosesus* morphologically also differ from *Narcissea* by truncate ellipsoid, obovoid to amygdaliform basidiospores and the absence of veil elements, respectively (Wächter & Melzer, 2020).

*N. lahorensis* has distinct features in comparison with other species of the genus, including a short stipe which is swollen at the base, and broader dimorphic basidia. Phylogenetically, most of the sequences included in the ITS analyses are obtained from soil samples via high-throughput sequencing (HTS) and directly submitted to the GenBank without any reference to a morpho-anatomical description and are known as dark sequences (Fig. 5 & Table 1). However, the sequence of the ITS region of *N. lahorensis* differs from the ITS sequences of *N. cordispora* (published by Örstadius, Ryberg & Larsson, 2015) and *N. patouillardii* (published by Nagy *et al.*, 2011) by 28 and 29 base pairs, respectively (Fig. 4).

*N. lahorensis* differs morphologically from the other species of the genus *Narcissea* by having a short stipe (13 – 20 mm) while both *N. patouillardii* and *N. cordispora* have longer stipe (up to 50 mm or more) (Uljé & Noordeloos, 1993; Uljé, 2005). Moreover, *N. cordispora* differs from *N. lahorensis* by larger spores (7.5 – 11.5 × 6.5 – 10.0 µm), a larger pileus (up to 25 mm diam. when expanded), and narrower basidia (7 – 10 µm broad) (Uljé & Noordeloos, 1993; Uljé, 2005). *N. lahorensis* is characterized by broad dimorphic basidia (15.6 – 28.8 × 8.6 – 11.5 µm), partly utriform pleurocystidia and polymorphic cheilocystidia (35.9 – 47.4 × 8.8 – 16.6 µm). *N. patouillardii* is also different from *N. lahorensis* by having ellipsoid to sub-globose cheilocystidia and pleurocystidia, up to 50 × 40 µm large (Uljé & Noordeloos, 1993; Uljé, 2005).

With respect to the habitat all these species also differ. *N. lahorensis* occurs on soil near sewage places while *N. cordispora* is recorded on dung of several animals, and *N. patouillardii* is reported as growing on vegetable debris (Uljé & Noordeloos, 1993; Vila & Rocabruna, 1996, 2002). This addition of *N. lahorensis* will increase the information about the genus *Narcissea* within the family *Psathyrellaceae*.

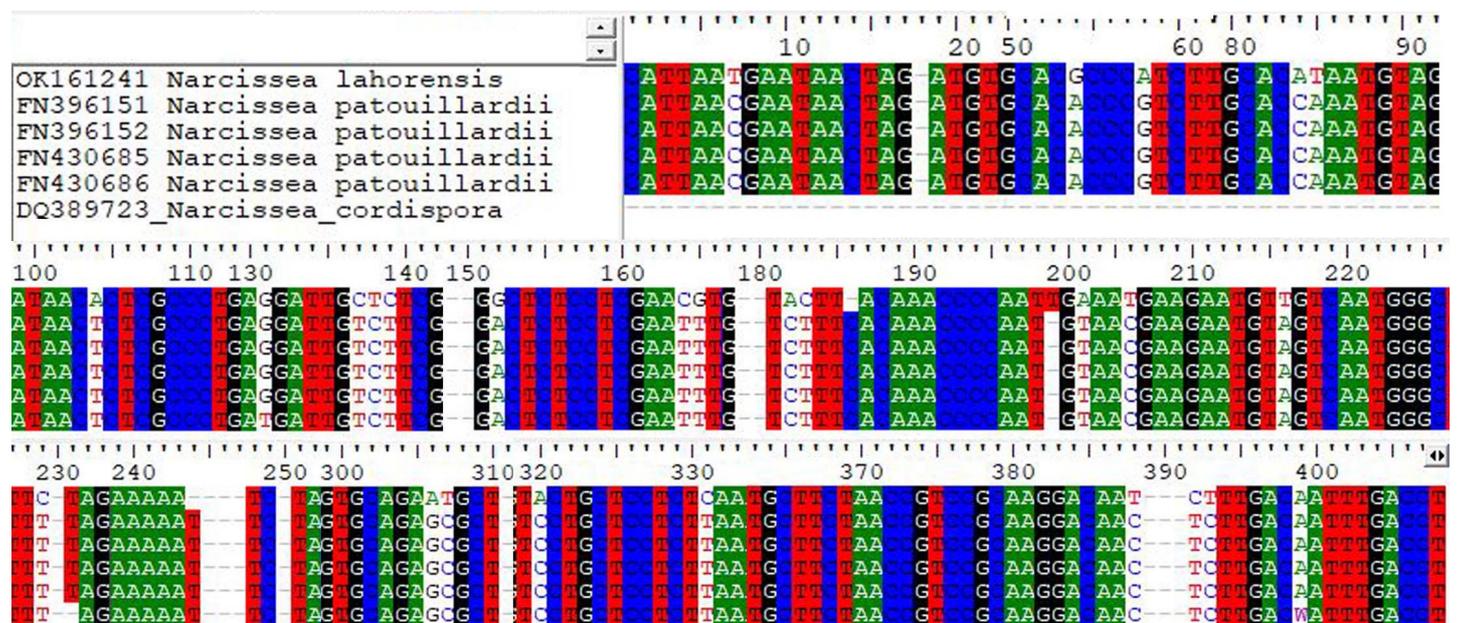
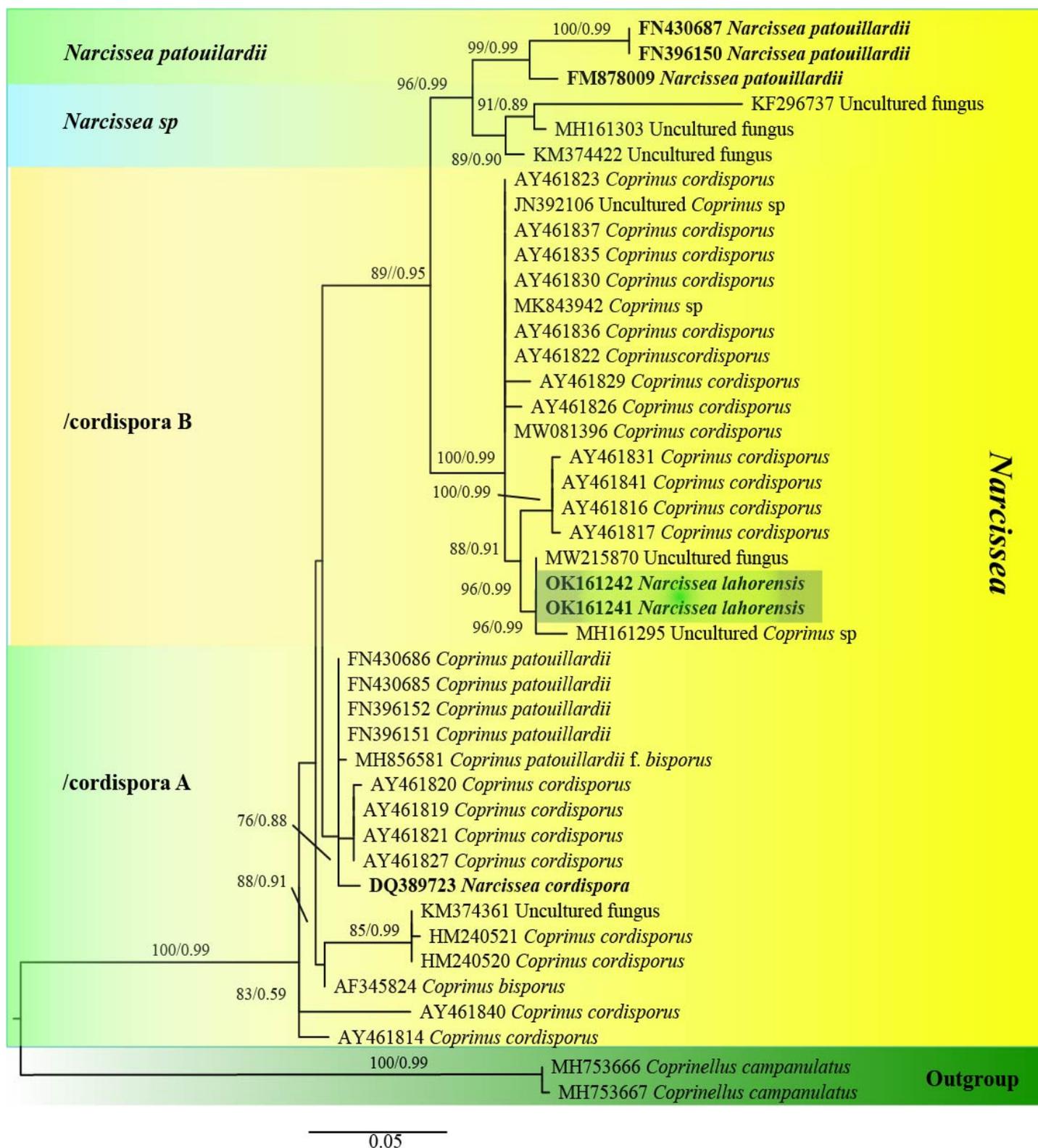


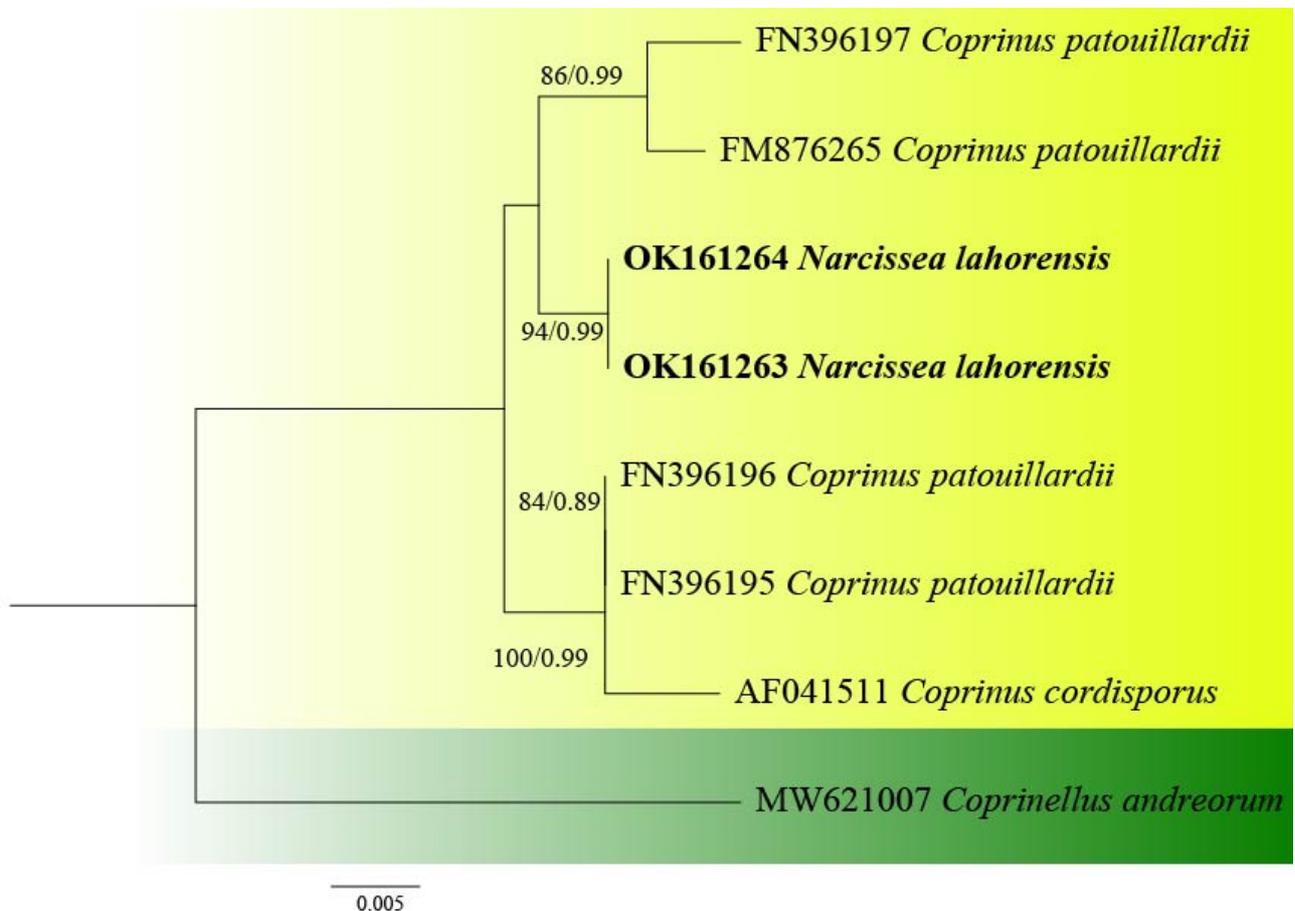
Fig. 4. Sequence key for *Narcissea lahorensis*, *N. patouillardii* and *N. cordispora* in Bioedit. Nucleotides differences along with positions in the final alignment of ITS dataset

Taxon	Voucher No.	GenBank	Accession	Reference
		ITS	LSU	
<i>Coprinus bisporus</i>	KACC49409	AF345824	-	Unpublished
<i>Coprinus cordisporus</i>	SFSU DEH2081	AY461830	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	5-F4	MW081396	-	Unpublished
<i>Coprinus cordisporus</i>	ROZ38	JN392106	-	Alvarado & Manjon (2013)
<i>Coprinus cordisporus</i>	SFSU DEH2046	AY461823	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	SFSU DEH1837	AY461822	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	SFSU MRK16	AY461837	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	SFSU MRK04	AY461835	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	SFSU DEH2074B	AY461829	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	SFSU DEH2071	AY461826	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	SFSU DEH2128B	AY461831	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	L Ulje1100	AY461841	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	SFSU DEH1084	AY461816	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	SFSU DEH1702	AY461817	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	SFSU DEH1813	AY461819	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	SFSU DEH1815	AY461820	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	SFSU DEH1829	AY461821	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	SFSU DEH2073	AY461827	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	UBC F19670	HM240520	-	Unpublished
<i>Coprinus cordisporus</i>	CCCM:108	HM240521	-	Unpublished
<i>Coprinus cordisporus</i>	L Ulje1058	AY461840	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	SFSU DEH000	AY461814	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	SFSU MRK06	AY461836	-	Keirle et al. (2004)
<i>Coprinus cordisporus</i>	-	-	AF041511	Hopple & Vilgalys (1999)
<i>Coprinus patouillardii</i>	SZMC-NL-1685	FN396151	FN396195	Nagy et al. (2011)
<i>Coprinus patouillardii</i>	SZMC-NL-1685	FN430686	-	Nagy et al. (2011)
<i>Coprinus patouillardii</i>	SZMC-NL-1695	FN396152	FN396196	Nagy et al. (2011)
<i>Coprinus patouillardii</i>	SZMC-NL-1695	FN430685	-	Nagy et al. (2011)
<i>Coprinus patouillardii</i> f. <i>bisporus</i>	CBS 172.50	MH856581	-	Vu et al. (2019)
<i>Coprinus</i> sp	SFC 2978	MK843942	-	Unpublished
<i>Narcissea cordispora</i>	LO41-010	DQ389723	-	Larsson & Örstadius (2008)
<b><i>Narcissea lahorensis</i></b>	<b>LAH36395</b>	<b>OK161241</b>	<b>OK161263</b>	<b>This Study</b>
<b><i>Narcissea lahorensis</i></b>	<b>LAH36970</b>	<b>OK161242</b>	<b>OK161264</b>	<b>This Study</b>
<i>Narcissea patouillardii</i>	SZMC-NL-1684	FN396150	FN396197	Nagy et al. (2011)
<i>Narcissea patouillardii</i>	SZMC-NL-1684	FN430687	-	Nagy et al. (2011)
<i>Narcissea patouillardii</i>	SZMC-NL-1687	FM878009	FM876265	Nagy et al. (2011)
Uncultured <i>Coprinus</i> sp	K-5-16-41	MH161295	-	Jeszeová et al. (2018)
Uncultured fungus	3993_2328	MW215870	-	Marčiulyrienė et al. 2021
Uncultured fungus	2_NA6_P31_C13	KF296737	-	Timling et al. (2014)
Uncultured fungus	K-7-16-45	MH161303	-	Jeszeová et al. (2018)
Uncultured fungus	-	KM374422	-	Johnson et al. (2014)
Uncultured fungus	-	KM374361	-	Johnson et al. (2014)
<b>Outgroup</b>				
<i>Coprinellus campanulatus</i>	SH144T	MH753667	-	Hussain et al. (2018)
<i>Coprinellus campanulatus</i>	SHP144	MH753666	-	Hussain et al. (2018)
<i>Coprinellus andreoreum</i>	CS1247	-	MW62100	Sammut & Karich (2021)

**Table 1.** Fungal taxa, Voucher numbers, GenBank accession numbers and references of the sequences used in the phylogenetic analyses of ITS and LSU



**Fig. 5.** Phylogram generated from Maximum Likelihood (ML) analyses and Bayesian Inference (BI) method based on ITS sequence data representing *Narcissea lahorensis* and its related species. Bootstrap values >70% based on 1000 replicates for ML and for BI  $\geq 0.80$  are shown at the branches as ML/BI



**Fig. 6.** Phylogram generated from Maximum Likelihood (ML) analyses and Bayesian Inference (BI) method based on LSU sequence data representing *Narcissea lahorensis* and its related species. Bootstrap values >70% based on 1000 replicates for ML and for BI  $\geq 0.80$  are shown at the branches as ML/BI

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