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A new species of *Lepiota, Lepiota lahorensis*, from Lahore, Pakistan

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Abstract: *Lepiota lahorensis* sp. nov. is described from Lahore, Pakistan. Its characterizing features are pinkish brown pileus with dark brown furfuraceous scales, oblong to ellipsoid spores, clavate cheilocystidia, and pileus covering made up of trichodermal elements without shorter elements at their base. The phylogenetic analysis based on nuclear ribosomal internal transcribed spacer sequence shows its unique position in sect. *Ovisporae*.

Key words: *Lepiota farinolens*, nrDNA, *Ovisporae*, phylogeny, Punjab plains

1. Introduction

The family Agaricaceae is a saprotrophic group of fungi exhibiting wide diversity in macro and microscopic features. With the advent of DNA ribotyping, some secotioid genera have also been included in this family, e.g., *Endoptychum agaricoides* Czern., *Podaxis pistillaris* (L.) Fr., and *Longula texensis* (Berk. & M.A. Curtis) Zeller. Morphologically the family was divided earlier in four tribes (Singer, 1986; Wasser, 2002), but later tribe Cystodermae was excluded, which has also been confirmed by molecular studies (Johnson and Vilgalys, 1998; Moncalvo et al., 2002). The other three tribes are Agariceae, Lepioteae, and Leucocoprinae (Singer, 1986; Vellinga, 2004).

Lepioteae comprises species with nonmetachromatic white spores and regular lamella trama, while Leucocoprinae is represented by taxa having trabecular lamellar trama and metachromatic spores, without or with a germ pore. Bon (1993) combined these two tribes in a separate family, Lepiotaceae, that differs from Agaricaceae in having white, or rarely green spore print (Vellinga, 2004). This separation is not borne out by the molecular-phylogenetic evidence and both are placed again in the Agaricaceae.

Morphological and molecular studies have shown that *Lepiota* is not monophyletic, but forms a monophyletic group together with *Cystolepiota*, *Melanophyllum*, and *Pulverolepiota* (Liang et al., 2011).

The morphological differences among *Lepiota* species are often subtle (Vellinga and Huijser, 1999). The analysis of the nrITS (nuclear ribosomal internal transcribed spacer) region has made identification of species much easier and more accurate. It has proven to be an influential tool to reveal specific and intraspecific variations within the species of *Lepiota* (Vellinga, 2003).

The genus *Lepiota* is represented by more than 400 species (Kirk et al., 2008; Kumar and Manimohan, 2009). These white-gilled mushrooms are well studied in Europe, but reports from tropical and subtropical regions are few (Sysouphanthong et al., 2011). In Asia, Pegler (1972) presented a revision of *Lepiota* species from Sri Lanka; Manjula (1983) and Kumar & Manimohan (2009) carried out studies in India; in Pakistan Ahmad et al. (1997) contributed to the exploration of lepiotaceous fungi. Recently efforts are being made to explore the diversity of these white-gilled species within Pakistan using molecular markers (Razaq et al., 2012a, 2012b; Nawaz et al., 2013; Qasim et al., 2015), and by now 30 species have been reported from Pakistan (Ahmad et al., 1997; Razaq et al., 2012a, 2012b; Nawaz et al., 2013; Qasim et al., 2015). Here, we describe a new species of *Lepiota* from Pakistan using nrITS analysis combined with morphological observations as part of our ongoing efforts to catalogue the species of Lahore.

2. Materials and methods

2.1. Macro- and microscopic analysis

The studied samples were collected and photographed in natural light using a Nikon D70s. Macromorphological studies were conducted with fresh specimens and

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photographs. Color codes were assigned to the morphological characters following Munsell's Soil Color Chart (1975). Microscopic characters were studied from material mounted in water, 5% KOH, phloxine or Melzer's reagent. Measurements were made at 40× and 100×. Twenty spores per basidiocarp were measured. The notation [n/m/p] indicates that measurements were made on n basidiospores, from m basidiocarps and p collections. The dimensions of the microscopic features are given according to the equation (a–) b–c (–d), extreme values are given in parentheses, and the range b–c indicates a minimum of 90% of the measured values. Drawings were made using a camera lucida. The following abbreviations were used for descriptions: avl = average length; avw = average width, Q = the quotient of length and width of spores. The terminologies used for descriptions follow Vellinga (2001). The specimens were deposited at the LAH Herbarium, University of Punjab, Lahore.

2.2. DNA extraction amplification and sequencing

Genomic DNA of specimens was extracted following a modified CTAB method (Bruns, 1995). About 1 mg of tissue was used for extraction. The ITS region of DNA was amplified using ITS1F/ITS4 primers (Gardes and Bruns, 1993). The amplified products were sequenced using Macrogen. The sequences were submitted to GenBank (information on the sequences is presented in the Table).

2.3. Sequence alignment, dataset assembly, and phylogenetic analysis

The sequences obtained were checked and compared with other sequences available in GenBank using the BLAST search tool. The top 100 sequences in BLAST analysis belonging to *Lepiota* were downloaded. Of these, 57 sequences representative of different sections were used for phylogenetic analysis. The sequences were aligned through the Muscle Multiple Sequence Alignment search tool and edited using BioEdit. The final aligned data set was used for phylogenetic analysis. A phylogenetic tree was constructed using the maximum likelihood method of the Jukes–Cantor model.

3. Results

Lepiota lahorensis T. Qasim and A.N. Khalid sp. nov. (Figures 1 and 2)

MycoBank # MB812877

Holotype: Pakistan. Punjab, Lahore, on rich loamy soil in lawns of the University of Punjab, alt. 217 m, T. Qasim & A. N. Khalid 21 September 2012 (T18, LAH. NO. 10002012).

Etymology: The specific epithet 'lahorensis' (*L.*) refers to the name of the city where the specimens are collected.

Pileus planoconvex, with pinkish brown central disc, dark brown and furfuraceous squamules, and appendiculate margin. Lamellae subdistant, alternate with lamellulae. Stipe equal to subequal, surface scaly, with white-flocculose scales. Basidiospores oblong to ellipsoid, dextrinoid. Basidia 4-spored, clavate. Cheilocystidia clavate. Pileus covering with long, trichodermal elements.

Pileus 10–30 mm in diameter, plane to planoconvex with obtuse to slightly distinct umbo, pinkish brown (10R4/8); central disc distinct from rest of pileus with dark brown furfuraceous squamules; scales continuous and smooth at center, becoming minute and sparse towards margin; background white; margin appendiculate; context white, moderately thick and fleshy. *Lamellae* free and approximate, subdistant, white to cream (7.5YR9/2), with entire edge; lamellulae present in 1–2 tiers. *Stipe* 42–48 × 3–4 mm, attached centrally, equal to sub equal, light brown to dark brown (7.5R3/6); surface scaly, white flocculose scales, base equal; *Volva* absent; context moderately thick, hollow. *Annulus* present, nonpersistent. *Odor* and *Taste* not recorded.

Basidiospores [80/4/4], (8.0–) 8.3–11.6 (–12.4) × (5.9–) 6.1–8.3 (–8.5) µm, avl × avw = 9.3 × 7.1 µm, Q = 1.36–1.47, avQ = 1.39, ellipsoid, thick-walled, with prominent apiculus, dextrinoid, smooth, monoguttulate. *Basidia* (26.4–) 27.1–29.7 (–32.6) × (11.8–)12.0–12.9(–13.7) µm, avl × avw = 28.8 × 12.3 µm, clavate, thin-walled, hyaline in KOH, guttulate, smooth, 4-spored. *Cheilocystidia* (22.2–) 24.5–29.9 (–31.4) × (10.4–) 10.9–12.9 (–13.5) µm, avl × avw = 26.5 × 11 .8 µm, clavate, thin-walled, hyaline in KOH, with oil-like contents, smooth. *Pileipellis* a trichoderm consisting of thin-walled, broadly clavate elements with clamp connection at base, (37.8–) 45.1–84.3 (–85.4) × (12.3–) 12.5–23.1 (–23.8) µm, avl × avw = 64.5 × 16.7 µm, hyaline to pale brown in KOH. *Stipitipellis* a cutis made of hyphae 4.9–7.8 µm wide, avw = 6.0 µm, smooth, thick-walled, hyaline to pale, parallel arranged, with ascending, clavate, long trichodermal elements, (27–) 44–73 (–74) × (14–) 15–21 (–26) µm, avl × avw = 54 × 17 µm. Clamp connections are present in all tissues.

Habitat and distribution: Saprotrophic and solitary to scattered on nutrient-rich loamy soil under planted angiospermic vegetation. So far only known from lowland northeastern Pakistan.

Additional specimens Pakistan, Punjab, Botanical Garden, University of Punjab, Lahore, at 217 m a.s.l., scattered on rich loamy soil on grounds of garden, 12 September 2014, A.N. Khalid (TQ2, LAH. NO. 10102014); University of Punjab, Lahore, on university grounds, 13 September 2014, A.N. Khalid & Tayyaba Qasim (TQ3, LAH 10112012); University of Punjab, Lahore, on rich loamy soil, 7 September 2014, A.N. Khalid & Tayyaba Qasim (TQ15, LAH. NO. 10122014).

3.1. Molecular and phylogenetic analysis

Polymerase chain reaction of the target region of extracted DNA yielded a fragment of 600–630 bp. The initial BLAST analysis showed maximum match with the sequence

Table. nrITS sequences of taxa included in the DNA analysis.

Species name	Geographic origin	ITS GenBank accession #
Lepiota sp.	Thailand	JN224828
Lepiota sp.	Thailand	JN224826
Lepiota sp.	Thailand	HQ647293
Lepiota sp.	USA	AY176478
Lepiota sp.	USA	AY176402
Lepiota sp.	USA	GQ203810
L. sp. *vellingana*	USA	AY176485
L. farinolens	USA	AY176368
Lepiota sp.	USA	AY176400
L. albogranulosa	Pakistan	LK932284
L. albogranulosa	Pakistan	LK932285
L. alopochroa	Thailand	HQ647294
L. andegavensis	USA	AY176461
L. boudieri	USA	AF391025
L. boudieri	USA	FJ998388
L. brunneoincarnata	USA	FJ998395
L. brunneoincarnata	China	FJ481017
L. brunneoincarnata	the Netherlands	AF482875
L. castanea	Canada	JN021056
L. coloratipes	China	KC819622
L. cristata	China	EU081944
L. cristata	China	EU081950
L. cristata	China	EU826490
L. cristata	USA	AF391051
L. cristata	USA	U85327
L. cystophoroides	USA	AF391031
L. cf. fraternal	Thailand	JN224823
L. erminea	the Netherlands	AY176470
L. elaiophylla	USA	AF391024
L. felina	USA	U85330
L. himalayensis	Pakistan	HE614898
L. ignicolor	the Netherlands	AY176472
L. lahorensis	Pakistan	KT182475

Table. (Continued).

L.lahorensis	Pakistan	KT186607
L. lahorensis	Pakistan	KT186608
L. lahorensis	Pakistan	KT186609
L. magnispora	Germany	AF391005
L. neophana	USA	GQ203809
L. pilodes	USA	AY176476
L. psalion	the Netherlands	AY176390
L. revelata	China	GU199359
L. rhodophylla	USA	EF080864
L. rhodophylla	USA	AY176480
L. rufipes	the Netherlands	AF391066
L. scaberula	USA	AF391029
L. scaberula	USA	AF391030
L. subalba	the Netherlands	AY176489
L. subgracilis	the Netherlands	AY176490
L. subincarnata	the Netherlands	AY176491
L. thiersii	USA	GQ203817
L. tomentella	USA	EF080868
L. vellingana	Pakistan	HE974765
L. vellingana	Pakistan	HE974764
L. vellingana	Pakistan	HE974766
L. xanthophylla	the Netherlands	AY176405
Cystolepiota cystophora	USA	GQ141550
Agaricus campestris	China	FJ223223

of *Lepiota farinolens* Bon & G. Riousset (GenBank AY176368).

The phylogenetic analysis showed that *Lepiota lahorensis* clusters with the sequences of *Lepiota* belonging to section *Ovisporae* (J.E. Lange) Kühner. In the maximum likelihood tree three clades are formed (Figure 3). All the taxa belonging to section *Ovisporae* with no short elements at the base of the long cells of the pileus covering clustered together in clade C. In this clade *Lepiota farinolens* is the closest relative of *Lepiota lahorensis.* Besides this, *Lepiota himalayensis* Khalid & Razaq (HE614898), *Lepiota vellingana* R. Nawaz & A.N. Khalid (HE974764, HE974765, HE974764), *Lepiota brunneoincarnata* Chodat & C. Martin (FJ99839, FJ481017, AF482875), and *Lepiota subincarnata* (AY176491) form a sister clade with *Lepiota lahorensis* (KT182475, KT186607, KT186608, KT186609). All the taxa present in this clade, reported from Pakistan, Europe, and North America, are characterized by pileal covering having only long elements. The species belonging to this clade are highly toxic (Gérault and Girre, 1975). Additionally, taxa with a pileal covering having shorter elements at the base of longer elements form a separate clade (Figure 3, clade A), whereas the taxa with hymeniform pileus covering cluster together in clade B.

4. Discussion

Lepiota lahorensis possesses a pinkish brown pileus with brown squamules, subdistant lamellae, a trichodermal

Figure 1. Basidomata of *Lepiota lahorensis* in their natural habitat. Scale bars: A–F = 1 cm.

pileus covering with longer clavate elements and without shorter elements at the base; scales at the stipe also possess trichodermal structure, oblong spores, clavate cheilocystidia, and a unique ITS sequence. *Lepiota lahorensis* can be placed in sect. *Ovisporae* based on this combination of characters (Singer, 1986; Vellinga, 2001; Kumar and Manimohan, 2009; Razaq et al., 2012a, 2012b).

Three different clades of *Lepiota* were recovered during maximum likelihood analysis. *Lepiota lahorensis* clusters in clade C (long elements only), which comprises members of sect. *Ovisporae*. These species feature longer pileal elements and oblong basidiospores. *Lepiota lahorensis* groups with *L. farinolens*, *L. brunneoincarnata*, *L. subincarnata*, *L. himalayensis*, and *L. vellingana* (Figure 3).

Lepiota lahorensis is close to *L. farinolens* and *L. himalayensis*. *Lepiota farinolens* has pileus with reddish brown coloration, a wide distinct umbo, and bulbous stipe base with scattered scales, and farinaceous smell, which is unusual in the genus (Salmon and Siquier, 2001).

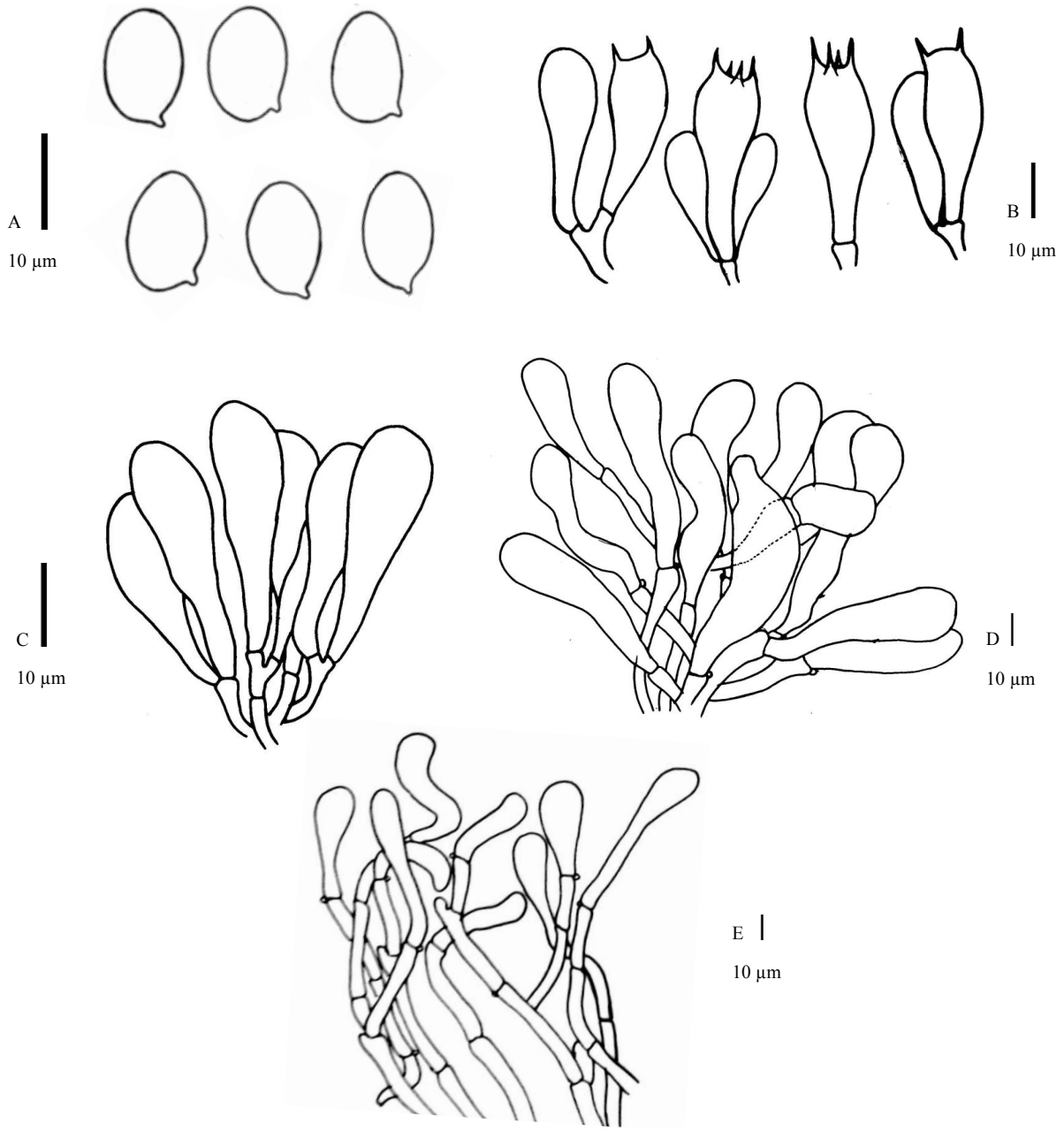

Figure 2. Microscopic structures of *Lepiota lahorensis*: A: Basidiospores; B: Basidia; C: Cheilocystidia; D: Pileus covering; E: Elements of scales on the stipe; Scale bars: A–E = 10 µm (Holotype).

Lepiota himalayensis from northeastern Pakistan is characterized by blackish scales on pileus, scales at the base of stipe, crowded lamellae, smaller spores [6.7–8.3 × 3.0–4.0 µm], clavate to narrowly clavate cheilocystidia, and longer trichodermal elements in the pileus covering.

Lepiota vellingana has also been reported from Lahore. It has brown scales on pileus, white to cream lamellae which make it close relative of *L. lahorensis*. However, there are certain features such as white pileus covering, crowded lamellae, pinkish brown scales at the stipe, smaller spores [6.3–8.5 × 4.2–6.6 µm], and longer trichodermal elements [80.5–197 × 6.5–8.5 µm], which make *L. vellingana* distinct from *L. lahorensis*. All Pakistani taxa of section *Ovisporae* cluster together in Clade C.

Lepiota revelata (Berk. & Br.) Sacc., described from the Sri Lankan section *Ovisporae*, has much smaller spores [3.5–5 × 2.3–3 µm (average 4.3 × 2.6 µm] and the elements of the pileus covering have distinctly rounded apices (Pegler, 1972).

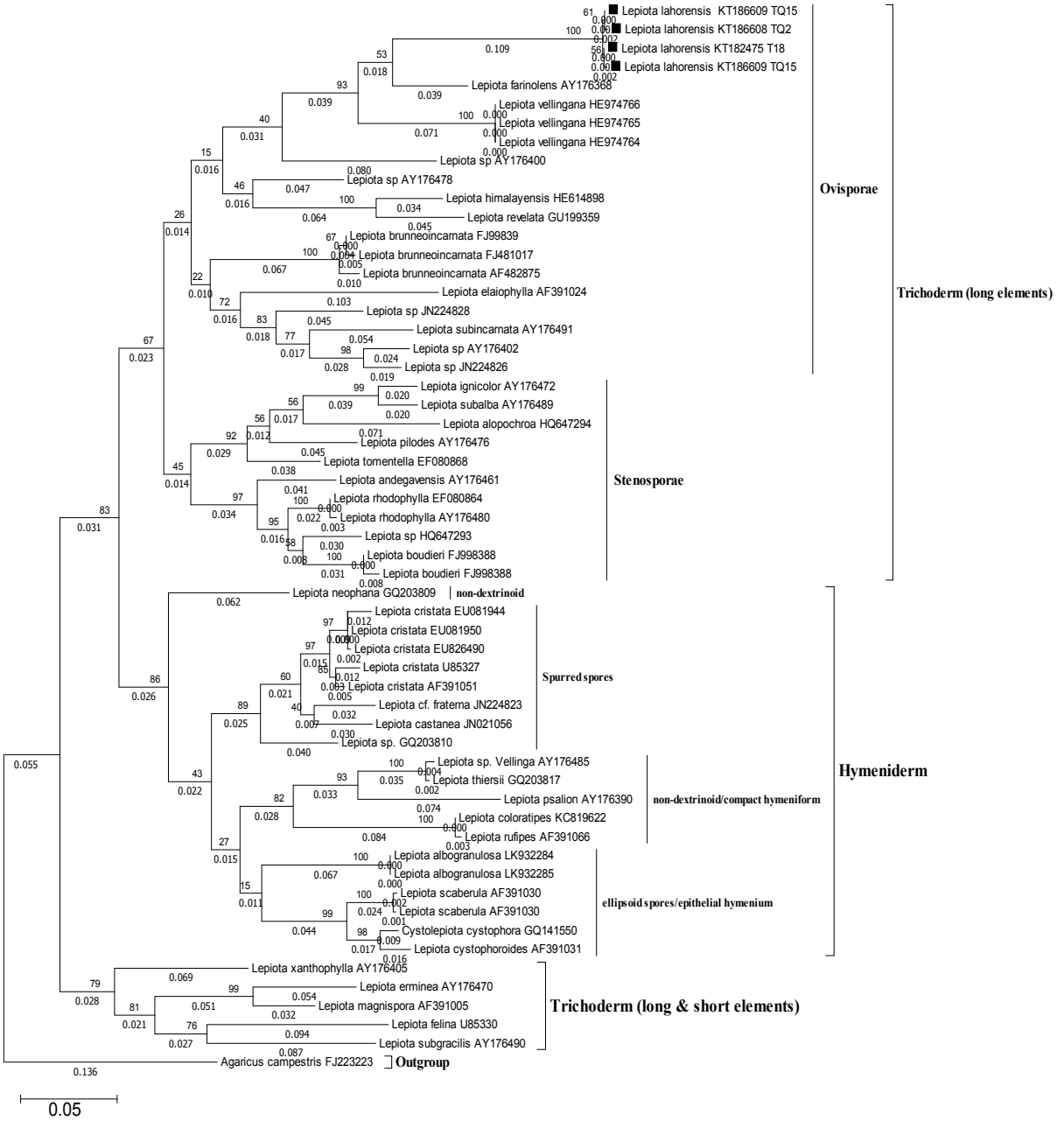

Figure 3. Molecular phylogenetic analysis by the maximum likelihood method, based on ITS sequences. The evolutionary history was inferred by using the maximum likelihood method based on the Jukes–Cantor model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The analysis involved 57 nucleotide sequences. There were a total of 766 positions in the final dataset. Evolutionary analyses were conducted in MEGA6. Bootstrap values >50% based on 1000 replicates are shown below the branches.

Lepiota brunneoincarnata, known from Europe (Vellinga, 2001), forms a sister clade to *Lepiota lahorensis*. This is a sturdy and relatively big species with distinct contrasting bands on the stipe. *Lepiota subincarnata* is a more pink-colored species, with a felted-squamulose pileus surface, and smaller spores than *L. lahorensis* [viz. (5.5) 6.0–7.5 (8.0) × 3.0–4.0(4.5) µm] (Vellinga, 2001).

In the phylogenetic tree, section *Stenosporae* also falls in clade C. The species in the respective section are distinguished from members of *Ovisporae* in having cylindrical spores with a spurred base (Vellinga, 2001). Section *Ovisporae* is divided into two subsections that are distinguished on the basis of the structure of pileal elements: subsection *Felininae* Bon harbors species with

long erect pileal elements with short clavate elements, while species whose pileus coverings are made up of long erect elements without short clavate elements belong to subsection *Helveolinae* Bon & Boiffard (Candusso and Lanzoni, 1990; Vellinga, 2001).

Thus *L. lahorensis* belongs to section *Ovisporae* and subsection *Helveolinae*. Phylogenetic analysis of the nrITS data supports this placement. Members of this subsection are notorious because of the presence of amanitins, which are highly toxic (Gérault and Girre, 1975).

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