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RESEARCH ARTICLE

Desert puffballs from Ladakh trans-Himalaya (J&K), India - the genus *Bovista* and *Calvatia*

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ABSTRACT: Present study emphasizes on the documentation of some hitherto interesting unrecorded puffballs from cold arid Ladakh region of Indian Trans-Himalaya, from where very few reports exist on the macrofungi. During the year 2011-13, five taxa of puffballs namely *Bovista minor*, *B. plumbea*, *B. plumbea* var. *dens-caulis*, *B. pusilla* and *Calvatia bovista* were recorded. Out of these, *B. minor* and *C. bovista* constitute new records for India whereas *B. plumbea* and *B. pusilla* were recorded for the first time from Ladakh region of Jammu and Kashmir State. In addition, *B. plumbea* var. *dens-caulis* is being proposed as a new variety. Detailed description of habitat, macro- and microscopic characteristics and their edibility have been provided with taxonomic key, photographs and illustrations.

Key words: Agaricomycotina, macrofungi, new records, taxonomy

Puffballs represent an important group of macromycetes having a closed basidiome, which either remains closed or opens only after the basidiospores mature. The spore producing tissue, the gleba, is enclosed by a wall or peridium, which may be composed of one or more distinct layers. The basidiospores are statismospores and are not forcibly ejected. Hawksworth *et al*. (1995) placed puffballs, as a distinct taxonomic group, in Gasteromycetes of subdivision Basidiomycotina within Basidiomycota. However, based on genetic and molecular studies, Kirk *et al*. (2008) treated them as a polyphyletic assemblage representing different orders within Agaricomycetes of subdivision Agaricomycotina and mentioned the existence of 55 (*Bovista*) and 40 (*Calvatia*) species worldwide while as Index Fungorum showed list of 195 and 138 records of these genera respectively. The five taxa described in the present communication include *Bovista minor*, *B. plumbea*, *B. plumbea* var. *dens-caulis*, *B. pusilla* and *Calvatia bovista*. Out of these, *B. minor* and *C. bovista* were recorded for the first time from India whereas *B. plumbea* and *B. pusilla* constituted first time record from Ladakh region of Jammu and Kashmir. A taxon, namely, *Bovista plumbea* var. *dens-caulis* var. nov. has been proposed as a new variety.

MATERIALS AND METHODS

Study area

Ladakh province of Indian Trans-Himalaya, representing one of the coldest and most elevated places on earth, is situated in Jammu and Kashmir state of Indian subcontinent. It is located between 32°15′ to 36° N Latitude and 75°15′ to 80°15′ E Longitude with an altitude ranging from 2,900 to 5,900 meter above mean sea level (Bhattacharyya, 1991). Covering an area of 82,665 sq. km in the inner belt of Karakoram and Greater Himalaya, Ladakh occupies more than half of the geographical area of Jammu and Kashmir. Having strategic geopolitical location, it shares international border with China (Aksai Chin) in northeast and Pakistan (POK) in northwest region where Line of control (LOC) separates India from two neighbouring countries (Fig. 1). Ladakh experiences extreme climate of temperate to alpine cold desert which is characterized by the intense heat and cold, barrenness and dryness, low oxygen and atmospheric pressure, fluctuating temperature (+35°C to –35°C) and low annual precipitation (snowfall/rainfall) of 80-300 mm, and consequently, the low relative humidity (<30%) making it cold arid region (Pal Murugan *et al*., 2010).

Methods

Surveys were conducted in various location of Leh district of Ladakh region in Jammu and Kashmir during 2011 to 2013. All the taxonomically important characters pertaining to gross morphology of sporocarps were recorded in the field. Sporocarps were collected and field records for habitats were made. For field photography, digital camera (DSC-W55, Sony cyber-shot 7.2 MP) was used. Microscopic observations were made by mounting the dried materials in 5% ethanol and 3% aqueous KOH solution, and then staining with 1% aqueous solution of Congo red. Besides, 1% Iodine and 5% KOH solutions were used for spore colour reactions. For microscopic size range, more than 30 basidiospores, basidia and other elements were measured under various magnifications. Microscopic characteristics were drawn with the help of Camera lucida fitted on compound microscope (Olympus). Identification and description was done by using relevant literature (Smith, 1951; Arora, 1986; Smith *et al*., 1981; Kirk *et al*., 2008). The examined samples have been deposited in the Herbarium of Botany Department, University of Jammu (HBJU).

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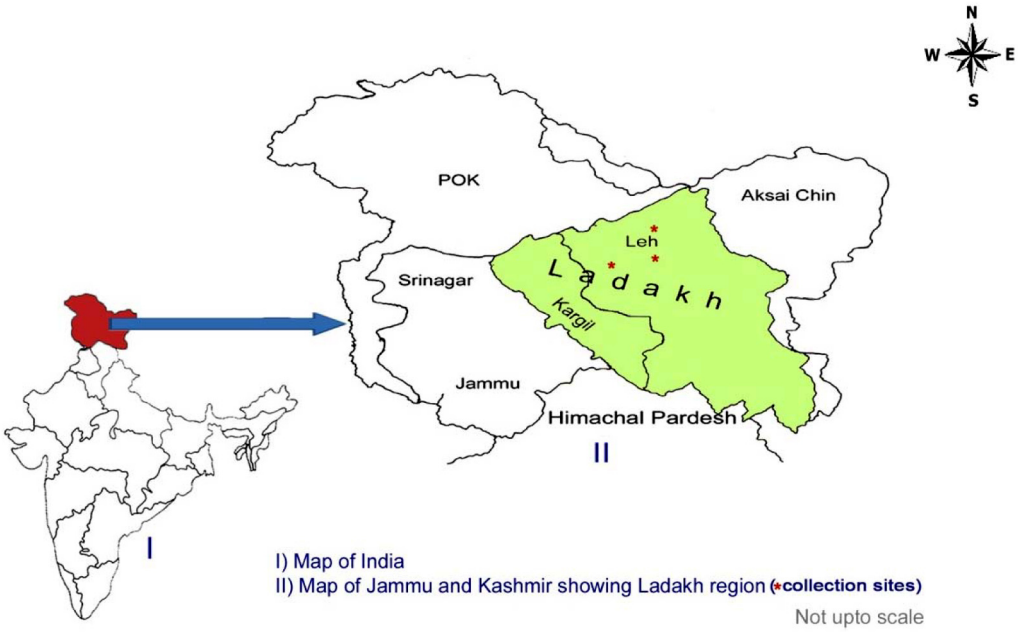

Fig. 1. Map of study area

RESULTS

Key to present species

1. Sporophore globose, basidiospores globose to subglobose with pedicel attached ……**2**

1´. Sporophore subglobose, basidiospores globose to subglobose without pedicel… … ***C. bovista***

2. Exoperidium easily peeling off at maturity exposing smooth endoperidium…..……...**3**

2´. Exoperidium remains unpeeled at maturity..... ……..**4**

3. Pedicel attached to basidiospores with pointed end ..... ***B. plumbea***

3´. Pedicel attached to basidiospores with blunt end…....***B. plumbea* var. *dens-caulis* var. nov.**

4. Pedicel up to 10.4-15.2 µm, capillitium threads 4.0-36.0 µm ……..... ***B. minor***

4´. Pedicel up to 8.0-12.8 µm, capillitium thread up to 2.4-11.2 µm…….. ***B. pusilla***

Bovista minor Morgan, *J. Cincinnati Soc. Nat. Hist.* 14: 147 (1892) Fig. 2a, Fig. 3-A

Sporophore: Globose, 2.5-4.0 cm in diameter, slightly embedded in substratum with minute stipe, whitish when young turning dark brown at maturity, surface smooth to irregularly ridged at maturity; Gleba: White when young turning dark brown at maturity; Odour: No particular odour; Basidia: clavate, 8.8-14.4 × 7.2-10.4 µm, thin walled, hyaline, tetrasterigmate; sterigmata 10.4-15.2 µm in length; Basidiospores: globose to subglobose, 4.0-5.6 × 3.2-4.8 µm (a_vL= 4.8, a_vW= 4.0, Q=1.2), thin walled, smooth, hyaline, mono- to biguttulate; pedicel 10.4-15.2 µm long attached to spore; Capillitium threads: 4.0-36.0 µm wide, thick walled, hyaline, highly ramified, septate, tapering towards tip; Exoperidial hyphae: 2.4-6.4 µm wide, thin walled, hyaline, filamentous, furcated, occasionally septate.

Reaction: Basidiopores turn light green but capillitium thread and exoperidium hyphae remain unchanged in 5% KOH. Basidiospores and capillitium thread turns pale green in 1% Iodine solution but exoperidium hyphae remain transparent.

Collection examined: India, Jammu and Kashmir, Ladakh, Leh, Phyang area, humicolous, solitary to scattered, deciduous forests of *Populus nigra* and *Salix alba*, Konchok Dorjey and Y.P. Sharma, HBJU 266, July 2012.

Edibility: Not consumed in Ladakh but edibility has been reported from other regions (Smith, 1951).

Distribution: The species has wide distribution across the world (Smith, 1951; Smith *et al*., 1981).

Remarks: The diagnostic characters of the above examined collection including macroscopic features, size and shape of basidiospores, shape of capillitium threads and habitat are typical of *Bovista minor* described by Smith, (1951). In present collection, basidiospore size ranges from 4.0-5.6 × 3.2-4.8 µm as compared to 4.0-5.0 × 3.5-4.0 µm in Michigan collection recorded by Smith (1951)

Bovista plumbea Pers., *Ann. Bot. (Usteri)* 15: 4 (1795) Fig. 2b-c, Fig. 3-B)

Synonymy: *Bovista plumbea* Pers., *Ann. Bot. (Usteri)* 15: 4 (1795) f. *plumbea*

Endonevrum suberosum (Fr.) Czern., *Bull. Soc. Imp. nat. Moscou* 18(2, III): 151 (1845)

Fig. 2. *Bovista minor*: a) sporophores in natural habitat; *Bovista plumbea*: b) sporophores in natural habitat c) sporophores showing inner gleba; *Bovista plumbea* var. *dens-caulis* var. nov.: d) sporophores in natural habitat e) sporophores showing inner gleba; *Bovista pusilla*: f) developmental stages of sporophores; *Calvatia bovista*: g) and h) sporophores in natural habitat

Globaria plumbea var. *suberosa* (Fr.) Quél., *Mém. Soc. Émul. Montbéliard*, Sér. 2 5: 371

Sporophore: Globose shaped, 2.5-3.5 cm in diameter, loosely attached to substratum by rhizoids, pure white when young turning dark brown at maturity; surface becomes slightly cracked, exoperidium easily peels off at maturity exposing smooth endoperidium; Gleba: pure white and fleshy when young turning into brown powdery mass at maturity. Basidiospores: Globose to sub-globose, 4.8-6.4 × 4.0-4.8 µm (a_vL= 5.6, a_vW= 4.4, Q=1.2-1.3), pale green (in Congo red), thick walled, monoguttulate; pedicel attached to spore, 9.6-14.4 µm long, pointed towards tip; Capillitium thread: 6.4-16.0 µm wide, thick and double walled, hyaline, branched dichotomously, aseptate; Exoperidial hyphae: 4.0-12.0 µm wide, thin walled, hyaline, branched, septate, sometimes swollen.

Reaction: The basidiospores and capillitium thread turns yellowish in 1% Iodine solution.

Collection examined: India, Jammu and Kashmir, Ladakh, Leh, Gangless area, humicolous, scattered, open grassland, Konchok Dorjey and Y.P. Sharma, HBJU 267, July 2012.

Edibility: Not edible in study area but reported to be edible from other regions (Phillips, 1981; Purkayastha and Chandra, 1985).

Distribution: Earlier reported from Uttar Pradesh, Himachal Pradesh and Jammu and Kashmir (Ahmad, 1941; Kaul *et al.,* 1978; Hennings, 1901; Thind and Thind, 1982; Kumar, 2009).

Remarks: The above examined collection has been identified as *Bovista plumbea* which is characterised by a typical globose sporocarp having a distinct white exoperidium which peels off in the form of thin sheets at maturity. The species typically fruits on open pastures, golf courses and similar substrates (Smith, 1951).

***Bovista plumbea* var. *dens-caulis* var. nov.** Fig. 2d-e, Fig. 3-C)

Sporophore: Globose, 2.5-3.2 cm in diameter, attached to substratum with rhizoids, pure white; surface smooth when young becoming reticulate at maturity, exoperidium easily peels off to expose the smooth endoperidium; Gleba: white at young turning to yellowish and then to brown spore mass after ages; Basidia: clavate to pyriform, 8.8-16.0 × 4.0-10.4 µm, thin walled, hyaline, 4-spored; Basidiospores: globose to sub-globose, 4.8-7.2 × 4.0-5.6 µm (a_vL= 6.0, a_vW= 4.8, Q=1.2-1.3), thick walled, hyaline, mono- to biguttulate; prominent pedicel (9.6-14.4 µm in length) attached to spore, blunt towards tip; Capillitium thread: up to 20 µm wide, thick walled, hyaline, ramified, aseptate; Exoperidial hyphae: 3.2-6.0 µm wide, thin walled, hyaline, straight, rarely branched, aseptate.

Reaction: The basidiospores and capillitium thread turns light greenish in Iodine solution

Collection examined: India, Jammu and Kashmir, Ladakh, Leh, Gangless area, humicolous, solitary to scattered, on open grassland, Konchok Dorjey and Y.P. Sharma, HBJU 268, August 2013.

Edibility: Not consumed in the study area, but edible (Purkayastha and Chandra, 1985).

Remarks: The morphological and microscopical detail of present specimen is in line with the description given for *Bovista plumbea* by Smith (1951) except for pedicel tip and spore colour reaction. In Smith's description, the pedicel attached to basidiospores has pointed tip as compare to blunt tip of the pedicel in our present collection. In addition, the basidiospores and capillitium thread in present collection turns light greenish in Iodine solution in contrast to yellow colouration observed by Smith (1951). In view of these differences, a new variety has been proposed.

Bovista pusilla (Batsch) Pers., *Syn. meth. fung.* (Gottingen) 1: 138 (1801) Fig. 2f, Fig. 3-D)

Synonymy: *Globaria pusilla* (Pers.) Quél., *Mém. Soc. Émul. Montbéliard*, Sér. 2 5: 371 (1873)

Pseudolycoperdon pusillum (Pers.) Velen., *Novit. Mycol. Nov.*, (Op. Bot. Æech.): 93 (1947)

Lycoperdon polymorphum var. *pusillum* (Pers.) F. Šmarda, *Fl. ÈSR*, B-1, Gasteromycetes: 238 (1958).

Sporophore: spherical, 2.0-3.5 cm in diameter, attached to substratum with few rhizomorphs, usually pure white in colour turning brownish and dries up after ages and release brown dusty spores; surface dry, smooth and devoid of any scales or spines; Gleba: whitish and then yellowish turning brown at maturity; Basidiospores: globose to subglobose, 2.4-4.8 × 1.6-4.0 µm (a_vL= 3.6, a_vW= 2.8, Q=1.5-1.2), thin walled, hyaline, mono- to biguttulate, pedicellate, pedicel up to 8.0-12.8 µm in length, attached to spore; Capillitium threads: 2.4-11.2 µm wide, thin walled, hyaline, highly dichotomously branched, aseptate with tapering ends; Exoperidial element: 3.2-14.4 µm wide, thin walled, hyaline, furcated, septate; Exoperidial hyphae: 2.4-3.2 µm wide, thin walled, hyaline, occasionally furcated, septate.

Reaction: Both spores and capillitium thread turns green but exoperidial hyphae remain unchanged in 5% KOH and 1% Iodine solution.

Collection examined: India, Jammu and Kashmir, Ladakh, Leh, Nubra, Khardong area, humicolous, scattered, open grassland, Konchok Dorjey and Y.P. Sharma, HBJU 269, August 2011.

Edibility: Not edible in study area but reported to be edible elsewhere (Garcha, 1980).

Distribution: Earlier reported from Dehradun, Pune and Jammu (Ahmad, 1941; Kumar, 2009).

Remarks: The specimen examined coincides with the description of *B. pusilla* by Smith *et al.* (1981). Microscopically, the species is characterised by globose to sub-globose (2.4-4.8 × 1.6-4.0 µm), thin walled, hyaline basidiospores with pedicel (8.0-12.8 µm long) attached; capillitium threads which are dichotomously branched, aseptate with tapering ends.

Calvatia bovista (L.) Pers., in Macbride and Allin, *Bulletin Labs. nat. Hist. St. Univ. Ia* 4: 41 (1896) Fig. 2g-h, Fig. 3-E)

Basionym: *Lycoperdon bovista* L., *Sp. pl.* 2: 1183 (1753)

Sporophore: Globose to subglobose with prominent stipe, 2.5-6.0 cm in diameter and 5.5-7.0 cm in length, purely white coloured; surface dry, shiny white, rough having small polygonal reticulations or warts, reticulations less conspicuous towards stipe; Gleba: purely white when young and fresh turning dark brown at maturity; Basidiospores: globose to subglobose, 3.2-4.8 × 2.4-4.0 µm in diameter, thin walled, hyaline, mono- to biguttulate; Capillitium thread: 4.0-8.0 µm wide, thin walled, hyaline, furcated, septate; Endoperidial element: globose to irregular, up to 44.0 µm wide, thin walled, hyaline, branched, highly septate; Exoperidial hyphae: 2.4-8.0 µm wide, thin walled, hyaline, branched, septate.

Reaction: Both spores and hyphae remains transparent in 5% KOH and 1% Iodine solution.

Collection examined: India, Jammu and Kashmir, Ladakh, Leh, Nubra, Sumoor area, humicolous, scattered, temperate forests of *Salix alba* and *Populus nigra*, Konchok Dorjey and Y.P. Sharma, HBJU 270, August 2011.

Edibility: Consumed in study area but only at young stage.

Distribution: Widely distributed around the world (Smith *et al.*, 1981).

Remarks: The examined collection is characterized in the field by large globose to sub-globose, whitish basidiocarps having more or less prominent irregular warts on surface and long sterile base. The macro- and microscopic details of present specimen is typical of *Calvatia bovista* described by Smith (1951) from Michigan, USA except for larger diameter of capillitium threads (7.0-11.0 µm; or upto 17.0 µm) as compared to 4.0-8.0 µm in present collection.

DISCUSSION

The principal objective of present study was to document the occurrence, distribution, taxonomy and edibility status of these hitherto unrecorded macrofungi existing in the extreme cold arid climate of Ladakh region of Indian Trans-Himalaya, from where only few reports on macrofungi are available (Dorjey *et al.*, 2013a, Dorjey *et al.*, 2013b). Survey of literature (Bilgrami *et al.*, 1979, 1981, 1991; Jamaluddin *et al.*, 2004) revealed that, of the five puffball taxa discussed, *Bovista minor*, *Calvatia bovista* and *Bovista plumbea* var. *dens-caulis* were new records for Indian sub-continent whereas *B. plumbea* and *B. pusilla* constitute first time record from Ladakh region of Jammu and Kashmir State.

Ladakh being geographically an isolated region with unusual mountainous terrain and harsh climatic conditions resulted in a unique ecosystem on earth's biosphere. The puffball species, described in present paper, inhabit mostly plain grassland areas in the cold desert. These grassland habitats have probably formed a unique ecosystem that provided adequate moisture and organic matter for growth and sustenance of these macromycetes in arid sandy soils. Basidiocarps of these macromycetes are strikingly radiating white coloured which is, perhaps, an adaptive strategy to reflect the intense solar radiation in study area. The existence of these puffball members in sandy desert of Ladakh indicates their adaptation and preferential growth in temperate and arid climatic conditions. This assumption is supported by several studies on puffball species growing in temperate areas, arid zones, high elevations and sandy deserts of North America including Beartooth Plateau, Arizona and Pacific Northwest regions (Norvell *et al.*, 2008; Bates *et al.*, 2009; Jalink, 2010).

This research document represents first attempt to present work on occurrence and taxonomy of puffballs

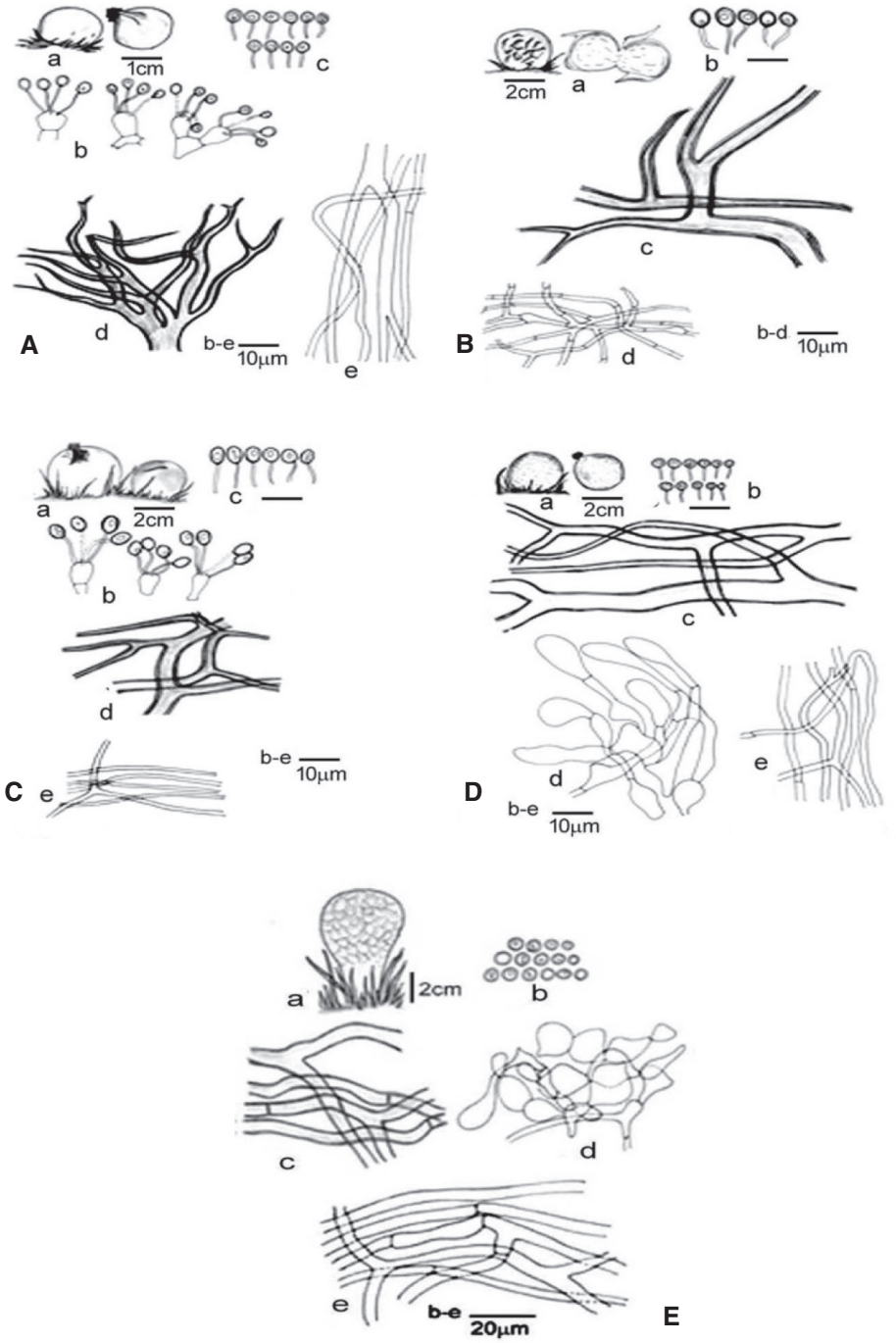

Fig. 3. A: *Bovista minor:* a) habit b) basidia c) basidiospores with pedicel d) capillitium threads e) exoperidial hyphae; **B: *Bovista plumbea:*** a) habit b) basidiospores with pedicel c) capillitium threads d) exoperidial hyphae; **C: *Bovista plumbea* var. *denscaulis* var. nov.:** a) habit b) basidia c) basidiospores with pedicel d) capillitium threads e) exoperidial hyphae; **D: *Bovista pusilla*** a) habit b) basidiospores with pedicel c) capillitium threads d) exoperidial hyphae e) endoperidial hyphae; **E: *Calvatia bovista*:** a) habit b) basidiospores c) capillitium threads d) exoperidial hyphae e) endoperidial hyphae

existing in the region and we do hope this investigation will inspire more researchers to undertake extensive studies on larger fungi of high-altitude Ladakh province, where harsh climate of freezing cold and high solar radiation pose challenges for every organisms to grow. Further studies on fungal phenology, habitats, adapting strategies used by these desert puffballs in freezing climate, their biochemical, cellular and molecular analysis could provide unique opportunity to know how these fungi are able to endure extreme climate and consequently, will help to identify some novel biochemical metabolites of medicinal importance.

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