



## Contributions to the family *Ascobolaceae* of India

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### Abstract

The present paper provides a checklist of reported species from genera *Ascobolus* Pers. and *Saccobolus* Boud. in India with one additional first record from India, *Saccobolus depauperatus*. In addition, *A. brassicae*, *A. furfuraceus*, *A. foliicola*, *A. saccharifereus*, *Saccobolus depauperatus* and *S. glaber* are reported for the first time from Kashmir, India and descriptions and photomicroplates of all said species are provided for their easy identification.

**Keywords** – Ascomata – episporium – operculate – saprobic

### Introduction

The family *Ascobolaceae* Boud. ex Sacc. of class Ascomycetes is comprised of 6 genera and about 129 species which are primarily saprobic species associated with herbivore dung (Kirk et al. 2008) or plant debris. The family *Ascobolaceae* is characterized by operculate asci and very thick walled uninucleate ascospores, which are hyaline when immature but become colored at maturity before discharge. Pezizoid ascomata, protruding asci from the hymenium, and colored episporium are the distinguishing characters of this family that make it different from the rest of Ascomycetes (Van Brummelen 1967). *Ascobolus* and *Saccobolus* are the most common genera of this family, and are some of the best-known coprophilous species of fungi.

The genus *Ascobolus* was first established by Gmelin in 1792, which is characterized by their mutually free ascospores inside the asci. The genus *Saccobolus* is comprised of about 25 species (Kirk et al. 2008) and is characterized by apotheciod ascomata, clustered ascospores which are usually arranged in clusters of a regular pattern within a common gelatinous membrane (Van Brummelen 1967) and by showing brown or purple brownish episporic pigment upon maturation (Van Brummelen 1967, Kaushal & Viridi 1986, Doveri 2004, Bell 2005). The ascospores of *Saccobolus* are similar to those of *Ascobolus* but are differentiated from the latter by their clustered arrangement and their shorter but broader asci and ascospores shapes (Van Brummelen 1967, Doveri 2004, Bell 2005).

Van Brummelen (1967) published the first monograph of the genera *Ascobolus* and *Saccobolus*, which also included four species from India. To date, about 15 species of *Ascobolus* and 7 species of *Saccobolus* have been reported from India by various researchers in this field. (Tables 1, 2).

The main objective of this study is to report six species new to Kashmir, India viz *A. brassicae*, *A. furfuraceus*, *A. foliicola*, *A. saccharifereus*, *Saccobolus depauperatus* and *S. glaber* of genera *Ascobolus* and *Saccobolus* as well as to provide checklist of species of genera *Ascobolus* and *Saccobolus* species reported from India to date. The knowledge of these genera in India is very

limited, and family Ascobolaceae has not been researched sufficiently in this part of the world.

**Table 1** List of *Ascobolus* species reported from India

No.	Species Name	Place of collection from India	References
1	<i>A. behnitziensis</i>	Kolhapur, Maharashtra	Ghandge & Patil (1988)
2	<i>A. brassicae</i> **	Varansi, Uttar Pradesh	Khare (1976)
3	<i>A. crenulatus</i>	Satara, Maharashtra	Ghandge & Patil (1988)
4	<i>A. denudatus</i>	Narkanda, MahasuUttarakhand	Thind&Waraitch (1974)
5	<i>A. foliicola</i> **	Radhanagari, Maharashtra	Ghandge & Patil (1988)
6	<i>A. furfuraceus</i> **	India, place not mentioned	Van Brummelen (1967)
7	<i>A. geophilus</i>	Mussoorie hills, Uttarakhand	Ghandge & Patil (1988)
8	<i>A. gollanii</i>	Saharanpur garden, Uttar Pradesh	Hennings (1901)
9	<i>A. hawaiiensis</i>	Kolhapur, Maharashtra	Ghandge & Patil (1988)
10	<i>A. immersus</i>	Patna	Ghandge & Patil (1988)
11	<i>A. indicus</i>	Delhi	Sanwal (1953)
12	<i>A. minutus</i>	Karad Maharashtra	Ghandge & Patil (1988)
13	<i>A. sacchariferus</i> **	Kolhapur, Maharashtra	Ghandge & Patil (1988)
14	<i>A. scatigenus</i>	Sheikhupura Dalhousie, Punjab Hyderabad, Punjab and Uttar Pradesh Hyderabad Saharanpur Graden, Uttar Pradesh	Van Brummelen (1967) Van Brummelen (1967) Leelavathy& Flower (1981) Van Brummelen (1967) Van Brummelen (1967)
15	<i>A. gomayapriya</i>	Andaman Islands	Niranjan&Sarma (2018)

**Table 2** List of *Saccobolus* species reported from India

No.	Species Name	Place of collection from India	References
1	<i>S. diffusus</i>	Kasauli, Himachal Pradesh	Kaushal & Virdi 1986
2	<i>S. glaber</i> **	Hyderabad	Van Brummelen (1967)
3	<i>S. humidicola</i>	Kasauli, Himachal Pradesh	Kaushal & Virdi 1986
4	<i>S. saccoboloides</i>	Kasauli, Himachal Pradesh	Kaushal & Virdi 1986
5	<i>S. thaxteri</i>	Patiala, Punjab	Kaushal & Virdi 1986
6	<i>S. versicolor</i>	Kasauli, Himachal Pradesh	Kaushal & Virdi 1986
7	<i>S. verrucisporus</i>	Kasauli, Himachal Pradesh	Kaushal & Virdi 1986
8	<i>S. depauperatus</i> *	Sopore, Kashmir	<b>Present study</b>

\*First report from India

\*\*First report from Kashmir

## Materials & methods

The study area, Kashmir has not been surveyed extensively for fungi, so various field trips were made to study its biodiversity. While doing so, some specimens from family *Ascobolaceae* were collected in the year 2019. Fresh specimens were collected in vials and then rehydrated in a damp/moist chamber to extend their life and allow us to regrow them. These specimens, along with their respective substrates were kept in a plastic box lined with wet tissue paper to retain moisture. The specimens were brought to the laboratory for further investigation. Specimens were air dried, at temperature less than 40°C, in a shady area to prevent any damage to them and were then deposited along with substrates in the herbarium of Government Degree College Sopore. Micro-slides were prepared either by making freehand sections of the specimens (In case of larger specimens viz *A. foliicola*, *A. furfuraceus* and *A. sacchariferus*), or by making a crush mount. Fresh/live material was examined under a Labomed CXL, mounted in sterile water. For the amyloidity test Lugol's Iodine (Baral's recipe) as well as Melzer's reagent were used separately, due to the fact that, although Lugol's Iodine is preferred for Ascomycetes (Baral 1987, Melzer's reagent was preferred by Van Brummelen (1967). Measurements were taken manually using an ocular micrometer calibrated with a stage micrometer. At least thirty mature spores from each specimen, all with well-developed epispodium, were measured to minimize the error.

The literature of Van Brummelen (1967), Kaushal & Viridi (1986) and Doveri (2014) were used for species identification. Facesoffungi numbers were registered as per Jayasiri et al. (2015). The first author Suhaib Firdous Yattoo has the copyright of all the photographs.

## Results

*Ascobolus brassicae* P. Crouan & H. Crouan, Ann. Sci. nat., Bot., ser. IV, 7:174 (1857) Fig. 2

Index Fungorum number: IF 245343; Facesoffungi number: 10090

Description – Apothecia gregarious, 0.3–0.7 mm in diameter, up to 0.3 mm in height, white at first, later becoming light pink due to protruding pink asci, margin furfuraceous. Asci cylindrical to clavate, with distinct large operculum, 140–160 × 22–28 μm, biserate packed spores. Paraphyses cylindrical branched with irregularly shaped tips, without pigment, 140–160 × 3–5 μm. Excipulum of *textura globulosa*, 15–40 μm. Spores globose, distinctly warty, episporium light pink, 12.8–13.4 μm.

Note – A rarely collected species from India, with only one record and representative collection, reported from Uttar Pradesh (Khare 1976). Niranjana & Sarma (2018) (*A. gomayapriya* sp. nov.) reported 13 species in India, with *A. brassicae* not being mentioned, perhaps by mistake.

Material Examined – India, Jammu and Kashmir, Sopore, Nowpora kalan, on rodent droppings, 01 Jul 2020, Suhaib Yattoo, GDC-BOT-030720 (Govt. Degree college Sopore), GDC-BOT-250220, 34.2926300, 74.4402760, 22.02.2020.

*Ascobolus foliicola* Berk. & Broome in J. Linn. Soc. (Bot.), Lond. 14: 109 (1873) Fig. 3

Index Fungorum number: IF 144172; Facesoffungi number: 10089

Description – Apothecia gregarious, stalked, yellow in color, upto 8 mm in diameter, 0.5 mm in height, margin furfuraceous. Hymenium 180–210 μm. Hypothecium well developed, cells up to 6–10 μm in diameter. Asci cylindrical, 180–220 μm, spores uniserate earlier later on becoming biserate. Paraphyses cylindrical, branched at tips, yellow pigmented. Excipulum with pyriform–subglobose cells, 10–30 μm × 15–60 μm. Spores with pink–brown episporium. Episporium with anastomosing ridges becoming reticulate in later stages. Spore size variable 15.5–22 μm × 7.5–12.7 μm, depending on the stage of maturation, exospore swollen in mature spores.

Material Examined – India, Jammu and Kashmir, Sopore, Nowpora kalan, on dead and rotten *Populus deltoides* leaves, 01 Jul 2020, Suhaib Yattoo, GDC-BOT-030720 (Govt. Degree college Sopore), 34.2926300, 74.4402760, 01.07.2020.

*Ascobolus furfuraceus* Pers., Neues Mag. Bot. 1: 115 (1794) Fig. 4

Index Fungorum number: IF 143631; Facesoffungi number: 10094

Description – Apothecia gregarious or solitary, sessile, 1–5 mm in diam., 0.5–1 mm high, closed subglobular at first, later becoming flat to concave disc shaped, lemon-yellow with pink protruding asci at first and becoming brown in final stages, with well-developed furfuraceous margin. Hymenium, 180–220 μm, flesh including the hypothecium of isodiametric cells up to 10–15 μm in diameter. Excipulum made of globular to sub globular cells up to 10–60 μm, margins with thick-walled pyriform cells upto 50–80 × 30–60 μm. Asci clavate (120–)170–240 μm × (20–)25–35 μm, 8-spored, with uniserate spore arrangement in early stages and biserate in later stages, light blue in Lugol's reagent. Spores ellipsoid, hyaline at first, then pinkish, finally purple–brown, 20–29 × 10–14 μm, ornamented with longitudinal, often anastomosing, striations. Paraphyses simple, rarely branched, enclosed within a lemon-yellow mucilage.

Material Examined – India, Jammu and Kashmir, Sopore, Nowpora kalan, on cow dung, 12 May 2020, Suhaib Yattoo, GDC-BOT-130520 (Govt. Degree college Sopore), 34.2932942, 74.4394454, 09.05.2020.

*Ascobolus sacchariferus* Brumm., Persoonia suppl. vol. 1: 122 (1967) Fig. 5

Index Fungorum number: IF 326522; Facesoffungi number: 10093

Description – Apothecia solitary or gregarious, sessile, 0.8–3mm in diam., 0.5–1mm high, finely furfuraceous, with a distinct margin, apothecia initially globular and white, later becoming hemispherical and purplish-brown due to maturation of ascospores. Hymenium up to 180µm thick, hypothecium of round cells up to 9 µm in diameter. Excipulum of *textura-globulosa* (up to 10µm) at the base but becoming *textura-angularis* (12–50µm×15–20µm) towards the margin. Asci 8–spored, narrowly clavate with uniseriate spore arrangement which later on becoming biseriate, 180–220×15–20µm, with curved base, slightly blue in Lugol’s reagent, spores 18–21×8.5–11.2µm.

Material Examined – India, Jammu and Kashmir, Sopore, Nowpora kalan, on cow dung and horse dung, 02 Mar 2020, Suhaib Yattoo, GDC-BOT-040320 (Govt. Degree college Sopore), 34.2877819, 74.4397236, 02.03.2020.

Notes – Variation in spore ornamentation was observed in one out of thirty specimens cultured in moist chamber. Episorium reticulated without deep crevices (Fig. 6), no more variations were observed.

***Saccobolus glaber*** (Pers.) Lambotte, Fl. Mycol. Belg., suppl. 1: 284 (1887)

Fig. 7

Index Fungorum number: IF 120544; Facesoffungi number: 10092

Description – Apothecia solitary to gregarious, sessile, concave, 0.3–1mm in diam., yellow at first, amber in later stages, margin undifferentiated. Hymenium 120–200µm thick, hypothecium poorly developed, flesh made of thin-walled isodiametric cells 10–15µm in diameter. Excipulum of globular to sub globular cells 10–18 µm×9–15 µm. Asci broadly clavate with a tapering short stalk, 150–240 × 30–45µm, tip flattened containing a large operculum, 8-spored, wall deep-blue to purple in Lugol’s reagent. Spores clustered within a common gelatinous envelope according to pattern–I (Van Brummelen 1967), each spore 20–30×10–15µm, slightly to strongly punctate. Paraphyses simple, unbranched with slightly swollen tips upto 9µm, containing yellow pigment in the upper part, enclosed within a colorless mucilage.

Material Examined – India, Jammu and Kashmir, Sopore, Nowpora kalan, on cow dung, sheep and goat droppings, 26 Feb 2020, Suhaib Yattoo, GDC-BOT-280220 (Govt. Degree college Sopore) 34.2916666, 74.4426312, 22.02.2020.

Note – White fruiting bodies (Fig. 8) were also observed, mostly found among the populations with adequate pigment but usually on the substrates that were exposed to direct sunlight. Upon re-growing in the moist chamber, they developed the normal pigmented fruiting bodies, suggesting that the variation was environmental.

***Saccobolus depauperatus*** Berk.&Broome., Ann. Mag. Nat. Hist. III 15: 448 (1865)

Fig. 9

Index Fungorum number: IF 148254; Facesoffungi number: 10091

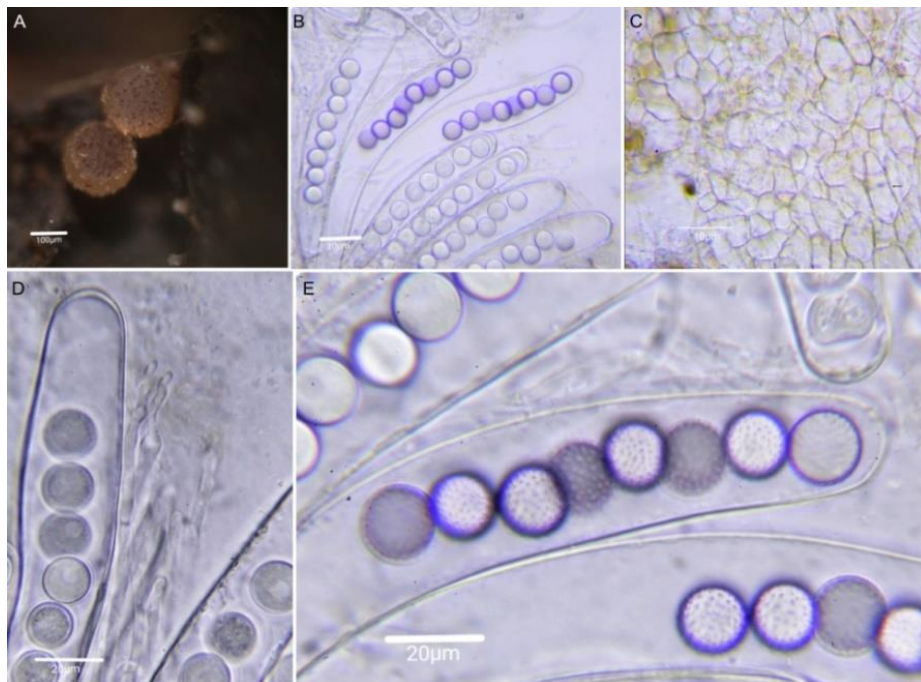
Description – Apothecia solitary, sessile, convex, 0.1–0.5 mm in diam., white at first, pink to purple in later stages, margin undifferentiated. Hymenium 70–120 µm thick, hypothecium poorly developed, flesh made of thin-walled isodiametric cells 10–15µm in diameter. Excipulum very thin, made of isodiametric cells (*textura globulosa*). Asci broadly clavate with a tapering short stalk, 70–90 × 20–24 µm, tip flattened containing a large operculum, 8-spored, wall deep-blue to purple in Lugol’s reagent. Spores clustered within a common gelatinous kidney shaped envelope according to pattern–II (Van Brummelen 1967), each spore 20–30×10–15µm, slightly to strongly punctate with slight grayish tinge. Paraphyses cylindrical, lacking amorphous pigment.

Material Examined – India, Jammu and Kashmir, Sopore, Nowpora kalan, on horse dung (in moist chamber), 06 Mar 2020, Suhaib Yattoo, GDC-BOT-080320 (Govt. Degree College Sopore), 34.2916666, 74.4426312, 05.03.2020.

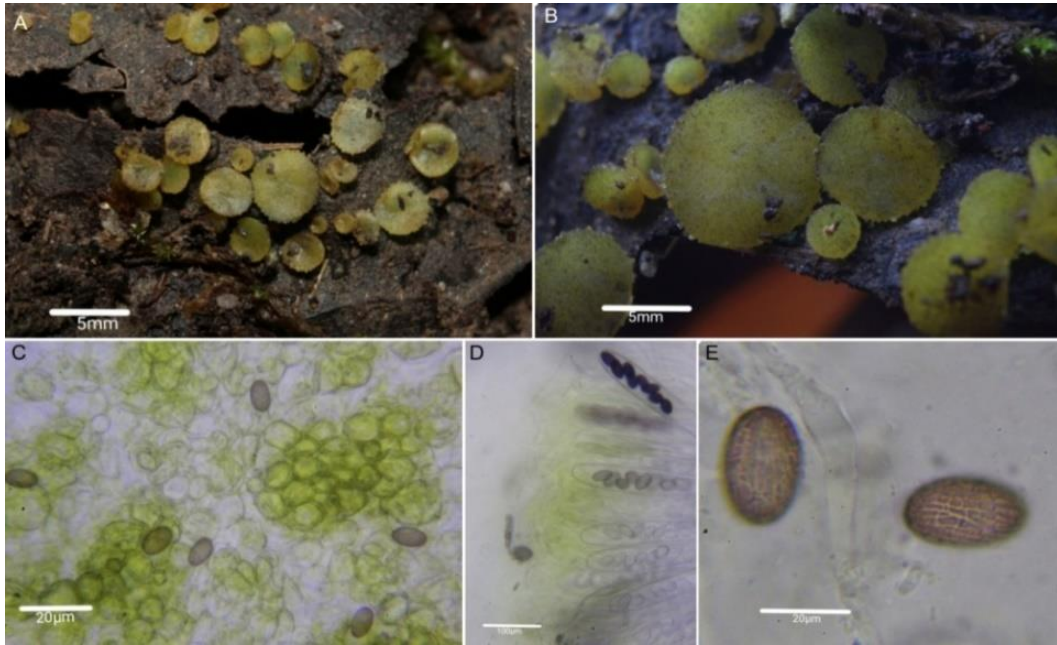
Note – Very small apothecia were preserved in Hoyer’s medium (although episorium coloration and other structures are preserved, spores show a slight difference in size).



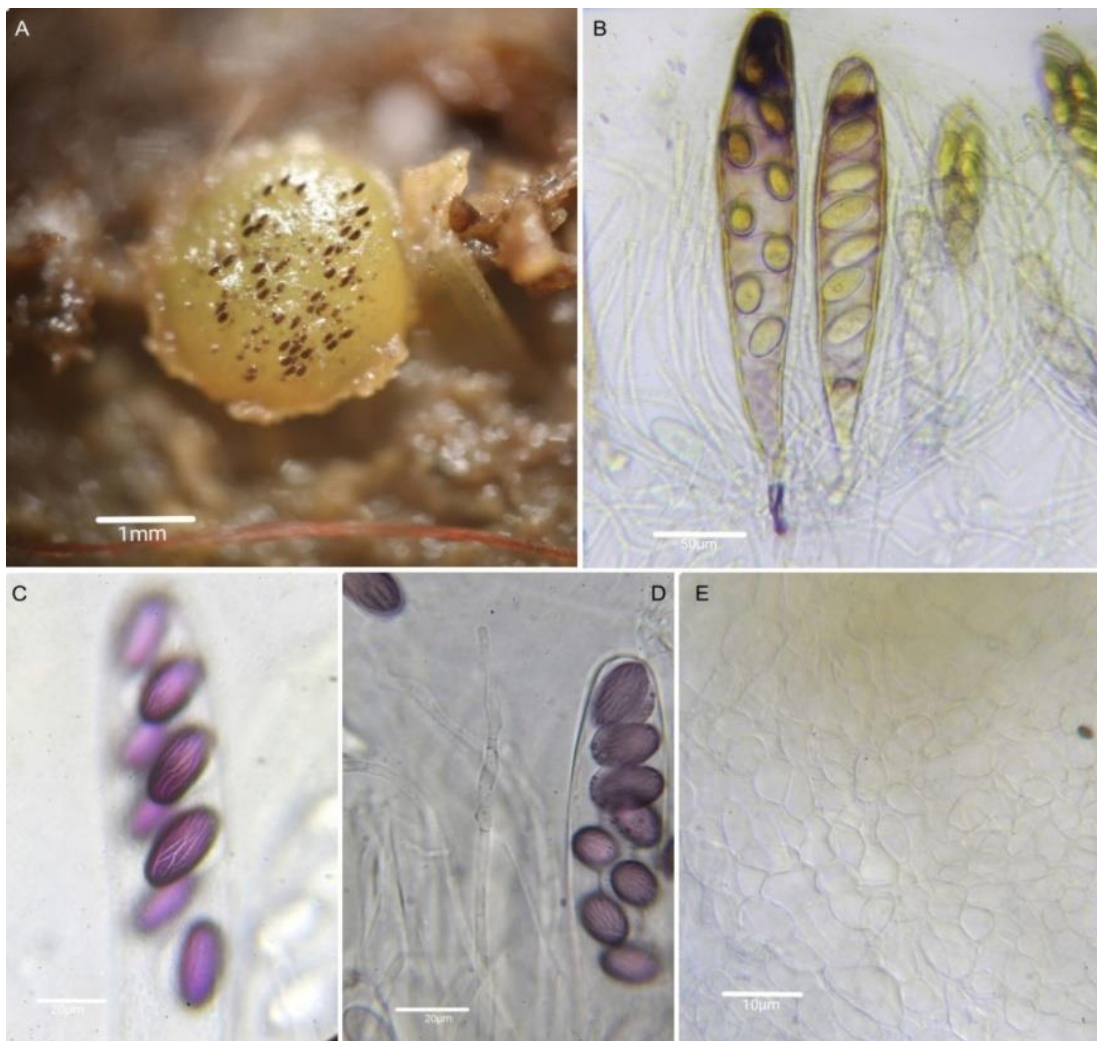
**Fig. 1** – Map of Kashmir, India showing study area Sopore.



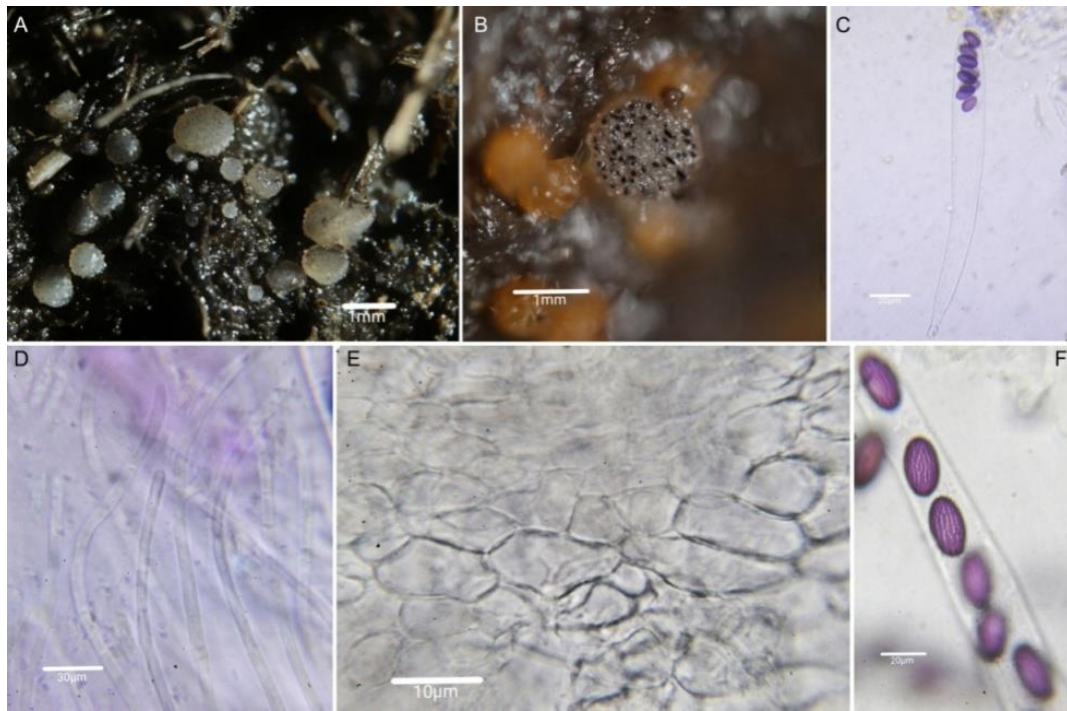
**Fig. 2** – *Ascobolus brassicae*. a Ascomata on rodent dropping. b Asci showing mature and immature spores. c Ectal excipulum. d Paraphyses. e Spores. Scale bars: a = 100 µm, b, d, e = 20 µm, c = 10µm.



**Fig. 3** – *Ascobolus foliicola*. a, b Ascomata. c Ectal excipulum. d Asci with biserrate arrangement of spores and paraphyses. e Spores. Scale bars: a, b = 5mm, c, d, e = 20 µm.



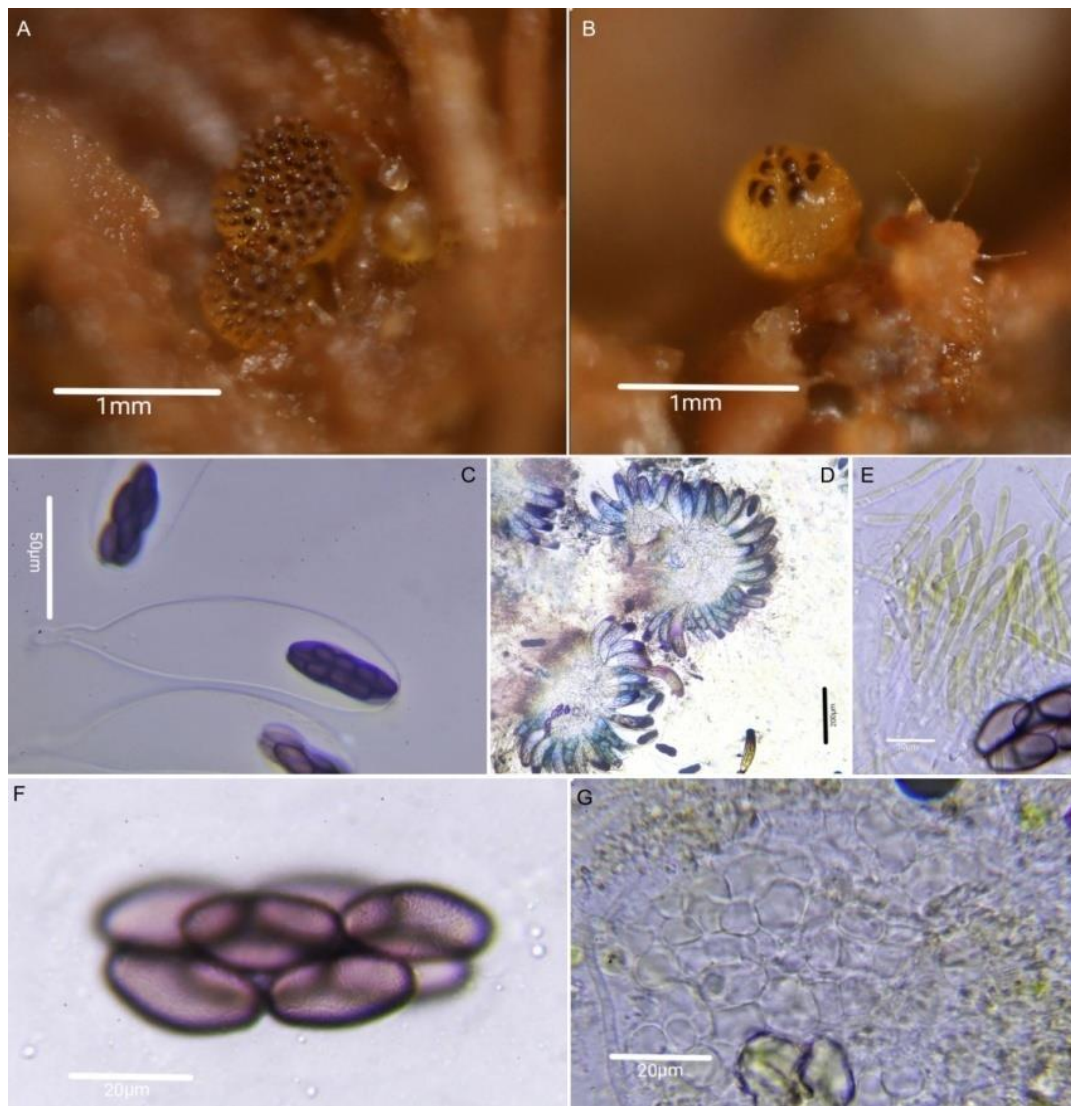
**Fig. 4** – *Ascobolus furfuraceus* a Ascomata. b Asci in Lugol's solution. c Asci with biserrate arrangement of spores. d Paraphyses. e Excipulum. a = 1mm, b = 50µm, c, d = 20µm, e = 10µm.



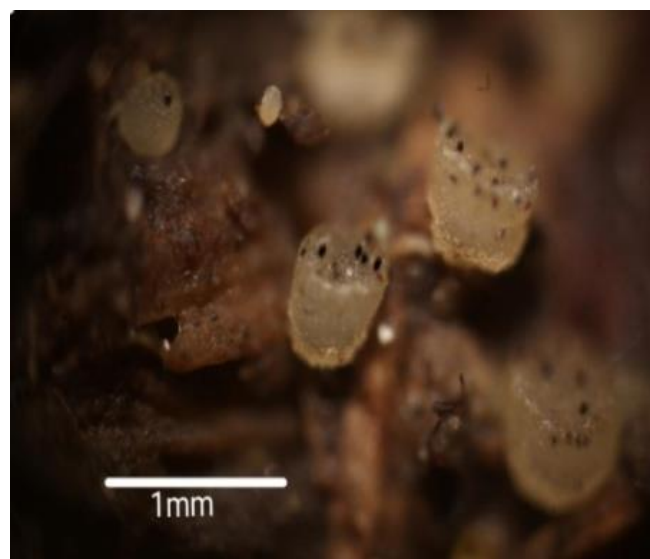
**Fig. 5** – *Ascobolus sacchariferus*. a, b Ascomata, c ascus with spores. d Paraphyses. e Ectal excipulum. Scale bars: a, b = 1 mm, c, e = 20  $\mu$ m, d = 10  $\mu$ m.



**Fig. 6** – Variation in spore ornamentation in *Ascobolus sacchariferus*. Scale bar = 10  $\mu$ m.

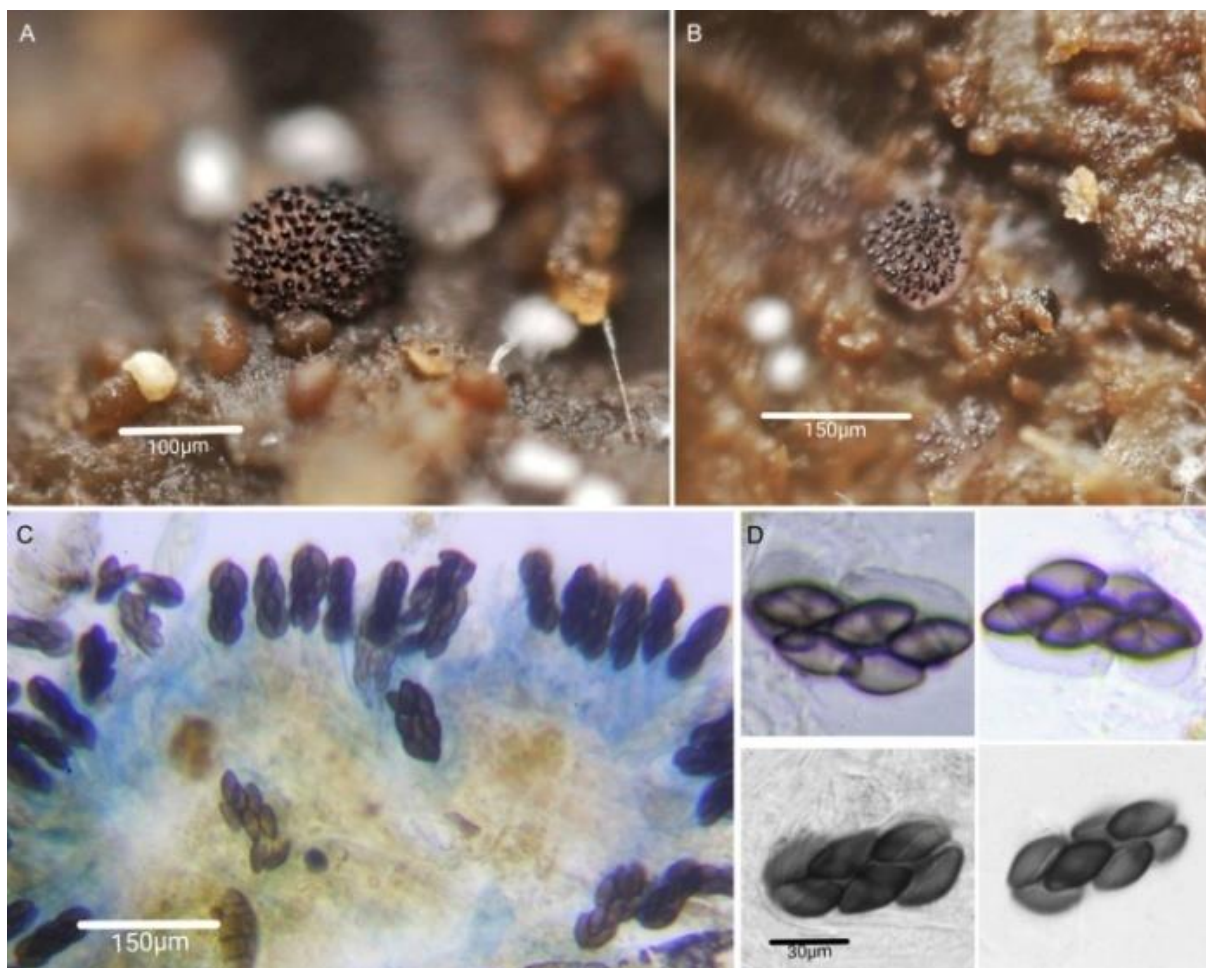


**Fig. 7** – *Saccobolus glaber*. a, b Ascomata. c Asci with spore clusters. d Squashed ascoma in Lugol's. e Pigmented Paraphyses. f Ascospores. g Excipulum. Scale bars: a, b = 1mm, c = 50 µm, d, f, g = 20 µm, e = 30 µm.



**Fig. 8** – Variation in fruiting bodies of *Saccobolus glaber*. Scale bars = 1 mm.





**Fig. 9** – *Saccobolus depauperatus*. a, b Ascomata. c Squashed ascoma in Lugol's. d Spore arrangement and shape of common mucilage. Scale bars: a-c = 150 µm, d = 20 µm

## Discussion

Kashmir is a fertile basin in India bordered by the Himalayas in the north and is one of the 26 biodiversity hotspots in India. Kashmir is one of the least explored biodiversity rich regions explored in India. The family *Ascobolaceae* mostly includes coprophilous fungi and is well represented by genera *Ascobolus* and *Saccobolus*. Both genera are closely related and differ mainly in their arrangement of ascospores within the asci.

The study provides the description of six species within genera *Ascobolus* and *Saccobolus* viz *Ascobolus brassicae*, *A. furfuraceus*, *A. foliicola*, *A. sacchariferus*, *Saccobolus depauperatus* and *S. glaber* with photomicrographs. A checklist of species of these genera reported from India to date is also provided. *Ascobolaceae* is represented by 6 genera and 129 species, although only 15 species of *Ascobolus* and 7 species of *Saccobolus* have been described from India. The present study is the first report of all described species from Kashmir, and the first report of *S. depauperatus* from India as a whole.

Some morphological variations were noted during the study, such as the spore ornamentation in some specimens of *Ascobolus sacchariferus*, however no other deviations from *A. sacchariferus* were noticed, so the specimens are considered just a variant to the typical *A. sacchariferus*. In addition, variations in fruiting body coloration in the *Saccobolus glaber* specimens were also observed. However, on regrowing the specimens in a moist chamber, no such variations presented, suggesting that the variable colorations completely environmental.

The present work contributes to knowledge of the family *Ascobolaceae* of India, increases knowledge about the genera *Ascobolus* and *Saccobolus*, and will help the researchers in this field to better understand their biology and distribution.

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