



## The Lichenicolous species of *Melaspilea* (Melaspileaceae) in India

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**ABSTRACT:** The lichenicolous species of genus *Melaspilea* has been studied. As a result, a new species *Melaspilea amarkantakensis* has been described and *M. insitiva* has been lectotypified. The new species grows on the thallus and ascomata of *Pertusaria* species in ‘sal’ forests of central India and is characterized by relatively small, blackish ascomata, I– and K/I+ pale blue hymenium, 4-spored, shorter, 25.4–33.2 × 14.3–20.2 μm asci and 1-septate, (12.1–)13.3–16.0(–17.5) × (5.0–)6.1–7.6(–8.9) μm ascospores lacking perispore.

**KEY WORDS:** *Arthoniomycetes*, *Ascomycota*, lectotypification, Madhya Pradesh, taxonomy.

### INTRODUCTION

Fungi obligately occurring on lichens as parasites, saprobes or commensals represent a major ecological niche for fungi. There are over 1800 species described as lichenicolous fungi out of estimated 3000 species (Lawrey and Diederich, 2003; 2011). These are poorly known group in India, represented by 42 species (Zhurbenko, 2013). Taxonomic study of *Melaspilea* would be a useful contribution towards understanding diversity of lichenicolous fungi in India.

The genus *Melaspilea* Nyl. is characterized by lirelliform apothecioid ascomata, an exciple composed of several cell layers and brown 1-septate ascospores (Flakus et al., 2014). It includes c. 66 species (Kirk et al., 2008). Index Fungorum shows 146 records for the genus but some are synonymised or transferred to another genus (<http://www.indexfungorum.org/names/Names.asp>). Majority of its species are lichenized, except eleven lichenicolous species viz. *M. cupularis* Müll.Arg. on *Pyrenula* sp., *M. diplasiospora* (Nyl.) Müll.Arg. on *Graphis elegans*, *M. epigena* Müll.Arg. on *Reimnitzia santensis*, *M. epigraphella* (Nyl.) Müll.Arg. on *Acanthothecis consocians*, *M. galligena* Zhurb. & I. Zhdanov on *Pertusaria* sp., *M. insitiva* Strit. on *Pertusaria leioplaca*, *M. leciographoides* Vouaux on *Verrucaria* sp., *M. lekae* Brackel & Kalb on *Sarcographa labyrinthica*, *M. lentiginosa* (Lyell ex Leight.) Müll.Arg. on *Phaeographis dendritica*, *M. tribuloides* (Tuck.) Müll.Arg. on *Anthracotheceum* sp. and *Pyrenula* sp. and *M. tucumana* Flakus et al. on *Pertusaria* sp. (Lawrey and Diederich, 2011; Kalb et al., 2012; Zhurbenko and Zhdanov, 2013; Flakus et al., 2014). Out of this, *M. insitiva* is known from India (Awasthi, 1991). Pérez-Ortega and Etayo (2010) already discussed taxonomic problems in *Melaspilea*

while describing a new genus *Labrocarpon* Etayo & Pérez-Ortega. Recently, Flakus et al. (2014) presented a key to lichenicolous species of *Melaspilea* s. lat. while describing a new species *M. tucumana* from Bolivia.

### MATERIALS AND METHODS

The specimens examined were housed at BM, BSA and LWG herbaria. Morphological and anatomical characters were studied by using Nikon SMZ 1500 and Nikon Eclipse 50i microscopes. Hand-cut sections were mounted in distilled water and lactophenol cotton blue. The amyloid reactions were tested in Lugol’s iodine solution (I) with and without pre-treatment of KOH. All measurements were made on material mounted in distilled water. The length, breadth, and length/breadth ratio (l/b) of ascospores are given as: (min–){ $\bar{X}$ –SD}–{ $\bar{X}$ +SD}{(–max), where min and max are the extreme values,  $\bar{X}$  the arithmetic mean, and SD the corresponding standard deviation followed by the number of measurements (n).

### TAXONOMIC TREATMENTS

*Melaspilea amarkantakensis* S. Joseph & G.P. Sinha, *sp. nov.*

MycoBank MB811922

Figs. 1. A–G & 2

Type: INDIA: Madhya Pradesh, Anuppur district, Amarkantak, Mai ki Bagiya, on *Pertusaria amarkantakana* growing on bark of *Shorea robusta*, 29 December 2004, A.N. Shukla 1461 B (BSA – holotype).

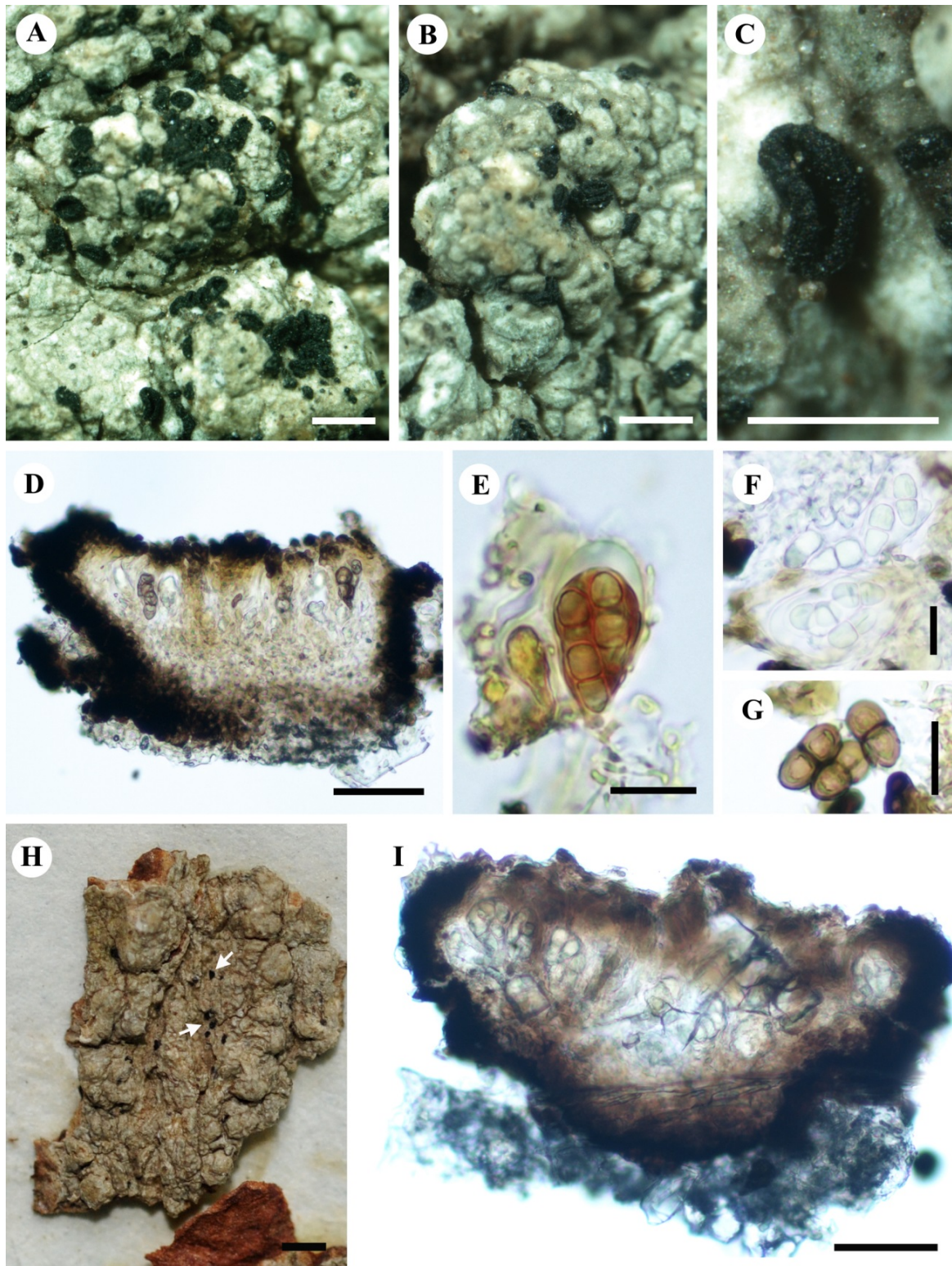


Fig. 1. A–G. *Melaspilea amarkantakensis* (holotype). A–C: Habit. D: Mature lirellae section. E: Ascus (in Lugol's iodine). F: Young ascospores. G: Mature ascospores. H–I. *M. insitiva* Stirt. (Lectotype). H: Habit (arrow showing ascomata). I: Ascoma section. Scale bar: A–C & H = 1 mm; D & I = 50  $\mu$ m; E–G = 15  $\mu$ m.



*Melaspilea amarkantakensis* is a lichenicolous fungus on *Pertusaria* and characterized by excipulum continuous to narrowly discontinuous or absent below the hypothecium, I– and K/I+ pale blue hymenium, strictly 4-spored, shorter,  $25.4\text{--}33.2 \times 14.3\text{--}20.2 \mu\text{m}$  asci and 1-septate,  $(12.1\text{--})13.3\text{--}16.0(\text{--}17.5) \times (5.0\text{--})6.1\text{--}7.6(\text{--}8.9) \mu\text{m}$  ascospores lacking perispore.

Lichenicolous fungus on thallus and ascomata of *Pertusaria*. Ascomata numerous,  $0.3\text{--}0.8 \times 0.1\text{--}0.2 \text{ mm}$ , lirellate to  $\pm$  rounded or irregular, black, more or less emerged, solitary or grouped; disc slit to  $\pm$  widely open, black, epruinose. Excipulum dark brown, continuous with stalk like extension when young, narrowly discontinuous to absent below the hypothecium at maturity,  $15\text{--}22 \mu\text{m}$  thick laterally,  $0\text{--}20 \mu\text{m}$  thick at base, K+ olivaceous. Epithecium brown to dark brown,  $6\text{--}15 \mu\text{m}$  thick, K+ olivaceous. Hymenium hyaline, rarely pale brown in upper part, not inspersed,  $30\text{--}52 \mu\text{m}$  high, K–, I– (or rarely I+ pale blue fading), K/I+ pale blue fading. Hypothecium hyaline or pale brown,  $20\text{--}45 \mu\text{m}$  thick, K– or K+ olivaceous, I–, K/I–. Paraphyses septate, shortly branched, with conspicuous dark brown pigmented apices. Asci broadly clavate, 4-spored,  $25.4\text{--}33.2 \times 14.3\text{--}20.2 \mu\text{m}$ , K–, I–, K/I–. Ascospores 1-septate, ovoid to oblong, at first hyaline, becoming dark brown at maturity,  $\pm$  constricted at the septum,  $(12.1\text{--})13.3\text{--}15.9(\text{--}17.5) \times (5.0\text{--})6.1\text{--}7.6(\text{--}8.9) \mu\text{m}$  ( $n = 63$ ),  $l/b = (1.7\text{--})1.9\text{--}2.3(\text{--}2.5) \mu\text{m}$ , with large guttules in each cell (observed in mature spores), without perispore.

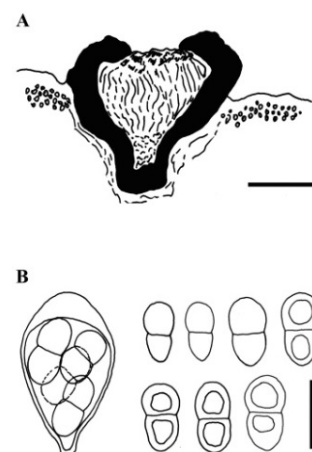
**Etymology:** The specific epithet ‘amarkantakensis’ refers to the type locality Amarkantak.

**Distribution and ecology:** Presently the species is known from Amarkantak (Mai ki Bagiya and Dhuni Pani) in Anuppur district and Kabir chabutra in Dindori district of Madhya Pradesh, India. It grows in dry deciduous forests with  $\pm$  pure strands of *Shorea robusta* along water reservoirs.

**Notes:** *Melaspilea amarkantakensis* is one of the four species of *Melaspilea* s. lat. occurring on *Pertusaria*. The species *M. insitiva*, growing on *Pertusaria*, can be distinguished from the new species by (6–) 8-spored asci with larger ascospores (details under the species). *M. galligena*, the other species occurring on *Pertusaria* induce gall formation on the host thallus and has larger ascospores [ $(20.7\text{--}) 22.5\text{--}25.9 (\text{--}30.0) \times (10.0\text{--}) 11.2\text{--}13.6 (\text{--}15.6) \mu\text{m}$ ]. The species, *M. tucumana*, growing on *Pertusaria* s. lat., characterized by inducing gall formation on the host thallus, presence of periphysoids in the inner part of exciple, and larger ascospores ( $18\text{--}25 \times 7\text{--}11 \mu\text{m}$ ). Further, *M. amarkantakensis* can easily be distinguished from other known lichenicolous *Melaspilea* by its strictly 4-spored asci and difference

in host species. It is close to *M. lekae* (on *Sarcographa labyrinthica*) which has ascomata with a reddish brown disc, a higher (c.  $60 \mu\text{m}$ ), K/I– hymenium, 4–8-spored, longer ( $35\text{--}40 \mu\text{m}$ ) asci and a different host.

The new species shows more similarities to *Labrocarpon*, a genus segregated from *Melaspilea* based on periphysoids covering inner part of the excipulum to accommodate *Melaspilea canariensis* D. Hawksw. on *Pertusaria*. However, this distinguishing character was not observed when specimens (topotype) of *Labrocarpon canariense* (D. Hawksw.) Etayo & Pérez-Ortega were examined by Zhurbenko and Zhdanov (2013). Recently, Flakus et. al. (2014) illustrated the presence of 1–3-septate periphysoids in the inner part of exciple in *M. tucumana*, previously reported by Pérez-Ortega and Etayo (2010) for *Labrocarpon canariense*. However, the authors place the species provisionally under *Melaspilea* s. lat., until the presence or absence of periphysoids are checked in all lichenicolous species of *Melaspilea* s. lat. including type of the genus. The present species lacks the presence of periphysoids in the inner part of exciple. *M. canariensis* [currently known as *Labrocarpon canariensis* (D. Hawksw.) Etayo & Pérez-Ortega] on *Pertusaria* can be distinguished from the new species by apothecia with slit like (never widely exposed) discs, excipular cells densely interspersed with dark brown granules, 8-spored, longer asci ( $40\text{--}60 \times 12\text{--}25 \mu\text{m}$ ) with slightly larger ascospores [ $(13.8\text{--})15.4\text{--}19.8(\text{--}21.0) \times (6.0\text{--})6.8\text{--}8.6(\text{--}9.5) \mu\text{m}$ ]. Zhurbenko and Zhdanov (2013) reported the hymenium of *Labrocarpon canariensis* I+ blue which is not mentioned in the protologue (Hawksworth, 1982) and confirmed the K/I+ blue reaction of the hymenium reported by Calatayud et. al. (1995). The hymenium in *M. amarkantakensis* is always I– or rarely in some lirellae sections I+ pale blue fading.



**Fig. 2.** *Melaspilea amarkantakensis*. A: Young lirellae section. B: Ascus and ascospores. Scale bar: A =  $50 \mu\text{m}$ ; B =  $15 \mu\text{m}$ .



*Melaspilea insitiva* Stirton, Proc. Roy. Soc. Glasgow 11: 314 (1879). Fig. 1. H & I

Type: INDIA: West Bengal, near Chinsurah, G. Watt s.n. (BM! – lectotype, designated here).

Lichenicolous fungus on thallus of *Pertusaria leioplaca*. Ascomata very small, few, scattered, minute, angular to rounded, blackish, c. 0.2 mm long, emerged, not forming gall; disc black, epruinose. Excipulum dark brownish, continuous below the hypothecium, 18–40  $\mu\text{m}$  thick laterally, 10–20  $\mu\text{m}$  thick at base, K+ olivaceous. Epithecium brownish, 10–15  $\mu\text{m}$  thick, K+ slightly olivaceous. Hymenium hyaline, not interspersed, 40–70  $\mu\text{m}$  high, I–, K–, K/I+ pale blue. Paraphyses branched and anastomosing, tips brown pigmented. Hypothecium hyaline to pale brownish, 13–25  $\mu\text{m}$  thick, K– or K+ slightly olivaceous, I–, K/I–. Asci (6–) 8-spored, 32–52  $\times$  16–23  $\mu\text{m}$ . Ascospores 1-septate, hyaline, brown at maturity, constricted at the septa, (16–)16.5–19.5(–22)  $\times$  (6.3–)7.4–9.6(–10.2)  $\mu\text{m}$ , l/b = (1.6–)1.8–2.3(–2.7)  $\mu\text{m}$  (n=17), guttules present in mature ascospores, without perispore.

**Distribution:** Presently known only from West Bengal (India) by type collection. The species appears to be endemic and not collected since 1879.

**Notes:** *Melaspilea insitiva* growing on *Pertusaria* was described by Stirton (1879) from West Bengal. It skipped attention of lichenicolous researchers (Clauzade et. al., 1989; Lawrey and Diederich, 2011) and was treated as a lichenized fungi (Zahlbruckner, 1923; Singh and Sinha, 2010), except Awasthi (1991) who keyed out the species as a parasitic fungi on *Pertusaria*. This led authors to study the type of *M. insitiva*. It is found to be a lichenicolous fungus on *Pertusaria leioplaca*. The species has also not been included in the recent key to the lichenicolous species of *Melaspilea* s. lat. by Flakus et. al. (2014).

This species shows more similarities with *Buelliella* owing to its almost rounded to angular ascomata. But it is difficult to take a decision to place it under *Buelliella* because of poor type material which has only few ascomata in scattered condition. *Buelliella minimula* growing on *Pertusaria*, with almost similar ascospores size (16–22  $\times$  6–12  $\mu\text{m}$ ) of *M. insitiva* but with distinct perispore.

Out of three ascomata examined, only one was found fertile. Though, an annotation label by Patwardhan and Makhija in 1980 exists with comment 'asci and ascospores not found' in the specimen packet. Therefore, till more collections are studied for its proper placement, the earlier placement under *Melaspilea* is retained. Lichenicolous researchers may take up the case more aptly.

Stirton (1879) not cited any herbaria or corresponding number in the protologue of *M. insitiva* except locality Chinsurah (see Fig. 3B). However, according to Singh and Sinha (2010) types are placed at two herbaria, BM and GLAM but without any type designation. The authors received a loan specimen from BM bearing the label 'Ex Herb. Stirton, Part of Type-specimen' and a hand written note '*Melaspilea insitiva* Stirton, India, near Chinsurah. D. G. Watt' (see Fig. 3A). Stirton (1879) mentioned the present collections by Dr. George Watt in the beginning of his paper. According to curator of GLAM, other part of type specimen is not available or missing. Therefore a type designation is required as per ICN Article 9.2 (McNeill et. al., 2012) and consequently a lectotype is designated from BM (BM001096048) herewith as its characters matches well with the protologue.



B

*Melaspilea insitiva* sp. nov.

Apothecia sessilia nigra minuta (latit.  $\approx$  2 mm. vel minora) planiuscula acute marginata; spore 8 n $\epsilon$  in thecis saccatis incolores obovate (uno apice acutiuscule), 1-septatae, 16–22  $\times$  6–12  $\mu\text{m}$ , paraphyses graciles irregulares satis distincte divaricatarum apicibus fusciscentibus; hypothecium infuscatum. Iodo gel. hym. non tineta nisi flavescens, theca pallide fulvescentes. Parasitica supra thallum *Pertusariae leioplacae* prope Chinsurah. The paraphyses are rendered distinct by K.

Fig. 3. A: Herbarium sheet of *Melaspilea insitiva*. B: Protologue of *M. insitiva*.

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**Zhurbenko, M. P. and I. S. Zhdanov. 2013.** *Melaspilea galligena* sp. nov. and some other lichenicolous fungi from Russia. *Folia Cryptogamica Estonica* **50**: 89–99.

## LITERATURE CITED

- Awasthi, D. D. 1991.** A Key to the microlichens of India, Nepal and Sri Lanka. *Bibliotheca Lichenologica* **40**: 1–337.
- Calatayud, V., V. Atienza and E. Barreno. 1995.** Lichenicolous fungi from the Iberian Peninsula and the Canary Islands. *Mycotaxon* **55**: 363–382.
- Clauzade, G., P. Diederich and C. Roux. 1989.** Nelikenigintajfungojlikenlogaj–Ilustritadeterminlibro. Bulletin de la Société Linnéenne de Provence, Numérospecial **1**: 1–142.
- Flakus, A., J. Etayo and M. Kukwa. 2014.** *Melaspilea tucumana*, a new gall-forming lichenicolous fungus from the tropical Andes in Bolivia. *Lichenologist* **46**: 657–662.
- Kalb, K., K. Buaruang, P. Mongkolsuk and K. Boonpragob. 2012.** New or otherwise interesting Lichens. VI, including a lichenicolous fungus. *Phytotaxa* **42**: 35–47.
- Kirk, P.M., P. F. Cannon, D. W. Minter and J. A. Stalpers. 2008.** *Ainsworth and Bisby's Dictionary of the Fungi*. 10<sup>th</sup>edn. CAB International, Wallingford, U.K. 771 pp.
- Hafellner, J. 2004.** *Buelliella*. In: Nash III, T.H. et. al. (eds.), *Lichen Flora of the Greater Sonoran Desert Region*. Vol. 2: 633–635. Tempe: Lichens Unlimited, Arizona State University, USA.
- Hawksworth, D. L. 1982.** *Melaspilea canariensis* sp.nov. and other lichenicolous fungi from Tenerife. *Lichenologist* **14**: 83–86.
- Ihlen, P. G. and M. Wedin. 2008.** An annotated key to the lichenicolous Ascomycota (including mitosporic morphs) of Sweden. *Nova Hedwigia* **86**: 275–365.
- Lawrey, J. D. and P. Diederich. 2003.** Lichenicolous fungi: interactions, evolution, and biodiversity. *Bryologist* **106**: 80–120.
- Lawrey, J. D. and P. Diederich. 2011.** Lichenicolous fungi–worldwide checklist, including isolated cultures and sequences available. Available from: <http://www.lichenicolous.net>. Accessed on 05 May 2014.
- McNeill, J. N., F. R. Barrie, W. R. Buck, V. Demoulin, W. Greuter, D. L. Hawksworth, P. S. Herendeen, S. Knapp, K. Marhold, J. Prado, W. F. Prud'Homme van Reine, G. F. Smith, J. H. Wiersema and N. J. Turland. 2012.** *International Code of Nomenclature for algae, fungi, and plants (Melbourne Code)*. Regnum Vegetabile 154. Koeltz Scientific Books, Koenigstein. 208 pp.
- Pérez-Ortega, S. and J. Etayo. 2010.** *Labrocarpon* gen. nov. for *Melaspilea canariensis*, with the description of *Buelliella protoparmeliopsis* sp. nov. from South America. *Lichenologist* **42**: 271–276.
- Singh, K. P. and Sinha, G. P. 2010.** *Indian Lichens: An Annotated Checklist*. Botanical Survey of India. 571 pp.
- Stirton, J. 1879.** New and rare lichens from India and the Himalayas. *Proceedings of the Royal Philosophical Society of Glasgow* **11**: 306–322.
- Zahlbruckner, A. 1923.** *Catalogus Lichenum Universalis*. Borntraeger, Leipzig.
- Zhurbenko, M. P. 2013.** A first list of lichenicolous fungi from India. *Mycobiota* **3**: 19–34.