



Endohyalina parmotrematis, a new species of lichenicolous fungi from India

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ABSTRACT: The new species *Endohyalina parmotrematis* R. Ngangom, Shweta Sharma, S. Joseph & Nayaka is described from the Western Himalaya of India. This is the first species of *Endohyalina* growing on the thallus of *Parmotrema*. The new species can easily be distinguished from other species of *Endohyalina* by having immersed to sessile apothecia with large *Mischoblastia*-type ascospores, and a different host.

KEY WORDS: Biodiversity, *Buellia sensu lato*, Caliciales, lichenicolous Ascomycota, *Mischoblastia*-type spores, taxonomy.

INTRODUCTION

The lichen genus *Endohyalina* belongs to the family Caliciaceae and is characterized by crustose, autonomous or obligately lichenicolous thalli on crustose or fruticose lichens; lecideine apothecia with *aethalea*-type exciple; with or without oil inspersion in hymenium; brown hypothecium; *Bacidia*-type asci; one-septate, brown ascospores mostly of the *Dirinaria*-type developing with type-B ontogeny, but grading into *Milvinia*-, *Physconia*- or *Pachysporaria*-type, bacilliform conidia and containing diploicin as the major secondary metabolite (Giralt *et al.*, 2010). At present the genus is represented by nine species with cosmopolitan distribution (Giralt *et al.*, 2010; Elix and Kantvilas, 2015, 2016).

The genus *Endohyalina* was established by Marbach (2000) to accommodate two species of *Buellia* with lecideine exciple having an outer carbonaceous zone and an inner broad hyaline zone. Later, Giralt *et al.* (2010) emended the current generic concept of *Endohyalina* to accommodate the additional species of the *Rinodina ericina*-group. Nadyeina *et al.* (2010) reconfirmed the position of the genus *Endohyalina* within group *Buellia sensu lato* based on phylogenetic studies.

During the studies of lichenicolous fungi inhabiting parmelioid lichens of India, a *Buellia*-like fungus was found on the thallus of *Parmotrema* spp. After a thorough examination of the specimen and comparing its characteristics with available literature, it was found that the species belongs to an undescribed species of *Endohyalina*. It is being described as a new species and also an updated world key is provided to facilitate the identification of all species of *Endohyalina*.

MATERIALS AND METHODS

The present study is based on specimens deposited in the herbarium LWG of CSIR-National Botanical

Research Institute, Lucknow. Their morphology was observed using a stereo zoom microscope (LEICA S8APO) and a digital camera (NIKON D90). Thin, hand cut vertical sections of apothecia were mounted in distilled water for anatomical observations and measurements were taken under a Leica DM500 compound microscope equipped with a digital camera (LEICA EC3). The measurements included the length, breadth and length/breadth ratio is given as (min–) $\{\bar{x}-SD\}$ – $\{\bar{x}+SD\}$ (max–), where ‘min’ and ‘max’ are the extreme values, \bar{x} is the arithmetic mean, SD is standard deviation and ‘n’ is the total number of measurements. The colour tests were carried out on the apothecial section using reagents K (5% KOH in water), I (Lugol’s reagent), K/I (I after pre-treatment with KOH). Lichen substances were analyzed using thin layer chromatography (TLC) with solvent system A. The mycological terminology generally follows Giralt *et al.* (2010), Rambold *et al.* (1994) for the asci, Scheidegger (1993) and Bungartz *et al.* (2004, 2007) for the proper exciple, and Giralt (2001) for the ascospore and ontogeny-types. The world distribution map of *Endohyalina* was prepared by using the software QGIS 3.6 (Fig. 1). The GPS coordinates were procured from the published references (Table 1). The species for which geographical coordinates were not available, were georeferenced by using the specimens examined information and Google Earth Pro 7.3.

TAXONOMIC TREATMENT

Endohyalina parmotrematis R. Ngangom, Shweta Sharma, S. Joseph & Nayaka, *sp. nov.* **Fig. 2**

MycoBank No.: MB845641

Type: India, Uttarakhand, Pauri district, Jaiharikhal, N 29°51'10.0", E 78°40'42.7", elev. 1689 m., on *Parmotrema austrosinense* (Zahlbr.) Hale growing on bark of *Pinus* tree, 15 April 2015, V. Shukla & R. Bajpai 015-034463 (LWG-holotype).

Table 1. The known distribution of the species of *Endohyalina* in the world.

Species	Known distribution	References
<i>E. arachniformis</i> Elix & Kantvilas	Australia	Elix & Kantvilas, 2015
<i>E. brandii</i> Giralt, van den Boom & Elix	Spain	Giralt <i>et al.</i> , 2010
<i>E. diderichii</i> Giralt, van den Boom & Elix	Spain	Giralt <i>et al.</i> , 2010
<i>E. ericina</i> (Nyl.) Giralt, van den Boom & Elix	Brazil, France, U.S.A., Portugal, Spain, Ireland	Giralt & Matzer, 1994; Marbach, 2000; Giralt <i>et al.</i> , 2010
<i>E. gillamsensis</i> Elix & Kantvilas	Australia	Elix & Kantvilas, 2016
<i>E. insularis</i> (Arnold) Giralt, van den Boom & Elix	France, New Zealand, Australia, Chile, Greece, Spain, Norway	Triebel, 1991; Giralt, 2001; Halici <i>et al.</i> , 2005; Kaschik, 2006; Hitch, 2006; Nadyeina <i>et al.</i> , 2010; Hafellner, 1993, 1995, 2018; Urbanavichus & Urbanavichene, 2019; John <i>et al.</i> 2020; Hafellner & Mayrhofer, 2007, 2021; Inashvili <i>et al.</i> , 2022
<i>E. interjecta</i> (Müll. Arg.) Giralt	France	Giralt <i>et al.</i> , 2010
<i>E. kalbii</i> (Giralt & Matzer) Giralt, van den Boom & Elix	Italy, Spain, Portugal	Giralt & Matzer, 1994; Giralt, 2001; Giralt <i>et al.</i> , 2010
<i>E. parmotremitis</i> sp. nov.	India	In this paper

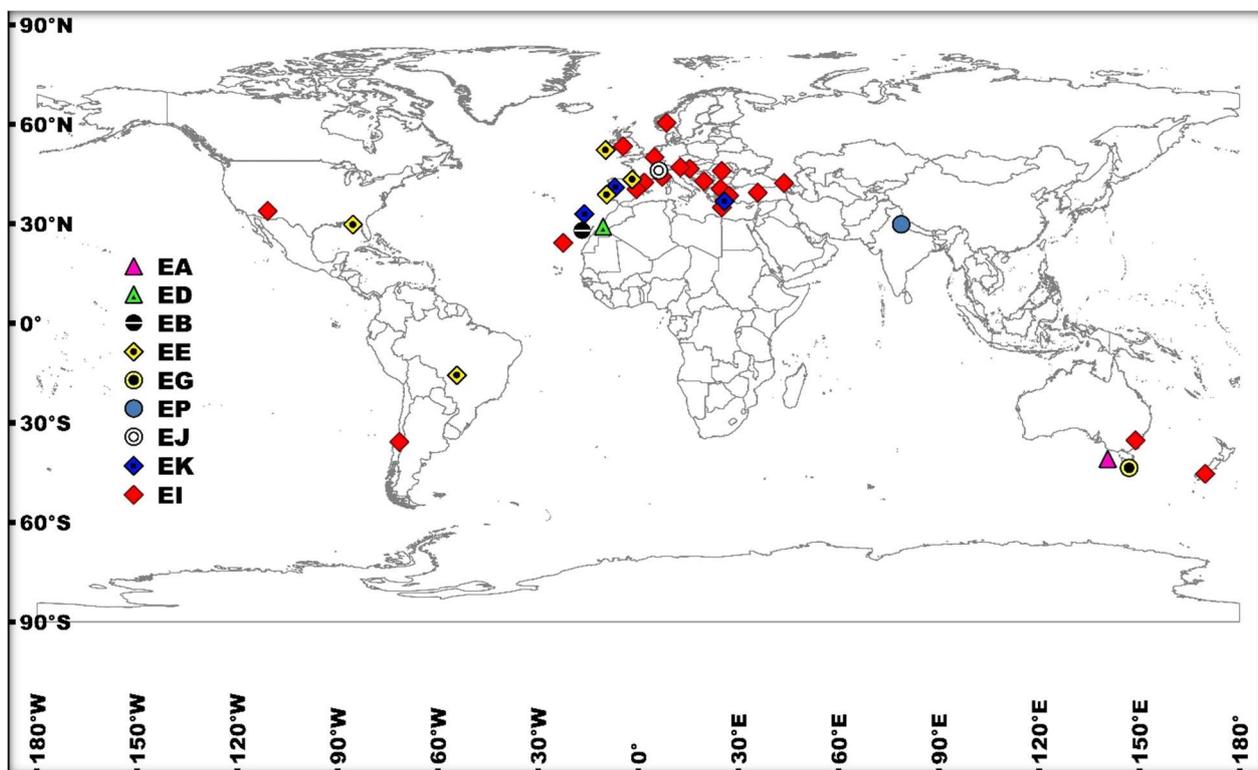


Fig. 1. World distribution map of different species of *Endohyalina*. EA – *Endohyalina arachniformis*, ED – *E. diderichii*, EB – *E. brandii*, EE – *E. ericina*, EG – *E. gillamsensis*, EP – *E. parmotremitis*, EJ – *E. interjecta*, EK – *E. kalbi*, EI – *E. insularis*

Diagnosis: Distinguished from other species of *Endohyalina* in having immersed to sessile apothecia, large *Mischoblastia*-type ascospores, and *Parmotrema* sp. as host.

Description: Thallus discontinuous, obligately lichenicolous on *Parmotrema* spp. The species develops endokapylic to epikapylic thallus, necrotic infection visible on host thallus. **Apothecia** lecideine with prominent thick margin, immersed in the thallus of the host, later becoming sessile and erumpent, 1.5–3.5 mm diam.; **exciple** distinct (*aethalea*-type), poorly differentiated, inner layer similar in structure and

orientation with paraphyses, transient with hyaline subhymenium and brown hypothecium, outer excipular hyphae moderately swollen and usually strongly carbonized with brown pigment, excipular ring absent, outer carbonaceous 19–25 μ m, inner hyaline 30–40 μ m, prosoplectenchymatous, 26–45 μ m wide, K–; **epihymenium** brown, continuous with outer exciple, 10 μ m thick, K–, I–; **hymenium** hyaline, oil inspersed, 60–80 μ m tall, K–, I+ blue; **hypothecium** hyaline to pale brown, 50–60 μ m tall, K–, I–; **paraphyses** simple to sparingly branched near tip, apically swollen with brown to dark brown pigmented caps of 3.0–3.5 μ m width; **asci**

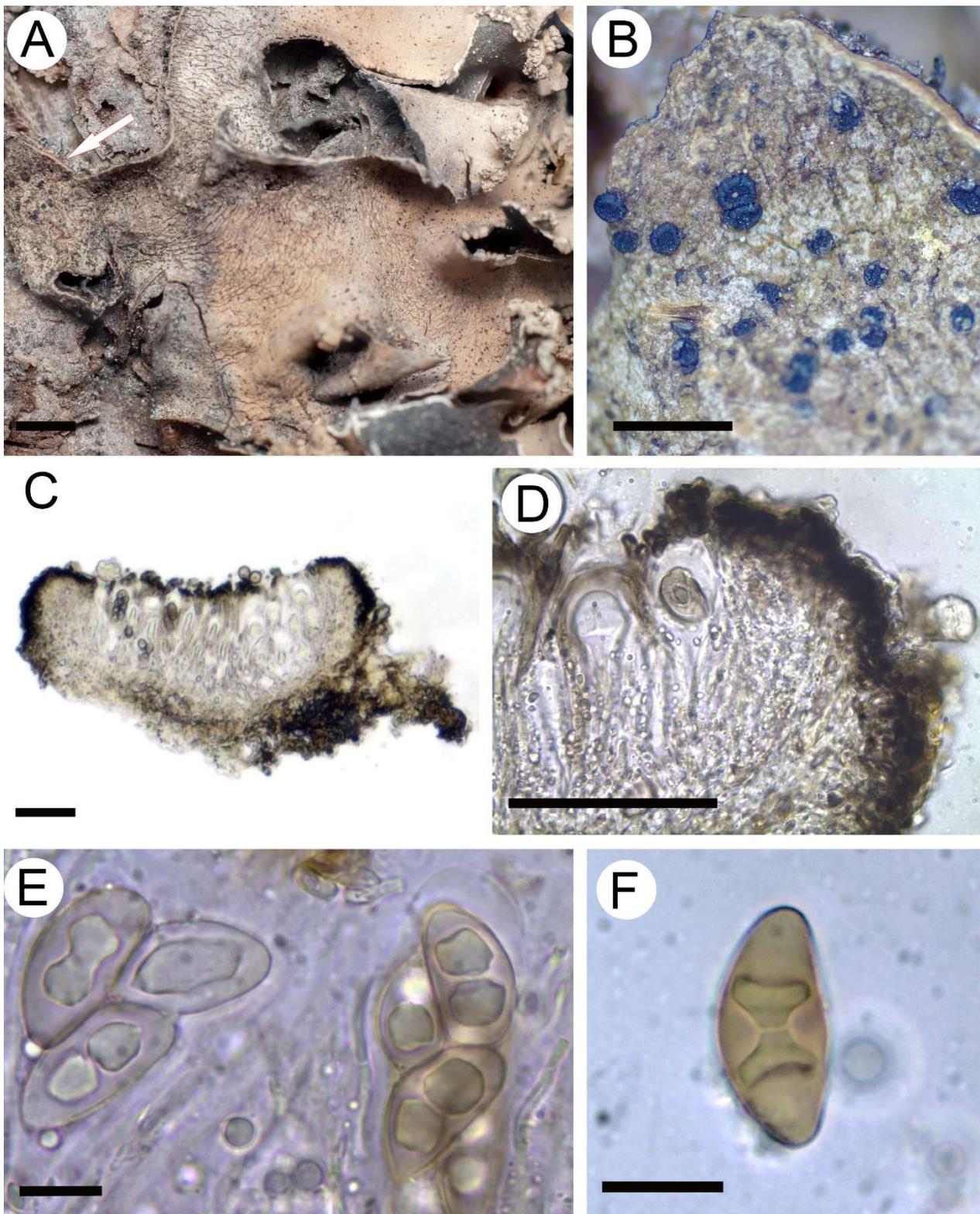


Fig. 2. *Endohyalina parmotremitis*. **A–B.** Habit showing black lecideine apothecia growing on the necrotic lichen thallus of *Parmotrema austrosinense*; **C–D.** Apothecial section showing reduced exciple with carbonaceous external layer; **E–F.** Ascospores ontogeny showing *Pachysporaria*- or *Dirinaria*-type grading into *Mischoblastia*-type at maturity. Scales: A = 2 mm, B = 1 mm, C–D = 50 μ m, E–F = 10 μ m.

**Table 2.** A comparison of *Endohyalina parmotremitis* with other lichenicolous species of the genus.

	<i>E. parmotremitis</i>	<i>E. diderichii</i>	<i>E. insularis</i>	<i>E. brandii</i>
Thallus	Absent or present (epikapylic)	Absent or present (epikapylic)	Absent	Present
Apothecia	Immersed to sessile	Immersed	Immersed to adnate	Sessile
Proper exciple	30–50 µm	30–40 µm	40–60 µm	30–50 µm
Hymenium	Inspersed	Inspersed	Not inspersed	Not inspersed
Hypothecium	Hyaline to pale brown, 50–60 µm tall	Brown, ca. 100 µm tall	Dark brown	Pale reddish brown, 70–150 µm tall
Ascospores type	<i>Dirinaria</i> -type becoming <i>Mischoblastia</i> -type	Ascospores fusiform, <i>Dirinaria</i> -type	Ascospores ellipsoid, <i>Dirinaria</i> -type	<i>Dirinaria</i> -type becoming <i>Milvina</i> - or <i>Physconia</i> -type
Ascospore size (µm)	17–21(–24) × 7–10(–13)	12–15(–17) × 5–6(–7)	11–15(–17) × 5.5–7.5(–9)	11–13(–14) × 6–7(–7.5)
Host	<i>Parmotrema austrosinense</i> , <i>P. reticulatum</i> and <i>P. subtinctorium</i>	<i>Ramalina</i> sp.	<i>Lecanora rupicola</i>	<i>Aspicilia intermutans</i>

clavate, *Bacidia*-type, 8-spored, I+ blue 47–55 × 15–19 µm; **ascospores** transversely one-septate, initially hyaline to light brown, soon becoming brown to dark brown at maturity, oblong to ellipsoid, no constriction near septum, not curved, with unevenly thickened wall, initially simple spore turning into *Pachysporaria*- or *Dirinaria*-type of type-B ontogeny in mid ontogeny and grading to *Mischoblastia*-type (with strongly thickened apical and septal wall and funnel-shaped cell lumina) at maturity, (14.96–) 17.56–21.54 (–24.44) × (5.04–) 7.7–10.78 (–13.09) µm, l/b ratio = (1.41–) 1.81–2.43 (–2.97), n = 50.

Etymology: The species epithet was derived from the host lichen genus.

Chemistry: Spot tests negative on apothecial section. Secondary metabolites not detected by TLC.

Host and ecology: *Endohyalina parmotremitis* is found to be lichenicolous on the thallus of *Parmotrema* species. Currently, the new species is known from Western Himalaya in Uttarakhand and Himachal Pradesh. The distribution of the new species along with other *Endohyalina* species is given in Fig. 1.

Discussion: *Endohyalina parmotremitis* is characterised by lichenicolous habitat having endokapylic to epikapylic thallus, densely inspersion hymenium, slightly enlarged apical cell of paraphyses, large *Mischoblastia*-type ascospores. The new species remarkably varies from *E. brandii* as the later has a subsquamulose thallus, non inspersion hymenium, smaller *Dirinaria*-type (11–14 × 6–7 µm) ascospores and *Aspicilia* as host genus. Among the other lichenicolous species of *Endohyalina*, *E. diderichii* differs from *E. parmotremitis* in its smaller, *Dirinaria*-type (12–15 × 5–6 µm) ascospores and *Ramalina* as host. *E. parmotremitis* also resembles *E. insularis*, but the later species differs in having larger apothecia, 0.2–0.4(–0.7) mm, non-inspersion hymenium, smaller *Dirinaria*-type (11–15 × 5.5–7.5 µm), ascospores and *Lecanora* as host. It is noteworthy to mention that one saxicolous lichenized *Endohyalina* species is known with *Mischoblastia*-type spores, *E. arachniformis* Elix & Kantvilas, which has a conspicuous dark brown to black, arachnoid prothallus with radiate margins (Elix & Kantvilas, 2015) but differs

in having an autonomous habitat, aeruginose-brown paraphyses apices, wider proper exciple (75–150 µm).

Additional specimens examined (Paratypes): India, Uttarakhand: Pauri district, Pauri-Devprayag road, near Circuit house, Pauri, elev. 1700 m. On the thallus of *Parmotrema reticulatum* (Taylor) M. Choisy. 24-06-2005. V. Shukla and Y. Joshi 05-005913 (LWG); Kiyankaleshwar, Pauri Garhwal, Pauri, elev. 1700 m. On the thallus of *Parmotrema reticulatum*. 24-06-2005. V. Shukla and Y. Joshi 05-005910 (LWG); Lansdowne, Tarkeshwar, elev. 1778 m. On the thallus of *Parmotrema reticulatum*. 14-04-2015. V. Shukla & R. Bajpai 15-026279 (LWG); Himachal Pradesh, Shimla district, Rohru, Chirgoan, Sandasu, elev. 1700 m. On the thallus of *Parmotrema subtinctorium* (Zahlbr.) Hale. 19-05-2002. S. Nayaka & R. Srivastava 02-81680 (LWG).

Key to the species of *Endohyalina* (modified from Giralt *et al.*, 2010)

- 1a. Thallus lichenized 2
- 1b. Thallus obligately lichenicolous 6
- 2a. Thallus whitish, composed of discrete to continuous verrucae; hymenium inspersion with few to numerous oil droplets; ascospores fusiform, 10–18 × (4.5–) 5–6 (–6.5) µm *E. ericina*
- 2b. Thallus yellow-green, ochraceous or brown; hymenium without oil droplets; ascospores ellipsoid 3
- 3a. Thallus brown, discontinuous, composed of subsquamulose areolae; ascospores throughout *Dirinaria*-type or grading into *Mischoblastia*-type or *Physcia*-type 4
- 3b. Thallus otherwise; ascospores either *Dirinaria*-type leading to *Pachysporaria*-type or *Serotina*-type finally becoming *Cratiria* or *Buellia*-type 5
- 4a. Areole lacking crystals (polarized light); conspicuous prothallus, radiate margins; ascospores 10–12 (–14) × 4.5–5.5 µm, *Dirinaria*-type grading into *Mischoblastia*-type or *Physcia*-type *E. arachniformis*
- 4b. Areole containing crystals (polarized light); no apparent prothallus; ascospores 10–12 (–14) × 4.5–5.5 µm, *Dirinaria*-type ... *E. interjecta*
- 5a. Thallus ochraceous, continuous, lacking crystals (polarized light); ascospores (*Dirinaria*-type leading to *Pachysporaria*-type) 11–16 × 6–8 µm; secalonic acids present *E. kalbi*
- 5b. Thallus bright yellow-green, continuous, ascospores (*Serotina*-type than becoming *Pachysporaria*-type, *Dirinaria*-type or *Physconia*-type later becoming *Cratiria* or *Buellia*-type) 14–20 × 8–10 µm; xantholepinone A present *E. gillamsensis*
- 6a. Hymenium inspersion with abundant oil droplets; thallus absent or composed of discrete, minute granules 7
- 6b. Hymenium not inspersion 8
- 7a. On *Ramalina*; thallus absent or when present, composed of whitish to pale greyish granules; apical cells of the paraphyses up to 4 µm diam.; ascospores fusiform *E. diderichii*
- 7b. On *Parmotrema*; thallus absent or when present not as above, brown; apical cells of the paraphyses 3–3.5 µm wide; ascospores ellipsoid *E. parmotremitis*



- 8a.** On *Lecanora rupicola*; thallus commonly absent; apothecia immersed in the thallus of the host to adnate, up to 0.7 mm diam.; ascospores 11–17 × 5.5–9 µm; hypothecium dark brown *E. insularis*
- 8b.** On *Aspicilia intermutans*; thallus present; apothecia sessile, up to 0.3 mm diam.; ascospores 11–13(–14) × 6–7 (–7.5) µm; hypothecium pale reddish brown *E. brandii*

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