Short Communication

New morphological and chemical data for Buellia imshaugii

While revising the saxicolous, xanthonecontaining Buellia species in the Iberian Peninsula (Giralt et al. 2009), we found that several specimens of Tetramelas concinnus (Th. Fr.) Giralt growing associated with Dimelaena oreina (Ach.) Norman were labelled as Buellia imshaugii Hafellner, an obligately lichenicolous species on D. oreina described from Canada (Hafellner 1979). To exclude the conspecificity of T. concinnus and B. imshaugii, the type specimens of both taxa were examined. The present study confirmed that they represent two distinct species. Further it provided interesting new morphological and chemical data for B. imshaugii which are presented below.

Buellia imshaugii Hafellner

Nova Hedwigia, Beih. 62: 58 (1979); type: Canada, South Saskatchevan, 13 July 1879, *J. Macoun* (as *Buellia lepidastra* Tuck.) (CANL 19326—holotype,19327 isotype!).

(Fig. 1)

Thallus growing parasitically on thalli of Dimelaena oreina, crustose, areolate, grey to greyish brown; epinecral layer present, 15–30 μ m thick; cortex paraplectenchymatous, 30–60 μ m thick, interspersed with abundant, small crystals (polarized light) which dissolve in K giving a yellow coloration (atranorin); algal layer 60–90 μ m thick; medulla I–, with abundant crystals, those adjacent to the algal layer reacting with PD forming small orange acicular crystals (pannarin), the remainder not reacting with any standard reagent, only partially dissolving in N; calcium oxalate crystals absent in H₂SO₄.



FIG. 1. *Buellia imshaugii* (isotype). A, ascosporeontogeny of *Callispora*-type with subapical wall thickenings appearing before the septum is inserted; B, young ascospores with weak subapical (*Callispora*-type) and septal wall thickenings; C, mature *Buellia*-type ascospores, lacking inner wall thickenings and slightly constricted at septum; D, *Physconia*- and *Buellia*-type ascospores in K both showing a thick proper wall and a thin perispore. Scale = 10 μm.

Apothecia lecideine, adnate to sessile, black, up to 0.8 mm diam., abundant and confluent; proper margin prominent, excluded with age; disc flat to convex, epruinose. Excipulum proprium of the dispersa-type (Scheidegger 1993), well developed, 50-80 µm thick, brown, N-, including crystals of pannarin (PD + orange), often surrounded externally by a colourless epinecral layer up to 5 µm thick. Epihymenium dark brown, N-, inspersed with some crystals (epipsamma). Hymenium colourless, 80-100 um high, without oil droplets. Hypothecium dark brown, up to 150 µm deep, upper part $(20-30 \,\mu\text{m})$ paler. Subapical and apical cells of the paraphyses dark brown, enlarged, 6-7(-8)um diam. and difficult to separate. Asci of the Bacidia-type (Rambold et al. 1994), often containing less than 8 well developed ascospores.

Ascospores of the *Physconia*-type with weak septal wall thickenings (Scheidegger 1993; Giralt 2001), $(13-)15-17(-18) \times 7-8 \cdot 5(-9) \mu m$; when young with tendencies to the *Callispora*-type (Fig. 1B) with weak subapical wall thickenings, when mature constricted at septum and *Buellia*type \pm lacking all inner wall thickenings (Fig. 1C); proper wall thick (Fig. 1D), smooth at ×1000 magnification. Ontogeny of the *Callispora*-type (Fig. 1A).

Conidia bacilliform $5-6(-7) \times 1 \mu m$.

Chemistry. HPLC analyses were carried out according to Elix *et al.* (2003). To detect the secondary substances of this lichenicolous species we followed Giralt *et al.* (2010). Spot tests: thallus: cortex K+ yellow; medulla PD+ orange; C-, KC-.

Buellia imshaugii contains atranorin [major] and pannarin [minor]; Dimelaena oreina contains usnic acid [major], gyrophoric acid [major], ovoic acid [minor] and lecanoric acid [trace] (= chemotype II, Calatayud & Rico 1999).

Discussion. Buellia imshaugii is characterized by its lichenicolous habit; the thallus containing atranorin and pannarin; the dispersa-type excipulum that is brown like the epihymenium (lacking an aeruginose pigment reacting N+ red-violet); the lack of hymenial oil droplets; the strongly enlarged apical cells of the paraphyses and by the *Physconia*-type ascospores, with slight callisporoid thickenings, a thick proper wall and a smooth surface at ×1000 magnification. This combination of diagnostic characters clearly separates *B. im*shaugii from all other lichenicolous species of *Buellia* s. lat. (cf. Hafellner 2004).

Buellia imshaugii is morphologically very close to the non-lichenicolous B. dispersa A. Massal. Both taxa develop a rather thick, areolate thallus, showing a well-developed epinecral layer and cortex which contains atranorin, a dispersa-type excipulum that is pigmented dark brown and is N- like the epihymenium; and both have Physconia-type ascospores. They clearly differ chemically since B. dispersa contains 2'-O-methylperlatolic and confluentic acids rather than pannarin. For comparison, see the detailed description of B. dispersa in Bungarz et al. (2002). Buellia imshaugii shares some important characters with species currently included in *Tetramelas* Norman (see Kalb 2004; Nordin 2004; Nordin & Tibell 2005; Giralt *et al.* 2009) including *callisporoid* ascospores with a thick proper wall. In our opinion, *B. imshaugii* could well be included in that genus. However, as we do not base our study on molecular data, and one of the main diagnostic characters used to separate *Tetramelas* from *Buellia* s. lat. is the presence of xanthones, we retain it in *Buellia* s. lat.

The saxicolous species of Tetramelas are T. concinnus and T. granulosus (Darb.) A. Nordin. The former, which might also grow on D. oreina, is distinguished from B. imshaugii in having longer ascospores [13- $21 \times 6.5 - 9(-10)$ µm versus $13 - 18 \times 7 8.5(-9) \mu m$, which sometimes become 2-3septate and have microrugulate to rugulate ornamentation, and in containing arthothelin, thiophanic acid and 4,5-dichloronorlichexanthone (cf. Bungartz et al. 2007; Giralt et al. 2009). Tetramelas granulosus, apart from its Antarctic distribution, it has (1-)3-septate, rugulate and larger ascospores $(17-28 \times 8-11.5 \ \mu m)$ and contains 6-Omethylarthothelin (cf. Nordin 2004). Finally, T. phaeophysciae A. Nordin & Tibell, which is obligately lichenicolous on saxicolous Phaeophyscia and Physcia species, is distinguished from B. imshaugii by the endokapylic thallus lacking secondary metabolites and the rugulate ascospores without inner wall thickenings (Nordin & Tibell 2005).

Within Buellia s. lat., B. imshaugii is the third species known to contain pannarin. This substance also occurs in B. cinnabarina U. Grube, a saxicolous Australian species characterized by a whitish, pruinose thallus, slightly pruinose apothecia, the presence of a bright red pigment (eumitrin U) and secalonic acid derivatives in the hypothecium and the excipulum, and by Buellia-type ascospores (Grube et al. 2004). According to Kaschik (2006), a further saxicolous Australian species, 'Rinodina' brattii H. Mayrhofer belongs to *Buellia* s. lat. and contains traces of pannarin, in addition to xanthones and atranorin.

Distribution. Buellia imshaugii is reported only from North America (Hafellner 1979; Esslinger 2009) and Europe (Scheidegger 1988, 1993). The North American records are from the type locality in Canada and from one additional locality in Colorado (Hafellner 1979, 2004). The European records are from Spain and Sardinia (Scheidegger 1988, 1993). The Spanish records (MUB-6895, 6983) refer to Tetramelas concinnus (see Giralt et al. 2009). The records of B. imshaugii from Colorado (USA) and Sardinia could not be located in GZU (W. Obermayer, personal communication). Calatayud & Rico (1999: 43) mention that Buellia aff. imshaugii grows on D. oreina in the Iberian Peninsula. The material (in MAF and VAB) is poorly developed and very scarce but it is unrelated to B. imshaugii s. str. and more closely resembles Buellia badia (Fr.) A. Massal., a rather polymorphic species (Bungartz & Nash 2004). Thus, B. imshaugii is actually known with certainty from the type locality only.

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