

# “*Buellia*” *lindingeri* and *Rinodina hallii* (Physciaceae), two closely related species

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**ABSTRACT.** A comparative study of the morphology, anatomy and chemistry of *Buellia lindingeri* and *Rinodina hallii* clearly shows that these taxa are very closely related but not conspecific. The unique epihymenia distinguishes them from most species of both *Buellia* and *Rinodina* but, as they are more closely related to *Rinodina*, *Buellia lindingeri* is transferred to that genus.

**KEYWORDS.** Macaronesia, North America, lichenized ascomycetes, morphology, chemistry, taxonomy, new combination.



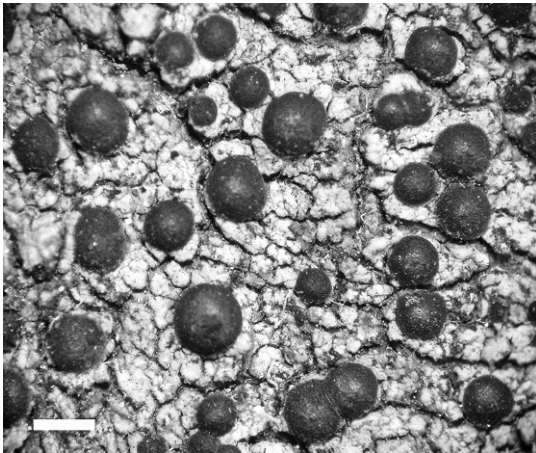
While compiling information on *Rinodina* from the Canary Islands (Boom van den et al. 2009), we were surprised to find the North American species *Rinodina hallii* Tuck. included in the Canarian checklists (Hafellner 1995, 2002, 2005; Hernández-Padrón 2001). This species is reported from El Hierro (Hernández-Padrón 1987), growing on the cortex and lignum of *Juniperus phoenicea*, but, unfortunately, the material in TFCM was not available for study (Hernández-Padrón pers. comm.). After studying the description given by Hernández-Padrón (1987), we suspected that the specimens were, in fact, referable to *Buellia lindingeri* Erichsen, a rather common and widely distributed species in Macaronesia. Moreover, a comparison of the diagnosis and additional descriptions of *B. lindingeri* and *R. hallii* (Giralt & Matzer 1994; Magnusson 1947;

Sheard 2004), indicated that these taxa were morphologically very similar. As a consequence, we performed a comparative study of the two species. The results have shown that they are closely related, with many morphological and chemical characters in common, but not conspecific. Further, both species have an almost plectenchymatic epihymenium (= epithecium) and seem to occupy an isolated position within both *Buellia* s. lat. and *Rinodina*. However, as they are most closely related to *Rinodina*, *B. lindingeri* is transferred to that genus.

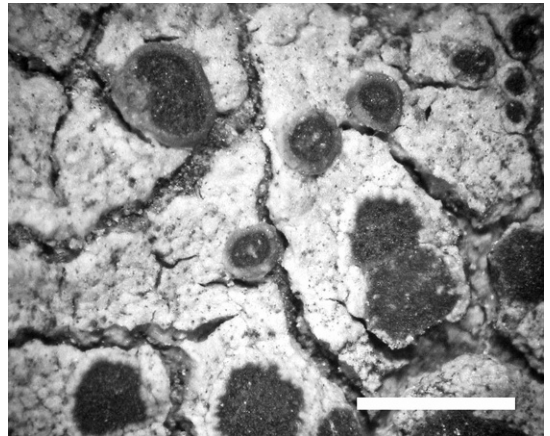
## MATERIALS AND METHODS

This study was based on herbarium material from ASU, BCN, FH and from the private herbaria of M. Brand and P. van den Boom. Lichen morphology was examined by standard techniques using stereo and compound microscopes. The terminology used for the apothecia follows Dughi (1952) and Giralt (2001), for the asci that of Rambold et al. (1994) and

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**Figure 1.** Thallus rimose-areolate with adnate, lecideine apothecia with excluded proper margins and convex discs of *Rinodina hallii* (FH lectotype). Scale = 1 mm.



**Figure 2.** Thallus with lecideine and apparently lecanorine apothecia (surrounded by a thalline collar) of *Rinodina hallii* (ASU 516301). Scale = 1 mm.

for the ascospore-type and ontogeny that of Giralt (2001). Chemical constituents were identified by high performance liquid chromatography (HPLC) (Elix et al. 2003).

**THE SPECIES**

**Rinodina hallii** Tuck., Bull. Torrey Bot. Club 5: 20. 1874. TYPE: U.S.A.. OREGON: upon bark, 1871, *E. Hall* (FH-259425!; lectotype, designated here). **Figs. 1–3**

**Description.** See Magnusson (1947); Sheard (2004).

**Discussion.** Characters separating *Rinodina hallii* from *R. lindingeri*: Thallus thin and smooth, often becoming thicker with age, rugose and rimose to rimose-areolate; apothecia lecideine but always containing at least some algal cells in the exciple (= pseudolecanorine) or apparently lecanorine, i.e., surrounded by a thalline collar, adnate; proper margin normally thin, often paler than the disc; proper exciple entirely colorless or pale brown in the outermost part; hypothecium colorless to yellowish, never deep brown (**Table 1**).

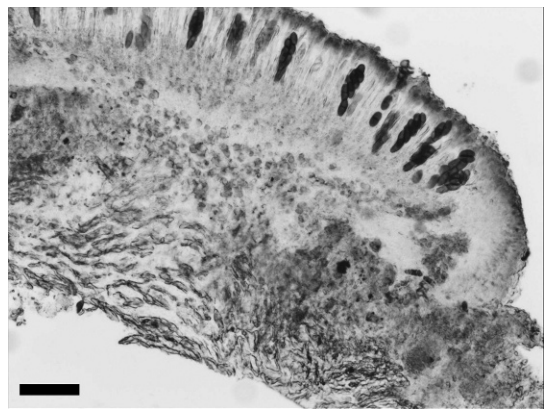
**Chemistry.** Zeorin [major] and variolaric acid [major]. Hypostictic acid [minor] was also detected in the specimen *Boom 29426*. Besides the lectotype, all specimens mentioned below were analysed by HPLC except that from BCN.

**Ecology and distribution.** Mostly on deciduous trees but also on conifers, at elevations of 395–

1675 m. A North American endemic species with an oceanic distribution (Sheard 2004).

**Additional specimens examined.** CANADA.

BRITISH COLUMBIA: Vancouver Island, Sidney, on maples, 19 November 1912, *Macoun* (Herbarium of G.K. Merrill, FH-259426, sub *Rinodina exigua* var. *pruinosa* Merrill). U.S.A. CALIFORNIA: upon redwood and oak (*Quercus sonomensis*), 1864 & 1865, *Bolander-A*, 239, 163 (Tuck. Herb. Exs. 2117, FH!-259422 259423, 259424, syntypes); Los Angeles Co., San Clemente Island, W-trending canyon running seaward toward Lost Point, W slope just NW of Thirst, ca. 1500 ft., the first large canyon N of the main largest canyon, 1966, *Weber & Santesson* (BCN);



**Figure 3.** Section of an apothecium of *Rinodina hallii* showing the colorless proper exciple containing algal cells and the colorless hypothecium (ASU 516301). Scale = 75 µm.

**Table 1.** Characters separating *Rinodina hallii* from *R. lindingeri*.

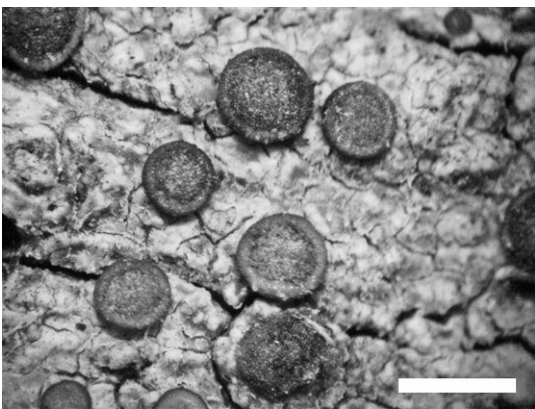
	Thallus	Apothecia	Proper margin	Proper exciple	Hypothecium	Chemistry
<i>R. hallii</i>	Becoming rugose, rimose to rimose-areolate	Lecideine, pseudolecanorine or apparently lecanorine, immersed to adnate	Normally thin and paler than the disc	Colorless entirely to pale brown in the outermost part	Colorless to yellowish	Variolaric, zeorin (majors); hypostictic acid normally absent
<i>R. lindingeri</i>	Persistently smooth to slightly rimose	Lecideine, sessile	Thick and prominent, $\pm$ concolorous with disc	Dark reddish brown entirely or in major part	Dark brown, at least in the lower part	Hypostictic acid, zeorin (majors); variolaric (minor)

Monterey Co., S of Carmel, S of Big Sur, Pfeiffer Big Sur State Park, "Quercus trail" with mature *Quercus agrifolia*, on *Quercus*, 121°46.6'W, 36°14.8'N, 30 m, 2002, *P. & B. v.d. Boom* (HB. BOOM 29426), between Big Sur and Morro Bay, Nacimiento, near crossing south coast/north coast trail, W sloping *Quercus* forest, on *Q. agrifolia*, 121°27.1'W, 36°00.6'N, 840 m, 2002, *P. & B. v.d. Boom* (HB. BOOM 29460), Hastings Ecological Preserve, near cabin and spring on road above Jean Knop's study site, forest with maples, 36°21'45"N, 121°34'0"W, ca. 800 m, 1989, *Ryan 27113* (ASU 527901); Santa Barbara Co., Santa Cruz Island, 7 km SSE of Prisoner's Harbor along road to the navy radar station, live oak stand, on *Q. agrifolia*, 34°0'0"N, 119°40'30"W, ca. 335 m, 1994, *Nash III 32515a* (ASU 527965a); Santa Clara Co., near Saratoga Gap at Skyline Blvd., just SW of San Jose,

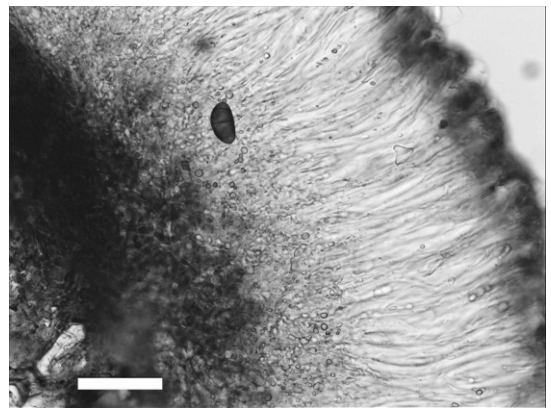
ca. 300 m, 1974, *Sigal 169* (ASU 516301); San Luis Obispo Co., 16 km E of San Simeon along route 46, on *Quercus*, ca. 90 m, 1973, *Nash III 8142a* (ASU 526294). OREGON: Benton Co., above Road 720 above Sulphur Springs McDonald-Dunn Forest just NW of Corvallis, 44°38'54"N, 123°19'24"W, ca. 309 m, 2000, *McCune 25747* (ASU 505197).

***Rinodina lindingeri*** (Erichsen) Giralt & van den Boom comb. nov.; *Buellia lindingeri* Erichsen, *Hedwigia* 66: 281. 1926. TYPE: MACARONESIA. CANARY ISLANDS. TENERIFE: Mesa Gallardina near La Laguna, on *Agave americana*, 750 m, 1917, *L. Lindinger* (HB!; holotype). **Figs. 4, 5**

**Description.** See Giralt and Matzer (1994); Kalb & Hafellner (1992); Tavares (1952).



**Figure 4.** Thallus slightly rimose with sessile, lecideine apothecia with prominent proper margins and pruinose discs of *Rinodina lindingeri* (*v.d. Boom 38157*). Scale = 1 mm.



**Figure 5.** Section of an apothecium of *Rinodina lindingeri* showing the brown lower part of the hypothecium with abundant oil droplets and the hymenium with oil paraphyses (*v.d. Boom 38193*). Scale = 50  $\mu$ m.

**Discussion.** Characters separating *Rinodina lindingeri* from *R. hallii*: Thallus persistently smooth to slightly rimose; apothecia lecideine, sessile and constricted at the base; proper margin thick and prominent,  $\pm$  concolorous with the disc; proper exciple entirely or in part dark reddish brown; hypothecium, at least in the lower part, dark brown (Table 1).

**Chemistry.** Hypostictic acid [major (minor in *Brand 13503*)], zeorin [major] and variolaric acid [minor (major in *Brand 13503*)]. Arthothelin [minor], 4-*O*-methylhypoprotocetraric acid [trace] and hypostictolide [trace] were also detected in the specimens *Boom 22459* and *Boom 38193*. All specimens mentioned below were analysed by HPLC.

**Ecology and distribution.** Mainly on smooth bark of broad leaved trees, but also on conifers or wood, at moderate altitudes (500–1100 m), in the laurel forest zone. *Rinodina lindingeri* is only known from Macaronesia (Canary Islands and Madeira).

**Additional specimens examined not reported in Giralt and Matzer (1994).** MACARONESIA. CANARY ISLANDS. LA PALMA: 8 km N of Santa Cruz, Bco. La Galga, Cubo de la Galga, Laurisilva forest in cleft with volcanic rock, on *Sorbus*(?), 28°45.5'N, 17°46.7'W, 550 m, 1999, *v.d. Boom* (HB. BOOM 22459); 2.2 km WSW of La Galga, Bco. La Galga, 28.7/84.5 FN 28E, on branches of fallen mature *Persea indica* in wood in cleft, 525 m, 1986, *Brand* (HB. BRAND 13352); Cumbre Nueva, W side, S of tunnel, 23.4/71.2, FN56, on *Castanea* in orchard on W-slope, 1120 m, 1986, *Brand* (HB. BRAND 13503). TENERIFE: N of Santiago del Teide, 1.5–2.5 km WSW of Erjos, open path to Las Portelas, in laurisilva, with a.o. *Laurus azorica*, *Erica arborea*, *Apollonias barbujuana*, some shrubs and outcrops, including a by-path young shrubs and trees and well-lit outcrops, on *Laurus*, 1050 m, 28°19.49'N, 16°43.84'W, 2007, *P. & B. v.d. Boom* (HB. BOOM 38193).

## DISCUSSION

Morphologically, *Rinodina lindingeri* and *R. hallii* differ mainly in the apothecium-type and development and in the color of the hypothecium. In *R. lindingeri* the apothecia are truly lecideine at all ontogenic stages, without any algal cell in the proper exciple. They are sessile, with a thick and prominent

proper margin that is concolorous with the disc, and the hypothecium is, at least in the lower part, dark brown. In *R. hallii* the apothecia are apparently lecideine but always contain at least some algal cells in the proper exciple (= pseudolecanorine). They are adnate, with a thin proper margin often paler than the disc and the hypothecium is hyaline to yellowish. Further, in *R. hallii* the apothecia are sometimes apparently lecanorine since a thalline collar plenty of algal cells surrounds them. This character was noted by Tuckerman who wrote in the sheet of the type specimen “apothecia plus minus thallo coronata”.

Otherwise the taxa are morphologically identical: thallus crustose, continuous, usually well delimited by a dark brown,  $\pm$  dendritic prothallus; algal cells small, to 10  $\mu\text{m}$  in diam., often in clusters; disc  $\pm$  dark brownish violet, covered or not by a whitish pruina; brown pigment in epihymenium, exciple and hypothecium reacting K+ olivaceous; hymenium colorless, lacking oil droplets but with abundant oil paraphyses; hypothecium with oil droplets; asci *Lecanora*-type, 8-spored; ascospores large, *Physcia*-type, 20–30  $\times$  9–12  $\mu\text{m}$ , with torus, microrugulate (visible at 1000 $\times$ ), developing after an ontogeny of type A; and conidia bacilliform, 4–6  $\times$  1–1.5  $\mu\text{m}$ .

Two additional interesting characters shared by these two taxa are found in the anatomy of the proper exciple and the epihymenium. The proper exciple is very thick, prosoplectenchymatic, anatomically uniform throughout (not differentiated into an inner and an outer part), composed of moderately thick-walled (= mesodermatous) hyphae, interwoven, thickly packed,  $\pm$  radially oriented, with colorless to brown cell-walls. However, the exciples and hypothecia of the two species differ in their pigmentation. In *R. lindingeri*, the proper exciple may be entirely dark reddish brown; dark reddish brown except in the innermost part; or, very rarely, only pigmented in the outermost part. The more extended the dark reddish brown pigmentation of the proper exciple, the more the dark brown pigmentation of the hypothecium extends into the hymenium. By contrast, in the pseudolecanorine apothecia of *R. hallii* the proper exciple is entirely colorless or only pale brown pigmented in the outermost part, and the hypothecium is colorless to yellowish, never deep brown.

Surrounding the proper exciple of the lecideine apothecia of *B. lindingeri* and the pseudolecanorine apothecia of *R. hallii*, there is an additional,  $\pm$  thick layer of loosely packed, mesodermatous, colorless to rarely pale brown hyphae immersed in a gelatinous extracellular matrix. This additional layer of hyphae corresponds macroscopically to a  $\pm$  visible whitish ‘collar’ surrounding at least the basal part of the proper margin of both species. This layer of hyphae or collar is very well developed and includes many algal cells in *R. hallii* whereas, in contrast, is less developed and lacks algal cells in *R. lindingeri*.

The epihymenium is formed by richly branched and interwoven paraphyses with not or only slightly enlarged, colorless to pale brown pigmented apical cells. It is always covered by a more or less abundant, coarsely granular, brownish orange epipsamma (crystals) that dissolves in K. Anatomically the epihymenium intergrades into a true epithecium since it forms a more or less separate layer of hyphae on top of the hymenium (Kalb & Hafellner 1992; Rambold et al. 1994).

Chemically, both species are also very similar. They contain zeorin and variolaric acid, always accompanied in *R. lindingeri* by hypostictic acid, a secondary substance only once detected in *R. hallii*. The combination of zeorin, hypostictic and variolaric acids is unique within *Buellia* and *Rinodina*. However, variolaric acid is also found in the terricolous *R. mniaraea* (Ach.) Körb. and *R. turfacea* (Wahlenb.) Körb. (and related taxa) and hypostictic acid in some Mediterranean populations of the silicicolous *R. luridescens* (Anzi) Arnold (according to Mayrhofer & Moberg [2002]). These terricolous species have large *Physcia*-type ascospores like *R. lindingeri* and *R. hallii*, while *R. luridescens* has pseudolecanorine apothecia and a  $\pm$  brownish pigmented hypothecium. According to Sheard (2004), *R. hallii* may be related to *R. trevisanii* (Hepp.) Körb., a species belonging to the *R. archaea*-group with *Physconia*-type ascospores and that contains zeorin.

Among *Buellia* s. lat., hypostictic acid is known in *B. sequax* (Nyl.) Zahlbr. aggr., *B. frigida* Darb. and *B. subfrigida* May (see Giralt et al. 2000, under *B. erubescens* Arnold), *B. argillicola* de Lesd., *B. mamillana* (Tuck.) W.A. Weber and *B. spuria*

(Schaer.) Anzi (Bungartz et al. 2007). These *Buellia* species have no others characters in common with *R. lindingeri* and *R. hallii*.

Several authors (Giralt & Matzer 1994; Kalb & Hafellner 1992; Rambold et al. 1994) have already commented on the uncertain taxonomic position of “*Buellia*” *lindingeri*. Its taxonomic assignment is controversial because it exhibits characters that exclude it from both *Buellia* (the *Lecanora*-type asci) and *Rinodina* (the lecideine apothecia and the brown hypothecium). An additional extraneous feature present in this species is its epithecium. In contrast, there has been no discussion to include *R. hallii* in *Rinodina*, probably due to its apothecia that are pseudolecanorine (always containing algal cells) or even apparently lecanorine and its colorless hypothecium, both characters fitting properly with the generic concept of *Rinodina*. Thus, in *R. hallii* the only foreign element to *Rinodina* constitutes its epithecium. This epithecium as well as several other characters (secondary chemistry, color and pruinosity of the apothecial discs, prosoplectenchymatic proper exciple, oil paraphyses, ascospore-type and size, ascospore-ontogeny and bacilliform conidia) are shared by *R. hallii* and *R. lindingeri*, again emphasizing that both species are closely related.

Due to the presence of a true epithecium, Kalb and Hafellner (1992) stated that *B. lindingeri* might be related to still unsettled taxa from the tropics. However, in Marbach’s (2000) monographic treatment of the corticolous/lignicolous species of *Buellia* s. lat. from subtropical and tropical areas, no taxon is mentioned possessing a true epithecium or other characters that could relate it with *R. lindingeri* and *R. hallii*. According to Etayo and Marbach (2003) all species of *Hafellia* have “... strongly branched paraphyses (forming an epithecium)...” In contrast, Bungartz (2004) affirms that the paraphyses of the genus *Hafellia* are not considerably different from other *Buellia* s. lat. species and a distinct layer on the surface of the hymenium cannot be observed (i.e., a distinct epithecium is absent). In any case, the two main diagnostic characters of the genus *Hafellia* (hymenium interspersed with oil droplets and *Callispora*-type ascospores) are absent in *R. hallii* and *R. lindingeri*.

Among the genera newly erected or resurrected by Marbach (2000), only *Sculptolumina* has rinodinooid ascospores (with pronounced inner wall thickenings). However, *Sculptolumina* has a hymenium interspersed with oil droplets, a poorly developed proper exciple composed of  $\pm$  carbonized cells and long filiform conidia (Giralt et al. 2009). Although all the genera treated in Marbach (2000) probably have *Bacidia*-type asci, this cannot be confirmed because the author does not distinguish between *Bacidia* and *Lecanora*-type asci and includes both ascus-types into the Physciaceae-type.

Although *R. lindingeri* and *R. hallii* seem to occupy an isolated position within *Buellia* or *Rinodina* we could find no evidence strong enough to accommodate them within a new, separate genus of the Physciaceae. Nevertheless, as molecular studies exclude *B. lindingeri* from the *Buellia*-group (Molina et al. 2002; Crespo et al. 2004) and relate it with the *Physcia*-group (Wedin et al. 2002)—exactly with *Rinodina lecanorina* (Massal.) Massal. and *R. luridescens* (Grube & Arup 2001; Grube & Hawksworth 2007; Helms et al. 2003; Kaschik 2006)—we transfer *Buellia lindingeri* to the genus *Rinodina*. Unfortunately no genetic data are yet available for *R. hallii*.

The molecular results (see the references mentioned above) which indicate that *R. lindingeri* and *R. lecanorina* constitute a well-supported monophyletic group are unexpected to us. Morphologically, *R. lecanorina* is not at all similar to *R. lindingeri* and *R. hallii*. It has cryptolecanorine to lecanorine apothecia and *Bicinta*-type ascospores. Chemically, it contains zeorin, as do many other *Rinodina* species. A monophyletic relation with *R. luridescens* appears more plausible because this is a species also characterized by pseudolecanorine apothecia, with a brown, well-developed proper exciple, a brownish hypothecium, rather large ascospores that are intermediate between the *Milvina*- and the *Physconia*-type (close to the *Physcia*-type) and a thallus that often contains hypostictic acid.

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