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**Abstract:** Two species of the lichen genus *Bacidia* (Ramalinaceae) with pruinose apothecia from Kangaroo Island, South Australia, are described and illustrated: *B. septosior* (Nyl.) Zahlbr., a long-overlooked species that is widespread in south-eastern Australia, and *B. brigitteae* Kantvilas, described as new and known only from Kangaroo Island where it occurs in coastal *Melaleuca*-dominated woodland.

Keywords: taxonomy, lichens, South Australia, Ramalinaceae

## Introduction

*Bacidia* De Not. (Ramalinaceae) is a large, cosmopolitan genus of lichens that occurs in virtually all vegetation and climatic zones of the world, ranges from sea-level to alpine elevations, and colonises a wide variety of substrata, including rock, wood, bark, soil and living leaves. The genus is well represented in Australia where 33 names in *Bacidia* have been recorded (McCarthy 2016), many based on Australian types, and all the major herbaria hold large numbers of specimens that are mostly unidentified or misidentified.

For decades, Bacidia in the traditional sense served as a useful "form" genus for crustose lichens with a green photobiont, biatorine or lecideine apothecia, eight-spored asci and hyaline, transversely multiseptate ascospores. However, as has occurred with most other form genera, such as Buellia De Not., Catillaria A.Massal., Lecanora Ach. and Lecidea Ach., new insights and the application of characters such as excipulum structure and, in particular, ascus type (Hafellner 1984) have led to the gradual segregation out of *Bacidia* of smaller, more natural units, sometimes classified in different families such as the Pilocarpaceae. Some of these smaller genera that occur in the Australian flora and that accommodate taxa formerly included under Bacidia include Arthrorhaphis Th.Fr. (Obermayer 2001), Bacidina Vězda (Vězda 1990), Bapalmuia Sérus. (Kalb et al. 2000), Bryobilimbia Fryday, Printzen & S.Ekman (Fryday et al. 2014), Fellhanera Vězda (Vězda 1990), Fellhaneropsis Sérus. & Coppins (Kantvilas & Lücking 2009), Jarmania Kantvilas (Kantvilas 2008), Sarrameana Vĕzda & P.James (Kantvilas & Vĕzda 1996) and *Scoliciosporum* A.Massal. (Kantvilas 2008).

In recent decades, there have been considerable advances in the study and delimitation of *Bacidia*, mainly in the Northern Hemisphere, and a large

body of literature pertaining to individual species or local regions now exists. Significant contributions of a broader application, and ones that assist understanding the species found in Australia, include the account for North American corticolous species (Ekman 1996), and flora treatments or keys for the Iberian Peninsula (Llop & Hladun 2002), Great Britain (Coppins & Aptroot 2009), the Sonoran Desert (Ekman 2004) and North America (B. McCune, unpublished mss). However, in Australia, the genus remains extremely poorly known and is, in some respects, the last of the "big genera" demanding detailed study and revision. It is noteworthy that since the resurgence of lichenology from the mid-20th Century onwards, the only contributions on Bacidia in Australasia remain the Flora account for New Zealand by Galloway (2007), and descriptions of new species by Elix (2009) and Coppins & Fryday (2007). In this author's considered view, the many taxa listed by McCarthy (2016) represent only the very tip of a very large and complex iceberg.

For some years, the author has been gradually examining the types and reliably identified specimens of many Bacidia species in the major herbaria of the world, with the intention of bringing some order to those taxa that occur in southern Australia, especially Tasmania and Kangaroo Island, where most of his taxonomic studies have been focused. This work has proved to be extremely challenging due to the large numbers of species and specimens involved, and the subtle variation in critical species-level characters such as apothecial pigmentation, excipulum structure and ascospore dimensions. A comprehensive account for the region is clearly some way off, even though a few particular species have now been resolved. In this paper, two species with pruinose apothecia, a feature that is rather restricted in the genus, are treated; the work represents the first hesitant steps towards a broader study.

## **Material and methods**

The study is based mainly on collections of the author housed in the Tasmanian Herbarium (HO), with additional material sourced from other herbaria as indicated in text. Anatomical and morphological observations were undertaken using light microscopy, with thin hand-cut sections mounted in water, 10% KOH, 50% HNO3, Lactophenol Cotton Blue, ammoniacal erythrosin and Lugol's Iodine, with and without pretreatment with KOH. Ascospore measurements are based on at least 50 observations for each taxon and are presented in the format: 5<sup>th</sup> percentile–*average*–95<sup>th</sup> percentile, with outlying values given in brackets. Chemical composition was investigated by thin-layer chromatography using standard methods (Orange *et al.* 2001). Nomenclature of apothecial pigments follows Ekman (1996).

# The species

### Bacidia septosior (Nyl.) Zahlbr.

*Catal. Lich. Univ.* 4: 240 (1926); *Lecidea septosior* Nyl. in Crombie, *J. Linn. Soc., Bot.* 17: 400 (1880). **Type:** [Australia, New South Wales] on bark of trees, near Port Jackson, *R. Brown 562* (holo: BM!).

*Thallus* crustose, whitish grey to grey green, rather scurfy, smooth to somewhat verruculose, or, more commonly, patchy and evanescent, to  $40-150 \mu m$  thick, forming irregular, undelimited patches to 15 cm wide or more; prothallus absent; photobiont chlorococcoid, with cells globose,  $7-13 \mu m$  diam. *Apothecia* biatorine, 0.6–1.6 mm diam., scattered, urceolate and strongly basally constricted to almost substipitate when young, soon becoming disciform; disc plane at first, often

persistently so, or sometimes becoming convex, black to brown-black, matt, occasionally bluish grey pruinose when very young but soon becoming epruinose; proper excipulum persistent, sometimes becoming reduced in the oldest, most convex apothecia, with outer parts glossy red-brown and the upper edge intensely bluish grey pruinose when young, later becoming brown-black to black and the pruina restricted to the upper inner edge or absent, in section cupulate, 90–160 µm thick, at the upper edge red-brown to brown, K± purple-brown, N+ orange, internally ± colourless, composed of radiating, branched and anastomosing hyphae 1-1.5 µm thick in a gel matrix, lacking enlarged terminal cells, inspersed with bands of angular crystals to c. 5 µm wide that are insoluble in K. Hypothecium 140-200 µm thick, colourless throughout, or pale yellowish, intensifying yellowish in K and N in the upper part. Hymenium 120–160 µm thick, sparsely inspersed with oil droplets; epithecium grey-green, brownish green or brownish, K± intensifying greenish, N+ purple, sometimes rather fleetingly so and then fading to pale orange, also often slowly forming a precipitate of minute, dark blue crystals; paraphyses 1.5-2 µm thick, simple, not or only weakly capitate, ± separating readily in K, but with apices remaining conglutinated in epithecial pigment and sometimes expanded to 3 (-4) µm wide; asci of the Bacidia-type, narrowly cylindrical, with an amyloid tholus not entirely pierced by a ± non-amyloid, conical masse axiale,  $90-110 \times 14-20 \mu m$ . Ascospores arranged side-by-side or very loosely coiled in the ascus, filiform to narrowly cylindrical with rounded apices, easily fractured, (55–) 64.5–77.6–96 (–104) × 4–4.5–5.5 (-6) µm, 20-35-septate. Pycnidia very rare, immersed; conidia filiform, curved, aseptate,  $15-20 \times 0.5$  (-1) µm. Chemistry: no substances detected by t.l.c. Figs 1, 2B.



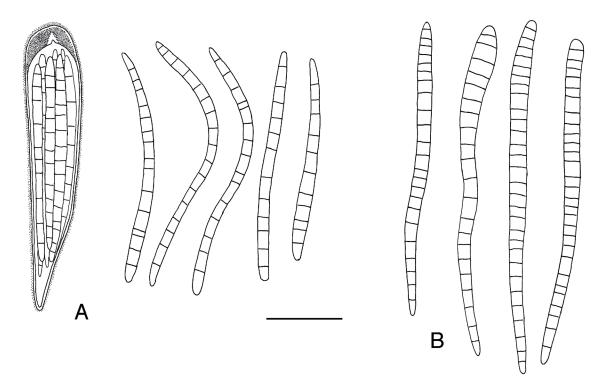
**Fig. 1.** *Bacidia septosior* habit (*Kantvilas 261/13*). Note the youngest (smallest) apothecia with a characteristically pruinose inner margin. Scale = 5 mm.

*Note on the type.* The sheet in BM bearing Brown's type specimen also has a second collection in very poor condition, annotated in the same hand with "on the bark of tree, S. Coast of Tasmania, R. Brown".

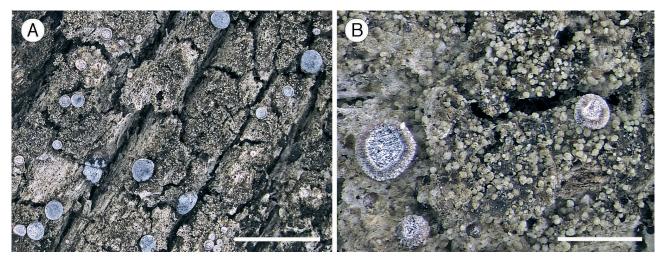
Discussion. This highly distinctive species is characterised by the presence of crystals in the excipulum in combination with the pruinose apothecial margin and the particular suite of apothecial pigments. The pruina are best seen in young apothecia, and older apothecia may be entirely epruinose or with the pruina reduced to just the inner upper edge of the excipulum. Whilst some specimens may be dominated by black epruinose mature apothecia, careful examination will usually reveal vestiges of pruina on at least some apothecia. The crystalline inclusions in the excipulum are invariably present although rarely they may be few in number. The excipular pigment is laurocerasi-brown of Ekman (1996). The epithecial pigment is more variable but, in most collections, the dominant colour in water is greenish or greenish grey or greenish brown. With the addition of KOH, the pigment remains unchanged or the greenish hints intensify, whereas with HNO<sub>3</sub>, it turns a vivid purple, then slowly fades to a pale orange; if this pigment is sufficiently concentrated, a precipitate of minute dark blue crystals may develop. The pigment appears to match Bacidia-green II of Ekman (1996).

Whilst this species, as defined above, is extremely clearcut, there are herbarium collections, particularly from Tasmania, that have essentially identical apothecial pigments and ascospores, but lack both the apothecial pruina and the crystalline excipular inclusions; such specimens are excluded from this species and require further study. The most similar species to B. septosior (Nyl.) Zahlbr., in that it has pruinose apothecia with crystals in the excipulum, is B. brigitteae, which is described and discussed below. Also extremely similar is Bacidia ceratina (Stirt.) Hellb., described from New Zealand (lectotype in GLAM examined). This name was synonymised incorrectly with B. laurocerasi (Delise ex Duby) Vain. by Galloway (1985). Although B. laurocerasi and B. ceratina have some superficial features in common, the latter has crystalline excipular inclusions and occasionally pruinose apothecial margins identical to what is seen in B. septosior. Interestingly, neither of these features is noted in the original description of Stirton (1875). I have considered the possibility that B. ceratina may be an earlier name for B. septosior, but have declined to synonymise these taxa. Brown's Australian type of *B. septosior* matches subsequent collections of this species exactly. In contrast, the New Zealand type specimen of B. ceratina has a very thick, papillate-vertuculose thallus that has never been observed in Australian specimens. Furthermore, no well-developed ascospores could be found in the B. ceratina specimen, but the hymenium is consistently thinner than that of B. septosior, and therefore presumably the ascospores may well be shorter.

**Distribution and ecology.** Bacidia septosior appears to be widespread in lowland, especially coastal areas of south eastern Australia (New South Wales, Victoria, Tasmania, the Bass Strait Islands and Kangaroo Island), where it occurs mostly on soft, spongy bark, such as on



**Fig. 2.** Anatomy of *Bacidia*, showing typical, *Bacidia*-type asci and filiform, multi-septate ascospores. **A** *B. brigitteae*; **B** *B. septosior*. Scale = 20 μm.



**Fig. 3.** *Bacidia brigitteae* (isotype). **A** Habit, showing persistently pruinose apothecia. Scale = 5 mm. **B** Detail, showing the characteristic, granular to goniocyst-like thallus. Scale = 1 mm.

the bases of eucalypts, *Callitris*, species of *Melaleuca*, or shrubs of the Asteraceae such as *Olearia* or *Bedfordia*. It appears to prefer marginally moister conditions, such as swampy *Melaleuca*-dominated woodland or older trees in locally damper microhabitats within dry eucalypt woodland. It is usually part of a rich association of epiphytic lichens, the more common of which include *Bacidia brigitteae* Kantvilas, *Megalaria grossa* (Pers. ex Nyl.) Hafellner, *Menegazzia caesiopruinosa* P.James, *M. subpertusa* P.James & D.J.Galloway, *Pannaria elixii* P.M.Jørg. & D.J.Galloway, *P. obscura* Müll. Arg., *Parmotrema perlatum* (Huds.) M.Choisy, *Physcia poncinsii* Hue and *Pseudocyphellaria aurata* (Ach.) Vain.

#### Specimens examined

SOUTH AUSTRALIA, Kangaroo Island: Cape du Couedic Road, 1 km S of Rocky River Settlement, 35°57'S 136°44'E, 70 m alt., 1994, H. Streimann 54997 (AD, CANB); Willson River, 35°52'S 137°56'E, 1997, R.J. Bates 48365 (AD); Moffatt Road, 35°49'S 138°00'E, 70 m alt., 2011, G. Kantvilas 251/11 & B. de Villiers (HO); West Bay, 35°53'S 136°33'E, 10 m alt., 2011, G. Kantvilas 299/11 (AD, HO); Creek Bay Farm, headwaters of Lubra Creek, 35°49'S 138°06'E, 40 m alt., 2011, G. Kantvilas 379/11 (AD, BM, HO); Brown Beach, 35°48'S 137°50'E, 10 m alt., 2012, G. Kantvilas 435/12 & B. de Villiers (AD, CANB, HO); North Cape Road, 35°36'S 137°35'E, 5 m alt., 2013, G. Kantvilas 261/13 & B. de Villiers (HO); the Old Cannery, American River, c. 1 km SW of Ballast Head, 35°46'S 137°48'E, 3 m alt., 2013, G. Kantvilas 347/13 & B. de Villiers (AD, HO); Rocky River, 35°56'S 136°37'E, 10 m alt., 2015, G. Kantvilas 518/15 (HO); South-West River, 36°01'S 136°52'E, 10 m alt., 2015, G. Kantvilas 423/15 (HO).

VICTORIA: Furnells Landing, 20 km S of Cann River township, 37°43'20"S 149°08'14"E, 1 m alt., 2008 *G. Kantvilas 155/08 & J.A. Elix* (HO).

TASMANIA: southern coast, [1804], *R. Brown s.n.* (BM); Marrawah to Montague Track, 40°48'S 144°50'E, 30 m alt., 1986, *G. Kantvilas 113/86* (HO); Swan Basin, 42°12'S 145°16'E, sea-level, 2000, *G. Kantvilas 32/00* (HO); Buckland Military Training Area, S of Bluestone Tier, 42°30'S 147°48'E, 300 m alt., 2003, *G. Kantvilas 325/03*  (HO); eastern side of Stanley Hwy, 40°47'S 145°16'E, 5 m alt., 2011, *G. Kantvilas 452/11* (HO); end of Bolduans Road, 40°47'S 145°02'E, 1 m alt., 2011, *G. Kantvilas 474/11* (HO); Stony Point, 40°45'S 144°59'E, 2 m alt., 2016, *G. Kantvilas 275/16* (HO). Bass Strait, Flinders Island: Patriarchs Inlet, 39°57'S 148°11'E, 1983, *J.S. Whinray s.n.* (HO, MEL).

### Bacidia brigitteae Kantvilas, sp. nov.

Bacidiae septosiori similis et item excipulo crystallis angularibus insperso sed thallo granuloso, apotheciis persistente griseo-pruinosis, hymenio tenuiore, 80– 90 µm crasso, ascis parvioribus, 60–75 µm longis, 12– 15 µm latis, et ascosporis brevioribus angustioribusque, 43–80 µm longis, 2.5–4 µm latis, modo 8–18-septatis praecipue differt.

**Typus:** South Australia, Kangaroo Island: Grassdale Lagoon, 36°00'S 136°53'E, 20 m alt., on *Melaleuca* in mallee woodland, 4 Oct. 2015, *G. Kantvilas 326/15 & B. de Villiers* (holo: HO 580598; iso: AD). **Mycobank number: MB821079.** 

Thallus granular or ± goniocyst-like, bright to dull green, less commonly becoming evanescent and endophloeodal, forming irregular, often discontinuous, undelimited patches to 10 cm wide or more; individual granules crowded together or dispersed, 30-60 (-80) µm wide, sometimes somewhat isidioid in appearance, comprising tightly clustered photobiont cells in a sheath of rather short-celled hyphae  $2.5-5 \ \mu m$ wide; prothallus absent; photobiont a unicellular green alga with globose cells 5-12 µm diam. Apothecia biatorine, 0.5–1.5 mm diam., scattered, superficial, basally constricted, disciform; disc mostly plane or occasionally becoming undulate or convex, pale brown but persistently and thickly pale grey pruinose from the earliest stages; proper excipulum persistent, somewhat inrolled when young and usually remaining ± taller than the disc, only rarely excluded when the disc is highly convex, at first pale grey to pale brown, soon becoming brown to dark brown to black, persistently grey pruinose in the upper part and concolorous with the disc, on the underside epruinose, in section

cupulate, 50–110 µm thick laterally, 90–150 µm basally, colourless within but sometimes pale brownish, K-, N± pale orange at the upper edge, composed of radiating, anastomosing hyphae 2.5-3.5 µm thick in a gel matrix, lacking enlarged terminal cells, inspersed with clusters of angular, rectangular or rhomboidal crystals up to 10 µm across that do not dissolve in K but dissolve in N. Hypothecium 100-180 µm thick, colourless throughout or pale yellowish grey in the upper part, weakly intensifying yellowish in K and N. Hymenium 80-90 µm thick, colourless, occasionally sparsely inspersed with oil droplets, overlain by a greyish brown epithecium, K+ purplish brown, N+ crimson and fading; asci of the Bacidia-type, elongatecylindrical, with an amyloid tholus not entirely pierced by a  $\pm$  non-amyloid, conical masse axiale, 60–75  $\times$ 12-15 µm; paraphyses simple or sparsely branched, 1.5 (-2) µm thick, with apices not capitate. Ascospores filiform with blunt or acute apices, side-by-side or, less commonly, coiled in the ascus, (43-) 50-60.1-77  $(-80) \times (2.5-) 3-3.3-4 \mu m$ , 8-18-septate. *Pycnidia* not found. Chemistry: no substances detected by t.l.c. Figs 2A, 3.

*Etymology.* The new species is named in honour of Brigitte de Villiers, who has accompanied me throughout my excursions on Kangaroo Island, with great patience strongly supported my lichen investigations there, and co-collected the type as well as many other specimens.

Remarks. When first collected, this taxon was shoehorned, with considerable reservations, into B. septosior on the basis of having apothecia with an excipulum inspersed with crystals, and generally filiform, multiseptate ascospores. Morphological differences such as the persistently pruinose, disciform rather than somewhat urceolate apothecia and the granular thallus were initially considered unusual variations of *B. septosior*. The opportunity to observe this species in the field and make subsequent, rich collections soon confirmed that this taxon was indeed distinct, and that the distinctive morphological thalline and apothecial characters mentioned above were consistently evident. There are further anatomical differences between the two species. In B. brigitteae, the hymenium is always thinner, with concomitantly shorter asci. Furthermore, the ascospores are consistently shorter and narrower, with fewer septations. Apothecial pigmentation appears superficially rather similar and at least some of the described differences may be due to differences in pigment concentration, especially with respect to the excipulum. However, the epithecial pigment of B. brigitteae is different but difficult to characterise: there is no hint of greenish coloration in either water or KOH, yet it reacts crimson in HNO3, but with no subsequent development of a dark blue precipitate.

According to Ekman (1996), a goniocyst-like thallus is unusual in *Bacidia*, and is more characteristic of the genus *Bacidina*. Yet the new species lacks the expanded, pseudoparenchymatous hyphae at the outer edge of the

excipulum that characterise *Bacidina*, and appears to belong comfortably in *Bacidia*.

**Distribution and ecology.** Bacidia brigitteae is known only from Kangaroo Island, where it grows on the soft, furrowed bark of *Melaleuca* in coastal old growth mallee woodland or *Melaleuca*-dominated woodland, in close proximity to rivers and lagoons where, presumably, humidity levels are higher, at least intermittently. Associated species include Bacidia septosior, Caloplaca kaernefeltii S.Y.Kondr., Elix & A.Thell, Leptogium crispatellum Nyl., Pannaria obscura Müll.Arg., Physcia rolandii Elix, Psoroglaena halmaturina P.M.McCarthy & Kantvilas, Rinodina australiensis Müll.Arg. and Strangospora pinicola (A.Massal.) Körb.

## Specimens examined

SOUTH AUSTRALIA, Kangaroo Island: Ravine des Casoars, along riverbank, c. 0.5 km inland from coast, 35°48'S 136°35'E, 15 m alt., 2012, *G. Kantvilas 482/12 & B. de Villiers* (AD, HO); South-West River, 36°01'S 136°52'E, 10 m alt., 2015, *G. Kantvilas 426/15 & B. de Villiers* (AD, HO).

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