

The identity of *Buellia sequax*

Mireia Giralt · Frank Bungartz · John A. Elix

Received: 19 May 2010 / Revised: 7 July 2010 / Accepted: 14 July 2010 / Published online: 11 August 2010
© German Mycological Society and Springer 2010

Abstract The study of the type specimens of *Buellia sequax* revealed that the current concept of this species is erroneous and instead corresponds to *B. abstracta*. By contrast, *B. sequax* s. str. is characterized by the same diagnostic characters as presently accepted for *B. excelsa*. Consequently, they represent one and the same taxon. Since *B. sequax* is the older name, *B. excelsa* is reduced to synonymy. *Buellia lepidastroidea* is also reduced to synonymy with *B. sequax* s. str. *Buellia sequax* s. str. (= *B. excelsa* and *B. lepidastroidea*) contains diploicin and atranorin, has a hymenium interspersed with oil droplets, and ascospores which develop with type-B ontogeny. This combination of characters suggests that *B. sequax* s. str. is closely related to the genera *Diploicia* and *Endohyalina* but *B. boergesenii* does not belong to this group. New data on the chemistry and distribution of *B. sequax* s. str. are provided and amended descriptions of this species and *B. abstracta* (= *B. sequax* auct.) are presented.

Keywords Species recircumscription · Taxonomy · Chemistry · Distribution · Physciaceae · Lichenized ascomycetes

Introduction

As part of a revision of the genus *Buellia* in the Iberian Peninsula and the Canary Islands, the first author (M.G.) studied many specimens previously identified as *Buellia sequax* (Nyl.) Zahlbr. As this material was very heterogeneous, the type specimens of *B. sequax* and of other taxa considered conspecific were examined, including *B. abstracta* (Nyl.) H. Olivier, *B. heteropsis* Müll. Arg., and *B. caloplacivora* Llimona & Egea. These studies established that the currently accepted concept of *B. sequax*, first promulgated by Scheidegger (1993) and followed by Bungartz et al. (2004), a concept that has subsequently been used in many local studies and lichen floras (e.g., Bungartz et al. 2007; Coppins et al. 2009), is erroneous.

According to Scheidegger (1993), *B. sequax* auct. is a rather polymorphic species concerning the thallus development (chasmolithic to epilithic from granular to areolate) and chemistry (without secondary metabolites or with norstictic acid), but readily recognized by a proper exciple of the *aethalea*-type, a hymenium which lacks oil droplets and by the presence of narrow, *Buellia*-type ascospores. In contrast, the three type specimens of *B. sequax* (H-NYL 9538!-lectotype, H-NYL 9539! and M 0023084!-isolectotypes) all have thallus a which contains atranorin and diploicin, a *dispersa*-type proper exciple, a hymenium with oil droplets and *Dirinaria*- or *Physconia*-type ascospores (at least when young), which develop with type B ontogeny. All these characters are consistent with the current accepted concept and type specimens of *B. excelsa* (Leight.) A.L. Smith.

M. Giralt (✉)
Departament de Bioquímica i Biotecnologia (Àrea de Botànica),
Facultat d'Enologia de Tarragona, Universitat Rovira i Virgili,
Marcel·lí Domingo s/n,
43007 Tarragona, Spain
e-mail: mireia.giralt@urv.cat

F. Bungartz
Botany Department, Charles Darwin Research Station,
Casilla 17-01-3891,
Quito, Ecuador
e-mail: fbungartz@fcdarwin.org.ec

J. A. Elix
Research School of Chemistry, Building 33,
Australian National University,
Canberra ACT 0200, Australia
e-mail: John.Elix@anu.edu.au

The previously widely employed concept of *B. sequax* auct. is consistent with the type of *B. abstracta*. As a consequence, *B. excelsa* (described in 1911) is regarded here as a synonym of *B. sequax* (described in 1875), and *B. sequax* auct. (Scheidegger 1993) identified as *B. abstracta* (described in 1903).

Materials and methods

This study is based on herbarium material from ASU, BCN, BM, H, M, MISC, MUB, PO and from the private herbaria of M. Mus (Mallorca). Lichen morphology was examined by standard techniques using stereo and compound microscopes. The terminology used for the proper exciple follows Bungartz et al. (2007), for the asci Rambold et al. (1994), and for the ascospore and ontogeny-types Giralt (2001).

Chemical constituents were identified by thin layer chromatography (e.g., Culberson and Ammann 1979; Culberson et al. 1981; Culberson and Johnson 1982) and high performance liquid chromatography (HPLC) (Elix et al. 2003).

Results

Emended description of *Buellia sequax* sensu stricto

Buellia sequax (Nyl.) Zahlbr. emend Giralt, Bungartz & Elix

Basionym *Lecidea sequax* Nyl., Flora 58: 302 (1875).
Buellia sequax (Nyl.) Zahlbr., Cat. Lich. Univ. 7: 410 (1931). Type: France, Hautes Alpes, Quartz sur les coteaux de la Vienne près du Moulin de l'Aiguille, 11 January 1872, Lamy 1083 (H-NYL 9538!-lectotype, selected by Scheidegger (1993), H-NYL 9539! and M-0023084!- isoelectotypes).

New Synonyms *Buellia excelsa* (Leight.) A. L. Smith, Monogr. Brit. Lich. 2: 174 (1911). *Lecidea excelsa* Leight., Grevillea 4: 78 (1876). Type: Ireland, Galway, Connemara, on the summit of Mt. Doughruaght, June 1875, Larbalestier (BM!-lectotype; BM!-isoelectotypes).

Buellia lepidastroidea Imshaug ex Bungartz, Mycotaxon 90: 85 (2004). Type: México, Baja California, Isla Cedros, ridge crest overlooking western shore and adjacent canyon to the E at the NW corner of the island, ca. 300 m, 19 March 1994, Nash 34458 (ASU!-holotype).

Description The following description is principally based here on the three type specimens of *B. sequax*. This material is very poorly developed and the thallus, where present, is clearly epilithic. However, since that material is

poorly developed and appears to be damaged, it is easy to understand why it was not previously interpreted correctly.

Thallus epilithic, poorly developed (as in type), discontinuous and visible only around the apothecia, or, when well developed, distinctly areolate to subsquamulose, whitish to grey, K⁺ yellow; cortex with small crystals which dissolve in K to give a yellow solution (atranorin); medulla with crystals insoluble in K, not amyloid (I⁻), lacking Ca-oxalate crystals (H₂SO₄⁻); prothallus absent (as in type) or present, greyish. Apothecia lecideine, initially adnate but soon becoming sessile, crowded, often coalescing, to 0.5–0.6(0.7) mm diam. Proper margin thick and visible in young apothecia, becoming thinner and finally disappearing. Disc plane to markedly convex, epruinose. Proper exciple well developed, brown throughout, N⁻, up to 80 µm thick, *dispersa*-type, inner part with some crystals insoluble in K. Epilhymenium brown, N⁻. Hymenium hyaline, 70–90 µm high, interspersed with numerous oil droplets. Hypothecium brown, up to 150 µm deep. Paraphyses agglutinated, very difficult to disaggregate. Apical cells of the paraphyses of 3–4(5) µm diam., with a brown cap. Asci 8-spored, *Bacidia*-type with an amyloid layer above the axial body, the axial body with converging flanks which are more distinctly amyloid than the surrounding parts of the tholus. Ascospores (11)13–15(18)×(5)6–7(8) µm; young ascospores with slight apical wall-thickenings and pronounced septal wall thickenings (*Dirinaria*-type) or more usually only with septal wall thickenings (*Physconia*-type); apical and septal wall thickenings almost disappearing in mature ascospores (±*Buellia*-type), apices mostly pointed, not constricted at septum, smooth at ×1,000. Ontogeny type B. Conidia bacilliform, of 4–5×1–1.2 µm (seen in the specimen hb. Mus, see below).

Chemistry The isoelectotype [M-0023084] of *Buellia sequax* contains atranorin [major], chloroatranorin [minor], brialmontin 1 [minor] and diploicin [minor].

Other specimens analyzed by TLC and HPLC *Buellia excelsa* (BM-000731409, isoelectotype): atranorin (major), diploicin (major), chloroatranorin (minor), isofulgidin (minor).

Buellia lepidastroidea (ASU-holotype): atranorin (minor), diploicin (major), chloroatranorin (minor), isofulgidin (minor), fulgidin (trace), 3-dechlorodiploicin (trace).

Buellia sequax (hb. Mus): atranorin (major), diploicin (minor), chloroatranorin (minor), isofulgidin (major), gyrophoric acid (minor); MUB: diploicin (major), isofulgidin (minor), atranorin (minor), chloroatranorin (minor), gyrophoric acid (minor), brialmontin 1 (minor).

Discussion The following characters are diagnostic for *B. sequax* s. str.: thallus epilithic, K⁺ yellow; medulla I⁻; proper exciple *dispersa*-type; hymenium with oil droplets; epilhymenium and hypothecium brown; ascospores *Dirinaria*-

to *Physconia*-type, of 11–18×5–8 µm, smooth; type B ontogeny. *Chemistry*: atranorin, chloroatranorin and diploicin usually accompanied by isofulgidin.

According to Bungartz (2004: p. 88), the North American *B. lepidastroidea* is closely related to *B. excelsa*, with the two taxa differing in chemistry (*B. excelsa* containing only atranorin and *B. lepidastroidea* atranorin, choroatranorin, diploicin, 3-dechlorodiploicin, fulgidin and isofulgidin). Previous analyses overlooked the fact that these two taxa are chemically identical, so *B. lepidastroidea* must also be considered conspecific of *B. sequax* s. str.

With the exception of the type specimens of *B. sequax* and *B. excelsa*, all additional specimens of *B. sequax* s. str. (including *B. lepidastroidea*) exhibited a well-developed, distinctly areolate thallus. The areoles are typically continuous, forming a warted to bullate or even subsquamulose crust (for an illustration, see Bungartz et al. 2007: color plate of *B. lepidastroidea*). The prothallus may vary from being inconspicuous to clearly visible as a greyish outline surrounding the thallus. Some thalli (holotype of *B. lepidastroidea*, Nash-13756, ASU) exhibit sublobulate, marginal areoles. Nevertheless, all specimens belong to *B. sequax* s. str. It is not unusual for thalline development in some *Buellia* species to be quite variable, so that the morphology of the thallus alone is not a reliable species character. The type specimens of *B. sequax* and *B. excelsa* are very scant and rather damaged, so they are not particularly representative for the species. Among the type specimens seen, the largest and best-developed ones are the isolectotypes M-0023084 for *B. sequax* and BM-000731409 for *B. excelsa*.

The presence of oil droplets in the hymenium, the content of diploicin and the ascospore ontogeny of type B, clearly indicate that *B. sequax* is related to the genera *Diploicia* and *Endohyalina* (Giralt et al. 2010). These two genera are separated by their different growth forms, *Diploicia* being typically placodioid with distinct marginal lobes whereas in *Endohyalina* the thallus is crustose or absent (endokapylic). *Buellia sequax* s. str. with a crustose thallus with marginal, ±sublobulate areoles seems to be intermediate between the two genera. This may well indicate that there is a transition in thallus growth from absent to crustose (*Endohyalina*), to effigurate (*B. sequax* s. str.) to placodioid with distinct marginal lobes (*Diploicia* s. str.). However, until macromolecular data are available we prefer to retain *B. sequax* in *Buellia* s. lat.

The species of *Buellia* s. str. (= *Hafellia*) differs from *B. sequax*, as well as from the genera *Diploicia* and *Endohyalina*, by the typically large, *Callispora*-type ascospores with variable septation (from uniseptate to muriform) which develop with a particular ontogeny (distinct from types A and B) where subapical wall thickenings become distinct before the septum is inserted (cf. Elix 2008).

According to Bungartz (2004), two further taxa belong to the *B. excelsa*-group (i.e., *B. sequax*-group), namely *B. boergesenii* Imshaug and *B. jorgensis* Zahlbr.

The tropical–subtropical (West Indies) *B. boergesenii* clearly differs from *B. sequax* s. str. in containing norstictic and connorstictic acids (HPLC: MSC-78329), the lack of hymenial oil droplets, a proper exciple similar to the *aethalea*-type, ascospores intermediate between the *Physconia*- and the *Orcularia*-type (cf. Imshaug 1955: 484, Fig. 2I: 512), with very pronounced inner septal wall thickenings throughout their development, and their type A ontogeny.

Buellia jorgensis was described from a coastal locality in northern Chile (Zahlbruckner 1926). The only specimen identified as such studied (Chile, M-0061335) proved to be *B. sequax* s. str. Although it was also collected on the coast of Chile (ca. 700 km north to the type locality), we have been unable to locate the type specimen. The conspecificity of these two taxa appears likely, but cannot be established with certainty at this time.

Specimens of *B. sequax* s. str. in MUB and hb. Mus were previously misidentified as *B. tergestina* J. Steiner & Zahlbr., a synonym of *B. dispersa* A. Massal. (Scheidegger 1993; Bungartz et al. 2002). Confusion of *B. sequax* s. str. and *B. dispersa* is not uncommon since both species share many diagnostic characters [thallus and apothecial habit, proper exciple (*dispersa*-type) and size and inner septal wall thickenings of the ascospores]. *Buellia dispersa* differs from *B. sequax* s. str. in the absence of oil droplets in the hymenium, in containing atranorin, 2'-*O*-methylperlatolic and confluent acids and by the ascospores with a type A ontogeny.

As outlined above, *B. sequax* s. str. has nothing to do with the current concept of *B. sequax* auct., a species characterized by a typically chasmolithic thallus which lacks lichen substances or contains norstictic acid (K+ yellow then red); a proper exciple of the *aethalea*-type; a hymenium without oil droplets; narrower *Buellia*-type ascospores without any inner wall thickening at any ontogenic stage and with type A ontogeny. These features are completely consistent with the type of *B. abstracta* (M), which consequently becomes the valid name for *B. sequax* auct.

Ecology and distribution In North America *B. sequax* s. str. is common along the coast of southern California and Baja California (Bungartz 2004; Bungartz et al. 2007; as *B. lepidastroidea*). If *B. jorgensis* can be confirmed as being synonymous, the record from northern Chile would extend its distribution far south into the southern hemisphere.

The distribution of this species is quite wide in Europe. Apart from the type localities of *B. sequax* and *B. excelsa*, in southern France (Provence, Hautes-Alpes) and western Ireland (west Galway, Connemara), respectively, the species is known from Sardinia, Denmark and Britain

(Scheidegger 1993; as *B. excelsa*). Surprisingly, the two latter records are not included in the *Nordic Lichen Flora* (Foucard et al. 2002) or the *Lichens of Great Britain and Ireland* (Coppins et al. 2009). This species has also been reported from two localities in Portugal (Paz-Bermúdez and Giralt 2010, as *B. excelsa*). Here, we add a new European record from Mallorca (Balearic Islands, hb. Mus) and a new North African record (Algeria, MUB). These specimens were collected on siliceous rocks in coastal mountains at mid altitudes, between 400 and 700 m. It seems to be a rare species, with a scattered distribution in the western Mediterranean and Atlantic areas.

Additional specimens of Buellia sequax s. str. examined
 NORTH AFRICA: Algeria, Jijel, Bosque de Texenna, 700 m, 1985, Egea (MUB, sub *Buellia tergestina*). BALEARIC ISLANDS: Mallorca, Serra de Tramuntana, Lluc, Clot d'Albarca, 1985, M. Mus (hb. Mus, two specimens sub *Buellia tergestina*). PORTUGAL: (PO, see Paz-Bermúdez and Giralt 2010). MEXICO: Baja California Sur, 15 mi W San Ignacio along Rte. 1, on shale, 5.26.1974, T.H. Nash-8947 (ASU, under *B. lepidastroidea*, not recorded in Bungartz 2004). Chihuahua, Along Rte. 10, ca. 25 mi. SE of Nuevo Casas Grandes, 4,830 ft., desert grassland, 30°10'N, 107°38'W, on rhyolite, 7.6.1976, Nash-13756 (ASU, under *B. lepidastroidea*, not recorded in Bungartz 2004). CHILE: Prov. Antofagasta, Cerro Moreno, 500 m, 1965, Follmann & Follmann-Schrag (M, under *Buellia jorgensis*).

Specimens of Buellia boergenesii examined for comparison
 WEST INDIES: St. Vincent, Leeward Cliffs (sea level) on Dike Island in Indian Bay, southeast of Kingston, Parish of St. George, 18.8.1963, Imshaug & Imshaug-30570 (MSC). Sea level, rocky point between Indian Bay and Greathead Bay, Kingstown, Parish of St. George, 31.8.1963, Imshaug & Imshaug-30881, 30890 (MSC-78330, 78331). Dominica, Scotts Head (Pte. Cachacrou), 100–226 ft., Parish of St. Mark, 15.12.1963, Imshaug & Imshaug-32998, 33001, 33006 (MSC-78134, 78135, 78136). Trinidad, Coast of Balandra Bay, 6 miles northeast of Matura, 18.10.1963, Imshaug & Imshaug-32080, 32084 (MSC-78424, 78425).

Buellia abstracta (Nyl.) H. Olivier

Bull. Acad. Intern. Géogr. Bot. 12: 176 (1903).
Basionym Lecidea abstracta Nyl., Flora Jena 66: 102 (1883). Type: France, Pyrénées, Cauterets, E. Lamy (M-0023906!-isotype).

Synonyms Buellia heteropsis Müll. Arg., Bull. Soc. Murithienne du Valais 10: 57 (1881). Type: Blöcke an

den Wänden nordwestl. der Rhonebrücke b. Brig, 15 September 1880, J. Müller Arg. (G!-holotype).

Buellia punctatula Malme, Arkiv for Botanik 21a(14): 9 & 14 (1927). Type: Paraguay, Asunción, “Zapitapunta”, on sunny sandstone, July 1893, Malme 1424 (S!-lectotype).

Brief description Thallus saxicolous, endolithic and indistinct to epilithic and inconspicuous, poorly developed, usually containing norstictic acid (K⁺ yellow then red crystals); medulla I–; apothecia adnate to sessile, up to 0.6(0.75) mm diam.; disc epruinose or slightly pruinose; proper exciple *aethalea*-type, dark brown to blackish brown, N–; epihymenium brown, N–; hypothecium brown; apical cells of the paraphyses up to 5.5 µm diam.; ascospores *Buellia*-type, 9–13 × (3.5)4–5(6) µm, pale, thin walled, usually straight, not constricted, smooth but often becoming microrugulate when overmature; conidia bacilliform, 2.5–4 × 1 µm.

The main diagnostic characters of *B. abstracta* are the narrow (usually 4–4.5 µm wide, or rarely and only when overmature, 5.5–6 µm wide), pale, thin walled *Buellia*-type ascospores, which are similar to those of *B. schaeereri* De Not.

Discussion The study of the type specimens of *B. heteropsis* (G!-holotype) and *B. caloplacivora* (BCN!-holotype) has confirmed that the former is conspecific with *B. abstracta* while the second belongs to the *Buellia subalbula*-group, a group of species clearly distinguished from *B. abstracta* (= *B. sequax* auct.) by their aeruginose proper exciple and epihymenium which react N⁺ red-violet. A detailed description of *B. caloplacivora* has recently been provided by Giralt and van den Boom 2010).

Another taxonomic synonym of *B. sequax* established by Scheidegger (1993) is *B. meiosperma* auct. However, the type material of this taxon has not been examined. The second author (F.B.) previously treated *B. punctatula* and *B. saxicola* de Lesd. (1932) as synonyms of *B. abstracta*. The type material of *B. punctatula* agrees very well with this concept, but the type of *B. saxicola* should be re-examined.

It should be noted that many specimens previously identified as *B. sequax*, clearly do not belong to *B. abstracta* s. str. Instead, they are usually characterized by a well-developed, chalky white thallus (±containing norstictic acid), have larger apothecia and significantly larger ascospores, (10–)11.5–13.5(–15) × (5.5–)6.0–6.5 (–7.5) µm. We have yet to find an appropriate name for these specimens.

Acknowledgements The authors are indebted to the curators of the herbaria of ASU, BCN, BM, G, H, M, MISC, MUB and PO for the loan of material used in this study. The first author thanks the Spanish Government for funding the project CGL2007-66734-C03-02/BOS. This publication is contribution number 2014 of the Charles Darwin Foundation for the Galapagos Islands.

References

- Bungartz F (2004) New and previously unrecorded saxicolous species of *Buellia* s. l. with one-septate ascospores from the Greater Sonoran Desert Region. *Mycotaxon* 90:81–123
- Bungartz F, Scheidegger C, Nash T III (2002) *Buellia dispersa* A. Massal., a variable lichen species from semiarid to arid environments of North America and Europe. *Bibl Lichenol* 82:19–35
- Bungartz F, Nash TH III, Ryan BD (2004) Morphology and anatomy of chasmolith versus epilithic growth: a taxonomic revision of inconspicuous saxicolous species from the Sonoran Desert Region generally ascribed to the “*Buellia punctata*” group. *Can J Bot* 82:540–562
- Bungartz F, Nordin A, Grube U (2007) *Buellia* De Not. In: Nash TH III, Ryan BD, Diederich P, Gries C, Bungartz F (eds) *Lichen flora of the Greater Sonoran Desert Region*, vol 3. *Lichens Unlimited*, Arizona State University, Tempe, pp 113–179
- Coppins BJ, Scheidegger C, Aptroot A (2009) *Buellia* De Not. In: Smith CW, Aptroot A, Coppins BJ, James PW, Wolseley PA (eds) *The lichens of Great Britain and Ireland*. *British Lichen Society*, London, pp 228–238
- Culberson CF, Ammann K (1979) Standardmethode zur Dünnschichtchromatographie von Flechtensubstanzen. *Herzogia* 5:1–24
- Culberson CF, Johnson A (1982) Substitution of methyl tert.-butyl ether for diethyl ether in the standardized thin-layer chromatographic method for lichen products. *J Chromatogr* 238:483–487
- Culberson CF, Culberson WL, Johnson A (1981) A standardized TLC analysis of β -orcinol depsidones. *Bryologist* 84:16–29
- Elix JA (2008) *Physciaceae* Zahlbr. In: *Flora of Australia*, vol 57, *Lichens 5*. Australian Biological Resources Study, CSIRO Publishing, Canberra, pp 494–533
- Elix JA, Giralt M, Wardlaw JH (2003) New chloro-depsides from the lichen *Dimelaena radiata*. *Bibl Lichenol* 86:1–7
- Foucard T, Moberg R, Nordin A (2002) *Buellia* De Not. In: Ahti T, Jørgensen PM, Kristinsson H, Moberg R, Søchting U, Thor G (eds) *Nordic lichen flora*, vol 2. Th-tryck, Uldevalla, pp 11–25
- Giralt M (2001) The lichen genera *Rinodina* and *Rinodinella* (lichenized Ascomycetes, Physciaceae) in the Iberian Peninsula. *Bibl Lichenol* 79:1–160
- Giralt M, van den Boom PPG (2010) The genus *Buellia* s.l. and some additional crustose genera of Physciaceae in the Canary Islands. *Nova Hedwigia* (in press)
- Giralt M, van den Boom PPG, Elix JA (2010) *Endohyalina*, the genus in the Physciaceae to accommodate the species of the *Rinodina ericina*-group. *Mycol Progr* 9:37–48
- Imshaug HA (1955) The lichen genus *Buellia* in the West Indies. *Farlowia* 4:473–512
- Paz-Bermúdez G, Giralt M (2010) The crustose specimens of the *Physciaceae*, *Caliciaceae* excluded, (lichenized ascomycetes) in the PO herbarium (Oporto, Portugal). *Sydowia* (in press)
- Rambold G, Mayrhofer H, Matzer M (1994) On the ascus types in the Physciaceae (Lecanorales). *Plant Syst Evol* 192:31–40
- Scheidegger C (1993) A revision of European saxicolous species of the genus *Buellia* De Not and formerly included genera. *Lichenologist* 25:315–364
- Zahlbruckner A (1926) *Chilenische Flechten*. *Medd Göteborgs Bot Trädgård* 2:1–26