New Species and New Records of *Buellia* (Lichenized Ascomycetes) from Jeju Province, South Korea

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**Abstract** A new species and 2 new records of lichen genus *Buellia* were discovered from Chuja-do Island in Jeju Province during a recent floristic survey: *B. chujana* X. Y. Wang, S. Y. Kondr., L. Lőkös & J.-S. Hur sp. nov., *B. halonia* (Ach.) Tuck., and *B. mamillana* (Tuck.) W. A. Weber. The new species is characterized by a brown, areolate thallus, the presence of perlatolic acid, and a saxicolous habitat. Together with previously recorded species, 10 *Buellia* species were confirmed from Jeju-do Island. Among these species, 3 growing in the exposed rocky area contained xanthone (yellowish lichen thallus, UV + orange), indicating that production of xanthone in this genus might be a defense strategy against the harm of UV light. Although the genus *Buellia* has been thoroughly studied in Korea before, novel species have been discovered continuously, and large species diversity has been found in this crustose genus, even from a small rocky island. This study indicates that the coastal area harbors a vast number of crustose lichen species, and there is great potential to discover unknown lichens in the coastal rocky area in Korea.

**Keywords** Coastal area, Lichenized fungi, New species, Taxonomy

The genus *Buellia* De Not. (Physciaceae), typed by *B. disciformis* (Fr.) Mudd, is characterized by single or pluriseptate brown ascospores, lecideine apothecium margins, and a brown to yellowish crustose thallus. It is a large genus containing c. 400 species worldwide [1]. It is morphologically similar to the genus *Rinodina* (Ach.) Gray, which usually has lecanorine apothecia and hyaline hypothecium, whereas *Buellia* has lecideine apothecia and deep brown hypothecium. Nine species have previously been reported in South Korea [2-7], most of which are from the rocky seaside area.

During a recent floristic survey of lichens from Chuja-do Island, one new species and two new records of *Buellia* have been found. Together with the previous records, 12 species are confirmed in South Korea now, and a key to all the known species are provided here. Studies have also been performed on the related genera from this area; our results on the genera *Amandinea*, *Hafellia*, and others are provided in another publication [8].

**MATERIALS AND METHODS**

**Morphological and chemical studies.** Approximately 140 *Buellia* specimens from South Korea were examined, all of which are deposited in the Korean Lichen Research Institute, Sunchon National University (KoLRI). Specimens were examined using standard microscopy techniques and were hand-sectioned under a dissecting microscope (SMZ 745T; Nikon, Tokyo, Japan). Anatomical descriptions are based on observations of these preparations under a microscope (GmbH 37081; Carl Zeiss MicroImaging, Göttingen, Germany) and photos were taken using a Nikon digital camera head DS-Fi2. Ascospore dimensions reflect ten measurements, typically from a single apothecium per specimen. Sizes are represented by the range between the smallest and largest single values.
Secondary metabolites of all the specimens were identified using spot tests (K = 10% aqueous KOH solution, C = saturated aqueous Ca(OCl)_2, KC = 10% aqueous KOH solution followed by saturated aqueous Ca(OCl)_2, P = 5% alcoholic p-phenylenediamine solution) and thin-layer chromatography (TLC) as described by Elix et al. [9] and Orange et al. [10]. Solvent C (toluene : acetic acid = 85 : 15) was used for TLC analysis.

DNA isolation, PCR, and DNA sequencing. Genomic DNA was extracted from freshly collected, frozen herbarium specimens using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer’s instructions. Sequences of the internal transcribed spacer (ITS) region, including the 5.8S ribosomal RNA gene, and partial (5' portion) large subunits of the ribosomal RNA (nrLSU) were generated for the new species and new records. Primers pairs ITS1F [11] and ITS4 [12], LR0R [13] and LRS [14], were used to amplify the ITS and nrLSU regions, respectively. Protocols for PCR amplification and sequencing were detailed by Liu et al. [15].

Sequence alignment. Newly generated ITS sequences were aligned with selected Buellia sequences from GenBank (Table 1). All raw sequences were assembled and edited using SeqMan (DNAstar packages) and BioEdit 7.09 [24], then were automatically aligned using MUSCLE v3.6 [25]. Ambiguous regions were identified and excluded using Gblocks [26] with the following parameters: for a conserved position, 15; minimum number of sequences for a flanking position, 16; maximum number of contiguous nonconserved positions, 6; minimum length of a block, 3; and allowed gap positions, with half.

Phylogenetic analysis. Maximum likelihood optimality criterion (ML) and Bayesian Inferences (BI) were used to construct the phylogenetic trees. Gaps were regarded as missing. GTR + I + G best-fitted substitution models were selected based on the Akaike Information Criterion (AIC) using jModelTest 3.7 [27]. BI analyses were performed

Table 1. GenBank accession numbers for the single-gene ITS dataset used in this study (newly generated’)

| Name of species | Accession No. | Locality | Collector/Voucher number/Herbarium | Reference |
|-----------------|---------------|----------|------------------------------------|-----------|
| *Diploicia canescens* | AJ421992      | Italy, Prov. di Livorno, Isola d'Elba | Triebel and Rambold, 6188 (M) | [16]      |
| *Buellia aethalea* | AF540496      | Sweden, Narke, Gotland Island Valen | Nordin A., 3256 (UPS) | [17]      |
| B. anisomera     | DQ354453      | Antarctica | | | |
| B. asterella     | AF250785      | -         | | | |
| B. capitis-regum | AF540497      | USA, CA, Santa Barbara Santa Rosa Island | Nash T. H., 31113 (UPS) | [17]      |
| B. chujana       | KT733597*     | Korea, Jeju Province, Chuja-do Island | Lőkös L., 140835-1 (KoLRI) Newly generated |
| B. chujana       | KT733598*     | Korea, Jeju Province, Chuja-do Island | Lőkös L., 140835-2 (KoLRI) Newly generated |
| B. dijiana       | AF250788      | -         | | | |
| B. disciformis   | AY143392      | Sweden, Jamtland, Klassasen, on Sorbus | Wedin M., 6155 (BM) | [20]      |
| B. erwescens     | AF250786      | -         | | | |
| B. frigida       | AF276069      | Antarctica, Boulder Hill, Vestfold Hills | IMI 384687 (CAB Bioscience [UK Centre] at Egham) | [21]      |
| B. georgei       | AF250787      | -         | | | |
| B. griseovirens  | AF540300      | Sweden, Uppland, Grasso Lonholmen | Nordin A., 4734 (UPS) | [17]      |
| B. halonia       | KT733595*     | Korea, Jeju Province, Mt. Dondae | Kondratyuk S. and Lőkös L., 140808 (KoLRI) Newly generated |
| B. halonia       | KT733596*     | Korea, Jeju Province, Mt. Dondae | Joshi Y. and So J., 140768 (KoLRI) Newly generated |
| B. insignis      | DQ198358      | Sweden | Nordin A., 5464 (UPS) | [22]      |
| B. lindingeri    | AF250789      | -         | | | |
| B. manillana     | KT733599*     | Korea, Jeju Province, Mt. Dondae | Kondratyuk S. and Lőkös L., 140792-1 (KoLRI) Newly generated |
| B. manillana     | KT733600*     | Korea, Jeju Province, Port of Yecho-ri | Halda J. P., 141100 (KoLRI) Newly generated |
| B. muriformis    | AF540301      | USA, OR, Oregon Dunes National Recreation Area | Nordin A., 5336a (UPS) | [17]      |
| B. ocellata      | AF540502      | Faroe Islands, Skovoy | Nordin A., 4284 (UPS) | [17]      |
| B. papillata     | AF250790      | -         | | | |
| B. penicrha      | AF540503      | USA, OR, Deschutes Co. Three Sisters Wilderness | Nordin A., 5322 (UPS) | [17]      |
| B. russa         | DQ354454      | Antarctica | Hur J.-S., ANT050942 (KoLRI) | [18]      |
| B. sachereri     | AF250791      | -         | | | |
| B. subdisciformis| AF352323      | Spain, Cap de Creus, Cataluna | 13750 (BCC) | [23]      |
| B. triphragmoides| AF540505      | Sweden, Torne Lappmark, Julkassjarvi | Nordin A., 4425 (UPS) | [17]      |
| B. triseptata    | AF540506      | USA, AR, Apache Co., White Mts, Apache National Forest, Mt Baldy Wilderness | Nordin A., 5229 (UPS) | [17]      |
with MrBayes v3.1.2 [28] using four chains and were run for 1 million generations. Trees were sampled every 100 generations. Chain convergence was determined using Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer/) to ensure sufficiently large effective sample size (ESS) values. The stop rule was used when parallel Markov Chain Monte Carlo runs converged (ESS value > 200). Phylogenetic trees were summarized using the sump and sumt commands with burn-ins discarded. Bayesian posterior probabilities (BPP) were estimated from the frequencies of branches among all trees, clades with BPP ≥ 0.95 were considered as significantly supported. ML inferences were made using RAxML v7.2.6 [29], with the GTR-GAMMA model. Bootstrap frequencies were estimated from the consensus tree built with 2,000 trees [29], with the GTR-GAMMA model. Bootstrap frequencies were estimated from the consensus tree built with 2,000 trees obtained from nonparametric bootstrapping pseudoreplicates. Diplócia canescens was selected as the out-group. Tree files were edited with FigTree v1.4.2 [30].

RESULTS AND DISCUSSION

Twenty-eight sequences were used in this study, with 6 of them (ITS) being newly generated (Fig. 1). The matrix contained 550 characters, of which 490 (89%) were conserved as seen in Gblocks, including 228 conserved, 262 variable, 213 parsimony-informative, and 49 singleton sites. The putative new species *B. chujana* formed a well-supported clade (bootstrap value = 100) in the phylogenetic tree. The new species clustered within the *Buellia* species as a sister species to *B. ocellata*, *B. russa*, *B. frigida*, and *B. aethalea*, sharing common characteristics such as immersed apothecia, saxicolous habitat, and 1-septate ascospores. The two new records also formed two monophyletic lineages (bootstrap value = 100). The high phylogenetic value supported the validity of the new species and new records. Together with the morphological analysis, a total of 12 species were confirmed in South Korea.

**Taxonomic treatment.**

**Key to the species of Buellia from Korea**

1. Habitat corticolous (growing on bark) .......................... 2
2a. Hymenium with numerous oil droplets; exciple dispersa-type; ascus 8-spored .......................... *B. discoformis*
2b. Hymenium without oil droplets; exciple aethalea-type; ascus 8- to 16-spored .......................... 3
3a. Thallus granular to subsquamulose; ascus 16-spored .......................... *B. polyspora*
3b. Thallus rimose to areolate; ascus 8-spored .......................... *B. punctata*
4a. Containing xanthone (thallus yellowish, UV + orange) .......................... 5
4b. Without xanthone (thallus UV −) .......................... 7
5a. Thallus forming patches, contains arthothelin, norstictic acid .......................... *B. halonia*
5b. Thallus continuous, without arthothelin .......................... 6
6a. Exciple mamillana-type, medulla I −⋯⋯⋯⋯⋯⋯*B. mamillana*
6b. Exciple aethalea-type, medulla I −⋯⋯⋯⋯⋯⋯*B. prospera*
7a. Thallus lichenicolous (growing over Aspicilia); lacking secondary metabolites .......................... *B. badia*
7b. Thallus not lichenicolous; secondary metabolites present .......................... 8
8a. Thallus deep brown to olive brown .......................... 9
8b. Thallus different shades of gray, never brown .......................... 10
9a. Thallus areolate, exciple mamillana-type .......................... *B. chujana*
9b. Thallus sub-squamulose, exciple dispersa-type .......................... *B. nashii*
10a. Thallus chalky, with a large amount of calcium oxalate crystals .......................... *B. maritima*
10b. Thallus without chalky consistency, without calcium oxalate crystals .......................... 11
11a. Medulla amyloid (I+ blue); contains norstictic and stictic acids .......................... *B. spuria*
11b. Medulla non-amyloid (I−); contains 2’-O-methylperlatolic and confluentic acids .......................... *B. stellulata*

The species.

*Buellia chujana* X. Y. Wang, S. Y. Kondr., L. Lőkös & J.-S. Hur, sp. nov. (Fig. 2A, 2B and 2E)

Mycobank: MB 814618.
Type: South Korea, Jeju Prov., Jeju-si, Chuja-do Island, seashore of Mojini-mongdol, 33°56’44.9” N, 126°20’31.1” E, alt. 57 m, on seaside rock growing together with Caloplaca aequata, 21 Jun 2014, L. Lőkös, 140835-1 (holotype: KoLRI 023326, accession number: KT733597 [ITS], KT733605 [nrLSU]); the same locality, growing together with Aspicilia sp. 140835-2 (isotype, KoLRI 023327 sub Aspicilia).

Etymology: The epithet refers to collection location in Jeju Province, Chuja-do Island.

Morphology and chemistry: Thallus crustose, rimose, continuous, dark greenish brown to dark brown; areoles 0.2–0.6 (~0.9) mm across, scattered and distant in the peripheral zone; prothallus grey or blackish grey, absent, or delimiting the thallus as a black outline; surface dull, smooth, and without pruina; medulla white, non-amyloid. Apothecia 0.2–0.3 (~0.6) mm in diam. from immersed to sessile; disc black, epruinose, planar; exciple narrow, mamillana-type, HNO, <; epihymenium brown, hymenium 50–60 μm tall, hyaline, without oil droplets or with scarce oil droplets 4–5 μm in diam.; paraphyses up to 2.5 μm diam., distinctly swollen towards the tips, with black caps 3.5–4 μm in diam.; hypothecium brownish, 20–30 (~70) μm; asci Bacidia-type, 8-spored, spores dark brown, (0–) 1-septate, ellipsoid, 6–8 × (6–) 7–9 (~11) μm. Pycnidia not seen.

Spot test: all negative (K−, P−, C−, KC−, CK−), UV−, containing perlatolic acid as the main compound (by TLC).

Notes: Buellia chujana is characterized by a smooth, rimose, and dark greenish-brown thallus, greyish hypothallus, saxicolous maritime habitat, growing on non-calcareous substrate, (HCl−) and small, 1-septate brown ascospores (less than 10 μm) long. It might be confused with B. nashii Bungartz, which has a brown thallus and grows on rock from the seaside, but the latter species differs by having subsquamulose thallus, dispersa-type apothecia, and atranorin and norstictic acid as its main compounds. B. badia is another species from South Korea with brown thalli which might be confusing, but it differs from this new species by...
the lichenicolous habitat (usually grows on *Aspicilia* sp. in Korea), squamulose thallus, and by having no secondary substances.

*Buellia ryanii* Bungartz, reported from America, has an olive brown thallus and spore size similar to *B. chujana*, but it has lecideine apothecia (*aethalea*-type), a distinct black prothallus, and pigment in the ephymenon (HNO3 + violet), furthermore, it does not have any secondary metabolites. *B. tergua* Bungartz from America also has a brown color ("leather colored"), similar spore size, and maritime saxicolous habitat, but it differs from this new species by its lecideine apothecia (*aethalea*-type), strongly carbonized exciple, pigmented ephymenon (HNO3 + violet), and the absence of substances.

**Additional specimens examined:** South Korea, Jeonbuk Prov., Gunsan city, Shinji Island, 35°49.084′ N, 126°28.159′ E, alt. 130 m, on rock, 21 Oct 2011, X. Y. Wang, J. A. Ryu, 110779 (KoLRI 013794); Jeju Prov., Jeju-si, Chujado-Island, seashore of Mojigi-mongol, 33°56′44.9″ N, 126°20′03.01″ E, alt. 57 m, on seaside rock, June 21 2014, L. Lőkös, 140835-2 (KoLRI 023327), accession number: KT733598 [ITS], KT733606 [nrLSU]).

*Buellia badia* (Fr.) A. Massal., Memor. Lich.: 124 (1853).

**Morphology and chemistry:** see Bungartz et al. [1].

**Notes:** This species is the only lichenicolous species of *Buellia* reported in Korea so far, characterized by a deep brown subsquamulose to squamulose thallus. It usually grows on the surface of *Aspicilia* sp. in Korea.

**Representative specimen examined:** South Korea, Jeju Prov., Jeju-si, Chujado-Island, Mt. Dondae, 33°56′53.9″ N, 126°19′26.7″ E, alt. 164 m, on rock, 21 Jun 2014, J. P. Halda, 140827 (KoLRI 023314).

*Buellia disciformis* (Fr.) Mudd, Man. Br. Lich.: 216 (1861).

**Morphology and chemistry:** see Bungartz et al. [1].

**Notes:** This species is characterized by a rimose, grayish-green thallus, and corticolous habitat, it contains atranorin as main compound.

**Representative specimen examined:** South Korea, Kangwon Prov., Mt. Duta, 37°26′36.8″ N, 126°59′35.3″ E, alt. 761 m, on bark, 5 Nov 2008, J.-S. Hur, 080106 (KoLRI 008350).

*Buellia halonia* (Ach.) Tuck., Lichens of California (Berkeley): 26 (1866) (new to Korea) (Fig. 2C and 2F).

**Morphology and chemistry:** see Bungartz et al. [1].

**Notes:** This species is unique by its yellowish-green, UV + orange and areolate thallus; apothecia small, *trachyspora*-type, up to 0.5 mm in diam.

**Ecology and distribution:** It has been reported in the Pacific coast of North and South America [1], and was newly recorded in the coastal area of South Korea.

**Representative specimens examined:** South Korea, Jeju Prov., Jeju-si, Chujado-Island, Mt. Dondae, 33°56′53.9″ N, 126°19′26.7″ E, alt. 164 m, on rock, 20 Jun 2014, Y. Joshi, J. So, 140768 (KoLRI 023242), the same locality, S. Y. Kondratyuk, L. Lőkös, 140808 (KoLRI 023292 KT733595 [ITS], KT733603 [nrLSU]), the same locality, J. P. Halda 140821 (KoLRI 023306).

*Buellia mamilanna* (Tuck.) W. A. Weber, Mycotaxon 27: 493 (1986) (new to Korea) (Fig. 2D).

**Morphology and chemistry:** see Bungartz et al. [1].

**Notes:** This species is characterized by a crustose and rimose thallus, continuous, yellowish-green color, and UV + orange; apothecia *mamilanna*-type, medulla I +.

**Ecology and distribution:** It has been reported in tropical to subtropical areas worldwide [1], and was newly recorded in the coastal area of South Korea.

**Representative specimens examined:** South Korea, Jeju Prov., Jeju-si, Chujdoi-Island, Mt. Dondae, 33°56′53.9″ N, 126°19′26.7″ E, alt. 164 m, on rock, 20 Jun 2014, S. Y. Kondratyuk, L. Lőkös, 140792-1 (KoLRI 023271, KT733599 [ITS], KT733601 [nrLSU]); Jeju-si, Yecho-ri, Yecho-ri Port, 33°57′23.5″ N, 126°20′03.02″ E, alt. 40 m, on rock, 21 Jun 2014, J. P. Halda, 141100 (KoLRI 023638, KT733600 [ITS], KT733602 [nrLSU]).

*Buellia maritima* (A. Massal.) Bagl., Sched. Critic. 8: 150 (1856).

**Morphology and chemistry:** see Bungartz et al. [1].

**Notes:** This species is characterized by a white to pale gray thallus, rimose to areolate, covered with dense pruina, and it contains norstictic and connorstictic acid as its main compounds.

**Representative specimens examined:** South Korea, Jeju Prov., Jeju-si, Chujado-Island, Mt. Dondae, 33°56′53.9″ N, 126°19′26.7″ E, alt. 164 m, on rock, 21 Jun 2014, J. P. Halda, 140768 (KoLRI 005404).

*Buellia nashii* Bungartz, Mycotaxon 90: 90 (2004).

**Morphology and chemistry:** see Bungartz et al. [1].

**Notes:** This species is unique by its brown sub-squamulose thallus, black apothecia sessile and lecideine, and it contains norstictic and stictic acid as its main compounds.

**Representative specimens examined:** South Korea, Jeju Prov., Jeju-si, Sinchang-ri, seashore road, on rock, growing together with *Endocarpon* and *Trapelia* sp., 33°20′31.6″ N, 126°10′12.08″ E, alt. 82 m, 18 Jun 2014, S. Y. Kondratyuk, 140270-1 (KoLRI 022632), the same locality, growing together with *Propotameliopsis chujensis*, *Verrucaria*, and *Buellia* spp., S. Y. Kondratyuk, 140280 (KoLRI 022462).

*Buellia polyspora* (Willey) Vain., Étud. Class. Lich. Brésil 1: 171 (1890).

**Morphology and chemistry:** see Bungartz et al. [1].

**Notes:** This species is distinct by having 16 spores in the ascus, oil-droplets in the hymenium, a thallus indistinct to granulose, lacking any secondary metabolites, and its corticolous habitat.

**Representative specimen examined:** South Korea, Jeju Prov., Seogwipo-si, Yeongto waterfall, 33°16′01.7″ N,
126°29′49.00″ E, alt. 210 m, on bark, 19 Jun 2014, Y. Joshi, J. So, 140576 (KoLRI 022969).

Buellia prospera (Nyl.) Riddle, Brooklyn Botanical Garden Memoirs 1: 114 (1918).

**Morphology and chemistry:**  see Zhang et al. [5].

**Notes:** This species is characterized by a thallus yellowish (UV + orange), continuous, smooth, and rinse; apothecia aethalea-type, and a coastal saxicolous habitat.

**Representative specimens examined:** South Korea, jeju Prov., jeju-si, Chuja-do Island, Mt. Dondae, 33°56′53.9″ N, 126°19′26.7″ E, alt. 164 m, on rock, growing together with Pertusaria and Ramalina, 20 Jun 2014, Y. Joshi, J. So, 140760 (KoLRI 023237), the same locality, growing together with Ramalina and Candelaria, 140765 (KoLRI 023240).

Buellia punctata (Hoffm.) A. Massal., Ric. Auton. Lich. Crost. (Verona): 81 (1852).

**Morphology and chemistry:**  see Bungartz et al. [1].

**Notes:** This species is characterized by a greenish brown thallus, aethalea-type apothecia, and corticulous habitat; it has no secondary metabolites and non-amyloid medulla (I−).

**Representative specimens examined:** South Korea, jeju Prov., jeju-si, Chuja-do Island, seashore of Mojini-mongdol, 33°56′43.70″ N, 126°19′53.52″ E, alt. 20 m, on bark, growing together with Amandinea sp. and Lecanora sp., 21 Jun 2014, S. Y. Kondratyuk, L. Lőkös, 140922-4 (KoLRI 023453), the same locality, growing together with Lecanora sp., 140934-4 (KoLRI 023473).

Buellia spuria (Schae.) Anzi, Cat. Lich. Sondr.: 87 (1860).

**Morphology and chemistry:**  see Bungartz et al. [1].

**Notes:** This species is characterized by its whitish gray thallus with black prothallus, apothecia aethalea-type, growing on rocks near the seaside, containing norstictic and stictic acid as its main compounds.

**Representative specimens examined:** South Korea, jeju Prov., jeju-si, Chuja-do Island, seashore of Mojini-mongdol, 33°56′44.9″ N, 126°20′03.01″ E, alt. 57 m, on rock, growing together with Biatora sp., 22 Jun 2014, S. Y. Kondratyuk, 141130 (KoLRI 023690), growing together with Biatora sp., 141117 (KoLRI 023677).

Buellia stellulata (Taylor) Mudd, Man. Brit. Lich.: 216 (1861).

**Morphology and chemistry:**  see Bungartz et al. [1].

**Notes:** This species is similar to B. spuria by its whitish gray thallus, black prothallus, and aethalea-type apothecia, but it differs by containing 2′-O-methylperlatolic and confluent acids, and its non-amyloid (I−) medulla.

**Representative specimens examined:** South Korea, jeju Prov., jeju-si, Sinchang-ri, seashore road, 33°20′31.6″ N, 126°10′12.08″ E, alt. 82 m, on rock, 18 Jun 2014, L. Gagarina, 140163 (KoLRI 022530), the same locality, Y. Joshi, J. So, 140172 (KoLRI 022541), the same locality, L. Lőkös, 140201 (KoLRI 022571).

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