Buellia epigaea (Pers.) Tuck, a new record of lichenized fungus species for Antarctica

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Abstract

Buellia epigaea, a terricolous lichenized fungal species known from numerous localities in Northern Hemisphere, but only from Australia in Southern Hemisphere, is reported from Antarctica for the first time. Here we provide morphological, anatomical, and molecular characteristics (nrITS) of this species. Besides, the differences of *B. epigaea* with morphologically, ecologically or phylogenetically related species are discussed.

Key words: Southern Hemisphere, lichens, *Caliciaceae*, Antarctic Peninsula, *Buellia epigaea*

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Introduction

One of the largest lichenized fungal genera *Buellia* is characterized by mostly black lecideine apothecia, oblong, ellipsoid or rarely citriform-shaped brown ascospores with one or more septa, and a reddish-brown or rarely hyaline hypothecium (Joshi et al. 2010). This genus is relatively well represented in Antarctica, as there were 28 species previously reported. The most comprehensive information about this genus in Antarctica was provided by Lamb (1968) and Øvstedal and Lewis-Smith (2001).

In the famous book of Lamb (1968) entitled "Antarctic Lichens II The Genera *Buellia* and *Rinodina*", species reported under the genus *Buellia* were: *Amandinea punctata, Buellia pycnogonoides, B. evanescens, B. illaetabilis, B. isabellina, A. augusta, A. latemarginata, A. babingtonii, B. fulvonitescens, B. frigida*, *Tetramelas anisomerus, T. inordinatus, T. nelsonii, T. granulosus, T. subpedicellatus, T. darbishirei, T. cladocarpizus*.

Øvstedal and Lewis-Smith (2001) reported that 26 species of *Buellia* are known from Antarctica. The current names of those species are: *Amandinea babingtonii*, *A. coniops, A. falklandica, A. latemarginata*, *A. petermanni, A. punctata, A. subplicata, Buellia aethalea, B. bouvetii, B. evanescens, B. frigida, B. illaetabilis, B. isabellina, B. lignoides, B. melanostola, B. pallida, B. perlata, B. pycnogonoides, B. perla, Tetramelas subpedicellatus, T. papillatus, T. graminicola, T. granulosus, T. grimmiae, T. cladocarpizus* and *T. nelsonii*. In addition to these species,

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Elix (2018) reported two new species from Antarctica: *Buellia minispora* and *B. rodseppeltii*.

In this paper we report *Buellia epigaea* for the first time from James Ross Island, Antarctic Peninsula (Antarctica).

Materials and Methods

Lichen samples were collected from 90-140 m altitude (the collection localities provided below) by spatula on soil and they were wrapped in toilet paper and put in paper bags. When brought to the Mendel Polar Station, they were left to dry for 3 days in a room with air flow. The studied samples are stored in the lichen section of ERCH.

The locality details of the studied specimens are given below:

JR 0.005: Antarctica, Antarctic Peninsula, James Ross Island, Dirty Valley (63° 48′ 38.1″ S , 57° 51′ 36″ W, alt. 90 m, on soil, Leg. M. G. Halıcı.

JR 0.199: Antarctica, Antarctic Peninsula, James Ross Island, Puchau (63° 48′ 24.9″ S, 57° 50′ 27.6″ W, alt. 140 m, on soil, Leg. M. G. Halıcı.

Morphological examinations were all carried under a stereo microscope. Sections were taken directly under the stereomicroscope by hand and anatomical characters were examined under a light microscope. Ascospores were measured in water. In addition, chemical reagents for spot tests were used to identify the sample species.

DNA isolation, PCR and sequencing

DNeasy Plant Mini Kit (Catalog No: 69104) produced by Qiagen company was used in the DNA isolation. The protocol given in the kit was followed during the isolation process.

Replication of the ITS gene region from the isolated DNA was performed under appropriate PCR conditions. PCR reaction mixture applied for gene regions 5 µl of 200 ng DNA, 5 µl of 10X reaction buffer, 5 µl of 25mM $MgCl_2$, 5 µl of 25µ dNTPs, 4 µl of 10 µM ITS1 Primer, 4 µl of 10 µM ITS Primer, 0.5 U of Taq DNA polymerase. PCR reaction was performed by adding 21.5 µl PCR water to complement the total volume to 50 µl. PCR amplifications of ITS were performed using fungal-specific primers ITS4 (TCCTCCGCTTATTGATATGC, White et al. 1990) and ITS1-F (CTTGGTCATTTA GAGGAAGTAA, Gardes and Bruns 1993).

PCR amplifications were carried out in a thermal cycler equipped with a heated lid, in the following conditions: an initial heating step for 5 min. at 95°C; 6 cycles with 1:30 min. at 94°C, 1:30 min. at 55°C, and 2 min. at 72°C; and 33 cycles with 1 min at 94°C, 1 min. at 52°C, and 2 min. at 72°C. A final extension step of 8 min. at 72°C was added, after which the samples were kept at 4°C.

After the PCR, amplificated samples were loaded on a 1% agarose gel with ethidium bromide dye added (5mg/ml) for electrophoretic separation. The DNA bands were detected under UV light at 100 watt after 60 min.

Sequence alignment and phylogenetic analysis

The sequencing was performed by ABI 3730 XL sequencer (applied Bioscience). ITS1F and ITS4 primers and the region containing the end of the small subunit, ITS1, 5.8 gene, ITS2 and the end of the large subunit were replicated and sequence analysis was performed.

Species	nrITS	Locality	Species	nrITS	Locality
JR 0.005 *B. epigaea*	**MW825639**	**JRI, Antarctica**	*Buellia triseptata*	AF540506	USA
JR 0.199 *B. epigaea*	**MW825640**	**JRI, Antarctica**	*Buellia halonia*	MG250193	China
Buellia aethalea	AF540496	Sweden	*Buellia halonia*	KT733595	South Korea
Buellia aethalea	AY143410	Italy	*Buellia lauricassiae*	AB971697	Japan
Buellia alboatra	AF224350	Fennoscandia	*Buellia lauricassiae*	AB971696	Japan
Buellia almeriensis	MF062520	Unknown	*Buellia ocellata*	AF540502	Fareo Adaları
Buellia arborea	KX132975	Switzerland	*Buellia mamillana*	KT733600	South Korea
Buellia asterella	AF250785	Unknown	*Buellia mamillana*	MF398995	South Korea
Buellia arnoldii	MK811634	Norway	*Buellia muriformis*	AF540501	USA
Buellia badia	MG250192	China	*Buellia numerosa*	LC153799	Japan
Buellia boseongensis	MF398999	South Korea	*Buellia numerosa*	LC153798	Japan
Buellia boseongensis	MF398998	South Korea	*Buellia schaereri*	MK778592	Russia
Buellia capitis-regum	AF250783	Unknown	*Buellia schaereri*	GU553288	Austria
Buellia dijiana	AF250788	Unknown	*Buellia penichra*	AF540503	USA
Buellia chujana	MG250191	China	*Buellia polyspora*	MK499345	Thailand
Buellia disciformis	AF250784	Unknown	*Buellia polyspora*	MK499346	Thailand
Buellia disciformis	FR799140	Unknown	*Buellia russa*	DQ534454	Antarctica
Buellia dives	MK811893	Norway	*Buellia subnumerosa*	LC153802	Japan
Buellia elegans	AY143411	USA	*Buellia subnumerosa*	LC153803	Japan
Buellia elegans	AJ421415	Unknown	*Buellia stellulata*	MF398996	South Korea
Buellia epigaea	KY266900	Norway	*Buellia subdisciformis*	MG551507	Unknown
Buellia erubescens	LC069373	Japan	*Buellia subdisciformis*	AF352323	Spain
Buellia erubescens	GU553289	Russia	*Buellia sublauri-cassiae*	MK499343	Thailand
Buellia frigida	JX036049	Antarctica	*Buellia sublauri-cassiae*	MK499344	Thailand
Buellia frigida	JX036048	Antarctica	*Buellia submuriformis*	AF540504	India
Buellia griseovirens	KC681817	Canada	*Buellia tesserata*	KX512904	Unknown
Buellia griseovirens	KC681816	Canada	*Buellia subsororioides*	KM044008	India
Buellia georgei	AJ421416	Australia	*Buellia taishanensis*	MG250190	China
Buellia georgei	AF250787	Unknown	*Diploicia canescens*	AF250793	Unknown
Buellia lindigeri	AF250789	Unknown			

Table 1. Sequences used in the analyses; newly produced ones are in bold and the others were downloaded from the Genbank. *Note*: JRI - James Ross Island.

Possible reading errors were corrected with the Cluster X function of MEGA 6.0 program and the sequences of the species obtained from the research area and the sequences of the genes downloaded from the GenBank (Table 1) were analyzed with Mega 6.0 program. The dendrograms were obtained with ML method and Tamura 3-parameter model. Pairwise deletion was applied to gaps in data, and the reliability of the inferred tree was tested by 1000 bootstrap replications for control. *Diploicia canescens* AF250793 was used as an outgroup.

Results and Discussion

Molecular results

The nrITS sequences of the Antarctic *Buellia epigaea* specimens collected from James Ross Island were blasted against database of ITS sequences of 34 *Buellia* species. The resulting phylogenetic tree shows that these specimens (JR 0.005 and JR 0.199) belong to *Buellia epigaea* which is new for Antarctica (Fig. 1).

Morphology

Thallus is crustose, creamy to chalky white, weakly rimose areolate or smooth. Photobiont is green, chlorococcoid. Apothecia are present, black, lecidein, smooth or weakly concave, the young ones almost sunk in thalli, non pruinose or slightly pruinose. Young apothecia have prominent black margin, in some of the older ones margin is almost excluded, 0.1 – 0.2 mm diam. (Fig. 2A). Epihymenium is brown or light brown, 5.5–30 µm, N - red or N + weakly reddish. Hymenium is hyaline, 60–130 µm. Paraphyses are branched or not branched, tips enlarged or almost capitate, some of them have oil droplets, tips 1.5–5 µm. Hypothecium brown, 55–230 µm, N-. Asci are 8-spored, 51–62 × 14–19 µm. Ascospores are 2-celled, brown, one septate, (15–)17–18.5–20(–21) × (5–)7–8.5–10(–10,5) µm and l/w (1.75–)1.82–2.25–2.69(–3.6) µm (n=21) (Fig. 2B). Perispore is present, areolate. Pycnidium was not observed. Spot tests: All negative.

Buellia epigaea group is characterized by white and often effigurate thalli that occur mainly on soil. Inside the group there are five species: *Buellia dijiana*, *B. georgei*, *B. lobata*, *B. epigaea* and *B. elegans*. These five species mainly known from Australia are morphologically and ecologically very similar to *Buellia epigaea*. *B. dijiana* has non effigurate thallus like *B. epigaea,* but it differs by ornamentation of perispore. *B. dijiana* has warty ornamentation. *B. epigaea* and *B. georgei* have similar ornamentation of perispore as both species have areolate perispores and they both grow on soil. *B. georgei* has mostly short marginal lobes. *B. lobata* and *B. elegans* have effigurate thalli unlike *B. epigaea* and also both species have secondary products differing with *B. epigaea* (Trinkaus et al. 2001).

Buellia epigaea is morphologically and ecologically also similar to *B. asterella*. Both species occur on soil. *B. epigaea* is distinguished by much larger thalli and 8-spored asci whereas *B. asterella* has 4 spored asci. Also *B. epigaea* has no atranorin unlike *B. asterella* (Kocourková-Horáková 1998). *B. asterella* is a critically endangered species growing on mosses and soil in dry grasslands of Europe.

Buellia epigaea is phylogenetically most related with *Buellia halonia* and *B. subsororioides* according to the data in GenBank (Fig. 1). These two species are saxicolous, have rimose-areolate thalli and they both have atranorin and norstictic acid (Nash et al. 2007, Shanmugam et al. 2016).

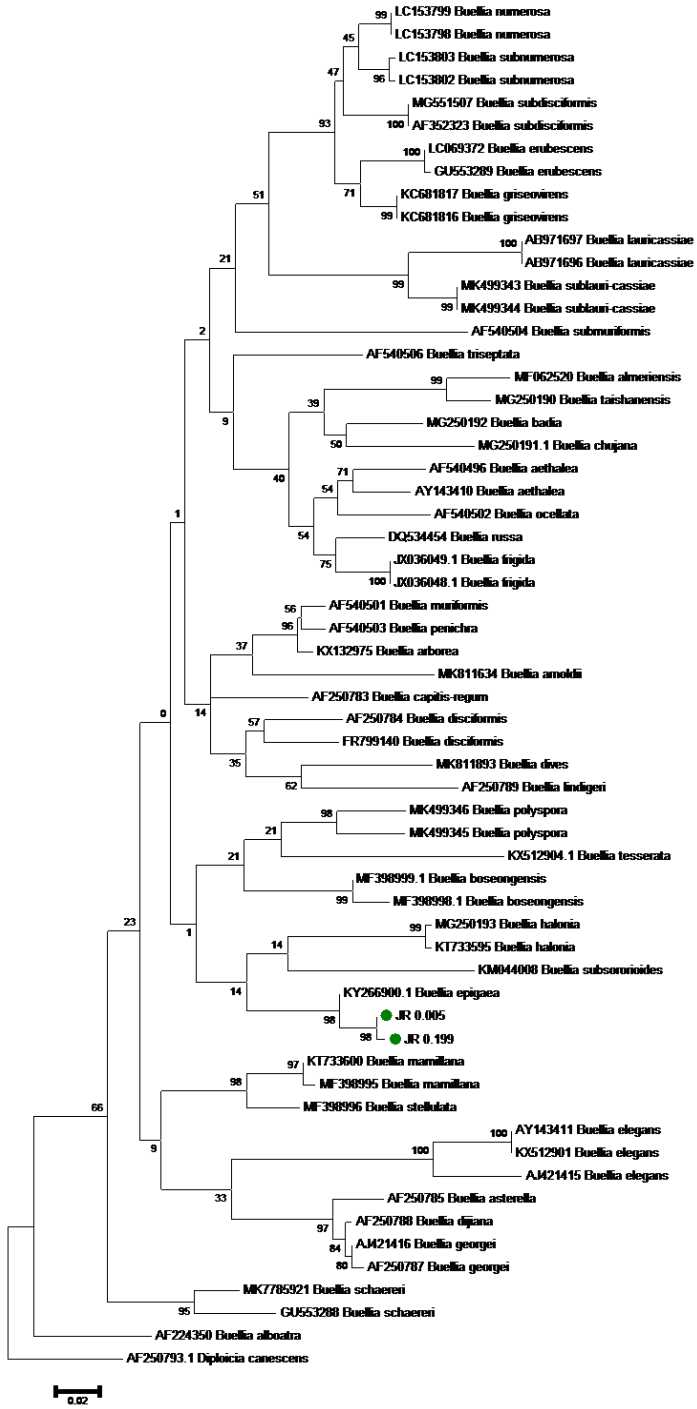

Fig. 1. Maximum Likelihood (ML) analysis inferred from ITS region sequences of *Buellia epigaea* and the other species of the genus.

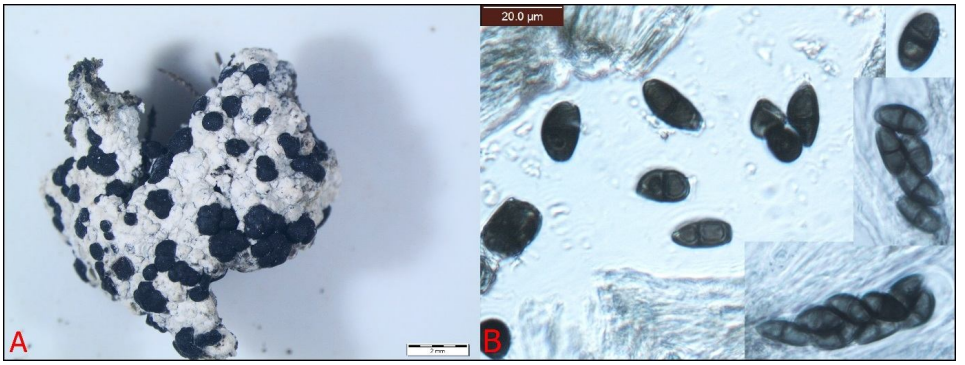

Fig. 2. *Buellia epigaea*. **A -** Habitus, **B -** Asci and ascospores.

Ecology and Distribution

Up to date, *Buellia epigaea* has been reported from Australia (Trinkaus et al. 2001), Europe (Germany, Spain and Czech Republic) (Kocourková-Horáková 1998, Cantón et al. 2004, Wirth et al. 2011), America (Looman 1964) including Mexico [1], Arctic (Powell 1967, Zhurbenko 1998, Hansen 2001, Urbanavichus 2015) and Middle East (Galun and Garty 2001) on soil, clay, humus, turf, detritus, dead leaves [1] (Fig. 3). This is the first report of this species from Antarctica. Antarctic specimens were collected on soil where the lichen vegetation is rich between 90-140 m altitude.

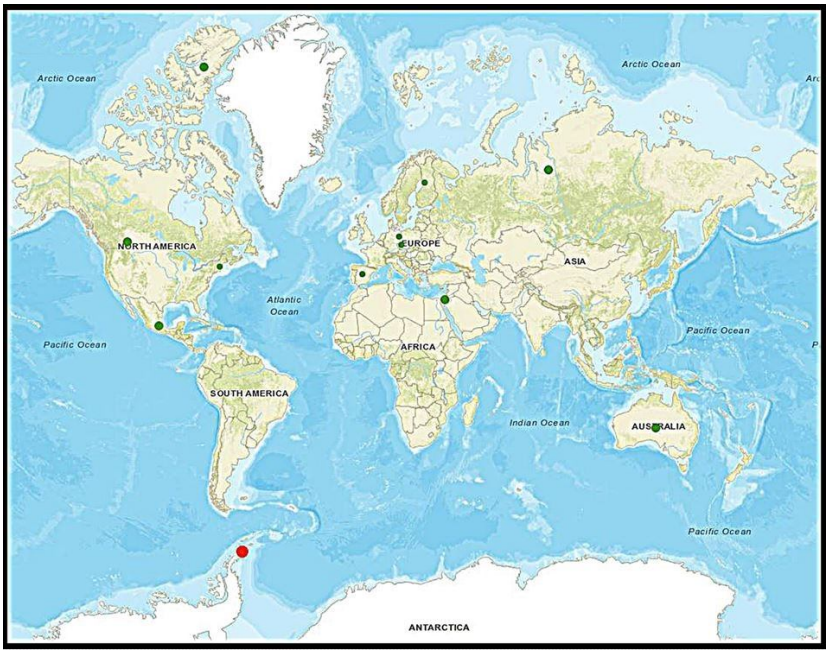

Fig. 3. World-wide distribution of *Buellia epigaea*.

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[1] Lias light - A Database for rapid Identification of Lichens
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