

A NEW LICHENIZED FUNGI RECORD FROM ANTARCTIC PENINSULA, ANTARCTICA ACCORDING TO nrITS PHYLOGENY: *Buellia badia* (FR.) A. MASSAL

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Abstract

Vegetation in the Antarctic Peninsula is quite poor due to the generally harsh conditions of Antarctica. Lichenized fungi are the dominant elements of the vegetation. There are about 450 species of lichenized fungi in Antarctica. About 65% of these lichenized fungi are distributed in the Antarctic Peninsula. *Buellia* is one of the most common genera in the Antarctic Peninsula. The genus *Buellia* is characterized by black lecideine apothecia, oblong or ellipsoidal and rarely citriform-shaped brown ascospores with one or more septa and a reddish-brown and rarely hyaline hypothecium. It is classified in the Caliciaceae family. The phylogeny of the heterogeneous genus *Buellia* is still not fully resolved today. There are about 400 species in the genus *Buellia* in the world. Only 20 of these species are distributed in Antarctica. In this study, we report *Buellia badia* as a new lichenized fungi record from Antarctic Peninsula, Antarctica according to its nrITS phylogeny.

Keywords: Antarctica, biodiversity, *Buellia*, *Buellia badia*, lichenized fungi

1. INTRODUCTION

Buellia is a lichenized fungi genus. It is characterized by its crustose thallus, mostly lecideine apothecia, one or more septate brown ascospores, *Bacidia*-type asci and brown or sometimes hyaline hypothecium (Bungartz et al., 2007). *Buellia* genus consist approximately 400 species around the world (Joshi et al., 2010). Only 20 are has a distribution in Antarctica: *Buellia aethalea* (Ach.) Th. Fr., *Buellia bouvetii* Øvstedal, *Buellia cycloplaca* Elix, *Buellia epigaea* (Pers.) Tuck, *Buellia evanescens* Darb., *Buellia frigida* Darb., *Buellia grisea* C.W. Dodge & G.E. Baker, *Buellia illaetabilis* I.M. Lamb, *Buellia lignoides* Filson, *Buellia melanostola* (Hue) Darb., *Buellia minispora* Elix, *Buellia pallida* C.W. Dodge & G.E. Baker, *Buellia perlata* Darb., *Buellia pycnogonoides* Darb., *Buellia rodseppeltii* Elix, *Buellia russa* (Hue) Darb., *Buellia soledians* Filson, *Buellia subfrigida* Mas. Inoue, *Buellia subtegens* Js. Murray, *Buellia vilis* Th. Fr.

In this paper we report *Buellia badia* for the first time from Antarctic Peninsula, Antarctica according to nrITS phylogeny.

2. MATERIALS AND METHODS

2.1. Collection and examination of lichen sample

Lichen sample was collected by the second author from James Ross Island (Antarctic Peninsula, Antarctica). The sample deposited in Erciyes University Lichen Herbarium (ERCH, Türkiye). For identification of the specimen, standart microscope techniques was used. Standart light microscope and stereomicroscope was used for morphological and anatomical examinations. Sections were taken in water and measurements were made from sections in water. The measurements are written in as “(smallest value) mean minus standard deviation-mean-mean plus standard deviation-(largest value)” format by calculating mean, standard deviation, maximum and minimum values. “n” was the total number of measurements for all samples of that species.

2.2. DNA isolation, extraction, amplification and sequencing

For total DNA isolation six apotjcia were taken and DNA was isolated by using a commercial DNA isolation kit (“DNeasy Plant Mini Kit; Qiagen: cat. no. 69104) according to manufacturer’s instructions. For PCR amplification, internal transcribed spacer region (ITS1-5.8S-ITS2 rDNA) (White et al., 1990). gene was used. Sample was prepared for a total of 50 µl of standard reactiontime amplification conditions were obtained with 25 µl of 2 × Taq PCR MasterMix in each tube with 2 µl of the primers ITS1F and ITS4, 2 µl of DNA extracts and 19 µl of distilled water. For PCR temperature profiles these conditions were employed: an initial denaturation step of “95°C for 5min”, followed by “35 cycles of 95°C for 45sec” (denaturation), “54°C for 45sec” (annealing), and “72°C for 60sec” (extension) followed by a final extension period of “72°C for 10min. The sequence analysis of the sample was performed by BM Labosis Laboratory (Ankara, Türkiye).

2.3. Phylogenetic analyses

For alignment and optimization of ITS sequences of all species, ClustalW option used in BioEdit V7.2.6.1. For analysis only parsimony-informative regions were used. Phylogenetic tree with bootstrap values was made with MEGA IX using the Maximum Likelihood (ML) method with a 1000 bootstrap replication as a rapid bootstrap (Tamura et al., 2021). *Diploicia canescens* (Dicks.) A. Massal. used as outgroup. New sequence were deposited in GenBANK (Table 1).

Table 1. ITS sequences used in the analysis. Newly generated data given bold.

Species	nrITS	Locality
<i>Buellia badia</i> (ERCH JR 0.229)	will be taken	James Ross Island, Antarctica
<i>Buellia aethalea</i>	AF540496	Sweden
	AY143410	Italy
<i>Buellia alboatra</i>	AF224350	Fennoscandia
<i>Buellia almeriensis</i>	MF062520	-
<i>Buellia arborea</i>	KX132975	Switzerland
<i>Buellia arnoldii</i>	MK811634	Norway
<i>Buellia asterella</i>	AF250785	-
<i>Buellia badia</i>	MG250192	China
<i>Buellia boseogensis</i>	MF398999	South Korea
	MF398998	South Korea
<i>Buellia capitis-regum</i>	AF250783	-
<i>Buellia chujana</i>	MG250191	China
<i>Buellia dijiana</i>	AF250788	-
<i>Buellia disciformis</i>	AF250784	-

	FR799140	-
<i>Buellia dives</i>	MK811893	Norway
<i>Buellia elegans</i>	AY143411	USA
	AJ421415	-
<i>Buellia epigaea</i>	MW825639	James Ross Island, Antarctica
	KY266900	Norway
<i>Buellia erubescens</i>	LC069373	Japan
	GU553289	Russia
<i>Buellia frigida</i>	JX036049	Antarctica
	JX036048	Antarctica
<i>Buellia georgei</i>	AJ421416	Australia
<i>Buellia georgei</i>	AF250787	-
<i>Buellia griseovirens</i>	KC681817	Canada
	KC681816	Canada
<i>Buellia halonia</i>	MG250193	China
	KT733595	South Korea
<i>Buellia lauricassiae</i>	AB971697	Japan
	AB971696	Japan
<i>Buellia lindigeri</i>	AF250789	-
<i>Buellia mamillana</i>	KT733600	South Korea
	MF398995	South Korea
<i>Buellia muriformis</i>	AF540501	USA
<i>Buellia numerosa</i>	LC153799	Japan
	LC153798	Japan
<i>Buellia ocellata</i>	AF540502	Fareo Islands
<i>Buellia penichra</i>	AF540503	USA
<i>Buellia polyspora</i>	MK499345	Thailand
	MK499346	Thailand
<i>Buellia russa</i>	DQ534454	Antarctica
<i>Buellia schaeferi</i>	MK778592	Russia
	GU553288	Austria
<i>Buellia stellulata</i>	MF398996	South Korea
<i>Buellia subdisciformis</i>	MG551507	-
	AF352323	Spain
<i>Buellia sublauri-cassiae</i>	MK499343	Thailand
	MK499344	Thailand
<i>Buellia submuriformis</i>	AF540504	India
	LC153802	Japan
<i>Buellia subnumerosa</i>	LC153803	Japan
<i>Buellia subsororioides</i>	KM044008	India
<i>Buellia taishanensis</i>	MG250190	China
<i>Buellia tesserata</i>	KX512904	-
<i>Buellia triseptata</i>	AF540506	USA
<i>Diploicia canescens</i>	AF250793	-

3. RESULTS AND DISCUSSIONS

3.1. Taxonomy

Buellia badia (Fr.) A. Massal. (Figure 1)

Description: Thallus lichenicolous, parasitic on *Aspicilia* sp., areolate, rusty brown. Apothecia black, lecideine, mostly semi-immersed or sometimes sessile, round or angular, 0.1–0.3 mm wide. Thalline margin present, mostly carbonized and very distinct. Epithecium brown, 30 μm , K-, N-. Hymenium hyaline, upper part sometimes brownish, 60 μm . Paraphyses simple, not branched, apices clavate, 2.5–3 μm . Hypothecium hyaline, 50 μm . Asci 8-spored, 52–62 \times 15–20 μm . Ascospores brown, one septate, oblong or sometimes with a narrow tips, *Buellia* type, (11–)11.5–12.5–13.5(–14) \times (5–)5.5–6–6.5(–8) μm (n=10) and l/w ratio: (1.63–)1.73–2.11–2.49(–2.8) μm (n=10). Pycnidia not seen.

Chemistry: All spot tests are negative.

Ecology: *Buellia badia* is a lichenicolous species on *Aspicilia* sp. recorded between 2-40 m altitude on Solorina Valley, James Ross Island (Antarctic Peninsula).

Specimen examined: Antarctic Peninsula, James Ross Island, Solorina Valley 63° 52' 39.0" S, 57° 46' 51.6" W, alt. 2 m, on *Aspicilia* sp., leg. M. G. Halıcı, 12.01.2017 (ERCH JR 0.229)

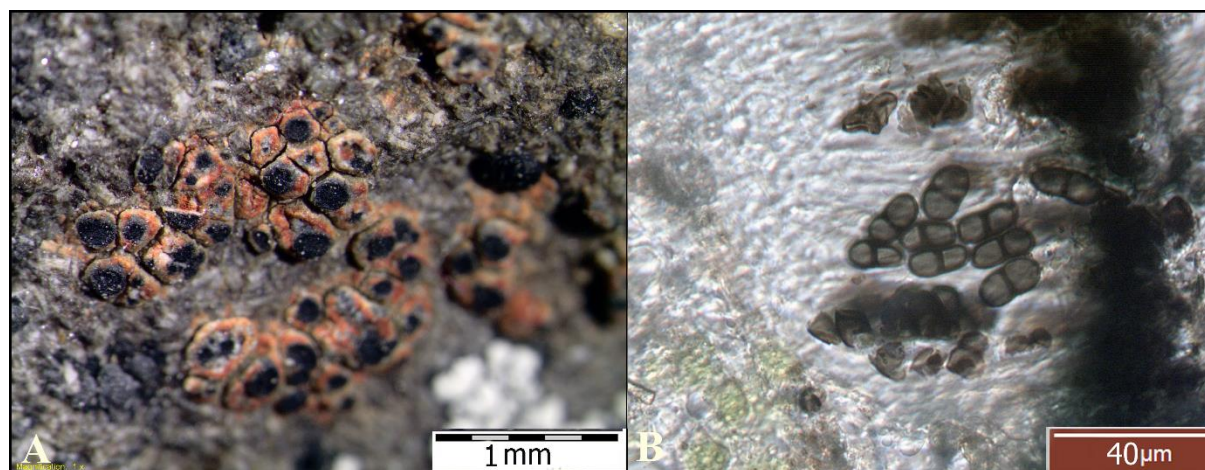


Figure 1. *Buellia badia*. A. Habitus (very young stage of development), B. Ascospores.

Buellia badia is similar to *Monerolechia californica* (H. Magn.) Elix anatomically and morphologically. They both have chocolate brown thallus and ascospores that are very similar in size, shape and structure. But they differ by their host preferences, apothecia exciple and secondary chemistry. *M. californica* is lichenicolous on *Dimelaena radiata* (Tuck.) Müll. Arg. and it has not been found on wood or bark unlike *B. badia*. Immature apothecia of *M. californica* has an initial formation of a thalline exciple unlike *B. badia*. And while *M. californica* has norstictic acid, in *B. badia* there is no secondary component (Nash et al., 2007).

3.2. Phylogenetic Results

As for *Buellia badia*, according to *ITS* phylogeny (Fig 2), the Antarctic specimen (JR 0.229) clearly matches up with *Buellia badia*. Anatomical, morphological and chemical characteristics of the sample also indicate that it is *B. badia*.

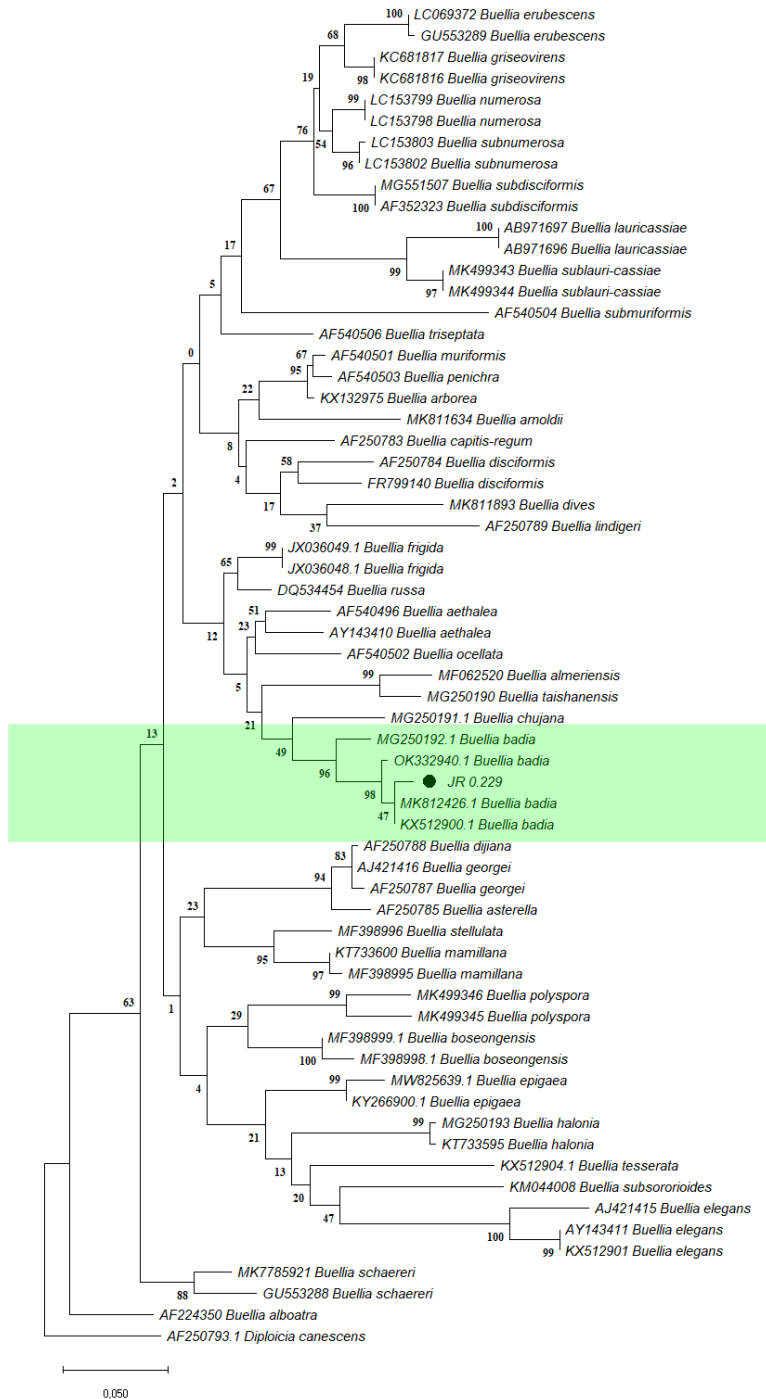


Figure 2. ITS ML phylogeny of *Buellia badia*

According to ITS ML phylogeny *Buellia badia* is closer to *Buellia chujana* Xin Y. Wang, S.Y. Kondr., Lőkös & Hur phylogenetically. While *B. badia* is lichenicolous on *Aspicilia* sp. *B. chujana* is not lichenicolous. While *B. chujana* has perlatolic acid, *B. badia* does not contain any secondary metabolites. And also *B. chujana* has dark greenish-brown areolate thallus unlike *B. badia* (Wang et al., 2016).

4. CONCLUSIONS

In this study we report *B. badia* for the first time from Antarctic Peninsula (Antarctica). *B. badia* has been previously reported from Europe, Macaronesia, North and South America, Asia, Africa, Australia, New Zealand (Elix, 2011) It is also reported for the first time from Antarctica within this study. And also detailed description, photos and phylogenetic tree were given in the study.

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