MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2022

October–December 2022—Volume 137(4), pp. 619–628

https://doi.org/10.5248/137.619 online publication date: 7 September 2023

Buellia parmigera sp. nov. from China

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Abstract—A new species, *Buellia parmigera,* was discovered from the Tibetan plateau, characterized by a crustose-subsquamulose thallus, immersed and lecanorine apothecia, a hyaline hypothecium, and *Buellia*-type ascospores. The new species is described, and compared with the other *Buellia* species with lecanorine apothecia.

Key words—lichenized fungi, *Ascomycota*, taxonomy, *Caliciaceae*

Introduction

Buellia De Not. (*Caliciales*, *Caliciaceae*) was introduced by De Notaris (1864), and Müller & von Arx (1962) designated *Buellia disciformis* (Fr.) Mudd as its type species. *Buellia* is characterized by a crustose thallus, black lecideine apothecia, *Bacidia*-type asci, brown ascospores with one or more septa, and a reddish-brown or rarely hyaline hypothecium. Since its establishment, approximately 400 species have been placed in the genus (Bungartz & al. 2007). Several other genera previously included in *Buellia* s.lat. (e.g., *Amandinea* Scheid. & H. Mayrhofer, *Diplotomma* Flot., and *Tetramelas* Norman) have since been segregated based on their macro- and micromorphology, chemistry, and ecological environment (Scheidegger 1993, Marbach 2000, Nordin 2000). However, due to the lack of phylogenetic studies, there remain many species

currently placed in *Buellia* s.lat. that may eventually be assigned to other genera. Over 64 species of *Buellia* s.lat. have been reported from China; these were mostly collected within the Tibetan Plateau region (Wei 2020, Wang & al. 2020). During the Second Tibetan Plateau Scientific Expedition and Research Program (STEP), more than 1200 specimens of *Buellia* were collected. Based on examination of morphology, coupled with chemistry and phylogenetic analyses, we propose *Buellia parmigera* as a new species. In this paper, we present a phylogenetic study of *Caliciaceae*, based on a nrITS matrix. There is no evidence that would support this new species being assigned to any genus separate from *Buellia* s.lat., so we have treated it as belonging to the broad concept of *Buellia*. Detailed descriptions and figures for the proposed new species are also provided.

Materials & methods

Morphological and chemical analyses

Specimens used in this study were collected from the Hengduan Mountains area and the Qinghai-Tibet plateau. They were all deposited in the Lichen Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China (KUN). Macromorphological studies were conducted under a dissecting microscope (Nikon SMZ 745T). Micromorphology was examined under an optical microscope (Nikon Eclipse Ci-S). Photographs were taken using a digital camera (Nikon DS-Fi2). The lichen secondary metabolites were detected and identified by spot tests [K (10% KOH), C ($Ca(OCl)_2$), KC, PD (P-phenylenediamine ethanol saturated liquid)] and thin-layer chromatography, using solvent systems C (toluene : acetic acid = 85 : 15) (Orange & al. 2001).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fresh materials using DNA secure Plant Kits (TIANGEN), according to the manufacturer's instructions. The primers were ITS1F & ITS4 (White & al. 1990, Gardes & Bruns 1993). PCR amplification reactions were performed in a 25 μL volume, containing 3 μL of genomic DNA, 1 μL of a 10 mM solution for each primer and 20 μL of 1.1 × T3 Super PCR Mix (TSINGKE). The PCR profile for the target region was as follows: initial denaturation at 98°C for 3 min, followed by 35 cycles of denaturation at 98°C for 10 s, annealing at 54–56°C for 10 s, elongation at 72°C for 15 s; and a final extension at 72°C for 2 min. PCR product sequencing was carried out with the same amplification primers, using Sanger technology by Tsingke Biotechnology Co., Ltd. (Kunming, China).

Phylogenetic analyses

Sixty-eight samples representing *Caliciaceae* Chevall. and *Physciaceae* Zahlbr. were downloaded from GenBank and eight samples of *Caliciaceae* were obtained from our materials. All sequences were edited manually using Geneious v8.0.2. The nrITS matrix was aligned using MAFFT v7 with the option of E-INS-I (Katoh & al. 2005). Ambiguous regions were excluded using Gblocks (Talavera & Castresana 2007) with the default settings. Phylogenetic relationships were inferred based on Bayesian inference (BI) and maximum likelihood (ML).

ML analyses were performed with RAxML v8.2.12 (Stamatakis 2006) with 2000 rapid bootstrap replicates. The best-fit partition substitution models were selected based on the lowest Bayesian information criterion (BIC) using Partition Finder 2 (Guindon & al. 2010, Lanfear & al. 2012, 2017): TIM2e+G4. Bootstrap support values (BS) were estimated from the 70% majority rule tree from all saved trees. BI analyses were performed with MrBayes v3.2.7 (Ronquist & al. 2012), running for 2 million generations. The trees were sampled every 100 generations and the first 25% of the trees were discarded as burn-in. Bayesian posterior probabilities (PP) were obtained from the 95% majority rule consensus tree of all saved trees. FigTree v1.4.0 (Rambaut 2012) and Adobe Illustrator were used to view and edit the phylogenetic tree.

Results

The nrITS matrix comprised 76 sequences including eight newly generated sequences (Table 1). Eight representative monophyletic genera were selected from *Caliciaceae* and several *Physciaceae* species were selected as the outgroup. The BI and ML trees showed similar topologies. Therefore, only the ML tree has been provided (Fig. 1). The results of the phylogenetic analyses showed that the specimens designated as *Buellia parmigera* were clustered within *Caliciaceae* with strong statistical support (BS = 96%; PP = 1) and they formed a highly supported monophyletic lineage (BS = 100%; PP = 1). There is no evidence that *B. parmigera* belongs to any genus separate from *Buellia* s.lat., so it is retained there. *Buellia parmigera* was sister to *B. aethalea* (Ach.) Th. Fr. with high support (BS = 95%). In addition, the clade including *B. parmigera* and *B. aethalea* was sister to the clade of *B. chujana* Xin Y. Wang & al. and *B. badia* (Fr.) A. Massal. with good support (BS = 91%; PP = 0.96). There were distinctively different micromorphological characteristics for *B. parmigera* versus other species in *Buellia* s.lat. We therefore recognize *B. parmigera* as a new species.

Table 1. Species, specimens, and sequences used in the phylogenetic analyses. Newly obtained sequences are in **bold** font.

Taxon	Voucher	GenBank (ITS)
Acolium inquinans	Wedin 6352 (UPS)	AY450583
A. karelicum	Hermansson 16472 (UPS)	KX512897
Amandinea lignicola	Toensberg 36426 (BG)	JX878521
A. punctata 1	AFTOL 1306	HQ650627.1
A. punctata 2	Nordin 5346	AF224353
A. punctata 3	000272563 (GZU)	GU553286
Anaptychia ciliaris	Wedin 6429 (UPS)	AY143391
Buellia aethalea 1	Per Johansson 5 (UPS)	AF540496
B. aethalea 2	F138222 (S)	JX000098
B. badia 1	Westberg 09-079 (S)	KX512900
B. badia 2	TS1767 (LCU)	MG250192
B. chujana 1	140835-1	KT733597
B. chujana 2	140835-2	KT733598
B. disciformis	Nordin 4429 (UPS)	AF540498
B. erubescens 1	Wetmore 95879 (S)	KX512902
B. erubescens 2	CBM: Watanuki: L01004	LC069373
B. erubescens 3	CBM: Watanuki: L01032	LC069374
B. erubescens 4	KW 63381	GU553289
B. griseovirens 1	Nordin 4734 (UPS)	AF540500
B. griseovirens 2	Lendemer 28500 (NY)	KC681819
B. griseovirens 3	Lendemer 28474 (NY)	KC681820
B. muriformis	Nordin5336a (UPS)	AF540501
B. numerosa 1	CBM: Watanuki: L01033	LC153798
B. numerosa 2	CBM: Watanuki: L01034	LC153799
B. parmigera	XY20-129 (KUN)	**ON166694**
B. parmigera	XY20-268 (KUN)	**ON166695**
B. parmigera	XY20-176 (KUN)	**ON166696**
B. parmigera	XY20-272 (KUN)	**ON166701**
B. parmigera	XY20-274 (KUN)	**ON166697**
B. parmigera	XY20-292 (KUN)	**ON166698**
B. parmigera	XY20-276 (KUN)	**ON166699**
B. parmigera	XY20-275 (KUN)	**ON166700**
B. penichra	Nordin5322 (UPS)	AF540503
B. subnumerosa 1	CBM: Watanuki: L01049	LC153800
B. subnumerosa 2	CBM: Watanuki: L01843	LC153803
B. subnumerosa 3	CBM: Watanuki: L01423	LC153801
Calicium nobile 1	Tibell 21968 (UPS)	KX512913

Taxon	Voucher	GenBank (ITS)
C. nobile 2	Tibell 23396 (UPS)	KX512914
Diplotomma alboatrum 1	18-60034 (KUN)	MN615696
D. alboatrum 2	18-60448 (KUN)	MZ224658
D. alboatrum 3	Prieto 3034 (S)	KX512924
D. alboatrum 4	Uppland, 2001 Nordin	AF408677
D. alboatrum 5	Nordin 5345	AF224351
D. alboatrum 6	Nordin 3205 (UPS)	DQ198357
D. pharcidium 1	—	FR799180
D. pharcidium 2	—	FR799314
D. venustum 1	18-58557 (KUN)	OL467349
D. venustum 2	18-58102 (KUN)	OL467350
D. venustum 3	XY19-252 (KUN)	OL467353
Heterodermia speciosa	Wetmore (S)	KX512927
H. vulgaris	Frisch 11/Ug1226 (UPS)	KX512928
Phaeophyscia ciliata	Prieto (S)	KX512929
Ph. orbicularis	Prieto 3012 (S)	KX512930
Physcia aipolia	Wedin 6145 (UPS)	KX512931
P. tenella	Odelvik & Hellström 0827 (S)	KX512932
Pseudothelomma ocellatum 1	Tehler 8063 (S)	KX512934
Pse. ocellatum 2	Hermansson 18662 (UPS)	KX512935
Pyxine cocoes	Prieto (S)	KX512936
Py. endochrysina 1	14-46462 (KUN)	KY611887
Py. endochrysina 2	14-46439 (KUN)	KY611888
Py. sorediata	Wetmore 91254 (S)	KX512937
Py. subcinerea	—	HQ650705
Rinodina cinnamomea 1	Spribille 19893 (GZU)	KX015688
R. cinnamomea 2	Spribille 20101 (GZU)	KX015689
R. degeliana 1	Tonsberg 41921	KX015674
R. degeliana 2	Thor 28169	KX015675
Tetramelas chloroleucus	Westberg 10–001 (S)	KX512938
T. geophilus	Nordin 4429 (UPS)	AF540499
T. insignis 1	Nordin 5664 (UPS)	DQ198358
T. insignis 2	ZT2013043	KP314327
T. pulverulentus	Nordin 6368 (UPS)	KX512940
T. triphragmoides	Nordin 4425 (UPS)	AF540505
Thelomma mammosum 1	Tibell 23775 (UPS)	KX512942
T. mammosum 2	Hernández et al. 2002 (UPS)	KX512943
T. santessonii 1	Nordin 4011 (UPS)	KX512944
T. santessonii 2	Nash 38262 (UPS)	KX512945

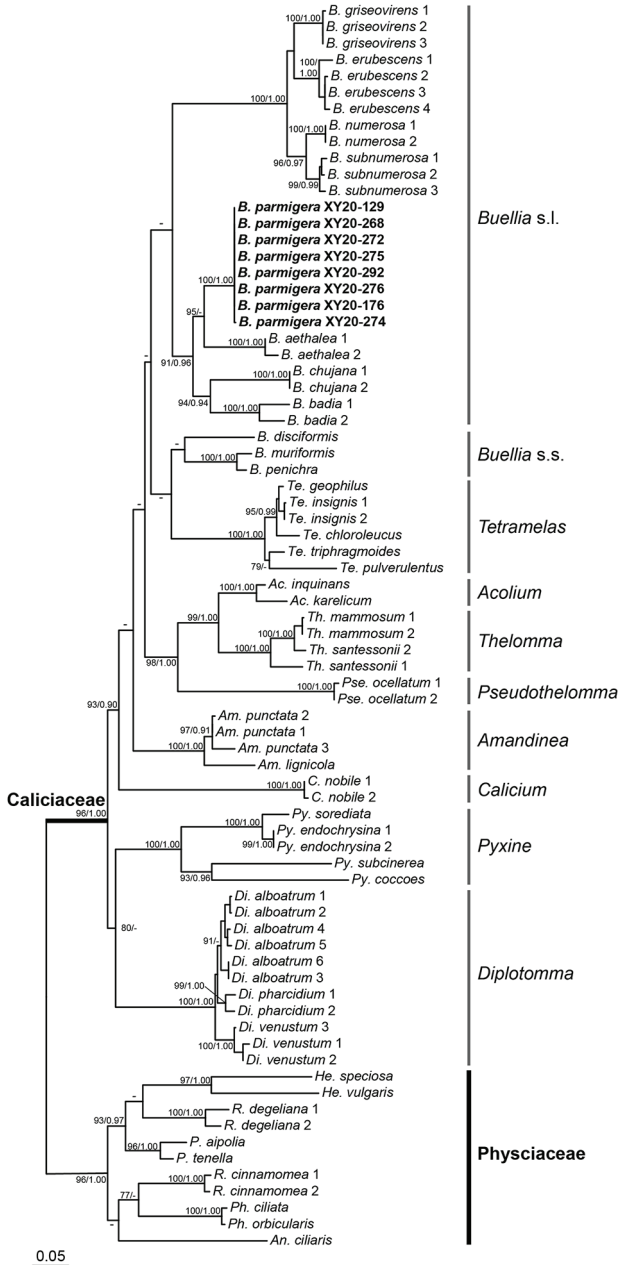

FIG. 1. RAxML tree of *Caliciaceae* based on analysis of nrITS region. Bootstrap support values for Maximum likelihood ≥ 75% and Bayesian posterior probabilities ≥ 0.90 are indicated near the nodes. The new species is shown in **bold**.

Taxonomy

Buellia parmigera M. Ai & Xin Y. Wang, **sp. nov.** Fig. 2.

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Differs from other Chinese *Buellia* species by its lecanorine apothecia and hyaline hypothecia; and from *Rinodina* spp. by its *Bacidia*-type asci and *Buellia*-type ascospores.

Type—China, Sichuan Province, Liangshan Yi Autonomous Prefecture, Yanyuan County, Weicheng town, 27.5015°N 101.6864°E, alt. 2814 m, on rock, 28 Jul. 2020, X.Y. Wang & al. XY20-268 (**Holotype**, KUN).

Etymology—*parmigera* (Latin = shield-bearer) refers to the shieldlike appearance of each areola and its single central apothecium, within the subsquamulose thallus.

Thallus crustose, areolate, closely adnate, up to 2 cm in diam., areolae irregular in shape, up to 0.5 mm in diam., sometimes subsquamulose, prothallus absent; upper surface yellow-brown to greenish brown, dull, without pruina; medulla white, non-amyloid (I–). Apothecia dense, usually aggregate in the central part, 1 per areola, lecanorine, immersed, disc dark brown to black , margin concolorous with the thallus, roundish to irregular, 0.1–0.5 mm in diam., margin persistent; thalline exciple lecanorine-type, algal layer continuous under the hypothecium; epihymenium yellowish brown to brown; hymenium hyaline, 50–60 µm tall, not inspersed, paraphyses simple to moderately branched, apically swollen, with a brown pigment cap; hypothecium hyaline, c. 40 µm tall; asci oval-clavate, *Bacidia*-type, 8-spored, spores 1-septate, hyaline when young, turning brown when mature, *Buellia*-type (Bungartz & al. 2007), ellipsoid, with obtuse ends, proper septum narrow, thickened when young, (9–)10–12(–13) × (4–)5–6(–7) µm. Pycnidia not seen.

Chemistry—Thallus K–, C–, KC+ pink, PD–, UV–, medulla I–; containing gyrophoric acid.

Distribution & ecology—This new species is mainly distributed in the Hengduan Mountains area, growing on exposed siliceous rock at elevations of (1200–)2500–3500 m.

Additional specimens examined (vouchered in KUN)—CHINA, Yunnan Province, **Shangri-la City**, Jiantang town, Potatso National Park, 27.8280°N 99.9784°E, alt. 3500 m, on rock, 25 Jul. 2020, X.Y. Wang & al. XY20-129; **Lijiang City**, Ninglang County, Xichuan Village, 27.0938°N 100.6070°E, alt. 2517 m, on rock, 26 Jul. 2020, X.Y. Wang & al. XY20-176. Sichuan Province, **Liangshan Yi Autonomous Prefecture**, Yanyuan County, Weicheng town, 27.5015°N 101.6864°E, alt. 2819 m, on rock, 28 Jul. 2020, X.Y. Wang & al. XY20-275, XY20-292; **Dechang County**, Leyue Town, 27.3190°N 102.3273°E, alt. 1268 m, on rock, 29 Jul. 2020, X.Y. Wang & al. XY20-353. Xizang Province, **Xiayadong Village**, 27.4313°N 88.9125°E, alt. 3187 m, on rock, 26 Jul. 2019, X.Y. Wang & al. XY19-2495.

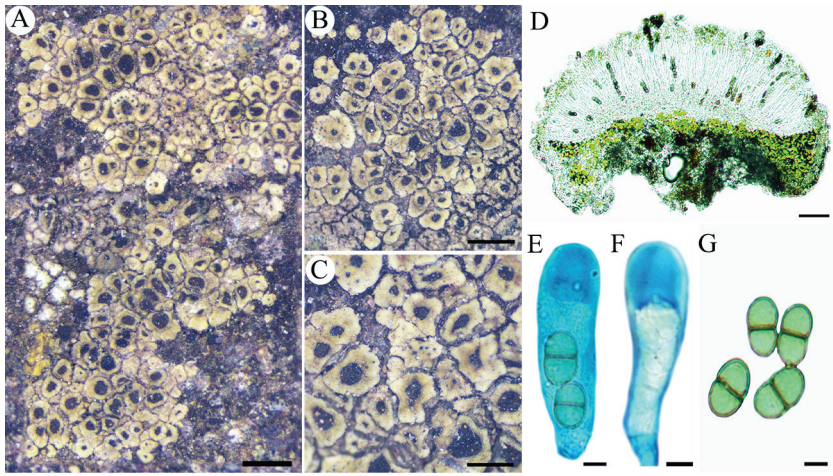

FIG. 2. *Buellia parmigera* (holotype, KUN–XY20-268). A–C. Subsquamulose thallus and immersed apothecia; D. The section of apothecium, showing lecanorine-type exciple and hyaline hypothecium; E, F. *Bacidia*-type asci; G. Ascospores, *Buellia*-type, 1-septate. Scale bars: A, B = 1 mm; C = 0.5 mm; D = 50 μm; E–G = 5 μm.

Notes—This new species could be distinguished from all other Chinese *Buellia* species by its saxicolous thallus, lecanorine apothecia, hyaline hypothecium and the presence of gyrophoric acid. It could potentially be misidentified as a *Rinodina* species due to its lecanorine apothecia and hyaline hypothecium, but it is confirmed as belonging to *Buellia* according to phylogenetic study and ascospore ontogeny.

Some *Buellia* species also have lecanorine apothecia at least during the early stage of development, such as *B. dakotensis* (H. Magn.) Bungartz, *B. endoferruginea* Bungartz, and *B. fouquieriensis* Bungartz. All these species have brown to reddish brown hypothecium and lecanorine apothecia which become lecideine when mature, and have a corticolous habit (Bungartz & al. 2007). The saxicolous species *Buellia mamillana* (Tuck.) W.A. Weber has lecanorine apothecia when young, but differs by having a pale greenish yellow upper surface, *mamillana*-type apothecia and the presence of xanthones (Bungartz & al. 2004).

Another species of *Buellia* containing only gyrophoric acid is *Buellia uberior* Anzi, with a wide distribution across the Northern Hemisphere (Bungartz & al. 2007), but it differs by having lecideine apothecia, deep reddish brown hypothecium, and epihymenium with cinereorufa-green pigments (HNO_3+ violet).

Acknowledgments

We are very grateful to Prof. Qiang Ren (Institute of Mycology, Chinese Academy of Sciences) and Prof. Xin Zhao (Liaocheng University) for pre-submission reviews. This study was supported by grants from the Flora Lichenum Sinicorum (31750001), the Second Tibetan Plateau Scientific Expedition and Research Program (STEP) (2019QZKK0503), Youth Innovation Promotion Association CAS (2020388), Yunnan Young & Elite Talents Project, National Natural Science Foundation of China (31970022) and State Key Laboratory of Phytochemistry and Plant Resources in West China (P2020-KF08).

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