

Brunnipila calyculiformis (Schumach.) Baral: A Novel Record for Türkiye

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ABSTRACT

The research subject of this study is a specimen of *Brunnipila* collected from the Biga (Çanakkale) district in 2024. The specimen, which is characterised by outward facing brown hairs and densely septate hairs with crystals on top, was identified as *B. calyculiformis* (Schumach.) Baral, as a new record for members of the Ascomycota in Türkiye, based on morphological characters in accordance with the data obtained from field studies and laboratory analyses. In this manuscript the macro- and micromorphological characteristics of the new record were described and illustrated. This new record will contribute to the macrofungal diversity of Türkiye and the distribution of the genus *Brunnipila*.

Keywords: Ascomycota, Mycobiota, Novel record, Türkiye.

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Introduction

The order *Helotiales* within the class *Leotiomycetes* is the most diverse order containing non-stromatic discomycetes with inoperculate asci [1-3]. Most members of *Helotiales* have very small apothecia, usually less than 2 mm in diameter. Apothecia may be sessile or stipitate, dark or brightly coloured, and superficial or erumpent along the plant host. The general shape of the apothecia is cupulate-discoïd, concha funnel-shaped or clavate [4]. Most are known to be saprophytic, living on fallen leaves and decaying wood, but some are pathogenic or symbiotic parasites with other organisms. This order is reported to include 11 families, about 500 genera and about 4000 species [1-2]. With the advent of molecular phylogeny, some families in the order *Helotiales* have been revised, such as members with stromata (*Sclerotiniaceae* and *Rutstroemiaceae*) or hairs (*Hyaloscyphaceae* and *Lachnaceae*) [5].

The *Lachnaceae* Raitv are a family of inoperculate discomycetes characterised by small, often stalked, hairy apothecia, usually brightly coloured, bowl-shaped. Most family members have been described as saprophytes on dead plant tissues. However, there are also species isolated as endophytes from living leaves and roots [6,7]. Some species within the family that are not known to pass to the sexual stage and remain only in the asexual stage have also been reported. Although these species show morphological similarities, they are phylogenetically positioned in different genera [7]. The most distinctive feature of *Lachnaceae* is that the ascocarp is hairy and the excipular cells are thin-walled. In 2004, *Lachnaceae* was separated from *Hyaloscyphaceae* sensu Nannf. and became a new family, supported by phylogenetic data [8-9]. Various researchers have placed totally ten genera

(*Albotricha*, *Brunnipila*, *Capitotricha*, *Dasyscyphella*, *Erioscyphella*, *Incrucipulum*, *Lachnellula*, *Lachnopsis*, *Proliferodiscus* and *Velebitea*) in the family *Lachnaceae* [10-13].

The genus *Brunnipila* Baral (*Lachnaceae*, *Helotiales*), which has unique characters that have long been overlooked, was introduced by Baral [14]. It was characterised by brown, moderately thick-walled, densely granulated hairs with a denser septation towards slightly capitate apex and firmly attached, flat or octahedral crystals on top. The lanceolate, strongly protruding paraphyses consistently lack refractive vacuolar bodies (Baral, pers. comm.). Octahedral crystals are less sharp than the crystals known from the genus *Incrucipulum* Baral, where they are more regular and sharper [15].

In recent years, valuable studies on Ascomycota have been carried out in Türkiye [3, 16-22]. In addition to these studies, [23] and [24] found that there is only one species (*Brunnipila clandestina* (Bull.) Baral) belonging to the genus *Brunnipila* in Türkiye. The aim of this study is to contribute to the Ascomycota of Türkiye and to determine the distribution of the genus *Brunnipila*.

Materials and Method

Fresh *Brunnipila* apothecia constituting the study materials were collected in Biga (Çanakkale) district on 23.03.2024. The external characteristics of the specimen were noted in the field notebook. It was subjected to photographic documentation in its natural environment. At the end of the day, they were transferred to Çanakkale Onsekiz Mart University Mycology Laboratory. The samples were dried in a non-illuminated environment and

were converted into fungarium material by the author for further use. For the determination of microscopic data, preparations from the dried sample were made in water, IKI or what and examined under a Leica DM500 (Germany) research microscope, first under a 4×10 objective and then under 10×10 and 40×10 objectives, respectively. Finally, immersion oil was dropped onto the preparation, it was analysed and photographed under a 100×10 objective. Microscopic characters (asci [IKI were used for the ascus reaction], ascospores, hairs and paraphyses) in the photographs were measured at least 20 times using Leica Application Suite (version 3.4.0). Macro- and micromorphological analyses were performed following the methods described by [14-15,25]. Microscopic drawings were prepared using CorelDRAW (64-bit) (Canada). This ensured accuracy and clarity in depicting the observed features of *Brunnipila*.

Result

Ascomycota Caval.-Sm.

Leotiomyces O.E. Erikss. & Winka

Helotiales Nannf.

Lachnaceae Raitv.

Brunnipila Baral

Brunnipila calyculiformis (Schumach.) Baral (Figure 1,2)

Syn: *Atractobolus calyculiformis* (Schumach.) Kuntze, Revis. gen. pl. (Leipzig) 3(3): 445 (1898); *Cyathicula calyculiformis* (Schumach.) P. Karst. [as 'calyculaeformis'], Not. Sällsk. Fauna et Fl. Fenn. Förh. 8: 207 (1866); *Dasyscyphus calyculiformis* (Schumach.) Rehm [as 'Dasyscypha'], Ascomyceten: no. 111b (1872); *Erinella calyculiformis* (Schumach.) Quél., Enchir. fung. (Paris): 301 (1886); *Lachnea calyculiformis* (Schumach.) Gillet [as

'calyculaeformis'], Champignons de France, Discom. (3): 69 (1880) [1879]; *Lachnella calyculiformis* (Schumach.) W. Phillips, *Man. Brit. Discomyc.* (London): 237 (1887); *Lachnum calyculiforme* (Schumach.) P. Karst., *Bidr. Känn. Finl. Nat. Folk* 19: 178 (1871); *Lachnum calyculiforme* var. *cyphelliforme* Rehm, in Strasser, *Verh. Kaiserl.-Königl. zool.-bot. Ges. Wien* 57(1): 338 (1907); *Peziza calyculiformis* Schumach. [as 'calyculaeformis'], *Enum. pl.* (Kjbenhavn) 2: 425 (1803); *Peziza calyculiformis* var. *gregaria* Berk. & Broome, *Ann. Mag. nat. Hist., Ser. 3* 7: 450 (1861); *Trichopeziza calyculiformis* (Schumach.) Rehm, *Ber. naturhist. Augsburg* 26: 53 (1881).

Apothecia 0.5 – 2 mm across, stipitate, outer surface brown; brown, beige-brown or olive-brown covered with hairs, often with pale (almost white) crystals at the tips of the hairs and hymenium whitish-gray. Discs pale yellowish brown. Hairs 130 – 175 \times 3.5 – 11 μm , warty, 5 – 12 septate, septa often denser towards apex, lateral walls 0.5–1 μm thick; cylindrical, brown, overall covered with minute, hyaline granules, at their tips with refractive matter or octahedral-shaped crystals, in their upper part the young hairs are paler. Stipe 0.3 – 0.7 mm long. Asci 45 – 60 \times 4 – 8 μm diam., hyaline, cylindrical, at apex tapering, arising from croziers, 8-spored, apex turning blue in IKI. Ascospores 7 – 11.5 \times 1.5 – 2 μm diam., usually irregularly biseriolate, hyaline, without septa, narrowly fusiform. Paraphyses 3 – 5.8 μm wide, lanceolate, exceeding the asci for 11–17 μm , septate towards the base. Ectal excipulum of textura prismatica - textura angularis, cells up to 15 μm diam.

Specimen examined: Biga (Çanakkale), near Kaldırımbaşı village, roadside on corticated branch fragments of *Corylus* sp. 40° 14'31"N, 27° 12'45"E, 73 m, 23.03.2024, Acar 1861.



Figure 1. *Brunnipila calyculiformis* a. Apothecia on bark of *Corylus* sp. b. Ascospores (in water). c. Asci (in IKI) d. Asci and paraphyses (in water). e. Fragments of hair (in IKI) f. Hairs lacking crystals. g. Hairs with crystals (in water). Scale bar: 10 μm

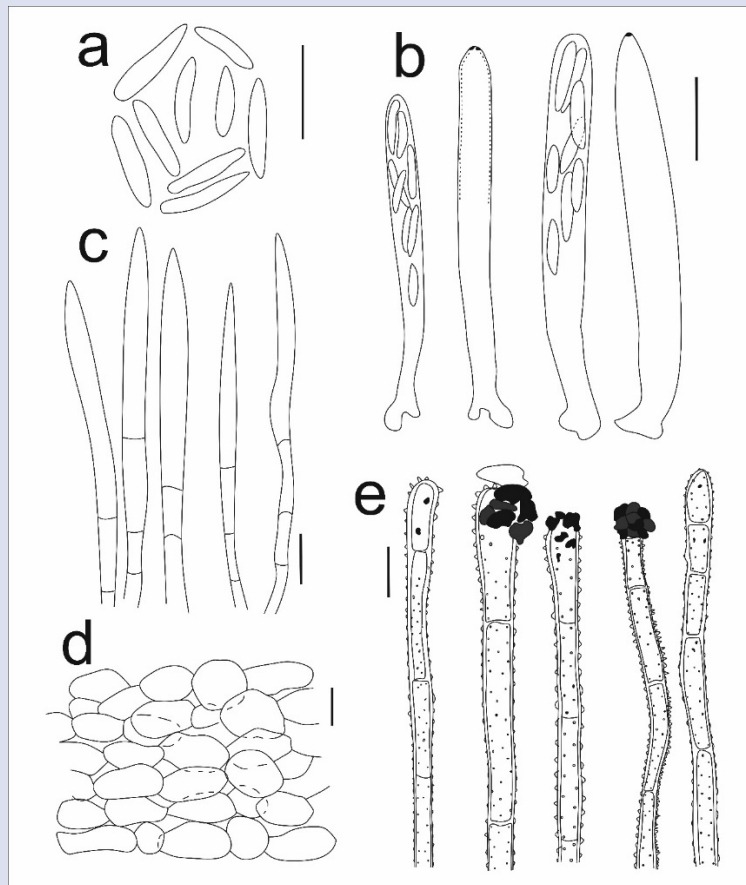


Figure 2. *Brunnipila calyculiformis* a. Ascospores b. Asci, c. Paraphyses, d. Ectal excipulum, e. Hair Scale bar: 10 µm

Discussion

Lachnum latebricola (Rehm) R. Galán & Raitv. (= *Lachnella calyculiformis* (Schum. ex Fr.) Phill. var. *latebricola* (Rehm) Phill.), previously recognised as a synonym of *Brunnipila calyculiformis*, was first discussed by [26] as a different species. Later this variety was elevated to the species level (separate due to smaller spores and partly specific substrates (*Ericaceae*) by [25,27]. *Brunnipila clandestina* (Bull.) Baral is among the species with similarities to *B. calyculiformis* in Baral. Both species have small, whitish or light-coloured apothecia. *Brunnipila calyculiformis* usually has flatter, disc-like apothecia, while the apothecia of *B. clandestina* are more hairy and prominent. Differences in substrate (*Rubus* L. and woody herbaceous stems of other dicots), larger paraphyses, smaller asci without croziers and ascospores are the distinguishing properties of *B. clandestina*. In addition, the paraphyses of *B. clandestina* are sometimes slightly yellowish in colour, whereas those of *B. calyculiformis* are hyaline [25]; [28] – here you find the statement “sans crochet”, Huhtinen apparently overlooked the value of croziers here.

The genus *Brunnipila*, which is represented by 11 species in the world [29], was previously now known with only one species in our country, *B. clandestina*. As a result of this study, the number of species belonging to the genus *Brunnipila* was increased to two and thus contributed to the mycobiota of the country.

Conflicts of interest

There are no conflicts of interest in this work.

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