New records of Helotiales in Turkey

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Received 5 Apr 2017 Accepted 15 Aug 2017

ABSTRACT: Species of *Ascocoryne sarcoides* (Jacq.) J.W. Groves & D.E. Wilson, *Chlorociboria aeruginascens* (Nyl.) Kanouse ex Ramamurthi, Korf & Batra and *Lachnellula agassizii* (Berk. & M.A. Curtis) Dennis, which are Helotiales, are described as new records for Turkey. Microscopic drawings and descriptions of the taxa are presented together with morphological photographs.

KEYWORDS: macrofungi, biodiversity, taxonomy, Ascomycota

INTRODUCTION

Helotiales Nannf. ex Korf & Lizon, with 13 families, 501 genera, and approximately 3880 species, is the largest and most varied order in the Leotiomycetes (Ascomycota)^{1–3}. Helotiales are a non-lichenized, inoperculate, and non-stromatic apothecial group of ascomycetes which show great morphological diversity because of their different nutritional strategies^{2,4,5}.

Morphological characteristics of the apothecia such as shape and colour, microscopic characteristics such as shape and size of asci and ascospores, apothecial ontogeny, and reaction of asci to Melzer's Reagent, ecological characteristics such as living on land or in water, and biological characteristics such as parasitic or saprophytic nutritional type have been used to identify and classify the families, genera, and species in the order Helotiales^{1,2}. In addition, members of this order have distinguishing characteristics such as apothecia generally small and often brightly coloured, sessile or stipitate, cupulate or discoid ascomata, asci which are mostly small, thin-walled, without separable wall layers, and with an apical pore, and ascospores which are simple or transversely septate, not quite longitudinally symmetrical, mostly hyaline, and often smooth^{3,6}.

Thanks to the geographical location and ecological, climatic, topographic, and geological factors, Turkey is as rich in fungal biodiversity as it is in plant species^{7,8}. Numerous studies have been performed recently on the macrofungi of Turkey, and approximately 2600 taxa have been identified, but in comparison with this, little work has been carried out until now on the Helotiales which are represented by 65 taxa within 15 families and 38 genera in Turkey $^{9-14}$.

The aim of this study was to explore the biodiversity of this country by reporting in Turkey for the first time the taxa of the order Helotiales *Ascocoryne sarcoides* (Jacq.) J.W. Groves & D. E. Wilson, *Chlorociboria aeruginascens* (Nyl.) Kanouse ex Ramamurthi, Korf & L. R. Batra and *Lachnellula agassizii* (Berk. & M. A. Curtis) Dennis, and to contribute to future studies on the biogeographical distribution of these taxa.

MATERIALS AND METHODS

The specimens were collected from the provinces of Artvin and Denizli in Turkey in 2014 and 2015. Field studies were performed mostly in the autumn and spring, the period during which the macrofungi produce fruiting bodies. Morphological and ecological characteristics of the samples were noted and photographed in their natural habitats. After field work, specimens were brought to the laboratory where they were examined. Microscopic characters were observed by light microscope using 5% KOH, Melzer's Reagent, and distilled water.

Identification of the taxa and descriptive terms for morphological and microscopic features were based on the current literature ^{15–26}. After viewing the samples using the latest studies of Helotiales in a checklist of Turkish mycota^{9–14}, they were considered as being new records for the macromycota of Turkey. Taxa, systematics, and author citations are quoted according to Kirk³, Index Fungorum (www.indexfungorum.org), and MycoBank (www.mycobank.org). The taxa investigated in this study are deposited in the fungarium of the Pamukkale University Mushroom Research and Application Centre (PAUMMER).



Fig. 1 Distribution of new records in different regions of Turkey according to the specimens examined. Square: *Ascocoryne sarcoides*; triangle: *Chlorociboria aeruginascens*; circle: *Lachnellula agassizii*.

RESULTS

The distribution of the specimens of fungi found in different regions of Turkey is given in Fig. 1. *Ascocoryne sarcoides, Chlorociboria aeruginascens,* and *Lachnellula agassizii* are presented together with descriptions, morphological photographs, and microscopic drawings.

Fungi Bartling Ascomycota Whittaker Pezizomycotina O.E. Erikss. & Winka Leotiomycetes O.E. Erikss. & Winka Helotiales Nannf. ex Korf & Lizoň Helotiaceae Rehm Ascocoryne J.W. Groves & D.E. Wilson

Ascocoryne sarcoides (Jacq.) J.W. Groves & D.E. Wilson, Taxon 16(1): 40 (1967) (Fig. 2)

Basionym: *Lichen sarcoides* Jacq. —Miscellanea austriaca ad botanicum, chemiam et historiam naturalem spectantia 2:20 (1781).

Synonyms:

Bulgaria sarcoides (Jacq.) Fr. —Systema Mycologicum 2:168 (1822).

Coryne sarcoides (Jacq.) Tul. & C. Tul. —(1865).

Helvella sarcoides (Jacq.) Dicks. —Fasciculus plantarum cryptogamicarum Britanniae 1:21 (1785).

Ombrophila sarcoides (Jacq.) W. Phillips. —A manual of the British Discomycetes, 323 (1887).

O. sarcoides (Jacq.) P. Karst. -86 (1871).

Pirobasidium sarcoides (Jacq.) Höhn. —Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 111: 1002 [16 of repr.] (1902).

Tremella sarcoides (Jacq.) Fr. —Syst. mycol. (Lundae) 2: 217 (1822).

Macroscopic description: Apothecia 5–15 mm in diameter, spherical when young, later flattening, edges becoming wavy, becoming upturned cupshaped, stalk absent or short and poorly developed,



Fig. 2 *Ascocoryne sarcoides*; (a) and (b) fresh apothecia, on natural substrate; (c) ascospores; (d) germinated ascospores with septum and long germ tubes; (e) asci; (f) ascus apices (immature and mature); (g) paraphysis apices; (h) medullary excipulum. Scala bars: (a) and (b) = 10 mm, (c) and (d) = 10 μ m, (e) = 15 μ m, (f) = 10 μ m, (g) and (h) = 5 μ m. All from *OKA* 298.

attached at the centre to the substrate, lobes at the edges frequently irregular. *Fleshy part* gelatinous. *Hymenium* smooth, slightly wrinkled when mature, reddish-pinkish or purplish.

Microscopic description: Ascospores $11-16 \times 4-5.5 \mu m$, ellipsoid with a smooth surface, hyaline, containing one or two drops of oil, with a single septum; white, creamy or yellowish tones. Asci 110– $150 \times 8-10 \mu m$ in size, threadlike-cylindrical, with 8 spores. Paraphyses numerous, filiform, cylindrical, 2– $5 \mu m$ at apex, multiguttulate, unbranched, generally forked at the base, with few septa, sometimes thickening towards the apex. Medullary excipulum made up 2– $4 \mu m$ wide, narrow interwoven hyphae. This fungus has an anamorphous conical or crater shape with irregular edges and a reddish or violet colour.



Fig. 3 *Chlorociboria aeruginascens*; (a) and (b) fresh apothecia developed on wood surface; (c) ascospores; (d) asci; (e) ascus apices (immature and mature); (f) paraphyses; (g) medullary excipulum with smooth-walled tomentum hyphae. Scala bars: (a) and (b) = 5 mm, (c) = 5 μ m, (d) and (f) = 10 μ m, (g) = 5 μ m. All from *OKA* 307.

Ecology: This species is widely distributed in late summer and autumn, particularly in damp areas, on logs and branches particularly of broadleaved trees rotting on the ground. The species is saprophytic and as well as being found on organic waste, and it can be seen on various trees.

Specimens examined: TURKEY, Artvin, Borçka, Camili biosphere reserve area, on the damp, fallen and well-rotted trunks of *Fagus orientalis* Lipsky, 1465 m, 07.10.2015, *OKA* 298; on damp decayed wood of *F. orientalis*, part of which was underground, 1470 m, 14.10.2015, *OKA* 306.

Chlorociboriaceae Baral & P.R. Johnst.

Chlorociboria Seaver ex Ramamurthi, Korf & Batra

Chlorociboria aeruginascens (Nyl.) Kanouse ex Ramamurthi, Korf & Batra, Mycologia 49(6): 858 (1958) [1957] (Fig. 3)

Basionym: Peziza aeruginascens Nyl. —(1869).

Synonyms:

Chlorosplenium aeruginascens (Nyl.) P. Karst. — Bidrag till Kännedom av Finlands Natus ock Folk 19:103 (1871).

Chlorosplenium aeruginosum var. aeruginascens (Nyl.) P. Karst. —233 (1870).

Peziza aeruginascens (Nyl.) —(1869).

Macroscopic description: Apothecia 2–7 mm in width, disk or cup-shaped, on maturing becoming almost flat and taking the shape of a shallow cup. *Hymenium* smooth, between blue and green in colour; outer surface whitish when young, later a turquoise colour between blue and green, finally taking a flaky appearance. *Stem* 2–5 mm in length, cylindrical, generally attached away from the centre, occasionally centrally, and of a similar colour to the hymenium. Smell and taste indeterminate.

Microscopic description: Ascospores $5-8 \times 1-2.5 \mu m$, fusiform to fusiform-elliptical, with an oil drop in the end part, surface smooth, hyaline and without septa. Asci $40-60 \times 3.5-4.5 \mu m$ in size, narrow clavate or cylindrical clavate, with eight ascospores. Paraphyses $60-90 \times 1.5-2.0 \mu m$, narrow clavate, threadlike, slightly widening at the ends, septate. Medullary excipulum textura intricate, numerous and intense, hyaline, narrow interwoven hyphae $2-4 \mu m$ wide, with walls thin, smoothwalled tomentum hyphae $1.5-2.5 \mu m$ diam, with short septate. Spore print varying from white to creamy.

Ecology: This species grows saprophytically solitary or in small groups on or in the wood of barked or rotted hardwood trees. It is easily seen on the forest floor because its mycelium appears green or light blue on the substrate on which it grows. The green mycelium can be seen all year round but the basidiomata generally appear in the autumn.

Specimens examined: TURKEY, Artvin, Borçka, Camili biosphere reserve area, on rotted and barked branches of *Pinus sylvestris* L. on the ground, 1380 m, 14.10.2015, *OKA* 307.

Hyaloscyphaceae Nannf.

Lachnellula P. Karst.

Lachnellula agassizii (Berk. & M.A. Curtis) Dennis, Persoonia 2 (2): 183 (1962) (Fig. 4)

Basionym: *Peziza agassizii* Berk. & M.A. Curtis, Grevillea —3 (28): 151 (1875).

Synonyms:

Atractobolus agassizii (Berk. & M.A. Curtis) Kuntze — Revisio generum plantarum 3 (2): 445 (1898).

Dasyscypha agassizii (Berk. & M.A. Curtis) Sacc. —(1889).



Fig. 4 *Lachnellula agassizii*; (a) and (b) fresh apothecia developed on wood surface; (c) ascospores; (d) asci; (e) apex of a mature ascus; (f) paraphyses; (g) excipular hairs. Scala bars: (a) and (b) = 10 mm, (c) and (f) = 10 μ m, (g) = 5 μ m. All from *OKA* 100.

Dasyscyphus agassizii (Berk. & M.A. Curtis) Sacc. —Sylloge Fungorum 8:438 (1889).

Lachnella agassizii (Berk. & M.A. Curtis) Seaver —The North American cup-fungi (Inoperculates) 3: 247 (1951).

Macroscopic description: Apothecia 3–7 mm in width, in the form of a shallow cup, with an incompletely developed white stalk; cap at first round then broadening, when wetted opens in a circular shape. *Hymenium* smooth, varying from bright yellow to yellowish orange; outer surface of the cap and its wavy edges densely covered with small flexible white hairs when young, on ageing the edges take on a fringed appearance, the outer surface is generally white or whitish. Has a short stalk and is attached to the substrate at its centre.

Microscopic description: Ascospores $6-9.5 \times 2.5-4 \mu m$, narrowly elliptical, surface smooth, uniseriate or biseriate, hyaline. Asci $50-75 \times 3-5 \mu m$, cylindrical to subcylindrical, eight spored. Paraphyses $55-80 \times 2-3.5 \mu m$, distinctly filiform, almost spatulate at apex, sharply tapering below, occasionally slightly

lanceolate, smooth, septate, sometimes branched at the base. *Excipular hairs* cylindrical, hyaline, relatively thick-walled, multiply septate by swollen or subacute, finely spiny, $2.5-4.0 \mu m$ diam.

Ecology: Generally found living saprophytically or parasitically in small groups on branches or pieces of wood of gymnosperms. A very small fungus, seen in spring or late autumn.

Specimens examined: TURKEY, Denizli, Buldan, Buldan upland lake area, or rotted and brokenup small branches or pieces of wood from *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe, 982 m, 02.10.2014, *OKA* 100.

DISCUSSION

Ascocoryne is a genus characterized by a gelatinous fruiting body and endophytic habits. When these fungi appear in a disorganized mass, they are similar to basidiomycete jelly fungi. Ascocoryne sarcoides is similar to A. cylichnium (Tul.) Korf in habitat and some macroscopic characteristics^{27–29}. However, it has been shown by many taxonomists that these two species differ³⁰. A. sarcoides has fruiting bodies which are bordered with light violet to greybrown, whereas A. cylichnium has fruiting bodies which are generally reddish purple to violet-pink. A. cylichnium has generally smaller apothecia (6-30 mm), ascospores $(18-30 \times 4-6 \mu m)$, and asci $(200-220 \times 10-12 \ \mu m)^{16, 19, 23, 25}$. Also, one of the most prominent characteristics of A. sarcoides is that asci and ascospores have one septum when they mature, the asci and ascospores of A. cvlichnium have more than one septum^{16, 19, 23–25}.

A. sarcoides is reported to have a wide distribution in forested areas of Europe (Finland, France, Britain, Iceland, Norway, Switzerland, and Germany), Australia, Asia (China), North America (Canada and Cuba), and South America (Chile)^{16, 19, 24, 25, 31}.

A. sarcoides forms colonies on the dead wood, fallen trunks, logs buried in the ground or pieces lying on the ground of deciduous trees, particularly *Carpinus, Fagus*, and *Quercus*^{19,24}. However, it has also been reported as growing apparently healthy on trees such as *Abies, Picea*, and *Pinus*^{19,31}. Also, it is reported that *A. sarcoides* has been found to have a protective characteristic as an endophyte against rotting fungi, and that it is found as much in the roots of trees as in the branches³². In our study, the *A. sarcoides* examined was identified on a rotting log of *Fagus orientalis*.

The mycelium of *Chlorociboria* species produces xylindein, a unique blue-green pigment, on the

substrate, making it one of the most recognizable fungi on the forest floor³³. Chlorociboria aeruginascens has morphological characteristics which make it confusable with such species as C. aeruginosa (Oeder) Seaver ex Ramamurthi, Korf & Batra, Chlorencoelia versiformis (Pers.) Dixon and Aeruginoscyphus sericeus (Alb. & Schwein.) Dougoud. However, when these species are examined microscopically, they can be easily distinguished by the dimensions of their ascospores. C. aeruginascens has smaller ascospores varying between 5 and $8 \,\mu$ m, while the ascospores of other species are larger: C. aeruginosa 9-15 µm, C. versiformis 9-15.5 μ m and A. sericeus 55–60 μ m^{15, 17, 19–21, 23, 25}. Also macroscopically, while C. aeruginascens has a number of fruiting bodies in one place, with asymmetric caps and generally stems which are away from the centre and only occasionally attached to the centre, C. aeruginosa has fruiting bodies which form separately, the caps are symmetrical and the stems are generally attached to the centre, and only occasionally away from the centre¹⁷.

C. aeruginascens is reported to have a wide distribution in forested areas in Europe, North America, and Asia^{15, 20, 21, 34}. This species grows particularly on many hardwood trees such as *Acer* sp., *Betula* sp., *Fagus* sp., *Populus* sp., *Ulmus* sp., and *Quercus* sp.^{17, 33, 35, 36}, but also on the well-rotted and damp logs and branches of the trees such as *Pinus* sp., *Tsuga* sp., and *Cedrus* sp. in groups or clusters, turning the substrate blue-green^{26, 37}. In this study, *C. aeruginascens*, which is a new record, was determined to be growing on pieces of the rotted branches of *P. sylvestris*.

The genus *Lachnellula* forms a natural group with a yellow disk and white excipular hairs, which generally grows saprophytically or parasitically on conifer wood. The species are macroscopically very similar to one another.

Because of the morphological similarity between *Lachnellula agassizii* and *L. ciliate* Dennis, *L. gallica* (P. Karst. & Har.) Dennis, *L. occidentalis* (G.G. Hahn & Ayers) Dharne, *L. suecica* (de Bary ex Fuckel) Nannf. and *L. willkommii* (R. Hartig) Dennis, it can be wrongly identified with confusion with these taxa. *L. occidentalis* is similar to *L. agassizii* in preferring *Pinus* and sometimes *Picea* as hosts. However, the upper surface of the cap of *L. occidentalis* is salmon-orange and has bigger ascospores of $11-20 \times 3.5-7.5 \ \mu m$ in size, while the colour of the upper surface of the cap of *L. agassizii* is bright orange-yellow to yellow, and has smaller ascospores which are $6-9.5 \times 2.5-4 \ \mu m$ in size. L. willkommii is similar to L. agassizii in that it is a parasite of Pinus, and the upper surface of the cap varies from yellow to orange. However, microscopically, L. willkommii can be easily distinguished from L. agassizii by its larger ascospores $(15-26 \times 6-10 \ \mu m)$ and asci $(125-170 \times 9-14 \ \mu m)$. L. suecica is similar to L. agassizii in its preference for Pinus and Abies and the yellowish orange colour of the upper surface of the cap. However, L. suecica is different from L. agassizii in having $4.5-7 \times 4-$ 5.5 µm globose type ascospores. Also, L. agassizii has $55-80 \times 2-3.5 \mu m$ spatulate paraphyses, while L. gallica and L. ciliate have larger filiform paraphyses (100–105 \times 1.5–2.5 μm and 85–145 \times 2–3 $\mu m,$ respectively), and so can be easily distinguished microscopically 18, 19, 22, 23, 25.

L. agassizii is reported to have a distribution in Europe, Asia, and North America^{18, 22, 25}. It is stated to grow saprophytically or weakly parasitically on the trunks or branches of gymnosperms such as *Abies, Pinus, Picea, Tsuga,* and *Larix,* forming small groups or clusters^{18, 22, 25}. In our study, *L. agassizii,* which is a new record, was determined on a small, damp, rotted piece of a branch of *P. nigra pallasiana*.

According to the current literature, one taxon each of the Helotiales genera *Ascocoryne* and *Chlorociboria*, and five taxa from the genus *Lachnellula*, have been reported in Turkey. In this study, the species *Ascocoryne sarcoides*, *Chlorociboria aeruginascens* and *Lachnellula agassizii* are reported in Turkey for the first time, bringing the number of Helotiales in Turkey to 68^{9-14} . Including the data which we have obtained, a contribution has been made to the biodiversity of the macromycota of Turkey, and findings have been reached which will be of use to future biogeographical distribution studies on these taxa, recorded in a different geographical region.

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