

# New records of two ascomycetes from the Republic of North Macedonia

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**Abstract.** *Nectria ganymede* on decaying ascomata of *Zeus olympius* is reported from the Republic of North Macedonia, thus significantly extending its geographic area westwards. *Gnomonia arnstadiensis* is collected on dead leaves of *Ostrya carpinifolia* at the Ohrid Lake. Concise descriptions and original illustrations of the studied materials are presented.

**Key words:** ascomycetes, Balkan mycota, *Gnomonia*, *Nectria*, *Pinus heldreichii*

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## Introduction

Fungi of genus *Gnomonia* Ces. & De Not. (*Gnomoniaceae*, *Diaporthales*) commonly develop on overwintered leaves and petioles of trees and shrubs, leaves, peduncles or stalks of herbaceous plants, with single, immersed or erumpent, black globose ascomata, without stroma (Barr 1978). According to the revised concept of the genus (Sogonov & al. 2008), perithecia are usually epiphyllous, with beaks, ascospores are hyaline, two-celled, seldom one-celled, mostly with appendages. *Gnomonia arnstadiensis* Auersw. is remarkable among the other *Gnomonia* species by its ascomata, with only an ostiole instead of a typical beak. Fifty-one species of gnomoniaceous fungi are known in Bulgaria, while in the adjacent Balkan countries knowledge of their diversity is scanty (Stoykov 2016). *Nectria ganymede* Minter & Diamandis (*Nectriaceae*, *Hypocreales*) is found on the old ascomata of *Zeus olympius* Minter & Diamandis, and so far has been known from Greece and Bulgaria (Minter & al. 1987; Stoykov & al. 2014). Data presented in this study expand the known distribution range of both species on the Balkan Peninsula.

## Material and methods

The two species were collected in October 2016, during a visit of the region of Ohrid Lake, along Metropol Hotel and along the road towards Galičica National Park. Air-dried specimens were kept at the Mycological Collection of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (SOMF). Colour photographs were taken by Canon PS A460, Canon PS A1400 HD and Olympus E330 digital cameras, under Boeco BM-180/T/SP, Olympus BX-41 LM and Boeco BOE 3500 dissecting microscope. Observation of the microstructures was carried out in water, and color reactions were studied additionally in lactophenol and in 5% KOH. Asci and ascospores were measured in water under LM, with specialized software for digital images Carnoy 2.0 (Schols & al. 2002).

The size of ascospores and their length/width (Q) ratio is presented in the descriptions as follows: (min–) mean±1standard deviation (–max), Q ratio, n, except for the description of *N. Ganymede*, where the spore measurements are presented as min–(mean<sub>min</sub>–mean<sub>max</sub>)–max, and ‘n’ denotes the number of meas-

ured spores. The size of ascomata and asci is given with its minimum and maximum values, n. Identifications are made according to Müller & von Arx (1962), Monod (1983), Minter & al. (1987), Stoykov (2012) and Stoykov & al. (2014).

## Results and discussion

*Nectria ganymede* Minter & Diamandis  
(Plate I, Figs 1-6)

**Ascomata** perithecia about 300 µm in size, pyriform, solitary, scattered or in small groups, semi-immersed in the decaying yellowish ascomata of *Zeus olympius*, orange-red. Ascomatal wall reacts by positive darker red in the solution of potassium hydroxide, yellowish in lactophenol, and in surface view often forms *textura epidermoidea*. **Ostiolum** lined with tightly packed, parallel tiny hyphae. **Asci** (55-) 65-70 × 11-13.5 µm, n = 5, thin-walled, 8-spored. **Ascospores** 15.7-(16.7-20.8)-28 × 4.7-(5.3-6.3)-7.3 µm, Q = (2.7-) 3.3±0.4 (-4.2), n = 16, irregularly biseriolate, ±tending to uniseriate towards the base of asci, hyaline to slightly brownish at maturity, 1-3-septate, usually with 2 guttules per cell, more guttulate if not fully ripe. **Paraphyses** not seen.

*Specimen examined.* The Republic of North Macedonia: above Lake Ohrid, along the road towards the high-mountain area of the Galičica National Park, 21.10.2016, leg. B. Assyov & D. Stoykov, on yellowish decaying ascomata of *Zeus olympius*, on fallen twigs of *Pinus heldreichii* Christ., SOMF 30222.

**Comments.** Germination of ascospores was observed after refrigerating the fresh collection of twigs holding ascomata for a couple of days. Data on the perithecia, asci and ascospores from the North Macedonian collection corresponded well to the known published descriptions of *Cosmospora ganymede* (Minter & Diamandis) Rossman & Samules: SOMF 29525, SOMF 29526, in Stoykov & al. (2014: 78) and *N. ganymede*: IMI 305041, in Minter & Diamandis (1987: 59), in spite of some differences observed for the minimum and maximum values of asci and spores. When compared to the minimum spore length in the original publication, the minimum spore length of the find exceeded it by almost 8 µm, and probably this was explained by the examination of spores out of the asci. The released spores were hyaline, initially non-septate and slender, subsequently ellipsoid and 1-3-septate.

The reaction of ascomatal wall to KOH was positive and recorded here as darker red, against the original description, which gave it as “darker red” in Minter & al. (1987), and “clear purple to violet” in Stoykov & al. (2014). The reaction of peridium tested here with lactophenol was yellowish, and was recorded as “yellow” in the original description (Minter & al. 1987).

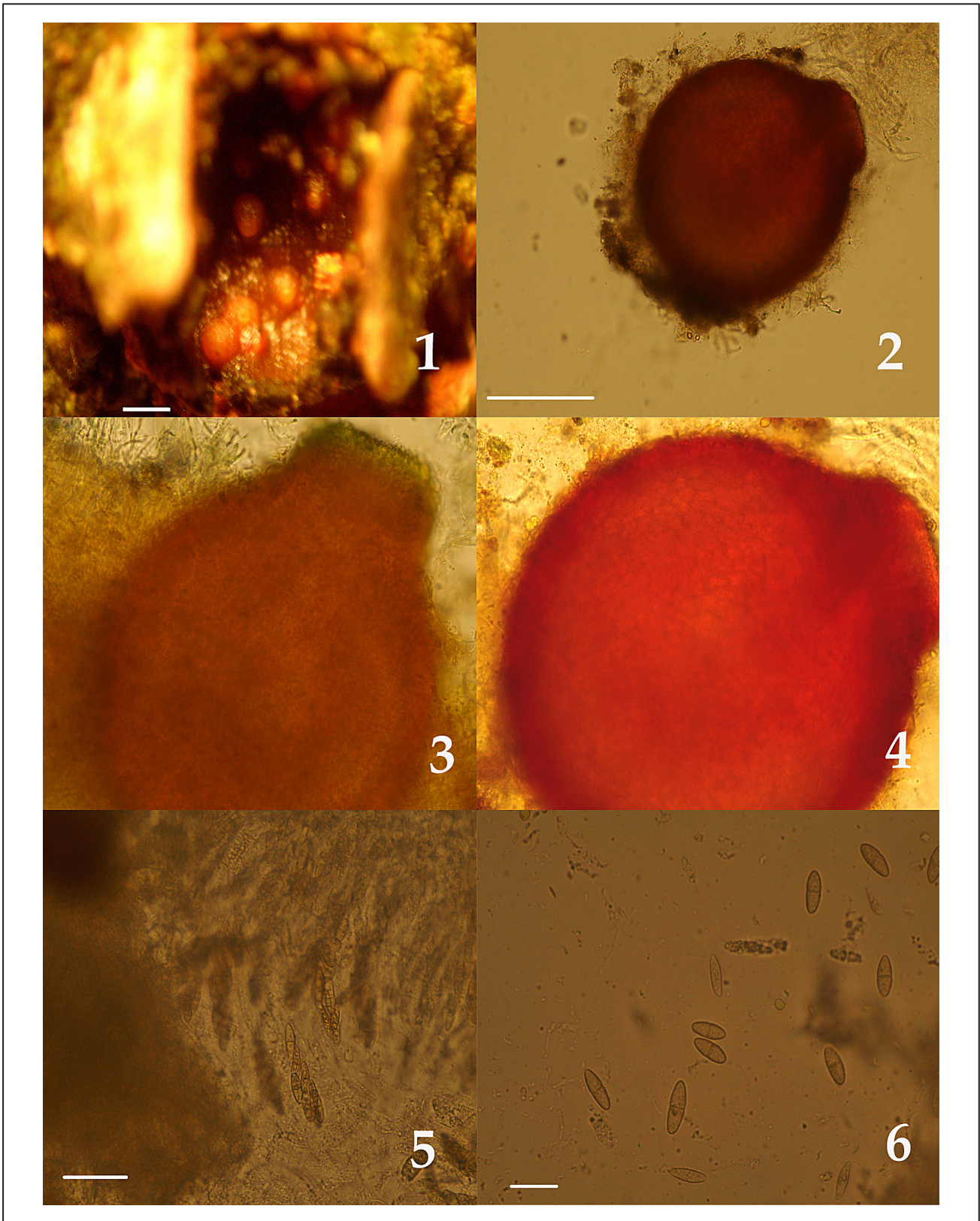
Some authors accepted the placement of *N. ganymede* in *Cosmospora* Rabenh. s. l. (Rossman & al. 1999; Stoykov & al. 2014). However, *Cosmospora* in the broad sense of Rossman’s findings was shown as polyphyletic in a number of recent studies (Gräfenhan & al. 2011; Lechat & al. 2019, and references therein). Its *Fusarium*-like anamorph, obtained in pure culture by Minter & al. (1987), excluded possible placement of *N. ganymede* in the restricted concept of *Cosmospora* (Gräfenhan & al. 2011). Furthermore, *N. ganymede* did not correspond to the proper characters of *Dialonectria* (Sacc.) Cooke, *Macroconia* (Wollenw.) Gräfenhan, Seifert & Schoers and *Stylonectria* Höhn. for reasons explained by Stoykov & al. (2014: 79). It did not fit the concept of *Fusicolla* Bonord. in all aspects, as defined by Gräfenhan & al. (2011), albeit some of its members were occasionally found on the stromata of other fungi and had yellow, pale-buff to orange ascomata, asci with apical ring, 1-septate ascospores, 1-3 (3-5)-septate macroconidia, exceptionally 10-septate. In the present collection, the ascomata were orange-red, asci were devoid of apical ring, ascospores were 1-3-septate, while conidia in the original description of Minter & al. (1987) were aseptate or 1-septate (only occasionally they were 3- or more septate). We have not been able to resolve yet the phylogenetic position of *N. ganymede* by means of DNA studies.

So far, *Nectria ganymede* has not been found in other localities than the ones known in Bulgaria (Pirin Mts, above Bansko town), Greece (Mt Olympus, Prionia area) and the new one in the Republic of North Macedonia (above Ohrid Lake, Galičica National Park).

*Gnomonia arnstadtiensis* Auersw. (Plate II, Figs 1-4)

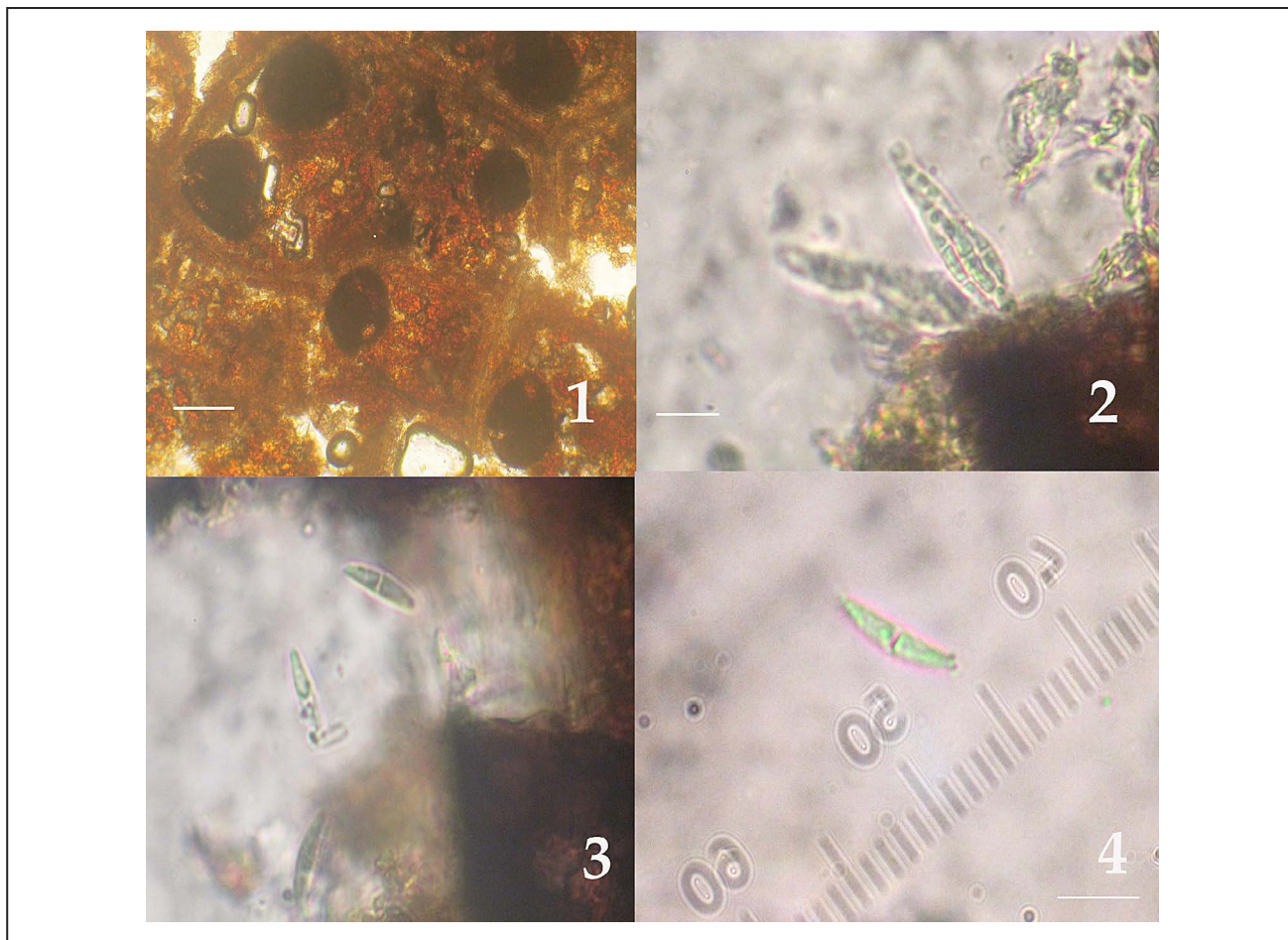
**Ascomata** perithecia (90-) 120-125 (-130) µm in size, n = 10, usually numerous, yellowish-brown to black under the dissecting stereomicroscope, ellipsoid to globose, immersed in the tissues of the leaves. Beak not present, ostiole lateral, seen as lateral transparence (Fig. 1). **Asci** 40-50 × 7-10 µm, n = 5, cylindrical, apical ring about 3.5 µm, with biserially overlapping ascospores. **Ascospores** (15-) 15.9±0.7 (-17.5) × (3.0-) 3.5±0.4



Plate I. *Nectria ganymede*

**Fig 1.** Perithecia on decaying ascomata of *Zeus olympius*. Scale bar = 400  $\mu$ m. **Fig. 2.** Perithecium with KOH + darker red reaction. Scale bar = 150  $\mu$ m. **Fig. 3.** Perithecium with lactophenol + yellowish reaction. **Fig. 4.** Perithecium with *textura epidermoidea*, in water. **Fig. 5.** Asci with guttulate spores, in water. Scale bar = 35  $\mu$ m. **Fig. 6.** Ascospores, in water. Scale bar = 20  $\mu$ m.



Plate II. *Gnomonia arnstadtensis*

**Fig 1.** Squash mount of perithecia from a leaf of *Ostrya carpinifolia* under LM, in water. Scale bar = 100 µm. **Fig. 2.** Ascus, in water. Scale bar = 10 µm. **Fig. 3.** Ascospores, in water. **Fig. 4.** Ascospore with hyaline appendage at the upper tip, in water. Scale bar = 10 µm.

(–4.5) µm, Q (3.7–) 4.6±0.5 (–5.2), n = 10, ellipsoid-fusoid, hyaline, straight or slightly curved, with median septum, non-constricted at septa, usually with hyaline appendages. Mature cells with two big guttules.

**Specimen examined.** The Republic of North Macedonia: along the road from Ohrid town towards Metropol Hotel, at the lakeside of Lake Ohrid, 20.10.2016, D. Stoykov, on dead leaves of *Ostrya carpinifolia* Scop., SOMF 30223.

**Comments.** Our data about the size of perithecia, length of asci and ascospores, obtained after examination under LM, recorded a smaller size than the one observed in the specimens studied by Monod (1983). Most probably, this was explained by the time of collection of the specimens, and the presence of unripened asci, examined in the squash water mounts. Generally, the size of ascumata, asci and ascospores of the material collected near the Ohrid Lake was within the min-

imal range known for the width and length of perithecia and ascospores in the studies of Müller & von Arx (1962), and close to the size of perithecia, asci and ascospores in Stoykov (2012).

The ascumata of *G. arnstadtensis* are non-stromatic, lack perithecial necks and are found on dead, usually overwintered leaves of *Carpinus betulus* L., *C. orientalis* Mill. and *O. carpinifolia* (Monod 1983; Stoykov 2012). Bulgarian materials, collected in 2005 by the author on overwintered leaves of *O. carpinifolia* (Belasitsa Mts) and *Carpinus betulus* (Eastern Forebalkan), have been characterized genetically and were proven to belong to *Gnomonia* (Sogonov & al. 2008). *Gnomonia arnstadtensis* was known in Europe from Bulgaria, Germany, Romania, Switzerland, and Turkey (Müller & von Arx 1962; Monod 1983; Stoykov 2004; Stoykov & Denchev 2007; Stoykov 2012).

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