Novel collections of *Zeus olympius* and *Cosmospora ganymede* (Ascomycota) from Bulgaria and Greece

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Summary: First Bulgarian records of *Zeus olympius* and *Cosmospora ganymede*, two unusual ascomycetes, described and so far known only from a single locality in Greece, are reported. *Zeus olympius* is related to Bosnian pine (*Pinus heldreichii*) and *Cosmospora ganymede* is associated with dead ascomata of the former. Newly collected specimens of both species from the *locus classicus* on the Mt Olympus, and of *Z. olympius* from Mt Pindus were also examined. Description of the Bulgarian and Greek materials is provided, including data from *in vivo* study, supplemented with illustrations of macroscopic and microscopic features, including the first colour photographs of *C. ganymede*. Germination of ascospores of *Z. olympius* was observed for the first time. The new collections significantly extend the geographic area of those apparently endemic ascomycetes. We also provide the first record of *Z. olympius* on artificially planted *P. heldreichii* outside its natural range.
Keywords: Balkan Ascomycota, endemic fungi, *Nectriaceae*, *Pinus heldreichii*, *Rhytismataceae*.

Résumé : premiers signalements bulgares de *Zeus olympius* et *Cosmospora ganymede*, deux ascomycètes peu courants, décrits et connus jusqu'à présent que d'une seule localité en Grèce, sont rapportés. *Zeus olympius* est lié au pin de Bosnie (*Pinus heldreichii*) et *Cosmospora ganymede* est associé aux ascocarpes morts du premier. De nouveaux spécimens des deux espèces recueillis du *locus classicus* sur le mont Olympe et de *Z. olympius* du mont Pinde ont également été examinés. Des descriptions des spécimens bulgares et grecques sont fournies, y compris les données de l'étude *in vivo*. Celles-ci sont complétées par des illustrations des caractéristiques macroscopiques et microscopiques, y compris les premières photographies en couleur de *C. ganymede*. L'observation de la germination des ascospores de *Z. olympius* est reportée pour la première fois. Les nouvelles récoltes étendent sensiblement la répartition géographique de ces ascomycètes apparemment endémiques. Nous fournissons également les premiers enregistrements de *Z. olympius* dans des plantations artificielles de *P. heldreichii* en dehors de son aire de répartition naturelle.
Mots-clés : Ascomycota des Balkans, champignons endémiques, *Nectriaceae*, *Pinus heldreichii*, *Rhytismataceae*.

Riassunto: Sono riportati primi ritrovamenti bulgari di *Zeus olympius* e *Cosmospora ganymede*, due ascomiceti insoliti, descritti e finora conosciuti solo da una singola località in Grecia. *Zeus olympius* è legato a pino bosniaco (*Pinus heldreichii*) e *C. ganymede* è associata ad ascomi morti del primo. Sono stati esaminati anche esemplari delle due specie provenienti da nuove raccolte dal *locus classicus* sul Monte Olimpo e di *Z. olympius* dal Monte Pindo. Sono fornite descrizioni del materiale bulgaro e greco, inclusi i dati provenienti da studi *in vivo*, integrate con le illustrazioni di caratteri macroscopici e microscopici, tra cui le prime fotografie a colori di *C. ganymede*. Le nuove raccolte estendono significativamente la distribuzione geografica di tali ascomiceti apparentemente endemici. La germinazione di ascospore di *Z. olympius* è stata osservata per la prima volta. Si registra per *Z. olympius*, la prima raccolta avvenuta su piante da impianto artificiale di *P. heldreichii*, fuori dal suo areale naturale.
Parole chiave: Ascomycota dei Balcani, funghi endemici, *Nectriaceae*, *Pinus heldreichii*, *Rhytismataceae*.

Introduction

Zeus olympius Minter & Diamandis is an unusual ascomycete, apparently restricted to its host, Bosnian pine (*Pinus heldreichii* Christ.; Fig. 1), and *Cosmospora ganymede* (Minter & Diamandis) Rossman & Samuels is yet another peculiar species, which seems associated with old ascomata of *Z. olympius*. Until recently they were only known from the *locus classicus* on the Mt Olympus in Greece (Minter *et al.*, 1987), and considered as a putative endemism of this area. However, intensive search revealed their presence in some localities in Bulgaria and also allowed the study of newly collected specimens from Mt Olympus and Mt Pindus. Detailed description and photographs of the new collections of the two species are presented herein, expanding the data about their morphology.

Materials and methods

Color photographs were taken from fresh collections, while these were later dried and preserved in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research (SOMF), Sofia. The description of colours of ascomata follows the British Fungus Flora Colour Chart (Anonymous, 1969) with some approximation. Fungi were examined microscopically in living state in water (Baral, 1992), and in dead state in 5% KOH, congo red in 10% ammonia, aqueous cotton blue and lactophenol with cotton blue (heating applied with the last two reagents). The amyloid reaction of the microscopic structures was tested in IKI (Baral, 1987) and Melzer's reagent, upon the recipe in Kirk *et al.* (2008), with and without KOH pretreatment. The microscopic study was conducted with the aid of Olympus BX-41 and AmScope T360B microscopes (oil immersion objectives 100×), equipped respectively with Olympus E330 and Amscope MU900 digital cameras. Measurements were performed on microphotographs with Piximetre v. 5.2 software. Spores were obtained from fresh mature ascomata and were always measured in water. From each ascoma 25 or 30 spores were measured, depending on the availability. Spore measurements are reported in the following format: (minimum–)mean±standard deviation(–maximum), Q = length/width ratio (n = sample size), except for the descriptions, where they are given in the following manner: minimum–($mean_{minimum}$–$mean_{maximum}$)–maximum. For the remaining microscopic structures, only minimum and maximum values are reported.

Taxonomy

Zeus olympius Minter & Diamandis, *Trans. Brit. Mycol. Soc.*, 88 (1): 55 (1987).

Illustrations: Minter *et al.* (1987: 56, habitus, ascomata and microscopic features, black and white drawing); Diamandis (1992: 498,

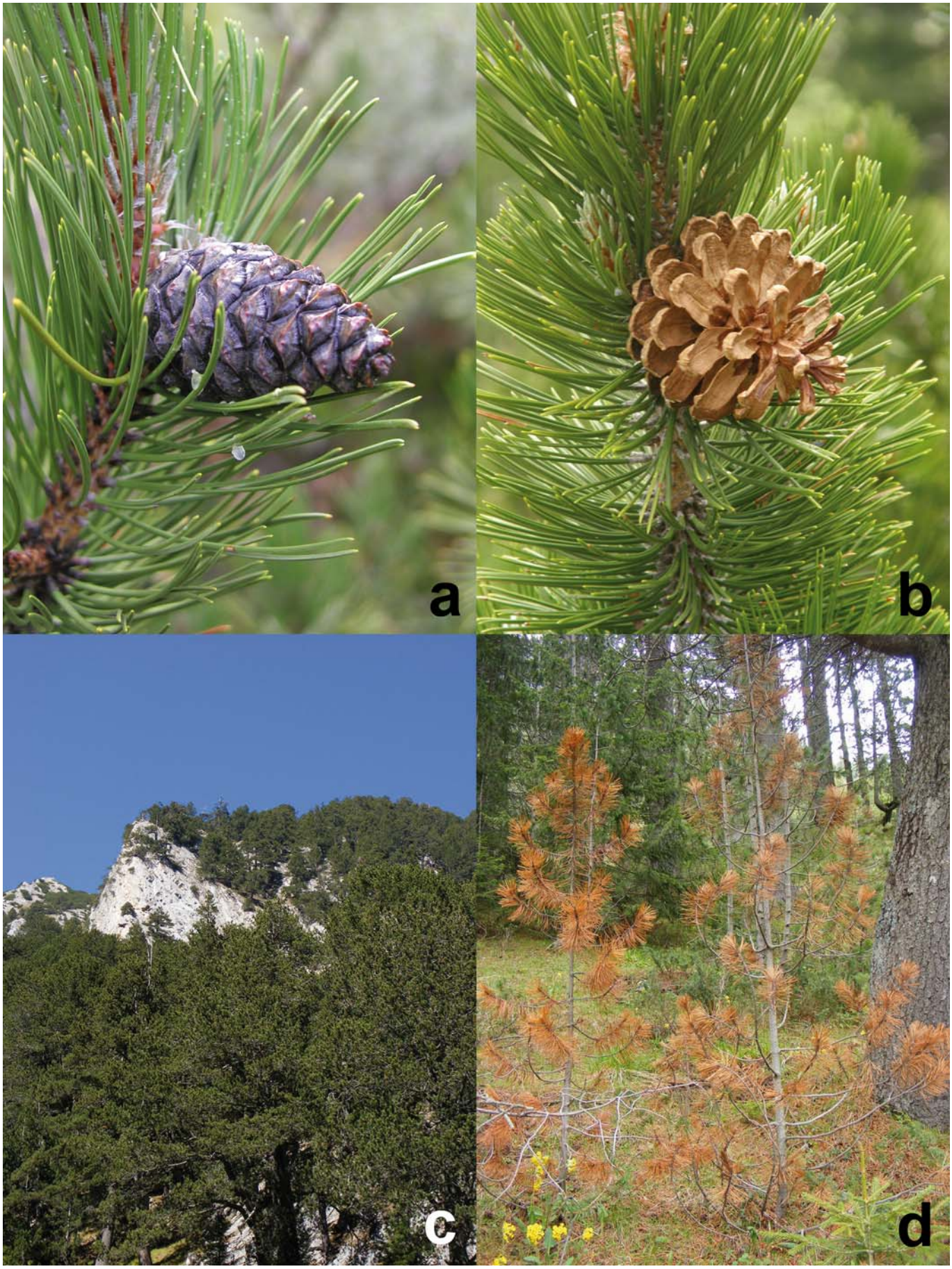

Fig. 1 — Host tree and habitat of *Zeus olympius*

a-b – leaves and cones of *Pinus heldreichii*; c – habitat; d – dead young trees with ascomata of *Z. olympius* developed on the main stem and lateral branches. Photos: B. Assyov.

ascomata, colour photograph); Minter (1996: 183, reproduction of the illustration from the protologue); Diamandis & Perlerou (2011: 30, ascomata, colour photograph); this paper (Figs. 2–3).

Original diagnoses

Generic diagnosis. *Hic fungus ascophorus, ad ordinem Rhytismatalium et familiam Rhytismatacearum pertinens, corticola, erumpens, clypeo nigro ascomatum sane statu tectus, ascosporas habet ellipsoideas vaginis indutas mirabilibus et ascos nil in iodo coerulescentes. Habet ascomata Therryae vel Coccomyceti aliquid similia, et ascos multarum Rhytismatacearum generibus similes, sed ascosporas adspectu mentem revocantes Ceratophacidium.*

Species diagnosis. *Haec species incolat lovis montem, virgas Pini leucodermis et ramulos mortuos habitans. Habet ipsis e virgis erumpentia ascomata quae corticem arboris reflectant ut aperiantur. Ascomata quodque diametrum circa* 0.5–2 *mm habet, et nigro a clypeo tegitur quod conpluribus in partibus statu maturo fissum est ut discum videatur. Asci plus minusve cylindrici, circa* 100–120 × 10–12.5 *µm magnitudinis respectu, nil in apicibus crassiores, sed saepius aliquanto curvati, seriatim maturant et ascosporas octo quique ferunt, fissura apicali quique dehiscentes ut ipsae liberentur. Ascosporae nec colore nec septis sese gaudentes, tenuitunicatae sunt et unicum habent parietem. In quibusque adest vagina mucosa sane aliquid eleganter et suaviter in apicibus fastigiata. Sporae autem, sine vaginis circa* 12–15 × 5–8 *µm magnitudinis respectu metiuntur.*

Description of the new specimens

Ascomata up to 8 mm in diameter, appearing scattered on dead twigs; an individual branch may carry hundreds ascomata. When immature the apothecium is immersed under the bark and covered by a black or sometimes beige layer of fungal tissue. During maturation, it becomes erumpent, breaking the fungal layer into several portions, and revealing the luteous, lemon chrome, yellowish orange to dirty orange coloured hymenium, in senescent ascomata color is fading to orange brown to brown, sometimes with olivaceous tinge. **Asci** develop from croziers or may occasionally have a bifurcate base, present a thin wall and rounded apex, and measure 105–195 × 10–17 µm (n=60). They are 8-spored, lacking a distinctive apical apparatus, mostly do not react with iodine solutions, but some show striking dextrinoid reaction in IKI. **Ascospores** biseriate in very young asci, becoming uniseriate at maturity, hyaline, smooth, ellipsoidal, aseptate or very rarely presenting a single septum (lactophenol), bearing a transparent mucous sheath somewhat constricted at the equator and extending up to 20 µm beyond the apices of the ascospore. Spore content is with numerous small guttules in water mounts, KOH, congo red in ammonia and aqueous Cotton blue, and more or less uniform in reagents with lactophenol and chloral hydrate. Spores measure 10.8–(12.7–15.6)–18.1 × 5.0–(6.2–7.9)–9.2 µm (excluding sheath), Q = 1.5–(1.9–2.1)–2.6 (n = 345). Germination occurs through a single germinative tube at one of the apices of the spore. **Paraphyses** measure up to 3.5 µm wide, and are thin-walled, filiform, mostly unbranched, sometimes with anastomoses close to the base, rarely septate, slightly widened at the apex up to 7 µm, in water mounts with yellowish globular content and vacuoles. **Hymenium** up to 200 µm thick in cross section. It arises from a yellowish brown subhymenium ca 40 µm thick, in turn over a colourless layer up to 400 µm thick, both presenting a poorly defined *textura intricata*. The dark covering layer of the immature ascoma is of up to 150 µm thickness and is formed of cells with thickened and heavily pigmented walls arranged as a *textura angu-*

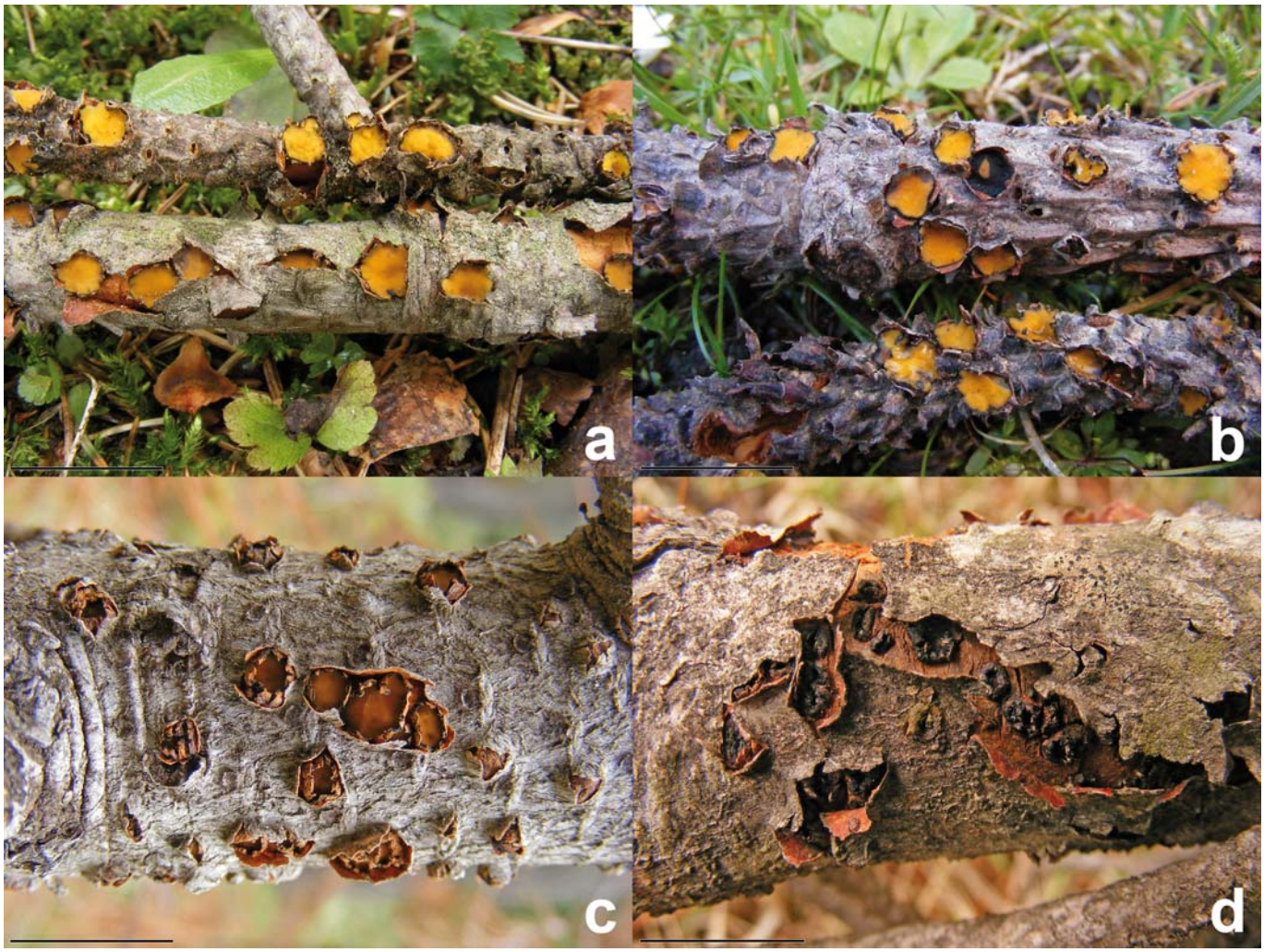

Fig. 2. — Ascomata of *Zeus olympius*
a-b – fully developed; c – developing; d – senescent (a, c & d – from Pirin Mts, *in situ*; b – from Mt Olympus, *ex situ*). Scale bars = 10 mm. Photos: B. Assyov.

laris or *textura globulosa*, turning into *textura epidermoidea* and *textura intricata* towards the torn edges.

Habitat. On dead stems of young trees, twigs and branches of *Pinus heldreichii* Christ.

Distribution. Bulgaria (Pirin Mts, Mt Slavyanka, Mt Vitosha), Greece (Mt Olympus, Mt Pindus).

Collections examined

Bulgaria: Mt Slavyanka, Hambar Dere ravine, 41°24′47.0″N 23°39′50.9″E, alt. ca 1076 m, 01.06.2012, R. Alexov & K. Grazdilov (SOMF 28178); above Goleshevo village, 41°24′46.1″N 23°36′41.9″E, alt. ca 1503 m, 17.08.2012, V. Alexandrov & T. Andreev (SOMF 29401); idem, 41°23′17.2″N 23°37′15.2″E, alt. ca 1847 m, 17.08.2012, V. Alexandrov & T. Andreev (SOMF 28179); Pirin Mts, Bunderitsa lo-

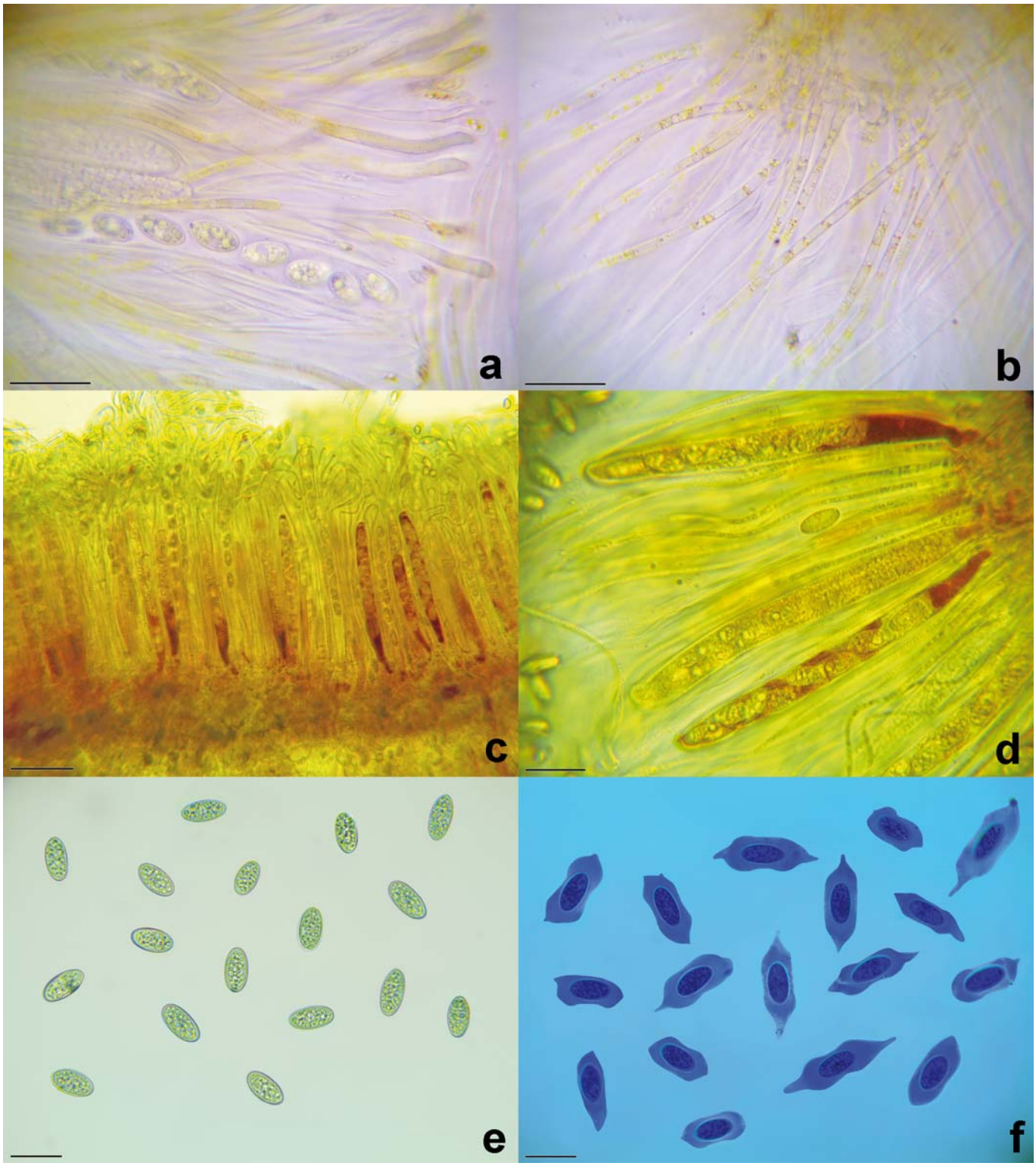

Fig. 3. — Microscopic features of *Zeus olympius*

a – asci and paraphyses (water); b – pigments distribution (water); c-d – dextrinoid reaction of asci (IKI); e – ascospores (water); f – ascospores (heated aqueous Cotton blue); spores on figs e & f were transferred without alteration on clear background. Scale bars: a, b, d, e & f = 20 µm; c = 30 µm. Photos: B. Assyov.

cality above Bansko town, 41°45′31.0″N 23°26′04.1″E, alt. ca 2480 m, 02.06.2012, R. Alexov & D. Vassilev (SOMF 29402); idem, 41°46′2.9″N 23°25′29.1″E, alt. ca 1855 m 06.07.2013 (old ascomata), R. Alexov, M. Slavova & I. Assyova (SOMF 29529); idem, 17.05.2014, B. Assyov & M. Slavova (SOMF 29528); Yavorov chalet, 42°35′32.5″N 23°17′45.1″E, alt. ca 1755 m, 28.06.2012, R. Alexov (SOMF 28180); Mt Vitosha, artificially planted trees below Aleko chalet, 41°42′30.9″N 23°19′18.6″E, alt. ca 1679 m, 22.05.2014, B. Assyov, I. Assyova & D. Stoykov (SOMF 29527); Greece: Mt Olympus, Prionia area, 40°5′7.5″N 22°24′25.0″E, alt. ca 1065 m, 12.04.2014, M. Slavova & I. Assyova (SOMF 29531); Mt Pindus, Metsovo municipality, in the vicinity of Aoos Dam, 39°48′41.9″N 21°6′37.0″E, alt. ca 1355 m, 15.06.2014 (old ascomata), B. Assyov (SOMF 29530); idem, 39°49′55.2″N 21°8′8.1″E, alt. ca 1390 m, 15.06.2014 (old ascomata), B. Assyov (SOMF 29536); all collections on branches or twigs of *P. heldreichii*.

Comments

Zeus olympius is easily and unambiguously identified due to its peculiar, bright colored ascomata, its specific substrate, as well as by its ascospores presenting characteristic mucous sheaths. These were observed by us in all media employed (albeit occasionally), but are best seen in heated aqueous cotton blue or cotton blue in lactophenol. Both the new collection from Mt Olympus and the Bulgarian specimens agree perfectly with the descriptions of the species (Minter *et al.*, 1987; Minter, 1996), although it was found that ascomata may reach larger size than mentioned in the original description. The new collections also show some differences in the minimum and maximum length and width of the ascospores from those cited in the protologue (Table 1), which we consider of no significance as such moderate discrepancies could derive from a larger set of samples from different localities. Other possible explanations of this fact may be individual variability or the use of mounting media different than water. Mean values for spore length and width, as well as their quotient ratio, are reported here for the first time (Table 1). It is notable that average values for both length and width vary significantly between different localities. The Bulgarian samples from Pirin and Vitosha mountains tend to have somewhat higher mean values, compared to those from the locus classicus, while lower average values for both length and width were recorded from a Bulgarian specimen from Mt Slavyanka (SOMF 28178). Unfortunately we were unable to obtain enough spores for characterization of the specimens from Epirus. The reason for this relatively high variability is as yet unclear, although genetic difference between ascomata from and within different localities is a possible explanation. The sampling size is likely not contributing as it was chosen to be relatively large and consistent methodology for measuring was used. Further study of the variability of the populations may shed light on this problem.

The abundant material allowed to compare the spore sizes of *Z. olympius* in different mounting media. For this particular test we chose a single large ascoma (7 mm across), which was divided in five parts, that were used further to obtain preparations in water, ammonia solution 10%, aqueous solution of Cotton blue, KOH 5% and IKI. It is presumed that using one single ascoma for all preparations helps to overcome the individual variability when spores derive from different fruitbodies. In all cases only normally developed ascospores, released from asci were measured. The results from the measurements are presented in Table 2. Comparing the mean values it is clear that in mountants other than water the ascospores show some degree of shrinking. For the spore length this is most visible in Cotton blue and IKI. For the spore width smallest values are obtained in KOH and IKI. As for the spore quotient, as expected, the differences are small and limited to 0.1–0.2 points. Most of the differences of means, when compared to the corresponding values obtained in water, were shown to be statistically significant by the unpaired *t* test.

We have successfully observed germination of ascospores in overmature ascomata, for which there are no data in the literature. Germination in hymenial mounts seems quite rare, we have encountered about ten spores in this stage. However, in all cases it appeared in a uniform way as noted in the description above.

The study of fresh specimens revealed one micromorphological peculiarity, not mentioned in the existing descriptions of the species. When mounted in IKI, most of the asci do not produce visible colour reaction. Individual asci (mostly developing) however immediately colour strikingly in red or blood red. The extent and the intensity of the reaction varies from one ascus to another, but most often it is located or is most intensive in the lower parts of asci, below the first ascospore. Application of KOH leads to complete discoloration and the red colour reappears when KOH is further replaced by IKI, thus pointing to a dextrinoid reaction. It also occurs with Melzer's reagent, but it is much more feeble, fades quickly and tends to become very easily obscured by the colour of the reagent.

Our observations so far confirm the suggestion of previous authors (Minter *et al.*, 1987; Minter, 1996; Diamandis & Perlerou, 2011) that the fungus is likely restricted to *Pinus heldreichii*. It has never been found growing on any other pine species, even though *P. heldreichii* occurs altogether with *P. peuce* Griseb., *P. sylvestris* L., *P. nigra* J.F. Arnold and *P. mugo* Turra in the Bulgarian localities studied, as it does with *P. nigra* on Mt Olympus. Nonetheless, the new collections from both Bulgaria and Greece suggest that *Z. olympius* may well be more widespread in the Balkan Peninsula, since the geographical range where *P. heldreichii* can be found includes also Albania, Bosnia and Herzegovina, Croatia, Kosovo, Macedonia, and Montenegro, reaching the Italian Peninsula to the west (Mt Pollino, Mt La Montea; Farjon & Filer, 2013). Diamandis & Perlerou (2011) noticed that despite of the purposeful research on fungi in

Specimens and literature data	Size of ascospores (µm)
Minter *et al.* (1987)	12–15 × 5–8
SOMF 29531 Mt Olympus	11.9–(14.4±1.1)–15.9 × 6.6–(7.7±0.5)–8.6; Q = 1.6–(1.9±0.1)–2.1
	10.8–(14.1±1.4)–15.6 × 6.8–(7.5±0.4)–8.3; Q = 1.5–(2.0±0.1)–2.1
	12.1–(14.1±1.0)–15.9 × 6.4–(7.4±0.4)–8.2; Q = 1.7–(1.9±0.2)–2.3
SOMF 28178 Mt Slavyanka	11.5–(13.5±1.1)–15.2 × 6.3–(7.0±0.4)–7.8; Q = 1.6–(1.9±0.1)–2.2
	11.5–(12.7±0.8)–14.4 × 5.6–(6.7±0.6)–8.0; Q = 1.6–(1.9±0.2)–2.2*
	11.2–(13.2±1.1)–15.5 × 5.2–(6.2±0.4)–6.7; Q = 1.7–(2.1±0.2)–2.6*
	11.8–(13.3±0.9)–15.1 × 5.0–(6.4±0.6)–7.7; Q = 1.6–(2.1±0.2)–2.6*
SOMF 29528 Pirin Mts	12.7–(15.1±1.2)–18.1 × 6.5–(7.4±0.4)–8.9; Q = 1.8–(2.0±0.2)–2.6
	12.5–(15.0±1.1)–17.5 × 7.1–(7.9±0.4)–9.2; Q = 1.6–(1.9±0.1)–2.2
	12.7–(15.6±1.1)–17.4 × 7.0–(7.8±0.3)–8.5; Q = 1.7–(2.0±0.2)–2.4
SOMF 29527 Mt Vitosha	12.9–(15.3±1.3)–17.6 × 6.8–(7.5±0.3)–8.0; Q = 1.7–(2.0±0.2)–2.3
	13.3–(15.3±1.1)–17.7 × 6.6–(7.5±0.4)–8.2; Q = 1.7–(2.1±0.2)–2.3

Table 1. Comparison of the size of ascospores of *Zeus olympius* according to literature and own measurements on specimens from Bulgaria and Greece. Spores of two to four ascomata were measured per a collection and 25 (marked with "*") or 30 spores were taken from each ascoma, which data are shown separately.

communities of *P. heldreichii* in Bosnia, *Z. olympius* has not been found there yet. However, it may be reasonably expected that further research within the geographic range of the host tree might reveal new, so far unknown, localities of the fungus.

An interesting fact is that during our searches, *Z. olympius* was found on planted Bosnian pine outside its natural range. The locality on Vitosha Mountain is artificially created about 60 years ago and is currently the only place, where the host tree successfully develops on non-calcareous ground (Panayotov & Yurukov, 2006). As far as known, seedlings came from a natural locality in Bulgaria. We were unable to trace the exact origin until now, but it might be assumed that source could have been some of the more accessible stands in Pirin Mountain.

Mounting medium	Spore length (µm)	Spore width (µm)	Quotient l/w
Water	11.9–(14.4±1.1)–15.9	6.6–(7.7±0.5)–8.6	1.6–(1.9±0.1)–2.1
Ammonia 10%	11.5–(14.0±1.4)–16.5	6.0–(6.9±0.4)–7.6[3]	1.7–(2.0±0.2)–2.5[1]
Cotton blue	11.8–(13.3±0.9)–15.2[3]	6.0–(7.4±0.5)–7.4[1]	1.6–(1.8±0.1)–2.1[2]
KOH 5%	12.0–(14.0±1.0)–15.7	6.2–(6.8±0.4)–7.6[3]	1.8–(2.1±0.2)–2.6[3]
IKI	10.8–(13.1±1.1)–14.9[3]	5.8–(6.5±0.5)–7.7[3]	1.6–(2.0±0.2)–2.4[1]

Table 2. Comparison by *t* test of spore measurements of *Zeus olympius* in different mounting media to values in water. P-values: [1] P<0.05, [2] P<0.0003, [3] P<0.0001.

The lifestyle of the fungus still remains unknown as it was at the time of its discovery (Minter *et al.,* 1987). We have observed ascomata of *Z. olympius* on main stems and twigs of standing dead young trees, dying twigs on living trees, as well as on branches, which have been cut down when still alive. Similarly to the type material, our collections come from twigs and individuals of different age. Screening for endophytic fungi by molecular tools, as well as cultural studies, could probably provide aid in resolving those questions.

The time of production of ascomata of *Z. olympius* apparently varies from one locality to another. The known Greek collections originate from April, while in the Bulgarian localities the fungus have been seen so far from mid of May to early June. The exact time span remains unknown and should be established by further observations as it apparently depends on both geographic longitude and elevation. Our observations at the locality on Mt Vitosha also show that ascomata on one branch are not produced simultaneously and this process may continue for more than 20 days. It has been noted that humidity probably has influence on the production of ascomata as in all cases the fungus was found in years and locations with somewhat higher humidity, always in shade, and never in exposed, sunny situations.

Cosmospora ganymede (Lowen & Minter) Rossman & Samuels, *Stud. Mycol*., 42: 121 (1999).

Basionym: *Nectria ganymede* Lowen & Minter, *Trans. Brit. Mycol. Soc.*, 88 (1): 59 (1987).

Illustrations: Minter *et al.* (1987: 58, ascomata and microscopic features, black and white drawing); this paper (Fig. 4).

Original diagnosis

Hic fungus ad genus Nectriae et subgenus episphaeriae pertinens, habet ascomata perithecialia, rubra, pyriformia, sparsa, circa 300 *µm diam, lateraliter collapsa. Asci adsunt clavati, apice et in fundamento rotundati,* 90–100 × circa 14 *µm, octospori, irregulariter biseriati. Ascosporae sunt* 8–23 × 6–8 *µm, ellipsoideae, primo sin colore et uniseptatae, deinde leviter brunneae et triseptatae. Paraphysae non visae sunt.*

Description of the new specimens

Ascomata pyriform, perithecia up to 300 µm in diameter and up to 350 µm high, appearing solitary, scattered or in groups of up to 20, semi-immersed in old and deteriorating hymenia of *Zeus olympius*, scarlet to red coloured, with some purple tints when old. Ascomatal walls collapsing laterally, in surface view forming *textura angularis* to *textura epidermoidea* and reacting positive (purple to violet) in solution of potassium hydroxyde. Ostiolum lined with tightly packed, parallel hyphae up to 2 µm wide. **Asci** 8-spored, thin-walled, 75–85 × 10–11 µm, not reacting with IKI or Melzer's reagent. **Ascospores** irregularly biseriate, tending to uniseriate towards the base of asci, 18.4–(22.4–23.3)–27.8 × 5.1–(6.4–6.9)–9.0 µm, Q = 2.8–(3.3–3.7)–4.6 (n=120); when immature hyaline and with one septum, to become pale brown before discharging from asci, at maturity almost exclusively 3-septate (spores with 1, 2 or 4 septa also seen) and with 1–2 guttules per cell, some ellipsoid, but most slightly curved, usually more or less constricted at the central septum; ascospore surface finely spinulose at higher magnifications. **Paraphyses** not seen.

Habitat. On dead ascomata of *Zeus olympius*.

Distribution. Bulgaria (Pirin Mts), Greece (Mt Olympus).

Collections examined

Bulgaria: Pirin Mts, Bunderitsa locality above Bansko town, 41°46′0.9″N 23°25′24.4″E, 17.05.2014, B. Assyov & M. Slavova (SOMF 29525); Greece: Mt Olympus, Prionia area, 40°5′7.5″N 22°24′25.0″E, alt. ca 1065 m, 12.04.2014, M. Slavova & I. Assyova (SOMF 29526); all collections on dead ascomata of *Z. olympius*.

Comments

These new collections seem to be the first after the discovery of the species in 1987 (Minter, online, a). Both specimens from Bulgaria and Greece seem to fit very well *Cosmospora ganymede*, although some differences in the minimum and maximum ascospore size were found in this study (Table 3). Especially striking is the minimum spore length, which is about 10 µm higher in our specimens, compared to that recorded in the original description. Those differences are probably explained by the fact that our measurements derive only from mature spores, released from asci. This assumption is likely confirmed by the fact that spores from asci measure 10.7–(18.5±2.7)–23.4 × 4.2–(5.6±0.7)–6.7 µm; Q = 2.5–(3.3±0.4)–4.5 (n=30).

Released mature ascospores are usually 3-septate, normally developed spores with one septum are rarely seen in mounts. In addition, in the studied ascomata extremely rarely spores with 2 or 4 septa occur. An exception is the specimen from Mt Olympus, which contains slightly over-ripe ascomata and 4-septate spores are more numerous.

Specimens and literature data	Size of ascospores (µm)
Minter *et al.* (1987)	8–23(–26) × 6–8
SOMF 29526, GRC	18.9–(22.4±2.3)–26.5 × 5.2–(6.5±0.8)–9.0; Q = 2.8–(3.5±0.4)–4.4
SOMF 29525, BGR	19.5–(23.3±1.8)–27.8 × 5.1–(6.4±0.6)–7.3; Q = 2.9–(3.7±0.4)–4.6
	18.4–(23.2±2.3)–27.0 × 5.8–(6.9±0.6)–8.2; Q = 2.8–(3.3±0.3)–4.2

Table 3. Comparison of the size of ascospores of *Cosmospora ganymede* according to literature and own measurements on specimens from Bulgaria (BGR) and Greece (GRC). Spores of one or two ascomata were measured per a collection, 30 spores were sampled per an ascoma and data are shown separately.

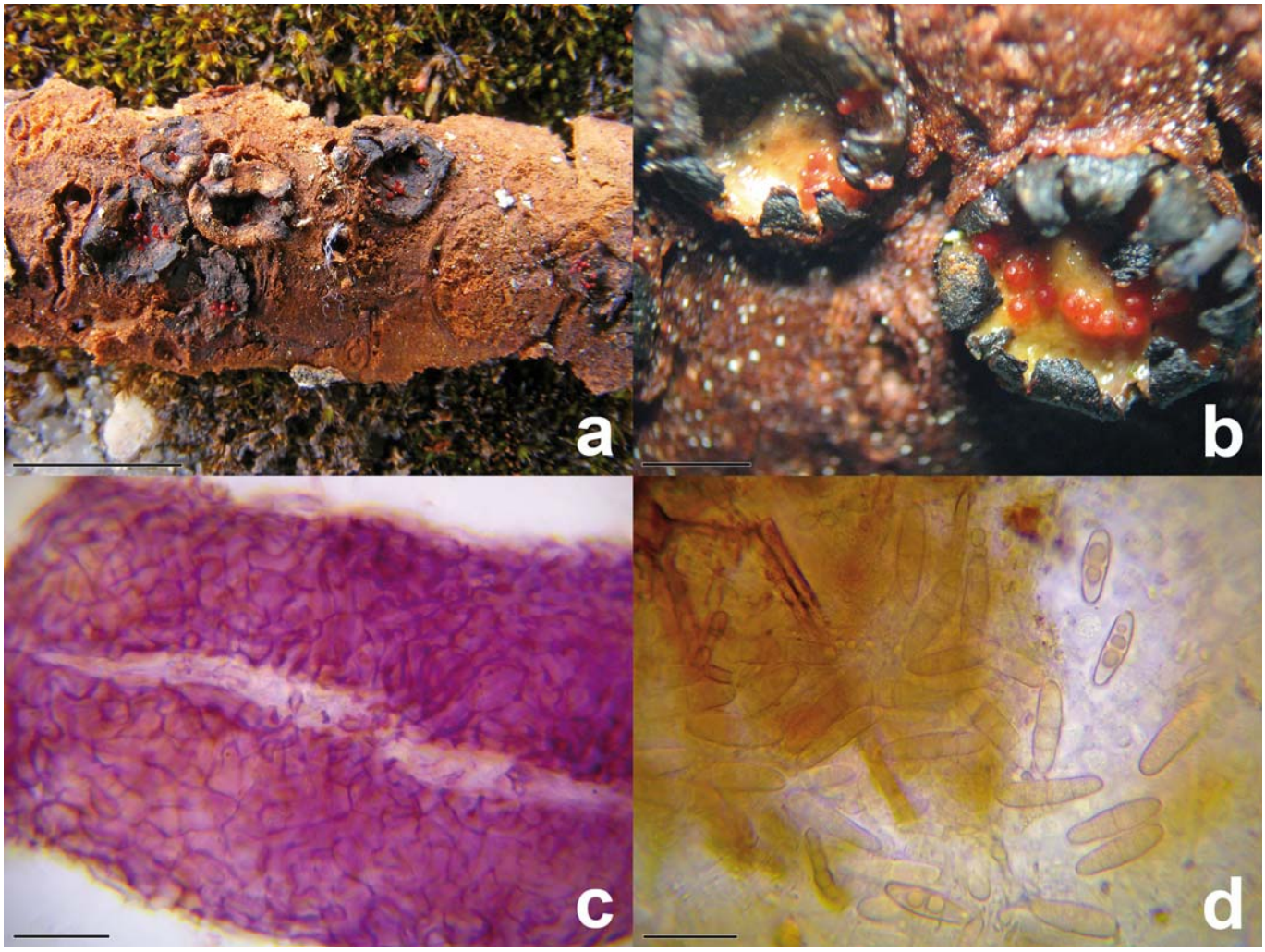

Fig. 4. — *Cosmospora ganymede*
a-b – perithecia on old ascomata of *Zeus olympius* (external bark layer removed); c – KOH+ reaction of ascomatal wall; d – ascospores in water. Scale bars: a = 5 mm; b = 1 mm, c & d = 20 µm. Photos: a, c & d – B. Assyov; b – D. Stoykov.

The reaction of ascomatal wall to KOH, which is positive for most members of *Cosmospora s. lat.* (Rossman *et al.*, 1999), was noted in the original description (Minter *et al*., 1987) as "deeper red", while our specimens demonstrated clear purple to violet colour reaction.

Although the species is presented here as *C. ganymede*, it should be noted that a number of studies have shown that the genus *Cosmospora* in the sense of Rossman *et al.* (1999) is clearly polyphyletic (see Gräfenhan *et al.*, 2011, and references therein). The authors were so far unable to assess the phylogenetic position of *C. ganymede* by means of DNA studies. However, Minter *et al.* (1987) obtained in pure culture and described the anamorph of the species, which is *Fusarium*-like. This information does allow partial narrowing the possible choices at generic level (as far as currently resolved). First of all, it may be considered that *C. ganymede* — although sharing KOH+ reaction, collapsing perithecia and tuberculate ascospores — could not fit the restricted concept of *Cosmospora*, proposed in Gräfenhan *et al.* (2011) because this latter is characterized by *Acremonium*-like anamorphs. Of the remaining possibilities most of the members of *Microcera* Desm. are known to be associated with insects. Three other genera, namely *Dialonectria* (Sacc.) Cooke, *Macroconia* (Wollenw.) Gräfenhan, Seifert & Schroers and *Stylonectria* Höhn. should be considered, as their members are characterized by KOH+ reaction, collapsing perithecia, *Fusarium*-like anamorphs and a preference for development on other fungi, ascomycetes in particular. The genus *Dialonectria* is known to have asci with an apical ring and predominantly 3–5-septate macroconidia, while in *C. ganymede* asci are devoid of apical ring and macroconidia are normally aseptate or with one septum. The genus *Macroconia* includes species with laterally collapsing perithecia, asci without apical ring, 3–7(–14) septate macroconidia and 1-septate smooth or striate ascospores, and in *C. ganymede* macroconidia are said to be generally aseptate or 1-septate, and ascospores are generally 3-septate, spinulose. Ascospores with similar ornamentation, combined with 0–1-septate macroconidia are known in the genus *Stylonectria*. However, the members of this genus are characterized by perithecia that collapse cupulate and have a 2-layered ascomatal wall. It is clear that for the time being the exact position of *C. ganymede* remains unknown and could only be revealed by molecular studies.

Similarly to *Zeus olympius*, *Cosmospora ganymede* has not been found so far elsewhere than in Bulgaria and Greece. We did not find this species on *Z. olympius* from localities on Mt Slavyanka, but this is most probably due to scarcity of the examined materials from this area. Despite of our attempts to find it on *Z. olympius* in artificial plantations, we were unable to prove this so far.

Conclusions

Zeus olympius and *Cosmospora ganymede*, so far thought to be local endemics for Mt Olympus in Greece, seem to exhibit wider endemic range, visibly related to the distribution of *Pinus heldreichii*, a subendemic species for the Balkan Peninsula and some areas of Italy. Further search will probably reveal those fungi in at least some of the countries, where this pine species occurs.

While the new collections of both species agree well with their original descriptions, *Z. olympius* shows high variability, which may even suggest some geographic pattern as seen from the data re-

ported in this paper. Further research, possibly by molecular means, is however necessary to show if such pattern really exists and to give satisfactory explanation of the variability. This may also allow the unequivocal establishing of the phylogenetic position of the two species, which the authors hope to be able to attempt later.

An interesting fact is that at least *Z. olympius* may occur in artificial plantations of Bosnian pine. Despite that the biology of the fungus remains practically unknown, this could have some practical value in the view of the conservation of this species, which currently attracts attention (Minter, online, b). Apart from this, *Z. olympius*, *C. ganymede* and *P. heldreichii* represent an interesting example for a three way association of endemic fungi and host, which understanding could have theoretical and practical value.

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