

**ZEUS OLYMPIUS GEN. ET SP. NOV. AND NECTRIA  
GANYMEDE SP. NOV. FROM MOUNT OLYMPUS, GREECE**

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*Zeus olympius* gen. et sp. nov. (Ascomycotina, Rhytismatales, Rhytismataceae) and *Nectria ganymede* sp. nov. (Ascomycotina, Hypocreales, Hypocreaceae) are described, illustrated and discussed, based on collections on dead branches of *Pinus leucodermis* on Mount Olympus in northern Greece. *Zeus olympius* appears to occur exclusively on *Pinus leucodermis* and *Nectria ganymede* exclusively on old ascomata of *Zeus olympius*.

During a recent visit by the senior author to Mount Olympus in northern Greece, two interesting fungi were collected. They are described, illustrated and discussed in the following paragraphs.

**Zeus Minter & Diamandis gen. nov.**

*Etym.*: named after the king of gods in ancient Greek mythology, who was believed to inhabit Mount Olympus

Hic fungus ascophorus, ad ordinem Rhytismatalium et familiam Rhytismatacearum pertinens, corticola, erumpens, clypeo nigro ascomatum sane statu tectus, ascoporas 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 ascoporas ad spectu mentem revocantes *Ceratophacidium*.

Species typica: *Zeus olympius* sp. nov.

**Zeus olympius Minter & Diamandis sp. nov.**

(Fig. 1)

Haec species incolat Iovis montem, virgas *Pini leucodermis* et ramulos mortuos habitans. Habet ipsis e virgis erumpentia ascomata quae corticem arboris reflectant ut aperiantur. Ascoma quodque diametrum circa 0.5-2 mm habet, et nigro a clypeo tegitur quod compluribus 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 ascoporas octo quique ferunt, fissura apicali quique dehiscentes ut ipsae liberentur. Ascosporae nec colore nec septis sese gaudent, 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.

Emortuis in virgis et ramulis *Pini leucodermidis* Antoine ad altitudinem circa 1300 m in Olympi monte collectus a D. W. Minter & S. Diamandis, Graecia, 2 April 1986, IMI 303384, holotypus.

*Ascomata* apothecial, scattered or in small groups in rather pale dead twigs and small branches of *Pinus leucodermis*, not closely associated with scars of short shoot needle bundles; when immature immersed below the bark and a black fungal covering layer; when mature circular or rather angular, 0.5-2 mm diam; becoming erumpent, throwing back the bark and breaking the black covering layer by several irregular radial splits to reveal the dark fawn-coloured hymenium (fragments of the bark often remaining attached to the black covering layer hiding its dark appearance), raising the substratum surface, but lacking a stalk, and so not protruding beyond the level of the dislodged bark covering-layers.

In vertical transverse section: the hymenium arising from a yellow-brown subhymenium about 15-30 μm thick composed of poorly defined *textura intricata* situated on a layer of colourless, poorly defined *textura intricata* embedded in mucus and about 100-200 μm thick; the blackened covering layer, approximately 30-80 μm thick, composed for the most part of cells 5-12 μm in diameter forming a *textura angularis* or *globulosa* with a dark brown pigment in the cell walls and between individual cells; these cells gradually intergrading via *textura epidermoidea* to become a *textura oblita* or *intricata* towards the torn edges of the covering layer; heavily tanned tissue of the substratum present below the ascoma, and on the outside of the blackened covering layer.

*Asci* developing from croziers, maturing in succession, with ascospores becoming visible a little before elongation of the ascus is completed, uniformly thin-walled, with only one wall layer visible at all stages of development, more or less cylindrical, slightly tapered towards the base, rounded at the apex, sometimes also curved characteristically towards the apex, not turning

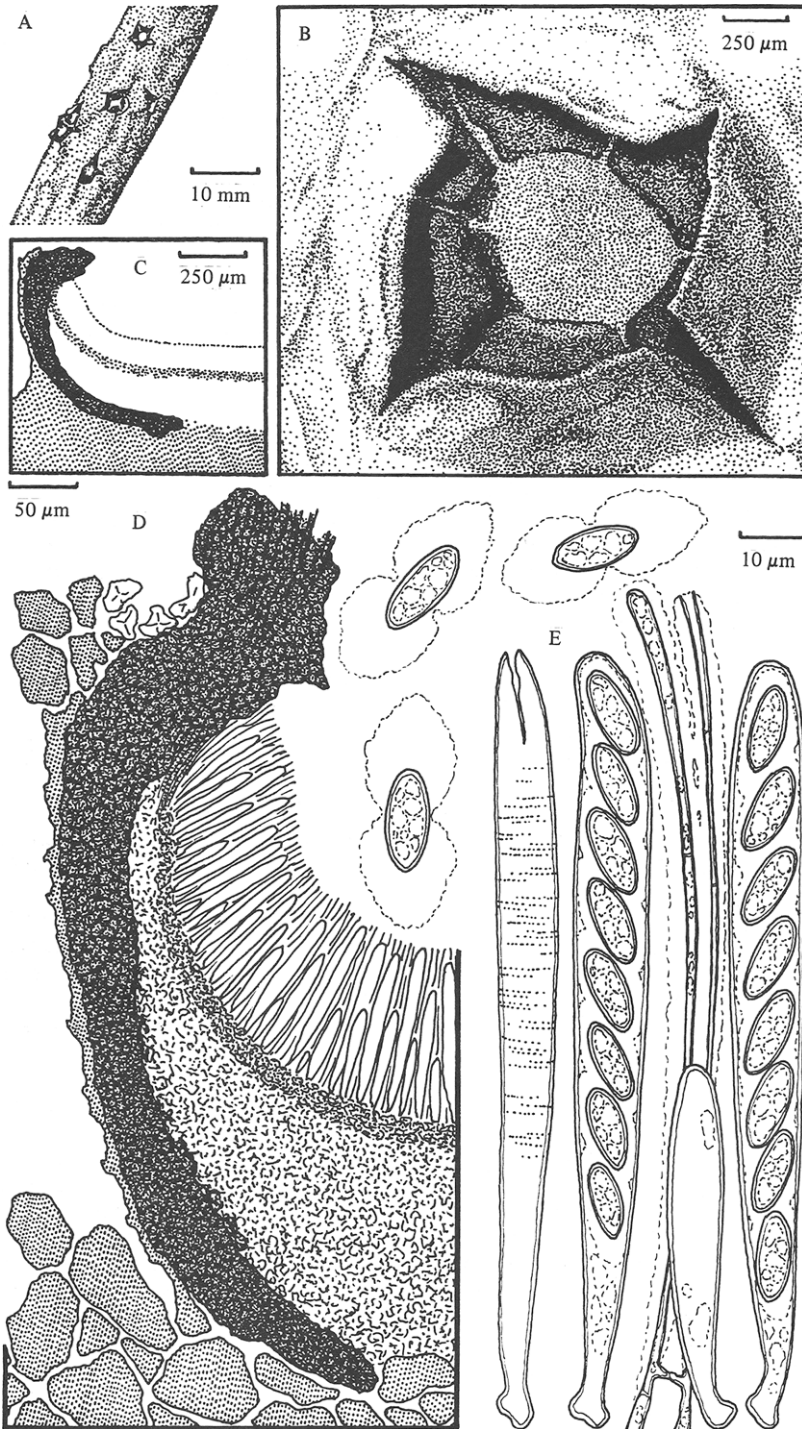


Fig. 1. *Zeus olympius*. (A) Habit view of ascomata on dead twig. (B) Detail of single ascoma as seen with the dissecting microscope. (C) Ascoma in vertical transverse section as seen at low power with a compound microscope. (D) Detail of part of ascoma in vertical transverse section as seen at a higher power with a compound microscope (random stippling indicates fungal pigments, linear stippling indicates tanned deposits in the substratum). (E) Asci, ascospores and paraphyses.

blue in iodine, with no discernible apical structures, containing eight ascospores arranged in a single file, opening by a single apical crack which may extend some way down the sides, becoming collapsed and bearing longitudinal creases and latitudinal striations after ascospore discharge, approximately  $110\text{--}120 \times 10\text{--}12.5 \mu\text{m}$  in size when mature and before ascospore discharge.

*Ascospores* colourless, thin-walled, smooth, more or less ellipsoidal, aseptate (perhaps sometimes becoming 1-septate around or after the time of discharge) bearing many guttules and surrounded by an inconspicuous but broad mucous sheath which is flared out and markedly wider beyond each end of the ascospore and constricted around its equator, thus appearing to be two separate sheaths, approximately  $12\text{--}15 \times 5\text{--}8 \mu\text{m}$  excluding sheath when mature; sheaths appearing similar in erythrosin in ammonia, cotton-blue and Meltzer's reagent (without pretreatment in KOH).

*Paraphyses* with unpigmented walls and yellowish globular contents, thin-walled, smooth, sparsely septate, covered in a mucous sheath visible in erythrosin in ammonia, cotton-blue and Meltzer's reagent (without pretreatment in KOH), filiform, apparently unbranched, slightly swollen at the apex which often appears ruptured in slide preparations, sometimes anastomosing near the base, arising (like the croziers) from the sub-hymenium.

*Specimens examined*: on dead twigs and small branches of *Pinus leucodermis* at an altitude of about 1300 m, above Prionia by the path to the mountain refuge on Mount Olympus, Greece, D. W. Minter & S. Diamandis, 2 April 1986, IMI 303384, holotype. Isotype in the herbarium of the Pathology Division of the Northern Forest Research Station, Loutra Thermi, Thessaloniki, Greece. On dead branches of *Pinus leucodermis* at an altitude of about 1300 m, above Prionia, on Mount Olympus, Greece, S. Diamandis, late April 1986, IMI 303844.

#### *Discussion of Z. olympius*

Many features of this fungus suggest it should be placed in the order Rhytismatales and family Rhytismataceae Chevallier (syn. Hypodermataceae Rehm): in particular the paler appearance of the twigs; the immersed apothecial ascomata, which open by splits in the blackened covering layer—swelling to reveal their discs in wet conditions and shutting again when dry; the iodine-negative asci with no apical modification, which open with irregular apical cracks (a particularly important feature (Minter & Cannon, 1984)); the probable violent spore discharge and the colourless ascospores with large mucous sheaths. All of such features of this family have been reviewed by Cannon & Minter (1986), and keys to genera have

been provided by Darker (1967), Korf (1973), Sherwood (1980) and Cannon & Minter (1986).

In making the following assessment, an extensive checklist of the families, genera, species and subspecific taxa of the Rhytismatales was consulted (Minter, unpubl.), and the recent monograph of the other similar family, Phacidiaceae Fries, by DiCosmo, Nag Raj & Kendrick (1984) was also scanned. Evidence relating to mucous sheaths around the ascospores and paraphyses was particularly carefully checked because such sheaths often take on different appearances when different stains are used in slide preparations (Punithalingam & Woodhams, 1984).

In external appearances the ascomata of *Zeus olympius* resemble those of *Colpoma* Wallroth, *Therrya* Saccardo or bark-inhabiting species of *Coccomyces* de Notaris and, to a lesser extent, species of *Cerion* Massee and *Ocotomyces* Evans & Minter. This general similarity with such taxa continues when the ascomata are examined in vertical transverse section, although on close inspection numerous small differences may be observed in tissue types and their extent and distribution. None of these genera, however, contains species with ellipsoid ascospores and, since ascospore shape remains an important criterion at generic level within the Rhytismataceae, the present species is clearly not congeneric with these taxa.

Ellipsoid or nearly ellipsoid ascospores are known from a rather limited number of genera within the Rhytismataceae, notably *Ceratophacidium* Reid & Pirozynski, *Myriophacidium* Sherwood, *Ploioderma* Darker and *Vladracula* Cannon, Minter & Kamal. Of these, *Ploioderma* contains strong needle pathogens, mainly of north American pines, while species of both *Myriophacidium* and *Vladracula* tend to be leaf-inhabiting and, in any case, lack the bulky nature of ascomata of the present species. *Ceratophacidium* is interesting because its only species also has ascospores with a mucous sheath drawn out at both ends. Asci of this species were, however, reported by Reid & Pirozynski (1966) to have each a minute apical ring turning blue in iodine (not noticed by Bonar (1942) when the type was first described), and the ascomata are much smaller, less pigmented, and less bulky than those of the present species. Furthermore, on careful inspection of Reid & Pirozynski's (1966) illustration and of a slide made from part of the type collection of the type species of *Ceratophacidium* (IMI 120921), the tapered mucous sheaths around ascospores in *Ceratophacidium* (which Reid & Pirozynski stated were examined in an iodine mountant) seem rather different from the flared and divided sheaths in *Zeus*.

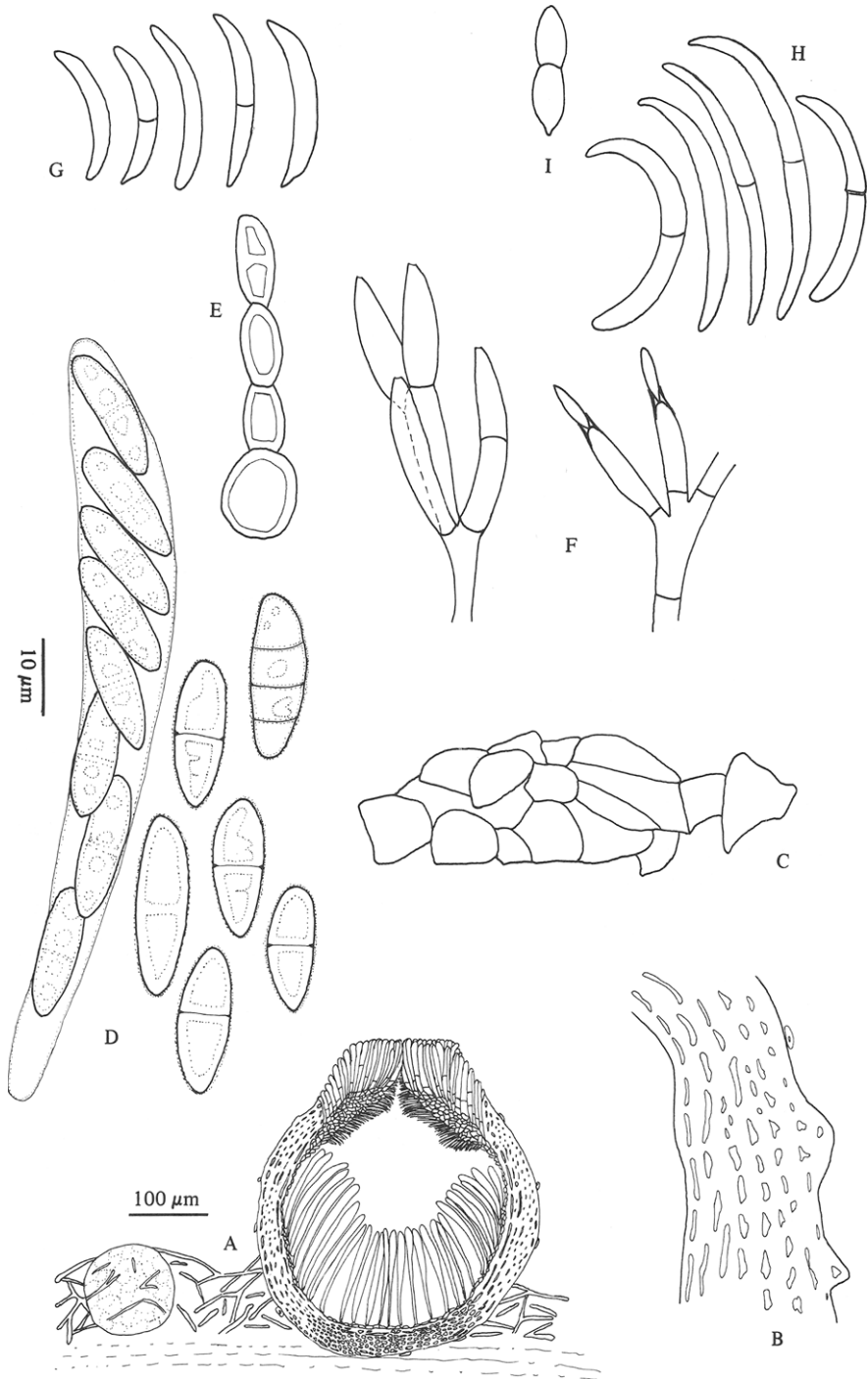


Fig. 2. *Nectria ganymede* (IMI 305041), mountants (A) Lactophenol, (B–I) lactophenol and cotton-blue. (A) Ascoma in vertical transverse section with immature ascoma and cottony mycelium. (B) Detail of wall of ascoma. (C) Cells of ascoma in surface view. (D) Ascus and ascospores. (E) Thickened hyphal cells. (F) conidiophore. (G) 'Macroconidia' from SNA after 1 month. (H) 'Macroconidia' from PSA after 1 month. (I) Distorted old conidium.

It is therefore concluded that the present species represents a new genus of the Rhytismataceae. Almost nothing is known of its biology: it fruits as a saprobe, producing asci and ascospores in spring and releasing them in wet conditions; it appears to be restricted to pine twigs; no anamorph has been observed and the mode of colonization of substrata is not known. It may inhabit living twigs as an endobiont, waiting until the death of the plant before fruiting, or its whole life cycle may be saprobic: ascomata were observed at the same stage of development on branches and twigs of different ages in the type collection, but that provides little further evidence for either an endobiotic or a saprobic existence.

**Nectria ganymede** Lowen & Minter sp.nov.

(Fig. 2)

*Etym.*: named after Ganymede, 'a beautiful youth of Phrygia who was taken up to heaven by Zeus to become cup-bearer to the gods and to sit on Zeus' lap' (Lemprière, 1788)

Hic fungus ad genus *Nectriae* et subgenus *episphaeriae* pertinens, habet ascomata perithecialia, rubra, pyriformia, sparsa, circa 300  $\mu\text{m}$  diam, lateraliter collapsa. Asci adsunt clavati, apice et in fundamento rotundati, 90–100  $\times$  circa 14  $\mu\text{m}$ , octospori, irregulariter biseriatii. Ascosporae sunt 8–23  $\times$  6–8  $\mu\text{m}$ , ellipsoideae, primo sin colore et uniseptatae, deinde leviter brunneae et triseptatae. Paraphyses non visae sunt.

Hymenio in *Zenos olympi* derelicto ad altitudinem circa 1300 m in Olympi monte collectus a S. Diamandis, Graecia, April 1986, IMI 305041, holotypus

*Ascomata* perithecial, translucent, colour no. 13, deep red (Kelly, 1965), plate 10, 8C (Kornerup & Wanscher, 1978), 290–300  $\mu\text{m}$  diam, pyriform, often collapsing laterally, scattered or in groups of up to 10, immersed at the base in the hymenial remains of ascomata of *Zeus olympius*, surrounded by a white, sparse cottony mycelium; ascomatal walls turning darker red in KOH then yellow in lactophenol (KOH+), composed of cells approximately 6–10  $\mu\text{m}$  diam forming a *textura angularis* to *epidermoidea* in surface view.

*In vertical transverse section*: ascomatal walls 10–20  $\mu\text{m}$  thick, forming a single layer several cells deep, composed of long thin cells 6–10  $\times$  2–4  $\mu\text{m}$  with thickened and fused walls about 1.5  $\mu\text{m}$  thick, and towards the base of the ascoma of more angular cells 6–10  $\times$  7–8  $\mu\text{m}$  with thinner walls; inner surface of ascomatal walls lined with colourless angular cells; papilla truncate at the apex, 100–150  $\mu\text{m}$  wide, the ostiole lined with slender hyphal cells which also extend sometimes downwards from the upper colourless angular cells; centrum expanding in water, causing sections to curl and break.

*Asci* arising mostly from the base of the ascoma,

uniformly thin-walled, with only one wall layer visible at all stages of development, clavate, rounded at both apex and base, not turning blue in iodine, with no apical structures visible, attaining full size, 90–100  $\times$  10–14  $\mu\text{m}$ , before ascospore discharge, 8-spored; the ascospores arranged unevenly within, uniseriate near the base and apex, but irregularly biseriate above the mid-point.

*Ascospores* 8–23 (–26)  $\times$  6–8  $\mu\text{m}$ , tending to deviate from ellipsoid, one side curved more than the other, often wider in the top half, colourless and 1-septate, becoming light brown, 3-septate and spinulose (visible with oil immersion) when mature; usually containing 1–2 guttules per cell, with a thickened ring visible at the edges of the central septum which is occasionally slightly constricted; germinating from all four cells.

*Paraphyses* not observed.

*Characteristics in pure culture*: very slow growing, attaining 3 mm diam on PCA+, OA+ and CMA after 7 days and on SNA after 19 days; slimy, with sparse aerial mycelium; colour no. 35, strong red orange (Kelly, 1965), plate 7, 8B (Kornerup & Wanscher, 1978), becoming colour no. 38, dark red orange (Kelly, 1965), plate 7, 6C, brownish orange (Kornerup & Wanscher, 1978), with a raised central area which is darker, and a reverse which is slightly lighter; producing a *Fusarium* 'macroconidial' anamorph, but no 'microconidial' anamorph, after 7 days.

*Mycelium* colourless, thin-walled, smooth, septate, branched at acute angles; producing thick-walled, enlarged, globose to ellipsoid or almost rectangular cells, 6–22  $\times$  4–6  $\mu\text{m}$ , in chains. *Conidiogenous cells* colourless, thin-walled, smooth, terminal and intercalary, produced directly or on short branches, sometimes in whorls on undifferentiated hyphae, 12–16  $\times$  2.5–3  $\mu\text{m}$ , more or less cylindrical, but tapered slightly at the base and strongly towards the apex, with apical wall-thickening sometimes visible. *Conidia* colourless, falcate, thin-walled, smooth, aseptate or 1-septate (occasionally 3- or more septate or swollen and distorted in cultures 6 weeks old), strongly curved, with a *Fusarium*-like foot cell sometimes visible, 9–18  $\times$  2–2.5  $\mu\text{m}$  on SNA, 16–28  $\times$  1.5–2.5  $\mu\text{m}$  on PSA after 1 month. *Conidial development* by a replacement wall-building apex system with percurrent, enteroblastic proliferation and schizolytic secession, without elongation of the conidiogenous cell.

*Specimen examined*: on the hymenial remains of *Zeus olympius* on dead twigs and small branches of *Pinus leucodermis* at an altitude of about 1300 m, above Prionia, on Mount Olympus, Greece, S. Diamandis, late April 1986, IMI 305041, holotype; isotype NY. Living cultures deposited in IMI 305041, ATCC, NY (CTR).

Discussion of *N. ganymede*

*Nectria* (Fr.) Fr. contains fungi with brightly coloured, fleshy, perithecial ascomata which may or may not be on or in a stroma, and with asci having a single thin wall. Saccardo (1878, 1883) restricted *Nectria* to species with 1-septate ascospores, and described several generic and sub-generic segregates based on single characters. Following Saccardo (1883), *Nectria ganymede* conforms to the subgenus *Hyphonectria* Sacc., comprising species with ascomata on a cottony mycelial base. It might equally be placed in *Calonectria* de Not. because of its multiseptate ascospores with a length-width ratio of less than 20:1 (Rogerson, 1970).

It has become clear in recent years, however, that such divisions of *Nectria* based on single characters have led to the placement of closely related species in diverse genera within the Hypocreales. Recent researchers have accordingly attempted to divide *Nectria* into more natural groups of species based on the correlation of many anamorphic and teleomorphic characteristics, with an emphasis on the structure of the ascomatal wall and the form of the anamorph (Booth, 1959, 1978; Rossman, 1983; Samuels, 1976, 1978; Samuels & Rossman, 1979). Following these authors, *N. ganymede* is best placed in *Nectria*, within the *episphaeria* group, because of its ascomatal characteristics (simple wall structure, lateral collapse of walls, KOH + wall-colour reaction), its light brown spinulose ascospores, its slow-growing *Fusarium* anamorph and its ecological association with other fungi.

*Nectria ganymede* may be distinguished from *N. episphaeria* (Tode:Fr.) Fr. by its larger, 3-septate ascospores, its larger asci and its unusual habitat. The *Fusarium* anamorph is close to *F. aquaeductuum* Lagerheim, with its characteristic 0- to 1-septate 'macroconidia' and slow growth; but it differs in its red to orange colony colour in pure culture (*F. aquaeductuum* is white), its apparent absence of 'microconidia' and its shorter 'macroconidia' and conidiogenous cells.

Although parasitism of members of the Rhytismatales by a wide range of other fungi (including at least one species of *Fusarium*) is well documented, this appears to be the first record of a species of *Nectria* living in association with and perhaps parasitizing a member of that order. The facts that ascomata of *N. ganymede* were observed only on ascomata of *Z. olympius* and not on surrounding pine tissues, and that *Z. olympius* was found only on *P. leucodermis* and not on closely adjacent trees of *P. nigra* (despite a careful search), suggest that there are strong ecological relationships between the two fungi and between *Z.*

*olympius* and its pine substratum. This fits well with what is known of several other fungal associates of the Rhytismatales (Darker, 1932; Mitchell *et al.*, 1976; Reid & Minter, 1979).

## CONCLUSIONS

At first sight it is remarkable that such a conspicuous fungus as *Zeus*, on such a well-collected substratum, in Europe, should appear to be both a new species and a new genus. It is, however, merely further evidence that the fungi of Greece are still very poorly known: this is, indeed, the second large and conspicuous ascomycete of this family to be described from pines from Greece within a decade, the other being *Elytroderma torres-juanii* Diamandis & Minter (1979). The genus *Nectria* is also usually considered to have been well studied in Europe, and the discovery of a new species from this continent is also rather surprising. The implication is clear: with further detailed studies of the Greek forests and National Parks, many beautiful and interesting new fungal species remain to be discovered.

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