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The genus *Rosellinia* (Sphaeriales) from Peninsular India

ALAKA PANDE and V. G. RAO

Mycology & Plant Pathology Group, Division of Plant Sciences, Agharkar
Research Institute,
G. G. Agharkar Road, Pune 411 004, India

Pande A. and Rao V. G. (1995): The genus *Rosellinia* (Sphaeriales) from Peninsular India – Czech Mycol. 48: 177–182

The paper describes four new species and three new records of *Rosellinia* (Sphaeriales, Xylariaceae) from Peninsular India.

Key words: *Rosellinia* acaciae, *R. aquiloidea*, *R. lakshadweepensis*, *R. petriniae*, Peninsular India.

Pande A. a Rao V.G. (1995): Rod *Rosellinia* (Sphaeriales) z indického poloostrova – Czech Mycol. 48: 177–182

Jsou popsány čtyři nové druhy a tři nové nálezy rodu *Rosellinia* (Sphaeriales, Xylariaceae) z indického poloostrova.

The genus *Rosellinia* de Not. is characterised in having mainly superficial, uniperitheciate stromata seated on a subiculum. The subiculum may be persistent or evanescent. Asci possess well developed amyloid plugs. Ascospores are surrounded by mucilaginous sheath or are provided with slimy caps on the spore ends. These characters separate *Rosellinia* from the allied genus *Hypoxyylon* where stromata are usually multiperitheciate, embedded at base in the host tissue/substratum and without mucilaginous sheath or slimy caps on ascospores. In India the genus *Rosellinia* is represented by 35 species. During our studies on the "Ascomycetes of Peninsular India", we collected and examined several fresh collections as well as herbarium specimens of *Rosellinia*, of which four were found to be undescribed species while three were collected for the first time from India, thus constituting new reports for the country (Bilgrami et al. 1979, 1981, 1991; Mukerji and Bhasin 1986). These are described and illustrated here along with Latin diagnoses.

1. *Rosellinia acaciae* sp. nov.

(Fig. 1a, 1b, 1c)

Subiculum heavy, brown, woolly. Stromata are with lower halves embedded in the subiculum, rounded, brown with dark conic ostiole, measure 1.0-1.2 mm in diam. Perithecia one per stroma, rounded. Ascal plugs prominent, amyloid, 4.2-7.0 μm in height with lower width 2.8-4.2 μm and upper width 4.2 μm . Ascospores brown, ellipsoid to lenticular with one end rounded other pinched, guttulate, mucilaginous sheath present, germ slit absent; measure 18.2-22.4 \times 5.6-8.4 μm .

Collected on *Acacia arabica* Willd. at Ahmedpur (Maharashtra) AMH 2021 (Holotype); 9.IX.1971.

Subiculum densum, laneum, constructum e fibrillis ramosissimis brunneis. Stromata rotundata, brunnea, ex parte immersa in subiculum, ostiolo atro, conico; stromata magnit. 1.0-1.2 mm in diametro. Perithecia singularia per stroma, globosa; Apparatu apicali prominenti, amyloideo, magnit. ad basim 4.2-7.0 μm crasso et ad apicem 4.2 μm crasso. Ascosporae brunneae, ellipsoidae vel lenticulares, uniguttulatae, uno apice rotundato, altero apiculato, magnit. 18.2-22.4 \times 5.6-8.4 μm ; rima germinativa absens.

Collecta in *Acaciam arabicam* Willd. AMH 2021 (Holotypus); ad locum Ahmedpur (Maharashtra); 9.IX. 1971

The present collection comes near to *R. glandiformis* Ell. et Ev. in general morphology but differs mainly in the dimensions of ascospores, in absence of germ slits, in possessing ascospores with one end rounded and the other pinched. Therefore the present collection is described here as a new taxon and the species is named as 'acaciae' denoting its host.

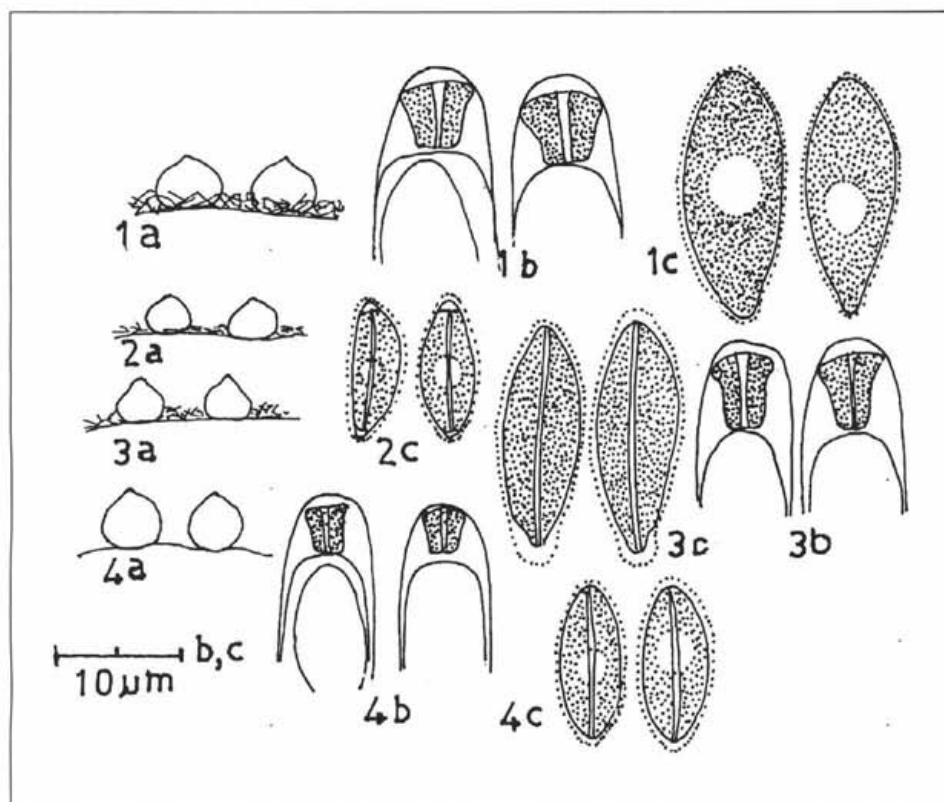
2. *Rosellinia aquiloidea* sp. nov.

(Fig. 2a, 2c)

Subiculum persistent, dark brown. Stromata seated on a subiculum, globose, minutely papillate, surface smooth, reddish brown to black, uniperitheciate, separate or aggregated in groups of 2-3; measure 0.5 - 0.8 mm in diam. and 0.5 - 1.0 mm in height. Perithecia globose, ostiolate, 480-600 μm in diam. Ascospores inequilateral with slightly pinched ends, brown, with a straight germ slit of nearly spore length, with small cellular appendages at both ends, with one guttule, mucilaginous sheath present, spores adhering, 7.0-11.2 \times 3.5-7.0 μm .

Collected on dead wood, at Molem (M.S.), 24.I.1982. AMH 5616 (Holotype).

Subiculum atro-brunneum, persistens. Stromata ex parte immersa in subiculum, globosa, minute papillata, laevia, rubigineo-brunnea vel fusca, uniperitheciata, segregata vel gregaria, magnit. 0.5-0.8 mm in diam. et 0.5-1.0 mm alta. Perithecia globosa, ostiolata, magnit. 480-600 μm in diam. Ascosporae inaequilaterales, brunneae, uniguttulatae, saepissime utroque apice appendiculo minuto hyalino ornatae, inter se adhaerentes, in muco involutae, magnit. 7.0-11.2 \times 3.5-7.0 μm , rima germinativa recta.



Figs. 1 - 4.
Rosellinia acaciae sp. nov. (1), *R. aquiloidea* sp. nov. (2), *R. lakshadweepensis* sp. nov. (3),
R. petriniae sp. nov. (4)
 a) stroma (diagrammatic representation), b) ascus plug, c) ascospores

Collecta ad culmos emortuos, ad Molem (M.S.), 24.I.1982. AMH 5616 (Holotypus).

The present collection compares with *R. aquila* (Fr.:Fr.) de Not., the type species, in having subcylindrical stromata, minutely apiculate ascospores with straight germ slits running the entire spore length. The stromata are rounded at the top and ascospores measure $7-11.2 \times 3.5-7.0 \mu\text{m}$. The apiculi are also very small in the present collection. These characters do not match with a typical 'aquila' where stromata are characterised by flattened top, much bigger apiculi on spore ends; [pencil-like, drawn out apiculi were described by Dargan (Dargan 1979)]; and ascospores measure $25-33 \times 6.3-9.0 \mu\text{m}$. These differences warrant our collection to be accommodated under a distinct taxon but showing resemblance to 'aquila' and thus a new name 'aquiloidea' is proposed to accommodate this collection.

3. *Rosellinia lakshadweepensis* sp. nov. (Fig. 3a, 3b, 3c)

Subiculum absent. Stromata almost globose or rounded, superficial, uniperitheciate, smooth, top rounded, ostiole broadly conic; 0.5-0.9 mm in diam. Perithecia globose, 400-500 μm in diam. Asci many, unitunicate, paraphysate, with rounded top and amyloid ascal plugs. Ascal plugs 2.8-3.8 μm in height and 2.8 μm wide along the entire length. Ascospores brown, ellipsoid, uniguttulate, with rounded spore ends, cellular appendages absent, mucilaginous sheath present, germ slit of nearly spore length; 10.5-17.5 \times 5.3-7.0 μm .

Collected on pericarp of *Cocos nucifera* L. at Kavaratti Island, Lakshadweep; 2.I.1988; Leg. V.D. Ranade; AMH 7601 (Holotype).

Subiculum nullum. Stromata globosa, superficialia, laevia, uniperitheciata, ad apicem rotundata, ostiolo late-conico; magnit. 0.5-0.9 mm in diam. Perithecia globosa, 400-500 μm in diam. Asci numerosi, unitunicati, paraphysibus circumdati, ad apicem rotundati, apparatu apicali amyloideo, magnit. 2.8-3.8 μm alto et 2.8 μm lato. Ascosporae brunneae, ellipsoideae, utroque apice rotundatae, uniguttulatae, in muco involutae, magnit. 10.5-17.5 \times 5.3-7.0 μm , rima germinativa recta.

Collecta ad pericapia *Cocos nuciferae* L. ad locum Kavaratti Island, Lakshadweep; AMH 7601 (Holotypus); dt. 2.I.1988;

Rosellinia sancta-cruciana, described as growing on petioles of *Cocos nucifera* L. from West Indies, differs from the present collection in arrangement of ascospores in asci, sub-navicular shape of ascospores, presence of hyaline appendage on spore ends, multiguttulate nature and larger dimensions (16.0-20.0 \times 6.0-7.5 μm). Therefore the present collection merits the status of a new taxon and is described as 'lakshadweepensis' named after the locality of its origin.

4. *Rosellinia petriniae* sp. nov. (Fig. 4a, 4b, 4c)

Subiculum brown, woolly, made-up of septate, brown, 1.5-2.25 μm broad hyphae of mycelium. Stromata black, carbonaceous, uni-peritheciate, globose, top rounded, ostiole papillate, upto 0.5 to 0.8 mm in diam. Perithecia globose, 340-500 μm in diam. Ascal plugs amyloid, prominent, 4.5-7.5 μm in height, 3.0-4.5 μm in width at base and 4.5-6.0 μm in width at top. Ascospores ellipsoidal with one end pinched, brown, mucilaginous sheath and slimy caps present, germ slit of entire spore length present, spores of adhering type, measure 15.0-21.0 \times 6.0-10.5 μm .

Collected on *Lantana camara* L.; 1.VII.1972, Maharashtra, AMH 2022 (Holotype).

Subiculum densum, laneyum, constructum e fibrillis brunneis, ramosissimis, septatis, 1.5-2.25 μm crassis; stromata atra, (carbonacea), uniperitheciata, globosa, ad apicem rotundata, ostiolo papillato; stromata magnit. 0.5-0.8 mm in diam. Perithecia globosa, 340-500 μm in diam. Asci apparatu apicali amyloideo, prominenti, magnit. 4.5-7.5 μm alto, ad basim 3.0-4.5 μm et ad apicem 4.5-6.0 μm

lato. Ascospores ellipsoidae, uno apice rotundato altero apiculato, in muco involutae, inter se adhaerentes, magnit. 15.0-21.0 x 6.0-10.5 μm , rima germinativa recta.

Ad culmos emortuos *Lantanae camarae* L.; 1.VII.1972. ad locum Maharashtra, AMH 2022 (Holotypus).

The present collection comes close to *R. bonarensis* Speg. which is a temperate species. Present collection, besides being collected in a dry deciduous forest of tropical zone (Marathwada, M.S., India), differs in absence of cellular appendages on spores and the dimensions of various structures. The species is named after Dr. L. E. Petrini, Switzerland, for her notable contributions to the genus *Rosellinia*.

5. *Rosellinia congesta* Hino et Katum.

(Bull. Fac. Agric. Yamaguchi Univ. 8: 656, 1957.)

Subiculum present, dark brown, woolly. Stromata 0.5-1.0 mm in diam.; 0.5-0.6 mm in height, globose with flat base, rounded top, papillate ostioles, surface smooth, 250-720 μm in diam and 300-400 μm in height, collared at base due to broken remnants of ectostromata. Asci with rounded apices, ascus plugs amyloid, 2.8-4.9 μm in height; 2.0-2.1 μm in width at base; 2.8-4.2 μm in width at top. Ascospores inequilateral to ellipsoid, ends rounded, brown, with a straight germ slit of nearly spore length, cellular appendages absent, mucilaginous sheath present. Spores adhering type; 10.5-17.5 x 3.5-7.0 μm .

Collected on bamboo sticks at (1) Anmod (Castle Rock, Karnataka) 20.1.1982; AMH 6294 (2) Chikhaldara (M.S.) 21.XII.1982; AMH 6499.

This species was recorded by Kar and Maity (1971) on bamboo from W. Bengal and later by Kumar and Sharma (1981) from Dehra Dun (U.P.) on *Eulaliopsis binata* (Retz.) C. E. Hubb. (fam. Graminae); however, their collection from Dehra Dun possessed slightly smaller ascospores. Here the species is reported for the first time from Peninsular India.

6. *Rosellinia dimidiata* Starb.

(Saccardo, P.A. Syll. Fung. XVI: 437, 1902)

Subiculum absent. Stromata dimidiate, base flattened, embedded in host tissue, up to 0.5-0.8 mm in diam. Perithecia 320-600 μm , dimidiate. Ascus plugs small, amyloid, 3.5-4.2 μm in height and 2.8 μm wide. Ascospores ellipsoid, ends rounded, rarely one end pinched, guttulate, dark brown, mucilaginous sheath present, slimy caps absent, spores adhering type, germ slit absent; 14.0-16.8 x 5.6-7.0 μm .

Collected on roots of *Tectona grandis* L. (M.S.); XII, 1970, AMH 2099.

The present collection is an addition to the *Rosellinia* spp. from India.

7. *Rosellinia sancta-cruciana* Ferd. et Winge

(Saccardo, P. A. Syll. Fung. XXII: 108, 1913)

Subiculum absent. Stromata globose, black, top rounded, ostiole conicopapillate; stromata upto 0.5 mm in diam., ectostromata breaking with remnants of globose perithecia at base. Ascus plugs prominent, amyloid, 4.2-7.0 μm in height and 2.1-2.8 μm in width at base, 2.8-3.5 μm in width at top; Ascospores ellipsoid with broadly rounded ends, mucilaginous sheath present, slimy caps absent, spores not adhering type, guttulate, germ slit present, of nearly spore length; 16.8-21.0 \times 5.6-8.4 μm .

Collected on *Zizyphus* sp. 1971. AMH 2101 (M.S.).

The present collection matches with the original description of the species. This is the first report from India.

A c k n o w l e d g e m e n t s

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Cryptococcus neoformans in the environment (a review)

ZDENKA JESENSKÁ

Institute of Preventive and Clinical Medicine,
Limbová 14, 833 01 Bratislava, Slovak Republic

Jesenská Z. (1995): *Cryptococcus neoformans* in the environment (a review).- Czech Mycol. 48: 183-198

The paper reviews the knowledge on the occurrence of *Cryptococcus neoformans*, the agent of human and animal cryptococcosis, in the environment (avian excreta, soil, air, water, plants, food, working and hospital environment), on the methods of its isolation and on some of its physiological characteristics associated with the survival *in vitro*.

Key words: *Cryptococcus neoformans*, environment, air, water, birds, soil, food, hospital environment, working environment, methods, media, survival.

Jesenská Z. (1995): *Cryptococcus neoformans* v prostredí (prehľad).- Czech Mycol. 48: 183-198

Článok prehľadne podáva poznatky o výskyte *Cryptococcus neoformans*, ktorý je dobre známy ako pôvodca mykóz človeka a zvierat, v prostredí v exkrementoch vtákov a v zemine, v ovzduší, vo vode, na rastlinách, v požívatinách a krmivách, v pracovnom a nemocničnom prostredí, o metódach izolácie a o niektorých jeho fyziologických vlastnostiach v súvislosti s prežívaním *in vitro*.

INTRODUCTION

Cryptococcus neoformans (Sanfelice) Vuillemin has two varieties: *Cryptococcus neoformans* (Sanfelice) Vuillemin var. *neoformans* (the heterobasidiomycetous teleomorph is *Filobasidiella neoformans* Kwon-Chung var. *neoformans*) and *C. neoformans* (Sanfelice) Vuillemin var. *gattii* Vanbreuseghem et Takashio (teleomorph: *F. neoformans* Kwon-Chung var. *bacillispora* Kwon-Chung). *C. neoformans* var. *gattii* was first described as a yeast with elongated cells in the cerebrospinal fluid of man and in the interior organs (brain, lungs) of experimentally infected animals. The isolate was also obtained from a patient in Zaire (De Vroey and Gatti 1989). A comparison of the electrophoretic karyotypes and chromosomal location of ten genes distinguished the two varieties: the varieties were distinct and consistent with the differences reported using other methods of classification (Wickes et al. 1994). According to McGinnis (1980), the differences presented originally by Kwon-Chung et al. have not been sufficient to justify the separation into two species (*F. neoformans*, *F. bacillispora*). Three other studies substantiated the unification of *F. neoformans* and *F. bacillispora* into one species (Schmeding et al. 1984, Mitchell et al. 1992, Fan et al. 1994).

The capsular polysaccharide of *C. neoformans* contains antigenic determinants that permit the classification into five serotypes - A, B, C, D and AD (Walter

and Coffee 1968, Ikeda et al. 1982). The serotypes A, D and AD belong to *C. neoformans* var. *neoformans*, while the serotypes B and C to *C. neoformans* var. *gattii* (Kwon-Chung 1975, 1976, Kwon-Chung et al. 1978 b, 1982).

C. neoformans s.l. may be commensal in healthy humans, but the reports on isolation of *C. neoformans* from the healthy persons are rare. It was found in 3 of 561 sputum specimens, in 1 of 820 otopharyngeal washings, in 6 of 723 interdigital areas of the feet and the fungus was not recorded from 162 samples of saliva nor from 320 fecal specimens (Howard 1973, Randhawa and Paliwal 1977).

Cryptococcal meningitis is the best known clinical form of cryptococcosis. Other forms include cutaneous, pulmonary, ocular or disseminated cryptococcosis. Some unusual or atypical cases of cryptococcosis were presented as cellulitis (Anderson et al. 1992), as molluscum contagiosum (Ghigliotti et al. 1992), bursitis (Faarr and Wright 1992), conjunctivitis (Balmes et al. 1992), tracheobronchitis (Beemer et al. 1972), arthritis (Levinson et al. 1974), osteomyelitis (Burch et al. 1975, Woolfitt et al. 1976) and many other diagnoses.

Cryptococcal meningitis in AIDS patients is liable to relapse and exhibits high mortality (Dupont et al. 1990, Laroche et al. 1992). The median value of overall survival for patients with AIDS and cryptococcal meningitis in New York was 9 months (White et al. 1992). Nearly 25% of the infections in Canada were observed in individuals having AIDS (Sekhon et al. 1990). Approximately 25,000 – 31,000 of patients with AIDS in the U.S.A. can be expected to develop clinically apparent cryptococcal infection, if the trends in AIDS will continue (Masci et al. 1992). The persistence of *C. neoformans* in the prostate despite of antifungal therapy was discussed by several authors (Bailly et al. 1991, Larsen et al. 1989, Staib et al. 1990). Bulmer (1990) believes that *C. neoformans* may live in a latent state within many people for a number of years. Not all people with AIDS raise pigeons or are in contact with them. It is also hard to believe that these patients acquired cryptococcosis after they have acquired AIDS.

In transplant patients, the development of cryptococcosis is often associated with a poor prognosis. The prevalence of cryptococcosis in renal transplant patients is 1 – 3% (Hellman et al. 1981, Kong et al. 1990, Mishima et al. 1977, Schröter et al. 1976).

C. neoformans var. *gattii* has not been found among isolates from Europe. However, this variety is prevalent in tropical and subtropical regions: 100% of the cultures from Austria, Belgium, Denmark, France, Germany, the Netherlands, Italy, Switzerland, Cuba and Japan belong to *C. neoformans* var. *neoformans*, while more than 85% of the isolates from Argentina, Canada and from the United States (except for southern California) were *C. neoformans* var. *neoformans*, the remainder being *C. neoformans* var. *gattii*. A high prevalence of *C. neoformans* var. *gattii* occurs in Australia, Brazil, Cambodia, Hawaii, southern California, Mexico, Paraguay, Thailand, Vietnam, Nepal, and countries in Central Africa. In these

isolates serotype B predominated, while serotype C was isolated exclusively in North America (Andreu et al. 1990, Ellis 1987, Imwidthaya et al. 1989, Kwon-Chung and Bennett 1984 a, b, Mendes et al. 1989, Mishra et al. 1981, Muchmore et al. 1980, Swine 1984). St-Germain et al. (1988) reported the isolation of *C. neoformans* var. *gattii* from a Canadian AIDS patient residing in Montreal. This patient was known to have travelled to Mexico six months before being diagnosed for AIDS. It is certainly possible that he had become infected during his stay in Mexico. Another patient lived in Los Angeles but had travelled extensively in Brazil (Clancy et al. 1990).

Natural infections by *C. neoformans* have been reported in a variety of animals, e.g. in horses, field mice, foxes, dogs, cats, etc. (Cho et al. 1986, Gargani et al. 1989, Krogh et al. 1974, Staib et al. 1985, Weitzman et al. 1973).

CRYPTOCOCCUS NEOFORMANS IN THE ENVIRONMENT

Avian excreta

Many studies have shown that pigeon excrements serve as natural reservoir of *C. neoformans*. Emmons in 1954 was the first to isolate *C. neoformans* from pigeon droppings and nests. Infusions of field-collected pigeon droppings were shown to be excellent media for the growth of *C. neoformans* (Walter et Yee 1968). In the Czech Republic, *C. neoformans* was isolated from pigeon excreta by Frágnér (1962) and Hubálek et al. (1971). The frequency of occurrence of *C. neoformans* in pigeon excreta can be high. For instance, it was isolated from 45% of pigeon faeces in Antwerp, Belgium (Swinne 1979), from 24% of pigeon droppings in Puerto Rico (Ruiz et al. 1989), from 59% of pigeon droppings samples in Japan (Toyazaki 1989), and from 18% of such samples in Germany (Weber et Schäfer 1991).

Nearly $10^5 - 10^6$ cells of *C. neoformans* were isolated per g of pigeon droppings in vacant towers (Hubálek 1975, Ruiz et Bulmer 1981). However, the fungus is not uniformly distributed. A significant correlation was found between the count of *C. neoformans* and the rate of uric acid in total nitrogen, creatinine concentration, sunlight and probably pH (Hubálek 1975). Death of the cells could be induced by lack of available intracellular water caused by low humidity and other factors (Ruiz et al. 1982a).

C. neoformans was also observed in canary droppings (Swinne-Descaïn 1975, Swine 1979), in 15 of 1046 fecal samples of avian species which were kept in the resident area of Munich, Germany (Kösters et al. 1991), in the zoological gardens of Berlin and Antwerp (Bauwens et al. 1986, Staib et Schulz-Dietrich 1984) and in excrements of exotic birds (Otčenášek et Ditrich 1985, Pal 1989, Weber and Schäfer 1991).

The study of Swinne (1979) proved the existence of an endosaprophytic phase of *C. neoformans*. Yeast cells can be carried by the pigeon in its crop for a long time without adverse effects. Pigeons can thus serve as carriers of the fungus (Khan et al. 1978).

Amoebas, mites and sow bugs may be the biotic factors for the control of *C. neoformans* in nature (Bunting et al. 1979, Ruiz et al. 1982 b). Two to four nonencapsulated yeast cells were observed in transverse histological sections of the intestinal tract of the sowbug *Metoponorthus pruinosis*, which were collected at night from a pigeon-inhabited tower (Ruiz et al. 1982 b).

Soil

It is very likely that the soil is not a primary habitat for *C. neoformans*. The soil becomes secondarily contaminated by pigeon excrements and the distribution of this organism is therefore not uniform.

While six out of 50 soil samples from pigeon nesting sites in Hannover, Germany, were positive for the fungus, none of other 120 samples from areas without pigeons were found to be positive (Böhm et al. 1970). *C. neoformans* was not isolated from soil samples in Georgia, U.S.A. (Bowman and Ahearn 1977), nor from the soil in Belgium (Swinne 1979), but it was found in 2 out of 25 soil samples from Alta Gracia, Argentina (Rubinstein et al. 1989).

Acanthamoeba polyphaga is a free-living amoeba found widely distributed in soil and water. Amoeba growing in the presence of *C. neoformans* killed most of the yeast cells. The lethal effect on *C. neoformans* may represent a biological control mechanism of this fungus in soil (Bunting et al. 1979, Neilson et al. 1978).

Air

C. neoformans was isolated from the air from sites marked by preponderance of pigeon excrements in numbers from 36 to 46 CFU/m³. The following numbers were reported from Oklahoma in a tower with pigeon excrements (in CFU/m³): before sweeping the floor debris with a broom - 15, after 1 min - 87, after 6 min - 188, after 12 h - 39 and after 24 h - 21 CFU/m³. 60% of the cells from the air in this tower were less than 4.7 µm in diameter. It was estimated that a human exposed to this atmosphere for 1 hour would have 41 cells of *C. neoformans* deposited in the lung (Khan et al. 1978, Ruiz and Bulmer 1981, Swinne-Descain 1975). The airflow played an important role in the dispersal of *C. neoformans* cells throughout the building, too (Bulmer 1990). 214 strains of *C. neoformans* were isolated from the air in a vacant tower in a large building complex in Oklahoma City. Hundreds of pigeons were residing in this tower. The results of a study of these strains imply that in the natural environment the infectious particles of *C. neoformans* are relatively small non-encapsulated yeast cells and not basidiospores (Jong et al. 1982).

C. neoformans was not isolated from air within the observation period of 2 years in a hospital for respiratory diseases in Delhi, India (Randhawa and Paliwal 1979), but it was demonstrated in Delhi repeatedly in the air of a bird veterinary hospital (Khan et al. 1978).

Water

Thirty-seven samples of drinking water in a dovecote contained *C. neoformans*: 27 of the samples were from the cages with pigeon droppings positive for *C. neoformans* (Swinne 1979). No other report of *C. neoformans* from water is known so far.

Plants

Several environmental studies suggest that *C. neoformans* var. *gattii* exhibits a specific ecological association with *Eucalyptus camaldulensis* in Australia, and the geographical distribution of this eucalypt appears to correspond to the epidemiologic distribution of cryptococcosis caused by *C. neoformans* var. *gattii*. The propagules may be spread from Australia via infected seeds containing dormant dikaryotic mycelium of the fungus (Ellis and Pfeiffer 1990 a, b, 1992). Five of six isolates tested in a hospital in Papua-New Guinea proved to be *C. neoformans* var. *gattii*. *Eucalyptus camaldulensis* does not occur in Papua-New Guinea. It is therefore possible that also other eucalypt species than *E. camaldulensis* are hosts to *C. neoformans* var. *gattii* (Currie et al. 1990). *C. neoformans* var. *gattii* isolated from eucalyptus was the same organism as the isolates from infected humans in San Francisco (Kwon-Chung et al. 1992). The first reported isolation of *C. neoformans* var. *gattii* from the environment outside Australia was from trees of *E. camaldulensis* growing at a site near Fort Point, San Francisco (Pfeiffer and Ellis 1991). In India, three patients were registered with *C. neoformans* var. *gattii* meningitis (Padhye et al. 1993). One isolate of var. *gattii* was identified in a bat guano collected in the attic of an old house in the city of Rio de Janeiro, Brazil (Lazera et al. 1993).

No *C. neoformans* germs could be isolated from the seeds of 117 species of 48 plant families from the Botanical Garden of Berlin, from 18 samples of wheat, oats and barley from a big mill in southern Germany or from seed mixtures used as bird feed. It must be assumed that birds do not come into contact with *C. neoformans* via infected plant seeds (Staib et al. 1978).

Foods

The type strain *C. neoformans* CBS 132 was isolated by Sanfelice from fermenting fruit juice (Kreger-van Rij 1984), other strains for example from apple fruits

in Canada, and from grapes in South Africa (Davenport 1976). The occurrence of *C. neoformans* was studied in 254 vegetable and 186 fruit samples collected from different markets in Delhi, India, from 1978 to 1980. *C. neoformans* was isolated from five sorts of products examined: apple, guava, papaya, carrot and potato. It appears from these findings that fruits and vegetables may not provide suitable conditions for the survival and multiplication of *C. neoformans* under natural conditions (Pal and Mehrotra 1985). The first isolation of *C. neoformans* from contaminated banana fruits was reported by Pal et al. (1990). *C. neoformans* was found among other pathogenic fungi from desiccated "black fungus" mushrooms – *Auricularia polytricha* (Mont.) Sacc. – imported from Taiwan to the USA (Kazanas 1987).

An examination of frozen chicken stored for a longer period of time in a cooling plant revealed that the surface was contaminated by *C. neoformans* in a high number (Muzikář and Tříška 1977).

Working environment

It is generally accepted that the lungs are the primary site of entry of *C. neoformans* into the human body. The accumulation of pigeon excrements in buildings is well known and therefore reconstruction of old buildings represents a health hazard for workers (Weeks et al. 1982). One case of pulmonary cryptococcosis was described in a 34 years old otherwise healthy sportsman after cleaning an old house (Marel et al. 1987).

Attention should be paid to the risk of infection in mycology laboratories when handling the sexual forms of *C. neoformans*. For safe operation, culture streaks on transparent media should be controlled microscopically for mycelium formation from the bottom of sealed Petri dishes. Chains of basidiospores of *Filobasidiella* sp. break into single spores by the slightest movement. The strictest rules of precaution must be followed (Staib and Blisse 1982).

Hospital environment

In a study on fungal contamination of 696 organ-cultured corneas only two were found contaminated by *C. neoformans* (Nelson et al. 1983).

SURVIVAL AND RESISTANCE IN VITRO

Twelve isolates of *C. neoformans* were strongly resistant to UV radiation in vitro (Toyazaki 1989). However, UV irradiation of pigeon excrements in vitro resulted in a germicidal effect on *C. neoformans*. It appears that UV radiation acts indirectly in pigeon excrements, by increasing the concentrations of toxic compounds like

peroxides (Hubálek and Příkazský 1975). Melanized *C. neoformans* cells were less susceptible than non-melanized cells to the fungicidal effects of UV light (Wang et Casadevall 1994).

In vitro inhibition of the growth of *C. neoformans* in chicken droppings appears to be the result of high alkalinity and of the presence of low molecular weight, thermostable growth-suppressing substances (Walter and Yee 1968). The fungus grows well in both sterile pigeon and chicken excrements but no viable cells of this organism were detected in the unsterile wet excrements in vitro after incubation at 25 °C for 4 weeks. The suspension of seven different species of bacteria (*Staphylococcus albus*, *Streptococcus fecalis*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Klebsiella aerogenes*), recovered from the intestinal contents of pigeons completely inhibited the growth of *C. neoformans* and no viable cells of any of the tested strains were detected after one week of incubation. It is not clear whether this inability of *C. neoformans* to survive in the presence of these bacteria can be attributed to the competition for space and/or nutrition, or is due to the effect of toxic metabolites (Aboul-Gabal and Atia 1978). Isolates of *Pseudomonas aeruginosa* and *Bacillus subtilis* from pigeon droppings demonstrated anticryptococcal activity in vitro but this activity was apparently dependent upon the medium on which the bacteria were grown. In addition, it appears that differences between various strains of one organism may exist. Certain bacteria occurring in nature have the potential to kill populations of *C. neoformans* on pigeon droppings. Ruiz et al. (1982 b) investigated the number of *C. neoformans* cells in pulverized pigeon dropping stored at room temperature for 9 months. They reported a general decrease in cell number, but the results differed for various cell sizes: no decrease in viability was recorded for airborne cells less than 3.3 µm in diameter, while the viability of cells greater than 3.3 µm in diameter decreased by 63% to 80%. Smaller cells appear to longer survive the conditions employed in this study. Storage at -4 °C did not affect the viability (Ruiz et al. 1982 a).

Trophozoites of *Acanthamoeba palestinensis* ingested and killed 99.9% of *C. neoformans* cells after 7 days of cultivation in vitro (Ruiz et al. 1982 b). A similar study with *Acanthamoeba polyphaga* yielded 78 - 97% killing in 7 days (Bunting et al. 1979).

C. neoformans strains were incubated in soil for 12 weeks at room temperature. The size of *C. neoformans* particles decreased with incubation time causing an increased number of isolated cells with diameters 0.65 to 2 µm. The heavily encapsulated strain lost its capsule during incubation in soil. This means that *C. neoformans* cells in nature exist in sizes that are capable of deep lung deposition. Upon entering the lung, non-encapsulated infection particles would start to produce the capsular material after only a few hours which would subsequently inhibit the phagocytic process (Bulmer 1990, Neilson et al. 1977). The maximum growth

temperature of *C. neoformans* was reported to be 39.8 °C (Madeira-Lopes et al. 1986), resp. 40 – 42 °C (Saez and Nguyen 1980). No growth was observed at 43.4 °C (Toyazaki 1989). The viability of *C. neoformans* cells adhering to the glass surface of dry Petri dishes was lost after 28 weeks, the maximum NaCl tolerance was 14% (Blaschke-Hellmessen et al. 1985).

C. neoformans was inhibited in basal media containing 0.1% sorbic acid at pH 4.5 (Bell et al. 1959). With the exception of 0.02 – 0.5% chlorhexidine gluconazole, the common disinfectants (ethylalcohol 40 – 70%, povidone iodine 3.0%, iodine tincture 4.0%, phenol 2.0% and saponated cresol solution 2.0%) were found to be effective in killing of isolates of *C. neoformans* in vitro (Toyazaki 1989). Weeks et al. (1982) recommended to use 5% formalin in a dosage of 1 gallon per 6 square feet for elimination of a potential health hazard by *C. neoformans* during renovation in historic buildings. Samples of the pigeon excreta were negative one week after the application of formalin. The entry of unauthorized individuals into buildings must be prevented during the decontamination procedure.

In Neill's medium, pH values above 8.0 are fungicidal, pH 8.0 is partially inhibitory and pH 7.5 and 6.5 permit relative good growth of *C. neoformans* (Walter and Yee 1968).

METHODS OF ISOLATION AND IDENTIFICATION

Staib (1962) introduced a new medium for the selective isolation of *C. neoformans*. The development of this medium was based on the observation that colonies of this yeast growing on media prepared from dung of canary birds and goldfinch were dark-brown pigmented if the birds were fed by *Guizotia abyssinica* (niger) seeds. The color of the colonies was preserved even if they were grown on membrane filters deposited on the agar medium (Staib 1963). This simple agar medium containing a water extract of *Guizotia abyssinica* seeds was later modified by supplementation with some salts, antibacterial antibiotics, diphenyl, creatinine and by reduction of the glucose content (Staib and Seeliger 1968), or by addition of 2 mg of methyl violet 2B per l of *Guizotia abyssinica* agar (Rubio et al. 1984). The medium prepared this way significantly inhibited the growth of bacteria and filamentous micromycetes. Racicot and Bulmer (1985) found that diphenyl inhibits the growth of *C. neoformans* and recommended the trypan blue medium. Since *C. neoformans* colonies develop later on a medium containing diphenyl, the lowest concentration should be used if necessary (Staib et al. 1987).

This complex medium was modified by a reduction of the glucose content and by omitting magnesium sulfate from the salt mixture (Staib et al. 1973). On the other hand, Salkin (1979) used the original recipe and reported that the simplest medium composed of disintegrated seed, water and agar is suitable for the selective isolation of *C. neoformans*. Paliwal and Randhawa (1978) reappraised

the medium composition and introduced a similar simplification. The production of dark pigments originally observed in *C. neoformans* colonies grown on media containing a water extract of the seed could be stimulated even in chemically defined media by addition of various derivatives of benzoic acid. 3,4-dihydroxycinnamic (caffeic) acid was found to be the best ingredient (Pulverer and Kroth 1971). Lütticken (1975) supplemented this medium with creatinine and Healy et al. (1977) by urea and inositol. Land et al. (1978) developed a multipurpose medium that enables the identification of brown-coloured colonies of *C. neoformans* together with the production of germ tubes and chlamydospores of *Candida albicans* strains. Fleming et al. (1977) introduced a medium for the presumptive identification of *Candida albicans* and *C. neoformans* as well. Staib's agar supplemented with penicillin (20 U/ml), streptomycin sulfate (40 U/ml) and gentamycin (40 µg/ml) was recommended for the detection of *C. neoformans* in clinical specimens, especially for the examination of specimens contaminated with gram-negative rod-like bacteria (Staib et al. 1989).

The brown colour of *C. neoformans* colonies produced during their growth on these media can be explained by the presence of phenoloxidase activity in these yeasts. This effect can be utilized even for the detection of *C. neoformans* in media containing extracts of other material of plant origin, such as *Vicia faba* beans (Shaw and Kapica 1972) or potatoes and carrots (Kapica and Shaw 1969), sunflower seeds (Rubinstein et al. 1989) or banana peels (*Musa paradisiaca*) (Paula et al. 1992). Occasional negative results in this isolation procedure can be caused by variability of the phenoloxidase activity in different strains of *C. neoformans* (Nurudeen and Ahearn 1979). A medium containing esculin (Edberg et al. 1980) is another example of a simple medium that can be used for selective isolation of *C. neoformans*.

Other isolation media such as the Littman medium containing bovine bile (Botard and Kelley 1968) have been used only sporadically for this purpose.

Two tests were developed for the rapid identification of *C. neoformans* based on pigment production by the phenoloxidase activity present in the organism. In the first test, cornmeal - Tween 80 agar is supplemented with the phenoloxidase substrate caffeic acid. This medium is prepared by dissolving 17 g of dehydrated cornmeal agar (DIFCO) in 900 ml of distilled water, adjusting the pH to 6.0, and by adding 10 ml of Tween 80. In 100 ml of distilled water, 0.3 g of caffeic acid is dissolved. The solutions are autoclaved separately at 121 °C for 15 min, cooled to 56 °C and mixed together. Colonies are cut off from the cornmeal-Tween-caffeic acid agar and after 18 to 24 h of incubation at 25 °C examined macroscopically for the brown pigment production and microscopically for yeast morphology. The second test - a phenoloxidase detection strip - is a test not based on a medium which allows the identification of *C. neoformans* on the same day. Strips of blotting paper are saturated with buffered L-β-3,4-dihydroxy-phenylalanine (L-DOPA - ferric citrate solution, prepared by mixing 1 ml of a freshly prepared L-DOPA

solution, 3.5 ml of a phosphate buffer and 0.5 ml of a ferric citrate solution). These solutions are prepared as follows: 1) L-DOPA is suspended in one to three drops of dimethyl sulfoxide and dissolved in water to a final concentration of 3 mg/ml. 2) The phosphate buffer (pH 6.8) is prepared by mixing equal volumes of 0.067 M Na_2HPO_4 and 0.067 M KH_2PO_4 . 3) Ferric citrate is dissolved in water to a concentration of 1 mg/ml by gentle heating. After saturation with the buffered L-DOPA-ferric citrate solution, the paper strips are held at 37 °C until dry and stored in a foil at -20 °C for up to 6 months. To perform the test, the strips are rehydrated with the phosphate buffer and inoculated by swabbing one to two colonies onto the surface of the strip. The strips are incubated at 37 °C in a moist chamber and examined at 30 min. intervals for the production of black pigment (a positive reaction). The tests appear to be specific for the identification of *C. neoformans* (Kaufmann and Merz 1982).

Serotypes and varieties of *C. neoformans* could be easily differentiated on CDG and more exactly on CGB media (Kwon-Chung et al. 1978b, 1982), and *Filobasidiella* development within 48 h at 26 °C on a natural medium closely resembling natural conditions (Staib 1981). The water soluble substances found in pigeon manure can offer optimal conditions for the formation of basidia and basidiospores if two sexually compatible strains are present. No formation of *Filobasidiella* was observed on concentrated pigeon manure (Staib and Blisse 1982).

Another medium was prepared by Salkin and Hurd (1982), which effectively differentiates B and C serotypes of *C. neoformans* from A and D serotypes. According to their experience, the medium of Kwon-Chung et al. (1978) is difficult to prepare and it occasionally yields ambiguous results. The new medium is based on the resistance of tested strains to low concentrations of cycloheximide and on assimilation of glycine as the single carbon source.

The easiest method is to make a test of the assimilation of D-proline: *C. neoformans* var. *neoformans* is able to utilize D-proline (Dufait et al. 1987).

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Pseudoomphalina kalchbrenneri (Agaricales) in the Czech Republic

In honour of Dr. Josef Herink, an octogenarian

FRANTIŠEK KOTLABA¹ and ZDENĚK POUZAR²

¹ Na Petřínách 10, 162 00 Praha 6, Czech Republic

² Mycological Department of the National Museum, Václavské nám. 68, 115 79
Praha 1, Czech Republic

Kotlaba F. and Pouzar Z. (1995): *Pseudoomphalina kalchbrenneri* (Agaricales) in the Czech Republic.— *Czech Mycol.* 48: 199–205

The authors have studied Bresadola's type material of *Omphalia kalchbrenneri* Bres. from Italy as well as fresh and dried herbarium specimens from Bohemian and Moravian localities. After studying the material and perusal of the literature, the conclusion was reached that fungi described by various authors as *Omphalia kalchbrenneri* Bres., *Agaricus compressipes* Peck and *Omphalia graveolens* Sev. Petersen are conspecific and the correct name for this fungus is *Pseudoomphalina kalchbrenneri* (Bres.) Sing. In the Czech Republic, this rare species is known from only six localities.

Key words: *Pseudoomphalina kalchbrenneri*, agaric, taxonomy, nomenclature, localities in the Czech Republic

Kotlaba F. a Pouzar Z. (1995): Kalichovka Kalchbrennerova (Agaricales) v České republice.— *Czech Mycol.* 48: 199–205

Autoři studovali Bresadolův typový materiál *Omphalia kalchbrenneri* Bres. z Itálie a živé i herbářové položky této houby z českých lokalit a z moravské lokality. Po studiu materiálu a literatury dospěli k závěru, že houby popsané různými autory jako *Omphalia kalchbrenneri* Bres., *Agaricus compressipes* Peck a *Omphalia graveolens* Sev. Petersen jsou totožné; správné jméno pro tuto houbu je *Pseudoomphalina kalchbrenneri* (Bres.) Sing. Tento vzácný druh je v České republice známý pouze ze šesti lokalit.

INTRODUCTION

Pseudoomphalina kalchbrenneri (Bres.) Sing. belongs to the fairly rare agarics and, whilst collected several times in Bohemia during the last thirty years, it has not been published for Czech Republic. It was first collected in this country by J. Kubička and J. Herink and determined by J. Herink (both Czech mycologists) in 1949 and determined by the latter, to whom we dedicate this paper. Our recent findings induced our interest in the variability and the delimitation of this species.

Various characters have been published in the literature which define *Pseudoomphalina kalchbrenneri* (Bres.) Sing. and *P. graveolens* (Sev. Petersen) Sing. as two distinct species. Moser (1983), for instance, separates *P. kalchbrenneri* (Bres.) Sing. from *P. compressipes* (Peck) Sing. (= *Omphalia graveolens* Sev. Petersen) on the grounds of the more reddish pileus surface and stipe, the smaller spores and the agreeable but different (not specially farinaceous) smell.

MATERIAL AND METHODS

We studied rather rich material – fresh as well as dried – from three mutually distant places in the western part of the Czech Republic (Northern, Central and Southern Bohemia). After a thorough study, we reached the conclusion that the above-mentioned characters are variable and, for this reason, cannot be used for distinguishing between the species.

For instance, the red-brown or reddish tint of the pileus surface was noted only in one collection from near Poříčko n. Sáz., Central Bohemia (PRM 710091) but the pileus surface in another collection from the same locality (PRM 829202), as well as from the remainder, was dark woody-brown when moist and pale when slightly dry.

Smaller spores were found in various collections with some from the same locality. Also, the farinaceous smell, whilst noted in some collections was absent from others. In the collection from the Krkonoše Mountains, made in 1993, the smell was inconspicuous whereas it was markedly farinaceous in the 1994 and 1995 collections. It may be that the intensity of the smell depends on the weather – moist or dry, cold or warm etc.

The spore size in material from Vítkovice in the Krkonoše Mountains (Northern Bohemia) in 1993 was (7-)8-10.5 × (3-)4.5-5(-5.3) μm (PRM 878727) but from "Žofínský prales" near Pivonice (Southern Bohemia) only (6.5-)7-7.5(-9) × 4-4.5 μm (PRM 710092).

From these facts, we conclude that the colour of the pileus, the spore size and the smell are variable and so cannot be retained as distinguishing features.

For the above-mentioned reasons, we consider *Omphalia kalchbrenneri* Bres. and *O. graveolens* Sev. Petersen to be conspecific. However, as the first name is much older (1883) than the second (1907), the correct name for this agaric is *Pseudoomphalina kalchbrenneri* (Bres.) Sing. However, in the same year as Bresadola, Peck (1883) published *Agaricus compressipes*, which is considered by some authors to be conspecific with Bresadola's fungus. It was recombined by Singer with *Pseudoomphalina* as *P. compressipes* (Peck) Sing., who considered it the correct name for the agaric under discussion. According to Stafleu and Cowan (1976, 1983) the first volume of Bresadola's *Fungi Tridentini* (dated 1881) appeared in January 1883, and not in 1881, whereas Peck's Report of the Botanist (Ann. Rep. New York State Mus. vol. 33) was issued as late as June 1883. Therefore, Bresadola's name *Omphalia kalchbrenneri* has nomenclatural priority.

The same opinion has been already reached by Knudsen and Hansen (1991), where the synonymy of *Pseudoomphalina kalchbrenneri* is treated in detail. There also exists another species in the genus *Pseudoomphalina*, viz. *P. pachyphylla* (Fr.) Knudsen, which is remarkable for the usually broad, ventricose, distant, emarginate lamellae and the subsquamulous pileal surface (see Knudsen and Hansen 1991).



Fig. 1 *Pseudoomphalina kalchbrenneri*. Mature carpophores in the locality near Vítkovice in the Krkonoše Mountains, 19.9.1993. x1.25 Photo F. Kotlaba.



Fig. 2 *Pseudoomphalina kalchbrenneri*. Left: view of the pileus surface, right: view of gills. Horní Vltavice, "Boubínský prales", 11.9.1949. Photo J. Herink.

We have studied Bresadola's type of *Omphalia kalchbrenneri* from the Forest of Birreni near Trento (Italy), Summer (aestate) 1883, coll. G. Bresadola (S 94/217). There were originally probably about three carpophores, now broken into many small pieces, and three fragments of stipes, 2.2, 1.4 and 1.3 cm long and about 1 mm thick with the spores measuring (7-)7.5-9.5(-10) \times 4-5 μ m. There is still another, much younger, Bresadola's collection of this species: Varena, August 1919, coll. G. Bresadola (S). It is also fragmented into small pieces and is identical with the type, with spores are of the same character and measuring 7-9.5 \times 4-5 μ m (both revised by us on 15th December, 1994).

Ballerio and Contu (1993) distinguish *Pseudoomphalina compressipes* (Peck) Sing. mainly by the orange-rouge colour of the pileus surface. In the original description of *Agaricus compressipes* Peck, however, no mention was made of any trace of this colour with Peck 1883 (p. 18) writing: "Pileus... brownish when moist, whitish or pale-alutaceous when dry...". We requested for study the loan of the specimens of *Pseudoomphalina compressipes* and *P. kalchbrenneri* collected in Sardinia (Italy) published by these authors and received four specimens (herb. CAG): 1. Pula, 14.10.1990, N. 13/32.3a; 2. M.te Limbara, 12.10.1984, N. 13/32.3b, both as *Pseudoomphalina compressipes* (but only the second collection, with amyloid spores measuring 7-8.5(-11) \times 4-5.5 μ), represents *P. kalchbrenneri*; 3. M.te Arci, 1.11.1989, N. 13/32.5c; 4. the same locality, 1.10.1989, N. 13/32.5a, both as *Pseudoomphalina kalchbrenneri*. The only collection published as *P. kalchbrenneri* in Ballero and Contu (1993) was from M. te Arci, 1.11.1989, and appeared to have large, shortly ovoid inamyloid spores; this is not *P. kalchbrenneri* but represent some *Omphalina* sp. The second specimen, determined as *P. compressipes*, was from Pula, 14.10.1990, but had the same microscopical characters and cannot therefore represent any *Pseudoomphalina*; it was also an *Omphalina* sp. (and the same as the other specimen send to us).

In our opinion, the distinguishing criteria given in the key to *Pseudoomphalina* by Ballero and Contu (1993) are partly incorrect, as some characters of *Omphalina* were involved. We are convinced that, taxonomically, *Pseudoomphalina compressipes* is not different from *P. kalchbrenneri*.

DESCRIPTION OF PSEUDOOMPHALINA KALCHBRENNERI

The following is prepared from fresh carpophores collected by the first author above Vítkovice in Krkonoše Mountains (North Bohemia) on 11. September 1994, supplemented by notes on fresh specimens from two other Bohemian localities (Poříčko n. Sáz. and Žofinský prales) and the descriptions of J. Herink, also based on fresh specimens, from the Boubín area in the Šumava Mountains (Southern Bohemia).

Pileus 11-51 mm in diam., at first appanate with an inflexed margin, deeply depressed at the centre, later (at maturity) broadly infundibuliform to infundibuliform, either smooth or sparsely radially costate with an undulated, smooth margin. Pileus surface otherwise glabrous (although faintly tomentose in one carpophore), slightly pruinose when young, especially on the crenate margin, where it forms a whitish zone; surface strongly hygrophanous with an almost cartilaginous consistency (the cuticle could be striped from the margin to the centre), colour woody brown when moist, rather dark without reddish tints, but becoming much paler when drying with the centre always darker. In some carpophores, the surface is translucently striate for over more than half of the pileus; pileal context 1.25-1.5 mm thick and rather silky fibrillose.

Lamellae 2.5-5 mm deep, 0.5-0.6 mm thick, with the entire edge (not dentate), deeply decurrent, white as a flour when young but soon becoming ivory and up to pale-butter-ochraceous when old.

Stipe central, slender, undulately uneven (slightly twisted), hollow, 30-65 mm long and 2-4(-5) mm broad, slightly glassy lustrous, elastic, slightly cartilaginous but little fragile, narrowly cylindrical, becoming grooved when old, smooth, woody brown; the base is slightly thickened, sometimes thin white felty, without any rhizoids.

Taste strongly cucumber-farinaceous, becoming remarkably bitter; smell also strongly cucumber-farinaceous.

Spores (6.5-)7-10(-11.5) × (3-)4-5(-5.5) μm, cylindrical ellipsoid with a distinct apiculus on the rounded base of the spore, mostly flat on the ventral but arched on the dorsal sides, thin-walled, smooth, hyaline, indextrinoid, amyloid in Melzer's reagent.

Carpophores appear in autumn, in the Czech Republic from September 3 to November 7.

LOCALITIES OF PSEUDOOMPHALINA KALCHBRENNERI IN THE CZECH REPUBLIC

The following specimens from the Czech Republic are preserved in the herbarium of the National Museum, Prague (PRM) and in the private herbarium of MUDr. J. Herink (Mnichovo Hradiště):

Bohemia

Vítkovice, distr. Jilemnice, montes Krkonoše, Bohemia septentr.; in margine silvae supra Vítkovice in fossa viae ad Rezek, 800 m alt., ad terram humosam sub *Athyrium filix-femina*, *Urtica dioica* etc., 19.IX.1993 (PRM 878727), 11.IX.1994 (PRM 883243) and 21.X.1995 (885420), leg. F. Kotlaba, det. F. Kotlaba et Z. Pouzar. - Poříčko n. Sáz., Boh. centr., in valle rivuli "Křešický potok", 320 m

alt.; ad terram (detritus), 13.X.1970, leg. et det. Z. Pouzar (PRM 710091); ib., in detr. *Carpini betuli* et *Piceae abietis*, 7.XI.1981, leg. J. Kubička, det. F. Kotlaba et Z. Pouzar (PRM 829202). – Horní Vltavice, vicus Kaplice, distr. Prachatice, montes Šumava, Boh. merid., vallis ad latera montium "Pažení" et "Boubín" (reservatio naturalis "Boubínský prales"), ca 950 m alt., 2.X.1952, leg. et det. J. Herink (herb. Herink 789/52). – Horní Vltavice, vicus Kaplice, distr. Prachatice, montes Šumava, vallis rivi "Kaplický potok", ca 910 m alt., ad terram humosam subarenosam, *Picea abies*, *Fagus sylvatica*, 11.IX.1949, leg. J. Kubička et J. Herink, det. J. Herink (herb. Herink 531/49). – Silva virginea "Žofínský prales" ap. Pivonice (Žofínské sruby), montes Novohradské hory, Boh. merid., ca 780 m alt.; in trunco valde putrido (detritus) *Piceae abietis*, 10.X.1968, leg. et det. Z. Pouzar (PRM 710460); ib., in detritu in *Fageto* sub *Urticis dioicae*, 3.IX.1970, leg. J. Kubička, det. Z. Pouzar (PRM 710092).

Moravia

Čeladná, pagulus Podolánky, distr. Frýdek-Místek, montes Moravskoslezské Beskydy, Silesia orient., reservatio naturalis "V Podolánkách", ca 680 m s.m., piceetum vetus umbrosum, solo turfoso... ad terram humosam adhaerentem ad radices *Piceae abietis* eversae, 22.IX.1982, leg. et det. J. Herink (herb. Herink 549/82).

ECOLOGY OF PSEUDOOMPHALINA KALCHBRENNERI IN CZECH REPUBLIC

None of the known localities in Czech Republic is situated on limestone with all are on acid, humous or clay-sandy soils, mostly on gneiss and granite. All localities are in the lower mountains (780 – 950 m alt.) except for Poříčko n. Sáz., which is in a deep brook valley in hilly country (320 m alt.).

In all localities, *Pseudoomphalina kalchbrenneri* occurs under spruce (*Picea abies*), sometimes together with frondose trees. In the mountainous localities "Boubínský prales", "Kaplický potok", "Žofínský prales" and "V Podolánkách", the spruce mixed forest is autochthonous, whilst the other two are secondary spruce plantations, probably since at least from the last century. In the locality near Vítkovice, the original vegetation was most probably mixed beech forest (*Fagus sylvatica* with *Picea abies*) whereas in Poříčko n. Sáz. at the bottom of the deep brook valley, it was probably brookside alder forest. The two last biotopes of *Pseudoomphalina kalchbrenneri* (Vítkovice and Poříčko n. Sáz.) have been influenced by man as they are on the forest roadside.

The first mentioned (Vítkovice) is on the margin of a road in a montane spruce forest (*Picea abies*) where the following plants occur: *Athyrium filix-femina*, *Urtica dioica*, *Senecio fuchsii*, *Oxalis acetosella*, *Petasites albus*, *Rubus idaeus*,

Silene dioica (= *Melandrium sylvestre*), young *Acer pseudoplatanus*, *Ajuga reptans*, *Sambucus racemosa* and *Myosoton* (*Malachium*) *aquaticum* (arranged according to their abundance in the locality). In association with *Pseudoomphalina kalchbrenneri* were *Clitocybe* sp., *C. fragrans* (With.:Fr.) Kumm., *Conocybe* sp., *Cystolepiota carcharias* (Pers.) Fayod, *Mycena sanguinolenta* (Alb. et Schw.:Fr.) Kumm. and *Tubaria* sp. whilst *Bjerkandera adusta* (Willd.:Fr.) P. Karst. and *Hymenoscyphus* sp. occurred on a small sycamore stump (*Acer pseudoplatanus*).

The Moravian locality ("V Podolánkách") is in a natural Carpathian moist spruce forest, with the following plants: *Calamagrostis villosa*, *Deschampsia flexuosa*, *Fagus sylvatica* (young), *Majanthemum bifolium*, *Oxalis acetosella*, *Rubus* sp., *Salix silesiaca*, *Senecio fuchsii*, *Viola palustris* etc.

We would like to draw the attention of mycologists to this interesting agaric (*Pseudoomphalina kalchbrenneri*) and allied species as there seem to be some problems which need further study and collaboration of mycologists from various countries.

A c k n o w l e d g m e n t

Thanks are due to Dr. Josef Herink for his kind cooperation in this paper, particularly for providing us his own description of the agaric concerned.

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Distribution and ecophysiological characteristics of the fungus *Tilletia controversa* in Slovakia

PETER PAULECH¹ and CYPRIÁN PAULECH²

¹ Institute of Experimental Phytopathology and Entomology, Slovak Academy of Sciences, 900 28 Ivanka pri Dunaji,

² Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 14, 842 23 Bratislava, Slovakia

Paulech P. and Paulech C. (1995): Distribution and ecophysiological characteristics of the fungus *Tilletia controversa* in Slovakia. – Czech Mycol. 48: 207–215

The distribution area of the fungus *Tilletia controversa* Kühn (dwarf bunt) and its host plants range in the Slovakian territory was studied. The mentioned fungus was detected on *Triticum aestivum* L. *Elytrigia repens* (L.) Desv. and on *Elytrigia intermedia* (Host.) Nevski. This contribution contains the basic morphological and ecophysiological characteristics of the fungus populations of infected plants. The fungus is wide-spread especially in central and eastern Slovakia. It was found on winter wheat stands at 777 locations (cadastral territories) and at 4 locations on species of the genus *Elytrigia*.

Key words: *Tilletia controversa* Kühn, distribution, Slovakia, ecophysiological characteristics

Paulech P. a Paulech C. (1995): Rozšírenie a ekofyziologická charakteristika huby *Tilletia controversa* na Slovensku. – Czech Mycol. 48: 207–215

Na území Slovenska bol vymedzený areál rozšírenia huby *Tilletia controversa* Kühn a okruh jej hostiteľských rastlín. Uvedenú hubu sme zistili na ozimnej pšenici (*Triticum aestivum* L.), na pýre plazivom (*Elytrigia repens* (L.) Desv.) a na pýre sivom (*Elytrigia intermedia* (Host.) Nevski). V práci je uvedená základná morfológická a ekofyziologická charakteristika populácie huby a infikovaných rastlín. Huba je rozšírená hlavne na strednom a východnom Slovensku. V porastoch pšenice sme ju zistili na 777 lokalitách (katastrálnych územiach) a na druhoch rodu *Elytrigia* na štyroch.

The first written report on the occurrence of the fungus *T. controversa* in Slovakia was given by Bäumler (1927). He found it on plants of couch grass (*Elytrigia repens* (L.) Desv.) by the riverside of the Danube in Bratislava. Later Paulech (1957) reported about the occurrence of the fungus on winter wheat (*Triticum aestivum* L.) in our country, too. The species *T. controversa* has been considered an important pathogen since, to which great attention was paid in our study. We mainly aimed at delimiting its distribution area and at ecophysiological characteristics of the fungus populations on our territory. This contribution contains a review of the obtained results.

MATERIAL AND METHODS

The occurrence and distribution of the fungus *Tilletia controversa* was found on winter wheat (*Triticum aestivum* L.), species of the genus *Elytrigia* Desv. and on other host plants (Paulech 1992) of the family *Poaceae* mentioned in the literature. Attention was also paid to the ability of the transfer of the mentioned fungus from *Elytrigia* to wheat. The occurrence of *T. controversa* on winter wheat was established during the years 1957-1993, that on *Elytrigia* and other grass species during the years 1978-1993.

The methodical procedure used for finding out the locations of the fungus in winter wheat stands in Slovakia has been described in our latest papers (Paulech et al. 1993a, Paulech et al. 1993b, Paulech and Paulech 1994). A description of the rules of finding out the occurrence of *T. controversa* on *Elytrigia* and possibly other grass species can also be found in previous papers (Paulech and Maglocký 1988, Paulech 1992). Cadastral territories (villages, communities) are identified as locations of *T. controversa* occurrence. The mentioned fungus was searched for in fields and fungus identification was performed in laboratory. The number of obtained locations is presented in the map of Slovakia by a grid method (Jasičová and Zahradníková 1976). The basal area on the map delimited by coordinates represents an area of circa 12.0 km x 11.2 km (134.4 km²). Cadastral territories situated in two or more neighbouring squares are noted only once either in the square to which the greatest part of its area belongs, or in the square with the highest intensity of dwarf bunt occurrence. The names of locations, intensity of the fungus' occurrence and other methodical data have been described in papers quoted in the methodical part of this work. Fungus identification was performed on the basis of visual symptoms of infected plants, by light microscopy of chlamydospores (spore diameter and thickness of their hyaline sheath in μm), by study of their shape in dried propanol (Trione and Krygier 1977) and since 1985 also by a method based on spore germination on rinsed out clayey soil (Paulech 1991).

The dormancy period of chlamydospores was studied in air-conditioned chambers (KTLK, ILKA, Germany) at a temperature of 8 °C for 12 hours of day light, at an illumination of 12 000 lux and 65 \pm 5% relative humidity.

A physiological race of the fungus *T. controversa* population was isolated from the cultivar 'Regina' growing at the location of Kotešová, district Žilina, in central Slovakia. Identification was performed on an American differential assortment (Hoffmann and Metzger 1976) in the garden of the Institute of Botany of the Slovak Academy of Sciences at Bratislava. The differential assortment was infected with a water suspension of fungus chlamydospores and reinfected with a suspension of the germinating chlamydospores and sporidia after two weeks (on germinating wheat).

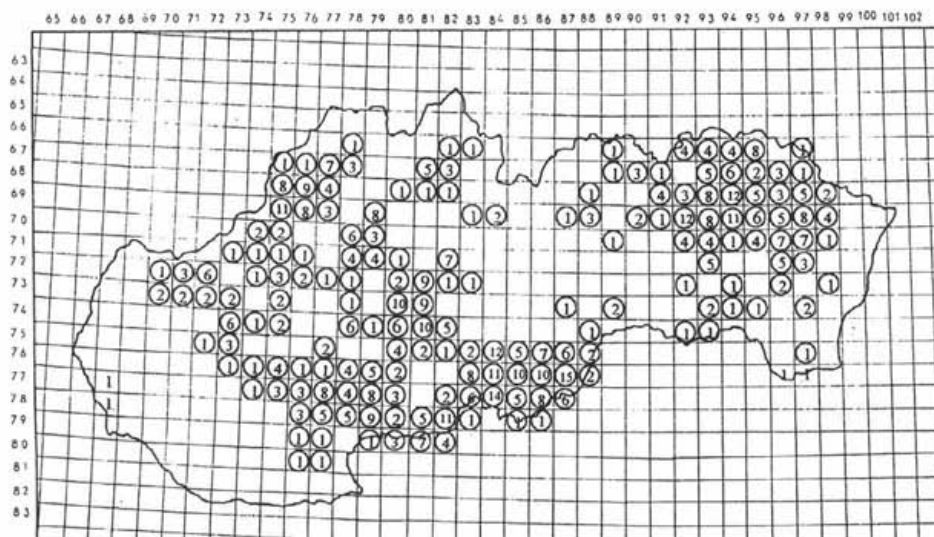


Fig.1. Distribution of the fungus *Tilletia controversa* on winter wheat (number of locations in circle) and on *Elytrigia* sp.(number of locations without circles) in Slovakia

In the experiment dealing with the transfer of the fungus from *Elytrigia* to wheat the germinating wheat was infected with germinated chlamydo-spores and sporidia of the fungus on young (ca 4 mm high) coleoptiles which tops had been cut by razor-blade. Afterwards infected plants were planted into a hot-bed.

Minimum, optimum and maximum temperatures for chlamydo-spore germination were studied in air-conditioned chambers. Microscopical measurements were performed by light microscopy and microphotography with a scan electron microscope Tesla BS 301. The whole methodical procedure has been the same as mentioned later in this publication, where details of data can be found.

RESULTS

The fungus *T. controversa* was found as a pathogen on winter wheat (*Triticum aestivum* L.), *Elytrigia repens* (L.) Desv. and *Elytrigia intermedia* (Host.) Nevski in Slovakia. Its occurrence on the species of the genus *Elytrigia* is rare, while the occurrence of the mentioned fungus on wheat is known from numerous locations, especially in middle and higher elevations. *T. controversa* does not or only sporadically occur on wheat in the lowland areas of Záhorská nížina, Podunajská nížina and Východoslovenská nížina. Its occurrence was established neither in wheat stands on the river Váh alluvium nor in the district Poprad.

T. controversa has so far been detected at 781 locations of western (101), central (373) and eastern (307) Slovakia in 35 districts (Fig. 1). A register of locations with the intensity of the fungus' occurrence is presented in the papers mentioned in the methodical part. At most of the locations the fungus occurred scattered. At strongly infected locations its distribution has adapted a more distinct area. Tab. 1 shows the ascertained intensities of the fungus occurrence from some strong infected locations.

Tab. 1. Mean number and percentage of smutty spikes in strong infested winter wheat stands in Slovakia

Location district(year)	Mean number of spikes/m ²				% of smutty spikes	n
	total	smutty				
	x	x	s _x	s		
Vyšný Mirošov Svidník 1982	697.60	143.40	21.03	47.03	26.58	5
Sverepec Pov.Bystrica 1988	695.50	245.80	15.88	50.21	35.55	10
-Poniky Ban.Bystrica 1988	459.20	232.20	17.61	39.38	50.11	5
Čerín Ban.Bystrica 1989	543.80	160.60	26.65	59.59	29.14	5
Hradná Žilina 1988	435.40	88.60	10.87	24.32	20.88	5
Súfov Žilina 1991	488.60	111.00	20.47	45.77	22.51	5
Kotešová Žilina 1991	493.00	89.80	13.14	29.50	17.60	5
Súfov Žilina 1993	599.40	131.40	30.42	68.02	22.27	5

Occurrence of *T. controversa* on species of the genus *Elytrigia* was detected at 3 locations: Malý Horeš and Malý Kamenec in the district of Trebišov (eastern Slovakia) and in the cadastral territory of the town Stupava (Mást 1) on the

southwestern slope of hill Vrchná Hora in the district of Bratislava-country (western Slovakia). These locations are of a xerothermic character and are currently uncultivated. During the last 15 years and at Vrchná Hora during the last 4 years, couch grass (*Elytrigia repens*) at these locations was strongly infected with *Tilletia* every year. At Vrchná Hora the centre of the fungus' occurrence was lately strongly affected when bungalows were built. *T. controversa* could not be found in wheat stands cultivated in the near and far distance from mentioned locations. Even host plants from the *Poaceae* family growing at the site among infected *Elytrigia* have been not infected.

Our experiments dealing with transfer of the fungus from *Elytrigia* to wheat by artificial infection were unsuccessful.

Morphological and ecophysiological characteristics of the fungus *T. controversa* population in Slovakia are represented in Tab. 2. Variability of chlamyospore diameter and thickness of the hyaline sheath are shown in Figs. 2 and 3. Fig. 4 presents the morphology of reticulate and smooth chlamyospores.

Tab. 2. Morphological and ecophysiological characteristics of the fungus *Tilletia controversa* Kühn in Slovakia

Characteristic	Values
spore shape	globose to subglobose
percentage of aspherical spores	0 - 13
spore diameter	17.70 - 22.23 μm
number of meshes per spore diameter	3 - 8
thickness of hyaline sheath	1.4 - 2.4 μm
percentage of hyaline spores	0 - 4
spore mass	semi-agglutinated
color of spore mass	brown to blackish brown
odour	of trimethylamin
dormancy period	28 - 30 days
temperature for germination	min. 1, opt. 5 - 8, max. 12°C
special condition for germination	light
spore vitality	6 - 8 years
identified physiological races	D - 16
symptoms of infected plants	host dwarfing

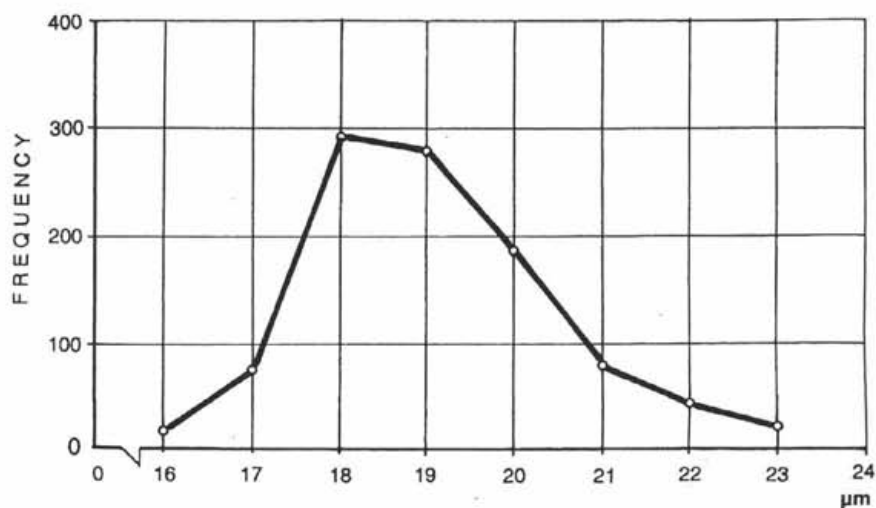


Fig. 2 Variability of chlamyospore diameter (*T. controversa*)

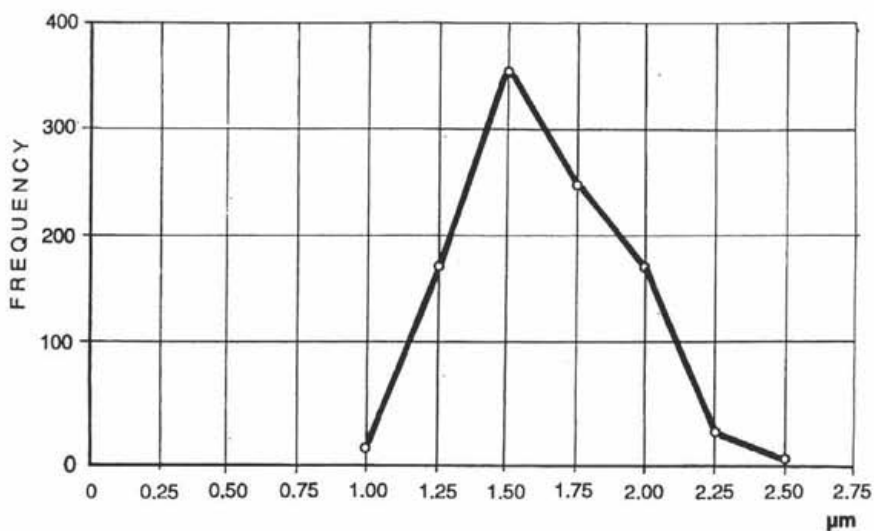


Fig. 3 Variability of thickness of hyaline sheath (*T. controversa*)

DISCUSSION

The great intensity of occurrence and the distribution of *T. controversa* in Slovakia enables us to express the opinion, that the mentioned species already occurred in our country in past times. According to the dwarf growth and the inclination to increased formation of offshoots of infected plants the fungus has been known to cultivators since the end of the last century or the beginning of this century (K. Klučka, 1958 – personal information). It was considered as common bunt (*Tilletia caries* (DC.) Tul.) recorded as *Tilletia tritici* (Bjerk.) Winter in the past. Chemical treatments used for wheat seed against the genus *Tilletia* have been effective only against *T. caries* (DC.) Tul. and *T. laevis* Kühn. This facilitated the mentioned fast spreading of the pathogen and an undisturbed development. The technology of wheat cultivation also contributed to its spreading widely in our country during the last decades (Paulech 1984). Its spreading was only limited by less suitable or unsuitable climatic conditions of some areas, or sometimes wheat growing sites (Paulech 1967).

In the mentioned lowlands of Slovakia free of bunt, wheat germinated at higher temperatures which are unsuitable for the germination of bunt chlamydospores. Higher soil humidity than on the neighbouring slopes of the river Váh alluvium speeds up the wheat germination and makes infection difficult. In areas with early winter frost (e.g. in the district of Poprad) infection is limited by frost drying out germinating chlamydospores or sporidia before they are able to penetrate into the tissues of germinating plants. For successful infection it is necessary that the infection stage of the fungus (germinating sporidia) matches the responsive stage of the host plants (wheat germination or a short time after germination) (Paulech 1967).

All four locations of *T. controversa* occurring on species of the genus *Elytrigia* (Bäumler 1927, Paulech and Maglocký 1988, Paulech 1992) described up to now are situated in districts without a common occurrence of the mentioned fungus on wheat. The ecophysiology of chlamydospore germination coming from *Elytrigia* sp. is similar to that of chlamydospores from infected wheat. Interactions between the fungus and its host plants and environmental conditions in our climate have not yet sufficiently been explained. From the results obtained till the present day follows that the populations of *T. controversa* in our country are made up of numerous specialized forms. We can state that on wheat another specialized form was found than on species of the genus *Elytrigia* (Paulech and Paulech 1991). Even lower systematic units – physiological races are formed on wheat. We have been able to identify the physiological race of D-16 (Paulech et al. 1993a) in Slovakia.

The ascertained morphological and ecophysiological characteristics of the studied species (Tab. 2) are basically similar to populations occurring in neighbouring countries (Pichler 1951, Podhradszky 1957, Uljanišev 1968, Kochman and Majew-

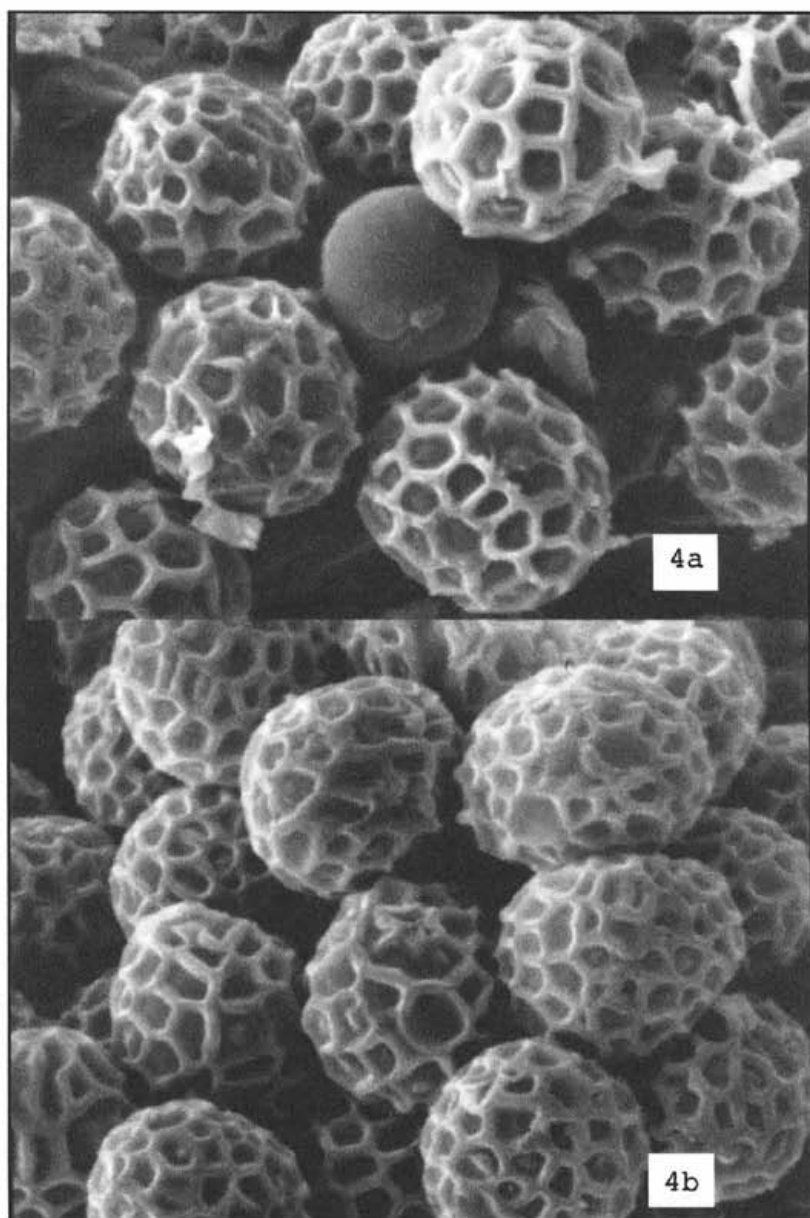


Fig.4 Morphology of *T. controversa* chlamydospore surface from *Triticum aestivum* (a) and *Elytrigia* sp. (b). SEM, Magnific. 3 000x

Photo by J. Blahutiaková

ski 1973, Jakubcová 1989). The general extension and the intensity of occurrence of *Tilletia controversa* on wheat and *Elytrigia* stands shows that soils of some locations indicate extensive chlamydospore infestation in Slovakia. Because of their many years' vitality (6-8 or more years) they are still a serious source of infection during the coming years. After the delimitation of its distribution area and obtaining basic knowledge on its characteristics it will be necessary to continue the study of *T. controversa* in our country. Further ecophysiological knowledge (e.g. the race spectrum of our populations) might be needed for possible wheat improvement with the respect to its resistance to the fungus.

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**Dacryonaema rufum (Basidiomycota)
neu für die Slowakei (Westkarpaten)**

EVA LISICKÁ

Slowakisches Nationalmuseum, Vajanského nábr. 2,
SK-814 36 Bratislava, Slowakei

Lisická E. (1995): *Dacryonaema rufum*, a new species for the Slovakia (Západní Karpaty Mts.– Czech Mycol. 48: 217–220

Dacryonaema rufum (Fr.) Nannf. (Basidiomycota, Dacrymycetaceae) is recorded for the first time from Slovakia (West Carpathians: Velká Fatra and Vysoké Tatry Mts.).

Key words: Basidiomycota, Dacrymycetaceae, *Dacryonaema rufum*, Slovakia

Lisická E. (1995): *Dacryonaema rufum* (Basidiomycota), nová pre Slovensko (Záp. Karpaty: Velká Fatra a Vysoké Tatry).– Czech Mycol. 48: 217–220

Dacryonaema rufum (Fr.) Nannf. sa zaznamenala prvýkrát na území Slovenska (Záp. Karpaty: Velká Fatra a Vysoké Tatry).

EINLEITUNG

Im frühen Herbst 1992 habe ich auf Holzbalken einer alten, halbverfallenen Heuscheune in dem Velká Fatra Gebirge Flechten gesammelt. Dabei wurde auch ein Pilz mitgenommen, dessen Fruchtkörper kleinen, zugespitzten Stiften ähnelten. Später wurde er von Prof. Dr. J. Poelt als *Dacryonaema rufum* (Fr.) Nannfeldt bestimmt.

Die Art wurde von E. Fries in 1823 als *Sphaeronaema rufum* und von Saccardo in 1884 als *Sphaeronaemella* klassifiziert (sec. Kennedy 1958). Nannfeldt (1947: 336, sec. Poelt and Michelitsch 1982) analysierte als erster fertile Fruchtkörper und konnte feststellen, daßes sich um einen Vertreter der Dacrymycetaceen handelt, den er *Dacryonaema rufum* nannte. Nach Jülich ("1981" 1982, sec. Poelt and Michelitsch 1982) wird die Familie in das System der Basidiomyceten folgendermaßen eingeordnet: Abt. Basidiomycota, 1. Kl. Heterobasidiomycetes, Unterkl. Dacrymycetidae, Ord. Dacrymycetales, Fam. Dacrymycetaceae.

BESCHREIBUNG

Die Morphologie und Anatomie von der monotypischen *Dacryonaema rufum* wird bei Nannfeldt 1947, und Poelt and Michelitsch 1982 (anhand der Proben aus den Alpen) eingehend beschrieben, illustriert und diskutiert. In den folgenden

Zeilen werde ich deshalb den Pilz nur kurz und stichhaltig beschreiben (anhand der fertilen Probe aus der Hohen Tatra: Bránka).

Junge Fruchtkörper zunächst in Form kleiner Pusteln, individuell im Substrat verankert, später schlank birnförmig, der basale Teil etwas in das Holz eingesenkt. Sterile Stiele bis 1 (-1, 1) mm hoch, Durchmesser des basalen Teiles bis 0, 5 mm, Durchmesser des apikalen Teiles bis 0,1 mm (Abb. 1. A.). Bei den fertilen Fruchtkörpern bilden sich die apikalen Köpfchenverdickungen schon sehr früh (Abb. 1. A.). Fruchtkörper knorpelig, homogen, von dickwändigen Hyphen mit engen Lumina. Im Stiel laufen die Hyphen \pm parallel, im Köpfchen biegen sie sich radial nach außen. Fruchtkörper rostrot bis rotbraun-schwärzlich, rötlich durchscheinend und – besonders in der oberen Hälfte – stark glänzend. Diese "lackierte" Oberfläche ist durch die Agglutination der gelatinösen Oberflächenhyphen verursacht (Kennedy 1958: 885) und bewirkt auch das häufige Zusammenkleben von mehreren Fruchtkörpern. Die Köpfchen tragen das Hymenium. Bei unserem Material waren die größeren, älteren Fruchtkörpern ohne Hymenium, die Oberfläche der Köpfchen verunebnet (Abb. 1. B.). Ich nehme an, daßes Fruchtkörper vom vorigen Jahr waren. Nur an einigen wenigen, kleineren, wahrscheinlich diesjährigen (=1992) Fruktifikationen konnte man ein spärliches Hymenium beobachten (etwas behaarte Köpfchen). Die Oberfläche eines voll entwickelten Hymeniums soll farinös erscheinen (Poelt and Michelitsch 1982). Das konnte man hier nicht sehen, da die Köpfchen das Vollreifestadium vermutlich noch nicht erreicht hatten. Basidiosporen gekrümmt-zylindrisch, dünnwandig, hyalin, mit mehreren Öltröpfchen, oder einem großen Öltropfen μm 6, 3-9,4 \times 2,4-3,1 μm messend (Poelt and Michelitsch 1982 geben größere Sporenmaße an: 8,6-12,3 \times 3,0-5,0 μm). Septierte Sporen konnte ich nicht beobachten (cf. Poelt and Michelitsch 1982).

VORKOMMEN UND VERBREITUNG

Dacryonaema rufum wächst auf angemorschten, rindenlosen Stümpfen und Stämmen von *Picea abies* und auf verarbeitetem Holz. Sie kommt an \pm lichten, jedoch vor Austrocknung geschützten Standorten vor. Als Begleiter habe ich folgende Flechten notiert: *Cladonia* cf. *digitata* (juv.), *Hypocenomycce caradocensis*, *H. praestabilis*, *H. scalaris*, *H. sorophora*, *lecanora conizaeoides*, *Parmeliopsis ambigua*, cf. *Porpidia tuberculata* (det. J. Poelt) und *Trapeliopsis granulosa*. Die Art wurde in der montanen Stufe der Karpaten (etwa 1100 – 1490 m) gesammelt. Bisher sind nur wenige Fundorte bekannt, ich nehme jedoch an, daßes, ähnlich wie in den Alpen, weitverbreitet, aber bisher übersehen wurde.

Der Pilz ist verbreitet in nordlichen Europa (Nannfeldt 1947), in British Columbia (Brough and Bandoni 1975, sec. Poelt and Michelitsch 1982) und 1982 wurde es für Mitteleuropa neu nachgewiesen (Poelt and Michelitsch 1982).

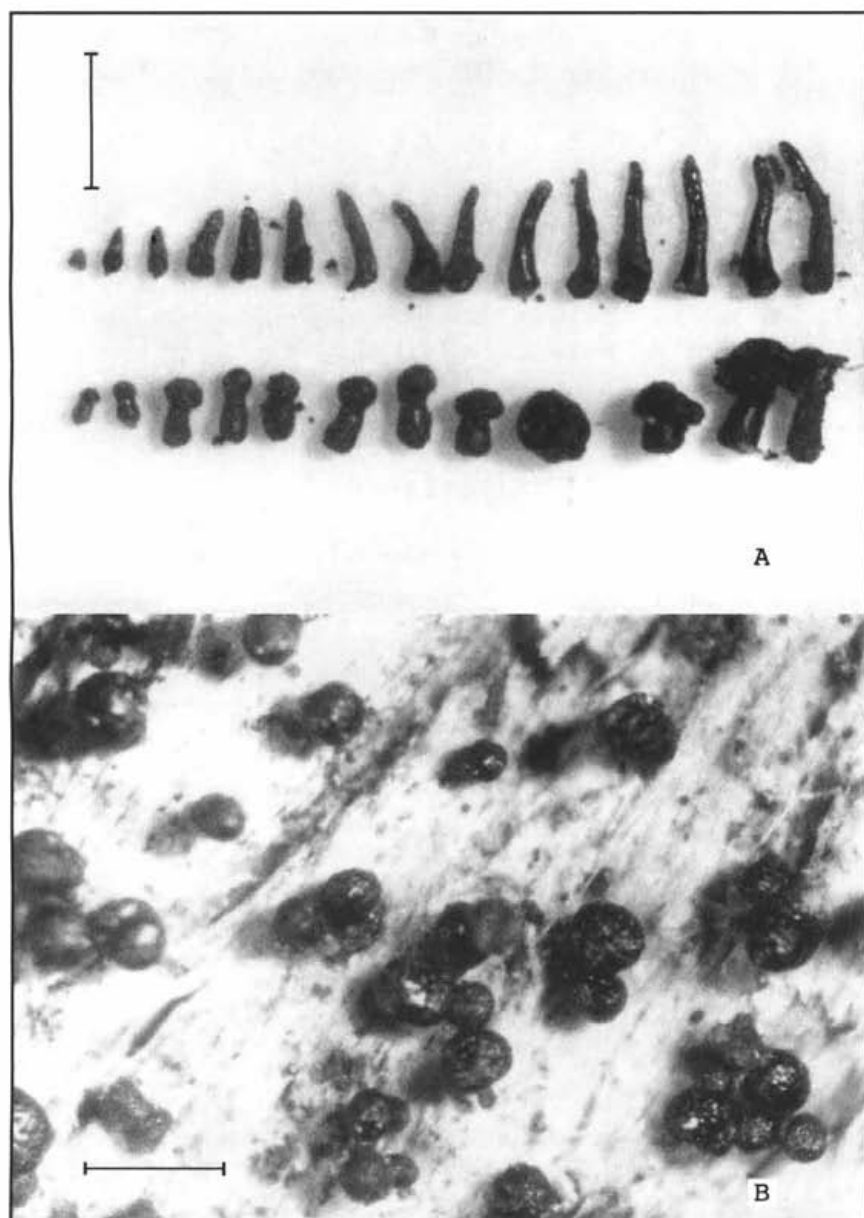


Abb. 1. *Dacryonaema rufum* (Fr.) Nannfeldt (Vysoké Tatry, Bránka). A. Sterile und fertile Fruchtkörper in Seitenansicht. B. Fruchtkörper mit Köpfchen von oben. Maßstrich 1 mm.

Slovakia centr.: Mts. Velká Fatra (6981): mons Tlstá hora, 1100 m, ad lignum faeniliorum, 3.9.1992 leg. E. Lisická, det. J. Poelt (BRA, GZU). **Slovakia sept.:** Mts. Vysoké Tatry (6787/c): Javorová dolina, loco Bránka dicto, ad truncum putridum (cf. *Picea abies*), 1200 m, 29.7.1994 leg. E. Lisická (BRA, PRM). – Ibid., Kolová dolina, ad truncum putridum (cf. *Picea abies*), 1490 m, 30.7.1994 leg. E. Lisická (BRA).

Die Nomenklatur der Flechten richtet sich nach Pišút et al. (1993).

D a n k s a g u n g

Diesen Beitrag widme ich dem Andenken an Prof. Dr. Josef Poelt (+ 3. VI. 1995). Ohne seine Hilfe wäre der Beitrag nicht entstanden.

Mein Dank gebührt auch Herrn Dr. Z. Pouzar, CSc. (Prag) für wertvolle Informationen bezüglich der Verbreitung und Fr. M. Škultétyová für die Anfertigung der Photographie.

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Actual state of the rust fungi systematics in the world

ZDENĚK URBAN

Department of Botany, Charles University Benátská 2, Praha 2, CZ 128 01

Urban Z. (1995): Actual state of the rust fungi systematics in the world.- Czech Mycol. 48: 221-224

The article was presented at the 7th International Congress of Mycology Division (IUMS-94) in Praha, July 3-8, 1994. The rust fungi are damaging for many important crops. Many rust species possess complicated life cycle. Up to now the most effective method has appeared to be the production of rust resistant cultivars and biological control on integrated basis. Therefore the thorough knowledge of taxonomy, biology and ecology of not only economically important rusts but also potentially harmful rust species on wild plants is highly desired. Especially in tropics and subtropics the rust taxonomy and ecology is known imperfectly. The author informs briefly on steps concerning the improvement of rust systematics organized at the rust symposia at International Mycological Congresses (IMC) in Tampa (USA) 1977, Tokyo, 1983 and Regensburg (BRD) 1990. In the international cooperation it should be prepared, through computers and videodisc technology, the "World data base for plant rust fungus species". Most limiting factors are: to find leaders in relatively rich and major institutions and funds from national and international organizations.

Key words: Rust fungi (Uredinales), systematics in the world, improvement.

Urban Z. (1995): Současný stav studia systematiky rzí (Uredinales) ve světě.- Czech Mycol. 48: 221-224

Článek je příspěvkem předneseným na 7. mezinárodním kongresu mykologického odboru (IUMS - 94) v Praze 3. až 8. července 1994. Rzi jsou paraziti mnohých plodin. Mnohé druhy mají komplikovaný životní cyklus. Nejúčinnější způsob boje je šlechtění k odolnosti a integrovaná ochrana. Proto je třeba dokonale poznat taxonomii, biologii a ekologii rzí nejen na plodinách, ale i na planých rostlinách. Taxonomie a ekologie rzí je nedokonale známá především v tropech a subtropích. Autor referuje o úkolech a možnostech uredinologů, které byly diskutovány na symposiích o rzích na Mezinárodních mykologických kongresech (IMC) v Tampě (USA) 1977, Tokiu, 1983 a v Řezně (BRD) 1990. V rámci mezinárodní spolupráce je třeba připravit, za pomoci počítačů a videodiskové technologie, „Banku základních údajů o rzích světa“. Největší problém je: získat vedoucí pracoviště a domácí i mezinárodní finanční podporu.

The rust fungi (Uredinales, Basidiomycetes) are obligate parasites of vascular plants. The mycelium is intercellular, well protected against the exterior environment. The life cycle of many species is complicated as displayed by the production of up to 5 various spore states. Moreover, there are many species which in their complete life cycle possess host alternation, i.e. their life cycle is completed on two host plants belonging to families which are very distant from each other. Especially the subtropics and tropics are full of rust species, the life cycle of which is, however, more or less shortened.

In the period 1903-1924 was published a monumental work by Paul and Hans Sydow: *Monographia Uredinearum*. It offers a survey of all so far described rust

species from over the world. For some rust species, most of all in heteroecious ones, very briefly their life cycle was given. These four volumes, in Latin and German, long out of date, still form the indispensable starting point for all serious taxonomic studies of rusts.

The rust fungi are serious pathogens in that they attack economically important crops such as cereals, legumes, coffee, timber etc. The losses on yield and profit amount to millions! With regard to the facts mentioned above rust control is very complicated and difficult. Up to now the most effective method has appeared to be the production of rust resistant cultivars of crops and trees. This aim can be attained only when the total of rust species living in all climatic belts but especially in the subtropics and tropics is thoroughly recognized. This is the objective of rust fungi systematics which must compile the basic knowledge of rust life cycles, their biology, ecology and mutual taxonomic relationships which is very often presented as infraspecific variability. A thorough knowledge of rusts, first of all those being parasites of wild plants, can provide the basis of a technology for controlling on a biological and integrated basis various stubborn weeds in crop plantations.

The first steps concerning the improvement of rust systematics study were made at the rust symposium organised at IMC 2 1977 in Tampa, Florida. Except for the points just mentioned the discussion concentrated on the urgency of international cooperation in preparing lists of uredinologists, major rust herbaria, rust genera and species including illustrations of these and lists of host plants. In addition to this it would be very important to increase the number of rust specialists working on rust taxonomy, phylogeny, ontogeny, and ecology of various important rust species and to interest additional workers to contribute to rust studies by using non-traditional investigation methods such as electron microscopy, cultivation and preservation of rust *in vitro*, molecular analyses etc. Moreover, it was stated that the rust exploration in the subtropics and tropics is completely insufficient and that the preparation of local, regional or subcontinental rust floras should be incited. When preparing such floras all data and experience concerning the geographic distribution and history of both rusts and their hosts and all ecological knowledge on relations of rusts to their environment should be exploited.

At IMC 3 1983 Tokyo not only a rust symposium was organized but also a special workshop for those interested in rust fungus systematics and phylogeny; among others, information was presented on what progress had been made in the questions discussed in Tampa 1977. The conclusion was that little progress had been achieved, if any. In addition the need to unify the terminology of rust life cycles was questioned. This is substantial when studying tropical and subtropical rusts in order to speak the same "language" in terms that cover not only the morphology but also the karyotic states of ontogenetic rust stages.

At IMC 4 1990 in Regensburg another workshop was organized under the title "Present activities and future networking of taxonomic work on Uredinales in the

world". Joe F. Hennen (Purdue, Lafayette, USA) and Yasu Hiratsuka (Edmonton, Canada) are to be praised for the preparation of the meeting. The latter opened the session with the paper "Current studies on taxonomy of Uredinales and future opportunities for world-wide networking". Very suggestive was the paper by M. Kakishima (Univ. Tsukuba, Japan): "Data base of Japan rust fungi"; this is the basic prerequisite for the research project "The rust flora of Japan". The discussion was supported by printed materials prepared by Y. Hiratsuka: List of monographs and descriptive manuals, List of major herbaria containing important rust collections, List of rust taxonomists of the world.

During the next year the aforementioned managers sent away invitations to cooperate on the project "World data base for plant rust fungus species". In addition to the programme earlier mentioned the call stresses the idea that through computer and perhaps videodiscs technology a contemporary work can be created containing descriptive, illustrative, nomenclatural, host, voucher (type) specimen and geographic distribution data on all known rusts. When completed, the work should stimulate further research by locating weaknesses and gaps in biosystematic uredinology.

Through the kindness of Y. Hiratsuka I got recent information on the current state of the project. The call mentioned received a fairly good response of possible cooperation and support. Unfortunately, most uredinologist interested stated that they cannot participate in a significant way because of their professional involvement in other activities. In the following I am giving a joint summary by Y. Hiratsuka and J. F. Hennen who discussed the matter a few times. Although reasonably good regional and national floristic treatments are available in Europe, North America, Japan and several countries in S. America and Asia, sufficient information is lacking in most of S. America, Mexico, Africa, the Carribean Is., the tropical Pacific Is., Southeast Asia and the Middle East. - Several projects using molecular-biological approaches in rust taxonomy and phylogeny are in progress and interesting results have been reported. However, these new approaches need to go hand-in-hand with classical morphology. In connection with this the cooperation of Purdue University (Arthur Herbarium) with the University of California (Berkeley), and Tel Aviv University with the University of Minnesota are encouraging. - Very few graduate students (if any) are now conducting taxonomic work on rusts in the world and few young uredinologists are interested in rust systematics. With the retirement of many active uredinologists in recent years and more to come in the next few years this situation will come critical. - However, with new interest in and emphasis on biodiversity and sustainable development of biota, floristic work on rusts of underexplored areas of the world can be supported well if good initiatives are proposed by strong groups. Several uredinologists in Japan (Kakishima, Ono etc.) are very active in collecting and studying rusts of various areas in Asia. Significant collections were also made in Brazil and other neotropical

regions in recent years. – The biggest problem to pursue the task of compiling world flora of rusts now is the leadership. We need to have strong leaders in major institutions such as Arthur Herbarium, IMI, New York Botanical Garden and some well established institutions who can devote enough time and effort to the project with funds acquired from national and international organizations. Unfortunately, Purdue University does not want manage the Arthur Herbarium in the future. Maybe, however, that a way will be found to move the Herbarium and the curator to another institution.

What to say at the end? Of all large groups of fungi the Uredinales are probably well documented and described. If the aforementioned target and long-term goals should be achieved only one very important condition must be fulfilled: have leaders in relatively rich and major institutions which will manage the international project "World monograph of Uredinales" with funds acquired from national and international organizations.

Present state, modern methods and perspectives in penicillium study

Abstracts from the Penicillium Seminar,
June 9, 1994, Prague, Czech Republic

The seminar was held by Czech Scientific Society for Mycology, Division of Micromycetes. At this one-day regional seminar ten Czech and Slovak scientists presented the papers cited below. The proceedings (in Czech with English abstracts) of "Present State, Modern Methods and Perspectives in Penicillium Study" are available at the secretary of the Division of Micromycetes: K. Prášil, Department of Botany, Faculty of Natural Sciences, Charles University, Benátská 2, 128 01 Praha 2, Czech Republic.

Study of the genus *Penicillium* – history and new approaches

Alena Kubátová

*Department of Botany, Faculty of Natural Sciences, Charles University,
Benátská 2, 128 01 Praha 2, Czech Republic*

The history of the study of *Penicillium* is presented. Study of herbarium specimens during the 19th century was followed by culture techniques (Brefeld, Dierckx, Sopp, Bainier, Westling, Biourge, Zaleski). A very important manual of penicillia was prepared by Raper and Thom (1949). The morphological taxonomic base was broadened by Abe (1956) using some physiological characters. The nomenclatural and taxonomic problems connected with Raper's and Thom's approach were resolved by Pitt (1979) and other taxonomists. Nevertheless, the problem of a species concept was not cleared. Great progress has been made from the 1980s. A multidisciplinary study of terverticillate penicillia started at CMI in Kew. The computer assisted key PENIMAT was developed. Frisvad and collaborators broadened the taxonomic base by adding other physiological characters and profiles of secondary metabolites. Collaborative studies on the international level were achieved. At the present time the taxonomic value of new biochemical and genetic methods is investigated. The current situation seems to lead towards a stability of names.

Nomen conservandum in the genus *Penicillium*

Olga Fassatiová

*Department of Botany, Faculty of Natural Sciences, Charles University,
Benátská 2, 128 01 Praha 2, Czech Republic*

During the workshop in Baarn (The Netherlands) in the year 1989 the specialists of the genus *Penicillium* and *Aspergillus* have designated the name *Penicillium*

chrysogenum Thom as nomen conservandum in view of its historic and economic importance. Otherwise, *Penicillium chrysogenum* ought to be classified as a synonym of the species *Penicillium griseoroseum* Dierckx.

Species concept in some *Penicillium* species

Alena Kubátová

Department of Botany, Faculty of Natural Sciences, Charles University, Benátská 2, 128 01 Praha 2, Czech Republic

The present species concept of some penicillia different from those of Pitt (1979) is discussed. Attention is paid to the paper of Pitt et al. (1990) dealing with closely related species *P. glabrum* (syn. *P. frequentans*), *P. spinulosum*, *P. purpurescens* and *P. montanense*. The present position of *P. janczewskii* (syn. *P. nigricans*) and *P. albidum* (nomen dubium) is pointed out. Some Czech isolates of "*P. albidum*" were re-identified as *P. janczewskii* and *P. daleae*. The species concept of *P. simplicissimum*, *P. brasilianum* and *P. janthinellum* is presented and compared with literature. The present species concept of *P. miczynskii*, *P. soppii* and *P. manginii* is discussed. Changes in the species concept of terverticillate penicillia are demonstrated (*P. aurantiogriseum*, *P. verrucosum*, *P. solitum*, *P. commune* etc.). The position of the species *P. minioluteum* is mentioned.

Study of the genus *Penicillium* in the Czech and Slovak Republics and survey of reported species

Alena Nováková

Institute of Soil Biology, Academy of Sciences, Na sádkách 702, 370 05 České Budějovice, Czech Republic

Alena Kubátová

Department of Botany, Faculty of Natural Sciences, Charles University, Benátská 2, 128 01 Praha 2, Czech Republic

Three lists of penicillia and associated teleomorphs reported from the Czech and Slovak Republics with their bibliography are presented.

The first list contains the findings of penicillia and teleomorphs from soils: 85 *Penicillium* species names in current use, 9 species of the genus *Talaromyces*, 6 species of the genus *Eupenicillium*, and other names of uncertain application. The most frequent species from soils appear to be *P. albidum*, *P. aurantiogriseum*, *P. brevicompactum*, *P. camembertii*, *P. canescens*, *P. chrysogenum*, *P. citrinum*, *P. commune*, *P. expansum*, *P. glabrum*, *P. janczewskii*, *P. purpurogenum*, *P. restrictum*, *P. rugulosum*, *P. simplicissimum*, *P. spinulosum*, and *P. variabile*. On

the other hand, *P. adametzii*, *P. arenicola*, *P. brasilianum*, *P. capsulatum*, *P. coprophilum*, *P. cyaneum*, *P. hordei*, *P. italicum*, *P. megasporum*, *P. rubefaciens*, *P. digitatum*, *P. nalgiovense*, *P. islandicum*, *P. scabrosum* and *P. soppii* were rarely isolated from soils. *P. brasilianum*, *P. italicum* and *P. melinii* represent species with an interesting occurrence.

The second list contains findings from other substrates (e.g. air, foods, feeds, etc.): 78 *Penicillium* names in current use, 6 species of *Eupenicillium*, 5 species of *Talaromyces*, several findings of other genera and other names of uncertain application. The most frequent species are the following: *P. chrysogenum*, *P. aurantiogriseum*, *P. expansum*, *P. glabrum*, *P. brevicompactum*, *P. citrinum*, *P. viridicatum*, *P. purpurogenum*, *P. variable*, and *P. janthinellum*. The following penicillia were recorded rarely: *E. crustaceum* (as *P. asperum*), *E. lapidosum* (as *P. lapidosum*), *E. ochrosalmoneum* (as *P. ochrosalmoneum*), *P. bilaiae*, *P. brasilianum*, *P. manginii* (as *P. atrosanguineum*), *P. melinii*, *P. piscarium*, *P. resedanum*, *P. sublateritium*, and *P. westlingii*. The first published find from this area is *P. expansum* by Opiz (1823). The oldest illustration of *Penicillium* species is probably that by Corda (1839), which represents *P. fieberi*.

A list of all *Penicillium* species finds including synonyms is added. Altogether 91 *Penicillium* species names in current use, 11 species of *Eupenicillium*, and 9 species of *Talaromyces* were recorded from the Czech and Slovak Republics.

Some rare penicillia and related genera

Ludmila Marvanová

Czech Collection of Microorganisms (CCM), Masaryk University,
Tvrdeho 14, 602 00 Brno, Czech Republic

The macro- and micromorphology of *Penicillium arenicola*, *P. brunneum*, *P. dupontii*, *P. inflatum*, *P. islandicum*, *P. minioluteum* and *P. olsonii* is briefly described. Differences between *Aspergillus*, *Eladia* (*E. saccula*), *Geosmithia* (*G. cylindrospora*, *G. emersonii*), *Gliocladium*, *Merimbla* (*M. ingelheimensis*), *Metarrhizium*, *Paecilomyces*, *Phialocephala*, *Scopulariopsis* and *Thysanophora* against *Penicillium* are pointed out.

Identification of *Penicillium* species using the production of mycotoxins

D. Veselá and D. Veselý

Institute of Experimental Medicine, Academy of Sciences, 517 83 Olešnice
v Orlických horách 14, Czech Republic

Penicillium is a widely distributed genus represented by more than 300 different species. A good guide for the difficult identification of *Penicillium* species and for

the assessment of the risk of their appearance is the production of mycotoxins. The method is illustrated by results of the monitoring of the *Penicillium* genus in the working environment of the Příbram uran mine. In 70 swabs of the workplace and 116 laryngeal swabs of miners 103 different penicillia belonging to 26 species were found. In 57 isolates the production of mycotoxins was found in liquid medium, as identified by thin layer chromatography (brevianamide A, citreoviridin, citrinin, curvularin, carolic acid, griseofulvin, chaetoglobosin A, mycophenolic acid, secalonic acid D, patulin, penicillic acid, rugulosin, xanthomegnin and viomellein). Chloroform extracts of 18 penicillia were toxic for 42 hours old chick embryos. However, toxic metabolites were not identified. The production of mycotoxins different from literature data was found in the case of *P. aurantiogriseum* (chaetoglobosin A), 7 isolates of *P. fellutanum* (carolic acid) and 15 isolates of *Penicillium* spp. (curvularin).

The genus *Penicillium* in the pathogenesis of some respiratory diseases

Alena Tomšíková

*Institute of Microbiology, Faculty of Medicine, Charles University,
Dr. E. Beneše 13, 305 99 Plzeň, Czech Republic*

As we have determined during 30 years investigation of patients from agriculture and different industries, many species of the genus *Penicillium* take part in the pathogenesis of some respiratory diseases (bronchitis, bronchial asthma, the farmer's lung syndrom) in our countries .

The sources were found partly in penicillia occurring out-doors, in-doors, on walls and objects of rooms (which was studied daily during 3 years), partly in penicillia occurring on the mucous membranes of men. *Penicillium decumbens* dominates in this study.

Penicillia has prevailed in previous years, especially in bronchial asthma, later in immunocompromised patients. The participation of penicillia in the pathogenesis of these diseases was verified partly according to immunological reactivity of patients, partly in experiments on rabbits. After the inhalation of hay particules contaminated by *P. decumbens*, severe bronchopneumonia accompanied by high levels of specific antibodies, as well as by allergic necrotising vasculitis, was developed in experimental animals.

For this reason we suppose that some species of the genus *Penicillium* should be considered as opportunistic pathogens.

However, there is also one strictly pathogenic species in the genus *Penicillium*, the dimorphic *P. marneffeii*, which is endemic in Southeast Asia. It has been recognised since 1973 as an agent of natural infections among immunocompromised patients travelling in Southeast Asia. It evokes a very severe disseminated, mostly

fatal disease, resembling disseminated histoplasmosis. This *Penicilliosis marneffeii* must be included in the clinical definition of AIDS, like other systemic mycoses (histoplasmosis, cryptococcosis, coccidioidomycosis) in defined endemic areas.

Some teleomorphs of *Penicillium* sp., their heat-resistance, occurrence and importance

Zdenka Jesenská and Elena Piecková

Institute of Preveance and Clinical Medicine, Limbová 14, 833 01 Bratislava, Slovak Republic

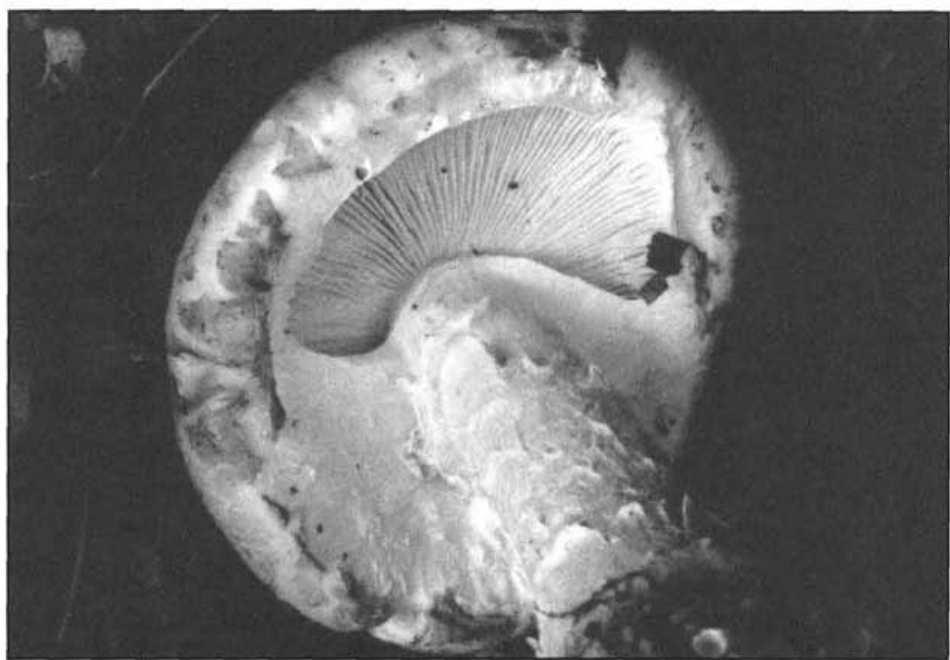
The paper deals with the modern taxonomy, occurrence and heat-resistance of *Penicillium* sp. which were introduced as *Penicillium vermiculatum*, *P. bacillosporium*, *P. avellaneum* and *P. baarnense* in the monography of Raper and Thom (1949). These species are new heat-resistant ones and the strains were isolated from soil. Heat-resistant fungi are a subject for scientific investigation.

Human illnesses caused by toxins of *Penicillium* species

Jan Šimůnek

Department of Preventive Medicine, Masaryk University, Joštova 10, 662 44 Brno, Czech Republic

Acute cardiac beri-beri is certainly caused by citreoviridin, the mycotoxin of *Penicillium citreoviride*. The significance of *Penicillium* mycotoxins in the ethiology of toxic hepatitis, pulmonary mycotoxicosis, Balcan endemic nephropathy, Danish nephropathy and some human tumors is probable, but not definitely demonstrated. The possible significance of cyclopiazonic acid in the ethiology of Reye's syndrome in sucklings is discussed.





Amanita strobiliformis (Paulet: Vitt.) Bertillon, growing on calcareous soil in deciduous forest in warm regions of Czech Rep. Loc.: Praha-Velká Chuchle, SPR Chuchelský háj (Carpinion), 10 Sept. 1990.

Photo by J. Klán

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INSTRUCTIONS TO AUTHORS

Preparation of manuscripts. Manuscripts are to be submitted in English, German or French. The text of the manuscript should be written on one side of white paper (A4, 210 × 297 mm) with broad margins (maximum 30 lines per page). Each manuscript must include an *abstract* (in English) not exceeding 300 words and a maximum of five key words. The paper will be followed by an abstract in Czech (or Slovak). The journal is responsible, however, for the translation of abstracts into Czech for foreign authors. Please send *two copies* of the typescript. The authors are asked to submit diskettes with the *accepted manuscripts* prepared on IBM-compatible personal computers. The files should be in ASCII under DOS. Both HD and DD/3.5" and 5.25" diskettes are acceptable.

Illustrations and tables. All tables, black and white photographs and figures (in black indian ink on a separate sheet) combined with the legends should be self-explanatory. Legends to the figures must be typed on a separate sheet. Colour photographs can be accepted but the authors will be responsible for the costs. All drawings or a photographs of microstructures should be provided with a scale. All illustration should be submitted as the *original drawing and one clear copy*. Output from computer graphics programmes produced on plotters or laser printers is quite acceptable. The dimension of any figure should not exceed 180 × 260 mm in size. References to illustrative matter in the text should normally in parentheses, e.g. ... spore sizes (Table 1) and ... as shown in Fig. 2 ...

Nomenclature. Latin names should conform to the International code of botanical nomenclature. New taxa must be substantiated by a Latin diagnosis including a reference to the public herbarium where the type specimen is deposited. The authors are asked to use only the acronyms listed in the Index Herbariorum.

References. References are to be listed in alphabetical order according to the surnames of the first authors. The bibliography should be written as follows:

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Ryvarden L. (1978): The Polyporaceae of North Europe, Vol. 2. Inonotus-Tyromyces. – Oslo, 507 pp. (book)

Tommerup I. C., Kuek C., and Malajczuk N. (1987): Ectomycorrhizal inoculum production and utilization in Australia. – In: Sylvia D. M., Hung L. L., and Graham J. H. (eds.) Proceedings of the 7th North American Conference on Mycorrhizae, pp. 93–295, Gainesville.

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