

## AMAUROASCUS KUEHNII AND OTHER FUNGI ISOLATED FROM A DEER HORN IN POLAND

ANDRZEJ CHLEBICKI & WOJCIECH SPISAK

**Abstract.** Four keratinophilic fungi isolated from deer horn collected in Gorce National Park (Poland) are reported: *Amauroascus kuehnii* Arx with its malbranchea-like anamorph, *Isaria fumosorosea* Wize, *Mortierella elongata* Linnem., and *Penicillium spinulosum* Thom s.l. The record of *Amauroascus kuehnii* is its second locality in Europe, and keratin deer horn is a new substrate for this fungus.

**Key words:** *Amauroascus*, ascomycete, dermatophilic fungi, distribution, *Malbranchea*, mitosporic and anamorphic fungi

Andrzej Chlebicki, W. Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, 31-512 Kraków, Poland; e-mail: a.chlebicki@botany.pl

Wojciech Spisak, Research & Development Centre „Alcor” Ltd, Kępska 12, 45-130 Opole, Poland; e-mail: spisak@alcor.pl

### INTRODUCTION

Soil is a reservoir of diverse symbiotic, saprobic and pathogenic fungi, including dermatophytes. That latter group is characterized by the ability to produce proteolytic and keratinolytic enzymes capable of decomposing various keratinized structures such as hair, horn, feathers, wool, bone, hoof, claws and cornified (keratinized) epidermis (Otčenašek *et al.* 1967; Garg *et al.* 1985; Wawrzkiwicz *et al.* 1987). They can infect animals, which are the source of secondary infection to man (Ajello 1974), and some can produce antibiotics (Uri *et al.* 1957; Wright 1956).

Decomposition of keratin into proteins results in alkalization (Faterpekar *et al.* 2008). Animal horns are sometimes colonized by specific fungi of the genus *Onygena* Pers., such as *O. corvina* Alb. & Schwein. and *O. equina* (Willd.) Pers. (Hansen 1998). These species have been reported from several localities in Poland (Gumińska 1977; Komorowska 1986). They belong to the keratinolytic fungi *sensu* Hubálek (2000): that is, keratin-decomposing fungi. Other fungi can grow on keratinized tissues but are not able to decompose keratin. Here we report four fungi isolated from a deer horn collected in Gorce National Park, Poland, including the very rare

species *Amauroascus kuehnii* Arx reported previously only from a few stations in North America and Europe.

### MATERIAL AND METHODS

The fungi were extracted from deer horn with a sterile scalpel and then grown on MEA and PDA media in Petri dishes. The inoculated media were incubated at 16°C in the dark. Dibromothymolsulfonophtalein (bromothymol blue) was used to indicate the pH of the fungal products diffused into the medium: 10 drops of pigment solution and 10 drops of 10% KOH were added to 250 ml PDA at 80°C. This indicator changes the medium color depending on pH: green for the neutral range, yellow for slightly acidic, and blue for slightly basic. The morphological characters of the living fungi were examined in water and Lactophenol Cotton Blue by light microscopy (Nikon SMZ 1500, Nikon Labophot 2, Nikon Eclipse 800). Micrographs were taken with these microscopes fitted with a digital camera. For scanning electron microscope (SEM) studies, mycelium was fixed in 3% buffered glutaraldehyde (pH 7), washed twice in buffer for 10 min and dehydrated in ETOH (ethanol) and acetone, coated with gold and photographed using a LEO 1430 VP Zeiss microscope with a working distance of *ca* 10 mm.

## RESULTS AND DISCUSSION

Four species of fungi were isolated from the deer horn. Only one of them (*Amauroascus kuehnii*) is a keratinolytic species. The other three keratinophilic fungi (*Isaria fumosorosea*, *Mortierella elongata*, *Penicillium spinulosum*) cannot decompose keratin.

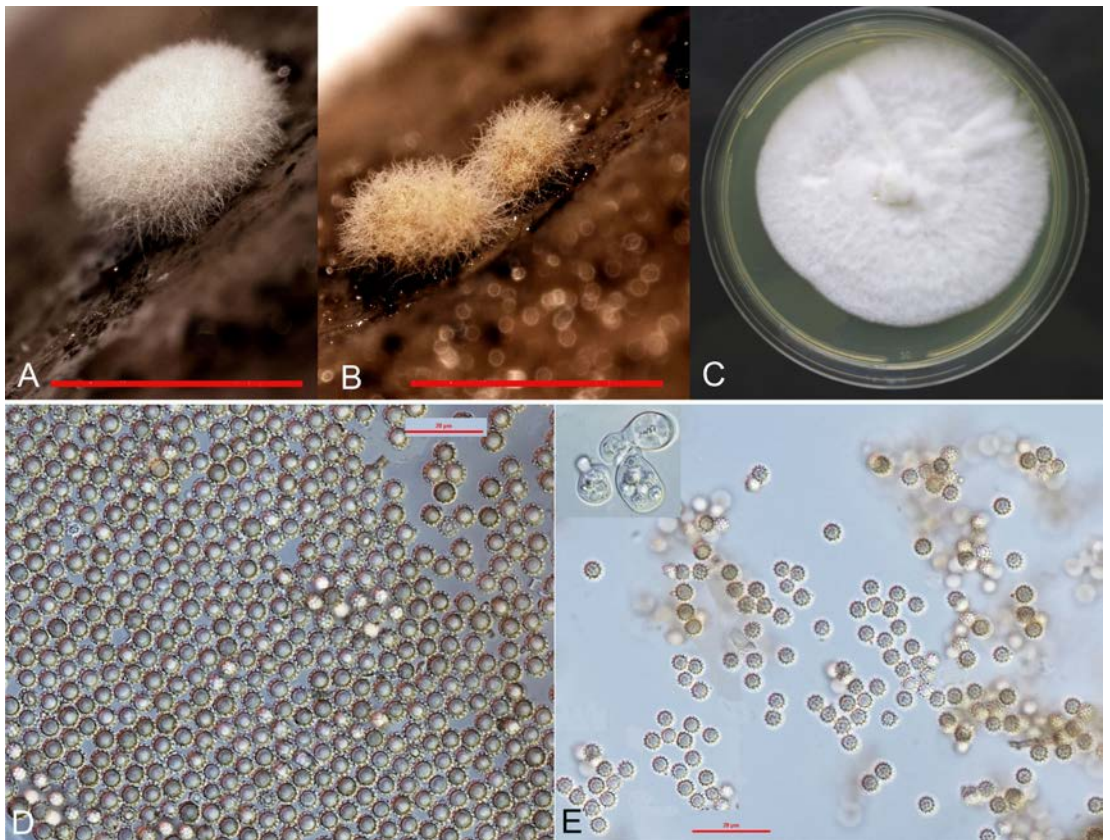
*Amauroascus kuehnii* Arx

Persoonia 6(3): 373. 1971. – *Auxarthron kuehnii* (Arx) M. Solé, Cano, Guarro, Mycol. Res. 106: 388. 2002.

TELEOMORPH. Ascomata gymnothecial, globose (Fig. 1A, B), pale yellow, superficial, solitary or rarely confluent, without distinct peridial elements, peridial hyphae thin-walled, pale brown, septate, encrusted with pigmented granules, branched, tips

blunt, asci saccate, (14–)15–17 × 9.5–11.5 μm, ascospores globose, alveolate, reticulate with raised bands and ± oval meshes with undulating surface, 4–5 μm diam. (Fig. 1D, E & 2), ca 13 bands per ascospore diameter.

ANAMORPH (malbranchea-like). Colonies on PDA at 18°C reaching 68 mm in 24 days, white on whole surface, aerial mycelium cottony, ca 2 mm high, after month ca 6 mm high (Fig. 4A), with small umbo in center, pale yellow droplets of exudates appear after two weeks, white sectors of dense mycelium present (Fig. 1C), reverse white to pale yellow (as color of medium), margin regular. Hyphae bearing lateral fertile branches straight, arthroconidia hyaline, cylindrical with truncate ends 3.7–4.3 × 2.5–3.1 μm (Fig. 3). Strain growing on PDA medium with dibromothymolsulfonophthalein



**Fig. 1.** *Amauroascus kuehnii* Arx: A – adult ascoma (scale bar = 1 mm), B – young ascomata (scale bar = 1 mm), C – colony on PDA after 24 days at 16°C in the dark, obverse, D – ascospores (LM), E – asci and ascospores (LM).

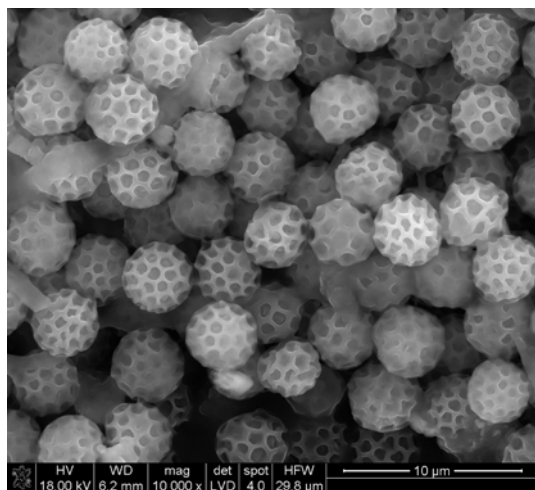


Fig. 2. *Amauroascus kuehnii* Arx, ascospores with thick bands and oval meshes (SEM).

as pH indicator did not show any change in color of medium, meaning that this strain not diffuse acidic products to the medium.

MATERIAL EXAMINED: POLAND, CARPATHIANS, Gorce National Park, 49°34'20"N, 20°12'10"E, in spruce forest, on deer horn wedged in soil, 25 July 2014, leg. A. Chlebicki (KRAM F 58021; malbranchea-like anamorph, strain PGH-24, deposited in KRAM F).

NOTES. The collected deer horn, preserved in a very damp container at room temperature (day/night) was first overgrown by white colonies of the malbranchea-like anamorph. The ascomata of *Amauroascus kuehnii* appeared after 3 months of storage. *Amauroascus kuehnii* is a rare fungus in North America and Europe. Kuehn (1957) noted it on bat dung in Georgia, United States. Currah (1985) reported its occurrence on lizard and mouse dung in the USA. Cano *et al.* (1987) isolated it from soil in Spain, and Guarro *et al.* (2012) reported it from Mexico. Keratin deer horn is a new substrate for this fungus.

*Amauroascus* J. Schröt. is related to *Auxarthron* G. F. Orr & Kuehn, and the distinction between these two genera has been suggested to be unclear (Currah 1985). Members of the genus *Auxarthron* have differentiated peridial hyphae and smaller punctae on the ascospore walls. Well developed reticuloperidia of ascospores of the genus *Au-*

*xarthron* are easily distinguished from those of *Amauroascus* (Sugiyama *et al.* 2002). According to Solé *et al.* (2002b) *Auxarthron* is a monophyletic genus while *Amauroascus* is clearly polyphyletic. Sugiyama *et al.* (2002) noted that *Amauroascus* is split into two lineages, but stated that the genera *Amauroascus* and *Auxarthron* are separate. *Amauroascus kuehnii* is morphologically intermediate between these two genera (Currah 1985). It has reticulate ascospores but does not have clearly differentiated peridial hyphae (Solé *et al.* 2002a). Solé *et al.* (2002b) recombined the species to the genus *Auxarthron*. Here we consider the species to be a member of the genus *Amauroascus*.

*Index Fungorum* ([www.indexfungorum.org](http://www.indexfungorum.org)) listed 15 species as accepted in the genus *Amauroascus* J. Schröt. Three of these species were previously reported from Poland: Schröter (1893) described *A. niger* J. Schröt. and noted *A. aureus* (Eidam) Arx, and *A. mutatus* (Quél.) Rammeloo (as *A. verrucosus* Eidam). *Amauroascus kuehnii* is new for Poland.

The anamorphs of *Amauroascus* were assigned to the genera *Chrysosporium* Corda and *Malbranchea* Sacc. Previously several *Chrysosporium* species as well as *Malbranchea pulchella* Sacc. & Penz. were reported from Poland (Mulencko *et al.* 2008). *Malbranchea pulchella*, noted in Poland by Gładoch (1978), is the anamorph of *Myxotrichum* Kunze. It has tightly coiled fertile branches which later disarticulate into conidia.

### *Isaria fumosorosea* Wize

Bull. Int. Acad. Sci. Lett. Cracovie, Cl. Sci. Math. Nat. Sér. B (1904): 721. 1904. – *Paecilomyces fumosoroseus* (Wize) A. H. S. Br. & G. Sm., Trans. Br. Mycol. Soc. 40: 67. 1957.

Mycelium flat, reddish-white (Fig. 4C), hyphae hyaline 3.0–3.5 µm wide, phialides smooth-walled, 5.0–6.5 × 2.5–3.0 µm, conidia ovoid, smooth-walled, 3.0 × 1.5–2.0 µm.

MATERIAL EXAMINED: POLAND, CARPATHIANS, Gorce National Park, 49°34'20"N, 20°12'10"E, in spruce forest, on deer horn wedged in soil, 25 July 2014, leg. A. Chlebicki (strain PGH-22 deposited in KRAM F).

NOTES. *Isaria fumosorosea* occurs on plant debris, soil and insects at many localities in Poland



**Fig. 3.** Malbranchea-like anamorph of *Amauroascus kuehnii* Arx: A – verruculose hyphae, B – septate and branched hyphae, C – arthroconidia, D – small “bulbs” attached to hyphae (top and intercalary position), probably bacteria. Photo A. Chlebicki.

(Kozak 2008, as *Paecilomyces fumosoroseus*). In view of the high morphological variability of the taxon it should be recognized as a species complex rather than a single species (Zimmerman 2008). *Isaria fumosorosea* is a well known entomopathogenic fungus (Xia *et al.* 2013).

### *Mortierella elongata* Linnem.

Mucor.-Gatt. *Mortierella* Coem. **23**: 21. 1941.

MATERIAL EXAMINED: POLAND, CARPATHIANS, Gorce National Park, 49°34'20"N, 20°12'10"E, in spruce forest, on deer horn wedged in soil, 25 July 2014, *leg. A. Chlebicki* (strain not preserved).

NOTES. *Mortierella elongata* was noted in Poland by Badurowa and Badura (1967), Dorenda (1986), Mańka *et al.* (1987) and Chlebicki *et al.*

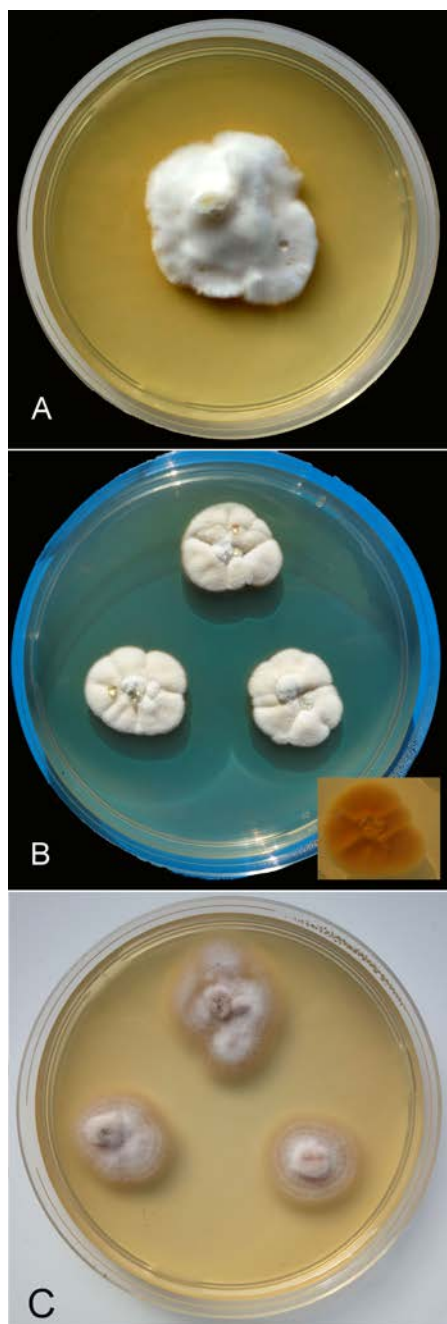
(2014). It is a well known soil fungus also noted as a bacterial endosymbiont (Dematheis *et al.* 2012).

### *Penicillium spinulosum* Thom *s.l.*

Bull. Bur. Animal Industr. U.S.D.A. **118**: 76. 1910. – *Penicillium trzebinskii* K. M. Zaleski, Bull. Acad. Polon. Sci., Math. et Nat., Sér. B (1927): 498. 1927.

MATERIAL EXAMINED: POLAND, CARPATHIANS, Gorce National Park, 49°34'20"N, 20°12'10"E, in spruce forest, on deer horn wedged in soil, 25 July 2014, *leg. A. Chlebicki* (strain PGH-23, deposited in KRAM F).

NOTES. This species belongs to the *Penicillium spinulosum* clade, section *Aspergilloides* sensu Houbraken *et al.* (2014). Our strain is characterized by its monoverticillate penicillia with occasionally branched stipes, yellow exudates and slightly orna-



**Fig. 4.** Malbranchea-like anamorph: A – colony on PDA after 2 months at 16°C in the dark, obverse. *Penicillium spinulosum* Thom: B – colonies on MEA after 10 days at room temperature, obverse and single reverse, day/night. *Isaria fumosorosea* Wize: C – culture on MEA after 10 days at room temperature, obverse, day/night (all Petri plates 90 mm diam.). Photo A. Chlebicki.

mented conidia. Colonies on PDA were gray, with an orange-brown to dark brown reverse. Colonies on MEA were white-rose to pale gray, with a pale brown reverse (Fig. 4B). *Penicillium spinulosum* has frequently been isolated from the rhizosphere and soil in Poland (Świdarska-Burek & Mamczarz 2008). The fungus was also the species most frequently isolated from wood elements of huts one year after construction (Seifert & Frisvad 2000). It produces fumonisine B1, which may be related to esophageal cancer in humans (Amadi & Adeniyi 2009).

**ACKNOWLEDGEMENTS.** We thank the anonymous reviewers for constructive remarks and suggestions on the manuscript. Financial support was provided by the W. Szafer Institute of Botany of the Polish Academy of Sciences through its statutory fund, and by the Ministry of Science and Higher Education under Project No. POIR.01.01.01-00-0004/15-00.

#### REFERENCES

- AJELLO L. 1974. Natural history of dermatophytes and related fungi. *Mycopathol. Mycol. Appl.* **53**: 93–110.
- AMADI J. E. & ADENIYI D. O. 2009. Mycotoxin production by fungi isolated from stored grains. *African J. Biotechnol.* **8**(7): 1219–1221.
- BADUROWA M. & BADURA L. 1967. Further investigations on the relationships between soil fungi and the macroflora. *Acta Soc. Bot. Poloniae* **36**: 515–529.
- CANO J., GUARRO J. & FIGUERAS M. J. 1987. Some keratinophilic fungi from Spain. *Mycopathologia* **100**: 163–167.
- CHLEBICKI A., ZIELENKIEWICZ U. & WILCZEK A. M. 2014. Fungi are not involved in biofilm formation on rock wall in subterranean arsenic mine in Poland. *Nova Hedwigia* **99**(1–2): 255–269.
- CURRAH R. S. 1985. Taxonomy of the Onygenales: Arthrodermataceae, Gymnoascaceae, Mycotrichaceae and Onygenaceae. *Mycotaxon* **24**: 1–216.
- DEMATHEIS F., KURTZ B., VIDAL S. & SMALLA K. 2012. Microbial communities associated with the larval gut and eggs of the western corn rootworm. *PLoS ONE* **7**(10): e44685.
- DOREND A. 1986. Mycoflora as a limiting factor for pathogenic fungi in red clover pure culture and its mixtures with cocksfoot. *Acta Mycol.* **22**: 15–34.
- FATERPEKAR S. K., JAIN S. K. & SHRIVASTAV A. 2008. Degradation of horse hair by soil inhabiting keratinophilic fungi. *Journal of Cell and Tissue Research* **8**(2): 1471–1476.

- GARG A. P., GANDOTRA S., MUKERJI K. G. & PUGH G. J. F. 1985. Ecology of keratinophilic fungi. *Proc. Indian Acad. Sci. Pl. Sci.* **94**(2): 149–163.
- GLADOCH M. 1978. Beitrag zu Untersuchungen über den Stickstoffumsatz einiger thermophiler und thermotolerant Bodenpilze. *Acta Mycol.* **14**: 157–161.
- GUARRO J., GENÉ J., STICHGEL A. M. & FIGUERAS M. J. 2012. *Atlas of soil Ascomycetes*. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.
- GUMIŃSKA B. 1977. The locality of *Onygena equina* (Willd. ex S.F. Gray) Pers. ex Fr. in the suburbs of Cracow. *Zesz. Nauk. Univ. Jagiellon. Prace Bot.* **5**: 153–156.
- HANSEN P. B. 1998. The genus *Onygena* Pers.: Fr.; species of Ascomycota decomposing keratin. *Svampe* **37**: 19–23 (in Dutch with English summary).
- HOUBRAKEN J., VISAGIE C. M., MEIJER M., FRISVAD J. C., BUSBY P. E., PITT J. I., SEIFERT K. A., LOUIS-SEIZE G., DEMIREL R., YILMAZ N., JACOBS K., CHRISTENSEN M. & SAMSON R. A. 2014. A taxonomic and phylogenetic revision of *Penicillium* section *Aspergilloides*. *Stud. Mycol.* **78**: 373–451.
- HUBÁLEK Z. 2000. Keratinophilic fungi associated with free-living mammals and birds. In: R. K. S. KUSHWAHA & J. GUARRO (eds), *Biology of dermatophytes and other keratinophilic fungi*. *Revista Iberoamericana de Micologia* **17**: 93–103.
- KOMOROWSKA H. 1986. *Onygena equina* (Willd.) Pers. Fr. in Poland. *Acta Mycol.* **22**(1): 49–52.
- KOZAK E. 2008. Hyphomycetes (genera from *Ozonium* to *Papulospora*) In: W. MULENKO, T. MAJEWSKI & M. RUSZKIEWICZ-MICHALSKA (eds), *A preliminary checklist of Micromycetes in Poland*, pp. 433–435. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.
- KUEHN H. H. 1957. Observation on Gymnoascaceae IV. A new species of *Arachniotus* and a reconsideration of *Arachniotus trisporus*. *Mycologia* **49**(1): 55–67.
- MAŃKA K., PRZEZBÓRSKI A., KWAŚNA H. & ŻÓŁTAŃSKA E. 1987. Effect of pH on the activity of some fungi isolated from forest and agricultural sites. *Zesz. Probl. Postępów Nauk Roln.* **307**: 175–196 (in Polish with English summary).
- MULENKO W., MAJEWSKI T. & RUSZKIEWICZ-MICHALSKA M. 2008. *A preliminary checklist of Micromycetes in Poland*. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.
- OTČENAŠEK M., HUDEC K., HUBÁLEK Z. & DVORAK J. 1967. Keratinophilic fungi from the nests of birds in Czechoslovakia. *Sabouraudia* **5**: 350–354.
- SCHRÖTER J. 1893. Gymnoascacei (Eidam 1880: Gymnoascen eingeschränkt). In: F. COHN, *Kryptogamen-Flora von Schlesien* **3**(2): 210–213. J. U. Kern's Verlag, Breslau.
- SEIFERT K. A. & FRISVAD J. C. 2000. *Penicillium* on solid wood products. In: R. A. SAMSON & J. I. PITT (eds), *Integration of modern taxonomic methods for Penicillium and Aspergillus classification*, pp. 285–298. Harwood Academia Publishers, Amsterdam, The Netherlands.
- SOLÉ M., CANO J., STICHGEL A. M. & GUARRO J. 2002a. Two new species of *Auxarthron* morphologically and genetically close to *A. kuehni*. *Stud. Mycol.* **47**: 103–110.
- SOLÉ M., CANO J. & GUARRO J. 2002b. Molecular phylogeny of *Amauroascus*, *Auxarthron*, and morphologically similar onygenalean fungi. *Mycol. Res.* **106**: 388–396.
- SUGIYAMA M., SUMMERBELL R. C. & MIKAWA T. 2002. Molecular phylogeny of onygenalean fungi based on small subunit (SSU) and large subunit (LSU) ribosomal DNA sequences. *Stud. Mycol.* **47**: 5–23.
- ŚWIDERSKA-BUREK U. & MAMCZARZ M. 2008. *Penicillium*. In: W. MULENKO, T. MAJEWSKI & M. RUSZKIEWICZ-MICHALSKA (eds), *A preliminary checklist of Micromycetes in Poland*, pp. 440–456. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.
- URI J., SZATHMARY S. & HERPAY Z. 1957. Production of an antibiotic by dermatophytes living in horn products. *Nature* **179**: 1029–1030.
- WAWRZKIEWICZ K., ŁOBARZEWSKI J. & WOLSKI T. 1987. Intracellular keratinase of *Trichophyton gallinae*. *J. Med. Veterin. Mycol.* **25**: 261–268.
- WRIGHT J. M. 1956. Production of gliotoxin in soils. *Nature* **177**: 896.
- XIA J., HUANG Z. & HU Q. 2013. Histopathological study of *Plutella xylostella* infected by three entomopathogenic fungal species. *Advances in Entomology* **1**(2): 15–19.
- ZIMMERMAN G. 2008. The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosa*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*): biology, ecology, and use in biological control. *Biocontrol Sci. Technol.* **18**(9): 865–901.