PANDORA UROLEUCONII SP. NOV. (ZYGOMYCETES: ENTOMOPHTHORACEAE), A NEW PATHOGEN OF APHIDS

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ABSTRACT

Pandora uroleuconii sp. nov. is described and illustrated. It possesses branched conidiophores, ellipsoid to ovoid monokaryotic and bitunicate primary conidia, pseudocystidia, and large nuclei staining distinctly with aceto-orcein. Rhizoids are absent. The pathogen differs from other species of the genus by absence of rhizoids, different size of primary conidia, growth on standard media and probably high host specialization. P. uroleuconii attacked aphid species Uroleucon aeneum during May and June in many localities in Slovakia. The species did not grow on standard media and did not exhibit infectivity to five common aphid species, Acrithosiphon pisum, Diuraphis noxia, Metopolophium dirhodum, Myzus persicae and Rhopalosiphum padi. The new fungal species is compared and contrasted with similar species of the genus.

Keywords: Entomophthorales, aphidophagous fungi, Uroleucon aeneum, Slovakia

During a survey of aphidophagous Entomophthorales in Slovakia, the aphid Uroleucon aeneum (Hille Ris Lambers) colonising Carduus nutans Linnaeus was found being attacked by fungus with specific taxonomic features allowing us to erect it as a new species. The fungus fits in almost all respects into genus Pandora (Humber 1989) by possessing elongate monokaryotic conidia with an outer wall, which may partially separate from an inner one, and by having large nuclei staining readily with aceto-orcein. Rather long tapering pseudocystidia at base 1.5 to twice as thick as conidiophores and branched conidiophores also support the placement in the genus Pandora. Lack of monohyphal rhizoids would seem to be the only obstacle in placing the fungus in Pandora, however, there is at least one further valid species devoid of rhizoids, Pandora delphacis (Hori) Humber, assigned to the genus. Rhizoids are also fungal structures, which might help readily distinguish Pandora from Erynia or Furia.

In the light of our observations the organism is described here as a new species. The description that follows is based on a general appearance of the pathogen as it occurs in naturally infected aphids and on its microanatomy as observed in aceto-orcein (AO).

Pandora uroleuconii Barta & Cagan, sp. nov.

Diagnosis: Corpora hyphalia elongata, hyphoidea vel irregulariter ramosa, uninucleata vel oligonucleata (1-6 nuclei praedita), 9.28 – 10.08 (7.92 – 12.87) μm
80


Holotypus: Specimen numero Cr 443 in collectione Instituti Botanici (Slovakum Musaeum Nationale) designatum, in Komjatice (Slovakia), die 25 mensis Iunii anno 1999, coll. et leg. M. Barta.


*Abbrevialione L/D utinum, quae in lingua Anglorum proportionem longitudinis ad crassitudinem signifcat.

Mycelium develops inside host in form of mono- to oligokaryotic, simple hyphae-like or irregularly shaped, sometimes ramified hyphal bodies 9.28 – 10.08 (7.92 – 12.87) μm thick (4x25 objects) and 120.6 (66.8 – 200.5) μm long (2x25 objects). Number of nuclei ranges from 1 to 6 per one hyphal body with average of 2 nuclei (4x25 objects). Pseudocystidia present but not prominent, simple and narrowing towards the obtuse apex, 15.46 (11.88 – 19.80) μm thick at base (13 objects), 9.5 (7.92 – 11.88) μm thick at the middle of their length and 5.99 (5.18 – 6.35) μm thick (25 objects) at the apex, average length 176.6 (138.6 – 207.9) μm (25 objects). Conidiophores branched interweaving to form a continuous hymenium more ore less completely covering pleural and dorsal parts of host’s body, conidiogenous cells slightly enlarged in subapical parts and constricted at their apex under forming conidia, with a diameter of 10.18 – 11.33 (9.90 – 12.87) μm (2x25 objects) and 5.84 – 6.39 (4.95 – 7.72) μm (2x25 objects) at their constricted necks. Primary conidia ellipsoid, broadly ellipsoid to ovoid, 24.71 – 33.03 (21.78 – 41.58) μm x 11.57 – 17.19 (9.90 – 22.77) μm L/D: 1.79 – 2.25 (10x25 objects), monokaryotic and bitunicate (with a separable outer wall layer except over the basal papilla); papilla rounded, centred on spore axis or displaced laterally. Secondary conidia formed singly on a short conidiophore arising laterally from a primary conidium and forcibly discharged, more or less similar to primary conidia 18.14 – 19.21 (15.84 – 27.72) μm x 9.31 – 9.62 (7.92 – 11.88) μm L/D: 1.93 – 2.01 (4x25 objects) or broadly ovoid 17.27 – 18.45 (11.88 – 27.72) μm x 9.85 – 10.18 (7.92 – 11.88) μm L/D: 1.70 – 1.93 (4x25 objects). Nuclei easily distinguished in vegetative or reproductive structures, staining readily with aceto-orcein and large with diameter of 5.46 – 7.09 (3.96 – 8.91) μm (8x25 objects) in primary spores. Rhizoids unobserved and probably not formed. Mouthparts and forelegs of cadavers were also carefully examined for rhizoid-like structures, but no monohyphal structures resembling
Figure 1. Morphology of *Pandora uroleuconii* sp. nov.

a) killed aphids adhered to the plant by their proboscises; b) cadaver with released spores around it; c) hyphal bodies (aceto-orcein “AO”); d) pseudocystidia (psc) protruding over a conidial layer (AO); e) conidiophores with forming primary conidia; f) primary conidia (left side - AO); g) secondary conidia (sc) forming on primary conidia (AO).
rhizoids were observed. No discoid-like holdfast typical to *P. neoaphidis* was noticed. Resting spores unobserved. The fungus does not grow on standard media (Sabourad-
dextrose agar enriched with milk and egg yolk – SEMA, egg yolk with milk – EYM, or
pure egg yolk – EY).

**HOLOTYPE:** Specimen No. Cr 443, air-dried cadavers of host aphid (exsiccatca),
Department of Botany, Slovak National Museum, coll. M. Barta, 25 June 1999,
Komjatice, Slovakia

**PARATYPE:** Specimen No. 25/2002, air-dried cadavers of aphids, Department of Plant Protection, Slovak Agricultural University, coll. M. Barta, 30 June 2002, Nitra,
Slovakia

**TYPE HOST:** *Uroleucon aeneum* (Hille Ris Lambers)

**TYPE LOCALITY:** Komjatice, Slovakia

**ETYMOLOGY:** *Uroleucon* – generic name of the type host species.

**DISTRIBUTION:** The species is known only from the type host in Slovakia. The fungus was observed from 19 localities in the country during late spring and early
summer (Table 1), and it is considered a frequent pathogen of the aphid species.

**Symptoms of disease:** Infected cadavers were easily recognised in aphid colonies by
their conspicuous posture on a host plant and by an obvious change of their colour
during fungal sporulation. With infection progress the mycosed aphids became
gradually extended and turgid but, in general, a shape of aphids’ bodies remained still
unchanged. Diseased cadavers were greyish-brown in colour with a pink tinge immediately after a death. In a final stage of disease development a conidiogenesis
appeared followed by a conidial release under humid conditions. At sporulation the
colour of cadavers changed from greyish-brown to silver. Due to a lack of rhizoids,
aphids killed by the pathogen were attached to the substrate by their proboscises, as well
as, by means of their forelegs. The attachment of the cadavers was rather weak and the
aphids were easily dislodged from the leaves when being collected. Under dry
conditions the cadavers became quite firm, did not sporulate and remained more or less
mummified. However, the mummies transferred to a humid chamber began to sporulate
readily. The mycelium inside cadavers preserves its viability up to fortnight after
storage in refrigerator and the sporulation started usually about two hours after
mummies were put on damp filter paper.

The infected aphids were for the first time observed on *C. nutans* in June 1999. Subsequent investigation of *U. aeneum* colonies in Slovakia revealed that the fungus
was regular pathogen of the aphid in the course of late spring and early summer,
although epizootic level of mycosis has yet not been observed. Table 1 shows localities
in Slovakia where the pathogen was recorded. According to our observations the “non-
rhizoidal fungus” (described here as *P. uroleuconii*) was the only pathogen of the aphid
colonies in 1999 and 2001 (In 2000 we did not observe the aphid colonies). On the
contrary, in 2002 besides this fungal species a pathogen with numerous unbranched
monohyphal rhizoids terminating in discoid holdfast was observed within the colonies,
as well. The “rhizoidal fungus” was identified as the most frequent aphidophagous
pathogen, *Pandora neoaphidis* (Rem. & Henn.) Humber. In this year the abundance of
*P. neoaphidis* cadavers even predominated over that of “non-rhizoidal fungus” in many
colonies and in some of them only *P. neoaphidis* occurred. Both fungi were easily
distinguishable *in situ* by external symptoms of mycoses: by a colouration of cadavers
and by a characteristic posture of dead aphids on a host plant associated with presence
Table 1. List of localities with *Pandora uroleuconii* records, together with coordinates and altitudes of the localities

<table>
<thead>
<tr>
<th>Locality</th>
<th>Date of record</th>
<th>Coordinates¹</th>
<th>Altitude²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Komjatice</td>
<td>25.6. 1999</td>
<td>48°09'20&quot; 18°10'00&quot;</td>
<td>130</td>
</tr>
<tr>
<td>Kamenica nad Hronom</td>
<td>23.5. 2001</td>
<td>47°49'50&quot; 18°43'30&quot;</td>
<td>117</td>
</tr>
<tr>
<td>Ruzovy Dvor</td>
<td>3.6. 2001</td>
<td>48°09'20&quot; 18°07'20&quot;</td>
<td>132</td>
</tr>
<tr>
<td>Rastislavice</td>
<td>12.6. 2001</td>
<td>48°08'20&quot; 18°04'30&quot;</td>
<td>124</td>
</tr>
<tr>
<td>Muzla</td>
<td>13.6. 2001</td>
<td>47°47'40&quot; 18°36'00&quot;</td>
<td>121</td>
</tr>
<tr>
<td>Dubno</td>
<td>5.7. 2001</td>
<td>48°11'20&quot; 20°00'20&quot;</td>
<td>234</td>
</tr>
<tr>
<td>Spisske Podhradie</td>
<td>6.7. 2001</td>
<td>49°00'00&quot; 20°45'00&quot;</td>
<td>435</td>
</tr>
<tr>
<td>Malý Cetín</td>
<td>30.5. 2002</td>
<td>48°14'20&quot; 18°10'50&quot;</td>
<td>135</td>
</tr>
<tr>
<td>Jatov</td>
<td>7.6. 2002</td>
<td>48°07'30&quot; 18°00'30&quot;</td>
<td>116</td>
</tr>
<tr>
<td>Velký Cetín</td>
<td>13.6. 2002</td>
<td>48°13'00&quot; 18°11'30&quot;</td>
<td>137</td>
</tr>
<tr>
<td>Klastor pod Znievom</td>
<td>17.6. 2002</td>
<td>48°58'30&quot; 18°48'00&quot;</td>
<td>500</td>
</tr>
<tr>
<td>Valca</td>
<td>17.6. 2002</td>
<td>49°00'00&quot; 18°51'00&quot;</td>
<td>450</td>
</tr>
<tr>
<td>Habovka</td>
<td>17.6. 2002</td>
<td>49°16'30&quot; 19°37'00&quot;</td>
<td>730</td>
</tr>
<tr>
<td>Oravská Polhora</td>
<td>18.6. 2002</td>
<td>49°31'30&quot; 19°26'00&quot;</td>
<td>890</td>
</tr>
<tr>
<td>Černík</td>
<td>23.6. 2002</td>
<td>48°09'20&quot; 18°13'30&quot;</td>
<td>129</td>
</tr>
<tr>
<td>Nitra</td>
<td>30.6. 2002</td>
<td>48°19'00&quot; 18°07'00&quot;</td>
<td>173</td>
</tr>
<tr>
<td>Zliechov</td>
<td>1.7. 2002</td>
<td>48°57'10&quot; 18°26'00&quot;</td>
<td>603</td>
</tr>
<tr>
<td>Korytnica-kupele</td>
<td>2.7. 2002</td>
<td>48°53'20&quot; 19°17'00&quot;</td>
<td>1075</td>
</tr>
<tr>
<td>Kostolany pod Tribečom</td>
<td>11.7. 2002</td>
<td>48°24'50&quot; 18°09'40&quot;</td>
<td>245</td>
</tr>
</tbody>
</table>

¹ North latitude and East longitude; ² metres above sea level

or absence of rhizoids. As aphids killed by the “non-rhizoidal fungus” (greyish-brown in colour) were weakly attached to plant with proboscises, individuals infected by *P. neaphidis* (ochre-red in colour) were quite firmly attached to plant surface by rhizoids emerging from the ventral part of abdomen.

Despite those dissimilarities in external symptoms, the “non-rhizoidal fungus” is microscopically very similar to *P. neaphidis* and other species closely related to *P. neaphidis*, namely *P. delphacis*. The new species, described here, is however distinguished from the both *Pandora* species by growth on standard media and probably high host specialization. Moreover it differs from *P. neaphidis* by absence of rhizoids and larger primary conidia (Table 2). Differences in conidial size between those pathogens are probably of low taxonomical value since the intraspecific variations in the conidial size of the species are rather great. We assume one source of the intraspecific variation might be a resporulation of some number of primary conidia deposited on microscopic preparations prepared for measurement, thus a certain amount of smaller secondary conidia might bias the results. Anyway, the species cannot be distinguished clearly by the spore size alone, and other taxonomic characters have to be applied in separating the species. Complete absence of rhizoids and pathobiology, especially host range or degree of fungal specificity, we consider a much more important taxonomic feature of *P. uroleuconii*. Taxonomic significance of presence/absence of rhizoids has been decisively discussed during a development of nomenclature of Entomophthorales. Some taxonomists, for delimiting entomophthoralean genera, put no weight on presence of rhizoids in certain species, as they are not always constant (Remaudière and Keller 1980), or others regarded a presence of rhizoids to be taxonomically significant while
Table 2. Gross taxonomical features of the *Pandora* species

<table>
<thead>
<tr>
<th>Fungal species</th>
<th><em>P. uroleuconii</em> sp. nov. ¹</th>
<th><em>P. neoaphidis</em> (Rem. &amp; Henn.) Humber ²</th>
<th><em>P. delphacis</em> (Hori) Humber ³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary spore size (μm) LxD; L/D</td>
<td>24.7-33.0 (21.8-41.6) x 11.6-17.2 (9.9-22.8); 1.8-2.3</td>
<td>21.0-32.0 (15.0-40.0) x 11.0-14.0 (9.0-16.0); 1.7-2.3 (1.4-2.9)</td>
<td>22.9-36.8 x 12.4-20.3</td>
</tr>
<tr>
<td>Rhizoids</td>
<td>absent</td>
<td>present</td>
<td>absent</td>
</tr>
<tr>
<td>Culture</td>
<td>no growth on SEMA, EYM, EY</td>
<td>growth on SDAEY, EYM, EY ⁴</td>
<td>fast growth on SEMA ⁵</td>
</tr>
<tr>
<td>Host spectrum</td>
<td><em>Uroleucon aeneum</em></td>
<td>plenty of aphid species from several families</td>
<td>plant- and leaf-hoppers (aphids artificially ⁵,⁶)</td>
</tr>
</tbody>
</table>

¹ authors’ observations, ² Remaudière and Hennebert 1980, ³ Hori 1906, ⁴ Keller 1991, ⁵ Milner et al. 1983, ⁶ Shimazu 1977

L – length, D – diameter, SDAEY – Sabourad-dextrose-agar enriched with egg yolk, SEMA – Sabourad-dextrose-agar enriched with egg yolk and milk, EYM – egg yolk with milk, EY – egg yolk

despite this absence was not considered a reliable criterion at a generic level (King and Humber 1981). Other authorities believe that rhizoids have a value at subgeneric level (Ben-Ze’ev and Kenneth 1981) or have value as a secondary generic character while certain exceptions are accepted, e.g. *P. delphacis* (Humber 1981, 1989). Balazy (1993) questioned the taxonomic significance of rhizoids for *P. neoaphidis* when he mentioned that rhizoids of the fungus might be sometimes on particular individuals underdeveloped or lacking in very dense aphid colonies. Further the author supposed that the *P. neoaphidis*, in the sense referred in current literature, is a species complex. This is in accordance with opinion of Humber (1983). Certain morphological and physiological variation among *P. neoaphidis* strains was highlighted by Keller (1991), as well. We assume that instability in presence of rhizoids observed by Balazy (1993) for *P. neoaphidis* could be one of signs of variability inside of the taxon. During our investigation the absence of rhizoids was unquestionably a constant character of *P. uroleuconii* as it was noticed on all cadavers in different localities all over the country during four consecutive years. This character was also occurred on cadavers of *U. aeneum* artificially inoculated by the pathogen in the laboratory. The absence of rhizoids was, moreover, associated with other physiological properties of the taxon which differentiated it from “rhizoidal” *P. neoaphidis*.

Besides morphological features discussed before, *P. uroleuconii* is close to *P. delphacis* by absence of rhizoids. However, both species differ in host specificity and growth in artificial cultures. *P. delphacis* is primarily a pathogen of leaf- and plant-hoppers in Asian rice paddies (Hori 1906; Holdom et al. 1989; Matsui et al. 1998), although the pathogen artificially infected some aphid species in laboratory (Shimazu 1977; Milner et al. 1983; Xu and Feng 2002). The pathogen exhibited even greater pathogenicity to aphids than *P. neoaphidis* (Xu and Feng 2000), however it has never been observed infecting aphids in nature and what is more the species has never been recorded from Europe. In our experiments all attempts for an artificial transmission of *P. uroleuconii* to five aphid species, *Acyrthosiphon pisum* Harris, *Diuraphis noxia* (Mordvilko), *Metopolophium dirhodum* (Walker), *Myzus persicae* (Sulzer) and *Rhopalosiphum padi* (Linnaeus), were unsuccessful (3 x 20 apterous aphids were tested against spores from freshly killed *U. aeneum* with a control of 20 individuals). The
pathogen infected only the type host, thus indicating what is probably a very narrow host specialization. *P. delphacis* grows more rapidly *in vitro* than *P. neoaphidis* and even it grows on a wider variety of media (nutritionally simple and complex) (Humber 1981; Milner et al. 1983). *P. uroleuconii* unlike those two species did not grow on artificial media. Any attempts to isolate the species failed. Spores of the pathogen apparently germinated after their deposition on media but a hyphal growth ceased shortly; this observation may support our supposition about pathogen’s high specificity. Two strains of *P. neoaphidis* were, however, isolated on SEMA from killed *U. aeneum*, and the strains showed pathogenicity to the five aphid species mentioned above. The inability to gain isolates of *P. uroleuconii* prevented us from testing the biochemical and molecular properties that might provide the clearest separation of those morphologically similar species.

*P. uroleuconii* has been defined by qualitative description of the external morphology, quantitative measurements of the main taxonomical structures, as well as, by observations on host preferences. Although the taxon shows a morphological resemblance in certain features to some representatives of *Pandora* genus, the results of examination presented here we regard to be substantial enough to justify the erection of the new species.

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**LITERATURE CITED**


