

## NEW DATA ON SPECIES OF *DISCOSIA*, *ROBILLARDA* AND *TRUNCATELLA* (XYLARIALES, ASCOMYCOTA) IN UKRAINE

*Key words:* anamorph, appendage-bearing conidia, Xylariales, Amphisphaeriaceae

### Abstract

Rare anamorphic fungi *Discosia circaeae* Lobik on *Circaea intermedia* Ehrh. and *Robillarda vitis* Prill. et Delacr. on *Parthenocissus quinquefolia* (L.) Planch. are first reported from Ukraine. A new host plant *P. quinquefolia* is first registered for the fungus *Truncatella angustata* (Pers.) Hughes. Detailed characters of morphology, geographic distribution, notes on their taxonomy and illustrations are provided for all studied fungi.

One of the manifestations of rich diversity in fungal spore morphology among Ascomycotina is characterized by formation of ancillary structures, recognized as «appendages». More than 150 genera of anamorphic coelomycetes produce appendage-bearing conidia and provide for investigators a workable approach to identification of these fungi (Nag Raj, 1993). Such morphological structures are most common for fungi adapted to specific ecological environment as fresh and salt water, dung, and to a lesser extent to terrestrial habitats. Among them are saprobes and hemibiotrophs which conidia are released passively under moist conditions.

During study of plant-associated anamorphic fungi in Ukraine, rare coelomycetes with appendage-bearing conidia were collected on living leaves of *Circaea intermedia* Ehrh. and *Parthenocissus quinquefolia* (L.) Planch. The anamorphic fungi were identified as *Discosia circaeae* Lobik, *Robillarda vitis* Prill. et Delacr., and are first reported from Ukraine. Another collected species is *Truncatella angustata* (Pers.) Hughes, quite rare anamorph on a new host plant. Discussed below are characters of their morphology and geographic distribution, notes on their taxonomy and illustrations.

1. *Discosia circaeae* Lobik, **Болезни Растений [Morgbi Plantarum], Leningrad 17 (3–4): 185. 1927 (1928).** Fig. 1.

*Leaf spots* first orbicular, about 1–2 mm diam., grayish brown; later orbicular or irregular, scattered, 3–6 mm

across, pale brown, with a centre pale yellowish brown, parchment, bearing conidiomata, and with a brown distinct narrow margin, slightly elevated and sharply delimited from other leaf tissues; lesions cover leaf surface between veins; leaf tending to perforate in the centre of lesions. *Conidiomata* eustromastic to subpycnidial, on both surfaces of leaf, scattered, dark brown, circular when viewed from above, reniform to subglobose, flattened, superficial or subepidermal, brown, 150–220 mm diam., 30–40 mm depth, unilocular; basal wall 10–20 mm diam., surrounded by one layer of dark brown cells; when absent, dehiscence by break down of upper wall. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* colourless, ampulliform to doliiform, 3–4(–8) × 1.5–2.0 μm, discrete or integrated, smooth, arising on hyphal layer of the conidiomatal cavity, holoblastic, with one apical proliferation, sometimes with a few repeated sequence of percurrent enteroblastic proliferations (annellations), followed by a replacement wall-building apex, with each conidium delimited at a point more distal than the previous, and with unthickened scars where conidia have seceded. *Conidia* with yellowish or greenish tint, central conidial cells more intensively coloured, cylindrical to oblong-elliptical, straight or curved, sometimes slightly flexuous, 15–20 × 2.5–3.2 μm, smooth, rounded at the apex, tapered and truncate at the base, 3-septate, thin-walled, in mucoid sheath; cells about equal or unequal, median cells cylindrical, adjacent to the apex middle cell the same size or shorter than middle cell closer to base, 4.0–4.5 μm long and 4.5–7.0 μm long, respectively; bearing two excentric, unbranched, flexuous, tubular appendages, showing protoplasmic continuity with conidial cells, 4.5–8.0 × 0.3–0.5 μm, joined on one line along inside of conidial curve, smooth, attenuated towards apex; apical appendage joined nearby to apical septa, basal appendage joint 1.0–1.5 μm next to the base of conidium; mean conidium body length to width ratio 6.1 : 1.

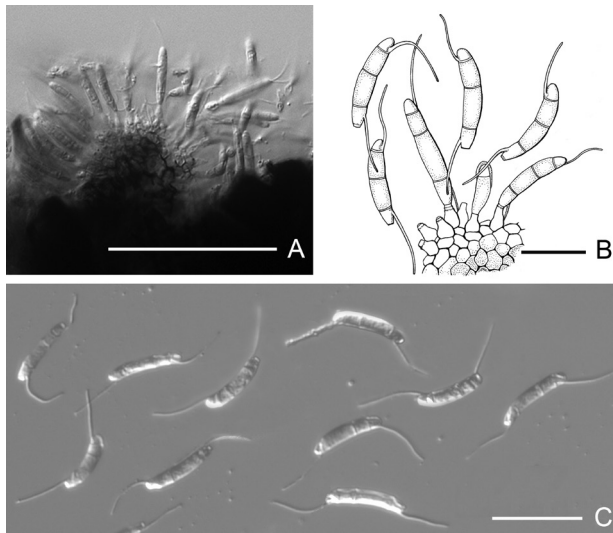


Fig. 1. *Discosia circaeae* Lobik. A. Transverse section of conidioma showing small conidiogenous cells and conidia. Scale bar = 50 µm. B. Conidiogenous cells and conidia. Scale bar = 10 µm. C. Variety of conidia with appendages. Scale bar = 20 µm.

**Substrata.** On living and withering leaves of *Circaea intermedia* Ehrh. (*Onagraceae*).

**Locality.** Mixed forest of *Fagus sylvatica* and *Quercus robur*, Stavchanske forestry, Roztochchya Nature Reserve (49°57'70" N, 23°65'44" E), Yavoriv district, Lviv oblast, Ukraine, 12.08.1989. New for Ukraine.

**Geographical distribution.** Europe: Russia (Stavropol' krai), Ukraine.

This fungus is a hemibiotroph that causes leaf spots of *Circaea* and only rarely can invade young upper leaves. The disease development ranges from weak to severe on different plants and it can cause earlier leaves fading and dying. The fungus is known from ecotopes with humid climatic conditions. First, *Discosia circaeae* was found and described on *Circaea lutetiana* L. in woods of the Caucasus Mountains (mountain Zheleznaya) near Pyatigorsk, Russia in July 1923 (Лобик, 1928). Our second collection of the fungus was done in mixed beech forests of Lviv oblast, Ukraine, during rather rainy vegetation season of 1989. Quite possible, *D. circaeae* has more wide distribution.

General morphological similarity of macrostructures among most species of the genus *Discosia* Lib., disregard of conidium and appendage proportions are the reasons of sometimes inaccurate information about these fungi geographic range. Modern molecular studies of the genus resulted in separation of the new genus *Immersidiscosia* Kaz. Tanaka, Okane et Hosoya and showed that species delineation remains a problem in *Discosia* (Tanaka et al., 2011). One of the

true *Discosia* species, *D. artocreas* (Tode) Fr., is a very common plurivorous saprobe described on *Fagus* sp. and occurring on a wide range of plants that number about 226 species (Andrianova, Minter, 2012). *Discosia artocreas* is well-known in Ukraine and recently was revealed by the author on the new host plants *Iris sibirica* L. (Sumy oblast, 20.09.2008) and *Parthenocissus quinquefolia* (Kyiv oblast, 30.08.2009). It was suggested that many specimens currently disposed as *D. artocreas* could, in fact, represent cryptic species (Tanaka et al., 2011). First of all, the reports on *D. circaeae* findings seem to be hidden under the name of *D. artocreas* in collections that were published without indication of associated organisms. The fungus is supposed to be prognostic to Bulgaria (Ванев и др., 1997) and, according to its current known range, it can occur in Armenia, Georgia and Turkey.

*Discosia circaeae* was accepted as a separate species by Subramanian and Chandra-Reddy (1974) in their extensive studies of the genus *Discosia* which included the examination of type material and numerous specimens collected worldwide. The monographer of anamorphic fungi with appendaged conidia, Nag Raj (1993), just indicated that the material of *D. circaeae* species was not examined in his study. Later Vanev (Vanev, 1991; Ванев и др., 1997) analyzed about 70 known species of *Discosia* and accepted only 31 species, including *D. circaeae*. The fungus was placed in section *Discosia* Vanev that incorporated species with distal appendages and unequal size of conidium cells, when the longest cell is the third one from the apical part of the conidium. The type of this section is *D. artocreas*. In fact, undertaken study has revealed that in *D. circaeae* the third cell is not significantly longer than the others. Presence of about equal median cells suggests re-allocation of the species *D. circaeae* to section *Laurina* Vanev. Other important differences which distinguish this species from *D. artocreas* are much smaller mean conidium body length to width ratio and location of the appendage and apical conidial cell junction point close to the upper septum of conidium. In *D. artocreas*, this appendage arises from almost the apex of the subtending cell (Nag Raj, 1993; Andrianova, Minter, 2012). Those characters place *D. circaeae* closer to *D. pleurochaeta* Durieu et Mont. (Ванев и др., 1997) and *D. brasiliensis* Nag Raj (Nag Raj, 1993) and separate this species from *D. artocreas*. Teleomorphs of above discussed *Discosia* species are not known, though some other species of the genus revealed to be connected with new genus *Adisciso* Kaz. Tanaka, Okane et Hosoya (Tanaka et al., 2011), placed in the ascomycete family *Amphisphaeriaceae* (*Xylariales*).

2. *Robillarda vitis* Prill. et Delacr., *Bulletin de la Société Mycologique de France* 5(4): 124. (1889) 1890.

Figs. 2, 3.

*Leaf spots* first angular or irregular, about 1–3 mm across, brown, with a centre pale brown, scattered between veins, sharply delimited from other leaf tissues; old lesions irregular, dark brown, slightly elevated and distinct, up to 4 mm across, bearing conidiomata, with broad reddish halo tending to spread along the leaf surface. *Conidiomata* subpycnidial or pycnidial, on upper surface of leaf, scattered, discrete, circular when viewed from above, globose to subglobose, ostiolate or later broadly opened, immersed, subepidermal, erumpent, light brown to brown, 70–100 µm diam., unilocular; with a wall 12–15 µm thick, composed of 6–7 layers of small *textura angularis* being pale brown internally and more coloured in the outer parts, coarse and elongated cells of 1–2 outer layers moderately thick-walled. *Ostiole* circular, central, 20–25 µm diam. or wide opened in actively sporulating conidiomata, surrounded by brown, thick-walled cells. *Conidiophores* reduced to conidiogenous cells lining the cavity of the conidioma. *Conidiogenous cells* colourless or with greenish tint, ampulliform to flat-globose, 4–5 × 3–5 µm, discrete, smooth, arising from the cells lining the conidiomatal cavity, holoblastic, each producing only one conidium or sometimes with a limited number of sympodial proliferations, with occasionally two conidia simultaneously attached to the conidiogenous

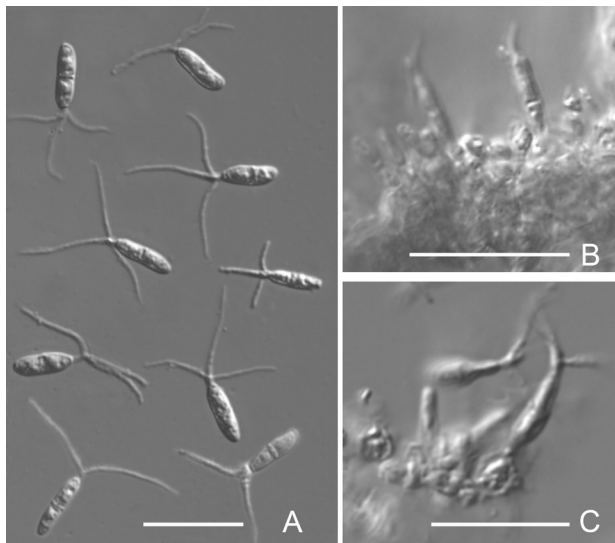


Fig. 2. *Robillarda vitis* Prill. et Delacr. A. Light coloured conidia with appendages. B. Cross-section through conidiomatal wall showing conidiogenous layer and juvenile appendaged conidia. C. Conidiogenous cells producing conidia. Scale bars = 20 µm.

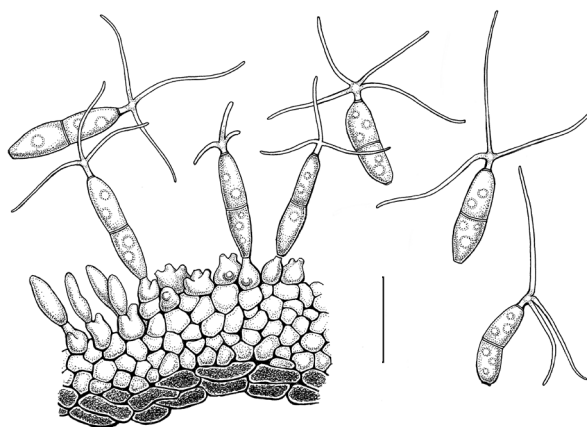


Fig.3. *Robillarda vitis* Prill. et Delacr. View of conidiogenous cells and conidia with appendages. Scale bar = 10 µm.

cell, secessional scars unthickened. *Conidia* fusiform to oblong ellipsoid, straight or somewhat curved, greenish olive, (10–) 11–13 × (3.3–) 3.5–4.0 µm, 1-septate, slightly narrower at the septum, smooth; apical conidial cell bearing colourless, 2.0–2.5 µm, cellular structure with a crest of 3–4 unbranched, colorless, apical appendages, 7.0–13.5(–16.6) × 1.0–1.5 µm, flexuous, smooth, tubular, having protoplasmic continuity with the subtending cell, attenuated toward the apex, always present; mean conidium body length to width ratio 3.2 : 1.

**Substrata.** On living and fading leaves of *Parthenocissus quinquefolia* (L.) Planch. (*Vitaceae*).

**Locality.** Forest of *Pinus sylvestris*, near Pliuty settlement (50°12'39" N, 30°39'15" E), Obukhiv district, Kyiv oblast, Ukraine, 23.08.2009. New for Ukraine.

**Geographical distribution.** Europe: France, Ukraine.

There are 35 names referred to *Robillarda* Sacc., 13 of which are well-defined species of this genus (Index Fungorum, 2013), about 15 other names are still without critical reappraisal, though used as species names in the genus *Robillarda*. Studied *R. vitis* is one of unexamined species. The fungus was first collected on leaves of *Vitis vinifera* L. in Gironde, France. It occurred on leaf lesions with red margins, formed immersed, pycnidial conidiomata that produced olivaceous, fusoid conidia, 10–11 × 4 µm, with 3 apical appendages, 8–15 × 1 µm (Prillieux, Delacroix, 1890). The type of *Robillarda* appendage structures were shown to consist of a short, nucleate, club-like cellular formation with several unbranched, tubular extensions, also each nucleate (Punithalingam, 1989). According original description the species *R. vitis* has some morphological features that partly resemble *R. sessilis* (Sacc.) Sacc., known from dead branches, bark, leaves, seeds and acorns of different plants, and *R. rhizophorae* Kohm., associ-

ated with roots of *Rhizophora*. The fungus *R. sessilis* is rarely detected in a few countries of Africa, America and Eurasia; it was recently registered in nature from Lithuania (Treigienė, Grigaliūnaitė, 2007) and Belarus (Yurchenko, Belomesyatseva, 2010). According the results of Nag Raj (1993) monographic study, *R. sessilis* conidia are of various shades of brown and about 3 µm in width, tending to have mean conidium body length to width ratio 4 : 1. Our study of Belarussian material of *R. sessilis* (Yurchenko, Belomesyatseva, 2010), due to kind permission of the authors, supported the view that *R. vitis* differs from the latter fungus by small-size conidioma, more globose conidiogenous cells and lesser conidial length to width ratio. The position of the genus *Robillarda* is not defined, the only species of this genus, *R. sessilis*, has undergone the molecular analysis of the LSU, SSU and ITS nrDNA and clustered with representatives of the ascomycete family *Amphisphaeriaceae* (*Xylariales*) without affinity with any genus in it (Rungjindamai et al., 2012).

**3. *Truncatella angustata* (Pers.) Hughes, Canadian Journal of Botany 36(6): 822. 1958. Fig. 4.**

*Leaf spots* on old living and withering leaves, lesions indistinct, few on a leaf, without clear outlines, indeterminate, minute, 2–3 mm across, starting as a change of tissue colour from greyish green to pale yellowish brown; with no obvious halo; plant tissues later becoming dry. *Conidiomata* scattered, acervular, at first subepidermal, later erumpent, circular when viewed from above, lenticular in vertical transverse section, 130–220 × 40–60 µm, unilocular, glabrous, brown; opening by a longitudinal split in the overlying host tissue; basal wall 25–30 µm thick, composed of small *textura angularis*, cells thick-walled, varying from brown in the outer parts to colourless internally. *Conidiophores* lining the cavity of the conidioma, erect, sparsely septate, unbranched or irregularly branched, colourless, smooth, 10–17(–20) × 1.7–2.0 µm, covered in mucus. *Conidiogenous cells* terminal, discrete or integrated, lageniform to cylindrical, 7–13 × 1.7–2.0 µm, colourless, thin-walled, smooth; holoblastic, with repeated percurrent enteroblastic proliferations by replacement wall-building apices, each conidium delimited at a point distal to the previous, resulting in annellations; secession schizolytic. *Conidia* fusiform, slightly curved, 3-septate, (13.5–)15.0–19.0 × (5.5–)6.0–7.5 µm, thick-walled, bearing appendages; basal cell obconic, 2–3 µm long, with a truncate base, colourless, smooth, narrower than the central cells; central cells both doliform, with unequal length

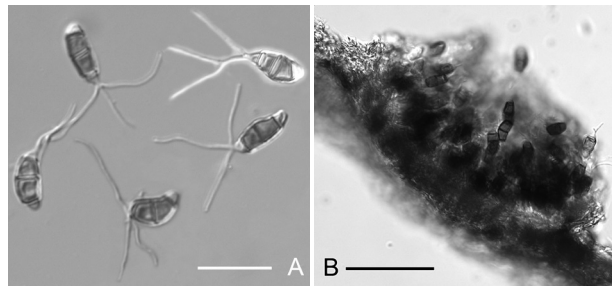


Fig. 4. *Truncatella angustata* (Pers.) Hughes. A. Variety of conidia with appendages. Scale bar = 20 µm. B. Transverse section of conidioma with conidia. Scale bar = 50 µm.

of cell side board, brown, without constrictions at the septa, together 10–12 µm long; apical cell conical, colourless, smooth, 1.0–1.5 × 1.5–2.5 µm; appendages on the apical cell arising in an apical crest of 2–3, tubular extensions having protoplasmic continuity with the subtending cell, sometimes irregularly or dichotomously branched, filiform, flexuous, 13–25 × 1.0 µm, smooth; mean conidium length to width ratio 2.7 : 1.

**Substrata.** On withering old leaves of *Parthenocissus quinquefolia* (L.) Planch. (*Vitaceae*). New host plant.

**Locality.** Planting, near Kozyn settlement (50°12'15" N, 30°39'57" E), Obukhiv district, Kyiv oblast, Ukraine, 30.08.2009.

**Geographical distribution.** Asia: Armenia, India, Iran, Japan, Kazakhstan; Australasia: Australia, New Zealand; Europe: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Lithuania, Norway, Poland, Portugal, Russia, Spain, Sweden, Switzerland, Ukraine, United Kingdom; North America: Canada, USA; South America: Brazil, Chili, Ecuador.

The anamorph *T. angustata* was registered in Ukraine on different trees of the genera *Acer*, *Carpinus*, *Fagus*, *Platanus* and *Robinia* since 1925. The fungus is widely distributed all over the world and known on many host plants from various families (Nag Raj, 1993). It colonises plant substrates and produces conidiomata on leaves, stems, twigs and fruits. *Truncatella angustata* was isolated as a fungal endophyte from different cultivated plants and from soils. Most recent investigations report on *T. angustata* as an agent that is associated with serious diseases of *Olea*, *Rosa*, *Vaccinium* and *Vitis* (Espinoza et al., 2008; Eken et al., 2009; Arzanlou et al., 2012, 2013). Thus, collection of the fungus on a new host plant from the family *Vitaceae*, *Parthenocissus quinquefolia*, may indicate its possible colonization of grapevine in Ukraine as well.

Species concept and nomenclature of *T. angustata* are problematic due to variability of the representatives of anamorphic pestalotioid genera. According Nag Raj (1993), conidia of *Truncatella* Steyaert species are characterized by lacking basal and branched apical appendages, concolours median cells; unlike *Pestalotiopsis* Steyaert species that differ by presence of basal appendage and apical crest of conidial unbranched appendages, versicoloured median cells. It is possible to distinguish *T. angustata* from rather similar morphologically *P. stevensonii* (Peck) Nag Raj by the complete absence of a basal appendage. Sequence data of ITS-rDNA region have demonstrated that *T. angustata* is rather allied but not identical with some species of the genera *Seimatosporium* Corda and *Pestalotiopsis* and clustered with representatives of Xylariales (Tanaka et al., 2011; Arzanlou et al., 2012). The teleomorph of *T. angustata* is reported by the Index Fungorum (2013) to be a member of the genus *Broomella* Sacc. (*Amphisphaeriaceae*, Xylariales). The presumptive teleomorph *Broomella acuta* Shoemaker et E. Müll. (Shoemaker, Müller, 1963) was proposed due to the same time collection with *Pestalotia truncata* Lévl. on *Clematis flammula* L., one of possible synonymous anamorphs of *T. angustata*, that needs further study.

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#### НОВІ ДАНІ ЩОДО ВИДІВ *DISCOSIA*, *ROBILLARDA* ТА *TRUNCATELLA* (XYLARIALES, ASCOMYCOTA) В УКРАЇНІ

Повідомляється про першу в Україні знахідку рідкісних анаморфних грибів *Discosia circaeae* Lobik на *Circaea intermedia* Ehrh. та *Robillarda vitis* Prill. et Delacr. на *Parthenocissus quinquefolia* (L.) Planch. Нова живильна рослина *P. quinquefolia* вперше зареєстрована для гриба *Truncatella angustata* (Pers.) Hughes. Представлені детальні описи морфологічних ознак, географічне розповсюдження, нотатки щодо таксономії та ілюстрації всіх вивчених видів.

Ключові слова: анаморфа, конідії з відростками, Xylariales, Amphisphaeriaceae.

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#### НОВЫЕ ДАННЫЕ О ВИДАХ *DISCOSIA*, *ROBILLARDA* И *TRUNCATELLA* (XYLARIALES, ASCOMYCOTA) В УКРАИНЕ

Сообщается о первой в Украине находке редких анаморфных грибов *Discosia circaeae* Lobik на *Circaea intermedia* Ehrh. и *Robillarda vitis* Prill. et Delacr. на *Parthenocissus quinquefolia* (L.) Planch. Новое питающее растение *P. quinquefolia* впервые зарегистрировано для гриба *Truncatella angustata* (Pers.) Hughes. Представлены детальные описания морфологических признаков, географическое распространение, заметки по таксономии и иллюстрации всех изученных видов.

Ключевые слова: анаморфа, конидии с отростками, Xylariales, Amphisphaeriaceae.