

MYCOTAXON

Volume XLIV, no. 2, pp.461-470

July-September 1992

COMPARATIVE MORPHOLOGICAL STUDIES OF DISCOSIA ARTOCREAS AND DISCOSIA FAGINEA

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ABSTRACT. Comparative morphological studies on the original specimens of Discosia artocreas (Tode) Fr. and D. faginea Lib. were carried out. The results proved that D. artocreas and D. faginea are two separate species. D. artocreas is a type of genus Discosia.

Genus Discosia was described by Libert in 1837 and it comprises imperfect fungi belonging to order Sphaeropsidales of class Coelomycetes. In the diagnosis of the original species Discosia faginea, Libert (1837) indicates Sphaeria artocreas Tode (a species described by Tode in 1791) as a synonym of D. faginea. Later Fries (1849), considering S. artocreas an individual species from genus Discosia, suggests the new combination Discosia artocreas (Tode) Fr.

While we revised taxonomically genus Discosia in the period 1975-1991, a number of obscure questions arose on the taxonomic status and the nomenclature of D. faginea and D. artocreas, bearing a direct relation to the determination of the type species of the genus Discosia. For example, in case Libert's position is accepted that D. faginea and D. artocreas are synonymous names of a common species, then, under article 55 of the International Code of the Botanical Nomenclature, priority is given to the older epithet of the species, i.e. "artocreas". On the other hand, Fries (l. c.), referring S. artocreas to genus Discosia was obviously acquainted with Libert's work, but in the original description of the new combina-

tion there is no mention of the indicated relation between D. faginea and D. artocreas, therefore it might be assumed he considers the two species to be independent.

Subramanian and Reddy (1974) have not studied the original specimens of D. faginea and D. artocreas, therefore they have no position as regards the taxonomy and the nomenclature of the two species.

The main difficulty when elucidating the taxonomic status of D. faginea and D. artocreas is due to the fact the original specimen of Sphaeria artocreas is considered to be destroyed or lost, that preventing the investigators, so far, from expressing an opinion on the problem of whether the two species are good ones, or we have to do with synonyms (Sutton, 1980).

In 1981, while revising herbarium materials from the Herbarium at the Royal Botanical Gardens, Kew, England (K), we came across the original specimen of S. artocreas from Fries's collection, marked: "Scleromyceti Sueciae No 151. Sphaeria artocreas Tode" (Fig. 1).

Having that material, together with the original specimen of D. faginea from Libert's Mycological collection, kindly placed at our disposal by the Herbarium at the National Botanical Garden in Brussels, Belgium (BR), we carried out comparative morphological studies on both specimens, the result of which provided us with a possibility to take a definite position with respect to the question discussed.

MATERIAL AND METHODS

In conformity with the modern taxonomic criteria, accepted in the systematics of the imperfect pycnidial fungi from class Coelomycetes (Sutton, l. c.) we based our comparative investigations on the morphology of the conidiogenous apparatus (pycnidia, conidiogenous cells), and the conidia of the original herbarium specimens of D. faginea (ex BR) and D. artocreas (sub Sphaeria artocreas-

ex K).

The dimension, the colour and the shape of the conidia as well as the exact position of the conidial septa and appendages were used as the basic systematic features in the comparative morphological characteristics of the studied specimens. The cited morphological elements were studied in lacto-phenol semistable preparations under Amplival light microscope, after the passing-light method. Conidiogenous cells and conidia were observed and photographed using Leitz-AMR-1000 A scanning electron microscope. For the precise investigation of the conidiogenous process and the structure of the generative organs, using a freezing microtome, thin cuts of pycnidia were made (having a thickness of about 10 μm), covered in paraffin.

Studied were also live liophylized specimens of D. faginea (strain CBS 443.67) and D. artocreas (strain CBS 241.66), preserved in the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. The live specimens were cultivated setting conidia cultures on oatmeal agar in Petri-dishes, and then exposed at a constant temperature of 24°C. The outward appearance and the diameter of the colonies were compared 3, 6, 9 and 12 days after setting the culture.

Traced out was the influence of the temperature on the growth of the colonies of both strains on oatmeal agar in Petri-dishes at a temperature of 3, 6, 15, 21, 24, 30, 33 and 36°C in a serial thermostat, as a result of which the temperature requirements were determined for the growth and spore-formation of the strains studied.

All the variants of the experimental investigations were carried out three times repeatedly.

RESULTS AND CONCLUSIONS

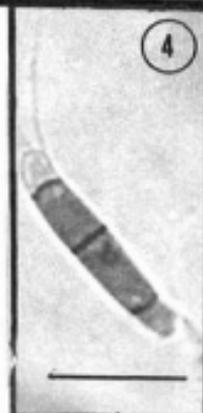
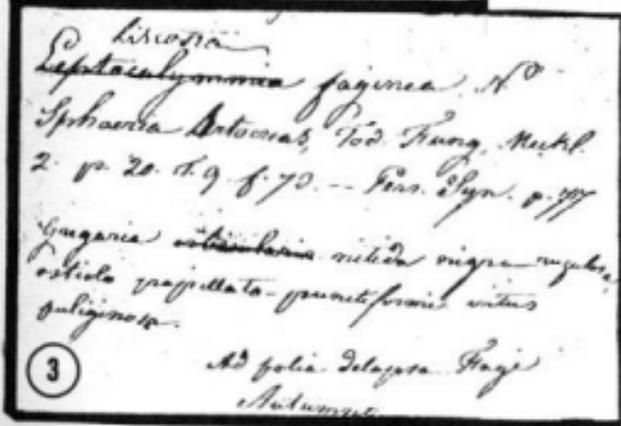
As a result of the comparative investigations carried out on the original herbarium specimens of D. faginea and D. artocreas, as well as on live cultures of these fungi,

we established considerable morphological, cultural and physiological differences between them, to be considered successively.

1. MORPHOLOGY OF THE CONIDIA. The conidia of the fungi of genus Discosia have characteristic shape, structure and dimensions, the genus being well differentiated as a separate group on the base of those features. On the other hand, within the limits of the genus to be observed are considerable differences in the structure of the conidia, the fact providing a possibility for the differentiation of intra-generic taxa of various ranks.

According to the position of the conidial appendages and the relative length of the conidial cells, 6 sections were differentiated within the limits of the genus (Vanev, 1991). It was established that the original specimens of D. faginea and D. artocreas share common features, referring them to the common Section I. Discosia, more particularly: the conidial appendages are adjacent to the apex and the base of the conidia, the two middle cells being of different length - the cell, adjacent to the base is always longer than the one adjacent to the apex. Regardless of the common features cited, others exist, in which the conidia of the studied specimens differ considerably from each other. On fig. 2 it is obvious that D. faginea has considerably wider conidia than the ones of D. artocreas. Differences are also to be observed in the shape - with D. artocreas predominating are cylindrical conidia having cells of equal width and colour, while with D. faginea the majority of the conidia are spindle-shaped, the two middle cells being wider and darker in colour than the two end cells. In the conidia of D. artocreas the middle cell adjacent to the base is always twice or more times longer than the other middle cell, adjacent to the apex, while in the conidia of D. faginea the difference in the length of the two middle cells never reaches 2 : 1 (Fig. 4).

2. MORPHOLOGY OF THE CONIDIOGENOUS CELLS AND THE PYC-



Figs. 1-2. Discosia artocreas: 1. Original specimen. 2. Conidium. Figs. 3-4: Discosia faginea: 3. Original description. 4. Conidium. Scale bars = 10 μ m.

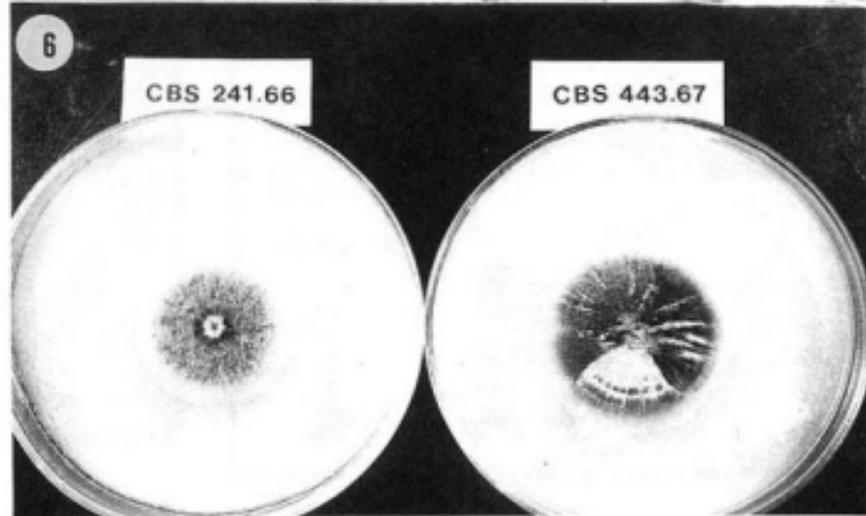


Fig. 5. Longitudinal pycnidial cut of *Discosia faginea*.
Fig. 6. Colonies of *Discosia artocreas* (CBS 241.66) and
Discosia faginea (CBS 443.67) at 24°C.

WIDIA. The conidiogenous cells of the fungi from genus *Discosia* form on a stromatic base within the pycnidia (Fig. 5). They are of a varying shape and length, altering within rather wide limits, due to which their taxonomic value is relatively limited. Studying completely developed pycnidia, certain differences were established in the structure of the conidiogenous cells of *D. artocreas* and *D. faginea*: in the pycnidia of the former species relatively short (up to 8 μ m) conidiogenous cells are formed, most often cone-shaped, while with the latter species those cells are longer (up to 15 μ m), being predominantly cylindrical or bottle-shaped.

Studying longitudinal pycnidial cuts from both species, it was established that with *D. artocreas* the pycnidia are most often pluriloculate, flat or slightly concave at the centre, with a convex margin and a relatively thin stromatic base, while with *D. faginea* the pycnidia are monolocate, disc-shaped, convex in the middle and having a thicker stromatic base.

3. CULTURAL CHARACTERISTICS. The data from fig. 6 show that the colonies of *D. artocreas* and *D. faginea* on oatmeal agar at 24°C have a varying rate of growth and a rather different outward appearance. The colony of *D. artocreas* (strain CBS 241.66) has a more retarded growth - on the 12th day after setting the culture it has a diameter of 48.5 mm (average for the 3 repetitions), while that of *D. faginea* (strain CBS 443.67) within the same time period reaches a diameter of 58 mm. Considerable are also the differences in the outward appearance of the colonies of both strains. Twelve days after setting the culture *D. artocreas* forms an indistinctly marked out yellowy-brown colony, having no concentric zonation and no radial rays, secreting a yellow pigment in the nutrient environment around the colony, in the form of a nimbus; the aerial mycelium is sparse, greyish-whity, cotton-like, placed predominantly in the centre; the formation of pycnidia is to be observed not earlier than nine days

after setting the culture. The colony of D. faginea is dark-olive-green to almost black, sharply outlined, having a number of concentric rings and well-seen whitish radial rays; the aerial mycelium is cobweb-like, grey, predominantly in the centre; no pigmentation of the environment surrounding the colony is to be observed; the formation of pycnidia starts after the 6th day following the setting of the culture.

4. TEMPERATURE REQUIREMENTS. It was experimentally proved that D. artocreas and D. faginea have different temperature requirements related to their growth and development. At 3°C, on the 12th day after setting the culture D. faginea forms a well shaped colony having a diameter of 3.1 mm (average for the 3 repetitions), while D. artocreas forms no colony at the same temperature and within the same time period. At 24°C both strains form the largest colonies, their conidiogenesis being most intensive. Considerable differences are to be observed in the requirements of both species towards high temperatures: at 30°C the growth of the colony and the formation of pycnidia of D. artocreas are almost normal, while at the same temperature the growth of the colony of D. faginea is highly suppressed, no pycnidia forming at that. At 33°C D. faginea forms no colony while D. artocreas develops successfully even at those relatively high temperatures.

The conclusion that should be drawn out these investigations is, the two species have different temperature requirements: D. artocreas develops more successfully at higher temperatures.

One of the goals of the experimental investigations carried out was to establish how and what an extent certain basic factors of the environment (nutrient substratum and temperature) effect the variability of the morphological features, on the base of which our classification scheme of genus Discosia is developed. For that purpose, the two strains were cultivated on different nutrient substrata (oatmeal agar, potato-dextrose agar and steri-

lized lupine stems) at different temperatures after the methods described.

The generalised results show that the position of the conidial appendages and the relative length of the conidial cells remain unchanged, i.e. they are not influenced by the composition of the nutrient environment and the changes in the temperature, while the dimension of the conidia vary within the limits established for each species.

Table 1. Comparison between D. artocreas and D. faginea.

Species	Dimensions of conidia (μm)
<u>D. artocreas</u>	(16,3-)18 \pm 1,2(-20) X (1,8-)2,1 \pm 0,2(-2,5)
<u>D. faginea</u>	(13,8-)18 \pm 2,7(-23) X (2,5-)2,9 \pm 0,2(-3,5)

It ensues from the cited results that the basic morphological features, on which we have founded the intra-generic classification of the fungi from genus Discosia, are characterized by insignificant variability amplitudes under changing environmental conditions, due to which their taxonomic value is relatively high.

In fine, the generalized conclusions may be drawn out that D. faginea and D. artocreas are two separate species having the right to independent existence. The cited conclusion provides us grounds to propound Discosia artocreas (Tode) Fr. (basionym Sphaeria artocreas Tode) as a type species for genus Discosia.

ACKNOWLEDGEMENTS

This research was carried out at the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands and was supported financially from the International Agricultural Centre, Wageningen, The Netherlands. The author wishes to thank the Curators of K and BR for the loan of the specimens examined.

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