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PHOMOPSIS (*COELOMYCETES*) SPECIES ON *COMPOSITAE* AND *UMBELLIFERAE*: A CRITICAL EVALUATION OF CHARACTERS WITH KEYS

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1. Introduction

In this study the type-species of the anamorphic genus *Phomopsis*, *P. lactucae* (SACC.) BUBAK, Oesterr.bot.Zeitschrift 55(2): 78 (February 1905) (see: RIEDL, WECHTL & SUTTON, 1981) (no.7) and other species occurring on *Compositae* and *Umbelliferae* are analyzed:

P. achilleae (SACC.) v. HOEHN., Fragm.z.Mycol.Nr.87, May 1906 (no.1),
P. albicans (ROB. & DESM.) DIED., Krypt.Fl.Brandenbg.IX, Pilze VII, 258, 1915 (no.2),
P. arctii (SACC.) TRAV., Flora Italica Crypt.2, 1. 226, 1906 (no.3),
P. asteriscus (BERK.) GROVE, Bull.Misc.Inf.53, 1917 (no.4),
P. cirsii GROVE, Brit.Coelomy.1, 456 + 177, 1935 (no.5),
P. inulina (SACC.) PETR., Engl.Bot.Jahrb.Beibl.Nr.142, 154, 1929 (no.6),
P. linearis (SACC.) TRAV., Flora Italica Crypt.1, 228, 1906 (no.8),
P. oblita SACC., Annal.mycol.8, 343, 1910 (no.9),
P. oleariae GROVE, Journ.Bot.London 60, 44, 1922 (no.10),
P. picea (PERS.) v. HOEHN., Fragm.z.Mykologie Nr.87, May 1906 (no.11)

A synoptic as well as a dichotomous key are offered. The numbers of species following the names in brackets are those used in the dichotomous key. Useful characters and other results are discussed. The so-called

"basal swelling-cone", the "stromatic pycnidia", the "endogenously" or "exogenously induced shape" of stromatic pycnidia, the "ostiolar channel", the "lateral" and "basal clypeus", and the "subconidiogenous basal cells" are used as new characters in *Coelomycetes*.

Phomopsis belongs to the *Phialostromatineae* (sensu SUTTON, 1980) as well as to the *Phialopycnidineae* (sensu SUTTON, 1980) of the *Coelomycetes*.

2. Material and Method

Earlier experiments on the genus *Phomopsis* using cultures had supplied no reliable characters for constructing a synoptical and dichotomous key from literature. Such experiments are handicapped by "petripatellisme" (GROVE, 1937). I tried to find a better basis by introducing additional characters. My own collections from the field and available herbarium-material were analyzed. It has been necessary to re-analyze the type specimens of BERKELEY (Kew), DESMAZIERES (herbarium SACCARDO, Padua), GROVE (Kew), LETENDRE (Padua), MALEBRANCHE (Padua), PERSOON (Leiden, Netherlands), ROUMEGUERE (Bruxelles and Padua), SACCARDO (Padua), SYDOW (Padua).

The specimens present at the Vienna National History Museum have been collected and identified by: DIEDICKE, ELIASON, HUBY, KABAT, LUDWIG, LUNDRELL, PISKORZ, RHODES, RIEDL, SMARODS, STRASSER, SYDOW.

As a rule, fruiting-bodies were disected by hand with a thin and sharp razor-blade because the use of a microtome leads to several disadvantages (no exact localisation, no possibility of observation during cutting, etc.).

3. Results and Discussion

1. My analysis about the occurrence of fruiting bodies throughout the year contradicted former opinions (KULIK, 1984; LI et al., 1985). Representatives of the genus *Phomopsis* can be collected in each month of the year, also during winter. Most specimens have been collected in April, May and June.
2. Here the fruiting bodies of *Phomopsis* are called "stromatic pycnidia". They look sometimes more like a stroma, sometimes more like pycnidia. Stroma and pycnidia cannot be separated exactly in the genus *Phomopsis*, but are significant for severals species.

3. In *P. asteriscus* the formation of a primary cavity is lysigen before an ostiolum is formed. Cavity-shape is determined very early in ontogenetic development.
4. The various shapes of cavity constitute to a significant character in the genus according to the autor's present experience:
   - 4.1. primary cavity-shapes:
     - 4.1.1. compressed-bottle-shaped 
     - 4.1.2. ellipsoidal
     - 4.1.3. cone-shaped
     - 4.1.4. obvers-patelliform 
     - 4.1.5. irregular
   - 4.2. secondary cavity-shapes:
     - 4.2.1. secundary obvers-patelliform 
   - 4.3. secondary compatimentation of cavity.

   The cavity-shape is determined by several different factors and varies during ontogeny. Therefore I did not use it in my keys.
5. The stromatic pycnidia of one and the same population of the species investigated may or may not be of the same size.
6. In the greater str. pycnidia of *P. achilleae* there are α - as well as β -conidia; in the little ones only α-conidia occur. In the type-species of the genus *P. lactucae*, however, there is no correlation between the size of str. pycnidia and the three types of conidia.
7. The walking-stick shape of some β-conidia is a result of great flexibility. It was found more often in older slide preparations which are already in a state of becoming more or less dry.
8. Guttulation in conidiogenous cells is observed in some cases.
9. The exact localisation of α - and β -conidiogenous cells in relation to "subconidiogenous basal cells", to α - and β -conidia, and of α - and β -conidiophor (one or many-celled) to stromatical pycnidial wall shows great variability within the genus.
10. Guttulation has been observed in β-conidia in a few cases.
11. Ontogenetic connectios between α - and β-conidia were observed for the first time: in *P. linearis*, *P. lactucae*, *P. albicans*, *P. arctii*. In *P. arctii* the α-conidiogenous cell is slightly s-shaped and guttulated. The subconidiogenous basal cell is much shorter and non-guttulated. Separation of α-conidia from the conidiogenous cell follows a preformed pattern, in which the conidiogenous cell is transformed to a conidium itself. These secondary conidia are separated

from the former subconidiogenous basal cell in the same way as primary conidia.

12. The analysis of the basipetal conidiogenesis was studied for the first time in *P. oblita*. After an α-conidium a β- or α-conidium follows in basipetal sequence first, then again an α- or β-conidium (in relation to physiological factors, see NITIMARGI, 1937). Many generations of conidia built up the great mass of conidia.
13. The filiform β-conidia are produced first in the "angles" of stromatical pycnidia.
14. Many correlation and intercorrelation analyses had been made in all characters investigated. Their results are the characters used in synoptic and dichotomous key.
15. The gradual similarity of pairs of species follows a Gaussian curve.
16. The synoptic key is based on analysis of correlation and intercorrelation of characters the significance of which has been evaluated. The following characters, some of which had to be redefined, proved most useful:
    - maximum length of the str. pycnidia seen from above;
    - shape of str. pycnidia as seen under the microscope (cylindrical, ellipsoidal, cone-shaped, irregularly hatshaped, hemisphaerical, or without definable shape);
    - papilla, "ostiolar channel";
    - "endogenously" versus "exogenously induced shape" (induction follows pressure from surrounding tissues);
    - relative thickness of stromatic pycnidial wall;
    - "basal swelling-cone";
    - "lateral and basal clypei";
    - maximum length of α- and β-conidia;
    - maximum breadth of α- and β-conidia;
    - shape of α-conidia (ends rounded or acute)
17. Aids for determination and standardisation of characters considering the great variability of the fungus are:
    - the number of guttulae in α-conidia
    - the relative quantity of α-, β- and γ-conidia.
18. For more exact descriptions of the species examined the following characters are useful as well:
    - change in colour of host surface;
    - location in host tissue;

- degree of protuding;
- relative density of fruiting bodies;
- shape of the str. pycnidia seen from above;
- melanisation;
- development of prominences into the interior;
- spreading of basal hyphae in relation to biochemical activities;
- locality and time of collecting;
- host;
- teleomorphs within D i a p o r t h e .

19. Most of the characters mentioned can only be recognized beyond doubt in collections from nature and are lost in artificial cultures (danger of petripatellism, GROVE, 1937).

20. There are four different hypotheses for the causes of different conidial shapes (α , β , γ):

20.1.1. The width of the space available above the conidiogenous cells arranged side by side in the centre of the basal and of the lateral wall equals that of their horizontal transect.
zontal transect.

20.1.2. In the "angles" between lateral and basal wall the free space is more narrow. Long filiform β-conidia can be produced there by all the conidiogenous cells standing side by side.

20.1.3. In the cavity near the ostiolum fusiform and ovoid α-conidia can arrange themselves very easily in a regular order. For inflexible stick-like conidia coming from different directions a regular arrangement would be very difficult near the ostiolum. *Flexible, filiform β-conidia are not handicapped in the same* way as they adapt their shape (cylindrical, s-shaped, curved, walking-stick-shaped).

20.2. An increase in volume can be achieved by elongation alone in α-conidia. Filiform β-conidia may grow wider and/or longer.

20.3. Rounded versus acute conidia: All young conidia are rounded with comparatively plastic conidial walls. Arrangement can change their shape very much with enough space around. them. Acute older α-conidia have more rigid walls with less available space around them as a consequence of the great mass of conidia. They can arrange themselves, therefore, better than rounded ones (although not all older α-conidia

of *Phomopsis* are acute).

20.4. Filiform β-conidia with their short diameters make better use of available space than the much wider α-conidia (they may occur exclusively in a cavity or mixed with β-conidia).

21. Relics of stromatic pycnidia are not rare in strongly melanized specimens. Strong pressing of fruiting-bodies in herbarium fascicles increases the number of damaged fruiting-bodies. Stromatic pycnidia in lateral position of the plant stems are complete. Abrasions of the apical part of str. pycnidia - such as the papilla, totally or partially - are found in herbarium material.

22. The variability of combinations among characters:

22.1. The following characters are combinded with all variations of other characters:

22.1.1. ellipsoidic and

22.1.2. cone-shaped str. pycnidia (seen under microscope)

22.1.3. medium lenth of α- and β-conida (-10μ and -12μ long α-conidia, 20-26μ long β-conidia)

22.1.4. medium width of α-conidia: 3μ

22.1.5. str. pycnidia more than 300μ long (seen from above)

22.2. The following characters of the species investigated are combined with certain expressions of characters:

22.2.1. In str. pycnidia with walls of the same thickness: very long α- and β-conidia do not exist; all α-conidia have rounded ends; α-conidia are much more noumerous than β-conidia

22.2.2. Broad α- and β-conidia are found only in ellipsoidic and cone-shaped str. pycnidia in the species investigated.

22.2.3. Str. pycnidia smaller than 300μ (seen from above) are always ellipsoidic in the species investigated.

22.2.4. β-conidia longer than 26μ do not exist in str. pycnidia with a basal swelling-cone in the species investigated.

22.3. Endogenous determination of shape of str. pycnidia and the basal swelling-cone do not show any correlation to other characters. Therefore I use them as primary characters in my dichotomous key.

23. The result of block-diagram analyses:

50-65 % of the species investigated have a diameter of less then than 500μ to 500μ, are lacking any clypeus, and have a distinct

papilla.

In about 50 % of the species a basal swelling-cone is present. In about 35 % of the species the wall is of nearly the same thickness apically, basally and laterally, in another 35 % there are great differences. In 30 % of the species an ostiolar channel (only seen under the microscope, with or without an additional papilla) is well developped. In all the species *investigated biguttulated α-conidia are present. More than half of the* species have fusiform α-conidia, up to 26μ long and up to 1μ wide filiform β-conidia, much more numerous α- than β-conidia. In about 50 % of the species α-conidia have a medium width of 3μ. In more than 1/3 of the species the α-conidia are up to 10μ long.

4. Synoptic key

1. The maximum of length of the stromatic pycnidia seen from above:
   up to 300μ: no. 1,4,8,
   up to 400μ: no. 6,
   up to 500μ: no. 2,3,5,7,9,10,11
   The measurements were taken without regard to any existing lateral or basal clypeus.
2. Their shape seen under the microscope:
   without regard to ostiolum, papilla, cavity, and lateral or basal clypei (with the exeption of F, +/- E).
   A. cylindrical ▭: no. 9,
   B. ellipsoidal ⬭: no. 1,3,4,5,8,9,
   C. cone-shaped △ : no. 6,11,
   D. hemishaerical ◠ : no. 5,9,10,
   E. without any definable shape  : no. 2,
   F. irregularly hat-shaped  , clypeus brim-shaped: no. 7
3. Relative thickness of stromatic pycnidial wall: without regard to the way of protruding and to the so-called "basal swelling cone". (Both characters are secondarily developped or characters of their own.) The relative thickness of the basal, lateral, and apical part of the wall is significant.
   3.1. all walls of the same thickness: no. 1,4,9,10
   3.2. area around ostiolum is thickened: no. 3,8,
   3.3. great differences in thickness of all parts of wall: no. 2,5,6, 9,11)

4. Endogenously versus exogenously induced shape: Endogenously induced shape is prevailing, when the fungus determines the shape of its fruiting body. In case of endogenously induced shape the inferior surface of the basal wall is even, not "wavy", nor irregular ... : no. 3,4,5,6,8,9,10. The shape of the fruiting body is exogenously induced, when the host tissues determine its shape. The inferior surface of the basal wall is "wavy", irregular, not flat ... : no. 1,2, 7,9,11.
5. Papilla and "ostiolar channel": The so-called "ostiolar channel" exists in every papilla of distinct length as well as in fruiting bodies lacking a distinct papilla, but with a very thick apical wall.
   5.1. stromatic pycnidia without papilla and without a comparatively significant "ostiolar channel": no. 5,8,10
   5.2. with a very short papilla and without a distinct "ostiolar channel": no. 1,8
   5.3. with distinct papilla and "ostiolar channel": no. 2,3,4,9
   5.4. without papilla, but with an "ostiolar channel" (only seen under the microscope): no. 6,7
   5.5. with strongly thickened upper wall and additional papilla, both penetrated by ostiolar channel: no. 11
6. Lateral and basal clypei (new definition): Lateral or basal clypei are wing-or root-shaped in optical transect. The basal clypeus is very rare. In about half of the analyzed species a clypeus was developed.
   6.1. without any clypeus ... : no. 1,4,5,8,9,10
   6.2. with a lateral clypeus ... : no. 2,7,9,10
   6.3. with a basal clypeus ... : no. 8
7. The so-called "basal swelling cone", a character used for the first time in fungi imperfecti: In the "basal swelling cone" the inner basal cell layers are pressed into the cavity like a cone. It consists of comparatively large, more or less dissolved, normally yellowish cells. This is different from a mere folding of the hole wall as well as from any outgrowths of the cell wall without specific anatomical differences. Young fruiting bodies may not yet have developed a swelling cone also in species for which it is characteristic as a rule. Shape and guttulation of α-conidia distinguish younger and older stages of the fruiting body. About half of the analyzed species have a "basal swelling cone".
   7.1. without "basal swelling cone": no. 1,2,3,4,5,10

7.2. with a "basal swelling cone" no. 6,7,8,9,11

8. Maximum length of α-conidia:
   - 8 μ: no. 4,6,10
   - 10 μ: no. 1,2,3,11
   - 12 μ: no. 8,9
   - 15 μ: no. 5,7

The length of α-conidia varies with several physiological factors and ontogenetic stages. (A very high number of guttulation drops helps to determine maximal length and to standardize various parameters. Non-guttulated α-conidia are smaller and younger in field collections.)

9. Maximum breadth of α-conidia:
   - 2 μ: no. 8,10
   - 3 μ: no. 2,4,6,7,9.11
   - 4 μ: no. 1,3,5

10. The shape of the α-conidia with rounded or acute ends: Younger conidia always have rounded ends. Older ones with two or more guttulation drops have rounded and/or acute ends (in field collections).
    10.1. ends of α-conidia rounded: no. 2,5
    10.2. ends of α-comidia rounded and acute, rarely acute, or intermediate shape of ends: no. 4,6,10
    10.3. ends of α-conidia acute: no. 1,3,6,7,8,9,10
    Many species have acute α-conidia.

11. The number of guttulation drops in α-conidia, a very good aid for determination: The number of guttulation drops (0,2,4, more) is induced by physiological factors, but correlates with age in field collections. It is a very significant aid for standardisation and therefore necessary for comparing conidial length, width, and shape. It is no valid taxonomic criterium, with one possible exception: the two bipolar endstanding guttulation drops are very significant in *P. albicans*. (One must have seen it, because the two drops are always more or less endstanding in *Phomopsis* species. In *P. albicans* they are extraordinary significant.)

12. Maximum length of β-conidia:
    - 20 μ: no. 4,6,8
    21-26 μ: no. 1,3,4,5,7,9,10
    27-32 μ: no. 2,5
    β-conidia: rarely are guttulated. Therefore guttulation was not taken into consideration.

β-conidia are 21-26 μ long in most species investigated

13. Maximum width of β-conidia:
    - 1 μ: no. 1,2,4,5,7,9,10
    - 2 μ: no. 3,4,6,8

    Maximum breadth of β-conidia show two significant groups of species which mostly are negatively correlated with maximum length of β-conidia.

14. Relative quantity of α-, β-, and γ-conidia, a very good aid for determination: The production of three different types of conidia α, β, and γ, is induced by physiological factors (NITIMARGI, 1937). It is a good aid for correlating and standardising with other characters
    - 14.1. significantly more α-conidia than β-conida ... $\alpha >> \beta$
    - 14.2. α-conidia rare, but many β-conidia ... $\alpha < < \beta$
    - 14.3. γ-conidia also developed

15. Host-plants: Host plants also may be an additional aid in identifying particular species.
    - 15.1. on *Compositae* exclusively (*Asteraceae*, *Cichoriaceae*): no. 1,2,3,5,6,7,8,9,10
    - 15.2. on *Umbelliferae* exclusively (*Apiaceae*): no. 4
    - 15.3. on *Compositae* as well as *Umbelliferae*: no. 11
    - 15.4. on *Compositae*: *Achillea*: no. 1
      Arctium: no. 3
      Artemisia: no. 9,11
      Cichorium: no. 2
      Cirsium: no. 5
      Inula: no. 6
      Lactuca: no. 7 (type of the genus)
      Olearia: no. 10
      Solidago: no. 8
      Tanacetum: no. 1,3
    - 15.5. on *Umbelliferae*: *Heracleum*: no. 4,11

5. Dichotomous key

1. Shape of stromatic pycnidia endogenously induced 2
1. Shape of stromatic pycnidia exogenously induced 7
2. Stromatic pycnidia with a basal swelling-cone 3
2. Stromatic pycnidia without a basal swelling-cone 4
3. Stromatic pycnidia (as seen under the microscope) cone-shaped; great differences in thickness of walls; ends of α-conidia rounded and/or acute, rarely acute, or intermediate ..... *P. inula*
3. Stromatic pycnidia (as seen under the microscope) ellipsoidal; wall round ostiolum thickened; ends of α-conidia acute ..... *P. linearis*
4. Ends of α-conidia rounded exclusively; stromatic pycnidia (as seen from above) up to 500 μ long; without papilla; walls of +/- equal thickness throughout; clypeus lacking ..... *P. cirsii*
4. Ends of α-conidia rounded and/or acute, predominately rounded, intermediate, or all acute 5
5. Stromatic pycnidia (as seen under the microscope) +/- hemisphaerical; (seen from above) broad elliptic and 250-500 μ long; walls of the same thickness throughout; clypeus lacking ..... *P. olearíae*
5. Stromatic pycnidia (as seen under the microscope) ellipsoidal or cone-shaped; without any clypei: with a distinct papilla 6
6. Wall of stromatic pycnidia of the same thickness throughout consisting of only three layers of cells, 10-20 μ thick; maximal diameter of stromatic pycnidia 300 μ; α-conidia ovoid, rarely acute at one end ..... *P. asteriscus*
6. apical wall surrounding ostiolum or as a whole very thick; maximal diameter of stromatic pycnidia about 500 μ; α-conidia with acute ends ..... *P. arctii*
7. Stromatic pycnidia with a basal swelling-cone 8
7. Stromatic pycnidia without a basal swelling-cone 9
8. Shape of stromatic pycnidia (in optical transect) irregularly hat-shaped; clypeus brim-shaped (lateral clypeus); without papilla, but with an ostiolar channel; great differences in thickness of walls; ends of α-conidia more or less acute and the ends more or less different from each other ..... *P. lactucae*
8. Str. pycnidia cone-shaped; great differences in thickness of walls; with an ostiolar channel running through papilla and tickened apical wall; with lateral clypeus; ends of α-conidia acute ..... *P. picea*

8. Shape of str. pycnidia cylindrical, ellipsoidal, more or less hemisphaerical; with a distinct papilla; with or without lateral clypeus; wall of the same thickness throughout; ends of α-conidia acute ..... *P. oblita*

9. Shape of str. pycnidia ellipsoidal; wall of the same thickness throughout; papilla very short; with distinct ostiolar channel; without any clypei; maximal diameter of str. pycnidia only about 300 μ; α-conidia with acute ends ..... *P. achilleae*

9. Str. pycnidia of irregular shape; great differences in thickness of walls; papilla distinct; with lateral clypeus; maximal diameter of str. pycnidia about 500 μ; α-conidia with rounded ends ..... *P. albicans*

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