

CONIDIAL APPENDAGES AS TAXONOMIC CRITERIA IN COELOMYCETES

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Abstract

Conidial appendages have been used as a taxonomic criteria in Coelomycetes. A modified conidial appendages classification has been proposed.

Introduction

Many fungal fruiting bodies are characterised by unicellular or multicellular appendage-like structures which are called setae as in *Chaetomium* Künze, *Erysiphe* Hedw.f., *Sphaerotheca* Lèv., *Podospora* Ces., *Uncinula* Lèv., *Phyllactinia* Lèv., *Chaetospora* Faurel & Schotter, *Chaetosticta* Petrak & H. Sydow, *Colletotrichum* Corda, *Stauronema* (Sacc.) H. Sydow, P. Sydow & Butler, *Chaetomella* Fuckel, *Myrothecium* Tode, *Dasysticta* Speg., *Chaetasbolisia* Speg., etc. These are quite different structurally and developmentally from the appendages found on spores as in *Hyalotiella* Papendorf, *Hyalotiopsis* Punith., *Robillarda* Cast, *Pseudorobillarda* (Cunell) Morelet, *Seimatosporium* Corda., *Seiridium* Nees, *Phytophthora* de Bary, *Olpidium* (Braun) Schröter, *Chytridium* Braun etc.

Hawksworth *et al.*, (1983) have defined appendages as a process (outgrowth) of any sort. According to Sutton (1973), many detached conidia bear apical, lateral or basal attenuated structures termed appendages. In a subsequent report Sutton (1980) defined appendages in conidia of Coelomycetes as cellular, setulose or mucilaginous ornamentation, usually filiform but sometimes infundibular or capitate.

There has been considerable confusion and controversy in the terminology applied by various authors to appendages in Coelomycetes. Sutton & Kobayashi (1969) and Sutton (1980) described *Strasseria* Bresad. & Sacc., and *Strasseriosis* Sutton & Kobayashi in which the only difference between conidia is the septum separating the main conidial body and basal filiform structure. In *Strasseria* the basal filiform structure is called an appendage while in *Strasseriosis* it is called a basal cell. Sutton (1980) has put appendages in parentheses. Nag Raj (1983) showed that in *Strasseria* a septum is also present between the appendage and the conidial body and also that an apical mucilaginous cap is also present. Hence, there is no difference in the two genera, except for the mucilaginous sheath. Similarly in *Scopaphoma* Dearn. & House, Sutton (1980) described conidia consisting of four cells, two relatively wide median cylindrical

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cells, and one apical and one basal cell. He called the apical cell an appendage, but did not use the term appendage for the basal cell. In *Pleurothyrium* Bubak, Sutton (1980) did not use the term appendage for the apical filiform cell, which is clearly different from the rest of the wider cell, whereas in *Heteropatella* Fuckel and *Monochaetielliosis* Sutton & Di Cosmo the term appendage has been used for the similar apical filiform cell. Similarly there are differences in opinion when unicellular conidial apices become filiform, as in *Chaetoconis* Clem., where Sutton (1980) considered it to be an appendage while Grove (1935) called it a filiform beak. Punithalingam (1984b) is of the opinion that it is inaccurate to call the filiform beak-like structure an appendage although subsequently Punithalingam (1989a, 1989b) did accept it as an appendage. Punithalingam (1989b) used the term arm for the filiform, septate, nucleate outgrowth in *Crucellisporium* spp. When a cell becomes modified or tapered into a filiform structure at what point in relation to the original cell should it be called an appendage? Reviewing the literature about the cellular, acellular tubular and solid appendages, it would appear that the term appendage can easily be used when a cell is modified or tapers in a way that it is less than 20% of the total width of the main cell.

In *Tunicago* an outer hard sheath is not called an appendage by Sutton (1980). Similarly according to Hawksworth *et al.*, (1983) the basal appendage in *Harknessia* does not fulfill the definition of an appendage since these structures are not outgrowths of conidia. The definition of appendages given by Sutton (1973) is adopted and modified in the present studies.

Pirozynski & Shoemaker (1971) gave a brief history of appendages in Coelomycetes and their importance in taxonomic work. Sutton (1973, 1980) and Nag Raj (1981) classified appendages in two categories: cellular and extracellular. Punithalingam (1981a, 1981b, 1982, 1984a, 1984b, 1989a, 1989b) and Punithalingam & Woodhams (1982, 1984, 1986) have contributed a great deal to elucidate the nature of appendages in various taxa, using the modified Leifson's bacterial flagella staining technique, initially described by Leifson (1930, 1938, 1951, 1958) and Conn *et al.*, (1962). In a subsequent report Punithalingam (1989a) has categorised conidial appendages as (i) Enucleate mucilaginous; (ii) Enucleate tubular and filiform; (iii) Enucleate tubular; (iv) Nucleate tubular; and v) Nucleate apical cell or appendages. The basis was morphology and presence or absence of nuclei.

Appendages in *Tiarosporella paludosa* described by different authors also vary. Pirozynski & Shoemaker (1971) and Whitney *et al.*, (1975) considered them to be flagellate or spirally twisted tentacles. Nag Raj (1973) called them mucilaginous undulate tentacles. Sutton (1980) considered them tentacular, developing from the sheath around the conidia. Using Leifson's flagella staining technique, Punithalingam (1981b) showed that they are formed from the splitting of the outer cell wall and not from the outer mucilaginous sheath. This has been classified as belonging to the enucleate mucilaginous group (Punithalingam, 1989a).

Generally it is thought that appendages in *Phyllosticta* spp., are extracellular and arise from a mucilaginous sheath around the conidia (Stewart, 1916, Sutton & Waterston, 1966), Ponnappa, 1970; Van der Aa, 1973). Punithalingam & Woodhams (1982) used Leifson's flagella staining technique for clarifying the nature of appendages in *Phyllosticta* spp., and demonstrated that appendages are cellular and had

protoplasmic connections with conidia at least in the early stages of development before the septa formation or plugging of the septal pore with protoplasm retracted into the conidial body. This has been classified in the Eucleate tubular and filiform group (Punithalingam, 1989a).

By using Leifson's method, Punithalingam (1983) showed that a mucilaginous sheath is present around the conidia of *Macrophomina phaseolina* (Tassi) Goid., which is the first report in this fungus. This has been classified in the Eucleate mucilaginous groups (Punithalingam, 1989a).

The position of appendages in *Pseudorobillarda phragmitis* (Cunnell) Morelet is also controversial. They were reported to be basal and developed from the outer sheath (Cunnell, 1958; Morelet, 1968; Nag Raj *et al.*, 1972, 1973). These studies depended upon conventional fungal staining techniques. However using the modified Leifson's method, Punithalingam & Woodhams (1986) were able to show that the appendages are apical and do not develop from an outer sheath but from the outer cell wall. Jones & Moss (1978) in *Haligena spartimae* and Nag Raj (1988) in *Clithramia elegantissima* Nag Raj reported a special type of appendage development which takes place through pores present in the outer conidial wall.

Reviewing the literature concerning appendage nomenclature it would appear that the scheme proposed by Sutton (1980) for classifying the appendages is not entirely satisfactory since there is probability of misunderstanding in the meaning of the term cellular and extracellular nature of appendages. Extracellular means outside the cell. Sutton (1973) defined cellular appendages as those having protoplasmic continuity with the conidial body at least in the early stages of development. Extra cellular appendages do not have such connections. According to Sutton (1973, 1980) appendages developed outside the conidial cell wall are extracellular because they have no cytoplasmic connection even in early stages of development. Since appendages developing from or composed of the conidiogenous cell as remnants are actually not the part of conidium, therefore should be considered as extracellular. It may be mentioned that Sutton (1980) referred to them as cellular as in *Harknessia* Cooke and *Lasmenia* Speg.

In the present report the term appendage is redefined in a simple way. It is the process or outgrowth on a cell or conidium which develops from the whole cell or conidia or any part of the same cell or conidia or develops from prolongation or secretion of other cell.

The classification of appendages as given by Punithalingam (1989a), is simple but has certain discrepancies. In the Eucleate mucilaginous types, *Macrophomina phaseolina* (Tassi) Goid., and *Tiarosporella paludosa* (Sacc. & Fiori) Höhn., have been grouped without consideration of the fact that the former develops from secretion of the outer mucilaginous sheath and the latter develops from the splitting of a wall. Similarly, *Phyllosticta* spp., and *Pseudorobillarda phragmitis* have been grouped together in the Eucleate tubular filiform type,. In *Phyllosticta theacearum* Van der Aa (IMI 310972 b), the appendage is cellular and not filiform since there is a very wide gap between the outer appendage wall suggesting it to be tubular according to Punithalingam (1989a). Similarly in *Pseudorobillarda phragmitis*, appendages develop from splitting of the cell wall (Punithalingam & Woodhams 1986). However scanning electron microscopy

Fig. 1. Classification of Appendages**CELLULAR**

Unicellular or Multicellular

Nucleate or Anucleate

Ablastic

Simple or Branched

Basal or Lateral

Solitary or Multiple

Blastic

Holoblastic or Enteroblastic

Simple or Branched

Basal

Hyaline or Pigmented

Solitary or Multiple

Exogenous or Endogenous

Centric or Ecentric

Apical*

Hyaline or Pigmented

Solitary or Multiple

Lateral*

Hyaline or Pigmented

Solitary or Multiple

ACELLULAR**Motile**

Whiplash or Tinsel or Both

Non-Motile

Parietal or Aparietal

Solid**Inclusive**

Hyaline or Pigmented

Partial

Simple or Branched

Basal

Hyaline or Pigmented

Solitary or Multiple

Exogenous or Endogenous

Centric or Ecentric

Apical*

Hyaline or Pigmented

Solitary or Multiple

Lateral*

Hyaline or Pigmented

Solitary or Multiple

Mucoid**Inclusive**

Hyaline or Pigmented

Remaining Soft or Becoming Hard

Partial

Simple or Branched

Basal / Apical / Lateral*

Hyaline or Pigmented

Solitary or Multiple

Capitate or Irregular or Infundibular

Note: Only one option is to be selected if more than one are provided.

* = If appendages are present at more than one positions, they will be characterized separately in composite form like apical and basal, apical and lateral, basal and lateral, apical-basal and lateral.

studies have shown that these are tube-like structures which arise from a wider tube situated at the apex. The appendage of *Tunicago* is another example where the outer mucilaginous wall becomes thick and hard surrounding the conidium, whereas in *Avetiaea* the conidium is surrounded by a mucilaginous sheath which remains soft. According to Punithalingam (1989a), *Tunicago* does not fit in any of his categories whereas *Avetiaea* can be put in the Enucleate mucilaginous type. However, Punithalingam (1989a) did not mention any appendages which completely surround the conidia. In the present paper a simplified system for the classification of appendages is proposed (Fig.1). The terms used for the classification of appendages are arranged alphabetically as follows:

Terminology used in appendage classification

Ablastic: Not developing in a holoblastic or enteroblastic manner but arising as remnants of conidiogenous cells carried away by conidia at secession or develop by splitting of conidial wall or from secretion of conidia or cell wall.

Acellular: Not of cellular nature. Developing from the activity of a non-living part of the cell. In this proposed classification, flagella have been placed as acellular appendages because under optical microscopy they look like solid outgrowths without protoplasm and in TEM studies there is no evidence of protoplasm or its connection. The simplest single oxonemic (tubule) flagellum is found in bacteria, whereas in the rest of the plant kingdom flagella consist of 2+9 Oxonemes, 2 central and 9 peripheral, becoming double in the basal region:- Kinetosome = blepharoplast, connected to the nucleus by a rhizoplast (Alexopoulos & Mims, 1979).

Aparietal: Not developing from the conidial wall.

Apical: Inserted at the apex.

Basal: Inserted at the base.

Blastic: Formed by inner or both inner and outer wall by apical wall building material.

Branched: Various types of branching can occur.

Cellular: Developing from a cell or any part of the cell and at least have protoplasm or its connection to the parent cell in early stages of development e.g., they can have cytoplasm or not.

Centric: Formed at the apex or base, generally along the main axis of the conidium.

Endogenous: Developing inside the conidiogenous cell.

Enteroblastic: Only the inner wall taking part in formation and appendages developing from the dissolution of the outer conidial wall by enzymatic activity or through a pore present in outer wall.

Enucleate: With no nuclei.

Excentric: Formed at the apex or base, generally away from the main axis of the conidium.

Exogenous: Developed outside the conidiogenous cell.

Holoblastic: Both inner and outer wall taking part in formation with an apical wall building apparatus (Minter *et al.*, 1983).

Hyaline:- Without pigmentation.

Inclusive:- Enclosing the conidia completely.

Lateral:- At the sides.

Motile:- Functions as a locomotive organ e.g., whiplash flagellum, tinsel flagellum.

Mucoid:- Mucilaginous in nature.

Mucoid hard:- Mucilaginous becoming hard,

Mucoid soft:- Mucilaginous remaining soft.

Multicellular:- Consisting of two or more cells.

Mutiple:- 2 or more formed.

Non-motile:- Not functioning as a locomotive organ.

Nucleate:- With a nucleus.

Parietal:- Developing from secretions or splitting of the cell wall itself.

Partial:- Enclosing the conidia partially.

Pigmented:- Coloured.

Setae:- Unicellular or multicellular, nucleate or enucleate appendages found on fruiting structures rather than spores.

Simple:- Unbranched.

Solitary:- Formed singly.

Unicellular:- Consisting of only one cell.

References

- Alexopoulos, C. J. and C.W. Mims. 1979. *Introductory Mycology* 3rd ed. John Wiley & Sons, New York, Chichester, Brisbane, Toronto. 632 pp.
- Conn. H.J., M.A. Darrow and V.M. Emmel. 1962. Staining procedure, used by the Biological Commission, 2nd. Edn. Within & Wilkins Co., Baltimore, USA., pp.289.
- Cunnell, G.J. 1958. On *Robillarda phragmitis*. sp. nov. *Trans. Br. mycol. Soc.*, 41: 405-412.
- Grove, W.B. 1935. British Stem and leaf fungi. 1. pp.448. CUP.
- Hawksworth, D.L., B.C. Sutton and G.C. Ainsworth. 1983. *Ainsworth & Bisby's Dictionary of the Fungi*. 7th ed. CAB, IMI Kew, Surrey, UK. 445 pp.
- Jones, E.B.G. and S.T. Moss. 1978. Ascospore appendages of marine Ascomycetes, an evaluation of appendages as taxonomic criteria. *Marine Biol.*, 49: 11-26.
- Leifson, E. 1930. A method of staining bacterial flagella and capsule together with a study of the origin of flagella. *J. Bact.*, 20: 203-211.
- Leifson, E. 1938. Staining of bacterial flagella. *J. Bact.*, 36: 656.
- Leifson, E. 1951. Staining, shape and arrangement of bacterial flagella. *J. Bact.*, 62: 377-389.
- Leifson, E. 1958. Timing of Leifson's flagella stain. *J. Bact.*, 33: 249.
- Morelet, M. 1968. De aliquibus in Mycologia Novitatibus. *Bull. Soc. Sci. Nat. Archeol. Toulon Var* no.175: 5-6.
- Nag Raj, T.R. 1973b. Genera Coelomycetum. X. *Ellisiella*, *Samukuta* and *Sakireeta*. *Can. J. Bot.*, 51: 2463-2472.
- Nag Raj, T.R. 1981. Coelomycetes Systematics pp 43-84. In: *The biology of conidial fungi*. Vol. 1. (Eds.) G.T. Cole and B. Kendrick. Academic Press, New York.
- Nag Raj, T.R. 1983. Genera Coelomycetum. XXI. *Strasseria* and two new anamorph genera, *Apostrasseria* and *Nothostrasseria*. *Can. J. Bot.*, 61: 1-30.
- Nag Raj, T.R. 1988. Genera Coelomycetum. XXIV. *Clithramia* anamorph gen. nov. *Can. J. Bot.*, 66: 903-906.

- Nag Raj, T.R., G. Morgan-Jones and B. Kendrick. 1972. Genera Coelomycetarum IV. *Pseudorobillarda* gen. nov., a generic segregate of *Robillarda* Sacc. *Can. J. Bot.*, 50: 861-867.
- Nag Raj, T.R., G. Morgan-Jones and B. Kendrick. 1973. *Pseudorobillarda* Nag Raj *et al.*, a later homonym of *Pseudorobillarda* Morelet. *Can. J. Bot.*, 51: 688-689.
- Pirozynski, K.A. and R.A. Shoemaker. 1971. Some Coelomycetes with appendaged conidia. *Can. J. Bot.*, 49: 529-541.
- Ponnappa, K.M. 1970. *Phyllostictina plumbaginis* sp. nov. *Mycologia*, 62: 395-401.
- Punithalingam, E. 1981a. Studies on Sphaeropsidales in culture III. *Mycol. Pap.* (CAB, IMI) Kew 149: 1-42 + 18 plates.
- Punithalingam, E. 1981b. Conidiation and appendage formation in *Tiarosporella paludosa* (Sacc. & Fiori) Höhnelt. *Nova Hedwigia*, 34: 539-566.
- Punithalingam, E. 1982. Conidiation and appendage formation in *Macrophomina phaseolina* (Tassi) Goid. *Nova Hedwigia*, 36: 249-290.
- Punithalingam, E. 1983. The nuclei of *Macrophomina phaseolina* (Tassi) Goid. *Nova Hedwigia*, 38: 339-367.
- Punithalingam, E. 1984a. The nuclei and conidial appendages of *Robillarda sessilis* (Sacc.) Sacc. *Nova Hedwigia*, 40: 45-58.
- Punithalingam, E. 1984b. The nuclei and 'conidial appendages' of *Chaetoconis polygoni* (Ell. & Ever.) Clem. *Nova Hedwigia*, 40: 99-112.
- Punithalingam, E. 1989a. Techniques for staining fungal nuclei and appendages. *Bot. J. Linn. Soc.*, 99: 19-32.
- Punithalingam, E. 1989b. The significance of nucleate conidial appendages in Coelomycetes. *Studies in Mycology*, 31: 113-121.
- Punithalingam, E. and J.E. Woodhams. 1982. The conidial appendage in *Phyllosticta* spp. *Nova Hedwigia*, 36: 151-198.
- Punithalingam, E. and J.E. Woodhams. 1984. A modified Leifson's flagella staining technique for revealing appendages in fungi. *Nova Hedwigia*, 40: 31-43.
- Punithalingam, E. and J.E. Woodhams. 1986. The conidial appendages and nuclei in *Pseudorobillarda* spp. *Nova Hedwigia*, 43: 485-498.
- Stewart, V.B. 1916. The leaf blotch disease of horse-chestnut. *Phytopath.*, 6: 5-19.
- Sutton, B.C. 1973. Ch.11. *Coelomycetes*, pp. 513-582. In: *The Fungi*, vol. IVA, (Eds) G.C. Ainsworth, F.K. Sparrow & A.S. Sussman. Academic Press, New York and London.
- Sutton, B.C. 1980. *The Coelomycetes* CAB, IMI Kew, Surrey, U.K.
- Sutton, B.C. and T. Kobayashi. 1969. *Strasseriosis* gen. nov., based on *Phellostroma tsugae*. *Mycologia*, 61: 1066-1071.
- Sutton, B.C. and J.M. Waterston. 1966. *Descriptions of Pathogenic fungi and Bacteria* no.88. (CAB, IMI) Kew, Surrey, U.K.
- Van der Aa, H.A. 1973. Studies in *Phyllosticta* 1. *Stud. Mycol. Baarn*, 5: 1-110.
- Whitney, H.S., J. Reid and K.A. Pirozynski. 1975. Some new fungi associated with needle blight of conifers. *Can. J. Bot.*, 53: 3051-3063.