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First record of *Pyxidiophora badiorostris* from France

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ABSTRACT

The authors, after introducing the systematics of *Pyxidiophorales* and comparing *Pyxidiophora* with *Kathistes* and *Mycorhynchidium*, describe *Pyxidiophora badiorostris* and provide an updated key to *Pyxidiophora* spp.

Key words: *Pyxidiophoraceae*, *Kathistes*, *Pyxidiophora*, *Pyxidiophora badiorostris*, key.

INTRODUCTION

Pyxidiophora Bref. & Tavel and *Mycorhynchidium* Malloch & Cain are the only two representatives of the *Pyxidiophoraceae* G.R.W. Arnold which, in turn, is the only family in *Pyxidiophorales* P.F. Cannon, an order related to *Laboulbeniales* Engler on account of a common association with arthropods for spore dispersion (Blackwell et al., 1988; Blackwell & Malloch, 1989; Blackwell, 1994): the hypophoretic spores of *Pyxidiophora* often possess an attachment apparatus (a dark, adhesive, apical or subapical area) which allows them to be carried by the mites linked to these insects (Lundqvist, 1980). Additional features, useful to delineate *Pyxidiophora*, are its long-beaked perithecia, the absence of an interascal tissue, a pseudoparenchymatous peridium, basally arranged, thin-walled, soon vanishing, clavate, 2-8-spored, sequentially maturing asci (Blackwell & Malloch, 1989). The spores are elongated (usually length/width ratio > 4), fusiform to clavate, hyaline, often transversely septate, clustered at maturity in a slimy mass above the beak apex, and the anamorphs are *Chalara*-like and *Thaxteriola* Speg. We refer to the key for other distinctive characteristics of this genus.

Kathistes Malloch & M. Blackw., in *Ophiostomatales* Benny & Kimbr., has many morphological features resembling *Pyxidiophora*, but, unlike the latter, lacks spores with an anchoring apparatus and has sporidiomata (immersed structures with a one-celled beak, associated with the ascomata, producing spore-like cells).

Pyxidiophora differs from *Mycorhynchidium* particularly in having perithecioid and long beaked rather than cleistothecioid ascomata. It includes about twenty species, mostly saprotrophic, growing on dung or decaying vegetable material, but a few [*P. asterophora* (Tul. & C. Tul.) Lindau; *P. lundqvistii* Corlett; *P. spinulorostrata* J. Webster & D. Hawksw.] are mycoparasites.

During one of our latest studies on herbivore dung in culture we have been lucky enough to observe several specimens of *Pyxidiophora badiorostris*, a curious and uncommon fimicolous pyrenomycetes, which we record as new for France.

MATERIALS AND METHODS

Dung was placed in a non-sterilised moist chamber, following the methods suggested by Richardson & Watling (1997) and Richardson (2001), slightly modified by Doveri (2004). We

examined the cultured material with a stereomicroscope (magnification x 7-45). The study of microscopic features has been carried out with Prior and Nikon Alphaphot-2 binocular microscopes with 10x eyepieces and 4x, 10x, 40x (phase contrast), 100x (oil immersion) lenses, using water as mounting medium, Melzer reagent, ammoniacal Congo red, and methyl blue in lactic acid as stains. The colour photos have been taken with a Nikon coolpix 4500 camera.

Spore size has been calculated on about fifty spores discharged from mature asci from several specimens of one collection.

Pyxidiophora badiorostris N. Lundq.

Original diagnosis: Lundqvist N., 1980. *Bot. Notiser* 133: 137.

Nonstromatica; perithecia semi-immersa, vulgo dispersa, 230-295 x 105-140 µm, globosa, longicollia, ± glabra; collum 95-145 x 21-28 µm, sursum decrescens, apice conico, valde transverse rugosum, badium. Peridium membranaceum, semipellucidum, flavidum, cellulis externis angulatis vel rotundatis, 10-23 µm diam.; cellulae externae colli elongatae, 10-24 x 4.5 µm, circa ostiolum longe acutae, cristis crassis, badiis, transversis, anastomosantibus obtectae. Paraphyses carentes. Asci trispori, 45-50 x 10-20 µm, subclavati, inamyloidei, cito deliquescentes. Sporae 38-52 x 4.5-5.5 µm, longe clavato-fusiformes, basim versus attenuatae, hyalinae; protoplastus 27-38 x 3-3.5 µm, utrinque acutus tum rotundatus, infra medium 1(-2)-septatus; paries sporarum praesertim apicaliter tumescens, appendicem basalem, 14-18 µm longam formans, maturitate corpore brunneo, applanato, rotundato, subapicali, 5-6 x 4.5 x 2 µm, instructus. Species coprophila.

Description

Perithecia non-stromatic, globose, membranous, yellowish or sometimes pale orange-yellowish, hairy, 120-150 µm diam., with a very long (180-260 µm), dark grey, crenulate, straight or slightly curved, somewhat pointed (conical at the apex), cylindrical beak, 15-20 µm diam. (25-30 µm at its base), sharply contrasting with the colour of the perithecial base, bearing a slimy globule at the tip.

Peridium thin, pseudoparenchymatous, of pale, thin-walled, polygonal to somewhat roundish cells, 12-20 x 8-17 µm, at the beak formed of parallel, very thick-walled, dark grey, cylindrical cells, 12-25 x 3-4 µm, converging and somewhat pointed at the apex. **Peridial hairs** particularly placed on the upper part of the perithecial base, 65-130 x 6-10 µm, smooth, hyaline, straight, fairly thick-walled (about 1 µm), 1-6-septate, tapering towards a pointed or slightly rounded tip, more curved to sinuous, sometimes ramified in the lower part. **Interascal tissue** absent. **Asci** numerous (several dozens), soon vanishing, about 60 x 12 µm, unitunicate, anamyloid, probably 3-spored (assumption based on the presence of 3-spore clusters above the beak tip, as the spore number inside the ascus is very hard to calculate), lacking an apical apparatus, with a flattened base. **Spores** fasciculate, 45-50 x 4-6 µm, hyaline, clavate-fusiform, straight or hardly curved, sometimes slightly asymmetrical, with a thread-like base and a fairly pointed apex, late 1-2-septate with septa placed in the lower part of the protoplast, thick-walled with walls swelling at maturity in the upper part, often with a subapical dark spot at maturity, containing greenish droplets, sheathed, particularly at the apex, by a gelatinous perisporium, often clustered at maturity in a slimy mass outside the beak tip. **Anamorph** not observed.

EXAMINED MATERIAL: FRANCE: 1) Soussouéou valley, Laruns commune (64), 1400 m, *MEN : 1547B, more than one hundred superficial to partly immersed, scattered or more often gregarious specimens, two to ten placed side by side on cattle dung in a moist chamber, crowded with arthropods, B. Coué, 11.08.05, Herbarium BC0104 and CLSM 013.05. 2) Col du Pourtalet, Laruns commune (64), 1900 m, *MEN : 1548B, some scattered specimens on cattle dung in a moist chamber, B. Coué, 16.08.05.

*MEN = Maille Élémentaire Nationale

RESULTS AND DISCUSSION

P. badiorostris was obtained from two samples of cattle dung collected at 1400-1900 m in the Pyrénées Atlantique. According to the literature *P. badiorostris* is obligatorily fimicolous, collected so far from domestic herbivore dung, particularly from cattle, in north- (Lundqvist, 1980; Richardson, *in litt.*) and south-temperate regions (Piontelli & Grixolli, 1997) as well as in the Mediterranean area (Barrasa & Moreno, 1982). Our French collections confirm its preference for cattle dung.

Macroscopically, *P. badiorostris* resembles *Kathistes* with the contrasting colour between the dark beak and the pale perithecial base (Malloch & Blackwell, 1990). Particularly, it is similar to *Kathistes fimbriata* (Barrasa & G. Moreno) Malloch & M. Blackw., but the latter, is distinguishable by its apically fimbriate, rather than conical, neck, formed of shorter and non-rugose cells, and by the somewhat smaller, 3-5 septate spores, which lack both a gelatinous sheath and swollen walls and become partly pale brown at maturity.

For the differences between *P. badiorostris* and all the other *Pyxidiophora* spp., see the key provided below.

The only real differences we have noticed between our collections and the original diagnosis (Lundqvist, 1980) are a taller beak and the presence of very long, straight, multiseptate peridial hairs in the former, whereas the hairs are scarcely mentioned in the latter and drawn as short, often curved, 2-septate at most. Richardson (*in litt.*) informs us that "he also found hairs like we describe in one of his samples, 135-150 μm long, with a slightly inflated lowest cell; the necks were also very long, up to 525 μm ". We think, however, these differences fall within the range of *P. badiorostris*, which is not fully known yet, as rare and often unidentified owing to its smallness.

According to Lundqvist (1980), who considered several genera (*Rhynchomyces* Sacc. & Marchal; *Mycorhyncus* Sacc.; *Treleasia* Speg.; *Copranophilus* Speg.; *Ascolanthanus* Cailleux; *Acariniola* T. Majewski & J. Wiśn.) to be synonymous with *Pyxidiophora*, the presence of an adhesive dark body at the distal spore end is not a constant feature in *Pyxidiophora*, so "there may be species that are unable to develop such a stage, but certainly others that regularly or only sporadically do it, [...]even the pigmentation and the septation may sometimes vary within the species, the former feature being particularly capricious.". On the basis of such a statement we attribute a scarce diagnostic value at specific rank to the dark spore spot and we do provide the following key, inspired by Hawksworth & Webster (1977), but revised and updated:

Updated world key to *Pyxidiophora* spp.

- | | |
|---|--|
| 1) Fimicolous species. | 2 |
| 1*) Non-fimicolous species | 10 |
| 2) Perithecial beak 350-700 μm tall, smooth, hyaline, of cylindrical cells, 30-60 x 10 μm . Perithecial base 100-120 μm diam., somewhat dark, embedded in a thin stroma. Asci 8-spored. Spores 60-70 x 5 μm , clavate, 0-1-septate, pointed at one end, blunt at the opposite. On cattle dung. | |
| | <i>P. spinuliformis</i> (Speg., 1909) N. Lundq. (1980) |
| N.B.: according to Lundqvist (1980) <i>Mycorhyncus subspinuliformis</i> Breton & Faurel (1967) could be a synonym of <i>P. spinuliformis</i> . | |
| 2*) Perithecial beak less than 350 μm tall, formed of smaller cells. Asci less than 8-spored. | 3 |
| 3) Perithecial beak 95-260 μm tall, rugose, "chestnut-brown" to dark grey, with cells 10-25 x 3-4.5 μm . Perithecial base 105-150 μm diam., non-stromatic, of yellowish cells, 10-23 μm diam. Spores 38-52 x 4.5-6 μm , clavate-fusiform, 1-2-septate, with a filiform base, often with a subapical dark spot at maturity. On cattle and horse dung. | |
| | <i>P. badiorostris</i> N. Lundq. (1980) |
| 3*) Perithecial beak not rugose, never so dark and so contrasting with the base colour. | 4 |
| 4) Spores more than 65 μm long, without a subapical spot. Perithecial base more than 100 μm diam. | 5 |
| 4*) Spores less than 65 μm long, with an inconstant subapical spot. Perithecial base less than 100 μm diam. | 6 |

- 5) Perithecial beak 255-265 µm tall. Perithecial base 110-125 µm diam., non-stromatic, hyaline to yellowish. Asci ?-spored. Spores fusiform, sometimes with a filiform base, 1-2-septate, 65-70 x 5.5-5.6 µm. On fox dung.
P. marchalii (Sacc. in Marchal, 1885) N. Lundq. (1980)
- 5*) Perithecial beak 250-320 µm tall. Perithecial base 120-160 µm diam., non-stromatic, reddish-brown. Asci 2-3-spored. Spores clavate-fusiform, with a strongly pointed base, 1-2-septate, 80-90 x 4.2-5 µm. On jackal dung.
P. bainemensis (Breton & Faurel, 1967) N. Lundq. (1980)
- 6) Spores 35-43 x 3.5-5 µm, fusiform, 1-septate, without a dark spot. Asci probably 4-spored. Perithecial beak 150-350 µm tall, of hyaline cells, 30 x 2-3.5 µm. Perithecial base 40-85 µm diam., hyaline, non-stromatic, with cells 7-10 µm diam. On dog, sheep, and deer dung.
P. microspora (D. Hawksw. & J. Webster, 1977) N. Lundq. (1980)
N.B.: according to Lundqvist (1980) *Mycorhyncus brunneocapitatus* D. Hawksw. & J. Webster [= *Pyxidiophora brunneocapitata* (D. Hawksw. & J. Webster, 1977) D. Hawksw. & J. Webster in Webster & Hawksworth (1986) could be a synonym of *Pyxidiophora microspora*, as the only effective difference is the presence of a dark spot in the spores of the former.
- 6*) Spores more than 45 µm long. 7
- 7) Perithecial beak 65-105 µm tall, of pale cells, 10-18 x 3-4.5 µm. Perithecial base 45-80 µm diam., non-stromatic, yellowish, with cells 7-18 µm diam. Asci not seen. Spores clavate-fusiform, with a filiform base, 1-2-septate, 48-57 x 3.7-7 µm, sometimes with a subapical spot. On reindeer, roe deer, hare, cattle, and horse dung.
P. arvernensis (Breton & Faurel, 1967) N. Lundq. (1980)
- 7*) Perithecial beak and its cells longer on average. 8
- 8) Perithecial beak 90-200 µm tall, of hyaline cells, 15-30 x 3-4 µm. Perithecial base 30-80 µm diam., stromatic, of hyaline cells, 7-10 µm diam. Asci probably 4-spored. Spores fusiform, with a pointed base, 1-septate, (45) 50-53 x 5-7 µm, occasionally with a subapical spot. On rabbit dung. *P. petchii* (Breton & Faurel, 1967) N. Lundq. (1980)
- 8*) Spores longer on average. 9
- 9) Perithecial beak 100-190 µm tall, of pale cells, 10-31 x 3-4 µm. Perithecial base 70-100 µm diam., stromatic, yellowish to pale ochraceous, of hyaline cells, 9-18 µm diam. Asci 3-7-spored. Spores clavate-fusiform, sometimes with a filiform base, 1-2-septate, 48-65 x 4-5 µm, with or without a subapical spot. On horse, sheep, and elk dung.
P. grovei (D. Hawksw. & J. Webster, 1977) N. Lundq. (1980)
- 9*) Perithecial beak 150-250 µm tall, of hyaline cylindrical cells, 35-37 x 5 µm. Perithecial base 35-60 µm diam., stromatic, hyaline to pale ochraceous. Asci ?-spored. Spores clavate-fusiform, with a pointed base, 1-septate, 53-65 x 6.2-7 µm, sometimes with a subapical spot. On jackal dung.
P. schotteriana (Breton & Faurel, 1967) N. Lundq. (1980)
- 10) Mycoparasitic species. 11
- 10) On other substrata 13
- 11) Perithecial beak 75-110 µm tall. Perithecial base 46-50 µm diam. Spores 1-septate, 47-53 x 5-5.5 µm, with a subapical spot. On *Inonotus obliquus* (Ach. ex Pers.) Pilát. *P. fusco-olivacea* (G.R.W. Arnold) N. Lundq. (1980)
- 11*) Perithecial beak shorter. Spores smaller.
- 12
- 12) Perithecial beak 17-23 µm tall. Perithecial base 33-40 µm diam. Asci 4-spored. Spores 1-septate, fusiform, 20-30 x 3-3.5 µm, with a filiform base, lacking a subapical spot. Associated with *Fusarium poae* (Peck) Wollenw.
P. lundqvistii Corlett (1986)
- 12*) Perithecial beak 40-60 µm tall. Perithecial base 70-90 µm diam. Asci 4-6-spored. Spores fusiform, with a pointed base, 1-septate, 25-35 x 5-6 µm, usually with a subapical spot. On *Nyctalis* and *Russula* spp.
P. asterophora (Tul. in Tulasne & Tulasne, 1860) Lindau (1897)
- 13) Upper peridial cells supporting sterile or fertile, coralloid outgrowths. 14
- 13*) Peridial cells lacking coralloid outgrowths. 15
- 14) Perithecial beak 38-56 µm tall, hyaline, of three different sequences of cells, the basal ones depressed. Perithecial base 50-80 µm diam., not stromatic, of hyaline cells, 9-16 µm diam., supporting some sterile, coralloid hairs, 19-27 µm long. Asci 3-spored. Spores fusiform, 0-4-septate, 48-57 x 5.5-7 µm, with a subapical spot. In galleries of bark beetles.
P. corallisetosa R. Kirschner (2003)
- 14*) Perithecial beak 100 µm tall, of the same colour as the base, of cylindrical, spinulose, serrate cells in rows. Perithecial base 110-125 µm diam., not stromatic, white, golden brown later, with cells 25-32 µm diam., upwards with coralloid conidiophores, up to 50 µm long. Asci 2-4-spored. Spores elongate-clavate, with a blunt apex and a pointed base, 1-septate, 47-50 x 5 µm, sometimes with a subapical spot. On submerged twigs.

P. spinulorostrata J. Webster & D. Hawksw. (1986)

- 15) Perithecial beak 85-155 µm tall, hyaline, of cylindrical, thin-walled cells in rows, 10-16 µm long. Perithecial base 70-100 µm diam., not stromatic, of hyaline cells, 8-13 µm diam. Asci 8-spored. Spores fusiform, with a filiform base, 1-3-septate, 50-75 x 5-8 µm, usually with a subapical spot. Associated with bark beetles.
P. kimbroughii M. Blackw. & T.J. Perry in Blackwell *et al.* (1986) **16**
- 15*) Spores smaller. Asci less than 8-spored.
- 16) Perithecial beak 90-200 µm tall, of hyaline cells, 15-30 x 3-4 µm. Perithecial base 30-80 µm diam., stromatic, of hyaline cells, 7-10 µm diam. Asci probably 4-spored. Spores fusiform, with a pointed base, 1-septate, (45) 50-53 x 5-7 µm, occasionally with a subapical spot. Caulicolous. *P. petchii* (Breton & Faurel, 1967) N. Lundq. (1980)
N.B.: We have placed *P. petchii* twice in key, because it is usually regarded as caulicolous (Breton & Faurel, 1967; Hawksworth & Webster, 1977; Lundqvist, 1980), but sometimes as fimicolous (Breton in Richardson, 1972). Richardson's record from Britain, however, was not recognised by Hawksworth & Webster (1977) to belong to *P. petchii*. **17**
- 16*) Spores smaller.
- 17) Perithecial beak 150-250 µm tall, of pale cylindrical cells, 20 x 3-4 µm. Perithecial base 100-125 µm diam., not stromatic, of hyaline to pale ochraceous cells, 10-15 µm diam. Asci 2-spored. Spores typically clavate, with a pointed base and a rounded apex, 1-septate, 40-49 x 4-5 µm, with or without a subapical spot. On dead herbaceous material. *P. caulicola* (D. Hawksw. & J. Webster, 1977) N. Lundq. (1980)
- 17*) Perithecial beak 80-266 µm tall, of hyaline cylindrical cells, 8-12 x 3-4 µm. Perithecial base 44-91 µm diam., not stromatic, of hyaline to pale ochraceous cells, 5-15 µm diam. Asci 2-3-spored. Spores fusiform, 1-2-septate, 33-46 x 5-6.5 µm, with a subapical spot. In galleries of bark beetles. *P. cuniculicola* R. Kirschner (2003)

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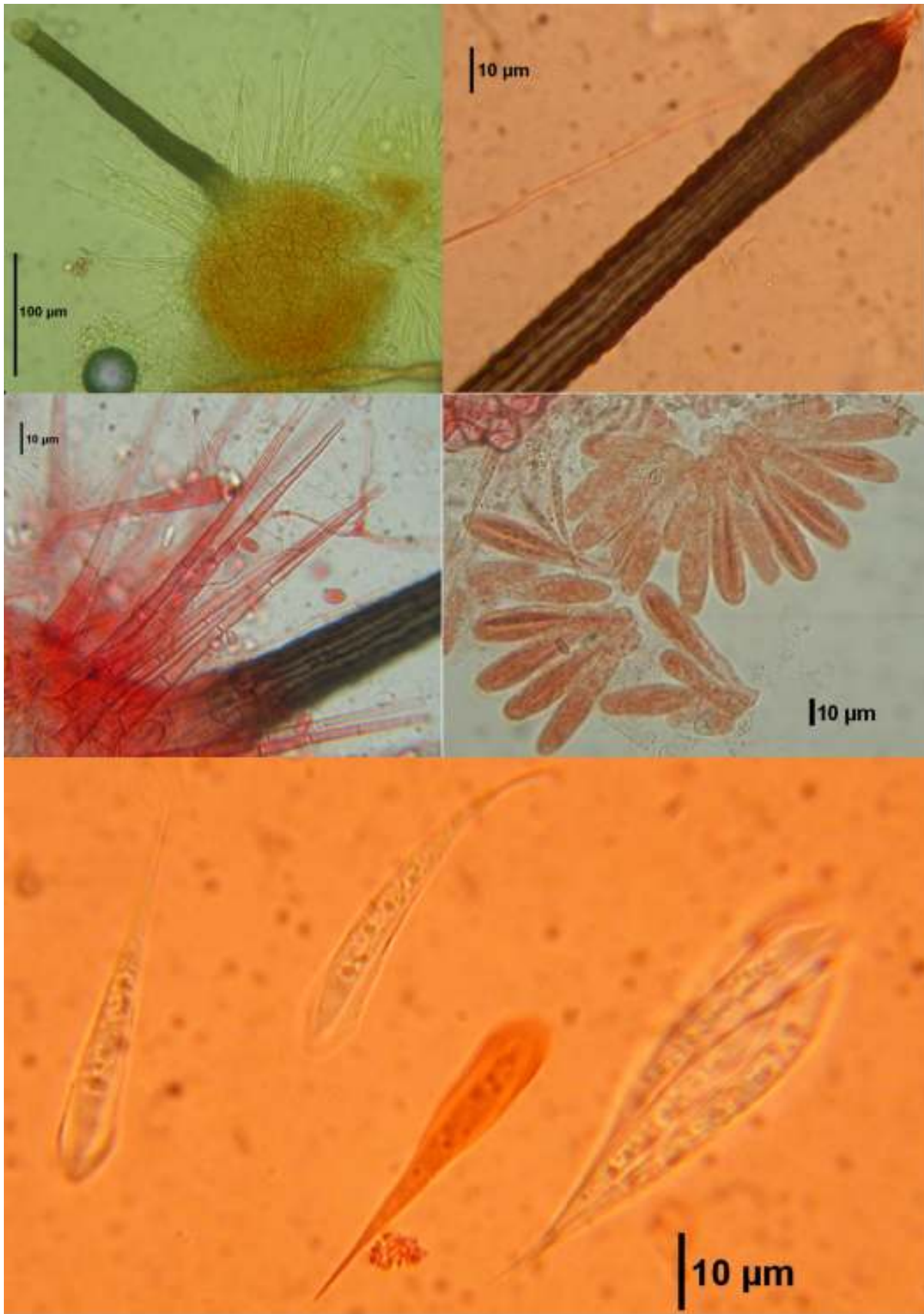
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LEGEND OF THE FREEHAND DRAWING

a = spores; b = spore cluster



LEGEND OF THE COLOUR PLATE
1 : Perithecium (Melzer reagent)
2 : Apex of the beak (Ammoniacal Congo red)

- 3 : Hairs and base of the beak (Ammoniacal Congo red)
- 4 : Asci (Ammoniacal Congo red)
- 5 : Spores and spore cluster (Ammoniacal Congo red)