Pilocintractia gen. nov. (Ustilaginomycetes)

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Abstract. Based on molecular sequence data and morphological characters of the sori a new genus, *Pilocintractia,* is described for *Cintractia fimbristylidicola.* The importance of sterile fungal filaments between mature spores for the classification of smut fungi is discussed. Presence of gelatinised fungal filaments between spores is a useful tool for separating *Pilocintractia* from other genera of the *Cintractia* sensu lato group.

Key words: *Cintractia* s. lat., Cyperaceae, *Fimbristylis, Pilocintractia,* smut fungi, taxonomy

Introduction

The genus *Cintractia* s. lat. was revised by Piepenbring *et al.* (1999). Based on molecular and morphological data it was split into four genera: *Cintractia* Cornu, s. str., *Gymnocintractia* M. Piepenbr., Begerow & Oberw. (= *Ustanciosporium* Vánky, emend. M. Piepenbr.), *Leucocintractia* M. Piepenbr., Begerow & Oberw., and *Stegocintractia* M. Piepenbr., Begerow & Oberw.

The unusual and unexpected placement of *Cintractia fimbristylidicola* Pavgi & Mundk. in the phylograms, published by Piepenbring *et al.* (1999: 493-494, Figs 37-38) was striking. This induced me to try to find morphological features to support its isolated place.

Materials and Methods

Materials studied: Dried specimens of *Cintractia axicola* (Berk.) Cornu on *Fimbristylis dichotoma* (L.) Vahl, Madagascar, Tamatave Prov., N of Fenerive, Manankinany River, *ca* 2 km from the coast, 26 Oct 1983, L. Johnsson 2029 (H.U.V. 11 313); *Cintractia fimbristylidicola* on *Fimbristylis complanata* Link, India, Orissa State, Ganjam, Chatrapur, 30 Aug 1904, E.J. Butler (isotype, H.U.V. 15 462); on *F. bisumbellata* (Forssk.) Bubani, Australia, Northern Territory, near Darwin, at Jabiru Road, near 'Window on the Wetlands', 12°39.566' S, 131°19.440' E, 13 Mar 1998, R.G. Shivas (H.U.V. 18 953 ex BRIP 25 453); on *F. spadicea* (L.) Wahl, Costa Rica, Prov. Guanacaste, La Cruz, Puerto Soley, alt. 1 m, 19 Oct 1992, M. Piepenbring 471 (H.U.V. 15 754); El Salvador, Dept. Usulután, Puerto El Triunfo, CREM, alt. *ca* 1 m, 22 Aug 1995, R. Esquivel *et al.* (BPI 749 399).

Mature sori were removed from the host plants and placed on microscope slides in a droplet of lactophenol with cotton blue. Several droplets of distilled water were added and the solution with the sori heated 2-3 times to boiling point. If needed, some additional droplets of distilled water were added. Under a stereo microscope, and using a razor blade, thin sections were made from each softened sorus after placing it between white, compact plastic foam. The sections were transferred into a small droplet of lactophenol with cotton blue on a microscope slide. A thin ring of pure lactophenol was drawn around the small droplet and the whole covered with a cover glass. The thin ring of lactophenol kept the slices together so they were not dispersed under the whole cover glass. This mounting method, recommended by Savile (1971: 42, 1987: 66), is especially useful when a small quantity of spores of type specimens are studied. By gently heating to boiling point, air bubbles were eliminated from the preparation, which was then studied by light microscopy (LM).

Results and Discussion

Sori of *Cintractia fimbristylidicola* (Fig. 1) are black, globoid to ovoid, compact, hard, not powdery, 0.5-1.5 × 1-2 mm in diameter, produced around some nuts in the spikelets, scattered in the inflorescence. Serial longitudinal and transverse sections (LS, TS) of the sori revealed the unique

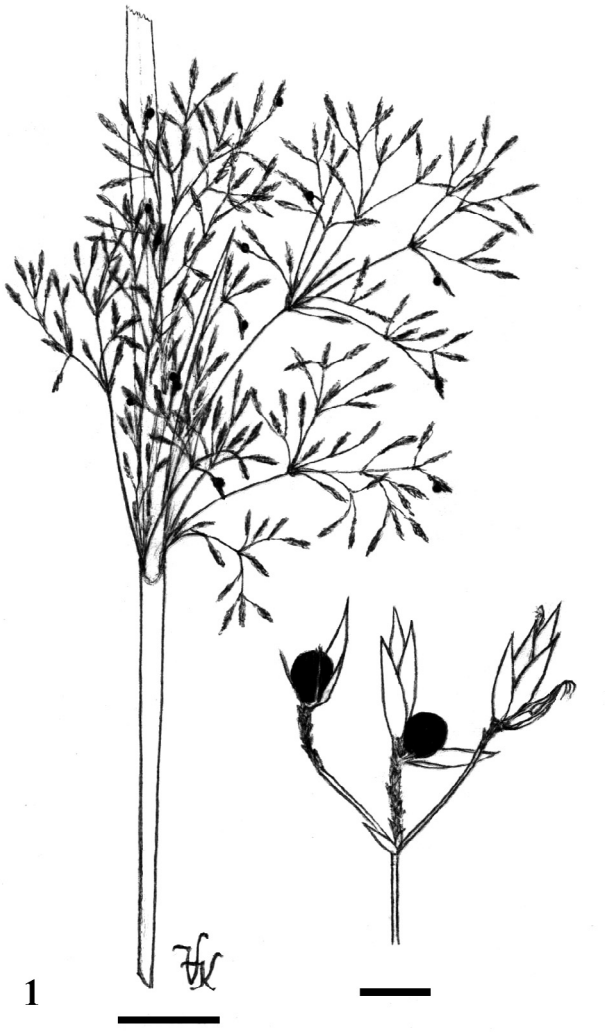
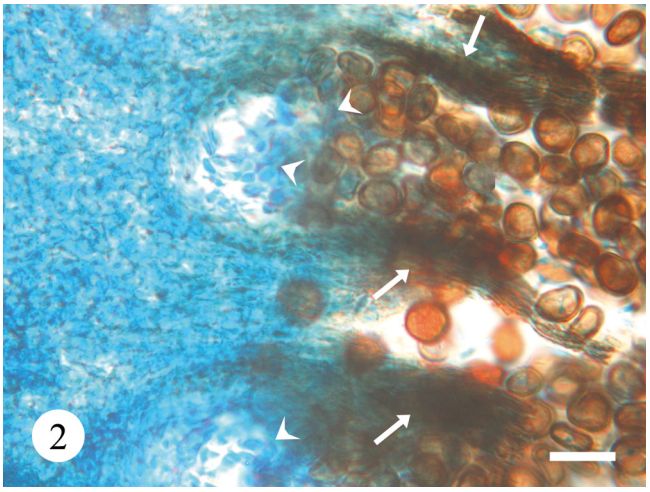


Fig. 1. Sori of *Pilocintractia fimbristylidicola* as black, globoid, hard, not powdery spore masses around some nuts of an inflorescence of *Fimbristylis complanata* (type). Habit, and enlarged some spikelets with two sori. Bars = 1 cm for habit, 2 mm for the detail drawing

morphology and histology in a three-dimensional view, as well as their probable development. In the centre of the sori, the stunted nuts are permeated by fungal hyphae. On the surface of these nuts, spores are produced successively in cup-shaped pockets (Fig. 2), in LS U-shaped, in TS circular, delimited by sterile, yellowish brown, fungal tissue, composed of tightly packed, uniformly thin, *ca* 1 µm wide, elongated cells. The content of these cells is not stained by cotton-blue, similarly to the content of the spores which have already brown coloured walls. The wall of the cup-shaped pockets is very thin, undifferentiated on the bottom, well developed at the side of the pockets (Fig. 2, arrows). It is *ca* 100-200 µm high, 15-40 µm wide (in LS appearing as fascicles), distally narrowing into a rounded edge. The pockets are arranged radially, open on their distal part, filled with young spores at the bottom, mature ones towards the periphery. On the bottom of the pockets, the cells of the sporogenous hyphae swell, and transform into young, polyangular, hyaline spore initials (Fig. 2, arrowheads) that soon become rounded, pigmented, mature spores. Distally, above the pockets, a thick mass of mature spores is formed, mixed with ramifying, hyaline, sterile hyphae with a gelatinised wall (Fig. 4). In addition to the relatively low sporogenous pockets, loose fascicles of hyaline, elongated, sterile fungal filaments develop from the surface of the nuts. They are arranged radially, first between the sporogenous pockets, and continuing beyond them into the spore masses (Fig. 3). Such fascicles are relatively few. More often, the filaments are solitary, interweaving the whole mass of mature spores (Figs 3-4). The filaments are 1.5-5 µm wide, with elongated, thin protoplasm and a relatively thick, gelatinised wall of variable thickness. Presence of gelatinised filaments between mature spore masses is a unique feature within the smut fungi. No trace of a peridium on the surface of the sori could be detected.

For comparison, a sorus of *Cintractia axicola* (Fig. 6), the type species of the genus, was studied. Around the host tissues of a thin, floral branch (Fig. 6, arrowheads), a 150-200 µm thick, brown layer is formed ('sterile' stroma), composed of

Fig. 2. Section of a sorus of *Pilocintractia fimbristylidicola* (type). U-shaped sporogenous pockets embedded in a stroma of thin-walled fungal cells with blue coloured, protoplasm rich content. The lateral wall of the pockets (arrows) is formed of brown, tightly packed, thick-walled, elongated cells. On the bottom of the pockets developing spores are seen (arrowheads), gradually maturing towards the opened end of the pocket. Bar = 20 µm. **Fig. 3.** Mature spores of *Pilocintractia fimbristylidicola* on *Fimbristylis spadicea* from the periphery of a sorus (Costa Rica, leg. M. Piepenbring 471, H.U.V. 15 754). Between two groups of spores there is a fascicle of hyaline, elongated, sterile, fungal filaments with protoplasm (blue) and thick, hyaline, gelatinised wall. Similar, but solitary filaments can be seen also between the spores (arrows). Bar = 20 µm. **Fig. 4.** Mature spores of *Pilocintractia fimbristylidicola* (type). Between the spores abundant sterile, fungal filaments (arrows), with elongated protoplasm (blue) and thick, hyaline, gelatinised, indistinguishable wall are present. Bar = 20 µm. **Fig. 5.** Spores of *Pilocintractia fimbristylidicola* (type), seen in SEM. The spore surface is irregularly verruculose-reticulate. Between and adhering to the spores, remnants of dried, sterile fungal filaments of various thickness can be seen (arrows). On one of these filaments the pattern of a spore surface is impressed (arrowheads). Bar = 5 µm. **Fig. 6.** TS of a sorus of *Cintractia axicola* on *Fimbristylis dichotoma* (Madagascar, H.U.V. 11 313), with radially arranged, U-shaped, sporogenous pockets (arrows) on the periphery of a thick, brown stroma of fungal cells, surrounding a floral branch (arrowheads). Distally of the sporogenous pockets the semiagglutinated mass of mature spores can be seen. Bar = 100 µm. **Fig. 7.** Mature spores of *Cintractia axicola* on *Fimbristylis dichotoma* from the periphery of a sorus (Madagascar, H.U.V. 11 313). Between the spores sterile, fungal filaments (arrows) with blue coloured protoplasm and thin, not gelatinised wall can be seen. Bar = 20 µm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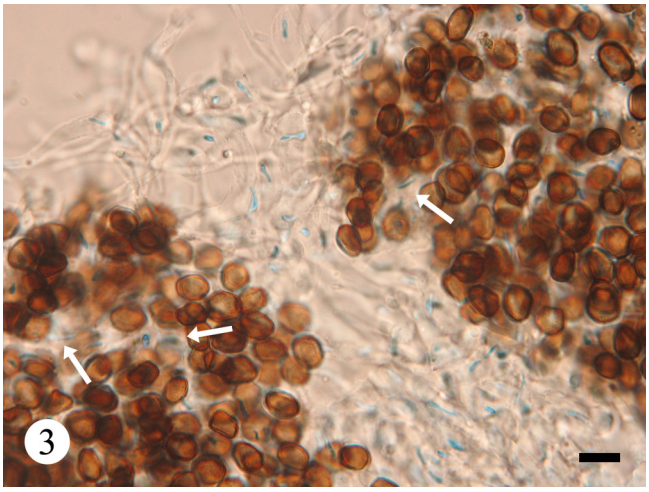
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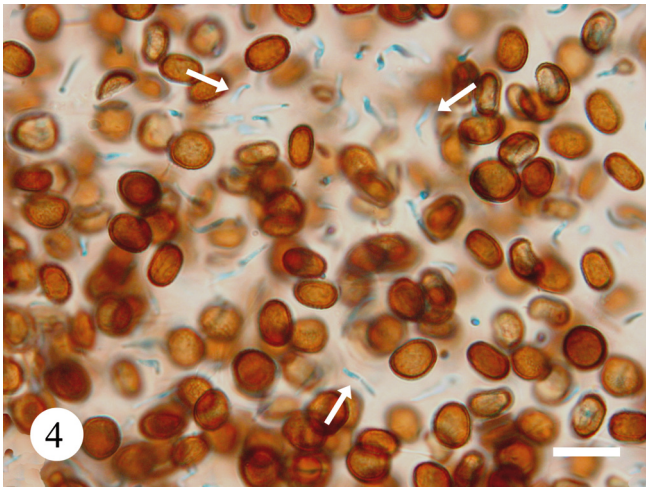
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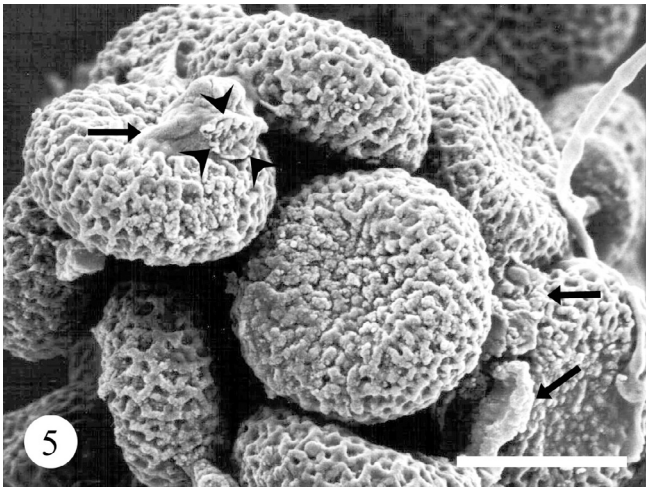
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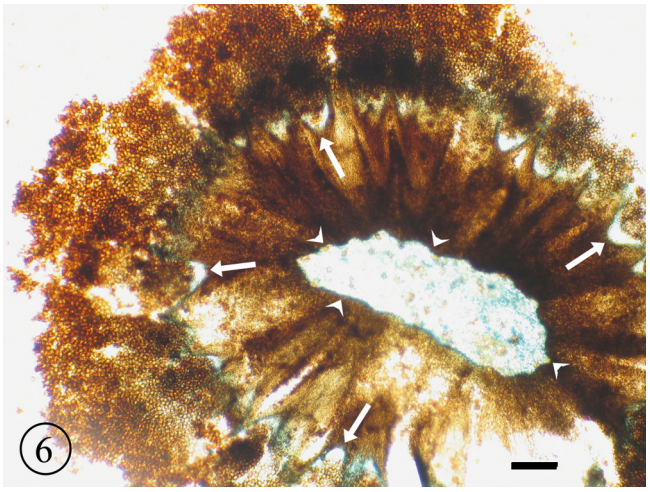
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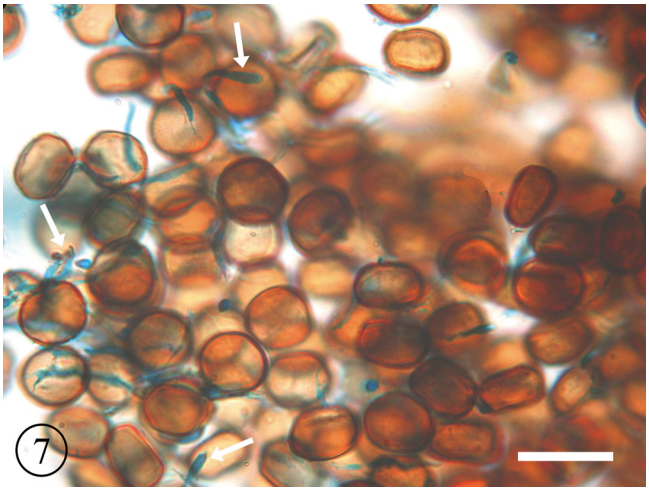
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irregular, pigmented, thin walled fungal filaments, arranged radially. Cup shaped sporogenous pockets are embedded in the distal part of this stroma (Fig. 6, arrows), with 50-200 µm high lateral walls, that gradually narrow into a subacute edge. The structure and colour of the cells forming these lateral walls are similar to those of the stroma. Distally from the pockets is a thick layer of mature spores (Fig. 7), interwoven by long, *ca* 0.5 µm thick, ramifying fungal filaments, with a sharply delimited, not gelatinised wall (Fig. 7, arrows). In young sori, the spore masses are covered by a thick fungal peridium. It is formed of barely distinguishable, sterile fungal cells, with a gelatinised, thick wall and some remnants of protoplasm. In young sori the peridium is white, soon turning dirty brown and flaking away in mature sori. Scattered between the spore masses are radially arranged fascicles of sterile fungal filaments of variable thickness. These connect the stroma with the peridium and are composed of the same kind of filaments that are present between the spores. The spore mass is rather compact but loose and powdery on the surface of the mature, naked sori.

Comparing the soral structure of *Cintractia fimbristylidicola* with that of *C. axicola,* the essential difference, excepting the absence of a peridium in *C. fimbristylidicola,* lies in the quality of the network of fungal filaments between the spores. Gelatinised filaments between the mature spores in *C. fimbristylidicola* are unique within the smut fungi. They are the reason mature sori of *C. fimbristylidicola* are not powdery, whereas those of *C. axicola,* with non-gelatinised filaments, are powdery on their surface. In addition, the ornamentation of the teliospores of *"Cintractia" fimbristylidicola* is not typical for the genus *Cintractia,* as was also remarked by Piepenbring *et al.* (1999: 496).

The stroma is a basal fungal layer in the sori of several smut genera, parasitising Cyperaceae and Juncaceae. It is on the surface of host plant tissues, beneath the spore masses. From the stroma sporogenous hyphae and often also sterile fungal elements develop. The anatomy, development, and function of the stroma is not sufficiently studied and understood. Often it is called 'sterile' stroma. Its value in the delimitation and characterisation of genera is therefore questionable. In the few observations I made on sori of *C. fimbristylidicola,* in some cases such a layer is present, in others it is lacking, perhaps consumed for spore building and forming of the hyaline filaments.

In the phylograms of Piepenbring *et al.* (1999, Figs 37-38), *Dermatosorus cyperi* Vánky (type on *Cyperus* aff. *cellulosoreticulatus* Böckeler, Venezuela) and *Trichocintractia utriculicola* (Henn.) M. Piepenbr. (type on *Rhynchospora gigantea* Link, Brazil) stand closest to *Cintractia fimbristylidicola. Dermatosorus,* with six known species, is a uniform group. The sori are produced in some ovaries of plants in Cyperaceae (*Bulbostylis, Cyperus, Eleocharis, Fimbristylis, Schoenoplectus,* and *Scirpus*) and are covered with a peridium. The reticulate spores are agglutinated into spore balls, which are surrounded by a cortex of sterile cells. *Trichocintractia* is a unispecific genus (comp. Piepenbring 1995). The sori develop in some spikelets of *Rhynchospora* (Cyperaceae) and are covered with a persistent, thick peridium, open on its top. From the soral base groups of long, thick-walled sterile cells radiate into the basal part of the powdery spore mass. *C. fimbristylidicola* has hardly any common morphological character with *Dermatosorus,* and in many respects differs also from *Trichocintractia.* The most important common character between *C. fimbristylidicola* and *T. utriculicola* is the presence of sterile fungal filaments between the spores. However, these are morphologically (and functionally?) different. In *T. utriculicola* they are restricted to the basal parts of the sori only. Furthermore, in *C. fimbristylidicola* there is no peridium, and the spores are not powdery but compact. This is because the spore masses are interwoven by the thin, hyaline, sterile fungal filaments with gelatinised walls, which hold the spores together even in the rehydrated, thin sections of the sori, which are elastic, not powdery. The filaments certainly do not belong to saprotrophic fungi, so often present between spores of smut fungi originating from wet, usually tropical countries, or when specimens were not dried within a few days.

Fascicles of chains of sterile fungal cells between spore masses also occur in species of *Farysia* and *Farysporium,* where they play an important role in the dispersal of the powdery spores or spore balls. The role of the hyaline, sterile filaments or fascicles of filaments in *C. fimbristylidicola* is not known. It seems that their role is to hold the spore masses together, close to the host plants, often growing in restricted, wet places. In this way, the sori fall off the perennial host plants in one piece, and remain on the ground until the next vegetation period. Until the new shoots flower, the sori are almost certainly decomposed into single spores by microbial activity, the thin, fungal, gelatinised filaments with protoplasm being consumed. The 'liberated' single spores beneath the host plant, under optimal conditions (temperature, humidity, presence of air, etc.) germinate about the flowering period of the young host plants, infecting them probably, through the stigmata, giving rise to new sori in some flowers.

In conclusion, *Cintractia fimbristylidicola* shows a combination of morphological characters which differentiates it from all other genera of the smut fungi including the *Cintractia* s. lat. group and related genera (comp. Vánky 2002). These characters support its isolated molecular biological position and make its separation under a new genus necessary:

Pilocintractia Vánky, **gen. nov.**

Sori *in nonnullis floribus spiculae eiusdem plantarum fam. Cyperaceae (Fimbristylis), circum nuces corpora nigra, globoidea, dura, sine peridio, cum vel sine stromate sed cum filamentis sterilibus formantes.* ***Teliosporae*** *pigmentiferae (brunneae, sine violaceo vel aurantiaco-flavido colore), agglutinatae, filamentis fungalibus, hyalinis, sterilibus, protoplasmaticis et pariete gelatinosis intertextae. Teliosporae in fundis cupulatis radiatim distributis (in sectionibus longitudinalibus litterae U-formibus), pariete ex cellulis fungalibus, pigmeniferis, sterilibus arcte contiguis et pariete crasso, non gelatinoso formatis, radiantibus a parte basali sori in massam sporarum, productae.* ***Germinatio***

teliosporarum *cum phragmobasidiis. Cellulae basidiorum basidiosporas vel hyphae producentes.* ***Interactio*** *inter plantam nutrientem et parasitum eius per hyphas intracellulares, matrice electronice opaca tunicatas.* ***Septa*** *matura sine poris.*

Typus generis: *P. fimbristylidicola.*

Sori in some flowers of a spikelet of plants in Cyperaceae (*Fimbristylis*), forming black, globoid, hard bodies around the nuts, without peridium, with or without stroma, but with sterile filaments. **Teliospores** pigmented (brown, without violet or orange-yellow tint), agglutinated, interwoven by hyaline, sterile fungal filaments with protoplasm and gelatinised wall. Teliospores produced in radially arranged, cup-shaped (in longitudinal section U-shaped) pockets with a wall formed of pigmented, tightly packed, sterile fungal cells with a thick, not gelatinised wall, radiating from the basal part of the sori into the spore masses. **Germination of teliospores** results in phragmobasidia. The cells of the basidia produce basidiospores or hyphae. **Host-parasite interaction** by intracellular hyphae, coated by an electron-opaque matrix. Mature **septa** are poreless. Typus generis:

Pilocintractia fimbristylidicola (Pavgi & Mundk.) Vánky, **comb. nov.**

Basionym: *Cintractia fimbristylidicola* Pavgi & Mundkur, Indian Phytopathol. **1**: 108, 1949 (as '*fimbristylicola*'). — Type on *Fimbristylis complanata,* India, Orissa State, Ganjam, Chatrapur, 30 Aug 1904, E.J. Butler (HCIO 1438; isotypes BPI 171 548, H.U.V. 15 462!).

Sori (Fig. 1) in some flowers of an inflorescence surrounding the ovaries, black, globoid to ovoid, compact, hard, not powdery, 0.5-1.5 × 1-2 mm, evident between the spreading glumes. Spore mass blackish brown, compact. Peridium lacking, basal stroma present or lacking. On the surface of the nuts, spores are produced successively in cup-shaped pockets (Fig. 2), delimited by sterile, yellowish brown, fungal tissue, composed of tightly packed, uniformly thin, *ca* 1 µm wide, elongated cells. These, in longitudinal sections, appear as 15-40 µm wide, *ca* 100-200 µm long, compact fascicles, distally narrowing into a conical tip. Distally from the pockets there is a thick layer of mature spores, permeated by hyaline, sterile hyphae (Fig. 2). Hyaline, radially arranged, long, loose fascicles of elongated, sterile, fungal filaments are also present between the spore masses (Fig. 3) but usually the filaments are solitary, interweaving the whole mass of mature spores. The filaments are 1.5-5 µm wide, with thin, elongated content of protoplasm (Fig. 4), and a relatively thick, more or less gelatinised wall, variable in thickness. **Teliospores** (Figs 4-5) flattened, in side view elliptic, no hyaline appendages, 6-8 µm wide, in plane view elliptic, ovate or subpolygonally irregular, 8.5-10.5 × 9-12(–13) µm, yellowish brown; wall even, 0.5-0.8 µm thick, in LM finely granular-verruculose, spore profile smooth to finely wavy, in SEM warts partly confluent forming a fine to rough, irregular reticulum. **Spore germination** (Piepenbring 1996: 32 & 34, Plate 8) results in phragmobasidia. The cells of the basidia produce basidiospores or hyphae.

On Cyperaceae: *Fimbristylis bisumbellata* (Forssk.) Bubani, *F. complanata* Link, *F. spadicea* (L.) Wahl; S. Asia, Australia, C. & S. America.

Etymology: *Pilo-* from Latin *pilus* = hair, referring to the sterile fungal filaments interweaving the spores, and *Cintractia,* the genus from which it was segregated.

Key to *Cintractia* and some related genera

1	Sori without peridium	**2**
1*	Sori with peridium (at least in young stage)	**5**
2	Spore balls present	**3**
2*	Spore balls absent (except for some *Ustanciosporium* species)	**4**
3	Pigmented spore balls mixed with hyaline spores	***Heterotolyposporium***
3*	Pigmented spore balls not mixed with hyaline spores	***Tolyposporium***
4	Mature spores mixed with gelatinised, fungal filaments; spore mass compact	***Pilocintractia***
4*	Mature spores not mixed with filaments; spore mass ± powdery	***Ustanciosporium***
5	Peridium present in young sori only; stroma lacking	***Stegocintractia***
5*	Peridium well developed; stroma with sporogenous pockets present	**6**
6	Old peridium white; spores with coarse warts forming typical rows	***Leucocintractia***
6*	Old peridium brown; spores finely warty	***Cintractia**** s. str.

*Except for *C. amazonica*, in which both peridium and stroma are lacking.

Pilocintractia has a character combination that differentiates it from all related genera. The presence of gelatinised, sterile, fungal filaments between the mature spores is especially typical for the genus. These filaments are responsible for the hard, compact sori when dried, and elastic or mucilaginous sori when wet. One or several of the characters of *Pilocintractia* may be found in related smut genera. For instance, cup-shaped pockets, in which the spores develop are also present in species of *Cintractia* and *Leucocintractia. Pilocintractia* shares the host plant genus *Fimbristylis* with some species of *Cintractia* (3 spp.), *Dermatosorus* (1 sp.), and *Moreaua* (2 spp.). Single, globoid, blackish brown sori in the flowers of Cyperaceae occur in several genera (i.e. in many species of *Anthracoidea, Cintractia, Ustanciosporium*) but they are often covered by a peridium and the spore mass is powdery or semiagglutinated with a powdery surface. Finally, molecular sequence data differentiates *Pilocintractia* from the related genera *Cintractia, Gymnocintractia* (= *Ustanciosporium*), *Leucocintractia, Stegocintractia,* and *Tolyposporium* (comp. Piepenbring *et al.* 1999).

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