
***Cintractiellaceae* fam. nov. (Ustilaginomycetes)**

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A new family, *Cintractiellaceae*, is proposed to accommodate the two peculiar smut fungi in the genus *Cintractiella*, *C. diplasiae* and *C. lamii*.

Key words: *Cintractiella*, *Cintractiella diplasiae*, *Cintractiella lamii*, *Cyperaceae*, Hypolytreae, Mapanioideae, smut fungi, taxonomy

Introduction

The classification of smut fungi and allied taxa has changed radically since publication of a paper by Bauer *et al.* (1997). Application of modern methods (ultrastructural studies and molecular analyses), has opened the way towards a better, phylogenetical classification, in which surprising relationships have become evident. The results were published, *i.a.*, in: Begerow *et al.* (1997/1998), Swann *et al.* (1999), Piepenbring *et al.* (1999), Bauer *et al.* (1999a,b, 2001), Vánky (1999b, 2000, 2001a), Begerow *et al.* (2000) and summarised by Bauer *et al.* (1998, 2000), Vánky (1999a, 2001b, 2002a,b).

The classification of the smut fungi is, however, not settled. Molecular analyses, classical, morphological investigations, studies of the parasites correlated with the systematics of their host plants, as well as new discoveries will certainly increase the number of higher taxa of smut fungi (*Microbotryales* and *Ustilaginomycetes*). At present, the *ca.* 1450 known "classical" smut fungi (those possessing teliospores), are classified into two classes, three subclasses, 8 orders, 26 families and 77 genera (Vánky, 2002b). To these, the new family for the genus *Cintractiella* Boedijn should also be added.

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The genus *Cintractiella*

Leaves of a *Hypolytrum* sp. (*Cyperaceae*, subfam. Mapanioideae, tribe Hypolytreae), with hypophyllous galls from which bundles of branches arise, were collected by Lam, in New Guinea, in 1920. The material was preserved in alcohol at Herb. Bogoriense (BO). K.B. Boedijn investigated the material thoroughly and described it as a new smut fungus in a new genus: *Cintractiella lamii* Boedijn, 1937: 370. Since that time the fungus was never collected again. Unfortunately, the type specimen in Bogor was lost; only the empty glass vessel and label is present. The description of this species, reproduced from the original (comp. Vánky, 2002b: 188) is:

***Cintractiella lamii* Boedijn, 1937: 370.**

Type: INDONESIA, Irian Jaya [= New Guinea], Prauwenbivak, on *Hypolytrum* sp., September 1920, Lam 1026 (BO).

Sori usually hypophyllous, appearing first as hemispherical, pustular galls composed of compact parenchymatous host cells and parasitic hyphae, bursting through the epidermis. From these galls bundles of straight or curved, 1-1.5 mm wide, 5-20 mm long, somewhat flattened "branches" arise, invested by pale green, scale-like leaves. From the branches black, hard, cylindrical bodies grow out measuring 0.5-0.75 × 8-12 mm, composed of agglutinated spore masses surrounded by a thin (8-12 µm), subhyaline or pale yellowish membrane composed of firmly interwoven, indistinct, 1-2.5 µm thick hyphae with gelatinous walls. The parasitic hyphae from the basal pustules invade the branches and in the axils of the last pair of scale-like leaves emerge from the host tissue and form a compact ring around the growing point. The spores are differentiating within this mass of sporogenous hyphae. Only the threads on the periphery are unaltered, of which the covering layer around the spore mass develops. The spores are at first small, colourless, thin-walled, irregular, often elongated and wholly filled with protoplasm. Gradually they increase in size, becoming globose, and the thickened wall takes a brown colour. Through the continuous spore formation and the fact that the spores strongly adhere to each other a column is formed, which projects far above the branch, and which is also firmly held together by the covering fungal membrane. *Spores* when mature single, globose, 29-36 µm in diameter, dark brown, provided with a single germ pore; wall 3-4 µm thick, minutely reticulate. *Spore germination* is not known. *Parasitic hyphae* strictly intercellular forming coiled, lobed or often branched haustoria into the host cells. (For illustrations see Boedijn, 1937: 369, 371, or Vánky, 2002b: 189).

Host: *Hypolytrum* sp.

Known distribution: Indonesia (Irian Jaya). Known only from the type collection.

Remarks: For a long time *Cintractia lamii* was considered an "interesting and enigmatic fungus", or even was suspected not to be a fungus. For example, when Kukkonen and Gjørnum (1977: 96) studied the *Cintractia* species of *Scleria*, they commented also on *Cintractiella lamii* writing: "this organism is not a smut fungus, but its place in the system should be more logically outside the Fungi - for example, among the epiphytic members of the family *Lejeunaceae* in *Hepaticae*". In the 2nd edition of the "Illustrated Genera of Smut Fungi" (Vánky, 2002b: 188), I wrote: "based on and judged from the accurate description and illustrations of Boedijn, I am rather convinced that *Cintractiella lamii* is a parasitic fungus, which could well be also an unusual smut fungus with isolated systematic position. Several of its characters remind of smut fungi in the *Cintractiaceae* but the presence of haustoria excludes it from this group."

From this impasse, an ingenious discovery (Piepenbring, 2001) brought progress in the enigma of *Cintractiella*. Namely, there is a further, curious, little known smut fungus, *Ustilago diplasiae* Henn., described on *Diplasia karataefolia* L.C. Rich. (*Cyperaceae*, subfam. Mapanioideae, tribe Hypolytreae), collected by Ule in Brazil. Groups or single sori of the type specimen have been deposited in several herbaria. Isotypes have been distributed in Ule, Mycotheca brasiliensis no. 2 (HUV 3625). In the United States National Herbarium (US), Smithsonian Institution, Washington, M. Piepenbring found a herbarium sheet (US 1497566) of *Diplasia karataefolia* from Venezuela, with witches' broom-like, dense groups of sori of *Ustilago diplasiae* around the basis of the floral peduncles. This material permitted Piepenbring (2001) an accurate description of the sori of this fungus, the discovery of the similarities with *Cintractiella lamii*, the transfer of *U. diplasiae* into *Cintractiella*, and the completion of the generic description of *Cintractiella*.

Cintractiella diplasiae (Henn.) M. Piepenbr., 2001: 120. (Figs. 1-3)

≡ *Ustilago diplasiae* Henn., 1904: 155.

≡ *Ustanciosporium diplasiae* (Henn.) M. Piepenbr., 2000: 335.

Type: BRAZIL, Amazonas, Rio Negro, São Joaquim, on *Diplasia karataefolia* L.C. Rich., January 1902, E. Ule 2779, isotypes in Ule, Mycotheca brasiliensis no. 2, HUV 3625!

Sori around the basal part of stunted floral peduncles of the inflorescence, forming witches' broom-like, dense groups of sterile, branching, modified, adventitious spikelets. Single sori (Fig. 1) correspond to sterile spikelets, are more or less cylindrical, ca. 1.5-2.5(-3) × 7-15 mm, surrounded by scale-like floral envelopes. Their distal part is filled with the black,

agglutinated or semi-powdery mass of spores. *Spores* (Figs. 2, 3) variable in shape and size, more rarely subglobose or ovoid, mostly ellipsoidal or slightly irregular, often elongated, $20\text{-}30 \times 22\text{-}44 \mu\text{m}$, reddish-brown; wall even, $(2.5\text{-})3\text{-}3.5\text{-}(4) \mu\text{m}$ thick, including the $1.5\text{-}2 \mu\text{m}$ high, blunt, rather densely situated, rarely confluent warts of variable sizes. *Spore germination* is unknown. *Host-parasite interaction* by intracellular haustoria.

Host: Diplasia karataefolia L.C. Rich.

Known distribution: South America (Brazil, Venezuela).

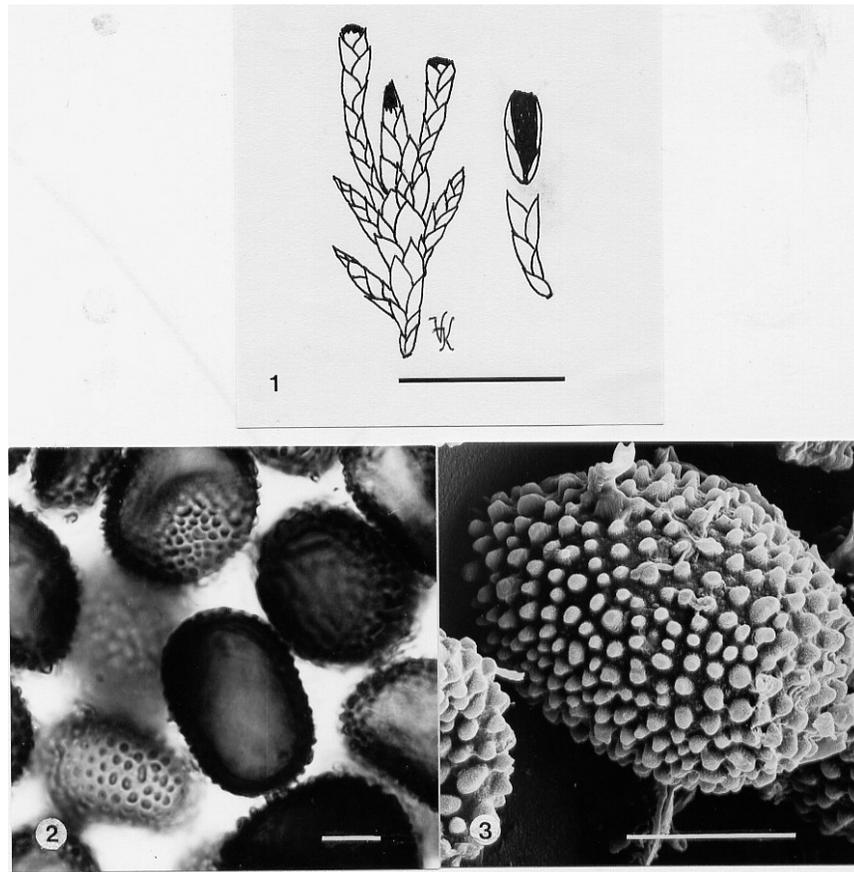


Fig. 1. Sori of *Cintractiella diplasiae* on the distal part of some sterile spikelets, originating from a branching witches' broom, produced adventitiously on floral peduncles of *Diplasia karataefolia* (from isotype). To the right a sterile spikelet of which the distal part is removed and opened, showing the black, semi-agglutinated mass of spores enclosed by scale-like floral envelopes. Bar = 1 cm.

Figs. 2, 3. Spores of *Cintractiella diplasiae* in LM and SEM (from isotype). Observe the different size of the warts in the SEM picture. Bars = $10 \mu\text{m}$.

Remarks: Study of hand-cut pieces from the basal part of a group of sori of *C. diplasiae*, in lactophenol with cotton blue, revealed abundant presence of globoid, lobed, ramified or coiled haustoria in parenchymatous cells between fascicles of vessels. The presence of haustoria not only confirms the relationship between *C. diplasiae* and *C. lamii*, discovered by Piepenbring (2001), but has also taxonomic significance. The presence of a "thin, evanescent layer of ramified, partly collapsed fungal hyphae enveloping the mass of teliospores", mentioned by Piepenbring (2001: 119), could not be confirmed from the material at hand. In contrary, fragments of septate, ramifying hyphae of variable thickness (0.5-2.5 μm) and colour (hyaline or pale yellowish-brown) were present between the spores. I strongly doubt that these belong to *C. diplasiae*. This, partly because of the heterogeneity of these hyphae, partly because in one case, even a hyaline, ovoid conidium, measuring *ca.* 1 \times 2 μm , was seen on a short sterigma on such a hyaline, hyphal fragment. I observed several times hyphae of saprobic fungi between spores of tropical smuts with opened sori, even if the specimens were dried within 2-3 days. A few, yellowish-brown, weakly-ornamented spores between mature spores in some slides, I interpret rather as immature spores than "sterile cells".

Piepenbring (2001), in her excellent paper, showed the relationship between *Cintractiella diplasiae* and *C. lamii*, and based on the common characters, gave an emended generic description of *Cintractiella* which I am reproducing in slightly altered form.

Cintractiella Boedijn, 1937: 268; emend. M. Piepenbring, 2001: 120.

Sori in adventitious spikelets on vegetative or generative organs of the host, occurring in groups ("witches' brooms"). *Spore mass* black, first agglutinated, enclosed in the distal part of sterile spikelets, at maturity exposed at the opened tip of the spikelet. *Spores* develop embedded in a hyaline, sporogenous fungal matrix, when mature solitary (not forming spore balls), relatively large and thick-walled, pigmented (reddish-brown, without tint of orange-red), ornamented. *Host-parasite interaction* by intracellular haustoria. On *Cyperaceae* (subfam. Mapanioideae).

Typus generis: C. lamii Boedijn.

Remarks: Cintractiella, with the enumerated, unusual characters and character-combination, cannot be included in any of the 26 known families of the smut fungi. Apparently, it is closest to the *Cintractiaceae* (including eight genera on *Cyperaceae* and/or *Juncaceae*), within the order *Ustilaginales*. However, members of the *Cintractiaceae* have intracellular hyphae and no haustoria. *Cintractiella* differs also from the four genera of the *Ustilentylomataceae*, within the order *Microbotryales* (on *Cyperaceae*,

Juncaceae and *Poaceae*) in which the spores are yellow or orange-red tinted, and haustoria are lacking.

To accommodate *Cintractiella*, I am proposing a new family. When molecular data and ultrastructure features become available, it is possible that description of suprafamiliar taxa will be necessary to classify *Cintractiella* into the correct place of the new, phylogenetic classification of smut fungi.

***Cintractiellaceae* Vánky, fam. nov.**

Membrum classis *Ustilaginomycetes*, cum characteribus generis *Cintractiella* Boedijn. *Sori* in spiculis adventitiis ad organa et vegetativa et generativa plantae nutrientis. *Massa sporarum* nigra, in matrice fungali hyalina, sporogena formata in parte distali spicularum sterilium inclusa. *Sporae* singulae, relative magnae, atro-pigmeniferae, ornamentis praedictae. *Interactio inter hospitem et parasitum* per haustoria intracellularia. In plantis familiae *Cyperaceae* (subfam. *Mapanioideae*).

Typus familiae: Cintractiella Boedijn, Bulletin du Jardin Botanique de Buitenzorg, Serie 3, 14: 268, 1937; emend. M. Piepenbr., Perspectives in Plant Ecology, Evolution and Systematics 4: 120, 2001.

Member of the *Ustilaginomycetes* having the characters of the genus *Cintractiella* Boedijn. *Sori* in adventitious spikelets on vegetative or generative organs of the host. *Spore mass* black, formed within a hyaline, sporogenous fungal matrix, enclosed in the distal part of sterile spikelets. *Spores* single, relatively large, darkly pigmented, ornamented. *Host-parasite interaction* by intracellular haustoria. On *Cyperaceae* (subfam. *Mapanioideae*).

Type of the family: Cintractiella Boedijn.

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